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Original Article

Influence of dietary fat sources on growth, bacterial resistance, and antioxidant ability of liver in common carp, *Cyprinus carpio*

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Abstract: The current study was conducted to determine the effect of dietary fat sources on fish growth, resistance, and antioxidant ability in common carp. The experimental diets were based on various fat sources, including fish oil (FO), linseed oil (LO), sesame oil (SO), and a mixture of LO and SO (SLO). The common carp (23.8±0.7 g) were distributed into a 100 L-tank system at a density of 25 fish per tank. Fish were fed experimental feed to satiation for seven weeks, and the consumable feed amount was recorded daily. After a 7-week trial, fish were infected with *Aeromonas veronii* at a dose of 0.43×10^6 CFU/mL and monitored for 14 days. The fish mortality was checked daily. Fish livers were sampled after feeding and on the second day post-bacterial injection to analyze the antioxidant parameters. The results indicated that the fat sources did not affect the fish growth, feed conversion rate, survival, and cumulative mortality in the challenge test but modified the antioxidant ability in fish liver. The malondialdehyde activity in SLO-fed fish was lower than that in FO group at the end of the feeding trial, while the glutathione activity in SO-fed fish was higher than those in other plant oil-fed fish after the bacterial challenge. The highest values of superoxide dismutase activity were recorded in LO fish after the nutritional trial and FO ones after the challenge test.

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Introduction

Lipid is one of the principal sources of nutrients in aquatic feeds. They have many roles, such as providing energy, participating in the cellular structure, dissolving vitamins, and joining the immune system (Alabdulkarim et al., 2012; Guo et al., 2020). Among their structural components, fatty acids are important molecules that play many essential functions in living organisms. Besides their nutritional and structural functions, fatty acids play important roles in fish immune responses and other physiological processes. They are classified into saturated fatty acids (SFA), mono-unsaturated FA (MUFAs), polyunsaturated FA (PUFAs), and highly unsaturated FA (HUFA) (Sargent et al., 2002; Tocher, 2003; Fahy et al., 2005). The n3 HUFAs, such as DHA and EPA induce antioxidant and anti-inflammatory effects (Anderson et al., 2014). Therefore, the dietary lipid sources enriched in these FAs, such as fish oil, are ideal for fish health and physiology. Besides fish oil, terrestrial vegetable oils are an abundant source of lipids used in aquafeeds, thanks to their great economic benefits. The plant-derived oils are generally enriched in PUFA, including linoleic acid, LA (C18: 2n-6) and α-linolenic acid, ALA (C18: 3n-3) (Nguyen et al., 2021) that are the precursors for n3 and n6 HUFA biosyntheses such as DHA, EPA, and ARA. As their chain structure contains double bonds, these fatty acids are often highly oxidized but do not contain the trans-fats found in animal fat products. In addition, vegetable oils also contain several natural antioxidants, such as vitamin E (Desai et al., 1988).

Some studies have previously shown that using different fat sources generally did not affect fish growth (Monge-Ortiz et al., 2018; Nguyen et al., 2019b, 2020; Sourabie et al., 2019) but influenced the tissue FA composition and fish health status.

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Indeed, the modifications in innate immune activities such as lysozyme, complement, peroxidase, and phagocytosis were observed in fish fed on various fat sources (Montero et al., 2010; Xu et al., 2015; Zuo et al., 2015; Tan et al., 2016, 2017; Conde-Sieira et al., 2018). However, the effect of dietary oils on the antioxidant ability in fish tissues is still limited (Peng et al., 2016; Fu et al., 2017). Oxidative stress is a general concept to describe a severe imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms. Under these conditions, ROS can induce damage in membrane lipids and DNA that affect the function of cellular proteins (Buico et al., 2009). ROS are free radicals, including superoxide anions, hydroxyl radicals, peroxyl radicals, and alkoxyl radicals. These radicals are increased during the bacterial infection or inflammatory response; and can inhibit the activity of pathogenic microorganisms that cause much damage to the host organism (Chelombitko, 2018). In normal conditions, the over-production of free radicals is a pathological state (Mittal et al., 2014). In these cases, the antioxidants could reduce free radical production and their negative effects on the body (Sardesai, 1995; Rani, 2017; Wilson et al., 2017).

Common carp, *Cyprinus carpio* Linnaeus, 1758 is an omnivorous fish widely cultured worldwide (Cai et al., 2019; Nguyen et al., 2019b). This species can bioconvert the PUFAs, such as ALA and LA, to several important HUFAs (DHA, EPA, and ARA) through the desaturation and chain elongation processes (Nguyen et al., 2022). Many studies have previously shown that the use of plant oils instead of fish oil, such as flaxseed/linseed oil, sesame oil, corn oil, canola oil, and sunflower oil, did not affect the growth performance and feed efficiency in common carp (Ren et al., 2012; Ljubojević et al., 2015; Nguyen et al., 2019a, 2021). The effects were observed on the FA profile or fish health status, such as immune response and fish resistance; however, no studies have reported the antioxidant capacity of common carp fed the plant oil compared to fish oil-fed ones. Some studies were conducted on other fishes, such as rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 (Kutluyer et al., 2017), black carp *Mylopharyngodon piceus* Richardson, 1846 (Sun et al., 2011), large yellow croaker *Larimichthys crocea* Richardson, 1846 (Li et al., 2021), and Nile tilapia *Oreochromis niloticus* Linnaeus, 1758 (Larbi Ayisi et al., 2018). Therefore, the current research was carried out to evaluate the effects of dietary fat sources on growth, feed utilization, resistance, and antioxidant capacity of the liver in common carp.

Materials and Method

The protocol of the feeding trial and bacterial challenge was approved by the Vietnam National University Animal Ethics Committee (T2020-03-03TĐ). The experimental design is briefly described in Figure 1. Common carp juveniles were collected from a local hatchery and acclimatized for two weeks in the experimental tank system before beginning the experiment. During this period, fish were fed on a commercial feed (Austfeed, 35% crude protein, 8% lipid). After two weeks, healthy fish were selected for the experiment.

Experimental diets: Four isoproteic (crude protein = 30.7%) and isolipididic (10%) diets were formulated based on three fat sources: cod liver oil (enriched in HUFA), linseed oil (enriched in α-linolenic acid, ALA, C18: 3n-3), sesame oil (enriched in linoleic acid, LA, C18: 2n-6), and a blend of linseed and sesame oil (*v:v*, *1:1*). The diets are denoted as follows: FO (Fish oil-based diet), LO (linseed oil-based diet), SO (sesame oil-based diet), and SLO (blend of sesame and linseed oil-based diet). The protein sources in experimental diets included fishmeal, wheat gluten, gelatin, and casein. In addition, the diets also contained other ingredients such as modified starch, mineral, and vitamin premix. The feed ingredients were chosen according to the nutritional requirements of common carp (Table 1) (NRC, 2011). All the powdered ingredients were well-mixed; then oil was added to the mixture, followed by water to produce a stiff dough. The dough was pelleted with a laboratory pellet mill, then air-dried and stored at 4ºC until use.

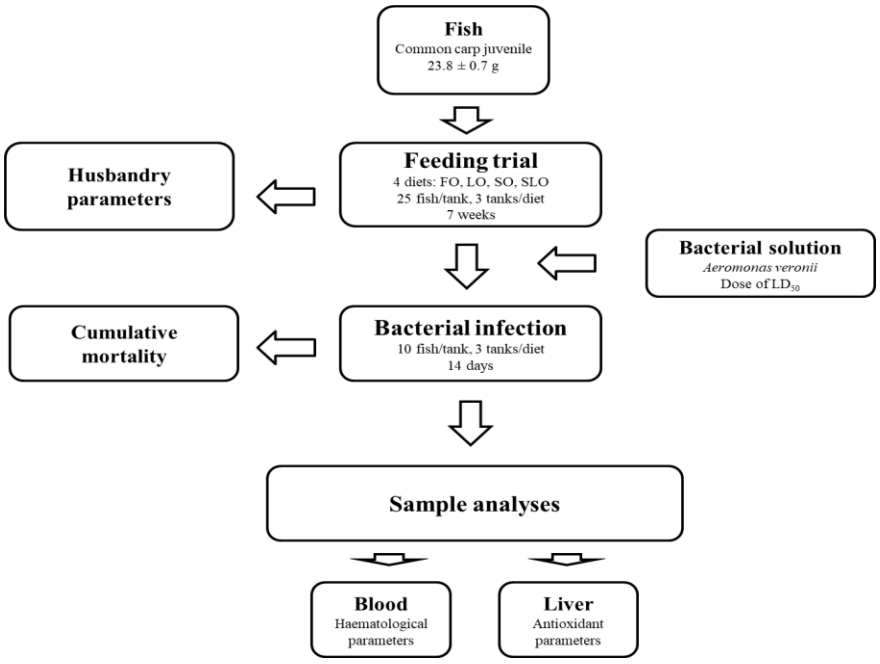

Figure 1. Brief diagram of experimental design.

Table 1. Ingredients and approximate composition of the experimental diets.

Ingredient (g.kg^{-1} diet, DM)	Diet			
	FO	LO	SO	SLO
Fish meal[1]	300.0	300.0	300.0	300.0
Wheat gluten[2]	120.0	120.0	120.0	120.0
Gelatin[3]	10.0	10.0	10.0	10.0
Casein[4]	25.0	25.0	25.0	25.0
Modified starch[5]	415.0	415.0	415.0	415.0
Cod liver oil (FO)[6]	100.0	--	--	--
Linseed oil (LO)[7]	--	100.0	--	50.0
Sesame oil (SO)[8]	--	--	100.0	50.0
Vitamin and mineral premix[9]	30.0	30.0	30.0	30.0
Total	1000.0	1000.0	1000.0	1000.0
Estimated crude protein (%)	30.7	30.7	30.7	30.7
Estimated crude lipid (%)	10.0	10.0	10.0	10.0
Estimated gross energy (GE, MJ.kg^{-1} DM)	18.3	18.3	18.3	18.3

Note: FO, fish oil; LO, linseed oil; SO, sesame oil; SLO, mixture of SO and LO. DM: dry matter. (1) Phuc Loc Vung Tau Co. Ltd., Hoang a road, Tan Hai Commune, Phu My Town, Ba Ria Vung Tau Provinc. (2)(3)(4) Sigma aldrich, St Louis, MO, USA, (5) Baaboo Food, Ho Chi Minh City, Vietnam, (6) Mollers Tran, Norway, (7)(8) Naturgreen, Spain, and (9) Nova-premix for fish, ANOVA, Vietnam.

Feeding trial: Seventy-five fish with an initial body weight (IBW) of 23.8±0.7 g were randomly allocated to each of 12 glass tanks (100 L holding capacity), resulting in triplicate tanks per dietary treatment. Fish were fed to satiation twice a day with the experimental diets for seven weeks. The consumable feed amount of each tank was recorded daily to calculate the feed conversion ratio (FCR). The environmental parameters in the RAS were maintained suitable for common carp, including temperature (from 22 to 25ºC); pH (7-7.5); dissolved oxygen (6-6.5 mg/L); and NO_2 (<0.05); NH_3/NH_4^+ (<0.05). The rearing tank was siphoned daily to clean the fish faeces and continuously aerated. After a 7-week trial, fish were weighed and counted to determine their growth rate and survival.

Bacterial challenge test: Before the bacterial challenge, a median lethal dose (LD_{50}) of *Aeromonas veronii* bacteria was determined in another group of carp juveniles (size of 32.8±5.2 g).

Briefly, the bacteria were isolated from diseased carp caused by *A. veronii* and stored in the laboratory of the Department of Environment and Aquatic Diseases, Faculty of Fisheries, Vietnam National University of Agriculture. The bacterial solution cultured at 30ºC for 24 hours was considered the stock solution (with OD = 1.374 at 610 nm), corresponding to a concentration of 10^9 CFU.mL^{-1}. The stock solution was diluted into concentrations of 10^8, 10^7, 10^6, 10^5, 10^4, and 10^3 CFU.mL^{-1}. Fish was injected with these bacterial concentrations at a volume of 0.1 mL per fish (ten fish for each concentration). Fish were then monitored for 14 days, where the number of dead fish in each tank with the specific disease symptom caused by *A. veronii* was recorded daily. The LD_{50} dose was calculated based on the cumulative mortality rate in each bacterial concentration.

At the end of the feeding trial, 30 fish in each experimental condition were intraperitoneally injected with *A. veronii* at a dose of LD_{50} and then randomly divided into a 120 L-tank system at a density of ten fish per tank. A group of ten fish from all experimental conditions was used as a control in which the fish were injected with a physiological saline solution instead of a bacterial one. The injected fish were then monitored for 14 days, and the number of dead fish in each tank were recorded daily. Dead fish with pathological symptoms caused by *A. veronii* bacteria in common carp, as described by Yu et al. (2010) were dissected and re-isolated to confirm the cause of death.

Sample collection and analysis: At the end of the nutritional trial and the second-day post-bacterial infection, fish were starved for 24 h, then anaesthetized with clove oil (50 mg/L) and used for sampling. Fish livers (three samples per tank) were then collected to measure the antioxidant parameters such as Malondialdehyde (MDA), Glutathione (GSH), and Superoxide dismutase (SOD) activities. The analysis protocols were detailed as follows:

Malondialdehyde activity (MDA): The MDA level in the liver homogenate was determined according to Wasowicz et al. (1993). Liver samples were first homogenized in phosphate buffer (100 mM, pH = 7.5) according to the ratio of 1 : 10 (w : v) at 4ºC. After cold centrifugation for 30 min, 150 µL of the solution was placed in a glass tube containing 1 mL of distilled water and 1 mL of 0.5 % thiobarbituric acid. The mixture was heated in a water bath at 99ºC for one hour. After cooling at room temperature, the mixture was added with 25 µL of 5 M HCl. The solution was then measured at 532 nm. In parallel, 150 µL of tetramethoxypropane solution was used as a standard. The MDA content (nmol.mL^{-1} of homogenate) was calculated according to the linear regression equation with the tetramethoxypropane standard, and the MDA content (nmol.mg^{-1} protein) of the fish liver was also considered.

Glutathione activity (GSH): GSH content in the liver was determined by Ellman reagent (Koh et al., 2012). The homogenate of the liver procedure was described as above. The homogenate was used for GSH quantification. The reaction mixture in each well of the 98-well plate was prepared as follows: 20 µL of homogenate solution, 160 µL of Tris-HCl buffer pH 7.5, and 20 µL of 5.5′-Dithiobis (2-nitrobenzoic acid) (DTNB) 10 mM solution. The absorbance of the product was immediately recorded at 412 nm. The GSH content (µg.mL^{-1}) in 1 mL of liver homogenate was determined based on a linear regression equation with the GSH standard. GSH content was expressed as µg reduced GSH.mg^{-1} protein liver.

Superoxide dismutase (SOD): SOD activity in liver homogenate was determined according to Marklund's method with modifications (Tung et al., 2017). The method is based on the ability of SOD to inhibit the auto-oxidation reaction of pyrogallol. The reaction mixture includes Tris-HCl buffer 100 mM, 1mM EDTA, pH 8.2; 10 µL homogenous, and 20 µL of 13 mM pyrogallol. The mixture solution was then measured at 420 nm. SOD activity is determined by U.min^{-1} per mg protein, where the unit (U) of SOD activity is defined as the total amount of enzyme that can inhibit the auto-oxidation of 50% of pyrogallol in the reaction.

Table 2. Growth, feed utilization, and survival of common carp used the experimental diets based on the fish oil (FO), linseed oil (LO), sesame oil (SO), and a mixture of SO and LO (SLO) after a 7-week feeding trial.

Variable	Experimental groups			
	FO	LO	SO	SLO
IBW (g.fish^{-1})	24.3±0.7	23.3±1.2	23.8±0.4	23.9±1.8
FBW (g.fish^{-1})	33.5±1.0	34.0±0.5	33.6±0.3	33.8±1.0
DWG (g.fish^{-1} per day)	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
SGR (%.day^{-1})	0.7±0.1	0.8±0.1	0.7±0.0	0.7±0.1
WG (%)	37.9±5.3	43.8±3.2	41.1±1.1	41.1±4.7
FI (g.fish^{-1} per day)	0.41±0.02	0.43±0.03	0.38±0.05	0.41±0.02
FCR	2.2±0.2	2.0±0.1	1.9±0.2	2.1±0.3
Survival rate (%)	100.0	100.0	100.0	100.0

Note: IBW: initial body weight; FBW: final body weight; DWG: daily weight gain; SGR: specific growth rate; WG: weight gain; FI: feed intake; FCR: feed conversion rate. Values are represented as mean±SD.

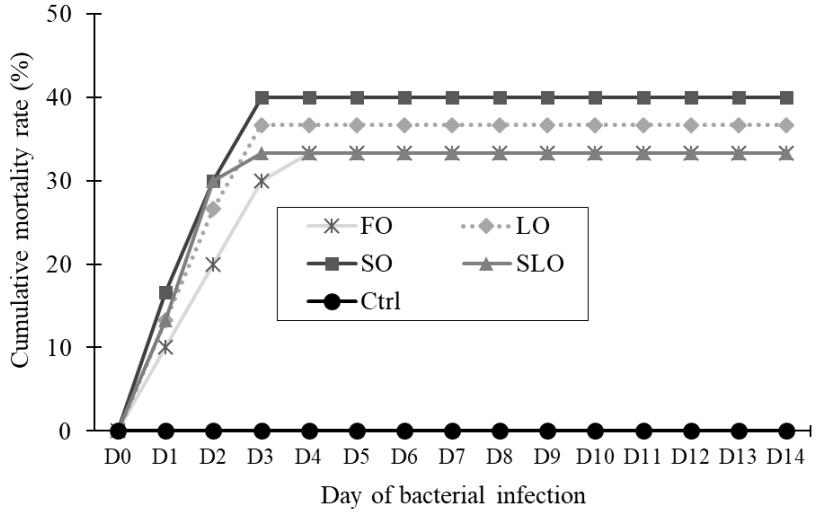

Figure 2. Cumulative mortality rate of infected fish after bacterial challenge (Note: FO, fish oil; LO, linseed oil; SO, sesame oil; SLO, mixture of SO and LO; Ctrl: the control without bacterial infection).

Data analyses: Mean values were first checked for homogeneity by the Univariate test. The data were then subjected to one-way ANOVA followed by a LSD post-hoc test using the replicate tank as a statistical unit for husbandry variables (n = 3) and the number of samples for each experimental condition for antioxidant parameters (n = 9). Differences between treatments were considered significant at $P<0.05$. All data were analyzed with the STATISTICA 10.0 software (Statsorf, Inc., East 14 Street, Tulsa, USA).

Results

Growth, feed utilization, and survival rate: After a 7-week feeding trial, the husbandry parameters were shown in Table 2, but no significant differences were observed between the experimental groups ($P>0.05$). The average fish weight increased from 23.84±0.73 to 33.70±0.69 g corresponding to 37.9±5.3 to 43.8±3.2% of the weight gain; the daily growth rate reached 0.2 g.fish^{-1} per day; the specific growth rate ranged from 0.7 to 0.8%.day^{-1}. The feed conversion ratio (FCR) varied from 1.9 to 2.1. The fish survival reached 100% in all of the experimental groups.

Cumulative mortality rate: Based on the cumulative mortality rate recorded in each tank, the LD_{50} dose of *A. veronii* in common carp juveniles was determined as $0.43x10^6$ CFU.mL^{-1}. After injection with LD_{50} dose, the fish mainly died from the first to the third day of the challenge experiment. The number of dead fish in each tank was daily recorded and the cumulative mortality results after a 14-day challenge are shown in Figure 2.

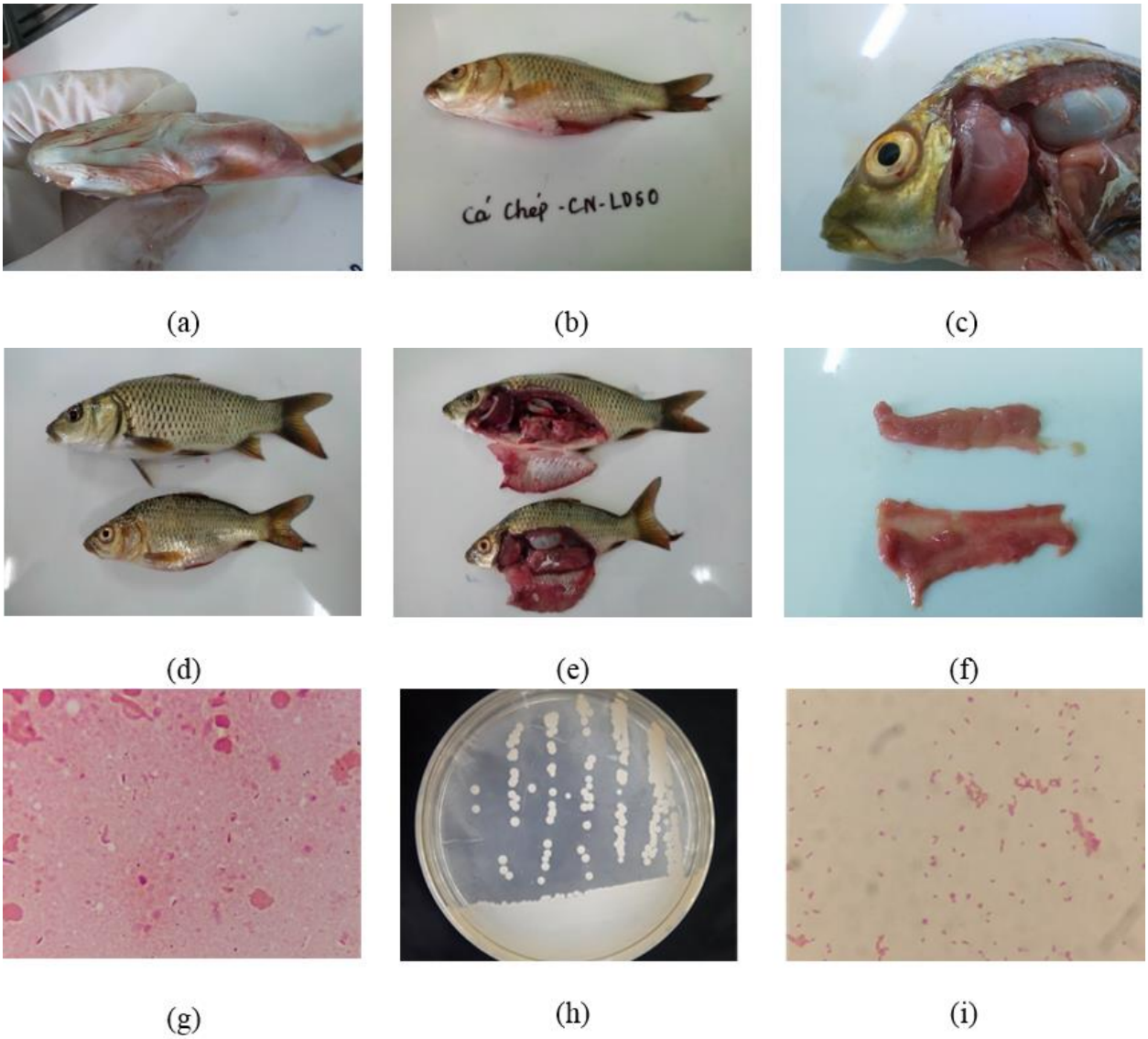

Figure 3. Images of dead fish after bacterial infection including external (a, b) and internal symptoms (c); comparison pictures between infected fish (above) and control fish (below) including external (d), anatomical (e), and intestinal symptoms (f); stained kidney tissue (g); bacterial colonies on nutrient agar medium (h); and gram-stained bacteria (i).

Accordingly, the cumulative mortality rate was not influenced by dietary fat sources (P>0.05) and ranged from 33.3±5.8 to 40.0±0.0 %.

The signs of dead fish included abdominal distension, red swelling, and bleeding in the skin, base of the fins, and anus. The internal organs, such as the liver and spleen, were pale, while the intestines were hemorrhagic. The kidney tissues were then stained and used for bacterial re-isolation. The results showed that *A. veronii* belonged to the negative gram and rod-shaped bacterium. The colony form is round and creamy yellow (Fig. 3).

Antioxidant capacity of liver: The activity of glutathione (GSH, Fig. 4), malondialdehyde (MDA, Fig. 5), and superoxide dismutase (SOD, Fig. 6) were measured in the common carp liver samples at the end of the nutritional trial and after bacterial infection. The GSH contents in the liver of carp dissected at the end of the feeding trial (from 35.23 to 39.83 μg.mg^{-1} protein) were not affected by the dietary fat sources (P>0.05). The differences between experimental conditions were displayed in the liver samples collected after bacterial infection (P<0.05, Fig. 4); accordingly, the GSH level in SO-fed fish (22.75±4.51 μg.mg^{-1} protein) was higher than those in other plant oil-fed groups and

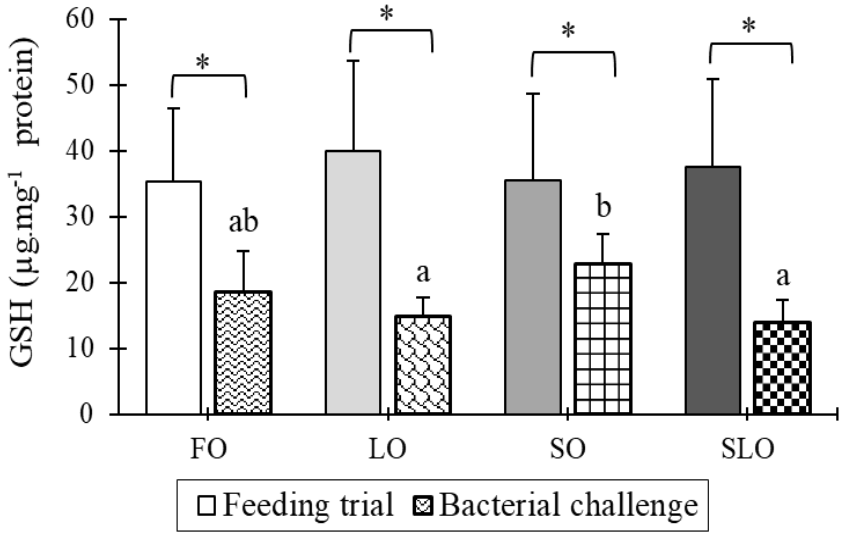

Figure 4. Glutathione (GSH) content in liver of carp fed on the different fat sources including fish oil (FO), linseed oil (LO), sesame oil (SO), and a mixture of SO and LO (SLO) for seven weeks and after infection with *Aeromonas veronii*. Data are represented as mean±SD. Values with common superscript letter denote non-significant differences (P>0.05).

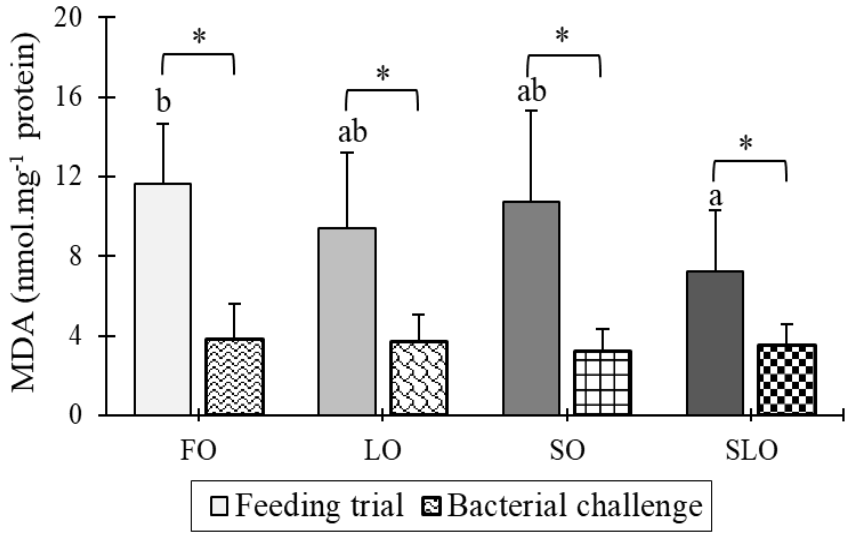

Figure 5. Malondialdehyde (MDA) content in liver of carp fed on the different fat sources including fish oil (FO), linseed oil (LO), sesame oil (SO), and a mixture of SO and LO (SLO) for seven weeks and after infection with *Aeromonas veronii*. Data are represented as mean±SD. Values with common superscript letter denote non-significant differences (P>0.05).

equivalent to the FO one (18.5±6.28 µg.mg^{-1} protein). Furthermore, the GSH activities in the fish livers after bacterial infection in all of the treatments were reduced in comparison with those at the end of the nutritional experiment (P<0.05).

Similar to GSH activity, the malondialdehyde (MDA) contents in the fish liver after bacterial infection were lower than those observed at the end of the feeding trial (P<0.05, Fig. 5). The influence of dietary fat sources on MDA level in carp livers was recorded at the end of the nutritional trial (P<0.05) while no differences were found after bacterial challenge (ranging from 3.25 to 3.80 nmol.mg^{-1} protein). Accordingly, the highest value of MDA activity was observed in the FO-fed fish (11.63±3.03 nmol.mg^{-1} protein) that was equivalent to LO-fed fish (9.40±3.80 nmol.mg^{-1} protein) and SO (10.72±4.58 nmol.mg^{-1} protein); the lowest level of MDA activity was found in SLO-fed ones (7.21±3.10 nmol.mg^{-1} protein).

SOD levels in carp livers also tended to decrease after bacterial challenge compared to those at the end of the feeding trial in almost all of the experimental groups (Fig. 6). The influence of dietary lipid

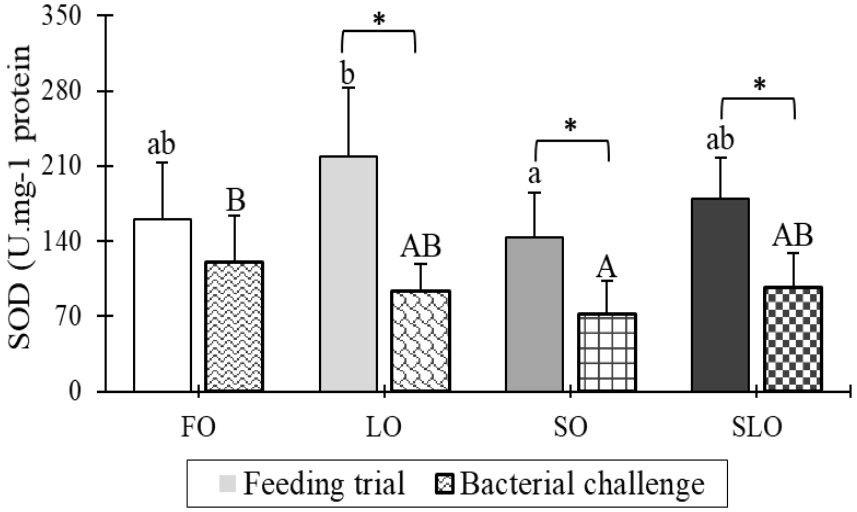

Figure 6. Superoxide dismutase (SOD) activity in liver of carp fed on the different fat sources including fish oil (FO), linseed oil (LO), sesame oil (SO), and a mixture of SO and LO (SLO) for seven weeks and after infection with *Aeromonas veronii*. Data are represented as mean±SD. Values with common superscript letter denote non-significant differences (P>0.05).

sources on SOD activity was displayed at the end of the nutritional trial as well as post-bacterial infection (P<0.05). Specifically, after a 7-week feeding trial, the highest value of SOD activity was found in LO-fed fish (218.92±52.15 U.mg^{-1} protein), while the lowest one was observed in the SO-fed group (143.78±41.10 U.mg^{-1} protein). Similarly, the lowest level of SOD activity was still recorded in SO-fed fish (71.97±31.40 U.mg^{-1} protein), whereas the highest value was observed in the FO-fed ones (120.32±43.09 U^{-1}mg protein). Furthermore, only the value of SOD activity in FO-fed fish was not reduced after bacterial infection.

Discussions

In the current experiment, plant oil instead of fish oil did not negatively affect fish growth and feed utilization (Table 2). This result is consistent with previous findings in common carp when no adverse effects of dietary plant oil on growth and feed performances were reported in the common carp diet (Ren et al., 2012; Nguyen et al., 2019b, 2020). The same data were also previously documented in other omnivorous fish such as triangular bream, *Megalobrama terminalis* Richardson, 1846 (Tian et al., 2018) and Nile tilapia (Teoh and Ng, 2016). On the other hand, the reductions in growth, feed efficiency, or survival rate were usually found in carnivorous/marine fish such as pike-perch, *Eurasian perch* Linnaeus, 1758 (Geay et al., 2015), turbot, *Scophthalmus maximus* Linnaeus, 1758 (Benedito-Palos et al., 2008; Montero et al., 2010), European seabass, *Dicentrarchus labrax* (Geay et al., 2011; Torrecillas et al., 2017), and rainbow trout, *Oncorhynchus mykiss* (Kutluyer et al., 2017; Mellery et al., 2017) fed on plant oil. The differences in fish growth performance and feed efficiency were generally observed in fish fed different levels of lipid or protein sources (Nguyen, 2020). In the current study, the experimental diets differed only in fat sources, while the lipid levels and protein ingredients were homogenous, leading to comparable results in fish growth and feed utilization for all of the conditions. Furthermore, reductions in fish growth were observed when the fish ingredients, including fishmeal and fish oil were totally replaced with plant-based ingredients. Conversely, no differences were found in the case of a low level of fish oil replacement and fish meal used as the main protein source (Nguyen et al., 2019b).

Influence of fat sources on fish survival and resistance to *A. veronii*: After the feeding trial, the fish survival reached 100% in all of the treatments, indicating that the dietary fat sources in the feed formulation did not affect the fish survival rate under rearing conditions. In addition, the suitable

environmental parameters for common carp during the experiment were also a reason for the absolute value of fish survival rate. After bacterial infection, the fish mortality was not significantly different between experimental groups, suggesting that the dietary lipid sources did not affect the resistance to *A. veronii*. These results were similar to the previous studies on common carp fed on the linseed and sunflower oil-based diets (Nguyen et al., 2019b), Nile tilapia fed on coconut oil (Apraku et al., 2017) and palm oil (Larbi Ayisi et al., 2018). Contrarily, some other studies have shown that fish mortality increased in carnivorous/marine fish using vegetable oils such as large yellow croaker (Tan et al., 2016) and Japanese sea bass, *Lateolabrax japonicus* (Tan et al., 2017). This difference can be explained by the different capacities of HUFA synthesis in these two fish groups. Generally, omnivorous / freshwater fish have a good ability to synthesize HUFAs from the PUFA precursors such as ALA and LA in plant oils, while this ability in carnivorous / marine fish is less efficient by the deficiency of several enzymes involved in this process (Oliva-Teles, 2012). In animals, some HUFAs such as ARA, EPA, and DHA are the precursors synthesizing several lipid mediators in the immune response (Nguyen et al., 2021). Therefore, the resistance to *A. veronii* of common carp, an omnivorous fish that can convert the PUFA precursors to HUFA, was not affected by the tested plant oils. The symptoms of dead fish after bacterial infection, tissue histopathological, bacteria, and colony characteristics in this study were similar to those in the disease common carp caused by *A. veronii* described in previous studies (Chen et al., 2019; Hoai et al., 2019).

Effect of dietary fat sources on the antioxidant ability in fish liver: In the body, free radicals are formed by a number of exogenous and endogenous processes. Their negative effects are generally neutralized by antioxidant defenses. Oxidative stress occurs due to an imbalance between the production of free radicals and antioxidant defenses. When the free radicals are generated more than the threshold that antioxidants can keep in balance, the free radicals begin to damage the fat tissue, DNA, and proteins in the body, consequently leading to numerous diseases, signs of aging, and reduced lifespan (Buico et al., 2009; Liguori et al., 2018). Therefore, increasing antioxidants by food intake is a great idea to maintain a good health for animals in general and aquatic animals in particular. To evaluate the antioxidant capacity of tissues; some parameters such as GSH, MDA, and SOD were generally used.

Regarding the results of GSH content, no differences were observed between experimental groups at the end of the feeding trial, indicating that the replacement of fish oil with linseed oil, sesame oil, or their mixture did not induce any negative effect on GSH activity in carp liver. Other studies have also previously shown that the use of dietary vegetable oils instead of fish oil did not reduce the antioxidant capacity in freshwater / omnivorous fish such as rockfish, *Sebastes schlegeli* (Aminikhoei et al., 2013), Nile tilapia (Larbi Ayisi et al., 2018), and *Onychostoma macrolepis* (Gou et al., 2021). In contrast, several studies demonstrated the decreases in the antioxidant capacity in the liver of fish using plant oil-based diets in marine fish like Japanese sea bass, *L. japonicus* (Tan et al., 2017), large yellow croaker (Mu et al., 2018, 2020; Li et al., 2019), and red Seabream, *Pagrus major* (Mzengereza et al., 2021). After bacterial infection, the GSH level in SO-fed fish was higher than that measured in fish fed on the other plant oil diets (LO and SLO), and comparable to the FO-fed group, indicating that the use of dietary sesame oil induced a higher antioxidant ability compared to those in other plant oil-fed carps. Glutathione is an antioxidant compound found in plants, animals, fungi, and some bacteria that can prevent the important cellular components against the damage caused by reactive oxygen species such as free radicals, peroxides, and heavy metals (Kerksick and Willoughby, 2005; Forman et al., 2009; Silvagno et al., 2020). During the bacterial infection or inflammatory process, the host body generally produces a number of strong oxidants that could destruct and inhibit the activities

of pathogens or other exogenous agents (Abdulkhaleq et al., 2018). These processes endamage the exogenous agents and negatively affect the host body if no regulatory mechanisms appear. In the current study, the common carp can convert LA, which is abundant in dietary sesame oil, to arachidonic acid (ARA). ARA contents in the liver (3.8 $mg.g^{-1}$) and dorsal muscle (1.9 $mg.g^{-1}$) were previously quantified (Nguyen, 2020) that were even higher than those measured in fish oil-fed fish (0.9 $mg.g^{-1}$). In the body, ARA is a precursor to produce some lipid mediators involved in the inflammatory response and the recovery process after inflammation (Nguyen et al., 2021), including antioxidant reaction. This argument may explain the highest antioxidant ability corresponding to the highest level of GSH was found in fish-fed sesame oil containing a high level of linoleic acid.

SOD is a special enzyme that could convert the destructive superoxides in the host body into non-toxic substances. Regarding the results of SOD activity, no influences of dietary fat sources were recorded at the end of the feeding period, suggesting that the dietary plant oil did not negatively affect the antioxidant ability in fish liver under normal conditions. However, this capacity in the LO-fed group was higher than that in SO-fed fish. Previous studies have also demonstrated the better antioxidant ability in fish fed linseed oil compared to other plant oil sources in rainbow trout (Kutluyer et al., 2017), large yellow croaker (Li et al., 2021), and *O. macrolepis* (Gou et al., 2021). Reported data have also demonstrated higher levels of DHA and EPA in fish-fed diet enriched in ALA, an n3 PUFA precursor. The supplementation of DHA and EPA in the fish diet has been previously documented to enhance the antioxidant ability in fish under basal conditions (Engstrm et al., 2009; Capó et al., 2015; Othman et al., 2015). Under the bacterial infection, the SOD activity in SO-fed fish was lower than that of FO-fed ones, while other plant oil-fed groups were comparable to FO-fed one, suggesting that SOD activity decreased in the SO group after bacterial infection. This result could be explained by the higher GSH content in SO-fed fish post-bacterial challenge compared to other plant oil-fed groups, leading to the decrease of SOD to keep the balance of the antioxidant process in the host body.

Contrary to GSH and SOD, malondialdehyde (MDA) is one of the products of lipid oxidation, known as an indicator of lipid oxidation degree in the body. The degree of oxidation in plasma and tissues is correlated with MDA concentrations (Ayala et al., 2014). The MDA level in SLO-fed fish at the end of the feeding trial was lower than that of FO-fed group, indicating that the lipid oxidation activity of the SLO fish was lower than that of the FO group under normal conditions; i.e. the antioxidant ability in the liver of fish fed on the mixture of linseed and sesame oil was higher than that in fish oil-fed fish. This result was probably explained by the balance of PUFA precursors in the SLO diet, including LA and ALA, which are converted to an amount enough of important HUFAs involved in immunoregulatory processes such as the redox reactions. Furthermore, the balance of the n3 / n6 FAs ratio in SLO probably positively affected the health status of experimental fish. As recommended, this ratio should be close to one (Simopoulos, 1991; Gómez Candela et al., 2011; Bhardwaj et al., 2016). Although the n3 / n6 ratio in fish oil control is close to one, the levels of ALA and LA in fish oil were much lower than those of the SLO, explaining why the antioxidant ability of SLO-fed fish was higher than that of the FO-fed group. No difference in MDA concentrations was observed after bacterial infection, indicating that terrestrial vegetable oils did not negatively affect the antioxidant ability of infected fish.

Conclusion

The replacement of fish oil with linseed oil, sesame oil, and their blends did not affect the growth, feed efficiency, survival, and resistance to *A. veronii* bacteria in common carp. Using these plant oil sources did not negatively affect the antioxidant ability in the liver of common carp. In addition, linseed oil and its mixture with sesame oil have improved the antioxidant capacity of common carp

under a basal condition, while sesame oil boosted this ability under a bacterial infection.

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