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ORIGINAL RESEARCH

Effects of partial replacement of fish oil by vegetable oil and animal fat on hematological and serum biochemical parameters, antioxidant capacity, lipase activity and intestine histology of rainbow trout (*Oncorhynchus mykiss*)

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Abstract This investigation assessed the effects of substituting canola oil (CO) and/or beef tallow (BT) for fish oil (FO) on a range of rainbow trout (*Oncorhynchus mykiss*) parameters. We randomly selected 250 fish (20 g) and allocated them to the following conditions: T_1 (control, 100% FO), T_2 (50% FO and 50% BT), T_3 (50% FO and 50% CO), and T_4 (25% FO, 25 5 BT, and 25 5 CO). T_1 and T_4 exhibited the highest mean final weight, weight gain, and survival rate, respectively, whereas T_2 demonstrated the lowest rates ($P<0.05$). When FO was switched out for CO, BT, or a mix of the two, the fish's red blood cell (RBC), hemoglobin, and hematocrit levels dropped significantly ($P<0.05$). Furthermore, statistically significant variations were observed in all other blood parameters ($P<0.05$), with the exception of monocytes, eosinophils, and immunoglobulin (Igm) ($P>0.05$). Furthermore, variations in serum biochemical factors were statistically significant ($P<0.05$), with the exception of lysozyme ($P>0.05$). In contrast to T_1, other samples exhibited histological indications of cellular injury. We suggest that rainbow trout diets incorporate an equal mixture of CO and BT in lieu of fish oil as a partial replacement.

Keywords Rainbow trout (*Oncorhynchus mykiss*) . Fish oil . Canola oil . Beef tallow . Growth parameters . Intestine histology

Introduction

Lipids are critical for numerous processes pertaining to fish development and metabolism, including nutrient transport and cell formation. (Turchini et al. 2009). Therefore, providing economically viable and nutrient-dense lipids in fish diets is a critical component of aquaculture diet preparation. Due to its status as a significant reservoir of polyunsaturated fatty acids (PUFAs) that positively impact fish growth and development, fish diet formulations frequently utilize fish oil as the lipid component (Yun et al. 2013). In contrast, an emerging pattern in fish feed formulation involves substituting fish oil with alternative sources as a means to promote sustainable aquaculture development (Gesto et al. 2021).

Because fish oil is inherently susceptible to oxidative reactions and quality degradation, the concept of substituting other lipid sources for fish oil in their diet has acquired traction (Ghelichi et al. 2018), This may have detrimental effects on the growth and development of fish, in addition to being less accessible than alternative lipid sources (Chen et al. 2020). The potential contamination of oil from marine fish in marine waters is another

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problem with the use of fish oil in fish feed preparation (Spilsbury et al. 2023), which might transfer marine contaminants to farmed fish ending up health issues in consumers. Oils from different sources including animal (Maldonado-Othón et al. 2022), plant (Qin et al. 2022), or both (Carvalho et al. 2020) have been considered for the replacement of fish oil in fish feed. However, substituting fish oil with alternative lipid sources, particularly when done entirely, could impede fish growth as a result of the deficiency of polyunsaturated fatty acids (PUFAs) in fish feed. Consequently, PUFA supplementation in fish diets was advised (Gesto et al. 2021). Nevertheless, the financial implications of this supplementation could potentially disrupt aquaculture operations, prompting the exploration of alternative approaches that are more economical, such as partial substitution. It is worth mentioning that the most favorable outcome could be attained by substituting all fish oil in the diet with alternative lipid sources without significantly impeding the growth and development of the fish.

Canola oil, also referred to as low erucic acid rapeseed oil, is frequently used as an alternative to fish oil in fish feed formulations. Its extensive global production rates, convenient accessibility, and PUFA content all contribute to its widespread acceptance as a substitute for fish oil in fish feed (Turchini et al. 2013). On the other hand, the elevated concentrations of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in tallow, compared to its diminished concentration of polyunsaturated fatty acids (PUFAs), distinguish it as an inexpensive and easily accessible lipid source. Consequently, fish feed formulations widely employ tallow as a partial or complete substitute for fish oil (Medagoda et al. 2022). There are several reports on the effects of replacing fish oil with canola oil (Castro et al. 2016; Gesto et al. 2021; Yıldız et al. 2018) and beef tallow (Gao et al. 2021; Lee et al. 2020; Pérez et al. 2014) Regarding various fish species, there is a scarcity of data regarding the effects of substituting fish oil in fish diets with these two lipid sources, both individually and in combination. As a result, the present investigation was designed to examine the effects of substituting a portion of the fish oil with the aforementioned lipid sources on various attributes of rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Experimental diets

Four diets were prepared in order to analyze the effect of replacing fish oil in the diet with separate and combined use of canola oil and beef tallow as follows: T1: 100% FO (fish oil); T2: 50% FO and 50% BT (beef tallow); T3: 50% FO and 50% CO (canola oil); and T4: 50% FO, 25% BT, and 25% CO. EPA, DHA, Σ SFA, Σ MUFA, Σ PUFA, Σ ω-3, Σ ω-6, Σ ω-9, Σ EPA+DHA, and Σ ω-3/ Σ ω-6 contents of FO were 7.24, 19.40, 26.30, 37.20, 32.1, 28.7, 3.08, 29.4, 26.7, and 9.34 g $100g^{-1}$, respectively. We produced the granules by combining the feed ingredients and moistening them with 300 ml of water per kilogram using a meat grinder. Subsequently, the mixture was dried. We produced beef tallow by thermally extracting animal oil from tallow from a nearby industrial slaughterhouse. Table 1 details the chemical composition and constituents of the diet.

Experimental protocol

The laboratory subsequently received juvenile rainbow trout from a nearby farm in Golestan Province, Iran. Subsequently, we selected 240 fish weighing less than 20 g and transferred them to 500-L aquariums, maintaining a density of 20 fish per tank (12 tanks for three replicates of each treatment). We quarantined the fish for one month and fed them a commercial diet (Faradaneh, Iran) for acclimatization. After determining the fish's length and weight, we randomly allocated twelve homogeneous groups of twenty fish each to each aquarium. We manually nourished the fish for ninety days, feeding them 3% of the aquarium's biomass at two meals. We adjusted the water discharge rate of each tank to 3 l/min. The pH and temperature of the water were found to be 7.06±0.76 and 15.07±0.24 ˚C, respectively. The dissolved oxygen content was in close proximity to saturation.

Growth performance calculations

Growth parameters including weight gain (WG), survival rate (SR), were calculated as follows:
WG = final weight (g) – initial weight (g)
SR = 100 × (number of final alive fish / number of initial fish)

Samplings

After a 24-hour fast and confirmation of the elimination of all digestive tract contents, the sampling process began. Six randomly selected fish from each treatment were captured using a dip net while under the anesthetic effects of a 200 mg/l clove extract solution. We obtained blood samples from a caudal vein using syringes and subsequently transferred them into plastic containers. Centrifugation at 3000 rpm for 5 minutes separated the blood serum, which we then stored at -20°C pending further analyses. After ventrally incising the fish specimens, the digestive tract was dissected in order to obtain the intestine (Nayak et al. 2003). Research Ethics Committee of Islamic Azad University approved all procedures.

Analyses

Proximate composition of the diets and fish fillets were analyzed according to AOAC (2006). We determined the moisture content by dehydrating the samples at 70 C for 48 hours. We used the Kjeldahl and Soxhlet assays to determine the crude lipid and protein contents of the samples, respectively.Crude ash percentage was determined by burning the samples in a furnace (550 ˚C) for 8 h. Crude fiber content was measured by acidic and alkali digestion. Lipid extraction from the formulated diets was performed via the method proposed by Folch et al. (1957).

Hematological parameters including red blood cell (RBC), white blood cell (WBC), hematocrit (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were analyzed according to the methods proposed by Feld-

Table 1 Ingredients (as g.kg^{-1}) and chemical composition of the experimental diets

	90 d	0 d	90 d	0 d	90 d	0 d	90 d	0 d
	T1	T1	T2	T2	T3	T3	T4	T4
Ingredients								
Fish meal[1]	360	100	360	100	360	100	360	100
Meat meal[2]	100	30	100	30	100	30	100	30
Soybean meal[3]	120	40	120	40	120	40	120	40
Wheat meal[4]	220	80	220	80	220	80	220	80
Fish oil[1]	150	50	75	50	75	50	75	50
Beef tallow	0	0	75	25	0	0	37.5	13.3
Canola oil	0	0	0	0	75	25	37.5	13.7
Sodium bentonite[5]	15	5	15	5	15	5	15	5
Vitamin premix[6]	15	6	15	6	15	6	15	6
Mineral premix[7]	15	5	15	5	15	5	15	5
Lysine[8]	1.5	0.5	1.5	0.5	1.5	0.5	1.5	0.5
Methionine[8]	1.5	0.5	1.5	0.5	1.5	0.5	1.5	0.5
Choline chloride[9]	2	1	2	1	2	1	2	1
Proximate composition	90 d	0 d	90 d	0 d	90 d	0 d	90 d	0 d
Moisture (%)	10.00	5.12	9.80	4.86	9.90	4.12	9.85	4.75
Crude ash (%)	8.00	2.32	7.90	2.64	8.10	3.12	7.98	3.34
Crude protein (%)	42.00	21.13	42.30	22.14	42.20	22.85	42.28	22.79
Crude lipid (%)	18.35	5.16	17.95	5.85	18.20	5.66	18.15	5.36
Crude fiber (%)	2.80	0.87	2.90	0.97	3.00	0.83	3.00	0.95
NFE (%)*	18.85	5.50	19.15	5.43	18.70	5.87	18.74	6.12
Gross energy (kJ g^{-1})**	20.51	8.13	20.48	8.43	20.48	8.88	20.48	8.76

Treatment abbreviations, T1: 100% fish oil; T2: 50% fish oil and 50% beef tallow; T3: 50% fish oil and 50% canola oil; and T4: 50% fish oil, 25% beef tallow, and 25% canola oil.

[1]Negin-Powder Co., Fisheries Industrial Complex, Amirabad Port, Neka, Iran.

[2]Tonekadasht-e-Shomal Co., Mazandaran, Iran.

[3]Kimiya Tejarat Zar Co., Tehran, Iran.

[4]Eris Trade Group, Tehran, Iran.

[5]Sina Tolid Co., Tehran, Iran.

[6]Sciance Laboratories, Alborz Industrial City, Qazvin, Iran. Provides the following (mg kg^{-1} diet): vitamin E (30), vitamin K (3), niacin (40), thiamine (2), riboflavin (7), pyridoxine (3), folacin (1.5), pantothenic acid (18), biotin (0.7), and cyanocobalamin (0.18).

[7]Sciance Laboratories, Alborz Industrial City, Qazvin, Iran. Provides the following (mg kg^{-1} food): Mg (100), Zn (60), Fe (40), Cu (5), Co (0.1), I (1), and antioxidant (100).

[8]Shimi Gostar Taban Co., Tehran, Iran.

[9]Alborz Gostar Darou, Karaj, Iran.

* NFE: nitrogen free extract = 100 – (moisture + crude ash + crude protein + crude lipid + crude fiber).

**Calculated based on 23.6, 39.5 and 17.2 kJ g^{-1} of protein, lipid and carbohydrate, respectively.

man et al. (2000). It should be noted that WBC count included neutrophil, lymphocyte, eosinophil, and monocyte counts. Blood biochemical parameters including albumin, cholesterol, triglyceride, glucose, cortisol, and protein were measured using Autoanalyser (Tajhizat Janjesh, Isfahan, Iran) according to the manufacturer's instructions and using commercial kits (Pars Azmun Co., Tehran, Iran). Cholesterol, triglyceride, glucose, and protein were analyzed through the procedures adopted by Burtis et al. (2001), Burtis et al. (2001), Trinder (1969), and Wootton (1964), respectively. Serum antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were measured according to Góth (1991), Marklund and Marklund (1974), and Paglia and Valentine (1967), respectively. Intestinal lipase activity was measured through the method applied by Iijima et al. (1998). We fixed the intestinal samples in Bouin solution and stained them using the hematoxylin and eosin method for histological examination. We used the ImageJ 1.45 software to quantify the muscle thickness, villi length, and villi girth. Additionally, we conducted the goblet cell count with a field of view 400 times smaller.

Statistical analyses

The Kolmogorov-Smirnov test revealed a normal distribution in the data. One-way ANOVA and Duncan's test were applied to the data in order to identify significant differences between the treatments ($P<0.05$). We performed every analysis using SPSS version 20.

Results

Growth performance and biological parameters

The total weight of the fish included in this investigation nearly tripled from its initial state to its final state, with initial and final weights varying between 22.39 and 22.93 g and approximately 66 and 71 g, respectively. T1 and T2 exhibited the most substantial and least substantial growth rates, respectively. The weight gain in T1 was notably greater than that of T2 and T3 ($P<0.05$); however, there was no statistically significant difference in weight gain between T1 and T4 ($P>0.05$). A comparable pattern was noted with regard to survival rates, with fish in treatments T1 and T4 exhibiting considerably higher rates of survival than those in treatments T2 and T3 ($P<0.05$). Nevertheless, there was no statistically significant distinction between treatments T1 and T4 in terms of survival rate ($P>0.05$).

Hematological parameters

For the purposes of this investigation, a summary of the hematological parameters of the fish fed diverse diets is provided in Table 3. Hematological parameters at day 0 did not exhibit any significant differences ($P>0.05$). When fish food was substituted with canola oil, beef tallow, or a combination of the two, RBC, hemoglobin, and hematocrit decreased significantly. Particularly diminished in comparison to the other regimens ($P<0.05$), T3 exhibited the lowest hematocrit. While a notable distinction was noted in RBC and hematocrit values between treatments T2 and T3, no such difference was found between treatment T4 and the aforementioned two regimes ($P>0.05$). Regarding MCV, MCH, and MCHC, the outcomes revealed no statistically significant variation across all treatments ($P>0.05$).

The sample with the lowest white blood cell count (WBC) was T4, which exhibited a significant decrease compared to T3 ($P<0.05$). However, no other samples differed substantially in terms of WBC ($P>0.05$). In

Table 2 Growth performance and biological parameters of rainbow trout after 90 days feeding with different regimens

	90 days	0 day	90 days	0 day	90 days	0 day	90 days	0 day
	T1	T1	T2	T2	T3	T3	T4	T4
Initial weight (g)	22.39±1.73a	5.39±1.78a	4.86±1.57a	2.86±1.59a	22.76±1.11a	2.76±1.14a	22.93±1.12a	3.98±1.19a
Final weight (g)	71.85±1.27a	7.86±1.28a	66.89±2.32a	6.99±2.82a	69.43±1.78ab	9.46±1.88ab	70.97 ±1.21c	7.87 ±1.31c
WG[1] (g)	49.34±1.38c	9.34±1.36c	43.89±1.35a	9.89±1.25a	47.11±1.21b	7.13±1.71b	47.91±2.52bc	7.91±2.62bc
SR[2]	97.22±1.21b	9.34±1.58c	92.43±2.15a	8.43±3.15a	91.01±1.72a	9.01±1.76a	96.88±1.05b	6.88±2.65b

Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).
[a] WG, Weight Gain
[b] SR, Survival Rate

contrast, there was no statistically significant variation in the percentage of lymphocytes among the other samples (P>0.05), whereas T1 had a substantially higher percentage of lymphocytes than the other samples (P<0.05). In contrast, the proportion of neutrophils in T1 was considerably reduced in comparison to the remaining samples. Furthermore, in terms of neutrophil percentage, no significant difference was observed among the remaining samples (P>0.05). Furthermore, there was no statistically significant variation observed in the proportions of monocytes and eosinophils across the regimens (P>0.05).

Serum biochemical parameters

Figures 1 and 2 depict the biochemical profiles of the fish's serum, which include protein, albumin, globulin, glucose, cholesterol, triglyceride, and cortisol. On day zero, there were no notable disparities observed in the serum biochemical parameters (P> 0.05). T1 and T4 contained the highest and lowest concentrations of blood serum protein, measuring 3.77±0.55 g/dL and 2.46±0.21 g/dL, respectively. Significant variations in serum blood protein levels were observed across all samples (P<0.05), with the exception of T3 and T4 (P>0.05). Additionally, blood serum albumin concentrations peaked at 2.41±0.07 g/dL in T1 and declined to 1.70±0.05 g/dL in T4. Significant variations were observed in the levels of serum blood albumin and globulin across all samples (P<0.05). The control group (T1) exhibited a substantially lower blood serum glucose level compared to the other three groups (P<0.05). Furthermore, it was observed that the cholesterol concentration in the blood serum of subjects T2 was considerably reduced compared to subjects T1 and T3 (P<0.05). Triglyceride levels in blood serum were greatest and lowest in T3 and T2, respectively. Triglyceride levels in the serum were notably reduced in T2 compared to the other samples (P 0.05); conversely, the concentration of triglyceride in T3 was substantially greater in T1 and T2 (p 0.05). In conclusion, the level of blood serum cortisol was notably reduced in the control group (T1) compared to the other groups (P<0.05). T2 had the highest concentration of cortisol in its blood serum, which was substantially greater than that of the other groups (P0.05).

Table 3 Hematological parameters of rainbow trout after 90 feeding with different regimens

	90 d	0 d	90 d	0 d	90 d	0 d	90 d	0 d
	T1	T1	T2	T2	T3	T3	T4	T4
RBC (10^6 mL^{-1})	2.347±0.068c	1.249±0.077c	2.067±0.040b	1.087±0.040b	1.920±0.030a	0.880±0.040a	1.997±0.074ab	0.888±0.094ab
WBC (cell/mm^3)	1.640±0.092ab	0.880±0.076ab	1.713±0.101ab	0.834±0.201ab	1.543±0.085a	0.463±0.095a	1.767±0.081b	0.967±0.061b
Neutrophil (%)	16.333±1.527a	16.333±1.527a	21.667±1.523b	17.667±1.723b	23.000±1.010b	14.000±1.010b	22.125±2.645b	16.125±2.645b
Lymphocyte (%)	82.333±2.082	21.432±2.091	76.333±2.082a	22.526±2.076a	74.667±2.082a	24.764±2.087a	75.667±2.082a	25.448±2.093a
Eosinophil (%)	0.333±0.113a	0.12±0.223a	0.333±0.150a	0.13±0.170a	0.333±0.022a	0.14±0.033a	0.667±0.333a	0.193±0.443a
Monocyte (%)	1.000±0.557a	0.098±0.657a	1.333±0.452a	0.54±0.532a	2.000±0.527a	0.800±0.667a	1.667±0.377a	0.657±0.397a
PCV (%)	50.333±1.055c	20.355±1.066c	46.001±1.000b	26.001±2.000b	42.667±1.527a	22.675±1.627a	45.010±1.001b	25.010±1.001b
Hb (g dL^{-1})	13.733±0.404c	03.543±0.123c	12.067±0.681b	02.123±0.781b	10.200±1.212a	4.300±1.812a	11.200±0.173ab	01.333±0.273ab
MCV (fl)	217.233±1.721a	111.233±2.221a	222.033±4.119a	100.033±5.119a	220.500±10.859a	118.700±10.859a	226.667±5.564a	116.647±4.564a
MCH (pg)	58.633±4.289a	27.555±3.117a	58.333±2.150a	28.435±1.850a	54.733±3.322a	24.765±1.4322a	57.967±4.627a	26.654±3.716a
MCHC (g dL^{-1})	27.300±1.758a	07.300±1.543a	26.200±0.954a	06.300±0.854a	24.300±2.029a	14.452±2.619a	25.367±2.059a	15.543±2.076a

Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05)

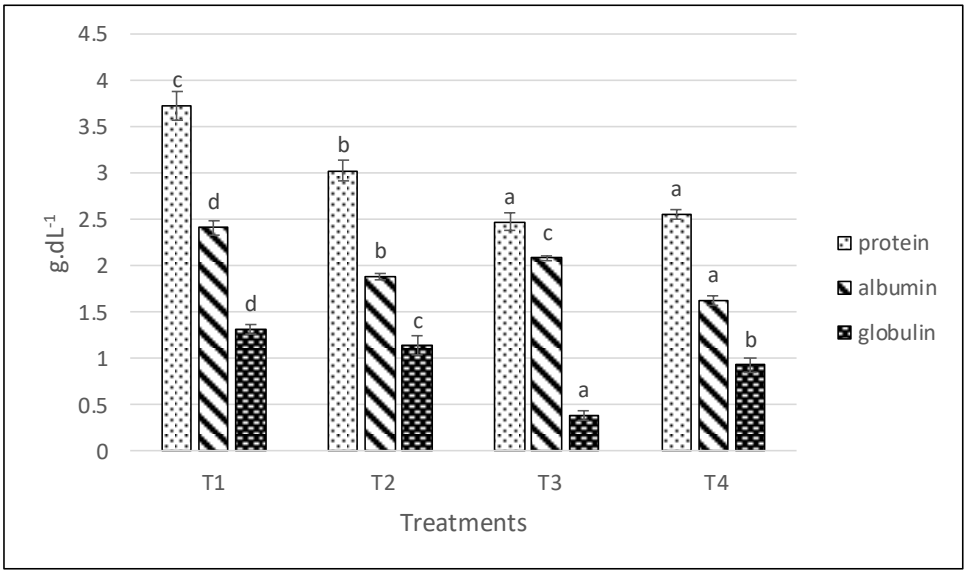

Fig. 1 Serum protein, albumin, and globulin of rainbow trout after 90 feeding with different regimens. Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).

Serum immune parameters, antioxidant enzymes and intestinal lipase activity

Serum immune parameters were analyzed by measuring blood serum immunoglobulin and lysozyme levels of the fish (Table 4). Subjecting the fish to different diets had no considerable effects on the serum immune parameters of the fish because no significant differences were seen in terms of serum immunoglobulin and lysozyme levels among the control and experimental groups (P>0.05). A similar trend was observed for serum antioxidant enzymes, where no significant differences were observed in terms of serum SOD, CAT, GPx levels among the control and experimental groups (P>0.05) (Table 4). Also, according to Table 4, there are no significant differences among different groups in terms of intestinal lipase activity (P>0.05).

Histological analysis of intestine

Figure 3 illustrates the histological examination of fish intestinal tissue. The T1 fish's intestine consisted of four healthy layers: submucosa, muscular tissue, serosa, and mucosa. However, T2 exhibited increased gaps in the basal portion of the intestinal villi and injury to the muscular layer, particularly longitudinal muscles. The intestinal histological status stayed the same in T3, but there were more goblet cells and bigger openings at the base of the intestinal villi compared to the control sample (T1). In addition, the quantity of lipid vacuoles was greater in T3 than in the remaining categories. In the end, the length of the intestinal villi increased in T4, but the histology of the intestine stayed the same, showing no signs of major damage to the muscular and serosa layers (Fig. 3).

Discussion

This study analyzed the effects of replacing fish oil in rainbow trout diet with canola oil and beef tallow

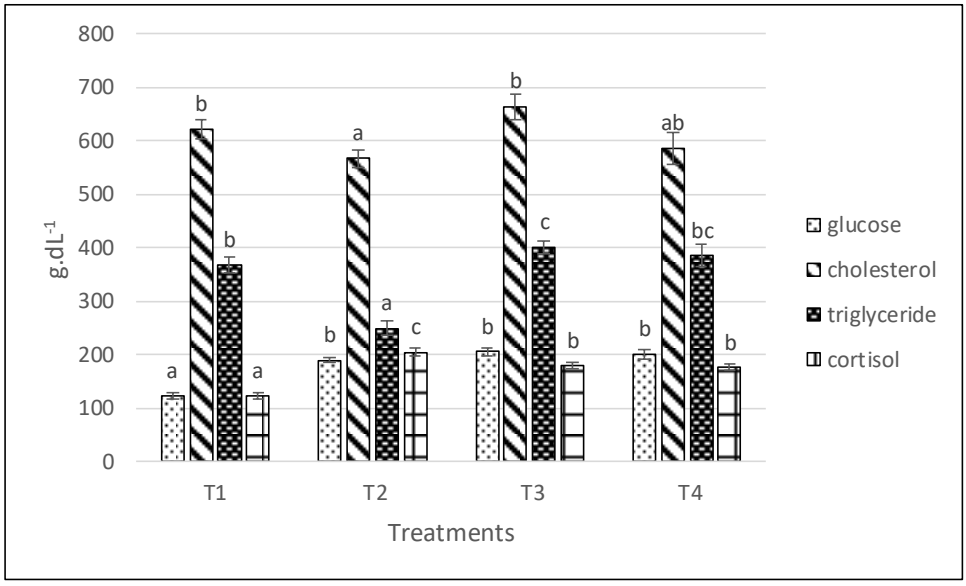

Fig. 2 Serum glucose, cholesterol, triglyceride, and cortisol of rainbow trout after 90 feeding with different regimens. Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).

Table 4 Serum immune parameters, serum antioxidant enzymes, and intestinal lipase activity of rainbow trout after 90 feeding with different regimens.

	90 d	0 d	90 d	0 d	90 d	0 d	90 d	0 d
	T1	T1	T2	T2	T3	T3	T4	T4
Immunoglobulin (mg.mL^{-1})	112.21±3.31a	52.35±4.51a	111.12±4.71a	41.22±3.78a	110.3±4.31a	40.3±3.21a	112.05±4.13a	42.05±2.13a
Lysozyme (µg.mL^{-1})	50.1±2.22a	20.1±2.32a	48.21±2.20a	48.21±2.20a	52.10±2.11a	22.11±2.16a	48.05±1.95a	28.05±1.75a
SOD (U.mL^{-1})	124.21±7.11a	28.21±7.31a	121.12±5.07a	32.12±6.07a	119.02±5.22a	29.02±6.22a	119.15±5.01a	29.15±5.01a
CAT (U.mL^{-1})	3.32±0.41a	1.42±0.61a	3.56±0.12a	1.26±0.12a	3.31±0.41a	1.31±0.51a	3.07±0.23a	1.07±0.33a
GPx (U.mL^{-1})	26.21±2.01a	11.31±2.01a	24.32±2.12a	12.32±4.56a	27.02±4.14a	14.02±3.14a	25.13±3.09a	12.16±3.09a
Intestinal lipase activity (U.mg^{-1} protein)	0.06±0.011a	0.01±0.015a	0.07±0.009a	0.01±0.008a	0.07±0.009a	0.02±0.008a	0.06±0.014a	0.03±0.017a

Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05)

on various parameters. The results showed that the highest final weight, weight gain, and survival rate belonged to T1 (100% FO) and T4 (50% FO + 25% CO + 25% BT), while the lowest final weight, weight gain, and survival rate were seen in T2 (50% FO + 50% BT). This is in contradiction with the results of Nogales-Mérida et al. (2011) who reported no significant difference in growth parameters of sheephead bream (Diplodus puntazzo) fed a diet with 25% FO and 75% animal fat. This discrepancy might be due to differences in the type of animal fat (beef tallow vs. pork fat), the percentage of FO replacement in fish diet, and the fish species studied. In general, lipid digestibility is dependent on fat source, fatty acid profile, level of saturation, fatty acid chain length, double bond location, placement of fatty acid in glycerol configuration, and fat melting point (Monteiro et al. 2018). Fatty acid digestibility in fish decreases with reduction in unsaturation degree (Olsen et al. 2004). Terrestrial animal fats, due to their elevated concentrations of saturated and monounsaturated fatty acids, exhibit a higher melting point than fish oil. Consequently, these fats remain solid at room temperature. Conversely, vegetable and fish oils, which are abundant in polyunsaturated fatty acids, remain liquid under comparable conditions. Unsaturated fatty acids are preferred by fish lipase, whereas SFAs are extremely resistant to lipolysis (Monteiro et al. 2018). Consequently, it appears that beef tallow decreased growth parameters in this study as a result of increased SFA in the fish diet, decreased fat digestibility, and decreased feed efficiency. The results of this research demonstrated that substituting FO with a combination of vegetable oil and animal fat resulted in more favorable effects on growth parameters and body weight gain when compared to using either vegetable oil or animal fat alone. The composition of blood serves as an indicator of aquatic organisms' physiological and environmental

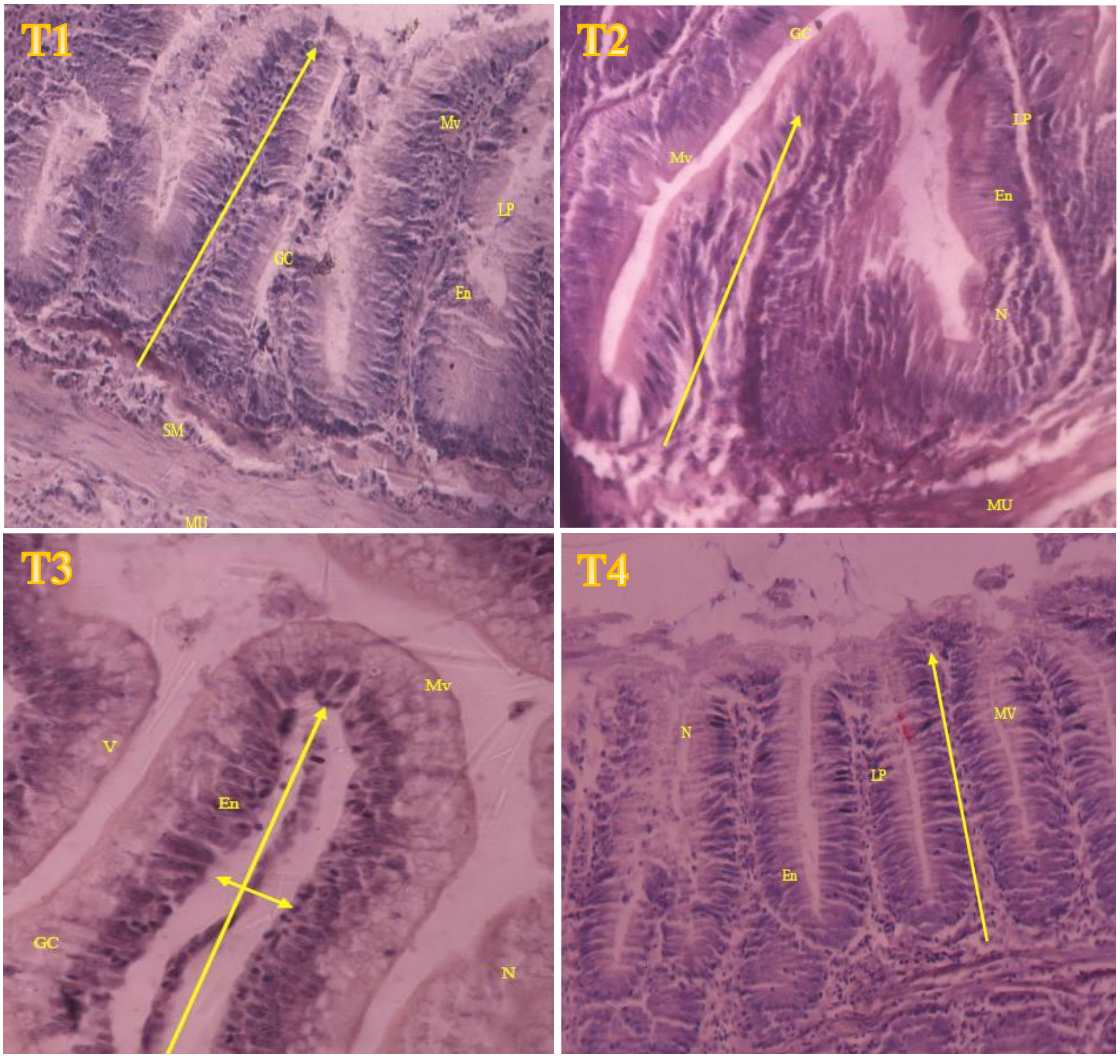

Fig. 3 Histological analysis of intestine sections from rainbow trout after two months feeding with different regimens (H&E, ×20). En: Enterocytes; GC: Goblet cells; SM: Submucosa layer; MU: Muscular layer; Arrow: intestine villi length; LP: Lamina propria; MV: Microvilli; N: Cellular necrosis; V: Enterocyte vacuoles.

well-being. Variations in hematological parameters may indicate unfavorable environmental circumstances or the presence of stressors in the fish's diet or culture media. Furthermore, dietary nutrients and the rate of diet uptake may also impact blood composition (Witeska et al. 2022).

In the diet of rainbow trout, substituting fish oil with beef tallow and/or canola oil resulted in a decrease in certain hematological parameters. These findings align with those reported in prior research pertaining to Nile tilapia. (Oreochromis niloticus) (Navarro et al. 2018) and African catfish (Heterobranchus longifilis) (Babalola et al. 2016) fed diet containing vegetable oils. In contrast, the results of this study are in contradiction with the results of Yildirim-Aksoy et al. (2007) who reported lower red blood cells in Nile Tilapia (Oreochromis niloticus) fed fish oil compared to the fish fed vegetable oil and animal fat. Furthermore, they mentioned that fish fed fish oil had significantly higher WBC and similar levels of hematocrit, which is not in agreement with the results of the current study. Additionally, Medagoda et al. (2022) stated that total replacement of fish oil with tallow in the diet of olive flounder (Paralichthys olivaceus) did not have a significant influence on hematocrit and hemoglobin of the fish, which is not consistent with the results of the present study. Hematological indicators, such as hematocrit, hemoglobin, and red blood cell depletion, are significant indicators of anemia in osteichthyes. This form of anemia has the potential to induce hemorrhage, accelerated erythropoiesis, and diminished oxygen reserves in fish, which subsequently restricts growth and compromises their overall health (Witeska et al. 2015). Differences in hematological parameters among fish fed diets containing different concentrations of fish oil may be attributed to the high unsaturated fatty acid content of fish oil, which increases the flexibility and permeability of cell membranes (Yildirim-Aksoy et al. 2009).

Replacement of fish oil with beef tallow and a mix of beef tallow and canola oil in this study did not significantly change circulating level of cholesterol compared with control, which is in line with the results of Monteiro et al. (2018) on the effect of replacing fish oil with terrestrial animal fats in European seabass. This could be related to high levels of cholesterol in both fish oil and beef tallow (Cheng and Hardy 2004). Conversely, yellowtail kingfish consuming a diet devoid of fish oil but supplemented with poultry oil and canola oil exhibited a substantial reduction in plasma cholesterol levels (Seriola lalandi) (Bowyer et al. 2012). A comparable finding was reported in the present investigation, wherein fish that were fed a diet consisting partially of canola oil substituted for fish oil had considerably reduced cholesterol levels compared to those that were fed a diet consisting solely of fish oil or fish oil plus animal fat. Plant-based sources' reduced cholesterol content could potentially explain this (Cheng and Hardy 2004). Generally, substituting fish oil with animal fat or a combination of animal fat and vegetable oil would not cause hypocholesterolemia in rainbow trout. In addition, substituting beef tallow for fish oil resulted in a substantial increase in plasma triglyceride levels, whereas substituting canola for oil led to a substantial decrease in the same. This contradicts the reports of Bowyer et al. (2012) and Monteiro et al. (2018) who did not witness significant changes in plasma triglyceride after replacing fish oil with other fats sources. Moreover, Pérez et al. (2014) stated that the replacement of fish oil with beef tallow in gilthead sea bream (Sparus aurata) diet reduced of triglyceride. The current research has observed hypertriglyceridemia resulting from the substitution of beef tallow for fish oil, which may indicate hepatic dysfunction or compromised nutritional status in the fish (Bowyer et al. 2012). Furthermore, substituting canola oil for fish oil led to a substantial reduction in plasma glucose levels. Conversely, substituting beef tallow for fish oil did not yield a statistically significant alteration in plasma glucose levels. This is in agreement with the results of Monteiro et al. (2018) who reported no significant difference in plasma glucose of European seabass after replacing fish oil with animal fats. Furthermore, replacing fish oil with canola oil, beef tallow, or a mix of them significantly increased plasma cortisol levels, which indicates considerable levels of stress (Benítez-Dorta et al. 2013).

Fish oil replacement had no significant effect on the activity of antioxidant enzymes SOD, CAT, and GPx, which is in contrast to the reports on Russian sturgeon (Acipenser gueldenstaedtii) (Li et al. 2017) and grouper (Epinephelus malabaricus) (Lin and Shiau 2007). Fish need antioxidant enzyme activity because pathogens they encounter induce the formation of reactive oxygen species (ROS). Antioxidant enzymes must regulate these reactive oxygen species (ROS) (Ishibe et al. 2008). In response to n-3 fatty acid peroxidation and the generation of free radicals in a diet rich in PUFA, antioxidant enzyme activity may increase (Kiron et al. 2011). Still, this study showed that eating a lot of PUFA didn't really change the activity of antioxidant enzymes compared to eating a lot of animal fat, which has a lot less PUFA. Overall, insignificant variations of antioxidant enzymes in the serums of the fish shows that the enzymes activity for detoxi-

fication of free radicals is not a function of PUFA content in the oil in diet, which is in contrast to the report of Köprücü et al. (2015). Furthermore, there were no big differences in the amounts of intestinal lipase between the groups. This is similar to what was found in a previous study on olive flounder, which found that replacing fish oil in the diet with tallow did not have a big effect on lipase activity (Medagoda et al. 2022).

Our results are consistent with those of another study in which the substitution of a variety of vegetable oils for diet fish oil did not yield a statistically significant impact on the activity of lipase in European seabass. This suggests that choosing an alternative oil source does indeed influence lipase activity (Castro et al. 2016).

Histological analysis revealed no significant impairment in the intestinal structure of fish fed a diet consisting solely of fish oil. This finding contradicts the results of a prior study that documented extensive intestinal structure injury in groupers fed a diet containing fish oil (Long et al. 2022). Although the free radicals produced by oxidative reactions in unsaturated fatty acid-rich diets may cause severe injury to the structure and function of the fish intestine (Song et al. 2019), The absence of intestinal injury in fish from the PUFA-rich diet may indicate that the fish oil utilized in this investigation was not oxidized prior to and throughout diet preparation. The presence of several lipid vacuoles in the fish fed the diet with partial replacement of fish oil with beef tallow could be attributed to the inefficiency in the synthesis of lipoproteins, because lipoproteins play a prominent role in the translocation of lipids from intestine to other organs, which is in agreement with the report of Caballero et al. (2002). In addition, the reduced quantity of lipid vacuoles observed in fish fed a diet consisting partially of canola oil substituted for fish oil may be attributable to the reduced digestibility of animal fats in comparison to vegetable oils (Caballero et al. 2002). Villi length and mucus layer thickness were not affected by high level of fish oil in control sample, which does not correspond to the report of Long et al. (2022) in groupers fed diets containing fish oil.

Conclusion

Based on the findings, substituting a portion of the fish oil in rainbow trout culture with a mixture of vegetable oil and animal fat could be regarded as an effective method to reduce the amount of fish oil used in the fish diet and, as a result, improve the operational economy and financial efficiency. A substantial disparity is evident when comparing the data retrieved from the Tables pertaining to days zero and ninety. Quantitatively, the data values on the 90th day are greater. This demonstrates that the number of days parameter is effective. On the contrary, economic assessments should be conducted before implementing the measures proposed in this research.

Competing interests The authors declare no competing interests.

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