PAPER

Effects of dietary lipid level on growth and feed utilisation of gilthead seabream (*Sparus aurata* L.) reared at Mediterranean summer temperature

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Abstract

We investigated the effects of different dietary lipid levels on gilthead seabream, *Sparus aurata*, reared at Mediterranean summer temperature. Sixty fish (average weight 75 g) per tank were randomly distributed, in triplicate groups, in a recirculating rearing system (27±1°C) and fed *ad libitum* five isonitrogenous (46% dietary protein) diets with increasing lipid level (16, 18, 20, 22 and 24% named D16, D18, D20, D22 and D24, respectively), over 89 days. Specific growth rate and final body weight were not affected by dietary lipid levels. Feed conversion ratio was significantly higher (P≤0.05) in D16 as compared to the other treatments, most likely due to the shortage of dietary energy supply, coped with a significantly higher voluntary feed intake. Consequently, we obtained a significantly lower protein efficiency ratio and gross protein efficiency in D16. Gross lipid efficiency was significantly higher in D16 and D18 than in the other treatments. Biometric parameters and lipase activity in gut content were not influenced by dietary treatments. In conclusion, D18 seems the most suitable diet for gilthead seabream reared at Mediterranean summer temperature, providing both the lowest fish in fish out (FIFO) ratio and a protein sparing effect, which makes gilthead seabream's production economically and environmentally more sustainable.

Introduction

Gilthead seabream, *Sparus aurata*, is a species of great interest in Europe, representing around the 51% of the total finfish marine and brackish water aquaculture production in the Mediterranean area (FAO, 2010).

Due to the current economic downturn and the fluctuation of gilthead seabream market, aquaculture producers are focusing to improve performances and reduce costs, where feed accounts for about 60 to 80% in intensive aquaculture (Hasan *et al*., 2007).

In this context, the optimisation of gilthead seabream farming by enhancing feed efficiency and the use of specific diets is a major factor in aquaculture and environmental sustainability (Bonaldo *et al*., 2010). Indeed, the optimal amount of dietary lipids would reduce the use of protein for energy production leading to a protein sparing effect, as already observed in gilthead seabream by Caballero *et al*. (1999) and to a decreased nitrogen excretion with environmental benefit (McGoogan and Gatlin, 1999). On the other hand, excess use of fish oil would lead to an increased feed price and to a worsening fish in fish out (FIFO) ratio. FIFO is defined as the efficiency at which aquaculture converts a weight-equivalent unit of wild fish into a unit of cultured fish (Merino *et al*., 2012).

Along with new feeding strategies, it is important to take into account the high water temperature of Mediterranean sea during summer period, especially as it is increasing the number of sea cages for cultured gilthead seabream. Furthermore, the average temperature of the Earth's surface has increased by about 0.8°C over the past 100 yr for the global warming and is projected to increase by between 1.8 to 4°C by the end of the 21st century (relative to the 1980 to 1999 average) (IPCC, 2007).

Most of the trials on growth performance and energy requirement in gilthead seabream have been performed between 21 and 24°C (Aksness *et al*., 1997; Deguara, 1997; Kissil *et al*., 2000; Lupatsch *et al*., 2001, 2003; Venou *et al*., 2003; Bonaldo *et al*., 2010), though, it should be considered that water temperature of the coastal surface in the Mediterranean basin is well above this range for several weeks during summer time, reaching 26 to 27°C or even more.

Fish are highly influenced by water temperature, which is known to affect ingestion, evacuation rate, metabolism and growth rate. The temperature at which fish growth is maximised is called the *optimum temperature for growth* and it should be noted that this optimal temperature is a few degrees lower than the temperature at which feed intake is greatest (Jobling, 1994). Over a certain temperature, rate of ingestion will decline steeply (Jobling, 1993) with a sharply increase of fish basal metabolism and the active metabolism raise even more than the standard one (Brett, 1964), generally with a consequent drop in growth (Calderer Reig, 2001). Up to a certain limit, a temperature raise, even of a few degree, has a positive effect on feed efficiency in gilthead seabream, with a much greater growth potential in spite of the increased energy requirement (Lupatsch *et al*., 2003). This latest concept has important implications in the formulations and feeding strategies in aquaculture, since a shortage of dietary energy would lead to a lower feed and protein utilisation efficiency. Therefore, being lipids the main energy source in diets for carnivorous fish species (National Research Council, 2011), their level should be carefully assayed.

Hence, the aim of our experiment was to assess the effect of different dietary lipid levels in gilthead seabream reared at 27±1°C with the less outlay of fish oil, thus maximising profits.

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Materials and methods

Experimental diets

Ingredients and proximate composition of the experimental diets are presented in Table 1. Five isonitrogenous (46% dietary protein) diets, formulated to contain increasing fat levels (16, 18, 20, 22 and 24% named D16, D18, D20, D22 and D24, respectively), have been provided by Skretting Aquaculture Research Centre, Stavanger, Norway.

Lipid levels were defined on the basis of a previous trial carried out in our laboratory highlighting the dietary lipid requirement in gilthead seabream fed *ad libitum*. Indeed, fish fed on 16% lipid displayed the best growth and feed utilisation at 24±1°C (Bonaldo *et al*., 2010), suggesting a range of 16 to 24% lipid inclusion to cope the enhanced energy requirement at higher temperature. All feeds were produced as extruded sinking pellets with a diameter of 4 mm.

Fish and feeding trial

The experiment was carried out at the Laboratory of Aquaculture, Department of Veterinary Medical Science (DIMEVET), Cesenatico (FC), Italy. Gilthead seabream, *S. aurata*, were obtained from the Panittica Pugliese hatchery, Torre Canne di Fasano (BR), Italy. Before starting the trial fish were acclimated at 27±1°C and fed with a mixture of the five diets for 14 days. Sixty fish per tank were weighed individually (75±1.4 g initial mean body weight) and randomly assigned to 800 L square tanks with a conical bottom. Each treatment was tested in triplicate tanks. All tanks were integrated in a recirculating rearing system with a flow of 16.6 L/min/tank. The overall water renewal of the system was 5% daily. Water temperature was maintained constant at 27±1°C to simulate the water temperature of the Mediterranean sea during summer periods. Photoperiod was held constant at a 12 h day length through artificial light (200 lx at the water surface) (Delta Ohm luxmeter HD-9221; Delta-Ohm, Padova, Italy). Dissolved oxygen level was kept at 100% saturation with a liquid oxygen system connected to a software controller [B&G Sinergia S.n.c., Chioggia (VE), Italy].

Feed was provided to approximately 5% overfeeding. Automatic feeders distributed the feed for seven days a week, over 89 days. The 60% of the daily ration was given in the morning between 08:30 and 09:30 and 40% in the afternoon between 15:30 and 16:30 for 1 h at a time. On Sundays, fish only get the 60% ration in the morning. During the meal, the uneaten feed was trapped by a feed collector put at the water output of tanks. In order to reduce feed leaching, collectors were emptied every 10 min. The uneaten pellets of each tank was daily gathered and dried overnight at 105°C. Thus, the actual voluntary feed intake (VFI) was determined daily. All experimental procedures were evaluated and approved by the Ethical-Scientific Committee for Animal Experimentation of the University of Bologna, in accordance with the European Community Council directive (86/609/ECC).

Sample collection and methods for chemical analysis

At the beginning and at the end of the experiment the fish were individually weighed and measured to determine specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF).

Ten fish from the initial bulk and ten fish from each tank at the end of the trial have been sampled after 1 day of starvation for chemical analyses of whole body composition and to calculate protein efficiency ratio (PER), gross protein efficiency (GPE) and gross lipid efficiency (GLE). All samples were stored at -20°C before analysis. Moisture content was obtained by weight loss after drying samples in stove at 105°C until constant weight. Crude protein was determined as total nitrogen (N) by using Kjeldahl method and multiplying N by 6.25. Total lipids were extracted according to Folch *et al*. (1957). Ash content was made by incineration to a constant weight in a muffle oven at 450°C. Gross energy was determined by calorimetric bomb (Adiabatic Calorimetric Bomb Parr 1261; PARR Instrument, Moline, IL, USA). Furthermore, at the end of the experiment ten more fish per tank have been sampled for wet weight, fat viscera weight, viscera and liver weight for the calculation of fat index (FaI), viscerosomatic index (VSI) and the hepatosomatic index (HSI). Then, five fillets and two skinned fillets from each of those ten fish were sampled for the determination of fillet yield (FY) and the muscle proximate analyses, respectively. At the end of the growth trial and all the associated samplings, the remaining groups of fish were used to determine the lipase activity from the gut content. This parameter was determined using the identical facilities and environmental conditions used in the growth trial. Gut content was collected 8 h after feeding by dissection of five animals per tank and by stripping the intestinal content into five different Eppendorf tubes (1.5 mL) respectively, prior to storage at -80°C until quantification of lipase activity using a Lipase Assay Kit (BioVision Inc., Milpitas, CA, USA). Briefly, lipase hydrolyses a triglyceride substrate to form glycerol, which is quantified enzymatically by monitoring a linked change in a OxiRed probe's absorbance (λ=500 nm). One unit of enzyme activity is defined as the amount of lipase that hydrolyses triglyceride to yield 1 μmol of glycerol per min at 37°C.

Statistical analysis

Data are presented as mean±SD of three replicate groups. All data were analysed by one-way ANOVA with Tukey's post-hoc test. All analyses were made using the statistical package R version 2.11.1 for Windows (Revolution Analytics, Mountain View, CA, USA). Significant differences were assumed when $P \leq 0.05$.

Results and discussion

High survival (93.3±0.0, 92.2±2.6, 92.2±4.2, 95.0±4.4 and 95.6±2.6% in D16, D18, D20, D22 and D24, respectively), with no significant differences, was reported in all treatments.

Water quality parameters as total ammonia nitrogen (≤0.1 mg/L), nitrite (NO^{2-}≤0.2 mg/L), nitrate (NO^{3-}≤50 mg/L), salinity (25 to 30 g/L) and pH (7.8 to 8.0) were held optimal and monitored daily during the whole experimental period.

The effects of different dietary lipid levels on gilthead seabream performances and nutritional indices are shown in Table 2. It is well known that at high water temperature there is an increase of fish's energy requirement, given the general effect on biochemical reactions (Eccles, 1985). In our trial the choice of setting the water temperature at 27°C has been taken in view of the work of Rigos *et al.* (2011), whilst ensuring good growth performances (Mozes *et al.*, 2011). However, with increasing temperature there is an increase in metabolic rate and, consequently, the amount of feed required for maintenance increases. Thus, as the ratio size is gradually increased, the scope for growth is lowest at progressively higher temperature and marked increase in energy requirements for maintenance, that accompanies rising temperature, accounts for the fact that fish lose weight when fed low rations at high temperatures (Jobling, 1994). Requena *et al*. (1997) demonstrated that the metabolic rate of gilthead seabream doubled when fish were reared at 28°C as compared to 20°C and that a shortage of dietary energy supply would reduce growth and increase nitrogenous output as a consequence of increased protein catabolism.

In the current study, increasing dietary lipid

level from 16 to 24% did not cause any significant differences in FBW and SGR. Those results are in agreement with other works (Velázquez *et al*., 2006; Bonaldo *et al*., 2010) conducted on gilthead seabream at lower temperatures, 26 and 24°C respectively, where dietary lipid content did not exert any effect on the FBW and SGR. Conversely, VFI showed a significant increment going from the highest lipid level toward the lowest one, as if it was adjusted on the basis of the dietary lipid level. Those results are in agreement with the thesis that fish, like homeothermic animals, adapt feed intake to meet their energy requirements (Kaushik and Médale, 1994; Lupatsch *et al*., 2001; Bonaldo *et al*., 2009).

The FCR resulted significantly lower in diets from D18 to D24 as compared to the lowest lipid level diet which could be related to a deficiency of dietary lipids in the last. Moreover, this datum is confirmed by a significantly reduced PER in D16 as compared to the other groups and a significantly lower GPE in D16 when compared to D20, D22 and D24. Indeed the lower dietary lipid level in D16 led to a subsequent higher utilisation of dietary protein for energy. Since protein retention is generally regulated by non-protein energy intake, PER is a good measure of the protein sparing effect of lipid (Lie *et al*., 1988). PER has been studied by several researchers in many fish species fed high energy diets where lipids represented the main energy source. In salmonids, up to 30% dietary lipid inclusion was found to improve feed and protein utilisation efficiencies and to reduce N excretion (Torstensen *et al*., 2001). This effect has also been reported in tilapia (Shiau and Peng, 1993), European seabass (Dias *et al*., 1998) and Atlantic halibut (Helland and Grisdale-Helland, 1998). Nevertheless, there are also some reports that have observed no protein-sparing effect of lipid in several species (McGoogan and Gatlin, 1999; Ozório *et al*., 2006) including gilthead seabream (Company *et al*., 1999; Vergara *et al*., 1999; Velázquez *et al*., 2006; Bonaldo *et al*., 2010). The results of our trial show that the increase of dietary lipids from D16 to D18 had a protein sparing effect, which did not increase further with lipid percentages beyond 18%. Consistently, significantly higher GLE values were found in fish fed the two lowest lipid diets over which any additional use of lipid resulted in a waste of energy.

FIFO ratio can be reduced both by substituting fish oil and fishmeal with plant based ingredients and/or by assessing the optimal

Table 1. Ingredients and proximate composition of experimental diets.

	Diet				
	D16	D18	D20	D22	D24
Ingredients, g/100 g					
Fishmeal North-Atlantic	20.00	20.00	20.00	20.00	20.00
Soy protein concentrate	20.00	20.00	20.00	20.00	20.00
Soybean meal	15.00	15.00	15.00	15.00	15.00
Wheat gluten	9.25	9.65	10.04	10.43	10.81
Sunflower meal	5.00	5.00	5.00	5.00	5.00
Corn gluten	2.00	2.00	2.00	2.00	2.00
Fish oil North-Atlantic	11.83	13.88	15.94	17.99	20.05
Wheat	15.92	13.47	11.02	8.58	6.14
Vitamin and mineral premix°	1.00	1.00	1.00	1.00	1.00
Proximate composition					
Moisture, %	7.55	7.26	6.48	7.13	6.55
Crude protein, %	46.03	46.31	45.81	46.16	46.94
Total lipids, %	17.69	18.92	22.17	23.17	26.33
Ash, %	5.61	5.66	5.70	5.65	5.74
Gross energy, MJ/kg	21.10	21.70	21.90	22.40	22.90
DE[#], MJ/kg	17.80	18.30	18.80	19.30	19.80

DE, digestible energy of feed ingredients. °Standard vitamin and mineral premix provided by Skretting Aquaculture Research Centre, Stavanger, Norway; [#]DE was determined according to Lupatsch *et al.* (1997), Ahmad *et al.* (2004), Kissil and Lupatsch (2004) and Lupatsch (2004).

Table 2. Growth performance and feed utilisation in gilthead seabream fed experimental diets over 89 days.

	Diet					
	D16	D18	D20	D22	D24	P
IBW, g	75.30±1.30	74.40±0.30	76.20±0.70	74.90±2.80	75.30±0.50	-
FBW, g	296.70±12.80	303.90±4.50	300.00±4.10	293.80±7.00	289.80±12.50	ns
SGR, %/day	1.54±0.05	1.58±0.02	1.54±0.02	1.54±0.03	1.51±0.04	ns
VFI, g	333.41±16.17^{a}	318.91±4.97^{ab}	309.73±7.31^{abc}	301.50±5.99^{bc}	293.41±8.98^{c}	≤0.01
FCR, g	1.53±0.03^{a}	1.38±0.03^{b}	1.37±0.03^{b}	1.38±0.00^{b}	1.38±0.06^{b}	≤0.001
PER, g	1.44±0.02^{b}	1.55±0.06^{a}	1.58±0.02^{a}	1.57±0.01^{a}	1.56±0.06^{a}	≤0.01
GPE, %/g	24.86±0.58^{b}	26.23±0.72^{ab}	27.33±0.55^{a}	27.31±0.56^{a}	26.69±0.68^{a}	≤0.01
GLE, %/g	69.86±0.93^{a}	69.66±3.00^{a}	56.63±4.08^{b}	55.96±6.27^{b}	49.76±3.24^{b}	≤0.001
FIFO, g/%	1.75±0.04^{c}	1.70±0.04^{c}	1.79±0.04^{bc}	1.91±0.00^{ab}	2.02±0.08^{a}	≤0.001
ECR, €/kg	1.52±0.02^{ab}	1.45±0.05^{b}	1.49±0.02^{ab}	1.53±0.01^{ab}	1.56±0.06^{a}	≤0.05

IBW, initial body weight; FBW, final body weight; ns, not significant; SGR, specific growth rate=100 x (ln final body weight - ln initial body weight)/[duration of experiment (days)]; VFI, voluntary feed intake=[total feed intake (g)/fish]; FCR, feed conversion ratio=dry feed consumed (g)/wet weight gain (g); PER, protein efficiency ratio=fish weight gain including weight of dead fish (g)/total protein intake (g); GPE, gross protein efficiency=100 x [(% final body protein x final body weight) - (% initial body protein x initial body weight)]/total protein intake (g); GLE, gross lipid efficiency=100 x [(% final body lipid x final body weight) - (% initial body lipid x initial body weight)]/total lipid intake (g); FIFO, fish in fish out ratio=fish oil content + fish meal (g)/yield of FO from wild fish + yield of fish meal from wild fish (%) x FCR (Jackson, 2010); ECR, economic efficiency ratio=[total feed intake (g) x feed cost (€ kg^{-1})]/weight gain (g). Each value is the mean±standard deviation of three replicates. $^{a-c}$Different letters in the same row indicate significant (P≤0.05) differences among treatments.

Table 3. Proximate composition of carcass, fillet and biometric parameters of gilthead seabream fed experimental diets over 89 days.

	Initial carcass composition	Diet					P
		D16	D18	D20	D22	D24	
Carcass, %							
Moisture°	65.86±0.31	62.38±0.28	62.85±0.28	62.46±0.53	62.97±1.10	63.18±0.56	ns
Crude protein	17.26±0.13	17.23±0.12	16.96±0.13	17.29±0.18	17.32±0.23	17.16±0.17	ns
Total lipids	15.46±0.27	17.80±0.27	17.59±0.14	16.87±0.92	17.23±1.52	17.27±0.60	ns
Ash	4.44±0.08	2.91±0.08	2.71±0.07	3.07±0.18	2.75±0.11	2.93±0.14	ns
Fillet, %							
Moisture#		69.69±0.50	68.62±0.04	69.47±0.94	69.28±0.65	69.09±0.66	ns
Crude protein		20.55±0.50	20.40±0.02	20.08±0.18	20.15±0.28	20.26±0.52	ns
Total lipids		8.64±0.87	9.47±0.34	8.64±0.83	9.45±1.16	9.37±1.22	ns
Ash		1.35±0.04	1.36±0.02	1.38±0.03	1.36±0.04	1.39±0.02	ns
Biometric parameters, %							
CF		1.77±0.17	1.79±0.15	1.76±0.21	1.77±0.17	1.79±0.17	ns
VSI		5.50±0.74	5.47±0.89	5.50±0.96	5.26±0.84	5.14±0.90	ns
HSI		1.40±0.32^{a}	1.37±0.31^{ab}	1.34±0.31^{ab}	1.18±0.31^{b}	1.19±0.29^{ab}	≤0.05
Fal		1.47±0.67	1.65±0.68	1.61±0.92	1.58±0.82	1.40±0.73	ns
FY		48.97±3.57	50.33±2.73	49.89±2.72	49.46±2.87	48.70±3.59	ns
Lipase activity§		0.70±0.36	0.54±0.24	0.51±0.26	0.40±0.24	0.50±0.25	ns

ns, not significant; CF, condition factor=100 x [body weight (g)/(body length (cm)]^{3}, n=one pool of 60 fish per tank; VSI, viscerosomatic index=100 x [viscera weight (g)/whole body weight (g)], n=ten fish per tank; HSI, hepatosomatic index=100 x [liver weight (g)/whole body weight (g)], n=ten fish per tank; Fal, fat index=100 x [visceral fat weight (g)/whole body weight (g)], n=ten fish per tank; FY, fillet yield=100 x [fillet weight (g)/whole body weight (g)], n=ten fish per tank. °Carcass moisture comprises protein, lipid and ash (% wet weight), n=one pool of ten fish per tank; #fillet moisture comprises protein, lipid and ash (% wet weight), n=one pool of two fish per tank; §one unit of enzyme activity is defined as the amount of lipase that hydrolyses trygliceride to yeld 1 mol of glycerol per min at 37°C, n=five fish per tank. Data are shown as mean±standard deviation. ^{a,b}Different letters in the same row indicate significant (P≤0.05) differences among treatments.

dietary lipid level. Replacing fish oil with vegetable oil in aquafeed may affect several aspects of fish lipid metabolism as reported in several trials on gilthead seabream (Montero *et al.*, 2003; Menoyo *et al.*, 2004; Fountoulaki *et al.*, 2009). Hence, we used fish oil as the only lipid source in order to avoid any interferences or bias in data interpretation. FIFO ratio in fish fed D16 and D18 was statistically improved as compared with fish fed the two highest lipid level diets. A significant difference in FIFO ratio was also registered between D20 and D24 with the worst value for the latter. However, when considering FIFO ratio the economic efficiency ratio (ECR) should be also taken into account, as to have an economic evaluation of the diets and improving fish feeding profitability (Martínez-Llorens *et al.*, 2012). Thus, accordingly to the trend of FIFO data, fish fed D18 gave the best ECR, which resulted significantly lower as compared to fish fed D24. Our results are consistent with those obtained in previous feeding trial on the same species (Martínez-Llorens *et al.*, 2012; Bonaldo *et al.*, 2010) and on sharpsnout seabream, *Diplodus puntazzo* (Hernández *et al.*, 2007).

Whole-body composition, fillet composition and biometric parameters are shown in Table 3. As reported in gilthead seabream (Vergara *et al.*, 1996, 1999) and in other marine fish species (Péres and Oliva-Teles, 1999; Luo *et al.*, 2005), high dietary lipid levels are known to increase fat deposition in visceral cavity and muscle tissues. However, this tenet is not in accordance with our results and those of Marais and Kissil (1979), Velázquez *et al.* (2006) and Bonaldo *et al.* (2010), where no correlation was observed between dietary lipid content and fat deposition in visceral cavity and muscle tissue. Neither the fillet nor the body composition were influenced by dietary treatments and fillet proximate composition resulted consistent with that found by Testi *et al.* (2006) and Bonaldo *et al.* (2010). In the present study, a slight negative correlation between HSI and dietary lipid content was observed, where only D16 was significantly different from D22, perhaps for the restrained range of variation. However, our results are in agreement with previous findings on the same species (Venou *et al.*, 2006; Couto *et al.*, 2008; Bonaldo *et al.*, 2010) and on other species like Sunshine bass (Hutchins *et al.*, 1998) and European seabass (Peres and Oliva-Teles, 1999). This pattern may be due to the higher content of starch in low lipid diets, which could stimulate a *de novo* lipid synthesis and deposition in the liver (Evans *et al.*, 2005) or increase hepatic glycogen deposition (Wilson, 1994; Couto *et al.*, 2008; Coutinho *et al.*, 2012). Nevertheless, the VSI was not significantly affected by dietary lipid levels even thought a slight sloping trend was observed from D20 towards D24, most likely correlate to the HSI values. The dietary treatments did not affect the CF, FaI and FY. Moreover, the FaI of our treatments was lower as compared to those found in previous trials on the same species (Martínez-Llorens *et al.*, 2007; Benedito-Palos *et al.*, 2007; Fountoulaki *et al.*, 2009; Bonaldo *et al.*, 2010) and on sharpsnout seabream (Piedecausa *et al.*, 2007), regardless of the lipid source and the inclusion level, whereas FY was consistent with those of Bonaldo *et al.* (2010) or higher as compared to Fountoulaki *et al.* (2009) and Martínez-Llorens *et al.* (2007). The lipase activity remained almost unchanged among the five treatments showing a slight inverse correlation with lipid level, as expected. Indeed, a similar pattern was found on sea bass larvae (Morais *et al.*, 2004) where it was suggested an adaptive response with lower lipase secretion in fish fed diets containing higher content of digestible lipids, whereas in Senegalese sole (Borges *et al.*, 2013) different dietary lipid levels did not exert any effect on lipase activity.

Conclusions

In conclusion, D18 seems the most suitable diet for gilthead seabream fed *ad libitum* at Mediterranean summer temperature. Indeed,

our results show that D16 and D18 allow a reduction in the FIFO ratio but an increase of lipids from 16 to 18% exerted a protein sparing effect making gilthead seabream's production economically and environmentally more sustainable.

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