



COMBINED EFFECTS OF BUTYL HYDROXYTOLUENE AND VITAMIN C ON THE GROWTH PERFORMANCE, BLOOD BIOCHEMISTRY, AND ANTIOXIDATIVE STATUS OF COMMON CARP (*CYPRINUS CARPIO*)

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Abstract

Common carp are proper finfish species for intensification, though their feeds should be reinforced with functional additives to ensure high growth performance and health status. Thus, this study tested the combined effects of butyl hydroxytoluene (BHT) and vitamin C (VC) on the growth performance, blood biomarkers, and antioxidative capacity of common carp. Fish were allotted into four groups and fed on four diets where the control diet was free of VC or/and BHT, while the second and third diets contained dietary VC or BHT at 500 or 150 mg/kg, respectively. The fourth diet was supplemented with both VC (500 mg/kg) and BHT (150 mg/kg) (BHT+VC). After 70 days, feeding VC or/and BHT markedly affected the growth performance of common carp. Carps fed VC, BHT, or BHT+VC had higher ($P<0.05$) FBW, WG, and SGR than fish fed the control diet, while the FCR was lower ($P<0.05$) in fish fed both VC and BHT than the control. In addition, feeding BHT or both BHT+VC markedly ($P<0.05$) reduced the AST, ALT, and urea. The blood protein, albumin, and globulin were higher ($P<0.05$) in fish fed VC or/and BHT than in the control. Fish fed dietary VC, or BHT+VC, had lower ($P<0.05$) total cholesterol (T-CHO) and triglycerides (TG) than those fed on the control or BHT. The SOD, CAT, and GPX were significantly higher ($P<0.05$) in carp fed on dietary VC or/and BHT than in fish fed on the control diet. Nevertheless, the malondialdehyde level was lower ($P<0.05$) in carp fed on VC or BHT+VC diets than fish on the control or BHT diets. The lysozyme activity was significantly higher ($P<0.05$) in carp fed on the BHT+VC diet than in the control or VC. In conclusion, the study suggests that dietary BHT or/and VC can be added to the diets of common carp to enhance the growth performance, feed digestion, blood biomarkers, and antioxidative capacity.

Key words: antioxidants, aquaculture, functional feed, immunostimulants, production

The aquaculture sector is defined nowadays as the “Blue Revolution” which essentially secures nutritious food and profitable business (FAO, 2022). Several hazards can limit aquaculture development to meet humanity’s seafood needs, such as low water quality, high temperature, and infectious diseases (Mook et al., 2012; Parvathy et al., 2023). In addition, aquafeed quality is assured by essential nutrients such as proteins, lipids, carbohydrates, minerals, vitamins, and functional additives (Schumann and Brinker, 2020; Somdare et al., 2023). Feed additives are increasingly applied in aquaculture because of their beneficial roles in the health status, productivity, and welfare of aquatic animals (Midhun and Arun, 2023; Ng et al., 2009). More specifically, feed additives are required to enhance fish health and well-being by modulating digestion capacity, activating immunity, enhancing antioxidative response, and combating harmful invaders (Ahmadniaye Motlagh et al., 2020; Emam et

al., 2022; Orso et al., 2022). Several natural and synthetic additives were recently included in aquafeed, such as antioxidants, immunostimulants, medicinal herbs, and probiotics (Bai et al., 2022; Yousefi et al., 2023). These additives could enhance feed utilization, intestinal health, metabolism, physiological behavior, antioxidative capacity, immunity, and resistance to biotic and abiotic stressors (García Beltrán and Esteban, 2022).

Butyl hydroxytoluene (BHT) is a synthetic antioxidant commonly used to prepare fat-rich materials, including aquafeed formulation (Yehye et al., 2015). Dietary BHT reduces oxidation caused by free radicals, thereby increasing the feed shelf-life. It is a lipophilic phenolic substance with anti-inflammatory, antiviral, and antibacterial effects (Lee et al., 2023; Sarmah et al., 2020). Further, BHT improves the lipid metabolism, odor, and flavor of feeds, leading to high-quality and efficient utilization (Aramli et al., 2014; Felter et al., 2021). The Eu-

ropean Union (EU) permitted BHT addition at 150 mg/kg in animal feeds, while overdosing may lead to toxicological impacts (EFSA, 2022). Several reports evaluated the addition of dietary BHT in aquaculture, where Merino et al. (2020) stated that dietary BHT enhanced sperm function and inhibited oxidative stress in coho salmon (*Oncorhynchus kisutch*). Further, Yu et al. (2016, 2018) illustrated that dietary BHT enhanced lipid metabolism and antioxidative capacity in largemouth bass (*Micropterus salmoides*). In common carp (*Cyprinus carpio*), dietary BHT did not affect the growth performance but severely deteriorated the hepatic tissue (Hwang et al., 1991). Therefore, in the current study, adding other functional additives may enhance the efficacy of BHT on the performances of common carp.

Vitamins are crucially needed for nutritionally balanced aquafeed (Dawood and Koshio, 2018). In this context, dietary vitamin C is known for its natural antioxidative capacity and several other biological and physiological roles (Darias et al., 2011). Vitamin C is water-soluble and contributes to forming proteins, hormones, collagen, and immune cells (Ai et al., 2006). The lack of exogenous supplementation of vitamin C induces severe immune and physiological disruption, resulting in low growth performance and high infection capability (Shahkar et al., 2015). The addition of vitamin C is tested in most of the farmed fish species, and their species-specific needs were determined (Liu et al., 2022). Vitamin C is recommended at 100 to 1000 mg/kg to enhance finfish species' growth performance and well-being depending on species, fish size, vitamin form, and feed formulation (NRC, 2011).

Common carp is a widely distributed finfish species with high commercial value (Chen et al., 2022; Hoseini et al., 2023). Therefore, feeds of farmed common carp should be fortified with functional additives to ensure high growth performance and health status under farming conditions (Kari et al., 2022). In this regard, dietary BHT and vitamin C have adequate antioxidative, metabolic, and physiological roles and can mitigate the impacts of lipid peroxidation that may occur in fish feed (Lee et al., 2023; Paduraru et al., 2021). Further, it has been illustrated that synergistic mixtures of feed additives cause influential roles compared to individual additions of feed additives in aquaculture (Gasco et al., 2018; Taherpour et al., 2023). Thus, the present study tested the combined effects of BHT and vitamin C on growth performance, blood biomarkers, and antioxidative capacity in common carp.

Material and methods

Test diets

Four diets were formulated to satisfy the nutritional requirements of common carp and contain dietary vitamin C (VC) or/and butylated hydroxytoluene (BHT) (Table 1). The basal diet contains fish meal, soybean meal, yellow corn, rice bran, corn gluten, wheat bran, and wheat middling as sources of proteins, lipids, and carbo-

hydrates. These ingredients were mixed gently with the minerals and vitamins. At the same time, VC (L-ascorbic acid, Sigma-Aldrich) and BHT (Free Trade Company, Al-Beheira, Egypt) were mixed with fish oil added to the dry ingredients. The control diet is free of VC or/and BHT, while the second and third diets contain dietary VC or BHT at 500 or 150 mg/kg, respectively. The fourth diet was supplemented with both VC (500 mg/kg) and BHT (150 mg/kg) (BHT+VC). The levels of VC and BHT were determined by following the recommendations of Ghafarifarسانی et al. (2022) and Hajirezaee et al. (2020) for VC and EFSA (2022) for BHT, respectively. Subsequently, all ingredients were well mixed, and water was added before pelletizing in the lab pelletizing machine. Pellets of 2–3 mm were produced, dried at room temperature, and kept in plastic bags until used in the feeding trial. The chemical composition of the diets was checked by using the standard method (AOAC, 2012).

Table 1. Composition of the basal diet and the chemical analysis

Ingredients (%)	Control	VC	BHT	BHT+VC
Fish meal (65% cp)	10	10	10	10
Soybean meal (44% cp)	35	35	35	35
Yellow corn	15	15	15	15
Corn gluten	7	7	7	7
Wheat bran	10	10	10	10
Rice bran	10	10	10	10
Wheat middling	5	4.95	4.985	4.935
Fish oil	5	5	5	5
Vitamin and mineral mix ¹	2	2	2	2
Dicalcium phosphate	1	1	1	1
Vitamin C (VC)	0	0.05	0	0.05
Butylated hydroxytoluene (BHT)	0	0	0.015	0.015
Total (%)	100	100	100	100
Chemical composition				
Crude protein (%)	30.11	30.09	30.14	30.19
Crude lipids (%)	7.55	7.43	7.38	7.52
Ash (%)	4.52	4.71	4.44	4.23
Fibers (%)	3.4	3.32	3.43	3.61
Gross energy (KJ/kg) ²	19.45	19.40	19.42	19.46

¹The mixture of vitamins and minerals is detailed by Dawood et al. (2020).

²Gross energy was calculated based on the values of protein, lipid, and carbohydrate as 23.6, 39.5, and 17.2 KJ/g, respectively.

Experimental conditions

Common carp juveniles were obtained from a private hatchery in Kafr El-Sheikh governorate (Baltim International Highway, El-Borsa area) and then gently transported to Laboratories and Greenhouses for the Faculties of Agriculture and Veterinary Medicine, Kafrelsheikh University, Egypt. Fish were then moved to 200 L plastic tanks full of fresh dechlorinated water and enriched with continuous aeration. Fish were acclimatized for two

weeks and then allocated to twelve glass aquaria (80 L). Each aquarium was stocked with fifteen fish with an initial weight of 4.25 ± 0.17 g/fish. The trial was conducted with four groups, with three aquaria for each group (triplicates). All aquaria were provided with continuous aeration, and the water was exchanged daily at 50% with new fresh dechlorinated water. Fish were offered their respective test diets up to apparent satiation level twice daily (08:00 h and 15:00 h). Thirty minutes later, the glass aquaria were checked visually to confirm the consumption of the offered feed. If any feed wastage was seen, considering that some fish may later expel pellets from their mouths, especially when they become full, it was collected and dried, then subtracted from the total feed intake in each aquarium to have the actual feed intake. The consumed feed amount was recorded in each aquarium to calculate the feed conversion ratio (FCR), protein efficiency ratio (PER), and feed intake. Fish were kept under the ordinary dark and light regime (12 h: 12 h), and the water quality was checked during the trial. The average values for the water temperature, dissolved oxygen, pH, and ammonia were 27°C, 5.63 mg/L, 8.17, and 0.03 mg/L, respectively.

Final sampling

The trial was terminated after 70 days, and all fish were starved for 24 hours before the sampling day. The following equations were used to calculate the weight gain (WG), specific growth rate (SGR), survival rate, FCR, PER, and feed intake.

$$WG = 100 \times (\text{final weight (FW, g)} - \text{initial weight (IW, g)}) / IW \text{ (g)},$$

$$SGR (\%/day) = 100 \times (\ln FW \text{ (g)} - \ln IW \text{ (g)}) / \text{days},$$

$$FCR = \text{total dry feed intake (g)} / (FW \text{ (g)} - IW \text{ (g)}),$$

$$PER = (FW \text{ (g)} - IW \text{ (g)}) / \text{dry protein intake (g)},$$

$$\text{Survival (\%)} = 100 \times \text{final fish number} / \text{initial fish number}.$$

Then, fish were anesthetized with 100 mg/L tricaine methane sulfonate (Sigma-Aldrich™), and blood was collected from 3 fish/aquarium using 2.5 ml gauge syringes from the caudal vein. The blood was kept in non-heparinized tubes for serum collection. After 2 hours, blood samples were centrifuged at $1008 \times g$ for 15 min at 4°C; then, serum was separated and kept at -20°C for further analysis. Moreover, three fish per aquarium were collected, washed with fresh water, and kept in the freezer (-20°C) until used for the carcass composition analysis.

Chemical analysis

The chemical composition of the test diets and the whole fish body was determined by following the standard method of AOAC (2012). In brief, the moisture content was detected using oven-drying at 110°C while the ash samples were detected using the muffle furnace at 550°C for 6 hours. The Kjeldahl method was used to analyze the crude protein content. In addition, the Soxhlet extraction method was applied to measure the crude lipids in the test diets and the whole fish body. The crude fiber in the test diets was analyzed by digestion of feed

samples for 15 minutes in a solution of 5% sulfuric acid and 5% sodium hydroxide.

Blood analysis

1. Blood biochemistry

Serum total proteins and albumins were determined according to Doumas et al. (1981) and Doumas and Biggs (1972), while globulin content was calculated mathematically. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, triglycerides, creatinine, and urea were detected by RA-50 chemistry analyzer (Bayer Diagnostics Manufacturing Ltd., Dublin, Ireland) using readymade chemicals (kits) supplied by Spinreact Co. (Girona, Spain), following the manufacturer's instructions.

2. Antioxidative capacity

Serum malondialdehyde (MDA) levels were determined by using the thiobarbituric acid method and were calorimetrically clarified using a commercial kit (LPO-586 Kit, Oxis International Inc., Portland, USA) as described by Ohkawa et al. (1979). The serum samples were used to detect superoxide dismutase (SOD) (McCord and Fridovich, 1969) and catalase (CAT) (Aebi, 1984) activities using colorimetric methods by commercial fish-specific kits (Biodiagnostic Co., Giza, Egypt).

3. Lysozyme activity

Serum lysozyme activity was determined using turbidimetric assay, according to the method described by Ellis (1990) based on the lysis of *Micrococcus lysodeikticus* (Sigma, USA). Briefly, a standard suspension of 0.15 mg/mL of *M. lysodeikticus* was prepared in 66 mM phosphate buffer (pH 6.0). Serum (50 µL) was added to 1 mL of the bacterial suspension, and the absorbance reduction was recorded at 30-s and 4.5-min intervals at 450 nm using a spectrophotometer (Shimadzu UV-1600PC). One unit of lysozyme was defined as a reduction in absorbance of 0.001/min.

Statistical analysis

Shapiro-Wilk and Levene tests confirmed normal distribution and homogeneity of variance. The obtained data were subjected to one-way ANOVA. Differences between means were tested at $P < 0.05$ level using the Duncan post-hoc test. All the statistical analyses were done via SPSS version 22 (SPSS Inc., IL, USA).

Results

Growth performance

Feeding VC or/and BHT markedly affected the growth performance of common carp (Table 2). The final weight (FBW), weight gain (WG), and specific growth rate (SGR) were meaningfully increased ($P < 0.05$) in fish fed VC or/and BHT-supplemented diets. In contrast, the feed conversion ratio (FCR) was decreased ($P < 0.05$) by dietary VC or/and BHT. The survival rate was 100% for all groups during the trial.

Carcass composition

The carcass composition of common carp fed dietary VC or/and BHT is presented in Table 3. The results illustrated that dietary VC or/and BHT has no marked effects on the moisture, protein, lipid, and ash contents ($P>0.05$). The moisture content was 76.69 ± 0.06 – $78.94\pm0.12\%$, while the protein content was 12.40 ± 0.03 – $13.52\pm0.02\%$. Besides, the lipid content was 5.62 ± 0.05 – $6.79\pm0.02\%$, while ash content was 2.43 ± 0.01 – $2.53\pm0.03\%$.

Blood biochemistry

The blood biochemical traits are shown in Table 4. Feeding BHT or both BHT+VC markedly ($P<0.05$) reduced the AST and ALT. Carps fed BHT, or both BHT+VC, had lower ($P<0.05$) AST than those fed the control or VC, and fish fed both BHT+VC had the lowest AST among the groups. Further, those fed BHT, or both BHT+VC, had lower ($P<0.05$) ALT than those fed the control. The blood protein, albumin, and globulin were higher ($P<0.05$) in fish fed VC or/and BHT than in the control. Fish fed dietary VC, or BHT+VC, had lower ($P<0.05$) total cholesterol (T-CHO) and triglycerides (TG) than those fed on the

control or BHT. The T-CHO was lower ($P<0.05$) in carps fed both BHT+VC than those fed VC only. Besides, the urea level was lower ($P<0.05$) in carps fed both VC or/and BHT than in the control. The results also show that dietary VC or/and BHT did not ($P>0.05$) affect the creatinine levels in the blood.

Antioxidative and immune responses

The antioxidative responses (SOD, GPX, and CAT), malondialdehyde (MDA) level, and lysozyme activity are presented in Table 5. The SOD was significantly higher ($P<0.05$) in carp fed on dietary VC or/and BHT than in fish fed on the control diet. In addition, the CAT was higher ($P<0.05$) in carp fed on BHT or BHT+VC diets than in the control or VC diets. The GPX was higher ($P<0.05$) in carp fed on dietary VC or/and BHT than the control, with the highest being in fish fed on dietary BHT. Nevertheless, the MDA level was lower ($P<0.05$) in carp fed on VC or BHT+VC diets than fish on the control or BHT diets. The lysozyme activity was significantly higher ($P<0.05$) in carp fed on the BHT+VC diet than the control or VC without marked differences ($P>0.05$) with BHT.

Table 2. Growth performance of common carp fed butyl hydroxytoluene or/and vitamin C for 70 days

Item	Control	VC	BHT	BHT+VC
IBW (g)	4.17 $\pm$ 0.10	4.18 $\pm$ 0.07	4.16 $\pm$ 0.14	4.33 $\pm$ 0.05
FBW (g)	18.80 $\pm$ 0.10 b	20.17 $\pm$ 0.10 a	20.50 $\pm$ 0.10 a	20.89 $\pm$ 0.11 a
WG %	351.66 $\pm$ 10.15 b	384.41 $\pm$ 8.88 a	392.63 $\pm$ 13.70 a	382.05 $\pm$ 2.57 a
SGR (%/day)	2.15 $\pm$ 0.03 b	2.25 $\pm$ 0.03 a	2.28 $\pm$ 0.04 a	2.24 $\pm$ 0.01 a
FCR	1.32 $\pm$ 0.02 a	1.25 $\pm$ 0.02 ab	1.26 $\pm$ 0.02 ab	1.21 $\pm$ 0.02 b
PER	1.68 $\pm$ 0.02	1.87 $\pm$ 0.02	1.89 $\pm$ 0.02	1.90 $\pm$ 0.02
Survival (%)	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00

Means $\pm$ SE ($n = 3$) with different letters in the same row show significant differences ($P<0.05$). IBW: initial body weight, FBW: final body weight, WG: weight gain, SGR: specific growth rate, FCR: feed conversion ratio, PER: protein efficiency ratio.

Table 3. Carcass composition of common carp fed on butyl hydroxytoluene or/and vitamin C for 70 days

Item	Control	VC	BHT	BHT+VC
Moisture (%)	76.86 $\pm$ 0.11	77.84 $\pm$ 0.08	76.69 $\pm$ 0.06	78.94 $\pm$ 0.12
Crude protein (%)	13.52 $\pm$ 0.02	13.48 $\pm$ 0.05	13.46 $\pm$ 0.24	12.40 $\pm$ 0.03
Ether extract (%)	6.47 $\pm$ 0.01	6.55 $\pm$ 0.05	6.79 $\pm$ 0.02	5.62 $\pm$ 0.05
Ash (%)	2.44 $\pm$ 0.02	2.45 $\pm$ 0.01	2.53 $\pm$ 0.03	2.43 $\pm$ 0.01

Means $\pm$ SE ($n = 3$). The absence of letters indicates no significant differences among the groups ($P>0.05$).

Table 4. Blood biochemical traits of common carp fed butyl hydroxytoluene or/and vitamin C for 70 days

Item	Control	VC	BHT	BHT+VC
ALT (U/l)	7.50 $\pm$ 0.87 a	6.50 $\pm$ 0.87 b	6.10 $\pm$ 0.06 b	6.33 $\pm$ 0.67 b
AST (U/l)	110.00 $\pm$ 2.89 a	114.00 $\pm$ 2.89 a	101.00 $\pm$ 9.24 b	77.67 $\pm$ 10.09 c
Total protein (g/dl)	3.20 $\pm$ 0.12 c	3.65 $\pm$ 0.03 b	3.80 $\pm$ 0.17 a	3.90 $\pm$ 0.25 a
Albumin (g/dl)	1.10 $\pm$ 0.05 b	1.05 $\pm$ 0.01 b	1.12 $\pm$ 0.04 a	1.32 $\pm$ 0.05 a
Globulin (g/dl)	2.10 $\pm$ 0.07 b	2.61 $\pm$ 0.04 a	2.69 $\pm$ 0.21 a	2.58 $\pm$ 0.21 a
Creatinine (mg/dl)	0.62 $\pm$ 0.01	0.64 $\pm$ 0.01	0.64 $\pm$ 0.03	0.60 $\pm$ 0.08
Urea (mg/dl)	9.00 $\pm$ 0.58 a	8.50 $\pm$ 0.29 b	8.00 $\pm$ 0.00 bc	7.83 $\pm$ 0.17 c
T-CHO (mg/dl)	152.00 $\pm$ 2.89 a	106.00 $\pm$ 6.35 b	148.50 $\pm$ 2.60 a	73.33 $\pm$ 5.36 c
TG (mg/dl)	240.50 $\pm$ 6.64 a	227.00 $\pm$ 9.81 b	262.50 $\pm$ 6.06 a	220.00 $\pm$ 12.17 b

Means $\pm$ SE ($n = 3$) with different letters in the same row show significant differences ($P<0.05$). ALT: alanine aminotransferase, AST: aspartate aminotransferase, T-CHO: total cholesterol, TG: triglycerides.

Table 5. Immunity and antioxidative traits of common carp fed butyl hydroxytoluene or/and vitamin C for 70 days

Item	Control	VC	BHT	BHT+VC
Superoxide dismutase (IU/L)	43.14±1.65 b	53.31±0.51 a	55.03±0.59 a	55.83±5.30 a
Catalase (IU/L)	56.64±3.24 b	56.40±3.34 b	70.80±1.13 a	69.83±4.95 a
Glutathione peroxidase (IU/L)	48.24±1.29 c	53.00±2.66 b	59.90±3.12 a	52.39±4.28 b
Malondialdehyde (nmol/mL)	6.39±0.32 a	5.07±0.39 b	6.95±1.25 a	5.86±0.27 b
Lysozyme activity (unit/ml)	30.51±0.38 b	31.25±1.20 b	32.79±1.53 ab	34.45±1.25 a

Means ± SE (n = 3) with different letters in the same row show significant differences (P<0.05).

Discussion

Aquafeed is susceptible to oxidation, which may occur during processing and storage (Sarmah et al., 2020). Subsequently, physical and chemical deteriorations affect the quality of formulated feeds and pellets, leading to low feed digestibility and consumption by fish (Rana et al., 2023). Therefore, it is necessary to prevent oxidation to guarantee high-quality feed and healthy fish (Ar-amli et al., 2014; Felter et al., 2021). Dietary butyl hydroxytoluene has been approved as a powerful synthetic antioxidant agent for high-quality feeds (EFSA, 2022; Fasihnia et al., 2020). In addition, vitamin C is recognized as a functional supplement known for its beneficial metabolic, physiological, and antioxidative roles (Darias et al., 2011). Therefore, the study hypothesized that BHT and vitamin C mixture could synergistically enhance the growth performance, blood biomarkers, and antioxidative capacity of common carp.

In this study, dietary BHT or/and vitamin C improved growth performance in common carp. Similarly, Yu et al. (2016, 2018) indicated that largemouth bass showed enhanced growth performance when fed BHT. Dietary BHT is an active antioxidant agent that inhibits oxidation in processed feed, ensuring high-quality feed and better feed efficiency (Yehye et al., 2015). Further, Wu et al. (2022) and Nasar et al. (2021) stated that gibel carp (*Carassius gibelio*) and grass carp (*Ctenopharyngodon idella*) fed vitamin C showed improved growth performance. Similar results were seen by Kaur et al. (2022), who stated enhanced growth performance in Amur carp (*Cyprinus carpio haematopterus*) fed dietary vitamin C. Vitamin C has a growth promotion role due to its role in the synthesized collagen involved in tissue formation (Tewary and Patra, 2008). Further, regulated metabolic and physiological functions resulting from vitamin C feeding may explain the enhanced growth performance in common carp (Chen et al., 2015). In addition to the role of BHT as an antioxidative agent, vitamin C also has a decisive antioxidative and immunomodulatory role, which causes healthy intestinal immunity (Dawood, 2021) and, thereby, efficient digestion. Enhanced growth performance by vitamin C can also be related to improved feed efficiency, which is reflected by improved FCR. Indeed, common carp fed BHT or/and vitamin C

showed enhanced FCR, referring to efficient feed utilization and absorption.

Blood biomarkers are usually applied to detect the feed digestion capacity in relation to fish's metabolic and physiological status (Seibel et al., 2021). The measurement of urea and creatinine levels in the blood can show the renal function and, thereby, the fish's health status and well-being (Adeshina and Abdel-Tawwab, 2021). Creatinine is a byproduct of muscle metabolism, and overproduction of creatinine released by the kidneys indicates renal malfunction (Kashani et al., 2020). In this study, no significant effects for BHT or/and vitamin C were observed on the creatinine levels, referring to the proper elimination of creatinine from the bloodstream in common carp. In addition, protein metabolism results in urea formation in the liver and excessive urea levels excreted by the kidneys (Lyman, 1986). The results demonstrated increased levels of urea in fish fed BHT or/and vitamin C-free diet (control), which indicated compromised renal function compared to common carp fed BHT or/and vitamin C. However, common carp fed dietary VC or/and BHT revealed increased total proteins, albumin, and globulin levels compared to the control. Indeed, both VC and BHT can effectively regulate the metabolic function in the animal's entire body. The results are in line with Yu et al. (2016), who stated that BHT did not affect the creatinine level in largemouth bass. Nevertheless, Yu et al. (2016) also reported reduced AST levels in largemouth bass fed dietary BHT, which agrees with our study. The low secretion levels of AST and ALT can be related to the positive role of dietary BHT or vitamin C in regulating fish's metabolic and physiological functions (Ai et al., 2006). In addition, the reduction in AST and ALT levels indicates healthy fish, regulated liver-related function secretions, and a lack of stress (Polakof et al., 2012). It is known that fish secrete high AST and ALT levels during stress as an indicator of liver function failure (López-Olmeda et al., 2009). The formation of protein in serum is one of the roles of the liver, which means that liver failure causes a low protein synthesis. However, in the current study, the levels of proteins, albumin, and globulin increased by BHT or/and vitamin C. Similar results were seen by Kaur et al. (2022), who increased blood protein, albumin, and globulin in Amur carp fed dietary vitamin C. This can be explained by the lack of liver fail-

ure and thereby regulated protein, albumin, and globulin secretions. In this context, the reduction of ALT and AST can also be related to the lack of liver damage since a damaged liver led to high secretion of ALT and AST (Vali et al., 2020). The results indicated a reduction in the T-CHO and TG in common carp fed BHT or/and vitamin C, which is in line with Yu et al. (2016), who indicated a reduction of T-CHO and TG in largemouth bass-fed BHT. Lowered T-CHO and TG in common carp can be explained by the role of BHT and vitamin C in regulating lipid metabolism and deposition while reducing fat levels in the blood and maintaining the entire body's health.

The deteriorated feed quality is a major cause of oxidative stress in finfish species (Birnie-Gauvin et al., 2017). During feed processing and stocking, there is a possibility of overoxidation involved in the oxidation of feed, thereby impairing feed utilization and antioxidative defenses (Aramli et al., 2014; Felter et al., 2021). Therefore, suitable antioxidative responses have evolved to defend against the overproduction of free radicals (ROS) (Santana et al., 2022). The continuous production of ROS results in lipid peroxidation, which can be expressed by the formation of malondialdehyde (MDA) (Garcia et al., 2020). The release of SOD, GPX, and CAT is developed when the MDA level surpasses the fish's antioxidative capacity (Shahidi and Ambigaipalan, 2015). In the current study, the activities of SOD, GPX, and CAT were increased while the level of MDA decreased in common carp fed BHT or/and vitamin C. In parallel, Yu et al. (2016) reported that largemouth bass fed dietary BHT had improved antioxidative response and low MDA levels. Further, common carp (Ghafarifarsani et al., 2022), Gibel carp (Wu et al., 2022), and grass carp (Nasar et al., 2021) fed dietary vitamin C had improved antioxidative capacity. Indeed, BHT addition is suggested mainly due to the substantial antioxidative role associated with the inhibition of ROS formation (Sarmah et al., 2020). Besides, the influential role of vitamin C as ROS scavenger reduces lipid peroxidation and activates the antioxidative capacity (Otten et al., 2021; Xu et al., 2022). In other words, vitamin C or BHT can deactivate the hazards of free radicals through electron transfer, then rapid and reversible deprotonation, leading to a robust antioxidative response (Yehye et al., 2015). The influential role of BHT or/and vitamin C as antioxidants in common carp may explain the enhanced growth performance, regulated blood biomarkers, and reduced blood lipids.

It is recommended to ensure aquafeed quality by fortifying suitable feed additives such as BHT or/and vitamin C to avoid oxidative stress and, thereby, impairment of immunity (Dawood et al., 2018). In this study, dietary BHT or/and vitamin C enhanced the lysozyme activity in common carp. Lysozyme activity protects against infectious bacteria by breaking the bacterial cells and inhibiting their harmful secretion in fish (Gannam and Schrock, 1999). Similar improvements in lysozyme activity were also observed in yellow catfish (*Pelteobagrus fulvidraco*) (Liang et al., 2017) and common carp (Ghafarifarsani et

al., 2022) fed dietary vitamin C. However, to the authors' knowledge, no studies investigated the role of BHT in activating lysozyme activity. Enhanced lysozyme activity in common carp confirms the protective role of BHT or/and vitamin C, which inhibit the attack of free radicals on the immune cells, thereby keeping fish immunized. Although the study lacks the pathogenic challenge, the enhanced antioxidative and lysozyme activities suggest that both BHT or/and vitamin C may contribute to the protection against invaders that may infect common carp during the farming season.

Conclusion

In summary, the study suggests that dietary BHT and vitamin C showed synergistic beneficial effects when added to the diets of common carp. Markedly, common carp fed dietary BHT and vitamin C exhibited high resistance against oxidative stress that may occur during the processing and storage of feed. Subsequently, fish revealed improved growth performance, feed digestion, blood biomarkers, and antioxidative capacity. Therefore, adequate mixtures of feed additives would be a potential strategy for ensuring the high productivity and well-being of common carp. However, future studies are suggested to better understand the mechanism of dietary BHT and vitamin C in improving the growth performance and health status of common carp.

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