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Reactive oxygen species (ROS)-mediated regulation of muscle texture in grass carp fed with dietary oxidants

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ABSTRACT

Natural oxidant faba beans (FB) improve the muscle textural quality of grass carp (*Ctenopharyngodon idellus*). This study further explored the role and spatiotemporal dynamics of ROS during the 120-day process of texture quality change in grass carp fed with four feeds including commercial feed, FB only, FB and antioxidants (vitamin C and vitamin E), and commercial feed and oxidant (vitamin K3). The results showed that ROS (total ROS, H_2O_2, $\cdot O_2^-$ and ·OH) time-dependently accumulated from intestine to blood, liver and muscle, improving the muscle texture by day 90 in grass carp fed solely with FB. Dietary antioxidants supplementation almost completely abolished the effect. It is notable that the textural quality and level of total ROS in the commercial feed and oxidant group supplemented vitamin K3 were higher than those of other three groups by day 60 ($p < 0.05$), suggesting that oxidant supplementation effectively improved the muscle textural quality of grass carp. Also, there was a positive feedback between ROS accumulation and production mainly in the mitochondria of tested organs as demonstrated by measuring the activity of the elevated electron transport chain (ETC) pathway, openness degree of mitochondrial membrane permeability transition pore (MPTP), and mitochondrial membrane potential ($\Delta\psi$). These results validated the regulatory role of ROS in the improvement of muscle texture in grass carp fed with the natural oxidants and also demonstrated that oxidant supplementations (vitamin K3, or/and FB) have the potential to be applied to aquafeed for the improvement of muscle textural quality in freshwater fishes.

Keywords:

Textural quality

Reactive oxygen species (ROS)

Grass carp

Mitochondria

Antioxidants

1. Introduction

Grass carp (*Ctenopharyngodon idellus*) is the largest aquaculture species, and it provides low-cost animal protein especially for developing and underdeveloped regions (FAO, 2020). With the development of intensive aquaculture, however, reduction of muscle quality has become one of the most important issues in grass carp culture. As a result of continuous efforts to overcome this problem, crisp grass carp (*Ctenopharyngodon idellus* C.et V), as the "China Geographical Indication Product", has been developed as a high-value product by feeding ordinary grass carp solely with faba beans. Compared to ordinary grass carp, crisp grass carp exhibits significantly improved muscle textural characteristics (e.g., hardness and chewiness), and its products are exported to North and Latin America as well as Southeast Asia with growing popularity (Chen et al., 2020). Compared to ordinary grass carp, the contents of crude protein and collagen were increased and crude fat and moisture contents were decreased in crisp grass carp muscle (Wang et al., 2015; Ma et al., 2020). Our previous study has shown that vicine (a pro-oxidant) may be responsible for the improvement of textural quality in crisp grass carp (Ma et al., 2020). Dietary vicine elevates lipid peroxide and glutathione levels and depresses the ratio of plasma vitamin E/lipid in chickens (Muduuli et al., 1981). The multi-omics analyses on the crisp grass carp model found that: 1) the dietary pro-oxidants faba beans (probable vicine and convicine) improve the textural quality of ordinary grass carp muscle, changing the expression of 25 ROS-related proteins during this process; and 2) reactive oxygen species (ROS, such as H_2O_2) are likely responsible for improving textural quality because tissue and plasma H_2O_2 concentrations are increased by faba bean feeding for about 200 days (Yu et al., 2017; Yu et al., 2020). However, the spatiotemporal dynamics of ROS during this process and the causal relationship remains unclear.

Generally, major ROS include total ROS, superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH) (Shadel & Horvath, 2015). ROS is generated by various biochemical reactions, but mainly as a by-product in the mitochondrial electron transport chain (ETC). Complex I/III are the sites with the greatest capacity to generate ROS in the mitochondrial ETC, and Complex IV also play important role for generating ROS (Kudin et al., 2004; Chen et al., 2010; Schofield et al., 2021). It is thus possible that the amount of these proteins affects the spatiotemporal dynamics of ROS in crisp grass carp. The main ROS produced by cells is a free

radical $\cdot O_2^-$, which is commonly generated as an intermediate product in several biochemical reactions. A positive effect of $\cdot O_2^-$ generated during exercise was found on skeletal muscle force generation (Gomez-Cabrera et al., 2010), but increased $\cdot O_2^-$ accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction degeneration (Jang et al., 2010). These facts suggest that different levels of $\cdot O_2^-$ production/accumulation cause divergent results in terms of changes in muscle structure and function. H_2O_2 is stable and permeable through cellular membranes. It can reduce myofibrillar Ca^{2+} sensitivity and affect structure of myofibrillar proteins (Andrade et al., 1998; Li et al., 2013). ·OH possesses a strong oxidizing potential and may be the most damaging ROS present in biological systems (Powers et al., 2011). ·OH can reduce the disulfide bonds in proteins, resulting in their unfolding and scrambled refolding into abnormal spatial configurations (Lipinski, 2011). So, different types of ROS have different biological functions, and determining the kinds and contents of ROS will help to clarify the role of ROS during muscle structure and function change. Thus, in addition to the problems of dynamics and causality described above, it is important to identify the types of ROS generated by pro-oxidant feeding along with their specific effects on grass carp physiology.

Improvements in the textural quality of grass carp are likely the consequence of complex physiological changes among in several tissues. The intestine and liver should be of particular relevance because dietary factors alter the gut microbiota and intestinal barrier function, affecting the interactions between the liver and intestine, i.e., the well-known “intestine-liver axis” (Kirpich et al., 2015). Liver metabolism is also closely related to muscle metabolism, especially to muscle mass and density (Yodershi et al., 2020). Dietary changes can increase skeletal muscle mass in mice in association with blood circulation and fat metabolism (including that in the liver) (Bilski et al., 2017), and changes in muscle protein and function are regulated by intestine (Andrea et al., 2019). Interaction between different organs and tissues also must be considered. So, identifying ROS contents in different tissues would help to further clarify the processes of ROS generation and accumulation.

In this study, we examined muscle textural quality (hardness, chewiness, etc.) as well as contents of various types of ROS (total ROS, $\cdot O_2^-$, H_2O_2 and ·OH) and the activity of ROS generation, transformation, and elimination systems (Complex I/III/IV, SOD, CAT, GSH,

NADPH) in four tissues (intestine, liver, serum and muscle) in four groups of grass carp fed with different feeds (commercial feed, faba beans only, faba beans and antioxidants, commercial feed and oxidant), on days 30, 60, 90 and 120. And then we explored the transmission of different ROS species in the four tissues and their role in the processes of improving muscle textural quality.

2. Materials and methods

2.1. Animals

Healthy ordinary grass carp (800–1000 g) were purchased from an aquaculture farm in Zhongshan, Guangdong Province, China. The fish were first temporarily cultured in a cement pond (5 m × 5 m × 1 m) for one week before being randomly divided into four groups [three ponds (2 m × 2 m × 1 m) per group, 15 individuals in each pond] for subsequent feeding experiments. The control group (C) was fed commercial feed that contained the following: fish meal, 5 g/kg; soybean meal, 215 g/kg; cottonseed meal, 80 g/kg; rapeseed meal, 200 g/kg; wheat flour, 180 g/kg; rice bran, 150 g/kg; leaf powder, 50 g/kg; malt root, 50 g/kg; choline chloride, 20 g/kg; mineral mixture 20 g/kg; and vitamin mixture, 30 g/kg. The faba beans group (FB) was fed solely with whole faba beans. The FB and antioxidants group (FB+AO) was fed FB supplemented with vitamin C (5 g/kg) and vitamin E (2.5 g/kg) (Table 1). These amounts were determined to be 10 times higher than those in Garcia et al. (2007) because fish in this group were fed the mixture of faba beans with the supplementation at the ratio of one to nine [i.e., (daily amounts of anti-oxidant feed) : (daily amounts of faba beans) = 10%: 90%]. The oxidant group (O) was fed the commercial feed containing vitamin K3 (20 g/kg) based on the contents of vicine and convicine (≈ 2%) in faba bean (Ma et al., 2020). The fish were fed at 09:00 and 17:00 every day, with a total daily feeding amount of 3%–5% of body weight. Culture conditions were the same in all tanks: water temperature 25–30 °C, pH 6.5–7.5, and dissolved oxygen >5.0 mg/L. The above experiments were conducted at Pearl River Fisheries Research Institute, Guangzhou, China.

The experimental protocols used in this study were approved by the Animal Ethics Committee of the Guangdong Provincial Zoological Society, China, under permit number GSZ-AW012.

2.2. Sample preparation

After fasting for 24 h, 6 fishes were randomly taken from each group on days 30, 60, 90 and 120. These specimens were individually euthanized in pH-buffered tricaine methanesulfonate (250 mg/L), and subjected to the measurement to body weight to calculate the weight gain rate (WGR, %) = (final weight – initial weight) / initial weight × 100. Blood samples were immediately collected from the tail prior to euthanization and centrifuged at 3500 rpm for 10 min at 4 °C after being held at low temperature for 1 h. A clean dissection tool was used to dissect each fish, and all sampling procedures were performed on ice. We collected a portion of back white muscle (junction of the fifth dorsal fin and lateral line scales, 2 cm × 2 cm × 1 cm) for textural analysis. We also collected appropriate samples of liver, intestine, and back white muscle, kept quick freezing in liquid nitrogen and then stored at −80 °C, for the ROS determinations (Wang et al., 2012).

2.3. Determination of muscle textural parameters

Muscle textural parameters (hardness, chewiness, springiness, gumminess and adhesiveness) were measured using a Universal TA-type texture analyzer from the Tengba Instrument Company (Shanghai, China). A 36-nm cylindrical probe from the analyzer was used to test the compression speed at a pre-test, post-test, and test speed of 1.0 mm/s. The compression interval was 5 s, with a compression ratio of 30%.

2.4. Dihydroethidium (DHE) staining

Dihydroethidium (DHE) has been considered to be the most sensitive and specific dye to detect superoxide radicals in living tissues, which emits blue fluorescence upon oxidation. DHE can also be used for fixed tissues with high sensitivity and specificity (Kalyanaraman et al., 2017). Muscle tissues were frozen in Tissue-Tek OCT embedding medium (Tissue-Tek 4583, Tokyo, Japan) and cut into 5-μm-thick sections. They were fixed with 4% paraformaldehyde for 10 min and washed with distilled water or PBS. Afterwards, they were dried slightly and stored at -20°C. Approximately 50 μL of DHE solution (Sigma-Aldrich, D7008, USA) was added dropwise to each tissue section, and the sections were placed in a humidifier to avoid light. The sections were kept at 24 °C for 1 h in the humidifier and then mounted with anti-fluorescence quenching mounting tablets (Southern biotech 0100-01), examined under a light microscope and

photographed (Olympus BX53).

2.5. Determination of ROS contents

Intestine, liver and muscle samples were thawed, chopped, and weighed. They were then mixed with an appropriate amount of phosphate-buffered saline (PBS, pH 7.2–7.4), homogenized with an automatic grinder and centrifuged at 2000–3000 rpm for 20 min at 4 °C to collect the supernatant. Blood samples were allowed to stand for 4 h and were then centrifuged (1000 g, 20 min, 4 °C) to obtain the serum. The Ultra-Sensitive Fish ELISA (enzyme-linked immunosorbent assay) Kits from Sino Best Biological Technology Co., Ltd. (Shanghai, China) were used to determine the contents of total ROS (a kind of one-electron reduction products of oxygen) (Kit No. YX-041-96), ·OH (Kit No. YX-040-96), $\cdot O_2^-$ (Kit No. YX-039-96), and H_2O_2 (Kit No. YX-038-96).

2.6. ROS generation, transformation and elimination systems

Intestine, liver, muscle and blood samples were processed as described above. The Ultra-Sensitive Fish ELISA Kits from Sino Best Biological Technology Co., Ltd. (Shanghai, China) were used to analyze the levels of proteins that are related to ROS generation: Complex I (Kit No. YX-042-96), Complex III (Kit No. YX-043-96), Complex IV (Kit No. YX-044-96), and MPTP (Kit No. YX-048-96), ROS transformation: SOD (Kit No. YX-046-96). The levels of nicotinamide adenine dinucleotide phosphate(NADPH), glutathione (GSH) and catalase (CAT), all of which are known to eliminate ROS (Mittler et al., 2017), were measured by the Ultra-Sensitive Fish ELISA Kits from Sino Best Biological Technology Co., Ltd. (Shanghai, China) for NADPH (Kit No. YX-045-96), GSH (Kit No. YX-046-96), and CAT (Kit No. YX-047-96).

2.7. Mitochondrial membrane potential (ΔΨ)

Mitochondria were isolated from the muscle on days 30, 60, 90 and 120 by a Tissue Mitochondria Isolation Kit No. C3606 (Beyotime, China). Fresh fish muscle sample was used to isolate mitochondria with the extraction process on ice completely according to the Kit instructions. The fluorescent, lipophilic and cationic probes were employed to measure the mitochondrial membrane potential (Δψ), following the instructions of mitochondrial membrane potential assay Kit No. C2006 with JC-1 (Beyotime, China).

2.8. Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics 23 (SPSS Inc., Chicago, IL, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test. P value of less than 0.05 was considered to be statistically significant. The results are presented as mean ± SD.

3. Results

3.1. Growth and muscle textural profile

After 120 days, there was no significant difference for weight gain rate among the control (36.60 ± 2.50%), oxidant (O) group (37.58 ± 3.06%), and FB + antioxidants (FB+AO) (36.95 ± 1.51%), and the weight gain rate of FB group was lower than other three groups ($p < 0.05$). There was no significant difference for survival rate among the control (97.7%), oxidant (O) (97.7%), FB+AO (100%), and FB (95%) groups.

The hardness, chewiness, springiness, adhesiveness and cohesiveness of muscles were measured in the four grass carp groups fed different diets (Fig. 1). The hardness and chewiness of FB and oxidant (O) groups were higher than those of the control and FB+AO groups on days 90 and 120 ($p < 0.05$). It is notable that the hardness of the oxidant group was higher than those of other three groups on day 60 ($p < 0.05$). We found no or minor effects of diets on springiness, gumminess, and adhesiveness of grass carp muscle. Notably, there were no differences in any muscle textural parameters between the control and FB+AO groups ($p > 0.05$), indicating that the addition of antioxidants completely abolished the effect of FB feeding on muscle textural parameters.

3.2. ROS content in four tissues

Muscle, intestine, liver, and serum samples collected on days 60, 90 and 120 were used for the measurement of ROS content (Fig. 2). On days 60, the total ROS content in the muscle of the oxidant group was higher than those of the control and FB + AO groups ($p < 0.05$). On days 90 and 120, the total ROS content in the muscle of the FB and oxidant groups was higher than those of the other two groups ($p < 0.05$) (Fig. 2A). The temporal dynamics of ROS in intestine, liver,

and serum showed similar tendencies, but in these organs most differences became significant on day 60 ($p < 0.05$) (Fig. 2B, 2C, 2D). The DHE staining visualized that the ROS content of the FB and oxidant groups were higher than those of the control on days 90 and 120 (Fig. 2E). It is of note, that the ROS content of oxidant group was higher than those of other three groups on day 60 (Fig. 2E), whereas no apparent difference was found among all groups on day 30 (Supplementary Fig. S2).

The contents of each ROS species are shown in Table 2, in which we found two general tendencies: 1) There was no significant difference in H_2O_2, $\cdot O_2^-$ and ·OH between the control and FB+AO groups on any of the measurement days; and 2) The FB and oxidant groups showed higher ROS accumulation compared to the other two groups, but in a different fashion depending on tissues. Specifically, H_2O_2 contents became significantly higher in the intestine and serum by day 60 and remained high in the FB and oxidant groups (Table 2). However, in muscle and liver, H_2O_2 contents of the FB group were not significantly different from those of the control group until day 90. The oxidant group, on the other hand, showed significant increase in H_2O_2 content in the muscle and liver by day 60 compared to the control group ($p < 0.05$). The temporal trajectory of the $\cdot O_2^-$ contents had a similar tendency to those of H_2O_2 (Table 2). There was no significant difference in $\cdot O_2^-$ contents of muscle, intestine, liver and serum between the four groups on day 30 (Supplementary Table S1); $\cdot O_2^-$ contents of the intestine and liver became significantly higher in the FB group by day 60 than those of the control and FB+AO groups ($p < 0.05$). However, $\cdot O_2^-$ contents in the muscle and serum of the FB group were significantly higher than those of the control and FB+AO groups only on days 90 and 120. The time-dependent, tissue-specific accumulation was also observed for the ·OH contents (Table 2). On days 60, 90 and 120, the ·OH contents of liver and serum were significantly higher in the FB and oxidant groups than the control group ($p < 0.05$). In muscle and intestine, the significant increase in ·OH contents in the FB group was observed only on days 90 and 120 ($p < 0.05$).

3.3. The ROS generation and transformation pathway in four tissues

Muscle, intestine, liver and serum samples were collected on days 60, 90 and 120, and key enzymes in ROS generation pathways were examined for their content (Table 3). Overall, there was no significant difference in the contents of Complex I, Complex III, Complex IV, and SOD, as

well as MPTP and ΔΨ, between the control and FB+AO groups. Similar to the time-dependent changes in ROS contents, there was no significant difference in Complex I content in the muscle, intestine, liver and serum between the four groups on day 30 (Supplementary Table S2); however, Complex I content of muscle became higher than that of the control after day 60 (oxidant group) and 90 (FB group) ($p < 0.05$). In intestine, liver and serum, Complex I contents in both FB and oxidant groups became significantly higher than those of the control group by day 60 (Table 3). For Complex III contents (Table 3), there was no significant difference in the muscle, intestine, liver and serum between the four groups on day 30 (Supplementary Table S2); however, Complex III contents of muscle and intestine became higher than that of the control after day 60 (oxidant group) and 90 (FB group) ($p < 0.05$). Complex III contents in the liver and serum were higher in the FB and oxidant groups than the control on days 60, 90 and 120 ($p < 0.05$). For Complex IV contents (Table 3), there was no significant difference in the muscle, intestine, liver and serum between the four groups on day 30 (Supplementary Table S2), but Complex IV content of muscle became higher than that of the control after day 60 (oxidant group) and 90 (FB group) ($p < 0.05$), while Complex IV contents of intestine, liver and serum in the FB and oxidant groups were more than those of the control after day 60 ($p < 0.05$).

SOD is an antioxidant enzyme, but we included this enzyme in the ROS generation and transformation pathway because SOD generates H_2O_2 from $\cdot O_2^-$ (Table 3). On day 30, there was no significant difference in the muscle, intestine, liver and serum between the four groups (Supplementary Table S2); SOD contents of muscle and serum became significantly higher than those of the control after day 60 (oxidant group) and day 90 (FB group) ($p < 0.05$). Furthermore, SOD contents in the intestine and liver of the FB and oxidant groups were higher than those of the control on days 60, 90 and 120.

The openness degree of mitochondrial MPTP (membrane permeability transition pore) in muscle was generally higher in the oxidant groups than the control and FB + AO groups after day 60 ($p < 0.05$) (Table 3). The MPTP of the FB group became comparable to that of the oxidant group after day 90. The mitochondrial membrane potential (ΔΨ) in muscle showed the opposite tendency, where those of the FB and oxidant groups were lower than those of the control after day 90 ($p < 0.05$) (Table 3).

3.4. The ROS elimination pathways in four tissues

Muscle, intestine, liver, and serum tissues were collected on days 60, 90 and 120 and tested for the content of ROS elimination pathways (CAT, GSH and NADPH). The results are shown in Table 4. The overall tendency was that the FB and oxidant groups showed lower values compared to the other two groups, and this trend became clear toward the end of the feeding trial. It is also noted that there was no significant difference in CAT, GSH and NADPH contents between the control and FB+AO groups on any of the measurement days. CAT contents of muscle and liver became significantly lower than that of the control group after day 60 (oxidant group) and 90 (FB group) ($p < 0.05$) (Table 4). In the intestine and serum, the CAT content started to decrease earlier in the FB and oxidant groups, resulting in the significant difference from the control after day 60 ($p < 0.05$).

GSH content of muscle also became significantly lower than that of the control group after day 60 (oxidant group) and 90 (FB group) ($p < 0.05$) (Table 4). In intestine and liver, GSH contents of both FB and oxidant groups became lower than those of the control group after day 60 ($p < 0.05$). GSH contents in the serum showed a slow response, where those of the FB and oxidant groups became lower than that of the control on day 120 ($p < 0.05$).

Similarly, NADPH contents of muscle and liver became lower than that of the control group after day 60 (oxidant group) and 90 (FB group) ($p < 0.05$) (Table 4). In the intestine and serum, NADPH contents of both FB and oxidant groups became lower than those of the control after day 60 ($p < 0.05$).

4. Discussion

4.1. ROS-mediated regulation of the textural quality of muscles

Texture is one of the most important quality indicators for fish products, and understanding the biological processes involved in fish muscle texture will increase the production of consumer-preferred fish products, improving profitability of fisheries and aquaculture. The texture improvement in grass carp muscle after feeding with faba bean is not a species-specific

phenomenon because similar increase in muscle texture has also been found in channel catfish (*Ictalurus punctatus*), crucian carp (*Carassius auratus*) and tilapia (*Oreochromis mossambicus*) (Zhu et al., 2012; Li et al., 2007; Lun et al., 2007). So, the mechanism deserves to be explored as it contributes to the effective application of this feeding protocol in aquaculture. The present study addressed the potential causal role of ROS in the improvement of muscle texture observed in crisp grass carp fed solely with faba beans by supplementing oxidants and antioxidants. Excitingly, dietary vitamin K3, which is conversed to semiquinone and hydroquinone capable of increasing the intracellular ROS levels (Yang et al., 2018), turned out to be more convenient food additive for the muscle texture improvement in grass carp compared to faba beans. With an appropriate safety evaluation, vitamin K3 will be a promising food additive that must be tested using other fish species. In order to further apply our findings to commercial setting, it is also necessary to evaluate the effect of dietary antioxidants and oxidants (e.g., FB and vitamin K3) on other important parameters such as growth and flesh proximate composition.

This study also illustrated the spatiotemporal dynamics of ROS in grass carp fed with oxidants. The temporal association of the ROS levels and muscle texture parameters suggest that each ROS concurrently contributes to the improvement of muscle texture. Indeed, elevated H_2O_2 levels likely decrease muscle fiber diameter and disrupt collagen turnover (Yu et al., 2020) and generate a high amount of ·OH in the presence of transition metals (Shadel & Horvath, 2015). ·OH is highly reactive and causes muscle damage that activates the proliferation of satellite cells, resulting in functional modification of the myocyte (Pietrangelo, 2009; Barclay et al., 1991). Excessive ·OH also increases the transcription and synthesis of collagen (Poli, 1997). It is thus likely that the elevated ·OH increased muscle hardness in crisp grass carp by promoting satellite cell proliferation or collagen synthesis (increased collagen level has been reported in crisp grass carp, Yu et al., 2020). Together with the clear abolishing effects of antioxidants, we conclude that the accumulation of ROS is responsible for the improvement of the textural quality in grass carp.

The increase of $\cdot O_2^-$ in mitochondria likely elevated the opening degree of MPTP, and then $\cdot O_2^-$ overflowed outside the mitochondria through MPTP, causing the mitochondrial membrane potential ($\Delta\Psi$) to decrease (Table 2 and 3). This, in turn, can promote the release of cytochrome c (Huang et al., 2016; Song et al., 2017). Mitochondrial cytochrome c release and oxidized

cytochrome c have been reported to play a significant role in yak meat tenderization (Wang et al., 2018). Also, cytochrome c can enter the cytoplasm and bind to Apaf-1 to activate Caspase-3/9 and inactivate Pax7, which is a key step in the initiation of muscle differentiation programs (Mohanty et al., 2010; Dick et al., 2015). The number and density of myofibers are directly proportional to muscle hardness in the Atlantic salmon (*Salmo salar* L.) (Johnston, 2000). Therefore, we infer that the accumulation of ROS could increase the opening degree of the MPTP and cause the spillover of cytochrome c, which enhances the proliferation of myofibers and increases the muscle hardness of crisp grass carp. However, the possible role of cytochrome c in the process of muscle hardness enhancement requires further investigation.

4.2. ROS generation by ETC and ROS accumulation

Increases in Complexes I, III, and IV in the mitochondrial ETC were found in the grass carp fed with faba beans and vitamin K3 (Table 3). Complex I is the major entry point for electrons into the respiratory chain, and it produces $\cdot O_2^-$ with Complexes III and IV by ETC (Sharma et al., 2009). An increase in $\cdot O_2^-$ to a threshold level triggers the opening of one of the requisite mitochondrial channels (i.e., MPTP or inner membrane anion channel) and causes $\cdot O_2^-$ efflux from the mitochondria; this leads to the simultaneous collapse of MPTP and $\Delta\Psi$ (Zorov, 2006). This is consistent with the MPTP increase and $\Delta\Psi$ decrease detected in our study (Table 3), and it can be further inferred that the production site of $\cdot O_2^-$ is the ETC in mitochondria. Excessive $\cdot O_2^-$ increases the opening degree of the MPTP, and $\cdot O_2^-$ is transmitted outside the mitochondria through MPTP. The $\cdot O_2^-$ is then converted into H_2O_2 by SOD, and the resulting H_2O_2 can be reduced by CAT and GSH (Mittler, 2017; Weisiger & Fridovich, 1973). In this study, the levels of CAT, GSH and NADPH in the grass carp fed with faba beans and vitamin K3 were lower than those of the grass carp fed with commercial feed and antioxidants (Table 4). We therefore infer that the reduction of ROS elimination and increase of ROS produced by ETC (Complex I, III, IV) together caused the ROS accumulation in various tissues examined when fed with dietary oxidants. Consistent with this idea, feeding with antioxidants vitamin C and vitamin E, which are proven to reduce the ROS levels and prevent oxidative stress (Gupta et al., 2003), decreased the ROS production (Complex I, III, IV) and promoted the ROS elimination, and did not cause ROS accumulation, compared to faba beans digestion (Table 3). Indeed, adding vitamins C and E to

fish diets has been known to improve the antioxidant capacity (Ortuño et al., 2001). However, the specific regulatory mechanism of ROS accumulation during muscle texture improvement remains further investigation.

The above discussion is generally consistent with the concept "ROS-induced ROS-release" (RIRR), which constitutes a positive feedback mechanism for enhancing ROS production that leads to potentially significant mitochondrial damage and stimulates the further generation of ROS in surrounding mitochondria (Zorov, 2006). In this study, the instantaneous ROS detection (DHE staining) confirmed that ROS did exist in various tissues, especially in the mitochondria of muscles. In shrimp, an increase in refrigeration storage time changes muscle hardness in associated with mitochondrial damage accompanied by the destruction of Z, I and M lines (Pornrat et al., 2007). Thus, one could speculate that in the crisp grass carp ROS produced in mitochondria negatively affect mitochondrial function (ETC, MPTP, $\Delta\psi$), causing further ROS production and changes in muscle structure and hardness.

4.3. Possible ROS transitions among tissues for muscle texture regulation

The increase in muscle hardness observed in crisp grass carp is the consequence of complex biological processes involving many intermediate connections and transmissions. In the present study, we found that ROS contents increased in a time-dependent manner (30 days to 120 days). Importantly, on days 60 and 90, ROS were mainly concentrated in the intestine, liver and blood; ROS levels gradually elevated in muscle and the difference became significant on days 90 and 120 (Fig. 3). The intestine, liver and muscle are major organs involved in the digestion, absorption, and metabolism of dietary nutrients (Jobgen et al., 2006), and it is reasonable that these organs show associated responses in this order. Feeding lentils to rats can induce changes in the protein synthesis of the intestines and stimulate corresponding changes in the protein synthesis function of the liver and muscle (Combe et al., 2004). Adding nutrients (polyunsaturated fatty acids) to the diet of cattle changes intestine function as well as the secretion of corresponding chylomicrons, and in turn stimulates liver function and affects muscle metabolism through blood transport (Hocquette & Bauchart, 1999). Also, ROS (H_2O_2) generated by the intestine can reach the liver through blood circulation, causing oxidative stress and increasing the expression of cystine/glutamate antiporters in the liver, thereby affecting the synthesis of skeletal muscle protein

(Bilinsky et al., 2015). It could be inferred that, in the crisp grass carp, certain pro-oxidants in faba beans (vicine and convicine) are first absorbed by intestine, causing the ROS accumulation and damage (Getachew et al., 2018), in the intestine and liver. The ROS (or oxidant substance) themselves or the "RIRR" effect of ROS (or oxidant substance) are then gradually transmitted to the muscle, resulting in changes in muscle texture. A similar phenomenon has been observed in mice, in which ingested nutrients (high-fat diet) are initially absorbed by the intestines and then transported to the liver through blood circulation, eventually resulting in changes in muscle metabolism (Dyar et al., 2018). The addition of selenium yeast to the diet of rainbow trout (*Oncorhynchus mykiss*) also up-regulated the expression of selenoproteins in the liver and muscle, which may be due to the intestinal absorption of selenium and blood circulation (Wang et al., 2018). However, in fish, the relations between ingested nutrients and digestive process have rarely been reported, and it deserves further exploration in the future.

4.4. Limitation

This study has several limitations. First, we used two different base diets, FB and commercial feed, to formulate the four experimental diets. Our major conclusion, the regulatory role of ROS in the improvement of muscle texture, must be still valid because it is derived from the comparison between the FB and FB + AO groups, and the comparison between the C and O groups. However, it is possible that some differences observed in this study are attributed to the nutritional difference between the base diets (i.e., nutritional deficiency of FB compared to the commercial diet). Another limitation is that we used frozen samples to quantify ROS levels. Because this is a protocol specified by the manufacturer, and because all samples were treated in the same way, the ROS quantification in this study must be robust against variations. However, we cannot rule out the possibility that the amount of ROS, especially highly unstable $\cdot O_2^-$ and ·OH, may be underestimated.

4.5. Conclusion

Texture improvement in grass carp fed with faba beans was associated with the increase in ROS content in muscle, and dietary antioxidants almost completely abolished the effect. Interestingly, oxidant supplementation effectively improved the textural quality and ROS levels, suggesting that vitamin K3 could be a functional feed additive for the improvement of muscle textural quality in

freshwater fishes. We also showed the possible transmissions of ROS from intestine, liver to muscle by blood circulation. The increase in muscle texture may be caused by the accumulation of different ROS species produced mainly by mitochondria. However, the specific mechanisms of muscle texture changes caused by ROS need further clarifications (e.g. Cytochrome c, Apaf-1, Caspase-3/9). Combined with cell culture technology, effect of ROS exposure should be examined using muscle cells to verify the proposed molecular mechanism.

Competing interests

The authors have declared no conflict of interests.

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Appendix A. Supplementary data

Supplementary Tables and Figures accompanies this paper were provided.

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Figure legends

Fig.1. Muscle textural profile analysis (A-E) of different treated groups on days 60, 90, 120, respectively. C, commercial feed group; FB, whole Faba beans group; FB+AO, Faba beans + antioxidants group; O, commercial feed + oxidant group. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's test (n = 6), * $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$.

Fig.2. Total reactive oxygen species (ROS) contents and Dihydroethidium (DHE) dyeing of ROS in the different treated groups on days 60, 90, 120, respectively. C, commercial feed group; FB, whole Faba beans group; FB+AO, Faba beans + antioxidants group; O, commercial feed + oxidant group. (A) Total ROS of the muscle; (B) Total ROS of the intestine; (C) Total ROS of the liver; (D) Total ROS of the serum; (E) DHE dyeing of ROS in the muscle on days 60, 90, 120, respectively. DHE ox (oxidized DHE) demonstrates high levels of ROS. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's test (n = 6), * $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$.

Fig.3. ROS generation and accumulation among the different tissues of the grass carp fed with faba beans. ·OH, hydroxyl radical, H_2O_2, hydrogen peroxide; CAT, catalase; GSH, glutathione; NADPH, reduced nicotinamide adenine dinucleotide phosphate; SOD, superoxide dismutase protein; $\cdot O_2^-$, superoxide, MPTP, mitochondrial membrane permeability pore; $\Delta\Psi$, mitochondrial membrane potential.

Table 1

The ingredients and proximal composition of experimental diets.

				FB+AO	
	C	FB	O	FB	AO
Faba beans	0	1000	0	1000	0
fish meal (g/kg)	50	0	50	0	50
soybean meal (g/kg)	240	0	240	0	240
rapeseed meal (g/kg)	200	0	200	0	200
cottonseed meal (g/kg)	80	0	80	0	80
rice bran (g/kg)	150	0	150	0	150
wheat flour (g/kg)	180	0	160	0	172.5
Soybean oil (g/kg)	30	0	30	0	30
choline chloride (g/kg)	20	0	20	0	20
mineral mixture (g/kg)	20	0	20	0	20
vitamin mixture (g/kg)	30	0	30	0	30
Vitamin K3 (g/kg)	0	0	20	0	0
Vitamin C (g/kg)	0	0	0	0	5
Vitamin E (g/kg)	0	0	0	0	2.5
Feeding amounts	100%	100%	100%	90%	10%
Crude protein (g/100g)	23.3	28	28.5	28	28.8
Crude fat (g/100g)	5	1.4	4.9	1.4	5

Note: C, commercial feed group; FB, Faba beans group; FB+AO, Faba beans + anti-oxidant feed group; O, commercial feed + oxidant group. Mineral mixture: iron 5 g/kg, copper 100 mg/kg, zinc 1.5 g/kg, manganese 0.5 g/kg, magnesium 20 g/kg, iodine 15 mg/kg, cobalt 5 mg/kg, selenium 5 mg/kg, and sodium, calcium, etc. Vitamin mixture: vitamin A 180000 IU/kg, vitamin D3 40000 IU/kg, vitamin E 1.6g/kg、vitamin K3 0.0.5g/kg, vitamin B1 0.2 g/kg, vitamin B2 0.25g/kg, vitamin B6 0.2g/kg, vitamin B12 0.65 mg/kg, vitamin C 2.5g/kg, niacin 0.65 g/kg, calcium pantothenate 0.65g/kg, folic acid 0.03g/kg, Inositol 1g/kg, Biotin H 8.3 mg/kg.

Table 2

Contents of H_2O_2, $\cdot O_2^-$ and ·OH of different issues in four groups on different periods.

		60 d				90 d				120 d			
		C	FB	FB + AO	O	C	FB	FB + AO	O	C	FB	FB + AO	O
H_2O_2 (nmol/L)	muscle	38.98±3.15^{b}	45±2.43^{b}	39.41±1.32^{b}	56.51±4.42^{a}	41.36±3.74^{b}	65.72±2.19^{a}	41.63±2.34^{b}	68.[illegible]±[illegible]^{a}	46.32±6.96^{b}	71.13±7.83^{a}	43.14±4.33^{b}	76.07±1.69^{a}
	intestine	52.46±1.11^{c}	64.83±3.34^{b}	47.1±5.01^{c}	79.14±5.27^{a}	49.66±4.17^{c}	72.35±6.27^{b}	45.93±4.72^{c}	8[illegible]±3.11^{a}	55.33±4.93^{c}	74.09±5.41^{b}	55.99±2.8^{c}	91.67±5.52^{a}
	liver	66.83±4.94^{c}	80.19±1.63^{bc}	69.55±6.8^{c}	96.44±3.53^{a}	72.85±4.24^{b}	95.37±3.08^{a}	72[illegible]^{b}	95.51±2.68^{a}	68.6±7.82^{b}	89.89±4.33^{a}	66.99±3.06^{b}	95±6.27^{a}
	blood	82.12±7.22^{b}	102.77±3.75^{a}	78.83±8.42^{b}	109.7±6.05^{a}	85.79±5.54^{ab}	107.3±10.01^{a}	[illegible]±4.91^{b}	115.62±9.39^{a}	84.19±6.25^{b}	109.9±5.64^{a}	84.93±3.15^{b}	122.82±15.07^{a}
$\cdot O_2^-$(nmol/L)	muscle	162.79±14.39^{a}	197.25±35.55^{a}	152.31±39.03^{a}	229.36±16.94^{a}	186.39±18.25^{b}	[illegible]±29.[illegible]^{a}	154.94±25.08^{b}	302.34±22.5^{ab}	161.57±16.64^{b}	286.27±37.81^{a}	160.87±16.07^{b}	300.92±12.6^{a}
	intestine	278.29±25.02^{b}	348.97±43.84^{a}	249.51±20.2^{ab}	415.98±35^{ab}	256.41±[illegible]6.9^{b}	[illegible]02.33±18.71^{a}	246.02±41.16^{b}	444.4±24.45^{b}	282.77±13.78^{b}	419.46±45.78^{a}	257.81±37.5^{b}	385.44±29.87^{b}
	liver	396.8±35.84^{b}	523.5± 18^{a}	417.11±65.32^{b}	470.75±13.08^{a}	396.[illegible]±[illegible]6.7^{b}	537.02±47.93^{a}	400.19±40.88^{b}	552±41.72^{a}	388.16±30.06^{b}	531.45±13.42^{a}	402.41±40.34^{b}	531.88±35.77^{a}
	blood	410.53±34.99^{a}	509.91±26.01^{a}	396.91±15.35^{a}	532.73±24.11^{a}	41[illegible]±[illegible]4.8^{c}	565.66±33.37^{a}	411.35±17.08^{c}	591.57±9.61^{ab}	398.08±31.13^{b}	564.69±22.7^{a}	410.68±28.17^{b}	565.48±35.97^{a}
·OH (nmol/L)	muscle	266.54±26.34^{a}	280.27±29.78^{a}	260.99±30.75^{a}	328.[illegible]±15.[illegible]3^{a}	250.15±23.86^{b}	382.35±28.53^{a}	274.86±39.02^{b}	352.48±48.33^{a}	261.32±21.16^{b}	375.25±58.45^{a}	247.66±27.23^{b}	374.36±49.46^{a}
	intestine	260.72±22.71^{b}	372.96±22.75^{a}	292.5 ± 15.[illegible]^{b}	284.[illegible]±52.29^{a}	249.49±34.09^{b}	406.57±26.95^{a}	267.58±30.97^{b}	299.27±35.29^{a}	271.02±9.87^{b}	439.43±44.18^{a}	270.87±25.11^{b}	311.7±8.9^{a}
	liver	253.25±12.72^{b}	393.64±21.73^{a}	266.24±36.14^{a}	381.68±20.84^{a}	269.56±15.64^{b}	430.14±45.91^{a}	280.82±36.32^{b}	411.43±16.6^{a}	269.92±13.86^{b}	433.34±35.64^{a}	286.88±19.09^{b}	454.3±46.05^{a}
	blood	268.46±31.24^{b}	359.84± 34.5^{a}	273.59±33.86^{b}	359.4±39.88^{a}	286.43±8.95^{b}	394.8±26.79^{a}	276.59±11.86^{b}	348.36±35.64^{a}	278.76±19.52^{b}	399.41±41.2^{a}	262.36±59.08^{b}	412.45±10.66^{a}

Note: C, commercial feed group; FB, Faba beans group; FB+AO, Faba beans + anti-oxidants group; O, commercial feed + oxidant group. (A) H_2O_2, hydrogen peroxide; (B) $\cdot O_2^-$, superoxide; (C) ·OH, hydroxyl radical. Values (mean ± SD) of the same row with different superscripts of the same period were significantly different ($p < 0.05$).

Table 3

Complexes contents, SOD, MPTP and ΔΨ in four groups on different periods.

		60 d				90 d				120 d			
		C	FB	FB +AO	O	C	FB	FB +AO	O	C	FB	FB +AO	O
Complex I (mmol/L)	muscle	28.55±2.49^{b}	36.33±4.89^{ab}	31.64±1^{ab}	37.97±2.08^{a}	29.01±1.27^{c}	42.77±6.68^{a}	31.27±2.2^{bc}	40.64±1.72^{ab}	29.38±1.73^{b}	42.93±3.35^{a}	32.33±1.98^{b}	48.83±3.01^{a}
	intestine	35.89±3.26^{b}	50±3.84^{a}	39.31±0.85^{b}	56.57±3.94^{a}	33.33±1.22^{c}	56.27±6.38^{a}	40.35±1.86^{bc}	44.54±3.92^{c}	38.01±1.37^{b}	60.34±4.12^{a}	39.93±2.69^{b}	63.34±3.91^{a}
	liver	40.43±2.64^{b}	56.21±3.66^{a}	43.63±4.06^{ab}	54.13±7.29^{a}	42.07±2.65^{c}	57.5±4.4^{ab}	46.96±3.05^{bc}	65.4±3.92^{a}	46.98±3.36^{b}	63.58±5.89^{ab}	50.1±3.56^{b}	71.14±9.96^{a}
	blood	58.34±3.28^{b}	77.76±4.38^{a}	60.53±6.3^{b}	79.14±5.3^{a}	61.44±3.81^{b}	80.83±4.42^{a}	61.1±4.51^{b}	75.57±4.15^{a}	62.37±4.82^{b}	85.64±4.24^{a}	61.82±3.3^{b}	81.4±3.58^{a}
Complex II (mmol/L)	muscle	8.63±1.09^{b}	10.64±1.09^{ab}	8.07±2.39^{ab}	12.65±1.38^{a}	9.1±0.31^{b}	14.32±0.96^{a}	11.56±1.46^{b}	15.65±0.97^{a}	9.33±1.35^{b}	15.22±1.29^{a}	11.43±0.67^{b}	16.7±0.79^{a}
	intestine	11.64±2^{b}	15.56±1.19^{ab}	13.43±1.68^{ab}	17.13±2.35^{a}	10.94±1.91^{b}	15.94±1.^{ab}	12.84±1.34^{ab}	20.04±3^{a}	11.37±1.84^{c}	16.78±1.64^{b}	12.2±0.51^{c}	20.61±0.56^{a}
	liver	14.5±1.66^{b}	19.1±1.21^{a}	16.41±1.6^{ab}	19.24±0.65^{a}	14.25±1.62^{b}	20.1±2.4^{a}	14.89±1.49^{b}	21.48±1.2^{a}	13.95±1.9^{b}	20.71±2.28^{a}	15.11±1.63^{b}	24.37±1.44^{a}
	blood	19.92±1.04^{b}	24.38±1.15^{a}	21.89±1.47^{ab}	24.75±1.18^{a}	19.93±1.38^{b}	25.49±1.98^{a}	20.75±0.51^{b}	26.56±1.26^{a}	20.07±1.26^{b}	26.18±0.97^{a}	21.28±0.94^{b}	26.59±1.04^{a}
Complex IV (mmol/L)	muscle	7.41±0.98^{b}	8.51±1.26^{ab}	8.04±1.29^{ab}	10.85±0.58^{a}	8.3±0.2^{b}	13.97±1.81^{a}	9.64±0.78^{b}	13.64±0.78^{a}	7.69±0.67^{b}	14.86±1.2^{a}	9.49±1.14^{b}	15.48±1.99^{a}
	intestine	11.44±0.68^{b}	15.53±1.1^{a}	11.95±1.75^{b}	16.22±0.59^{a}	11.38±0.75^{b}	17.07±1.44^{a}	12.31±1.08^{b}	18.18±1.66^{a}	11.29±1.18^{b}	18.89±1.03^{a}	11.67±1.4^{b}	19.12±1.74^{a}
	liver	10.7±1.84^{b}	15.8±0.22^{a}	11.64±1.3^{b}	17.73±0.38^{a}	11.8±0.89^{c}	16.7±1.72^{a}	12.32±1.92^{b}	17.86±1.11^{a}	10.79±1.66^{b}	17.88±1.46^{a}	11.78±1.34^{b}	20.44±1.03^{a}
	blood	17.67±0.57^{c}	22.2±0.61^{a}	19.56±0.85^{b}	21.84±1.13^{a}	18.1±0.73^{b}	24.96±1.95^{a}	19.4±0.82^{b}	24.38±0.64^{a}	17.71±0.96^{b}	25.18±1.86^{a}	19.27±2.2^{b}	25.56±1.14^{a}
SOD (mmol/L)	muscle	198.84±2.99^{b}	206.38±5.52^{b}	193.75±5.96^{b}	220.16±3.^{a}	199.53±7.76^{b}	237.02±5.9^{a}	203.44±7.03^{b}	239.2±7.6^{a}	200.8±5.21^{b}	241.73±6.99^{a}	199.2±9.33^{b}	254.67±7.93^{a}
	intestine	180.36±8.05^{b}	211.68±3.56^{a}	183.23±6.02^{b}	210.58±3.^{a}	174.39±4.92^{b}	226.85±6.24^{a}	182.51±6.18^{b}	233.28±9.26^{a}	187.63±9.21^{b}	240.32±6.36^{a}	189.49±4.75^{b}	248.02±6.43^{a}
	liver	181.18±3.27^{b}	210.27±8.53^{a}	182.08±7.51^{b}	223.82±6.66^{a}	176.44±2.69^{b}	217.77±6.66^{a}	180.69±1.86^{b}	218.37±2.91^{a}	183.33±5.61^{b}	228.41±5.83^{a}	184.7±6.12^{b}	230.56±3.24^{a}
	blood	131.9±3.67^{b}	142.91±4.92^{ab}	134.82±4.59^{b}	148.39±3.66^{a}	127.03±4.14^{c}	148.36±5.34^{ab}	137.2±4.92^{bc}	155.94±5.33^{a}	129.95±3.55^{b}	153.77±6.68^{a}	132.11±2.26^{b}	155.67±4.5^{a}
MPTP	muscle	192.05±14.46^{b}	253.8±33.34^{b}	218.3±31.15^{b}	355.3±24.64^{a}	187.66±8.36^{b}	283.18±11.3^{a}	157.98±36.44^{b}	247.94±31.02^{a}	188.72±9.11^{b}	297.87±14.11^{a}	171.38±23.48^{b}	311.28±44.01^{a}
ΔΨ	muscle	19444.51±235.79^{b}	18325.15±794.43^{b}	19837.91±199.52^{b}	17958.34±782.39^{a}	19506.62±373.54^{a}	18361.46±339.05^{b}	19381.93±443.37^{a}	17795.13±201.52^{b}	19338.72±443.02^{a}	17882.19±207.8^{b}	19733.42±470.18^{a}	17274.86±298.87^{b}

Note: C, commercial feed group; FB, Faba beans group; FB+AO, Faba beans + anti-oxidants group; O, commercial feed + oxidant group. SOD, superoxide dismutase; MPTP, mitochondrial membrane permeability pore; ΔΨ, mitochondrial membrane potential. Values (mean ± SD) of the same row with different superscripts of the same period were significantly different ($p < 0.05$).

Table 4

Contents of GSH, CAT, and NADPH of different tissues in four groups on different periods.

		60 d				90 d				120 d			
		C	FB	FB + AO	O	C	FB	FB + AO	O	C	FB	FB + AO	O
GSH (mmol/L)	muscle	10.01±0.77^{a}	8.72±1.02^{a}	9.51±1^{a}	7.22±0.44^{b}	9.95±0.79^{a}	7.64±0.46^{b}	10.15±0.52^{a}	7.16±0.[illegible]^{b}	9.79±0.33^{a}	7.21±0.37^{b}	9.97±0.95^{a}	6.53±0.3^{b}
	intestine	10.87±0.53^{ab}	8.69±0.98^{b}	11.37±0.75^{a}	8.26±1.1^{b}	10.76±0.39^{a}	8.21±0.6^{b}	10.89±0.78^{a}	8.[illegible]9±0.47^{b}	10.96±0.52^{a}	8.93±0.26^{b}	10.94±0.89^{a}	8.3±0.77^{b}
	liver	14.71±0.87^{a}	11.55±0.44^{b}	14.88±0.76^{a}	11.4±0.76^{b}	15.6±1.41^{a}	11.95±0.88^{b}	15.[illegible]±0.5^{a}	10.82±0.96^{b}	14.69±1.37^{a}	10.57±1.05^{b}	14.9±0.28^{a}	11.01±0.99^{b}
	blood	12.08±0.53^{a}	11.57±0.86^{a}	10.82±0.6^{a}	10.69±0.4^{a}	12.01±0.97^{a}	9.99±0.76^{ab}	11.[illegible]±0.96^{a}	8.61±0.79^{b}	11.75±1.61^{a}	8.81±0.71^{b}	12.49±0.53^{a}	7.71±0.82^{b}
CAT (mmol/L)	muscle	47.02±2.48^{a}	44.19±2.96^{a}	43.84±3.73^{a}	35.62±2.85^{b}	46.67±2.74^{a}	35.[illegible]±2.[illegible]^{b}	47.37±2.4^{a}	34.2±4.2^{b}	44.48±2.27^{a}	33.95±2.09^{b}	45.48±2.44^{a}	30.55±3.34^{b}
	intestine	61.63±2.22^{a}	51.43±2.25^{b}	64.66±4.58^{a}	52.08±1.27^{b}	58.82±1.61^{a}	46.38±1.17^{b}	60.35±1.53^{a}	45.81±2.88^{b}	60.22±2.86^{a}	49.63±2.96^{b}	61.73±4.77^{a}	42.92±3.67^{b}
	liver	73.48±4.44^{a}	65.21±3.72^{ab}	75.02±3.1^{a}	65.18±2.19^{b}	7[illegible]±2.[illegible]^{ab}	61.17±4.11^{b}	75.32±5.04^{a}	57.77±2.9^{c}	72.99±4.54^{a}	61.94±2.42^{b}	75.99±4.64^{a}	54.61±2.25^{b}
	blood	82.48±4.09^{a}	70.4±3.63^{b}	80±4.58^{ab}	68.42±4.0[illegible]^{b}	80.[illegible]±4.85^{a}	67.78±4.69^{b}	81.27±6.28^{a}	63.56±2.77^{b}	80.3±5.6^{a}	63.68±0.44^{b}	81.9±5.06^{a}	59.2±5.1^{b}
NADPH (mmol/L)	muscle	12.37±0.78^{a}	10.56±0.87^{a}	12.09±1.75^{a}	9.14[illegible]^{b}	12.37±0.65^{a}	8.34±0.78^{b}	12.34±0.84^{a}	7.99±1.1^{b}	12.2±1.15^{a}	9.57±1.03^{b}	12.17±0.65^{a}	8.83±0.72^{b}
	intestine	12.85±0.86^{a}	9.51±1.24^{b}	12.05[illegible]93^{a}	9.32±1.01^{b}	13.7±1.11^{a}	10.68±0.92^{b}	13.34±0.49^{a}	10.32±0.52^{b}	13.14±1.58^{a}	10.42±0.56^{bc}	12.43±0.72^{ab}	9.68±0.18^{c}
	liver	12.42±0.58^{a}	10.27±1^{ab}	11.96±0.9[illegible]^{a}	9.57±0.67^{b}	11.81±0.7^{a}	9.42±0.69^{b}	11.74±0.68^{a}	9.2±1.08^{b}	12.03±0.57^{a}	9.07±0.63^{b}	11.51±0.54^{a}	9.06±0.35^{b}
	blood	20.14±0.77^{a}	16.04±0.76^{b}	19.92±0.55^{a}	16.23±1.14^{b}	20.33±1.15^{a}	16.72±1.25^{b}	20.28±0.65^{a}	15.93±0.51^{b}	20.07±1.53^{a}	16.83±0.82^{b}	20.15±0.94^{a}	16.51±0.68^{b}

Note: C, commercial feed group; FB, Faba beans group; FB+AO, Faba beans + anti-oxidants group; O, commercial feed + oxidant group. GSH, glutathione; CAT, catalase; NADPH, reduced nicotinamide adenine dinucleotide phosphate. Values (mean ± SD) of the same row with different superscripts of the same period were significantly different ($p < 0.05$).

Author statement

Lunjian Chen: Writing-original draft, Data curation, Formal analysis. **Gen Kaneko**: Writing-original draft, Writing-review and editing. **Yichao Li**: Writing-original draft, Formal analysis. **Jun Xie**: Funding acquisition. **Guangjun Wang**: Investigation. **Zhifei Li**: Data curation. **Jingjing Tian**: Methodology. **Kai Zhang**: Investigation. **Wangbao Gong**: Formal analysis. **Yun Xia**: Methodology. **Ermeng Yu**: Conceptualization, Writing-original draft, Writing-review and editing.

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Dietary oxidant effectively improved the muscle textural quality of grass carp
- Dietary antioxidants almost abolished the texture improvement induced by oxidants
- ROS time-dependently accumulation from four tissues improved the textural quality
- Positive feedback was found between ROS accumulation and production in mitochondria

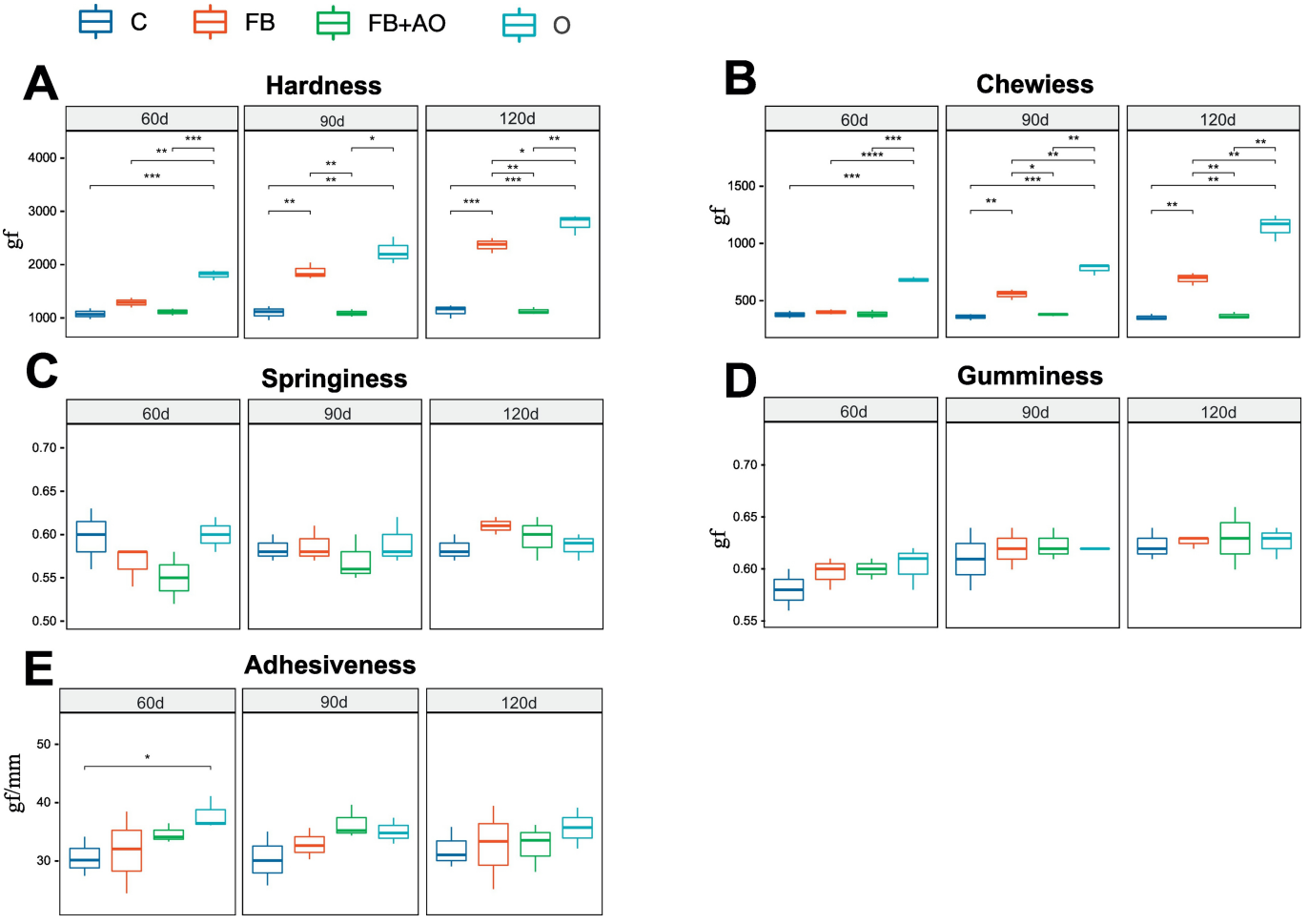
C
FB
FB+AO
O
A
Hardness
60d
90d
120d
gf
4000
3000
2000
1000
B
Chewiess
1500
1000
500
C
Springiness
0.70
0.65
0.60
0.55
0.50
D
Gumminess
E
Adhesiveness
gf/mm
50
40
30

Figure 1

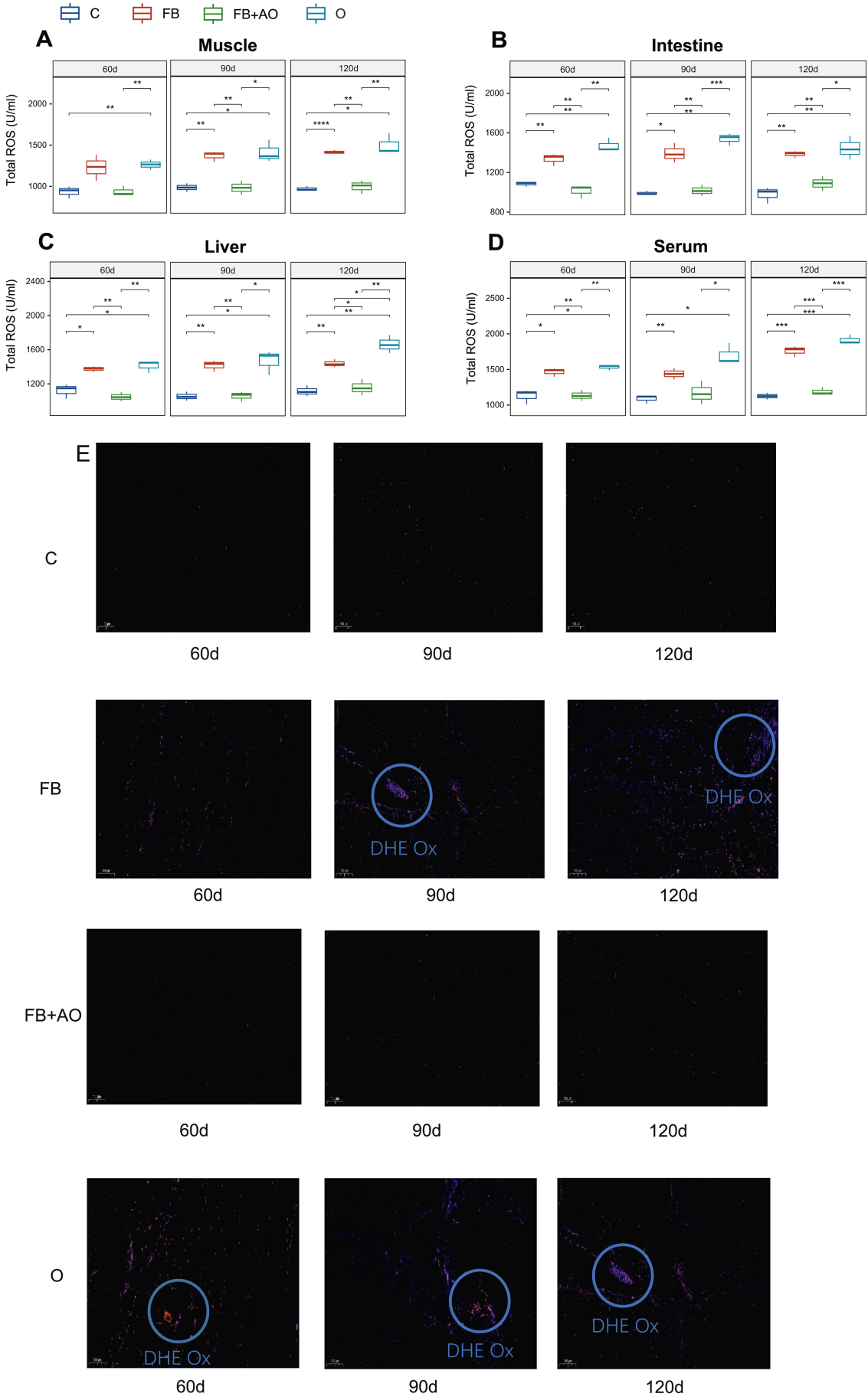
C
FB
FB+AO
O
A
Muscle
B
Intestine
C
Liver
D
Serum
60d
90d
120d
Total ROS (U/ml)
E
DHE Ox

Figure 2

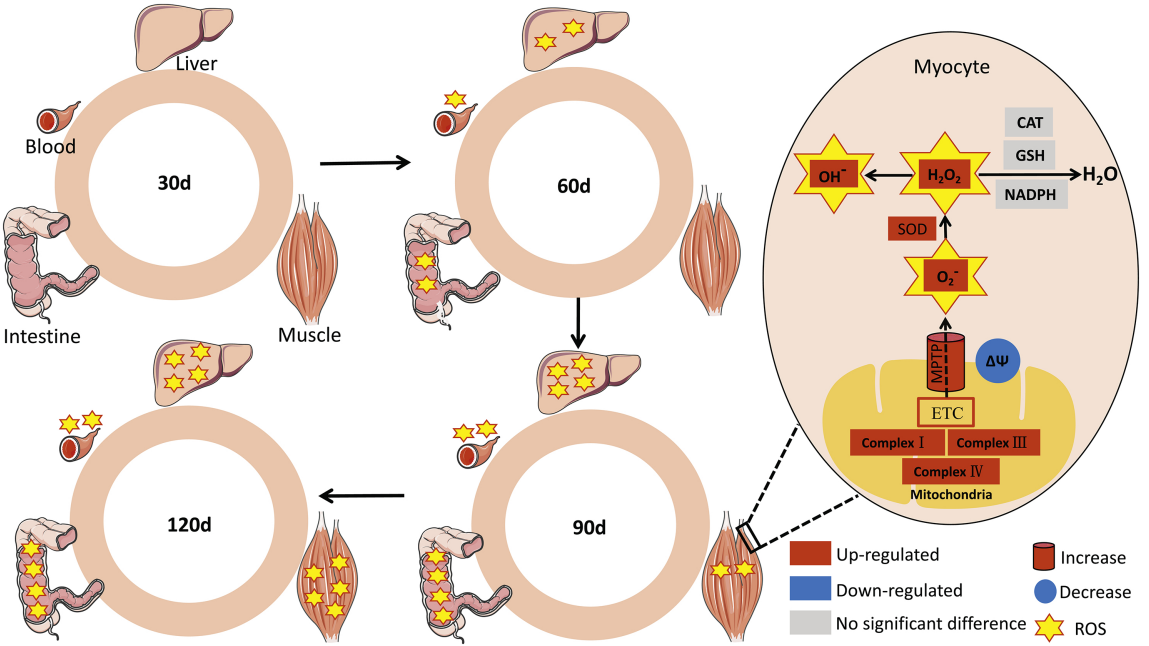
Liver
Blood
30d
Intestine
Muscle
60d
90d
120d
Myocyte
CAT
GSH
NADPH
OH^-
H_2O_2
H_2O
SOD
O_2^-
MPTP
ΔΨ
ETC
Complex Ⅰ
Complex Ⅲ
Complex Ⅳ
Mitochondria
Up-regulated
Down-regulated
No significant difference
Increase
Decrease
ROS

Figure 3