Effects of Feed Quality on Growth and Feed Utilization of Gilthead Seabream (*Sparus aurata*, L.) at Low Temperatures

Utjecaj kvalitete hrane na rast i iskoristivost hrane za podlanicu (Sparus aurata, L.) pri niskim temperaturama

Slavica Čolak
University of Zadar
Department of Ecology, Agronomy and Aquaculture
Zadar, Croatia
E-mail: slavica.colak@unizd.hr

Silvia Križanac
Cromaris d.d.
Zadar, Croatia
E-mail: silvia.krizanac@cromaris.hr

Božena Vitlov*
Cromaris d.d.
Zadar, Croatia
E-mail: bozena.vitlov@cromaris.hr

Josipa Raljević
University of Zadar
Department of Ecology, Agronomy and Aquaculture
Zadar, Croatia
E-mail: jraljevic.98@gmail.com

Renata Barić
Cromaris d.d.
Zadar, Croatia
E-mail: renata.baric@cromaris.hr

Lav Bavčević
University of Zadar
Department of Ecology, Agronomy and Aquaculture
Zadar, Croatia
E-mail: lbavcevic@unizd.hr

DOI 10.17818/NM/2024/3.3
UDK 639.2/.3
Original scientific paper / *Izvorni znanstveni rad*
Paper received / *Rukopis primljen:* 30. 8. 2024.
Paper accepted / *Rukopis prihvaćen:* 19. 11. 2024.

Abstract

The gilthead seabream (*Sparus aurata*) is cultured throughout the Mediterranean; the sea temperature most significantly determines the duration of farming. Sea temperatures reduce gilthead seabream's need for feed and fish growth is not expected in these conditions. It is hypothesized that improving feed formulation could improve growth during the winter months. For the research, two groups were formed, and fed for a month with two types of fish feed of different nutritional composition at an average temperature of 13.9 ± 0.90 °C. The fish from cage A (21.53 ± 1.53 cm; 173.77 ± 38.24 g) were fed fish feed containing protein and lipid 100% from marine origin (H1). The fish from cage B (22.17 ± 1.43 cm; 181.08 ± 43.05 g) were fed with commercial feed for gilthead seabream, whose proteins and lipids were partially replaced by plant raw materials (H2). The temperature was measured daily and samples were taken at the beginning and end of the experiment for biometrics, which included the fish's total length, total weight, and condition factor. This study showed that fish fed with a modified formula achieved higher growth parameters than fish fed with commercial feed. The data obtained indicate that gilthead seabream can grow at low temperatures and there is a need for further research to ensure the correct selection of raw materials for the testing and production of feed for the period of low sea temperatures.

KEY WORDS
sea cage
gilthead seabream
sea temperature
feed

Sažetak

Podlanica (Sparus aurata) se uzgaja diljem Sredozemlja, u uzgoju temperatura mora određuje duljinu uzgoja. Na temperaturama mora ispod 15 °C podlanica smanjuje svoju potrebu za hranom i u uzgoju na tim temperaturama ne očekuje se rast ribe. Pretpostavka je da bi poboljšanje formulacije hrane moglo poboljšati rast tijekom zimskih mjeseci. Za potrebe istraživanja formirane su dvije skupine koje su hranjene mjesec dana dvjema vrstama riblje hrane različitog nutritivnog sastava na prosječnoj temperaturi od 13,9 ± 0,90 °C. Riba u kavezu A (21,53 ± 1,53 cm, 173,77 ± 38,24 g) hranjena je hranom za ribe s proteinima i lipidima koji su u potpunosti potjecali iz morskih sirovina (H1). Riba u kavezu B (22,17 ± 1,43 cm, 181,08 ± 43,05 g) hranjena je komercijalnom hranom za podlanicu u kojoj su proteini i lipidi djelomično zamijenjeni sirovinama biljnog podrijetla (H2). Temperatura se mjerila svakodnevno, a uzorci za biometriju uzimali su se na početku i na kraju pokusa te uključivali ukupnu duljinu, ukupnu masu i faktor kondicije ribe. Rezultati ovog istraživanja pokazali su da su ribe hranjene modificiranom recepturom postigle veće parametre rasta u odnosu na ribe hranjene komercijalnom hranom. Dobiveni podaci ukazuju na potrebu daljnjih istraživanja kako bi se osiguralo pravilno testiranje i odabir sirovina za proizvodnju riblje hrane za razdoblje niskih temperatura mora.

KLJUČNE RIJEČI
uzgoj
podlanica
temperature mora
hrana

[*] Corresponding author

1. INTRODUCTION / *Uvod*

Fish farming in the Mediterranean is highly dominated by gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) production. The gilthead seabream production was 282 thousand tonnes in 2020 [1]. The sea temperature most significantly determines the duration of cultivation, which usually takes between 18 and 24 months to reach 400 g from 3 g with FCR from 1.5 to 2 kg per kilogram of growth [2].

The feed for farmed fish is a complete diet with a high proportion of proteins and lipids, of animal or plant origin, which ensures production success. Fishmeal is an optimal source of protein for fish, and currently, about 75% of fishmeal is used in the production of fish feed worldwide [3, 4]. Considering the limited resources and high price of fishmeal, it is expected that fishmeal will be replaced by proteins from alternative protein sources [5, 6]. Numerous studies have been conducted to explore the effect of replacing raw materials in fish feed. Some amounts of fishmeal can be replaced with alternative protein sources, but individual species needs should be considered [7]. Research on gilthead seabream indicates good possibilities for replacing raw materials that ensure good growth and health [8, 9, 10, 11, 12, 13]. However, little research has been conducted to determine whether alternative protein and lipid sources can promote fish growth and health at low temperatures. The existing ones support the possibility of substitution if additives have been added to such food to improve appetite, health, and growth. Such feeds generally provide the same results as feeds with 15-30% fishmeal and are considered commercial feeds [14, 15]. The choice of feed affects the farming's financial results, but it is also necessary to observe the impact of feed on the environment. The effect on the environment increases with lower utilization of feed, which can be caused by the fish's inability to digest certain raw materials and the inability to digest at certain temperatures [16]. Feed utilization efficiency is usually determined with a calculation of feed conversion ratio (FCR), protein efficiency ratio (PER), and energy efficiency ratio (EER). These data are well known for salmon, while data for gilthead seabream are few and mostly data for cultivation at high temperatures [17, 18, 19].

Temperature affects fish metabolism and feed intake decreases outside the species optimal temperature range [20]. Climatically, the Mediterranean is predominantly a warm sea with a temperature range along the coast of 13 - 29 °C [21]. The cultivated individuals are exposed to seasonal temperature fluctuations during the cultivation period. The optimum sea temperature for the cultivation of sea bream is 24 – 26 °C. Wild gilthead seabream can tolerate temperatures from 11 °C in the winter to 23 °C in the summer [22]. In unfavorable condition, fish migrate to more favorable areas. The main disadvantage in farming gilthead seabream in floating cages is the inability to migrate to a more favourable thermal area, i.e., the inability to migrate to depth when surface temperatures begin to decrease [23]. At temperatures below 15 °C, the fish's feeding requirements drop considerably, and at temperatures below 13 °C fish completely stop eating [24, 25, 26, 14]. Due to reduced appetite and metabolism, fish growth below 15 °C is minimal [24, 27]. At temperatures below 14 °C, a health condition known as "Winter Disease" occurs. Current data suggest the disease is caused an immunological suppression and metabolic disorder at low temperatures [28, 29, 27, 30, 31]. During the disease, reduced feeding or periodic starvation is often recorded. As a result of reduced feeding, the fish use energy reserves and lose weight [32, 27, 33]. The functional feeds for the winter period and feeding protocols help reduce the disease's incidence [27, 34, 35]. In addition, during spawning, which lasts from October to March, the gilthead seabream needs energy and nutrients for the growth and development of the gonads and produces an egg biomass greater than its body weight [36]. When temperatures increase at the beginning of the spring, the feed intake of the gilthead seabream is still weak and it takes time to return to normal intake [15]. Moreover, the fish attempts to regain the lost weight and begins compensatory growth, which is most often associated with an increased appetite, i.e., hyperphagia. The fish cannot successfully compensate for the lost length and growth and therefore needs more time to reach market size which causes an increase in production cost [37].

The Adriatic Sea is shallow and the temperature in winter can be significantly lower than in the rest of the Mediterranean [38]. Farmed gilthead seabream in cage farming is faced with lower sea temperatures, which significantly affects the growth of the farmed fish, and thus the financial result of the production. There is interest in feeds with adapted formulations for low temperatures to prevent the negative effects of low temperatures on farmed fish. Such feed would ensure growth in conditions where the fish do not grow or where this growth is insignificant, thus reducing the duration of cultivation and improving production efficiency. This paper aims to determine the growth of fish fed a diet of marine ingredients under conditions of low sea temperature to set more objective performance targets for diets with alternative protein sources.

2. MATERIALS AND METHODS / *Materijali i metode*

The experiment was carried out in March/April 2023, on a farm in the Middle Eastern Adriatic Sea 44°01'23.6"N 15°13'09.5" E (Figure 1). The fish originated from the same batch and were randomly distributed in two floating cages measuring 9 x 5 x 5 meters (volume 225 m^3). A total of 1.000 individuals of gilthead seabreams were distributed in two cages. The fish in cage A (21.53 ± 1.53 cm, 173.77 ± 38.24 g) were fed a diet containing 100 % crude lipids from fish oil and fishmeal; 100% crude protein from a fishmeal; 100% carbohydrates were wheat starch (H1). The fish in cage B (22.17 ± 1.43 cm, 181.0 ± 43.05 g) were fed with commercial feed for gilthead seabream with reduced raw materials of marine origin, protein (fish meals 15%) and lipid (fish oil 3%) (H2).

The proximal composition of the diets was determined in triplicate following standard AOAC procedures [39]. The proximate composition of diets is shown in Table 1. The fish were fed once a day by hand to apparent satiation. The experiment lasted for one month. Amounts of feed, mortality, temperature, and oxygen were monitored and measured daily.

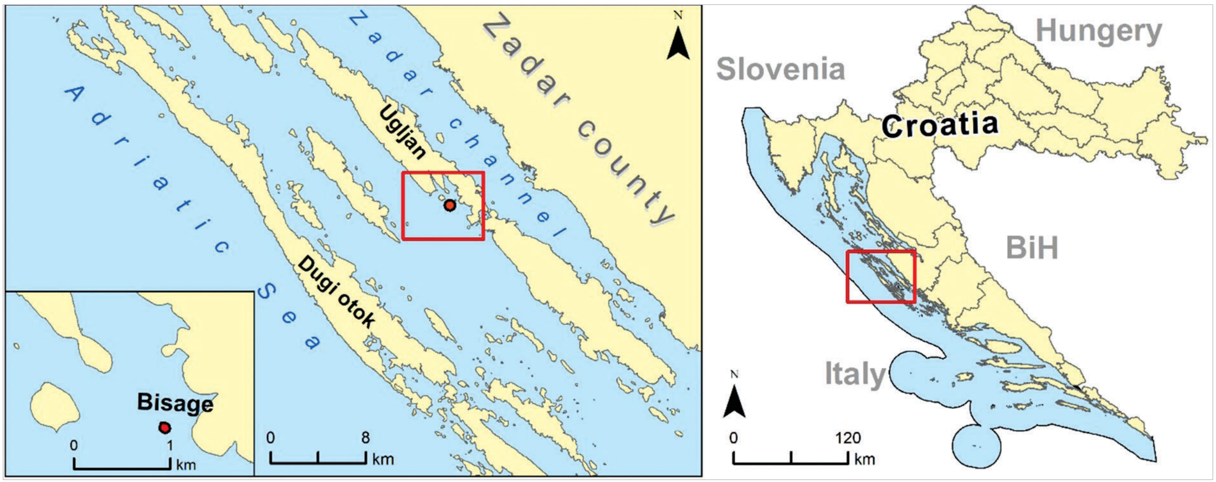

Figure 1 Study area

Slika 1. Područje u kojem je obavljeno istraživanje

Table 1 Proximal composition of experimental feed (H1); commercial feed for gilthead seabream (H2).

Tablica 1. Sastav pokusne hrane (H1) i komercijalne hrane (H2)

Proximal composition (% of diet)	H1	H2
Crude protein (%)	52.0	42.0
Crude lipid (%)	21.0	17.0
Moisture (%)	7.0	7.0
Crude fiber (%)	0.7	3.3
NFE* (%)	7.9	23
Ashes (%)	11.4	7.2
Gross energy (MJ/kg)****	22.0	20.7
DE** (MJ/kg)	20.0	16.0
DE*** (MJ/kg)	19.3	16.4
DP/DE** (g/MJ)	24.3	22.5

*NFE - nitrogen free extract
**DE – included proteins, lipids, and starch, data from food manufacturers
***DE – included proteins, lipids, and CHO, data from food manufacturers
****The diet's gross energy (GE) was calculated using 23.9 kJ/g proteins, 39.8 kJ/g lipids, and 17.6 kJ/g carbohydrates [40]

At the end of the experiment, 50 fish from each group were sampled. The fish were anesthetized with benzocaine 30-40 mg/L (Aquacen benzocaine 200 mg/ml, Cenavisa, S.L.). The total weight and total length were measured for all sampled individuals. The total length of the fish (L) was measured to the nearest 0.1 cm, and the total weight of the fish (W) was measured to the nearest 0.1 g.

Data of L and W were used to calculate Fulton's condition factor (K):

$$K = \frac{W}{L^3} * 100$$

$$Feed\ conversion\ ration\ (FCR) = \frac{feed\ (g)}{weight\ gain\ (g)}$$

$$Specific\ growth\ rate\ (SGR) = \frac{\ln\left(\frac{final\ W}{initial\ W}\right)}{duration\ of\ the\ experiment\ (days)} * 100$$

$$Daily\ feeding\ ratio\ (DFR) = \frac{apparent\ daily\ feed\ intake\ \left(\frac{g}{day}\right)}{average\ biomass\ of\ fish\ per\ group} * 100$$

where apparent daily feed intake is the total amount of food divided by the number of days

$$Thermal\ growth\ coefficient\ (TGC) = \left(\frac{final\ weight^{\frac{1}{3}} - initial\ weight^{\frac{1}{3}}}{\sum T}\right) * 100$$

where *T* is the seawater temperature in °C.

$$Protein\ efficiency\ ratio\ (PER) = \frac{weight\ gain\ (kg)}{amount\ of\ protein\ consumed\ (kg)}$$

$$Energy\ efficiency\ ratio\ (EER) = \frac{weight\ gain\ (kg)}{amount\ of\ gross\ energy\ consumed\ (MJ)}$$

The growth prediction was made using the model according to [41].

Statistical analysis was conducted by TIBCO Statistica 14.0.0.15. computer software. The total length, total weight, and condition factor between experimental groups were compared using Student's t-test for independent samples (p<0.05).

3. RESULTS / *Rezultati*

3.1. Temperature / *Temperatura*

The sea temperature range during the experiment was between 12.6 °C and 15.9 °C. The average temperature was 13.9 ± 0.90 °C. No mortalities were reported.

Table 2 shows the growth parameters at the beginning and the end of the experiment. The testing carried out showed that at the beginning of the experiment, fish from cage B were significantly longer and had a significantly lower condition factor than fish from cage A. At the end of the experiment testing the mean values of length, weight, and condition factor among the cages at the end of the experiment determined that fish from cage A had a significantly higher condition factor than fish from cage B.

Table 2 Growth and feeding efficiency parameters of gilthead seabream in cage A and cage B

Tablica 2. Parametri rasta i učinkovitosti hranidbe kod podlanice u kavezu A i kavezu B

	Cage A	Cage B
Initial W (g)	173.77 ± 38.24	181.08 ± 43.05
Final W (g)	197.03 ± 42.16	185.81 ± 40.33
Initial L (mm)	21.53 ± 1.53	22.17 ± 1.43*
Final L (mm)	22.64 ± 1.31	22.48 ± 1.36
Initial K	1.71 ± 0.12*	1.63 ± 0.18
Final K	1.67 ± 0.16*	1.61 ± 0.15
DFR	0.63	0.64
FCR	1.52	7.47*
SGR	0.42	0.09
TGC	0.57	0.12
PER	1.26	0.32
EER	0.03	0.006

* indicate the statistically significant differences between cages (P < .05)

In addition to the comparison of differences in total length, total weight, and condition factors between cages, differences within individual cages were also examined. Testing the differences in mean values of total length, total weight, and condition factor of gilthead seabream at the beginning and the end of the experiment for fish within individual cages is shown in Table 3. At the end of the experiment, the fish in cage A were significantly longer, significantly heavier, and with an insignificantly lower condition factor compared to the beginning of the experiment. The fish from cage B at the end of the experiment were insignificantly longer, insignificantly heavier, and had insignificantly lower condition factors at the end of the experiment.

Table 3 Result of t-test for total length (L), total weight (W), and fitness factor (K) of gilthead seabream at the beginning and end of the experiment within an individual cage

Tablica 3. Rezultat t-testa za ukupnu duljinu (L), ukupnu masu (W) i faktor kondicije (K) podlanice na početku i na kraju pokusa unutar pojedinačnog kaveza.

Cage		t	df	p
A	L	-4.33	116	0.000032
	W	-3.19	116	0.0018
	K	1.39	116	0.17
B	L	-1.44	144	0.15
	W	-1.02	144	0.31
	K	0.39	144	0.69

A model developed for fish fed commercial diets (Figure 2) predicted the growth of fish fed H1 and H2 diets. The initial weight in the model is the final weight in the experiment, and the temperatures used for the model are the average sea temperature for the location of the experiment. Fish fed with diet H1 reached consumption weight (400 g) earlier than fish fed with diet H2.

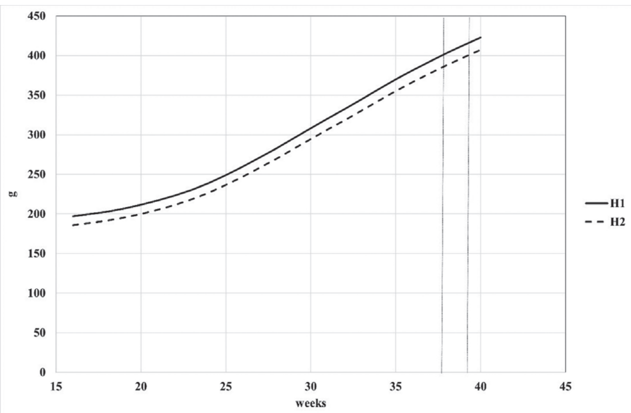

Figure 2 Growth predictions for fish feed with feed H1 and H2 after the experiment based on a model adapted to estimate growth on commercial gilthead seabream feed. The vertical lines intersect the growth lines at 400 g

Slika 2. Predviđanja rasta hrane za ribe s hranom H1 i H2 nakon pokusa na temelju modela prilagođenog za procjenu rasta komercijalne hrane za podlanicu. Okomite linije sijeku linije rasta na 400 g

4. DISCUSSION / *Rasprava*

The duration of the growing cycle usually depends on the temperature of the marine environment [2, 42]. The experiment was conducted in a period when the sea temperatures were stably low, the average was 13.9 °C with the lowest temperature of 12.6 °C. According to the previous knowledge about growth, based on which the models for predicting growth are made, the growth of the sea bream is not expected at these temperatures. Reduced growth is accompanied by increased FCR [43, 44].

The average FCR in our study for fish from cage A was 1.52. For fish from cage B, it was significantly higher and was 7.47, while the DFR was almost the same. Food quality can be further validated through the protein efficiency ratio and energy efficiency ratio. Fish fed H1 achieved 1.26 kg of body weight per 1 kg of protein and 0,03 kg/ MJ GE, while fish fed H2 achieved 0.32 kg of body weight per 1 kg of protein and 0,006 kg/MJ GE. The obtained results show a lower utilization of feed in cage B compared to cage A and explain the high FCR in cage B. A trial on protein utilization in gilthead seabream (75 g) feed with five isonitrogenous (46% protein) diets with increasing lipid levels (16-24%) was performed at 27 °C where PER was 1.44 - 1.58 g of body weight per 1 g of protein [19]. Our results are similar to [18] where fingerlings (1.5 g) were fed with 50% protein and a 10% lipid diet reared at 23. 5 °C, protein efficiency ratio was 1.22 g of body weight per 1 g of protein. But we must emphasize that in their experiment the fish were younger and that the temperature was optimal, which underlines the good performance of the H1 food.

A previous report [30] examined the influence of two types of food on the growth and health of fish during low temperatures. A commercially formulated feed with 15% protein of marine origin also caused fish to have a higher FCR (3.9-5.2) than a diet with 45% protein from marine origin (2.0-2.8). In the current experiment, fish fed H1 (100% proteins of marine origin) had an even lower FCR than those from [45] which were fed diets with 45% proteins of marine origin. The increased FCR on our commercial feed compared to theirs which had the same proportion of protein from marine sources is probably due to the different cultivation methods during the experiment, tanks compared to floating cages. Our results are similar to those in trials carried out in cages [45] where the FCR of commercial feed (20MJ GE) was 11.02 in an experiment that lasted 56 days at a temperature of 12 - 17 °C. In our study, TGC was higher in fish fed with diet H1. The obtained results indicate that the fish from cage A achieved considerable growth, while the fish from cage B stagnated in growth, which is in line with previous production results at low temperatures [27]. The comparison of SGR growth rate among cages leads to the same conclusions as the previous comparison using the TGC coefficient.

Due to the statistically significant differences in total length and condition factor between fish from cages A and B at the beginning of the experiment, an additional analysis of the increase in total length, total weight, and change in condition factor was performed for each cage. In fish from cage A, the difference in initial and final total length and total weight was significant. The condition factor dropped, but the difference is not significant. In contrast, in cage B there were no statistically significant changes in total length, total weight, and condition factor. Comparative analysis of growth and FCR results indicates the impact of using feed with different compositions. The experimental (H1) feed was designed to provide energy and protein in surplus as determined in previous research on gilthead seabream [46, 47]. Fish fed with this feed had significant growth at low temperatures while the fish on H2 grew insignificantly. Growth predictions were obtained based on a model adapted to estimate growth on commercial bream feeds [41]. However, the commercial feed has resulted in standard production results, comparable to the data obtained from growth models for predicting the growth of gilthead seabream in production. The model was used to predict fish growth after the experiment, in production feed with commercial feed. The fish from cage A would reach a final weight of 400 g 15 days before the fish from cage B. A shorter production cycle would have a favorable financial effect, especially considering that the amount of food consumed in one winter month would be small compared to two weeks of summer temperatures.

Feed for cage A had proteins and fats exclusively of fish origin. Since there are significant differences in the raw material and chemical composition between H1 and H2, it is not possible to determine with precision which factor influenced the significant differences in the performance of both groups. These results indicate the possibility of improving the growth of gilthead seabream in the cold months through changes in the feed formulation. We can assume that in the winter period, the choice of raw materials and formulations is the limiting factor for fish growth, which specifically refers to protein digestibility and fat quality. Formulations of feed to support growth during the winter months should consider alternative protein sources to achieve maximum growth. The feed must be produced with low-cost and sustainable raw materials to achieve better financial results, without jeopardizing the fish's maximum growth. To achieve this, in experiments formulations of control feed must ensure maximum growth and health to evaluate better the results of future inclusions of alternative protein sources in fish formulations as previously proposed. We must also emphasize that feeding strategies that include more digestible raw materials such as fishmeal during the winter months do not necessarily mean that the share of fishmeal in the diet for the entire production cycle has increased.

5. CONCLUSION / *Zaključak*

Our research was conducted from the beginning of March to the beginning of April when the fish did not make significant progress in growth due to the colder sea temperature. Nevertheless, an increase was recorded in cage A which can be attributed to the feed with a higher quality nutrient composition than commercial feed. The fish growth recorded in cage B shows the standard growth of the gilthead seabream during this period.

An important difference in the composition of the feed is based on the proportion of fish raw materials. The protein and fat sources of the experimental feed are fish oil and fishmeal compared to commercial feed, which contains significant amounts of raw materials of terrestrial origin. The choice of feed raw materials could be a limiting factor for the growth of the fish in the colder season. Better protein and energy efficiency of feed H1 indicates that if the feed quality is increased following the nutritional and digestibility requirements of the sea bream, the growth rate can be increased even during low sea temperatures.

Future studies should last longer with the same or a similar improved feed formulation to determine whether the increase in growth due to the improved feed formulation is economically justified.

There is a need to set standards for control feed that would give us an insight into the real growth potential and additionally encourage the better development of formulations.

Author Contribution: Conceptualization, L. B., S. Č., B. V., S. K.; Methodology, L. B., S. Č., B. V., S. K.; R. B. Formal Analysis, L. B., S. Č., B. V., J. R.; Investigation, L. B., S. Č., B. V., J. R.; Resources, L. B., S. Č., B. V., S. K., R. B., J. R.; Writing-Original Draft, S. Č., L. B., B. V., S. K.; Writing-Review & Editing, L. B., S. Č., B. V., S. K.; Visualization Preparation, L. B., S. Č., B. V.; Supervision, L. B.

Funding: The research presented in the manuscript did not receive any external funding

Conflict of interest: None

Acknowledgment: We thank the staff at Bisage Farm for helping us during the trial.

REFERENCES / *Bibliografija*

[1] European Commission, Joint Research Centre, Scientific, Technical and Economic Committee for Fisheries (2023, January). Guillen, J., Virtanen, J., & Nielsen, R. (eds.), *Economic report on the EU aquaculture (STECF-22-17)*. Publications Office of the European Union. Retrieved from: https://data.europa.eu/doi/10.2760/51391

[2] Pavlidis, M. A., & Mylonas, C. C. (2011). *Sparidae: Biology and aquaculture of gilthead sea bream and other species* (pp. 4-10). Blackwell Publishing, United Kingdom.

[3] IFFO – The Marine Ingredients Organization (2017, February). *The benefits of fishmeal and fish oil in swine and poultry diets*. Retrived from: iffo.com/node/338.

[4] Bavinck, M., Ahern, M., Hapke, H. M., Johnson, D. S., Kjellevold, M., Kolding, J., Overå, R., Schut, T., & Franz, N. (2023). Small fish for food security and nutrition. *FAO Fisheries and Aquaculture Technical Paper,* 694. FAO, Rome,

[5] Hua, K., Cobcroft, J. M., Cole, A., Condon, K., Jerry, D. R., Mangott, A., Praeger, C., Vucko, M.J ., Zeng, C., Zenger, K., & Strugnell, J. M. (2019). The Future of Aquatic Protein: Implications for Protein Sources in Aquaculture Diets. *One Earth*, *1* (3), 316-329. https://doi.org/10.1016/j.oneear.2019.10.018

[6] Hussain, S. M., Bano, A. A., Ali, S., Rizwan M., Adrees, M., Zahoor, A. F., Sarker, P. K., Hussain, M., Arsalan, M. Z. H., Yong, J. W. H., Naeem, A. (2024). Substitution of fishmeal: Highlights of potential plant protein sources for aquaculture sustainability. *Heliyon*, 10, e26573. https://doi.org/10.1016/j.heliyon.2024.e26573

[7] Daniel, N. (2018). A review on replacing fish meal in acqua feeds using plant protein sources. *International Journal of Fisheries and Aquatic Studies*, *6* (2), 164-179. Retrieved from: https://www.fisheriesjournal.com/archives/2018/vol6issue2/PartC/6-1-35-823.pdf

[8] Carvalho, M., Torrecillas, S., Montero, D., Sanmartín, A., Fontanillas, R., Farías, A., Moutou, K., Velasquez, J. H., & Izquierdo, M. (2023). Insect and single-cell protein meals as replacers of fish meal in low fish meal and fish oil diets for gilthead sea bream (*Sparus aurata*) juveniles. *Aquaculture*, 566, 739215. https://doi.org/10.1016/j.aquaculture.2022.739215

[9] Savonitto, G., Barkan, R., Harpaz, S., Neori, A., Chernova, H., Terlizzi, A., & Guttman, L. (2022). Author Correction: Fishmeal replacement by periphyton reduces the fish in fish out ratio and alimentation cost in gilthead sea bream *Sparus aurata*. *Sci Rep*, 11, 20990 https://doi.org/10.1038/s41598-022-11695-7

[10] 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. https://doi.org/10.3390/ ani11071919

[11] Kissil, G. W., & Lupatsch, I. (2004). Successful replacement of fishmeal by plant proteins in diets for the gilthead seabream. *Sparus aurata*. *The Israeli Journal of Aquaculture – Bamidgeh*, *56* (3), 188-199. https://doi.org/10.46989/001c.20378

[12] De Francesco M, Parisi G, Pérez-Sanchez J, Gomez-Réqueni P., Medale F., Kaushik S., Mecatti, M., & Poli, B. M. (2007). Effect of high-level fish meal replacement by plant proteins in gilthead sea bream (*Sparus aurata*) on growth and body/fillet quality traits. *Aquaculture Nutrition*, *13* (5), 361-372. https://doi.org/10.1111/j.1365-2095.2007.00485.x

[13] Sitjà-Bobadilla A., Peña-Llopis S., Gómez-Requeni P., Médale F., Kaushik S., & Pérez-Sánchez J. (2005). Effect of fish meal replacement by plant protein sources on nonspecific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). *Aquaculture*, *249* (1), 387-400. https://doi.org/10.1016/j.aquaculture.2005.03.031

[14] Schrama, D., Richard, N., Silva, T. S., Figueiredo, F. A., Conceição, L.E., Burchmore, R., Eckersall, D., & Rodrigues, P. M. (2016). Enhanced dietary formulation to mitigate winter thermal stress in gilthead seabream (*Sparus aurata*): a 2D-DIGE plasma proteome study. Fish Physiol. *Biochem*, 43, 603-617. https://doi.org/10.1007/s10695-016-0315-2

[15] Teodosio, R., Aragao, C., Colen, R., Carrilho, R., Dias, J., & Engrola, S. (2021). A nutritional strategy to promote gilthead seabream performance under low temperatures. *Aquaculture*, 537, 736494. https://doi.org/10.1016/j.aquaculture.2021.736494

[16] Halver, J. E., & Hardy, R. W. (2013). *Fish Nutrition*. Third Edition. Academic Press, London, United Kingdom.

[17] Aas, T. S., Ytrestøyl, T., & Åsgård, T. (2022). Utilization of feed resources in the production of Atlantic salmon (*Salmo salar*) in Norway: An update for 2020. *Aquaculture Reports*, 26, 101316. https://doi.org/10.1016/j.aqrep.2022.101316

[18] El-Husseiny, O. M., Elhammady, A. K. I., Tolba, S. M., & Ashraf, S. (2013). Lipid and protein utilization by gilthead sea bream (Sparus aurata L.) under flow-through system with regards to environmental impact. *Journal of the Arabian Aquaculture Society*, *8* (2). Retrieved from: https://www.arabaqs.org/journal/vol_8/2/Text%2013-28.pdf

[19] Mongile, F., Bonaldo, A., Fontanillas, R., Mariani, L., Badiani, A., Bonvini, E., & Parma, L. (2014). Effects of dietary lipid level on growth and feed utilization of gilthead seabream (*Sparus aurata* L.) reared at Mediterranean summer temperature. *Italian Journal of Animal Science*, 13, 2999. https://doi.org/10.4081/ijas.2014.2999

[20] Volkoff, H., & Ronnestad, I. (2020). Effects of temperature on feeding and digestive processes in fish. *Temperature*, *7* (4), 307-320. https://doi.org/10.1080/23328940.2020.1765950

[21] Haberle, I., Hackenberger, D. K., Djerdj, T., Bavčević, L., Geček, S., Hackenberger, B. K., Marn, N., Klanjšček, J., Purgar, M., Pečar Ilić, J., & Klanjscek, T. (2024). Effects of climate change on gilthead seabream aquaculture in the Mediterranean. *Aquaculture*, 578, 740052. https://doi.org/10.1016/j.aquaculture.2023.740052

[22] Barnabe, G. (1990). Rearing bass and gilthead sea bream. *Aquaculture*, 2, 647-686.

[23] Angeles Gallardo, M., Sala-Rabanal, M., Ibraz, A., Padros, F., Blasco, J., Fernandez-Borras, J., & Sanchez, J. (2003). Functional alterations associated with "winter syndrome" in gilthead sea bream (*Sparus aurata*). *Aquaculture*, *223* (1-4), 15-27. https://doi.org/10.1016/S0044-8486(03)00164-9

[24] Ibraz, A., Fernández-Borràs, J., Blasco, J., Gallardo, M. A., & Sánchez, J. (2003). Oxygen consumption and feeding rates of gilthead sea bream (*Sparus aurata*) reveal lack of acclimation of cold. *Fish Physiol Biochem*, 29, 313-321. https://doi.org/10.1007/s10695-004-3321-8

[25] [25] Ibraz, A., Blasco, J., Sala-Rabanal, M., Gallardo, A., Redondo, A., & Fernandes-Borras, J. (2007). Metabolic rate and tissue reserves in gilthead sea bream (*Sparus aurata*) under thermal fluctuations and fasting and their capacity for recovery. *Canadian Journal of Fisheries and Aquatic Sciences*, *64* (7), 1034-1042. https://doi.org/10.1139/f07-079

[26] Remen, M., Nederlof, M. A. J., Folkedal, O., Thorsheim, G., Sitja-Bobadilla, A., Perez-Sanchez, J., Oppedal, F., & Erik Olsen, R. (2015). Effect of temperature on the metabolism, behavior and oxygen requirements of *Sparus aurata*. *Aquacult Environ Interact*, 7, 115-123. https://doi.org/10.3354/aei00141

[27] Bavčević, L., Petrovic, S., Crnica, M., & Corazzin, E. (2006). Effects of feeding strategy on growth of sea bream (*Sparus aurata*). *Ribarstvo*, 64, 1-3. Retrieved from: https://hrcak.srce.hr/4666

[28] Tort, L., Padros, F., & Rotllant, J. (1998). Winter syndrome in the gilthhead seabream *Sparus aurata*. Immunological and histopathological features. *Fish Shellfish Immunol*, 8, 37-47. https://doi.org/10.1006/fsim.1997.0120

[29] Šarušić, G. (1999). Clinical signs of the winter disease phenomenon in sea bream (*Sparus aurata*, L.). *Bulletin of the European Association of Fish Pathologists*, *19* (3), 113. Retrieved from: https://www.cabidigitallibrary.org/doi/full/10.5555/19992211755

[30] Silva, T. S., da Costa, A. M. R., Conceicao, L. E. C., Dias, J. P., Rodrigues, P. M. L., & Richard, N. (2014). Metabolic fingerprinting of gilthead seabream (*Sparus aurata*) liver to track interactions between dietary factors and seasonal temperature variations. *PeerJ*, 2, e527. https://doi.org/10.7717/peerj.527

[31] Ibraz, A., Padros, F., Gallardo, M. A., Fernandez-Borras, J., Blasco, J., Tort, L. (2010). Low temperature challenges to gilthead sea bream culture: review of cold-induced alterations and „Winter Syndrome". *Reviews in Fish Biology and Fisheries*, 20, 539-556. https://doi.org/10.1007/s11160-010-9159-5

[32] Šarušić, G., & Bavčević, L. (2000). Nutrition as possible ethiological agent of Winter disease syndrome in sea bream (*Sparus aurata* L.). *Ribarstvo*, *58* (4), 153-161. Retrieved from: https://hrcak.srce.hr/4465

[33] Ibraz, A., Beltran, M., Fernandez-Borras, J., Gallardo, M. A., Sanchez, J., & Blasco, J. (2007). Alterations in lipid metabolism and use of energy depots of gilthead sea bream (*Sparus aurata*) at low temperatures. *Aquaculture*, 262, 470-480. https://doi.org/10.1016/j.aquaculture.2006.11.008

[34] Ibarz, A., Blasco, J., Gallardo, M. A., & Fernández-Borràs, J. (2010). Energy reserves and metabolic status affect the acclimation of gilthead sea bream (*Sparus aurata*) to cold. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *155* (3), 319-326. https://doi.org/10.1016/j.cbpa.2009.11.012

[35] Schrama, D., Richard, N., Silva, T. S. Figueiredo, F. A., Conceição, L. E. C., Burchmore, R., Eckersall, D., & Rodrigues, P. M. L. (2017). Enhanced dietary formulation to mitigate winter thermal stress in gilthead sea bream (*Sparus aurata*): a 2D-DIGE plasma proteome study. *Fish Physiol Biochem*, 43, 603-617. https://doi.org/10.1007/s10695-016-0315-2

[36] Alamansa, E., Perez, M. J., Cejas, J. R., Badia, P., Villamandos, J. E., & Lorenzo, A. (1999). Influence of broodstock gilthead seabream (*Sparus aurata* L.) dietary fatty acids on egg quality and egg fatty acid composition throughout the spawning season. *Aquaculture*, 170, 323-336. https://doi.org/10.1016/S0044-8486(98)00415-3

[37] Bavčević, L., Klanjšček, T., Karamarko, V., Aničić, I., & Legović, T. (2010). Compensatory growth in gilthead sea bream (*Sparus aurata*) compensates weight, but not lenght. *Aquaculture*, 301, 57-63. https://doi.org/10.1016/j.aquaculture.2010.01.009

[38] Bonacci, O., & Vrsalović, A. (2022). Differences in Air and Sea Surface Temperatures in the Northern and Southern Part of the Adriatic Sea. *Atmosphere 2022*, *13* (7), 1158. https://doi.org/10.3390/atmos13071158

[39] Association of Official Analytical Chemists (AOAC) (2005). *Official methods of Analysis of AOAC International*. 18th Edition. Gaithersburg, Maryland 208777-2417, USA. Retrieved from: https://www.researchgate.net/publication/292783651_AOAC_2005

[40] Steffens, W. (1989). *Principles of fish nutrition*. Ellis Horwood, Chichester, UK.

[41] Burić, M., Bavčević, L., Grgurić, S., Vresnik, F., Križan, J., & Antonić, O. (2020). Modelling the environmental footprint of sea bream cage aquaculture in relation to spatial stocking design. *Journal of Environmental Management*, 270, 110811. https://doi.org/10.1016/j.jenvman.2020.110811

[42] Alvarez, A., Garcia, B., Jesus Cerezo Valverde, J. C., Felipe Aguado Gimenez F. A., & Hernandez, M. D. (2010). Gastrointestinal evacuation time in gilthead seabream (*Sparus aurata*) according to the temperature. *Aquaculture research*, 41, 1101-1106. https://doi.org/10.1111/j.1365-2109.2009.02391.x

[43] Mayer, P., Estruch, P., Blasco, J., & Jover, M. (2008) Predicting the growth of gilthead sea bream (*Sparus aurata* L.) farmed in marine cages under real production conditions using temperature- and time-dependent models. *Aquaculture Research*, 39, 1046-1052. https://doi.org/10.1111/j.1365-2109.2008.01963.x

[44] Seginer, I. (2016). Growth models of gilthead sea bream (*Sparus aurata* L.) for aquaculture. *Aquacultural Engineering*, 70, 15-32. https://doi.org/10.1016/j.aquaeng.2015.12.001

[45] Bavčević, L., Čolak, S., Luzzana, U., Petrović, S., Couzzeau, P., & Burlini, M. (2007). Spring feeding protocols in cage cultured sea bream. *Krmiva*, 49, 37-44. Retrieved from: https://hrcak.srce.hr/28590

[46] Lupatsch, I., & Kissil, G. W. (2003) Defining energy and protein requirements of gilthead seabream (*Sparus aurata*) to optimize feeds and feeding regimes. *The Israeli Journal of Aquaculture – Bamidgeh*, *55* (4), 243-257. https://doi.org/10.46989/001c.20354

[47] Lupatsch, I. (2005). Protein and energy requirements in Mediterranean species. *Cahiers Options Méditerranéennes*, 63. Retrieved from: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=0383b6267cd14ef2e04e4be1368402a75f0ce076