

Nile Tilapia and Gilthead Seabream Dietary Self-Selection of Alternative Feeds with Spirulina and Quinoa

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Abstract

Classical assessments of new fish feeds are anthropocentric, focusing on growth. Although this methodology is accurate, it does not consider the fish' perspective. This study aimed to investigate the behavioural responses and feed preferences of Nile tilapia - *Oreochromis niloticus* and gilthead seabream - *Sparus aurata*, in two self-selection trials (self-feeders and diet encapsulation). Using self-feeders, both species were offered three feeds: a control (PD) and two diets (ORG1 and ORG2) containing non-conventional ingredients, including spirulina (*Spirulina platensis*) and quinoa (*Chenopodium quinoa*). Three groups of tilapia with an average weight of $163.0 \text{ g} \pm 4.3 \text{ g}$ (mean $\pm$ SD) and four groups of seabreams with $174.7 \text{ g} \pm 27.0 \text{ g}$ were tested. To investigate the role of olfactory factors in dietary selection, three other diets were encapsulated and offered to tilapia: Diet A, a purified feed, Diet B that contained predominantly spirulina and Diet C which had a mixture of spirulina and quinoa. Seven individual tilapia of $331.9 \text{ g} \pm 31.4 \text{ g}$ were used. Using self-feeders, tilapia exhibited a preference for ORG2 (46.5%), which was influenced by the sensory properties of feeds and post-ingestion signals, as their choice for ORG2 persisted during diet encapsulation using Diet C, which was also formulated with quinoa and spirulina. Seabream did not show a preference for any feed. These findings highlight the effectiveness of self-selection experiments in allowing fish to express their feeding behaviour and preferences. Therefore, this approach should be considered in the initial screening and design of new aquaculture feeds and ingredients.

1. Introduction

In the wild, since no single feed supplies all essential nutrients, most fish show dietary selection and pick up different items to create a complete and balanced diet according to their physiological needs to survive (Huntingford, 2020). Fish indeed are able to select and regulate the intake of macronutrients and energy (Luz et al., 2018). This selection and regulation occurs through a process known as “nutritional wisdom” (Raubenheimer and Simpson, 1997; Simpson and Raubenheimer, 2001). In order to restore the metabolic balance as a result of a nutritional challenge, “specific hungers” have the ability to sense and ingest particular nutrients and/or substances in diets (White et al., 2000). Therefore, fish select their feed based on a series of complex regulatory mechanisms, associated with physiological, learning, and behavioural processes, involving hormonal and neural activities in the brain, gastrointestinal tract and liver (Comesaña et al., 2020; Forbes, 2001; Fortes-Silva et al., 2016; Otero-Rodino et al., 2016; Richter, 1943; Simpson and Raubenheimer, 2001). Accordingly, fish feeding behaviour is a relevant characteristic that should be considered when farming species, especially to assess which feed fish would eat more or less.

Aquaculture plays a major role in society by providing to the growing world population a vital source of animal protein. However, its development can be hampered by the environmental sustainability of the feeds used (FAO, 2022). Common ingredients (e.g., fishmeal and fish oil) used in feed formulation provide a valuable nutritional value to farmed organisms and most of them, in the European Union, are derived from well-regulated wild fish stocks and can even be certified (Hilmarsdottir et al., 2021). However, in

some production areas, though inclusion rates of marine resources has dropped considerably over the past years, they remain widely used during the juvenile stages of carnivorous species, being sourced from non-sustainable stocks (Little et al., 2016). Therefore, the use of alternative ingredients, such as algae and plants may reduce the dependency of fish feed on traditional resources. Spirulina is a microalgae with a high protein content and a nutritionally reasonable fatty acid profile being produced by low-cost open pond technologies (Mosha, 2019). Quinoa is a plant with a high protein, fibre, and carbohydrate contents together with low fat (Pellegrini et al., 2018). Both ingredients possess bioactive compounds that regulate physiological processes and exhibit biological activities, which could improve weight gain, feed efficiency, and disease resistance in fish while reducing the environmental impacts of the sector (Balakrishnan and Schneider, 2022; Han et al., 2021). In order to achieve all these benefits, thus improving feed sustainability in aquaculture, it is necessary to address the inclusion of these functional ingredients in novel feed formulations and their effects on fish acceptability and intake, especially when knowledge about these alternatives is much lower compared with traditional ingredients (Carlberg et al., 2015; Pratiwy et al., 2017).

The classic approach to investigate the effects of new diets occurs through growth experiments mainly based on physiological mechanisms (Roy et al., 2020). Although this methodology is accurate and have yielded considerable knowledge on animal production, it may not be suitable in the short-term, involves a large number of animals, it is time-consuming, expensive and more importantly it does not consider fish preferences, since they are unable to choose what feed to eat (Brännäs and Strand, 2015; Filho et al., 2018; Fortes-Silva et al., 2016). Conversely, self-selection methods allow fish to freely and voluntarily accept the given diet, while taking into account fish feeding behaviour and learning processes (Fortes-Silva et al., 2016). Furthermore, these methodologies allows fish to choose which feeds better suits their nutritional and energetic needs (Fortes-Silva et al., 2016). Additionally, it can also be used to investigate the detection and acceptance/rejection of feed additives, toxic substances and antinutritional factors (e.g., as phytic acid and phytate) present in plants and vegetables (Costa et al., 2022; Fortes-Silva et al., 2016). Therefore, to provide a more complete perspective on the potential of novel diets, growth experiments may be complemented with self-selection methods.

Encapsulated diets and self-feeders are some of self-selection methodologies that can be used to investigate feed intake regulation and dietary preferences. Diet encapsulation consists on packaging experimental feeds inside coloured edible gelatine capsules, avoiding the interference from the organoleptic characteristics of pelleted diets, with the colour of the capsules being the only external cue (Almaida-Págan et al., 2006; Rubio et al., 2003). Therefore, fish will select their diets based only on the nutritional content of the feeds, without the effect of chemosensitivity and olfactory factors, but rather mainly based on post ingestion and absorption metabolic signals (Almaida-Págan et al., 2006; Rubio et al., 2003; Ruohonen and Grove, 2001; Santos et al., 2019). Self-feeders consist of electronic devices that are placed on top of fish tanks, with a switch below the water level that when pulled and triggered by fish, allow different feeds, with distinct chemosensory properties, to fall from the dispenser into the tank (Jobling et al., 2001; Pratiwy et al., 2021; Sánchez-Vázquez et al., 1994). Therefore, contrary to capsules, the taste and texture of pelleted feeds that fall from the self-feeders is evaluated and sensed by the fish

allowing them to make their selection based on post-ingestion and absorption signals coupled with orosensory feed properties (Filho et al., 2018; Fortes-Silva et al., 2016; Raubenheimer et al., 2012). Both methods have not only been used and validated for Nile tilapia (*Oreochromis niloticus*) and gilthead seabream (*Sparus aurata*), but also for several other fish species, such as perch (*Perca fluviatilis*), tambaqui (*Colossoma macropomum*), sharpsnout seabream (*Diplodus puntazzo*) and rainbow trout (*Oncorhynchus mykiss*) (Atienza et al., 2004; Brännäs and Strand, 2015; Costa et al., 2022; Filho et al., 2018; Fortes-Silva and Sánchez-Vázquez, 2012; Montoya et al., 2012; Puchol et al., 2022; Yamamoto et al., 2001). Although self-selection research in fish has been evaluated in several species, making them viable options to address behaviour, information about the topic remains scarce compared to what is known regarding terrestrial animals. Based on this, knowledge about fish behaviour and its controls have not yet been totally understood and should be further explored, especially when considering the initial screening and design of potential new aquaculture feeds (Fortes-Silva et al., 2012; Pratiwy and Kohbara, 2018; Puchol et al., 2022).

The present work aims to investigate the acceptability, behavioural response, feed intake regulation and dietary preferences of two commercially important fish species - freshwater (Nile tilapia) and marine (gilthead seabream) – using the self-selection methods of self-feeders and diet encapsulation, to test the acceptance of non-conventional diets based on spirulina (*Spirulina platensis*) and quinoa (*Chenopodium quinoa*) as functional ingredients.

2. Materials and Methods

2.1. Formulation of self-feeder diets

For the self-feeders, three experimental diets (PD, ORG1 and ORG2) for each species (Nile tilapia and gilthead seabream) were prepared by SPAROS Lda (Olhão, Portugal) with a pilot-scale twin-screw extruder (CLEXTRAL BC45, France) equipped with a screw diameter of 55.5 mm. A temperature range of 105–110°C was used for the extrusion process. All batches of extruded feeds were dried in a convection oven (OP 750-UF, LTE Scientifics, United Kingdom). Pellet size was 4mm. A control diet (PD) was formulated to mimic current commercial feeds for each species. The remaining two diets (ORG1 and ORG2) were formulated to include spirulina (3.5% and 7%, respectively) and quinoa (5% in both), as well as other organic alternatives (e.g., pea protein concentrate, corn meal) to address some of the current environmental concerns and/or ethical issues often associated with ingredients present in traditional commercial formulations. The ingredient selection (Tables 1 and 2) was chosen based within an organic framework, on market availability and nutritional composition. The inclusion levels were adjusted for each species, according to existing knowledge on tolerance to different ingredients as well as their nutritional and especially amino acid requirements, without compromising fish growth, development, and welfare.

Tilapia feeds had similar protein, lipid, and energetic contents with around 33.8% WW crude protein, 7.6% WW crude lipid and 18.9 KJ gross energy/g WW (Table 1). Seabream diets also showed identical

proximal compositions, having on average 41.7% WW crude protein, 16.8% WW crude lipid and 22.2 KJ gross energy/g WW (Table 2). Amino acid profiles of the experimental diets given to both species are presented in Tables 3 and 4. Although in ORG1 the diets exhibited lower methionine levels, this amino acid requirement was fulfilled.

Table 1
Diet formulation (inclusion levels %) and proximate composition (% as fed) of the experimental diets (PD, ORG1 and ORG2) in self-feeders for Nile tilapia (*Oreochromis niloticus*).

<i>Ingredients (inclusion levels %)</i>	PD	ORG1	ORG2
Fishmeal	5.00		
Poultry meal	5.00		
Brewer's yeast	5.00	10.00	10.00
<i>Spirulina</i>		3.50	7.00
Pea protein concentrate		5.50	2.25
Wheat gluten		3.50	7.00
Corn gluten meal	11.60		
Soybean meal	18.00	18.00	
Rapeseed meal	6.50	6.50	13.00
Sunflower meal	3.25	7.50	15.00
Wheat meal	27.75	6.70	9.35
Rice bran full fat	10.00	10.00	10.00
Corn meal		6.95	6.95
Quinoa		5.00	5.00
Faba beans		9.00	7.00
Vitamin and mineral premix	1.00	1.00	1.00
Choline chloride	0.20	0.20	0.20
Antioxidant powder (Verdilox)	0.20	0.20	0.20
Mono-calcium phosphate	1.80	2.15	2.05
L-lysine	0.30		
DL-methionine	0.10		
Fish oil	0.90	1.00	1.00
Soybean oil	3.40	3.30	3.00
Proximate Composition (% as fed)			
Dry matter (DM)	92.10	91.74	90.58

<i>Ingredients (inclusion levels %)</i>	PD	ORG1	ORG2
Ash	6.27	6.33	6.22
Crude protein	33.50	34.06	33.75
Crude lipid	8.43	7.63	6.75
Gross energy (kJ/g ⁻¹)	19.33	19.24	18.18

All values are reported as mean of duplicate analysis.

Table 2
Diet formulation (inclusion levels %) and proximate composition (% as fed) of the experimental diets (PD, ORG1 and ORG2) in self-feeders for gilthead seabream (*Sparus aurata*).

<i>Ingredients (inclusion levels %)</i>	PD	ORG1	ORG2
Fishmeal Super Prime	20.00	20.00	20.00
Poultry meal	10.00		
Brewer's yeast		5.00	5.00
<i>Spirulina</i>		3.50	7.00
Pea protein concentrate		16.50	11.50
Wheat gluten			3.00
Corn gluten meal	8.00		
Soybean meal	16.00	16.00	
Rapeseed meal	3.30		3.30
Sunflower meal	6.00		10.00
Wheat meal	5.90	5.55	
Wheat bran		5.00	5.00
Quinoa		5.00	5.00
Faba beans	7.00	7.00	7.00
Whole peas	7.00		7.00
Vitamin and mineral premix	1.00	1.00	1.00
Choline chloride	0.20	0.20	0.20
Antioxidant powder (Verdilox)	0.20	0.20	0.20
Mono-calcium phosphate	0.70	1.05	1.00
L-lysine	0.30		
DL-methionine	0.10		
Fish oil	4.60	4.60	4.60
Soybean oil	9.70	9.40	9.20
Proximate Composition (% as fed)			
Dry matter (DM)	94.22	95.11	91.50

<i>Ingredients (inclusion levels %)</i>	PD	ORG1	ORG2
Ash	7.07	6.36	6.33
Crude protein	40.91	43.03	41.01
Crude lipid	18.43	16.63	15.22
Gross energy (kJ/g ⁻¹)	22.20	22.47	22.00

All values are reported as mean of duplicate analysis.

Table 3
Amino acid profile (g/100g fed basis) of the experimental diets
in self-feeders for Nile tilapia (*Oreochromis niloticus*).

<i>Amino acids (g/100g fed basis)</i>	PD	ORG1	ORG2
Arginine	1.77	2.19	2.01
Histidine	0.74	0.77	0.76
Lysine	1.79	1.77	1.46
Threonine	1.24	1.25	1.27
Tryptophan	0.36	0.41	0.40
Isoleucine	1.32	1.36	1.30
Leucine	3.11	2.42	2.36
Valine	1.55	1.58	1.60
Methionine	0.75	0.52	0.59
Phenylalanine	1.62	1.55	1.53
Cysteine + Cystine	0.52	0.53	0.54
Tyrosine	1.14	1.16	1.12
Aspartic Acid	2.68	2.98	2.54
Glutamic Acid	6.46	6.32	6.95
Alanine	1.95	1.55	1.60
Glycine	1.63	1.48	1.54
Proline	2.22	1.93	2.04
Serine	1.62	1.61	1.60
Taurine	0.03	< 0.002	< 0.002

All values are reported as mean of duplicate analysis.

Table 4
Amino acid profile (g/100g fed basis) of the experimental diets in self-feeders for gilthead seabream (*Sparus aurata*).

Amino acids (g/100g fed basis)	PD	ORG1	ORG2
Arginine	2.80	2.75	2.69
Histidine	0.93	1.01	0.94
Lysine	2.69	2.84	2.55
Threonine	1.59	1.61	1.63
Tryptophan	0.47	0.49	0.50
Isoleucine	1.62	1.78	1.68
Leucine	3.36	3.15	2.96
Valine	1.97	2.07	2.08
Methionine	0.96	0.74	0.92
Phenylalanine	1.88	1.88	1.77
Cysteine + Cystine	0.52	0.51	0.49
Tyrosine	1.39	1.42	1.34
Aspartic Acid	3.69	4.31	3.74
Glutamic Acid	6.63	6.66	6.76
Alanine	2.41	2.17	2.15
Glycine	2.36	2.00	2.02
Proline	2.18	1.74	1.82
Serine	1.81	1.88	1.84

All values are reported as mean of duplicate analysis.

2.2. Formulation for diet encapsulation

For the diet encapsulation three experimental diets (A, B and C) for Nile tilapia were prepared at the University of Murcia (Murcia, Spain) with distinct proximal composition (Table 5). Diet A had casein and dextrin (34% and 30%, respectively) as main ingredients and served as a proxy for a purified diet. Diet B was mainly composed of spirulina (58%), while Diet C had different doses of spirulina and quinoa (46% and 20%, respectively). Other ingredients present in the diets include fish oil, soy oil, cellulose, alginate, and vitamin premix. The spirulina and quinoa used in the supplemented diets B and C were provided by

TILAMUR S.L (Murcia, Spain). Encapsulation occurred by filling eatable gelatine capsules (No. 4; FAGRON, S. A., Barcelona, Spain) with previous produced diets grounded, using a semi-automatic encapsulator - Tencyfarma, Miranda de Ebro, Barcelona, Spain). All capsules were weighed after manufacture ($\pm 0.17\text{g}$ filled with feed) and stored (at 4°C) in a common and single plastic bag for all the days of the experiment until use, to avoid any external contamination which might have allowed the fish to distinguish their content by their external chemical properties. Three colorations of capsules were used (red, green, and yellow), each corresponding to a specific diet, to help fish discriminate between them. Feed samples were grounded and analysed for dry matter (105°C for 24 h), crude protein calculated by the Kjeldahl method (automatic flash combustion; Leco FP-528, Leco, St. Joseph, USA) ($\text{N} \times 6.25\%$), lipid content by diethyl ether extraction (Soxtherm Multistat/SX PC (Gerhardt, Königswinter, Germany; 150°C) and ash content by heating in an oven at 450°C for 24 h. Diets proximal composition varied between 23.9% – 32.4% wet weight (WW) crude protein and 16.6% – 19.9% WW crude lipid (Table 5).

Table 5
Diet formulation (inclusion levels %) and proximate composition (% as fed) for the experimental diets (A, B and C) in diet encapsulation for Nile tilapia (*Oreochromis niloticus*).

<i>Ingredients (% as is)</i>	A	B	C
Casein	34.17		
Dextrin	30.00	2.85	3.23
Gelatin	6.83		
Fish oil	14.63	18.91	16.60
Soy oil	4.67	6.30	5.53
Cellulose	5.00	6.84	0.81
Alginate	4.60	6.55	7.43
Vitamin Premix	0.10	0.14	0.16
Quinoa			19.87
<i>Spirulina</i>		58.41	46.37
Proximate Composition (% as fed)			
Dry matter (DM)	93.50	90.80	90.30
Ash	6.92	6.90	9.84
Crude protein	32.44	30.15	23.93
Crude lipid	16.64	19.89	18.87

All values are reported as mean of duplicate analysis.

2.3. Fish and husbandry conditions

Fish were reared and handled by trained scientists and following the Spanish legislation on Animal Welfare and Laboratory Practices, while the experimental protocol was approved by the National Committee of the University of Murcia on Ethics and Animal Welfare under the Guidelines of the European Union Council on the protection of animals used for experimental purposes (Directive 2010/63/EU).

Nile tilapia was used for self-feeder and encapsulation experiments. The latter was used as a follow up experiment to discard the effects of oral factors on the selection of the feeds. Both experiments were conducted in the chronobiology laboratory at the University of Murcia. Individual fish and groups were weighed right before the start and at the end of each experiment to not compromise behaviour. Nile tilapia with an average initial individual weight of $163.0 \text{ g} \pm 4.3 \text{ g}$ (mean $\pm$ S.D.; in groups for self-feeder studies) and $331.9 \text{ g} \pm 31.4 \text{ g}$ (mean $\pm$ S.D.; individual for capsules studies) were provided by the University of Murcia, from a mono-sex male population (offspring tilapia, GMT®). The tanks with 300 L were maintained in an aquaculture recirculating system, with a protein skimmer, as well as mechanical, biological, UV filtered and aerated water. Fish were allowed to acclimate to laboratory conditions for at least 2 weeks, during which time they were fed a commercial diet (Skretting TI-3 (3.2mm); with % DM: 32.0% crude protein, 6.0% crude fat and 5.8% crude fibre), which was supplied by hand to visual satiety once a day. Abiotic parameters were measured, and mortality was recorded daily. The photoperiod was 12L:12D (lights on at 09:00). Values of $29.0 \pm 1.0^\circ\text{C}$ for water temperature, 7.2 ± 0.2 for pH, 6.9 ± 0.4 ppm for dissolved oxygen and $0.7 \pm 1.0 \text{ mg/l}$ ammoniac nitrogen were maintained.

Groups of gilthead seabream were only used for the self-feeder experiment performed at the Aquaculture Laboratory located in Algameca (Cartagena, Spain). Fish had an average initial individual weight of $174.7 \text{ g} \pm 27.0 \text{ g}$ (mean $\pm$ S.D) and were provided by IMIDA from San Pedro del Pinatar (Spain). The tanks with 150 L were maintained in a flow-through system, with a protein skimmer, as well as mechanical, biological, UV filtered and aerated water. Fish were allowed to acclimate to laboratory conditions for at least 2 weeks, during which time they were fed a commercial diet (Skretting L-4 Alterna 2P; with % DM: 46.5% crude protein, 20.0% crude fat and 3.4% crude fibre), which was supplied by hand to visual satiety once a day. Abiotic parameters were measured, and mortality was recorded daily. The animals were kept in a natural photoperiod with values of $27.0 \pm 1.0^\circ\text{C}$ for water temperature, 37 ± 1.0 ppm for salinity, 7.6 ± 1.0 for pH and 6.3 ± 0.5 ppm for dissolved oxygen.

2.4. Experimental setup

2.4.1. Self-feeders

The self-feeder experiment was performed in accordance with Fortes-Silva et al. (2012). For Nile tilapia, it lasted 36 days and 30 fish were conditioned as groups in three plastic tanks (each with 11 fish), while for seabream it lasted 67 days and 32 fish were divided in four plastic tanks (each with 8 fish) (Figs. 1 and 2). Three self-feeders provided by the University of Murcia were equipped in each tank. The position of

each diet (PD, ORG1 and ORG2) on the feeders also varied between tanks, to avoid a possible positional effect. The feeding systems were connected with an electric transformer (one for five self-feeders). Each of them was composed of a trigger (a switch with rubber tip), actuated by the fish, placed 2 cm above the water surface, connected to an electromagnet, and a feeder (EHEIM 3581, Deizisau, Germany) that delivered a predetermined amount of feed (1 pellet = 0.04 g) after each trigger actuation and electromagnet activation. To determine the daily intake, every day the feed remaining in the feeder was weighed at the same time (11:30) and subtracted with the total number of grams given the previous day, before refilling the feeder recipient for the next day. After percentages of the offered diets exhibited a statistically significant difference for one feed, diets were switched between feeders to provide a challenge for the fish, reduce the possible preference and influence for a particular string sensor or relative position of the self-feeders.

Figure 1. Experimental setup for self-feeders trial using Nile tilapia (*Oreochromis niloticus*). Each of the three feeders inside each tank, contained a specific feed (PD, ORG1 and ORG2).

2.4.2. Encapsulated diets

The experiment with encapsulated diets was performed according to Fortes-Silva et al. (2012). It lasted 35 days and 7 fish were housed in 4 plastic tanks, divided in half separated with a net, thus single individual fish were used (Fig. 3). Ten capsules of each colour (30 in total) were mixed and given to each fish daily at the same time (11:30h) and left in the water for 20 min. After this period, to determine the daily intake, the total number of uneaten capsules corresponding to each diet were removed from the tanks, counted, and subtracted with the total number of capsules given capsules. After a determined diet was preferred and exhibited a statistically significant difference over the others, feeds were switched between capsule' colours to reduce the possible preference for a specific one.

2.5. Data analysis and statistics

The statistical analysis was performed with the SPSS software, version 23.0. For the diet encapsulation the experimental unit was the number of fish ($n = 7$), while for the self-feeders was the tank ($n = 3$ or 4). The relative selection of each diet is expressed as a percentage of the total number of capsules or feed consumed, considering the total diets as 100%. The feed and capsules intakes were expressed as total grams of feed ingested/% of body weight and number of capsules/100g of body weight, respectively. Further, in the days where a specific diet was mostly selected, the percentages of capsules ingested and feed consumed were compared by one-way ANOVA, followed by a Tukey's post-hoc test to examine significant pair-wise comparisons, before meeting criteria for normality and homogeneity using Shapiro – Wilk and Levene's test, respectively. *Arcsine* transformations of capsules and feed intake percentages were performed to achieve homogeneity of variance. The statistical significance was considered at $P < 0.05$.

3. Results

3.1. Self-feeders

Nile tilapia reached a final mean weight of 194.7 ± 3.9 g (mean $\pm$ S.D.), all fish survived and on average feed consumed daily represented 0.75% of average body weight/day. As reported by Fortes-Silva et al., 2012, uneaten and wasted feed was negligible, only around 2% of the total given feed, thus the amount of feed demanded by the fish was almost entirely ingested. The dietary preference (Fig. 4) in self-feeders initially demonstrated an adaptation period to the feeders of around 5 days. During this time, fish preferred the position of specific feeders, rather than the inserted feed, but quickly changed their behaviour. All diets were chosen similarly for several days before an increase in preference for diet ORG2 was observed during three consecutive days (with an average of 46.5%; $p < 0.05$). Throughout the same period diets PD and ORG1 were preferred on average 28.9% and 24.7%, respectively. Diets were switched between feeders on day 22, another period of equal preference remained, while from day 30 until the end of the experiment, diet ORG2 was mainly chosen (between 40.7% and 56.0%; $p < 0.05$).

Diets PD (0.24 grams/% BW; $p < 0.05$) and ORG1 (0.21 grams/% BW; $p < 0.05$) were consumed 38.5% and 46.2%, respectively, less than diet ORG2 (0.39 grams/% BW; $p < 0.05$) during days with statistically significant differences (Fig. 5).

Gilthead seabream final weight was 264.4 ± 29.5 g (mean $\pm$ S.D.), no mortality was recorded and average feed consumption was 1.21% of the animal's biomass. From the initial days of the study, fish exhibited a clear preference for diet ORG1 (between 65.50% and 83.45%; $p < 0.05$). However, after diets were switched between feeders, the preference for diet ORG1 fell, while for diet PD and ORG2 increased (Fig. 6). For several days, no statistically significance was achieved (except only for one day). Then, fish were fasted for 10 days as a challenge test to motivate them to choose a diet, according to Aranda et al. (2001). Nevertheless, even after this approach, a consistent preference was not achieved ($p > 0.05$).

3.2. Encapsulated diets

When fed capsules, tilapia exhibited a mean final weight of 362.6 ± 33.6 g (mean $\pm$ S.D.), and no mortality was observed. The average feed consumption was 0.51% of the average body weight/day. Not all fish started to ingest capsules from the first day, but progressively all of them successfully swallowed the whole capsules without breaking the covering. In terms of dietary selection (Fig. 7), after 10 days of ingesting similar diet preferences, fish progressively increased their intake and selection of capsules containing diet C, while simultaneously decreasing that of diets A and B. When the preference for diet C reached values (maximum of 44.0%) that showed statistically significant differences ($p < 0.05$) for several days (day 11–16) over diets A and B (remained at an average value of 28.9% and 30.5%, respectively), the colour of the capsules associated with each diet rotated (day 17). Initially, this change decreased the ingestion of diet C, while conversely increasing diet A and B. Up to day 29, the preference of fish towards the diets remained at a steady state, until a clear consistency and preference for diet C was re-established (between 41.0% and 47.2%; $p < 0.05$) during the rest of the experimental period.

During days with statistically significant differences between intake of feeds, diets A (1.11 capsules/100g BW) and B (1.12 capsules/100g BW), were ingested 32.7% and 32.1%, respectively, less than diet C (1.65 capsules/100g BW; $p < 0.05$) (Fig. 8).

Figure 8. Average daily intake (number of capsules ingested per 100g of body weight) of three diets (A, B and C) by Nile tilapia during days with statistically significant differences. Bars represent the mean counts $\pm$ SD ($n = 7$ individual fish). The star represents significant differences (one-way ANOVA, $p < 0.05$).

4. Discussion

The main purpose of this research was to assess the feeding behaviour and ability of Nile tilapia and gilthead seabream to self-select their preferred diets. Fish choose feeds and regulate their feeding behaviour based on homeostatic and hedonic mechanisms. The homeostatic pathway maintains normal energy balance homeostasis and it takes over in response to nutritional demands and metabolic needs. The hedonic control is related to the brain reward system, where fish mainly sense the orosensory, pellet quality and palatable aspects of feed, being independent on nutritional requirements (Kulczykowska and Sánchez Vázquez, 2010; Volkoff, 2019). Thus, fish can choose which feed items to ingest mainly based on size, palatability and nutritional properties (Raubenheimer et al., 2012). All feeds offered had the same size, thus fish choice was only based on the other two parameters. For this reason, fish tested with self-feeders and diet encapsulation, which enabled the isolation of palatability and nutritional parameters, allowed for a better understanding of the effects of “nutritional wisdom”.

Fish feeding is modified by both Pavlovian (to find the feed) and operant learning (to catch and manipulate the feed) behaviour (Millot et al., 2014). Operant learning is the process that is associated between a behavioural action and its outcomes. If fish are rewarded enough times, fish will learn the relationship and will increase the probability and frequency of repeating the same action. In a population, there may be only one dominant fish that is curious enough to pull the trigger, but if rewarded, this information may be socially transmitted and become more common in all individuals (Millot et al., 2014). On the initial days of the self-feeding experiment tilapia showed a preference for one feeder, which changed after some time after assessing the content of the other feeders, demonstrating their exploratory and learning behaviour, as shown by Figueiredo et al. (2023). A similar situation was recorded using European seabass, that when fish were fed with the standard diet they exhibited a preference for one of the self-feeders (Aranda et al., 2000). Although tilapia kept in groups developed strong social hierarchies that could affect feeding behaviour (Toguyeni et al., 1997), this was not observed as fish did not show an aggressive behaviour and still choose a feed on both types of experiment and regardless being kept alone or in groups. Similarly, Fortes-Silva et al. (2012) showed that tilapia were not aggressive and exhibited a similar pattern of diet selection whether maintained isolated or in groups, when offered diets with a balanced or unbalanced composition of essential amino acids. Indeed, in our self-feeders experiment, tilapia were not isolated as in diet encapsulation, meaning that social learning may not play a significant role in the feeding and behavioural mechanism as suggested by Vivas et al. (2006). Using self-feeders, Nile tilapia chose diet ORG2 with an intake 0.75% of fish weight/day. Similarly to other studies, it was

possible to observe that tilapia consumed most diet pellets and that the feed waste (less than 2% of the total given feed) that remained in the tanks was residual. For example, Fortes-Silva et al. (2012) reported a negligible food waste of 1% with tilapia. To compare the intake, Pratiwy et al. (2017) tested the growth performance of Nile tilapia reared under self-feeding systems and showed feed intake values of around 1.85%/body weight. The choice for diet ORG2 was presumably based on tilapia nutritional needs (post-ingestive) coupled with feed organoleptic characteristics. Likewise, other studies with European seabass (*Dicentrarchus labrax*) and tilapia reported a similar behaviour (Fortes-Silva et al., 2016, 2012; Rubio et al., 2006). It is important to note since fish required almost three weeks to exhibit a preference and no feed was predominantly chosen from the beginning of the study, this can reflect the less clear taste differences between feeds. In a study by Carlberg et al. (2015), Arctic charr (*Salvelinus alpinus*) took 9 days, while Fortes et al. (2010) noted that tilapia clearly preferred since the beginning of the experiment diets containing phytase with an intake and that after switching feeds, the pattern was re-established only after 3 days. In the present study, after feed was switched between feeders, tilapia also resumed, re-established and sustained the previous pattern of selection of diet ORG2, while maintaining a constant consumption of other diets, meaning that the fish established levels of consumption for each feeds. However, once again they took some time (9 days), pointing out the effect of the minor differences between the diets. These findings are in accordance with Fortes et al. (2010), who reported that a diet with 1500 IU kg⁻¹ phytase was preferred throughout the trial, even after switching feeders. The same author showed that tilapia retained and maintained the intake of specific levels of protein, fat and carbohydrate when feeds were switched over (Fortes-Silva and Sánchez-Vázquez, 2012). Fish are able to identify and evaluate distinct amino acid profiles between diets (Fortes-Silva et al., 2012). All feeds were formulated to contain the minimum requirements of every essential amino acid (EAA). However, diet ORG1 presented the lowest levels of methionine. Although methionine was near the lowest requirement in ORG1, it still was enough to fulfil the species physiological state for normal growth (NRC, 2012). Overall, the self-feeder experiment with tilapia resulted in a preference for diet ORG2, which was influenced by a combination of several factors including learning-reward behaviour, nutritional requirements, as well as the orosensory properties of the diets.

To discard the effects of olfactory factors on the selection of the feeds, Nile tilapia were fed encapsulated diets in a second experiment. Tilapia preferred diet C (with spirulina and quinoa), after 11 days and after colour rotation, and presented an intake of 0.51% of their body weight. While individual differences between fish of the same species can exist, in our case the preference was unanimous for all fish. The same species with a final body weight of around 53g required 15 days to distinguish between two vegetable oil blends at 30°C and after switching capsules' colours only 3 days, with an average intake of 1.12% between all treatments (de Almeida et al., 2021). In another study, the feed intake of tilapia with around 80g was 1.36% of the animal's biomass and required 7, 11 and 23 days to differentiate diets with distinct protein levels (0, 25 and 42%) and 6, 10 and 4 days after inverting the content of the capsules (Costa et al., 2022). Capsule colour used in this study did not affect our results. In other studies, using tilapia, by Fortes-Silva et al. (2011) and de Almeida et al. (2021) coloration also did not affect fish preference and only served as a "reference" for the fish to identify which diet was associated with each

colour. Therefore, in the present study it was clear that by randomizing capsules' colours for each individual and after changing the colour paired to each diet, tilapia continued to choose capsules with diet C, meaning that the content, rather than the capsule colour, was the main factor considered for their preference. Tilapia is able to address the content based in a wide range of physiological processes. All capsules presented the same chemosensory properties at oropharyngeal level, meaning that palatability, texture, flavour and odour associated with traditional pelleted diets were negligible for the fish (Rubio et al., 2006). Moreover, it is important to note that neither the capsules or pellets leached nutrients into the water, which could act as attractants to fish (Busti et al., 2022). Therefore, fish had to evaluate the quality and nutritional composition of the content of the capsules and learn to associate this information with the capsules' colour (Almaida-Págan et al., 2006; Rubio et al., 2003). Accordingly, tilapia opted for the diet C, which after evaluation, was probably more able to satisfy their physiological state, based on post-ingestive and/or post-absorption processes as suggested by Fortes-Silva et al. (2016) and Rubio et al. (2003). However, it is important to note that in the present study, besides the constituting ingredients, the formulations of the three experimental diets, mainly protein and lipid, were different, thus they could have affected fish feeding behaviour. For the experimental size (around 300g) of Nile tilapia used, the recommend percentage of these macronutrients on the feeds is between 32–36% for proteins and 8–12% for lipids (NRC, 2012). Our results were in accordance with Pereira-da-Silva et al. (2004) that registered a crude protein feed intake of 24% by Nile tilapia, when given the possibility to self-select between distinct protein dietary levels. Although diet A (mainly with casein and dextrin) and B (rich in spirulina) had more similar nutritional profiles to these values than diet C (mixture of spirulina and quinoa), the latter was still preferred. Since fish had at their disposal other feeds, they also consumed them in a different proportion, possibly to create a balanced diet and compensate the lack of some essential nutrients from diet C (Simpson and Raubenheimer, 2001). A similar situation was found by Costa et al. (2022), who reported that when tilapia of 80g were offered two rations, with different crude protein and amino acids levels, it showed a significant preference for the consumption of one of the feeds, while also eating at lower levels the other one. In the present study, the intake of diet C, which had the lowest protein content (23.93%) increased, could mean that this defect was mild for the fish, perhaps due to their size, so they increased its intake, and possibly also consumed other feeds available, to reach their level of requirement, especially in the case of protein (Henry, 1985). This scenario was also confirmed by Fortes et al., (2011), who noted that when protein was restricted, tilapia increased its intake by consuming more capsules with this nutrient to maintain their energy intake (Fortes-Silva et al., 2011). Similarly, Almaida-Págan et al. (2006) and García-Meilán et al. (2013) showed that when given a diet with lower protein to sharpsnout and gilthead seabream, respectively, the fish increased its intake. Indeed, studies have shown that fish have the ability to regulate its consumption and defend a given nutritional intake target (Almaida-Págan et al., 2006; Brännäs and Strand, 2015; Fortes-Silva et al., 2016; Rubio et al., 2003). Nile tilapia exhibited a consistent preference for diet C driven by post-ingestive and post-absorption processes, even in the absence of olfactory factors present in the self-feeder experiment, reflecting that they are able to identify and select a feed with spirulina and quinoa as functional ingredients.

Conversely to tilapia, gilthead seabream did not show a consistent preference for a particular feed using self-feeders. The experiment was performed with rapidly growing juveniles at high water temperature in summer (since it was a flow-through aquaculture system), a scenario which could have made that the homeostatic system, associated with high energetic demands, override the hedonic regulation of feeding behaviour, not allowing seabream to discriminate diets efficiently (Puchol et al., 2022). Another possible explanation for the lack of diet discrimination is because all three experimental diets were nutritionally similar to the previously fed commercial feed, meaning that fish were familiar with it and did not notice enough differences (Pulido-Rodriguez et al., 2021). In our study, gilthead seabream wasted and rejected more feed than tilapia, while the intake was at an average of 1.21%/body weight. In addition, there was a much higher variation on the daily intake of feeds compared with tilapia, which could be related with the more curious and aggressive behaviour of seabream towards feed, especially when limited and defendable as in self-feeders (Puchol et al., 2022). There are few studies available regarding seabream using self-feeders with similar methodology to compare results. Nevertheless, in a previous investigation with seabream, it was shown that fish with 254g could select a diet with distinct oxidation levels of dietary lipids after 10 days with a preference of 82% and 7 days after switching feeders with an average intake of 1.57%/body weight (Montoya et al., 2011). Similarly, to what occurred with tilapia, it is possible that on the initial days seabream were preferring a specific feeder on each tank that, by coincident, contained diet ORG1. A specific feeder was also selected on the initial experimental days with European seabass and gilthead seabream by Aranda et al. (2000) and Montoya et al. (2011), respectively. The preference for a particular feeder can then indirectly affect fish' choice based on pre-ingestive parameters (Montoya et al., 2011). Therefore, it was necessary to change the positions of the feeders to assert dietary preferences and avoid any preference for a specific position as it was noted by Puchol et al. (2022). Indeed, after changing the position of the feeders, seabream decreased their intake for ORG1 and never achieved a clear preference for any of the given feeds, suggesting that both pre- and post-ingestive signals were involved in diet selection (Montoya et al., 2011). Montoya et. al. (2011) observed two selection patterns after changing the position of the feeders: some fish groups resumed their selection for a specific diet, while the other groups did not show a clear preference for any diet until they were subjected to a 3-week fasting period, after which they shortly resumed their dietary preferences. In the present study, seabream were also fasted, for a duration of 10 days in order to present them with a challenge, aiming to define a feeding pattern, as the physiological state of fish caused by oxidative stress due to fasting would reinforce their selection behaviour (Montoya et al., 2011). However, seabream were not observed to demand more feed and define a pattern, as the compensatory bite activity increase was not enough to attain a sufficient feed intake level to reach a preference. Conversely, European seabass fasted for 6 and 15 days, increased demand for diets to recover metabolic status with hyperphagia, mainly for protein used as energy source (Aranda et al., 2001; Vivas et al., 2003). Since seabream were unable to exhibit a preference for any feed, it was not necessary to conduct a capsule experiment.

The feed intake and growth rates obtained in all our studies were in general lower compared to other performance experiments, as it was expected. It should be noted that fish sizes differ among experiments, which in turn directly affects their intake requirements and growth rates. Moreover, the diets were not

formulated with the goal of optimizing fish growth but rather to study fish behaviour. Indeed, experiments on dietary selection do not necessarily correlate the most selected diet with optimal performance (Fortes-Silva et al., 2012). Even in growth experiments, although a diet is formulated to provide maximum growth, when given the opportunity, fish might not prefer that formula (de la Higuera, 2001). When using capsules, tilapia “feel” that it is a “stranger” method of feeding, which can lead to a decrease in the intake. It is important to note that although tilapia were isolated in the diet encapsulation experiment, it should not represent a factor of stress that would have affected feed intake (Fortes-Silva et al., 2012). Despite it has been seen that animals in a self-feeding scheme can perform well (in terms of weight gain, dietary intake, etc), in some species, these method can impair growth and decrease feeding efficiency (Gélineau et al., 1998; Montoya et al., 2011; Santos et al., 2019; Tidwell et al., 1991). These differences are related to the adaptation of self-feeders by fish, where some animals in the same group assimilate the self-feeding system more than others (Ferrari et al., 2014; Tidwell et al., 1991). Nevertheless, the lower performance indicators obtained in our experiments were not a concern, especially as no mortality occurred and fish still gained weight.

5. Conclusions

In one hand, tilapia was able to show a preference and selected one of the given feeds (with spirulina and quinoa) by sensing the orosensory properties, in the case of self-feeders, but also based only on post-ingestion and absorption signals, for diet encapsulation, confirming their ability to choose a specific feed. On the other hand, gilthead seabream did not show a consistent preference for any diet. Accordingly, self-selection studies based on fish “nutritional wisdom”, allow fish to exhibit their behaviour, thus it may be considered in the initial screening and design of potential new aquaculture feeds and ingredients, before they are commercially available.

Declarations

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Animal Welfare Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Fish were reared and handled by trained scientists and following the Spanish legislation on Animal Welfare and Laboratory Practices, while the experimental protocol was approved by the National Committee of the University of Murcia on Ethics and Animal Welfare under the Guidelines of the European Union Council on the protection of animals used for experimental purposes (Directive 2010/63/EU).

Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

R.M: Conceptualization, Methodology, Investigation, Resources, Data Curation, Formal Analysis, Writing - original draft, Writing - review & editing. L.C: Conceptualization, Writing-review & editing, Supervision, Funding acquisition. J.D: Conceptualization, Methodology, Resources. S.E: Writing-review & editing, Supervision, Funding acquisition. F.V: Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Funding acquisition. The first draft of the manuscript was written by Rodrigo Mendes and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are not publicly available due to privacy concerns but are available from the corresponding author on reasonable request.

References

1. Almada-Págan, P., Rubio, V., Mendiola, P., Decosta, J., Madrid, J., 2006. Macronutrient selection through post-ingestive signals in sharpsnout seabream fed gelatine capsules and challenged with protein dilution. *Physiology & Behavior* 88, 550–558. <https://doi.org/10.1016/j.physbeh.2006.05.027>
2. Aranda, A., Sanchezvazquez, F., Madrid, J., 2001. Effect of short-term fasting on macronutrient self-selection in sea bass. *Physiology & Behavior* 73, 105–109. [https://doi.org/10.1016/S0031-9384\(01\)00464-4](https://doi.org/10.1016/S0031-9384(01)00464-4)
3. Aranda, A., Sánchez-Vázquez, F.J., Zamora, S., Madrid, J.A., 2000. Self-design of fish diets by means of self-feeders: validation of procedures. *Journal of Physiology and Biochemistry*. 56, 155–166. <https://doi.org/10.1007/BF03179782>
4. Atienza, M.T., Chatzifotis, S., Divanach, P., 2004. Macronutrient selection by sharp snout seabream (*Diplodus puntazzo*). *Aquaculture* 232, 481–491.
5. Balakrishnan, G., Schneider, R.G., 2022. The Role of Amaranth, Quinoa, and Millets for the Development of Healthy, Sustainable Food Products—A Concise Review. *Foods* 11, 2442. <https://doi.org/10.3390/foods11162442>
6. Brännäs, E., Strand, Å., 2015. A test of “nutritional wisdom” in perch (*Perca fluviatilis*) by self-selection of encapsulated macronutrients. *Applied Animal Behaviour Science* 171, 219–225. <https://doi.org/10.1016/j.applanim.2015.08.009>
7. Busti, S., Bonaldo, A., Diana, A., Perfetti, S., Viroli, C., Fontanillas, R., Eriksen, T.B., Gatta, P.P., Parma, L., 2022. The incidence of different pellet size on growth, gut evacuation, feed digestibility and feed waste in gilthead sea bream (*Sparus aurata*). *Aquaculture* 555, 738204.
8. Carlberg, H., Cheng, K., Lundh, T., Brännäs, E., 2015. Using self-selection to evaluate the acceptance of a new diet formulation by farmed fish. *Applied Animal Behaviour Science* 171, 226–232. <https://doi.org/10.1016/j.applanim.2015.08.016>
9. Comesaña, S., Conde-Sieira, M., Velasco, C., Soengas, J.L., Morais, S., 2020. Oral and pre-absorptive sensing of amino acids relates to hypothalamic control of food intake in rainbow trout. *Journal of experimental biology* 223, jeb221721.
10. Costa, L.S., Guilherme, H.O., Bahiense, R.N., Santos, F.A.C., Gamarano, P.G., Olmeda, J.F.L., Ribeiro, P.A.P., Luz, R.K., 2022. Effect of protein on the post-ingestive response of tilapia fed encapsulated diets. *Aquaculture* 556, 738289. <https://doi.org/10.1016/j.aquaculture.2022.738289>
11. de Almeida, C.A.L., de Almeida, C.K.L., de Fátima Ferreira Martins, E., Bessonart, M., Pereira, R.T., Paulino, R.R., Rosa, P.V., Fortes-Silva, R., 2021. Coping with suboptimal water temperature: modifications in blood parameters, body composition, and post-ingestive-driven diet selection in Nile

- tilapia fed two vegetable oil blends. *Animal* 15, 100092.
<https://doi.org/10.1016/j.animal.2020.100092>
12. de la Higuera, M., 2001. Effects of Nutritional Factors and Feed Characteristics on Feed Intake, in: Houlihan, D., Boujard, T., Jobling, M. (Eds.), *Food Intake in Fish*. Blackwell Science Ltd, Oxford, UK, pp. 250–268. <https://doi.org/10.1002/9780470999516.ch11>
 13. FAO, 2022. The State of World Fisheries and Aquaculture 2022. FAO.
<https://doi.org/10.4060/cc0461en>
 14. Ferrari, S., Benhaïm, D., Colchen, T., Chatain, B., Bégout, M.-L., 2014. First links between self-feeding behaviour and personality traits in European seabass, *Dicentrarchus labrax*. *Applied Animal Behaviour Science* 161, 131–141.
 15. Figueiredo, L.G., Santos, F.A.C., Torres, I.F.A., Boaventura, T.P., Luz, R.K., 2023. Behaviour of personality-screened juvenile *Oreochromis niloticus* in different challenges: Bold fish present more exploratory capacity and learning to face imposed challenges. *Anais da Academia Brasileira de Ciências* 95, e20210976. <https://doi.org/10.1590/0001-3765202220210976>
 16. Filho, E.C.T.N., Mattos, B.O., Santos, A.A., Barreto, K.A., Albinati, R.C.B., Oliveira Vidal, L.V., Sánchez-Vázquez, F.J., Fortes-Silva, R., 2018. Geometric approach to evaluate the energy/protein balance in tambaqui (*Colossoma macropomum*): Can fish ensure nutritional intake targets by postingestion signals? *Aquaculture Nutrition* 24, 741–747. <https://doi.org/10.1111/anu.12602>
 17. Forbes, J., 2001. Consequences of feeding for future feeding. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 128, 461–468.
 18. Fortes-Silva, R., Kitagawa, A., Sanchez Vazquez, F.J., 2016. Dietary self-selection in fish: a new approach to studying fish nutrition and feeding behavior. *Reviews in Fish Biology and Fisheries* 26. <https://doi.org/10.1007/s11160-015-9410-1>
 19. Fortes-Silva, R., Martínez, F.J., Sánchez-Vázquez, F.J., 2011. Macronutrient selection in Nile tilapia fed gelatin capsules and challenged with protein dilution/restriction. *Physiology & Behavior* 102, 356–360. <https://doi.org/10.1016/j.physbeh.2010.11.036>
 20. Fortes-Silva, R., Rosa, P.V., Zamora, S., Sánchez-Vázquez, F.J., 2012. Dietary self-selection of protein-unbalanced diets supplemented with three essential amino acids in Nile tilapia. *Physiology & Behavior* 105, 639–644. <https://doi.org/10.1016/j.physbeh.2011.09.023>
 21. Fortes-Silva, R., Sánchez-Vázquez, F., 2012. Use of self-feeders to evaluate macronutrient self-selection and energy intake regulation in Nile tilapia. *Aquaculture* 326, 168–172.
 22. García-Meilán, I., Valentín, J.M., Fontanillas, R., Gallardo, M.A., 2013. Different protein to energy ratio diets for gilthead sea bream (*Sparus aurata*): Effects on digestive and absorptive processes. *Aquaculture* 412–413, 1–7. <https://doi.org/10.1016/j.aquaculture.2013.06.031>
 23. Gélineau, A., Corraze, G., Boujard, T., 1998. Effects of restricted ration, time-restricted access and reward level on voluntary food intake, growth and growth heterogeneity of rainbow trout (*Oncorhynchus mykiss*) fed on demand with self-feeders. *Aquaculture* 167, 247–258.

24. Han, P., Li, Jingjing, Zhong, H., Xie, J., Zhang, P., Lu, Q., Li, Jun, Xu, P., Chen, P., Leng, L., Zhou, W., 2021. Anti-oxidation properties and therapeutic potentials of spirulina. *Algal Research* 55, 102240. <https://doi.org/10.1016/j.algal.2021.102240>
25. Henry, Y., 1985. Dietary factors involved in feed intake regulation in growing pigs: A review. *Livestock Production Science* 12, 339–354. [https://doi.org/10.1016/0301-6226\(85\)90133-2](https://doi.org/10.1016/0301-6226(85)90133-2)
26. Hilmarsdottir, G.S., Ogmundarson, Ó., Arason, S., Gudjónsdóttir, M., 2021. Efficiency of fishmeal and fish oil processing of different pelagic fish species: Identification of processing steps for potential optimization toward protein production for human consumption. *Journal of Food Processing and Preservation*. 45. <https://doi.org/10.1111/jfpp.15294>
27. Huntingford, F.A., 2020. Fish Behaviour: Determinants and Implications for Welfare, in: Kristiansen, T.S., Fernö, A., Pavlidis, M.A., van de Vis, H. (Eds.), *The Welfare of Fish, Animal Welfare*. Springer International Publishing, Cham, pp. 73–110. https://doi.org/10.1007/978-3-030-41675-1_4
28. Jobling, M., Covès, D., Damsgård, B., Kristiansen, H.R., Koskela, J., Petursdottir, T.E., Kadri, S., Gudmundsson, O., 2001. Techniques for measuring feed intake. *Food intake in fish* 827, 49–87.
29. Kulczykowska, E., Sánchez Vázquez, F.J., 2010. Neurohormonal regulation of feed intake and response to nutrients in fish: aspects of feeding rhythm and stress. *Aquaculture Research* 41, 654–667. <https://doi.org/10.1111/j.1365-2109.2009.02350.x>
30. Little, D.C., Newton, R.W., Beveridge, M.C.M., 2016. Aquaculture: a rapidly growing and significant source of sustainable food? Status, transitions and potential. *Proceedings of the Nutrition Society* 75, 274–286. <https://doi.org/10.1017/S0029665116000665>
31. Luz, R.K., Boluda-Navarro, D., Sánchez-Vázquez, F.J., Fortes-Silva, R., Portella, M.C., Zamora Navarro, S., Madrid, J.A., 2018. European seabass (*Dicentrarchus labrax*) ability to discriminate between diets made with different dietary fat sources. *Aquaculture Nutrition* 24, 65–73. <https://doi.org/10.1111/anu.12534>
32. Millot, S., Nilsson, J., Fosseidengen, J.E., Bégout, M.-L., Fernö, A., Braithwaite, V.A., Kristiansen, T.S., 2014. Innovative behaviour in fish: Atlantic cod can learn to use an external tag to manipulate a self-feeder. *Animal Cognition* 17, 779–785.
33. Montoya, A., Martins, D.A., Yúfera, M., Sánchez-Vázquez, F.J., 2011. Self-selection of diets with different oil oxidation levels in gilthead seabream (*Sparus aurata*). *Aquaculture* 314, 282–284. <https://doi.org/10.1016/j.aquaculture.2011.01.041>
34. Montoya, A., Zamora, S., Sánchez-Vázquez, F.J., 2012. Dietary selection by gilthead sea bream (*Sparus aurata*) provided with unbalanced mixed-macronutrient feeds dispensed from self-feeders. *Aquaculture* 358–359, 35–40. <https://doi.org/10.1016/j.aquaculture.2012.06.008>
35. NRC, 2012. National Research Council (NRC): Nutrient requirements of fish and shrimp: The National Academies Press, Washington, D.C., 2011, 376 + XVI pp (Hardback), ISBN: 978-0-309-16338-5. *Aquaculture International* 20, 601–602. <https://doi.org/10.1007/s10499-011-9480-6>
36. Otero-Rodino, C., Libran-Perez, M., Velasco, C., Alvarez-Otero, R., Lopez-Patino, M.A., Míguez, J.M., Soengas, J.L., 2016. Glucosensing in liver and Brockmann bodies of rainbow trout through

- glucokinase-independent mechanisms. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 199, 29–42.
37. Pereira-da-Silva, E.M., Orsoli, D.N., Araújo, L.F., Cantelmo, O.Â., Merighe, G.K.F., 2004. Regulação da ingestão protéica na tilápia do Nilo, *Oreochromis niloticus*. *R. Bras. Zootec.* 33, 1921–1927. <https://doi.org/10.1590/S1516-35982004000800001>
38. Pratiwy, F., Kohbara, J., Dhahiyat, Y., Sunardi, S., 2017. Effectiveness of the self-feeding method on growth performance of Nile tilapia, *Oreochromis niloticus*. *Aquaculture Science* 65, 367–375.
39. Pratiwy, F.M., Kohbara, J., 2018. Dualistic feeding pattern of Nile tilapia (*Oreochromis niloticus*, L.) reared under different self-feeding system conditions. *Aquaculture Research* 49, 969–976. <https://doi.org/10.1111/are.13544>
40. Pratiwy, F.M., Pratiwi, D.Y., Kohbara, J., 2021. Machinery feeding development for sustainable aquaculture: Self-feeding system. *International Journal of Fisheries and Aquatic Studies* 9, 212–216. <https://doi.org/10.22271/fish.2021.v9.i4c.2537>
41. Puchol, S., Leal, E., Angotzi, R., Rosel, J., Morais, S., Cerdá-Reverter, J.M., 2022. Dietary discrimination using a dual-choice self-feeding system in seabream (*Sparus aurata*). *Aquaculture* 559, 738449. <https://doi.org/10.1016/j.aquaculture.2022.738449>
42. Pulido-Rodriguez, L.F., Cardinaletti, G., Secci, G., Randazzo, B., Bruni, L., Cerri, R., Olivotto, I., Tibaldi, E., Parisi, G., 2021. Appetite regulation, growth performances and fish quality are modulated by alternative dietary protein ingredients in gilthead sea bream (*Sparus aurata*) culture. *Animals* 11, 1919.
43. Raubenheimer, D., Simpson, S., Sánchez-Vázquez, J., Huntingford, F., Kadri, S., Jobling, M., 2012. Nutrition and diet choice. *Aquaculture and Behavior*. Wiley-Blackwell 150–82.
44. Raubenheimer, D., Simpson, S.J., 1997. Integrative models of nutrient balancing: application to insects and vertebrates. *Nutrition Research Reviews* 10, 151–179. <https://doi.org/10.1079/NRR19970009>
45. Richter, C.P., 1943. Total self-regulatory functions in animals and human beings. *Harvey Lecture Series* 38, 1942–1943.
46. Roy, J., Mercier, Y., Tonnet, L., Burel, C., Lanuque, A., Surget, A., Larroquet, L., Corraze, G., Terrier, F., Panserat, S., Skiba, S., 2020. Rainbow trout prefer diets rich in omega-3 long chain polyunsaturated fatty acids DHA and EPA. *Physiology & Behavior* 213, 112692. <https://doi.org/10.1016/j.physbeh.2019.112692>
47. Rubio, V.C., Sánchez-Vázquez, F.J., Madrid, J.A., 2006. Influence of nutrient preload on encapsulated macronutrient selection in European sea bass. *Physiology & Behavior* 89, 662–669. <https://doi.org/10.1016/j.physbeh.2006.08.005>
48. Rubio, V.C., Sánchez-Vázquez, F.J., Madrid, J.A., 2003. Macronutrient selection through post-ingestive signals in sea bass fed on gelatine capsules. *Physiology & Behavior* 78, 795–803. [https://doi.org/10.1016/S0031-9384\(03\)00082-9](https://doi.org/10.1016/S0031-9384(03)00082-9)

49. Ruohonen, K., Grove, D., 2001. In situ study of gastric emptying using X-radiography and macrocapsules. *Aquaculture Research* 32, 491–493.
50. Sánchez-Vázquez, F.J., Martínez, M., Zamora, S., Madrid, J.A., 1994. Design and performance of an accurate demand feeder for the study of feeding behaviour in sea bass, *Dicentrarchus labrax* L. *Physiology & Behavior* 56, 789–794. [https://doi.org/10.1016/0031-9384\(94\)90244-5](https://doi.org/10.1016/0031-9384(94)90244-5)
51. Santos, F.A.C., Fortes-Silva, R., Costa, L.S., Luz, R.K., Guilherme, H.O., Gamarano, P.G., Oliveira, C.G., Santos, W.M., Ribeiro, P.A.P., 2019. Regulation of voluntary protein/energy intake based practical diet composition for the carnivorous neotropical catfish *Lophosilurus alexandri*. *Aquaculture* 510, 198–205. <https://doi.org/10.1016/j.aquaculture.2019.05.038>
52. Simpson, S.J., Raubenheimer, D., 2001. A framework for the study of macronutrient intake in fish: A framework for nutrient regulation. *Aquaculture Research* 32, 421–432. <https://doi.org/10.1046/j.1365-2109.2001.00593.x>
53. Tidwell, J., Webster, C., Knaub, R., 1991. Seasonal production of rainbow trout, *Oncorhynchus mykiss* (Walbaum), in ponds using different feeding practices. *Aquaculture Research* 22, 335–342.
54. Toguyeni, A., Fauconneau, B., Boujard, T., Fostier, A., Kuhn, E.R., Mol, K.A., Baroiller, J.-F., 1997. Feeding Behaviour and Food Utilisation in Tilapia, *Oreochromis Niloticus*: Effect of Sex Ratio and Relationship With the Endocrine Status. *Physiology & Behavior* 62, 273–279. [https://doi.org/10.1016/S0031-9384\(97\)00114-5](https://doi.org/10.1016/S0031-9384(97)00114-5)
55. Vivas, M., Rubio, V.C., Sánchez-Vázquez, F.J., Mena, C., García García, B., Madrid, J.A., 2006. Dietary self-selection in sharpsnout seabream (*Diplodus puntazzo*) fed paired macronutrient feeds and challenged with protein dilution. *Aquaculture* 251, 430–437. <https://doi.org/10.1016/j.aquaculture.2005.06.013>
56. Vivas, M., Sánchez-Vázquez, F.J., García García, B., Madrid, J.A., 2003. Macronutrient self-selection in European sea bass in response to dietary protein or fat restriction: Protein or fat restriction in macronutrient self-selection in sea bass. *Aquaculture Research* 34, 271–280. <https://doi.org/10.1046/j.1365-2109.2003.00799.x>
57. Volkoff, H., 2019. Fish as models for understanding the vertebrate endocrine regulation of feeding and weight. *Molecular and Cellular Endocrinology* 497, 110437. <https://doi.org/10.1016/j.mce.2019.04.017>
58. White, B.D., Porter, M.H., Martin, R.J., 2000. Protein selection, food intake, and body composition in response to the amount of dietary protein. *Physiology & Behavior* 69, 383–389. [https://doi.org/10.1016/S0031-9384\(99\)00232-2](https://doi.org/10.1016/S0031-9384(99)00232-2)
59. Yamamoto, T., Shima, T., Furuita, H., Suzuki, N., Sánchez-Vázquez, F.J., Tabata, M., 2001. Self-selection and feed consumption of diets with a complete amino acid composition and a composition deficient in either methionine or lysine by rainbow trout, *Oncorhynchus mykiss* (Walbaum): Self-feeding of amino acid-deficient diets by trout. *Aquaculture Research* 32, 83–91. <https://doi.org/10.1046/j.1355-557x.2001.00007.x>

Figures

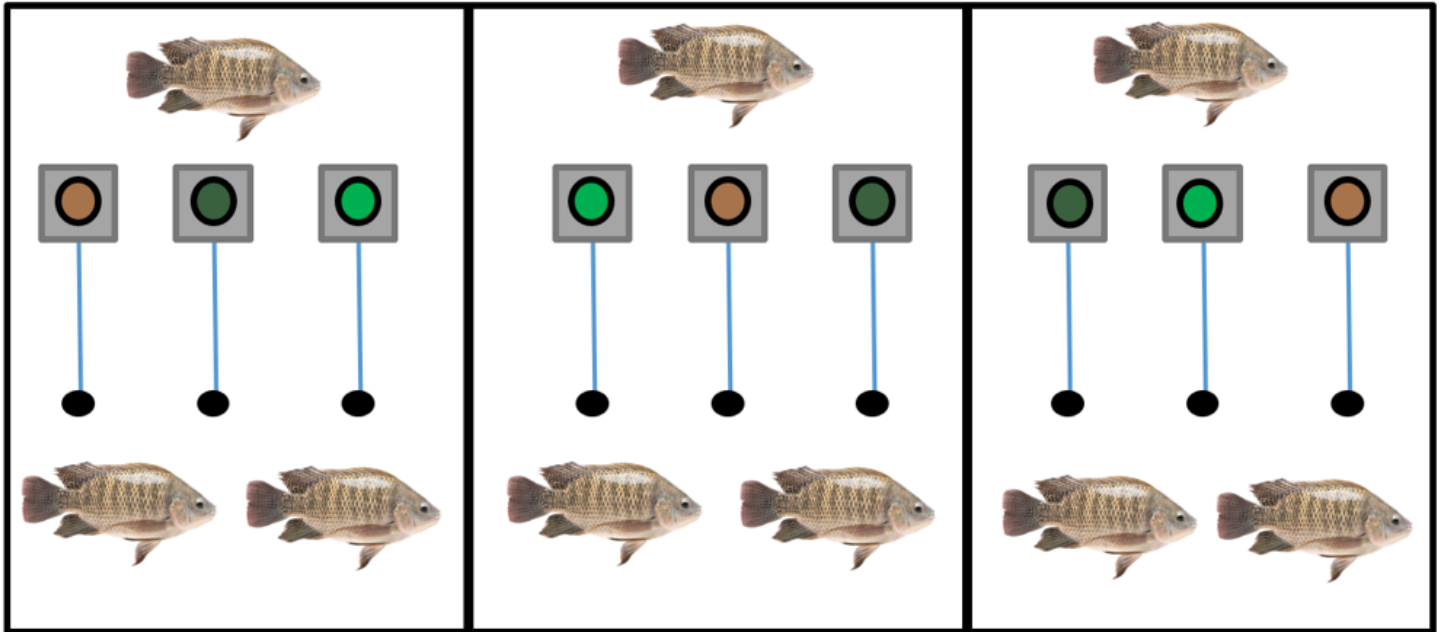


Figure 1

Experimental setup for self-feeders trial using Nile tilapia (*Oreochromis niloticus*). Each of the three feeders inside each tank, contained a specific feed (PD, ORG1 and ORG2).

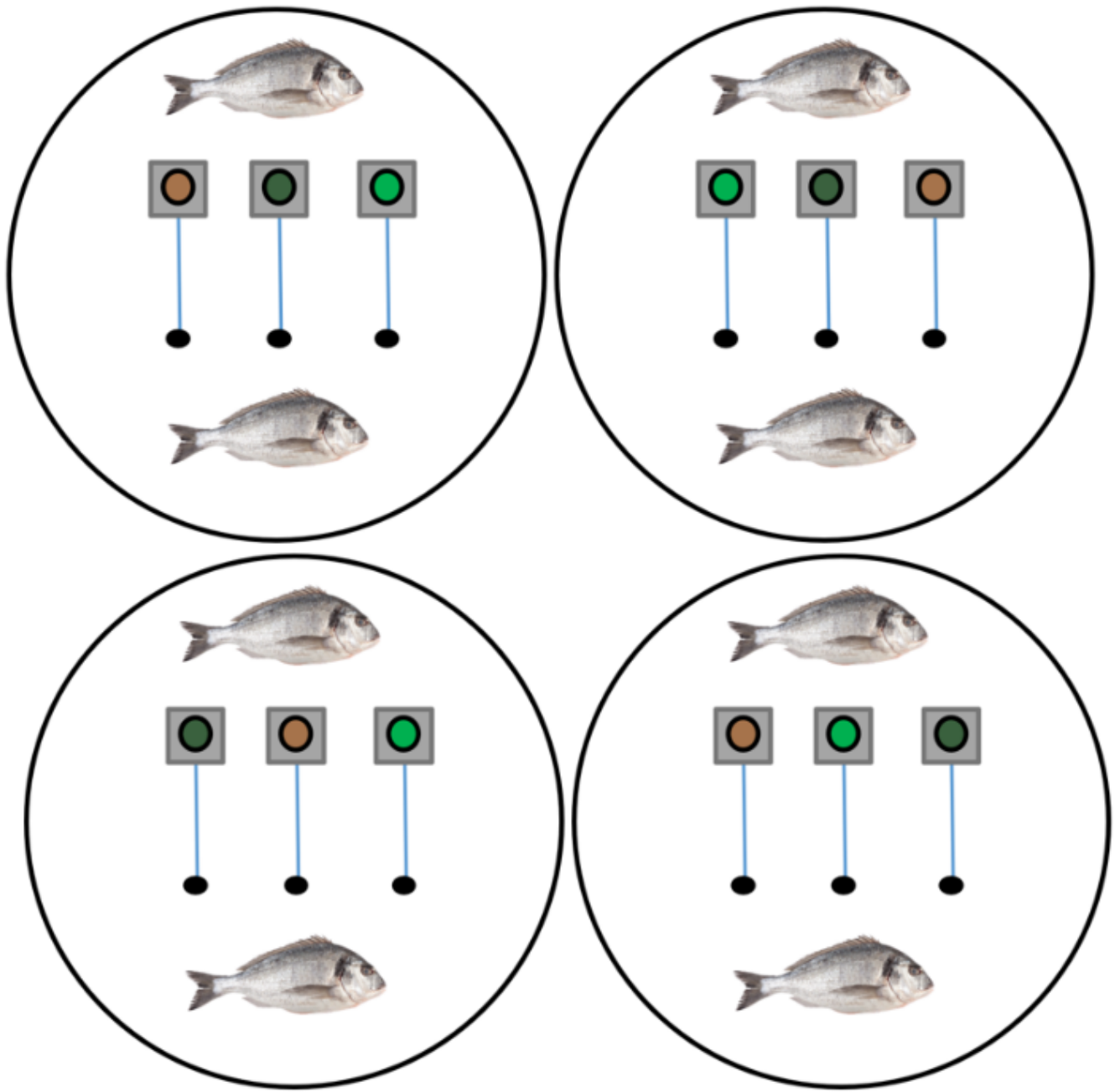


Figure 2

Experimental setup for self-feeders trial using gilthead seabream (*Sparus aurata*). Each of the three feeders inside each tank, contained a specific feed (PD, ORG1 and ORG2).

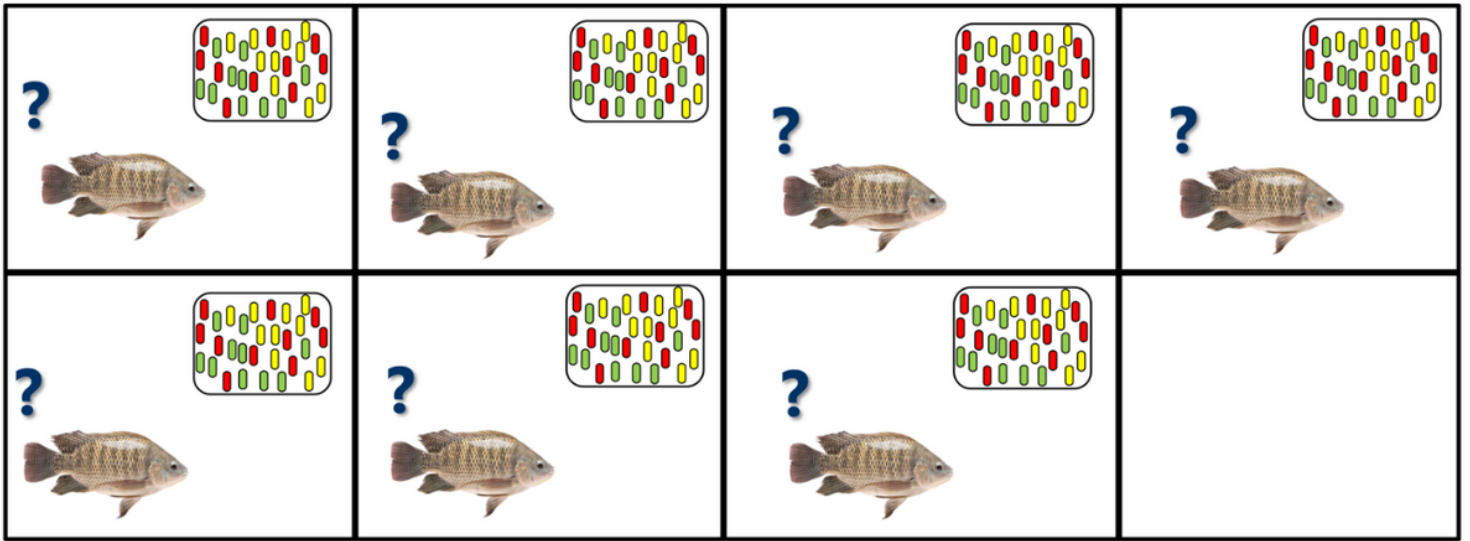


Figure 3

Experimental setup for diet encapsulation trial using Nile tilapia (*Oreochromis niloticus*). Each capsule colour represent a specific feed (A, B and C).

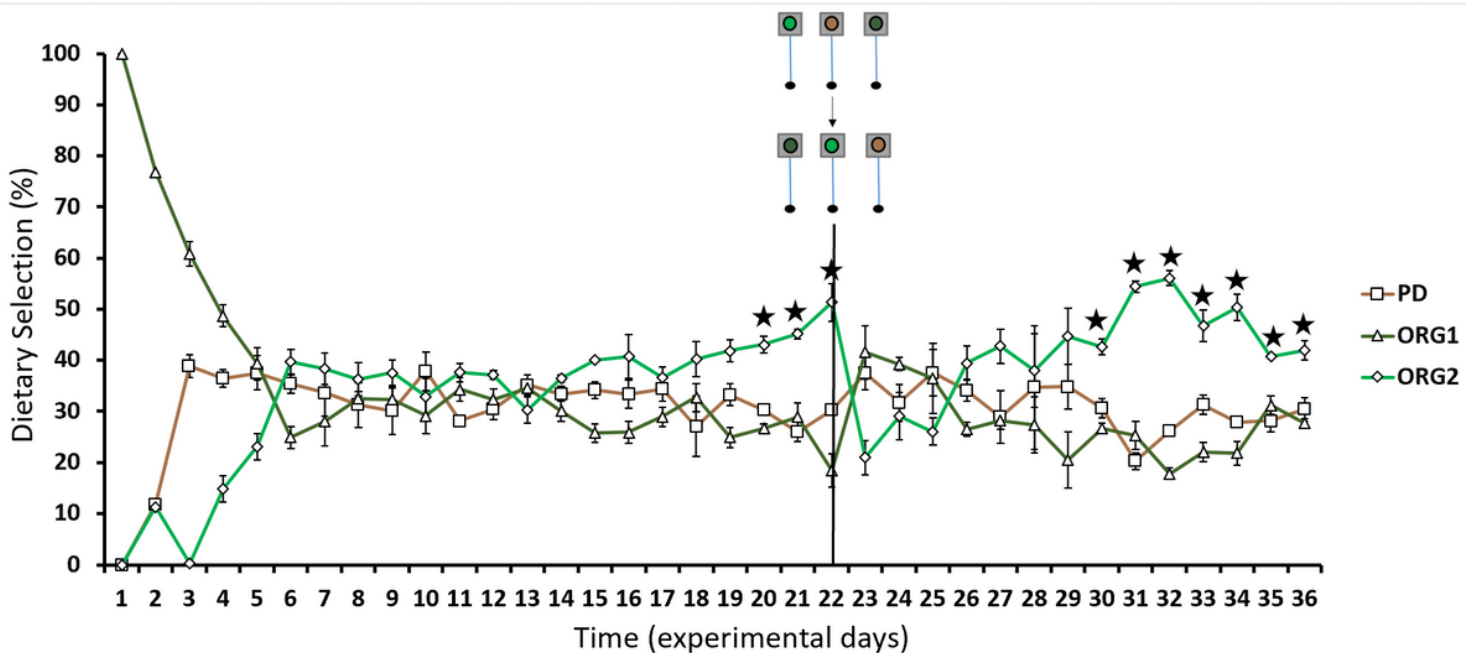


Figure 4

Evolution of average daily intake (% total grams of feed ingested) of three diets (PD, ORG1 and ORG2) by Nile tilapia over 36 days. Diets were changed between feeders on day 22. Lines represent the mean counts $\pm$ SD ($n=3$ tanks). Stars represent significant differences (one-way ANOVA, $p < 0.05$).

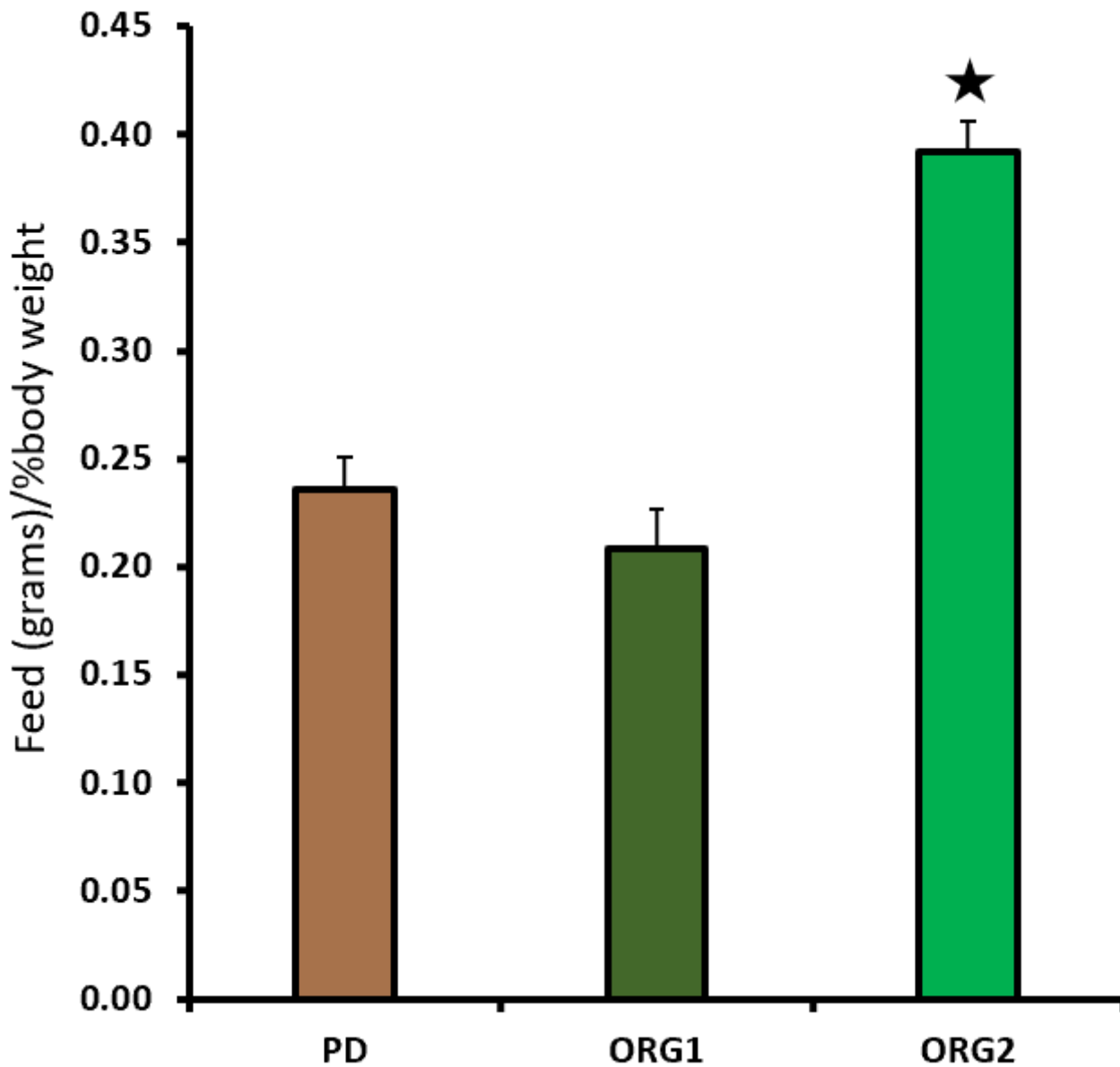


Figure 5

Average daily intake (total grams of feed ingested per percentage of body weight) of three diets (PD, ORG1 and ORG2) by Nile tilapia only during days with statistically significant differences. Bars represent the mean counts $\pm$ SD ($n=3$ tanks). The star represents significant differences (one-way ANOVA, $p < 0.05$).

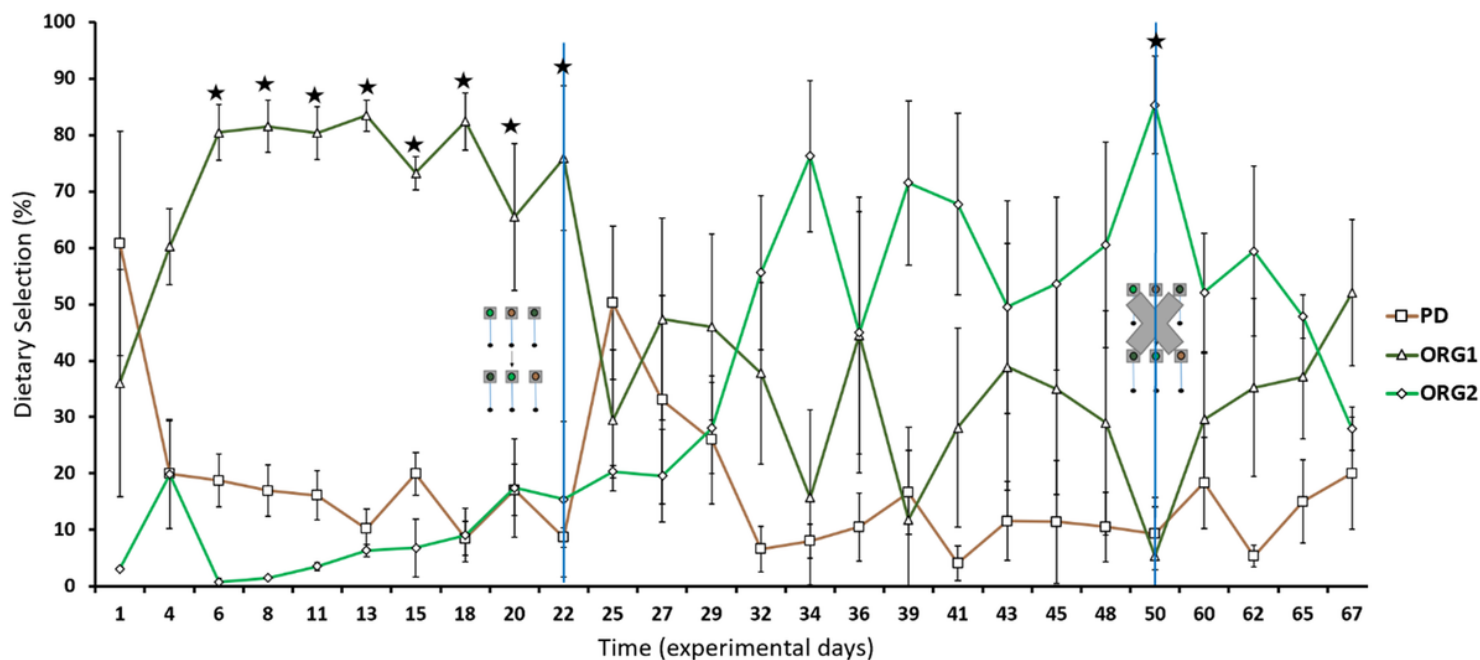


Figure 6

Evolution of average daily intake (% total grams of feed ingested) of three diets (PD, ORG1 and ORG2) by gilthead seabream over 67 days. Diets were changed between feeders on day 22 and fish were fasted on day 50. Lines represent the mean counts $\pm$ SD ($n=4$ tanks). Stars represent significant differences (one-way ANOVA, $p < 0.05$).

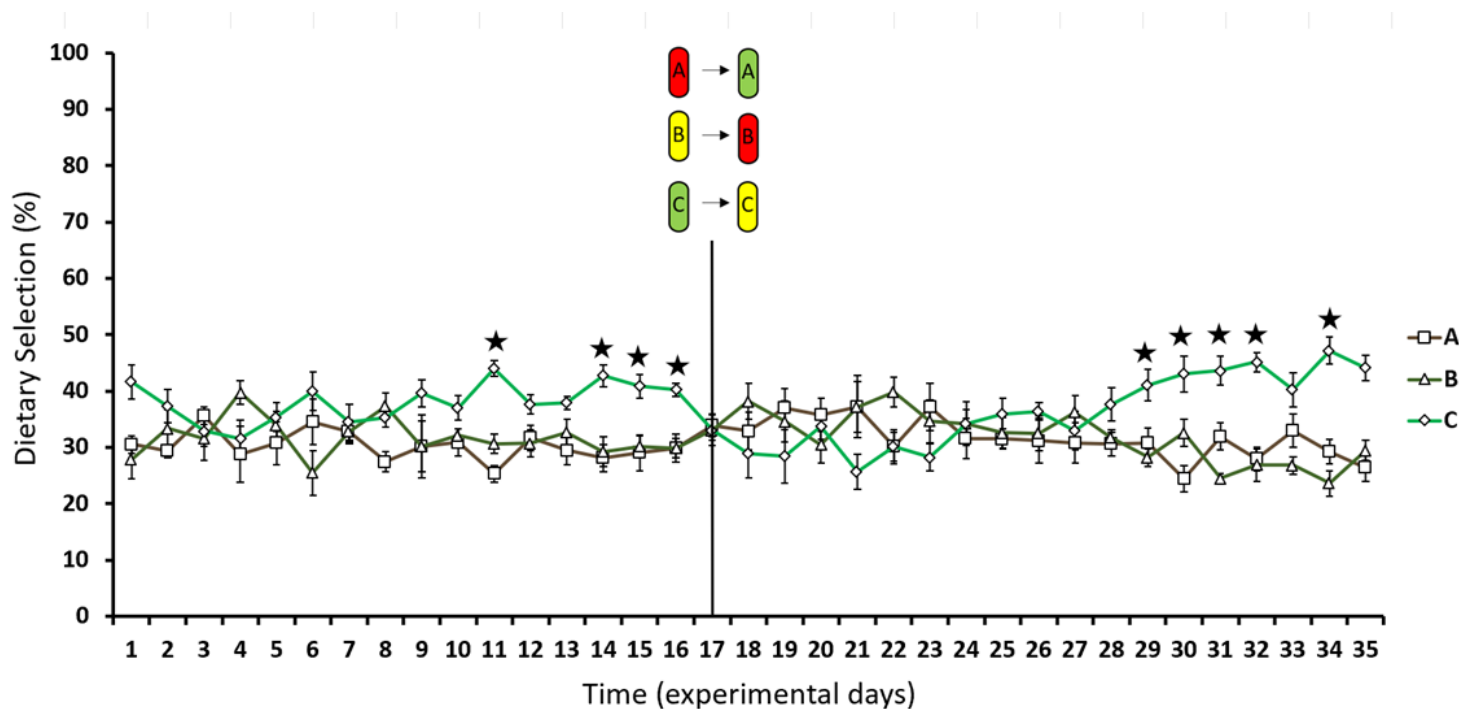


Figure 7

Evolution of average daily intake (% total number of capsules ingested) of three diets (A, B and C) by Nile tilapia over 35 days. The content of the capsules changed on day 17. Lines represent the mean counts $\pm$ SD ($n=7$ individual fish). Stars represent significant differences (one-way ANOVA, $p < 0.05$).

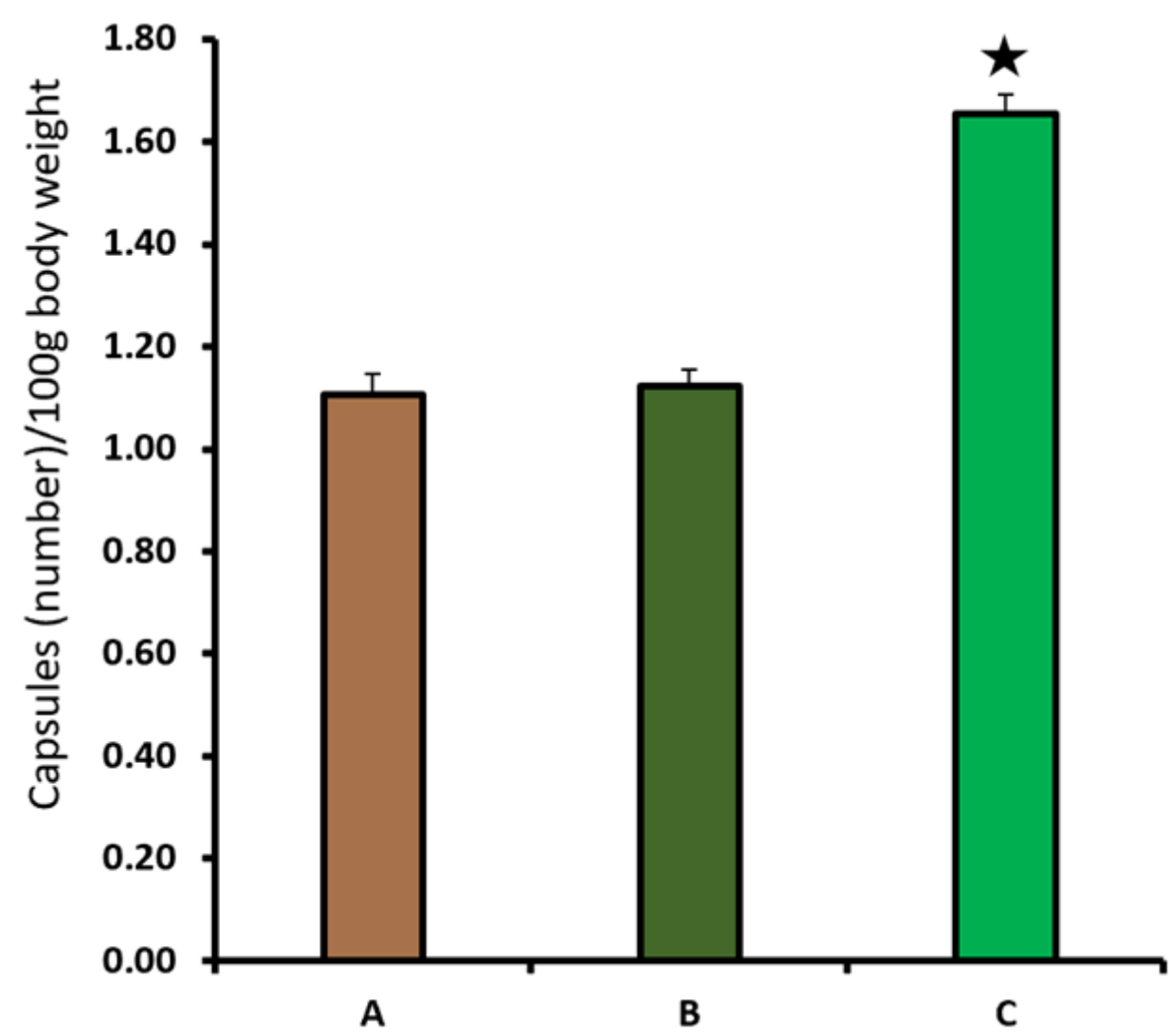


Figure 8

Average daily intake (number of capsules ingested per 100g of body weight) of three diets (A, B and C) by Nile tilapia during days with statistically significant differences. Bars represent the mean counts $\pm$ SD ($n=7$ individual fish). The star represents significant differences (one-way ANOVA, $p < 0.05$).