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Effect of masking organoleptic properties of fat on diet self-selection by the sparid *Diplodus puntazzo*

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Abstract

Fish are able to select a balanced diet according to their nutritional needs by choosing among incomplete feeds or even pure macronutrients. However, the relevance of both the organoleptic properties of diet and the postingestive signals that they produce remains unclear. Thus, sharpsnout seabream were allowed to select between diets containing different edible oils with their organoleptic properties masked by using gelatine capsules. Fish were fed capsules of two different colours so that they could associate the capsule colour with its corresponding postingestive effect. The longitudinal experiment included a first phase during which the fish were adapted to consuming the gelatine capsules. In a second phase, the fish were challenged with two different encapsulated diets: one comprising a complete diet containing fish oil and the other a fat-free diet. Sharpsnout seabream showed a preference for the fish oil capsules (3.8 ± 1.1 g kg^{-1} body weight (BW), 66.8% of total intake) over the fat-free capsules, showing that they were able to associate the colour of the capsule with their nutritional content through postingestive signals. After that, the fish were challenged to select between the capsules containing the fish oil diet and capsules containing a vegetable oil (linseed or soybean), in which case they showed no preference between diets (2.4 ± 0.3: 2.1 ± 0.5 g kg^{-1} BW of fish oil versus linseed oil capsules and 2.2 ± 0.2: 1.8 ± 0.6 g kg^{-1} BW of fish oil versus soybean oil capsules), indicating that the fatty acid composition of the different oils was not sufficient to affect dietary selection through postingestive signals. So, in conclusion, when orosensorial information from food is absent, the fish are able to select between diets at a macronutrient level by using postingestive information. However, this information is not sufficient for distinguishing between diets that differ in the type of oil used.

KEY WORDS: *Diplodus puntazzo*, edible oils, feeding behaviour, fish, nutrient selection, taste

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Introduction

Marine fish meals and oils have long been the main ingredients in feeds manufactured for intensively farmed carnivorous fish, although recent years have seen an intensified search for alternative protein and lipid sources. Several plant protein sources and oils have been investigated as potential candidates to replace fish products, although both have properties that make them inferior to fish products. For example, plant oils are poor in the n-3 highly unsaturated fatty acids (n-3 HUFAs) that are essential nutrients for carnivorous marine fish (Montero *et al.* 2003; Mourente *et al.* 2005).

Much of the research developed in this area focuses on analysing the effects on fish nutrition and final product quality of replacing fish oil by vegetable oils. For this reason, a variety of sources and mixtures of vegetable oils (soybean, corn, sunflower, rapeseed, palm, olive, borage, etc.) and their replacement levels have been tested in different cultured species (Turchini *et al.* 2009). Nevertheless, few data exist in fish literature on the dietary preferences for oils, especially in marine fish. This information would be of interest because such knowledge might enable feeding strategies to be adapted to the feeding behaviour of the fish, with the corresponding economic and environmental consequences (Geurden *et al.* 2007).

Depending on their origin, fats contain fatty acids of different chain lengths and degrees of unsaturation, both of these factors may affect feed intake in mammals (Friedman *et al.* 1983; Langhans & Scharrer 1987; Lawton *et al.* 2002). In fish, recent studies have shown that European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus* *mykiss*) are capable of discriminating between two diets containing different feed oils (fish versus vegetable oils), and it has been suggested that different feed oils might exert different postprandial feedbacks on appetitive feeding behaviour in these fish species (Luz *et al.* 2004; Geurden *et al.* 2005).

When allowed simultaneous access to feeds of unbalanced nutrient composition, using multiple on-demand feeders, fish of several species will consume a diet that matches their nutritional needs and feeding habits (Sánchez-Vázquez *et al.* 1998, 1999; Aranda *et al.* 2000, 2001; Rubio *et al.* 2004). However, on-demand feeding protocols are not ideal for investigation of the nutritional signals (orosensory or postingestive) that may underlie the dietary choice. This can be tested by masking the flavour by putting the feed in gelatin macrocapsules, which can be entirely swallowed by the fish (Ruohonen & Grove 2001; Rubio *et al.* 2003; Almaida-Pagán *et al.* 2006). With this technique, animals can be provided with diets of different compositions but similar physical and organoleptic characteristics. The selection observed would imply postingestive and/or postabsorptive mechanisms in which orosensory factors do not intervene.

The present study has as its main objective to determine the ability of sharpsnout seabream, a marine fish of interest for Mediterranean aquaculture, to select among complete encapsulated diets containing different oils (fish, linseed or soybean). Possible selection patterns were studied in individually kept fish, which were offered the choice between two colours of capsule (with similar palatability characteristics) and challenged to discriminate between experimental diets (with different composition) in a three phase trial.

Materials and methods

Animal housing

The experiments were carried out at the Aquaculture facilities of the Institute of Agricultural and Food Research and Development (IMIDA) in San Pedro del Pinatar (Murcia, Spain). Sharpsnout seabream (*Diplodus puntazzo*, Cetti 1777) from Valle Ca Zuliani Societá Agricola S.R.L. (Pila di Porto Tolle, Italy) were acquired and then acclimatized to the experimental installations in 93 L cylindrical-conical tanks, which formed part of a closed saltwater circuit (salinity: 37 g L^{-1}; NO_2^-<0.1 mg L^{-1}; NO_3^-<0.1 mg L^{-1}; NH_3 < 0.5 mg L^{-1}; pH 7.7). They were fed a commercial diet for gilthead seabream (*Skretting* D2 Excel). The water circuit was equipped with a biological filter, an ultraviolet lamp and a thermostat to maintain the temperature at 21 ± 1 °C. However, after 3 weeks of experimentation, a malfunction occurred in the thermal control system of the water circuit which resulted in a decrease in water temperature during the following 3 weeks (see Fig. 2). The water flow was regulated to maintain dissolved oxygen at 70% of the saturation level. The animals were kept in experimental conditions for 90 days (from October to January), with a natural photoperiod (37°50′N, 0°46′W) and were fed experimental diets once a day, six times per week.

Encapsulated diet production

The experimental diets were packaged in No. 4 gelatin capsules with a volume of 0.2 mL (Roig Farma S.A., Barcelona, Spain), using a semi-automatic encapsulator (Tecnyfarma, Miranda de Ebro, Spain). Different diets were prepared, each with the same proportion of each of the macronutrients and differing only in the component that represented the fat percentage (see Table 1). These diets included a complete standard diet (D), containing cod liver oil and soybean oil in a ratio of 3 : 1; a fish oil diet (F), containing the fish oil as the only lipid source; a fat-free diet (FF), containing cellulose; a soybean oil diet (S) and a linseed oil diet (L). All diets included a 5 : 1 mixture of casein and gelatin as protein source, dextrin as source of carbohydrates, a vitamin and mineral complex (Dibaq Diproteg, S.A., Fuentepelayo, Spain), sodium alginate as a binding agent and cellulose as a filler (Table 1). Two colours of capsule (orange and yellow) were used, as these colours are best accepted by fish (Almaida-Pagán *et al.* 2006). All capsules used in the same tank were first stored inside a plastic bag for at least 10 days, to mix any possible outside contaminants that would allow the fish to distinguish the capsule content.

Samples of each encapsulated diet were taken for a composition analysis based on AOAC procedures (AOAC 1997) (Table 1). The moisture content was determined by oven drying at 105 ± 1ºC for 24 h, until a constant weight was reached. The gross protein amount was calculated using the Kjeldahl method, with a nitrogen to protein conversion factor of 6.25. The gross fat amount was determined by the ethyl ether extraction method, using a Soxhlet extractor. Overall ash was determined by incineration at 450 ± 2 °C for 24 h in a muffle furnace, until a constant weight was obtained. Finally, the nitrogen-free extract (NFE) was calculated as 100 minus the sum of the gross protein, gross fat, ash and moisture. All analyses were performed in triplicate.

Table 1 Ingredients of diets and capsule composition by proximate analysis (n = 3)

Diets	D	F	L	S	FF
Capsule content ingredients (g kg^{-1} of dry weight)					
Casein	391.4	391.4	391.4	391.4	391.4
Gelatin	78.3	78.3	78.3	78.3	78.3
Cod liver oil	104.1	138.8	–	–	–
Soybean oil	34.7	–	–	138.8	–
Linseed oil	–	–	138.8	–	–
Dextrin	130.5	130.5	130.5	130.5	130.5
Minerals and Vitamins	20.0	20.0	20.0	20.0	20.0
Cellulose	155.0	155.0	155.0	155.0	293.8
Sodium alginate	46.0	46.0	46.0	46.0	46.0
$CaCO_3/CaPO_4$	40.0	40.0	40.0	40.0	40.0
Proximate analysis of the capsules (g kg^{-1} of dry matter)					
Dry matter	900.1	910.3	890.9	880.5	920.1
Gross protein	570.9	550.6	580.9	560.2	570.5
Ether extract	110.5	120.7	120.6	130.3	10.1
NFE	240.2	260.3	220.9	240.4	350.6
Ash	60.4	50.4	50.6	60.1	50.8
Gross energy (MJ kg^{-1})[1]	22.2	22.5	22.6	22.5	19.9

D, complete standard diet; F, complete fish oil diet; L, complete linseed oil diet; S, complete soybean oil diet; FF, fat-free diet; NFE, nitrogen-free extract.

[1] Calculated from the macronutrient percentage mean using the following energy coefficients: 23.6 MJ kg^{-1} for protein; 38.9 MJ kg^{-1} for fat; and 16.7 MJ kg^{-1} for carbohydrates (Miglavs & Jobling 1989).

Experimental design

Twelve sharpsnout seabream (initial weight: 330.8 ± 45.0 g, expressed as mean ± SD) were used, each individually housed. The same animals were subjected to three consecutive experimental phases for a total experimental duration of 90 days, as shown in Fig. 1. In each phase, the fish were fed two types of capsule, one of each colour (yellow or orange), so that they could associate the capsule colour with its corresponding postingestive effect. In Phase 1 (control phase, 29 days), the two colours of capsule contained the same standard diet (D), with a composition similar to that selected by this species in previous self-selection studies (Almaida-Pagán *et al.* 2006) (Table 1). No colour preferences were found in Phase1. Besides, to prevent any possible influence of capsule colour preferences, which has previously been observed in this species (Almaida-Pagán *et al.* 2006, 2008), the capsule colour and the type of diet relationship were fixed for each fish however modified among fish. In Phase 2, which lasted 32 days, the least selected colour of capsule for each fish was used to package a complete diet, with fish oil as the only lipid source (F), whereas the most selected colour of capsule contained a diet whose fat proportion was replaced with cellulose, representing a fat-free diet (FF). In this manner, the animals were induced to select between the two most extreme diets as far as the lipid content was concerned. Finally, in Phase 3 (28 days), the 12 sharpsnout seabream were divided into two experimental groups (see Fig. 1). One group, consisting of 7 animals, was fed one colour of capsule containing the F diet, and another colour of capsule with the linseed oil diet (L). The other group, with 5 animals, was fed F capsules and soybean oil

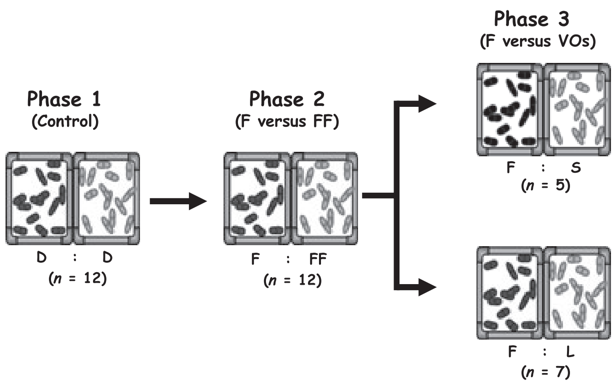

Figure 1 Schematic drawing of the different experimental phases, according to the main objectives and feeding conditions. The same fish were subjected to every experimental phase. In all phases, capsules of two colours (yellow and orange) were provided separately in two floating containers. Phase 1 (29 days): adaptation to the complete encapsulated diet (D), to determine the daily energy intake and possible capsule colour preferences. Phase 2 (F versus FF, 32 days): ability to discriminate between diets with very high differences in lipid composition. Phase 3 (F versus vegetable oils diets, 28 days): ability to discriminate between different oil sources.

diet (S) capsules. The same colour–diet relationship that had been used for each fish during Phase 2 was maintained, replacing the capsules containing the FF diet in each fish with those containing the corresponding vegetable oil diet (L or S). The objective in this phase was to determine whether sharpsnout seabream are capable of selecting between diets which only differed in the origin of the oil used and, therefore, their fatty acid composition, in the absence of any difference in orosensory factors.

The capsules were always made available in the tank at the same time (12:00–12:30 pm), with each colour placed in a different floating feeder. After 30 min, the uneaten capsules were collected and counted to calculate the feed intake.

The animals were weighed at the beginning and the end of the experiment.

Data analysis

The gross energy intake (GE) calculated for the fish was based on capsule intake and food composition, using the following coefficients: 23.6 MJ kg^{-1} for protein; 38.9 MJ kg^{-1} for fat and 16.7 MJ kg^{-1} for carbohydrates (Miglavs & Jobling 1989). GE was expressed as kJ kg^{-1} BW/day (BW = initial body weight).

In this study, the same animals were successively subjected to the different experimental situations, and the selection pattern from each phase acted as a control for the following one. For this purpose, the capsule intake for each phase was represented with the capsule intake of the same colour for the previous phase.

Statistical analyses were performed to determine any difference in average energy intake and capsule ingestion between experimental phases. A Student's *t*-test for dependent samples, with a level of significance of 0.05, was used to compare means from 1) energy intake at the end of Phases 2 and 3 for groups F versus L (n = 7) and F versus S (n = 5); 2) food intake (in grams) of F capsules and fat-free capsules (FF) in the different weeks of Phase 1 (corresponding colour) and Phase 2 (n = 12); and 3) the food intake (in grams) of F capsules and vegetable oil capsules (L or S) in the last week of Phase 2 and the different weeks of Phase 3.

These analyses were performed using the SPSS statistical package, version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

During the early part of Phase 1, energy intake gradually increased; although, after 3 weeks, temperature decreased because of a malfunction of the control unit, and this had an effect on intake (Fig. 2). Energy intake was relatively low during the latter part of Phase 1 and early in Phase 2. Once temperature had been restored to 21 °C, energy intake increased and was almost 90 kJ kg^{-1} BW during the latter stages of Phase 2 (Fig. 2).

In Phase 3, when the sharpsnout seabream were divided into two experimental groups (F versus L group and F

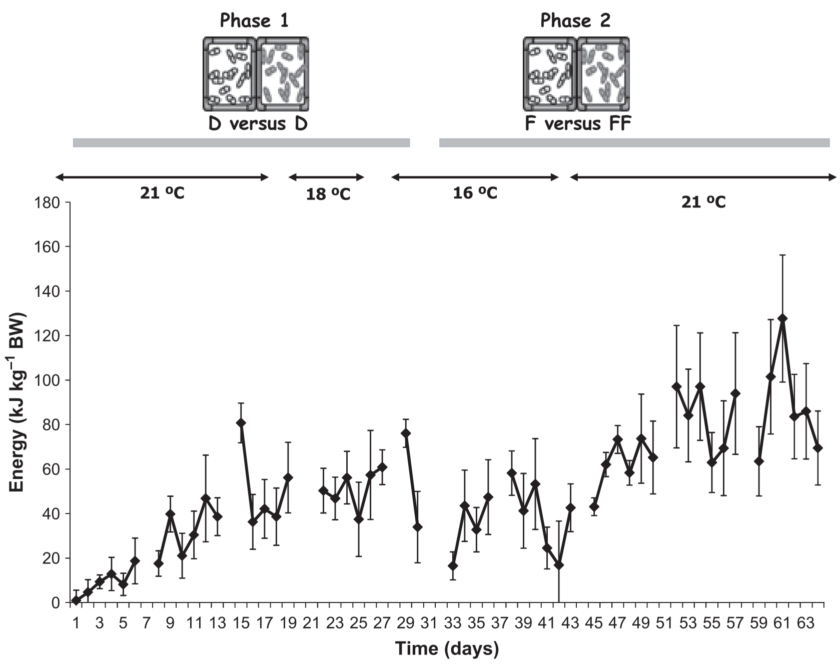

Figure 2 Average daily energy intake (kJ kg^{-1} body weight) of sharpsnout seabream fed the same standard complete diet (D versus D, Phase 1) and a complete fish oil diet *versus* a fat-free diet (F versus FF, Phase 2) in differently coloured capsules placed separately in two floating containers. Values represent the mean ± SEM of 12 fish. Horizontal arrows represent temperature values throughout the experimental phases.

versus S group), the average energy intake showed no significant variations from the last week in Phase 2 (Fig. 3). The animals in the F versus L group reached an average intake of 113.0 ± 30.0 kJ kg^{-1} BW at the end of Phase 2 and 101.7 ± 59.0 kJ kg^{-1} BW (P = 0.324) in Phase 3. Something similar occurred in the F versus S group, with an average intake of 89.0 ± 27.0 kJ kg^{-1} BW and 77.1 ± 44.0 kJ kg^{-1} BW, respectively (P = 0.653) (Fig. 3).

In Fig. 4, the food intake means (g kg^{-1} BW) along the three weeks of Phase 1 and the five weeks of Phase 2 are depicted, applying in Phase 1 the same colour–diet relationship as in the following phase so that they could be used as a control. In Phase 1, the 12 sharpsnout seabream were fed orange and yellow capsules, each containing the same standard diet (D) while, in Phase 2, the 12 fish were induced to select between capsules with a fish oil diet (F) and capsules with a fat-free diet (FF). A progressive increase in food intake would be expected, but the decrease in environmental temperature (earlier mentioned) prevented it. Even though the response of the animals to the dietary change could be clearly seen (Fig. 4). They modified their behavioural

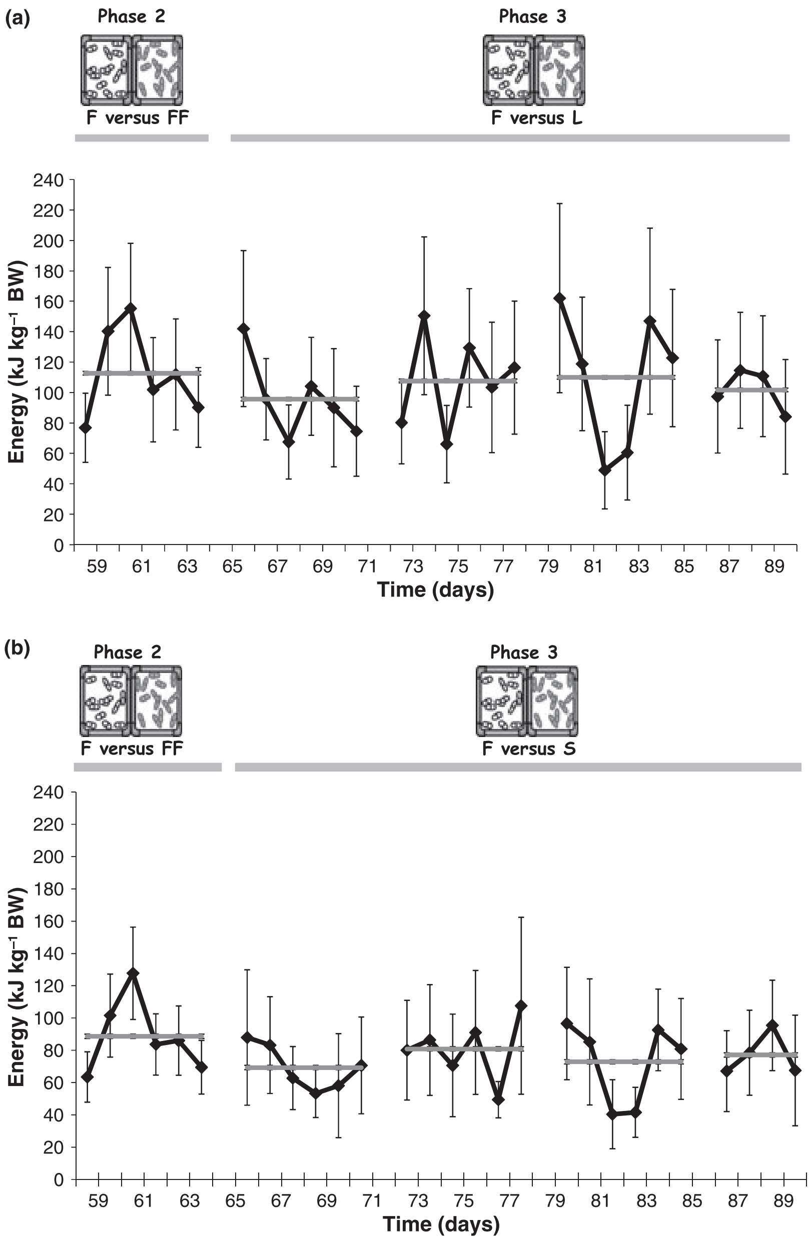

Figure 3 Average daily energy intake (kJ kg^{-1} body weight) of sharpsnout seabream fed capsules of two colours containing fish oil diet (F) *versus* a fat-free diet (last week of Phase 2) or F *versus* vegetable oil diet (Phase 3): (a) group provided F and linseed oil diet (L) and (b) group provided F plus soybean oil diet (S). Values represent the mean ± SEM of 7 fish (a) and 5 fish (b).

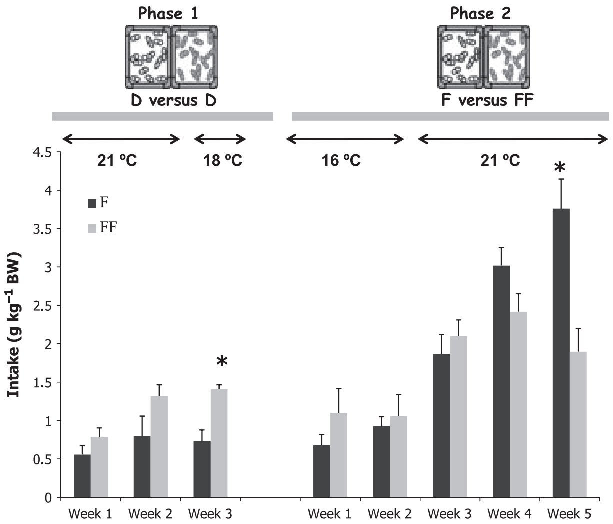

Figure 4 Food intake means expressed as g kg^{-1} of body weight for sharpsnout seabream fed two colours of capsule containing both a standard complete diet (D) (Phase 1) and a complete fish oil diet (F) *versus* a fat-free diet (FF) (Phase 2) during the different weeks of each phase. Data from Phase 1 and Phase 2 were represented in the same way, to analyse changes in capsule intake patterns between them. Values represent the mean ± SEM of 12 fish for each week. Asterisks represent the statistical differences between the two capsule intake means for a given week. Horizontal arrows represent temperature values throughout the experimental phases.

pattern, and their intake of F capsules reached the 66.8% of total grams intake (over the 33.3% of the same-coloured capsules in Phase 1).

Figure 5 represents the capsule intake means for both experimental groups (F versus L group and F versus S group) in the four weeks of Phase 3. To appreciate the change in the selection pattern for these fish, the pattern from the last week of Phase 2 is also shown, assigning the same colour–diet relationship to the data that was used in Phase 3. During the first week of Phase 3, the F versus L group of animals maintained an intake pattern very similar to that which they had been exhibiting since Phase 2 (Fig. 5). However, this pattern began to change during the second week, reaching similar intake means, which were not significantly different (P = 0.937), for both encapsulated diets (2.4 ± 0.3 and 2.1 ± 0.5 g kg^{-1} BW for F and L capsules, respectively) (Fig. 5a). On the other hand, in the F versus S experimental group, the fish quickly changed their intake pattern, consuming the same number of capsule of both types in the first week of Phase 3 (Fig. 5b). During the last week of this phase, no significant differences were found between the capsule intake means of either capsule type (2.2 ± 0.2 and 1.8 ± 0.6 g kg^{-1} BW for F and S capsules,

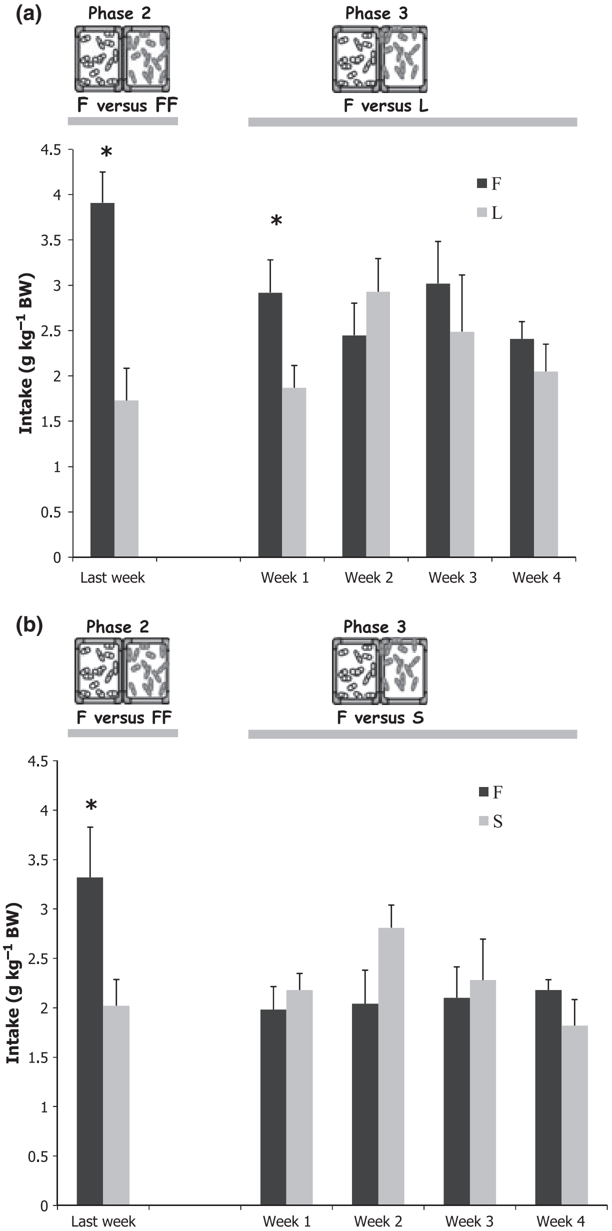

Figure 5 Food intake means expressed as g kg^{-1} of body weight for sharpsnout seabream fed two colours of capsule containing a complete fish oil diet (F) *versus* a fat-free diet (FF) (last week of Phase 2) and F *versus* a vegetable oil diet (Phase 3). In this last phase, the vegetable oil was provided by linseed (L) (n = 7) or soybean (S) (n = 5). Values represent the mean ± SEM of the same fish from the last week of Phase 2 as control and the four weeks of Phase 3. Data from the previous phase were represented in the same way as in Phase 3, to analyse changes in capsule intake patterns between them. Asterisks represent the statistical differences between the two capsule intake means for a given week.

respectively) (P = 0.703). The animals were weighed at the end of the experiment, showing the weight of 370.8 ± 69.0 g (mean ± SD).

Discussion

Sharpsnout seabream are capable of distinguishing between capsules containing, respectively, a complete diet with fish oil (F capsules) and a fat-free diet (FF) capsules. When fish were offered the two types of capsule, they changed their previous capsule colour selection and began to demonstrate a preference for F capsules (66.8% of the total intake in grams). Because these are encapsulated diets, with similar physical and organoleptic characteristics, but with different compositions (Ruohonen & Grove 2001), this selection could only result from a learning process through which the fish came to associate the capsule colour with their postingestive effects (Rubio *et al.* 2003; Almaida-Pagán *et al.* 2006). It has been suggested that in mammals, chemoreceptors exist in the gastrointestinal tract and associated viscera that are capable of detecting not only the energy content of the diet, but also its nutritional composition during the digestive process (Badman & Flier 2005). Appetite control would seem to begin at a peripheral level, with a complete signalling network connecting the gastrointestinal tract, the visceral organs and the central nervous system. New studies indicate that this system would also function in the case of fish, with a similar degree of complexity (Volkoff 2006).

Once it became evident that sharpsnout seabream had the capacity to discern the absence of fat in the diet, fish were allowed to select between encapsulated complete diets that only differed in the type of oil they contained (fish, linseed or soybean oil). Previous studies using pelleted diets showed the ability of two species of fish, rainbow trout (*Oncorhynchus mykiss*) (Geurden *et al.* 2005) and European seabass (*Dicentrarchus labrax*) (Luz *et al.* 2004), to select diets containing fish oil in preference of those containing vegetable oils. Furthermore, these authors also observed differences in fish preferences for different types of vegetable oils. While the trout showed a greater preference for rapeseed oil, the seabass showed a preference for both soybean and rapeseed oils. These observations suggest that vegetable oils contain components that could affect feed acceptance by fish. But if these components (based on differences in oil composition) act throughout orosensorial or postingestive mechanisms is unclear. The predominant C_{18} PUFA from vegetable oils may affect the sensorial characteristics of food. Linolenic and linoleic acids appear to differentially affect the taste receptor cell activity in mammals (Gilbertson *et al.* 1997; Tsuruta *et al.* 1999). On the other hand, feeding fish diets containing vegetable oils rich in 18-carbon fatty acids (C_{18} PUFA) and poor in long-chain, highly unsaturated fatty acids (C_{20} and C_{22} HUFA) may result in functional alterations caused by changes in the fatty acid metabolism, as well in membrane composition and lipid accumulation (Turchini *et al.* 2009). Although little is known on the orosensory recognition of lipids in fish (Lamb 2001), it would be necessary to exclude such information in self-selection experiments and thus, be able of distinguishing between the fish capacity to select between diets according to their nutritional value and the simple preference of the fish for a particular smell, taste or texture.

In the present study, an encapsulation protocol was used to bypass the fish orosensory barrier. When two types of capsule were administered to sharpsnout seabream, one with F and the other with a vegetable oil (linseed or soybean oil), no selection preference was observed in any case. The fish ingested both types of capsule equally after 28 days of experimentation. This means that, if the orosensory properties related to the diets are masked, sharpsnout seabream show no preference for either diet, which would indicate the crucial role played by the chemosensory information from the feed on the short-term selection that occurs in fish fed pelleted diets.

Moreover, these observations, together with the fact that sharpsnout seabream maintained their energy intake when they were fed vegetable oil diets, would agree with the good acceptance of vegetable oil diets observed in fish when these were offered in the absence of any choice (Bell *et al.* 2003; Geurden *et al.* 2007; Piedecausa *et al.* 2007).

In summary, when orosensory information from the feed is masked, sharpsnout seabream are capable of discriminating between a complete diet with fish oil and a fat-free diet (FF), associating the capsule colour with its nutritional content through postingestive signals. While these mechanisms have been shown to be effective for discrimination at the macronutrient level, they are not sufficient for distinguishing, within the timeframe of this experiment, among complete encapsulated diets that differ in the type of oil used.

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