Effects of Temperature and Feeding Regime on the Fatty Acid Composition of the Mediterranean Mussel (*Mytilus galloprovincialis*)

Utjecaj temperature i hranidbenog režima na sastav masnih kiselina mediteranske dagnje (Mytilus galloprovincialis)

Petar Zuanović*
PhD student
University of Zadar
Department of Ecology, Agronomy and Aquaculture
Croatia
E-mail: pzuanovic22@unizd.hr

Zoran Zorić
University of Zadar
Department of Ecology, Agronomy and Aquaculture
Croatia
E-mail: zzoric@unizd.hr

Ivan Župan
University of Zadar
Department of Ecology, Agronomy and Aquaculture
Croatia
E-mail: zupan@unizd.hr

Tomislav Šarić
University of Zadar
Department of Ecology, Agronomy and Aquaculture
Croatia
E-mail: tosaric@unizd.hr

Tibor Janči
Faculty of Food Technology and Biotechnology
Laboratory for Meat and Fish Technology
Croatia
E-mail: tjanci@pbf.hr

Ana Dobrinčić
Faculty of Food Technology and Biotechnology
Laboratory for Biochemical Engineering, Industrial Microbiology and Malting and Brewing Technology
Croatia
E-mail: adobrincic@pbf.hr

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Abstract

Mussels (*Mytilus galloprovincialis*) were held in a recirculating aquaculture system at 12, 18 and 24°C and fed the microalga *Tisochrysis lutea* at three rations (3.0, 4.5 and 6.0% of dry matter) for 15 days, following a 7-day unfed acclimation. Fatty acids of the diet and of the mussel soft tissue were determined by gas chromatography. The diet was rich in polyunsaturated fatty acids (PUFA, 42.88% of total fatty acids), dominated by docosahexaenoic acid (22:6n-3, DHA; 13.48%) and linoleic acid (18:2n-6; 15.21%), but contained only 0.69% eicosapentaenoic acid (20:5n-3, EPA). The proportions of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), n-3 and n-6 PUFA and the n-6/n-3 ratio differed among the temperature × ration combinations; as each combination was represented by a single tank, the results are presented descriptively. The highest PUFA (51.41%) and n-3 (47.81%) and the lowest saturated fatty acid (23.84%) proportions occurred at 18°C with the 3.0% ration. Higher feeding rations were associated with higher proportions of n-6 fatty acids (1.06–7.38%), a higher n-6/n-3 ratio and lower DHA levels. EPA was below the limit of quantification in all tissue samples and arachidonic acid (20:4n-6) never exceeded 0.11%, mirroring the composition of the diet. A moderate temperature combined with a restricted feeding ration therefore appeared to yield the most favourable n-3 profile, but the nutritional quality of the product may also depend on the fatty acid composition of the microalgal diet.

Sažetak

Mediteranske dagnje (Mytilus galloprovincialis) držane su u recirkulacijskom sustavu pri 12, 18 i 24 °C te hranjene mikroalgom Tisochrysis lutea (3,0, 4,5 i 6,0% suhe tvari) tijekom 15 dana, nakon sedmodnevne aklimatizacije bez hranjenja. Masnokiselinski sastav hrane i mekog tkiva određen je plinskom kromatografijom. Hrana je bila bogata višestruko nezasićenim masnim kiselinama (PUFA, 42,88%), s dokozaheksaenskom (22:6n-3, DHA; 13,48%) i linolnom kiselinom (18:2n-6; 15,21%) kao dominantnima, uz svega 0,69% eikozapentaenske kiseline (20:5n-3, EPA). Udjeli zasićenih (SFA), mononezasićenih (MUFA) i višestruko nezasićenih masnih kiselina (PUFA), n-3 i n-6 PUFA te omjer n-6/n-3 razlikovali su se među kombinacijama temperature i obroka; budući da je svaka kombinacija bila zastupljena jednim spremnikom, rezultati su prikazani deskriptivno. Najviši udjeli PUFA (51,41%) i n-3 (47,81%) te najniži udio zasićenih masnih kiselina (23,84%) zabilježeni su pri 18 °C uz obrok od 3,0%. Veći obroci bili su povezani s višim udjelom n-6 masnih kiselina (1,06 – 7,38%), višim omjerom n-6/n-3 i nižim udjelom DHA. EPA je u svim uzorcima tkiva bila ispod granice kvantifikacije, a arahidonska kiselina (20:4n-6) nije prelazila 0,11%, što odražava sastav hrane. Umjerena temperatura uz ograničenu količinu hrane dala je najpovoljniji n-3 profil, no kvaliteta konačnog proizvoda mogla bi ovisiti i o sastavu masnih kiselina mikroalge.

KEY WORDS

Mytilus galloprovincialis
Tisochrysis lutea
fatty acids
recirculating aquaculture system
feeding ration

KLJUČNE RIJEČI

Mytilus galloprovincialis
Tisochrysis lutea
masne kiseline
recirkulacijski sustav
režim hranidbe

* Corresponding author

1. INTRODUCTION / *Uvod*

The Mediterranean mussel, *Mytilus galloprovincialis*, represents one of the most important bivalve species in coastal ecosystems and Mediterranean aquaculture. As a sessile filter-feeding organism, it links primary production, suspended organic matter and higher trophic levels, while its growth, physiological condition and biochemical composition are strongly influenced by food availability and environmental conditions. Due to its ecological relevance, commercial importance and frequent use in biomonitoring, *M. galloprovincialis* is a suitable model species for investigating how environmental factors affect the nutritional and physiological quality of marine organisms [1, 2].

Temperature is one of the key environmental factors regulating metabolism, filtration activity, energy allocation and adaptive capacity in bivalves. Changes in seawater temperature may affect physiological stability, including digestive and metabolic processes, while elevated temperatures can induce stress responses associated with reduced survival and shifts in the mussel microbiome. In the context of increasing thermal variability in coastal environments, understanding temperature-driven changes in the biochemical composition of mussels is relevant for both aquaculture management and the assessment of climate-related impacts on marine organisms [3, 4].

Fatty acids are important components of lipid metabolism in bivalves because they contribute to membrane structure, energy storage and physiological responses to environmental variation. Particular attention is given to saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), including long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Ratios such as PUFA/SFA and n-3/n-6 are commonly used as indicators of nutritional value, physiological status and possible changes in dietary sources [4, 5, 6].

The diet of bivalves is directly related to their fatty acid profile, since many essential fatty acids are transferred from microalgae to the tissues of filter-feeding organisms. Microalgae are primary producers of EPA and DHA in marine food webs, and their fatty acid composition may vary considerably depending on species and culture conditions. Therefore, the type and quantity of microalgal food supplied to mussels can influence the incorporation of individual fatty acids into mussel tissue and consequently affect their nutritional quality [7, 8].

Tisochrysis lutea was used in the present experiment as the microalgal food source for mussels, making its fatty acid composition particularly relevant for interpreting dietary effects on mussel tissue. Species and strains commonly used within the *Isochrysis*/T-Iso group are widely applied in bivalve nutrition because they can provide nutritionally important polyunsaturated fatty acids, especially DHA, which may be transferred through feeding and incorporated into bivalve tissues. Previous studies on microalgae cultivated for bivalve larval feeding and broodstock conditioning have shown that *Isochrysis galbana* T-Iso can produce relatively high levels of linolenic acid and DHA, while fatty acid production may vary depending on culture conditions such as nitrate availability and irradiance [7, 9].

Temperature has been shown to drive membrane lipid remodelling and stress responses in bivalves [3, 10], while the quality and quantity of the microalgal diet directly determine the incorporation of essential fatty acids into mussel tissue [8, 9]. The aim of this study was to explore the individual and combined effects of temperature and feeding level on the fatty acid composition of *M. galloprovincialis*. Special emphasis was placed on changes in the main fatty acid groups, selected biologically important individual fatty acids and lipid quality ratios reflecting the nutritional and physiological status of mussels [4, 8].

The effects of temperature and dietary microalgae on bivalve lipids have mostly been studied in isolation, either in the field [5, 6, 11], where temperature co-varies with seasonal changes in the natural seston [8,12], or at a single feeding level. It therefore remains unclear whether a given feeding ration produces the same lipid response across the thermal range experienced in Mediterranean mussel farming. The present study addresses this gap by crossing three water temperatures (12, 18 and 24°C) with three feeding rations (3.0, 4.5 and 6.0% of dry matter) in a recirculating system, using a single, chemically characterised diet (*Tisochrysis lutea*), allowing the combined response to temperature and ration, which has rarely been examined in *M. galloprovincialis*, to be explored.

2. MATERIALS AND METHODS / *Materijali i metode*

2.1. Experimental organisms and acclimation / *Pokusni organizmi i aklimatizacija*

The Mediterranean mussel, *Mytilus galloprovincialis*, a species of major ecological and aquaculture importance in coastal marine ecosystems, was used in this study. At the beginning of the experiment, approximately 110 individuals per tank were introduced into the experimental recirculating aquatic system and acclimated for 7 days. During acclimation the water temperature was gradually adjusted from the ambient temperature of 18 °C to the target treatment temperatures of 12, 18 and 24 °C at a rate of 0.5 °C every 12 h, while salinity was maintained at 35 ‰ in all experimental tanks throughout the experiment. Mussels were not fed during acclimation in order to standardise their nutritional status and to empty the digestive tract before the start of the feeding treatment. After the seven-day acclimation period, 30 individuals per tank were sampled and divided into three pooled samples of 10 individuals for the initial fatty acid analysis.

2.2. Experimental system and design / *Pokusni sustav i plan pokusa*

The experiment was conducted in an experimental recirculating aquatic system. Each tank had a volume of 120 L, and three tanks maintained at the same temperature were connected to one biofilter. Mussels were exposed to three temperature treatments: 12, 18 and 24 °C. Water temperature was maintained using a TANK Chiller Line TK2000 unit. After the initial sampling, the remaining 80 individuals per tank were exposed to the feeding treatments for 15 days. This number ensured a sufficient amount of soft tissue for the fatty acid analysis at the end of the experiment. As each of the nine tanks represented one unique temperature × feeding combination, this full factorial design allowed the individual and combined effects of both factors on the fatty acid composition of mussel tissue to be explored.

2.3. Cultivation of the microalga Tisochrysis lutea / *Uzgoj mikroalge Tisochrysis lutea*

The microalga *Tisochrysis lutea* (ISO), used as the dietary source in the experimental feeding of mussels, was cultivated under controlled conditions in an Industrial Plankton PBR1250 photobioreactor. The culture was maintained at 22 °C and pH 7.95, while light intensity was adjusted according to culture density to maintain suitable growth

conditions. The photoperiod was set to 20 h light and 4 h dark. Cultivation was performed using a batch model, with the addition of a nutrient medium prepared according to an in-house formulation containing macroelements, trace elements and vitamins, including thiamine, biotin and vitamin B12 complex.

The cultivated microalgal biomass was used as a controlled food source for mussels during the 15-day feeding treatment.

2.4. Feeding regime / *Režim hranidbe*

Mussels were fed once daily with a live culture of *Tisochrysis lutea* harvested directly from the photobioreactor and delivered as a cell suspension in seawater; no concentrated paste or dried biomass was used. The cell density of the harvested culture was determined before each feeding, and the volume added to each tank was calculated so that the daily ration corresponded to 3.0, 4.5 or 6.0% of the dry matter of the mussels in that tank. The application of different feeding levels enabled the assessment of the effect of food availability on the fatty acid composition of mussel tissue [7, 8, 9].

2.5. Sampling and tissue preparation / *Uzorkovanje i priprema tkiva*

Initial sampling was performed after acclimation and before the beginning of the feeding treatment, and final sampling after 15 days of feeding. At each sampling, 30 individuals were collected from each tank and divided into three pooled samples of 10 individuals, one from each of three vertical levels of the rearing lantern placed in the tank. The soft tissue was excised from the shell with a scalpel, the byssus threads were removed and the tissue was placed on a sieve to drain the intervalvular water. Each pooled sample was stored separately at -80 °C until fatty acid analysis.

2.6. Fatty acid analysis / *Analiza masnih kiselina*

Fatty acid methyl esters (FAME) were prepared directly from the homogenised soft tissue by one-step direct transesterification according to Lepage and Roy, which allows the simultaneous extraction and methylation of fatty acids from all lipid classes without a separate lipid extraction step. The FAME were then separated and quantified by gas chromatography [13].

The FAMEs were determined according to the method ISO 12966 (2017) [14] using a Shimadzu GC-2030 P gas chromatograph equipped with autoinjector AOC 20i Plus and flame ionization detection unit (Shimadzu, Kyoto, Japan). A high-polarity capillary column TR-FAME (30 m × 0.222 mm × 0.20 µm, Thermo Fisher, Waltham, Massachusetts, USA) was used for chromatographic determination of the FAMEs. The carrier gas was nitrogen (99% purity) at the flow rate 0.7 mL/min. The parameters of gas chromatography were as follows: injector temperature, 245 °C; detector temperature, 245 °C; split mode, 1:30; and total flow, 95.5 mL/min. The initial temperature program of the column started from 120 °C for 5 min, then increased by 4 °C/min to 160°C and then by 10 °C/min to 190°C and held for 30 min. The injection volume was 0.6 µL. Total time of analysis was 48 min. Individual fatty acids were identified by comparison with appropriate FAME standards (37-component FAME Mix, Supelco, Merck, Darmstadt, Germany).

2.7. Data processing / *Obrada podataka*

Fatty acid composition data were processed both individually and according to the main fatty acid groups. Individual fatty acids were grouped into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 and n-6 fatty acids. The results are reported as one value per tank, i.e. per temperature × ration combination. All values are expressed as a percentage of the total identified fatty acids. Because the number of chromatographic peaks that could be assigned to a fatty acid differed between samples (14–36), the data set was additionally re-normalised on the seven fatty acids identified in every sample (16:0, 16:1, 18:0, 20:1n-9, 20:3n-3, 22:1n-9 and 22:6n-3), and this reduced data set was used as a sensitivity check of the treatment patterns.

Particular attention was given to biologically important fatty acids, including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), as well as ratios reflecting nutritional quality, such as PUFA/SFA and n-6/n-3.

2.8. Data analysis / *Analiza podataka*

Because each temperature × ration combination was represented by a single tank, samples taken from the same tank are not independent replicates of the treatment (pseudoreplication [15]), and tank effects cannot be separated from treatment effects. Inferential statistical tests (analysis of variance and post-hoc comparisons) were therefore not applied, and the results are presented descriptively. Differences between treatments are described as observed differences and trends, with emphasis on patterns that were consistent across temperatures or rations. The initial fatty acid composition of each temperature line was compared descriptively with the post-feeding composition at the same temperature, calculated as the mean of the three rations. Data were processed in Statistica ver. 14.0 (TIBCO Software Inc., Santa Clara, CA, USA).

3. RESULTS / Rezultati

3.1. Fatty acid profile of the *Tisochrysis lutea* diet/ *Sastav masnih kiselina mikroalge Tisochrysis lutea korištene u hranidbi*

The fatty acid profile of the freeze-dried sample of the *T. lutea* culture used as feed was dominated by polyunsaturated fatty acids (PUFA, 42.88% of total fatty acids), followed by monounsaturated (MUFA, 34.82%) and saturated fatty acids (SFA, 22.29%) (Figure 1).

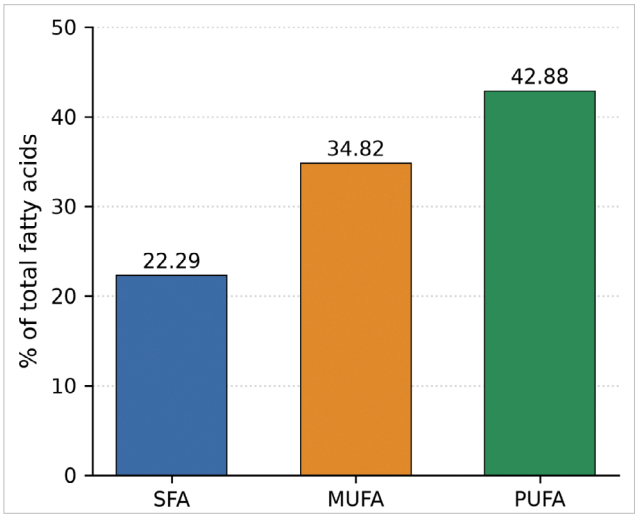


Figure 1 Fatty acid groups (% of total fatty acid) in *Tisochrysis lutea*
Slika 1. Skupine masnih kiselina (% ukupnih masnih kiselina) u mikroalgi Tisochrysis lutea
Source: authors (data processed in Statistica ver. 14.0, TIBCO Software Inc.)

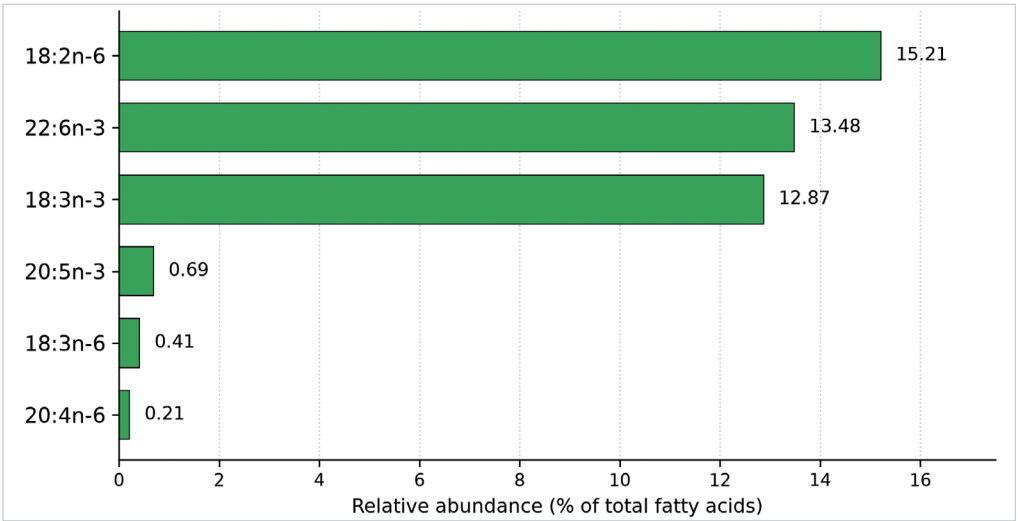


Figure 2 Individual polyunsaturated fatty acids (% of total fatty acids) in *Tisochrysis lutea*

Slika 2. Pojedinačne višestruko nezasićene masne kiseline (% ukupnih masnih kiselina) u mikroalgi Tisochrysis lu tea

Source: authors (data processed in Statistica ver. 14.0, TIBCO Software Inc.)

Among the polyunsaturated fatty acids, linoleic acid (18:2n-6; 15.21%), docosahexaenoic acid (22:6n-3, DHA; 13.48%) and α-linolenic acid (18:3n-3; 12.87%) were dominant, whereas eicosapentaenoic acid (20:5n-3, EPA; 0.69%), γ-linolenic acid (18:3n-6; 0.41%) and arachidonic acid (20:4n-6, ARA; 0.21%) were present only in small amounts (Figure 2). Total n-3 fatty acids reached 27.04% and total n-6 fatty acids 15.83%, giving an n-6/n-3 ratio of 0.59.

3.2. Fatty acid groups in mussel tissue after the feeding trial / *Skupine masnih kiselina u tkivu dagnji nakon pokusa hranidbe*

After 15 days of feeding, the proportions of all three fatty acid groups differed among the temperature × ration combinations, and the response to ration was not the same at the three temperatures (Figure 3).

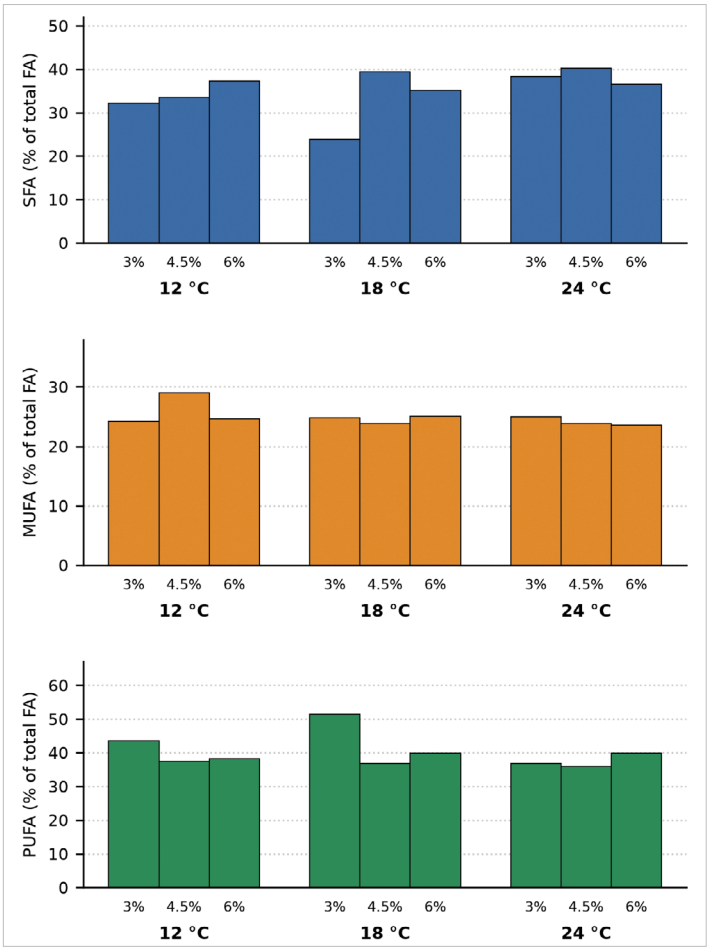


Figure 3 Proportions of SFA, MUFA and PUFA in the soft tissue of *Mytilus galloprovincialis* after 15 days of feeding

Slika 3. Udjeli SFA, MUFA i PUFA u mekom tkivu dagnje Mytilus galloprovincialis nakon 15 dana hranjenja

Source: authors (data processed in Statistica ver. 14.0, TIBCO Software Inc.)

3.3. The n-3 and n-6 fatty acids and the n-6/n-3 ratio / *n-3 i n-6 masne kiseline te omjer n-6/n-3*

The n-3 and n-6 groups and the n-6/n-3 ratio also differed among the temperature × ration combinations (Figure 4).

The n-3 proportion was highest at 18 °C/3.0% (47.81%), followed by 12 °C/3.0% (42.48%), whereas all remaining combinations showed similar values between 30.90% and 32.37%. The n-6 proportion showed the clearest dose response of all variables measured, rising from 1.06% at 12 °C/3.0% to 7.38% at 18 °C/6.0% and increasing with ration at every temperature. As a consequence, the n-6/n-3 ratio increased with ration at all three temperatures (from 0.025 at 12 °C/3.0% to 0.233 at 18 °C/6.0%), while remaining far below the value of 1 in every treatment.

3.4. Comparison of fatty acid composition before and after feeding / *Usporedba sastava masnih kiselina prije i nakon hranjenja*

Because the rations had not yet been applied at the initial sampling, the pre-feeding composition can be compared with the post-feeding composition per temperature but not per ration (Figure 5). SFA was slightly lower after feeding at all three temperatures (by 1.9–2.6 percentage points), and PUFA changed by less than 3.5 percentage points (slightly higher after feeding at 12 and 18 °C, lower at 24 °C). Total n-3 fatty acids were almost unchanged at 12 and 18 °C, but lower after feeding at 24 °C (39.29% vs 31.82%).

MUFA showed the largest change at 24 °C, where it increased from 18.99% to 24.14%, while remaining almost unchanged

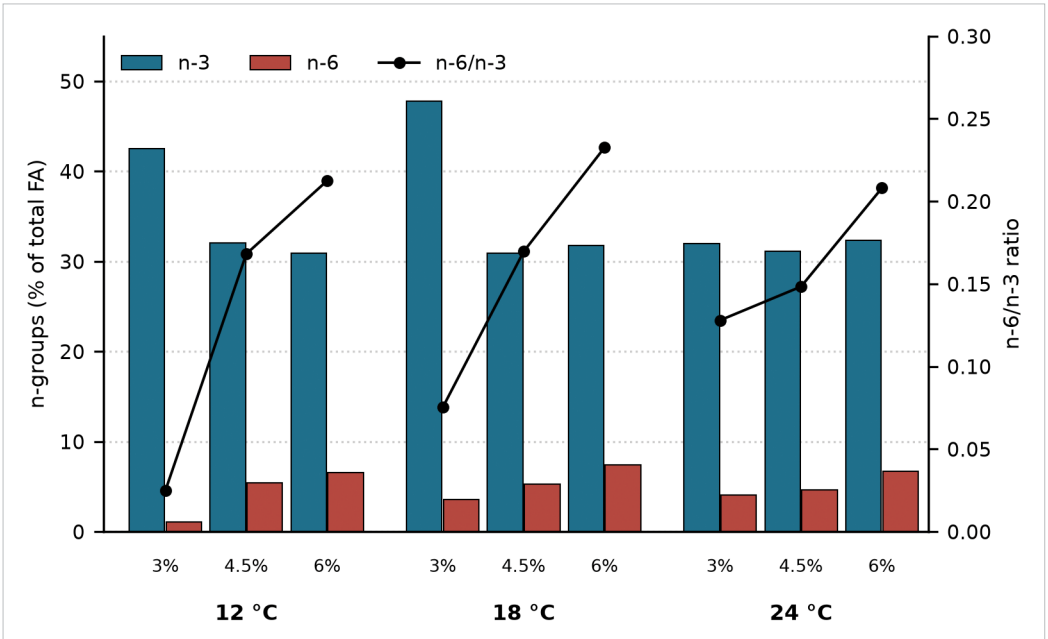


Figure 4 Proportions of n-3 and n-6 fatty acids (left axis, bars) and the n-6/n-3 ratio

Slika 4. Udjeli n-3 i n-6 masnih kiselina (lijeva os, stupci) i omjer n-6/n-3

Source: authors (data processed in Statistica ver. 14.0, TIBCO Software Inc.)

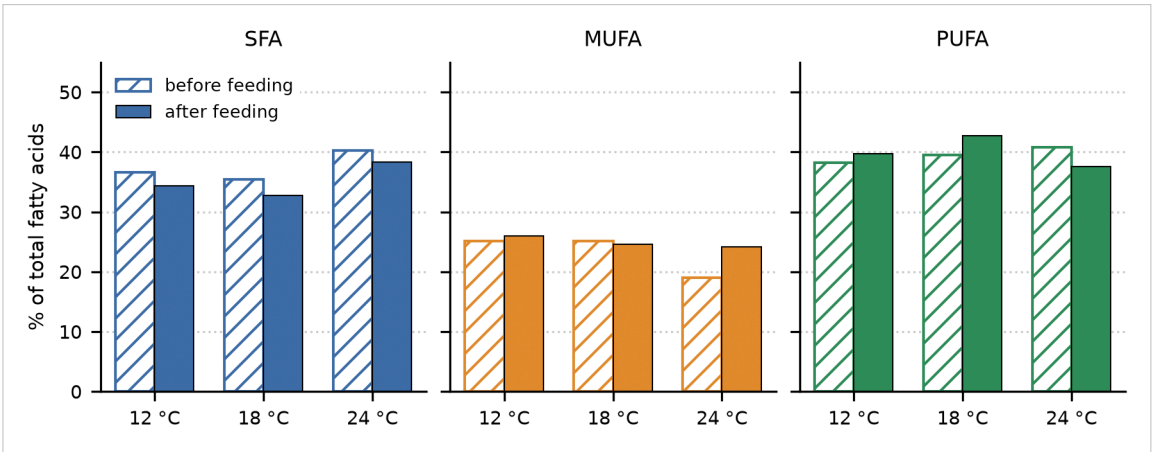


Figure 5 Fatty acid groups in the soft tissue of *M. galloprovincialis* at the end of the acclimation period (before feeding, hatched bars) and at the end of the 15-day feeding trial (after feeding, solid bars; mean of the three rations)

Slika 5. Skupine masnih kiselina u mekom tkivu dagnje M. galloprovincialis na kraju razdoblja aklimatizacije (prije hranjenja, šrafirani stupci) i na kraju 15-dnevnog pokusa hranidbe (nakon hranjenja, puni stupci; srednja vrijednost za tri obroka)

Source: authors (data processed in Statistica ver. 14.0, TIBCO Software Inc.)

at 12 and 18 °C. The n-6 group was higher after feeding at all temperatures, from 1.48–2.70% before to 4.34–5.41% after, reflecting the linoleic acid content of the diet.

3.5. Dominant individual fatty acids after exposure to different feeding levels / *Dominantne masne kiseline nakon izlaganja različitim razinama hranidbe*

Seven fatty acids (16:0, 16:1, 18:0, 20:1n-9, 20:3n-3, 22:1n-9 and 22:6n-3) were identified in every tissue sample, and the dominant individual fatty acids after feeding are shown in Figure 6 and Figure 7. Palmitic acid (16:0) was the dominant saturated fatty acid, ranging from 14.59% at 18 °C/3.0% to 27.01% at 12 °C/3.0%, and stearic acid (18:0) ranged from 4.77% to 7.71%. Among monounsaturated fatty acids, oleic acid (18:1n-9; 4.16–8.42%) and gondoic acid (20:1n-9; 2.93–6.18%) were the most abundant, the latter being lowest at the 6.0% ration at every temperature. DHA (22:6n-3) was by far the most abundant polyunsaturated fatty acid in mussel tissue and largely determined the behaviour of the PUFA and n-3 groups. Its proportion was highest at 18 °C/3.0% (41.45%) and at 12 °C/3.0% (35.31%), and fell to 21.90–24.35% in all other treatments, so that DHA was highest at the 3.0% ration at each temperature. Linoleic acid (18:2n-6; 1.06–6.66%) showed the opposite trend and was the main contributor to the increase of the n-6 group with ration, while eicosatrienoic acid (20:3n-3) decreased from 7.17% to 3.98% as the ration increased.

EPA (20:5n-3) was below the limit of quantification in all mussel tissue samples, whereas ARA (20:4n-6) was quantified only in three treatments (18 °C/6.0%, 24 °C/4.5% and 24 °C/6.0%), at trace levels never exceeding 0.11% of total fatty acids (Figure 6). Because both fatty acids were present only in small amounts in the *T. lutea* diet (EPA 0.69%, ARA 0.21%), only a minimal dietary supply of C20 highly unsaturated fatty acids was available during the trial; over 15 days, EPA did not reach quantifiable levels in mussel tissue and ARA remained at trace levels. In the sensitivity check on the seven fatty acids identified in every sample (Section 2.7), the ranking of treatments was preserved: the 18 °C/3.0% treatment retained both the highest PUFA proportion (53.46% on the core set vs 51.41% on the full profile) and the lowest SFA proportion (23.71 vs 23.84%). The observed treatment rankings were therefore similar in the full and reduced fatty acid datasets.

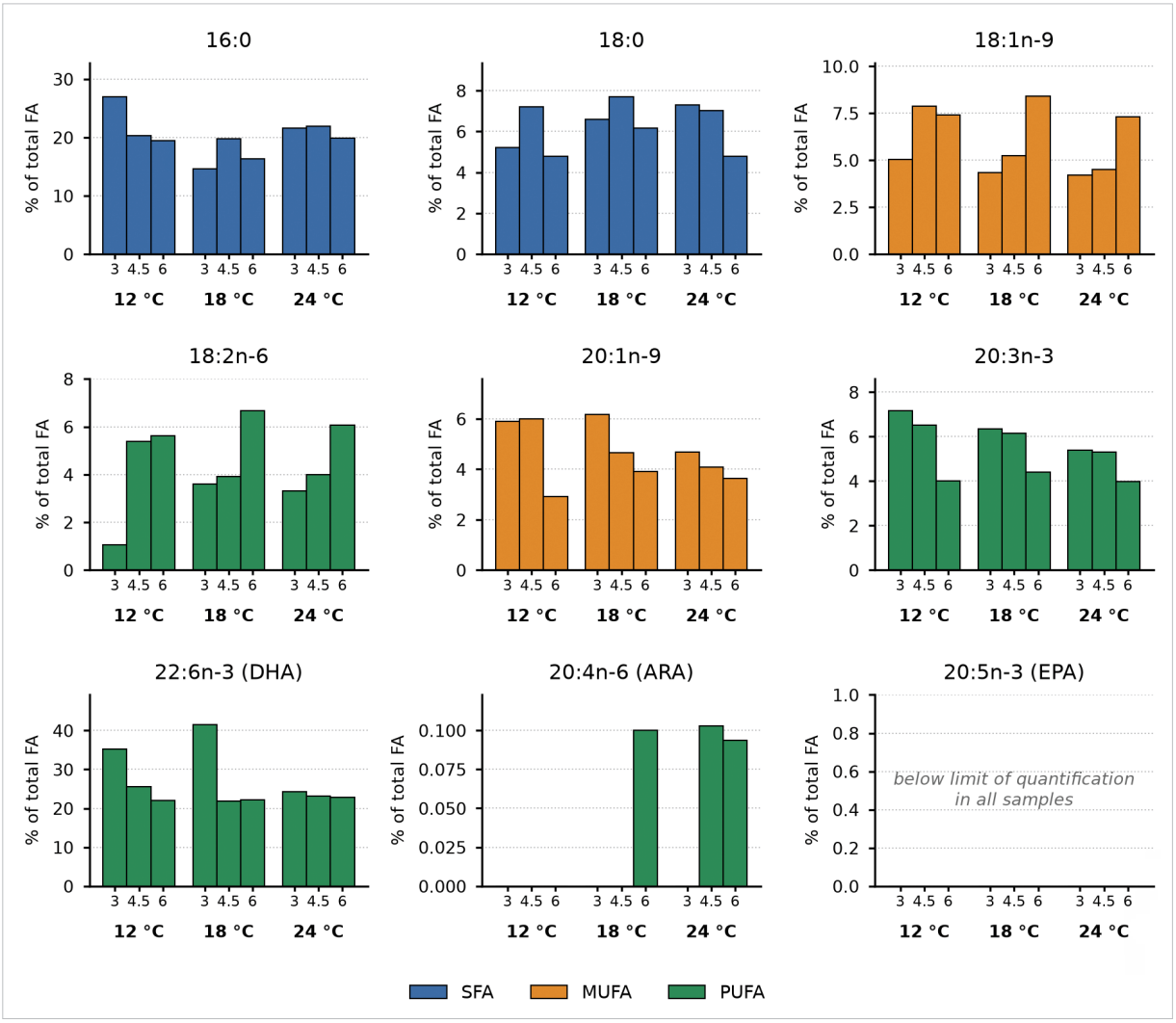


Figure 6 Dominant individual fatty acids in the soft tissue of *M. galloprovincialis* after 15 days of feeding

Slika 6. Dominantne pojedinačne masne kiseline u mekom tkivu dagnje M. galloprovincialis nakon 15 dana hranjenja

Source: authors (data processed in Statistica ver. 14.0, TIBCO Software Inc.)

3.6. Nutritional quality indices / *Indeksi nutritivne kvalitete*

The PUFA/SFA ratio was highest at 18 °C/3.0% (2.16), followed by 12 °C/3.0% (1.35), whereas all remaining treatments fell between 0.90 and 1.14. The n-6/n-3 ratio remained between 0.025 and 0.233 in all treatments, i.e. one to two orders of magnitude below the upper limit of 4 recommended for human nutrition.

3.7. Overall comparison of treatments after feeding / *Ukupna usporedba tretmana nakon hranjenja*

The heat map of individual fatty acids (Figure 7) showed that the treatments separated along two opposing gradients: high DHA (22:6n-3) at the 3.0% ration at 12 and 18°C, and increasing 18:2n-6 with decreasing 20:3n-3 at higher rations (Section 3.5). This pattern is consistent with selective retention of long-chain n-3 fatty acids in membrane phospholipids when food is limited, while higher rations shift the tissue towards a diet-driven profile, since 18:2n-6 was abundant in the *T. lutea* diet (15.21%). At 18 °C with the lowest ration, PUFA reached 51.41% and SFA fell to 23.84%, the most extreme values of all combinations; raising the ration to 4.5% reversed the pattern (SFA 39.39%, PUFA 36.81%), whereas at 24 °C the profile remained comparatively stable across rations. MUFA varied over a much narrower range (23.57% to 29.05%). The 18 °C/3.0% treatment thus combined the highest PUFA, n-3 and DHA proportions with the lowest SFA proportion and the most favourable PUFA/SFA ratio.

4. DISCUSSION / *Rasprava*

The results of this study indicate that the fatty acid composition of *M. galloprovincialis* differed among the combinations of temperature and feeding regime. After 15 days of feeding, clear differences were observed in the main fatty acid groups, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the n-3 and n-6 fractions. These observations suggest that the lipid profile of mussel tissue cannot be explained only by food availability, but may reflect combined nutritional and temperature-dependent physiological responses [3, 4, 12]. The use of a one-step direct transesterification procedure, which allows efficient recovery of fatty acids from all major lipid classes, increases the reliability of the total fatty acid profiles reported in this study [13].

The observation that the same food quantity did not produce the same lipid response at 12, 18 and 24 °C suggests that temperature may modify the way mussels utilize, retain or redistribute dietary fatty acids. Such a response would be consistent with the ability of bivalves to remodel membrane lipids under changing thermal conditions, particularly through changes in the degree of fatty acid unsaturation. Temperature may therefore act not only as an external environmental variable, but also as a factor affecting lipid metabolism and tissue composition.

Among the experimental treatments, the most favourable fatty acid profile was observed at 18 °C and 3% dry matter feeding.

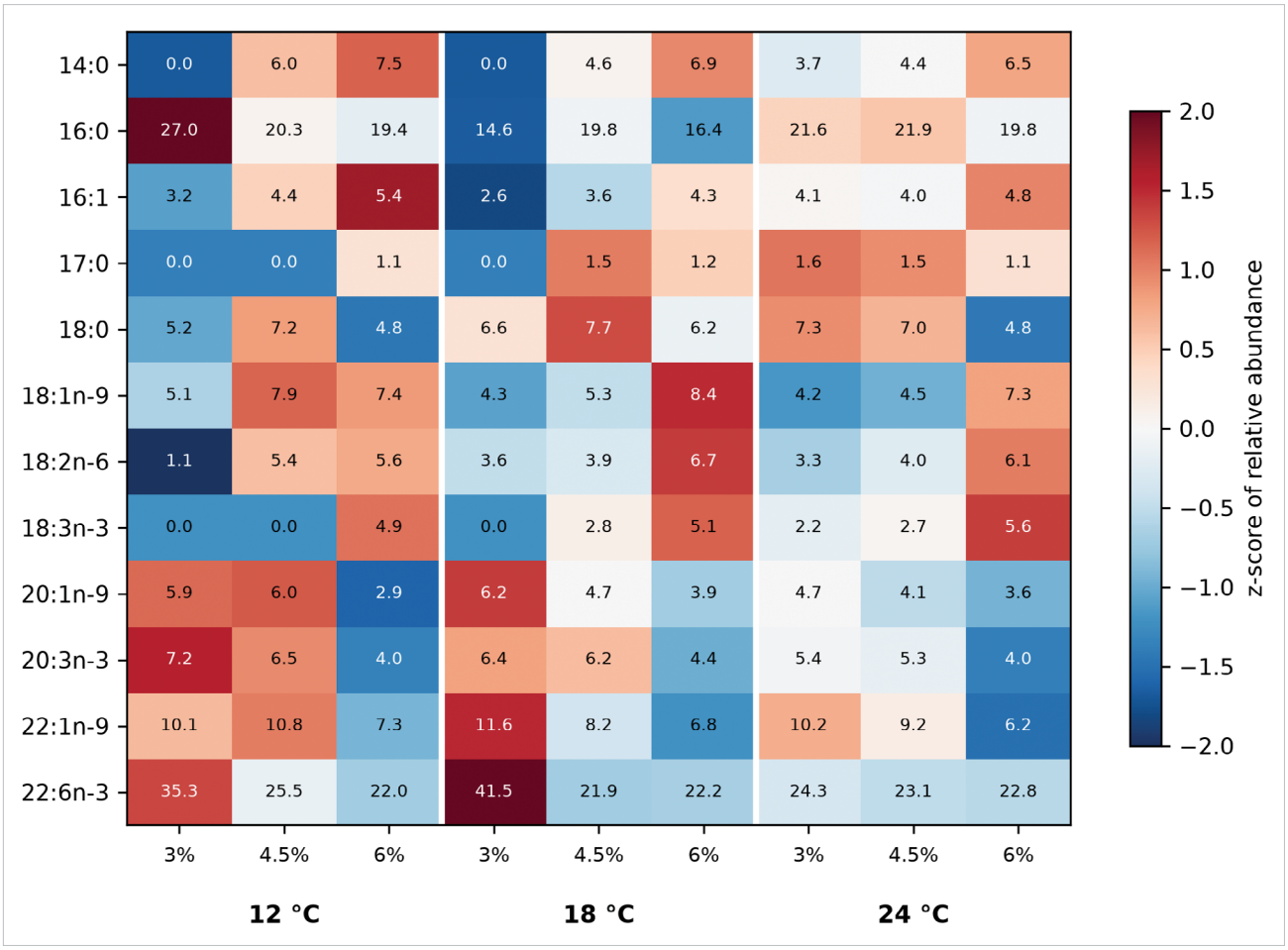


Figure 7 Heatmap of the most variable individual fatty acids in *M. galloprovincialis* tissue

Slika 7. Toplinska karta najvarijabilnijih pojedinačnih masnih kiselina u tkivu dagnje M. galloprovincialis

Source: authors (data processed in Statistica ver. 14.0, TIBCO Software Inc.)

This treatment was associated with higher proportions of PUFA and n-3 fatty acids and lower proportions of SFA. These results suggest that moderate temperature and lower feeding intensity may provide more favourable conditions for maintaining the biochemical quality of mussel tissue. In contrast, increasing the feeding level did not necessarily improve the fatty acid profile, indicating that higher food availability does not automatically result in higher nutritional quality [4, 10].

The fatty acid composition of the microalga *T. lutea* provides an important context for interpreting the mussel response. The ISO microalga was characterized by a high proportion of PUFA and a particularly high DHA content, whereas EPA was present at a much lower level. This indicates that the diet supplied during the experiment was a relevant source of unsaturated fatty acids, especially DHA. Since microalgae are the primary source of long-chain n-3 fatty acids in marine food webs, their biochemical composition can directly influence the fatty acid profile of filter-feeding organisms such as mussels [7, 9].

However, the presence of DHA in the diet does not necessarily imply its equal incorporation into mussel tissue under all experimental conditions. The efficiency of fatty acid incorporation may depend on temperature, feeding intensity, initial lipid status and the balance among individual fatty acids. Previous studies have shown that dietary DHA can affect growth, fatty acid composition and physiological processes in bivalves, but that the optimal response also depends on the balance among DHA, EPA and ARA [12].

The results also illustrate the importance of analysing fatty acids both as groups and as individual compounds. Group-level analysis provided a general overview of nutritional and biochemical changes, whereas individual fatty acids showed more pronounced treatment-specific responses. Several fatty acids, including caprylic acid (8:0), trans-linoleic acid (trans-18:2n-6), butyric acid (4:0), elaidic acid (trans-18:1n-9), behenic acid (22:0) and arachidic acid (20:0), showed marked changes between the initial condition and the condition after feeding. These changes suggest that individual fatty acids may be more sensitive indicators of physiological response than broad fatty acid groups alone.

The n-6/n-3 balance further indicated that nutritional quality varied among treatments. The highest n-3 values were observed at 18 °C and 3% feeding, whereas the highest n-6 values were observed at 18 °C and 6% feeding, indicating that increasing the feeding level alters the balance between the two fractions, but not necessarily in a nutritionally favourable direction. The individual fatty acid data (Figure 6) clarify which compounds drive this pattern. The high n-3 and PUFA proportions at 18 °C and 3% feeding were largely driven by DHA (22:6n-3), which reached 41.45% of total fatty acids in that treatment, roughly twice the value recorded at the higher rations. The opposite behaviour of linoleic acid (18:2n-6), which rose from 1.06% at 12 °C/3% to 6.66% at 18 °C/6%, explains the strong association between ration and the n-6 fraction and reflects direct dietary transfer, since 18:2n-6 was the most abundant polyunsaturated fatty acid of the *T. lutea* diet. Figure 6 also shows that EPA (20:5n-3) remained below the limit of quantification in every treatment and that ARA (20:4n-6) never exceeded 0.11%, suggesting that the increase in the n-6 fraction was primarily associated with changes in C18 fatty acids rather than with changes in long-chain n-6 fatty acids. The decrease of 20:3n-3 and 20:1n-9 with increasing ration, with the lowest proportions at the 6.0% ration at all temperatures, may be consistent with a shift from mobilisation of stored lipid reserves under restricted feeding towards greater incorporation of dietary lipids at higher feeding levels. Since mussels are generally considered an important source of long-chain n-3 fatty acids, particularly EPA and DHA [16], these changes are relevant both for mussel physiology and for the assessment of their nutritional value. In the present trial, DHA was the predominant long-chain n-3 fatty acid, since the mono-specific *T. lutea* diet supplied a very small amount of EPA (0.69% of total fatty acids) [12].

A limitation of this study is that the initial and final samples were not paired samples from the same individuals. The initial samples represented the biochemical condition after acclimation, whereas the final samples represented the response after 15 days of exposure to different temperature and feeding treatments. Therefore, comparisons before and after feeding should be interpreted as comparisons between experimental conditions rather than as individual temporal changes. In addition, the results are expressed as relative fatty acid composition, meaning that changes in the proportion of one fatty acid may also reflect changes in other components of the total profile. In addition, each temperature × ration combination was applied to a single tank, and the three pooled samples per treatment were collected from different levels of the same lantern; tank effects therefore cannot be separated from treatment effects, and no inferential statistical tests were applied. The differences described here should therefore be regarded as observed trends; their consistency across temperatures and rations and their agreement with the dietary profile support their biological relevance, but they should be confirmed in a design with replicated tanks.

Taken together, the most informative outcome of the trial was that the same ration did not produce the same lipid response at 12, 18 and 24°C, and the most favourable profile was obtained at 18°C under a restricted ration of 3% dry matter. Because the tissue profile closely followed the profile of the diet, these observations suggest that the fatty acid composition of the microalgal diet, particularly its EPA content, may be an important consideration for the nutritional quality of mussels reared in recirculating systems.

5. CONCLUSION / *Zaključak*

This study indicated that temperature and feeding regime were associated with differences in the fatty acid composition of *M. galloprovincialis* under controlled experimental conditions. The lipid response of mussels did not appear to reflect temperature or food availability alone. These findings suggest a possible association between the observed fatty acid profiles and the combinations of environmental temperature and feeding intensity. As each combination was represented by a single tank, these findings should be confirmed in experiments with replicated tanks.

The most favourable fatty acid profile was observed at 18°C and 3% dry matter feeding, when higher proportions of PUFA and n-3 fatty acids and lower proportions of SFA were recorded. In contrast, increasing the feeding level did not necessarily improve the nutritional quality of mussel tissue, suggesting that higher food availability does not always lead to a more desirable fatty acid composition.

The fatty acid profile of the microalga *T. lutea* confirmed that the diet used in the experiment was an important source of PUFA, particularly DHA. However, the response of mussel tissue varied among treatments, suggesting that the incorporation and retention of dietary fatty acids may be influenced by temperature and feeding regime.

Overall, the findings highlight the importance of considering both temperature and feeding level when evaluating fatty acid composition in *M. galloprovincialis*. These results may contribute to the optimization of feeding strategies and environmental conditions in mussel aquaculture, with the aim of improving the biochemical and nutritional quality of mussel tissue.

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