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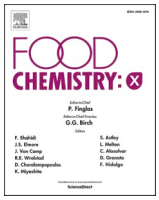

Dietary iron affects lipid deposition, nutritional element, and muscle quality in coho salmon (*Oncorhynchus kisutch*)

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ABSTRACT

We investigated effect of dietary iron (Fe) on the lipid deposition, nutritional element, and muscle quality in coho salmon. Four level Fe diets at 23.7, 46.4, 77.3, and 127.7 mg/kg were fed to the post-larval coho salmon for 12 weeks. Our results showed that dietary Fe decreased the content of triglyceride and the activity of fatty acid synthetase, ATP-citrate lyase, and acetyl-CoA carboxylase. The content of Fe in muscle was increased with increasing dietary Fe levels, and dietary Fe affected the content of nutritional elements. In addition, dietary Fe levels affected the composition of fatty acids and the content of free amino acids, and increased muscle fiber size. The lower dietary Fe levels also affected the hardness, chewiness, resilience, springiness, cohesiveness, and gumminess of salmon muscle. In all, dietary Fe inhibited the lipid deposition and affected the content of nutritional element and muscle quality in coho salmon.

Introduction

As one of the nutritional metal elements, iron (Fe) is essential for various physiological functions in animals, especially for energy production and oxygen transport (Loréal et al., 2014; Marku et al., 2021). In addition, Fe is a component of functional proteins and a key element for ferritin formation, hemoglobin, and transferring (Marku et al., 2021; Vogt et al., 2021). Fe also plays a role in the fish immune system and protects fish species from disease and infection (Chandrapalan & Kwong, 2021; Guo et al., 2019). However, the overload of Fe is toxic for aquatic fish. It has been found that Fe overload induced lipid peroxidation and decreased the activity of super oxide dismutase and catalase in freshwater fish *Labeo rohita* (Singh, Barman, Devi, Devi, & Pandey, 2019).

It is known that the nutritional requirement of Fe is mainly from diet to keep the normal growth of fish (Isibor et al., 2020; Oğuz & Yeltekin, 2014). Then, the assimilated Fe combines with transferrin and is transported to the target organs of fish (Bury & Grosell, 2003). Nevertheless, excessive Fe in diet may lead to the over-accumulation of Fe in fish body (Yadav, Sinha, Egnew, Romano, & Kumar, 2020). The over-accumulation of Fe may cause tissue damage and immune response in fish species (Singh et al., 2019). In addition, the deficiency of Fe also has negative effects on the immune function and growth of fish (Guo et al., 2017, 2019). It is confirmed that Fe deficiency significantly affects the growth and feed utilization of rainbow trout (Evliyaoğlu et al., 2022).

Moreover, the lipid content in muscle has a remarkable influence on the muscle quality of fish. Previously, we have observed that dietary Fe affected the growth performance and body composition of coho salmon (Yu et al., 2021). Nevertheless, the effect of Fe on lipid deposition, nutritional element, and muscle quality remains unknown in coho salmon. Thus, the aims of our present study were to examine effect of dietary Fe on the lipid deposition, nutritional element, and muscle quality in coho salmon *Oncorhynchus kisutch*.

Materials and methods

Experimental diets

The ingredients were ground into fine powder through 88 μm mesh, added into four levels of ferrous sulphate ($FeSO_4 \cdot 7H_2O$), and mixed

Abbreviations: ACC, acetyl-CoA carboxylase; ACL, ATP-citrate lyase; FAS, fatty acid synthetase; TG, triglyceride.

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according to Table 1. Four levels of Fe diets were selected according to our previous study on coho salmon (Yu et al., 2021). We produced the stiff dough by blending fish oil and soybean oil, then added the suitable water (350 g/kg ingredients) into the diets and made pellets. The pellets were dried at room temperature, ground and sieved into particle size of 2.0 × 3.0 mm, and stored at −20 °C for later usage. The final dietary Fe concentration was 23.7, 46.4, 77.3, and 127.7 mg/kg diet after analyzed with an inductively-coupled plasma emission spectrometer (Agilent 5100, USA), respectively.

Animals and experimental procedure

We obtained coho salmon from a hatchery in Linyi municipality, Shandong Province, China. Then we put 1200 coho salmon (0.35 ± 0.02 g) into twelve plastic tanks (80 × 60 × 60 cm, L × W × H, 240 L) and there were 100 fish in each tank. A flow-through rearing system was used for fish culture with a natural light–dark cycle and continuous aeration. The temperature was 15.5 ± 0.5 °C, dissolved oxygen was 9.5 ± 0.8 mg/L, and water pH was 6.9 ± 0.3. We fed the salmons four times daily with apparent satiation at 7:00, 10:30, 14:00 and 17:30 for 12 weeks.

Sampling procedures

12 weeks later, 16 fish in each tank were euthanized with 150 mg/L MS222. The MS-222 is one of the most commonly used euthanasia agents in fish species, and it is listed as an approved euthanasia method by American Veterinary Medical Association. After fish were euthanized, five dorsal muscle samples (each sample pooled from 3 fish) were sampled from 15 fish, which were used for the following biochemical index analysis, fatty acid analysis, free amino acid analysis, nutritional metal element analysis, and texture analysis, respectively. In addition, another one dorsal muscle sample was sampled from one fish in each tank for the histological analysis. The protocols were approved by the Animal Care and Use Committee of Shandong University of Technology (No. 20200601).

Table 1
Formulation and proximate composition of the experimental diets (% dry matter).

Ingredients (g)	Dietary iron level (mg/kg)			
	23.7	46.4	77.3	127.7
Casein[1]	40	40	40	40
Gelatine[1]	10	10	10	10
Dextrin[1]	16	16	16	16
α-Cellulose[1]	9.5	9.38	9.23	8.98
Fish oil[2]	7.5	7.5	7.5	7.5
Soybean oil[2]	7.5	7.5	7.5	7.5
Mineral mix[3]	6	6	6	6
Vitamin mix[4]	1	1	1	1
L-Arg	1	1	1	1
Antioxidant	0.6	0.6	0.6	0.6
Met DL-Met	0.5	0.5	0.5	0.5
Choline chloride	0.3	0.3	0.3	0.3
Vc phosphate	0.05	0.05	0.05	0.05
Glycine Betaine	0.05	0.05	0.05	0.05
Ferrous Sulphate	0	0.12	0.27	0.52
Proximate composition (%)				
Crude protein	41.76	41.59	41.71	41.61
Crude fat	12.11	12.12	12.11	12.03
Ash	5.52	5.41	5.40	5.88
Moisture	7.46	7.29	7.39	7.39
Fe	23.7	46.4	77.3	127.7

[1] Ingredients were obtained: Casein from Sigma Chemical, St. Louis, MO., USA (crude lipid 0.84%, crude protein 92.24%); Dextrin from Shandong Xiwang Sugar Co., ltd., Shandong, China; Gelatin from Shandong Yixin Biological Technology Co., ltd., Shandong, China; α-Cellulose from Shanghai Lanping Industrial Co., ltd., Shanghai, China.

[2] Provided by Shandong Conqueren Marine Technology Co., ltd., Weifang, China.

[3] Composition (g kg^{-1} mineral premix): $KAl(SO_4)_2{\cdot}12H_2O$, 124.0; $CoCl_2{\cdot}6H_2O$, 49.0; $CaCl_2$, 17880.0; $FeSO_4{\cdot}7H_2O$, 707.0; $MgSO_4{\cdot}7H_2O$, 4317.0; KCl, 1192.0; KI, 5.0; NaCl, 4934.0; $MnSO_4{\cdot}4H_2O$, 31.0; $ZnSO_4{\cdot}7H_2O$, 177.0; $Ca(H_2PO_4)_2{\cdot}H_2O$, 12457.0; $Na_2SeO_3{\cdot}H_2O$, 3.0; KH_2PO_4, 9930.0.

[4] Composition (IU or g kg^{-1} vitamin premix): cholecalciferol, 4,000 IU; α-tocopherol, 75.0 IU; retinal palmitate, 10,000 IU; thiamine-HCl, 40.0 g; riboflavin, 30.0 g; menadione, 22.0 g; pyridoxine-HCl, 20.0 g; D-biotin, 1.0 g; D-calcium pantothenate, 150.0 g; ascorbic acid, 200.0 g; *meso*-inositol, 500.0 g; folic acid, 15.0 g; niacin, 300.0 g; cyanocobalamin, 0.3 g.

Biochemical index analysis

The content of triglyceride (TG) and the activity of fatty acid synthetase (FAS), ATP-citrate lyase (ACL), and acetyl-CoA carboxylase (ACC) were detected with commercial kits. The kits TG (ZC-S0411), FAS (ZC-S0597), ACL (ZC-S0861), and ACC (ZC-S0775) were obtained from Shanghai Zhuocai biology Co., ltd (Shanghai, China). In addition, we determined the protein concentration with the method of coomassie brilliant blue G250 staining, and Bradford assay was performed following with a curve of bovine serum albumin (BSA).

Fatty acid analysis

The fatty acids in supernatant were analyzed according to a previous method (Zuo et al., 2012). The muscle samples were freeze-dried for 48 h. Then we placed 0.1 g sample into a 12 mL glass tube, added 3 mL potassium hydroxide methanol solution (1 mol/L), and heated at 75 °C for 25 min in a water bath. Secondly, we added 3 mL 2 mol/L methanol hydrochloride solution and heated at 75 °C for another 20 min in a water bath. Finally, we added 1 mL hexane and oscillated for extraction. The fatty acids in supernatant were analyzed with a gas chromatograph (Agilent 7890, USA) and a flame ionization detector. The column temperature was programmed to increase from 150 °C to 200 °C at 15 °C/min, from 200 °C to 250 °C at 2 °C/min. The temperature of injector and detector was 250 °C, respectively.

Free amino acid analysis

According to a previous method (Xu et al., 2016), the muscle samples (~500 mg) were homogenised in 10 % sulfosalicylic acid solution and centrifugated at 15,000 rpm for 15 min. The supernatant was filtered through 0.22-μm filters for amino acid detection. Then, an automatic amino acid analyzer (Hitachi L-8900, Japan) was used to detect free amino acids content.

The nutritional metal element analysis

The muscle samples were put into the nitric acid for 24 h and heated at 90 °C for another 4 h in the closed polypropylene tubes with a previous method (Hauser-Davis et al., 2016). The volume of various samples was diluted with ultrapure water after cooling. The inductively-coupled plasma emission spectrometer (Agilent 5100, USA) was used to determine the content of metal ions. In addition, ^{103}Rh was used for the internal standard, and quality control was performed by the blank control.

Texture analysis

The muscle texture was analyzed with a TA-XT Plus Micro TPA device (Stable Micro Systems, Godalming, United Kingdom) according to a previous method (Zhang et al., 2019). The dorsal muscle samples (2 cm × 2 cm × 2 cm) were used for the texture profiles analysis (TPA) measurements. The test speed was 5 mm/s with 5 s stay time at 25 °C. In addition, three fish were used for the analysis of texture curves and indexes.

Histological analysis

We firstly fixed the muscle samples for 24 h with Davidson's solution. The gradient ethanol was used to dehydrate the muscle samples. Then the samples were transparented with xylene, embedded in paraffin, and sectioned by using a microtome at 4 μm (Leica, RM2016, Germany). Finally, hematoxylin and eosin (HE) was used to stain the sections and a light microscopy was used to obtain the images (Olympus, Japan).

Statistical analysis

Data were shown as mean values ± standard error of mean (s.e.m). After testing the homogeneity and normality of variances among various Fe treated groups, one-way analysis of variance (ANOVA) followed by Tukey's test was used to show the differences at $P < 0.05$ by using SPSS 25.0 software.

Results

Effect of dietary Fe on the indexes of lipid deposition in coho salmon

The activity of FAS was remarkably decreased by Fe treatment at 77.3 and 127.7 mg/kg compared to the control group (Fig. 1A). Dietary Fe treatment at 46.4 and 77.3 mg/kg significantly decreased ACC activity (Fig. 1B). Furthermore, dietary Fe treatment at 46.4, 77.3, and 127.7 mg/kg remarkably decreased the activity of ACL (Fig. 1C). TG content was also significantly decreased by 77.3 and 127.7 mg/kg Fe treatment (Fig. 1D).

Effect of dietary Fe on the fatty acid composition in coho salmon

The level of C14:0 and C16:0 fatty acids was remarkably reduced by 46.4 and 77.3 mg/kg Fe treatments (Table 2). Dietary Fe treatment at 77.3 mg/kg significantly reduced C18:0 fatty acid amount (Table 2). Moreover, the level of C16:1n-7 and C20:1n-9 fatty acids was remarkably increased by 77.3 mg/kg Fe treatment, but the level of C16:1n-7 was significantly decreased by 127.7 mg/kg Fe treatment (Table 2). Dietary Fe treatment at 46.4 mg/kg enhanced C18:1n-9 fatty acid level (Table 2). In addition, dietary Fe treatment at 77.3 mg/kg significantly increased the amount of C20:5n-3 and C18:3n-3 fatty acids (Table 2).

Effects of dietary Fe on the amino acid content in the muscle of coho salmon

The level of essential amino acids, including Thr, Leu, Val, Met, Phe, Lys, His, and Arg, was remarkably increased by 77.3 mg/kg Fe treatment, while the level of Ile, Leu, His, and Arg was significantly decreased by 127.7 mg/kg Fe treatment (Table 2). The content of non-essential amino acids Pro, Glu, and Asn was remarkably increased by 46.4 mg/kg Fe treatment. In addition, dietary Fe treatment at 77.3 mg/kg significantly increased the amount of non-essential amino acids Tyr, Ser, Ala, Pro, Asp, Glu, Asn, and Gln, but Fe treatment at 127.7 mg/kg decreased the amount of Asp, Glu, and Asn (Table 2).

Effect of dietary Fe on the content of nutritional elements in coho salmon

The amount of Fe was remarkably increased by Fe treatments at 46.4, 77.3, and 127.7 mg/kg (Table 3). The accumulation of Fe was increased with increasing Fe concentration in diets (Table 3). Dietary Fe treatment at 77.3 mg/kg significantly increased the content of K and Ca (Table 3). However, no remarkable difference was found under 46.4 and 127.7 mg/kg Fe treatments (Table 3). Dietary Fe treatment at 46.4, 77.3, and 127.7 mg/kg did not significantly affect Mg content, and no remarkable difference was found on Cu content (Table 3). Furthermore, the amount of Na and Zn was significantly increased by 77.3 and 127.7 mg/kg Fe treatments (Table 3).

Effect of dietary Fe on the muscle texture of coho salmon

The muscle hardness was significantly increased by 77.3 mg/kg Fe

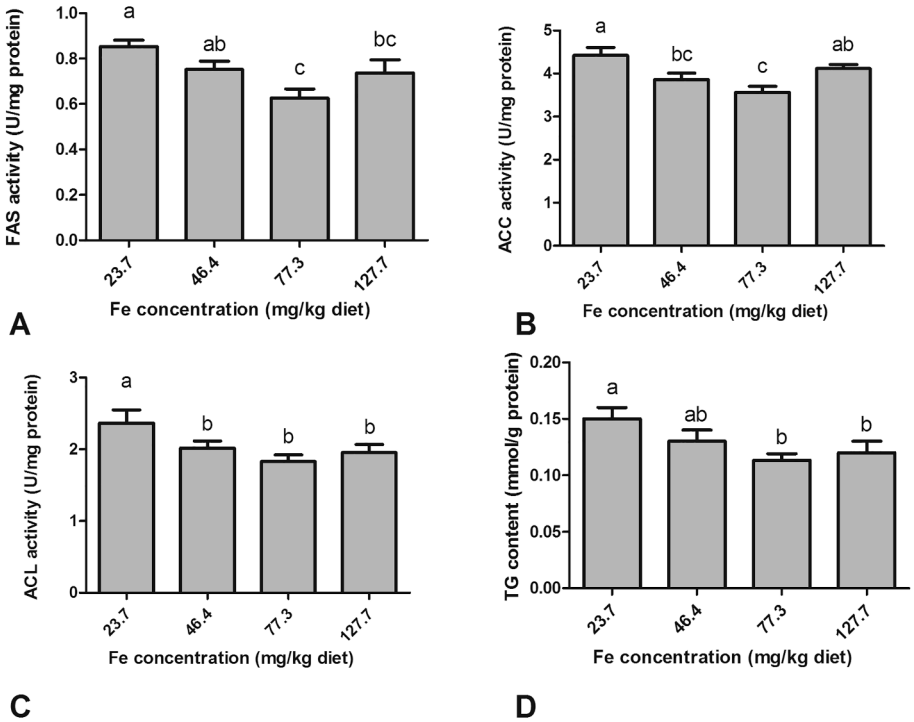

Fig. 1. Effect of dietary Fe on the indexes related to lipid deposition in coho salmon. (A) FAS activity; (B) ACC activity; (C) ACL activity; (D) TG content. Values are expressed as means ± s.e.m. (n = 3). Statistically significant differences are denoted by different letters ($P < 0.05$).

Table 2
Effect of dietary iron on the fatty acid composition and the content of amino acids in the muscle of coho salmon.

	Fe content (mg/kg)			
	23.7	46.4	77.3	127.7
fatty acid composition (% total fatty acids)				
C14:0	2.41 ± 0.27^{a}	1.90 ± 0.12^{bc}	1.68 ± 0.11^{c}	2.30 ± 0.17^{ab}
C16:0	20.79 ± 0.30^{a}	18.97 ± 0.81^{b}	17.70 ± 0.43^{b}	21.19 ± 0.61^{a}
C18:0	7.40 ± 0.43^{a}	6.78 ± 0.24^{a}	5.89 ± 0.23^{b}	7.54 ± 0.36^{a}
∑SFA	30.61 ± 1.00^{a}	27.65 ± 1.16^{b}	25.27 ± 0.76^{c}	31.03 ± 1.14^{a}
C16:1n-7	2.99 ± 0.12^{b}	2.85 ± 0.14^{b}	3.48 ± 0.17^{a}	2.27 ± 0.16^{c}
C18:1n-9	17.01 ± 0.67^{ab}	17.49 ± 0.45^{a}	15.78 ± 0.11^{b}	16.72 ± 0.65^{ab}
C20:1n-9	1.92 ± 0.19^{b}	2.13 ± 0.17^{b}	2.64 ± 0.10^{a}	1.85 ± 0.07^{b}
C22:1n-9	1.90 ± 0.19^{a}	2.16 ± 0.25^{a}	2.08 ± 0.20^{a}	2.12 ± 0.20^{a}
∑MUFA	23.82 ± 1.16^{a}	24.63 ± 1.00^{a}	23.98 ± 0.58^{a}	22.96 ± 1.08^{a}
C18:2n-6	6.65 ± 0.41^{a}	6.84 ± 0.54^{a}	7.46 ± 0.24^{a}	7.42 ± 0.35^{a}
C18:3n-3	1.89 ± 0.23^{b}	2.03 ± 0.11^{b}	2.66 ± 0.21^{a}	2.39 ± 0.25^{ab}
C20:5n-3	6.93 ± 0.33^{b}	7.55 ± 0.30^{ab}	8.38 ± 0.44^{a}	6.66 ± 0.41^{b}
C20:4n-6	7.57 ± 0.36^{ab}	7.60 ± 0.36^{ab}	8.48 ± 0.48^{a}	7.36 ± 0.30^{b}
C22:6n-3	22.78 ± 0.71^{a}	23.73 ± 0.94^{a}	24.04 ± 0.27^{a}	22.98 ± 0.39^{a}
∑PUFA	45.82 ± 2.04^{b}	47.75 ± 2.26^{ab}	51.02 ± 1.64^{a}	46.82 ± 1.70^{b}
Essential amino acids (μg/g wet weight)				
Thr	102.52 ± 7.63^{bc}	113.74 ± 6.88^{ab}	118.55 ± 4.30^{a}	97.29 ± 4.54^{c}
Ile	15.60 ± 2.22^{a}	16.03 ± 1.52^{a}	18.75 ± 0.99^{a}	10.11 ± 1.28^{b}
Leu	35.88 ± 1.96^{b}	37.03 ± 2.61^{b}	42.99 ± 1.65^{a}	25.29 ± 1.69^{c}
Val	23.52 ± 1.96^{bc}	28.44 ± 2.43^{ab}	33.46 ± 2.21^{a}	21.97 ± 1.05^{c}
Met	15.55 ± 2.18^{bc}	20.07 ± 2.99^{ab}	23.56 ± 2.27^{a}	13.48 ± 2.17^{c}
Phe	15.42 ± 1.86^{b}	21.34 ± 1.06^{a}	23.22 ± 2.24^{a}	11.18 ± 2.34^{b}
Lys	35.56 ± 2.01^{c}	44.38 ± 2.33^{b}	51.99 ± 2.59^{a}	33.82 ± 2.31^{c}
His	23.48 ± 2.28^{b}	25.62 ± 2.17^{b}	31.60 ± 1.69^{a}	15.45 ± 1.90^{c}
Arg	77.92 ± 2.06^{b}	91.43 ± 1.75^{ab}	104.96 ± 9.76^{a}	61.60 ± 6.12^{c}
Non-essential amino acid (μg/g wet weight)				
Tyr	25.56 ± 2.20^{b}	29.64 ± 5.88^{ab}	37.55 ± 2.04^{a}	23.36 ± 2.07^{b}
Ser	427.55 ± 3.85^{bc}	444.57 ± 12.75^{ab}	464.15 ± 12.20^{a}	400.52 ± 12.19^{c}
Gly	672.87 ± 8.13^{ab}	687.16 ± 15.54^{a}	700.52 ± 17.28^{a}	645.71 ± 11.11^{b}
Ala	447.87 ± 7.97^{bc}	467.07 ± 5.51^{ab}	480.20 ± 8.29^{a}	432.95 ± 12.26^{c}
Pro	492.23 ± 15.87^{b}	543.38 ± 14.58^{a}	560.46 ± 12.78^{a}	476.05 ± 11.33^{b}
Asp	47.48 ± 3.35^{b}	57.80 ± 3.07^{b}	68.70 ± 6.27^{a}	33.64 ± 2.63^{c}
Glu	475.66 ± 10.77^{b}	523.49 ± 10.83^{a}	539.48 ± 7.41^{a}	444.80 ± 9.85^{c}
Asn	35.16 ± 2.63^{b}	42.23 ± 2.16^{a}	45.39 ± 2.02^{a}	23.83 ± 2.71^{c}
Gln	125.69 ± 7.16^{b}	135.04 ± 10.09^{ab}	147.94 ± 7.96^{a}	115.97 ± 3.78^{b}

Values are expressed as means ± s.e.m. (n = 3). Statistically significant differences were done by rows and denoted by different letters ($P < 0.05$).

treatment, but 46.4 and 127.7 mg/kg Fe treatments had no significant difference (Table 4). Dietary Fe treatment at 46.4 and 77.3 mg/kg significantly increased the muscle springiness (Table 4). In addition, the cohesiveness and gumminess of muscle was also significantly increased by 46.4 and 77.3 mg/kg Fe treatments (Table 4). However, 77.3 mg/kg Fe treatment remarkably increased the chewiness and resilience of muscle (Table 4).

Table 3
Effect of dietary Fe on the content of nutritional metal elements in coho salmon.

Nutrient element content	Fe content (mg/kg)			
	23.7	46.4	77.3	127.7
Fe (mg/kg FW)	74.64 ± 9.84^{d}	142.27 ± 15.47^{b}	195.18 ± 14.71^{c}	258.47 ± 15.63^{a}
K (mg/kg FW)	4101.11 ± 80.05^{b}	4165.17 ± 44.04^{ab}	4313.06 ± 64.93^{a}	4241.06 ± 41.61^{ab}
Ca (mg/kg FW)	212.40 ± 8.44^{c}	243.18 ± 10.42^{ab}	267.87 ± 7.83^{a}	234.91 ± 11.15^{bc}
Mg (mg/kg FW)	331.15 ± 22.99^{a}	326.52 ± 15.19^{a}	343.01 ± 9.83^{a}	363.34 ± 10.22^{a}
Cu (mg/kg FW)	35.88 ± 4.04^{a}	35.89 ± 3.05^{a}	36.90 ± 4.50^{a}	35.55 ± 5.04^{a}
Na (mg/kg FW)	407.52 ± 14.07^{b}	413.79 ± 19.76^{b}	469.21 ± 11.08^{a}	495.01 ± 20.04^{a}
Zn (mg/kg FW)	67.77 ± 4.97^{b}	70.30 ± 2.63^{b}	87.56 ± 4.48^{a}	91.03 ± 5.72^{a}

Values are expressed as means ± s.e.m. (n = 3). Statistically significant differences were done by rows and denoted by different letters ($P < 0.05$).

Table 4
Effect of dietary Fe on the muscle texture in coho salmon.

Texture parameter	Fe content (mg/kg)			
	23.7	46.4	77.3	127.7
Hardness (g)	1475.96 ± 83.02^{bc}	1629.40 ± 52.09^{b}	1824.88 ± 62.37^{a}	1432.34 ± 40.47^{c}
Springiness	0.48 ± 0.03^{c}	0.61 ± 0.04^{ab}	0.68 ± 0.06^{a}	0.50 ± 0.04^{bc}
Cohesiveness	0.34 ± 0.03^{c}	0.41 ± 0.02^{ab}	0.47 ± 0.02^{a}	0.37 ± 0.03^{bc}
Gumminess (g)	449.61 ± 25.20^{b}	552.72 ± 32.67^{a}	612.96 ± 30.77^{a}	436.41 ± 21.12^{b}
Chewiness (g)	138.25 ± 7.23^{b}	157.92 ± 8.79^{ab}	172.80 ± 9.16^{a}	138.15 ± 9.52^{b}
Resilience	0.23 ± 0.02^{b}	0.29 ± 0.02^{ab}	0.33 ± 0.04^{a}	0.24 ± 0.03^{b}

Values are expressed as means ± s.e.m. (n = 3). Statistically significant differences were done by rows and denoted by different letters ($P < 0.05$).

Effect of dietary Fe on the muscle micro-structure in coho salmon

The histological muscle structure was affected by the different dietary Fe level treatments (Fig. 2A ~ D). The muscle fibers became larger in coho salmon treated by 46.4, 77.3, and 127.7 mg/kg Fe diets (Fig. 2A ~ D).

Discussion

The fatty acids synthesis enzymes, mainly including ACC, ACL and FAS, play a crucial role in regulating lipid metabolism and lipid deposition (Gu et al., 2019; Liu et al., 2019). In this study, we discovered that the suitable dietary Fe content decreased ACC, ACL, and FAS activity as well as TG content. Our results showed that dietary Fe inhibited the lipid deposition by decreasing the activities of fatty acids synthesis enzyme ACC, ACL, and FAS, which resulted in the decrease of TG content.

Previously, it is found that Fe deficiency affected the composition of fatty acids in the brain of neonatal rats during lactation and pregnancy (Rees et al., 2020). In addition, Fe affects the availability of fatty acids, lipid metabolism, and polyunsaturated fatty acids (Rees et al., 2020). In the guinea pig offspring fed with Fe-deficient diets, the cerebral fatty acids and eicosanoids were also significantly affected (Jougleux, Rioux, Fiset, Boudreau, & Surette, 2019). In this study, we found that the lower Fe level treatment decreased the content of C16:0 and C14:0 fatty acids, but enhanced the content of C20:1n-9, C16:1n-7, C20:5n-3, C18:3n-3, and C20:4 fatty acids. It clearly showed that dietary Fe levels affected the composition of unsaturated and saturated fatty acids in the salmon

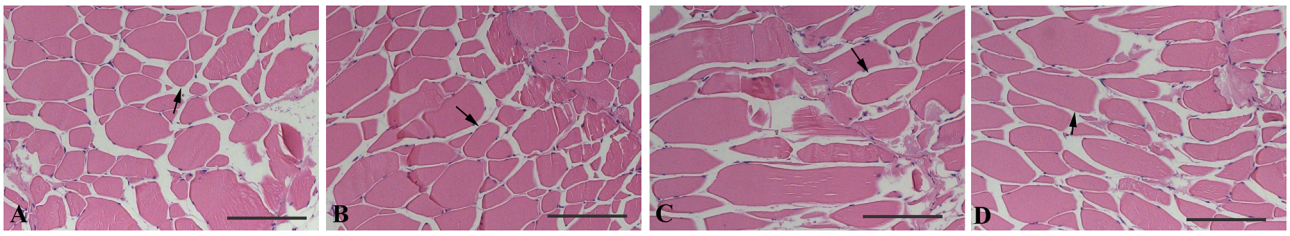

Fig. 2. Effect of dietary Fe on the muscle section of coho salmon. (A) 23.7 mg/kg Fe; (B) 46.4 mg/kg Fe; (C) 77.3 mg/kg Fe; (D) 127.7 mg/kg Fe. Bar: 100 μm. Arrow shows the cross-section of muscle fibers.

muscle. Iron deficiency affects lipid metabolism for iron is one of an essential cofactors in various key enzymes (Rees et al., 2020). In this study, we also observed that dietary Fe levels affected FAS, ACC, and ACL activity, which may result in the change of composition of fatty acids.

It has been found that the disruption of metal ion homeostasis affects lipid peroxidation and protein modification (Jomova & Valko, 2011; Valko, Jomova, Rhodes, Kuča, & Musílek, 2016). In our present study, the accumulation of Fe in salmon muscle was enhanced with increasing Fe concentration in diets. Dietary Fe significantly affected the content of K, Ca, Na, and Zn. Our results showed that the absorption of nutritional elements was influenced by Fe treatments. The over-accumulation of Fe in fish tissues may affect the absorption of some other nutritional metal elements. Furthermore, compared to the lower Fe level treatment, the histological muscle structure was influenced by the higher dietary Fe content. The muscle fibers seemed become larger when treated by the higher dietary Fe content in coho salmon. Thus, the accumulation of Fe in the muscle may affect the size of muscle fibers in coho salmon.

In addition, the muscle texture is one of key indexes to show the characteristics of muscle quality in fish species. The texture characteristics of muscle is closely related to the nutritional composition, protein structure, and the diameter and density of muscle fibers (Wang et al., 2021). In addition, the muscle hardness and springiness are the main factors affecting the meat quality (Pałka, Otwinowska-Mindur, Migdał, Kmiecik, & Wojtysiak, 2021). In this study, the hardness, chewiness, and resilience of muscle were significantly increased by 77.3 mg/kg Fe treatment, but Fe treatment at 46.4 and 127.7 mg/kg had no significant difference on these indexes. Thus, it indicates that the fish treated by 77.3 mg/kg Fe diet has the stronger muscle resistance to maintain integrity, which makes muscle taste better as chewing. Moreover, the springiness, cohesiveness, and gumminess of muscle was also significantly increased by the lower Fe level treatment. It shows that the dietary Fe affected the muscle texture of coho salmon. Previously, it is found that the content of fatty acids and nutrient elements affects the muscle texture of fish (Santos et al., 2019; Zhao et al., 2018). In our study, the content of nutritional elements and fatty acids was increased by the lower dietary Fe level treatments, which may further result in the change of the muscle texture.

In addition, the content of some essential and non-essential amino acids (Thr, Leu, Val, Met, Phe, Lys, His, Arg, Tyr, Ser, Pro, Asp, Glu, Asn, and Gln) was remarkably increased by 77.3 mg/kg Fe treatment, but the content of Ile, Leu, His, Arg, Asp, Glu, and Asn was significantly decreased by 127.7 mg/kg Fe treatment. Previously, it is found that selenium affected amino acid and lipid metabolism in yeast cells (Kieliszek, Błażejak, Bzducha-Wróbel, & Kot, 2019). Free fatty acids have the protein-sparing effects, particularly during fasting and metabolic stress (Gormsen et al., 2008). For lipid metabolism is related to the metabolism of free amino acids, the change of fatty acid composition under Fe treatments may further lead to the change of free amino acid levels. In addition, the content of amino acids was affected by dietary Fe treatment. The amino acid intake affects the protein synthesis in the intact rat, especially the amino acid leucine (Stipanuk, 2007). Thus, the content of amino acids increased by appropriate Fe level treatment may affect the process of protein synthesis.

In summary, effects of dietary iron on the lipid deposition, nutritional element, as well as muscle quality was detected in the salmon muscle. It indicated that dietary Fe decreased TG content and the activities of ACC, FAS and ACL. Moreover, the accumulation of Fe was increased with increasing Fe concentration in diets, and dietary Fe affected the content of K, Ca, Na, and Zn in salmon muscle. In addition, dietary Fe affected the size of muscle fibers, and the composition of unsaturated and saturated fatty acids was affected. The level of amino acids was also increased by the lower dietary Fe level treatments. The lower dietary Fe level treatment at 77.3 mg/kg is an optimal dose to balance the levels of various indicators. In conclusion, dietary Fe affected the lipid deposition, nutritional element, and muscle quality in coho salmon.

CRediT authorship contribution statement

Dongwu Liu: Conceptualization, Funding acquisition, Investigation, Formal analysis, Methodology, Writing - original draft, Writing – review & editing. **Lingyao Li:** Investigation, Formal analysis, Methodology. **Lingling Shan:** Investigation, Formal analysis, Methodology. **Qin Zhang:** Investigation, Formal analysis, Methodology. **Hairui Yu:** Conceptualization, Funding acquisition, Writing - original draft, Writing – review & editing.

Declaration of Competing Interest

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.

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The authors have nothing to disclose.

Consent to participate

All authors read and approved the final manuscript.

Consent for publication

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Not applicable.

Code availability

Not applicable.

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The present study was carried out strictly according to the recommendations in the Guide for the Use of Experimental Animals of Shandong University of Technology (No. 20200601).

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