

Research Article

Effects of Dietary Oregano (*Origanum vulgare*) Essential Oil Supplementation in Hybrid Red Tilapia (*Oreochromis spp.*) Diet on Growth Performance, Survival, Intestinal Health, Reproductive and Serum Biochemical Indices

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Feed additives are fundamental components of aquaculture, modulating growth performance, nutrient utilisation and physiological responses in cultured fish. Phytochemical compounds, particularly plant-derived essential oils (EOs), are increasingly explored for their functional benefits in fish. Although commonly used oils such as sunflower, cottonseed and corn oil have been widely studied, oregano EO (OEO) remains less investigated, despite evidence of its positive effects in zebrafish, Nile tilapia and rainbow trout. Its efficacy in hybrid red tilapia (*Oreochromis* sp.) has not been documented. This study evaluated dietary OEO supplementation at 0, 0.4, 0.8 and 1.2 g/kg feed on growth, survival, fish health and gut morphology in hybrid red tilapia fingerlings with a mean weight of 9.5 g. A completely randomised research design with three replicates of 15 fish per treatment was employed, and fish were fed to satiety twice daily for 13 weeks. The results indicate that fish-fed 1.2 g/kg OEO demonstrated significantly higher survival, weight gain (WG) and feed efficiency indices (feed conversion ratio [FCR] and protein efficiency ratio [PER]) compared to controls ($p < 0.05$). Haematological analysis revealed elevated haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and MCH concentration (MCHC), alongside reduced aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, indicating improved metabolic and liver function. Serum biochemical parameters, including alkaline phosphatase (ALP), albumin (ALB), globulin (GLO), total protein and cholesterol, were markedly enhanced at 1.2 g/kg feed OEO inclusion. Organ indices (hepatosomatic index [HSI], viscerosomatic index [VSI], stomach index [SI] and gonadosomatic index [GSI]) were significantly elevated, while gut histomorphology showed longer, wider and more branched villi with increased goblet cell counts and intervilli spacing in the highest OEO treatment group (1.2 g/kg feed). The study concludes that dietary OEO at 1.2 g/kg significantly improved growth performance, feed utilisation, blood health and gut morphology in hybrid red tilapia. Its gonad-stimulating properties further highlight its potential application in broodstock diets to support sustainable fingerling production.

Keywords: aquaculture; hybrid red tilapia growth performance intestinal health; oregano essential oil; reproductive indices; serum biochemistry

1. Introduction

The global expansion of aquaculture, driven by increasing demand for fish, has highlighted the sector as a viable source of affordable protein [1]. Tilapia farming has expanded rapidly

over the past three decades, with Nile tilapia (*Oreochromis niloticus*) representing the most widely cultured species due to its fast growth, adaptability and established production systems [2, 3]. However, red tilapia, a hybrid strain developed from crosses involving *Oreochromis mossambicus*, *O. niloticus*,

and *Oreochromis urolepis hornorum* [4, 5] has gained increasing attention in global aquaculture. Red tilapia production is expanding in Asia, the Middle East and Latin America, supported by its favourable flesh quality, consumer appeal and higher market value.

Globally, tilapia production surpassed 6 million tonnes in 2023 [6], with red tilapia accounting for a smaller but fast-growing share. In Asian markets such as China, Thailand, the Philippines, Malaysia and Taiwan, red tilapia commands premium prices compared to Nile tilapia. This price advantage is largely attributed to its attractive reddish-pink skin and white flesh, which resemble high-value marine fish such as snapper, making it particularly desirable in live and whole-fish markets [7]. In many regions such as North America and East Asia, consumers are willing to pay 20%–30% more for red tilapia relative to Nile tilapia, providing farmers with stronger profit margins [8–10]. Beyond market value, red tilapia demonstrates favourable traits such as tolerance to brackish water, adaptability to cage and pond systems and resilience under intensive farming conditions [11, 12]. These features, combined with its premium consumer preference, make red tilapia a strategic species for diversification of tilapia aquaculture. Thus, while Nile tilapia dominates global output, red tilapia represents a high-value niche product with growing importance in sustaining profitability and meeting diverse market demands. Hence the choice species for this study.

In aquaculture, feed additives and supplements play a critical role in enhancing the growth of farmed fish [13]. Numerous studies have explored the use of plant-based fish feed additives and oils. For instance, soybean oil has been used as a dietary supplement in Atlantic cod (*Gadus morhua*) feeds [14], spirulina has been used as major plant based ingredient (5%–10%) in the fish diet to promote growth in three-spot gourami (*Trichopodus trichopterus*) and common carp (*Cyprinus carpio*) [15, 16]. Similarly, *Azolla microphylla* as fed ingredient have been documented to have enhanced growth and immunity in common carp (*C. carpio* L.) and Nile tilapia [17, 18]. Sunflower oil inclusion in the fish diet has also been reported to promote growth in Nile tilapia [19, 20], blended cottonseed oil has been used as feed supplement to improved growth and feed efficiency in Nile tilapia [21–23] and *Carica papaya* pulp and seeds have been shown to enhance growth and immunity in Mozambique tilapia (*O. mossambicus*) and African catfish (*Clarias gariepinus*) [24, 25].

Among plant-based dietary supplements, oregano (*Origanum vulgare*) essential oil (OEO) has emerged as a standout in aquaculture due to its multifaceted applications. OEO is not only essential for aquaculture but also widely used as essential oil (EO) in livestock to enhance animal health [26]. In aquaculture, OEO has been widely used as an anaesthetic during fish handling in hatcheries as an alternative to clove oil. Its anaesthetic properties have been effectively documented in Mozambique tilapia (*O. mossambicus*). Gabriel et al. [27], gilthead seabream (*Sparus aurata*) [28], and Nile tilapia [29]. OEO's anaesthetic efficacy is often preferred over clove oil, with fish exhibiting shorter induction and recovery times at optimal dosages [29, 30]. Despite its wide usage and potential in aquaculture as an anaesthesia, the use of OEO and its constituents as

dietary additives in aquaculture remains underexplored even though OEO has proved to be effective and positively influences fish growth, and health while increasing survival in European sea bass (*Dicentrarchus labrax*) [31, 32]. OEO is considered a safe, ecological alternative to synthetic antibiotics and other veterinary medicines Not only in aquaculture but also in livestock [26]. OEO has also been reported to enhance gut microbiota and improve intestinal functions in common carp [33]. Additionally, in different studies this EO has been used as a preservative agent, as it was used to preserve fish fillets by preventing lipid oxidation and bacterial spoilage while enriching them with health-promoting bioactive compounds [34]. OEO have been found to be rich in bioactive compounds such as carvacrol and thymol, which make it best used as a safe food additive, and for this reason it is widely used as a growth promoter and anaesthetic/health enhancer in farmed Nile tilapia [35].

Recent studies have also demonstrated that OEO can improve growth performance and health in farmed fish. For instance, OEO, has been documented to promote growth and enhance intestinal histomorphology in salmon trout [36], channel catfish (*Ictalurus punctatus*) [37] and Neotropical catfish (*Lophiosilurus alexandri*) [38].

Beyond aquaculture, oregano plant leaf extracts are recognised for their high functionality as medicinal agents, with applications in human as medicine [39] and to boost immunity and health in livestock [26]. OEO biochemical compositions such as carvacrol and thymol, are known for their antibacterial, anti-inflammatory, antioxidative and immune-modulating properties [40]. Furthermore dietary supplementation with OEO has been documented to enhance immune function and antioxidative stress in koi carp (*Cyprinus rubrofasciatus*) [33], Black Sea salmon (*Salmo labrax*) [41], *Catla catla* [42] and gilthead seabream (*Sparus aurata* L.) [28]. Similar findings were reported in Nile tilapia and zebrafish (*Danio rerio*)-fed dietary inclusion of OEO [43, 44]. Despite extensive research on the use of OEO as a dietary supplement and anaesthetic in various fish species, its efficacy and effects on physiological parameters in hybrid red tilapia (*Oreochromis* spp.) remain unexplored despite high demand of red tilapia in local and global market especially in South East Asian countries and North America. And also, being that red tilapia is tolerant to high salinity levels of up to 60 ppt, it is considered the best tilapia species for mariculture [45, 46]. Notably, there no similar study on the effects of OEO on growth performance, survival, reproductive indices, haematological parameters and gut histomorphology that has been done and documented in red tilapia. This study therefore hypothesis that oregano essential supplementation in the diet of red tilapia significantly influence physiological parameters such as growth performance, survival, gut histomorphology, reproductive indices and serum biochemical markers in dosage dependant mannar.

2. Materials and Methods

2.1. Sources of Experimental Fish and Culture Conditions. Hybrid red tilapia fingerlings, with an average weight of 9.54 ± 0.15 g, were harvested from the rearing tank at the

TABLE 1: Composition of formulation diets used in the study.

Ingredients	Control (0 g/kg)	TA (0.4 g/kg)	TB (0.8 g/kg)	TD (1.2 g/kg)
Rice powder	57.4	57.4	57.4	57.4
Soya bean powder	31	31	31	31
Fish meal	3	3	3	3
Oregano essential oil	0	0.4	0.8	1.2
Sun flower oil	5.9	5.5	5.1	4.9
Tapioca powder	1.2	1.2	1.2	1.2
Vitamin premix (VP10)	1.	1	1	1
Mineral premix (Mp5)	0.5	0.5	0.5	0.5
Total (g)	100	100	100	100

Note: Feed ingredients were procured from Broadway Market, Ernakulam, Kerala, India, while the vitamin and mineral premixes were sourced from Shrimpex Aquashop.

Hatchery Complex at the School of Industrial Fisheries, Cochin University of Science and Technology. A total of 300 fingerlings were transferred to a 1000 L tank filled with 700 L of freshwater for a 7-day acclimatisation period. During acclimatisation, the fish were fed on a formulated basal diet at 5% of their body weight twice daily. Water was aerated constantly, and uneaten feed and excreta were syphoned daily, accompanied by partial water exchanges to maintain water quality. After acclimatisation, the fish were randomly distributed into 12 experimental tanks (100 L capacity, each containing 80 L of fresh water), with 15 fingerlings per replicate tank. The experiment followed a completely randomised research design with three replications in each treatment level.

Four treatment levels were set, testing four dietary OEO supplementations in the fish diet. The treatment groups were denoted as TC (Control, 0 g/kg), TA (0.4 g/kg), TB (0.8 g/kg) and TD (1.2 g/kg). Different experimental diets for each treatment group were formulated as indicated in Table 1. During the treatment period, 10% of the water in each tank was exchanged daily, and uneaten feed and waste were syphoned out after feeding daily. Fish were fed to satiation with close monitoring of fish while feeding to ascertain that all feeds supplied were consumed by the fish with no feeds going to waste.

Water quality parameters, were measured throughout the treatment period daily. This included temperature, dissolved oxygen (DO) and pH, temperature was measured using a digital thermometer (AQUANEAT Digital Thermometer (Model A045) made in China), DO was determined via the Winkler titration method, and pH was assessed using a digital pH metre, (AMPEREUS Digital pH Metre, made in India, Kavita Corporation). Water quality was maintained at pH, 7.5 ± 0.40 ; DO, 6.7 ± 0.05 mg/L; temperature, $28.4 \pm 0.38^\circ\text{C}$. Light conditions followed the natural photoperiod. Total ammonia (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were maintained below detectable levels throughout the treatment period across all experimental groups.

2.2. Experimental Diet Preparation and OEO Inclusion Levels. Isonitrogenous and isolipidic experimental diets was formulated to meet the nutritional requirements of tilapia as recommended by Lim et al. [47] and Zuluaga-Hernández et al. [48] and divided into four portions based on the treatment

TABLE 2: Chemical constitution of *Origanum vulgare* L. essential oil used.

Chemical and mineral constituents of <i>Origanum vulgare</i> L. essential oil	
Chemical compound	Concentration
Hydrocarbons	15.20%
Citral	0.49%
Geraniol	0.21%
Limonene	0.28%
Linalool	0.28%
Eugenol	0.20%
Arsenic	<1 mg/Kg
Carvacrol	61.20%
Beta-caryophyllene	2.4%–8.2%
Mineral composition	Concentration
Lead	<1 mg/Kg
Mercury	<1 mg/Kg
Cadmium	<1 mg/Kg
Total heavy metals	<10 mg/Kg
Density of oregano EO	0.913 g/mL

Note: Oregano vulgare essential oil was supplied by TSBT International, Akshaya Nagar, Bangalore, India, (chemical compositions are based on manufacturers descriptions and analysis).

groups. The ingredients used in the diets included rice powder, soya bean meal, fish meal, sunflower oil, OEO tapioca as a binder and a mineral and vitamin premix. The feed ingredients were procured from Broadway Market (Ernakulam, Kerala, India), whereas the vitamin and mineral premixes were obtained from Shrimpex Aqua shop, and included in the basal feed in the proportions detailed in Table 1, after determining ingredient proportions using the Pearson square method of feed formulation.

OEO used in this experiment was purchased from TSBT International, Akshayanagar, Bangalore, India, and its chemical composition as per the manufacturer manual was as provided in Table 2. The experimental diets incorporating OEO per treatment group followed established protocols described in previous studies conducted in Nile tilapia [31–33, 38].

In the preparation of OEO supplemented diet based on treatment groups, OEO was dissolved in absolute ethanol in

TABLE 3: Proximate composition analysis of formulated basal diet.

Biochemical composition of basal feed (%)	Mean % $\pm$ SE
Moisture content	9.27 $\pm$ 0.092
Ash	9.54 $\pm$ 0.071
Crude protein	44.96 $\pm$ 0.748
Crude lipid	11.98 $\pm$ 0.031
Carbohydrates	24.25 $\pm$ 0.286

Note: The proximate composition of the basal diet was identical across all treatment groups, as the same basal feed was used.

a proportion of 0.1–10 mL of absolute ethanol to prepare suspensions at varying concentrations based on the experimental treatment groups. The formulated basal feeds were then mixed with the OEO suspensions based on the dosage inclusion and treatment groups. The mixture of oregano-supplemented feed was then open air dried under shade for 3 days to allow the strong smell associated with oregano to fade away from the feeds which may otherwise affect the palatability of feeds for the fish. Exposure of the feed to strong sunlight was avoided since strong light can degrade EOs. The feeds were then pelleted and oven-dried. The oregano dietary implementation dosages were as follows: Control TC (0 g/kg), TA (0.4 g/kg), TB (0.8 g/kg) and TD (1.2 g/kg).

The proximate composition of the formulated feed was analysed and found to contain variable nutrient compositions, as indicated in Table 3. The proximate composition of the basal diet was identical across all treatment groups, as the same basal feed was used. The analysis was conducted following standard procedures for biochemical composition analysis as outlined by AOAC [49] and Feldsine et al. [50]. Each diet was tested in triplicate (three tanks per treatment).

The fish were fed twice daily at 5% of their bodyweight for 13 weeks, with feeding rations provided to fish until apparent satiety while the fish were being monitored closely to ensure that there was no wastage of feed during the treatment period per treatment level. Sampling was done after every 3 weeks with a minimal sample size of 7 fish per replicate (sample size $n = 21$ per treatment group). This sample size was arrived at based on other related work that emphasises the need for a reduced sample frame to minimise stress and health risks. All protocols involving fish welfare during the treatment and handling period complied with the ethical guidelines approved by the Institutional Animal Ethics Committee, Department of Biotechnology, Cochin University of Science and Technology, Kerala, India (Ethical Approval Number: 363/GO/Re/S/01/CCSEA/90). The research also adhered to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.3. Fish Growth and Feed Utilisation Parameters Analysis. Fish sampling was done every 3 weeks during the treatment period, where four sampling sessions were conducted throughout the 13 weeks of treatment. Growth parameter data were obtained per treatment. Seven fish from each replicate per treatment were sampled ($n = 21$) per treatment group. Fish weight (g) and total length were recorded for each fish at each sampling time. At the end of the 13 weeks treatment period, the final weight and total length of each fish in each replication were recorded to calculate growth performance, feed conversion ratio (FCR), survival rate, weight gain (WG, %), fish condition factor (K), body somatic index, protein efficiency and feed conversion efficiency. These metrics were calculated using formulas adopted from Haque et al. [51]; Hosseinpour Zelaty et al. [52] and Somdare et al. [53].

Average weight gain, AWG = Final weight of fish – Initial weight at stocking/number of days cultured,

Specific growth rate, SGR (%) = $\text{Log (final fish weight)} - \text{log (initial fish weight)} / \text{number of culture days} \times 100$,

Feed conversion ratio, FCR = Feed consumed/fish weight gain,

where feed consumed is the quantity of feeds given (g) during the culture period.

Weight gain = Final weight of fish (g) – initial fish weight,

Protein efficiency ratio, PER
= Weight gain/crude protein fed,

Total feed consumed (g)

= (Weight of feed container before feeding)
– (weight after feed) $\times$ total number of days cultured,

Fulton's condition factor (K)

= $100 \times (\text{Weight of fish}) / (\text{length of fish})^3 (100 \times W/L^3)$.

2.4. Body Indices Analysis Procedures.

Stomach index, SI = Weight of the stomach/fish weight $\times$ 100,

Gonadosomatic index, GSI

= Weight of the gonads/fish weight $\times$ 100,

Hepatosomatic index, HSI

= Weight of the Liver/fish weight $\times$ 100,

Visceral somatic index, VSI

= Total visceral weight/fish weight $\times$ 100.

2.5. Haematology and Serum Enzyme Biochemical Indices Analysis.

Blood samples were collected at the end of the 13 weeks treatment period. Three fish from each replicate per treatment ($n=9$) were used. Nine fish from each treatment were sampled for haematology and serum enzyme analysis. Fish were anaesthetised using OEO at a concentration of 0.25 mL/L of water, which allowed for rapid induction and prolonged recovery during the blood collection process following the recommended dosage in previous study in Mozambique tilapia [30]. Blood was drawn from the caudal vein using a 5 mL syringe fitted with a 24-gauge needle, following the procedure adapted from [7, 54].

Blood samples were allocated into different tubes for specific analyses: EDTA tubes for haematological analysis, sodium fluoride tubes for glucose testing, and serum tubes for serum enzyme function tests. Haematological parameters were analysed within 1 h of collection using an automated haematological analyser (HeCo Vet C, SEAC, Florence, Italy), following protocols established by Fazio [55] and Fazio et al. [54]. The parameters assessed included red blood cell count (RBC), haematocrit (Hct), haemoglobin (Hb) concentration, white blood cell (WBC) count, platelet count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and MCH concentration (MCHC). A specialised lysing reagent (SEAC code 71010460), containing potassium cyanide, quaternary ammonium salts and surfactants, was used to prepare samples. The analyser employed customised software specific for fish blood to account for nucleated RBCs, ensuring accuracy by subtracting their nuclei from WBC counts. Human blood with stabilised platelets was used as a control for instrument calibration.

For serum analysis, blood samples in non-EDTA tubes were centrifuged at $3500 \times g$ for 15 min to separate the supernatant. Biochemical parameters, including total protein, albumin (ALB), globulin (GLO), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol, were measured using an automated UV spectrophotometer following protocols established for Nile tilapia [56]. Total protein was quantified using the biuret method with an automated UV spectrophotometer (SEAC Slim, Florence, Italy). Alkaline phosphatase (ALP) activity was determined by incubating plasma with para-nitrophenyl phosphate in ammonium bicarbonate buffer (pH 7.9, 32°C) and measuring absorbance

at 450 nm, as described by Juma Syanya et al. [4], Abo-Taleb et al. [57] and Adham et al. [58].

2.6. Intestinal Morphometry Analysis. Histological and histometric analyses of the fish gut were conducted on fish intestines. Three fish per treatment group ($n=3$) were dissected, and intestinal sections from anterior, mid-section and hind section were extracted and fixed in 10% formalin for 24 h. The fixed tissues underwent standard paraffin embedding procedures adopted from Adeoye et al. [59] and Ali et al. [60], where the sample underwent dehydration in graded series of alcohol baths (70%, 95% and 100% alcohol), followed by clearing in xylene and infiltration in molten paraffin wax (58–60°C). After embedding in paraffin, tissues were blocked, cut into 5-micrometre sections using a microtome, floated on warm water and placed on slides. Section samples (three cross-sections) were waxed, hydrated and stained using haematoxylin-eosin stain according to Fischer et al. [61] and Taddese et al. [62]. Finally, slides were dehydrated, cleared in xylene and mounted with DPX, preparing six slides per treatment for analysis.

Intestinal morphology features such as villus length, villus width and villus crypt length were measured using ImageJ software following the procedure described by Collins [63]. A total of nine wells and random villi and villus-associated crypts were determined for each intestinal cross-section. Goblet cells were stained with periodic acid–Schiff (PAS) and counted according to the same-sized villi ($n=9$). Goblet cell quantification was achieved by a 300-point grid overlay in ImageJ software, counting intersecting cells across 400 mm^2 at $\times 200$ magnification, following methods by Loureiro Paschoalini et al. [64] and Palladino et al. [65].

2.7. Statistical Analysis. All statistical analyses were conducted using SPSS version 24 software. Data normality was assessed using the Kolmogorov-Smirnov test, while homogeneity of variance was evaluated using Levene's test. A one-way analysis of variance (ANOVA) was performed to compare means across groups, followed by Tukey's HSD (Honestly Significant Difference) pairwise tests to identify significant differences. A threshold of $p < 0.05$ was used to determine statistical significance. Results are presented as mean $\pm$ standard error (SE). The SE of the mean (SEM) was preferred for this study because of its ability to assess the precision of a sample mean as an estimate of the population mean.

3. Results

3.1. Growth Performance and Feed Utilisation Indices. The growth performance of red tilapia subjected to varying supplementation levels of OEO varied significantly over the 13-week culture period. Initial weights (Week 1) across treatments showed no significant differences ($p = 0.9061$, $p > 0.05$) (Figure 1A). From Week 4 onwards, fish-fed higher OEO supplementation levels (1.2 g/kg) showed significantly higher WG compared to fish from the control group ($p < 0.05$) (Figure 1B). Higher OEO inclusion levels (1.2 g/kg feed) consistently resulted in superior growth at Week 7 (Figure 1C) and Week 10 (Figure 1D) compared to the control (0.0 g/kg feed) and the lower inclusion level (0.4 g/kg feed) ($p < 0.001$). By the end of

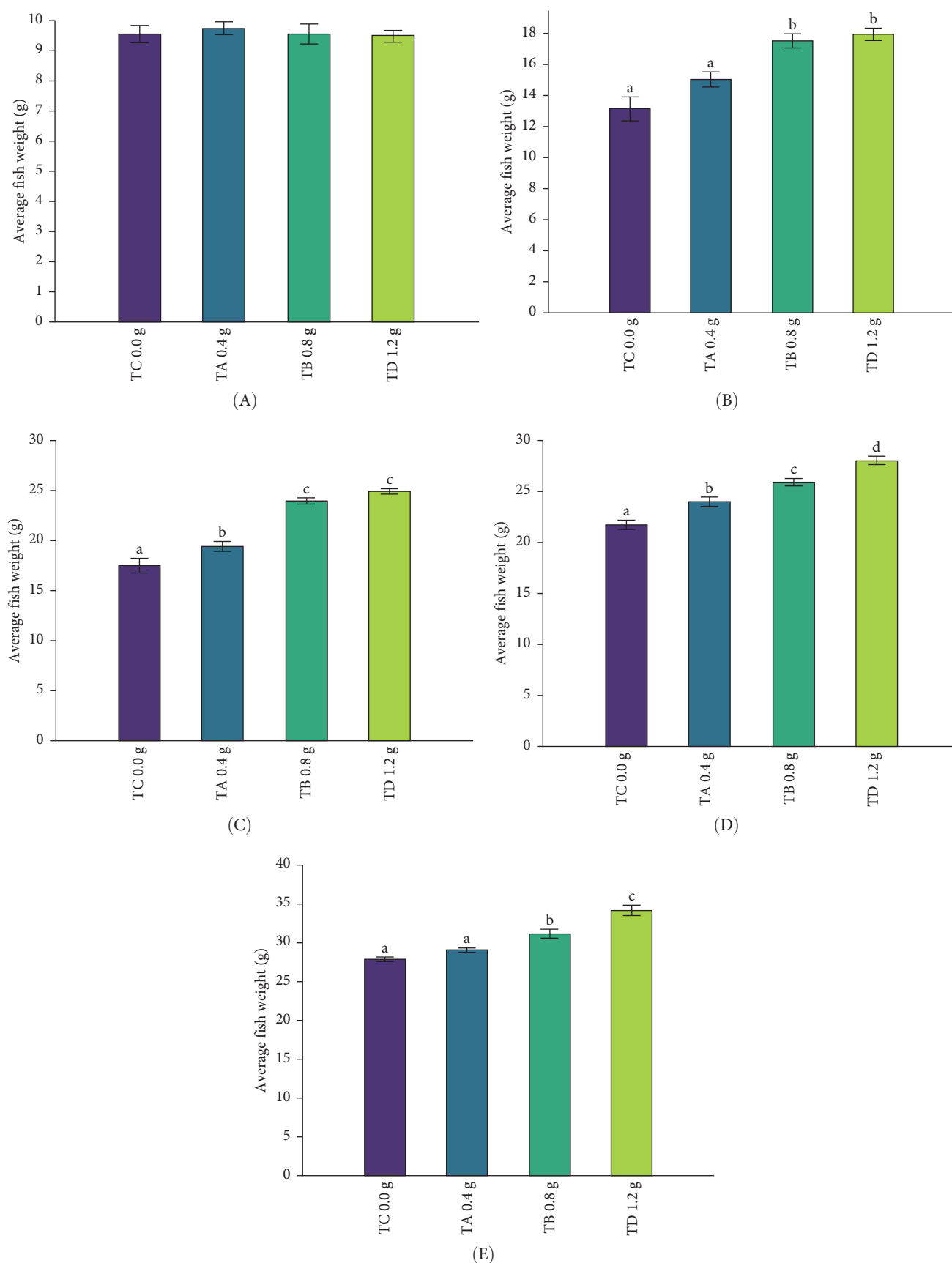


FIGURE 1: Growth variation of fish at different OEO supplementation levels between week 1 and 13. (A) Week 1, (B) Week 4, (C) Week 7, (D) Week 10 and (E) Week 13. Growth parameters are presented as mean $\pm$ standard error (S.E.) where one-way ANOVA indicated significant differences ($p < 0.05$), Tukey's pairwise test was performed. Bars with different lowercase letters within treatment groups across weeks indicate significant differences at ($p < 0.05$).

TABLE 4: Effects of different inclusion levels of oregano essential oil on growth performance and feed utilisation in hybrid red tilapia.

Growth parameters	Treatment groups (oregano EO dosage inclusion level)			
	Control 0.0 g/kg feed	TA 0.4 g/kg feed	TB 0.8 g/kg feed	TD 1.2 g/kg feed
Initial weight (g)	9.570 ± 0.281	9.766 ± 0.210	9.577 ± 0.336	9.501 ± 0.203
Final weight (g)	27.874 ± 0.256 ^a	29.076 ± 0.238 ^a	31.175 ± 0.545 ^b	34.187 ± 0.663 ^c
Average weight gain, AWG (g)	27.715 ± 0.255 ^a	28.913 ± 0.239 ^a	31.015 ± 0.546 ^b	34.081 ± 0.664 ^c
Specific growth rate (%/day)	1.788 ± 0.047 ^a	1.821 ± 0.041 ^a	1.974 ± 0.069 ^b	2.134 ± 0.061 ^b
Feed consumed (g/fish)	50.168 ± 1.440	50.275 ± 1.900	48.676 ± 1.0363	48.183 ± 1.856
FCR	1.8 ± 0.048 ^a	1.727 ± 0.058 ^b	1.563 ± 0.033 ^d	1.409 ± 0.045 ^d
PER	0.522 ± 0.009 ^a	0.551 ± 0.010 ^a	0.617 ± 0.019 ^b	0.705 ± 0.023 ^c
Fish condition factor, <i>K</i>	0.936 ± 0.030 ^c	0.939 ± 0.052 ^c	1.130 ± 0.037 ^d	1.227 ± 0.045 ^d
Survival rate (%)	90.333 ± 0.881	93.000 ± 0.577	95.666 ± 0.333	97.000 ± 0.000

Note: Growth parameters are presented as mean ± standard error (S.E.) based on three determinations. Where one-way ANOVA indicated significant differences ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript lowercase letters within each row indicate significant differences at ($p < 0.05$).

Abbreviations: FCR, feed conversion ratio; PER, protein efficiency ratio.

TABLE 5: Haematological parameters across different oregano essential oil supplementation treatment levels in hybrid red tilapia.

Haematological parameters	Treatment groups (oregano EO dosage inclusion level)			
	Control 0.0 g/kg feed	TA 0.4 g/kg feed	TB 0.8 g/kg feed	TD 1.2 g/kg feed
Haemoglobin, Hb (g/dL)	9.06 ± 0.05 ^a	9.40 ± 0.06 ^b	9.57 ± 0.05 ^c	9.69 ± 0.08 ^c
Haematocrit, Hct (%)	26.10 ± 0.07 ^a	26.66 ± 0.16 ^a	27.56 ± 0.18 ^b	27.30 ± 0.16 ^b
Mean corpuscular volume, MCV (femtoliters, fL)	152.12 ± 0.26 ^a	154.16 ± 0.23 ^b	156.58 ± 0.13 ^c	159.32 ± 0.27 ^d
Mean corpuscular haemoglobin MCH (femtoliters, fL)	51.76 ± 0.12 ^a	52.90 ± 0.27 ^a	56.71 ± 0.26 ^b	59.66 ± 0.23 ^c
Mean corpuscular haemoglobin concentration, MCHC (%)	35.90 ± 0.14 ^a	36.91 ± 0.52 ^b	36.97 ± 0.26 ^b	38.94 ± 0.50 ^c
Platelets/microlitre (μL)	329.66 ± 0.38 ^a	327.13 ± 0.48 ^a	329.25 ± 0.33 ^a	336.86 ± 0.26 ^b
White blood cells, WBCs (10 ⁹ cells/μL)	95.78 ± 0.43	96.17 ± 0.26	96.14 ± 0.17	97.14 ± 0.34
Lymphocytes (×10 ³ cells/μL)	85.43 ± 0.19 ^a	86.69 ± 0.14 ^a	92.60 ± 0.13 ^b	95.80 ± 0.74 ^c
Monocytes (%)	3.42 ± 0.06 ^a	3.52 ± 0.10 ^b	3.60 ± 0.16 ^b	3.53 ± 0.18 ^b
Eosinophils (%)	2.16 ± 0.01 ^a	2.55 ± 0.05 ^b	2.57 ± 0.07 ^b	2.97 ± 0.05 ^c

Note: Haematological parameters are presented as mean ± standard error (S.E.) based on three determinations. Where one-way ANOVA results were significant ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript lowercase letters within each row indicate significant differences at $p < 0.05$.

Week 13, fish from a higher OEO inclusion level (1.2 g/kg feed) achieved significantly greater WG and growth (34.187 g) compared to fish from the control group (27.874 g) ($p < 0.001$) (Figure 1E).

The findings further reveal significant dosage dependant effects of OEO inclusion levels on red tilapia's growth parameters and feed utilisation. Initial weights were statistically similar across all treatment groups ($p > 0.05$). Final weights, average WG (AWG) and specific growth rate (SGR) significantly improved in fish from higher OEO inclusion levels (1.2 g/kg), with WG of (34.187 g) compared to fish from the control group ($p < 0.05$; Table 4). FCR was significantly better in fish from the higher OEO inclusion group (1.2 g/kg) diet ($p < 0.05$). Similarly, protein efficiency ratio (PER) was significantly enhanced in fish from higher OEO inclusion in the fish diet compared to fish from the control group ($p < 0.05$). However, there was no significant difference in the feed consumed per fish across all treatment groups compared to the control group ($p > 0.05$). Fish condition factor (*K*) was markedly improved in fish

from higher OEO levels (1.2 g/kg feed) with a *K* value of 1.22 compared to the control group with a *K* value of 0.939 ($p < 0.05$). Survival rates were significantly improved in fish from the higher OEO (1.2 g/kg feed) treatment group, showing 97% survival compared to fish from the control group with a 90.33% survival rate ($p < 0.05$; Table 4).

3.2. Haematological Parameter Analysis. The findings on the effects of OEO on haematological parameters of fish indicate that there was significant improvements in haematological parameters in hybrid red tilapia in dosage dependant manner. Hb and Hct levels were significantly elevated in fish from higher OEO (1.2 g/kg) compared to fish from the control group ($p < 0.05$; Table 5). Similarly, MCV, MCH and MCHC were significantly elevated in fish fed higher OEO (1.2 g/kg feed) compared to fish from the control group ($p < 0.05$) and better RBC maturation and Hb synthesis. Fish from the 1.2 g/kg OEO supplementation group showed increased platelets. Similarly, higher OEO supplementation in the fish diet boosted

TABLE 6: Serum biochemical parameters of hybrid red tilapia fed different dietary supplementation of oregano essential oil for 13 weeks.

Parameter	Treatment groups (oregano EO dosage inclusion level)			
	Control TC 0.0 g/kg	TA 0.4 g/kg feed	TB 0.8 g/kg feed	TD 1.2 g/kg feed
Aspartate amino transferase, AST (μL)	29.993 $\pm$ 0.428 ^a	28.516 $\pm$ 0.364 ^a	24.628 $\pm$ 0.560 ^b	22.695 $\pm$ 1.495 ^b
Alanine aminotransferase, ALT (μL)	23.661 $\pm$ 0.516 ^a	20.775 $\pm$ 0.364 ^b	19.151 $\pm$ 0.419 ^b	18.195 $\pm$ 0.464 ^c
Alkaline phosphate, ALP (μL)	22.928 $\pm$ 0.581 ^a	26.965 $\pm$ 0.351 ^b	29.806 $\pm$ 0.579 ^c	32.875 $\pm$ 0.710 ^d
Albumin, ALB (g/dL)	3.121 $\pm$ 0.055 ^a	3.490 $\pm$ 0.041 ^c	3.716 $\pm$ 0.023 ^f	3.973 $\pm$ 0.046 ^g
Globulin, GLO (g/dL)	3.053 $\pm$ 0.059 ^a	3.305 $\pm$ 0.104 ^a	4.463 $\pm$ 0.067 ^b	4.961 $\pm$ 0.057 ^d
Serum cholesterol (Chol) (mg/dL)	109.333 $\pm$ 0.843 ^a	121.500 $\pm$ 1.118 ^b	127.000 $\pm$ 0.577 ^c	138.666 $\pm$ 0.714 ^d
Total protein (g/dL)	4.101 $\pm$ 0.007 ^a	4.280 $\pm$ 0.025 ^b	4.581 $\pm$ 0.081 ^c	4.768 $\pm$ 0.023 ^d

Note: Blood serum parameters are presented in mean $\pm$ standard error (S.E.). Where one-way ANOVA was significant at ($p < 0.05$), a Turkey's pairwise test was performed. Means with different superscript lowercase letters in each row indicate significant differences at ($p < 0.05$).

TABLE 7: Fish organ indices parameters of hybrid red tilapia across different supplement levels of oregano essential oil.

Parameters	Treatment groups (oregano EO dosage inclusion level)			
	Control TC 0.0 g/kg	TA 0.4 g/kg feed	TB 0.8 g/kg feed	TD 1.2 g/kg feed
Hepatosomatic index	2.404 $\pm$ 0.136 ^a	2.787 $\pm$ 0.091 ^b	3.166 $\pm$ 0.048 ^c	2.932 $\pm$ 0.052 ^c
Stomach index	1.082 $\pm$ 0.055 ^a	1.389 $\pm$ 0.046 ^b	1.578 $\pm$ 0.080 ^b	1.829 $\pm$ 0.068 ^c
Viscerosomatic index	3.491 $\pm$ 0.057 ^a	3.577 $\pm$ 0.032 ^a	3.894 $\pm$ 0.056 ^b	3.907 $\pm$ 0.108 ^b
Gonado somatic index	1.544 $\pm$ 0.079 ^a	1.760 $\pm$ 0.035 ^a	1.961 $\pm$ 0.043 ^c	2.116 $\pm$ 0.111 ^c

Note: Fish organ indices are presented in mean $\pm$ standard error (S.E.). Where one-way ANOVA was significant ($p < 0.05$), a Turkey's pairwise test was performed. Means with different superscript lowercase letters in each row indicate significant differences ($p < 0.05$).

lymphocytes and eosinophils. Lymphocytes and eosinophils were markedly enhanced in fish from the higher OEO treatment group ($p < 0.05$; Table 5).

3.3. Blood Serum Enzyme Activity Analysis. On serum biochemical composition, AST and ALT were markedly reduced in fish from higher OEO supplementation levels (1.2 g/kg feed) compared to fish from the control group ($p < 0.05$). However, ALP levels was significantly elevated in fish from the higher OEO supplementation (1.2 g/kg feed) groups compared to the control group ($p < 0.05$).

ALB and GLO levels were markedly elevated in fish fed 1.2 g OEO/kg feed treatment groups compared to fish from the control group ($p < 0.05$). Serum cholesterol levels were significantly increased in fish from the higher OEO inclusion of 1.2 g/kg feed compared to the control group ($p < 0.05$; Table 6). Total protein was significantly elevated in fish from higher OEO supplementation (1.2 g/kg feed), showing 4.768 g/dL compared to fish from the control group with total protein of 4.101 g/dL (Table 6).

3.4. Fish Organ Biometric Indices Analysis. Significant variations were observed in fish organ indices such as the hepatosomatic index (HSI), stomach index (SI), gonadosomatic index (GSI), and viscerosomatic index (VSI) in fish-fed different supplementation levels of OEO. Fish-fed higher OEO diets supplements of 1.2 g/kg exhibited significantly higher HSI and VSI compared to the fish from the control group ($p < 0.05$; Table 7). The SI was significantly elevated in fish-fed higher OEO (1.2 g/kg feed), with an index value of 1.829, compared to fish from the control group with an index of 1.082 ($p < 0.05$; Table 7). Similarly, the GSI, a measure of reproductive gonadal development,

was significantly higher in fish-fed higher OEO-supplemented diets (1.2 g/kg feed) with a GSI of 2.116 compared to fish from the control group with a GSI of 1.544 ($p < 0.05$; Table 7).

3.5. Intestinal Histomorphology and Gut Health Analysis. Different supplementation levels of OEO in hybrid red tilapia diets significantly affected fish intestinal histomorphometry and gut health. The findings in Table 8 affirms that the intestinal cross-section of the fish from the control group exhibited a shorter villi length compared to fish from the higher oregano supplementation group (1.2 g/kg feed) ($p < 0.05$). This was evident across all gut regions (anterior, mid and posterior gut; Table 8). Across the three intestinal segments, villi length, width, inter-villi distance and the number of goblet cells significantly increased in fish from the higher OEO treatment group compared to the control group ($p < 0.05$; Table 8). The highest values for villi length, width, inter-villi distance (crypt) and the number of goblet cells were observed in the midgut section of the intestine of fish across all treatment groups. However, fish from higher OEO (1.2 g/kg feed) showed significantly elevated villus length, width, villus crypts and the number of goblet cells within the midsection of the intestine compared to the hind section and anterior gut regions ($p < 0.05$). Conversely, the lowest values were recorded in fish fed the control diet ($p < 0.05$; Table 8).

Inter-villi distance (crypt) (μm) was significantly elevated in the hindgut of fish across all treatment groups. Fish treated with higher OEO (1.2 g/kg feed) showed significantly higher inter-villi distance (crypt) than those in lower OEO inclusion and control treatment levels ($p < 0.05$; Table 8). The number of goblet cells was markedly higher in the foregut across all treatment groups, with fish from the higher OEO supplementation

TABLE 8: Intestinal morphometry of hybrid red tilapia-fed different oregano essential oil supplementation levels.

Items	Treatment groups (oregano EO dosage inclusion level)			
	Control TC 0.0 g/kg	TA 0.4 g/kg feed	TB 0.8 g/kg feed	TD 1.2 g/kg feed
Anterior part of intestine				
Villi length (μm)	293.857 $\pm$ 0.737 ^a	361.714 $\pm$ 7.190 ^b	442.571 $\pm$ 5.375 ^c	503.428 $\pm$ 1.913 ^d
Villi width (μm)	166.142 $\pm$ 0.799 ^c	186.285 $\pm$ 0.918 ^d	188.285 $\pm$ 1.442 ^d	195.000 $\pm$ 0.786 ^e
Inter villi distance (crypt) (μm)	118.428 $\pm$ 1.250 ^a	143.714 $\pm$ 1.614 ^b	160.857 $\pm$ 1.100 ^c	170.000 $\pm$ 0.816 ^d
Goblet cells/mm ²	5.857 $\pm$ 0.508 ^a	9.428 $\pm$ 0.649 ^b	11.428 $\pm$ 0.571 ^b	15.142 $\pm$ 0.508 ^c
Mid part of intestine				
Villi length (μm)	419.428 $\pm$ 0.972 ^a	523.142 $\pm$ 2.971 ^b	615.285 $\pm$ 2.446 ^c	709.285 $\pm$ 1.442 ^d
Villi width (μm)	109.285 $\pm$ 0.680 ^b	121.142 $\pm$ 0.911 ^c	128.857 $\pm$ 0.594 ^d	141.142 $\pm$ 0.911 ^e
Inter villi distance (crypt) (μm)	51.142 $\pm$ 1.183 ^a	78.142 $\pm$ 0.508 ^b	102.857 $\pm$ 1.223 ^d	122.428 $\pm$ 0.996 ^e
Goblet cells/mm ²	1.142 $\pm$ 0.260 ^a	2.000 $\pm$ 0.308 ^a	2.000 $\pm$ 0.308 ^a	2.857 $\pm$ 0.404 ^b
Posterior part of intestine				
Villi length (μm)	174.000 $\pm$ 1.414 ^d	215.000 $\pm$ 4.488 ^e	280.714 $\pm$ 0.680 ^f	309.714 $\pm$ 4.109 ^g
Villi width (μm)	163.571 $\pm$ 1.087 ^a	181.000 $\pm$ 0.816 ^b	191.857 $\pm$ 0.884 ^c	108.857 $\pm$ 0.911 ^d
Inter villi distance (crypt) (μm)	122.428 $\pm$ 0.996 ^a	196.428 $\pm$ 0.649 ^b	216.000 $\pm$ 1.154 ^c	229.428 $\pm$ 0.841 ^d
Goblet cells/mm ²	2.571 $\pm$ 0.368 ^a	3.714 $\pm$ 0.420 ^a	6.285 $\pm$ 0.285 ^b	7.571 $\pm$ 0.368 ^b

Note: Intestinal histometric parameter are presented in mean $\pm$ standard error (S.E) Where one-way ANOVA was significant at ($p < 0.05$), a Turkey's pairwise test was performed and means with different superscript lowercase letters in each row indicate significant differences at ($p < 0.05$).

level (1.2 g/kg feed) treatment group showing a significantly greater number of goblet cells compared to the control and other treatment groups ($p < 0.05$). The midgut exhibited the lowest number of goblet cells across all treatment groups, although fish-fed on 1.2 g/kg feed OEO still showed significantly higher goblet cell counts in this region compared to the control ($p < 0.05$).

4. Discussion

Aquaculture faces a myriad of challenges that compromise fish health and growth, ultimately reducing overall productivity [66–69]. While commercial feeds provide balanced nutrition and are widely utilised in aquaculture production, the incorporation of functional supplements has been proposed, particularly in tilapia farming, to enhance growth performance, feed efficiency and immunity in farmed fish [38, 70, 71]. In this context, phytochemical additives, including plant-based feed supplements and EOs, have gained significant attention in aquaculture due to their ability to enhance growth performance, fish digestive activities, antioxidative, immunostimulant and stress-resistance properties.

Among the plant-based feed additives commonly used in fish farming, such as *C. papaya* seeds and leaves [53, 72], Neem (*Azadirachta indica*) leaves [73, 74], soya-based oil [75], Mucuna (*Mucuna pruriens*) [76–78] and *Aloe vera* [79, 80], OEO is increasingly being used as phytochemical supplements in farmed tilapia feeds though on experimental basis. The efficacy of OEO as a feed supplement has been validated in various farmed fish species such as Zebrafish (*Danio rerio*) [81], Nile tilapia [32], rainbow trout [82] and Mozambique tilapia [27].

Previous studies on the application of OEO in Nile tilapia diets have demonstrated its beneficial effects on growth enhancement [31, 32]. Building on these findings, the present study hypothesised that dietary OEO supplementation in fish

diet could similarly improve growth performance, feed efficiency, gut morphology, reproductive health, survival and immunity of hybrid red tilapia fingerlings at varied dosage levels. The findings provide evidence supporting this hypothesis.

The results indicate that OEO positively influences the growth performance of hybrid red tilapia fingerlings, with effects becoming apparent from Week 4 of treatment in dosage dependant manner. However, the significant growth improvements in fish at higher OEO supplementation levels (0.8 and 1.2 g/kg feed) highlight OEO's potential as a growth promoter in fish. These findings are in tandem with Abdel-Latif et al. [33] on the effects of different dosage levels of OEO in common carp diet, whose effect was dosage dependent. Similarly, Singh et al. [83] and Zheng et al. [37] in their findings documented that OEO contains bioactive compounds which contains antimicrobial and antioxidant properties. These compounds were reported to enhance fish gut health by modulating gut microbiota, improving nutrient absorption and boosting immune responses, leading to better fish growth and health.

The superior growth performance of fish-fed higher OEO at 1.2 g/kg feed is an indicator as optimal dose for maximising growth in hybrid red tilapia, aligns with studies of Shourbela et al. [32], who documented similar growth enhancements pattern with plant-based OEOs in Nile tilapia where fish from the higher dosage levels of OEO showed enhanced growth performance. Our study further indicates that fish that were fed higher OEO supplementation (1.2 g/kg feed) showed improved final weights and SGR. This improvement in these growth parameters can be attributed to the bioactive compounds in OEO which enhance gut health, boost nutrient absorption and improve metabolic efficiency, hence better feed utilisation and growth. Similar finding were documented in channel catfish (*Ictalurus punctatus*), [37] Nile tilapia [32], common carps [33], salmon trout (*Salmo trutta*) and rainbow trout [36, 82]

where higher OEO inclusion levels in the diet improved SGR and AWG and FCR.

Studies have further indicated that OEO has an aromatic flavour, which has been observed to increase feed palatability among other fish species, leading to voluntary feed intake and consequently enhanced WG and SGR, especially in Nile tilapia and common carp [33, 84]. Our study further revealed similar improvement in FCR, and PER in fish fed higher OEO supplementation an indicator of better feed utilisation and efficient feed conversion into body mass by the fish.

This finding aligns with Faisal et al. [42] study in *Catla Catla*, where FCR was significantly improved in fish-fed higher OEO inclusions, hence improving growth performance. The bioactive compounds in OEO were reported to enhance growth, FCR and feed efficiency in common carp [33, 85], yellowtail tetra (*Astyanax altiparanae*) [86], livestock [26] and European sea bass (*Dicentrarchus labrax*) [31].

These studies are in tandem with our study where fish fed higher OEO had significantly enhanced growth performance, possibly due to the bioactive compounds in OEO. This study also revealed that, fish condition factor markedly improved in fish from the higher OEO supplementation group (1.2 g/kg feed) compared to the control group. This suggests improved fish health and proportionate fish growth relative to body shape changes, potentially due to energy allocation towards both growth and somatic reserves by the fish-fed a higher OEO supplementation diet. A similar observation was reported in channel catfish (*I. punctatus*)-fed higher OEO [37].

Importantly, survival rates significantly improved in fish-fed higher OEO supplementation in the diet. The high fish survival reveals the safety and palatability of OEO in red tilapia diets and the ability of OEO to enhance fish immunity and health. Improvements in feed intake and a higher survival rate have also been observed in sea bass (*Dicentrarchus labrax*)-fed diets supplemented with different OEOs [31].

OEO has been documented to improve serum biochemical composition parameters and liver enzyme activities in *Catla catla* [42], salmon trout [36], rainbow trout [82], and sea bass [31]. Our findings concur with the previous studies where there was a significant reduction in AST and ALT levels in red tilapia blood-fed higher dietary OEO supplementation (1.2 g/kg feed). This is an indication of improved liver health reduced oxidative stress and general health among fish fed a higher OEO-supplemented diet compared to fish from the control group and lower dosage levels, which recorded markedly higher AST and ALT levels in their blood. AST and ALT enzymes are markers of liver function in fish, and their decrease indicates reduced cellular damage in the liver of fish and health-enhanced liver enzyme activities [87–90]. This is likely due to the antioxidant properties of OEO compounds like carvacrol and thymol in the oil.

Similar findings were also documented in by Dokou et al. [82], where higher OEO supplementation in the diet mitigated oxidative stress in rainbow trout fish. However, a significant increase in ALP levels in fish-fed with higher OEO inclusion (1.2 g/kg feed) was observed in our study. This could be attributed to enhanced metabolic activity and bone development, as ALP is involved in phosphate metabolism and mineralisation.

This finding concurs with the previous study done by Ghafar-farsani et al. [85] in the common carp.

The study further indicates elevated levels of ALB, GLO and total protein in fish-fed higher OEO supplementation compared to those from the control group. Elevated ALB, GLO and total protein levels indicate improved protein synthesis and enhanced fish immunity and immune response and are an indication of better fish health. These findings are in tandem with previous studies that were conducted by Abdel-Latif et al. [33], Mabrok and Wahdan [91] and Shourbela et al. [32], who all documented enhanced fish immunity and gut health in fish-fed higher inclusions of OEO diets in common carp and Nile tilapia.

Higher serum cholesterol levels indicated in fish-fed higher OEO (1.2 g/kg feed) are an indication of increased lipid metabolism as an influence of oregano supplementation in the fish diet. Similar findings were documented by Carneiro et al. [38] in Neotropical catfish (*Lophiosilurus alexandri*)-fed different inclusion levels of oregano essential in the fish diet. Based on these findings, it is a proof that OEO contain bioactive compounds that enhance protein metabolism, fat absorption and utilisation in fish. Our study further indicated significant improvement in the haematological parameters of fish-fed different OEO supplements; the effects were dosage dependent. The significant improvements in haematological parameters observed in hybrid red tilapia-fed higher dietary OEO supplementation (1.2 g/kg) can be attributed to several bioactive compounds in OEO, such as carvacrol and thymol, which enhance nutrient absorption, reduce oxidative stress and stimulate haematopoiesis [27]. Elevated Hb and Hct in fish from higher OEO supplementation diets suggest improved oxygen-carrying capacity, likely due to enhanced iron absorption and utilisation, as OEO's antioxidant properties mitigate erythrocyte damage, as was documented in broilers-fed OEO-supplemented diets [92]. The study further indicate that fish-fed higher OEO showed significantly elevated MCV, MCH and MCHC compared to fish from the control groups. Increased MCV, MCH and MCHC parameters with increased OEO in the fish diet indicate better RBC maturation and Hb synthesis and oxygen transportation literally due to OEO bioactive compound role in improving dietary mineral elements such as iron bioavailability in fish blood. These findings are in line with studies done on Nile tilapia and common carp [29, 93]. The elevated levels of platelets in fish-fed higher OEO suggest enhanced fish blood clotting ability, aligning with studies showing OEO's anti-inflammatory and wound-healing properties in livestock [26]. Similarly, lymphocytes and eosinophils were significantly increased in fish from higher OEO supplementation. The boost in lymphocytes and eosinophils in fish from higher OEO supplementation reflects immune modulation, as OEO stimulates leukocyte proliferation and enhances disease resistance in gilthead seabream (*Sparus aurata*) and common carp (*C. carpio* L.) [28, 85, 94]. Similar findings were also documented in Nile tilapia-fed feed containing OEO showed improved immune responses [95, 96].

Our study further indicates that OEO significantly enhanced fish organ indices, the process that was dosage dependant. The HSI, an indirect measure of energy status due to the liver's role as an energy reserve [97, 98], significantly

improved in fish-fed higher dietary OEO inclusions. These findings contradict those of Chen et al. [99], who reported a reduction in HSI in yellow catfish fed dietary arginine but align with reports of Dinardo et al. [31] where OEO in the diet of sea bass significantly increased the liver HSI. Similarly, the SI was markedly higher in fish-fed higher OEO (1.2 g/kg feed), an indication of enhanced digestive system health and capacity, likely due to the OEO's role in stimulating digestive enzymes [85]. The GSI was significantly elevated in fish-fed higher OEO (1.2 g/kg feed). This is an indication that OEO enhances early fish gonadal development, possibly due to the antioxidant and hormonal regulation properties within the endocrine system of the fish. This finding contradicts the previous studies on the dietary supplementation of *C. papaya* seed powder in the diet of Mozambique tilapia, red tilapia and Nile tilapia, where high dosage levels of *C. papaya* seed powder significantly reduced the gonadal somatic index due to enhanced masculinisation effects, which resulted in suppression in gonadal development [100–104].

These findings are a clear indicator that OEO is best suited for fish feed supplementation in the diet of broodstock, which requires faster gonadal development and replenishment for subsequent breeding seasons, as it supports early gonad maturation and development in fish. By supporting rapid gonad development and overall health, OEO can improve the reproductive potential of broodstock fish, making them better prepared for spawning. Although no literature directly corroborates our finding of a significant, dose-dependent increase in the GSI with higher oregano supplementation as reproductive parameters, our results are consistent with the principle that dietary additives can enhance reproductive performance. This aligns with the related findings of Zou et al. [105] where fish oil supplementation improved growth and reproductive parameters in female *Haliotis discus hannai* broodstock.

Above all, the intestinal histomorphometry features of hybrid red tilapia across different OEO supplementation levels displayed increased villus height, width and the number of goblet cells within all sections of the gut system (foregut to midgut and hindgut sections of the intestinal gut system). However, the midgut displayed significantly higher proportions of both villus height and width, crypt intestinalis and the number of goblet cells across all treatment groups. These findings are in tandem with the previous studies by Heluy et al. [95] and Magouz et al. [96], who both reported increased intestinal villi length and width in Nile tilapia fed higher dietary inclusion levels of OEO. This is an indication of improved fish intestinal health, gut mucus secretion and digestive activities, which were more concentrated along the midgut of the fish across all organo-EO treatment levels.

Based on these findings, there is a significant impact of OEO inclusion on the gastrointestinal tract morphology of hybrid red tilapia, especially at higher levels. A similar finding was also documented by Trejo-Ramos et al. [106] in striped bass (*Morone saxatilis*) where higher oregano levels were reported to have improved Prokaryote Microbiota of the fish gut system. Enhanced intestinal histomorphometry features were believed to enhance food absorptive surface area and nutrient uptake capacity, which further promoted growth

due to efficiency in food assimilation by the fish body. Goblet cell density increased significantly, reflecting enhanced mucin secretion for gut protection with increased OEO in the fish diet.

According to Carneiro et al. [38], Dokou et al. [82] and Ran et al. [107], bioactive compounds in OEO, particularly carvacrol and thymol, likely contributed to epithelial proliferation, mucosal immunity and gut integrity in fish such as sea bass, rainbow trout and red tilapia. These results, coupled with our findings, reveal that OEO has a vital role in aquaculture, as it stands out as the best phytogetic plant-based EO, fish feed additive that has a myriad of functions in the fish body system hence promoting growth and fish health. Finally, the study reveals that dietary supplementation with OEO plays a significant role in enhancing fish health by promoting growth, improving feed utilisation and nutrient absorption, supporting gut health and intestinal mucosal defence mechanisms and stimulating gonad development, thereby contributing to better overall health performance in fish.

5. Conclusion and Recommendations

Based on the key findings of this study, it is evident that dietary supplementation with OEO positively influences multiple physiological parameters in hybrid red tilapia. The effectiveness of OEO as a feed additive for sustainable aquaculture depends on its inclusion level. This study demonstrated that higher supplementation (1.2 g/kg feed) significantly enhanced growth performance, FCR, PER, intestinal histomorphology and survival rates. At this level, HSI, GSI and VSI indices were also elevated, highlighting OEO's role in promoting both overall health and reproductive organ development. These metabolic and physiological effects are likely attributable to its major bioactive compounds, carvacrol and thymol, which possess immunostimulant, antioxidant, anti-inflammatory and immune-modulating properties.

The findings suggest that incorporating OEO at 1.2 g/kg feed in red tilapia diets can improve nutrient absorption, feed utilisation, growth and reproductive performance, supporting its use as a natural alternative to synthetic antibiotics and chemotherapeutics. Due to its gonad-stimulating effects, OEO is particularly recommended for broodstock diets even though no previous studies corroborate this finding. Importantly, its phytochemical nature and local availability make it a practical substitute for conventional fish oils, with potential application across both intensive and extensive production systems. Although this study focused on hybrid red tilapia, evidence from other fish species indicates similar beneficial effects, revealing OEO's wider applicability in aquaculture. A dosage level of 1.2 g/kg feed is therefore recommended for hybrid red tilapia. Further research should evaluate long-term impacts of OEO at levels beyond 1.2 g/kg and investigate potential ecological effects on aquatic biodiversity and ecosystem health.

6. Limitations of the Study

This study was conducted exclusively on hybrid red tilapia, which limits the generalisability of the findings to other fish species, particularly non-cichlids such as cyprinids and crustaceans. Further research is, therefore, required to validate the

effects of OEO in diverse aquatic species, especially with respect to gonadal development. The experiment was also restricted to a 13-week period, and long-term trials are necessary to evaluate the sustained effects of OEO supplementation on growth, reproduction and overall fish health across the full production cycle up to harvest. Although OEO demonstrated clear benefits, the study did not include a cost–benefit analysis of its dietary inclusion in commercial feeds, leaving a gap in assessing its economic feasibility for large-scale aquaculture. Moreover, potential environmental impacts of OEO discharge through fish excreta or uneaten feed were not examined. Future studies should therefore investigate the ecological footprint of OEO supplementation to ensure its sustainable use in aquaculture.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

The study protocol for handling fish in this research was ethically approved by the Institutional Animal Ethics Committee, Department of Biotechnology, Cochin University of Science and Technology, Kerala, India (Ethical Approval Number: 363/GO/Re/S/01/CCSEA/90). The research adhered to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Disclosure

All the authors have accepted the final version of this manuscript. Following this assistance, the authors have carefully reviewed and further edited the content as needed, taking full responsibility for the final publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Fredrick Juma Syanya, Megha Lovejan and A.R. Nikhila Khanna: conceptualisation, data methodology, image analysis, data curation, analysis, writing – original draft. **Harikrishnan Mahadevan:** supervision, guiding formal data validation and final editing and review. **Zachariy Oreko Winam and Paul Mumina:** reviewing, manuscript preparation, data analysis, proofreading and final editing.

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