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Growth performance, biochemical parameters, and digestive enzymes in common carp (*Cyprinus carpio*) fed experimental diets supplemented with vitamin C, thyme essential oil, and quercetin

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ABSTRACT

Herbal additives and vitamins have gained considerable attention to improve fish health. This study investigates the effects of vitamin C (VC), *Thymus vulgaris* L. essential oil (TE), and quercetin (QR) supplementation on growth performance, digestive enzyme, body composition, and biochemical parameters of common carp (*Cyprinus carpio*). Four hundred and twenty fish weighing 20.46 ± 0.07 g were randomly divided into seven experimental treatments in triplicates. Experimental diets were containing as T1 (0, control), T2 (500 mg/kg VC), T3 (1000 mg/kg VC), T4 (1% TE), T5 (2% TE), T6 (200 mg/kg QR), and T7 (800 mg/kg QR). Fish were fed 3% of body weight daily for 60 days. According to the results, the groups fed with experimental diets showed the higher final weight, weight gain (WG), specific growth rate (SGR), and survival rate (SR) and lower feed conversion ratio (FCR) compared to the control group ($p < .05$). Regarding biochemical indices results, T5, T6, and T7 significantly had the higher serum total protein (TP) than the control ($p < .05$). Meanwhile, albumin (ALB) showed no significant difference in all groups ($p > .05$). All the supplemented groups were found to have significantly lower creatinine (CRT), glucose (GLU), and urea (UR) and higher globulin (GLO) content compare to the control ($p < .05$). Moreover, T3, T4, and T5 showed a significant decrease in triglyceride (TRIG) levels compared to the control ($p < .05$). Cholesterol (CHOL) activity in T4 supplemented group was significantly lower than the control ($p < .05$). Also, cortisol (CORT) recorded a significant decrease in T6 and T7 compared to the control ($p < .05$). Lactate dehydrogenase (LDH) had a significantly lower level in T4, T6, and T7 compared to the control ($p < .05$). The whole fish body composition was not affected by the feed additives and the control ($p > .05$). Besides, significant enhancements were observed in cases of intestine protease, amylase, and lipase enzymes in all the supplemented groups compared to the control ($p < .05$). In conclusion, the present results demonstrated that VC, TE, and QR could effectively improve survival, growth performance, and biochemical indices in *C. carpio*.

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Introduction

The rapid expansion of modern aquaculture due to the global population growth has resulted in the occurrence of immune depression and outbreaks of infectious diseases (Gobi et al. 2017). Accordingly, several bacterial, parasitic, fungal, and viral diseases, as well as harmful environmental conditions, make fish more vulnerable and exclude aquaculture industry development (Devi et al. 2019; Al-Mofarji et al. 2021). In this regard, the overuse of antibiotics and chemotherapeutics in

disease prevention and growth promotion not only caused consequent detrimental effects on aquatic animals and environmental health but also led to the promotion of drug-resistant pathogen and the accumulation of toxic residues (Carnevali et al. 2014; Ahmadifar et al. 2019; El Basuini et al. 2020, 2021).

Immunostimulants are known as an ideal alternative to antibiotics and chemotherapeutics (Nawaz et al. 2018). Several natural or synthetic immunostimulant compounds improve diseases resistance of aquatic

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animals by enhancing the innate immune system (Harikrishnan et al. 2020). The natural, safe, and cost-effective food supplements as eco-friendly treatments (Harikrishnan et al. 2021) must be considered to apply in aquaculture to regulate the immune system (Abdel-Tawwab et al. 2020; Santhosh and Umesh 2021).

Vitamins are recommended to be used as supplements in the diets of many aquatic animals. Vitamin C (VC, ascorbic acid), a water-soluble vitamin, has vital roles in immunomodulatory and antioxidant properties (Chen et al. 2015), which prevent lipoproteins peroxidation, reduces harmful oxidants in the stomach, and boosts iron absorption (Darias et al. 2011; Liu et al. 2011), may reduce the risk of cancer. Most aquatic organisms cannot synthesise ascorbic acid (El Basuini et al. 2021) from D-glucose due to the absence of gluconolacton oxidase enzyme (Fonseca et al. 2013). Ascorbic acid deficiency developed growth depression, anorexia, anaemia, scoliosis, lordosis, and immunity depression (Tewary and Patra 2008; Xiao et al. 2010). Therefore, constant supplies of VC are required in the fish diet to elevate growth and strengthen the immune system function of fish (Chen et al. 2015; Asaikkutti et al. 2016; Koshio and Angeles 2017).

Due to modern trends, considerable interest has been reported towards the administration of medicinal plants as an immunostimulant and growth enhancer in the fish diet (Van Hai 2015; Abarike 2020; Reverter et al. 2021). Besides, they are biodegradable without any environmental hazard compared to synthetic agents (Kostaki et al. 2009; Van Hai 2015). Phytochemical studies have shown that depending on the herbal bioactive compounds, different modes of action and microbial targets are noticed (Haute et al. 2016; Hoseinifar et al. 2019). *Thymus vulgaris* L. (thyme), which belongs to the Lamiaceae family, is an aromatic herb (Lee et al. 2005). The antimicrobial capacity of thyme essential oil (TE) is based on phenolic compounds (terpenes) (Benavides et al. 2020). Carvacrol and thymol are the most active constituents of TEs (Kostaki et al. 2009) and applied as affordable antibacterial, antispasmodic, antifungal, and antioxidant attributes (Rota et al. 2008; Kostaki et al. 2009; Kykkidou et al. 2009; El-nekeety et al. 2011). Recent investigations have considered the effects of thyme on an innate and acquired immunity in rainbow trout (*Oncorhynchus mykiss*) (Ghafarifarsani et al. 2021), common carp (*Cyprinus carpio*) (Mohiseni et al. 2019), and sea bass (*Dicentrarchus labrax*) (Kostaki et al. 2009).

Moreover, several epidemiological research has found that flavonoids, such as the flavonol quercetin (QR), have a broad range of beneficial antioxidant and

antibacterial activities (Review 2014). It accumulated and maintained in the body more quickly than other flavonoids (Choi et al. 2003). QR hinders mammalian tumour expansion and carcinogen, also induces apoptosis (Weber et al. 2002). QR is found in a most edible variety of food and feed plants (Luehring et al. 2011). Although the biological effects of QR have investigated in a limited number of species like rainbow trout (*Salmo gairdneri*) (Plakas 1985), medaka (*Oryzias latipes*) (Weber et al. 2002), and pigs (Luehring et al. 2011), there are a few investigations about its nutritional role in fish biochemical parameters.

As an important economically freshwater fish species, common carp (*Cyprinus carpio*) is the main farmed species worldwide (Modanloo et al. 2017). Thus, the present study was conducted to assess the comparison of the efficiency of VC, TE, and QR on growth, digestive enzyme, and some biochemical parameters in *C. carpio*.

Material and methods

Experimental animals and conditions

A total of 420 healthy common carp of average body weight of 20.46 ± 0.07 g (Mean \pm SE) obtained from a local fish farm in Karaj, Iran. The fish was stocked in a private farm. Fish had randomly distributed into 21 tank indoor cylindrical polyethylene tanks of 300-l containing 200l well water. Fish were fed with the commercial diet for 14 days to acclimatise to experimental conditions. Fish health status visually checked their physical appearance, normal colouration, and their movements all over the body and fins. Then, the experiment started with seven experimental groups, each treatment repeated in triplicates, with a density of 20 fish per tank. The water quality parameters were measured regularly. All the experimental tanks contained aerated freshwater through sea star aquarium purification filter (HX-1180F2) (pH 7.32 ± 0.68 , dissolved oxygen level 6.58 ± 0.46 mg/l and temperature 23.5 ± 1.18 °C). Constant aeration was supplied with air-stones connected to an air pump. Daily changing water was about 50% (Ahmadifar et al. 2019; Mohammadi et al. 2020). The light regime was set at 12 h light: 12 h darkness. Fish fed the experimental diet for 60 days.

Diet preparation and feeding trial

Basal diet formulation is shown in Table 1, which used as a control. Three experimental diets formulated by supplementation of the VC, TE, and QR. First, a basal

diet with two levels of VC (ACROS, USA, purity: 99%) as a powder, in the form of L-ascorbyl-2-polyphosphate (T2 and T3 containing 500 and 1000 mg/kg). Other experimental groups received TE supplementation (Maleki Commercial Company, Fars, Iran; Table 2) dissolved in methanol solution (Ghafariarsani et al. 2021) and sprayed on the diets (T4 with 1% TE and T5 with 2% TE). Then, the diets dried at room temperature in the darkness. Besides, QR (> 95% purity, Sigma Chemical Co., USA) supplementation was achieved by manually mixing the adequate amount of QR (T6 containing 200 and T7 containing 800 mg/kg) into the meals.

Such concentrations were chosen based on previous studies using these dietary supplementations for other fish species (El-nekeety et al 2011; Abdel Rahman et al 2018; Wang et al 2020). During the period, the diets were stored in sealed plastic bags at 4 °C until use. Fish were hand-fed two times daily, 3% of body weight. Based on regular biometry (every two weeks), the feeding ratio was corrected. Utmost care was taken to avoid feed loss. However, uneaten food and faeces were removed by siphoning.

Sample collection

Twenty-four hours starved fish anaesthetised (100 mg/L eugenol) randomly taken by scoop-net at the end of the feeding trial. Blood was withdrawn from the caudal vein using 2-mL syringes and collected in plastic Eppendorf tubes. Serum samples were separated at 1600 g for 10 min centrifugation and stored at -20 °C for further analysis. To measure the activity of the digestive enzymes, (amylase, protease, and lipase) anaesthetised fish killed. Then, the intestine tissues were dissected and removed. Samples homogenised

Table 1. Analysis of the commercial feed for *C. carpio* (Faradaneh Co. Shahrekord, Iran).

Analyses	Composition
Crude protein	37%
Crude lipid	6%
Crude fibre	6%
Digestible phosphorus	1.25%
Moisture	7%

Table 2. Chemical composition of the essential oil of *Tymus vulgaris*.

Compound name	Percentage
Thymol	37–55
Carvacrol	0.5–5.5
p- Cymene	14–28
γ- Terpinene	4–12
Linalol	1.5–6.5
β- Myrcene	1–3
α- Terpinene	0.9–2.6

by adding cold saline solution (0.85% NaCl) and centrifuged at 9300 x g under 4 °C for 20 min. The resulting supernatant was used for estimation (Asaikkutti et al. 2016) and the supernatant was then kept at 4 °C for assessing digestive enzyme activities. For proximate body composition analysis, fish bodies were frozen, and later frozen-dried, ground, and the whole homogenised bodies were used.

Analytical methods

Growth performance

The weight and length of each fish were measured separately. Survival rate and food index parameters calculated using the following equations:

$$\text{Weight gain (WG; g)} = \text{final body weight} - \text{initial body weight}$$

$$\text{Feed conversion rate (FCR)}$$

$$= \text{feed intake (g)/weight gain (g)}$$

$$\text{Specific Growth Rate (SGR; \% / day)}$$

$$= [(\ln (\text{final body weight}) -$$

$$\ln (\text{initial body weight})) / \text{trial period}] \times 100$$

$$\text{Survival rate (SR; \%)} = (\text{final number of fish} / \text{initial number of fish}) \times 100$$

Body composition assay

The fish's whole body was analysed for moisture, protein, lipid, and ash according to the standard procedures AOAC (1995). Moisture analysed by oven drying at 110 °C to constant weight, crude protein (N × 6.25) was measured by the Kjeldahl method after acid digestion, crude lipid was determined by the ether-extraction method using a Soxtec System HT (HT6, Tecator, Sweden), and ash was acid-digested by combustion in Muffle furnace at 550 °C for 4 h.

Digestive enzyme analysis

Intestine amylase, protease, and lipase were determined according to Zamani et al. (2009). The total protein determined by the Bradford method (Bradford et al. 1976).

Biochemical analysis

Total protein (TP), albumin (ALB), globulin (GLO), glucose (GLU), creatinine (CRT), cholesterol (CHOL), triglyceride (TRIG), cortisol (CRT), urea (UR), and lactate dehydrogenase (LDH) measured using an automatic biochemical analyser (Roche Hitachi 911 Chemistry Analyser, Tokyo, Japan) with Commercial kits (Pars

Azmun Co., Tehran, Iran) according to Yarahmadi et al. (2016).

Statistical analysis

Normality and homogeneity of variance were checked with the Kolmogorov–Smirnov test. Statistical analysis performed using SPSS software version no. 20.00 (SPSS Inc., Chicago, IL, USA) and represents the mean \pm SE (standard error). Differences in parameters processed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test considering $p < .05$ as the significance level.

Results

Growth performance

The results of growth performance indices and survival rate after feeding for 60 days are summarised in Table 3. There was no significant difference ($p > .05$) for initial weight (IW) in fish treatments. On the other hand, the value of the final weight (FW) and WG were significantly higher in fish fed experimental diets than fish fed in the control group (T1) ($p < .05$). Meanwhile, no significant differences were found among fish fed experimental treatments ($p > .05$). The FCR both in the control group and T2 were recorded significantly higher than the other groups ($p < .05$). There were no significant differences noticed between other treatments. FCR in the control treatment was significantly higher compared with T2 ($p < .05$). Although the SGR revealed no significant differences among treated fish ($p > .05$), the control group significantly had the lowest SGR ($p < .05$). Moreover, the SR was recorded 100% in those fish who received experimental

treatments, and the control group had the lowest SR significantly ($p < .05$).

Body composition assay

The results of the proximate body composition of the *C. carpio* are presented in Table 4. Compared with the control group, no significant effects were observed on the content of moisture, crude protein, crude lipid, and ash in the whole fish body among the VC, TE, and QR supplementary groups ($p > .05$).

Digestive enzyme analysis

As shown in Figure 1, the mean digestive enzyme activities of all treatment groups were significantly increased than the control ($p < .05$). The average values of amylase activity were observed to significantly increase in all the experimental groups than the control group ($p < .05$). As for the lipase activity, assays showed a significant increase in fish-fed supplemented diets as compared with the control ($p < .05$), although there were no significant differences detected among tested groups ($p > .05$). Furthermore, protease activity was significantly lower in fish fed the basal diet (T1) compared to the other treated groups ($p < .05$).

Biochemical analysis

According to Table 5, the serum TP levels were significantly higher in the T5, T6, and T7 compared to the control group ($p < .05$), other treatments (T2, T3, and T4) had shown no significant difference compared to the control treatment (T1) ($p > .05$). Serum ALB content remains unaffected by experimental diets ($p > .05$). Serum GLO assessment showed a significant increase in all dietary groups compared to the T1

Table 3. Growth performance of *C. carpio* fed different experimental diets.

Parameters	T1(control)	T2	T3	T4	T5	T6	T7
IW (g)	20.51 \pm 0.19	20.53 \pm 0.24	20.42 \pm 0.14	20.48 \pm 0.18	20.61 \pm 0.26	20.35 \pm 0.21	20.31 \pm 0.23
FW (g)	50.06 \pm 0.79 ^b	57.66 \pm 0.83 ^a	59.95 \pm 0.93 ^a	59.15 \pm 0.60 ^a	61.34 \pm 1.01 ^a	60.56 \pm 1.23 ^a	61.26 \pm 0.55 ^a
WG (g)	29.55 \pm 0.62 ^b	37.12 \pm 1.05 ^a	39.53 \pm 0.78 ^a	38.67 \pm 0.76 ^a	40.73 \pm 0.90 ^a	40.20 \pm 1.03 ^a	40.95 \pm 0.36 ^a
FCR	1.98 \pm 0.026 ^a	1.88 \pm 0.012 ^b	1.81 \pm 0.011 ^c	1.79 \pm 0.012 ^c	1.75 \pm 0.010 ^c	1.78 \pm 0.008 ^c	1.76 \pm 0.008 ^c
SGR (% d ⁻¹)	1.48 \pm 0.013 ^c	1.72 \pm 0.042 ^b	1.79 \pm 0.013 ^{ab}	1.76 \pm 0.030 ^{ab}	1.81 \pm 0.024 ^{ab}	1.81 \pm 0.019 ^{ab}	1.83 \pm 0.009 ^a
SR (%)	95.66 \pm 0.33 ^b	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a

IW: initial weight; FW: final weight; WG: weight gain; FCR: feed conversion ratio; SGR: specific growth rate; SR: survival rate. T1: control; T2: 500 mg/kg VC; T3: 1000 mg/kg VC; T4: 1% TE; T5: 2% TE; T6: 200 mg/kg QR; T7: 800 mg/kg QR.

Different letters (a–c) in the same row indicate significant differences ($p < 0.05$). Data represent as mean \pm SE of triplicate observations.

Table 4. Body composition of *C. carpio* fed different experimental diets.

Parameters	T1(control)	T2	T3	T4	T5	T6	T7
Moisture	69.49 \pm 0.31	70.63 \pm 1.03	68.97 \pm 0.58	69.09 \pm 0.53	69.26 \pm 1.06	70.26 \pm 0.38	70.21 \pm 0.52
Crude protein	15.05 \pm 0.37	16.03 \pm 0.15	16.28 \pm 0.22	15.65 \pm 0.57	16.23 \pm 0.34	15.20 \pm 0.63	15.77 \pm 0.32
Crude lipid	4.53 \pm 0.11	4.24 \pm 0.12	4.21 \pm 0.12	4.35 \pm 0.19	4.08 \pm 0.08	4.24 \pm 0.15	4.19 \pm 0.21
Ash	3.21 \pm 0.13	3.32 \pm 0.16	3.29 \pm 0.15	3.30 \pm 0.17	3.40 \pm 0.26	3.29 \pm 0.18	3.42 \pm 0.12

T1: control; T2: 500 mg/kg VC; T3: 1000 mg/kg VC; T4: 1% TE; T5: 2% TE; T6: 200 mg/kg QR; T7: 800 mg/kg QR.

Data represent as mean \pm SE of triplicate observations, $n = 3$.

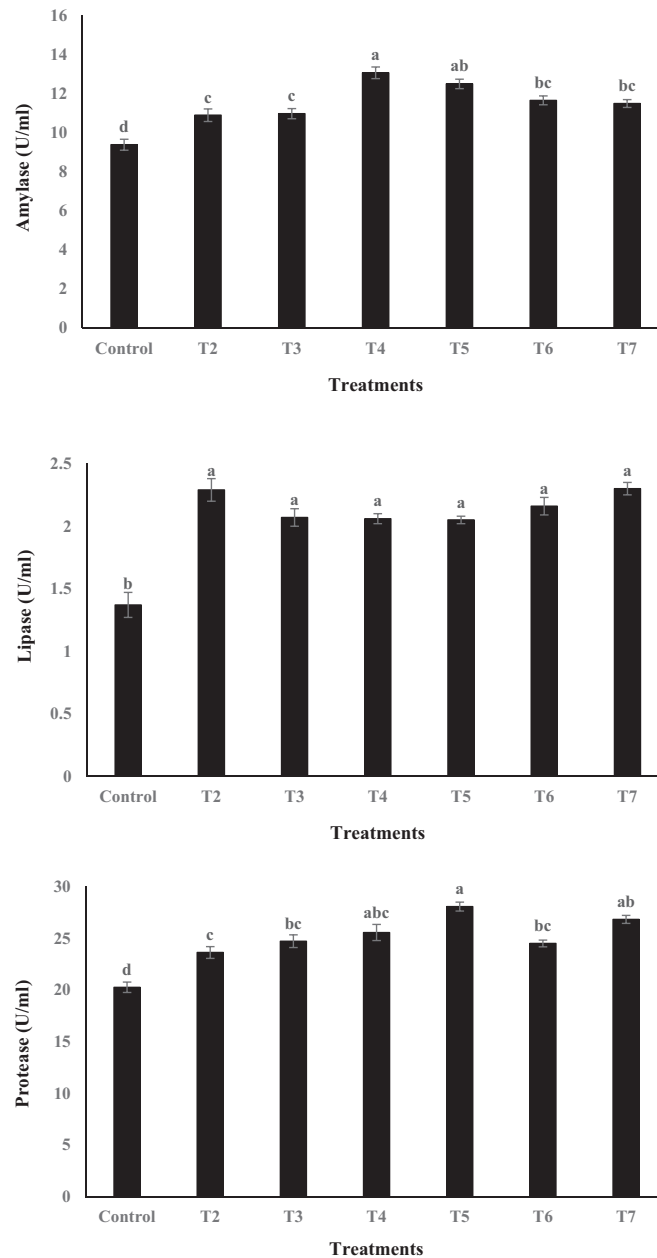


Figure 1. Digestive enzymes of *C. carpio* fed different experimental diets. T1: control; T2: 500 mg/kg VC; T3: 1000 mg/kg VC; T4: 1% TE; T5: 2% TE; T6: 200 mg/kg QR; T7: 800 mg/kg QR. Data represent as mean \pm SE of triplicate observations, $n = 3$. Different letters (a–d) in the same row indicate significant differences ($p < .05$).

Table 5. Biochemical parameters of *C. carpio* fed different experimental diets.

Parameters	T1(control)	T2	T3	T4	T5	T6	T7
TP (g/dL)	1.88 \pm 0.02 ^b	2.05 \pm 0.03 ^{ab}	2.04 \pm 0.03 ^{ab}	2.05 \pm 0.03 ^{ab}	2.08 \pm 0.04 ^a	2.10 \pm 0.04 ^a	2.07 \pm 0.04 ^a
ALB (g/dL)	1.20 \pm 0.01 ^a	1.26 \pm 0.01 ^a	1.28 \pm 0.03 ^a	1.27 \pm 0.02 ^a	1.29 \pm 0.03 ^a	1.29 \pm 0.02 ^a	1.27 \pm 0.03 ^a
GLO (g/dL)	0.67 \pm 0.01 ^b	0.78 \pm 0.01 ^a	0.76 \pm 0.02 ^a	0.77 \pm 0.01 ^a	0.79 \pm 0.01 ^a	0.81 \pm 0.01 ^a	0.79 \pm 0.01 ^a
TRIG (mg/dL)	166.26 \pm 3.01 ^a	152.66 \pm 3.38 ^{ab}	150.08 \pm 2.05 ^b	149.12 \pm 3.11 ^b	141.79 \pm 2.99 ^b	153.54 \pm 2.71 ^{ab}	153.65 \pm 3.80 ^{ab}
CHOL (mg/dL)	225.59 \pm 4.31 ^a	207.56 \pm 3.74 ^{ab}	208.52 \pm 4.39 ^{ab}	198.12 \pm 6.07 ^b	205.39 \pm 6.55 ^{ab}	205.94 \pm 3.99 ^{ab}	203.69 \pm 5.93 ^{ab}
GLU (mg/dL)	95.10 \pm 2.10 ^a	77.17 \pm 2.09 ^b	78.72 \pm 2.66 ^b	78.54 \pm 2.81 ^b	78.77 \pm 4.31 ^b	79.80 \pm 2.73 ^b	78.48 \pm 3.66 ^b
CORT(nmol/L)	64.87 \pm 1.71 ^a	55.99 \pm 1.70 ^{ab}	54.27 \pm 1.66 ^{ab}	54.55 \pm 2.58 ^{ab}	54.12 \pm 2.86 ^{ab}	52.87 \pm 2.89 ^b	53.70 \pm 1.88 ^b
CRT (mg/dL)	0.88 \pm 0.02 ^a	0.68 \pm 0.02 ^b	0.71 \pm 0.03 ^b	0.61 \pm 0.02 ^b	0.62 \pm 0.03 ^b	0.66 \pm 0.02 ^b	0.65 \pm 0.03 ^b
UR (mg/dL)	1.42 \pm 0.04 ^a	1.24 \pm 0.02 ^b	1.20 \pm 0.02 ^{bc}	1.09 \pm 0.02 ^{bcd}	1.11 \pm 0.01 ^{bcd}	1.05 \pm 0.03 ^{cd}	1.03 \pm 0.04 ^d
LDH (U/L)	142.15 \pm 2.76 ^a	130.49 \pm 2.38 ^{ab}	131.83 \pm 2.27 ^{ab}	124.73 \pm 2.30 ^b	131.69 \pm 2.73 ^{ab}	119.98 \pm 3.32 ^b	121.59 \pm 2.92 ^b

TP: total protein; ALB: albumin; GLO: globulin; TRIG: triglyceride; CHOL: cholesterol; GLU: glucose; CORT: cortisol; CRT: creatinine; UR: urea; LDH: lactate dehydrogenase. T1: control; T2: 500 mg/kg VC; T3: 1000 mg/kg VC; T4: 1% TE; T5: 2% TE; T6: 200 mg/kg QR; T7: 800 mg/kg QR.

Data represent as mean \pm SE of triplicate observations, $n = 3$. Different letters (a–d) in the same row indicate significant differences ($p < 0.05$).

($p < .05$); however, no significant differences were observed among all dietary groups ($p > .05$). Results showed that serum TRIG levels in T3, T4, and T5 were significantly lower than the control group ($p < .05$). There were no significant differences between other treatments (T2, T6, and T7) and the control group ($p > .05$). All experimental groups, except T4, were not modified in CHOL level by the tested diet ($p > .05$). Determination of serum GLU levels showed significant decrease values in fish-fed experimental diets as compared to the control ($p < .05$), whereas no statistical variations were recorded among the fish fed experimental groups ($p > .05$). Significantly higher CORT values were found in T6 and T7 treatments compared with the control groups ($p < .05$); however, there were no significant differences between other treatments (T2, T3, T4, and T5) than the control ($p > .05$). Besides, results exhibited a significantly lower amount of UR and CRT in fish-fed supplementation diets than in the control group ($p < .05$). Fish treated with T4, T6, and T7 have significantly lower LDH activity than T1 ($p < .05$); moreover, other dietary groups (T2, T3, and T5) have shown no significant differences than T1 ($p > .05$).

Discussion

The current study compared the effects of herbal and vitamin additives. The available results demonstrated beneficial properties in improving the growth performance, digestive enzymes as well as biochemical parameters in *C. carpio* organisms.

Supplementation of *C. carpio* diet with VC, TE, and QR improved FW, WG, FCR, and SGR. Moreover, there were no mortalities or abnormalities among various experimental groups that received the experimental diet. These findings are in accordance with the results presented VC on Nile tilapia (*Oreochromis niloticus*) (Abdel Rahman et al. 2018; El Basuini et al. 2021), and large yellow croaker (*Pseudosciaena crocea*) (Ai et al. 2006), and herbal immunostimulant diets such as thyme on rainbow trout (*O. mykiss*) (Ahmadifar et al. 2011; Sönmez et al. 2015), Starry sturgeon (*Acipenser stellatus*) (Dorojan et al. 2015), common carp (*C. carpio*) (Mohiseni et al. 2019), and also QR on Olive Flounder (*Paralichthys olivaceus*) (Kim et al. 2015), blunt snout bream (*Megalobrama amblycephala*) (Jia et al. 2019), and grass carp (*Ctenopharyngodon idella*) (Xu et al. 2019). However, no significant changes were found in WG and SGR of Nile tilapia fed thyme powder (Khalil et al. 2020). Also, some studies reported no significant effects of QR on the growth performance of

Olive Flounder (Xu et al. 2019) and tilapia (Zhai and Liu 2013). The effects of phytobiotics on growth performance indices might depend on several factors. For example, animals have different susceptibility and tolerance to dietary flavonoids (Xu et al. 2019). VC makes a great surface area in intestinal villi and goblet cells (Abdel Rahman et al. 2018). Also, VC has an essential role in metabolising lipid, protein, and carbohydrate (Liu et al. 2011; Asaikkutti et al. 2016). Although the growth-promoting mechanism of QR is still unclear (Xu et al. 2019), it changes into aglycone form in the body with pharmacological effects after absorption (386 and enhances intestinal enzyme activity (Xu et al. 2019). Besides, TE with stimulating the secretion of pancreatic enzymes can improve feed digestibility (El-Ghousein and Al-Beitawi 2009; Mohiseni et al. 2019; Xu et al. 2019). The digestive enzyme evaluation (protease, amylase, and lipase) confirm this hypothesis. Furthermore, Phytotherapy improves the digestibility and absorption process (Xu et al. 2019), gut morphology, microbial community (Yousefi et al. 2021), and overall metabolic processes. Moreover, reducing the impact of undesirable bacteria (Khalil et al. 2020) and aid gain more nutrients in fish (Ahmadifar et al. 2011). However, future studies need to examine this option.

Our results demonstrated that no significant differences in body composition notice in supplementary groups, which is in agreement with the levels of proximate body composition of tilapia fed QR and freshwater prawn (*Macrobrachium malcolmsonii*) fed diets containing VC (Asaikkutti et al. 2016). In contrast, Liu et al. (2011) recorded body protein and lipid contents increased significantly in *C. carpio* fed with VC (Liu et al. 2011). Furthermore, an increase in protein and lipid and a decrease in moisture of Starry sturgeon fed thyme (1%) and vitamin E (500 mg/kg) as dietaries was recorded (Dorojan et al. 2015). Meanwhile, studies recorded that QR affected lipid metabolism-related gene expression in the liver (Zhai and Liu 2013). Also, VC plays an essential role in protein and lipid metabolism (Awad et al. 2013). Carvacol and thymol direct diet energy towards protein production and cause higher protein sedimentation (Zheng et al. 2009). Additionally, herbal additives modulate the secretion of pancreatic enzymes, the main factors in nutrient digestion and assimilation, lead to increase muscle protein (Yılmaz 2012). Various parameters like dose and type of plants, agronomic, fish species, and age can change fish body composition.

Analysis of digestive enzyme content can provide information on the potential effects of nutrients on digestive function and nutritional absorption (Javahery

et al. 2019). Medicinal plants and vitamins make a chance to improve overall body metabolism, growth rate, and feed utilisation. According to the results, amylase, lipase, and protease levels increase in all the experimental groups. Similarly, digestive enzyme activity improved in Nile tilapia treated with thyme powder (Khalil et al. 2020) as well as *M. malcolmsonii* (Asaikkutti et al. 2016) and Jian carp (*C. carpio*) fed VC (Liu et al. 2011). There are a few studies analyses the influence of QR on the *C. carpio* digestive enzyme. Liu et al. reported that the reason VC increases the digestive enzyme values may be due to the role of VC to act as an extracellular scavenger. This might prevent lipid peroxidation in the hepatopancreas (Liu et al. 2011). Besides, VC improves the intestinal microflora population, and the number of intestinal villi and goblet cells (Abdel et al. 2018). Carvacrol and thymol as active constituents of TE were reported to have beneficial effects on the nutritional digestibility, activity of digestive enzymes, gut microbiota, and reducing the impact of undesirable bacteria (Khalil et al. 2020). More production of endogenous enzymes increases cholecystokinin and exocrine pancreatic secretion. These changes modulate digestive physiology and promote digestion and absorption of feed and supplements in the host (Li et al. 2009; Dong et al. 2018). TE as an herbal additive, QR as flavonoid, and VC might improve the growth performance and overall health with modulating digestive enzymes by improving gut microbiota (Khalil et al. 2020), increasing the energy required for nutrients digestion, and development of digestive organs like brush borders to make the greater surface area (Liu et al. 2011).

Orally functional feed additives, like herbal products and vitamins, commonly well known as fish performance and health improvement by modulating metabolic or endocrine pathways. The evaluation of biochemical blood indicators emphasises physiological condition, health status, reaction to external stimuli and stressors, and disease resistance (El Basuini et al. 2020).

TP is an important indicator for fish health, nutritional state, and liver function (Dorojan et al. 2015). Fish fed with a high level of TE (T5) and QR supplements (T6 and T7) had higher TP levels. TP elevation may due to improving liver and other organs functions, which synthesised serum protein (Metwally 2009) as well as the contribution of important liver defense protein molecules (Keiko et al. 2015; Hoseini and Yousefi 2019) and antibodies (Devi et al. 2019) like agglutinins, lecithins, and immunoglobulins which are important defense molecules. Therefore, An

increase in TP level improves fish innate immunity and stress-reducing (Keiko et al. 2015). Likewise, rainbow trout fed basal diet incorporated with thyme extract (Hoseini and Yousefi 2019) and QR showed TP elevation (Awad et al. 2013).

ALB and GLO are two important parts of total protein (Dorojan et al. 2015) and the main resource for immunoglobulins production (Ahmadifar et al. 2019). ALB manages lipids transportation and general metabolism (Dorojan et al. 2015). In the present study, with any supplementation diet, the ALB ratio did not change in *C. carpio*. In contrast, some studies recorded enhanced ALB level with medicinal plants such as sweet orange (*Citrus sinensis*) oil extract and green tea (*Camellia sinensis*) leaves powder both in Nile tilapia (Kuebutornye and Abarike 2020) as well as thyme oils in rainbow trout (Ghafariarsani et al. 2021). Different fish species, ages, sex, and health condition are also responsible for these differences.

Values of serum GLO in fish treated with supplementations were significantly higher than its content in the control group. The same results observed in juvenile tilapia fed with ginseng herb (*Panax quinquefolius*) and TE supplementation (Kuebutornye and Abarike 2020) as well as broiler chickens fed TE diet (El-Ghousein and Al-Beitawi 2009). Meanwhile, Dorojan et al. (2015) recorded no significant effect on GLO levels in Starry sturgeon fed with QR diet (Dorojan et al. 2015).

The most important function of TRIG is to store and provide primary metabolic and cellular energy (Pourmozaffar et al. 2018). Evaluation of TRIG reflects nutritional status and lipid metabolism (El Basuini et al. 2020). As a result, serum TRIG decreased significantly after feeding VC (T3) and TE diet (T4 and T5). Carvacrol in TE can elevate the emulsification of lipids. Therefore it facilitates lipid absorption into the blood (Mohiseni et al. 2019). Meanwhile, the reduced lipid content may store more energy for a higher metabolic rate, lead to better growth performance (Xu et al. 2019), and avoiding fatty liver disease (Pês et al. 2016). The same findings were reported for other animal species, including a decreasing trend in Nile tilapia fed dietary coenzyme Q10 with VC (El et al. 2021) and broiler chickens fed dietary TE (El-Ghousein and Al-Beitawi 2009). Also, in this study, no significant difference was observed in QR treatments, which was inconsistent with the result observed in silver catfish (*Rhamdia quelen*) (Pês et al. 2016).

CHOL is a major structural component of biomembrane, the outer layer of serum lipoproteins (Dorojan et al. 2015), and precursor of steroid hormones

(Pourmozaffar et al. 2018). A significant difference in CHOL concentration was found only between the control group and the highest level of dietary TE (T4). This may be linked to the hypocholesterolemia impact of TE at this dose which reflects fish health improvement. Also, thymol and carvacol might limit the HMG-CoA reductase responsible for CHOL biosynthesis (El-Ghousein and Al-Beitawi 2009). Meanwhile, Hayek et al. (1997) reported that QR bounds to low-density lipoprotein hinder its oxidation and decrease serum CHOL value in mice (Hayek et al. 1997). Moreover, herbal compounds like phytosterols facilitate lipid metabolism by transcription and inhibiting apolipoprotein secretion as well as sterol biosynthesis in hepatocytes (Dossou et al. 2018; Kesbiç et al. 2020). Our findings are supported by some literature, El-Ghousein and Al-Beitawi (2009) reported that TE (0.5, 1, 1.5, and 2%) as an immunostimulant substance could adversely affect CHOL levels (El-Ghousein and Al-Beitawi 2009). Similarly, no changes in CHOL level recorded for red sea bream (*Pagrus major*) followed by treatment with VC (Dawood et al. 2017) and silver catfish with QR dietary. Therefore, natural plant extracts, including essential oils, may prevent the accumulation of fatty liver disease in fish (Fonseca et al. 2013).

In this study, GLU stress indicator hormone, decreases in all the experimental groups compared to the control. VC might stimulate hypoglycaemic hormone (insulin) in the pancreas and reduce GLU uptake (El Basuini et al. 2020; Al-Obaidi et al. 2021; El et al. 2021). Also, TE proved to decrease the effects of stress factors (Gulec et al. 2013). Moreover, QR can inhibit GLU absorption in adipocytes (Jia et al. 2019). In stress conditions, hypothalamus–pituitary–interrenal (HPI) stimulates (Hoseini and Yousefi 2019), and the levels of catecholamines, corticosteroid, and hyperglycaemia increase (Yousefi et al. 2019). Also, GLU elevates through either glycogenolysis (breakdown of glycogen to glucose) or gluconeogenesis (break down of proteins to glucose) to make energy (Mohammadi et al. 2020). Therefore, in this study, GLU reduction showed that these supplements did not cause stress. The same results were observed in the Nile tilapia fed dietary VC (El et al. 2021), and rainbow trout fed TE levels (Hoseini and Yousefi 2019). However, QR had no significant effect on blood GLU level on blunt snout bream (*M. amblycephala*) (Jia et al. 2019).

CORT, a stress-related marker, participates in fish growth and metabolism (Pês et al. 2016). The present study showed a decrease in CORT in experimental treatments, but only QR treatments (T6 and T7) showed significant differences. QR affects a

hypothalamic-pituitary-adrenal axis, which decreases stress by reducing CORT activity and elevating hypoglycaemic hormone (insulin) (El Basuini et al. 2020). Therefore, QR can protect organisms against environmental stress (Park et al. 2010). Also, VC prevents steroidogenesis, related to cortisol production during stress (Ai et al. 2006). Likewise, there was no significant difference in serum CORT levels between the control and TE treatments in rainbow trout, recorded by Hoseini and Yousefi (2019). Moreover, Park et al. (2010) found that the serum CORT concentrations in Olive Flounder were significantly lower after treatment with QR (Park et al. 2010).

UR and CRT use as an indicator of gill and kidney functions (Yılmaz 2012). The CRT prevents nitrogenous waste and renal disease (Yang and Chen 2003), and provides amino acid requirements, also helps feed utilisation in fish (Yılmaz 2012). In this study, CRT decreased in fish fed all the experimental diets. Nevertheless, Yılmaz et al. (2012) found that herbal supplements like TE, rosemary (*Rosmarinus officinalis*), and fenugreek (*Trigonella foenum graecum*) did not change serum CRT in sea bass (*D. labrax*) (Yılmaz 2012). Also, Shirazi thyme (*Zataria multiflora Boiss*) showed no effects on CRT levels in *C. carpio* (Mohiseni et al. 2019). These differences may depend on some factors like stress, sex, herbal and fish species, and environmental conditions.

Blood UR is a nitrogen metabolic product of protein catabolism (Yang and Chen 2003). In the present study, fish fed the basal diet had the most UR level among treatments, indicating fish fed nutritional additive have more efficient nitrogen utilisation and better performance. In contrast, some research recorded no significant differences in UR levels, such as Starry sturgeon fed with TE (Dorojan et al. 2015) and silver catfish fed QR supplementation (Pês et al. 2016).

LDH plays an important role in anaerobic glycolysis (Yang et al. 2019) and conversion of lactate to pyruvate and NAD⁺ to NADH (Modanloo et al. 2017). The data showed that serum LDH concentrations decline in fish fed TE (T4) and QR (T6 and T7) than in control fish. In contrast to our study, some research recorded no significant differences in *C. carpio* fed TE (Mohiseni et al. 2019) and silver catfish fed QR (Pês et al. 2016). The differences might cause by species, dietary lipid level, sources, and experimental duration. Less information is available regarding the role of VC/TE/QR on CRT, UR, and LDH levels.

Conclusion

In conclusion, the present findings proved VC, TE, and QR supplementation not only provided better

serum biochemical parameters but also improved growth indices. Moreover, oral administration of optimal concentration of VC, TE, and QR which enhance fish performance, is recommended. Studies on the effects of these compounds in *C. carpio* immunity are scarce; therefore, more investigations are required to determine fish treated tolerance against infections.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Disclosure statement

The authors declare that they have no conflict of interest.

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Data availability statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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