Dietary tributyrin supplementation enhances the immune and antioxidant responses of rainbow trout (Oncorhynchus mykiss) without changes in fish performance

Leonardo Julian Magnoni, Francisca Silva-Brito, Thais Cavalheri, Carlos Espirito-Santo, Mariana Palma, Rodrigo Ozório, Stéphane Panserat, Sofia Morais, Ivan Viegas

Aquaculture Reports 32 (2023) 101735

Contents lists available at ScienceDirect

Aquaculture Reports

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

Dietary tributyrin supplementation enhances the immune and antioxidant responses of rainbow trout (*Oncorhynchus mykiss*) without changes in fish performance☆

Leonardo Julian Magnoni [a,b,*], Francisca Silva-Brito [b], Thais Cavalheri [b], Carlos Espirito-Santo [b,c], Mariana Palma [d], Rodrigo Ozório [b], Stephane Panserat [e], Sofia Morais [f], Ivan Viegas [d]

[a] The New Zealand Institute for Plant and Food Research Limited, Port Nelson, Nelson, New Zealand
[b] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Matosinhos, Portugal
[c] Faculty of Sciences, University of Porto, Porto, Portugal
[d] University of Coimbra, Centre for Functional Ecology, Department of Life Sciences, Coimbra, Portugal
[e] INRAE, Universitéde Pau et des Pays de l'Adour, Saint-Pee-sur-Nivelle, France
[f] Lucta S.A., Innovation Division, Animal Science Unit, UAB Research Park, Bellaterra, Spain

ARTICLE INFO

Keywords:
Aquafeeds
Plant-derived ingredients
Butyric acid
Short-chain fatty acid
Growth performance
Fish welfare

ABSTRACT

Dietary supplementation with tributyrin (TBT), a source of butyric acid, has been shown to improve growth performance and health status when high levels of plant ingredients are included in aquafeeds. Here, we investigated the response of rainbow trout (*Oncorhynchus mykiss*) to a plant-based diet supplemented with TBT aiming to improve fish performance and welfare. Juvenile rainbow trout (19.0 ± 0.19 g, mean ± SEM) were divided into 12 tanks (210 L, 40 fish/tank) connected to a RAS system and fed four experimental diets in triplicate to apparent satiety. Diets tested comprised a basal diet (CTR) including 10% fishmeal and 10% fish oil, plus 83% plant-derived ingredients (44% crude protein, 18% crude fat, gross energy 21.8 MJ kg^{-1}) with increasing inclusion levels of TBT (55% purity), supplying 0.05% (TBT1), 0.1% (TBT2) and 0.2% (TBT4) of this compound. Fish performance was evaluated after 44 days. Following the growth trial, the effect of the diets on selected plasma innate immune and hepatic antioxidant parameters of trout was investigated at 3 h and 24 h after feeding. TBT supplementation had no significant impact on growth performance, feed efficiency, feed intake, or proximal composition in rainbow trout ($P > 0.05$). However, the plasma lysozyme activity was higher in fish fed the TBT2 diet than in those fed CTR or TBT4 diets after 3 h ($P = 0.021$). In addition, the plasma antiprotease activity in fish fed the TBT2 and TBT4 diets was higher than those fed the CTR diet after 24 h ($P = 0.010$ and 0.013, respectively). Catalase activity in the liver of trout fed the TBT4 diet was higher than in fish fed CTR or TBT2 diets after 3 h ($P = 0.016$ and 0.019). Results showed that effective levels of dietary TBT supplementation at 0.1% and 0.2% could result in improved immune and antioxidant responses in trout, respectively. Therefore, dietary TBT supplementation in this carnivorous species may result in enhanced welfare when high levels of plant-derived ingredients are used in aquafeeds.

1. Introduction

Aquaculture production is projected to steadily increase in the future, although it will encounter some challenges. One of the major concerns is the stable quantity of fishmeal (FM) and fish oil (FO) generated, which have traditionally been essential ingredients for aquafeed production (FAO, 2020). To tackle this growing concern, one of the focuses of aquaculture nutrition research has been the

Abbreviations: FO, Fish oil; FM, Fish meal; SBM, Soybean meal; SCFA, Short-chain fatty acids; TBT, Tributyrin.

☆ The manuscript has not been published elsewhere, accepted for publication elsewhere, or under editorial review for publication elsewhere. All the authors are revised and approved the submission of this manuscript.

* Corresponding author at: The New Zealand Institute for Plant and Food Research Limited, Port Nelson, Nelson, New Zealand.
E-mail address: Leonardo.Magnoni@plantandfood.co.nz (L.J. Magnoni).

https://doi.org/10.1016/j.aqrep.2023.101735
Received 13 June 2023; Received in revised form 10 August 2023; Accepted 11 September 2023

procurement of alternative ingredients (Glencross et al., 2020; Turchini et al., 2019). Plant-based diets have been tested and optimized for several fish species, decreasing the use of FM and FO in aquafeeds. However, the use of full plant-based diets in some cultured fish seems to be linked with reduced growth and higher disease outbreaks due to potential amino acid profile imbalance (Gatlin et al., 2007), inadequate fatty acid profile (Glencross, 2009) and/or the presence of anti-nutritional factors (Francis et al., 2001; Kokou and Fountoulaki, 2018), which may result in functional changes (Dhanasiri et al., 2020; Panserat et al., 2009), and may involve alterations in the gut microbiota (Pérez-Pascual et al., 2021; Ringø et al., 2016).

Previous studies in rainbow trout (*Oncorhynchus mykiss*) have shown that diets with total or partial replacement of FM or FO by plant-based ingredients result in impaired growth, reduced feed intake, and lower feed efficiency and protein retention (Lazzarotto et al., 2018; Le Boucher et al., 2011; Sadoul et al., 2016; Véron et al., 2016). Feeding rainbow trout plant-based diets resulted as well in alterations of several metabolic pathways (Lazzarotto et al., 2018; Panserat et al., 2009; Véron et al., 2016), and adverse effects on various immune-related parameters (Burrells et al., 1999; Jalili et al., 2013; Rumsey et al., 1994). In particular, the dietary replacement of FM by soybean meal (SBM) in rainbow trout has been shown to trigger an overall pro-inflammatory status, inducing gut inflammation and intestinal impairment (Blaufuss et al., 2020; Palma et al., 2021; Seibel et al., 2022).

Short-chain fatty acids (SCFA), including butyric acid, have been used as dietary supplements to reverse or ameliorate the potential negative effects of plant-derived ingredients in aquafeeds (Abdel-Latif et al., 2020; Estensoro et al., 2016; Rimoldi et al., 2016; Tran et al., 2020), improving the immune status (Abd El-Naby et al., 2019; Liu et al., 2019; Yamamoto et al., 2021). Butyric acid is a biologically active molecule, and its derived salt forms, particularly sodium butyrate, have been well-investigated as an aquafeed supplement (Abdel-Latif et al., 2020). Previous studies have shown a beneficial effect of sodium butyrate in rainbow trout when supplemented at 0.25% and 0.5% in a diet containing 18% SBM, increasing growth rate and feed efficiency (Mirghaed et al., 2022), as well as boosting the immune response and the fish resistance to disease (Mirghaed et al., 2019).

Tributyrin (TBT; or glyceryl tributyrate; CAS Number: 60–01–5) is a triglyceride composed of butyric acid and glycerol that presents several advantages over butyrate in aquafeeds (Palma et al., 2022). In particular, the active compound is released after the gastric passage, and each TBT mole can deliver up to three moles of butyric acid after complete hydrolysis (Hou et al., 2018; Newmark and Young, 1995; Volatiana et al., 2020a; Volatiana et al., 2020b). In contrast to the use of butyrate and its derived salt forms, dietary inclusion of TBT in aquaculture species is recent and has been less studied, although results suggest that it may be highly advantageous when supplemented in the diet of carnivorous species between 0.05% and 0.2% (Palma et al., 2022).

A previous study in juvenile yellow drum (*Nibea albiflora*) fed diets with FM substituted by SBM showed improved growth performance, suppression of pro-inflammatory genes, and improved intestinal morphology when TBT was supplemented at 0.1% (Tan et al., 2020). Similarly, juvenile black seabream (*Acanthopagrus schlegelii*) fed diets including SBM supplemented with TBT at 0.05% and 0.2% had an improved growth performance (Volatiana et al., 2020a), as well as a more healthy intestinal morphology with changes in the humoral antioxidant activity (Volatiana et al., 2020b). Therefore, assessing the effects of dietary TBT supplementation on plant-based diets is key for improving aquafeed formulations, as this compound could be used to optimize fish production and enhance animal welfare. This is particularly interesting for carnivorous species such as rainbow trout, requiring high protein diets.

Despite the importance of rainbow trout for freshwater aquaculture worldwide, no previous studies have reported on the potential effects of TBT when supplemented in a diet including 83% plant-derived ingredients. This study investigated the potential use of dietary TBT supplementation in this species aiming to improve fish performance and welfare by studying changes in growth rate, feed intake, feed conversion ratio, and selected parameters related to the innate immune and antioxidant responses.

2. Materials and methods

2.1. Experimental design

Juvenile rainbow trout were produced by Truticultura do Minho (Portugal), transported to the CIIMAR facilities, and quarantined. During this period, fish were fed twice a day with a commercial diet. Thereafter, 480 juvenile trout (19.0 ± 0.19 g, mean ± SEM) were randomly distributed in 12 tanks (210 L, 40 fish per tank, density 3.62 Kg m^{-3}) connected to a RAS system under controlled conditions and daily monitored (16 ± 1 °C; 0.3 ± 0.1‰; pH 6.0 ± 0.5; >95% air saturation; 12 h:12 h photoperiod). Water ammonium and nitrite levels were below 0.05 mg mL^{-1} and 0.5 mg mL^{-1}, respectively.

Four experimental diets, extruded pellets of 3 mm, were formulated and manufactured by Sparos Lda. (Portugal), consisting of a basal diet including 10% FM and 3% FO, plus 83% plant-derived ingredients with increasing inclusion levels of TBT in replacement of silica. The diets included 0% (CTR), 0.1% (TBT1), 0.2% (TBT2), and 0.4% (TBT4) of a TBT product (55% purity, Lucta S.A., Spain), resulting in effective concentrations of 0.05%, 0.1%, and 0.2%, respectively. The ingredients and proximal composition of the diets used in this trial are provided in Table 1. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed twice a day to apparent satiety at 9:00 h and 16:00 h. After 44 days, fish were feed deprived for 24 h and growth performance was evaluated. For this procedure, each group of fish was anesthetized with MS-222 (0.1 g L^{-1}) buffered with $NaHCO_3$ (0.2 g L^{-1}) in well-oxygenated water, individually weighted. Fish were returned to their original tanks for recovery and the previously described feeding regime was resumed. Six days after this procedure, nine fish per treatment (n = 3 per tank) were sampled to evaluate the impact of dietary TBT supplementation on innate immune parameters in plasma and antioxidant

Table 1
Ingredients and proximal composition of the experimental diets.

Ingredients (%)	CTR	TBT1	TBT2	TBT4
Fishmeal	10.0	10.0	10.0	10.0
Soy protein concentrate	12.5	12.5	12.5	12.5
Pea protein concentrate	12.5	12.5	12.50	12.5
Wheat gluten	13.0	13.0	13.0	13.0
Corn gluten meal	7.5	7.5	7.5	7.5
Soybean meal	5.0	5.0	5.0	5.0
Wheat meal	9.35	9.35	9.35	9.35
Wheat bran	4.0	4.0	4.0	4.0
Potato starch gelatinized	6.35	6.35	6.35	6.35
Vit. & Min. Premix	1.0	1.0	1.0	1.0
Vitamin E	0.05	0.05	0.05	0.05
Betaine	0.05	0.05	0.05	0.05
Antioxidant	0.2	0.2	0.2	0.2
MAP	1.5	1.5	1.5	1.5
L-Lysine	0.5	0.5	0.5	0.5
DL-Methionine	0.2	0.2	0.2	0.2
Silica	0.4	0.3	0.2	0.0
Tributyrin (TBT)	0.0	0.1	0.2	0.4
Fish oil	3.0	3.0	3.0	3.0
Rapeseed oil	12.9	12.9	12.9	12.9
Total	100.0	100.0	100.0	100.0
Diet proximal composition				
Dry matter (DM, %)	92.0	92.0	92.0	92.0
Crude protein (% DM)	44.0	44.0	44.0	44.0
Crude fat (% DM)	18.0	18.0	18.0	18.0
Ash (% DM)	5.2	5.2	5.2	5.2
Gross energy (MJ/kg)	21.8	21.8	21.8	21.8

CTR control diet. Diets TBT1, TBT2, and TBT4 included a product with TBT at 55% purity (Lucta S.A., Spain) resulting in effective inclusion levels of 0.05%, 0.1%, and 0.2%, respectively.

response in the liver at 3 h or 24 h post-feeding, being these sampling times representative of absorptive and post-absorptive states, respectively. Fish were anesthetized as described before and blood was collected from the caudal vein with heparinized syringes. Plasma was obtained immediately after blood centrifugation at 10.000 xg for 5 min. Fish were euthanized and the liver was dissected. Samples were stored at −80 °C until analyses.

2.2. Feed and whole-body composition analysis

Fish were sampled at the end of the growth trial (three fish per tank) and frozen at −20 °C. Whole animals were pooled per tank and ground. All the samples were freeze-dried and kept at −20 °C before analysis. Samples were analyzed for dry matter (105 °C for 24 h), ash by combustion using a muffle furnace (500 °C for 5 h), crude protein content (N × 6.25, Leco N analyzer, Model FP-528, Leco Corporation, St. Joseph, USA), and crude fat content by petroleum ether extraction (at 140 °C) in a Soxttherm Multistat/SX PC apparatus (Gerhardt, Germany).

2.3. Lysozyme activity

Lysozyme activity was measured in plasma using a turbidimetric assay as described by Costas et al. (2011). A suspension of *Micrococcus lysodeikticus* was prepared at a concentration of 0.5 mg mL^{-1} in phosphate buffered saline (PBS, Na_2HPO_4 0.05 M and pH 6.2). The sample (15 μL) and 250 μL of the bacterial suspension were added to each well of a plate in triplicate. The reaction was carried out at 25 °C and the absorbance at 450 nm was measured between 0.5 and 4.5 min in a microplate spectrophotometer (BioTek Instruments, Inc., USA). Lyophilized hen egg-white lysozyme (Sigma) was serially diluted in Na_2HPO_4 buffer (0.05 M, pH 6.2) and used to calculate the lysozyme activity in μg mL^{-1}.

2.4. Peroxidase activity

The peroxidase activity in plasma was quantified according to Quade and Roth (1997) with some modifications. Briefly, 15 μL of the sample was transferred to each well of a plate in triplicate. Then 50 μL of 3,3′,5,5′-tetramethylbenzidine hydrochloride (TMB, 20 mM), and 50 μL of H_2O_2 (5 mM) were added as substrates. The reaction was stopped after 2 min by adding 50 μL of H_2SO_4 (2 M). The OD was read at 450 nm in a microplate spectrophotometer (BioTek Instruments, Inc., USA). Hanks' balanced salt solution (HBSS) instead of plasma was used as a blank. One unit of activity was defined as a change of 1 unit of absorbance and expressed per mL^{-1} of plasma.

2.5. Antiprotease activity

Total antiprotease activity in plasma was measured according to Machado et al. (2015) with some modifications. Briefly, samples diluted with PBS (115 mM and pH 7.0) were incubated for 10 min at 25ºC with 10 μL of 5 mg mL^{-1} trypsin solution (Sigma-Aldrich). Then, 80 μL of PBS was added along with 125 μL of azocasein (Sigma-Aldrich) and incubated for 1 h at 22 °C. The reaction was stopped by adding 250 μL of trichloroacetic acid (TCA, 4.6%). The mixture was incubated for 30 min at 25 °C and centrifuged for 10 min at 10,000 xg. An aliquot of 100 μL of the supernatant was transferred along with 100 μL of NaOH 1 N to each well of a plate in triplicate. The OD was read at 450 nm in a microplate spectrophotometer (BioTek Instruments, Inc., USA). The sample was replaced by PBS in positive controls (100% protease), and both sample and trypsin were replaced by PBS in negative controls (0% protease). The percentage of inhibition of trypsin activity for each sample was calculated.

2.6. Protease activity

Protease activity in plasma was measured according to Guardiola et al. (2016) with some modifications. Samples and 125 μL of 2% azocasein (Sigma-Aldrich) were incubated for 24 h at 25 °C. The reaction was stopped by adding 250 μL of TCA (4.6%). The mixture was incubated for 30 min at 25 °C and centrifuged for 10 min at 10,000 xg. Aliquots of 100 μL of the supernatant was transferred to each well containing 100 μL of NaOH 1 N in triplicate. The OD was read at 450 nm in a microplate spectrophotometer (BioTek Instruments, Inc., USA). Samples were replaced by PBS and 10 μL of trypsin (5 mg mL^{-1}, Sigma) or just PBS to evaluate 100% and 0% of protease activity, respectively.

2.7. Oxidative stress parameters

Livers were homogenized in 0.1 M phosphate buffer (pH 7.4) in a ratio of 1:10. The protein concentration in the homogenates was quantified using bovine serum albumin as a standard (Bradford, 1976). Lipid peroxidation (LPO) was quantified by using thiobarbituric acid as a substrate (TBA test) (Ohkawa et al., 1979). Catalase (Cat, EC 1.11.1.6.) activity was quantified using hydrogen peroxide (30%) as a substrate (Claiborne, 1985). Glutathione reductase (GR, EC1.8.1.7) and glutathione peroxidase (GPx, EC 1.11.1.9.) activities were measured based on NADPH (Sigma, Portugal) oxidation at 340 nm (Cribb et al., 1989; Mohandas et al., 1984). Glutathione S-transferase (GST, EC 2.5.1.18) activity was measured using 1-chloro-2,4-dinitrobenzene as a substrate (Habig et al., 1974). Total and oxidized glutathione levels (TG and GSSG) were measured by the concomitant reaction of the reduced glutathione (GSH) with 5,5′-dithiobis-(2-nitrobenzoic acid; DTNB) and read at 412 nm (Baker et al., 1990). In the GSSG assay, 2-Vinyl-pyridine was used to remove all the free thiols present in the sample leaving only GSSG (Griffith, 1980). The GSH level was calculated as the result of subtracting the amount of GSSG from the TG. All the changes in absorption were measured at 25 °C in a microplate spectrophotometer (BioTek Instruments, Inc., USA), and reactions were performed in duplicates.

2.8. Calculations

Fish performance parameters were calculated as follows:

Weight gain (WG, in g) = Final body weight (g) – Initial body weight (g)

Daily growth index (DGI, as %) = $100 \times [(FBW)^{1/3} - (IBW)^{1/3}] \times$ time (days)

Feed conversion ratio (FCR) =Dry feed intake (g) / Wet weight gain (g)

Feed intake (FI, as % BW) = [Dry feed intake (g) / Mean body weight (g)] *100

2.9. Statistical analysis

Statistical analysis was performed using Sigmaplot software version 14.5 (2020 Systat Software, Inc.). Data were checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene test). A one-way ANOVA was performed to access the effects of diets on the fish performance at the end of the growth trial. A two-way ANOVA was performed to analyse the effect of diets (D) and time post-feeding (T) on humoral innate immune and oxidative stress parameters. When the effects of the factors were significant, the Tukey test was applied. Significant differences were considered when $P < 0.05$. The results were expressed as mean ± standard error of the mean (SEM).

3. Results

3.1. Fish growth performance

The effects of dietary TBT inclusion on the growth performance of rainbow trout are presented in Table 2**.** Despite tripling the initial body weight, feeding the experimental diets to rainbow trout at apparent satiation for 44 days did not result in significant differences in final body weight, weight gain, daily growth index, feed intake, or feed conversion ratio ($P > 0.05$, one-way ANOVA). Similarly, experimental diets had no significant impact on proximal body composition (Table 3, dry matter, protein, lipid, and ash contents) ($P > 0.05$, one-way ANOVA).

3.2. Humoral innate immune-related parameters

Innate immune response parameters in the plasma of rainbow trout fed either experimental diet at 3 h and 24 h post-feeding is presented in Fig. 1. Lysozyme activity was higher in the TBT2 group than in CTR or TBT4 groups after 3 h of feeding ($P = 0.021$ for both, two-way ANOVA). In addition, plasma antiprotease activity was higher in both TBT2 and TBT4 groups than in the CTR group 24 h after feeding ($P = 0.010$ and 0.013, respectively, two-way ANOVA). On the other hand, peroxidase and protease activities measured in plasma were not affected by the diets ($P > 0.05$, two-way ANOVA). Protease activity in plasma was significantly higher in trout sampled at 24 h than at 3 h post-feeding in both CTR and TBT4 groups ($P < 0.001$ and $P = 0.006$, respectively, two-way ANOVA). Antiprotease activity in plasma in the TBT4 group was also significantly higher at 24 h than at 3 h post-feeding ($P = 0.004$, two-way ANOVA).

3.3. Markers of oxidative stress in liver

The response of several markers of oxidative stress in the liver of rainbow trout to dietary TBT inclusion at 3 h and 24 h post-feeding is shown in Fig. 2. Higher catalase activity was displayed by the TBT4 group than the CTR or TBT2 groups at 3 h post-feeding ($P = 0.016$ and 0.019, respectively, two-way ANOVA). Other markers, including lipid peroxidation, total and oxidized glutathione, glutathione-S-transferase, glutathione reductase, and glutathione peroxidase activities remained similar between dietary groups either at 3 h or 24 h post-feeding ($P > 0.05$, two-way ANOVA). No significant differences were detected between trout at 3 h or 24 h post-feeding for a given dietary group ($P > 0.05$, two-way ANOVA).

Table 2
Growth performance parameters of rainbow trout fed diets supplemented with tributyrin (TBT) for 44 days.

Parameters	Dietary groups				
	CTR	TBT1	TBT2	TBT4	P-value
IBW	18.96 ± 1.01	18.48 ± 0.80	19.12 ± 0.35	19.39 ± 0.35	0.478
FBW	63.58 ± 6.42	63.13 ± 8.57	65.58 ± 2.56	60.63 ± 2.62	0.765
WG	44.62 ± 7.14	44.65 ± 7.79	46.45 ± 2.88	41.24 ± 2.47	0.724
FCR	0.79 ± 0.07	0.80 ± 0.08	0.79 ± 0.03	0.82 ± 0.06	0.933
DGI	3.00 ± 0.39	3.03 ± 0.32	3.09 ± 0.16	2.82 ± 0.12	0.658
FI	1.80 ± 0.15	1.82 ± 0.18	1.79 ± 0.07	1.86 ± 0.12	0.928

CTR control diet. Diets TBT1, TBT2, and TBT4 included TBT at 0.05%, 0.1%, and 0.2%, respectively. IBW, Initial body weight (g); FBW, Final body weight (g); WG, Weight gain (g); FCR, Feed conversion ratio; DGI, Daily growth index (%); FI, Feed intake (%). Values represent mean ± SEM (n = 3). The absence of letters indicates no significant differences between groups (P > 0.05, one-way ANOVA).

Table 3
Body proximal composition of rainbow trout fed the experimental diets for 44 days.

Proximal composition	Initial	Dietary groups CTR	TBT1	TBT2	TBT4
Dry matter (DM, %)	98.29 ± 0.45	98.94 ± 0.13	98.58 ± 0.13	98.99 ± 0.26	98.98 ± 0.28
Ash (% DM)	7.59 ± 0.34[a]	6.11 ± 0.07[b]	6.09 ± 0.24[b]	6.00 ± 0.21[b]	5.95 ± 0.34[b]
Lipid (% DM)	31.10 ± 1.60[a]	39.72 ± 1.82[b]	39.91 ± 0.52[b]	38.84 ± 0.19[b]	40.47 ± 1.91[b]
Protein (% DM)	60.39 ± 1.08[a]	53.70 ± 1.14[a,b]	53.06 ± 0.58[b]	54.03 ± 0.63[a,b]	52.51 ± 2.05[b]

CTR control diet. Diets TBT1, TBT2, and TBT4 included TBT at 0.05%, 0.1%, and 0.2%, respectively. Values are presented as mean ± SEM (n = 3). Different letters indicate a significant difference between groups ($P < 0.05$, one-way ANOVA).

4. Discussion

TBT supplementation has shown positive effects on the performance of cultured fish species when fed plant-based diets. Previous studies suggest that supplementation of TBT at 0.1% in plant-based diets resulted in improved growth performance in several fish species such as snakehead (*Channa argus*), yellow drum (*Nibea albiflora*), black sea bream (*Acanthopagrus schlegelii*), turbot (*Scophthalmus maximus*), and hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) (Hou et al., 2018; Tan et al., 2020; Volatiana et al., 2020b; Yang et al., 2021; Yin et al., 2021). The increase in fish growth appears to be at least partly linked to improved digestive and absorptive processes by modulating intestinal tract functions (Palma et al., 2022).

In this study, feeding juvenile rainbow trout a diet formulated with 83% of plant-derived ingredients to apparent satiation for 44 days did not result in improved growth performance or decreased FCR when TBT was supplemented at an effective quantity of 0.05–0.2%. These results were unexpected, as levels of butyric acid released from TBT in the digestive tract of rainbow trout by these diets may have reached between 0.165% and 0.66% after complete hydrolysis. In a previous study performed with juvenile rainbow trout for 45 days fed with a diet including 18% SBM supplemented with sodium butyrate (54% purity), growth was improved at effective concentrations of 0.135% and 0.27%, along with decreased FCR at 0.135% compared with the non-supplemented diet (Mirghaed et al., 2022). The disparity in the results could be due to the different FM and FO inclusion levels used, as well as the type and proportion of plant-based ingredients chosen for the replacement, as have been described for aquaculture species when butyric acid, butyrate, or TBT are supplemented in aquafeeds (Abdel-Latif et al., 2020; Palma et al., 2022). However, it could be possible that partial hydrolysis of TBT in the present study may result in lower effective concentrations of butyric acid released into the gastrointestinal tract. Interestingly, feeding adult rainbow trout for 51 days on diets supplemented with a blend of sodium formate (~0.6%) and sodium butyrate (~0.3%) did not improve growth or feed utilization in FM-based (~55%) or plant-based (~38%) diets (Gao et al., 2011).

Despite the lack of differences between treatments, rainbow trout tripled their initial BW by the end of the trial and the observed growth rate was consistent with values reported for this species under similar rearing conditions. For example, Rasmussen et al. (2007) showed that feeding juvenile rainbow trout a commercial high-quality energy-rich diet to apparent satiation for 42 days resulted in a similar growth rate to that reported in this study (~3% BW day^{-1} at 17.7°C). Because the basal diet reported here and used as a control (CTR) contained relatively low inclusion levels of FM (10%) and FO (3%), the lack of significant effect of TBT supplementation on growth performance was unexpected. This contrasts with the previously mentioned study in which

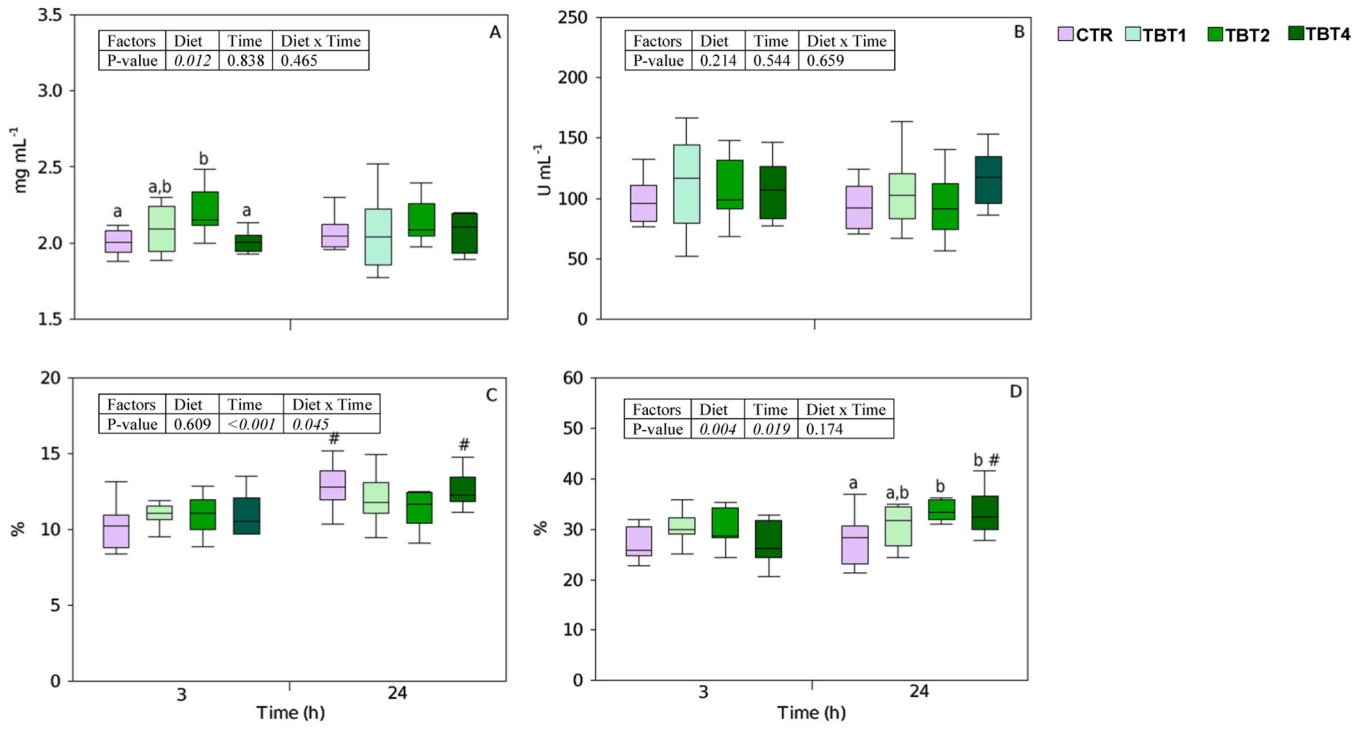

Fig. 1. Innate immune parameters in plasma of rainbow trout fed a plant-based diet supplemented with tributyrin (TBT). A, Lysozyme; B, Peroxidase; C, Protease; and D, Antiprotease. Fish were fed diets without (CTR) or with increasing inclusion levels of a product (55% purity) supplying 0.05% (TBT1), 0.1% (TBT2), and 0.2% (TBT4) of TBT for 50 days and sampled 3 h or 24 h after feeding. Boxes represent the upper 75th and lower 25th percentiles. Within each box, the black line represents the median. The whisker bars indicate the 90th and 10th percentiles (n = 9). Different superscript letters indicate significant differences between diets for a given time, whereas a different symbol indicates significant differences between time for a given diet ($P < 0.05$, two-way ANOVA outcome included in inset tables).

supplementation of butyric acid at 0.135% and 0.27% in a diet containing high levels of FM (45.5%) and 18% SBM resulted in increased growth of rainbow trout (Mirghaed et al., 2022). However, it should be highlighted that despite the high level (83%) of plant-based ingredients and low levels of FM (10%) and FO (3%) used to formulate the diets in our study, several good quality plant ingredients, which are unlikely to contain important amounts of anti-nutritional factors, were used at relatively low levels, rather than performing a replacement of FM with a single ingredient. In particular, the basal diet used in this study included 12.5% soy protein concentrate, 12.5% pea protein concentrate, 13% wheat gluten, 7.5% corn gluten meal, 9.35% wheat meal, 5% wheat bran, 6.35% starch, 12.9 rapeseed oil, and only 5% SBM. Similar growth rate without significant changes in feed intake, and lack of differences in the proximal body composition between the experimental groups, suggests that the metabolic utilization of the nutrients included in the feed was not altered by the TBT supplementation.

Despite the lack of changes in fish performance parameters, the current study provided important evidence suggesting a positive modulation of the innate immune response by dietary TBT supplementation, which could potentially revert into a better performance in situations when fish culture is performed in more stressful conditions. In this regard, changes detected in markers related to humoral immune response and oxidative stress in the liver suggest a beneficial effect of TBT on fish health and welfare. This is supported by the increased antiprotease activity detected in the plasma of fish 24 h after being fed TBT2 and TBT4 diets compared to the group fed the CTRL diet, suggesting an improved innate immune response. Host protease inhibitors are important non-specific humoral factors in the defence system, which are broadly present in fish and control several protease-mediated processes (Ellis, 2001). One of the functions proposed for this enzyme is the inactivation of proteases (toxins) secreted by pathogenic bacteria (Ellis, 2001).

Another immune-related marker in the plasma of rainbow trout showing a positive modulation by dietary TBT supplementation was lysozyme activity. Lysozyme is important in mediating protection against microbial invasion, not only by its lytic activity against bacterial walls (Saurabh and Sahoo, 2008; Smith et al., 2019; Uribe et al., 2011) but also by activating phagocytes and the complement system of fish (Saurabh and Sahoo, 2008; Smith et al., 2019). Our study shows an increase in the activity of this enzyme 3 h after being fed the diet-supplemented TBT at 0.1% (TBT2) compared to the groups fed the CTRL or TBT4 diets. A similar response for this humoral innate immune parameter was observed in the intestine of common carp (*Cyprinus carpio*) fed all-plant diets, which showed a higher lysozyme activity when TBT was supplemented in the basal diet above 0.1% (Xie et al., 2021). Interestingly, plasma lysozyme activity was increased in juvenile rainbow trout fed a diet supplemented with sodium butyrate at 0.081% and 0.135%, but a higher level (0.27%) resulted in a contrary response (Mirghaed et al., 2022). This suggests that lysozyme activity in trout might display a quadratic response to butyric acid levels, as evidenced also by the present study showing no effect of the TBT4 diet.

Free radicals and reactive oxygen species (ROS) are generated under basal and even more during stressful conditions. Fish health status is closely related to free radicals and ROS formation, being the immune response constrained to some extent to avoid oxidative stress (OS) (Biller-Takahashi and Takahashi, 2018; Hoseinifar et al., 2021). To avoid and/or repair the damage that these compounds cause when generated in excess, organisms possess antioxidant defence systems that function to keep a balance between prooxidants and antioxidants, minimizing the risk of OS (Hoseinifar et al., 2021). Therefore, an increase in the efficiency of the antioxidant defence system is suggested to be a key mechanism displayed by the animals (Gora et al., 2018; Kamunde et al., 2019; Magnoni et al., 2017; Sotoudeh and Mardani, 2018). In the present study, we have shown an improved response in the antioxidant system displayed by an increase in catalase activity in the liver of rainbow trout 3 h after being fed the TBT4 diet. Catalase has a key role in the defence of organisms against OS (Ighodaro and Akinloye,

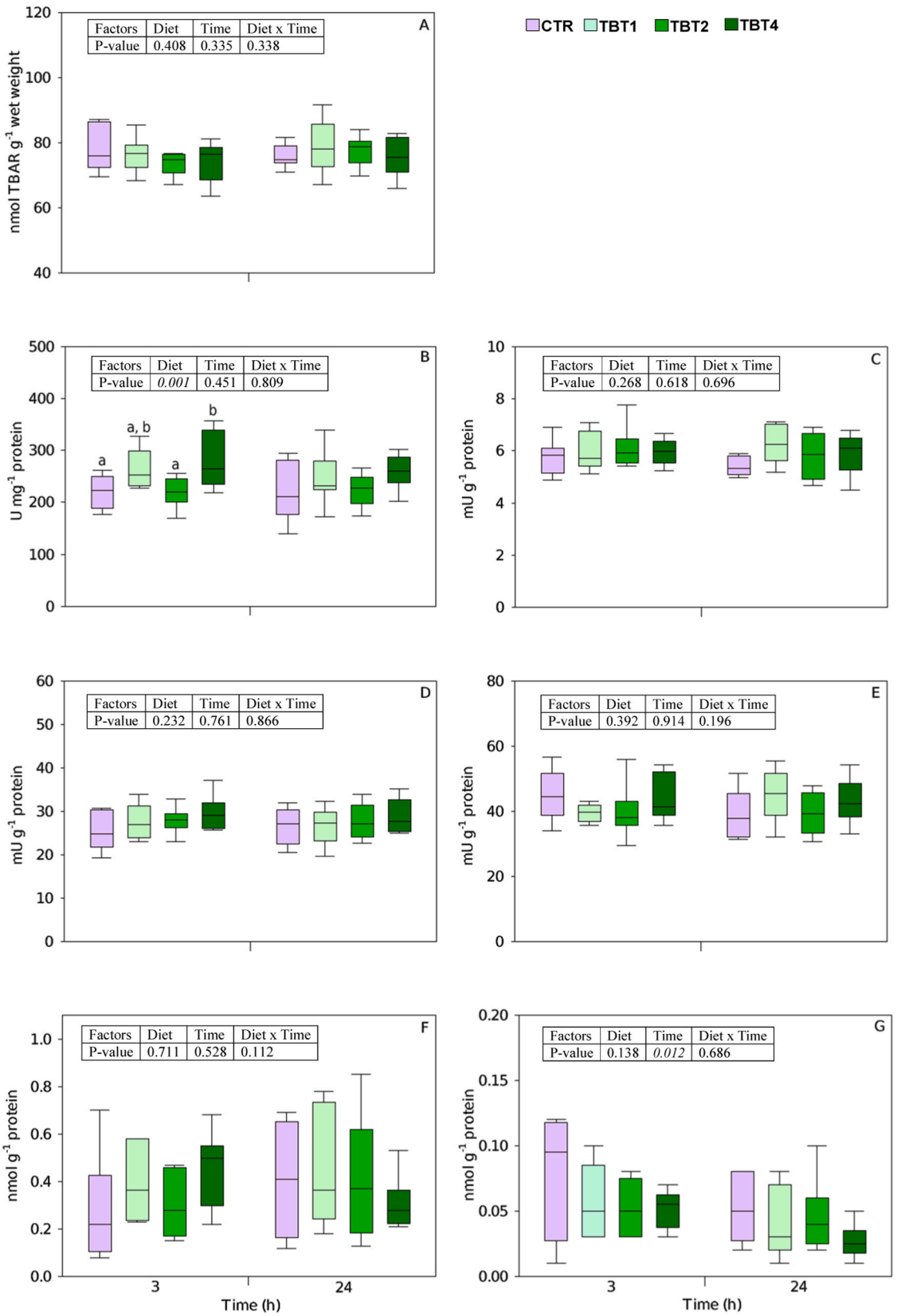

Fig. 2. Markers of oxidative stress in the liver of rainbow trout fed a plant-based diet supplemented with tributyrin (TBT). A, lipid peroxidation; B, catalase; C, glutathione reductase; D, glutathione peroxidase; E, glutathione-S transferase; F, total glutathione; G, oxidized glutathione. Fish were fed diets without (CTR) or with increasing inclusion levels of a product (55% purity) supplying 0.05% (TBT1), 0.1% (TBT2), and 0.2% (TBT4) of TBT for 50 days and sampled 3 h or 24 h after feeding. Boxes represent the upper 75th and lower 25th percentiles. Within each box, the black line represents the median. The whisker bars indicate the 90th and 10th percentiles (n = 9). Different superscript letters indicate significant differences between diets for a given time ($P < 0.05$, two-way ANOVA outcome included in inset tables).

2018), as it is required to decompose hydrogen peroxide (H_2O_2), which can be generated in abundance when the immune response is activated (Biller-Takahashi and Takahashi, 2018). A similar effect on catalase activity has been described in plasma of blunt snout bream fed a plant-based diet supplemented with TBT between 0.03% and 0.15%, showing as well an increased catalase gene expression in the intestine 24 h after feeding (Liang et al., 2021). Increased catalase activity and gene expression has been also shown in the intestine of common carp 24 h after being fed a plant-based diet supplemented with TBT between 0.02% and 0.04% (Xie et al., 2021).

Even though changes were detected in catalase activity in the liver of rainbow trout, several components of the glutathione antioxidant system analysed in this study, as well as lipid peroxidation, all remained similar between the groups. In contrast, TBT supplementation in snakehead fish fed a plant-based diet appeared to produce significant effects on markers of OS and antioxidant capacity linked to the glutathione system in the intestine (Hou et al., 2018). TBT supplementation at an effective concentration of 0.1% in a diet containing 10.5% SBM resulted in a significant increase in glutathione peroxidase activity in this tissue 24 h post-feeding, and in a decrease of malonaldehyde levels. Different organs might have different responses or sensitivities than that detected in the intestine, as this organ acts as a body barrier, and may have an important role in controlling ROS formation locally before systemic effects on OS become visible. Therefore, it seems possible that the challenge imposed by the diet in this study was not intense enough to induce changes in OS and antioxidant capacity linked to the glutathione system in liver of rainbow trout. Glutathione peroxidase is associated with antioxidant capacity, as it reduces free radical and ROS formation, whereas malonaldehyde levels are used to determine the degree of OS as they are produced from peroxidation of polyunsaturated fatty acids in tissues, in a similar way as the quantification of lipid peroxidation by the TBA test is often used, as in the present study. Other study has shown that glutathione peroxidase activity remained similar 24 h post-feeding in the serum of grass carp fed a plant-based diet supplemented with TBT at 0.05%, 0.10%, and 0.15%, although serum malonaldehyde levels were reduced at 0.10% and 0.15% TBT supplementation (Hu et al., 2021). In blunt snout seabream fed a plant-based, dietary TBT supplementation did not affect total glutathione levels in plasma 24 h post-feeding, but at 0.03% and 0.09% glutathione peroxidase activity was increased, and malondialdehyde levels were reduced at 0.06% (Liang et al., 2021).

5. Conclusions

In the present study, with a diet containing 83% plant-based ingredients, the supplementation with TBT at the levels investigated did not improve fish growth or feeding efficiency. However, the results suggest that dietary TBT supplementation at 0.1% could result in improved immunity as indicated by the increased lysozyme and anti-protease activities in plasma. In addition, increased catalase activity in the liver of trout fed the diet-supplemented TBT at 0.2% can result in improved antioxidant capacity. Therefore, dietary supplementation with this triglyceride supplying butyric acid, a short-chain fatty acid, may result in enhanced welfare when FM and/or FO are substantially reduced and replaced by plant ingredients in aquafeeds.

6. Institutional review board statement

This study was conducted under the supervision of accredited experts in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGAV-Portugal, following FELASA category C recommendations), according to the guidelines on the protection of animals used for scientific purposes from the European directive 2010/63/UE. The fish trial took place at the aquaculture facilities of the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR). The fish trial was carried out following the ARRIVE (Animal Research: Reporting of In Vivo Experiments) Guidelines for Reporting Animal Research. The experimental protocol was approved by the Animal Welfare and Ethics Body committee of CIIMAR with the final authorization from the General Directorate for Food and Veterinary Medicine (ID: 0421/000/000/2020).

Funding

This work was supported by Fundação para a Ciência e Tecnologia (FCT; Portugal), co-funded by the European Regional Development Fund (ERDF/FEDER) through the Programa Operacional Factores de Competitividade (POFC) in the form of a research grant to I.V. (ConTribuT: PTDC/BAA-AGR/3550/2020); and structural funds to R&D Unit Center for Functional Ecology - Science for People and the Planet (CFE), with reference UIDB/04004/2020, financed by FCT/MCTES through national funds (PIDDAC).

CRediT authorship contribution statement

Conceptualization: **L.J. Magnoni**, **S. Panserat**, **S. Morais**, **I. Viegas**; Formal analysis: **L.J. Magnoni**, **F. Silva-Brito**, **C. Espírito Santo**, **T. Cavalheri**, **R.O.A. Ozório**; Funding acquisition: **L.J. Magnoni**, **I. Viegas**; Methodology: **L.J. Magnoni**, **F. Silva-Brito**, **C. Espírito Santo**, **T. Cavalheri**, **R.O.A. Ozório**, **I. Viegas**; Resources: **L.J. Magnoni**, **R.O.A. Ozório**, **I. Viegas**; Supervision: **L.J. Magnoni**, **R.O.A. Ozório**, **I. Viegas**; Writing - original draft: **L.J. Magnoni**; Writing - review & editing: **F. Silva-Brito**, **R.O.A. Ozório, M. Palma**, **S. Panserat**, **S. Morais**, **I. Viegas**.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Acknowledgments

The authors are grateful to Wesley Ferreira (University of Porto-CIIMAR) for assistance in the trials and during sampling. Thanks to the personnel at the aquatic care facilities of BOGA (CIIMAR) for support during the fish quarantine and the trial.

References

Abd El-Naby, A.S., Khattaby, A.E.-R.A., Samir, F., Awad, S.M.M., Abdel-Tawwab, M., 2019. Stimulatory effect of dietary butyrate on growth, immune response, and resistance of Nile tilapia, Oreochromis niloticus against Aeromonas hydrophila infection. Anim. Feed Sci. Technol. 254, 114212.

Abdel-Latif, H.M.R., Abdel-Tawwab, M., Dawood, M.A.O., Menanteau-Ledouble, S., El-Matbouli, M., 2020. Benefits of dietary butyric acid, sodium butyrate, and their protected forms in aquafeeds: a review. Rev. Fish. Sci. Aquac. 28, 421–448.

Baker, M.A., Cerniglia, G.J., Zaman, A., 1990. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal. Biochem. 190, 360–365.

Biller-Takahashi, J., Takahashi, L., 2018. Oxidative stress and fish immune system: phagocytosis and leukocyte respiratory burst activity. An. da Acad. Bras. De. Ciências 90, 3403–3414.

Blaufuss, P.C., Bledsoe, J.W., Gaylord, T.G., Sealey, W.M., Overturf, K.E., Powell, M.S., 2020. Selection on a plant-based diet reveals changes in oral tolerance, microbiota and growth in rainbow trout (Oncorhynchus mykiss) when fed a high soy diet. Aquaculture 525, 735287.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Burrells, C., Williams, P.D., Southgate, P.J., Crampton, V.O., 1999. Immunological, physiological and pathological responses of rainbow trout (Oncorhynchus mykiss) to increasing dietary concentrations of soybean proteins. Vet. Immunol. Immunopathol. 72, 277–288.
Claiborne, A., 1985. Handbook of methods for oxygen radical research (Florida). CRC Press,, Boca Raton.
Costas, B., Conceição, L.E., Dias, J., Novoa, B., Figueras, A., Afonso, A., 2011. Dietary arginine and repeated handling increase disease resistance and modulate innate immune mechanisms of Senegalese sole (*Solea senegalensis* Kaup, 1858). Fish. Shellfish Immunol. 31, 838–847.
Cribb, A.E., Leeder, J.S., Spielberg, S.P., 1989. Use of a microplate reader in an assay of glutathione reductase using 5,5′-dithiobis(2-nitrobenzoic acid). Anal. Biochem. 183, 195–196.
Dhanasiri, A., Chen, X., Dahle, D., Siriyappagouder, P., Fæste, C.K., Fernandes, J.M.O., 2020. Dietary inclusion of plant ingredients induces epigenetic changes in the intestine of zebrafish. Epigenetics 15, 1035–1051.
Ellis, A.E., 2001. Innate host defense mechanisms of fish against viruses and bacteria. Dev. Comp. Immunol. 25, 827–839.
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, Á., Øverland, 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, e0166564.
FAO, 2020. The State of World Fisheries and Aquaculture 2020, Sustainability in action. FAO,, Rome.
Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227.
Gao, Y.L., Storebakken, T., Shearer, K.D., Penn, M., Overland, M., 2011. Supplementation of fishmeal and plant protein-based diets for rainbow trout with a mixture of sodium formate and butyrate. Aquaculture 311, 233–240.
Gatlin III, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38, 551–579.
Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquac. 1, 71–124.
Glencross, B.D., Baily, J., Berntssen, M.H.G., Hardy, R., MacKenzie, S., Tocher, D.R., 2020. Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. Rev. Aquac. 12, 703–758.
Gora, A.H., Sahu, N.P., Sahoo, S., Rehman, S., Ahmad, I., Agarwal, D., Dar, S.A., Rasool, S.I., 2018. Metabolic and haematological responses of *Labeo rohita* to dietary fucoidan. J. Appl. Anim. Res. 46, 1042–1050.
Griffith, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem. 106, 207–212.
Guardiola, F.A., Cuesta, A., Esteban, M.Á., 2016. Using skin mucus to evaluate stress in gilthead seabream (*Sparus aurata* L. Fish. Shellfish Immunol. 59, 323–330.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.
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. Aquac. 29, 198–217.
Hou, Y., Hou, Y., Yao, L., Chen, S.Y., Fan, J., Qian, L., 2018. Effects of chromium yeast, tributyrin and bile acid on growth performance, digestion and metabolism of Channa argus. Aquac. Res.
Hu, Y., Shi, Y., Liu, Y., Zhang, J., Xie, S., Liu, Z., Wei, Z., Zhong, L., 2021. Effects of tributyrin on growth performance, immune response and intestinal barrier function of juvenile grass carp (*Ctenopharyngodon idellus*) fed diets with high cottonseed and rapeseed meal. Aquac. Nutr. 27, 2468–2480.
Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex. J. Med. 54, 287–293.
Jalili, R., Tukmechi, A., Agh, N., Noori, F., Ghasemi, A., 2013. Replacement of dietary fish meal with plant sources in rainbow trout (Oncorhynchus mykiss); effect on growth performance, immune responses, blood indices and disease resistance. Iran. J. Fish. Sci. 12, 577–591.
Kamunde, C., Sappal, R., Melegy, T.M., 2019. Brown seaweed (AquaArom) supplementation increases food intake and improves growth, antioxidant status and resistance to temperature stress in Atlantic salmon, *Salmo salar*. PLOS ONE 14, 1–24.
Kokou, F., Fountoulaki, E., 2018. Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. Aquaculture 495, 295–310.
Lazzarotto, V., Médale, F., Larroquet, L., Corraze, G., 2018. Long-term dietary replacement of fishmeal and fish oil in diets for rainbow trout (*Oncorhynchus mykiss*): effects on growth, whole body fatty acids and intestinal and hepatic gene expression. PLOS ONE 13, e0190730.
Le Boucher, R., Quillet, E., Vandeputte, M., Lecalvez, J.M., Goardon, L., Chatain, B., Médale, F., Dupont-Nivet, M., 2011. Plant-based diet in rainbow trout (Oncorhynchus mykiss Walbaum): are there genotype-diet interactions for main production traits when fish are fed marine vs. plant-based diets from the first meal. Aquaculture 321, 41–48.
Liang, H., Ji, K., Ge, X., Xi, B., Ren, M., Zhang, L., Chen, X., 2021. Tributyrin plays an important role in regulating the growth and health status of juvenile blunt snout bream (Megalobrama amblycephala), as evidenced by pathological examination. Front. Immunol. 12.
Liu, Y., Chen, Z., Dai, J., Yang, P., Xu, W., Ai, Q., Zhang, W., Zhang, Y., Zhang, Y., Mai, K., 2019. Sodium butyrate supplementation in high-soybean meal diets for turbot (Scophthalmus maximus L.): effects on inflammatory status, mucosal barriers and microbiota in the intestine. Fish. Shellfish Immunol. 88, 65–75.
Machado, M., Azeredo, R., Díaz-Rosales, P., Afonso, A., Peres, H., Oliva-Teles, A., Costas, B., 2015. Dietary tryptophan and methionine as modulators of European seabass (*Dicentrarchus labrax*) immune status and inflammatory response. Fish. amp Shellfish Immunol. 42, 353–362.
Magnoni, L.J., Martos-Sitcha, J.A., Queiroz, A., Calduch-Giner, J.A., Gonçalves, J.F.M., Rocha, C.M., Abreu, H.T., Schrama, J.W., Ozorio, R.O., Pérez-Sánchez, J., 2017. Dietary supplementation of heat-treated *Gracilaria* and *Ulva* seaweeds enhanced acute hypoxia tolerance in gilthead sea bream (*Sparus aurata*). Biol. Open 6, 897–908.
Mirghaed, A.T., Yarahmadi, P., Soltani, M., Paknejad, H., Hoseini, S.M., 2019. Dietary sodium butyrate (Butirex (R) C4) supplementation modulates intestinal transcriptomic responses and augments disease resistance of rainbow trout (Oncorhynchus mykiss). Fish. Shellfish Immunol. 92, 621–628.
Mirghaed, T.A., Yarahmadi, P., Soltani, M., Paknejad, H., Pirali Kheirabadi, E., 2022. Beneficial effects of a sodium butyrate source on growth performance, intestinal bacterial communities, digestive enzymes, immune responses and disease resistance in rainbow trout (*Oncorhynchus mykiss*). J. Surv. Fish. Sci. 8, 1–15.
Mohandas, J., Marshall, J.J., Duggin, G.G., Horvath, J.S., Tiller, D.J., 1984. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: possible implications in analgesic nephropathy. Biochem. Pharmacol. 33, 1801–1807.
Newmark, H.L., Young, C.W., 1995. Butyrate and phenylacetate as differentiating agents: practical problems and opportunities. J. Cell. Biochem. 22, 247–253.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Palma, M., Bledsoe, J.W., Tavares, L.C., Romano, N., Small, B.C., Viegas, I., Overturf, K., 2021. Digesta and plasma metabolomics of rainbow trout strains with varied tolerance of plant-based diets highlights potential for non-lethal assessments of enteritis development. Metabolites 11.
Palma, M., Magnoni, L.J., Morais, S., Viegas, I., 2022. Tributyrin supplementation in fish and crustacean nutrition: a review. Rev. Aquac. n/a.
Panserat, S., Hortopan, G.A., Plagnes-Juan, E., Kolditz, C., Lansard, M., Skiba-Cassy, S., Esquerré, D., Geurden, I., Médale, F., Kaushik, S., Corraze, G., 2009. Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (Oncorhynchus mykiss) liver. Aquaculture 294, 123–131.
Pérez-Pascual, D., Pérez-Cobas, A.E., Rigaudeau, D., Rochat, T., Bernardet, J.-F., Skiba-Cassy, S., Marchand, Y., Duchaud, E., Ghigo, J.-M., 2021. Sustainable plant-based diets promote rainbow trout gut microbiota richness and do not alter resistance to bacterial infection. Anim. Micro 3, 47.
Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet. Immunol. Immunopathol. 58, 239–248.
Rasmussen, R.S., Larsen, F.H., Jensen, S., 2007. Fin condition and growth among rainbow trout reared at different sizes, densities and feeding frequencies in high-temperature re-circulated water. Aquac. Int. 15, 97–107.
Rimoldi, S., Finzi, G., Ceccotti, C., Girardello, R., Grimaldi, A., Ascione, C., Terova, G., 2016. Butyrate and taurine exert a mitigating effect on the inflamed distal intestine of European sea bass fed with a high percentage of soybean meal. Fish. Aquat. Sci. 19, 40.
Ringø, E., Zhou, Z., Vecino, J.L.G., Wadsworth, S., Romero, J., Krogdahl, Å., Olsen, R.E., Dimitroglou, A., Foey, A., Davies, S., Owen, M., Lauzon, H.L., Martinsen, L.L., De Schryver, P., Bossier, P., Sperstad, S., Merrifield, D.L., 2016. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story. Aquac. Nutr. 22, 219–282.
Rumsey, G.L., Siwicki, A.K., Anderson, D.P., Bowser, P.R., 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Vet. Immunol. Immunopathol. 41, 323–339.
Sadoul, B., Foucard, A., Valotaire, C., Labbé, L., Goardon, L., LeCalvez, J.M., Médale, F., Quillet, E., Dupont-Nivet, M., Geurden, I., Prunet, P., Colson, V., 2016. Adaptive capacities from survival to stress responses of two isogenic lines of rainbow trout fed a plant-based diet. Sci. Rep. 6, 35957.
Saurabh, S., Sahoo, P., 2008. Lysozyme: an important defence molecule of fish innate immune system. Aquac. Res. 39, 223–239.
Seibel, H., Chikwati, E., Schulz, C., Rebl, A., 2022. A multidisciplinary approach evaluating soybean meal-induced enteritis in rainbow trout oncorhynchus mykiss. Fishes 7, 22.
Smith, N.C., Rise, M.L., Christian, S.L., 2019. A comparison of the innate and adaptive immune systems in cartilaginous fish, ray-finned fish, and lobe-finned fish. Front. Immunol. 10, 1–23.
Sotoudeh, E., Mardani, F., 2018. Antioxidant-related parameters, digestive enzyme activity and intestinal morphology in rainbow trout (*Oncorhynchus mykiss*) fry fed graded levels of red seaweed, *Gracilaria pygmaea*. Aquac. Nutr. 24, 777–785.
Tan, P., Wu, X., Zhu, W., Lou, B., Chen, R., Wang, L., 2020. Effect of tributyrin supplementation in high-soya bean meal diet on growth performance, body composition, intestine morphology and microbiota of juvenile yellow drum (Nibea albiflora). Aquac. Res. 51, 2004–2019.
Tran, N.T., Li, Z., Wang, S., Zheng, H., Aweya, J.J., Wen, X., Li, S., 2020. Progress and perspectives of short-chain fatty acids in aquaculture. Rev. Aquac. 12, 283–298.
Turchini, G.M., Trushenski, J.T., Glencross, B.D., 2019. Thoughts for the future of aquaculture nutrition: realigning perspectives to reflect contemporary issues related to judicious use of marine resources in aquafeeds. North Am. J. Aquac. 81, 13–39.

Uribe, C., Folch, H., Enríquez, R., Moran, G., 2011. Innate and adaptive immunity in teleost fish: a review. Vet. Med. 56, 486–503.
Véron, V., Panserat, S., Le Boucher, R., Labbé, L., Quillet, E., Dupont-Nivet, M., Médale, F., 2016. Long-term feeding a plant-based diet devoid of marine ingredients strongly affects certain key metabolic enzymes in the rainbow trout liver. Fish. Physiol. Biochem. 42, 771–785.
Volatiana, J.A., Wang, L., Gray, N., Tong, S., Zhang, G., Shao, Q., 2020a. Tributyrin-supplemented high-soya bean meal diets of juvenile black sea bream, *Acanthopagrus schlegelii*: study on growth performance and intestinal morphology and structure. Aquac. Res. 51, 135–146.
Volatiana, J.A., Sagada, G., Xu, B., Zhang, J., Ng, W.-K., Shao, Q., 2020b. Effects of butyrate glycerides supplementation in high soybean meal diet on growth performance, intestinal morphology and antioxidative status of juvenile black sea bream, *Acanthopagrus schlegelii*. Aquac. Nutr. 26, 15–25.
Xie, D., Dai, Q., Xu, C., Li, Y., 2021. Dietary tributyrin modifies intestinal function by altering morphology, gene expression and microbiota profile in common carp (*Cyprinus carpio*) fed all-plant diets. Aquac. Nutr. 27, 439–453.
Yamamoto, F.Y., Older, C.E., Hume, M.E., Hoffmann, A.R., Gatlin, D.M., 2021. Effects of butyrate, propionate, and their combination in vitro, and the impacts of their supplementation in high-plant-protein diets to the production performance, innate immune responses, and intestinal microbiota of red drum (Sciaenops ocellatus). Aquaculture 545, 737225.
Yang, P., Zhang, Y., Sun, J., Liu, H., 2021. Effects of tributyrin supplemented in a high-soybean meal diet on the growth performance and intestinal histopathology of juvenile Scophthal musmaximus L. Isr. J. Aquac. - Bamidgeh.
Yin, B., Liu, H., Tan, B., Dong, X., Chi, S., Yang, Q., Zhang, S., 2021. Supplementing tributyrin to cottonseed protein concentrate-based diets can improve growth performance, lipid metabolism and distal intestinal immunity in hybrid grouper (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂). Aquac. Nutr. 27, 2378–2391.