***Microchloropsis gaditana*, *Schizochytrium* sp., *Phaeodactylum tricornutum*, AND *Tisochrysis lutea* AS n-3 PUFA SOURCES IN THE DIET OF JUVENILE GILTHEAD SEABREAM (*Sparus aurata*)**

E.Z. Gkalogianni[1]*, P. Psofakis[1], A. Asimaki[1], E. Fountoulaki[2], M. Henry[2], I.T. Karapanagiotidis[1]

[1] Department of Ichthyology and Aquatic Environment, University of Thessaly, Fytoko Street, 38446, Volos, Greece, egkalogianni@uth.gr
[2] Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Anavyssos, Attika, Greece

Introduction

In recent years microalgal biomass has become one of the most promising sources of bioactive compounds in aquafeeds, especially in terms of lipids and fatty acids (Soto-Sánchez et al. 2023). Among the several species *Microchloropsis gaditana*, *Phaeodactylum tricornutum*, *Schizochytrium* sp. and *Tisochrysis lutea* are of great interest to aquaculture as the first two are well known sources of EPA, while the other two as rich sources of DHA. That being the case, a dietary combination of these four species could potentially substitute fish oil in fish diets satisfying the essential fatty acids requirements. Ergo, the aim of this study was to evaluate the fish oil substitution by different blends of these four species on the diet of Gilthead seabream (*Sparus aurata*).

Materials and Methods

Juvenile seabreams of 8.77±0.01 g initial mean weight were obtained from a commercial fish hatchery and distributed after an acclimatization period of 15 days in triplicate to 18 closed seawater circulation system tanks (125L) (27 individuals/tank, 3 reps/dietary group). The groups were fed six different isoenergetic (21 MJ/Kg), isonitrogenous (48% CP) and isolipidic (15.5%) diets that satisfied the EPA+DHA requirements of the species (>1.8% of diet). The control diet (C) contained 8% fish oil, 4% soybean oil and 25% fishmeal resembling a commercially available seabream diet. Four other diets were formulated replacing 50% of the dietary fish oil of the control diet by a blend of microalgae biomasses of the species: *Schizochytrium* sp. and *M. gaditana* (SM), *Schizochytrium* sp. and *P. tricornutum* (SP), *P. tricornutum* and *T. lutea* (PT) and *M. gaditana* and *T. lutea* (MT). A sixth diet was also used as a reference containing 12% of fish oil as the sole dietary oil. The inclusion level of each microalgae contributed a certain amount of proteins in the diet and as such fishmeal protein was also subsequently substituted. Fish were hand-fed to apparent satiation twice a day for 11 weeks.

Results and Discussion

The SM, SP and MT groups of fish had similar final body weight, weight gain, SGR and FCR with both control groups (C, FO) (Table 1) indicating that dietary fish oil can be partially replaced by the blends of *M. gaditana* and *Schizochytrium* sp., *Schizochytrium* sp. and *P. tricornutum*, *M. gaditana* and *T. lutea* without impairing the growth of *S. aurata* and feed efficiency. The growth retardation occurred in the PT fish was due to their lower feed intake, which denotes a lower acceptability of either *P. tricornutum* or *T. lutea* by *S. aurata*. A lower feed intake was also obvious in the SP group but not in the MT group, implying that probably the inclusion of *P. tricornutum* was the significant factor.

Up to date, the strategy of mixing different microalgae species in order to balance dietary fatty acids and to replace dietary fish oil has not been adequately studied. The blend of *Microchloropsis sp.* and *Schizochytrium* sp. has been previously proved as a successful fish oil replacer for *S. aurata* (Karapanagiotidis et al. 2022) as well as in another species

(Qiao et al. 2014; Seong et al., 2021., Sarker et al., 2020a). A blend of *Tisochrysis lutea* with *Tetraselmis suecica* successfully replaced 36% of dietary fish oil in *Dicentrarchus labrax* without adversely affecting fish growth performance (Cardinaletti et al. 2018). Sarker et al. (2020) using different combinations of *Microchloropsis* sp., *Isochrysis* sp., and *Schizochytrium* sp. in the diet of *Oncorhynchus mykiss* reported that *Schizochytrium* sp. and *Isochrysis* sp. are good candidates for DHA supplementation and that the latter. is better than *Nannochloropsis* sp. as a substitute for fish oil.

The present study showed that blends of specific microalgae species is a promising strategy for further fish oil replacement in the diet of *S. aurata*, that in turn can enhance the eco-efficiency of its aquaculture production. Certainly, the effectiveness of such dietary manipulation for increasing the n-3 fatty acids in fish tissues should be further investigated.

Table 1. Growth performance and feed utilization of *S. aurata* fed with the experimental diets.

Parameters / dietary groups	C	FO	SM	SP	PT	MT
Final weight (g/fish)	35.93±0.87[a]	35.40±3.53[a]	33.81±2.06[ab]	30.83±2.86[ab]	27.98±1.29[b]	32.27±2.49[ab]
Feed intake (g/fish)	33.39±0.80[ab]	34.43±0.67[a]	34.04±0.89[a]	30.80±1.18[bc]	29.17±1.10[c]	33.10±1.29[ab]
Weight gain (g/fish)	27.17±0.85[a]	26.64±3.53[a]	25.03±2.07[ab]	22.06±2.86[ab]	19.22±1.30[b]	23.50±2.50[ab]
SGR (%/day)	1.79±0.03[a]	1.76±0.13[a]	1.71±0.08[ab]	1.59±0.12[ab]	1.47±0.06[b]	1.65±0.10[ab]
FCR	1.23±0.01[b]	1.31±0.16[ab]	1.36±0.09[ab]	1.41±0.14[ab]	1.52±0.06[a]	1.42±0.11[ab]
Survival (%)	100.0±0.00	100.0±0.00	98.77±2.14	98.77±2.14	100.00±0.00	98.77±2.14
Hepatosomatic Index (%)	1.41±0.14	1.35±0.14	1.08±0.21	1.19±0.08	1.25±0.22	1.24±0.17
Viscerosomatic Index(%)	7.58±0.47	7.20±0.14	6.57±0.42	7.55±0.84	7.67±0.40	7.42±0.07
Condition factor	1.38±0.04	1.33±0.02	1.36±0.02	1.38±0.01	1.34±0.03	1.35±0.02

Note. Values represent means ± standard deviation of triplicates. Values within each row not sharing a common superscript letter are significantly different ($P < 0.05$).

Acknowledgements

This research has been co-financed by the European Union and Greek national funds through the Operational Programme Competitiveness, Entrepreneurship and Innovation-EPAnEK 2014-2020 (project code: MIS 5045804).

References

Soto-Sánchez, O., Hidalgo, P., González, A., Oliveira, P. E., Hernández Arias, A. J., & Dantagnan, P. (2023). Aquaculture Nutrition 2023:5110281. https://doi.org/10.1155/2023/5110281

Karapanagiotidis, I.T., Metsoviti, M.N., Gkalogianni, E.Z., Psofakis, P., Asimaki, A., Katsoulas, N., Papapolymerou, G., Zarkadas, I. (2022). Aquaculture 561, 738709. https://doi.org/10.1016/j.aquaculture.2022.738709

Seong, T., Uno, Y., Kitagima, R., Kabeya, N., Haga, Y., Satoh, S. (2021). Aquaculture Research 52, 6025–6036. https://doi.org/10.1111/are.15463.

Qiao, H., Wang, H., Song, Z., Ma, J., Li, B., Liu, X., & Zhang, L. (2014). Aquaculture nutrition 20, 646-653. https://doi.org/10.1111/anu.12127.

Sarker, P.K., Kapuscinski, A.R., McKuin, B., Fitzgerald, D.S., Nash, H.M., Greenwood, C. (2020a). Sci. Rep. 10, 19328. https://doi.org/10.1038/s41598-020-75289-x.

Sarker, P.K., Kapuscinski, A.R., Vandenberg, G.W., Proulx, E., Sitek, A.J. (2020b). Elementa 8, 5. https://doi.org/10.1525/eleme nta.404.

Cardinaletti, G., Messina, M., Bruno, M., Tulli, F., Poli, B. M., Giorgi, G., & Tibaldi, E. (2018). Aquaculture, 485, 173-182.

Η ανακοίνωση εγκρίθηκε για παρουσίαση στο AQUACULTURE EUROPE 2023, September 18 - 21, Vienna, Austria.