

Beneficial effects of some selected feed additives for European seabass (*Dicentrarchus labrax* L.): a review

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Abstract European seabass (*Dicentrarchus labrax* L.) is one of the most commercially important marine fish species and is widely farmed in several Mediterranean regions. For a long period, researchers have focused on finding functional feed supplements that maximize the productivity and profitability of seabass farming. This article will primarily focus on reviewing the beneficial effects of selected promising feed additives and their possible application in the aquaculture of European seabass, such as organic acid salts (sodium butyrate, sodium propionate, potassium diformate, and sodium diformate), taurine, astaxanthin, and some selected macroalgae such as *Ulva* spp., *Gracilaria* spp., and *Pterocladia capillacea*. This review also highlights the optimal dietary supplementation dose for different sizes of European seabass. Their beneficial effects on growth, feed utilization, biochemical composition, health status, immune responses, gut microbiome, intestinal integrity/histomorphology, and skeletal muscle cellularity were also discussed. The literature data will spotlight the application of these dietary supplements for farmed European seabass, which may facilitate monitoring of adequate fish health status, exploit their advantageous effects and potentially assist in the formulation of aquaculture feeding strategies.

Keywords Feed additives · Seabass · Acidifiers · Taurine · Astaxanthin · Macroalgae

Introduction

With the rapid expansion of aquaculture industry worldwide, special attention is now being paid to the application of efficient feeding strategies for faster and better yields and efficiency of aquaculture operations. Envisioning a more sustainable aquaculture development, several efforts have further been made to minimize the amount of the fishmeal ingredient in aquafeeds. Thus, feed industry was forced to explore multiple alternative protein sources and or feed additives (Turchini et al. 2019). In several countries, aquaculture activities are exposed to the risk of diseases or reduced productivity because of the intensified use of plant-based diets as alternatives to fish meal (FM)-based diets (Colombo et al. 2022). Developing fish feeds, particularly for carnivorous species with low levels of FM and/or fish oil (FO), without compromising performance or health, is a crucial issue to consider. Moreover, feeds is the most expensive component in aquaculture. Therefore, in recent decades, there has been growing interests in using different feed additives/supplements to enhance growth performance and control infectious diseases (Açar et al. 2019; Yilmaz et al. 2012, 2016, 2022) which have positive effects on the productivity of aquatic-farm. Feed supplements represent various components that promote ingestion, nutrient absorption and assimilation, growth, and health status by influencing physiological functions such as immune competence, disease resistance, stress tolerance and reproduction (Bai et al. 2015). Feed additives are also required for prophylactic usage to

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avoid disease incidence by supporting and improving the immune system to fight infections and emergent pathogens (Encarnação 2016; Alemayehu et al. 2018). Functional feed additives can be described as dietary components that provide health benefits, and maximize the performance or product quality of target aquatic animals (Irkin 2019) even when using fish meal-based diets (Saleh et al. 2020).

There is currently active research in several countries on a wide variety of fish/shrimp feed additives as natural growth promoters, due to the ban on the use of antibiotics in fish production (Van Doan et al. 2023). Natural growth promoters may be supplemented to promote gut health and maximise nutrient utilization, leading to cost-effective, safe, and profitable production (Nuez-Ortín 2013).

The European Sea bass (*Dicentrarchus labrax* L.) is a strictly carnivorous marine fish, highly market valued, and commercially cultured in the Mediterranean region (Vásquez and Muñoz-Cueto 2014). Owing to the continuous demand for seafood, it is expected that the production rates of these fish species will continue to increase in the foreseeable future. The use of feed supplements in the diet of seabass is a promising avenue for enhancing feed and nutrient utilization, leading to improved growth and immunity (Abdel-Mohsen et al. 2018; Wassef et al. 2017, 2020, 2021; Saleh et al. 2018, 2020; Martins et al. 2023). In sea bass farming, similar to other cultured marine species, feed cost represents the largest proportion of production expenses. Therefore, minimizing feed costs without compromising fish growth performance is essential. Thus, the use of certain feed additives will allow farmers to maximize the performance and productivity of European seabass and improve the economics of its farming.

This review article was prepared to light on and summarise the results from the published literature on selected feed additives for European seabass, including some organic-acid salts, taurine, astaxanthin and selected macroalgae, such as the green: *Ulva* spp. and the red algae: *Gracilaria* spp. and *Pterocladia capillatae*. These potentially important feed supplements were chosen among the wide range of functional feed supplements because of the substantial amount of information available on their use in European seabass farming. The optimal dietary inclusion dosage and the effects of these feed supplements on growth, feed utilization, health status, biochemical composition, immune responses, gut microbiome, intestinal morphology, and muscle cellularity are summarized in Table 1.

Organic-acid salts

In general, most organic acids are short-chain fatty acids (SCFAs), their salts are chelated to minerals (Ng and Koh 2017), and they can be used as acidifiers. SCFAs or their salts can increase the bioavailability of dietary minerals by chelating them. They show antimicrobial activity, and accordingly, they are considered feed preservatives (Hoseinifar et al. 2017; Tran et al. 2020). Although dietary acidifiers have been extensively investigated in various terrestrial animals, studies on their use in aquatic animals have intensified only during the last two decades (Ng and Koh 2017). The use of acidifiers in aquaculture nutrition has been the focus of nutrition research and commercial practice (Abdel Mohsen et al. 2018; Dawood et al. 2018; Abdel-Latif et al. 2020; Wassef et al. 2017, 2020, 2021; Van Doan et al. 2023; Martins et al. 2023). In the near future, their commercial use is expected to significantly increase due to the worldwide ban on the use of antibiotics (Van Doan et al. 2023; Tran et al. 2020).

Previous trials on using organic acids/salts as feed additives revealed their ability to reduce the pH of both the feed and the animal stomach (which leads to increased release of proteolytic enzymes in the stomach and the small intestine), and impair the growth of pathogenic organisms (Ng and Koh 2017; Hoseinifar et al. 2017). Moreover, acidifiers can act as an energy source in the small intestine and beneficially modulate gut microbiota (Nuez-Ortín 2013; Abdel-Mohsen et al. 2018), by reducing pathogenic bacteria, and increasing lactic acid bacteria (LAB) colonization (Lückstädt 2008a, b). Acetate, propionate, and butyrate are the dominant SCFAs in the fish gut, and are important metabolites in energy homeostasis, metabolism, and maintenance of gut health (Tran et al. 2020). The stimulation and activation of digestive enzymes, improving the absorption of nutrients, and antimicrobial effects are the most important modes of action of dietary acidifiers (Sardar 2020; Tran et al. 2020). Various acidifiers have been used as growth promoters because they can promote higher protein digestibility, better feed conversion efficiency, boost the immune response, and reduce mortality rates (Nuez-Ortín 2013). This implies that the acidifiers used in aquafeeds can enhance animal health status and performance, reduce antibiotic usage, and finally maintain resource efficiency, farm profit, and therefore aquaculture



Table 1 Summary of the beneficial effects of some selected functional feed supplements for European seabass (*Dicentrarchus labrax*).

Items	Initial weight of fish (g)	Tested doses	Optimal dietary dose	Beneficial effects	Reference
Sodium butyrate (SB)	~0.45	0, 1, 2, 3 g Kg ⁻¹	2 g Kg ⁻¹	Increased immunoglobulins, respiratory burst activity, phagocytosis, LYZ, myeloperoxidase and bactericidal activities	Abdel-Mohsen et al. (2018)
	~5.14	2 g Kg ⁻¹	2 g Kg ⁻¹	Positively modulated distal-intestine microbiota	
	~1.5	2 g Kg ⁻¹	2 g Kg ⁻¹	Increased cell-proliferation in intestinal crypts	Rimoldi et al. (2016)
Sodium propionate (SP)	~0.45	0, 1, 2 and 3 g Kg ⁻¹	2 or 3 g Kg ⁻¹	Normalized the intestinal abnormalities induced by feeding soybean meal (SBM)-diets	
	~4.75	0, 3, 4 and 5 g Kg ⁻¹	3 g Kg ⁻¹	No significant differences in WG or SGR	Terova et al. (2016)
	~13.71	3 g Kg ⁻¹	3 g Kg ⁻¹	Improved mucosal protection and inflammatory responses	Wassef et al. (2020)
Sodium diformate (SDF, KDF)	~5.5	0, 2, 3, and 4 g Kg ⁻¹ diet	2 g Kg ⁻¹	Improved growth performance	Wassef et al. (2017)
	~55	2, 3, 4, 5, 7, 9, 12 and 17 g Kg ⁻¹	4.7–5.0 g Kg ⁻¹	Enhanced general health status and immune response	
	~9.4	10 g Kg ⁻¹	10 g Kg ⁻¹	Improved intestinal functions (increased villus height and number of goblet cells)	Hussein et al. (2020)
Taurine (TRN)	~55	0, 2, 5, 7 and 12 g Kg ⁻¹	4.7–5.0 g Kg ⁻¹	Highest WG, SGR and feed utilization indices	Martins et al. (2018)
	~92.57	15 g Kg ⁻¹	15 g Kg ⁻¹	Increased WBCs, lymphocytes, monocytes, phagocytosis, serum proteins and LYZ activities	(Martins et al., 2019)
	~6.9	10 g Kg ⁻¹	10 g Kg ⁻¹	Promoted growth	Martins et al. (2021)
			Normalized physiological conditions	Ceccotti et al. (2019)	
			Improved Kg (Capital K), Kg -1 (Superscript) muscle structure	Coutinho et al. (2017)	
			2 or 3 g kg-1 served as a growth promoter and a basic defense module		
			Increased values of RBCs, Hb and HTC		
			TRN up to 7 g Kg ⁻¹ improved growth performance		
			Maximum feed efficiency and protein efficiency ratios at 12 g Kg ⁻¹ TRN		
			Decreased whole-body lipid content in 12 and 17 g Kg ⁻¹ TRN-fed fish than other groups		
			Whole-body protein and ash contents, HSI and VSI were not affected		
			Improved growth performance		
			Enhanced antioxidant responses (↓ hepatic GPX and lipid peroxidation)		
			Decreased intestinal inflammation and apoptosis (↓ Casp3 and Casp9 gene-expression)		
			Increased total bile acid content in the liver with increasing TRN levels		
			Decreased glucose, total cholesterol, HDL, and LDL with increasing dietary TRN level		
			Decreased hepatic CAT, GPX, GR, and SOD activities		
			Decreased hepatic lipid peroxidation with increasing TRN levels		
			Enhanced growth and swimming performance		
			Enhanced antioxidant status of fish		
			Protect against oxidative stress induced by forced swimming		
			No effects on growth, feed utilization, and whole-body composition		
			Decreased intestinal glutathione GSH and oxidized (GSSH) glutathione content, the activity of GPX both in liver and intestine, and glucose-6-phosphate dehydrogenase (G6PDH) activity in the liver		



Table 1 Continued

	~85	0, 2, 5 and 10 g Kg ⁻¹	2 and 10 g Kg ⁻¹	HSP, MAPK levels, L-LDH activity levels up-regulated some genes in the liver and the anterior intestine	Feidantis et al. (2014)
	N.M.	0, 5, 10 and 20 g Kg ⁻¹	10 g Kg ⁻¹	10 and 20 g Kg ⁻¹ TRN in a diet containing high SBM content promoted the feed intake, feed efficiency and growth	Kotzamanis et al. (2012)
	~78	0, 5, 10 and 20 g Kg ⁻¹ in high plant protein diets	20 g Kg ⁻¹	Improved growth Enhanced organoleptic properties of fish filets	Kotzamanis et al. (2020a)
	~86	0, 2, 5 and 10 in high soy meals	10 g Kg ⁻¹	No effect on growth, feed efficiency, fish body or muscles composition and intraperitoneal fat deposition	Kotzamanis et al. (2020b)
	~0.81	0, 1, 2 and 3 g Kg ⁻¹	2 g Kg ⁻¹	No differences in the histology of the gut and liver among tested groups	Martinez et al. (2004)
	~514	2 g Kg ⁻¹	2 g Kg ⁻¹	10 g Kg ⁻¹ TRN might have a pronounced effect on flesh quality Better growth when fish meal and SBM are the primary sources of dietary protein	Rimoldi et al. (2016)
	~5	0, 4, 7 and 10 g Kg ⁻¹	10 g Kg ⁻¹	Normalize SBM-induced intestinal abnormalities Increased lymphocytes, monocytes, serum phagocytic and LYZ activities	Saleh et al. (2020)
	~13.71	10 g Kg ⁻¹	10 g Kg ⁻¹	1% TRN boost growth, and enhanced muscle function and firmness Promoted growth	Wassef et al. (2021)
	~0.4	0, 0.06, 0.08 and 0.10 g Kg ⁻¹	0.10 g Kg ⁻¹	Normalized physiological conditions, and improved the striated muscle structure Enhanced growth, feed utilization, survival rate and fish protein content	Saleh et al. (2018)
AstAaxanthin (AST)	0.3	AST: 0.05, 0.1 g CM: 5, 10, 15 g ML: 0.1, 0.2, 0.3 g Kg ⁻¹	Crab waste meal (CM): 10 Marigold flower meal (ML): 0.2g Kg ⁻¹	Reduced SOD and GPx levels Increased intestinal mucosal phagocytic and lysozyme activities. Improved fish tolerance against osmotic stress. Improved growth, total carotenoid content, antioxidant activities and stress resistance as efficiently as the commercial synthetic astaxanthin.	Goda et al., (2018)
<i>Ulva lactuca</i> <i>Pterocladia capillacea</i>	~0.23	0, 50, 100, and 150 g Kg ⁻¹	50 g Kg ⁻¹	Enhanced growth performance and fish nutrients composition Increased stress resistance against air-exposure test	Wassef et al. (2013)
<i>Gracilaria</i> spp. Extract	~11.95	50 g Kg ⁻¹	50 g Kg ⁻¹	Revealed higher fish antioxidant capacity than CTRL fish Induced a down-regulation of hepatic stress-responsive heat shock proteins (grp-78, grp-170, grp94, grp-75) Down-regulated the transcription levels of genes associated with oxidative stress response (prt3.5 and gpx4) in liver. Led to an up-regulation of genes associated with inflammation (il34, ccr9, cd33) and a down-regulation of genes related to cytokine signaling (<i>mif</i> , <i>il1b</i> , <i>def</i> , <i>a2m</i> , <i>myd88</i>) in head-kidney. Positively modulated seabass resistance to bacterial infection (<i>Phdp</i>)	Peixoto et al. (2019)
<i>Gracilaria bursariensis</i> (GP), <i>Ulva rigida</i> (UR)	~4.7	0, 50 and 100 g Kg ⁻¹	50 g Kg ⁻¹	The dietary inclusion of GP and UR up to 10% showed no negative consequences on growth performance, nutrients utilization or body composition	Valente et al. (2006)

Table 1 Continued

<i>G. cornea</i> (GC)			GC should be limited to 5% of the diet.
<i>Gracilaria gracilis</i>	N.D.	0, 25, 50 g Kg ⁻¹ and algal extract at 3,5 g Kg ⁻¹	No negative impact on growth or feed utilization, haematological profile or some metabolic parameters of fish. Supplementation with 25g Kg ⁻¹ boosted immune response and protected against oxidative damages by improved enzymatic and non-enzymatic responses of fish. Passos et al. (2021)

Table abbreviations

CAT: Catalase, FBW: Final body weight, G6PDH: Glucose-6-phosphate dehydrogenase, GPX: Glutathione peroxidase, GR: Glutathione reductase, Hb: Hemoglobin, HDL: High density lipoprotein, HSI: Hepato-somatic index, HSP: Heat shock proteins, HTC: Hematocrit, LDL: Low density lipoprotein, L-LDH: Lactate dehydrogenase, LYZ: Lysozyme, MAPK: Mitogen-activated protein kinase, RBCs: Red blood cells, SGR: Specific growth rate, SOD: Superoxide dismutase, SR: Survival rate, VSI: Viscerosomatic index, WBCs: White blood cells, WG: Weight gain, *Phdp*: *Photobacterium damsela* subsp. piscicida. N.M.: Not mentioned. CTRL: control fish.



sustainability (Abdel-Tawwab et al. 2021; Abdel-Latif et al. 2020).

It is noteworthy that using optimum levels of acidifiers in aquafeeds is extremely important, because excess dietary levels of acidifiers can reduce both feed palatability and feed intake, and may, therefore, lead to adverse effects on animal performance (Ng and Koh 2017). Typically, organic acids/salts are supplemented in the developed feed at a dosage between 0.1% and 1.0% (Hoseinifar et al. 2017). Other researchers have defined the dietary dosages of organic acids/salts to be up to 2% (De Schryver et al. 2010; Rimoldi et al. 2016), however, this might have been reasonable for research purposes. Nonetheless, many factors such as the fish (species, size, and physiological status), organic acid salt (form and inclusion levels), diet composition, and rearing conditions may affect the growth-promoting effects of acidifiers used in fish nutrition (Alemayehu et al. 2018; Ng and Koh 2017).

A limited number of acidifiers have been tested for European seabass, to determine their effects on the fish performance and general health status. Among the most evaluated organic salts for European seabass are: sodium butyrate, sodium and calcium propionate, potassium and sodium diformate. Positive performance results for these organic salts have been documented in several previous trials (Rimoldi et al. 2016; Abdel-Mohsen et al. 2018; Wassef et al. 2017; Hussein et al. 2020; Wassef et al. 2020, 2021). These studies suggest that these organic salts are palatable, digestible, and suitable acidifiers for this fish species, and accordingly, as a promising alternative to antibiotics in aquafeeds (Sardar 2020; Tran et al. 2020). Therefore, the use of these acidifiers in European seabass feeds could contribute to improvement in fish performance, fish health, farm profit, and resource efficiency. In this section, we will focus on the effects of dietary supplementation with these specific organic salts on European seabass performance.

Sodium butyrate

Sodium butyrate (SB) is a salt of SCFA butyric acid and one of the most commonly used organic acid salts in animal feeds because it is stable and has much less odour (Ng and Koh 2017). It is produced after the fermentation of carbohydrates by anaerobic microflora in the hindgut and is considered an important energy source for the epithelial cell lining of the gastro-intestinal tract (GIT) of animals and humans (Hamer et al. 2008). The uptake of dietary SB produce multiple nutritional functions, particularly in the GIT of animals, induces differentiation of epithelial cells, inhibits mucosal apoptosis and, raises the proliferation index of the intestinal crypts (Hamer et al. 2008; Robles et al. 2013). Results from the published literature have indicated that the gut health of farmed fish/shrimp is of vital importance for its well-being and performance; hence, butyrate has received significant consideration for its multiple advantageous effects on host energy metabolism, anti-inflammatory properties, and being a potential immunomodulatory substance (Liu et al. 2017; Ahmed and Sadek 2015; Abdel-Mohsen et al. 2018).

It has been reported that using butyrate as a feed supplement improves the availability of many indispensable amino acids and nucleotide derivatives and enhances fish growth (Robles et al. 2013). Recently a published report showed that dietary supplementation with SB nanoparticles also stimulates intestinal functions and health of Nile tilapia, because of improved intestinal villi and increased absorptive surface area (Abdel-Tawwab et al. 2021). In addition, SB dietary supplementation has resulted in better nutrient utilization, enhanced health status and improved fish growth performance (Abdel-Mohsen et al. 2018; Luz et al. 2019). Hamer et al. (2008) showed that butyrate exhibits potential anti-inflammatory properties in the hind intestine of animals by modifying transcription factors, which in turn controls the expression of inflammatory response genes and exerts other advantageous effects on animal energy metabolism.

Another form of salt butyrate is poly- β -hydroxybutyrate (PHB), the polymer of the SCFA β -hydroxy-butyrate (β -HB) is a bacterial energy storage compound (De Schryver et al. 2010, 2011; Franke et al. 2017). After dietary uptake of PHB, polymers are degraded into oligomers and monomers, reducing the pH in the host gut (Defoirdt et al. 2009), which may aid beneficial bacteria to flourish and inhibit the growth of pathogenic bacteria, thereby enhancing the gut health of the treated animals (Cotter and Hill 2003).

In several fish species, the results have indicated positive effects of SB on weight gain, feed utilization efficiency, nutrient composition, the intestinal microbiota composition, and trophic effects on the intestinal epithelium by increasing crypt depth and villi length. Among the previously published studies, dietary butyrate has positive effects on European seabass gut health or intestinal integrity (Rimoldi et al. 2016; Abdel-Mohsen et al. 2018). De Schryver et al. (2010) declared that diets containing 2% and 5% PHB resulted



in the highest weight gain and survival rate and positively modulated the intestinal microbiota of juvenile European seabass. In contrast, Rimoldi et al. (2016) showed no significant differences in growth or condition factors between European seabass fed 0.2% SB-supplemented diet and those fed a non-supplemented control diet. However, those authors mentioned that SB exerts an alleviating effect on the inflamed distal intestine of seabass fed a 30% soybean meal diet (SBM-induced distal bowel enteritis). Terova et al. (2016) emphasized that SB exhibits anti-inflammatory and immune-modulatory properties in European seabass.

Similarly, PHB has been investigated in feeds for juveniles of European seabass, and it was observed to enhance growth, survival, disease resistance, and positively altered the microbial composition of their GIT (De Schryver et al. 2010; Terova et al. 2016; Franke et al. 2017). This enhancement may be attributed to that the fish enzymes in the gastrointestinal tract (partially) degrade PHB into β -hydroxybutyrate oligomers and monomers, which inhibit growth of harmful bacteria (De Schryver et al. 2010; Şahin et al. 2021). Moreover, the effect of feeding European seabass post larvae (28-d-old) with *Artemia* nauplii enriched with PHB on survival, growth, and immune response was investigated by Franke et al. (2017). These authors demonstrated that PHB stimulated the expression of some immune-related genes such as antimicrobial peptides including *dicentracin* and *hepcidin* genes in post larva-stage, thereby protecting the larvae against pathogens.

The efficacy of microencapsulated-SB as a functional diet acidifier was evaluated, for European seabass with an emphasis on the effects of SB on growth, nutrient utilization, immuno-competence, gut microbiota, and histomorphological features of the intestine (Abdel-Mohsen et al. 2018). The results demonstrated that 2 g microencapsulated-SB kg^{-1} diet exerted multiple positive effects, including promotion of growth, survival and feed utilization and elevation of protein deposition, and reduction of lipid content of fish. In addition, there was an appreciable enhancement in the general health status and immunology indicators such as immunoglobulin, respiratory burst activity, phagocytosis, myeloperoxidase, bactericidal, and lysozyme activities as compared with the control fish indicating better fish resistance against surrounding pathogens (Abdel-Mohsen et al. 2018). Furthermore, measurement of the intestinal sections of seabass revealed that the 0.2% SB-supplemented diet increased the muscular layer thickness, villi length, and width, and furthermore, the goblet cells count. These findings demonstrate the beneficial effects of SB on gut health, inducing better disease resistance and growth performance for European seabass fry. In brief, the inclusion of 2 g of microencapsulated SB kg^{-1} feed could maintain fish health status, modulate distal intestinal microbiota, and increase cell proliferation in the intestinal crypts of seabass (Abdel-Mohsen et al. 2018). Although the mechanisms of action of butyrate are not fully understood, it is assumed that butyrate is involved in the complex interaction between fish diet, gut microbiome, and energy metabolism (Robles et al. 2013).

For other finfish, several studies reported significant improvement in growth and feed conversion ratios and other physiological functions when SB or PHB was included in their diets, Robles et al. (2013) for gilthead seabream (*Sparus aurata*); Ahmed and Sadek (2015) for Nile tilapia (*Oreochromis niloticus*); Rodriguez et al. (2017) for red drum (*Sciaenops ocellatus*); Liu et al. (2017) for grass carp (*Ctenopharyngodon idella*); Zhou et al. (2019) for golden pampano (*Trachinotus ovatus*); Luz et al. (2019) for Pirarueu (*Arapaima gigas*).

Sodium/ calcium propionate

The organic salts sodium propionate (SP) and calcium propionate (CaP) are considered natural growth promoters, health enhancers, and immuno-stimulators, that can effectively support hygiene and feeding in aquatic organisms (Hosseini et al. 2011; Hoseinifar et al. 2017; Tran et al. 2020). However, only a few studies have emphasized their beneficial effects on the intestinal microbiota (Ringø 1991; Ringø et al. 2016; Wassef et al. 2020) or the intestinal histo-morphological features of fish (Wassef et al. 2020, 2021). The composition of gut microbiota is an essential factor for evaluating fish health status, because it is related to many biological processes that positively affect the nutritional conditions and immunity of the host (Hasan and Yang 2019).

Regarding the European seabass, a recent study on the fry stage, indicated that the addition of 0.2% or 0.3% SP to the diet could improve growth performance, general health, immunity, and intestinal function of fish (Wassef et al. 2020). These authors reported a 34% increase in weight gain in fish fed a dietary level of 0.3% SP associated with a decline in feed conversion ratio, compared with the control fish (CTR). Moreover, dietary administration of 0.3% SP increased the carcass protein content and improved immunity of



European seabass compared with CTR fish. European seabass fed the 0.2% or 0.3% SP-added diets had also showed a 95% microbial count reduction in their distal intestine compared with CTR fish. *Vibrio* spp. disappeared (zero or negligible) in fish fed with 0.2% and 0.3% SP diets, and the faecal coliform bacteria count gradually diminished to zero in the 0.3% SP-fed fish. In addition, there was enhancement in the fish intestinal histo-morphology in proximal, mid, and distal sections. The largest villi area and goblet cell counts were observed in fish fed with 0.3% SP or 0.2% SP diet compared with CTR fish (Wassef et al. 2020).

For other fish species, several studies demonstrated the beneficial effects of dietary propionic acid/salts supplementation on growth, feed utilization, immune response, and expression of immune-related and growth-related genes. Among them may be mentioned: Ringo (1991) for Arctic Charr (*Salvelinus alpinus* L.); Hassaan et al. (2014) for Nile tilapia (*Oreochromis niloticus*); Hoseinifar et al. (2016) for Caspian white fish (*Rutilus frisitikutum*); Safari et al. (2016) for Zebra fish (*Danio rerio*).

Sodium and potassium diformate

Among acidifiers, formic acid is produced physiologically through animal metabolism and has corrosive properties and stronger acidity than other SCFAs (Luise et al. 2020). Formic acid and its salts may accidentally enter aquaculture diets as a component of preserved feed items such as fish silage or when they are added for a specific purpose, such as acting as a non-antibiotic growth stimulator (Ng and Koh 2017; Hoseinifar et al. 2017). Recently, formic acid and its salts, especially sodium and potassium diformate (NaDF, KDF), have been evaluated as beneficial and promising dietary supplements in aquafeeds (Hussein et al. 2020; Tran et al. 2020). Both NaDF and KDF are widely used as feed acidifiers because of their active ingredients, relative stability, and ease of handling in extruded feeds (Ng and Koh 2017).

Previous studies have explained that dietary NaDF and KDF can decrease the pH of gut digesta (Lückstädt 2008a), thus providing energy for renewing the intestinal epithelia which may improve the digestibility of proteins and minerals (Hoseinifar et al. 2017). NaDF increased feed intake, enhanced the bioavailability of amino acids, and increased the digestibility of lipids, proteins, and amino acids (Morken et al. 2011). These effects are the primary reason for enhancing growth and feed utilization in fish (Yúfera et al. 2012). Formate salt can improve animal health because of its strong antimicrobial effects on pathogens and positive modulation of the gut microbiome (Lückstädt 2008b; Defoirdt et al. 2009; Luise et al. 2020). This beneficial modulation of microbiota and improvement of gut health may be an additional reason for improving the growth performance and feed efficiency of fish (Hoseinifar et al. 2017). NaDF growth-promoting effect is mainly based on its strong antimicrobial effect against several bacteria, including total anaerobic, lactic acid, and coliform bacteria (Øverland et al. 2000), and based on its potential to maximize the digestion, absorption, and utilization of ingested nutrients (Lückstädt 2008a, b).

There is limited information on the effects of NaDF or KDF inclusion in European seabass feeds, with only three recent reports published in this context (Wassef et al. 2017; Hussein et al. 2020; Wassef et al. 2021). In a dose/response growth trial on NaDF supplementation in the feed of juvenile European seabass, Wassef et al. (2017) concluded that 3 g/kg is the best economical and cost-effective inclusion dosage for fish weighing approximately 5 g. The addition of 0.3% NaDF promoted growth, improved feed utilization efficiency and survival, and enhanced basal health status and, consequently, overall fish performance. However, the major nutrient composition and the amino acid profile of European seabass were not significantly altered by the addition of dietary NaDF. On the basis of these results, this dose (0.3% feed) of NaDF was administered to juvenile seabass (with an initial weight of 14 g) fed a reduced amount of FM (20%) within a high plant-mix diet (Wassef et al. 2021). Their results suggest that supplementation of a plant-based diet with 0.3% NaDF significantly improved the growth and striated muscle structure of juvenile European seabass. Similar results have shown the potential for KDF application in diets for juveniles' seabass to serve as a growth promoter and a general health booster (Hussein et al. 2020).

Several previously published studies have reported the potential efficacy of dietary NaDF- or KDF-supplementation in improving growth performance, feed efficiency, and modulating the immune competence indicators in other fish species: Zhou et al. (2009) for hybrid tilapia (*Oreochromis niloticus* ♀ X *O. aureus* ♂); Abu Elala and Ragaa (2015) for Nile tilapia (*Oreochromis niloticus*); Morales et al. (2016) for rainbow



trout (*Oncorhynchus mykiss*); Rodriguez et al. (2017) for red drum (*Sciaenops ocellatus*).

It is noteworthy to mention that most organic acids commercially used in the food and feed industry are produced synthetically by manufacture worldwide and are available in the market at reasonable prices. For example, ADDCON Co., Norway for Na/K diformate and Na/Ca propionate and Shandong Kejian Chemicals, Co., China, for sodium butyrate, among others.

Taurine

Taurine (TRN) (2-amino-ethane-sulfonic acid) is a neutral β -amino acid freely distributed throughout most of the tissues of living organisms. Taurine exists naturally, in animals, including mammals, birds, fish, and aquatic invertebrates such as oysters and mussels. Although plants contain less than 1% of the taurine levels found in animals, the most taurine-rich plants are algae, followed by fungi and other terrestrial plants (Salze and Davis 2015). High taurine levels naturally occur in seafood and meat, and many vertebrates can synthesize taurine (Salze and Davis 2015). TRN is derived from methionine via cysteine, and generally, the liver is the primary organ capable of synthesizing TRN in fish (Sampath et al. 2020a). Taurine can be produced either by extraction and purification from taurine-rich sources or by chemical synthesis (El-Sayed 2014; Salze and Davis 2015). Historically, TRN has been listed as an approved ingredient for human and animal feeds in several countries.

TRN has several benefits, for example, free TRN can be considered as a fish olfactory stimulus, which increases feed consumption and is therefore widely used as a feed attractant (Carr 1982). Huxtable (1992) mentioned that TRN is also well known for its vital role in lipid metabolism, where it improves fat absorption in the intestine by inducing bile acid synthesis and cholesterol degradation. Numerous studies on fish have also shown that TRN has an effective activity in eyesight (Kuzmina et al. 2010; Shen et al. 2018, 2019). TRN is known as a functional nutrient based on studies that have emphasized that it exhibits several biological and physiological roles in osmoregulation, membrane stabilization, and modulation of cellular calcium levels in fish, and it acts as an anti-inflammation and anti-oxidation agent (Salze and Davis 2015). The effects of TRN on fish and shrimp nutrition, metabolism, and physiology have been extensively investigated (Huxtable 1992; Kuzmina et al. 2010; El-Sayed 2014; Shen et al. 2018, 2019; Sampath et al. 2020a; Kotzamanis et al. 2020a, b; Wassef et al. 2021).

The beneficial effects of TRN as a dietary additive to promote the growth of cultured aquatic organisms have been evidenced in several studies (Kim et al. 2005; Gaylord et al. 2006, 2007; Qi et al. 2012; El-Sayed 2014; Salze and Davis 2015; Sampath et al. 2020a), as well as on the health, muscle hardness and reproduction of fish (Al-Feky et al. 2016a, b; Shen et al. 2018, 2019; Saleh et al. 2020; Kotzamanis et al. 2020a, b; Sampath et al. 2020b; Wassef et al. 2021). In particular, TRN has also been investigated as a functional dietary supplement for its positive effects on fish well-being, immunity competence, and gut health (El-Sayed 2014; Salze and Davis 2015; Sampath et al. 2020a). However, the advantages of TRN are species-specific and dose-dependent, relative to its dietary concentration and period of feeding (Salze and Davis 2015; Shen et al. 2018). Furthermore, the response of marine fish to dietary TRN is affected by fish size (life stage), assuming that TRN is considered an essential amino acid for larvae and small juvenile fish that consume foods rich in TRN during their early life stages (Sampath et al. 2020a). Dietary TRN supplementation may result in optimum growth performance during the juvenile stage of fish because of the high metabolic functions related to growth during this period (Salze and Davis 2015).

Approximately 0.2%-1.0% dietary TRN have been reported for the optimum performance of European seabass, depending on fish size and age. Juvenile seabass require 1% TRN content in their diet (Feidantsis et al. 2014; Martins et al. 2019; Kotzamanis et al. 2020a, b; Saleh et al. 2020), whereas Martinez et al. (2004) and Rimoldi et al. (2016) used a dose of 0.2% and/or 0.3% for fry and larger fish, respectively. The addition of dietary TRN is also beneficial (at 1%) for European seabass weighing 5 g fed FM-based diets (Saleh et al. 2020), and in a reduced-FM plant-based diet for juvenile seabass (Wassef et al. 2021). Several studies have demonstrated that dietary TRN addition to plant-based or FM-based diets for seabass might have a positive impact on growth performance and general health status of fish (Martinez et al. 2004; Rimoldi et al. 2016; Martins et al. 2018, 2019, 2021, 2023; Ceccotti et al. 2019). In addition, Wassef et al. (2021) suggested that TRN has immuno-regulatory properties in juvenile seabass when administrated at the appropriate level (1%). Feidantsis et al. (2014) showed that TRN-enriched feeds (up to 1%) had



highly protective mechanisms, particularly in the liver of seabass. It has been reported that dietary TRN supplementation can reduce intestinal inflammatory and apoptotic processes in seabass and improved the antioxidant response (Martins et al. 2019; Sampath et al. 2020b). For example, Rimoldi et al. (2016) found that 0.2% TRN led to some mitigating effects on the inflammation of the distal intestine of seabass formed after being fed a 30% soy protein diet

In contrast, other studies have illustrated that reduced-FM diets supplemented with 1% TRN did not significantly improve juvenile seabass growth or feed utilization efficiency (Feidantsis et al. 2014; Coutinho et al. 2017; Kotzamanis et al. 2020a, b).

A high volume of work has been published on the beneficial effects of TRN-enriched diets on the growth and efficiency of feed utilization for other fish species, particularly for fish fed with high plant protein-containing diets. Among them: Atlantic salmon (*Salmo salar*) (Espe et al. 2012), turbot (*Scophthalmus maximus*) (Qi et al. 2012); Nile tilapia (Al-Feky et al. 2016a, b; Shen et al. 2018), white seabream (*Diplodus sargus*) (Magalhães et al. 2019), grouper (*Epinephelus icoioides*) (Shen et al. 2019); and African catfish (Adeshina and Abdel-Tawwab 2020).

Astaxanthin

Astaxanthin (AST) is a naturally occurring carotenoid in the marine environment, classified as a xanthophyll that provides the rich pink colour observed in various plant and aquatic animal species, including salmon muscles and the carapace of shrimps, crabs, and lobsters (Naguib 2000; Yamashita 2013). The green microalgae *Haematococcus pluvialis* and *Monoraphidium* spp. and the red yeast *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* are among the microorganisms that can biosynthesize AST in appreciable amounts, and they have gained considerable interest as sources of natural AST (Akhavan and Jafari 2017). However, the highest level of AST produced by the yeast was lower than the amount found in microalgae or crustaceans (Yamashita 2013), so these microalgae may offer a continuous source of AST, when mass produced. AST is a nutrient (an oxidized form of β -carotene) with unique cell membrane actions and diverse benefits and therefore commercially used in various industries such as poultry, aquaculture, food, cosmetics, pharmaceuticals, and nutraceuticals (Yamashita 2013; Lim et al. 2018).

Rahman et al. (2016) and Lim et al. (2018) reported that fish, like other animals, have poor ability to biosynthesize their own AST demand *de novo* and must obtain this pigment from their diet. AST is perhaps the best-known pigmentation agent routinely added to certain aquafeeds to boost and enhance the coloration of some farm-raised species such as ornamental fish (Bjerkeng 2008) and shrimp (Zhang et al. 2013; Wade et al. 2017). The natural pigmentation of the flesh and skin of farmed fish caused by AST is considered an important consumer criterion or quality and price.

Numerous published studies have shown that AST, as a carotenoid, is a potent antioxidant equal to or greater than that of vitamin E when supplemented to fish diets, and exhibits various benefits, among which improving flesh or skin colour (Naguib 2000; Yamashita 2013; Lim et al. 2018; Yilmaz et al. 2013). AST, predominantly through its antioxidant effects, enhances the immune response and protects fish vision (Higuera-Ciapara et al. 2006; Li et al. 2007; Yamashita 2013; Lim et al. 2018). Because AST has unique bioactive properties, it exerts several functions in fish, including growth enhancement (Storebakken and Goswami 1996), improvement of broodstock performance (Ahmadi et al. 2006; Palma et al. 2017), immune function, and increased resistance to disease (Amar et al. 2012; Yamashita 2013; Lim et al. 2018).

Due to the high need for AST in the animal feed sector, particularly aquaculture and poultry, AST is chemically synthesized on a large scale. In terms of pigmentation, the microbial sources of carotenoids are comparable to those from synthetic sources (Gomes et al. 2002). It is worth noting that some authors suggest that esterified AST sourced from algae could be twice as effective as synthetic AST for the pigmentation of red seabream (*Pagrus major*) (Higuera-Ciapara et al. 2006), in addition to providing a better growth rate in European seabass (Saleh et al. 2018). Nevertheless, the interest shown by the aquaculture industry for natural sources of AST has been growing because of the increasing demand for fish fed on natural pigments (Lim et al. 2018).

Saleh et al. (2018) investigated the effect of feeding European seabass with three concentrations of AST (60, 80 and 100 mg/kg diet) for 60 days. The results indicated that fish fed diets with 100 mg of AST exhibited a significant improvement in growth rate, feed utilization efficiency, percentage survival, and total



protein content. Furthermore, as the amount of dietary AST increased, the activity of the hepatic anti-oxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx) decreased. The results also revealed that by increasing the AST inclusion levels in diet, an increase in the intestinal mucosal phagocytic and lysozyme activities was observed, which indicate the efficiency of AST as an immuno-stimulant agent in seabass diet. Goda et al. (2018) compared the effects of three dietary concentrations of two natural carotenoid sources, namely marigold flower meal and crab waste meal, with two synthetic AST-supplemented diets. Their results indicated that the dietary inclusion of crab waste meal or marigold flower meal at levels of 10 or 0.2 g/kg diet, respectively, could improve growth, increase total carotenoid content, increase antioxidant biomarkers and reduce the stress of European seabass fry as efficiently as the commercial synthetic AST. They concluded that the total carotenoid content in seabass muscles significantly increased with increasing dietary carotenoid levels, regardless of the carotenoids source. Both studies have demonstrated that the improvement in growth performance parameters of seabass fed with diets supplemented with AST may be due to its major role as an antioxidant and its involvement in intermediary metabolism of fish (Yamashita 2013; Lim et al. 2019). AST may further adjust the capabilities of intestinal microflora to break down the indigestible components to extract more nutrients and increase the activity of digestive enzymes (James et al. 2006).

The advantages of using AST-enriched diets were also evidenced for other fish species, including rainbow trout juveniles (Rahman et al. 2016), Asian seabass (*Lates calcarifer*) (Lim et al. 2019; Lim et al. 2021), red porgy (*Pagrus pagrus*) (Kalinowski et al. 2011), gilthead seabream (*Sparus aurata*) (Wassef et al. 2010; Li et al. 2014; Ritu et al. 2023).

Marine macroalgae

Algae (micro- and macro-algae) are natural aquatic plants with great potential as aquafeed ingredients, because of their high contents of nutrients and bioactive compounds (Ferreira et al. 2021). Therefore, the use of algae in livestock and human nutrition was emphasized several years ago (Montgomery and Gerking 1980). Global production of macroalgae, in general, has been dominated by marine species that grow in both marine and brackish waters and show highly variable nutritional value (Wan et al. 2019; Camarena-Gómez et al. 2022).

Marine macroalgae are rich sources of several nutritional components with great potential to increase the health status of farmed fish (Naiel et al. 2020; Ferreira et al. 2021; Fernandes et al. 2022). They contain various bioactive compounds such as carotenoids, polyunsaturated fatty acids, alginate, agar, carrageenan, laminarin and fucoidan and peptides such as taurine, carnosine, and glutathione (Naiel et al. 2021; De Alencar et al. 2018). They are also rich in several vitamins such as B1, B12, A, C, E, D, pantothenic acid, riboflavin, niacin, and folic acid and several minerals (iodine, zinc, sodium, calcium, manganese, iron, potassium, phosphorus, and selenium) (Øverland et al. 2019; Morais et al. 2020; Naseem et al. 2021; Ferreira et al. 2021). Primarily, marine macroalgae constitute one of the richest natural sources of antimicrobial and antioxidant (Wan et al. 2019; Silva et al. 2020; Naiel et al. 2021; Ferreira et al. 2021). Therefore, the use of macroalgae as feed additives in fish feeds has been reviewed by many authors (Øverland et al. 2019; Wan et al. 2019; Morais et al. 2020; Naiel et al. 2020; Saleh 2020; Fernandes et al. 2022; Ferreira et al. 2021; Camarena-Gómez et al. 2022; Abel-Latif et al. 2022) among others.

Although macroalgae are promising ingredients for aquafeeds, their complex polysaccharide structure limits their wide use, particularly for strictly carnivorous fish species (Fernandes et al. 2022). In addition, macroalgae have complex cell walls, which may contain antinutritional factors that may reduce nutrient uptake or result in inflammation of the intestinal tract of fish (Batista et al. 2020b). Castillo and Gatlin (2015) recommended the addition of exogenous carbohydrases to plant-based fish diets, to improve nutrient digestibility and reduce nutrient excretion in aquatic species. However, Fernandes et al. (2022) suggested three pretreatments techniques (physical, chemical and biological) to disrupt algal polysaccharide structure, to enhance macroalgal bioavailability in fish, such as European seabass. Overall, the results published on the use of macroalgal meals as feed ingredients for finfish have varied, presumably due to variation in macroalgal species, nutritional contents during harvest season, geographical origin, processing, and experimental design (Øverland et al. 2019). Some species of macroalgae can also accumulate large amounts of heavy metals (such as arsenic, cadmium or lead) which may limit their use in aquafeeds (Abdallah 2008).



However, the measured levels of these heavy metals in many macroalgae species are generally below the feed safety limits (Holdt and Kraan 2011; Irkin 2019).

Nutritional studies have also been performed on some farmed fish species, to unravel the impact of supplementation of some selected marine macroalgae and their beneficial effects were reported in terms of growth performance, immune and antioxidant status, and resistance to stress and bacterial diseases. *Ulva* species (sea/water lettuce) (Chlorophyta), red *Gracilaria* spp., and *Pterocladia capillacea* (Rhodophyta) are among the promising marine macroalgae that are distributed worldwide (Khalafalla et al. 2015; Ismail et al. 2020; Patarra et al. 2019), and have been tested as feed supplement in aquafeeds for many fish species (Wassef et al. 2001, 2005, 2013; Fernandes et al. 2022).

Regarding European seabass, the possibility of using *Ulva* spp., *Gracilaria* spp., and *Pterocladia capillacea* in their compounded feeds was investigated earlier, and several reports have been published (Valente et al. 2006; Wassef et al. 2013; Peixoto et al. 2016; Peixoto et al. 2019; Batista et al. 2020a, b; Fernandes et al. 2022). Two studies determined the nutritional value of dried *Ulva lactuca* and *P. capillacea* (protein, lipid, carbohydrates, and fiber contents; fatty acid and amino acid profiles) and tested three dietary inclusion levels (5, 10, 15%) for fish fry (Wassef et al. 2013). They suggested the dietary inclusion of 5% dried *U. lactuca* or *P. capillacea* to promote growth, feed utilization efficiency, and reinforce stress resistance of European seabass fry (0.5 g). Recently, the same supplementation level of 5% of dried *Ulva* spp. was assessed by Morais et al. (2020) to be adequate in complete pelleted fish diets. However, Valente et al. (2006) recommended that *Ulva* sp. and *Gracilaria* spp. can be incorporated up to 10% into feeds of European seabass (IW, ca 5g) without affecting the performance of fish. Peixoto et al. (2016) tested a control diet against six practical diets supplemented either with *Ulva* spp., *Gracilaria* spp., or *Fucus* spp., at 2.5 or 7.5% levels, plus an additional diet with a blend of the three seaweeds, each supplemented at 2.5% (*Mix*). Their results indicate that the use of dietary seaweed supplementation alone or in combination improves immune and antioxidant responses in European seabass without compromising growth performance. In another study, Peixoto et al. (2019) investigated the effect of dietary supplementation of 5% *Gracilaria* spp. aqueous extract (GRA) on European seabass antioxidant capacity and resistance against bacterial infection (*Photobacterium damsela*). They recorded a lower mortality rate in fish fed GRA, increased lysozyme activity levels, and decreased lipid peroxidation, suggesting higher antioxidant capacity than in fish fed the control diet. They added that dietary GRA induced the down-regulation of hepatic stress-responsive heat shock proteins (*grp-78*, *grp-170*, *grp-94*, *grp-75*), and positively modulate gut health and potentiate fish digestive capacities. More recently, Bassos et al. (2021) evaluated the inclusion of either dried algae *Gracilaria gracilis* at 2.5% and 5% or algal extract at 0.35% levels in the diet of European seabass and concluded that the supplementations did not affect growth or feed utilization, haematological profile or some metabolic parameters. Nevertheless, dried algae powder (*G. gracilis*), with a 2.5% inclusion, boosted the immune response and protected against oxidative damages by improving enzymatic and non-enzymatic responses. However, the ability of European seabass to effectively digest nutrients from macroalgae species (e.g., *Ulva rigida*) is hampered by the high carbohydrate content which negatively influences fish digestive enzyme activity (Fernandes et al. 2022). Accordingly, these later authors applied chemical, physical and biological treatments on *U. rigida*, before feeding it to European seabass, which successfully increased the protein content and hydrolyzed the polysaccharides structure of the tested alga. They concluded that solid state fermentation (SSF) followed by sequential hydrolysis was the more efficient treatment.

Detailed information on the effects of numerous marine macroalgae on other finfish species has been previously reported in many comprehensive reviews (Øverland et al. 2019; Van Doan et al. 2023; Ritu et al. 2023).

Conclusions and future recommendations

The inclusion of organic acids/salts as acidifiers in European seabass aquafeeds can exert multiple beneficial effects. Therefore, these acidifiers could provide a safer and potent feed supplement to benefit health status, reinforce the immune response, enhance nutrients utilization and survival, implying a strong basal health-promoting effect on European seabass. The recommended doses are: 2g microencapsulated sodium butyrate/kg diet, 3g sodium propionate or sodium/potassium diformate/kg diet. However, the appropriate dietary-supplementation level of taurine was evidenced to be 1.0% to promote juveniles seabass perfor-



mance and improve muscle quality even when fed a FM-based diet. Astaxanthin was proven to be a qualified feed additive for seabass fry when added at 0.1 g/kg diet, to promote growth, increase survival, induce health benefits and reduce the impact of stressors with no adverse effects on fish performance. With regards to marine macroalgae, dried *Ulva lactuca* and *Pterocladia capillacea* could be used as feed supplements (at 5%) to enhance seabass fry performance, nutrient composition, and stress resistance. Dried algae *Gracilaria gracilis* powder, with a 2.5% inclusion, boosted the immune response and protected against oxidative damages by improving enzymatic and non-enzymatic responses of fish. Solid state fermentation (SSF) of *U. rigida* was evidenced to be an efficient pretreatment to improve feed efficiency of seabass juveniles without affecting growth performance. Finally, the aforementioned studies, in feed-supplements in seabass aquaculture, emphasized the need for further research to better understand their use and potential for enhancing the sustainability and efficiency of aquaculture operations.

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