Aquaculture Reports 24 (2022) 101175

Contents lists available at ScienceDirect

Aquaculture Reports

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

Responses of growth, blood health, pro-inflammatory cytokines genes, intestine and liver histology in Red Seabream (*Pagrus major*) to camelina meal

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ARTICLE INFO

Keywords:
Plant-origin protein
Fish meal substitutes
Interleukin 1 beta
Tumor necrosis factor-alpha
Blood biochemical Parameters

ABSTRACT

The current work assessed the inclusion impacts of oilseed camelina meal (CM) as a protein source in red sea bream (*Pagrus major*) diets. A 45 day assessment period with 180 juveniles (6.47 ± 0.17 g) were allocated in triplicates to 4 experimental groups and fed formulated diets in which fish meal (FM) was subrogated at graded series of 0% (T1), 20.5% soybean meal (T2), 20.5% camelina meal (T3), and 33% camelina meal (T4). No noticeable alterations were observed in specific growth rate, feed intake, survival rate, hepatosomatic index, and Fulton's condition factor among the experimental groups. Fish fed the T4 diet showed considerably reduced ($P < 0.05$) final weight and body protein content when compared to those fed T2, T3, and basal diets. Plasma biochemical parameters show no differences ($P > 0.05$) in glucose, total bilirubin, total protein, total cholesterol, triglyceride, glutamyl oxaloacetic transaminase, and glutamic pyruvate transaminase. Hematocrit levels decreased noticeably ($P < 0.05$) in fish groups fed camelina meal in comparison to the control group. Liver and intestinal histology showed a healthy status and an increase in villus length and goblet cell number in camelina groups. Fish fed the T4 diet displayed higher expression levels ($P < 0.05$) of relative mRNA interleukin 1 beta (IL-1b) and tumor necrosis factor-alpha (TNF-α) in comparison to other groups. The inclusion of camelina meal (up to 20%) in red sea bream diets produced similar outcomes to specimens fed fish meal and soybean meal.

1. Introduction

Fish meal (FM) is recommended as the chief dietary protein supply in practical diets of cultured animals on account of its high digestibility, the rich proportion of amino acids, and the presence of essential fatty acids (Dossou et al., 2018a). However, perennial increases in FM prices

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https://doi.org/10.1016/j.aqrep.2022.101175

and ecological bottlenecks coupled with the fast growth of the aquaculture sector have triggered worldwide research for affordable non-conventional sources. Camelina (*Camelina sativa*) is an oilseed from the Brassicaceae family and has been recognized as a source of green energy food for people and livestock (Berti et al., 2016). Camelina meal (CM) offers an economic benefit as it costs around US$260–300/metric ton (Natelson et al., 2015; Li and Mupondwa, 2016) whereas fish meal costs around US$1385/metric ton (World Bank, 2020). CM also has a high yield per unit area and is rich in oil content (90%) making the oilseed the next potent crop for agriculture purposes and the biofuel industry (Moser and Vaughn, 2010). The lipid fraction in oil seed Camelina sativa contains approximately 40% linolenic acid (ALA, 18:3n-3) which is an omega − 3 precursor and also contains other essential amino acids such as 15% linoleic acid (LNA, 18:2n-6) (Hixson et al., 2014). A recent study recommended that it is feasible to fully substitute fish oil with camelina oil without having any adverse implications on red sea bream (Mzengereza et al., 2021a).

CM is rich in residual oils that contain high amounts of monounsaturated and polyunsaturated fatty acids (18:3n-3 and 18:2n-6) (Krzyżaniak et al., 2019; Wei et al., 2020). A study by Waraich et al. (2013) highlighted that high oil residuals gained after low temperature pressing of camelina seed contained about 45% crude protein, 10% remaining oil, and 12% crude fiber. These results suggest that CM could be a versatile source of protein and lipids in aquaculture feed. Previous studies on Rainbow trout (*Oncurkycuss mykiss*) have shown that growth performance was not affected when the camelina meal was used as a feed substitute at a 20% and 16% ratio of the FM protein (Bullerwell et al., 2016). Atlantic salmon (*Salmo salar*) also remained unaffected when 14% of fish meal protein was substituted by camelina meal (Wei et al., 2020). Meanwhile, studies by Hixson et al. (2016) show that fish fed excess camelina meal ($\geq$ 30%) diets registered reduced growth response, a trend likely to have been caused by the general presence of secondary plant metabolites in plant protein meals. Camelina seed contains glucosinolates (GLS) and phytates antinutrients that are detrimental to protein digestibility and synthesis (Pedroche et al., 2004). It is therefore incumbent to carefully formulate camelina meal diets to contain a balanced amino acid, fatty acid, lipid, and protein fraction. Furthermore, studies have also shown that partial substitution of FM by plant meals in diets for marine species may impact feed utilization, growth, and fish health (Dossou et al., 2021, 2018b).

The impacts of dietary plant meal on different components of the fish immune system have been documented for several species (Dossou et al., 2018a; El Basuini et al., 2022; Paray et al., 2021). However, literature on the potential of camelina meal on immune-related genes expression in red sea bream is still lacking (Mzengereza et al., 2021b). The red sea bream (*Pagrus major*) is considered a commercially valuable species for marine aquaculture (Dossou et al., 2018a; El Basuini et al., 2016). Therefore, studies on finding appropriate feed ingredients for red sea bream that are both economically and nutritionally viable are worthwhile. Thus, this study elucidates the role of dietary camelina meal on growth response, blood biochemical parameters, the expression of Tumor Necrosis Factor- α (TNF- α) and Interleukine 1b (IL-1b), and histology of intestine and liver of red sea bream.

2. Materials and methods

2.1. Test diets

Formulated diets were isolipidic (14% crude lipid) and isonitrogenous (49% crude protein), in tandem with nutrient requirements of red sea bream. Fish meal was partially replaced as a graded series of 0% (T1), 20.5% soybean meal (T2), 20.5% camelina meal (T3), and 33% camelina meal (T4).

All diets were prepared at the Faculty of Fisheries at Kagoshima University, Kagoshima City, Japan. A mixer (CA-4488Z, Kaijo Corp., Tokyo, Japan) was used to combine the constituents of the feed with that addition of water to form a mash, which was eventually steam pelleted in (Motor number:154718, Royal Meat Choper, Japan). Pellets were dehydrated in a mechanical convection oven (DK 400, Yamato Scientific, Tokyo, Japan) at 60 °C for a minimum of 3 h. Dried pellets were stored at − 20 °C until use. At the commencement of the experiment, 1.2 mm feed pellets were fed correspondingly to fish size, and it increased to 1.5 mm and 1.6 mm towards the final phase of the trial.Table 1.

2.2. Proximate composition and amino acid profile

Samples (fish and diets) were dried using a freeze drier (Eleya, FDU-1100 Rikakikai Co.LTD., Tokyo, Japan) for a minimum of 3 days. Dry matter was assessed by taking weight samples prior to freeze-drying samples and at the end of the freeze-drying process. Feeds and fish were evaluated for moisture, ash, crude lipid, and crude protein contents following AOAC (2000) (Table 2). Dietary total amino acid proportions were defined using the method depicted by Kader et al. (2010). Total amino acid (TAA) quantification was carried out using high-performance liquid chromatography (HPLC, Shimadzu Corp. Kyoto, Japan) (Table 2). 2 mg of sample was mixed with a calculated concentration of norleucine as an internal control, and to it, a known 4 N methane sulfonic acid was added and placed on a heater for 22 h under 110 °C hydrolyses. Sample preparation was finished by adjusting the pH of the hydrolysate to 2.2 ± 0.05 using 30% perchloric acid and 4 N sodium hydroxide. Samples were eventually filtered and stored at 4 ℃ until injection into the HPLC. The chromatography assessment and separation of amino acids were done using an HPLC ion exchanging

Table 1
The basal diet fed to red sea bream for 45 days.

Ingredient	%
Brown Fish meal[1]	59.0
Soybean meal[2]	–
Camelina meal[3]	–
Casein[4]	1.0
Squid meal[5]	5.0
Krill meal[6]	4.0
Soybean lecithin[7]	3.0
Pollack liver oil[8]	2.0
Wheat flour	1.4
Activated gluten[9]	5.0
Vitamin mix[10]	3.0
Mineral mix[11]	3.0
stay C4[12]	0.08
Alpha-cellulose[13]	13.52

[1] Nippon Suisun Co. Ltd., Tokyo, Japan.
[2] Riken Vitamin, Tokyo, Japan
[3] Tokai Seapro Co., Ltd. (Fukuoka, Japan)
[4] Wako Pure Chemical Industries, Ltd. Osaka, Japan.
[5] Nippon Suisun Co. Ltd., Tokyo, Japan
[6] Nippon Suisun Co. Ltd., Tokyo, Japan
[7] Kanto Chemical Co., Inc. Tokyo, Japan.
[8] Riken Vitamin, Tokyo, Japan
[9] Glico Nutrition Company Ltd. Osaka, Japan. The commercial name "A-glu SS".
[10] Vitamin mixture (mg kg^{-1} diet): ß-carotene (0.10), vitamin D3 (0.01), menadione NaHSO3·3H2O (K3) (0.05), DL-α-tocopherol acetate (E) (0.38), thiamine-nitrate (B1) (0.06), riboflavin (B2) (0.19), pyridoxine-HCl (B6) (0.05), cyanocobalamin (B12) (0.0001), biotin (0.01), inositol (3.85), niacin (nicotinic acid) (0.77), Ca Pantothenate (0.27), folic acid (0.01), choline chloride (7.87), ρ-aminobenzoic acid (0.38), and cellulose (1.92).
[11] Minerals mixture (mg kg−1 diet): MgSO4 (5.07), Na2HPO4 (3.23), K2HPO4 (8.87), Fe Citrate (1.1), Ca Lactate (12.09), Al (OH)3 (0.01), ZnSO4 (0.13), MnSO4 (0.03), Ca (IO3)2 (0.01), and CoSO4 (0.04).
[1] Stay-C 35: L-Ascrobil-2-phosphate-Mg.

Table 2
Chemical composition of tested diets fed to red sea bream for 45 days.

Items	T1	T2	T3	T4
Moisture (g/kg)	110.2 ± 1.0	100.86 ± 0.3	100.95 ± 0.6	110.26 ± 0.5
Protein (g/kg)	496 ± 0.4	499 ± 0.2	482 ± 0.9	486 ± 0.1
Total Lipid (g/kg)	95 ± 0.1	94 ± 0.01	90 ± 0.0	87 ± 0.0
Ash (g/kg)	104 ± 0.3	125 ± 0.3	116 ± 0.3	95 ± 0.1
Amino acid profile (g/100 g of a dry ingredient)				
Essential Amino acid				
Arginine	2.84	2.70	2.74	2.54
Histidine	2.27	1.57	2.25	2.12
isoleucine	1.95	1.38	1.95	1.99
Leucine	4.05	4.40	4.21	4.77
Lysine	3.50	3.40	3.42	3.05
Methionine	1.06	1.06	1.07	1.03
Phenylalanine	2.78	2.87	2.84	2.74
Threonine	2.19	2.12	2.20	2.17
valine	2.14	2.51	2.14	2.52
Tryptophan	0.45	0.39	0.37	0.38
Non-essential Amino acid				
Taurine	0.69	0.72	0.68	0.70
Aspartic acid	5.21	5.18	5.23	5.09
Glutamic acid	8.21	8.20	8.25	8.06
Serine	2.08	2.05	2.09	2.06
Porine	2.92	3.02	3.04	2.96
Glycine	2.75	2.85	2.77	2.68
Alanine	2.15	1.85	1.89	1.76
Tyrosine	0.92	0.87	0.96	0.83

resin column.

2.3. Animal maintenance and acclimatization

Fish were purchased from a commercial facility located in Kumamoto prefecture, Japan. Fish were conditioned for 3 weeks to 6.3 mg L^{-1} dissolved oxygen level and water temperatures averaging 17.6 °C. Red seabream juveniles (6.47 ± 0.17 g) were randomly placed into twelve 100 L fiberglass circular tanks. Each diet was triplicated and each treatment was stocked with 15 fish, each receiving marine water flows with natural photoperiod (24 h:12 L,12D). Fish were fed twice daily until visible satiation is observed (0800, 1500) for 45 days.

2.4. Plasma biochemical and hematology assay

Blood was drawn from the caudal vein of six fish per triplicate. Syringes with heparin were used to collect blood for plasma analysis. For plasma separation, samples were centrifuged at 3000g for 15 min at 4 °C using (MX-160; Tomy Tech USA Inc., Tokyo, Japan) and kept at − 80 °C for later use. Hematocrit was assayed in a microhematocrit machine using whole blood. Plasma parameters were assessed using a spectrophotometer (SPOTCHEMTM EZ model SP-4430, Arkray, Inc. Kyoto, Japan) with a commercial kit from Arkay Inc, 57 Nishi Aketa-Cho, Higashi–Kujo, Minami-Ku, Kyoto, Japan (bar code: 4987486773252; QR code: (17) 190800 (10) MD8H20, (01) 0487486773252).

2.5. Histomorphological Evaluation

Fish were ice killed and eviscerated to collect internal organs which were then placed in Bouin solution. Samples were desiccated using graded levels of alcohol baths (70%, 80%, 90%, 100%) every 24 h and finally rinsed of alcohol using xylene. Tissues were then saturated with paraffin wax overnight (24 h). Rotary microtome (RM 2135, Leica, Nussloch, Germany) was used to cut embedded samples into surgical form with a thickness of 5 μm, put in a warm water bath (40 ^{0}C), fitted on a slide, and dyed with hematoxylin and eosin. The slide was mounted properly and evaluated (Entellan, EMD Millipore, Billerica, MA, USA). Histological slides were photographed using a Nikon Coolscan 4000ED (Nikon Inc., Japan). Images were obtained using Nikon Scan 4.0.2 (Nikon Inc., Japan) and viewed using NIS, Elements: Advanced Solutions Imaging Software (Version 5.30.00, Japan).

2.6. Real-time PCR (qPCR)

The liver was detached from fish samples, dipped in RNAlater (Invitrogen; Thermo Fisher Scientific, Vilnius, Lithuania), and kept at a −80 °C regime before analysis. Approximately 30 mg of liver samples were partitioned and transferred in a tube (SARSTEDT A 0.200.01 S) homogenized before being subjected to a centrifuge process at 12,000 ×rpm for 15 min. The resulting supernatant was mixed with 70% alcohol prior to RNA isolation using RNeasy Mini Kit 50 (Qiagen; Hilden, Germany) as outlined by the kit manufacturer. After RNA extraction, cDNA was isolated using the Prime Script™ RT Master mix Kit (Takara, Japan) following the manufacturer's recommendations. Integrity and proportions of RNA were measured using a Lite spectrophotometer (Thermal Fisher Scientific, USA). Analysis of Real-time PCR was performed by employing the SYBR Select Master Mix kit (Thermo Fisher Scientific, Japan) following the primer guidelines (Table 3). Elongation factor (β-Actin) was employed as the housekeeping gene (Table 2). Amplification was done using the CFD-3120 Mini Opticon Real-Time PCR System (BIO-RAD, Singapore) as recommended by the following protocol: First 2 min denaturation at 95 °C, 40 cycles of 95 °C for 15 s, and 65 °C for 30 s. Quantification of samples was triplicated in each treatment.

2.7. Statistical analyses

Super ANOVA 1.11 (Abacus Concepts, Berkeley, California, USA) was used to analyze data following the completely randomized design. Mean differences were computed using Duncan's multiple range test. Probability < 0.05 highlighted significant differences observed between the means.

3. Results

3.1. Growth indices and carcass composition

Growth variables of red sea bream fed experimental diets are provided in Table 4. After 45 days of the feeding test, no significant differences were found in SGR, FI, SR%, HSI%, and Fulton's condition factor (K) among experimental groups. Fish fed the T4 diet showed significantly reduced final weight ($P < 0.05$) when compared to those fed T2, T3, and basal diets. Meanwhile, fish fed the control diet (T1) had a substantially reduced ($P < 0.05$) FCR compared to those in other groups. Carcass composition of red sea bream fed experimental diets for 45 days is stated in Table 5. Moisture, total lipids, and ash levels were unaffected ($P > 0.05$) at the end of the feeding trial while fish fed the T4 diet exhibited the lowest protein content compared to other groups.

3.2. Plasma biochemical parameters

Table 6 represents the plasma biochemical constituents in the red sea bream specimen from the trial. No significant differences ($P > 0.05$)

Table 3
Forward and Reverse primers for immune-related genes.

Gene		Primer Sequence (5–3′) [F] = Forward – [R] = Reverse	Accession number	Reference
β-actin	**[F**	TCTGTCTGGATCGGAGGTC	JN226150.1	(Shadrack et al., 2022)
	[R]	AAGCATTTGCGGTGGACG		
TNF-α	**[F]**	CCAAACAGAAGCACTAACCAAGA	AY314010.1	
	[R]	CTAAATGGATGGCTGCCTTG		
IL-1β	**[F]**	CGAGTACCAAACAGCATGGA	AY257219.1	
	[R]	GTGTAGGGGGCAGGTAGGTC		

ß-actin: housekeeping gene; TNF-a: tumor necrosis factor; IL-1b: interleukim-1b

Table 4
Growth response of red sea bream (*Pagrus major*) after 45 days feeding trial.

Growth parameter	Experimental diets			
	T1	T2	T3	T4
Initial weight (g/fish)	6.55	6.50	6.40	6.43
Final weight (g/fish)	24.3 ± 0.3[a]	23.5 ± 0.9[a]	23.0 ± 0.8[a]	21.6 ± 0.3[b]
Specific growth rate (SGR)	2.95 ± 1.2	2.86 ± 0.3	2.91 ± 0.2	2.73 ± 0.5
Feed intake (FI, g/fish/45 days)	17.8 ± 1.3	17.5 ± 0.8	17.6 ± 2.3	17.5 ± 0.9
Feed conversion ratio (FCR)	0.9 ± 0.0[b]	1.04 ± 0.03[a]	1.03 ± 0.02[a]	1.2 ± 0.05[a]
Survival rate (SR %)	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Hepatosomatic index (HSI %)	1.9 ± 0.2	1.73 ± 0.1	1.81 ± 0.09	1.9 ± 0.1
Fulton's condition factor (K)	2.0 ± 0.2	2.0 ± 0.5	1.83 ± 0.5	2.5 ± 0.2

Values are expressed as mean ± SEM (n = 3). Numbers with the same alphabets are not significantly different (P < 0.05)
Specific growth rate (SGR) = [ln Final weight- ln Initial weight / 45 days] × 100
Feed intake (FI, g/fish/45 days) = total feed intake in 45 days feeding period (g) / number of fishes.
Feed conversion ratio (FCR) = dry feed intake (g)/weight gain (g)
Survival rate (SR%) = [Initial fish number-dead fish number / initial fish number] × 100
Hepatosomatic index (HSI%) = [liver weight/body weight] × 100
Fulton's condition factor (K) = [total fish weight, g/ total fish length^3, cm^3] × 100.

Table 5
Carcass composition of red sea bream fed experimental diets for 45 days.

Items	T1	T2	T3	T4
Moisture (g kg^{-1})	690.6 ± 0.5	680.0 ± 1.2	690.4 ± 0.3	690.1 ± 0.5
Protein (g kg^{-1})	219 ± 0.8[a]	209 ± 0.1[a]	203 ± 1.7[a]	183 ± 0.5[b]
Total Lipid (g kg^{-1})	166 ± 0.4	178 ± 2.4	181 ± 0.1	182 ± 0.2
Ash (g kg^{-1})	42 ± 0.09	57 ± 0.07	46 ± 0.1	32 ± 0.05

Values are displayed as mean ± SEM (n = 3). Data with the same alphabets are not significantly different (P < 0.05).

Table 6
Plasma biochemical parameters in juvenile red sea bream fed test diets.

Items	Experimental diets			
	T1	T2	T3	T4
Hematocrit (%)	34 ± 0.3[a]	33 ± 0.5[b]	34 ± 0.2[c]	28 ± 2.1[d]
Glucose (mg/dl)	76.6 ± 5.8	67.5 ± 5.7	76.3 ± 4.3	79.3 ± 8.1
Total bilirubin (mg/dl)	5.3 ± 0.3	7.3 ± 0.6	5.6 ± 0.3	6.6 ± 0.8
Total protein (mg/dl)	3.6 ± 0.1	3.5 ± 0.3	3.5 ± 0.1	3.7 ± 0.2
Total cholesterol (mg/dl)	188.3 ± 5.4	180.3 ± 12.0	181.3 ± 4.3	190.0 ± 2.8
Triglyceride (mg/dl)	129 ± 14.6	134.3 ± 38.8	112.6 ± 20.1	143.6 ± 20.2
GOT (IU/L)	72.3 ± 20.3	100.0 ± 45	110.6 ± 46.3	116.6 ± 30.7
GPT (IU/L)	17.3 ± 7.3	26 ± 16	48.6 ± 34.2	26.6 ± 5.7

Values are displayed as mean ± SEM (n = 3). Data with common alphabets are not significantly different (P < 0.05)
GOT = Glutamyl oxaloacetic transaminase
GPT = Glutamic pyruvate transaminase

were found in glucose, total bilirubin, total protein, total cholesterol, triglyceride, glutamyl oxaloacetic transaminase (GOT), and glutamic pyruvate transaminase (GPT). Hematocrit levels significantly decreased ($P < 0.05$) in fish groups fed T2, T3, and T4 compared to the control group.

3.3. Intestinal and Liver morphology

Intestinal morphometrics in red sea bream fed test diets for 45 days are indicated in Table 7 and Fig. 1. The lowest ($P < 0.05$) villous length and goblet cell count were noticed in the control group while no alteration in crypt depth among all groups. Fig. 2 shows healthy livers of red sea bream with no inflammation signs in all groups.

3.4. Immune-related gene expression

Fig. 3 presents immune-related gene expression (IL-1b and TNF-a) of red sea bream fed experimental diets for 45 days. Fish fed the T4 diet displayed significantly higher expression levels ($P < 0.05$) of relative mRNA IL-1b and TNF-α in comparison to other groups.

4. Discussion

The growth trends observed in this study highlight that fishes fed a diet with 20.5% CM as a protein source responded similarly to fish-fed control diets, suggesting that red sea bream can utilize relatively high CM proportions. Results in the current research also corroborate a study conducted by Koskela et al. (2021) on rainbow trout (*Oncorhynchus mykiss*) fed 20% camelina seeds and where it performed similarly to control diets. Moreover, Pan et al. (2011) concluded that the addition of high CM proportions amounting to 160 g Kg^{-1} hardly affected the growth response or carcass contents of rainbow trout. However, other earlier authors have reported lower acceptance implementation rates of CM in the diets between 40 g Kg^{-1} to 100 g Kg^{-1} (Brown et al., 2016; Hixson et al., 2016; Wei et al., 2020). The disparities in the rate of CM level inclusion may be attributed to species-specific differences in feed acceptance or intake, nutrient digestibility efficacies, absorption, and assimilation.

On the other hand, our present study has shown that elevated CM up to 33% decreased the final body weight. The diminishing growth patterns are associated with the unpalatability of plant protein meals due to high-level secondary plant metabolites including glucosinolates and phytates which reduce feed consumption and bio-availability of nutrients (Ye et al., 2016). Feed palatability is one of the main influencers of fish growth when considering important nutritional requirements such as essential amino acids (Espe et al., 2006). Moreover, the existence of anti-nutritional secondary plant metabolites could be linked to the slight variation in FCR of fishes fed on plant-based protein sources compared to the control. In the future, bioprocessing feed technological research may enhance the nutritive value of camelina meal supplemented at high proportions in aquaculture diets. Secondary plant metabolites can be removed, and reduced through natural approaches e.g. fermentation with biological terms (yeast, probiotics, prebiotics, and enzymes), changing to different forms like concentrates, and physical methods like soaking in water or subjecting to heat (Dawood et al., 2020; Dossou et al., 2018a, 2021).

Blood chemistry is an important sign of the stress reactions, underlying health status, and general homeostasis of fish in response to

Table 7
Intestinal morphometric in red sea bream fed experimental diets for 45 days.

Items	Experimental diets			
	T1	T2	T3	T4
Villous length (Vl), μm	214.37 ± 17.70[b]	294.32 ± 22.10[a]	279.30 ± 27.40[a]	288.08 ± 23.40[a]
Cryptal depth (Cd), μm	135.24 ± 12.22	118.28 ± 25.31	124.34 ± 14.39	112.89 ± 27.30
Goblet cell count per villi	12.99 ± 1.32[b]	14.22 ± 1.90[ab]	18.17 ± 2.21[a]	17.62 ± 1.27[a]

Values (mean ± SEM, n = 3) within a row with various superscripts are different (P < 0.05).

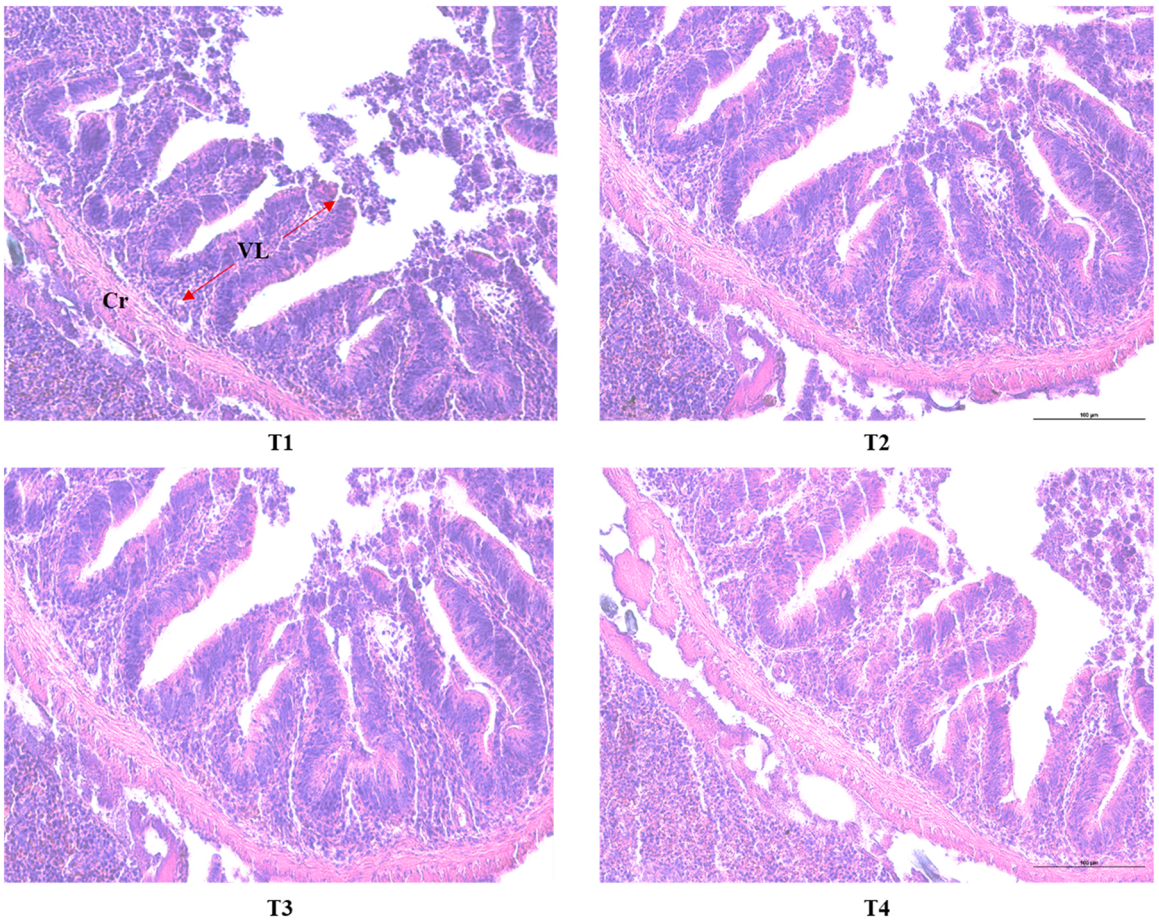

Fig. 1. Intestine histomorphology of red sea bream fed experimental diets for 45 days. VL = Villus length, Cr = Crypt depth.

nutrition and other environmental cues (El Basuini et al., 2021; Gewaily et al., 2021). Plasma constituents in the current feed trial are within those reported by earlier authors working on red sea bream (Dawood et al., 2016). Haematocrit levels decreased with a corresponding increase in camelina meal. Low haematocrit is an indication of the inhibitory impact of phytate and glucosinolates on the bioavailability of important minerals for cell synthesis e.g. iron and zinc, as previously reported by Lall and Kaushik (2021).

Carcass chemical analysis of red sea bream showed that trends in total lipid and protein constituents of the fish were a reflection of total lipids and protein contents of the diets. Crude protein proportions of the diets ranged from 496 g Kg^{-1} (control) to 486 g Kg^{-1} (T4) with increasing camelina addition. A similar trend in crude protein levels was obtained in the fish (from 219 g Kg^{-1} to 183 g Kg^{-1}). Furthermore, the total lipid content of diets ranged from 193 g Kg^{-1} (control) to 175 g Kg^{-1} (300 g Kg^{-1} CM) while for fish carcass lipid ranged from 161 g Kg^{-1} (control) and 188 g Kg^{-1} in the T4 diet. Diets fed to red sea bream were uniform in their nutritive score across the treatment. The difference in carcass proximate composition among treatments was noticeable for protein content with high inclusion (33%) of CM. The lower protein deposition in the carcass of fish fed a high level of CM (T4 diet) may be linked to protein poor digestion and assimilation rates due to the presence of anti-nutritional substances. In this line, Pedroche et al. (2004) stated that camelina seed contains glucosinolates (GLS) and phytates antinutrients that are detrimental to protein digestibility and synthesis. Data presented in a study by Wei et al. (2020) using high oil CM showed that differences in tissue lipid and protein in Atlantic salmon were more pronounced than in the current study. Moreover, the differences are attributed to different rates of nutrient deposition in tissues among different species and the age or size of fish (El Basuini et al., 2017). Also, slight changes that were reported for protein levels in red sea bream are in line with earlier studies on the utilization of plant protein in red sea bream (Dossou et al., 2018a, 2018b).

Histological assessment in the current study revealed that the hepatocyte morphology of red sea bream was unaltered in fish fed up to 33% CM as there was no sign of inflammation compared with control-fed fish (P>0.05). There were considerable changes in intestinal morphology, especially for fish red sea bream fed the basal diet, which showed shorter and narrower villi with smaller areas and lower goblet cells in comparison with red sea bream fed other diets. Previous works have suggested that plant-based protein in fish feeds could increase the goblet cell population and villus length (Bansemer et al., 2015; Matulić et al., 2020; URÁN et al., 2008). The increased goblet cells and villus length are an adaptive and protective response of the guts to the anti-nutritive factors in plant-based protein sources to increase utilization or absorption of nutrients (adaptive) as well as mucus secretion (protective). Gene expression is linked to physiological features associated with good aquaculture output (Nielsen and Pavey, 2010). IL-1β and TNF-α are important mediators of inflammation in the infection response (Zou and Secombes, 2016). Previous research has demonstrated that substituting a fish meal with plant protein can produce intestinal inflammation in fish by inducing inflammatory factor expression and activating inflammatory pathways. This could be due to unbalanced amino acid composition and the high presence of antinutritional elements found in plant materials (Yin et al., 2020, 2018). In the same context, Chen et al. (2021) reported that IL-1β and TNF-α were upregulated in the cottonseed protein concentrate (CPC) group, showing that substitution of fish meal with CPC resulted in inflammatory stimuli in the gastrointestinal tract of grouper. qPCR analysis shows that red sea bream fed with higher camelina level (T4), showed significantly higher

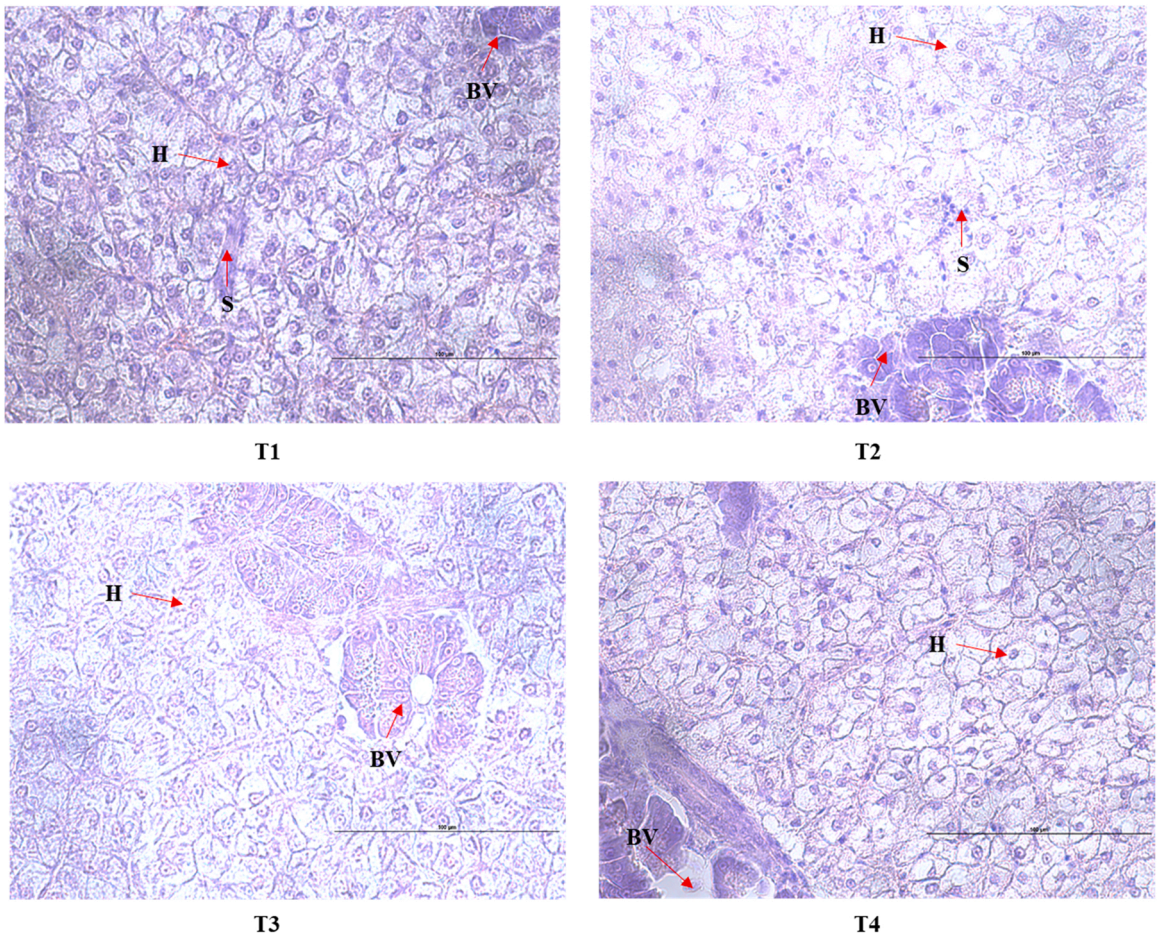

Fig. 2. Liver histomorphology of red sea bream fed experimental diets for 45 days. BV = Blood vessel, H = Hepatocytes, S = Sinusoids.

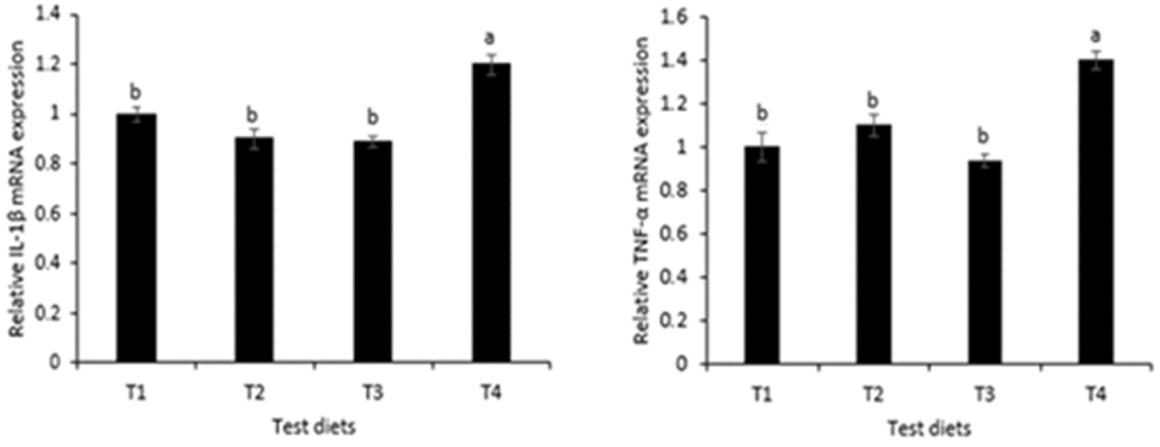

Fig. 3. Immune-related gene expression (IL-1b and TNF-a) of red sea bream fed experimental diets for 45 days. Bars (mean ± SEM, $n = 3$) with various superscripts are different ($P < 0.05$).

($P < 0.005$) TNF-α and IL-1b expression in comparison to other groups. The elevated gene performance could verify that there was an inflammatory reaction in fish fed the T4 diet with a degree of suppression in the immune system, possibly indicating a stress response. Previous research on several species has revealed a dramatic modification of inflammatory and immunological pathways due to the high supplementation of plant protein in diets (Martin and Król, 2017; Sahlmann et al., 2013; Sitjà-Bobadilla et al., 2005).

5. Conclusion

Results from the present study highlight that 20.5% FM protein could be substituted by Camelina meal without significant negative impacts on growth performance, blood plasma chemistry, feed utilization, histology of intestines and liver as well as immune-related TNF-α and IL-1b genes expression, and body composition. The findings of the present feed trial will enable feed manufacturers to incorporate plant proteins more effectively in generating affordable and healthy aquaculture feeds.

Authors statement

The authors have contributed equally to this work.

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.

Acknowledgement

The work was funded by Taif University Researchers Supporting Project number (TURSP-2020/76), Taif University, Taif, Saudi Arabia. Support for this research was made possible through the Ministry of Education, Culture, Sports, Science and Technology of Japan (Monbukagakusho) to whom the authors are grateful. The authors thank Mr. Mio Kenjiro and Dr. Oki Hayasaka for their assistance in histology analysis. The authors are indebted to Tokai Seapro Co., Ltd. (Fukuoka, Japan) for supplying camelina meal.

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