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Growth, appetite regulation, intestine health, and functionality of gilthead seabream (*Sparus aurata*) fed different oilseed-based diets

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ABSTRACT

The growing inclusion of plant feedstuffs (PF) in aquafeeds has driven the partial replacement of fishmeal, with oilseed meals such as soybean, rapeseed, and sunflower among the most widely used, although they differ markedly in nutritional composition, particularly in their carbohydrate fractions. Within this context, this study investigated the effects of different oilseed-based diets on gilthead seabream (*Sparus aurata*) growth performance, feed utilization, liver composition and index, diets' apparent digestibility, plasma metabolites, gut morphology and immune-related gene expression, digesta short-chain fatty acids, and appetite-related gene expression. For that purpose, fish were fed diets containing either 30% soybean, rapeseed, sunflower, a mix of the three, or a control diet without oilseed ingredients (fish meal, corn gluten, wheat meal and gluten) for 65 days. The experimental diets did not affect growth, feed efficiency and body composition. Fish fed the Sunflower diet exhibited the lowest hepatosomatic index, liver lipid and glycogen levels, plasma glucose, and apparent digestibility coefficients for energy and lipids. Plasma triglycerides were higher in fish fed the Soybean and Rapeseed diets, while plasma cholesterol was increased in fish fed the Soybean and Sunflower diets. Digestive enzyme activity (amylase and lipase) was higher in fish fed the Rapeseed diet. While immune-related gene expression was not affected by dietary treatment, intestine morphology presented minimal changes, namely decreased goblet cell number and increased supranuclear vacuolization in fish fed the Soybean diet. Acetate concentration was higher in the gut of fish fed Sunflower and Soybean than in other diets. Intestine *peptide YY* and hypothalamus *cocaine- and amphetamine-regulated transcript* (*cart*) expression were increased in fish fed Soybean, Sunflower, and Mix diets (except for *cart* in Soybean diet).

In conclusion, diets with up to 30% oilseed inclusion supported normal growth of gilthead seabream juveniles and did not induce gut health disruption. However, the dietary carbohydrate profile influenced hepatic energy storage, nutrient utilization, and selected appetite-related molecular responses. These findings provide insight into potential diet–host interactions associated with oilseed-based formulations.

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1. Introduction

The need for alternative ingredients to fish meal (FM) in aquafeeds formulation has led to an increase in the use of plant feedstuffs (PF) as the primary suitable alternatives (Naylor et al., 2021). Among PF, oilseed meals such as soybean, rapeseed, and sunflower are widely used in aquafeeds and contribute substantially to FM replacement (Hussain et al., 2024). Nevertheless, PFs have some disadvantageous characteristics when compared to FM, such as antinutritional factors (ANF), amino acid imbalances, lower nutrient digestibility, and lower palatability (Francis et al., 2001; Hua et al., 2019; Glencross, 2020; Naylor et al., 2021).

In aquafeed formulation, combining different PF and supplementation with amino acids and attractants is a strategy to mitigate ingredient-specific limitations (Duodu et al., 2020) and meet amino acid requirements (Gatlin et al., 2007). PF have different carbohydrate profiles (Fig. 1), particularly the non-starch polysaccharides (NSPs) fraction (Knudsen, 2014; Navarro et al., 2019). For instance, while cereals have high starch content and low levels of NSPs, consisting mainly of cellulose, mixed linked β-(1,3) (1,4)-D-glucans, and arabinoxylans, oilseeds have low starch content and high levels of NSP, consisting mainly of cellulose, pectin, lignin, and xyloglucans (Knudsen, 2014; Navarro et al., 2019). Further differences exist in the nutritional profile of oilseed. Soybean and sunflower have high levels of acetic xylans and acetic pectic substances, while sunflower also presents significant amounts of xyloglucan (Düsterhöft et al., 1991; Knudsen, 2014; Navarro et al., 2019). Arabinans and arabinogalactans are significant constituents in rapeseed, with arabinogalactan being present also in soybean, either free or linked to rhamnogalacturonans (Knudsen, 2014; Navarro et al., 2019).

Starch is an available energy source for fish, since they produce digestive amylases, although with high differences between species, with herbivorous and omnivorous fish using starch more efficiently than carnivores (Enes et al., 2011). However, NSPs are an unavailable energy source since fish lack endogenous NSPs-degrading enzymes (Enes et al., 2011; Oliva-Teles et al., 2025) and can affect fish performance and feed utilization (Francis et al., 2001; Sinha et al., 2011; Maas et al., 2020; Porcino and Genovese, 2022). Further, dietary NSPs can be soluble (sNSP) and insoluble (iNSP), and this affects chyme viscosity due to the water-binding characteristics of sNSP (Wang et al., 2024b). While the growth of fish fed PF-rich diets may not be compromised in the short term, they may experience intestinal inflammation or impaired immune response (Sitjà-Bobadilla et al., 2005; Bonaldo et al., 2008; Kokou et al., 2012), affecting fish welfare and growth performance in the long term.

In carnivorous species, such as European seabass (*Dicentrarchus labrax)*, dietary NSPs drive putative selective pressure to favor gut microbiota more fit to metabolize NSPs (Serra et al., 2019, 2021). Fermentation of NSPs by intestinal microbiota produces short-chain fatty acids (SCFAs), which have been associated with improved fish growth performance and intestinal health (Tran et al., 2020). In addition, SCFAs are pointed out as key players in appetite regulation (van de Wouw et al., 2017; Han et al., 2021b).

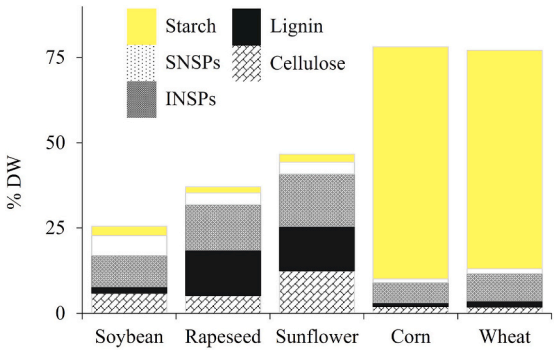

Fig. 1. Difference in starch and main dietary fiber components between oilseed and cereal ingredients. Note: Data adapted from Knudsen, 2014 and Navarro et al., 2019. All data are expressed as % of dry weight (%DW). Non-starch polysaccharides (NSP) include Cellulose, Klanson lignin, insoluble non-cellulosic polysaccharide (INCP) and soluble non-cellulosic polysaccharide (SNCP).

Appetite is affected by the energy intake/expenditure balance and the production/release by central and peripheral tissues of orexigenic and anorexigenic hormones that stimulate and inhibit feed consumption (Volkoff, 2016). Some studies have already evaluated the effects of diets totally or partially composed of PF mixtures on appetite-related gene expression (Hevrøy et al., 2008; Van Nguyen et al., 2013; Abasubong et al., 2021; Basto-Silva et al., 2021, 2022; Pulido-Rodriguez et al., 2021; Lorenz et al., 2022). However, studies evaluating the effect of specific PF on feed intake (FI) regulation are scarce and only available for soy protein concentrate (Volkoff et al., 2017; Sabioni et al., 2022; Calo et al., 2024).

The relevance of gut microbiota metabolism on appetite regulation through the microbiota-gut-brain axis has already been highlighted in humans and other mammals (van de Wouw et al., 2017; Martin et al., 2019; Romaní-Pérez et al., 2021). However, such evidence in fish is still scarce (Butt and Volkoff, 2019). Evaluating diet-induced gut microbiota NSPs fermentation and its effects on the microbiota-gut-brain axis in fish may unravel aspects of appetite regulation.

This study aimed to evaluate the effects of dietary inclusion of three oilseeds (soybean, rapeseed, and sunflower), differing in NSPs content, while maintaining constant FM inclusion to isolate the effects associated with the different oilseed ingredients, on growth performance, feed utilization, appetite-related gene expression, and gut health and functionality (digestive enzymes activity, histomorphology, immune-related gene expression, diet digestibility, and SCFAs production) in gilthead seabream (*Sparus aurata*), juveniles, an important aquaculture species in the Mediterranean. Understanding how oilseed-derived NSPs influence nutrient utilization, gut physiology, and appetite regulation may provide new insights into diet–host–microbiota interactions in fish and contribute to the optimization of plant-based aquafeeds for sustainable aquaculture.

2. Materials and methods

2.1. Experimental diets

Five isolipidic (18% lipids) and isoproteic (45% protein) diets were formulated to include 30% of soybean (Soybean diet), rapeseed (Rapeseed diet), sunflower (Sunflower diet), or 10% of each oilseed (Mix diet). A diet without oilseed meal was used as a control. For the digestibility assay, chromium oxide was added at 0.5% as an inert, indigestible tracer. All feeds were manufactured by grounding and mixing all the ingredients and were pelleted using a laboratory-scale pelletizer (California Pellet Mill, CPM Crawfordsville, IN, USA) passing through a 2 mm matrix and dried in a forced-air oven for 48 h at 45 °C. The formulation and proximate composition of the diets are summarized in Supplementary material.

2.2. Growth trial

The feeding trial was carried out by certified personnel (FELASA category C) in compliance with Directive 2010/63/EU on the use of animals for scientific purposes. The experimental protocol was reviewed and approved by the CIIMAR ORBEA Animal Welfare Committee (authorization ORBEA_CIIMAR_27_2019).

Gilthead seabream (*Sparus aurata*) juveniles obtained from IPMA (Olhão, Portugal) were acclimated for two weeks and fed a commercial diet (44% protein; 18% lipid; Aquasoja Sustainable Feed, Sorgal, Ovar, Portugal) to ensure uniform nutritional conditioning prior to the

experimental treatments and to prevent early adaptation to any of the experimental diets. After acclimation, fish were transferred to the experimental set up constituted by a recirculating aquaculture system (RAS) composed of 15 cylindrical fiberglass tanks (500 L each), maintained at 22.5 ± 0.4 °C, with filtered seawater (35.3 ± 0.8 g L^{-1} salinity) and dissolved oxygen levels of 7.5 ± 0.2 mg L^{-1}.

Fish with an initial body weight of 95.0 ± 0.1 g were randomly allocated in groups of 18 fish to each tank in triplicate. Fish were hand-fed to apparent satiation twice daily, six days per week, for a total of 65 days, ensuring minimal feed waste.

At the end of the trial, fish were starved for 24 h and then bulk-weighed after anaesthesia with ethylene glycol monophenyl ether (0.3 mL L^{-1}). Three fish per tank were sampled for visceral and hepatic indices and for whole-body composition. Additionally, nine fish were collected at day 0 to determine the initial whole-body composition.

Following sampling, the remaining fish were fed for an additional five days. Five hours after the morning feeding nine fish from each tank were euthanized in compliance with Directive 2010/63/EU. Blood was collected from 3 fish, and plasma was obtained by centrifugating at 3000 ×g for 10 min. Plasma was stored at −20 °C for subsequent metabolite analyses. The nine fish were also sampled to collected tissues as follows: (i) liver to determine lipid and glycogen content; (ii) whole intestine digesta (obtained by squeezing) for short chain fatty acid analysis (2 fish/tank); (iii) anterior intestine, stomach hypothalamus, and liver for appetite-related gene expression analysis (2 fish/tank). Three other fish were sampled to collect: (i) distal intestine for morphological evaluation, and (ii) anterior intestine for immune-related gene expression (2 fish/tank). Lastly, intestine with content were collected by three additional fish for digestive enzymes analysis.

Gene expression samples were stored in RNA later at 4 °C overnight and then stored at −80 °C with the other sample (plasma, liver, whole intestine, and digesta samples). Distal intestine sample were left for 24 h in phosphate-buffered formalin (4%, pH 7.4) before being conserved in ethanol (70%) until further processing.

Concerning the growth trial, the following parameters were calculated:

Daily growth index (DGI): ((final body $weight^{1/3}$ – initial body $weight^{1/3}$) / days) × 100.

FI : (total dry FI/ABW)/(duration of the trial), where average body weight (ABW) was calculated as: (initial body weight + final body weight)/2.

Feed efficiency (FE): wet weight gain/dry feed intake.

Protein efficiency ratio (PER): weight gain/crude protein intake.

Nitrogen retention (%NI): (NR / NI) × 100, where nitrogen retention (NR, g kg^{-1} day^{-1}) was calulated as: ((final body weight × % final whole-body protein) – (initial body weight × % initial whole-body protein)/6.25 × 1000) / ABW × time in days, and nitrogen intake (NI, g kg weight $gain^{-1}$) was calculated as: (protein intake / 6.25 × 1000) / weight gain.

Energy retention (%EI): (ER / EI) × 100, where energy retention (ER, kJ kg^{-1} day^{-1}) was calculated as: (((final body weight × % final whole-body energy) – (initial body weight × % initial whole-body energy)) × 1000)/ (average body weight × days), and energy intake (EI, kJ kg weight $gain^{-1}$) was calculated as: (energy intake × 1000)/weight gain.

Hepatosomatic index = (liver weight / body weight) × 100.

Visceral somatic index = (viscera weight / body weight) × 100.

2.3. Digestibility trial

The digestibility experiment was conducted in a recirculating aquaculture system consisting of 60 L fiberglass tanks (two replicate tanks per dietary treatment), configured following the design described by Cho et al. (1982). The system operated with a continuous supply of seawater maintained at 22.3 ± 1.6 °C, a salinity of 33.0 ± 0.6 g L^{-1}, and dissolved oxygen levels of 6.1 ± 0.4 mg L^{-1}. Fish (initial body weight of 93.3. ± 0.3 g) were randomly distributed in groups of 7 fish on ten tanks. Throughout the experiment, fish were hand-fed to apparent visual satiation twice daily, seven days per week. An adaptation period of eight days was allowed before fecal collection commenced. Thereafter, feces were collected once per day for 47 days prior to the morning feeding. Immediately after sampling, fecal material was centrifuged (3000 ×*g* for 10 min) The pellets collected were stored at −20 °C. Pooled feces from each tank were dried at 60 °C to and subsequently kept at room temperature until further analysis.

Apparent digestibility coefficient (ADC) of dry matter, proteins, lipids and energy was calculated as follows:

$$ADC: \left[1-\left(\frac{Cr_2O_3\ \text{level}^{\ \text{diet}} \times \text{nutrient or energy level}^{\ \text{feces}}}{Cr_2O_3\ \text{level}^{\ \text{feces}} \times \text{nutrient or energy level}^{\ \text{diet}}}\right)\right] \times 100$$

2.4. Proximate composition analysis

Proximate composition of the ingredients, experimental diets, whole-body samples, and feces was determined following standard procedures described by the Association of Official Analytical Chemists (AOAC, 2000). Dietary carbohydrates were assessed as follows: starch according to Beutler (1984); NDF (neutral detergent fiber), ADF (acid detergent fiber) and ADL (acid detergent lignin) according to Mertens (2002) and to Möller et al. (2009). NDF, ADF, and ADL analyses were performed using a VELP Scientifica FIWE 6 Fiber Analyzer (Code F30520200). Gross energy content was measured by combusting the samples in an adiabatic bomb calorimeter (PARR model 1261, PARR Instruments, Moline, IL, USA). Liver lipid was determined according to Folch et al. (1957), and glycogen according to Plummer (1987). Chromic oxide in diets and feces was determined according to Furukawa and Tsukahara (1966).

2.5. Plasma metabolites and digestive enzyme activities

The quantification of plasma metabolites was performed using commercial kits from (cholesterol, kit ref.: 1001090; triglycerides, kit ref.: 1001312, glucose kit ref.: 41010; lactate, kit ref.: 201001330; total protein, kit ref.: 1001290; total lipids, kit ref.: 1001270; Spinreact, S.A., Girona, Spain). To quantify the plasma metabolites a Multiskan GO microplate reader (Model 5111 9200; Thermo Scientific, Nanjing, China) was used to perform the absorbance reading. Intestinal samples with content were homogenized and centrifugated according to Fernandes et al. (2022) and stored in aliquot at −80 °C until assessment. Total alkaline protease activity, α-amylase (EC 3.2.1.1), and lipase (EC 3.1.1.3) activities were performed as described in Couto et al. (2016) and detected with a Multiskan GO microplate reader (Model 5111 9200; Thermo Scientific, Nanjing, China).

2.6. Gene expression

Samples were homogenized in TRIzol reagent using a Precellys Evolution homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France), adjusting manufacturer recommendations to the specific tissue homogenized. RNA was extracted using an extraction kit (DirectzolTM RNA Miniprep, Zymo Research) and quantified by spectrophotometry (μDrop™ plate, ThermoScientific, Waltham, VA, USA) Quality was assessed through electrophoresis on 1% agarose gel and through the 260/280 and 260/230 absorbance ratios. After adjusting RNA concentration to 0.5 μg/8 μL H_2O, complementary DNA (cDNA) was synthesized utilizing the NZY First-Strand cDNA Synthesis Kit (NZYTech. MB12502, Lisbon, Portugal).

Quantitative real-time PCR (qPCR) was performed using a CFX Connect™ Real-Time PCR Detection System (Bio-Rad, California, USA). Reaction mixture and PCR reaction was performed according to Santos et al. (2022) with slight modifications. PCR reaction set up consisted of a 30s incubation at 95 °C followed by 40 cycles of 15 s at 95 °C and 30s at

the annealing temperature to the specific primer (Table 1). Primer amplification efficiency was verified using a series of twofold cDNA dilutions and calculated from the slope of the standard curve plotting Ct values against relative cDNA concentrations, following the method described by Pfaffl (2001). Primer efficiency was between 90 and 110%, except in the intestine for *18 s*, *il10,* and *cck* (Table 1).

2.7. Volatile fatty acids in the digesta

SCFAs and branched-chain fatty acids (BCFAs) were extracted from fish digesta following the method described by Scortichini et al. (2020). After extraction, the organic phase was injected into an Agilent 8860 gas chromatograph equipped with an LL autosampler, a split/splitless injection system, and a flame ionization detector (Agilent, Santa Clara, CA, USA). Separation of the compounds was achieved on a CP-Wax 58 (FFAP CB) capillary column (50 m × 0.25 mm, 0.20 μm film thickness; Agilent, Santa Clara, CA, USA). Equipment set up and the quantification of acetate, propionate, and butyrate, isobutyrate, isovalerate, and valerate was performed according to De Carvalho et al., 2024. Sample peak areas were compared with those of standard solutions prepared in diethyl ether. (Sigma-Aldrich, St. Louis, MO, USA).

2.8. Histological analysis

The distal intestine samples were processed and sectioned using standard histological techniques and stained with hematoxylin and eosin. Stained slides were scanned using a Hamamatsu NanoZoomer C13140–01, and images were viewed with NDP.View2 software (Hamamatsu, Japan).

Sections of the distal intestine were evaluated in a double-blind manner using a semi-quantitative scale from 1 (normal morphology) to 5 (marked structural alteration). The assessment followed the descriptors outlined by Penn et al. (2011), considering parameters such as the height of the mucosal folds, the thickness and cellularity of the lamina propria and submucosa, and the extent of supranuclear vacuolization (SNV). Goblet cell presence was scored from abundant (1) to low (5).

2.9. Statistical analysis

SPSS version 27 for Windows (IBM® SPSS® Statistics, New York, USA) was used for all the statistical analyses. The experimental unit was defined according to the parameter analyzed: tank for growth performance, feed utilization, and digestibility data, and individual fish for plasma metabolites, digestive enzyme activities, histological evaluation, SCFAs quantification, and gene expression analyses. Fish were randomly sampled from replicate tanks within each dietary treatment. Data were first examined for normal distribution using the Shapiro–Wilk test and for homogeneity of variances using Levene's test; when these assumptions were not met, appropriate data transformations were applied. Except for the histological variables, all datasets were evaluated using one-way ANOVA, adopting a significance threshold of $p < 0.05$. When

Table 1
Appetite regulation- and immune-related genes and primers used for qPCR.

Gene	5′-3′ Sequence primer	Tissue	Annealing temperature (°C)	PCR Efficiency (%)	Accession number [a]	Reference
Appetite-related gene						
ghrelin	F:CCCGTCACAAAAACCTCAGAAC R:TTCAAAGGGGGCGCTTATTG	Stomach	64	91.1	MG570187	Forner-Piquer et al. (2018)
leptin	F:TCTCTTCGCTGTCTGGATTCCTGGAT R:CTCCTTCTTGCTCTGTAGCTCTT	Liver	64	107.9	KP822924	Babaei et al. (2017)
cck	F:CTGTGTACGAGCTGTTTGGGG R:AGCCGGAGGGAGAGCTTT	Intestine	60	85.8	KP822925	Babaei et al. (2017)
pyy	F:GATCGTGGATGATGCTCGC R:GTGATGAGGTTGACGTAATGCC	Intestine	59	94.4	XM030400857	This study
npy	F:AAACCGGAGAACCCCGGGGAGG R:CTGGACCTTTTTCCATACCTCTG	Hypothalamus	64	98.4	KP822926	Babaei et al. (2017)
cart	F:CTGA GGA GCA AAG AGA TGC CCT TAG AGA AA R:GCG TCA CAC GAA GGC AGC CA	Hypothalamus	64	94.4	MG570186	Forner-Piquer et al. (2018)
agrp	F:CCAACAGTCCTGTCTGGGTTA R:CAGTAGCAGATGGCGTTGAA	Hypothalamus	64	103.3	KX015827	Koch et al. (2019)
Immune-related gene						
il1β	F: GGG CTG AAC AAC AGC ACT CTC R: TTA ACA CTC TCC ACC CTC CA	Intestine	64	100.6	AJ277166	Cerezuela et al. (2013)
il10	F: TGG AGG GCT TTC CTG TCA GA R: TGC TTC GTA GAA GTC TCG GAT GT	Intestine	60	83.1	FG261948	Angosto et al. (2014)
cox 2	F: GAGTACTGGAAGCCGAGCAC R: GATATCACTGCCGCCTGAGT	Intestine	60	90.6	AM296029	Cerezuela et al. (2013)
tnf α	F: TCG TTC AGA GTC TCC TGC AG R: CAT GGA CTC TGA GTA GCG CGA	Intestine	60	103.5	AJ413189	Angosto et al. (2014)
Reference genes						
18 s	F: AGG GTG TTG GCA GAC GTT AC R: CTT CTG CCT GTT GAG GAA CC	Stomach	64	94.7	AM490061.1	Chaves-Pozo et al. (2008)
		Liver	64	94.6		
		Intestine	64	85.6		
		Hypothalamus	64	98.2		
Ef1α	F: CTG TCA AGG AAA TCC GTC GT R: TGA CCT GAG CGT TGA AGT TG	Stomach	64	101	AF184170	Cerezuela et al. (2013)
		Liver	64	105		
		Intestine	60	105.7		
		Hypothalamus	64	97.3		

18 s: ribosomal Protein S18; agrp: agouti related proteins; cart: cocaine- and amphetamine-regulated transcript; cck: cholecystokinin; cox2: cyclooxygenase 2; Ef1α: elongation factor 1 α; il1β: interleukin 1β; il10: interleukin-10; pyy: peptide y; npy: neuropeptide y; tnfα: tumor necrosis factor alpha. F: Forward; R: Reverse.

[a] From the GenBank database (https://www.ncbi.nlm.nih.gov).

significant effects were detected, Tukey's *post hoc* test was applied to identify differences among treatment groups. Histological data were analyzed using the non-parametric Kruskal–Wallis test, followed by pairwise comparisons with Bonferroni-adjusted significance levels.

3. Results

Experimental diets were well accepted, and no mortality was observed during the trials. At the end of the growth trial, no significant differences were observed between groups in growth performance, FI, feed efficiency, protein efficiency ratio, nitrogen and energy retention (as a percentage of intake), whole-body composition, and visceral somatic index (VSI) (Table 2).

The hepatosomatic index (HSI) ($p < 0.001$) and liver glycogen ($p < 0.05$) were lower in fish fed the Sunflower diet than in the Control diet, and liver lipid content ($p < 0.05$) was lower in fish fed the Sunflower and Mix diets than in the Control diet (Table 2).

Table 2
Growth performance, feed utilization, whole-body and liver composition (% wet weight), hepatosomatic (HSI), and visceral somatic (VSI) indices of gilthead seabream fed the experimental diets.

Diets	Control	Soybean	Rapeseed	Sunflower	Mix	*p-value*
Growth performance and feed utilization						
Final body weight (g)	177.9 ± 2.29	179.2 ± 11.64	167.9 ± 7.49	168.8 ± 4.44	170.4 ± 12.36	0.388
DGI	1.63 ± 0.04	1.65 ± 0.19	1.47 ± 0.12	1.48 ± 0.08	1.51 ± 0.20	0.395
FI (g kg ABW^{-1} day^{-1})	12.36 ± 0.73	12.84 ± 1.30	11.66 ± 1.05	11.51 ± 0.45	11.29 ± 1.22	0.352
FE	0.76 ± 0.05	0.73 ± 0.01	0.73 ± 0.05	0.75 ± 0.01	0.77 ± 0.02	0.489
PER	1.55 ± 0.09	1.54 ± 0.02	1.54 ± 0.10	1.59 ± 0.01	1.63 ± 0.04	0.384
NR (% NI)	26.46 ± 2.09	28.62 ± 0.77	30.9 ± 6.92	31.23 ± 2.07	31.18 ± 1.52	0.402
ER (% EI)	34.32 ± 3.06	31.15 ± 0.57	33.38 ± 3.30	31.31 ± 1.37	36.1 ± 2.41	0.128
Whole-body composition						
Protein	16.08 ± 0.67	16.83 ± 0.33	17.23 ± 1.38	17.18 ± 0.59	16.94 ± 0.48	0.428
Lipid	14.03 ± 1.04	12.81 ± 0.77	12.82 ± 1.13	11.96 ± 0.95	13.21 ± 0.67	0.175
Energy	8.71 ± 0.22	8.54 ± 0.13	8.74 ± 0.16	8.53 ± 0.21	8.85 ± 0.21	0.249
Ash	4.15 ± 0.10	4.03 ± 0.10	4.21 ± 0.37	4.16 ± 0.23	4.14 ± 0.37	0.940
Dry matter	34.38 ± 0.70	34.06 ± 0.82	34.89 ± 1.11	33.4 ± 0.47	34.38 ± 0.38	0.243
Liver composition						
Lipid	9.50 ± 1.20^{b}	7.10 ± 2.70^{ab}	8.70 ± 2.50^{ab}	6.60 ± 1.10^{a}	6.6 ± 2.10^{a}	0.013
Glycogen	8.34 ± 0.81^{b}	8.12 ± 1.42^{ab}	7.84 ± 1.09^{ab}	6.78 ± 1.1^{a}	7.75 ± 0.81^{ab}	0.041
Indexes						
HSI	1.48 ± 0.16^{b}	1.23 ± 0.17^{ab}	1.21 ± 0.15^{ab}	1.00 ± 0.12^{a}	1.15 ± 0.20^{ab}	0.000
VSI	5.58 ± 0.82	5.15 ± 0.72	5.21 ± 0.73	4.66 ± 0.68	5.61 ± 1.03	0.099

Mean values and standard deviation (±SD) are presented for each parameter (Growth performance, feed utilization, whole-body composition $n = 3$; Liver composition and Indexes n = 9). Different letters in the same row indicate significant differences between diets ($P < 0.05$). ABW: average body weight; CP: crude protein; DGI: daily growth index; DM: dry matter; HSI: hepatosomatic index; EI: energy intake; ER: energy retention; FE: feed efficiency; FI: feed intake; NI: nitrogen intake; NR: nitrogen retention; PER: protein efficiency ratio; VSI: visceral index.

The dietary treatments did not affect plasma protein, lipid, and lactate levels (Table 3). Plasmatic glucose ($p < 0.05$) was lower in fish fed the Sunflower and Mix diets than in the Control diet, while triglycerides ($p < 0.001$) were lower in the Sunflower diet than in the Soybean and Rapeseed diets. On the other hand, cholesterol was ($p < 0.001$) lower in fish fed the Rapeseed diet than in the Soybean and Sunflower diets.

Total alkaline protease activity was not affected by dietary treatment. In contrast, α-amylase activity ($p < 0.05$) was higher in fish fed the Rapeseed diet than in the Control diet, and lipase activity ($p < 0.001$) was higher in fish fed the Rapeseed diet than in the other diets (Table 3).

Propionate, butyrate, isobutyrate, isovalerate, and valerate were not detected in the digesta samples, while acetate ($p = 0.001$), the only SCFA detected, was higher in the digesta of fish fed the Sunflower and Soybean diets than in those fed the Control diet (Table 3).

Dietary treatment did not affect the ADC of dry matter and protein, while the ADC of energy ($p < 0.01$) and lipid ($p < 0.05$) was lower in fish fed the Sunflower diet than in the Control, Soybean, and Rapeseed diets (Table 4).

The distal intestine histomorphology evaluation (Table 5 and Fig. 2) showed no significant alterations in intestinal fold height, lamina propria, and sub-mucosa width and cellularity. On the other hand, a decrease in goblet cell number ($p < 0.01$) was observed in fish fed the Soybean diet compared to the Control, Rapeseed, and Sunflower diets (Table 5). SNV ($p < 0.01$) was higher in fish fed the Soybean diet compared to the Mix diet (Table 5).

The relative gene expression of intestinal inflammatory response biomarkers measured, namely *cyclooxygenase 2 (cox2), interleukin-10 (il10)*, *interleukin 1β (il1β),* and *tumor necrosis factor alpha (tnfα),* was not affected by dietary treatments (Fig. 3).

The relative gene expression of hypothalamus *agouti-related peptide* (*agrp*) and *neuropeptide y* (*npy*), liver *leptin*, stomach *ghrelin,* and intestine *cholecystokinin* (cck) was not affected by the diet composition (Fig. 4). However, the gene expression of hypothalamic *cocaine- and amphetamine-regulated transcript* (*cart*) ($p < 0.05$) was higher in fish fed the Sunflower and Mix diets than the Soybean diet. The relative gene expression of the intestine *peptide yy* (*pyy*) ($p < 0.01$) was higher in fish fed Soybean, Sunflower, and Mix diets than in the Control diet.

4. Discussion

Soybean, rapeseed, and sunflower meals are widely used as ingredients for aquafeeds (Kaiser et al., 2022; Shi et al., 2023; Qian et al., 2024). Nonetheless, their nutritional value can be compromised by the high content of NSPs and other ANF that might affect fish growth and gut health (Francis et al., 2001; Sinha et al., 2011; Porcino and Genovese, 2022). The present study, however, showed that when incorporated into diets at up to 30%, these oilseeds did not affect the growth performance on the model organism used in this study. Previously, no effects on gilthead seabream growth performance were observed when fed diets incorporating 20% rapeseed meal (Omnes et al., 2015), 30% soybean meal (Parma et al., 2016), or 24% sunflower meal (Sánchez Lozano et al., 2007). These results align with the reported adaptability of gilthead seabream to PF-based diets (Menoyo et al., 2004; Monge-Ortiz et al., 2016; Perera et al., 2019; Porcino and Genovese, 2022).

Nonetheless, while not compromising gilthead seabream growth performance, PF-rich diets were shown to induce intestinal inflammation and/or impair immune response (Sitjà-Bobadilla et al., 2005; Bonaldo et al., 2008; Kokou et al., 2012). In the present study, however, except for the reduction in goblet cell number in fish fed the Soybean diet, no significant alterations in gut morphology and immune response were detected when comparing the oilseed-based diets with the Control diet. Dysfunctional mucus and altered goblet cell profile are associated

Table 3
Plasma metabolites (mg dl^{-1}), digestive enzyme (mU mg^{-1} protein) and digesta short-chain fatty acid (mM) of gilthead seabream fed the experimental diets.

Diets	Control	Soybean	Rapeseed	Sunflower	Mix	*p-Value*
Plasmatic metabolite						
Glucose	101 ± 17[b]	87 ± 12[ab]	93 ± 16[ab]	78 ± 10[a]	80 ± 13[a]	0.011
Triglycerides	417 ± 48[ab]	504 ± 113[b]	538 ± 136[b]	332 ± 65[a]	447 ± 107[ab]	< 0.001
Cholesterol	241 ± 34[ab]	262 ± 24[b]	194 ± 38[a]	262 ± 50[b]	219 ± 30[ab]	< 0.001
Lipids	1391 ± 120	1646 ± 348	1526 ± 175	1396 ± 160	1425 ± 278	0.116
Proteins	3832 ± 367	3872 ± 352	3704 ± 254	3506 ± 394	3556 ± 280	0.096
Lactate	25 ± 10	27 ± 10	24 ± 9	24 ± 6	25 ± 8	0.936
Digestive enzyme						
Total alkaline protease	750.5 ± 259.9	683.9 ± 263.7	674.8 ± 155.7	746.7 ± 143	598.6 ± 75.3	0.499
α-amylase	533.9 ± 183.2[a]	647.3 ± 236.6[ab]	870 ± 261.8[b]	792.1 ± 259.1[ab]	674.4 ± 200.3[ab]	0.036
Lipase	21.1 ± 4.6[a]	24.3 ± 4.5[a]	35.6 ± 9.2[b]	24.1 ± 3.9[a]	23.2 ± 3.5[a]	< 0.001
Digesta short-chain fatty acid						
Acetate	1.36 ± 0.33[a]	2.12 ± 0.33[bc]	1.62 ± 0.29[ab]	2.18 ± 0.37[c]	1.82 ± 0.26[abc]	0.001

Mean values and standard deviation (±SD) are presented for each parameter of plasma and digestive enzyme ($n = 9$) and short-chain fatty acid ($n = 6$). Different letters in the same row indicate significant differences between diets ($P < 0.05$). Enzyme activities are expressed per mg of hepatic soluble protein (specific activity). Protein concentration was determined according to Bradford (1976) using bovine serum albumin as a standard.

Table 4
Apparent digestibility coefficients (ADC) of gilthead seabream fed the experimental diets.

Diets	Control	Soybean	Rapeseed	Sunflower	Mix	*p-value*
ADC dry matter	50.4 ± 6.6	57.5 ± 2.7	38.2 ± 11.2	34.5 ± 4.8	32.3 ± 5.0	0.049*
ADC Protein	84.6 ± 0.7	88.5 ± 0.8	82.7 ± 5.4	90.5 ± 0.5	85.8 ± 0.8	0.127
ADC Lipid	91.1 ± 0.4[b]	93.6 ± 0.0[b]	92.9 ± 3.2[b]	81.0 ± 3.6[a]	87.4 ± 1.4[ab]	0.013
ADC Energy	56.6 ± 3.4[bc]	69.4 ± 0.7[c]	54.6 ± 8.5[bc]	35.1 ± 2.6[a]	47.2 ± 4.8[ab]	0.007

Mean values and standard deviation (±SD) are presented for each parameter of plasma ($n = 2$). Different letters in the same row indicate significant differences between diets ($P < 0.05$). "*" Indicate that post-hoc test did not differ between diet.

Table 5
Score-based evaluation of the distal intestine histology of gilthead seabream fed the experimental diets.

Diets	Control	Soybean	Rapeseed	Sunflower	Mix	*p-value*
Fold height	1.18 ± 0.24	1.18 ± 0.25	1.27 ± 0.44	1.27 ± 0.44	1.56 ± 0.62	0.760
Lamina propria [a]	1.33 ± 0.43	1.5 ± 0.37	1.35 ± 0.4	1.33 ± 0.43	1.44 ± 0.39	0.820
Sub mucosa [a]	1.27 ± 0.44	1.5 ± 0.46	1.41 ± 0.42	1.24 ± 0.29	1.22 ± 0.26	0.598
Goblet cell	1.54 ± 0.36[a]	2.75 ± 0.65[b]	1.48 ± 0.4[a]	1.66 ± 0.35[a]	1.7 ± 0.2[a,b]	0.002
SNV [b]	1.46 ± 0.47[a,b]	3.08 ± 1.32[b]	1.57 ± 0.6[a,b]	1.48 ± 0.43[a,b]	1.16 ± 0.25[a]	0.005

Mean values and standard deviation (±SD) are presented for each parameter of plasma (n = 9). Different letters in the same row indicate significant differences between diets ($P < 0.05$).
[a] Width and cellularity.
[b] Supranuclearr vacuolization.

with inflammatory conditions, with impairments in the mucus system preceding inflammation (Gustafsson and Johansson, 2022), and the reduction in goblet cell number may indicate initial intestinal alterations in fish fed the Soybean-based diet, potentially associated with early intestinal stress responses, although no clear activation of inflammatory cytokines was observed. Moreover, supranuclear hyper vacuolization was also observed in the distal intestine of fish fed the Soybean diet, as also previously reported in gilthead seabream fed high dietary levels of PFs (Sitjà-Bobadilla et al., 2005). In the present study, acetate was the only SCFA detected, and it was higher in the digesta of fish fed the Soybean, Sunflower and Mix diets than the Control. Acetate is a major SCFA produced by gut microbiota and is involved in host–microbiota interactions, energy homeostasis, and the maintenance of intestinal health (Tran et al., 2020). For instance, SCFAs have been described as functional compounds modulating intestinal immune and physiological responses in European seabass (Fontinha et al., 2024), and dietary sodium acetate has been associated with improved intestinal status, reduced inflammatory markers, and modulation of the microbiota in juvenile yellow catfish (*Pelteobagrus fulvidraco*) (Wang et al., 2024a). Accordingly, the increased acetate levels observed in fish fed the Soybean diet may reflect a microbiota-mediated adaptive response contributing to intestinal homeostasis under dietary stress.

Compared to the Control diet, fish fed the Sunflower diet presented the lowest plasmatic glucose level, liver glycogen accumulation, and the lowest HSI. This was not unexpected since the Sunflower diet had the highest fiber and the lowest starch content, while the Control diet presented opposite characteristics. Previous studies in gilthead seabream have reported that high dietary starch levels are associated with increased glycemia levels (Couto et al., 2008; Basto-Silva et al., 2021). It is well established that the liver can store glycogen or convert it to lipids under condition of excess glucose (Enes et al., 2009). Moreover, a positive correlation between digestible starch, HSI, and glycogen content has been reported (Venou et al., 2006; Ekmann et al., 2013).

Although variations in PF sources and dietary carbohydrate content, namely in starch and NSPs content, have been reported to influence fish whole-body composition (Sinha et al., 2011; Liu et al., 2022a, 2022b; Qian et al., 2024; Han et al., 2021a; Horstmann Zuther et al., 2024), this was not observed in the present study. Similarly, dietary inclusion of up to 30% gelatinized starch did not affect gilthead seabream whole-body composition, although differences in HSI, liver composition, and

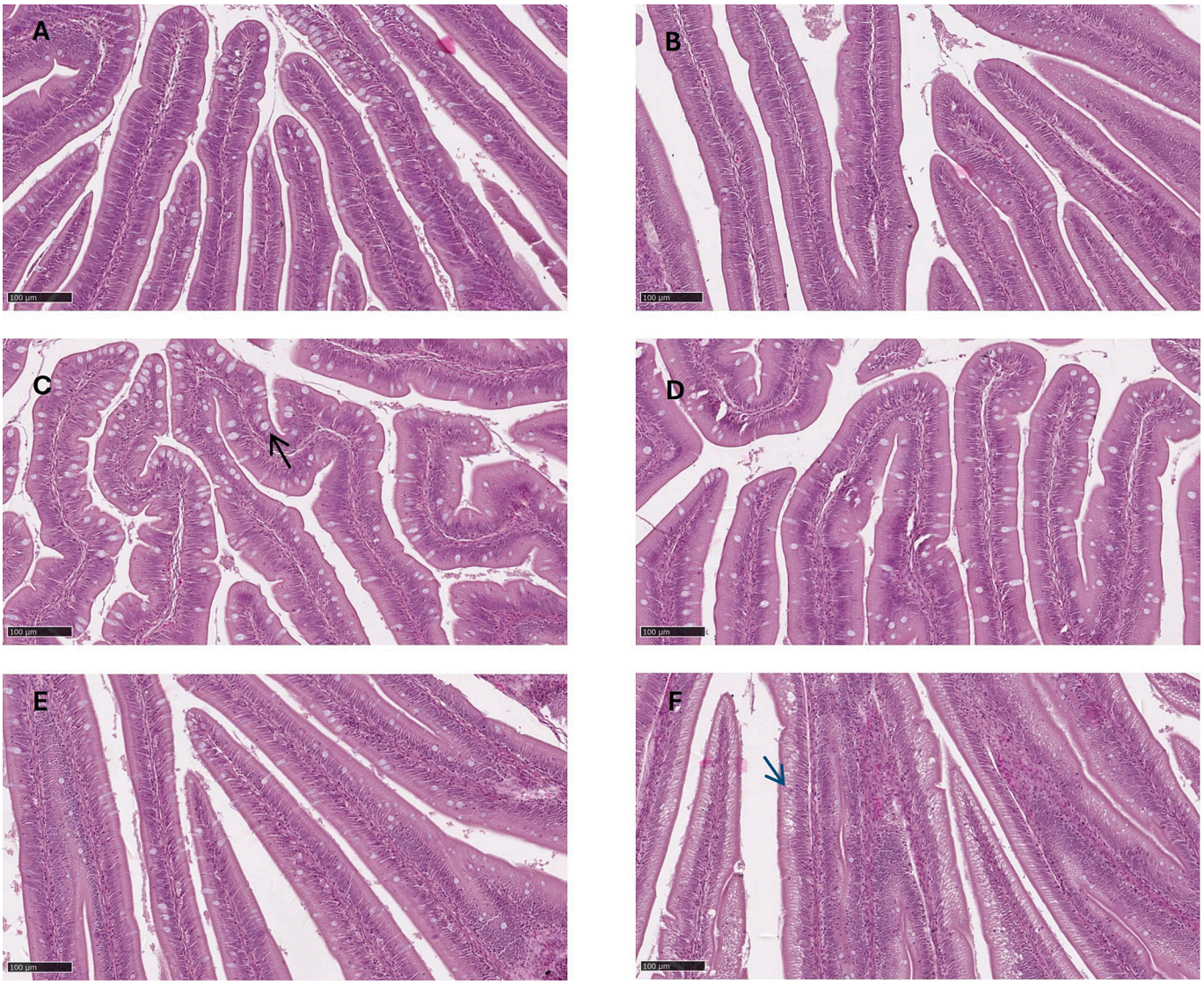

Fig. 2. Distal intestine of gilthead seabream fed the Control (A), Soybean (B), Rapeseed (C), Sunflower (D), and Mix (E) diets. Blue arrows indicate supranuclear hyper vacuolization highlighted in 40% of the distal intestine of fish fed Soybean diet (F). Black arrow indicates an example of goblet cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

plasmatic glucose were reported (Couto et al., 2008). However, it cannot be ruled out that differences in whole-body composition might have emerged over a more extended feeding period. Interestingly, the absence of changes in whole-body lipid content may be directly linked to the lack of differences in growth and FI, as body composition plays an important role in regulating energy balance, appetite, and growth performance in fish (Breck, 2014).

Dietary oilseed inclusion did not affect the diet's ADC, except for the Sunflower diet, which showed decreased ADC of lipids and energy. This diet had the highest amount of NSPs, and it is known that dietary NSPs content and profile influence gut transit time and limit nutrient absorption (Sinha et al., 2011; Prakash et al., 2025). Moreover, Sunflower diet had the highest NSP: starch ratio (6.1) in comparison to the other oilseed diets (Soybean diet = 1.3; Rapeseed diet = 2.8; Mix diet = 2.4), which may have increased the proportion of non-digestible carbohydrates relative to digestible energy substrates. On the other hand, fish fed the Rapeseed diet presented higher amylase and lipase activities than the fish fed the Control and all other diets, respectively. This could be related to the rapeseed NSPs profile. In pigs, the uronyl-rich pectic structures present in rapeseed were reported to form complex matrices with cellulose and lignin (Lannuzel et al., 2022). These structures may increase the bulk and surface area of digesta and interact with digestive enzymes, substrates, and allochthonous microbiota, potentially explaining the increased digestive enzyme activity observed in fish fed with the Rapeseed diet. Although it is well known that digesta viscosity is affected by NSPs solubility (Sinha et al., 2011; Wang et al., 2024b) and specific NSPs, such as arabinoxylans, galactomannans, and pectin (Sinha et al., 2011), further research is needed to clarify how changes in the NSPs profile affect viscosity and structural characteristics of fish digesta.

In the present study, plasma triglycerides and cholesterol of the control group were not different from those of the oilseed-based diets. However, within the experimental diets, plasma triglycerides were lower in fish fed the Sunflower diet than the Soybean and Rapeseed diets, and plasma cholesterol was lower in fish fed the Rapeseed diet than the Soybean and Sunflower diets.

While it is known that plasma cholesterol and triglyceride levels in fish are decreased by dietary fiber and NSPs in a dose-dependent manner (Ren et al., 2020; Zhong et al., 2020; Wang et al., 2024b), the results of the present study suggest that the effects are linked to the NSP profile, as also reported in Nile Tilapia (*Oreochromis niloticus*) (Jiang et al., 2022).

Higher triglyceride levels induced by rapeseed have also been reported in previous studies in largemouth bass (*Micropterus salmoides*)

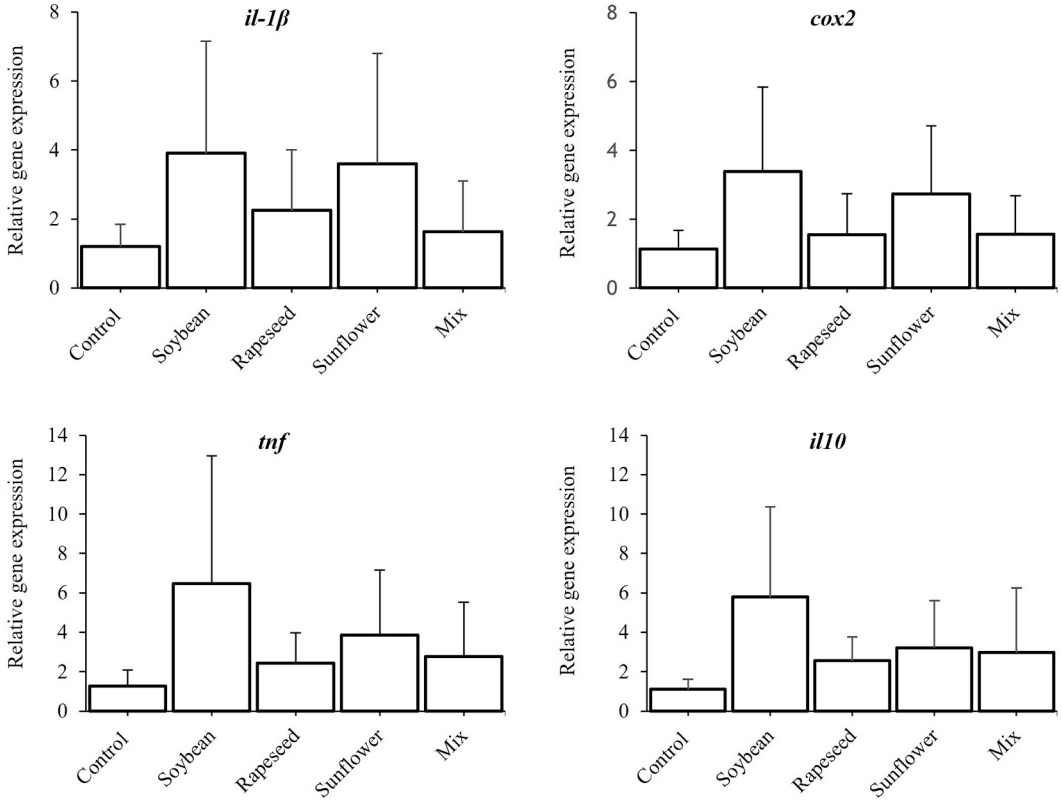

Fig. 3. Immune-related gene expression in the anterior intestine of gilthead seabream fed the experimental diets. Mean values and standard deviation (±SD) are presented for gene expression (n = 6). cox2: cyclooxygenase 2; il1β: interleukin 1β; il10: interleukin-10; tnfα: tumor necrosis factor alpha.

when rapeseed meal was included at levels exceeding 15% (Kang et al., 2025), as well as in other carnivorous species (Qian et al., 2024). Although the precise mechanisms remain unclear, it is possible that the unique NSP profile of rapeseed, particularly its uronyl-rich pectic structures, contributes to elevating plasma triglyceride levels through the previously discussed increase in digestive lipase activity.

In the case of the Soybean diet, plasma triglyceride levels may also be associated with soybean-specific ANF. Some studies have reported decreased triglyceride levels in fish fed with soybean due to impaired lipid absorption caused by soya saponins (Gu et al., 2014; Knudsen et al., 2008). Recent evidence also suggests that soybeans may affect lipid metabolism beyond simple malabsorption. For instance, treatment with soybean trypsin inhibitor in mice subjected to metabolic stress led to increased plasma triglycerides, suggesting an upregulation of lipid and energy metabolism as a compensatory mechanism (Kuzmina et al., 2025). In zebrafish, soybean meal-based diets supplemented with saponins were shown to upregulate key components of the sterol regulatory element-binding proteins gene family, which regulate lipogenesis (Valenzuela et al., 2021). In carp, lipid metabolism under soybean meal diets appears to be modulated through the AMP-activated protein kinase signaling pathway (Zhao and Xu, 2022). In gilthead seabream, soya saponins were reported not to affect growth performance but they can disturb the intestinal mucosal structure, potentially compromising gut functionality (Couto et al., 2014). In the present study, the Soybean diet was associated with reduced goblet cell numbers, higher SNV, and a trend toward increased expression of immune-related genes. These alterations may reflect mild intestinal morphological changes potentially associated with early intestinal stress responses, potentially reducing lipid absorption efficiency. As a compensatory mechanism, hepatic *de novo* lipogenesis could have been upregulated, possibly explaining the observed elevation in plasma triglyceride levels. However, this remains speculative, and further analysis is needed to determine whether the increased triglyceride levels in the Soybean diet group originated from endogenous synthesis rather than direct dietary absorption.

Other antinutrients may have influenced plasma metabolite dynamics. For instance, the lower plasmatic cholesterol level in fish fed the Rapeseed diet than those fed the Sunflower and Soybean diets might be due to its high phytosterol levels (Sujith Kumar et al., 2017). Phytosterols are known to reduce cholesterol uptake by competing with cholesterol for enterocyte absorption sites, as demonstrated across species, including mice (Feng et al., 2018), broiler (Ding et al., 2021), and fish (Couto et al., 2015; He et al., 2022).

Despite appetite being affected by diet composition (Bertucci et al., 2019), few studies in fish evaluated the effect on appetite of specific PF-ingredients, except for soybean concentrate, which was studied in dourado (*Salminus brasiliensis)* and pacu (*Piaractus mesopotamicus*) (Volkoff et al., 2017; Sabioni et al., 2022) and fermented soybean wich was investigated in turbot (*Scophthalmus maximus*) (Dan et al., 2022) and in Chinese perch (Feng et al., 2022). In gilthead seabream, a study shown that the expression of some appetite-related genes were affected by the usage of PF as replacement of FM (Basto-Silva et al., 2021).

In the present study, diet composition did not affect FI, although some FI-related genes were expressed differently between groups. Intestinal *pyy* expression was increased in fish fed the Soybean, Sunflower, and Mix diets compared with the Control group, suggesting an anorexigenic role of these oilseeds. An anorexic role of pyy in fish has been suggested by studies showing that its administration reduced FI in goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*) (Velasco et al., 2018; Gonzalez and Unniappan, 2010). Previously, it was also observed that *pyy* expression was increased by soy protein concentrate (Sabioni et al., 2022) but it was not affected by swine liver hydrolysate (Lorenz et al., 2022) in dourado.

On the other hand, hypothalamic *cart* expression was higher in fish fed the Sunflower and Mix diets than in fish fed the Soybean diet. In another study with gilthead seabream, diets including 20–25% soybean meal also induced a decrease in *cart* expression compared to a FM-based

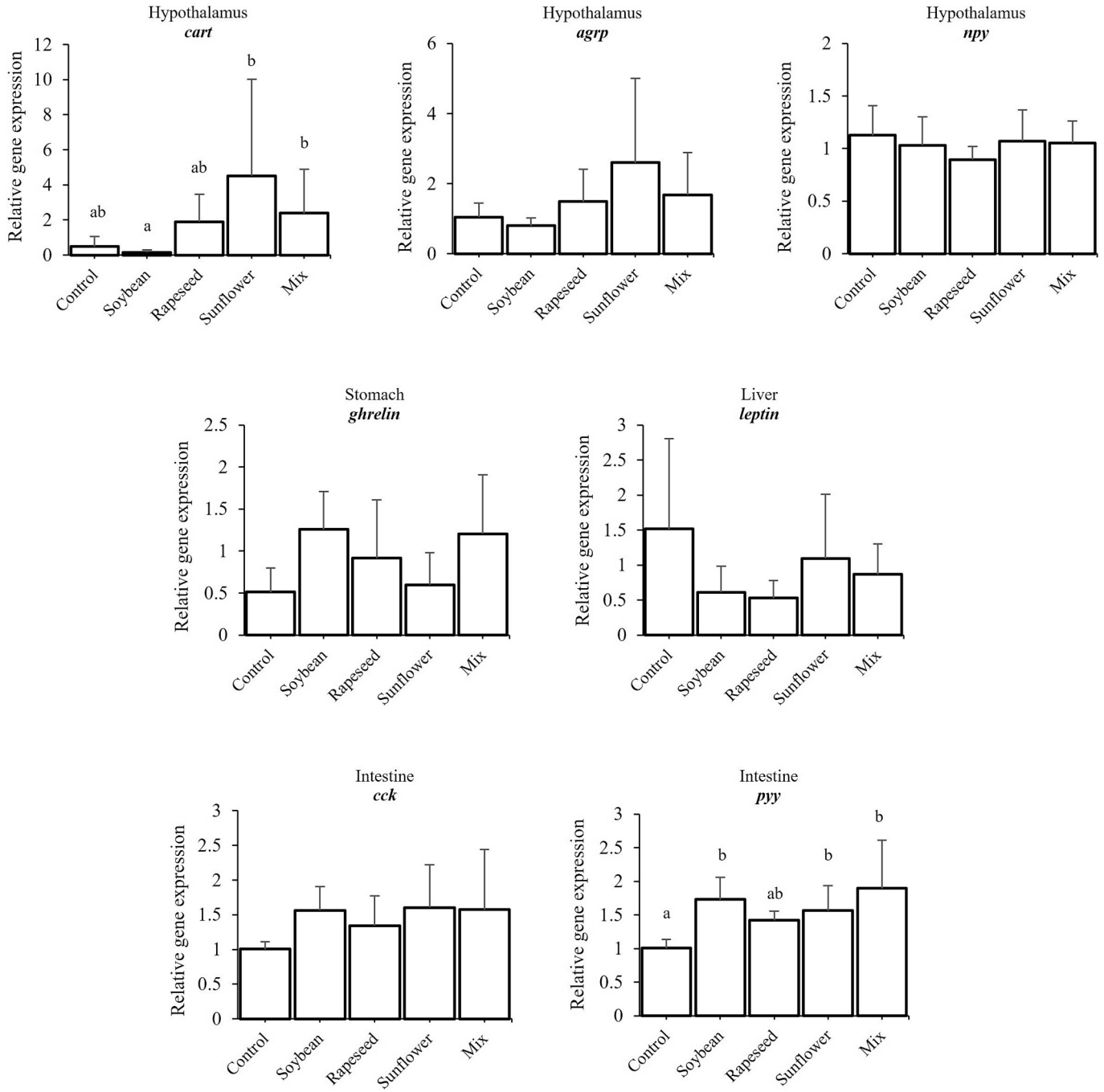

Fig. 4. Postprandial (5 h after meal) appetite-related gene expression in target tissues of gilthead seabream fed the experimental diets. Mean values and standard deviation (±SD) are presented for gene expression (n = 6). Different letters indicate significant differences between diets (cart, $p < 0.05$; pyy, $p < 0.01$). agrp: agouti-related peptide; cart: cocaine- and amphetamine-regulated transcript; cck: cholecystokinin; pyy: peptide yy; npy: neuropeptide y.

diet, supporting an effect of dietary protein source (Basto-Silva et al., 2021) albeit if in other studies, *cart* expression was not affected by PF-based diets in other teleost such as Atlantic salmon (*Salmo salar* L.) (Hevrøy et al., 2008), Cobia (*Rachycentron canadum*) (Van Nguyen et al., 2013) and in pacu (Volkoff et al., 2017).

The higher intestinal *pyy* expression in the Sunflower and Mix diets could potentially have contributed to the observed hypothalamic *cart* expression. Indeed, while the prevailing mammalian model indicates that pyy reduces appetite by inhibiting *npy* expression and subsequently activating POMC neurons (Bauer et al., 2016) in fish the effects of intestinal pyy on central nervous system remain unclear (Rønnestad et al., 2017). For instance, in channel catfish (*Ictalurus punctatus*), no change in hypothalamic *npy* and *pomc* mRNA levels was observed after intraperitoneal administration of *pyy* (Schroeter et al., 2015). Thus, a different *pyy*-mediated mechanism in fish can occur (Blanco et al., 2021).

In rainbow trout, oral administration of amino acids led to intestinal *cck* upregulation, inducing a hypothalamic *cart* co-expression (Comesaña et al., 2020). In the present study *cck* expression showed a trend similar to that of *pyy* (although not statistically significant), and an increase in *cart* expression was observed in fish fed the Sunflower and Mix diets. However, fish fed Soybean diet, despite showing an increased expression of *pyy* and *cck* (non-significant), did not present an increase of hypothalamic *cart* expression as the one observed in fish fed the Sunflower and Mix diets. This suggests that in fish fed the Soybean diet,

other factors besides the co-expression of *pyy/cck* and *cart* influenced *cart* expression and potential gut-brain interaction.

SCFAs were reported to interact with the enteroendocrine L cells containing pyy hormone, influencing its release in rats (Tolhurst et al., 2012; Nishida et al., 2021), pigs (Zhang et al., 2022), and humans (Larraufie et al., 2018). A similar mechanism was also suggested in fish (Butt and Volkoff, 2019). In the present work, increased gut acetate levels in fish fed the Sunflower and Soybean diets mirrored that of intestinal *pyy* expression, further supporting the potential role of SCFAs in pyy release, as reported in mammals. Nevertheless, further investigation is required to reveal the potential interconnection of acetate-pyy/cck-cart as an anorexigenic pathway of the microbiota-gut-brain axis in fish.

The distinct appetite-related gene expression patterns observed across oilseed-based diets suggest that diet-specific components, particularly the quantity and fermentability of NSPs, may differentially modulate appetite-regulatory pathways. The Sunflower diet, characterized by a higher proportion of insoluble NSPs (highest ADF), is likely associated with reduced digesta viscosity and faster gastrointestinal transit. Thus, at the sampling time (5 h post-feeding), fish may have been at a more advanced stage of digestion, with a greater proportion of digesta reaching the intestine, consistent with gut transit models in salmonids (Aas et al., 2017). This could have increased enteroendocrine stimulation, contributing to the upregulation of *pyy* expression. In parallel, the higher NSP inclusion may have increased substrate availability for acetate fermentation.

In contrast, Soybean NSP, particularly pectic polysaccharides, are highly fermentable and have been associated with increased SCFA production, including acetate (Tian et al., 2019), supporting a potential role of fermentability in the observed pyy response. In addition, fermented soybean-based diets have been associated with downregulation of anorexigenic markers such as cart (Dan et al., 2022), suggesting that factors beyond SCFAs-mediated signaling may be involved. In this context, inflammation should be considered as a potential co-factor since it has been suggested to affect *cart* expression in mice (Burgos et al., 2019). While the present study indicates a possible influence of soybean-induced inflammatory responses, though not statistically significant, further research is required to determine whether chronic and acute inflammation exert distinct effects on *cart* expression and how these effects may be associated with specific soybean characteristics.

Although the orexigenic role of npy has been established in several fish (Volkoff, 2016, 2019), in this study, hypothalamic *npy* expression was not affected by the PF-based diets, a result like the ones observed in another study in gilthead seabream (Basto-Silva et al., 2021) and in cobia (Van Nguyen et al., 2013). While dietary carbohydrate levels were shown to increase *npy* expression in goldfish (Narnaware and Peter, 2002) and Chinese perch (*Siniperca chuatsi*) (Peng et al., 2022), results of the present study and other studies (Babaei et al., 2017; Basto-Silva et al., 2021) indicate that in gilthead seabream, dietary carbohydrate levels do not affect *npy* brain expression.

In fish, agrp has been generally reported as an orexigenic hormone (Volkoff, 2016). However, in the present study no significant differences were observed, consistent with the absence of a stimulus to feed and, consequently, difference in FI.

Despite the stomach being the tissue where ghrelin is most consistently secreted in both mammals (Sakata and Sakai, 2010) and fish (Calo et al., 2021), in this study, *ghrelin* gene expression was not influenced by dietary treatment, evidence supported by other works that also used gilthead seabream as experimental organism (Basto-Silva et al., 2021, 2022). Moreover, the lack of changes in *ghrelin* expression supports the observed unaltered FI among dietary treatments.

Although studies in mammals strongly support leptin's anorexic role, in fish the effect of diet composition on leptin expression presents great variability (Volkoff, 2016). In fact, leptin expression appears to be tissue- and species-specific, contributing to the reported variability (Basto-Silva et al., 2021). PF-based diets did not induce changes in *leptin* expression in Atlantic salmon (Sissener et al., 2013), in pacu (Volkoff et al., 2017), or in dourado (Lorenz et al., 2022). In gilthead seabream PF replacement to FM decreased brain *leptin* expression and increased hepatic *leptin* expression (Basto-Silva et al., 2021). Moreover, a lower protein/carbohydrate ratio induced higher liver *leptin* expression in gilthead seabream (Basto-Silva et al., 2022). Nonetheless, in the present study, neither the diet's ingredients nor its macronutrient composition affected the level of *leptin* expressed in the liver. Nevertheless, although the absence of differences in FI among dietary treatment supports liver *leptin* expression results of this study, adipose tissue might be a more suitable tissue for the detection of changes induced by carbohydrates dietary levels, since in gilthead seabream fed different dietary macronutrient composition no effect on leptin expression was observed in liver, but an increase was observed in the adipose tissue of fish fed high protein/low carbohydrate diets (Babaei et al., 2017).

5. Conclusion

This study demonstrates that diets incorporating up to 30% oilseed meals (soybean, rapeseed, sunflower, or their mixture) can sustain adequate growth, without having major alterations on intestine histomorphology and inflammatory status of gilthead seabream juveniles. Nonetheless, some caution is needed with soybean since it caused minor histological effects, potentially linked to its antinutrients. Differences in carbohydrate profile, particularly in the ratio of starch to fiber and the NSPs composition, influenced nutrient utilization, hepatic energy storage, and plasma parameters, underscoring the importance of ingredient-specific evaluation in aquafeed formulation. The observed potential link between NSPs fermentation (acetate level) and the activation of gut-brain anorexigenic signaling pathway (upregulation of *pyy* and *cart*) in fish fed Sunflower diet highlights the need for further research to better understand the role of dietary fiber and fermentation products in appetite regulation in fish.

CRediT authorship contribution statement

Paolo Guttuso: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rafaela A. Santos:** Methodology, Investigation. **Rui Magalhães:** Methodology, Investigation. **Sara Moutinho:** Methodology, Investigation. **Ana Couto:** Writing – review & editing, Methodology, Formal analysis. **Margarida Gamboa:** Methodology, Investigation. **Pedro Pousão-Ferreira:** Resources. **Nelson Mota de Carvalho:** Investigation, Formal analysis. **Ana Raquel Madureira:** Resources, Funding acquisition. **Cláudia R. Serra:** Writing – review & editing, Supervision, Funding acquisition. **Aires Oliva-Teles:** Writing – review & editing, Validation, Supervision, Conceptualization. **Inês Guerreiro:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Paolo Guttuso and Inês Guerreiro report financial support was provided by Foundation for Science and Technology. Given Aires Oliva-Teles role as Associate Editor, he had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. The other 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2026.744032.

Data availability

Data will be made available on request.

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