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Evaluation of dietary protein and lipid requirements of two-banded seabream (*Diplodus vulgaris*) cultured in a recirculating aquaculture system

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Abstract The objective of this study is to investigate the effects of dietary protein and lipid levels on the growth performance and bioeconomic benefits of two-banded seabream (*Diplodus vulgaris*) juveniles, a candidate species for aquaculture sector. Eight experimental diets were formulated with four protein (50, 45, 40 and 35 %) levels for each of the two lipid levels (15 and 10 %). Triplicate groups of juvenile fish with an average initial body weight of ~3.64 g were reared in a recirculating aquaculture system and hand fed twice a day until satiation for a period of 60 days. In the experiment, no difference in survival rate was found between the different groups. Relative growth rate (RGR), specific growth rate (SGR), feed conversion ratio (FCR) and daily feed intake were not significantly affected by increasing protein and/or lipid treatments in this present study. However, the RGR, SGR and FCR values showed slightly better efficiency in the experimental group (35/15) fed with lower protein content (35 %) and higher lipid level (15 %) compared with those fed other diets. According to bioeconomic analyses results, the diet with the 35 % protein and 15 % lipid generated the best profit. The results suggest that two-banded seabream can be accepted as a promising alternative species for the aquaculture industry and optimum growth of two-banded seabream fingerlings can be obtained when they are fed a diet containing 35 % crude protein and 15 % crude lipid.

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Keywords Two-banded seabream (*Diplodus vulgaris*) · Protein · Lipid · Growth performance

Introduction

The increase in production and supply of seabream and sea bass, the main two marine species for the Turkey aquaculture industry, has caused a decline in the market price for these two species. In order to solve the problem with the market demand, fish culturists have tended to work on new alternative marine fish species. The alternative species in aquaculture provide the diversity for extending the market opportunity for their product.

Two-banded seabream is a sparid fish, a candidate for aquaculture sector due to both high market value, and adapts easily to production system (Jug-Dujakovic and Glamuzina 1988). *Diplodus vulgaris* has a broad range of salinity tolerance compared with other marine fish (Horta et al. 2004). Guidetti (2004) and Pallaoro et al. (2006) investigated the stomach contents of two-banded seabream to reveal the feeding habits, and they pointed out that algae and sea urchin were important prey for this species. *D. vulgaris* is an omnivorous sparid species. Omnivorous species need less animal-based protein in their diets; therefore, the diet costs are lower. These characteristics make the two-banded seabream a good candidate for the aquaculture industry (Ozório et al. 2009). Nutrient requirements of this species are still unknown and not well studied. Nutritionally balanced diets contribute to high growth performance.

A well-balanced diet in terms of protein-to-lipid ratio is one of the keys for the successful and effective feeding in fish nutrition. Furthermore, well-balanced diets are important to support growth performance, fish welfare and fish health. Among nutrients, protein and lipid are given more attention as main nutrients affecting fish growth. Proteins are the most expensive nutrient sources in fish diets and may cause to increase in N excretion, when they are over of requirement level (Wagner et al. 1995; Brunty et al. 1997; Yigit et al. 2002). Optimization of protein and lipid levels in fish diets in terms of economic and environmental perspective is also an important factor for an environmental friendly aquaculture industry (Ozório et al. 2009; Yigit et al. 2002, 2004).

The aim of the present study is to evaluate the suitable levels of dietary protein and lipids for maximum growth, feed utilization and cost of feed production for two-banded seabream (*D. vulgaris*).

Materials and methods

A total of 480 two-banded seabream (*D. vulgaris*) with a mean initial weight of 3.64 ± 0.016 g were captured in the Strait of Canakkale (formerly Dardanelles) (Çanakkale, Turkey) and kept at the Marine Aquaculture Research and Development Center of Faculty of Marine Science and Technology (formerly, Faculty of Fisheries) of Canakkale Onsekiz Mart University (Dardanos, Canakkale, Turkey). Fish were held in two circular tanks for 15 days to adapt to the new rearing conditions (750 L max volume) and during the adaptation stage; fish were fed to satiety twice a day, for 7 days a week with commercial gilthead seabream diets (49 % crude protein; Bioaqua, Turkey). After the acclimatization period, 20 fish were weighed and distributed into each of 24 aquariums of

84 L (35 cm × 45 cm × 60 cm deep) in a recirculation seawater system equipped with aeration and filtration systems. Fish were kept under a constant photoperiod of 12 h light/12 h dark period. The experiment was conducted in three replicates per treatment. Water quality parameters were periodically measured throughout the study, and the following parameters were recorded: temperature 23.1 ± 0.4 °C, salinity 29.8 ± 0.4 ppm, pH 8.19 ± 0.3, pH 8.19 ± 0.3, dissolved oxygen 7.08 ± 0.37 mg L^{-1}, nitrite 0.08 ± 0.02 mg L^{-1} and total ammonia 0.4 ± 0.1 mg L^{-1}.

Experimental procedures and analyses

Eight fish meal-based diets with decreasing protein levels of 50, 45, 40 and 35 % at two lipid levels of 15 and 10 %, respectively, were formulated using commercially available ingredients (Table 1).

All ingredients were well mixed using a laboratory food mixer. The mixtures were primed with water to yield a suitable pulp. Wet diets were made into 2-mm pellet size using a meat grinder and dried at 40 °C in a drying chamber. The diets were stored at −20 °C until use.

During the feeding experiment, fish were hand fed twice a day until satiety for 60 days. Feeding was carefully monitored in order to ensure an even distribution of the feed offered among all experimental fish. Feeding activity in one tank was continued for about 20 min, and special attention was given to avoid overfeeding. Feed intake data were recorded daily by subtraction of feed distributed from the initial feed weight. Fish were accepted as satiated when they refused feeding.

Growth performance, feed utilization and protein utilization of two-banded seabream fed with different dietary protein and lipid levels were evaluated by calculating relative growth rate (RGR), specific growth rate (SGR), feed conversion rate (FCR) and protein efficiency rate (PER), using the following equations:

$$\mathrm{RGR}(\%) = [(\text{final weight(g)} - \text{initial weight(g)})/\text{initial weight(g)}] \times 100$$

$$\mathrm{SGR}(\%\ \text{day}^{-1}) = [(\mathrm{l_n}\ \text{final weight(g)} - \mathrm{l_n}\ \text{initial weight(g)})/\text{days}] \times 100$$

$$\mathrm{FCR} = \text{feed intake(g)}/\text{weight gain(g)}$$

$$\mathrm{DFI}(\text{daily feed intake}) = (\text{air dry feed intake/number of fish})/\text{days}$$

$$\mathrm{DPI}(\text{daily protein intake}) = (\text{feed intake} \times \text{crude protein in diet}/100)/\text{days}$$

$$\mathrm{PER}(\text{protein efficiency rate}) = \text{wet weight gain/protein intake}$$

Experimental fish were individually weighed at the beginning and at the end of the experiment. The fish were weighed in mass every 20 days during the trial. Prior to sampling in each period, fish were deprived of feed for 1 day. In order to avoid stress during handling, fish were anesthetized with a benzocaine solution of 5 %; 100 mL ethyl alcohol (94 %) was mixed with 5 g crystalline benzocaine, and 1 mL of the solution was made up to 1 L with water (Allen 1988; Allen et al. 1994).

All experimental diets were analyzed for proximate composition according to AOAC (2000). Samples were frozen at −20 °C prior to analyses. Dry matter was detected after drying at 105 °C until a constant weight was achieved. Ash content was measured in a muffle furnace at 525 °C for 12 h. The amount of crude protein was analyzed by the Kjeldahl method. Lipid extractions were determined by the SOXTEC system.

Table 1 Feed ingredients (g/100 g) of experimental diets

Ingredients (g/100 g)	50/15	45/15	40/15	35/15	50/10	45/10	40/10	35/10
Experimental diet								
Fish meal[a]	80	73	64	56	80	73	64	57
Corn starch	9.6	15.6	22.6	30.6	14.6	20.6	28.6	35.6
Fish oil	6	7	9	9	1	2	3	4
Vit-C	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin[b]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral[c]	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Binder (CMC)[d]	1	1	1	1	1	1	1	1
Cholin chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100	100
Nutritional composition (%dry matter, except for moisture)								
Moisture	8	8	8	8	8	8	8	8
Crude protein	50.1	45.08	40.05	35.06	50.07	45.01	40.06	35.03
Crude lipid	15.06	15.1	15	15.05	10.09	10.1	10.1	10.1
Crude ash	11.6	10.6	9.1	8.2	11.4	10.4	9.2	8.1
NFE	15.24	21.22	27.85	33.69	20.44	26.94	32.73	38.77
GE (kJ/g diet)[e]	20.36	20.21	20.11	19.94	19.27	19.19	19.00	18.84
P:E (mg/kJ)[f]	2.46	2.23	1.99	1.76	2.60	2.35	2.11	1.86

[a] Produced by Sibal Fish Feed and Fish Meal Company, Sinop, Turkey

[b] Per mg mixture: vitamin A, 65,000 IU; vitamin D3, 45,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B1, 12.5 mg; vitamin B2, 12.5 mg; vitamin B6, 15 mg; vitamin B12, 0.025 mg and ascorbic acid, 120 mg

[c] Per mg mixture: Ca, 100 mg; P, 50 mg; K, 30 mg; Na, 20 mg; Mg, 10 mg; Fe, 22 mg; Zn, 3 mg; Mn, 3 mg; Cu, 1.8 mg; Co, 0.15 mg; I, 0.12 mg; Se, 0.05 mg; DL-calcium pantothenate, 40 mg; niacin, 50 mg; folic acid, 2.5 mg; biotin, 0.08 mg and inositol, 75 mg

[d] Carboxymethhyl cellulose

[e] Energy values are estimated based on 23.6 kJ/g for protein, 39.5 kJ/g for lipid and 17 kJ/g for NFE

[f] Protein-to-energy ratio in mg/kJ

Statistical analysis

Results were expressed as mean ± standard deviation (SD), and group mean differences were compared using one-way ANOVA. Data in percentage were arc-sin transformed prior to analysis. A significant level of $p < 0.05$ was employed at all cases.

Results

During the experiment, survival rate was 100 % in all experimental groups, showing that all fish were acclimated to experimental conditions and trial diets well. Overall, the growth performance and feed utilization were lower in the low-lipid diets compared to the high-lipid diets at all protein levels. When fish meal inclusion levels were decreased in order to produce low-lipid diets, NFE incorporation levels increased 5 % in all low-lipid diets compared to the high-lipid ones (Table 1).

Best growth performance was recorded in the group fed with 35 % CP and 15 % CL diet, while the poorest growth rate was obtained in the fish fed with diets with lowest protein level (35 % CP) and lower lipid diet (10 % CL) (Fig. 1). However, these differences were visible differences and not significant between groups ($p > 0.05$) (Table 2). Although there were no significant differences, better FCRs were obtained for high-lipid diets compared to the low-lipid ones. DFI values were similar in all experimental groups; this led to increasing DPIs with the increase in dietary protein levels in both high- and low-lipid diets. In the high-lipid groups, a general improvement in PERs was recorded with the decline in dietary protein levels. However, this was not obtained in the low-lipid diets, where PER value was best in the low-protein–low-lipid diet. Furthermore, in the high-lipid diet groups, higher DPIs did not improve PERs; in contrast, PERs improved when DPIs declined. This was reflected by a better RGR and SGR in the lowest dietary protein (35 %) with high lipid level (15 %). Fish growth performance and feed utilization data are shown in Table 2.

As expected, the cost of diets was increased with the increase in dietary protein level (Table 3). Overall, the gross income (GI) values in the low-lipid groups were lower than the high-lipid groups. The highest GI was obtained in the group with lowest dietary protein (35 %) and high lipid level (15 %). Similarly, profit and feeding cost as percentage of profit was also highest in the 35 % protein and 15 % lipid groups. Even though the differences were not significant among all experimental groups, economic analyses (Table 3) also confirmed the growth-related experimental results in terms of best profit obtained with the 35 % protein and 15 % lipid inclusion diet.

Discussion

Two-banded seabream is a potential marine fish species to be used as an alternative commercial species in aquaculture industry. Nevertheless, the nutritional requirement of this species is scarce.

In the present study, survival was 100 % in all experimental groups in contrast to other sparid studies (Sá et al. 2008; Zhang et al. 2010). From the present results, diet containing 35 % crude protein had shown maximum growth of juvenile two-banded seabream. Carnivorous species such as gilthead seabream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*) and rainbow trout (*Onchorhynchus mykiss*) require 45–55 % protein (Sabaut and Luquet 1973; Hidalgo and Alliot 1988; Kim et al. 1991). The optimal protein level in fish diet is influenced by the amino acid composition of the test proteins (Wilson 1989). In the present study, fish meal was the main protein source.

The optimum dietary protein results in the present study agreed with Ozório et al. (2009); for the best growth performance two-banded seabream need 36 % protein and 18 % lipids in its diets. Our results were compared with *Diplodus puntazzo*, *Dentex dentex* and *Pagrus pagrus* due to the existence of very limited data on *D. vulgaris*. Atienza et al. (2004) expressed that diet containing 47 % protein is needed to obtain the maximum growth performance for *D. puntazzo,* and this value is higher than *D. vulgaris*. Espinós et al. (2003) and Schuchardt et al. (2008) indicated that the dietary protein demand of *D. dentex* and *P. pagrus* was 50 %. Similarly, it is higher than *D. vulgaris*. In this study, the best FCR was obtained in high-lipid group. On the other hand, SGR and RGR decreased with decreasing lipid level.

Not significantly, but a general decrease was observed in PERs as dietary protein level increased in both high- and low-lipid groups. Similar results were reported in European eel

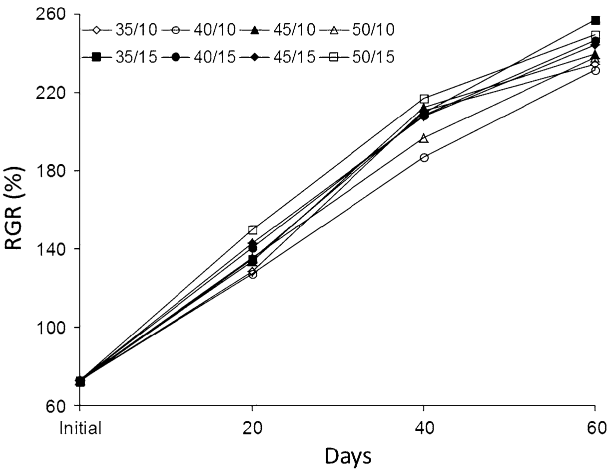

Fig. 1 Variation of biomass of two-banded seabream fed with different level of protein and lipid for 60 days

Table 2 Growth performance and feed utilization efficiency of *D. vulgaris* juveniles fed with different diets

Groups	SGR (%/day)	FCR	DFI (g)	DPI (g)	PER
50/15	2.05 ± 0.06^{a}	1.51 ± 0.03^{a}	0.22 ± 0.005^{a}	$2.22 \pm 0.050^{a,b}$	$1.32 \pm 0.034^{a,b}$
45/15	2.01 ± 0.03^{a}	1.65 ± 0.12^{a}	0.23 ± 0.022^{a}	$2.12 \pm 0.200^{a,b}$	$1.35 \pm 0.102^{a,b,c}$
40/15	2.03 ± 0.03^{a}	1.53 ± 0.21^{a}	0.22 ± 0.029^{a}	1.64 ± 0.346^{a}	1.82 ± 0.437^{c}
35/15	2.10 ± 0.08^{a}	1.50 ± 0.33^{a}	0.22 ± 0.044^{a}	1.60 ± 0.311^{a}	1.73 ± 0.367^{b}
50/10	1.97 ± 0.04^{a}	1.80 ± 0.13^{a}	0.24 ± 0.011^{a}	2.47 ± 0.114^{b}	1.56 ± 0.008^{a}
45/10	1.98 ± 0.05^{a}	1.66 ± 0.05^{a}	0.23 ± 0.004^{a}	$2.07 \pm 0.040^{a,b}$	$1.39 \pm 0.092^{a,b,c}$
40/10	1.92 ± 0.12^{a}	1.66 ± 0.12^{a}	0.21 ± 0.009^{a}	1.74 ± 0.075^{a}	$1.42 \pm 0.052^{a,b,c}$
35/10	1.95 ± 0.03^{a}	1.77 ± 0.28^{a}	0.23 ± 0.031^{a}	1.89 ± 0.009^{a}	$1.62 \pm 0.123^{a,b,c}$

Values with different superscript letters are significantly different ($p < 0.05$)

RGR, relative growth rate (%) = [(final wet weight − initial wet weight)/initial wet weight] × 100

SGR, specific growth rate (%/day) = [(ln final wet weight − ln initial wet weight)/days] × 100

FCR, feed conversion rate = feed consumed/weight gain

DFI, daily feed intake (g) = (air dry feed intake/number of fish)/days

DPI, daily protein intake (g) = (feed intake × crude protein in diet/100)/days

PER, protein efficiency rate = wet weight gain/protein intake

(*Anguila anguila*) (De La Higuera et al. 1989) and in Yellow snapper (*Lutjanus argentiventris*) (Maldonado-García et al. 2012), in terms of decreasing PERs with increasing dietary protein levels.

The lower growth performance and feed utilization in low-lipid diets compared to the high-lipid diets in the present study could be attributed to the higher NFEs and lower GE levels in the low-lipid diets. It is interesting to see the best performance of fish with the lowest P:E ratio (1.76) diet (35/15). This shows that low-protein but high-energy diets demonstrate better performance in two-banded seabream diets. In the present study, the dietary protein requirement of *D. vulgaris* is considerably higher than white sea bream

Table 3 Bioeconomic analyses of two-banded seabream juveniles fed diets with different levels of protein and lipid during the course of the study (mean ± SD for triplicate groups)

	High lipid level (15 %)				Low lipid level (10 %)			
	50	45	40	35	50	45	40	35
Feed cost ($/kg)	1.62	1.57	1.52	1.45	1.57	1.52	1.46	1.40
Price of Fish ($/kg)	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40
FS (kg/fish)	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}
MWG (kg)	0.18 ± 0.08^{a}	0.17 ± 0.03^{a}	0.17 ± 0.05^{a}	0.18 ± 0.12^{a}	0.16 ± 0.07^{a}	0.16 ± 0.08^{a}	0.16 ± 0.18^{a}	0.16 ± 0.05^{a}
FC ($/kg)	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}
GI ($/fish)	$0.78 \pm 0.04^{a,b}$	$0.75 \pm 0.01^{a,b}$	$0.77 \pm 0.02^{a,b}$	0.81 ± 0.06^{b}	$0.73 \pm 0.03^{a,b}$	$0.73 \pm 0.03^{a,b}$	$0.70 \pm 0.08^{a,b}$	0.71 ± 0.02^{a}
TIBC ($)	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}
TFBC ($)	$0.59 \pm 0.01^{a,b}$	$0.58 \pm 0.00^{a,b}$	$0.59 \pm 0.01^{a,b}$	0.61 ± 0.03^{b}	$0.57 \pm 0.01^{a,b}$	$0.57 \pm 0.01^{a,b}$	0.55 ± 0.04^{a}	$0.56 \pm 0.01^{a,b}$
Profit ($/kg)	$0.40 \pm 0.02^{a,b}$	$0.39 \pm 0.00^{a,b}$	$0.40 \pm 0.01^{a,b}$	0.42 ± 0.03^{b}	$0.38 \pm 0.02^{a,b}$	$0.38 \pm 0.02^{a,b}$	0.36 ± 0.04^{a}	$0.37 \pm 0.01^{a,b}$
FC as % profit	55.76 ± 2.86	48.28 ± 0.99	53.68 ± 1.61	56.33 ± 4.18	54.05 ± 2.32	47.77 ± 2.28	61.84 ± 7.02	57.28 ± 1.79

Values with different superscript letters are significantly different ($p < 0.05$)

FC, feeding cost ($/kg) = feed supply (kg/fish) × feed cost ($/kg)

GI, gross income ($/fish) = mean weight gain (kg) × price of fish ($/kg)

TIBC, total initial biomass cost ($) = initial fish weight (kg) × price of fish ($/kg)

TFBC, total final biomass cost ($) = final fish weight (kg) × price of fish ($/kg)

Profit ($/kg) = (total final biomass cost-total initial biomass cost) − feeding cost

MWG (kg) = mean weight gain

(*Diplodus sargus*) (27 % protein; Sá et al. 2008), similar with two-banded seabream (*D. vulgaris*) (36 % protein; Ozório et al. 2009) and lower than sharpsnout sea bream (*D. puntazzo*) (43 % protein; Coutinho et al. 2012), gilthead seabream (*S. aurata*) (50–54 % protein; Lupatsch et al. 2003). The recorded protein level for best growth performance of *D. vulgaris* in the present study is also lower than those reported for other marine species such as yellow tail (*Seriola dumerilii*, 50 % protein, Jover et al. 1999), Japanese flounder (*Paralichthys olivaceus*, 50 % protein, Yigit et al. 2004) and dentex (*D. dentex*, 50 %, Espinós et al. 2003). A reason for the lower protein requirement of *D. vulgaris* compared to other sparid fishes or other marine species could be due to the feeding habits of *D. vulgaris*, which is an omnivorous species, whereas the other fishes recorded with higher protein requirements in earlier reports are carnivorous species.

Dietary lipids are important nutrients, and fatty acids are needed for normal growth and development. Marine fish need eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) for good growth performance in the diets (NRC 1993). The lipids of the test are obtained from fish meal and fish oil. The dietary lipid level results in this study showed differences between 10 and 15 % levels; growth was low with 10 % lipid than 15 % lipid which are very close to the requirements of marine species such as *D. labrax* 12–14 % lipid (Pérez et al. 1997), *D. dentex* 12–17 % lipid (Espinós et al. 2003), *P. pagrus* 15 % lipid (Schuchardt et al. 2008).

Regarding economic efficiency of experimental diets, the reduction or increase in cost of diets is related to fish meal levels in the diet. Overall, the experimental diets used for two-banded seabream in the present study gave lower GI results compared to the values reported for gilthead seabream diets in a previous study by Yigit et al. (2012). Similarly, the profit and feed costs as percentage of profit values in the present study were also lower than those reported for gilthead seabream diets by Yigit et al. (2012). These differences could be attributed to the lower fish meal contents in their diets for gilthead seabream.

The economic analyses performed supported the growth parameters found in the present study with best GI and profit obtained in diets containing 35 % protein and 15 % lipid level. At all protein levels, the diets with higher lipid levels showed better profit compared to those with lower dietary lipid levels. Considering the importance of profitability of fish diets, high performance with less cost is the main target for the environment-friendly and successful aquaculture operations.

As a conclusion, based on the results in the present study, it is recommended that the use of practical diets containing 35 % protein and 15 % lipid provides efficient and cost-effective production of two-banded seabream juveniles. The fact that growth performance of fish was virtually unchanged in the present study, even when protein inclusion levels were as low as 35 %, arises a question "what would happen with protein levels below this value?" Further researches are encouraged to investigate nutritional studies on two-banded seabream with lower protein levels and different lipid contents.

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