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Application of microencapsulated postbiotic in Gilthead Sea Bream (*Sparus aurata*) farming: focus on lipid metabolism

Applicazione di postbiotici microincapsulati nell'allevamento di orata (*Sparus aurata*): focus sul metabolismo dei lipidi

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RIASSUNTO

L'orata, *Sparus aurata*, è la principale specie allevata in Mediterraneo, di conseguenza è soggetta alle conseguenze negative delle pratiche di acquacoltura intensive, come l'insorgenza di frequenti focolai di malattie infettive. L'amministrazione di postbiotici a specie allevate rappresenta una pratica sostenibile e sicura con proprietà ben note per il potenziamento del sistema immunitario. Questo studio ha indagato gli effetti secondari dell'acido butirrico (comunemente usato come postbiotico), incapsulato in una capsula a base di amido, sul metabolismo lipidico di *Sparus aurata*. I giovanili di orata sono stati suddivisi in tre gruppi sperimentali e alimentati per 12 settimane con tre diete sperimentali: un gruppo di controllo (CTRL) alimentati con una dieta commerciale, un gruppo POP alimentati con la stessa dieta commerciale del gruppo di controllo, ma con l'aggiunta di microcapsule vuote, e un gruppo POP+POST alimentati con la stessa dieta commerciale del gruppo di controllo, ma con l'aggiunta di microcapsule contenenti acido butirrico. L'analisi dei parametri di crescita (peso corporeo finale, FCR, SGR e assunzione giornaliera di mangime) non ha rivelato differenze significative tra i pesci dei tre gruppi sperimentali. Le analisi molecolari hanno evidenziato un possibile effetto della capsula a base di amido sul metabolismo lipidico. Al contrario, l'amministrazione di POP+POST non ha alterato le vie molecolari coinvolte nel metabolismo lipidico rispetto al gruppo di controllo.

ABSTRACT

The gilthead seabream, *Sparus aurata*, is one of the main reared species in the Mediterranean region, and therefore it is subjected to the negative consequences of intensive aquaculture practice such as the occurrence of infectious disease outbreaks. The administration of postbiotics to farmed species represented a sustainable and safe practice with well-known immune-busting properties. This study investigated the secondary effects of butyric acid (commonly used as postbiotic), encapsulated in a starch-based capsule, on sea bream lipid metabolism. Juvenile gilthead seabreams were divided into three experimental groups and fed for 12 weeks three experimental diets: a control group (CTRL) of fish fed the commercial diet, a POP group of fish fed the same commercial diet of the control group added with empty microcapsules and a POP+POST group of fish fed the same commercial diet of the control group added with butyric acid microencapsulated.

Analysis of growth parameters (final body weight, FCR, SGR, and daily feed intake) revealed no significant differences among fish from the three experimental groups. Molecular analysis revealed a possible effect of the starch capsule on lipid metabolism. Differently, the administration of POP+POST did not alter the molecular pathways involved in lipid metabolism if compared to the control group.

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1. INTRODUCTION

1.1 Concept of ‘biotics’

In the feed industry, the term "biotics" denotes dietary practices aimed at modifying the composition of gut microbiota to promote the organism’s health (Wegh et al., 2019). Prebiotics, probiotics, and synbiotics are the main components of the “biotics category” playing a crucial role in modulating vertebrates’ gut microbiota, affecting its activity and indirectly influencing the immune response (Figure 1). The beneficial properties of “biotics” are related to their ability to create a favorable intestinal environment by strengthening the intestinal barrier and the immune system (Hill et al., 2014; Kantal et al., 2024). Due to these characteristics, biotics have been used for several years in various sectors, mainly in the nutritional industry for food products and dietary supplements for human consumption (Meybodi and Mortazavian, 2017), and more recently in the animal health sector, both for pets and farm animals (Grześkowiak et al., 2015; Bhogoju and Nahason, 2022).

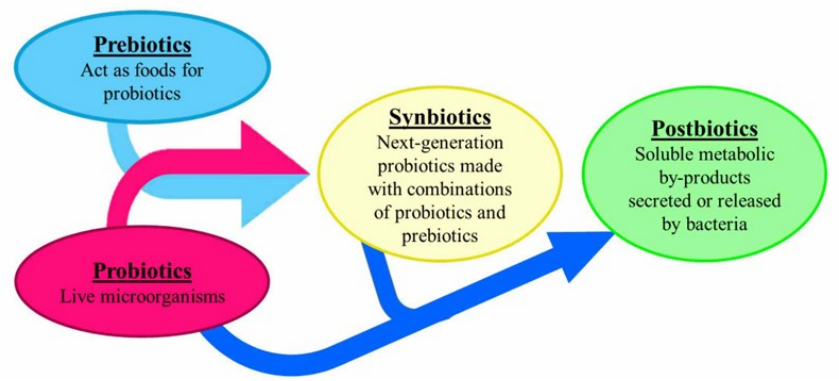

Figure 1. *Definitions of different types of "biotics" (Kantal et al., 2024).*

Probiotics are "live organisms that confer a health benefit to the host when administered in adequate amounts" (Hill et al., 2014).

Prebiotics, differently, can be considered as "substrates selectively utilized by host's microorganisms (usually as nourishment) conferring a health benefit", which can alter and modify the composition of the intestinal microflora (Gibson et al., 2017; Wegh et al., 2019). Prebiotics can nourish the intestinal microbiota, and their breakdown products (such as short-chain fatty acids like lactic acid, butyric acid, and propionic acid) are released into the bloodstream reaching other organs where they can exert their effects (Davani-Davari et al., 2019). Synbiotics are "a mixture of live microorganisms and substrates selectively used by host organisms", essentially, they are a combination of prebiotics and probiotics (Kantal et al., 2024, Wegh et al., 2019).

The potential therapeutic role of probiotics and prebiotics has been demonstrated by different clinical studies that observed their function in treating several disorders such as antibiotic-associated diarrhea (Can et al., 2006), irritable bowel syndrome (Brenner et al., 2009), Crohn's disease (Schultz et al., 2004), and in the treatment of infections like *Clostridium difficile* (Hickson et al., 2007) and *Helicobacter pylori* (Cremonini et al., 2002).

Recently, a new class of “biotic” compounds has been discovered consisting of bioactive compounds produced by beneficial microorganisms during natural fermentation processes defined as postbiotics (Kantal et al., 2024).

1.2 Postbiotics

The word “postbiotic” derives from the Greek words "post" (after) and “bios" (life), it was first introduced in 2012 to define preparations of inactive microorganisms or their components that exert beneficial effects once in the gut environment of the host (Kantal et al., 2024; Tsilingiri et al., 2012). The International Scientific Association of Probiotics and Prebiotics (ISAPP) has officially defined a postbiotic as “a preparation

of inanimate microorganisms and/or their components that confers a health benefit to the host" (Salminen et al., 2021).

Postbiotics include enzymes, proteins, peptides, amino acids, fatty acids, vitamins and, other organic compounds produced by specific microorganisms (Nataraj et al., 2020; Ang et al., 2020; Abdel-Latif et al., 2022). They were observed to exhibit a range of diverse effects, including immunomodulatory, anti-inflammatory, and antioxidant properties (Zółkiewicz et al., 2020) (Figure 2). Among the numerous potential impacts of postbiotics, it was observed, for example, that postbiotics derived from *Streptococcus thermophilus* showed an increase in lymphocyte (T-helper1 cells) response in mice mesenteric lymph nodes (Ménard et al., 2005). Apoptosis events, induced in cancerous gastric cells exposed to propionate produced by *Propionibacterium freudenreichii*, were also observed to reduce the inflammation linked to carcinogenesis (Cousin et al., 2012). Finally, postbiotics have been shown to induce antioxidant responses in post-weaning lambs after the supplementation of cell-free supernatant collected from *Lactobacillus plantarum* resulting in a reduction of lipid peroxidation in the serum (Izuddin et al., 2020).

At date, the use of postbiotics is expanding thanks to their advantages with respect to probiotics since they do not require the viability of microorganisms, making them easier to use on a larger scale (Tao et al., 2024). Moreover, these compounds reduce the risk of complications such as bacteraemia (the presence of bacteria in the bloodstream) and probiotic translocation that lead to the migration of viable microbes from the gastrointestinal tract to other organs (Holmes et al., 2021; Salminen et al., 2021; Kothari et al., 2019). This phenomenon can occur when the intestinal barrier is weakened, potentially leading to clinical infections (Kothari et al., 2019). Additionally, a collateral risk exists in transferring resistance genes during translocation events with further complications in health outcomes (Holmes et al., 2021; Salminen et al., 2021).

Postbiotics can be composed of one or multiple substances and they exhibit complex interactions with the host. One postbiotic can have multiple effects, depending on different technological factors such as the identification of the microorganism, the inactivation technique (since different processes can lead to variations in postbiotic composition and effects), and the description and quantification of the final postbiotic composition (Salminen et al., 2021) and different postbiotics may have overlapping functionalities (Tao et al., 2024).

1.2.1 Main categories of postbiotics

All microorganisms selected that synthesize postbiotic compounds have been established as safe for the host through rigorous safety evaluations encompassing both bacterial and fungal strains. Notable bacterial genera included in the postbiotic category are: *Lactobacillus* spp. (e.g., *L. plantarum, L. fermentum, L. helveticus, L. casei, L. rhamnosus, L. brevis, L. xylosus*), *Bifidobacterium* spp. (e.g., *B. bifidum, B. animalis*), *Enterococcus* spp. (e.g., *E. faecalis, E. faecium*), *Bacillus* spp. (e.g., *B. coagulans, B. subtilis*), and *Pseudomonas* spp. (e.g., *P. stutzeri, P. putida*), while yeasts are the primary contributors from the fungal kingdom (Tao et al., 2024).

Postbiotics, deriving from the above-mentioned strains, can be divided into three main categories: inactivated microbial cells, bacterial cell components, and crude by-products generated from microbial metabolism.

Inactivated microbial cells

Microbial cells are inactivated, through specific methods, while maintaining their structural integrity (Tao et al., 2024). They are also called ghosts/inactivated probiotics or, preferably, paraprobiotics

(Aguilar-Toalá et al., 2018). Studies conducted on different experimental models have shown that inactivated microbial cells retain certain biological properties similar to those of viable cells, such as scavenging oxygen radicals, reducing inflammatory markers, and modulating host physiology (Tao et al., 2024; Aggarwall et al., 2022). In this regard, Chung et al. (2019) demonstrated that heat-killed *Enterococcus faecalis* cells exhibit anti-inflammatory effects, including the ability to suppress inflammatory markers such as IL-6 and TNF-α, while also enhancing the production of anti-inflammatory cytokines. Similarly, Jang et colleagues (2018) showed that heat-killed probiotic strains of *Lactobacillus* have anti-oxidative effects, specifically the ability to scavenge free radicals, in HT-29 cells (human colon adenocarcinoma).

Bacterial cell components

The components of a bacterial cell that belong to this category are pili proteins, peptidoglycan, teichoic acids, and cell surface proteins (Tao et al., 2024).

Pili are located on the surface of certain bacteria and form long fibrous filamentous structures principally composed of proteins. They play a key role in cell adhesion, migration and proliferation for wound healing

(Wang et al., 2024). Pili proteins exert their function as postbiotics cooperating with host cells to modulate inflammatory and immune responses (Wang et al., 2024).

Peptidoglycan is the fundamental component of the bacterial cell wall, in both Gram-positive and Gram-negative bacteria (Wang et al., 2024). Fragments of peptidoglycan that are released by the action of endogenous peptidoglycan hydrolases contribute to the modulation of the host's immune response (Aggarwall et al., 2022). Peptidoglycan extracted from probiotic bacteria has demonstrated anti-cancer effects both *in vitro* and *in vivo (*Wang et al., 2018; Aintablian et al., 2017). It also showed an immunomodulatory activity, enhancing the immune response in the lungs and respiratory tract by increasing the production of both pro-inflammatory and anti-inflammatory cytokines in mice (Clua et al., 2017; Li X. et al., 2017). Additionally, it exhibited anti-inflammatory effects in a colitis mouse model (Shida et al., 2009).

Furthermore, it has been observed that also peptidoglycan hydrolases (derived from *Lactobacillus rhamnosus*) may have a beneficial effect as postbiotics since they were observed to participate in maintaining the integrity of gut lining and suppress programmed cell death in mice (Yan et al., 2006).

Teichoic acid is an important component of the Gram-positive bacterial cell wall, they are grouped into two types: (i) wall teichoic acid and (ii) lipoteichoic acids based on their different anchoring to peptidoglycan and cell membranes respectively (Tao et al., 2024; Aggarwall et al., 2022). Specifically, wall teichoic acid is important for preserving the cell shape, regulating bacterial colonization and adhesion, and influencing resistance to antimicrobial peptides. Lipoteichoic acid is crucial for maintaining cation balance in the cell wall, reducing inflammation, and facilitating recognition by host cell receptors (Tao et al., 2024). Several studies (Claes et al., 2012; Ahn et al., 2019; Shiraishi et al., 2016) have shown that *Lactobacillus's* lipoteichoic acid (LTA) possesses anti-inflammatory properties, as seen in a study conducted by Pradhan et al. (2023), who demonstrated that *in vivo* mouse models, the use of *Lactobacillus* LTA reduced colitis-related symptoms and other inflammatory markers such as gut permeability, myeloperoxidase activity, an enzyme produced by neutrophils involved in the immune response, and histopathological damages in colon.

Finally, bacteria cell surface proteins, such as surface (S) layer protein, mucus binding protein, fibronectin-binding protein and collagen-binding protein serve as the primary point of contact, during interactions with the

host, activating a range of cellular processes (signal transduction pathways) in the intestinal cell (Nataraj et al, 2020; Sudhakaran et al., 2022). These proteins, when isolated and administered as postbiotics, promote the adhesion of beneficial bacteria of the gut microbiota to the intestine and modulate the immune response, preventing damage caused by pathogens. A study demonstrated how surface proteins extracted from *Enterococcus faecium* reduced apoptosis induced by *Listeria monocytogenes* in Caco-2 cells, a human colon carcinoma cell line (Y. He et al., 2018). Gao K. and colleagues (2017) showed how surface proteins of *Lactobacillus rhamnosus* reduced inflammatory cytokines and stimulated the expression of Toll-Like receptors in an *in vitro* model using porcine intestinal epithelial cells.

Microbial metabolites

Bacteria produce small molecular weight metabolites that are crucial for regulating their growth, development, and reproduction (Aguilar-Toalá et al., 2018). These metabolites (Figure 2) encompass a variety of substances including lipids, in particular short-chain fatty acids (butyrate, propionate and dimethyl acetyl-derived plasmalogen), proteins (lactocepin and p40 molecule), carbohydrates (galactose-rich

polysaccharides, teichoic acids and exopolysaccharides), vitamins/co-factors (B-group vitamins, vitamin K), organic acids (propionic and 3-phenyllactic acid), and complexes molecules (peptidoglycan-derived muropeptides and lipoteichoic acids) (Aguilar-Toalá et al., 2018).

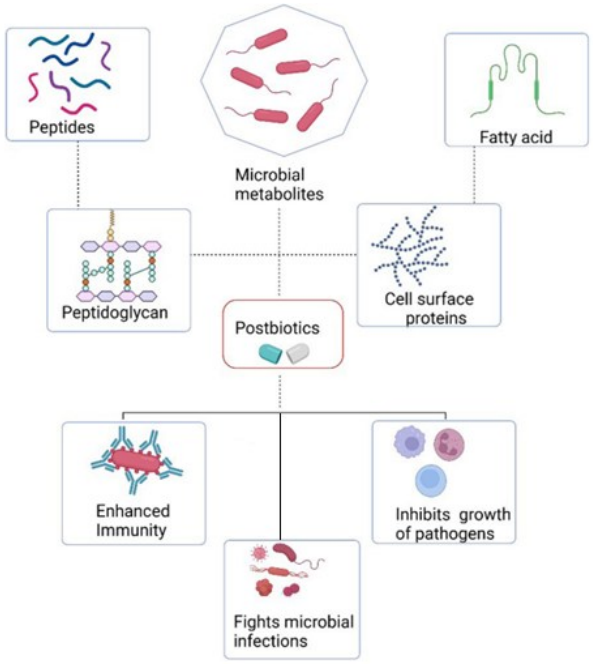

Figure 2. *Different postbiotics produced by a microorganism (Sudhakaran et al., 2022 - modified).*

These compounds may be secreted by living bacteria or released after bacterial lysis within the host gut, providing additional physiological benefits such as cell-to-cell communication and helping to protect the microflora stability against stress factors (Aguilar-Toalá et al., 2018). For example, some bacteria strains (particularly those from the *Lactobacillus* and *Bacillus* genera) synthesize bacteriocins, a term that includes proteins, peptides, and their precursor forms (Khochamit et al., 2015;

Nataraj et al., 2020). These compounds specifically contribute to the inhibition of pathogenic bacteria and regulate immune functions (Tao et al., 2024).

1.2.2 Isolation and production of postbiotics

Actual methodologies employed in postbiotics production include operations in both laboratory and industrial settings. Factors such as the fermentation medium, bacterial growth, harvesting, concentration methods and preservation are all key elements in postbiotics production (Sudhakaran et al., 2022). After the selection of the target bacteria strain (based on its established health benefits or its ability to produce specific beneficial metabolites) the production process normally follows different specific stages (Figure 3):

- Fermentation and bacterial propagation: selected strains are cultured in a specific growth medium within a controlled environment represented by a laboratory setting or a fermentation tank.
- Harvesting of the bacteria: the bacteria are collected once they have achieved the ideal growth density. This can be achieved through centrifugation or filtration.

- Inactivation and cell disruption: the bacteria are inactivated through different methods such as heat treatment, irradiation, or chemical treatment (Kantal et al., 2024). Successively, common techniques like heat and enzymatic treatments, solvent extraction, and sonication are used to extract postbiotic compounds from the bacterial cells. This extraction process is usually followed by further purification and cleanup steps, which may include techniques like centrifugation, dialysis, freeze-drying, and column purification (Kantal et al., 2024).

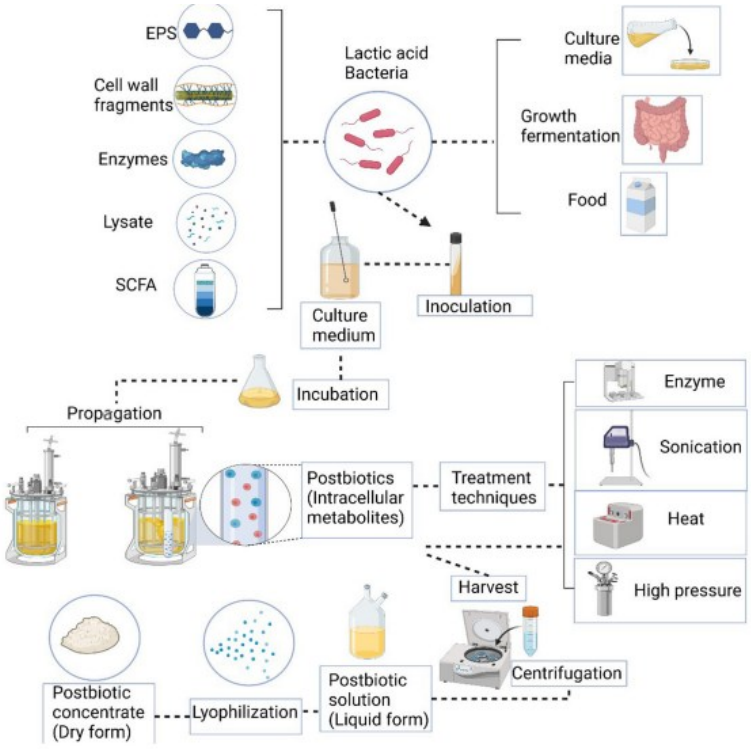

Figure 3. *Example of the production and isolation process of postbiotics from lactic acid bacteria (Sudhkaran et al., 2022).*

At the end of the extraction procedures, isolated postbiotics are characterized to identify their composition and potential health benefits, with different techniques depending on the specific objectives and type of characterization (qualitative or quantitative) (Kantal et al., 2024). Common methods for this purpose include chromatography coupled with tandem mass spectrometry, Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, and direct infusion techniques. Ultra-performance liquid chromatography (UPLC) is particularly recommended due to its high efficiency, resolution, low solvent consumption, and excellent sensitivity and precision (Kantal et al., 2024; Sudhakaran et al., 2022).

1.3 Application of postbiotics in aquaculture

At present, intensive aquaculture practices are the predominant form of fish farming at global scale thanks to their high biomass production and a cost-to-profit ratio that favors the latter. Unfortunately, these farming methods have increased the possibility of infectious disease outcomes, resulting in the massive use of antibiotics to prevent significant biomass loss, both in marine and land-based farms (Assefa and Abunna, 2018). One of the direct consequences of antibiotic overuse on fish health is the

phenomenon of antibiotic resistance, which is particularly detrimental, it also affects the organisms living in the surrounding environment, thus compromising biodiversity (Braña et al., 2021; Martinez-Porchas and Martinez-Cordova, 2012). Furthermore, the massive use of antibiotics indirectly concerns the consumers' health, due to the ingestion of fish with a high amount of antibiotics in their organism (Milijasevic et al., 2024).

This situation necessitates the development of alternative sustainable solutions to maintain high-rate productivity and at the same time ensure the health of farmed species. In recent decades, bacterial-derived compounds such as prebiotics, probiotics and postbiotics have emerged as a promising approach to address this issue. Promoting the use of these feed additives in fish would enhance their growth performance and feed efficiency, ultimately leading to increased fish production and contributing to the protection of human health (El-Saadony et al., 2021).

Probiotics in aquaculture are being administered, more and more frequently, in alternative to the use of antibiotics to improve the health of the organisms and the quality of the water, while preventing infectious outbreaks while the use of postbiotics is still in its early stages (Chauhan et al., 2018; Tao et al., 2024). The majority of probiotics commercialized

are lactic acid bacteria, bifidobacteria, *Bacillus* spp., and yeasts (Todorov et al., 2024). The main species treated with probiotics are *Oncorhynchus mykiss* (Panigrahi et al., 2010), *Sparus aurata* (Salinas et al., 2008), *Cyprinus carpio* (Wang et al., 2021) and *Dicentrarchus labrax* (Frouël et al., 2007). Concerning postbiotics' potential, it has been demonstrated their properties as growth enhancers, stress reducers, agents for mitigating viral diseases, and promoters of animal health in shrimps (Wang et al., 2023), bullfrogs (Tao et al., 2023) and fish (Yu et al., 2023; Jafarzadeh et al., 2024). For instance, a study by Li and colleagues (2023) demonstrated that the administration of supernatant postbiotic of *Cetobacterium somerae* and *Lactococcus lactis*, for over six weeks, increased the expression of genes related to lipid metabolism in *Danio rerio*, enhancing microbiota balance and correcting lipid metabolic disorders. Furthermore, diet administration of postbiotics, derived from *Bacillus*, *Lactobacillus*, and yeast strains such as *Saccharomyces*, have been observed to strengthen gut defense increasing mucosal thickness and elongating microvilli as shown in a study conducted by Wang et al. (2023) on juvenile specimens of *Macrobrachium nipponense*. Postbiotics, by supporting gut health, help enhance the absorption of essential nutrients, thereby promoting overall growth and development

(Tao et al., 2024). This connection between gut health and growth is further supported by scientific findings, such as the study by Wang et al. (2023), which demonstrated that the improvement in growth performance observed in *Macrobrachium nipponense* was significantly linked to enhanced gut health, resulting in more efficient nutrient absorption.

In addition to their role in gut health, several studies have highlighted the potential of postbiotics to enhance the overall immune status of aquatic animals by strengthening both innate and adaptive immune responses. As an example, a study demonstrated that feeding shrimp (*Panaeus vannamei*) with *Pediococcus pentosaceus* postbiotics continuously for 12 weeks significantly enhanced the lysozyme levels and phagocytic activity, thereby boosting their innate immune response (Ballantyne et al. 2023). Furthermore, the study conducted by Sun et al. (2014) showed how *Psychrobacter sp.* postbiotics provided for 60 days led to a significant increase in the expression of IgM and Epinecidin-1, an antimicrobial peptide, in grouper (*Epinephelus coioides*).

Beyond their role in promoting gut health and modulating immune responses, postbiotics can significantly have preventive effects on pathogenic infections and improve disease resistance in aquatic species

(Tao et al., 2024; Chadravanshi et al., 2024). A study found that adding *Clostridium butyricum* postbiotics to the diet of shrimp (*Panaeus vannamei*) can boost their resistance to *Vibrio parahaemolyticus* infections (Luo et al., 2021). Additionally, incorporating *Bacillus cereus* postbiotics into the diet has been shown to decrease cumulative mortality in *Litopenaeus vannamei* during challenge tests with *Vibrio parahaemolyticus*, compared to the control group (Li et al., 2021).

Lastly, another significant benefit linked to postbiotics administration is related to the acquisition of environmental adaptability. Research indicates that adding postbiotics to the diets of aquatic species can reduce stress effects induced by different environmental factors, such as high stocking density, hypoxia, and decreased salinity (Danwood et al., 2016). This property has been observed in a 56-day study on *Pangrus major* fed a diet implemented with *Lactobacillus plantarum*-derived postbiotics. After the postbiotics administration fish showed an enhanced tolerance to low salinity and increased anti-stress capabilities (Dawood et al., 2015).

1.4 Postbiotics encapsulation

Encapsulation refers to the process of compartmentalizing a bioactive compound (the active agent) by isolating it from its surrounding environment. This process involves enclosing or coating the compound with a carrier material (often called wall materials or encapsulants) to form capsules or microcapsules, typically at the micro- or nanometer scale (Bamidele and Emmambux, 2021; Dezfooli et al., 2018). Postbiotics can be encapsulated to preserve their structural and functional integrity during storage (Bamidele and Emmambux, 2021).

Moreover, microencapsulation appears as a more effective method to deliver postbiotic compounds specifically to target organs. Microencapsulation ensures better protection of the encapsulated against environmental factors and degradation, offering a more controlled release (Dezfooli et al., 2018). The use of different materials with selective properties (such as those that dissolve at specific pH levels or temperatures) has enabled the design of systems that target specific sites within the organism. Encapsulation ensures the controlled and prolonged release of molecules in fish digestive tract (Dezfooli et al., 2018; Cattaneo et al., 2024).

Specifically considering the use of postbiotics in aquaculture practices, selecting the appropriate encapsulation material is crucial to ensure that microcapsules do not dissolve in water before ingestion or degrade in the stomach's acidic environment before reaching the target site. Choosing the most suitable material should consider the target species' physiology, its living environment, and practical commercial considerations, including farming conditions (such as pH, temperature, and salinity) that can affect the stability and release of microcapsules (Dezfooli et al., 2018). Additionally, the feeding behavior of the target species should be considered: sinking particles are more suitable for sessile organisms like shellfish while floating particles are more effective for fish.

The majority of encapsulation wall materials are biopolymers, which can form a matrix with bioactive compounds through molecular bonds. Common encapsulants include: (i) carbohydrates, which are readily found in plants, such as cellulose, gum, alginate, chitosan, and maltodextrin (Bamidele and Emmambux, 2021).

(ii) proteins, which offer good functional properties and are found in both plants and animals, such as collagen, gelatine, and whey protein.

(iii) lipids, which can be classified as polar and non-polar. Like proteins, lipids exhibit strong functional capacities, they are particularly effective

for stabilizing, protecting, and ensuring the controlled release of bioactive compounds at their active sites (Bamidele and Emmambux, 2021).

1.5 Gilthead seabream *(Sparus aurata)*

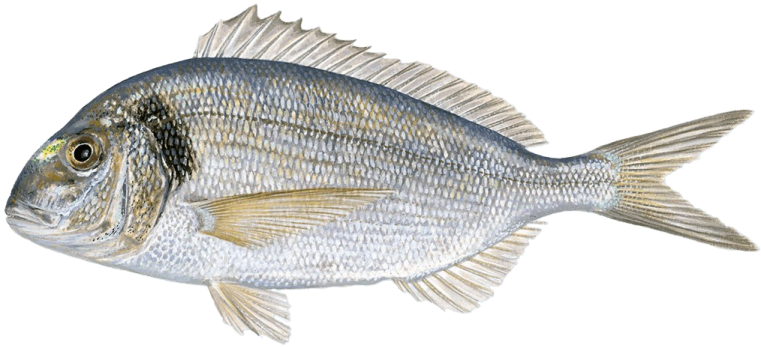

Figure 4. *Sparus aurata* (Linnaeus, 1758).

The gilthead seabream (*Sparus aurata*) (Figure 4) is a marine species that belongs to the superclass Actinopterygii, class Osteichthyes, and order Perciformes (Mhalhel et al., 2023). The gilthead seabream is characterized by a silvery-grey, leaf-shaped body. Its distinctive features are the prominent black spot located at the origin of the lateral line, extending along the upper margin of the opercle, the gently curved head profile and the golden band on the forehead between the two small eyes (Mhalhel et al., 2023). The species' name derives from this last feature, and it comes from the Latin term "auratus" which means golden.

The gilthead seabream is a eurythermal and euryhaline species able to tolerate a wide range of temperatures and salinity levels (Mhalhel et al., 2023). It is an omnivorous opportunistic feeder which adjusts its feeding habits based on the availability of food (Mhalhel et al., 2023). Its diet consists of bivalves and gastropods, but also includes polychaetes, echinoderms, teleosts and occasionally ascidians, algae, and bryozoans, (Sola et al., 2007; Mhalhel et al., 2023).

The seabream exhibits protandrous hermaphroditism. During the first two years of its life, it's a functional male. After reaching a length of more than 30 cm, it undergoes a sex change and becomes female. Females are batch spawners that can lay 20.000-80.000 eggs per day during the reproductive season (Sola et al., 2007; Mhalhel et al., 2023).

1.5.1 Gilthead seabream farming in the Mediterranean

Over the years, marine aquaculture in the Mediterranean basin has evolved significantly. In the early 1980s, intensive gilthead seabream farming was introduced in the Mediterranean, utilizing marine cages or tanks and recirculating inland aquaculture systems (Mhalhel et al., 2023). Nowadays, almost the entire production consists of intensive farming while extensive practices still exist as a traditional activity in

some regions, but with a minimal impact on the market (Sola et al., 2007). At date, gilthead seabream is the main reared species in the Mediterranean region, with an annual production reaching 281.914 tonnes between 2020-2021 (34.1% of the total production) (FAO, 2023). Unfortunately, this consistent production is often accompanied by the more frequent occurrence of infectious disease outbreaks. In this context, the use of postbiotics could offer a promising solution to prevent these pathological situations by strengthening the immune system and different vital metabolic functions including lipid metabolism which plays a key role in enhancing the health and resilience of the organism (Ang et al., 2020).

1.6 Lipid metabolism in fish

Lipids are a highly efficient source of energy, providing twice as many calories per gram compared to carbohydrates due to their high-energy bonds (Birsoy et al., 2013). Lipids are also essential building blocks for membrane biosynthesis, precursors for the synthesis of other cellular products, and molecules involved in intracellular signaling (Birsoy et al., 2013).

Teleost fish store lipids primarily as triglycerides in various anatomical compartments, including visceral organs, liver, subcutaneous tissue, and muscles (both red and white) (Weil et al., 2013). The specific sites of lipid accumulation vary depending on factors such as species, nutritional condition, life stage, and physiological state (Weil et al., 2013; Salmerón, 2018).

Dietary lipids are a crucial source of essential fatty acids, particularly polyunsaturated fatty acids (PUFAs) and highly unsaturated fatty acids (HUFAs), which are vital for health, reproduction, optimal growth, and other processes in teleost fish (Ayisi et al., 2017).

Marine fish, differently from freshwater species, have a limited capacity to biosynthesize long chain (LC)-PUFAs starting from C18 precursors (linoleic and α-linolenic acid) due to a scarce enzymatic activity involved in this metabolic pathway (Monroing et al., 2011). In the marine environment, rich in LC-PUFAs produced by microalgae, fish assimilate them through diet making these bioconversion pathways not necessary (Xu et al., 2020). The limited ability to biosynthesize these macromolecules in marine farmed species could be particularly problematic during the early developmental stages, when the physiological demand for LC-PUFA, especially docosahexaenoic acid

(DHA), is high due to the rapid growth and maturation of neural tissues (Monroing et al., 2011), although they play important physiological roles throughout the entire life cycle.

The main site where lipids are synthesized is the liver, where the activity of lipogenic enzymes is significantly higher compared to the adipose tissue (Ayisi et al., 2017).

The primary carbon source for the synthesis of new lipids is acetyl-CoA, which is produced in mitochondria through the oxidative decarboxylation of pyruvate or the breakdown of some amino acids (Tocher, D. R., 2003). Although acetyl-CoA is generated in mitochondria, the key site for the synthesis of fatty acids is the cytosol (Figure 5) (Ayisi et al., 2017).

One important multienzyme complex is the cytosolic fatty acid synthetase (FAS), which catalyzes the synthesis of palmitate (C16:0). All known organisms, including fish, can biosynthesize *de novo* this compound (Tocher, D.R., 2003).

For the biosynthesis of 16:0 fatty acid, eight two-carbon acetyl units are required, with one provided by acetyl-CoA as a primer. The remaining seven acetyl units are first converted into malonyl-CoA through carboxylation by acetyl-CoA carboxylase, and then they are combined

through the fatty acid synthesis process in a series of condensation reactions that require NADPH (Tocher, D.R., 2003; Ayisi et al. 2017).

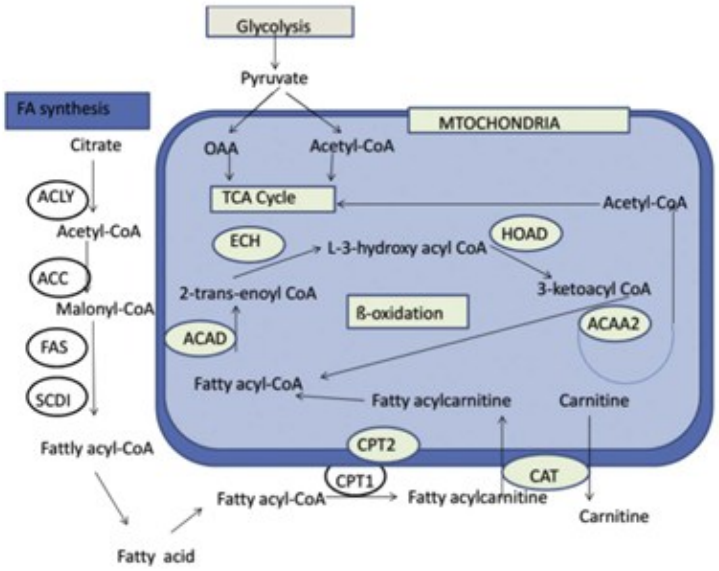

Figure 5. *Lipogenesis pathways in fish (Ayisi et al., 2017).*

Another important process is the mobilization of lipids stored in cellular lipid droplets across various storage tissues, which involves the breakdown of triglycerides to release energy. This process involves different lipase enzymes and starts with the transport of triglycerides mediated by very low-density lipoproteins (VLDL), which act as the primary carriers in the blood (Ayisi et al., 2017). Once triglycerides are broken down by lipase enzymes into fatty acids, these bind to albumin to be transported to muscles and adipose tissues. The formation of the albumin-fatty acid complex facilitates the entry of the fatty acids into the

mitochondria where β-oxidation will occur to generate energy (Ayisi et al., 2017).

β-oxidation consists of four cyclic reactions, in which the starting fatty acid is shortened by two carbon atoms. These two carbon atoms are converted into acetyl-CoA, which then enters the Krebs cycle (Ayisi et al., 2017). This process occurs in two different organelles, mitochondria and peroxisomes, involving different enzymes and mechanisms. The peroxisome proliferator-activated receptors (*ppars*), particularly *pparα* and *pparβ*, are key regulatory receptors that modulate this pathway by activating the genes responsible for encoding proteins involved in the metabolic process (Ayisi et al., 2017).

A significant issue in aquaculture is the use of high-fat diets that can compromise normal liver function. Indeed, these diets usually lead to abnormal lipid accumulation in the liver (steatosis), causing inflammation and oxidative stress (Meng et al., 2023). Recent studies demonstrated the beneficial effects of postbiotics supplementation in farming diets on liver health.

Meng et al. (2023) for example, observed a significantly lower hepatic lipid content and lower structural damages in the liver of common carp (*Cyprinus carpio*) fed high-fat diets supplemented by increasing

concentrations of postbiotic compared to fish fed the same high-fat diet not implemented with the postbiotic obtained from the culture medium of various bacteria.

Similarly, Li et al. (2023) observed that liver steatosis was reduced in zebrafish (*Danio rerio*) fed postbiotic-supplemented diets compared to a control group. The postbiotic significantly decreased liver TAG content. Additionally, these diets increased the expression of genes associated with the fat breakdown (*pparα*, *cpt1*, *ucp2*), enhancing the body's ability to utilize fats and decreasing the expression of genes involved in fat synthesis (*pparγ*, *cebpα*, *dgat-2*, *acc1*) suggesting a reduction in fat production and accumulation.

2 AIM OF STUDY

Sparus aurata, commonly known as gilthead seabream, is one of the key species in Mediterranean aquaculture exerting a high impact on economic and social aspects of many coastal countries. Although intensive farming represents the only effective solution to meet the increasing market demand, it is often challenged by the negative consequences of high-density farming practices such as disease outbreaks. At present, the use of antibiotics to defeat animal diseases is unsustainable due to the well-known association with antibiotic resistance phenomena.

In this regard, different strategies have been applied to prevent the worsening of farming conditions while simultaneously strengthening the health of the farmed fish. Postbiotics are the most recent products that offer promising solutions to regulate the immune function, enhance animals' health to reduce the probability of disease outbreaks.

The present study used butyrate as a postbiotic compound encapsulated in a starch capsule to test the effects of postbiotic administration on lipid metabolism-related pathways in *Sparus aurata.* The possible side effects of the starch capsule were also investigated.

3. MATERIALS AND METHODS

3.1 Ethics

The experimental trial, performed at the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Center for Marine Research (Heraklion, Greece), was authorized by the ethics committee of the region of Crete, Greece (license No EL91-BIObr-03) and conducted following the guidelines established by the EU Directive 2010/63/EU.

3.2 Postbiotic encapsulation

Butyrate, used in the present study as a postbiotic compound, was microencapsulated with a capsule (POP) composed of Arabic gum (55%), starch (22%), and a lower percentage of cellulose, sodium ascorbate, and Vitamin E. The encapsulation process was performed by STM Aquatrade S.r.l. (Castel Raimondo, Macerata, Italy) through a new technology called Co.M.E. (Coating Made Easy) within the +POP (Powder on Pellets) concept, a private project of STM Aquatrade S.r.l. according to Zarantoniello et al. (2024). Due to intellectual property protection, the specifics of the microcapsule preparation process cannot be fully disclosed.

The resulting complex and butyrate and capsule (POP+POST) contained 0.1 g per kg of feed.

3.3 Experimental diets preparation

Experimental diets were obtained from the same commercial diet (provided by Zoonomi S.A., Greece) used as the control diet (CTRL). The proximate composition of this commercial diet is reported in Table 1. The other two experimental diets were obtained by adding to the control diet the POP capsule (5 g per kg of feed) and the complex POP+POST (5 g per kg of feed) respectively.

Proximate composition (%)	
Crude protein[1]	45%
Crude fat[2]	17%
Crude ash	10%
Crude fiber	1.9%
Ca	1.2%
P	1.2%
Na	0.5%
Antioxidants	
BHA	30 ppm
BHT	45 ppm
Additives	
Vit A	11.250 UI/Kg
Vit D3	1.500 UI/Kg
Vit E	290 mg/Kg
Fe ($FeSO_4$)	150 ppm
Fe	99 ppm
Zn (ZnO)	37 ppm
Zn	18 ppm
$MnSO_4$ H_2O	38 ppm

Mn	9 ppm
$CuSO_4$ $5H_2O$	1.5 ppm
Cu	4.5 ppm
I [$Ca(IO_3)_2$] (Calcium iodate)	0.75 ppm
$C_5H_{11}NO_2Se$ (L-Selenomethionine)	0.3 ppm
Preservatives	
Citric acid	30 ppm

Table1. *Proximate composition (%) and ingredients of the CTRL diets. [1]Fish meal, wheat flour, sunflower meal, fermented soybean meal, corn gluten. [2]Fish oil, soybean oil.*

3.4 Experimental design

Juvenile gilthead sea breams were obtained from the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) hatchery of the Hellenic Centre for Marine Research (Heraklion, Greece).

After a period of acclimatation, 225 seabream juveniles were individually weighed (54.07 ± 0.16 g) and randomly distributed (25 fish per tank) in 9 open circulation indoor tanks (3 tanks per experimental group, 500 L each) according to three experimental groups: a control group (CTRL) of fish fed the commercial diet, a POP group of fish fed the same commercial diet of the control group added with postbiotic microencapsulated and a POP+POST group of fish fed the same commercial diet of the control group added with empty microcapsules.

The duration of the experimental trial was three months, during which fish were hand-fed until apparent satiation, two times a day, 6 days per

week. During the whole experimental trial, the environmental parameters were daily monitored and kept at optimal values.

3.5 Sampling

At the end of the feeding trial, fish were fasted 24 h prior to organs sampling to reduce handling stress and ensure an empty gastrointestinal tract. All the fish were anesthetized with a lethal dose of anaesthetic (phenoxyethanol, 500 ppm) and individually weighed. In addition, the liver was also weighed before collecting individual samples of liver and intestine from a subsample of fish (5 fish per tank, 15 per experimental group) and properly stored in a solution of RNAprotect tissue reagent (Qiagen) at -20 °C to perform further molecular analyses.

3.6 Growth parameters and Hepatosomatic index

The total biomass of each tank has been calculated at the beginning and the end of the experimental trial.

- The feed conversion ratio (FCR) was calculated using the following formula:

Total feed given (g)/WG,

where WG is the weight gain (g) obtained by subtracting the final body weight (g) from the initial body weight (g)

- Specific growth rate (SGR, %/day) was calculated through the following equation:

100(ln FBW – ln IBW)/days fed*,

where lnFBW is the natural logarithm of final body weight, lnIBW is the natural logarithm of initial body weight.

- Daily feed intake (% body weight/day) was obtained using the equation:

100(total feed given (g)*days fed)/((IBW + FBW) * 0.5),*

where IBW and FBW are the initial and final body weight respectively.

Finally, the hepatosomatic index (HSI) was calculated with the formula:

*LW/iFBW*100*,

where LW is the individual liver weight (g), iFBW is the final individual body weight (g).

3.7 Molecular analyses

3.7.1 RNA extraction and cDNA synthesis

Total RNA was extracted from liver and intestine tissues using RNAzol RT reagent (Sigma-Aldrich, St. Louis, MO, USA). The extracted total

RNA was diluted in 20 µL of RNAse-free water (Qiagen). Final RNA concentration was quantified with a NanoPhotometer P Class spectrophotometer (Implen, München, Germany) and RNA integrity was verified by GelRed™ staining by highlighting 28S and 18S ribosomal RNA bands on 1% agarose gels. RNA was stored at -80 °C until use.

After RNA extraction and before the retrotrascriptase in cDNA, 1 µg of total RNA underwent DNase I treatment (Sigma-Aldrich) to digest and remove residual genomic DNA and was reverse transcribed into cDNA using the iScript ™ cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). The resulting cDNA was diluted to 1:10 and stored at -20 ◦C.

3.7.2 Real-Time PCR

PCR analyses were performed with the SYBR green method in an iQ5 iCycler (Bio-Rad). Reactions were set on a 96-well plate mixing, for each sample, 1 µL cDNA diluted 1:10, 9 µL of 2× concentrated iQ ™ Sybr Green (Bio-Rad) as fluorescent intercalating agent, 0.2 µL of forward primer and 0.2 µL of reverse primer. The thermal profile for all reactions was 3 min at 95 °C and then 45 cycles of 20 s at 95 °C, 20 s at the specific annealing temperature of each primer, and 20 s at 72 °C. At the end of each cycle, fluorescence was monitored, and one single peak

was detected in all cases in the melting curve analyses. Relative quantification of the expression of genes involved in liver lipid metabolism was performed: acyl-CoA oxidase 1 (*acox1*), fatty acid binding protein 1 (*fabp1*), fatty acid synthase (*fasn*), lipoprotein lipase a (*lpl*), lipase hepatic a (*HL*), peroxisome proliferator-activated receptor alpha (*pparα)*, peroxisome proliferator-activated receptor beta (*pparβ)*, peroxisome proliferator-activated receptor gamma (*pparγ),* phospholipase A2 (*pla2*) and sterol regulatory element binding transcription factor 1 (*srebf1*).

Elongation factor 1-alpha (*EF1-alpha*) and L27 ribosomal protein (*rpl27*) were used as internal standards in each sample to standardize the results by eliminating variation in mRNA and cDNA quantity and quality. Amplification products were sequenced, and homology was verified, while no amplification products were observed in negative controls and no primer-dimer formations were observed in control templates. Data obtained were analyzed using the iQ5 optical system software version 2.0 (Bio-Rad) including GeneEx Macro iQ5 conversion and GeneEx Macro iQ5 files. Primer sequences used in the present study are reported in Table 2. Primers were used at a final concentration of 10 pmol/ μL.

ID genes	Forward primer (5'-3')	Reverse primer (5'-3')
ef1alpha	AGTCCACCTCCACCGGTCAT	AGGAGCCCTTGCCCATCTC
rpl27	AAGAGGAACACAACTCACTGCCCCA	GCTTGCCTTTGCCCAGAACTTTGTAG
HL	TTGTAGAAGGTGAGGAAAACTG	GCTCTCCATCAGACCATCC
fabp1	GTCCTCGTCAACACCTTCACCAT	CGCCTTCATCTTCTCGCCAGT
pparα	GCAGCCTGTGAGTCTTGTGAGTGA	CTCCATCAGGTCTCCACACAGC
pparβ	CGTGTTCGGGATTCGGGACT	CACCCTGTCGTGCTGCTCTGTA
pparγ	GACATGCAAACACCAAGCAGA	TGGAGGTGTAGTCCAACGTG
srebf1	CACCTGGGATCTGACGCTTT	CGGACTGGGTCAAACAAGGA
acox1	GGCCAACCAAGAGCAAATGG	CCCGGATGGGGACAATGAAA
lpl	GAGCACGCAGACAACCAGAA	GGG GTAGATGTCGATGTCGC
pla2	CCAGACCATCTTCACCATCC	CACCCAATCCACAGGAGTTC
fasn	TGGCAGCATACACACAGACC	CACACAGGGCTTCAGTTTCA

Table 2. Primers sequences and genes ID.

3.8 Statistical analysis

All data of the gene relative expression were analyzed using GraphPad Prism 8 software. The normal distribution of the variables was verified through the Shapiro–Wilk test of normality. Statistical differences among experimental groups were assessed using one-way ANOVA analyses followed by Tukey's post hoc test. Unpaired, two-tails Student's *t*-test was used to determine significant differences considering two groups at a time. Significance was set at $p < 0.05$.

4. RESULTS

4.1 Growth parameters and HSI

No significant differences ($p > 0.05$) in all growth parameters evaluated (final body weight, FCR, SGR, daily feed intake) were observed among experimental groups (Table 3). HSI values did not differ among experimental groups as well (Table 3).

Parameters	CTRL	POP	POP + POST
Initial weight (g)	1347 ± 29.00	1354 ± 24.88	1354 ± 26.13
Final weight (g)	3415.33 ± 79.64	3380.28 ± 82.72	3416.33 ± 45.65
FCR	1.13 ± 0.03	1.15 ± 0.02	1.13 ± 0.03
SGR (%/ day)	0.98 ± 0.02	0.96 ± 0.01	0.97 ± 0.02
Daily feed intake (%BW/day)	1.31 ± 0.01	1.31 ± 0.01	1.30 ± 0.01
Hepatosomatic index (HSI)	1.546 ± 0.30	1.508 ± 0.23	1.534 ± 0.13

Table 3. *Growth performance indexes and hepatosomatic index (HSI) among the experimental groups. FCR, feed conversion ratio, SGR, specific growth. Data are expressed as mean value ± SD Significance was set at $p < 0.05$.*

4.2 Genes expression

Lipid metabolism

A significant difference ($p \leq 0.05$) was observed for *srebpf1*, POP group was characterized by higher expression values than the CTRL group (Figure 6a). No significant differences were observed for *fasn* expression among the experimental groups (Figure 6b).

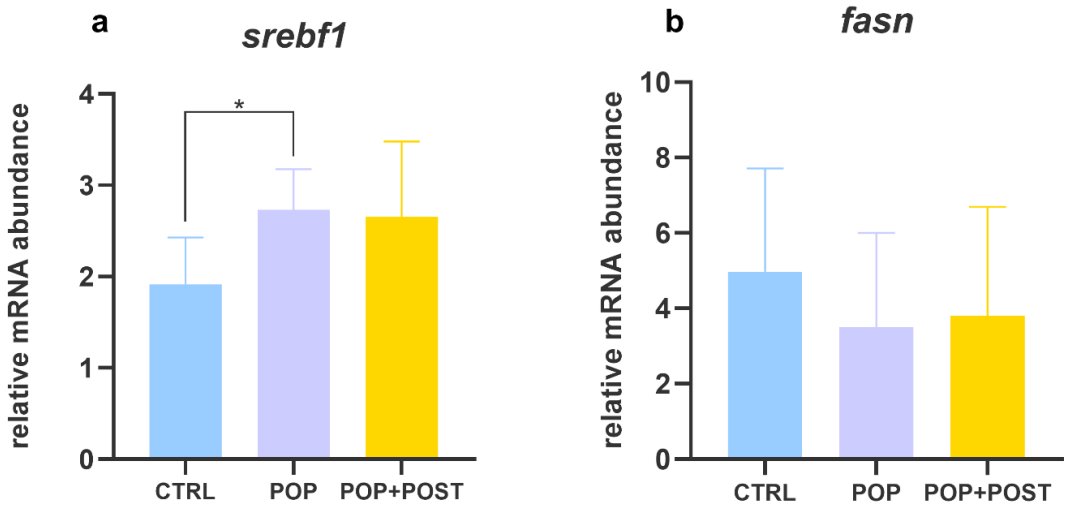

Figure 6. *Relative mRNA expression values of srebpf1(a) and fasn (b). The asterisk (*) indicates statistical significance ($p < 0.05$) determined by t-test analysis.*

Concerning the mRNA relative abundance of *ppars*, only *pparα* has a significantly lower expression level in the POP group compared to CTRL and POP+POST groups (Figure 7a). The mRNA levels of *pparβ* and *pparγ* did not significantly change among the three experimental groups (Figure 7b, c).

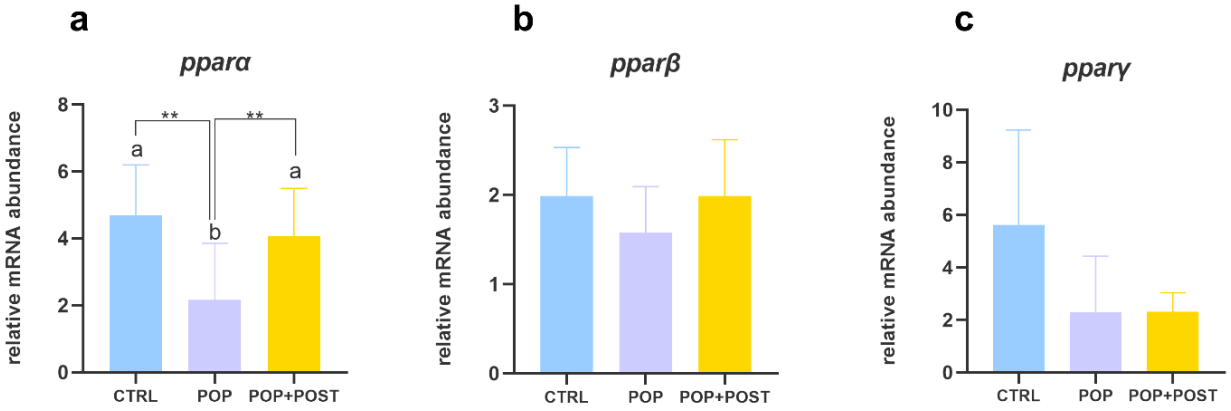

Figure 7. *Relative mRNA expression values of pparα (a), pparβ (b) and pparγ (c). Different letters indicate significant differences at ($p < 0.05$) determined by one-way ANOVA analysis. Asterisks (**) indicate statistical significance ($p < 0.01$) determined by the t-test analysis.*

The gene expression level of *fabp1* is significantly lower in the POP+POST group compared to both the CTRL and POP groups (Figure 8).

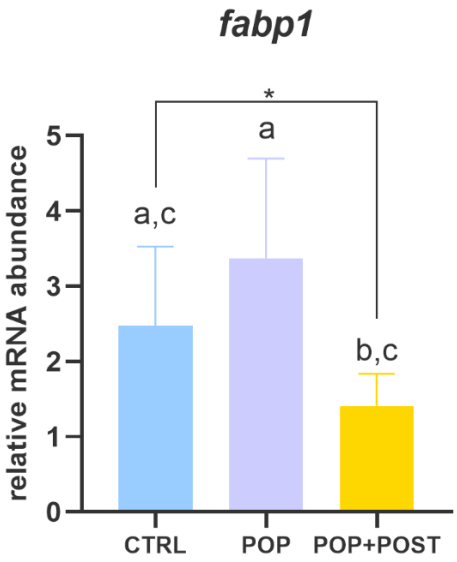

Figure 8. *Relative mRNA expression value of fabp1. Different letters indicate significant differences at ($p < 0.05$) determined by the one-way ANOVA analysis. Asterisk (*) indicates statistical significance ($p < 0.05$) determined by the t-test analysis.*

Significant differences in mRNA levels of *acox1* were noted between the CTRL and POP groups (Figure 9).

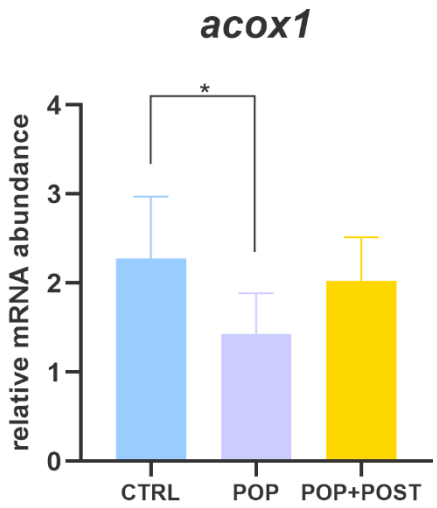

Figure 9. *Relative mRNA expression values of acox1. The asterisk (*) indicates statistical significance ($p < 0.05$) determined by the t-test analysis.*

The relative mRNA expression of *HL* was significantly lower in POP group compared to both CTRL and POP+POST groups (Figure 10a).

Relative expression values of *lpl* in the CTRL group were significantly higher than those in the POP group (Figure 10b).

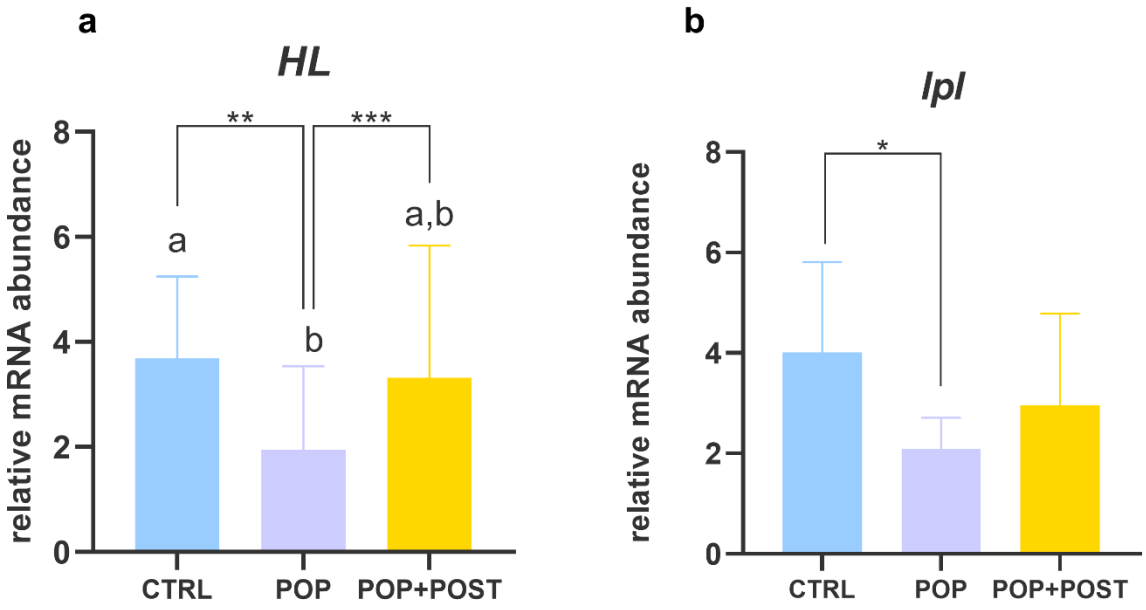

Figure 10. *Relative mRNA expression values of lpl and HL. Different letters indicate significant differences at (p< 0.05) determined by the one-way ANOVA analysis. Asterisks (**) (***) indicate statistical significance (p < 0.01; p < 0.001) determined by the t-test analysis.*

A significantly higher level of expression of *pla2* was observed in CTRL and POP+POST groups compared to the POP group (Figure 11).

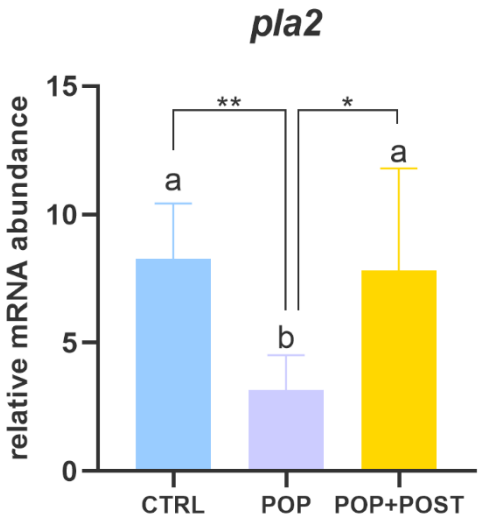

Figure 11. *Relative mRNA expression value of pla2. Different letters indicate significant differences at ($p < 0.05$) determined by the one-way ANOVA analysis. Asterisks (*) (**) indicate statistical significance ($p < 0.05$; $p < 0.01$) determined by the t-test analysis.*

5. DISCUSSION

Postbiotics administration in aquaculture can enhance gut health by modulating the composition of beneficial microbiota and boosting mucosal thickness thereby contributing to gastrointestinal health of aquatic species (Tao et al., 2024, Abbasi et al., 2022). Despite postbiotics offer several advantages over probiotics, their absorption rate could be improved through microencapsulation procedures (Dezfooli et al., 2018).

The administration of microencapsulated butyric acid did not influence the growth of juvenile sea bream analyzed in the present study.

Despite the mode of administration (free or encapsulated), different studies have investigated the effects of butyric acid as a postbiotic in aquaculture species with conflicting results. Some data observed that butyric acid can improve growth performance and feed intake efficiency in different farmed species, including Asian seabass (*L. calcarifer*) (Aalamifar et al., 2020), African catfish (*G. gariepinus*), and Nile tilapia (*O. niloticus*) (Omosowone et al., 2018). Other studies have reported dissimilar results, for instance, no effects were observed in terms of growth and feed assimilation in the Atlantic salmon (butyric acid with propionic and acetic acids), gilthead seabream and grass carp fed

different concentrations of butyric acid (Bjerkeng et al., 1999; Zhou et al., 2018; Estensoro et al., 2016). In particular, according to our results, Estensoro et al. (2016b) did not observe any improvement of final weight or the HSI in juvenile gilthead seabream.

Different results could be related to several factors such as the diet composition, life-stage and health conditions of farmed animals, and the concentration of butyric acid administered (Zhou et al., 2019).

In particular, the effective dose of butyric acid can vary among fish species, as reported by several studies (Aalamifar et al., 2020; Omosowone et al., 2018; Zhou et al., 2019).

Considering the relatively low dose of butyric acid used in the present study, it is not surprising the absence of effects on fish growth parameters, therefore its well-known impact on lipid metabolism was investigated.

This study represented the first attempt to apply microencapsulation technology in postbiotic dietary administration and to evaluate the possible effects of the microcapsule provided alone. In particular, the expression of genes involved in lipid synthesis (*srebf1, fasn*), transport

(*fabp1, HL and lpl*), oxidation (*ppars*) and other related processes, was evaluated.

The results obtained highlighted that the microcapsule alone appeared to influence lipid metabolism more significantly when complexed with butyric acid. Indeed, the composition of the microcapsule (starch and Arabic gum mainly), characterized by a high carbohydrates content could have modulated the expression of the above-mentioned genes. Carbohydrates and lipids are closely interconnected in the process of glycolipid metabolism (Qu et la. 2022). A study, conducted by Castro et al., (2016a) demonstrated that high levels of dietary carbohydrates enhanced the activity of enzymes and/or gene expression associated with lipogenesis in fish. Additionally, carbohydrates intake was found to influence circulating triglyceride and cholesterol levels, thereby affecting lipid transport and increasing lipid deposition (Castro et al., 2016b, Chen et al., 2012).

In the present study, starch microcapsule enhanced the expression of *srebf1,* a key transcription factor in fatty acid synthesis (Minghetti et al., 2011). A similar result was observed in a study conducted by Li et al. (2019) on Prussian carp (*Carassius gibelio*) fed a carbohydrate-based diet. The upregulation of *srebf1*, observed in both our study and the case

discussed by Li and colleagues, can be related to the enhancement of the lipogenic potential in the liver induced by the carbohydrate's assimilation. It is well known that in the liver carbohydrates are usually converted into lipids to be stored for long terms energy needs (Uyeda and Repa, 2006). As a consequence, it is not surprising the downregulation of *pparα* expression observed in the POP group. *Pparα* is a key transcription factor in fatty acid β-oxidation, a decrease in its expression can be therefore related to an increase in lipid accumulation (Lefebvre et al., 2006).

Indeed, the expression of *pparα* in the liver, according to a study conducted on juvenile gilthead seabream in 2016 by Castro and colleagues is downregulated by a carbohydrate-rich diet (Bonacic et al., 2016; Zheng et al., 2014).

The pattern of relative mRNA expression shown by *pparα* was in line with the lower expression of *lpl and HL* in the POP group compared to the control. These two genes induce the hydrolyzation of triglycerides during lipolysis for their mobilization as free fatty acids and glycerol. Inhibition of *lpl* and *HL* expression could be a consequence induced by the starch stimulation of insulin production which reduces lipolysis in favor of lipid storage (Choi et al., 2010).

Pparα influences also *acox1* which is an essential gene for beta oxidation process in peroxisome and it was observed to be downregulated as well in the POP group (Carcoran et al., 2015).

Finally, *pla2* expression, was significantly lower in POP group respect to the control in line with the other genes above discussed. This gene encodes for enzymes belonging to the phospholipase A2 class, which releases arachidonic acid (ARA) from membrane phospholipids (Miao et al., 2022). ARA is a precursor to various bioactive mediators such as prostaglandins and leukotrienes. Arachidonic acid (ARA) plays important roles in regulating lipid metabolism, biological processes and immune response. The possible relation between pla2 expression and carbohydrates assimilation is not clear yet, therefore a deeper investigation is required to understand the observed effects on sea bream.

The only significant reduction in gene expression observed in the POP+POST group respect to the control was associated with *fabp1*. This gene is involved in the intracellular transport and storage of fatty acids as triglycerides (Houston et al., 2017). Unfortunately, no study explained the potential link between *fabp1* expression and butyric acid in fish. From the existing literature, it is known that butyric acid reduces lipid

storage in young broilers (Yin et al., 2016). Thus, further research is needed to fully understand the effects observed in gilthead sea bream.

6. CONCLUSION

In conclusion, this study demonstrated that the administration of microencapsulated butyric acid did not significantly affect growth parameters in juvenile sea bream, as no notable differences were observed in body weight, feed conversion ratio, specific growth rate, or hepatosomatic index across the experimental groups. However, the study provided valuable insights into the influence of the microcapsule on lipid metabolism. Gene expression analysis revealed that the microcapsule, composed mainly of starch and Arabic gum, had a more pronounced effect on lipid-related genes than the butyric acid itself. Specifically, the microcapsule influenced the expression of key genes involved in fatty acid synthesis, transport, and oxidation, suggesting that carbohydrate intake, via the starch component, could modulate lipid metabolism. Notably, the study also revealed a reduction in the expression of *fabp1* in the POP+POST group, highlighting the potential effects of butyric acid on lipid storage. Although the mechanisms behind these findings require further investigation, the results underline the complexity of dietary interventions in aquaculture and suggest that microencapsulation could offer a promising approach for modulating metabolic pathways in fish. Further research is necessary to fully understand the interactions

between microencapsulated compounds and lipid metabolism in aquaculture species.

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