



# Modelling the effect of prebiotics, probiotics and other functional additives on the growth, feed intake and feed conversion of European sea bass (*Dicentrarchus labrax*) juveniles

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## ABSTRACT

Dietary supplementation of aquafeeds with functional additives is a commonly employed strategy in order to reduce the potential negative effects associated to fishmeal (FM) and fish oil (FO) replacement by alternative protein and oil sources. Nevertheless, the wide variety of functional ingredients with different bioactive properties hinders the selection of appropriate dietary supplementation strategies on feed formulation. The present study aimed to develop an observational multiple-linear regression (MLR) model to identify the effects of a variety of functional ingredients supplementation on European sea bass juveniles (*Dicentrarchus labrax*) growth performance and feed utilization. A literature survey was conducted gathering a total of 61 dietary treatments. The functional ingredients were classified in three main groups, namely, “probiotics”, “prebiotics” or “others” (including plant derived compounds such as essential oils, extracts and powders). Three different MLR were obtained and validated, allowing to describe the effects of functional ingredients supplementation on fish specific growth rate (SGR) (with a final R-squared (R<sup>2</sup>) = 0.96, adjusted R-squared (adj R<sup>2</sup>) = 0.92 and a p-value= 7.21E-08), fish feed intake (FI) (R<sup>2</sup> = 0.97, adj R<sup>2</sup> = 0.95 and a p-value= 5.42E-12) and fish feed conversion ratio (FCR) (R<sup>2</sup> = 0.90, adj R<sup>2</sup> = 0.80 and a p-value= 2.02E-05). MLR model trimming, allowed the detection of a significant positive correlation (cor) between dietary prebiotics supplementation and SGR (cor= 0.32, p-value= 8.52E-04). On the contrary, prebiotic supplementation presented a negative correlation with fish FI (cor= -0.44, p-value= 6.27E-05) and FCR (cor= -0.41, p-value= 8.96E-05).

## 1. Introduction

The aquaculture industry has been facing new challenges in recent years to achieve a more economically and environmentally sustainable production. In this sense, a great effort has been made in the development of dietary strategies based on alternative protein sources that aim to reduce the dependence on marine raw materials (Fiorella et al., 2021). Nevertheless, feed formulation with terrestrial raw materials or other alternative protein sources such as terrestrial by-products and insects (Luthada-Raswiswi et al., 2021), may induce nutritional imbalances negatively affecting feed utilization and thus fish growth and health (Montero and Izquierdo, 2010; Schreck and Tort, 2016). Altogether, can lead to increased production costs, lower growth yields and higher amounts of feed requirements in a production cycle.

The deleterious effects associated to the reduction of fishmeal (FM) and fish oil (FO) in fish diets are of relevance in carnivorous fish species (Naylor et al., 2021), such as the European sea bass (*Dicentrarchus labrax*). Previous studies have reported a suitable reduction of up to 7.5 % FM on European sea bass diets without impairing growth performance compared to a control diet with 31.5 % FM (Campos et al., 2017). Nevertheless, fish health and welfare may also be compromised by FM and FO replacement, reducing pathogen resistance (Torrecillas et al., 2017a) and fish stress tolerance (Torrecillas et al., 2017b).

Dietary supplementation with functional additives is a well-known strategy to offset those negative effects (Kader et al., 2010; Estensoro et al., 2016; Torrecillas et al., 2018, 2019). Functional additives are compounds with the ability to enhance fish growth performance, health, and welfare by increasing, for example, nutrient digestibility and

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**Table 1**  
Explanatory quantitative variables employed for full linear regression models fitting.

Abbreviation	Quantitative variable	Units
Temp	Temperature	°C
Oxygen	Dissolved oxygen	gpm
ABW	Average body weight	g
FI_norm	Normalized individual feed intake	% body weight/day
diet_CP	Dietary crude protein	g/kg dry weight
diet_GE	Dietary crude energy	MJ/kg
diet_CP/GE	Dietary Protein to Energy level	g/MJ
diet_CL	Dietary crude lipid	g/kg dry weight
diet_moisture	Diet moisture	%
diet_ash	Dietary ash content	g/kg dry weight
diet_Prebiotics	Dietary prebiotics	g/kg dry weight
diet_Probiotics	Dietary probiotics	g/kg dry weight
diet_Others	Dietary "others" additives	g/kg dry weight

bioavailability, stimulating fish immune response and tissue integrity, and increasing fish stress resistance (Hoseinifar et al., 2021). Among the wide range of active substances, which can be used as functional additives, probiotics are live microbes with the ability to promote fish health by enhancing the internal microbiome balance. Several studies have investigated the effects of dietary probiotics supplementation reporting positive effects on fish growth (Geng et al., 2011; Ramos et al., 2017) and nutrient utilization (Sáenz de Rodríguez et al., 2009; Liu et al., 2012; Bunnay et al., 2019; Hooshyar et al., 2020). However, there is a high variability on the reported effects regarding fish growth performance depending on the probiotic supplemented, dose, intake duration as well as the target fish species (Aly et al., 2008; He et al., 2013; Liu et al., 2013; Adeoye et al., 2016).

Other functional additives used in fish dietary supplementation are the prebiotics. Prebiotics are plant derived indigestible fibers that could selectively enhance a limited number of intestinal bacteria species (Hoseinifar et al., 2015), directly benefiting host health and feed utilization (Mazurkiewicz et al., 2008; Ebrahimi et al., 2012; Gültepe et al., 2011; Wu et al., 2013). Prebiotic supplementation has also been reported to promote growth of a wide variety of fish species (Torrecillas et al., 2007; Zhou et al., 2009; Şara et al., 2010; Soleimani et al., 2012; Guerreiro et al., 2018; Torrecillas et al., 2018). Meanwhile, other studies did not detected positive effects on fish growth performance (Grisdale-Helland et al., 2008; Řehulka et al., 2011; Serradell et al., 2020), again depending mainly on the prebiotic, dose, period of supplementation and fish species studied. Among the plant derived bioactive compounds a third group of functional ingredients can be found, the phytonutrients, which include essential oils, extracts and powders. Those functional additives contain high concentrations of secondary metabolites with beneficial effects, enhancing fish health and improving growth in different fish species (Bello et al., 2012; Abdel-Latif et al., 2020; Rashidian et al., 2021; Yousefi et al., 2021). On the other hand, as occurs with pro- and prebiotics, there is a wide range of results observed depending on the phytonutrient studies, fish species, dose and feeding strategy tested in terms of growth performance, including neutral or non-beneficial ones (Motlagh et al., 2020; Tasa et al., 2020; Fernández-Montero et al., 2021).

The wide variety of functional ingredients, the different effects associated with the level of inclusion and their still unclear mechanisms hinder the selection of appropriate strategies for dietary supplementation. Additionally, the experimental conditions in the different studies that analyze the effects of functional ingredients on fish health and growth are highly variable, preventing the direct comparison of the results obtained. In this sense, mathematical modeling has been identified as a powerful tool to analyze fish growth and feed utilization in response to different variations in diet composition and culture conditions (Van Dam, 1990; Galkanda-Arachchige et al., 2020; Luthada-Raswiswi et al., 2021). For example, regression models can be used to deduct the effects of factors that vary between studies (confounding

variables) and therefore obtain normalized estimates of fish responses to different inclusion levels of functional ingredients. Thus, the objective of the present study was to develop an observational multiple-linear regression model to robustly isolate the effects of dietary functional ingredients may have on growth and feed utilization parameters of European sea bass juveniles by simultaneously considering the results of several growth trials performed under different contexts.

## 2. Materials and methods

### 2.1. Literature survey and selection criteria

A literature survey was conducted employing the bibliographic databases Web of Science (-, 2022) and Scopus (-, 2022). The search for relevant bibliography was carried out following the title, abstract and keywords search strings: ("Dicentrarchus labrax" OR "European sea bass" OR "European seabass") AND ("juveniles" OR "fry") AND ("functional feeds" OR "functional ingredients" OR "functional diets" OR "diet supplementation" OR "probiotics" OR "prebiotics" OR "phytonutrients" OR "essential oils" OR "plant derived compounds" OR "phytonutrient feed additives" OR "synbiotics").

To be selected for compilation, studies had to meet the following criteria: (I) experiments carried out with European sea bass juveniles; (II) studies focused on dietary supplementation with probiotics, prebiotics and/or plant derived compounds; (III) at least one of the following growth and feed utilization parameters had to be reported in the study: specific growth rate (SGR), feed conversion ratio (FCR) and/or feed intake (FI); (IV) fish growth information, including initial (IBW) (g) and final body weight (FBW) (g), and a measure of dispersion in relation to the mean value (e.g., standard deviation [SD]); (V) feeding trial duration; (VI) dietary treatments composition or at least proximal composition, including dietary protein (diet\_CP) and energy (diet\_GE) concentrations; (VII) culture conditions information (at least mean water temperature and dissolved oxygen).

### 2.2. Data conditioning and analysis

Prior to data analysis, all the information gathered from the different studies was converted to standard units, expressing the different variables as: fish body weight (g); fish body length (cm); average body weight (ABW) (g); individual feed intake (g per fish day<sup>-1</sup>); water temperature (°C); water dissolved oxygen (ppm); tank volume (L); duration (days); dietary ingredients (% diet); dietary chemical composition (% diet); diet gross energy (MJ/kg). To facilitate data analysis, the different functional additives were classified in three global groups, namely, "probiotics", "prebiotics" or "others" (including plant derived compounds or symbiotic compounds).

Since fish are poikilothermic animals (Bell et al., 1986), important traits as feed intake and growth rate are conditioned by water temperature. Thus, in order to evaluate the effect of the inclusion of functional additives on fish growth and conversion efficiency, relevant responses were normalized to remove the effect of important confounding variables (i.e., fish size and temperature) employing the formula:

$$\text{normalized trait} = \frac{\text{measured raw trait}}{\text{maximum trait value}}$$

where the *measured raw trait* consisted of the value obtained from the direct calculation of the different parameters as follows:

$$\text{SGR (day}^{-1}\text{)} = [\ln(\text{FBW}) - \ln(\text{IBW})] / \text{days},$$

$$\text{FI (\% body weight/day)} = [(\text{individual feed intake}) / (\text{IBW} + \text{FBW}) / 2] \times 100,$$

$$\text{FCR (g feed intake/g weight gain)} = \text{individual feed intake} / (\text{FBW} - \text{IBW}),$$

and the *maximum trait value* consisted of the value obtained from three

**Table 2**  
Effects of different functional ingredients on growth performance and feed efficiency of European sea bass (*Dientrarchus labrax*) juveniles.

Group	Functional additive	Inclusion method	Dose	Duration	Dietary crude protein content (%)	Dietary Protein/Energy ratio	Effects	Reference
Probiotics	Bactocel PA10 (Lamelland SAS, Canada) ( <i>Pediococcus acidilactici</i> , strain CNCM I-4622)	Grounded and mixed before extrusion	2, 2.5 or 3 g/kg $\sim 1 \times 10^{10}$ CFU	100 days	45.5	2.25	– ↑ FBW, WG, SGR – No differences in FCR	Eissa et al. (2022)
	AquaStar Growout (BIOMIN Holding GmbH) (commercial probiotic blend of <i>Bacillus</i> sp., <i>Lactobacillus</i> sp., <i>Enterococcus</i> sp. and <i>Pediococcus</i> sp.)	Added as mash feed	3 g/kg $\sim 5.23 \times 10^8$ CFU/kg.	100 days	47.3	2.14	– No differences on FBW, DGI or protein efficiency ratio	Pereira et al. (2018)
	MIX-AVI® pro( IVS-Wynco LLC, Springdale, AR, USA) ( <i>L. plantarum</i> )	Sprayed after extrusion	Sprayed $10 \times 10^9$ CFU/ kg	90 days	42.25	2.10	– ↑ Survival – No differences on FBW, SGR or FCR	Piccolo et al. (2015)
	Bactocel PA10 (Lamelland SAS, Canada) ( <i>Pediococcus acidilactici</i> , strain CNCM I-4622)	-	-	60 days	47.20	1.97	– No differences on FBW and length	Torreillas et al. (2018)
Prebiotics	Fructo-oligosaccharide or xylo-oligosaccharide	-	1 g/kg	49 days	45.95	2.09	– ↑ FBW – ↑ WG on low protein diets	Guerreiro et al. (2015)
	Mannan-oligosaccharides (Bio-Mos, Alltech Inc.)	-	2,4 or 6 g/kg	60 days	48.71	-	– No differences on FBW, RG, K or SGR – ↓ FCR on fish fed 4 and 6 g/kg – ↓ FI	Torreillas et al. (2011)
	MOS (Bimos® and Actigen® second and generation of MOS; Alltech, Inc., Kentucky, USA)	-	3 or 6 g/kg MOS	60 days	47.20	1.97	– ↑ FBW and length	Torreillas et al. (2018)
	Galactomannan-oligosaccharides (GMOS) (Delacon, Austria)	Grounded and mixed before extrusion	5 g/kg	63 days	47.87	2.04	– No differences on FBW, SGR or FCR – ↑ Resistance <i>V. anguillarum</i>	Torreillas et al. (2019)
Others	Anise ( <i>Pimpinella anisum</i> L.)	Powdered and mixed with fish oil	1.5, 2.5 and 3.5 g/kg	120 days	44.06	2.06	– ↑ FBW, WG and SGR – ↑ FBW, WG and protein efficiency ratio with increasing levels of supplementation	Ashry et al. (2022)
	SSF-BSG (solid-state fermentation of brewer's spent grain) (Unicer-Bebidas de Portugal, S.A. (Matosinhos, Portugal))	Grounded and mixed before extrusion	4 and 8 g/kg	64 days	47.90	1.98	– No differences on FBW or body composition – Lower feed intake	Fernandes et al. (2022)
	Digestarom PEP MGE150 (Biomin Holding GmbH, Austria) (Anise, citrus and oregano essential oils)	Added as mash feed	2 g/kg	60 days	47.30	2.14	– ↑ SGR	Gonçalves et al. (2019)
	Cinnamon	Powder	10, 15 or 20 g/kg	90 days	46	2.15	– ↑ FBW, WG, and protein efficiency ratio – ↑ SGR on fish fed 10 g/kg – ↓ FCR on fish fed 15 and 20 g/kg	Habiba et al. (2021)
	Garlic meal	Grounded and mixed before extrusion	20, 40 and 60 g/kg	60 days	43.03	2.25	– ↑ FBW on fish fed 40 g/kg	İrkin and Yigit (2015)
	<i>Yucca schidigera</i>	Grounded and mixed before extrusion	0.25, 0.5 or 1 g/kg	45 days	44.82	2.21	– ↑ FBW, WG and SGR – ↓ FCR with increasing supplementation	Mansour et al. (2021)
	Synbiotic [(MOS, Bimos® and Actigen® (second generation of MOS; Alltech, Inc., Kentucky, USA) + <i>Pediococcus acidilactici</i> (BAC, Bactocel®; Lallemand Inc., Cardiff, UK)	-	3 or 6 g/kg Bimos	60 days	47.20	1.97	– ↑ FBW and length	Torreillas et al. (2018)
	Mixture of garlic and labiate plant essential oils (PHYTO) (Delacon, Austria)	Vacuum coating	5 g/kg	63 days	47.87	2.04	– No differences on FBW, SGR or FCR – ↑ Resistance <i>V. anguillarum</i>	Torreillas et al. (2019)
Carvacrol (5-isopropyl-2-methylphenol) (cod. 282197; Sigma-Aldrich, Milan, Italy)	Diluted in fish oil	2.5 and 5 g/kg	63 days	51.30	2.30	– No differences on FBW, WG, SGR or FCR	Volpatti et al. (2013)	

(continued on next page)

Table 2 (continued)

Group	Functional additive	Inclusion method	Dose	Duration	Dietary crude protein content (%)	Dietary Protein/Energy ratio	Effects	Reference
	Dried leaf powder of thyme ( <i>Thymus vulgaris</i> L.) or rosemary ( <i>Rosmarinus officinalis</i> L.) or seed powder of fenugreek ( <i>Trigonella foenum graecum</i> L.)	Powder	10 g/kg	45 days	48.43	2.23	– No differences on FBW, SGR, FCR or fat retention – Thyme increased protein efficiency ratio, protein retention, energy retention and fillet protein composition	Yilmaz et al. (2012)

↑ increased in comparison to not supplemented diet. ↓ reduced in comparison to not supplemented diet. FBW (final body weight); WG (weight gain); SGR (specific growth rate); FCR (feed conversion ratio); DGI (daily growth index); FI (feed intake)

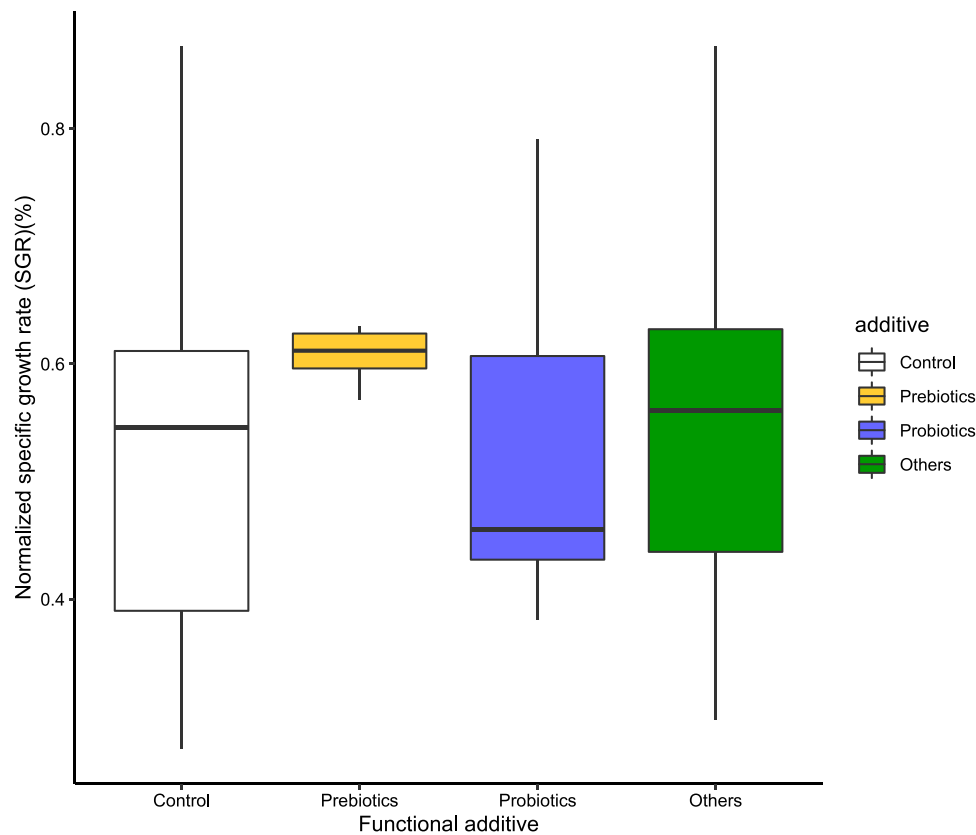


Fig. 1. Boxplot of functional ingredients dietary inclusion effects on normalized specific growth rate values.

Table 3

Coefficients of linear regression obtained for the normalized specific growth rate (SGR\_norm) observational models.

Specific growth rate model	Equation	R <sup>2</sup>	Adj R <sup>2</sup>	Variable p-value	Model p-value
Simple model <sup>a</sup>	Exp (5.65e <sup>-2</sup> *(Temp) + 6.94e <sup>-5</sup> * (norm_FI) <sup>2</sup> + 0.71 * (diet_CP /diet_GE))	0.67	0.65	-	9.20e <sup>-10</sup>
Simple model w/ Prebiotics <sup>b</sup>	Exp (6.1e <sup>-2</sup> *(Temp) + 7.1e <sup>-5</sup> * (norm_FI) <sup>2</sup> + 0.81 * (diet_CP /diet_GE) + 0.32 * (diet_Prebiotics))	0.75	0.73	8.52e <sup>-4</sup>	5.3e <sup>-9</sup>
Simple model w/ Probiotics <sup>c</sup>	Exp (5.65e <sup>-2</sup> *(Temp) + 6.94e <sup>-5</sup> * (norm_FI) <sup>2</sup> + 0.71 * (diet_CP /diet_GE) - 4.22e <sup>-3</sup> * (diet_Probiotics))	0.67	0.63	0.96	5.3e <sup>-9</sup>
Simple model w/ Others <sup>d</sup>	Exp (6.58e <sup>-2</sup> *(Temp) + 7.1e <sup>-5</sup> * (norm_FI) <sup>2</sup> + 0.73 * (diet_CP /diet_GE) - 0.1 (diet_Others))	0.7	0.66	0.11	1.49e <sup>-9</sup>

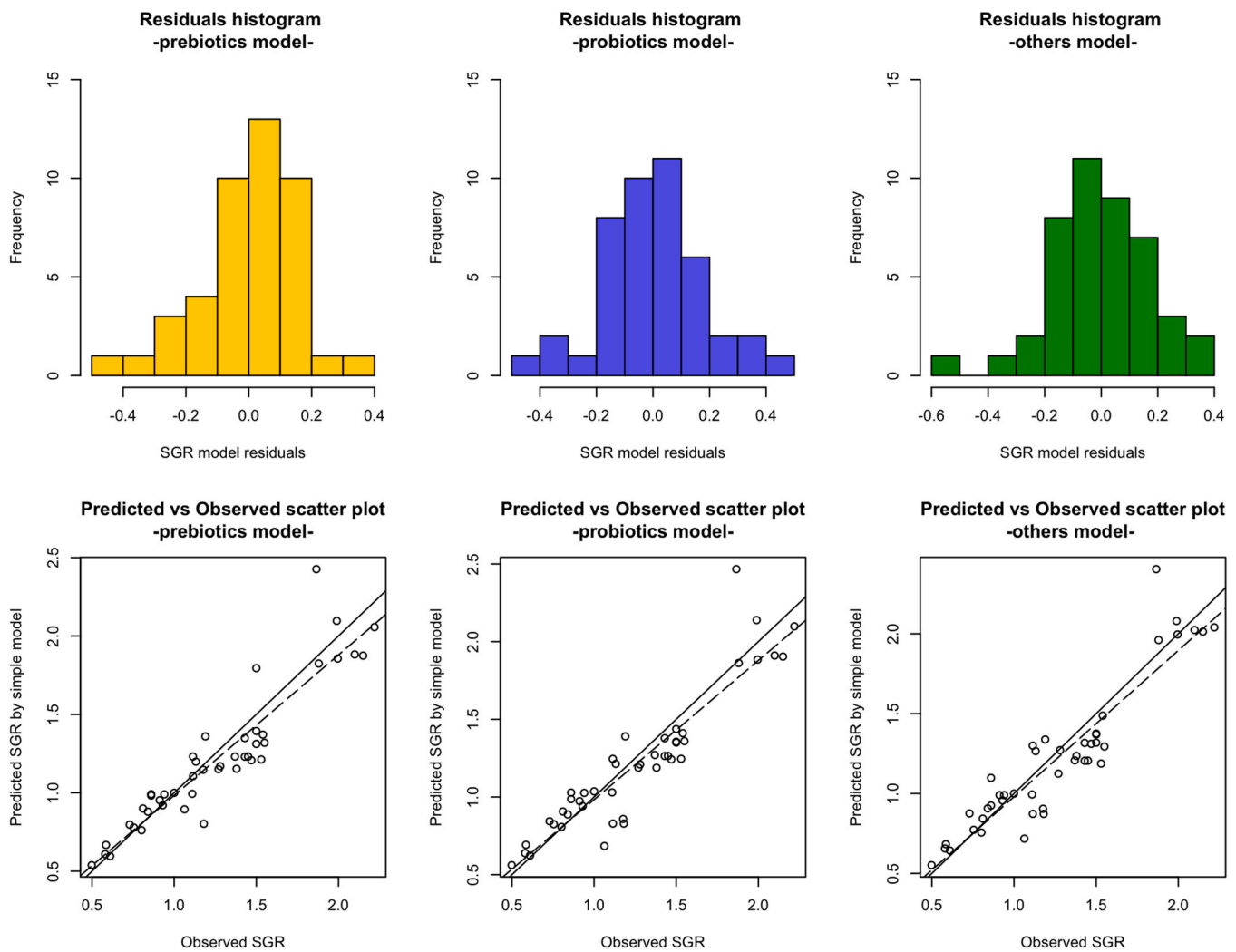
Temp (water temperature °C); norm\_FI (normalized fish individual feed intake (g /fish per day)); diet\_CP /diet\_GE (Protein – Energy ratio (g /MJ)); diet\_Prebiotics (dietary prebiotics content (g /kg)); diet\_Probiotics (dietary probiotics content (g /kg)); diet\_Others (dietary Others content (g /kg)).

<sup>a</sup> SGR simple model (trimmed simple model).

<sup>b</sup> SGR simple model with prebiotics dietary inclusion as quantitative variable.

<sup>c</sup> SGR simple model with probiotics dietary inclusion as quantitative variable.

<sup>d</sup> SGR simple model with Others dietary inclusion as quantitative variable.



**Fig. 2.** European sea bass (*Dicentrarchus labrax*) specific growth rate (SGR) distribution for a) prebiotics simple model residuals; b) probiotics simple model residuals; c) others simple model residuals; d) prebiotics simple model fitted values; e) probiotics simple model fitted values; f) others simple model fitted values.

reference models developed in the context of the AquaIMPACT EU project (Horizon 20/20) for the prediction of European sea bass growth, feed intake and feed conversion, which were provided by Sparos Lda. (Olhão, Portugal). The models used are described by the following equations:

$$\ln(\text{SGR}_{\max}) = -7.93079 + [0.50781 \times \ln(\text{ABW})] - [0.00133 \times \text{ABW}] - [0.09766 \times (\ln(\text{ABW})^2)] + [0.2524 \times \text{Temp}] - [0.0041 \times (\text{Temp})^2],$$

$$\ln(\text{FI}_{\max}) = 5.11608 + [0.61529 \times \ln(\text{ABW})] + [0.14896 \times \text{Temp}] - 0.00136 \times (\text{Temp})^2,$$

$$\text{FCR}_{\text{typical}} = 0.9036676 \times (\text{ABW})^{0.1082725}.$$

\*ABW (average body weight (g)) =  $(\text{IBW} \times \text{FBW})^{0.5}$ .

\*Temp (temperature (°C)).

These models were obtained using quantile regression (to estimate quantiles 0.95 for  $\text{SGR}_{\max}$  and  $\text{FI}_{\max}$ , and quantile 0.50 for  $\text{FCR}_{\text{typical}}$ ) of log transformed responses, based in an aggregated data base including information about European sea bass growth trials from 37 sources.

After model fitting employing the explanatory quantitative variables presented in Table 1, a stepwise backward selective regression was performed by iteratively adding and removing coefficients in order to find the simplest and best performing model (Agostinelli, 2002; Wang et al., 2007). The obtained equations were evaluated in reference to the

model selection criteria and residuals analysis. (Sanquetta et al., 2018).

### 3. Results

#### 3.1. Data base overview

Fifteen studies (Table 1) passed the minimum requirements in order to be eligible for the model database, adding up to a total of 61 dietary treatments to be used to define the present descriptive model. From the total 61 dietary treatments registered, 12 addressed the study of prebiotics, 13 of probiotics, 21 of “others” group and 15 treatments were void of supplementation (control diets). The data set covered a wide range of culture conditions, with temperatures ranging between 17 and 28 °C and oxygen concentrations ranging from 5.4 to 9.7 ppm. The database presented a range of fish body weight values between 4.69 and 130.30 g. The chemical composition of the diets used in the listed studies presented the following ranges of values: crude protein (42.00–51.30 % dry weight); crude lipids (12.56–28.90 % dry weight); gross energy (18.30–24.46 MJ/kg dry weight) and moisture (2.92–12.00 % wet weight). The duration of the experiments ranged from 45 to 120 days.

The final database included four studies focusing on the effects of dietary inclusion of probiotics (Piccolo et al., 2015; Pereira et al., 2018; Torrecillas et al., 2018; Eissa et al., 2022), four studies focusing on the

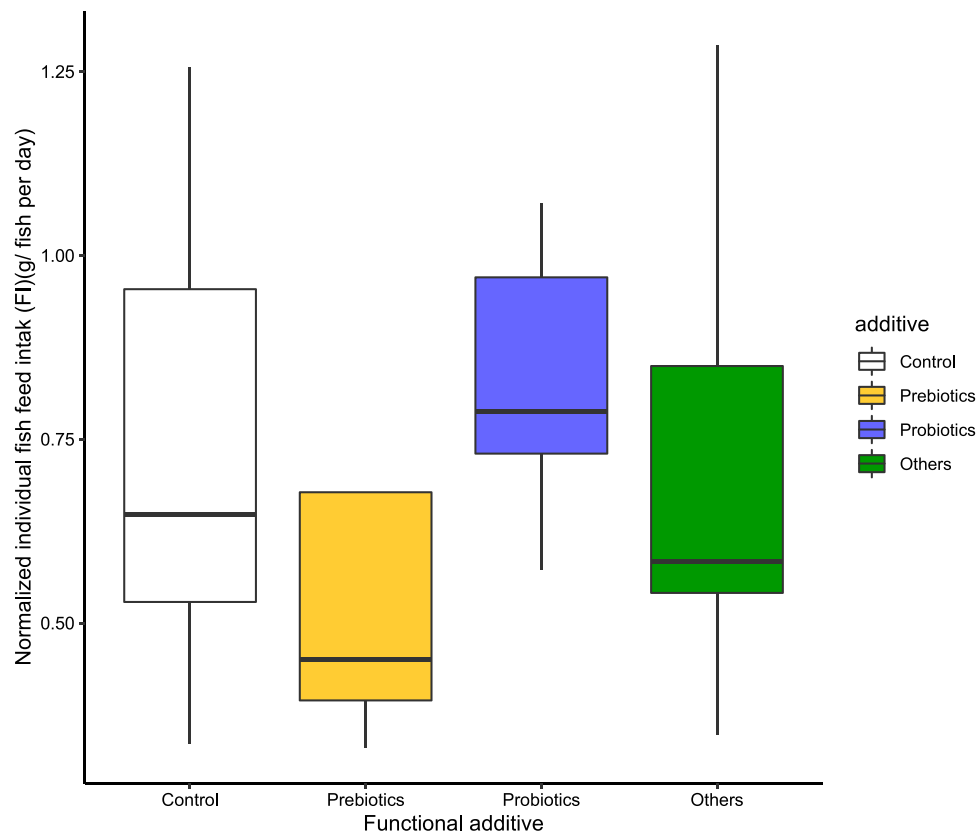


Fig. 3. Boxplot of functional ingredients dietary inclusion effects on normalized fish individual feed intake values.

Table 4

Coefficients of linear regression obtained for the normalized feed intake (FI\_norm) observational models.

Feed intake model	Equation	R <sup>2</sup>	Adj R <sup>2</sup>	Variable p-value	Model p-value
Simple model <sup>a</sup>	Exp (- 5.1e <sup>-2</sup> * (Temp) - 4.14e <sup>-3</sup> * (ABW) + 1.91 * (diet_CP) - 3.54 * (diet_GE) - 38.1 * ( diet_CP /diet_GE) + 5.75e <sup>-2</sup> * (diet_moisture))	0.75	0.7	-	6.45e <sup>-8</sup>
Simple model w/ Prebiotics <sup>b</sup>	Exp (- 3.85e <sup>-2</sup> * (Temp) - 3e <sup>-3</sup> * (ABW) + 2,1 * (diet_CP) - 4.28 * (diet_GE) - 41.5 * (diet_CP /diet_GE) + 5.33e <sup>-2</sup> * (diet_moisture) - 0.44 * (diet_Prebiotics))	0.86	0.82	6.27e <sup>-5</sup>	1.1e <sup>-10</sup>
Simple model w/ Probiotics <sup>c</sup>	Exp (- 4.37e <sup>-2</sup> * (Temp) - 4.37e <sup>-3</sup> * (ABW) + 1.87 * (diet_CP) - 3.84 * (diet_GE) - 37.13 * (diet_CP /diet_GE) + 5.12e <sup>-2</sup> * (diet_moisture) + 0.2 * (diet_Probiotics))	0.80	0.72	0.13	8.6e <sup>-8</sup>
Simple model w/ Others <sup>d</sup>	Exp (- 5.51e <sup>-2</sup> * (Temp) - 4.1e <sup>-3</sup> * (ABW) + 1.91 * (diet_CP) - 4 * (diet_GE) - 38.21 * (diet_CP /diet_GE) + 5.73e <sup>-2</sup> * (diet_moisture) + 3e <sup>-2</sup> * (diet_Others))	0.75	0.7	0.70	2.5e <sup>-7</sup>

Temp (temperature °C); ABW (average body weight (g)); diet\_CP (dietary protein content (%)); diet\_GE (dietary energy content (MJ/kg)); diet\_CP /diet\_GE (Protein – Energy ratio (g /MJ)); diet\_moisture (diet moisture content (%)); diet\_Prebiotics (dietary prebiotics content (g/kg)); diet\_Probiotics (dietary probiotics content (g/kg)); diet\_Others (dietary Others content (g/kg)).

<sup>a</sup> FI simple model (trimmed simple model).

<sup>b</sup> FI simple model with prebiotics dietary inclusion as quantitative variable.

<sup>c</sup> FI simple model with probiotics dietary inclusion as quantitative variable.

<sup>d</sup> FI simple model with Others dietary inclusion as quantitative variable.

effects of dietary inclusion of prebiotics (Torrecillas et al., 2011; Guereiro et al., 2015; Torrecillas et al., 2018; Torrecillas et al., 2019), and eleven studies focusing on the effects of dietary inclusion of plant derived compounds or synbiotics (Torrecillas et al., 2011; Yilmaz et al., 2012; Volpatti et al., 2013; İrkin and Yiğit, 2015; Torrecillas et al., 2018; Torrecillas et al., 2019; Habiba et al., 2021; Mansour et al., 2021; Ashry et al., 2022; Fernandes et al., 2022).

Half of the studies reported positive effects on fish growth parameters, associated to dietary supplementation with probiotics, prebiotics or others (i.e., plant derived compounds or synbiotics) (Table 2). Regarding feed utilization, 37.5 % of the selected studies described beneficial effects of functional ingredients on feed intake or FCR. Two studies reported lower feed intake rates associated to dietary supplementation (Torrecillas et al., 2011; Fernandes et al., 2022).

### 3.2. Correlation between dietary supplementation and specific growth rate (SGR)

The experiments employing prebiotics as functional ingredients typically presented higher normalized SGR than those employing other dietary treatments (Fig. 1).

Normalized individual SGR modelling with a complex multiple-regression obtained a total R-squared (R<sup>2</sup>) = 0.96, an adjusted R-squared (adj R<sup>2</sup>) = 0.92 and a p-value = 7.213e-08. The full model followed the equation:

$$\ln(\text{SGR}_{\text{norm}}) = 0.44 * (\text{Temp}) - 1.52e-2 * (\text{Temp})^2 + 0.33 * (\text{Oxygen}) - 5.91e-3 * (\text{ABW}) + 0.28 * \ln(\text{ABW}) - 1.46e-2 * (\text{FI}_{\text{norm}}) + 1.49e-4 * (\text{FI}_{\text{norm}})^2 + 0.5 * (\text{diet}_{\text{CP}}) - 1.12 * (\text{diet}_{\text{GE}}) - 9.8 * (\text{diet}_{\text{CP}} / \text{diet}_{\text{GE}}) - 0.16 * (\text{diet}_{\text{CL}}) +$$



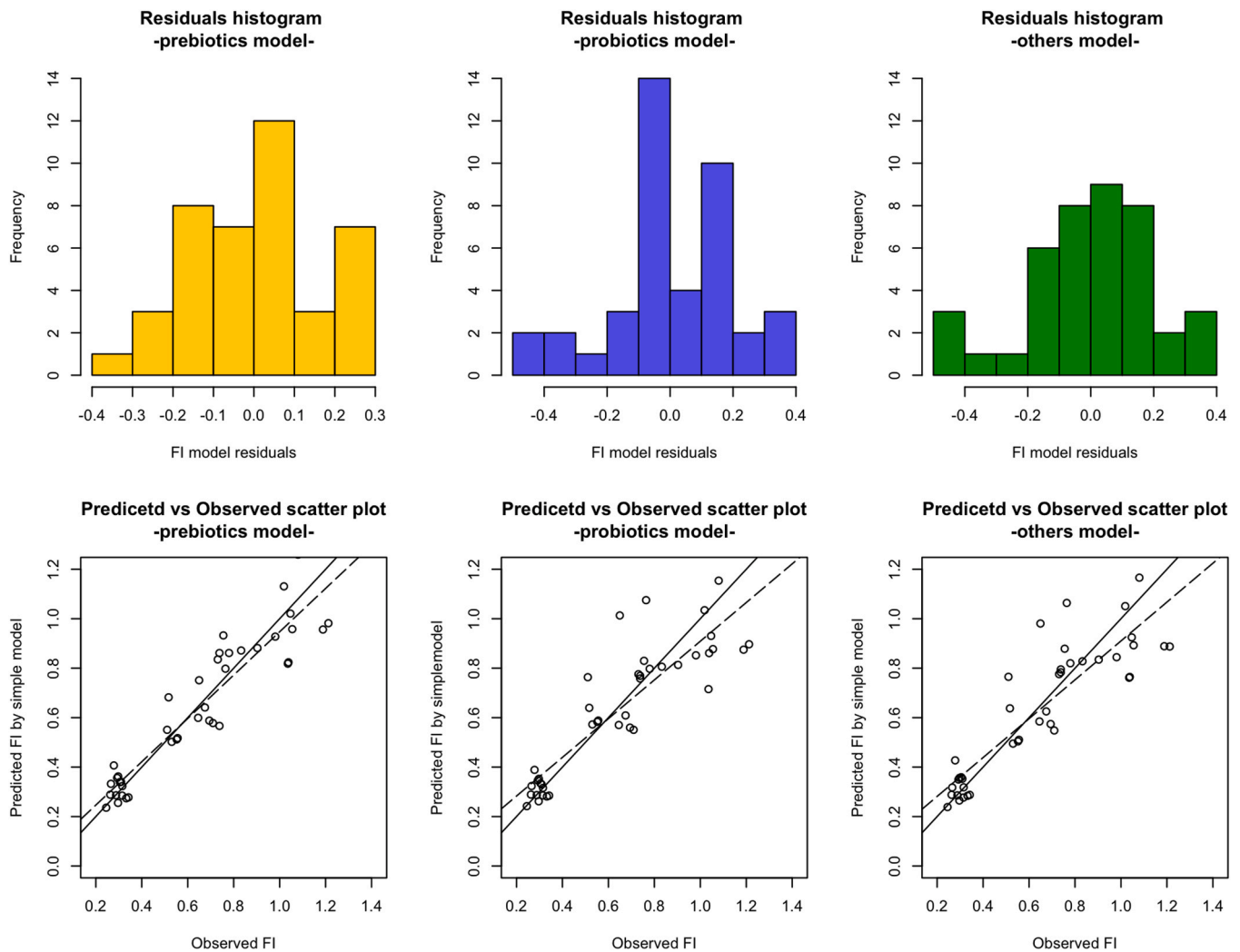


Fig. 4. European sea bass (*Dicentrarchus labrax*) individual feed intake (FI) distribution for a) prebiotics simple model residuals; b) probiotics simple model residuals; c) others simple model residuals; d) prebiotics simple model fitted values; e) probiotics simple model fitted values; f) others simple model fitted values.

$$3.1 e^{-2}*(diet\_moisture) - 2.23e^{-2}*(diet\_ash) + 8.57e^{-2}*(diet\_Probiotics) + 0.12*(diet\_Prebiotics) + 7.02e^{-2}*(Others)$$

in which “diet\_Probiotics”, “diet\_Probiotics” and “diet\_Others” are quantitative variables.

Model trimming resulted in a *Simple model* with a ( $R^2$ ) = 0.67, an adjusted R-squared ( $adj R^2$ ) = 0.65 and a  $p$ -value = 9.20E-10 (Table 3). The addition of dietary prebiotics (“diet\_Probiotics”) as descriptive variables significantly improved the *Simple model* selection criteria (Table 3) (Fig. 2).

### 3.3. Correlation between dietary supplementation and individual feed intake (FI)

The experiments employing prebiotics as functional ingredients presented significantly lower normalized FI values ( $p < 0.05$ ; one way ANOVA (presence /absence)) than those employing dietary treatments supplemented with probiotics (Fig. 3).

Normalized individual FI modelling with a complex multiple-regression obtained a total R-squared ( $R^2$ ) = 0.97, an adjusted R-squared ( $adj R^2$ ) = 0.95 and a  $p$ -value = 5.42E-12. The full model followed the equation:

$$\ln(FI\_norm) = -1.88*(Temp) + 4e-2*(Temp)^2 + 0.21*(Oxygen) - 9.1e^{-3}*(ABW) + 0.23*\ln(ABW) + 0.7*(diet\_CP) - 1.22*(diet\_GE) - 13.5(diet\_CP$$

$$/diet\_GE) + 0.16*(diet\_CL) + 0.06*(diet\_moisture) + 0.13*(diet\_ash) + 1.76*(diet\_Probiotics) - 0.16*(diet\_Prebiotics) - 0.07*(Others)$$

in which “diet\_Probiotics”, “diet\_Probiotics” and “diet\_Others” are quantitative variables.

Model trimming resulted in a *Simple model* with a ( $R^2$ ) = 0.75, an adjusted R-squared ( $adj R^2$ ) = 0.7 and a  $p$ -value = 6.45E-08 (Table 4). The addition of dietary prebiotics (“diet\_Probiotics”) as a model descriptor, significantly improved the *Simple model* selection criteria (Table 4) (Fig. 4).

### 3.4. Correlation between dietary supplementation and feed conversion ratio (FCR)

The experiments employing prebiotics as functional ingredients typically presented lower normalized FCR than those employing the other dietary treatments (Fig. 5).

Normalized feed conversion ratio modelling with a complex multiple-regression obtained a total R-squared ( $R^2$ ) = 0.90, an adjusted R-squared ( $adj R^2$ ) = 0.80 and a  $p$ -value = 2.02e-05. The full model followed the equation:

$$\ln(FCR\_norm) = 0.46*(Temp) - 6.7e-3*(Temp)^2 - 0.31*(Oxygen) - 1.3e^{-2}*(ABW) + 0.2*\ln(ABW) + 0.3*(diet\_CP) - 0.6*(diet\_GE) - 5.25(diet\_CP$$

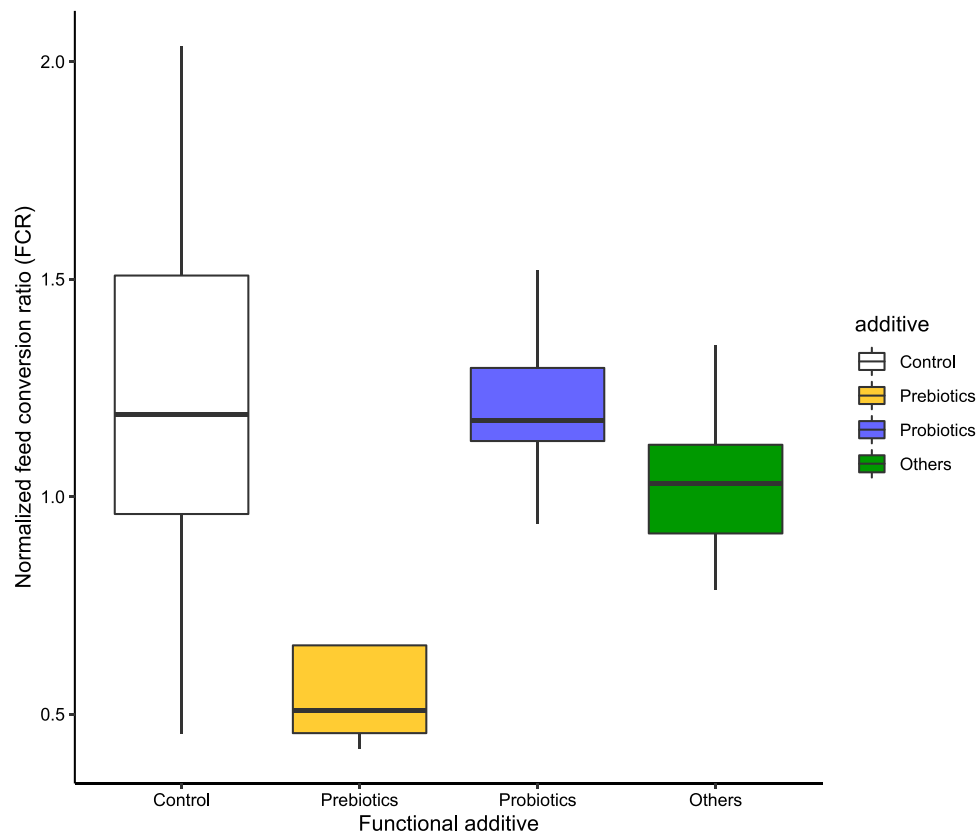


Fig. 5. Boxplot of functional ingredients dietary inclusion effects on normalized feed conversion ratio values.

$$/diet\_GE) + 0.13*(diet\_CL) - 3.4e^{-2}*(diet\_moisture) + 4.3e^{-2}*(diet\_ash) - 6.13e^{-2}*(diet\_Prebiotics) - 0.27*(diet\_Probiotics) - 0.13*(Others)$$

in which “diet\_Prebiotics”, “diet\_Probiotics” and “diet\_Others” are quantitative variables.

Model trimming resulted in a *Simple model* with a ( $R^2$ ) = 0.63, an adjusted R-squared ( $adj R^2$ ) = 0.54 and a *p-value* =  $1.01e^{-4}$  (Table 5). The addition of dietary prebiotics (“diet\_Prebiotics”) as a model descriptor, significantly improved the *Simple model* selection criteria (Table 5) (Fig. 6).

#### 4. Discussion

The models developed in the present study presented significant *p-values*, validating the regression coefficients between the descriptor variables and the different modelled traits (Sanquetta et al., 2018). The experimental models presented acceptable  $R^2$  scores (between 0.90 and 0.97), adjusted  $R^2$  scores (between 0.80 and 0.95) and normal residuals distribution. SGR was positively correlated (0.71 regression coefficient) to the dietary protein to energy ratio, meanwhile FI was negatively correlated (−38.1 regression coefficient) to this descriptive variable. The FCR was negatively correlated (−0.14 regression coefficient) to the dietary energy contents. In this sense, the linear models confirmed the elevated importance of dietary nutrient and energy balance on fish growth and feed utilization (Azevedo et al., 2002; Oliva-Teles, 2012; Méndez-Martínez et al., 2021).

Regarding the inclusion of functional additives, the analysis performed in the present study showed a pattern by which those dietary treatments employing prebiotics as functional additives presented higher normalized SGR values (Fig. 1) and lower individual FI and FCR normalized values (Figs. 3 and 5) than those employing probiotics or other functional additives. Model trimming revealed that prebiotics were the only functional additives group inducing significant effects on

SGR, FI and FCR models outcome. Interestingly, prebiotic inclusion presented the same patterns of correlation with the modeled traits as those presented by the dietary energy contents.

The prebiotic inclusion was positively correlated to SGR (0.32 regression coefficient) and negatively correlated to FI (−0.44 regression coefficient) and FCR (−0.44 regression coefficient), altogether pointing to a beneficial effect of prebiotic supplementation on fish growth and feed efficiency as suggested Torrecillas and co-authors in 2011. After feeding European sea bass juveniles with diets supplemented with 4 and 6 g/kg mannan-oligosaccharides (MOS), the authors observed a significant improvement on fish FCR, with lower feed intake and similar growth performance than fish fed a diet void of supplementation. MOS dietary inclusion led to reduced fish liver lipid vacuolization and decreased glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME). The authors proposed an effect of dietary prebiotic inclusion on the promotion of hepatic glycolytic activity, providing internal energy for body tissues and reducing feed intake through the induction of neural signals modulating appetite and satiation systems (Torrecillas et al., 2011). Similarly, in 2005 Laiz-Carrión and co-authors reported increased hepatic glycogenolysis and gluconeogenesis together with a reduced G6PD activity in sea bream (*Sparus aurata*) fed with immunostimulants (Laiz-Carrión et al., 2005).

Several studies have reported beneficial effects of prebiotic functional ingredients on fish growth and feed utilization. A study carried out with rainbow trout (*Oncorhynchus mykiss*) juveniles reported better growth performance in groups of fish fed diets supplemented with inulin or fructo-oligosaccharides (FOS) at either 5 or 10 g/kg than those fish fed not supplemented diets (Ortiz et al., 2013). Similarly, Soleimani and collaborators (Soleimani et al., 2012) fed Caspian roach (*Rutilus rutilus*) with diets supplemented with FOS at a concentration of 20 and 30 g/kg. After a 7-week feeding trial, fish fed the functional diets presented higher growth and better FCR compared to fish fed a reference diet. This increased growth performance could be associated to the increased



**Table 5**  
Coefficients of linear regression obtained for the normalized feed conversion ratio (FCR\_norm) observational models.

Feed conversion ratio model	Equation	R <sup>2</sup>	Adj R <sup>2</sup>	Variable p-value	Model p-value
Simple model <sup>a</sup>	Exp (3.7e <sup>-2</sup> *(Temp) – 0.12 *(Oxygen) – 4.5e <sup>-3</sup> *(ABW) – 1.23e <sup>-2</sup> *(diet_CP) – 0.14 *(diet_GE) – 0.11 *(diet_moisture))	0.63	0.54	-	1.01e <sup>-4</sup>
Simple model w/ Prebiotics <sup>b</sup>	Exp (2.45e <sup>-2</sup> *(Temp) – 0.1 *(Oxygen) – 3.34e <sup>-3</sup> *(ABW) – 1.61e <sup>-2</sup> *(diet_CP) – 0.1 *(diet_GE) – 9.33e <sup>-2</sup> *(diet_moisture) – 0.41 *(diet_Prebiotics))	0.80	0.75	8.96e <sup>-5</sup>	2.22e <sup>-7</sup>
Simple model w/ Probiotics <sup>c</sup>	Exp (3.57e <sup>-2</sup> *(Temp) – 0.13 *(Oxygen) – 4.6e <sup>-3</sup> *(ABW) – 1.47e <sup>-2</sup> *(diet_CP) – 0.14 *(diet_GE) – 0.11 *(diet_moisture) – 6.74e <sup>-2</sup> *(diet_Probiotics))	0.63	0.53	0.61	2.87e <sup>-4</sup>
Simple model w/ Others <sup>d</sup>	Exp (2.38e <sup>-2</sup> *(Temp) – 0.12 *(Oxygen) – 4.18e <sup>-3</sup> *(ABW) – 2.13e <sup>-2</sup> *(diet_CP) – 0.13 *(diet_GE) – 0.11 *(diet_moisture) + 0.11 *(diet_Others))	0.66	0.56	0.17	1.33e <sup>-4</sup>

Temp (water temperature °C); Oxygen (dissolved oxygen (ppm)); ABW (average body weight (g)); diet\_CP (dietary protein content (%)); diet\_GE (dietary energy content (MJ/kg)); diet\_moisture (diet moisture content (%)); diet\_Prebiotics (dietary prebiotics content (g/kg)); diet\_Probiotics (dietary probiotics content (g/kg)); diet\_Others (dietary Others content (g/kg)).

<sup>a</sup> FCR simple model (trimmed simple model).

<sup>b</sup> FCR simple model with prebiotics dietary inclusion as quantitative variable.

<sup>c</sup> FCR simple model with probiotics dietary inclusion as quantitative variable.

<sup>d</sup> FCR simple model with others dietary inclusion as quantitative variable.

concentrations of short chain fatty acids (SCFAs) as by-product of prebiotic fermentation by intestinal bacteria (Rastall and Gibson, 2015; Rivera-Piza and Lee, 2020). Between the different SCFAs derived from these indigestible fibers, butyrate and propionate are known to stimulate the intestinal gluconeogenesis leading to metabolic advantages on host intestinal health and growth. The propionate is directly absorbed, triggering the *de novo* synthesis of glucose acting as internal energy source and thus enhancing host growth performance. Butyrate meanwhile, can be metabolized by the intestinal cells, playing an important role stimulating the growth and differentiation of enterocytes and colonocytes leading to higher absorptive surface and an enhanced gut homeostasis (Rivera-Piza and Lee, 2020). Zhou et al. (2010), reported increased pyloric caeca and intestinal microvilli height in red drum (*Sciaenops ocellatus*) fed 4 different functional diets supplemented with prebiotics (Zhou et al., 2010). Similarly, Torrecillas and co-authors (2013) reported significant longer and more densely distributed microvilli on the posterior intestinal enterocytes surface in European sea bass juveniles fed with a 4 g/kg MOS supplemented diet in comparison to a control diet void of supplementation (Torrecillas et al., 2013).

An enhanced intestinal health and functionality will directly benefit host by increasing nutrient absorption (Butt and Volkoff, 2019; Dawood, 2021) even under unfavorable conditions such as those derived from the nutritional imbalances derived from high FM/FO replacement on diet formulation. As reported by Guerreiro and co-authors in 2015, the inclusion of either 1 g/kg of xylo-oligosaccharides (XOS) or 1 g/kg of fructo-oligosaccharides (FOS) led to higher body weight in European sea bass fed low protein based diets (Guerreiro et al., 2015). Similarly, in

2018, Torrecillas and co-authors studied the effects of prebiotics (MOS), probiotics (*Peridococcus acidilactici*) and their combination in fish growth and immune response of European sea bass juveniles fed low FM (5 %) and FO (6 %) based diets. The authors reported higher final body weight in those fish fed prebiotics and the symbiotic compound (MOS + *P. acidilactici*). On the contrary the probiotic alone did not induced significant differences on fish growth in comparison to the control diet, void of supplementation. Nevertheless, the symbiotic compound attenuated the MOS-induced gut humoral pro-inflammatory response. Those results may suggest a role of probiotic compounds as immune modulators rather than as growth enhancer products (Nayak, 2010; Lazado and Caipang, 2014; Huang and Lee, 2018; Firmino et al., 2021), supporting the lack of influence of this compounds inclusion in the final outcome of the models developed in the present study. In the same way, Pérez-Sánchez et al., analyzed the effects of a combination of phytogetic compounds in the growth performance of the sea bream. The phytogetic compounds did not induced significant effects on fish growth performance, but reduced their gut inflammatory response leading to an improved absorptive capacity (Pérez-Sánchez et al., 2015).

The observational models developed in the present study met the conditions necessary to be validated. Nevertheless, full models required a trimming treatment in order to split the full model into local models with lower R<sup>2</sup> values but simpler equations (Chicco et al., 2021). Model simplification allowed the identification of dietary prebiotics inclusion as a determinant factor on fish growth and feed utilization. Nevertheless, considering the wide variety of functional ingredients and their ways of action, further studies are required in order to clarify these mechanisms and employ prebiotics as effective tools in order to increase aquaculture production yields.

#### CRedit authorship contribution statement

All authors contributed to the study conception and design. Data collection and curation was performed by Antonio Serradell. Data validation was performed by Tomé Silva and Filipe Soares. The first draft of the manuscript was written by Antonio Serradell and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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#### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antonio Serradell Pastor reports financial support was provided by University of Las Palmas de Gran Canaria. Daniel Montero Vitores reports financial support was provided by European Commission. Daniel Montero Vitores reports a relationship with Sparos Lda that includes: non-financial support.

#### Data Availability

The datasets generated during and/or analyzed during the current study are publicly available at <https://data.mendeley.com/datasets/bhgmxs3g2d>.

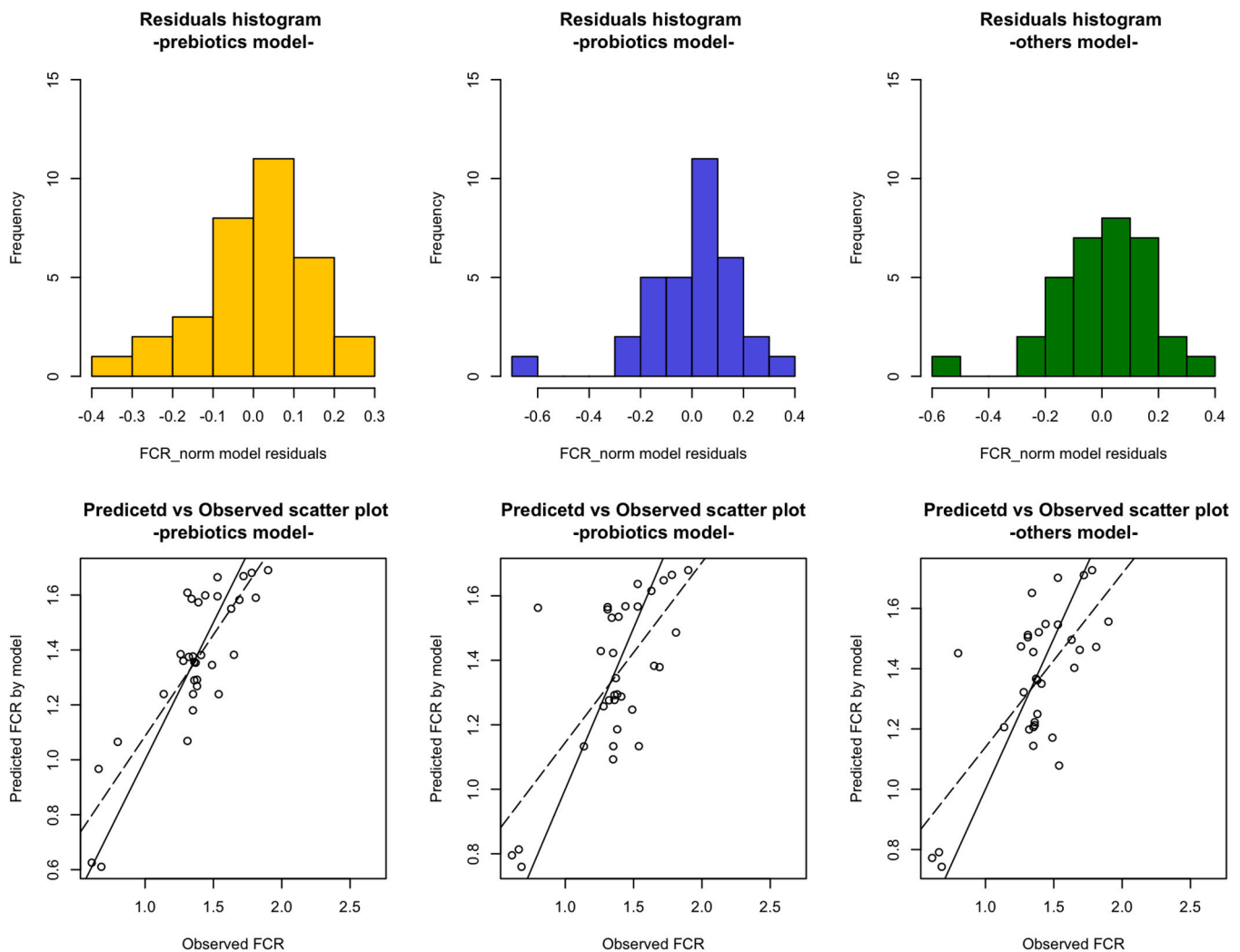


Fig. 6. European sea bass (*Dicentrarchus labrax*) feed conversion ratio (FCR) distribution for a) prebiotics simple model residuals; b) probiotics simple model residuals; c) others simple model residuals; d) prebiotics simple model fitted values; e) probiotics simple model fitted values; f) others simple model fitted values.

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