Aquaculture Reports 32 (2023) 101729

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

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

A. Serradell [a,*], S. Torrecillas [a,b], F. Soares [c], T. Silva [c], D. Montero [a]

[a] *Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de las Palmas de Gran Canaria, Muelle de Taliarte s/n. 35214 Telde, Las Palmas, Canary Islands, Spain*
[b] *Aquaculture Program, Institute of Agrifood Research and Technology (IRTA), La Ràpita, Tarragona, Spain.*
[c] *Sparos Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal*

ARTICLE INFO

Keywords:
European sea bass (*Dicentrarchus labrax*)
Functional additives
Multiple lineal-regression
Specific growth rate
Feed utilization

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 (R2) = 0.96, adjusted R-squared (adj R2) = 0.92 and a p-value= 7.21E-08)), fish feed intake (FI) (R2 = 0.97, adj R2 = 0.95 and a p-value= 5.42E-12)) and fish feed conversion ratio (FCR) (R2 = 0.90, adj R2 = 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

---

[*] Corresponding author.
E-mail address: tonetser2@gmail.com (A. Serradell).

https://doi.org/10.1016/j.aqrep.2023.101729
Received 3 March 2023; Received in revised form 14 June 2023; Accepted 8 September 2023

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 Rodrigáñez et al., 2009; Liu et al., 2012; Bunnoy 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 phytogenics, 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 phytogenic 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 "*phytogenics*" OR "*essential oils*" OR "*plant derived compounds*" OR "*phytogenic 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^{-1}); 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:

$$normalized \quad trait = \frac{measured \quad raw \quad trait}{maximumtrait \quad value}$$

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

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

FI (% body weight/day) = [(individual feed intake)/ (IBW + FBW)/2/days] x 100,

FCR (g feed intake/g weight gain) = individual feed intake / (FBW – 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) (*Pediococcus acidilactici*, 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 *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp. and *Pediococcus* 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) (*L. plantarum*)	Sprayed after extrusion	Sprayed 10×10^{9} CFU/ kg	90 days	42.25	2.10	– ↑ Survival – No differences on FBW, SGR or FCR	Piccolo et al. (2015)
	Bactocell PA10 (Lamelland SAS, Canada) (*Pediococcus acidilactici*, strain CNCM I-4622)	-	-	60 days	47.20	1.97	– No differences on FBW and length	Torrecillas 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	Torrecillas et al. (2011)
	MOS (Biomos® 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	Torrecillas 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 *V.anguillarum*	Torrecillas et al. (2019)
Others								
	Anise (*Pimpinella anisum* 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 Yiğit (2015)
	Yucca schidigera	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, Biomos® and Actigen© (second generation of MOS; Alltech, Inc., Kentucky, USA) + *Pediococcus acidilactici* (BAC, Bactocell®; Lallemand Inc., Cardiff, UK)		3 or 6 g/kg Biomos	60 days	47.20	1.97	– ↑ FBW and length	Torrecillas 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 *V.anguillarum*	Torrecillas 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 (*Thymus vulgaris* L.) or rosemary (*Rosmarinus officinalis* L.) or seed powder of fenugreek (*Trigonella foenum graecum* 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	Yılmaz 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)

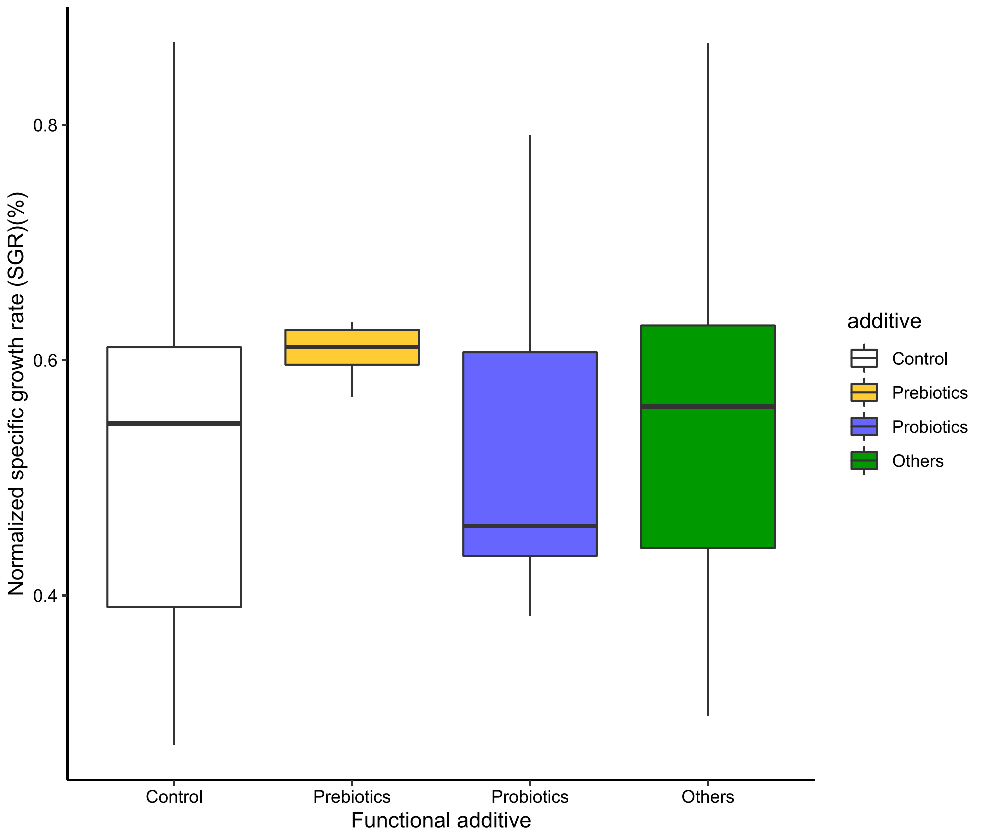


Fig. 1. Boxplot of functional ingredients dietary inclusion effects on normalized specific growth rate values.

Table 3
Coefficients of linear regression obtained for the normalized specific growth rate (SGR_norm) observational models.

Specific growth rate model	Equation	R^2	Adj R^2	Variable *p*-*value*	Model *p*-*value*
Simple model[a]	$\text{Exp}\ (5.65\text{e}^{-2} \ast (\text{Temp}) + 6.94\text{e}^{-5} \ast (\text{norm\_FI})^2 + 0.71 \ast (\text{diet\_CP}/\text{diet\_GE}))$	0.67	0.65	-	9.20e^{-10}
Simple model w/ Prebiotics[b]	$\text{Exp}\ (6.1\text{e}^{-2} \ast (\text{Temp}) + 7.1\text{e}^{-5} \ast (\text{norm\_FI})^2 + 0.81 \ast (\text{diet\_CP}/\text{diet\_GE}) + 0.32 \ast (\text{diet\_Prebiotics}))$	0.75	0.73	8.52e^{-4}	5.3e^{-9}
Simple model w/ Probiotics[c]	$\text{Exp}\ (5.65\text{e}^{-2} \ast (\text{Temp}) + 6.94\text{e}^{-5} \ast (\text{norm\_FI})^2 + 0.71 \ast (\text{diet\_CP}/\text{diet\_GE}) - 4.22\text{e}^{-3} \ast (\text{diet\_Probiotics}))$	0.67	0.63	0.96	5.3e^{-9}
Simple model w/ Others[d]	$\text{Exp}\ (6.58\text{e}^{-2} \ast (\text{Temp}) + 7.1\text{e}^{-5} \ast (\text{norm\_FI})^2 + 0.73 \ast (\text{diet\_CP}/\text{diet\_GE}) - 0.1\ (\text{diet\_Others}))$	0.7	0.66	0.11	1.49e^{-9}

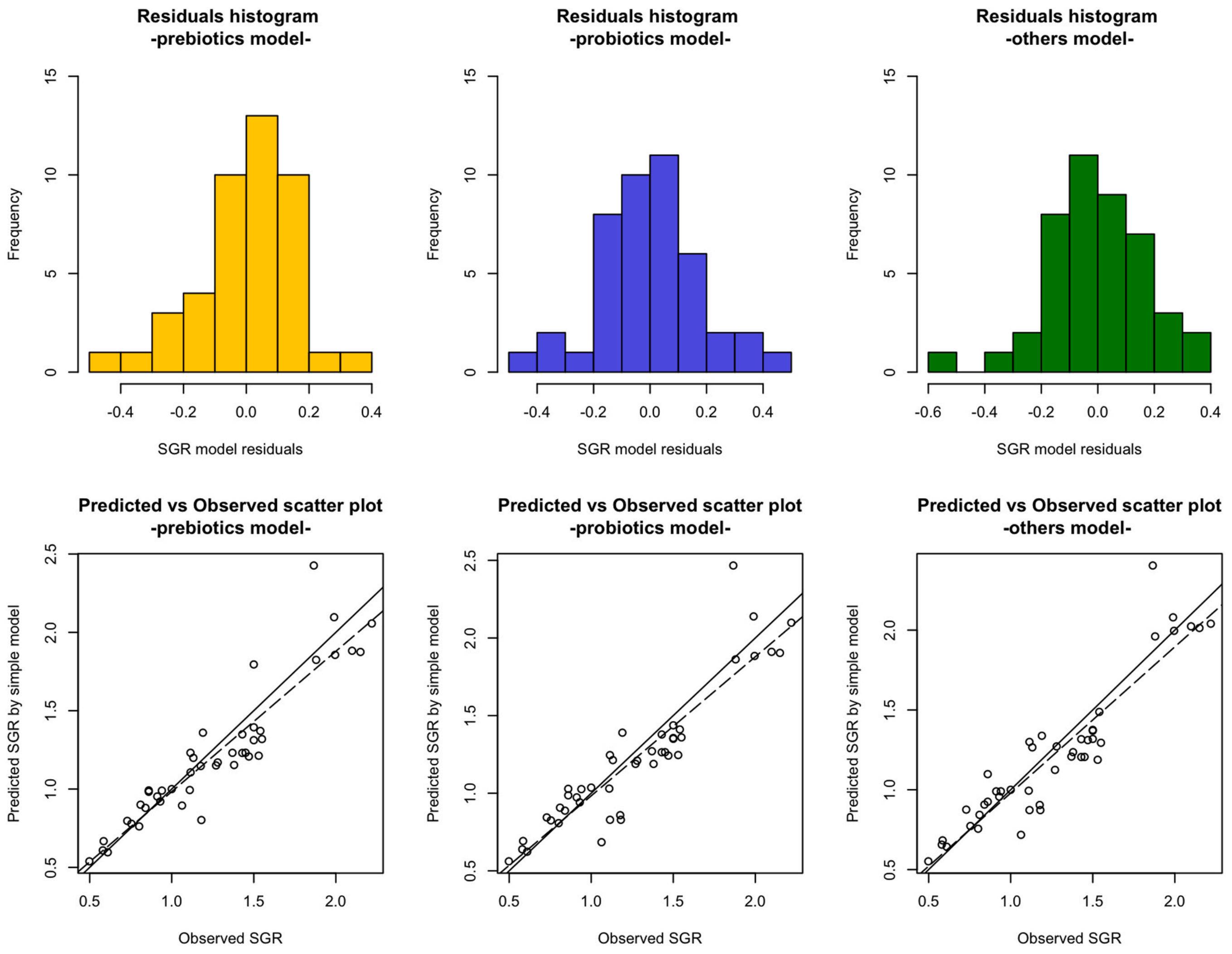
Temp (water temperature °C); norm_FI (normalized fish individual feed intake (g /fish per day)); diet_CP /diet_GE (Protein – Energy ratio (g /MJ)); diet_Prebiotics (dietary prebiotics content (g /kg)); diet_Probiotics (dietary probiotics content (g /kg)); diet_Others (dietary Others content (g /kg)).

[a] SGR simple model (trimmed simple model).

[b] SGR simple model with prebiotics dietary inclusion as quantitative variable.

[c] SGR simple model with probiotics dietary inclusion as quantitative variable.

[d] SGR simple model with Others dietary inclusion as quantitative variable.


Residuals histogram -prebiotics model-

Residuals histogram -probiotics model-

Residuals histogram -others model-

Predicted vs Observed scatter plot -prebiotics model-

Predicted vs Observed scatter plot -probiotics model-

Predicted vs Observed scatter plot -others model-

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:

$\ln(SGR_{max}) = -\,7{\cdot}93079 + [\,0{\cdot}50781 \times \ln(ABW)] - [0{\cdot}00133 \times ABW] - [0{\cdot}09766 \times (\ln(ABW)^2)] + [0{\cdot}2524 \times Temp] - [0{\cdot}0041 \times (Temp)^2]$,

$\ln(FI_{max}) = 5{\cdot}11608 + [0{\cdot}61529 \times \ln(ABW)] + [0{\cdot}14896 \times Temp] - 0{\cdot}00136 \times (Temp)^2]$,

$FCR_{typical} = 0{\cdot}9036676 \times (ABW)^{0{\cdot}1082725}$.

*ABW (average body weight (g)) = $(IBW * FBW)^{0.5}$.
*Temp (temperature (°C)).

These models were obtained using quantile regression (to estimate quantiles 0.95 for SGR_max and FI_max, and quantile 0.50 for FCR_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

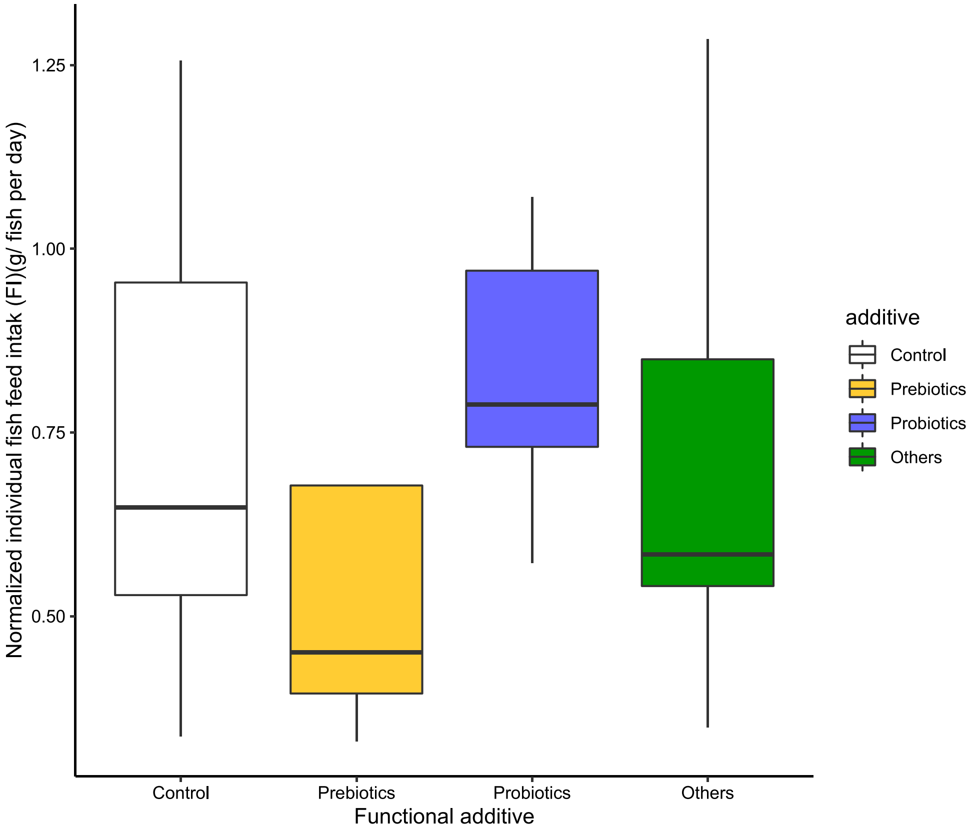


Fig. 3. Boxplot of functional ingredients dietary inclusion effects on normalized fish individual feed intake values.

Table 4
Coefficients of linear regression obtained for the normalized feed intake (FI_norm) observational models.

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

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 mositure content (%)); diet_Prebiotics (dietary prebiotics content (g/kg)); diet_Probiotics (dietary probiotics content (g/kg)); diet_Others (dietary Others content (g/kg)).

[a] FI simple model (trimmed simple model).

[b] FI simple model with prebiotics dietary inclusion as quantitative variable.

[c] FI simple model with probiotics dietary inclusion as quantitative variable.

[d] FI simple model with Others dietary inclusion as quantitative variable.

effects of dietary inclusion of prebiotics (Torrecillas et al., 2011; Guerreiro 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; Yılmaz 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^2) = 0.96, an adjusted R-squared (*adj* R^2) = 0.92 and a *p-value* = 7.213e-08. The full model followed the equation:

Ln (SGR_norm) = 0·44 *(Temp) - 1·52e-2*(Temp)^{2} + 0·33*(Oxygen) – 5·91 e^{-3}*(ABW) + 0·28*ln (ABW) - 1·46e^{-2}*(FI_norm) + 1·49e^{-4}* (FI_norm)^{2} + 0·5*(diet_CP) – 1·12*(diet_GE) – 9·8 (diet_CP /diet_GE) – 0·16*(diet_CL) +

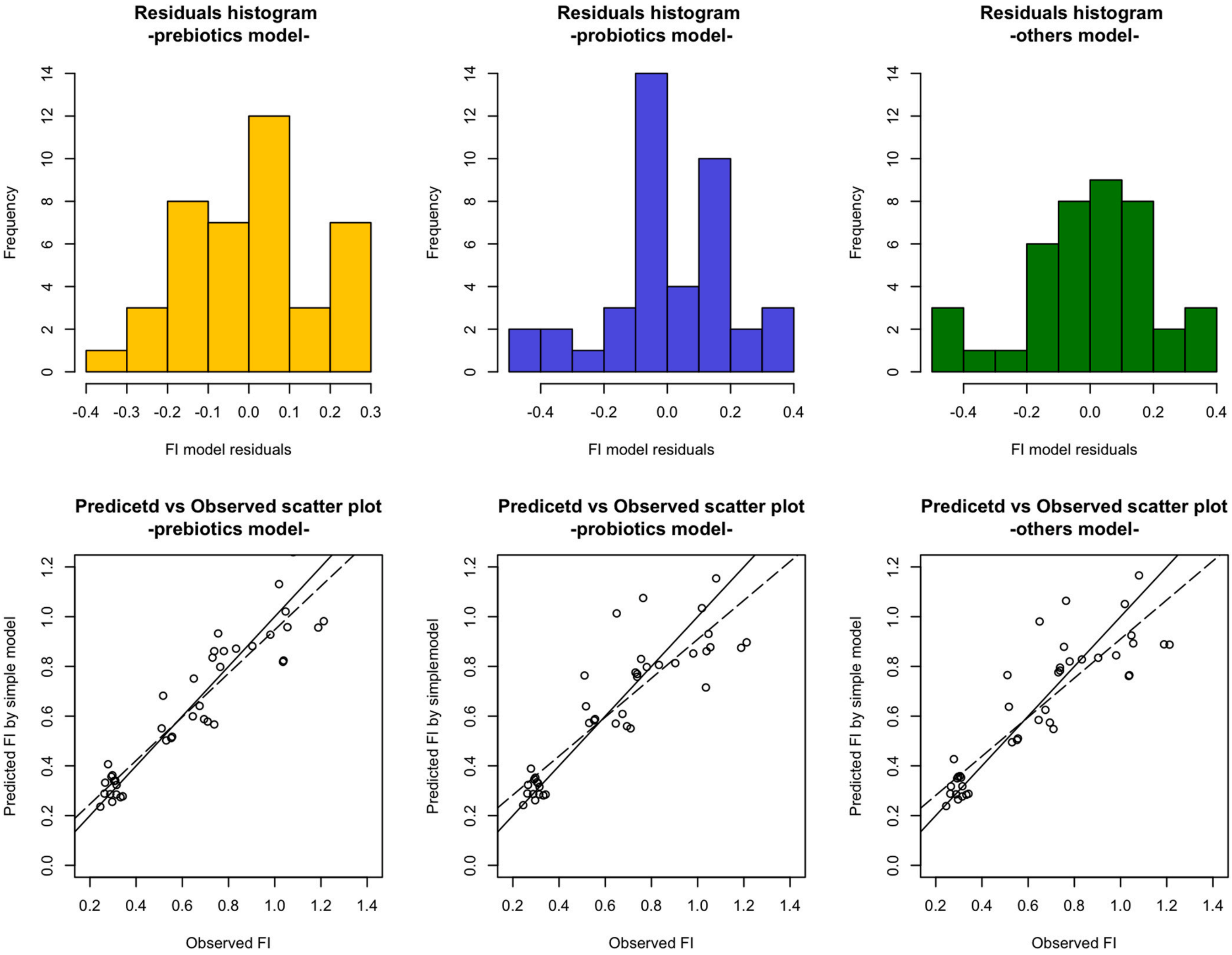


Fig. 4. European sea bass (*Dicentrarchus labrax*) individual feed intake (FI) distribution for a) prebiotics simple model residuals; b) probiotics simple model residuals; c) others simple model residuals; d) prebiotics simple model fitted values; e) probiotics simple model fitted values; f) others simple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

$3{\cdot}1\ e^{-2}*(\text{diet\_moisture}) - 2{\cdot}23e^{-2}*(\text{diet\_ash}) + 8{\cdot}57e^{-2}*(\text{diet\_Probiotics}) + 0{\cdot}12*(\text{diet\_Prebiotics}) + 7{\cdot}02e^{-2}*(\text{Others})$

in which “diet_Prebiotics”, “diet_Probiotics” and “diet_Others” are quantitative variables.

Model trimming resulted in a *Simple model* with a (R^2) = 0.67, an adjusted R-squared (*adj* R^2) = 0.65 and a *p-value* = 9.20E-10 (Table 3). The addition of dietary prebiotics (“diet_Prebiotics”) 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:

$\text{Ln}\ (\text{FI\_norm}) = -1{\cdot}88*(\text{Temp}) + 4e\text{-}2*(\text{Temp})^2 + 0{\cdot}21*(\text{Oxygen}) - 9{\cdot}1e^{-3}*(\text{ABW}) + 0{\cdot}23*\ln\ (\text{ABW}) + 0{\cdot}7*(\text{diet\_CP}) - 1{\cdot}22*(\text{diet\_GE}) - 13{\cdot}5\ (\text{diet\_CP}/\text{diet\_GE}) + 0{\cdot}16*(\text{diet\_CL}) + 0{\cdot}06*(\text{diet\_moisture}) + 0{\cdot}13*(\text{diet\_ash}) + 1{\cdot}76*(\text{diet\_Probiotics}) - 0{\cdot}16*(\text{diet\_Prebiotics}) - 0{\cdot}07*(\text{Others})$

in which “diet_Prebiotics”, “diet_Probiotics” and “diet_Others” are quantitative variables.

Model trimming resulted in a *Simple model* with a (R^2) = 0.75, an adjusted R-squared (*adj* R^2) = 0.7 and a *p-value* = 6.45E-08 (Table 4). The addition of dietary prebiotics (“diet_Prebiotics”) 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:

$\text{Ln}\ (\text{FCR\_norm}) = 0{\cdot}46\ *(\text{Temp}) - 6{\cdot}7e\text{-}3*(\text{Temp})^2 - 0{\cdot}31*(\text{Oxygen}) - 1{\cdot}3e^{-2}*(\text{ABW}) + 0{\cdot}2*\ln\ (\text{ABW}) + 0{\cdot}3*(\text{diet\_CP}) - 0{\cdot}6*(\text{diet\_GE}) - 5{\cdot}25\ (\text{diet\_CP}$

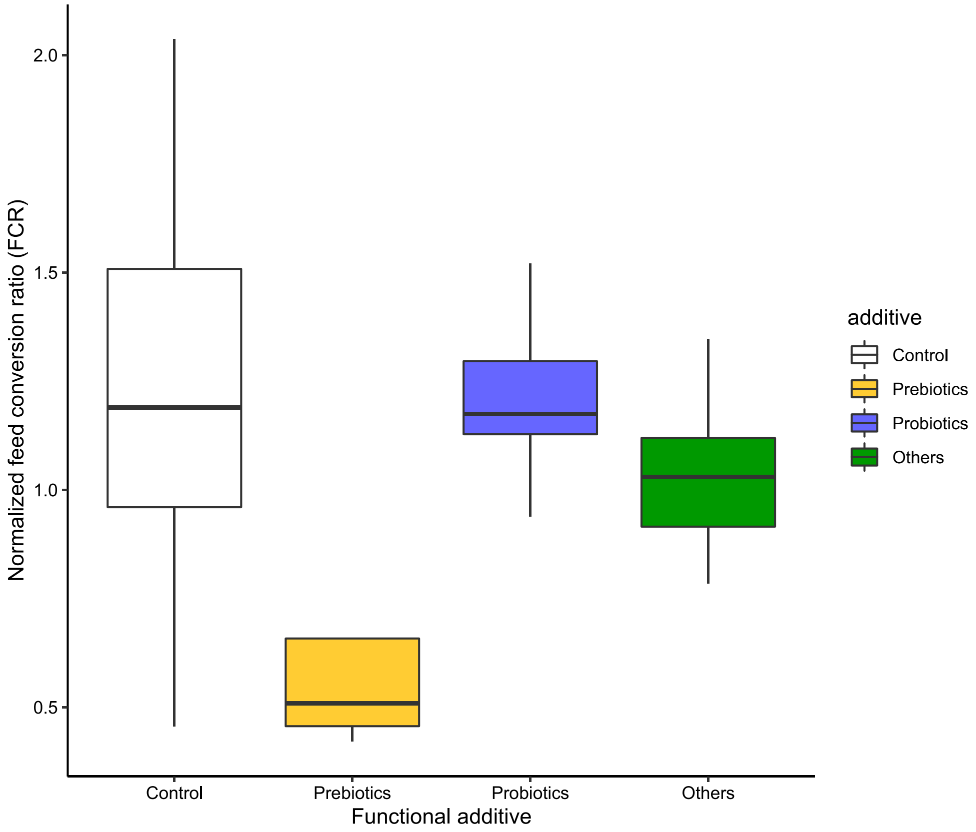


Fig. 5. Boxplot of functional ingredients dietary inclusion effects on normalized feed conversion ratio values.

/diet_GE) + 0·13*(diet_CL) – 3·4e^{-2}*(diet_moisture) + 4·3e^{-2}*(diet_ash) – 6·13e^{-2}*(diet_Probiotics) – 0·27*(diet_Prebiotics) – 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 Laíz-Carrion 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^2	Adj R^2	Variable *p-value*	Model *p-value*
Simple model[a]	Exp (3.7e^{-2} *(Temp) – 0.12 * (Oxygen) – 4.5e^{-3} * (ABW) – 1.23e^{-2} * (diet_CP) – 0.14 * (diet_GE) - 0.11 * (diet_moisture))	0.63	0.54	-	1.01e^{-4}
Simple model w/ Prebiotics[b]	Exp (2.45e^{-2} *(Temp) – 0.1 * (Oxygen) – 3.34e^{-3} * (ABW) – 1.61e^{-2} * (diet_CP) – 0.1 * (diet_GE) - 9.33e^{-2} * (diet_moisture) – 0.41 * (diet_Prebiotics))	0.80	0.75	8.96e^{-5}	2.22e^{-7}
Simple model w/ Probiotics[c]	Exp (3.57e^{-2} *(Temp) – 0.13 * (Oxygen) – 4.6e^{-3} * (ABW) – 1.47e^{-2} * (diet_CP) – 0.14 * (diet_GE) - 0.11 * (diet_moisture) – 6.74e^{-2} * (diet_Probiotics))	0.63	0.53	0.61	2.87e^{-4}
Simple model w/ Others[d]	Exp (2.38e^{-2} *(Temp) – 0.12 * (Oxygen) – 4.18e^{-3} * (ABW) – 2,13e^{-2} * (diet_CP) – 0.13 * (diet_GE) − 0.11 * (diet_moisture) + 0.11 * (diet_Others))	0.66	0.56	0.17	1.33e^{-4}

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)).

[a] FCR simple model (trimmed simple model).

[b] FCR simple model with prebiotics dietary inclusion as quantitative variable.

[c] FCR simple model with probiotics dietary inclusion as quantitative variable.

[d] 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-Sanchez et al., analyzed the effects of a combination of phytogenic compounds in the growth performance of the sea bream. The phytogenic 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^2 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.

Funding

This work was founded by the European Union's Horizon 2020 research and innovation program under grant agreement no. 818367; AquaIMPACT—Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture. Authors want to thank the University of Las Palmas de Gran Canaria (ULPGC) for the funding for Antonio Serradell through the call "ERASMUS + PRÁCTICAS (SMT)" 2020-2021.

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.

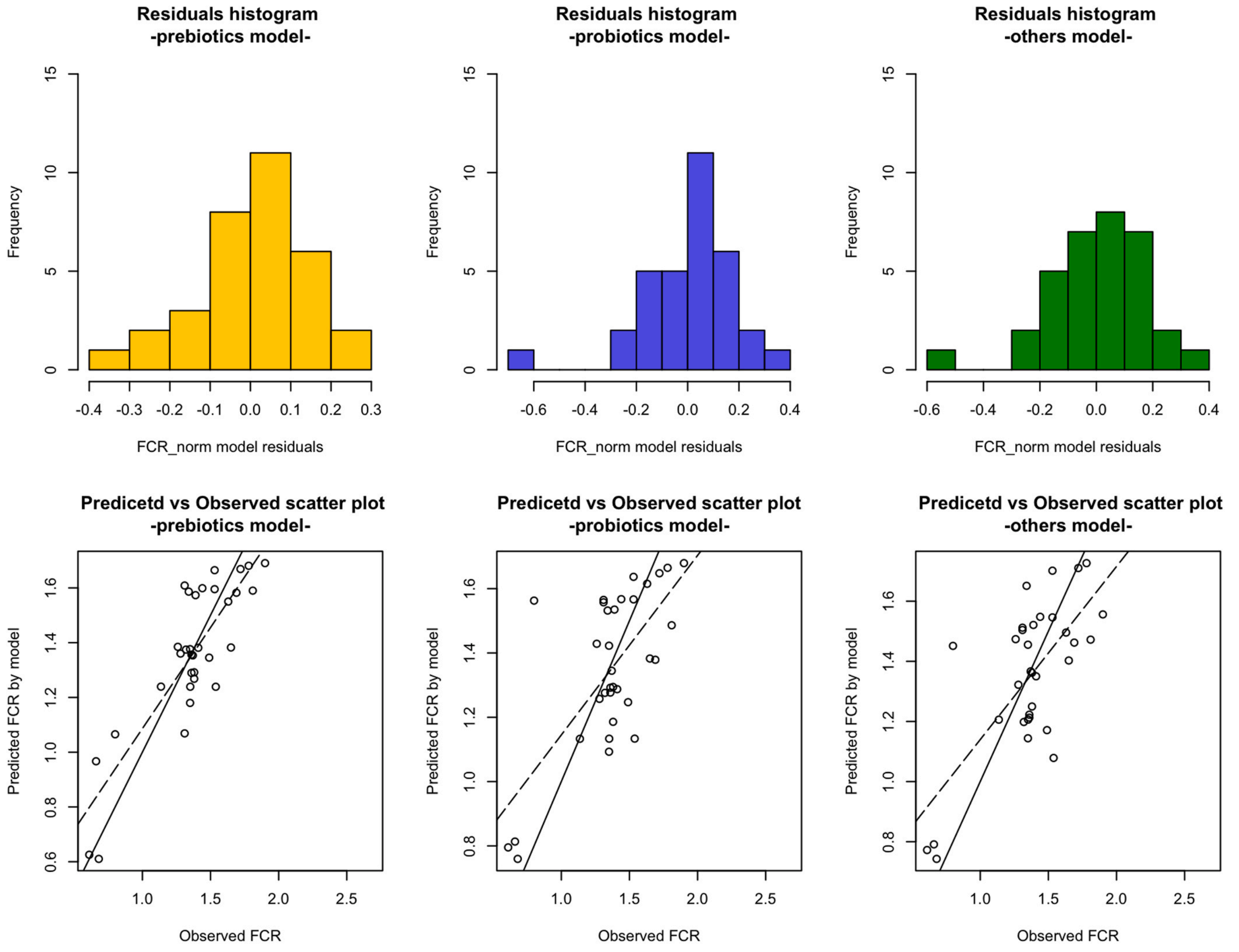


Fig. 6. European sea bass (*Dicentrarchus labrax*) feed conversion ratio (FCR) distribution for a) prebiotics simple model residuals; b) probiotics simple model residuals; c) others simple model residuals; d) prebiotics simple model fitted values; e) probiotics simple model fitted values; f) others simple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

Acknowledgments

Authors want to thank the European Union's Horizon 2020 research and innovation program under grant agreement no. 818367; AquaIMPACT—Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture. Authors want to thank to the University of Las Palmas de Gran Canaria (ULPGC) for the funding for SA through the call "ERASMUS + PRÁCTICAS (SMT)" 2020–2021.

References

Abdel-Latif, H.M., Abdel-Tawwab, M., Khafaga, A.F., Dawood, M.A., 2020. Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture 526, 735432. https://doi.org/10.1016/j.aquaculture.2020.735432.

Adeoye, A.A., Yomla, R., Jaramillo-Torres, A., Rodiles, A., Merrifield, D.L., Davies, S.J., 2016. Combined effects of exogenous enzymes and probiotic on Nile tilapia (*Oreochromis niloticus*) growth, intestinal morphology and microbiome. Aquaculture 463, 61–70. https://doi.org/10.1016/j.aquaculture.2016.05.028.

Agostinelli, C., 2002. Robust stepwise regression. J. Appl. Stat. 29 (6), 825–840. https://doi.org/10.1080/02664760220136168.

Aly, S.M., Mohamed, M.F., John, G., 2008. Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). Aquac. Res. 39 (6), 647–656. https://doi.org/10.1111/j.1365-2109.2008.01932.x.

Ashry, A., Habiba, M., Desouky, M., El-Zayat, A., Moonmanee, T., Van Doan, H., Dawood, M., 2022. The effects of coriander seeds (*Coriandrum sativum*) on the growth performance, growth hormone, antibacterial capacity, and immune response of European sea bass (*Dicentrarchus labrax*). Ann. Anim. Sci. 22 (4), 1273–1280. https://doi.org/10.2478/aoas-2022-0021.

Azevedo, P.A., Bureau, D.P., Leeson, S., CHO, C.Y., 2002. Growth and efficiency of feed usage by Atlantic salmon (Salmo salar) fed diets with different dietary protein: energy ratios at two feeding levels. Fish. Sci. 68 (4), 878–888. https://doi.org/10.1046/j.1444-2906.2002.00506.x.

Bell, M.V., Henderson, R.J., Sargent, J.R., 1986. The role of polyunsaturated fatty acids in fish. Comp. Biochem. Physiol. Part B: Comp. Biochem. 83 (4), 711–719. https://doi.org/10.1016/0305-0491(86)90135-5.

Bello, O.S., Emikpe, B.O., Olaifa, F.E., 2012. The body weight changes and gut morphometry of Clarias gariepinus juveniles on feeds supplemented with walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb residues. Int. J. Morphol. 30 (1), 253–257. https://doi.org/10.4067/S0717-95022012000100045.

Bunnoy, A., Na-Nakorn, U., Srisapoome, P., 2019. Probiotic effects of a novel strain, *Acinetobacter* KU011TH, on the growth performance, immune responses, and resistance against Aeromonas hydrophila of bighead catfish (*Clarias macrocephalus* Günther, 1864). Microorganisms 7 (12), 613. https://doi.org/10.3390/microorganisms7120613.

Butt, R.L., Volkoff, H., 2019. Gut microbiota and energy homeostasis in fish. Front. Endocrinol. 10, 9. https://doi.org/10.3389/fendo.2019.00009.

Campos, I., Matos, E., Marques, A., Valente, L.M., 2017. Hydrolyzed feather meal as a partial fishmeal replacement in diets for European seabass (*Dicentrarchus labrax*) juveniles. Aquaculture 476, 152–159. https://doi.org/10.1016/j.aquaculture.2017.04.024.

Chicco, D., Warrens, M.J., Jurman, G., 2021. The coefficient of determination R-squared is more informative than SMAPE, MAE, MAPE, MSE and RMSE in regression analysis evaluation. PeerJ Comput. Sci. 7, e623 https://doi.org/10.7717/peerj-cs.623.

Dawood, M.A., 2021. Nutritional immunity of fish intestines: Important insights for sustainable aquaculture. Rev. Aquac. 13 (1), 642–663. https://doi.org/10.1111/raq.12492.

Ebrahimi, G.H., Ouraji, H., Khalesi, M.K., Sudagar, M., Barari, A., Zarei Dangesaraki, M., Jani Khalili, K.H., 2012. Effects of a prebiotic, Immunogen®, on feed utilization, body composition, immunity and resistance to *Aeromonas hydrophila* infection in the common carp *Cyprinus carpio* (Linnaeus) fingerlings. J. Anim. Physiol. Anim. Nutr. 96 (4), 591–599. https://doi.org/10.1111/j.1439-0396.2011.01182.x.

Eissa, E.S.H., Baghdady, E.S., Gaafar, A.Y., El-Badawi, A.A., Bazina, W.K., Al-Kareem, O.A.M., Nadia, N.B., 2022. Assessing the influence of dietary Pediococcus acidilactici Probiotic Supplementation in the Feed of European Sea Bass (*Dicentrarchus labrax* L.) (Linnaeus, 1758) on farm water quality, growth, feed utilization, survival rate, body composition, blood biochemical parameters, and intestinal histology. Aquac. Nutr. 2022. https://doi.org/10.1155/2022/5841220.

Estensoro, I., Ballester-Lozano, G., Benedito-Palos, L., Grammes, F., Martos-Sitcha, J.A., Mydland, L.T., Calduch-Giner, J.A., Fuentes, J., Karalazos, V., Ortiz, A., Overland, M., Sitjà-Bobadilla, A., Pérez-Sánchez, J., 2016. Dietary butyrate helps to restore the intestinal status of a marine teleost (*Sparus aurata*) fed extreme diets low in fish meal and fish oil. PLoS One 11 (11), e0166564. https://doi.org/10.1371/journal.pone.0166564.

Fernandes, H., Castro, C., Filipe, D., Ferreira, P., Salgado, J.M., Moyano, F., Oliva-Teles, M., Belo, I., Peres, H., 2022. Application of Fermented Brewer's Spent Grain Extract in Plant-Based Diets Improves Pre-and Post-mortem Oxidative Status of European Seabass (*Dicentrarchus labrax*). Aquac. Nutr. 2022. https://doi.org/10.1155/2022/2629052.

Fernández-Montero, Á., Torrecillas, S., Acosta, F., Kalinowski, T., Bravo, J., Sweetman, J., Roo, J., Makol, A., ocando, J., Carvalho, M., Izquierdo, M.S., Montero, D., 2021. Improving greater amberjack (*Seriola dumerili*) defenses against monogenean parasite *Neobenedenia girellae* infection through functional dietary additives. Aquaculture 534, 736317. https://doi.org/10.1016/j.aquaculture.2020.736317.

Fiorella, K.J., Okronipa, H., Baker, K., Heilpern, S., 2021. Contemporary aquaculture: implications for human nutrition. Curr. Opin. Biotechnol. 70, 83–90. https://doi.org/10.1016/j.copbio.2020.11.014.

Firmino, J.P., Galindo-Villegas, J., Reyes-López, F.E., Gisbert, E., 2021. Phytogenic bioactive compounds shape fish mucosal immunity. Front. Immunol. 12, 695973 https://doi.org/10.3389/fimmu.2021.695973.

Galkanda-Arachchige, H.S., Wilson, A.E., Davis, D.A., 2020. Success of fishmeal replacement through poultry by-product meal in aquaculture feed formulations: a meta-analysis. Rev. Aquac. 12 (3), 1624–1636. https://doi.org/10.1111/raq.12401.

Geng, X., Dong, X.H., Tan, B.P., Yang, Q.H., Chi, S.Y., Liu, H.Y., Liu, X.Q., 2011. Effects of dietary chitosan and Bacillus subtilis on the growth performance, non-specific immunity and disease resistance of cobia, Rachycentron canadum. Fish. Shellfish Immunol. 31 (3), 400–406. https://doi.org/10.1016/j.fsi.2011.06.006.

Gonçalves, R.A., Serradeiro, R., Machado, M., Costas, B., Hunger, C., Dias, J., 2019. Interactive effects of dietary fishmeal level and plant essential oils supplementation on European sea bass, *Dicentrarchus labrax*: Growth performance, nutrient utilization, and immunological response. J. World Aquac. Soc. 50 (6), 1078–1092. https://doi.org/10.1111/jwas.12616.

Grisdale-Helland, B., Helland, S.J., Gatlin, D.M., 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galsctooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar* L.). Aquaculture 283, 163–167. https://doi.org/10.1016/j.aquaculture.2008.07.012.

Guerreiro, I., Couto, A., Pérez-Jiménez, A., Oliva-Teles, A., Enes, P., 2015. Gut morphology and hepatic oxidative status of European sea bass (*Dicentrarchus labrax*) juveniles fed plant feedstuffs or fishmeal-based diets supplemented with short-chain fructo-oligosaccharides and xylo-oligosaccharides. Br. J. Nutr. 114 (12), 1975–1984. https://doi.org/10.1017/S0007114515003773.

Guerreiro, I., Oliva-Teles, A., Enes, P., 2018. Prebiotics as functional ingredients: focus on Mediterranean fish aquaculture. Reviews in aquaculture 10 (4), 800–832.

Gültepe, N., Salnur, S., Hoşsu, B., Hisar, O.L.C.A.Y., 2011. Dietary supplementation with Mannanoligosaccharides (MOS) from Bio-Mos enhances growth parameters and digestive capacity of gilthead sea bream (*Sparus aurata*). Aquac. Nutr. 17 (5), 482–487. https://doi.org/10.1111/j.1365-2095.2010.00824.x.

Habiba, M.M., Hussein, E.E., Ashry, A.M., El-Zayat, A.M., Hassan, A.M., El-Shehawi, A.M., Sewilam, M., Van Doan, H., Dawood, M.A.O., 2021. Dietary cinnamon successfully enhanced the growth performance, growth hormone, antibacterial capacity, and immunity of European sea bass (*Dicentrarchus labrax*). Animals 11 (7), 2128. https://doi.org/10.3390/ani11072128.

He, S., Zhang, Y., Xu, L., Yang, Y., Marubashi, T., Zhou, Z., Yao, B., 2013. Effects of dietary *Bacillus subtilis* C-3102 on the production, intestinal cytokine expression and autochthonous bacteria of hybrid tilapia *Oreochromis niloticus*♀ × *Oreochromis aureus*♂. Aquaculture 412, 125–130. https://doi.org/10.1016/j.aquaculture.2013.06.028.

Hooshyar, Y., Abedian Kenari, A., Paknejad, H., Gandomi, H., 2020. Effects of *Lactobacillus rhamnosus* ATCC 7469 on different parameters related to health status of rainbow trout (*Oncorhynchus mykiss*) and the protection against Yersinia ruckeri. Probiotics Antimicrob. Proteins 12 (4), 1370–1384. https://doi.org/10.1007/s12602-020-09645-8.

Hoseinifar, S.H., Mirvaghefi, A., Amoozegar, M.A., Sharifian, M., Esteban, M.Á., 2015. Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. Fish. Shellfish Immunol. 45 (1), 27–32. https://doi.org/10.1016/j.fsi.2015.03.029.

Hoseinifar, S.H., Yousefi, S., Van Doan, H., Ashouri, G., Gioacchini, G., Maradonna, F., Carnevali, O., 2021. Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic and synbiotics. Rev. Fish. Sci. Aquacture 29 (2), 198–217. https://doi.org/10.1080/23308249.2020.1795616.

Huang, C.M., Lee, T.T., 2018. Immunomodulatory effects of phytogenics in chickens and pigs—a review. Asian-Australas. J. Anim. Sci. 31 (5), 617. https://doi.org/10.5713/ajas.17.0657.

İrkin, L.C., Yiğit, M., 2015. The use of garlic (*Allium sativum*) meal as a natural feed supplement in diets for European seabass (*Dicentrarchus labrax*) juveniles. J. Aquac. Eng. Fish. Res. 2 (3), 128–141. https://doi.org/10.3153/JAEFR16015.

Kader, M.A., Koshio, S., Ishikawa, M., Yokoyama, S., Bulbul, M., 2010. Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. Aquaculture 308 (3–4), 136–144. https://doi.org/10.1016/j.aquaculture.2010.07.037.

Laiz-Carrión, R., Martín-del-Río, M.P., Soengas, J.L., Mancera, J.M., 2005. Efectos de una dieta inmunoestimuladora y de la permanencia en temperatura constante sobre el metabolismo hepático de carbohidratos en dorada *Sparus aurata* L., 1758. Bol. Inst. Esp. De. Oceanogr. 21, 83–89. ⟨http://hdl.handle.net/10508/7286⟩.

Lazado, C.C., Caipang, C.M.A., 2014. Mucosal immunity and probiotics in fish. Fish. Shellfish Immunol. 39 (1), 78–89. https://doi.org/10.1016/j.fsi.2014.04.015.

Liu, C.H., Chiu, C.H., Wang, S.W., Cheng, W., 2012. Dietary administration of the probiotic, *Bacillus subtilis* E20, enhances the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. Fish. Shellfish Immunol. 33 (4), 699–706. https://doi.org/10.1016/j.fsi.2012.06.012.

Liu, W., Ren, P., He, S., Xu, L., Yang, Y., Gu, Z., Zhou, Z., 2013. Comparison of adhesive GUT bacteria, immunity, and disease resistance in juvenile hybrid tilapia fed different Lactobacillus strains. Fish. Shellfish Immunol. 6 (34), 1661. https://doi.org/10.1016/j.fsi.2013.04.010.

Luthada-Raswiswi, R., Mukaratirwa, S., O'Brien, G., 2021. Animal protein sources as a substitute for fishmeal in aquaculture diets: a systematic review and meta-analysis. Appl. Sci. 11 (9), 3854. https://doi.org/10.3390/app11093854.

Mansour, A.T., Fayed, W.M., Elkhayat, B.K., Omar, E.A., Zaki, M.A., Nour, A.A.M., Morshedy, S.A., 2021. *Yucca schidigera* extract dietary supplementation affects growth performance, hematological and physiological status of European Seabass. Ann. Anim. Sci. 21 (3), 1043–1060. https://doi.org/10.2478/aoas-2021-0007.

Mazurkiewicz, J., Przybył, A., Golski, J., 2008. Usability of Fermacto prebiotic in feeds for common carp (*Cyprinus carpio* L.) fry. Nauka Przyr. Technol. 2 (3), 15. ⟨http://www.npt.up-poznan.net/tom2/zeszyt3/art_15.pdf⟩.

Méndez-Martínez, Y., Ceseña, C.E., Luna-González, A., García-Guerrero, M.U., Martinez-Porchas, M., Campa-Cordova, Á.I., Cortés-Jacinto, E., 2021. Effects of different dietary protein-energy ratios on growth, carcass amino acid and fatty acid profile of male and female *Cherax quadricarinatus* (von Martens, 1868) pre-adults. Aquac. Nutr. 27 (6), 2481–2496. https://doi.org/10.1111/anu.13379.

Montero, D., Izquierdo, M., 2010. Welfare and health of fish fed vegetable oils as alternative lipid sources to fish oil. Fish. oil Replace. Altern. Lipid Sources Aquac. Feeds.

Motlagh, H.A., Safari, O., Selahvarzi, Y., Baghalian, A., Kia, E., 2020. Non-specific immunity promotion in response to garlic extract supplemented diets in female Guppy (*Poecilia reticulata*). Fish. Shellfish Immunol. 97, 96–99. https://doi.org/10.1016/j.fsi.2019.12.007.

Nayak, S.K., 2010. Probiotics and immunity: a fish perspective. Fish. Shellfish Immunol. 29 (1), 2–14. https://doi.org/10.1016/j.fsi.2010.02.017.

Naylor, R.L., Hardy, R.W., Buschmann, A.H., et al., 2021. A 20-year retrospective review of global aquaculture. Nature 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6.

Oliva-Teles, A., 2012. Nutrition and health of aquaculture fish. J. Fish. Dis. 35 (2), 83–108. https://doi.org/10.1111/j.1365-2761.2011.01333.x.

Ortiz, L.T., Rebolé, A., Velasco, S., Rodríguez, M.L., Treviño, J., Tejedor, J.L., Alzueta, C., 2013. Effects of inulin and fructooligosaccharides on growth performance, body chemical composition and intestinal microbiota of farmed rainbow trout (*Oncorhynchus mykiss*). Aquac. Nutr. 19 (4), 475–482. https://doi.org/10.1111/j.1365-2095.2012.00981.x.

Pereira, L.F., Peixoto, M.J., Carvalho, P., Sansuwan, K., Santos, G.A., Gonçalves, J.F.M., Ozório, R.O.A., 2018. Cross-effects of dietary probiotic supplementation and rearing temperature on growth performance, digestive enzyme activities, cumulative mortality and innate immune response in seabass (*Dicentrarchus labrax*). Aquac. Nutr. 24 (1), 453–460. https://doi.org/10.1111/anu.12578.

Pérez-Sánchez, J., Benedito-Palos, L., Estensoro, I., Petropoulos, Y., Calduch-Giner, J.A., Browdy, C.L., Sitjà-Bobadilla, A., 2015. Effects of dietary NEXT ENHANCE® 150 on growth performance and expression of immune and intestinal integrity related genes in gilthead sea bream (*Sparus aurata* L.). Fish. Shellfish Immunol. 44 (1), 117–128. https://doi.org/10.1016/j.fsi.2015.01.039.

Piccolo, G., Bovera, F., Lombardi, P., Mastellone, V., Nizza, S., Di Meo, C., Marono, S., Nizza, A., 2015. Effect of *Lactobacillus plantarum* on growth performance and hematological traits of European sea bass (*Dicentrarchus labrax*). Aquac. Int. 23 (4), 1025–1032. https://doi.org/10.1007/s10499-014-9861-8.

Ramos, M.A., Batista, S., Pires, M.A., Silva, A.P., Pereira, L.F., Saavedra, M.J., Ozório, R.O.A., Rema, P., 2017. Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. Animal 11 (8), 1259–1269. https://doi.org/10.1017/S1751731116002792.

Rashidian, G., Boldaji, J.T., Rainis, S., Prokić, M.D., Faggio, C., 2021. Oregano (*Origanum vulgare*) extract enhances zebrafish (*Danio rerio*) growth performance, serum and mucus innate immune responses and resistance against *Aeromonas hydrophila* challenge. Animals 11 (2), 299. https://doi.org/10.3390/ani11020299.

Rastall, R.A., Gibson, G.R., 2015. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. Curr. Opin. Biotechnol. 32, 42–46. https://doi.org/10.1016/j.copbio.2014.11.002.

Řehulka, J., Minařík, B., Cink, D., Žalák, J., 2011. Prebiotic effect of fructooligosaccharides on growth and physiological state of rainbow trout, Oncorhynchusmykiss(WALBAUM). Acta Univ. Agric. Et. Silvic. Mendel. Brun. 59 (5), 227–235.

Rivera-Piza, A., Lee, S.J., 2020. Effects of dietary fibers and prebiotics in adiposity regulation via modulation of gut microbiota. Appl. Biol. Chem. 63 (1), 1–12. https://doi.org/10.1186/s13765-019-0482-9.

Sáenz de Rodrigáñez, M.A., Díaz-Rosales, P., Chabrillón, M., Smidt, H., Arijo, S., León-Rubio, J.M., Alarcón, F.J., Balebona, M.C., Moriguiño, M.A., Moyano, F.J., 2009. Effect of dietary administration of probiotics on growth and intestine functionality of juvenile Senegalese sole (*Solea senegalensis*, Kaup 1858). Aquac. Nutr. 15 (2), 177–185. https://doi.org/10.1111/j.1365-2095.2008.00581.x.

Sanquetta, C.R., Dalla Corte, A.P., Behling, A., de Oliveira Piva, L.R., Péllico Netto, S., Rodrigues, A.L., Sanquetta, M.N.I., 2018. Selection criteria for linear regression models to estimate individual tree biomasses in the Atlantic Rain Forest, Brazil. Carbon Balance Manag. 13 (1), 1–15. https://doi.org/10.1186/s13021-018-0112-6.

Şara, A., Barbu, A., Ani, A., Benţea, M., 2010. The effects of some additives on the bioproductive indices and meat quality of brook trout (Salvelinus fontinalis M.). Anim. Sci. Biotechnol. 43, 1.

Schreck, C.B., Tort, L., 2016. The concept of stress in fish. In: Fish Physiology, vol. 35. Academic Press, pp. 1–34. https://doi.org/10.1016/B978-0-12-802728-8.00001-1.

Serradell, A., Torrecillas, S., Makol, A., Valdenegro, V., Fernández-Montero, A., Acosta, F., Izquierdo, M.S., Montero, D., 2020. Prebiotics and phytogenics functional additives in low fish meal and fish oil based diets for European sea bass (Dicentrarchus labrax): Effects on stress and immune responses. Fish & shellfish immunology 100, 219–229.

Soleimani, N., Hoseinifar, S.H., Merrifield, D.L., Barati, M., Abadi, Z.H., 2012. Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (*Rutilus rutilus*) fry. Fish. Shellfish Immunol. 32 (2), 316–321. https://doi.org/10.1016/j.fsi.2011.11.023.

Tasa, H., Imani, A., Moghanlou, K.S., Nazdar, N., Moradi-Ozarlou, M., 2020. Aflatoxicosis in fingerling common carp (*Cyprinus carpio*) and protective effect of rosemary and thyme powder: Growth performance and digestive status. Aquaculture 527, 735437. https://doi.org/10.1016/j.aquaculture.2020.735437.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L., Izquierdo, M.S., 2007. Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish. Shellfish Immunol. 23, 969–981. https://doi.org/10.1016/j.fsi.2007.03.007.

Torrecillas, S., Makol, A., Betancor, M.B., Montero, D., Caballero, M.J., Sweetman, J., Izquierdo, M., 2013. Enhanced intestinal epithelial barrier health status on European sea bass (Dicentrarchus labrax) fed mannan oligosaccharides. Fish & shellfish immunology 34 (6), 1485–1495.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Ginés, R., Sweetman, J., Izquierdo, M., 2011. Improved feed utilization, intestinal mucus production and immune parameters in sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS). Aquac. Nutr. 17 (2), 223–233. https://doi.org/10.1111/j.1365-2095.2009.00730.x.

Torrecillas, S., Caballero, M.J., Mompel, D., Montero, D., Zamorano, M.J., Robaina, L., Rivero-Ramírez, F., Karalazos, V., Kaushik, S., Izquierdo, M., 2017a. Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets. Fish. Shellfish Immunol. 67, 302–311. https://doi.org/10.1016/j.fsi.2017.06.022.

Torrecillas, S., Mompel, D., Caballero, M.J., Montero, D., Merrifield, D., Rodiles, A., Robaina, L., Zamorano, M.J., Karalazos, V., Kaushik, S., Izquierdo, M., 2017b. Effect of fishmeal and fish oil replacement by vegetable meals and oils on gut health of European sea bass (*Dicentrarchus labrax*). Aquaculture 468, 386–398. https://doi.org/10.1016/j.aquaculture.2016.11.005.

Torrecillas, S., Rivero-Ramírez, F., Izquierdo, M.S., Caballero, M.J., Makol, A., Suarez-Bregua, P., Fernández-Montero, A., Rotllant, J., Montero, D., 2018. Feeding European sea bass (*Dicentrarchus labrax*) juveniles with a functional synbiotic additive (mannan oligosaccharides and *Pediococcus acidilactici*): An effective tool to reduce low fishmeal and fish oil gut health effects? Fish. Shellfish Immunol. 81, 10–20. https://doi.org/10.1016/j.fsi.2018.07.007.

Torrecillas, S., Terova, G., Makol, A., Serradell, A., Valdenegro, V., Gini, E., Izquierdo, M. S., Montero, D., 2019. Dietary phytogenics and galactomannan oligosaccharides in low fish meal and fish oil-based diets for European sea bass (*Dicentrarchus labrax*) juveniles: Effects on gut health and implications on in vivo gut bacterial translocation. PLoS One 14 (9), e0222063. https://doi.org/10.1371/journal.pone.0222063.

Van Dam, A.A., 1990. Multiple regression analysis of accumulated data from aquaculture experiments: a rice-fish culture example. Aquac. Res. 21 (1), 1–15. https://doi.org/10.1111/j.1365-2109.1990.tb00377.x.

Volpatti, D., Chiara, B., Francesca, T., Marco, G., 2013. Growth parameters, innate immune response and resistance to Listonella (*Vibrio anguillarum*) of *Dicentrarchus labrax* fed carvacrol supplemented diets. Aquac. Res. 45 (1), 31–44. https://doi.org/10.1111/j.1365-2109.2012.03202.x.

Wang, Q., Koval, J.J., Mills, C.A., Lee, K.I.D., 2007. Determination of the selection statistics and best significance level in backward stepwise logistic regression. Commun. Stat. -Simul. Comput. 37 (1), 62–72. https://doi.org/10.1080/03610910701723625.

Wu, Y., Liu, W.B., Li, H.Y., Xu, W.N., He, J.X., Li, X.F., Jiang, G.Z., 2013. Effects of dietary supplementation of fructooligosaccharide on growth performance, body composition, intestinal enzymes activities and histology of blunt snout bream (*Megalobrama amblycephala*) fingerlings. Aquac. Nutr. 19 (6), 886–894. https://doi.org/10.1111/anu.12033.

Yılmaz, S., Sebahattin, E., Celik, E., 2012. Effects of herbal supplements on growth performance of sea bass (*Dicentrarchus labrax*): Change in body composition and some blood parameters. J. Biosci. Biotechnol. 1 (3), 217–222.

Yousefi, M., Ghafarifarsani, H., Hoseinifar, S.H., Rashidian, G., Van Doan, H., 2021. Effects of dietary marjoram, *Origanum majorana* extract on growth performance, hematological, antioxidant, humoral and mucosal immune responses, and resistance of common carp, *Cyprinus carpio* against *Aeromonas hydrophila*. Fish. Shellfish Immunol. 108, 127–133. https://doi.org/10.1016/j.fsi.2020.11.019.

Zhou, C., Liu, B., Wang, G.T., Xie, J., Ge, X.P., Su, Y.T., 2009. Effects of the compound of oligosaccharide and Chine medicines and flavomycin on growth disease resistance of allogynogenetic crucian carp (Carassius auratus gebelio var. E`erqisi, Bloch). Freshw. Fish. 39, 44–51.

Zhou, Q.C., Buentello, J.A., Gatlin III, D.M., 2010. Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture 309 (1–4), 253–257. https://doi.org/10.1016/j.aquaculture.2010.09.003.