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Original Research Article

Dietary sanguinarine supplementation recovers the decrease in muscle quality and nutrient composition induced by high-fat diets of grass carp (*Ctenopharyngodon idella*)

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ABSTRACT

The intake of high-fat diets (HFD) has been shown to diminish the muscle quality of aquatic animals. Sanguinarine, as an excellent additive, exhibits the capability to reduce fat deposition and alleviate inflammation. However, its role in the muscle quality reduction caused by HFD remains unclear. An eight-week trial was conducted to investigate the impacts of dietary supplementation of sanguinarine at 1200 μg/kg (HFDS; crude fat = 10%) on the muscle quality of grass carp (*Ctenopharyngodon idellus*) in comparison to a basic diet (CON, crude fat = 5%). Each group had 3 replicates, with 40 fish per replicate. This experiment employed one-way ANOVA and Duncan's multiple comparisons of the means. The results showed that the HFD exhibited lower growth performance, reduced protein deposition, myofiber diameter, and muscle hardness, coupled with higher levels of fat deposition and inflammation when compared with the CON. However, HFDS improved growth performance ($P < 0.05$), fat metabolism (*ppar-α* ($P = 0.001$), *lpl* ($P < 0.001$), *atgl* ($P < 0.001$), and *cpt1* ($P = 0.001$) expression exhibited a significant elevation), protein deposition (the protein and mRNA levels of AKT ($P = 0.004$), PI3K ($P = 0.027$), TOR ($P = 0.005$), and P70S6K ($P = 0.007$) demonstrated a marked increase), myofiber diameter, muscle hardness, and the total content of eicosapentaenoic acid and docosahexaenoic acid. Furthermore, the HFDS reduced oxidative damage caused by fat deposition by significantly downregulating *nf-κb* ($P < 0.001$), *il-1β* ($P < 0.001$), *il-6* ($P < 0.001$), *il-8* ($P = 0.003$), and *tnf-α* ($P < 0.001$) expression and markedly upregulated *nrf2* ($P < 0.001$), *gpx4* ($P < 0.001$), *cat* ($P < 0.001$), *sod* ($P < 0.001$), and *gr* ($P = 0.003$) expression. The findings from this study suggest that sanguinarine has the potential to alleviate the adverse effects of HFD on growth and muscle quality, providing a theoretical foundation for its practical implementation.

1. Introduction

In recent years, there has been a surge in the aquaculture industry, presenting unparalleled opportunities for development stemming from the widespread adoption of intensive and large-scale farming models. The aim is to enhance aquaculture efficiency and minimize feed expenditures, mainly by reducing protein usage and increasing fat levels in the diet. Furthermore, numerous studies have shown that fat has a protein-saving effect, so that the utilization of high-fat diets (HFD) is prevalent in the intensive aquaculture of largemouth bass (*Micropterus salmoides*) and grass

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carp (*Ctenopharyngodon idellus*) (Du et al., 2006; Li et al., 2020). However, HFD can result in excessive fat deposition in the body and induce diseases of rice field eel (*Monopterus albus*) such as inflammation (Shi et al., 2022a). Fat deposition in muscle not only leads to inflammation and a decrease in antioxidant capacity, but also affects muscle quality, including muscle texture and fatty acid composition. Studies in rats have shown that myofiber diameter is negatively correlated with dietary fat content (Trovato et al., 2018). In the study of Atlantic salmon (*Salmo salar* L.), it was found that suitable dietary fat can improve muscle quality, while high dietary fat levels result in high muscle fat content and reduced meat quality (Johnston et al., 2006). In addition, prolonged consumption of HFD has been shown to improve muscle pH value and fat content, decrease myofiber diameter, and reduce polyunsaturated fatty acid content in Nile tilapia (*Oreochromis niloticus*) (Lv et al., 2021; Zhang et al., 2022). These results suggest that the prolonged consumption of HFD is closely related to muscle quality, which affects myofiber growth and development, muscle fatty acids, and muscle texture. Fish quality is also a major concern of consumers. Therefore, it becomes imperative to enhance the muscle quality of farmed fish fed with HFD.

Sanguinarine is a quaternary benzo phenanthridine alkaloid derived predominantly from the Papaveraceae family. It has been isolated from various plant sources, including *Argemone mexicana* L., *Macleaya cordata* (Willd.) R. Br., *Sanguinaria canadensis* L., and *Chelidonium majus* L. (Wu et al., 2020). Currently, sanguinarine is also considered a green feed additive. Our laboratory has carried out many studies on sanguinarine, and the results showed that incorporating a suitable dosage of sanguinarine into the diets comprised of cottonseed and rapeseed meals has demonstrated the capacity to enhance the gut health of grass carp (Liu et al., 2020). Additionally, the inclusion of sanguinarine in the diet has been found to alleviate the inflammation and oxidative stress in rice field eel caused by hydrogen peroxide (H_2O_2) and lipopolysaccharide (LPS) (Shi et al., 2020, 2022b). Furthermore, the incorporation of sanguinarine into diets has been shown to stimulate digestive capacity, bolster immunity, and ameliorate the intestinal microbiota composition in Koi carp (*Cryprinus carpio*) (Zhang et al., 2019). These findings collectively suggest that the utilization of sanguinarine as an aquatic feed additive confers a protective effect on the aquatic animal. However, comprehensive studies on the impacts of sanguinarine on the muscle quality of fish have not been thoroughly conducted. Previous experiments have shown that sanguinarine possesses anti-inflammatory and antioxidant properties, etc. Therefore, it is speculated whether sanguinarine can improve muscle quality by alleviating muscle inflammation caused by HFD.

Grass carp holds significant economic importance as an aquatic animal in China, consistently securing the top position in annual freshwater fish production and having widespread popularity as an aquatic product. Previous experimental results have demonstrated that 10% fat content in grass carp feed significantly increases fat deposition (Du et al., 2006). Thus, the objective of this experiment was to assess the impacts of sanguinarine supplementation to HFD on the growth and muscle quality of grass carp. This study provides novel insights into the utilization of sanguinarine as a secure feed supplement to enhance muscle quality under conditions of HFD in grass carp.

2. Materials and methods

2.1. Animal ethics statement

The present study adhered to the ethical guidelines stipulated by the Committee on Ethics of Animal Experiments at Hunan Agricultural University.

2.2. Experimental diets and feeding trial

Three diets were prepared (Table 1): a control diet containing 4.95% crude fat (CON), a HFD containing 10.27% crude fat, and a HFD with 1200 μg/kg sanguinarine supplementation (HFDS). The sanguinarine (purity > 95%) utilized in this experiment was provided by Hunan Provincial Key Laboratory of Chinese Veterinary Medicine. Prior to mixing, all feed ingredients were ground to pass through an 80-mesh sieve. Subsequently, water and soybean oil were gradually added and homogeneously blended. The mixture was then subjected to the diet pelleting process to obtain pellet sizes of 1.5 and 2.0 mm, followed by an oven-drying procedure at 55 °C for 10 h.

The fish were procured from an aquafarm located in Changde, China. Following a 24 h fasting period, 360 healthy and comparably sized fish were randomly distributed among 9 cages, each measuring 2.0 m × 2.0 m × 2.0 m. The experimental setup involved 40 fish per cage, with three cages allocated to each dietary condition. During the 56-day breeding experiment, the feeding regimen involved providing the fish with 3% to 5% of their body weight three times daily (07:00, 12:00, and 18:00). The physicochemical parameters of the water, including the temperature (25–30 °C, measured using a thermometer), dissolved oxygen (6–8 mg/L), and ammonia and nitrate (≤0.4 mg/L, measured using an LH-M900 portable colorimeter, Zhejiang Lohand Environment Technology Co., Ltd., Zhejiang, China), were kept stable during the experimental period.

2.3. Sampling

Following the breeding process, the fish underwent a 24-h fasting period, after which they were anesthetized using eugenol (1:12,000, Sinopharm Chemical Reagent Co., Ltd, Beijing, China).

Table 1
Composition and nutrient levels of experimental diets (%, dry matter).

Item	CON	HFD	HFDS
Fish meal[1]	6.00	6.00	6.00
Soybean meal[1]	26.00	26.00	26.00
Cottonseed meal[1]	10.00	10.00	10.00
Rapeseed meal[1]	15.00	15.00	15.00
Flour[1]	27.00	27.00	27.00
DDGS[1]	4.00	4.00	4.00
Microcrystalline cellulose[1]	6.06	1.06	1.06
Choline chloride[1]	0.20	0.20	0.20
Soybean oil[1]	3.20	8.20	8.20
$Ca(H_2PO_4)_2$[1]	1.50	1.50	1.50
Premix[2]	1.00	1.00	100
Mould inhibitor[1]	0.03	0.03	0.03
Antioxidants[1]	0.01	0.01	0.01
Sanguinarine[3], μg/kg	0	0	1200
Nutrient levels[4], %			
Crude protein	31.72	31.32	31.75
Crude fat	4.95	10.27	10.09
Ash	6.42	6.35	6.64
Carbohydrate	34.27	34.32	34.26
Crude fiber	12.48	7.17	7.21
Gross energy, MJ/kg	19.6	20.8	20.9

[1] Provided by Hunan Aohua Agriculture and Animal Husbandry Technology Co. Ltd, China.
[2] Provided the following per kilogram of premix: vitamin A, 120,000 IU; vitamin D_3, 40,000 IU; vitamin E, 480 mg; vitamin B_1, 200 mg; vitamin B_2, 280 mg; vitamin B_8, 240 mg; vitamin K_3, 200 mg; vitamin B_{12}, 0.6 mg; calcium pantothenate, 720 mg; nicotinic acid, 1000 mg; folic acid, 60 mg; biotin, 1.2 mg; VC phosphatase, 6850 mg; acid, 3200 mg; iron, 4800 mg; magnesium, 4000 mg; zinc, 2000 mg; manganese, 800 mg; copper, 160 mg; cobalt, 12 mg; selenium, 4 mg; and iodine, 40 mg.
[3] Provided by Hunan Provincial Key Laboratory of Chinese Veterinary Medicine, China.
[4] Nutrient levels were measured values.

Within a 12-h timeframe, muscle samples were extracted from three randomly selected fish from each cage for texture analysis. For qRT-PCR and Western blot analysis, muscle samples were collected from six fish per cage, with three fish contributing to each combined sample, which were subsequently stored at −80 °C. Additionally, back muscles were collected from five fish per cage and preserved at −20 °C for the purpose of detecting muscle fatty acid content and proximate composition.

2.4. Growth parameters

The following formulas were used to calculate growth performance indices:

Weight gain rate (WGR, %) = $100 \times (W_t - W_0)/W_0$;

Survival rate (SR, %) = $100 \times N_t/N_0$;

Feed conversion ratio (FCR) = $F/(W_t - W_0)$;

Feed intake (FI, g/fish) = dry diet fed/fish number;

where W_0 and W_t are the initial and final body weights (g); N_t is the final number of fish; N_0 is the initial number of fish; F is the total amount of the feed consumed (g).

2.5. Proximate composition

Diet and muscle proximate compositions were determined following the methods outlined by the Association of Official Analytical Chemists (AOAC, 2006). Briefly, the moisture content was assessed by subjecting a specific sample weight to consistent drying at 105 °C until a stable weight was achieved. The determination of the crude protein content was carried out utilizing the Dumas combustion method with a protein analyzer (FP-528, Laboratory Equipment Corporation, Michigan, USA). The determination of crude fat contents was executed using the Soxhlet extraction technique (SOX406, Hanon Future Technology Group Co., Ltd., Jinan, China). The determination of the crude ash was conducted through combustion in a muffle furnace (FO610C, Yamato Scientific Co., Ltd., Japan) at 550 °C for 8 h. The gross energy was determined using an oxygen bomb meter (ZDHW-HN7000A, Hebi Huaneng Electronic Technology Co., Ltd., Hebi, China).

2.6. Muscle fatty acid composition

Gas chromatography was employed to determine the fatty acid content in grass carp muscle. An appropriate amount of mixed samples was taken into a 10-mL sample tube. An aliquot of 4 mL acetone methanol solution (vol/vol = 2:1) was combined with 50 mg Amberlyst-A26 of anion (Xi'an Kaixin Biotechnology Co., Ltd., Xi'an, China) exchange resin and allowed to react in a constant temperature oscillator for 2 h. The resulting solution was then separated, and the exchange resin was washed thrice with acetone methanol solution and subsequently dried under a stream of nitrogen gas. Three milliliters of 0.5 mol/L sodium hydroxide methanol solution was added into the bottle and mixed well before connecting the reflux device. The reaction was initiated by adding 1 mL of boron trifluoride methanol solution and was allowed to proceed for 5 min. At room temperature, 2 mL dichloromethane and 0.5 mL internal standard solution were then added for a 3-min extraction. After stratification, the lower layer was carefully collected and subjected to filtration through a 0.45-μm filter membrane. The fatty acid content was detected by a gas chromatograph (7890A, Agilent, USA), where the temperatures of the detector and the sampler were set at 280 and 240 °C, respectively.

2.7. Histopathological analysis

Muscle tissue samples were obtained from 3 fish for each replicate. The samples were fixed in paraformaldehyde and embedded in paraffin wax. Following a previously established experimental method, the steps of hematoxylin-eosin (H&E) staining were as follows: 8-μm tissue sections were prepared on the glass slide with a slicer (RM 2016, Leica Instruments Co., Ltd, Shanghai, China), and then stained with hematoxylin. The stained tissue sections were then observed under an electron microscope (NikonNi-U, Nikon Corporation, Tokyo, Japan) for analysis (Shi et al., 2019).

2.8. Textural analysis

Back muscles from grass carp measuring (1.0 cm × 1.0 cm × 1.0 cm) were collected for analysis. Muscle hardness, resilience, gumminess, cohesiveness, chewiness, and springiness were assessed using a texture analyzer (TMS-PRO, FTC, USA) in the TPA mode, with the P36/R probe. The primary test parameters were as follows: the initial speed prior to the test was maintained at 1 mm/s, followed by a post-test speed of 2 mm/s, while the actual test was conducted at a consistent speed of 1 mm/s, and the compression ratio was set at 50%.

2.9. qRT-PCR analysis

RNA was extracted from intestinal tissue samples using TRIzol reagent (Carlsbad, USA). The RNA extraction procedure, cDNA synthesis, and qRT-PCR procedures followed the protocols outlined in a prior study (Shi et al., 2022a). Shenggong Bioengineering Co., Ltd. (Shanghai, China) provided all primers used in the study (Table 2). All target genes exhibited amplification efficiencies ranging from 0.95 to 1.10. Gene expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method.

2.10. Western blot

Protein extraction from intestinal tissue was performed using RIPA lysate (Beyotime Biotechnology, Shanghai, China). The extraction process for intestinal nucleoproteins was based on methods employed in a previous study (Shi et al., 2022a). Experimental techniques for gel electrophoresis, membrane transfer, and antibody incubation were consistent with those detailed in the earlier study (Shi et al., 2022a). Primary antibodies used included β-actin, p-AMPK, AMPK, PPARα, TOR, NF-κB p65, Nrf2, p70S6K, PI3K, and AKT (rabbit, 1:1000), with item no. AF7018, AF3423, AF6423, AF5301, AF6308, AF5006, AF0639, AF6226, AF6241, and AF6261, respectively (Affinity Biosciences, Jiangsu, China). IgG (rabbit, 1:2000, item no. S0001, Affinity Biosciences, Jiangsu, China) served as the secondary antibodies in the experiment. BeyoECL plus (Beyotime Biotechnology, Shanghai, China) was employed for protein band detection, and visualization was accomplished using the Genesys imaging system (Alcatel, Nanterre, France). Subsequently, Image J software (v1.54, Bethesda, MD, USA) was utilized to assess the gray value.

2.11. Statistical analysis

Mean ± standard error (SE) were used to present the data. Statistical analyses were performed using SPSS 24.0 (IBM SPSS Statistics, Version 24.0, Armonk, NY, USA). Distinct superscripts

Table 2
List of primers used in this study.

Gene	Forward primer sequence (5'→ 3')	Reverse primer sequence (5'→ 3')
pi3k	TGCCAGACGCAAGAACGATA	TCTCTCTGTAGTCTGCCGCT
akt	GGCTTGTGTAAGGAGGGCAT	TCTGGTGTCTGTTTCTGAGGAC
tor	GCCACGCAAACTCACCATAA	CGTAAGGAGGCTGGGTCATT
s6k	TGGCTGGGGTGTTCGAC	CATTGATCTGAGCCTCCTCCA
4ebp1	CTGCTGCCTCCCTGACATTC	GGGGGCTGATGCGGTTATTAT
ppar-α	CGCTGAGGTTCGGATATTT	ACGTCACCTGGTCATTTAAG
lpl	TACAGCGGCGTTCACACTTG	CTACATGAGCACCAAGACTG
atgl	TCGTGCAAGCGTGTATATG	GCTCGTACTGAGGCAAATTA
hsl	CACTCCGACAAGGACAGGAC	GCAACAAGCCTCCACCAT
cpt1	GCATCCATGACACGTTTATTC	GAAGTTTCTCTTCTCTCGTCTC
myog	CCCTTGCTTCAACACCAACG	TCTCCTCTCCCTCATGGTGG
myod	TGAGGGAGAGGAGACGACT	GCTCCAGAACAGGGTAGTAGT
mrf4	TCATTCAACTTGTGCCCTCC	GCCCACTTTGCGATACCC
mrf5	GGAGAGCCGCCACTATGA	GCAGTCAACCATGCTTTCAG
mstn1	GCAGGAGTCACGTCTTGGCA	GAGTCCCTCCGGATTCGCTT
pax	CAAGAAACTGGCTCAATCCG	TCGCAATCGTCCTCATCG
nrf2	CTGGACGAGGAGACTGGA	ATCTGTGGTAGGTGGAAC
keap1	TTCCACGCCCTCCTCAA	TGTACCCTCCCGCTATG
gpx1	GGGCTGGTTATTCTGGGC	AGGCGATGTCATTCCTGTTC
gpx4	TACGCTGAGAGAGGTTTACACAT	CTTTTCCATTGGGTTGTTCC
gr	GTGTCCAACTTCTCCTGTG	ACTCTGGGGTCCAAAACG
cat	GAAGTTCTACACCGATGAGG	CCAGAAATCCCAAACCAT
sod	CGCACTTCAACCCTTACA	ACTTTCCTCATTGCCTCC
tnf-α	CGCTGCTGTCTGCTTCAC	CCTGGTCCTGGTTCACTC
il-1β	AGAGTTTGGTGAAGAAGAGG	TTATTGTGGTTACGCTGGA
il-6	CAGCAGAATGGGGGAGTTATC	CTCGCAGAGTCTTGACATCCTT
il-8	ATGAGTCTTAGAGGTCTGGGT	ACAGTGAGGGCTAGGAGGG
il-15	CCTTCCAACAATCTCGCTTC	AACACATCTTCCAGTTCTCCTT
tgf-β1	TTGGGACTTGTGCTCTAT	AGTTCTGCTGGGATGTTT
nf-κb	GAAGAAGGATGTGGGAGATG	TGTTGTCGTAGATGGGCTGAG
β-actin	GGCTGTGCTGTCCCTGTA	GGGCATAACCCTCGTAGAT

pi3k, phosphoinositide-3 kinase; *akt*, protein kinase B; *tor*, mammalian target of rapamycin; *s6k*, S6 kinase; *4ebp1*, 4E-binding protein 1; *ppar-α*, recombinant peroxisome proliferator activated receptor alpha; *lpl*, lipoprteinlipase; *atgl*, adipose triglyceride lipase; *hsl*, hormone-sensitive lipase; *cpt1*, carnitine palmitoyltransferase 1; *myog*, myoglobin; *myod*, myogenic determinant; *mrf4*, myogenic regulatory factor 4; *mrf5*, myogenic regulatory factor 5; *mstn1*, myostatin 1; *pax*, paired box; *nrf2*, nuclear factor erythroid 2-related factor 2; *keap1*, kelch like ECH associated protein 1; *gpx1*, glutathione peroxidase 1; *gpx4*, glutathione peroxidase 4; *gr*, glutathione reductases; *cat*, catalase; *sod*, superoxide dismutase; *tnf-α*, tumor necrosis factor alpha; *il-1β*, interleukin-1 beta; *il-6*, interleukin-6; *il-8*, interleukin-8; *il-15*, interleukin-15; *tgf-β1*, transforming growth factor beta 1; *nf-κb*, nuclear transcription factor-κB; β-actin, beta-actin.

($P < 0.05$) were used to indicate the presence of significant differences. Assessments of homogeneity and normality were performed using the Levene tests and Shapiro-Wilk, respectively. Statistical significance was assessed using one-way ANOVA, followed by Duncan's multiple comparisons of means.

3. Results

3.1. Growth and biochemical composition in muscle

No significant differences were observed in survival rate, feed intake, muscle moisture, dripping loss, and cooking loss among all groups (Table 3, Fig. 1A, E, and F). When compared to the HFD group, the WGR, SGR, and muscle crude protein in the CON and HFDS groups were significantly increased ($P < 0.05$) (Table 3 and Fig. 1B). The HFD group resulted in significantly higher ($P < 0.05$) FCR, muscle crude fat, and pH values when compared to the CON diet (Table 3, Fig. 1C and D). When compared to the HFD group, the FCR, muscle crude fat, and pH values for the HFDS group were significantly lower ($P < 0.05$), but not significantly lower than those in the CON group (Table 3, Fig. 1C and D). When compared with the CON and HFDS groups, the CF, HSI, and VSI in the HFD group were significantly higher ($P < 0.05$) (Table 4).

Table 3
The effects of different diets on the growth performance of grass carp.

Index	CON	HFD	HFDS
Initial weight, g	49.99 ± 0.12	50.52 ± 0.42	50.19 ± 0.55
Final weight, g	278.41 ± 5.99^{b}	232.76 ± 5.42^{a}	274.57 ± 6.04^{b}
WGR, %	456.93 ± 12.12^{b}	360.60 ± 7.20^{a}	446.95 ± 7.32^{b}
SGR, %/d	3.07 ± 0.04^{b}	2.73 ± 0.03^{a}	3.03 ± 0.03^{b}
FI, g/fish	301.89 ± 0.37	301.96 ± 2.93	302.84 ± 2.78
FCR	1.32 ± 0.04^{a}	1.66 ± 0.06^{b}	1.35 ± 0.02^{a}
SR, %	95.00 ± 2.89	91.67 ± 8.33	93.33 ± 4.41

CON, a control group (containing 4.95% crude fat); HFD, a high-fat diet (containing 10.27% crude fat); HFDS, a high-fat diet supplemented with 1200 μg/kg sanguinarine; WGR, weight gain rate; SGR, specific growth rate; FI, feed intake; FCR, feed conversion ratio; SR, survival rate.
The data are presented as mean ± standard error (SE). Values with different superscripts in the same row indicate significant differences ($P < 0.05$).

3.2. Morphological characteristics of myofiber and myogenic regulatory factor

A significant decrease in the myofiber diameter of grass carp due to prolonged consumption of HFD was revealed by muscle histology analysis, whereas a notable increase in myofiber diameter was shown in the HFDS group compared to the HFD group (Fig. 2A and B). Further analysis showed significantly higher numbers of myofibers with a diameter below 60 μm and a significantly lower numbers of myofibers with a diameter above 60 μm were observed in the HFD group when compared to the CON and HFDS groups ($P < 0.05$); however, no obvious difference was found between the CON and HFDS groups (Fig. 2C and D). No significant variations in expression levels of *myod* and *mstn1* were exhibited among the different groups (Fig. 2E). In comparison to the CON group, a notable elevation of *myog*, *mrf4*, *myf5*, and *pax* expression was exhibited in the HFD group ($P < 0.05$). The expression of *myog*, *mrf4*,

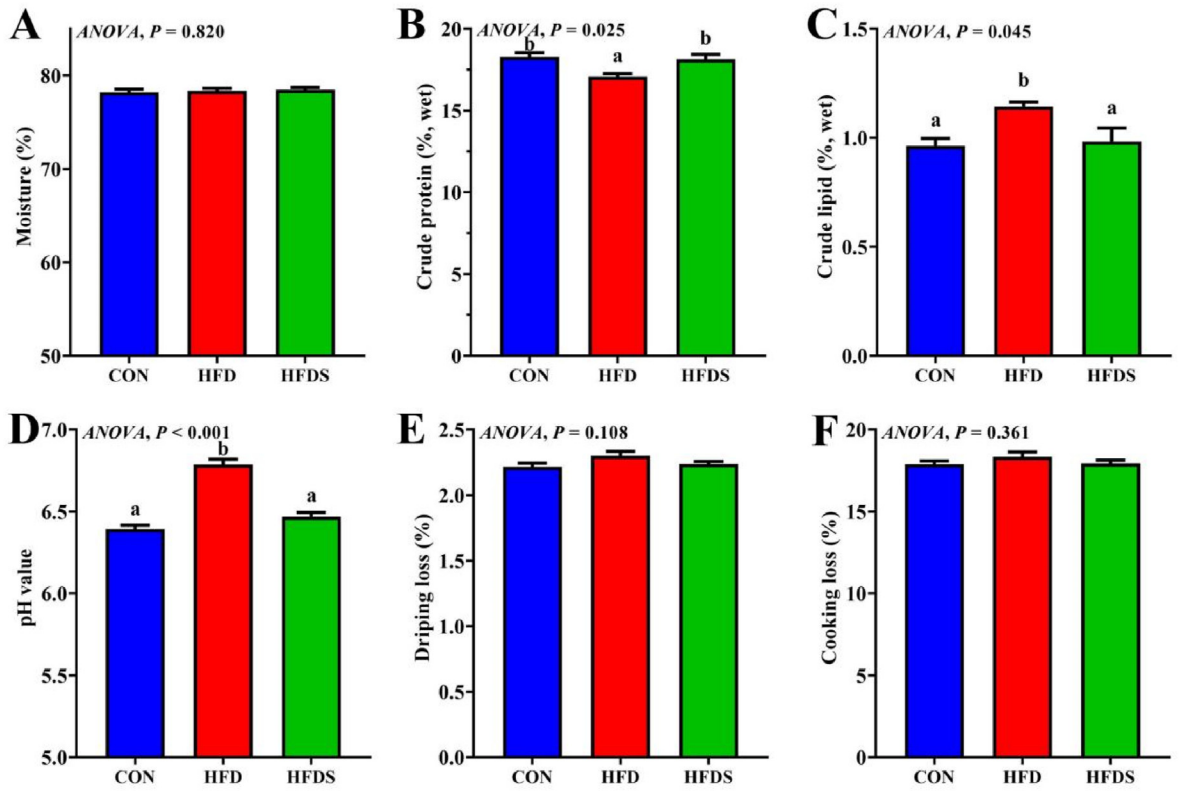

Fig. 1. The effects of different diets on biochemical composition in muscle of grass carp. (A) Moisture in muscle; (B) crude protein in muscle; (C) crude fat in muscle; (D) pH value in muscle; (E) dripping loss in muscle; (F) cooking loss in muscle. The bars indicate the mean ± standard error (SE). Different superscripts denote significant differences ($P < 0.05$). CON = a control group (containing 4.95% crude fat); HFD = a high fat diet (containing 10.27% crude fat); HFDS = supplementing 1200 μg/kg sanguinarine to HFD.

Table 4
The effects of different diets on physical indicators of grass carp.

Index	CON	HFD	HFDS
CF, g/cm^3	1.86 ± 0.02^a	2.18 ± 0.02^c	1.93 ± 0.02^b
HSI, %	1.92 ± 0.12^a	2.32 ± 0.01^b	1.72 ± 0.06^a
VSI, %	16.63 ± 0.34^a	19.57 ± 0.19^b	16.74 ± 0.15^a

CON, a control group (containing 4.95% crude fat); HFD, a high-fat diet (containing 10.27% crude fat); HFDS, a high-fat diet supplemented with 1200 μg/kg sanguinarine; CF, condition factor; HSI, hepatosomatic index; VSI, viserosomatic index. The data are presented as mean ± standard error (SE). Values with different superscripts in the same row indicate significant differences ($P < 0.05$).

myf5, and *pax* was significantly attenuated in the HFDS group ($P < 0.05$) when compared to the HFD group (Fig. 2E).

3.3. Textural parameters

No significant differences were shown in cohesiveness and resilience among all groups (Fig. 3A, D, and G). Compared to the CON and HFDS group, hardness and chewiness in the HFD group were significantly lower ($P < 0.05$), whereas springiness and gumminess were opposite (Fig. 3A, B, C, E, and F). Compared to the CON group, chewiness was significantly decreased in the HFDS group ($P < 0.05$) (Fig. 3F).

3.4. Fatty acid composition of muscle

The predominant constituents of muscle fatty acids in grass carp included C16:0, C18:0, C18:1n9c, and C18:2n6c (Table 5 and Fig. 4A). Compared to the CON group, prolonged consumption of the HFD significantly decreased the contents of C20:4n6, C23:0, C24:0, and C22:6n3 ($P < 0.05$) and increased the content of C20:2. However, the HFDS group showed a tendency to elevate the contents of C20:4n6, C23:0, C24:0, and C22:6n3, and the contents of C20:4n6, C23:0, and C24:0 demonstrated no significant discrepancy when compared to the CON group (Table 5). Across all experimental groups, there were no statistically significant distinctions in ∑SFA, ∑MUFA, ∑PUFA, ∑n-3, ∑n-6, and ∑n-3/∑n-6 (Fig. 4B, C, D, F, G, and H). The total content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was significantly decreased ($P < 0.05$) in the HFD group (Fig. 4E) compared to the CON group, and it was significantly increased ($P < 0.05$) in HFDS group when compared to the HFD group (Fig. 4E).

3.5. Expression of genes and proteins related to muscle protein synthesis and lipolysis

According to the data presented in Fig. 5A, there was no statistically significant influence on *4ebp1* expression across the different diets. The expression levels of muscle *pi3k*, *akt*, *tor*, and *s6k* in the HFD group were significantly lower than in the CON group, while they were significantly up-regulated in the HFDS group ($P < 0.05$) (Fig. 5A). No obvious influence was observed in *hsl* expression across the different groups (Fig. 5B). The expression levels of muscle *ppar-α*, *lpl*, *atgl*, and *cpt1* in the HFD group were significantly lower than in the CON group, while they were significantly up-regulated ($P < 0.05$) in the HFDS group (Fig. 5B). Results depicted in Fig. 5C and D indicated that protein expression levels of p-AMPK, PPARα, TOR, and PI3K were significantly diminished ($P < 0.05$) in the HFD group compared to the CON group. Adding sanguinarine to HFD significantly increased ($P < 0.05$) protein expression levels of p-AMPK, PPARα, TOR, p70S6K, PI3K, and AKT when compared to the HFD group (Fig. 5C and D).

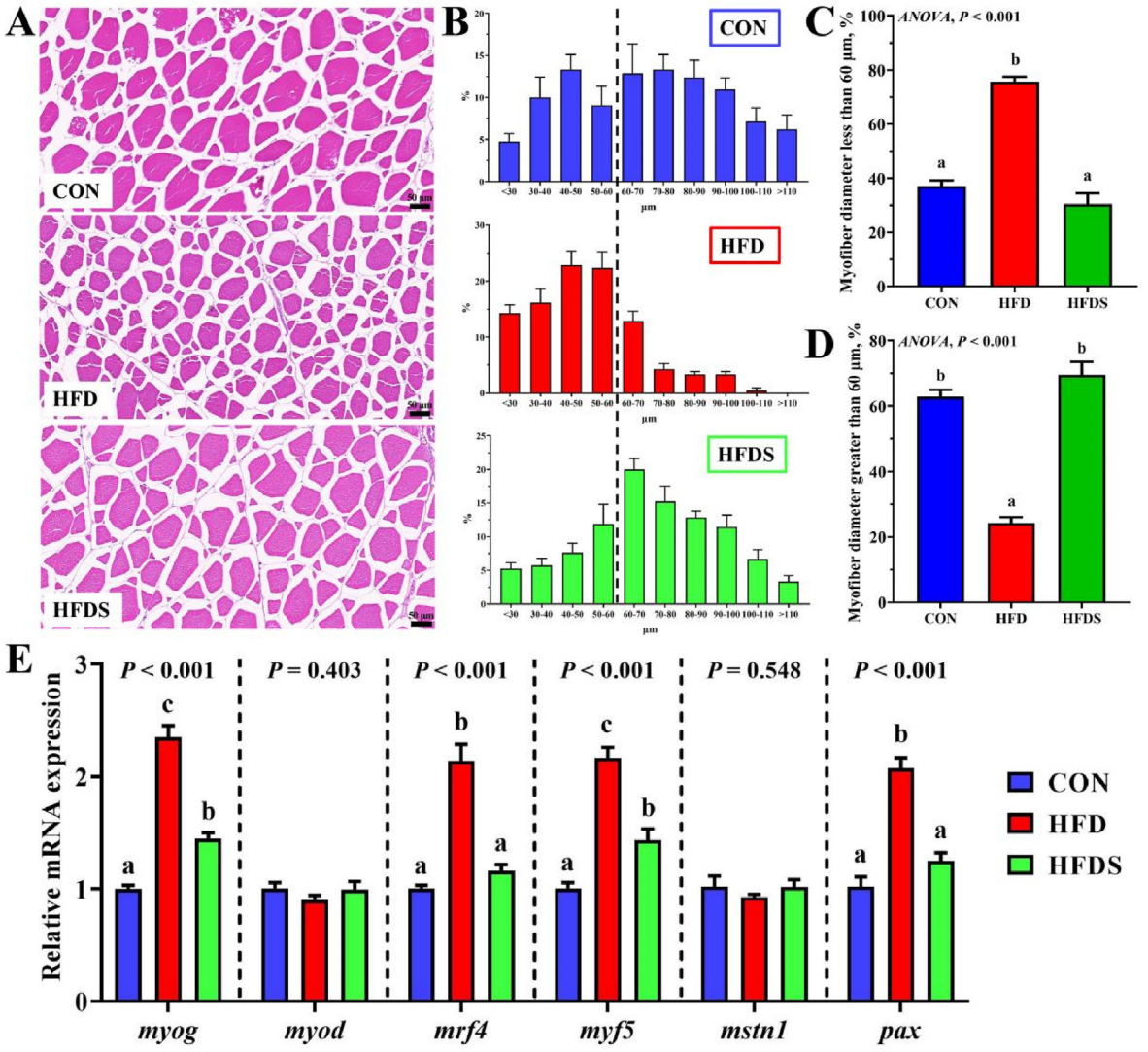

Fig. 2. The effects of different diets on morphological characteristics of myofiber and myogenic regulatory factor of grass carp. (A) Morphological characteristics of myofiber. (B) Myofiber diameter distribution. (C) Proportion of myofiber diameter less than 60 μm. (D) Proportion of myofiber diameter greater than 60 μm. (E) Myogenic regulatory factor gene expression. The bars indicate the mean ± standard error (SE). Different superscripts denote significant differences ($P < 0.05$). CON = a control group (containing 4.95% crude fat); HFD = a high fat diet (containing 10.27% crude fat); HFDS = supplementing 1200 μg/kg sanguinarine to HFD.

3.6. Expression of genes and proteins related to inflammation and antioxidants in muscle

Compared to the CON group, the expression levels of muscle *nf-κb*, *il-1β*, *il-6*, *il-8*, and *tnf-α* in the HFD group were significantly higher ($P < 0.05$), while *tgf-β1* and *il-15* expression showed the opposite trend (Fig. 6A). The HFDS group significantly down-regulated *nf-κb*, *il-1β*, *il-6*, *il-8*, and *tnf-α* expression ($P < 0.05$) and up-regulated *il-15* and *tgf-β1* expression when compared to the HFD group (Fig. 6A). No significant differences were observed in *gpx1* expression across the different groups (Fig. 6B). In comparison to the fish consuming the CON diet, a distinct reduction ($P < 0.05$) was evident in the expression levels of *nrf2*, *gpx4*, *cat*, *sod*, and *gr* in the muscle of fish consuming the HFD, whereas *keap1* expression displayed a converse pattern (Fig. 6B). The HFDS group resulted in a notable modulation of antioxidant-related mRNA expression when compared with the HFD group (Fig. 6B). Fig. 6C and D indicated that Nf-κB p65 protein expression was significantly higher in the HFD group ($P < 0.05$) when compared to the CON and HFDS groups. No statistically significant variance was observed in Nrf2 protein levels across all experimental groups; however, the HFD group showed a tendency to decrease (Fig. 6C and E).

3.7. Multidimensional correlation analysis

The correlation analysis indicated that the transcriptional levels of muscle development, muscle protein synthesis, muscle lipolysis, inflammation, and antioxidants were significantly correlated ($P < 0.05$) to growth performance, muscle characteristics, myofiber characteristics, and muscle texture (Fig. 7).

4. Discussion

4.1. Adding sanguinarine to HFD increased the growth performance of grass carp

Compared with protein, fat are considered a more economical source of energy and a key nutritional factor affecting the growth performance of fish and shrimp. This study revealed that prolonged consumption of HFD resulted in a significant decline in the WGR

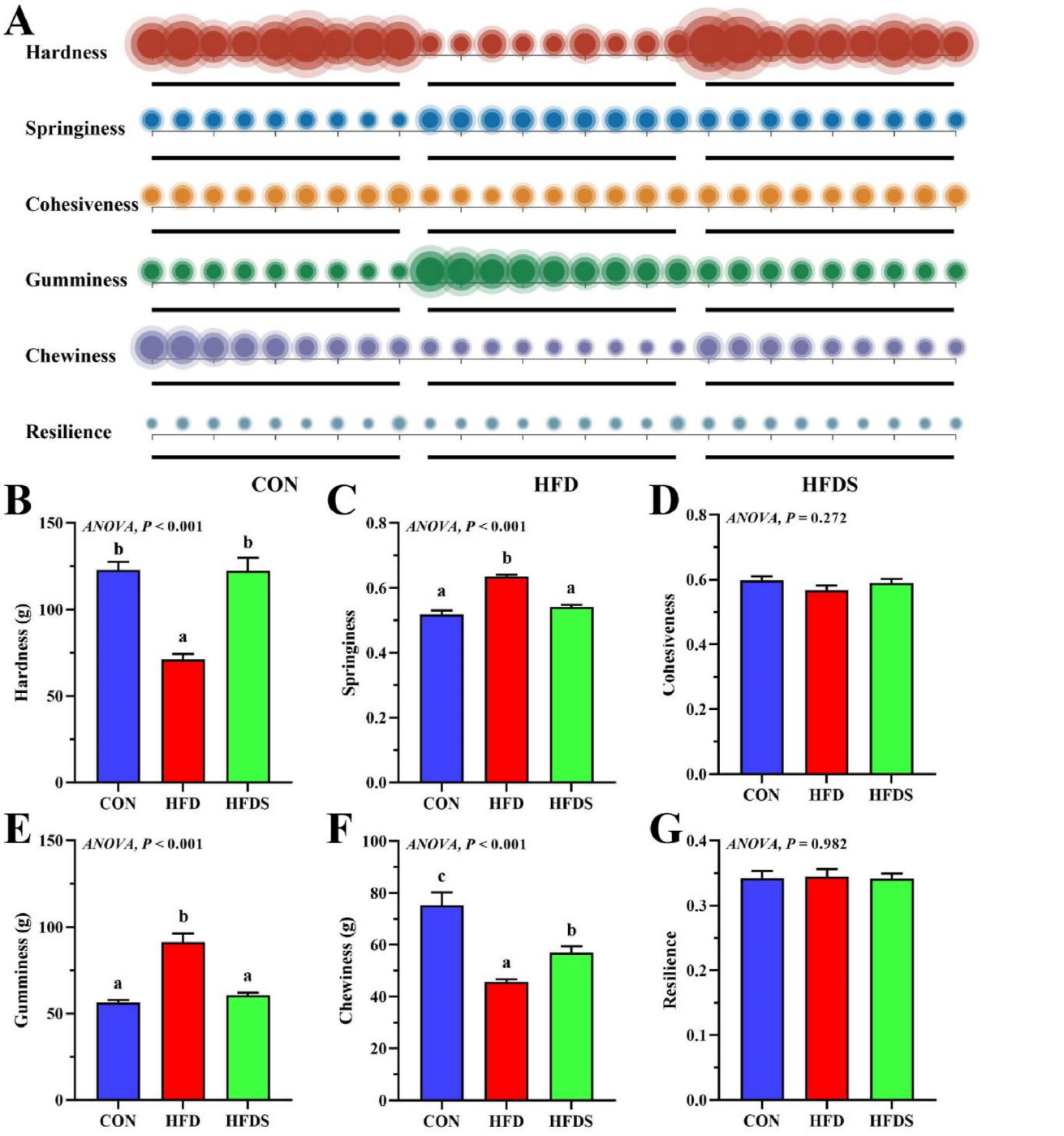

Fig. 3. The effects of different diets on textural parameters in muscle of grass carp. (A) Bubble diagram; (B) hardness; (C) springiness; (D) cohesiveness; (E) gumminess; (F) chewiness; (G) resilience. The bars indicate the mean ± standard error (SE). Different superscripts denote significant differences ($P < 0.05$). CON = a control group (containing 4.95% crude fat); HFD = a high fat diet (containing 10.27% crude fat); HFDS = supplementing 1200 μg/kg sanguinarine to HFD.

and feed utilization rate of grass carp. This finding aligns with a previous study conducted on grass carp (Li et al., 2016), and similar results have been found in other aquatic animals, such as large-mouth bass (Yin et al., 2021), golden pompano (Li et al., 2021), and blunt snoutbream (Dong et al., 2020). It is possible that the energy supplied by HFD may surpass the growing needs of the aquatic animal, leading to metabolic imbalance and reduced feed utilization, which in turn results in reduced utilization of other nutrients (Ding et al., 2020). Sanguinarine, as a botanical extract, possesses distinctive attributes including adaptability, absence of drug resistance, and minimal occurrence of toxic side effects. The results indicated that the addition of 1200 μg/kg sanguinarine to HFD improved growth performance to levels similar to that of the CON group, without any significant variance observed. One possible explanation for this enhancement may be linked to the ability of sanguinarine to enhance the utilization of amino acids (Drsata et al., 1996), such as phenylalanine and tryptophan, thereby promoting growth and development. Additionally, the capacity of

Table 5
Fatty acid (FA) composition (%) in the muscle of grass carp.

Item	CON	HFD	HFDS
C12:0	0.13 ± 0.03	0.07 ± 0.01	0.10 ± 0.01
C14:0	1.01 ± 0.12	0.88 ± 0.05	0.95 ± 0.02
C14:1	0.06 ± 0.01	0.05 ± 0.00	0.06 ± 0.00
C15:0	0.25 ± 0.02	0.21 ± 0.01	0.23 ± 0.01
C15:1	0.09 ± 0.02	0.05 ± 0.00	0.07 ± 0.00
C16:0	25.11 ± 1.37	21.79 ± 0.81	23.76 ± 0.46
C16:1	2.87 ± 0.37	2.58 ± 0.16	2.55 ± 0.13
C17:0	0.34 ± 0.03	0.27 ± 0.01	0.30 ± 0.01
C18:0	7.15 ± 0.24	6.41 ± 0.14	6.98 ± 0.18
C18:1n9c	29.93 ± 1.43	31.43 ± 0.53	31.02 ± 0.40
C18:2n6c	19.59 ± 2.61	25.09 ± 1.54	22.01 ± 0.77
C20:0	0.38 ± 0.01	0.36 ± 0.01	0.40 ± 0.03
C18:3n6	0.28 ± 0.02	0.29 ± 0.02	0.28 ± 0.02
C18:3n3	1.54 ± 0.22	2.05 ± 0.14	1.73 ± 0.07
C20:1	0.82 ± 0.06	0.75 ± 0.01	0.74 ± 0.02
C20:2	0.91 ± 0.04^{a}	1.03 ± 0.01^{b}	1.10 ± 0.01^{b}
C22:0	0.19 ± 0.02	0.15 ± 0.01	0.17 ± 0.01
C20:3n6	1.44 ± 0.10	1.28 ± 0.06	1.30 ± 0.02
C20:3n3	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
C22:1n9	0.54 ± 0.09	0.31 ± 0.03	0.39 ± 0.05
C20:4n6	4.35 ± 0.43^{b}	2.93 ± 0.09^{a}	3.46 ± 0.13^{ab}
C23:0	0.09 ± 0.01^{b}	0.05 ± 0.00^{a}	0.08 ± 0.00^{b}
C22:2	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
C20:5n3	0.22 ± 0.01	0.21 ± 0.03	0.27 ± 0.03
C24:0	0.13 ± 0.01^{b}	0.08 ± 0.01^{a}	0.11 ± 0.01^{ab}
C24:1	0.10 ± 0.01	0.09 ± 0.01	0.12 ± 0.01
C22:6n3	2.23 ± 0.26^{b}	1.36 ± 0.02^{a}	1.57 ± 0.06^{a}

CON, a control group (containing 4.95% crude fat); HFD, a high fat diet (containing 10.27% crude fat); HFDS, supplementing 1200 μg/kg sanguinarine to HFD.
The data are presented as mean ± standard error (SE). Values with different superscripts in the same row indicate significant differences ($P < 0.05$).

sanguinarine to bolster immunity and enhance intestinal health, including promoting intestinal structural stability, mitigating intestinal inflammation, and optimizing intestinal microbiota, may also contribute to its growth-promoting effects (Liu et al., 2020).

4.2. Dietary sanguinarine alleviated the negative effects of HFD on fat deposition and muscle composition of grass carp

The elevated fat content within the musculature of aquatic organisms is considered a significant factor contributing to meat texture deterioration. Numerous studies have reported that prolonged consumption of HFD can lead to muscle fat deposition in aquatic animals such as grass carp and Nile tilapia (*Oreochromis niloticus*), resulting in the deterioration of fish meat quality (Lv et al., 2021; Yuan et al., 2016). In the present study, HFD exhibited a pronounced impact on the augmentation of fat accumulation within the muscle tissue of grass carp, primarily manifesting as a significant increase in the content of muscle crude fat, leading to a decrease in the hardness of the grass carp muscle. However, supplementation of sanguinarine to HFD remarkably reduced the crude fat content in muscle, significantly increased muscle chewiness, and decreased muscle springiness and gumminess. This phenomenon may also be one of the reasons why sanguinarine enhances the muscle hardness.

With the aim of elucidating the underlying molecular mechanism through which sanguinarine mitigates muscle fat deposition, this investigation assessed the mRNA and protein expression profiles of genes associated with fat metabolism in the muscle tissue. Adenosine 5'-monophosphate (AMP)-activated protein kinase

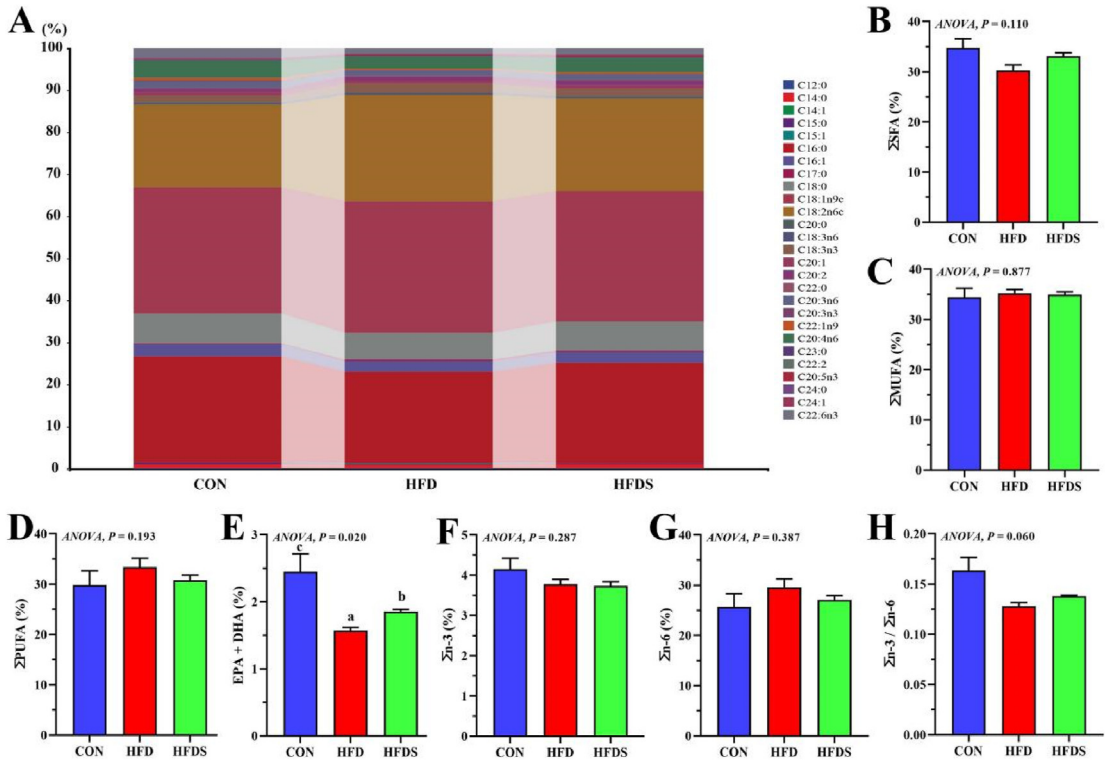

Fig. 4. The effects of different diets on muscle fatty acid composition of grass carp. (A) Interactive ribbon bar chart; (B) ∑saturated fatty acid (∑SFA); (C) ∑monounsaturated fatty acids (∑MUFA); (D) ∑polyunsaturated fatty acids (∑PUFA); (E) eicosapentaenoic acid + docosahexaenoic acid (EPA + DHA); (F) ∑n-3 fatty acids (∑n-3); (G) ∑n-6 fatty acids (∑n-6); (H) ∑n-3/∑n-6. The bars indicate the mean ± standard error (SE). Different superscripts denote significant differences ($P < 0.05$). CON = a control group (containing 4.95% crude fat); HFD = a high fat diet (containing 10.27% crude fat); HFDS = supplementing 1200 μg/kg sanguinarine to HFD.

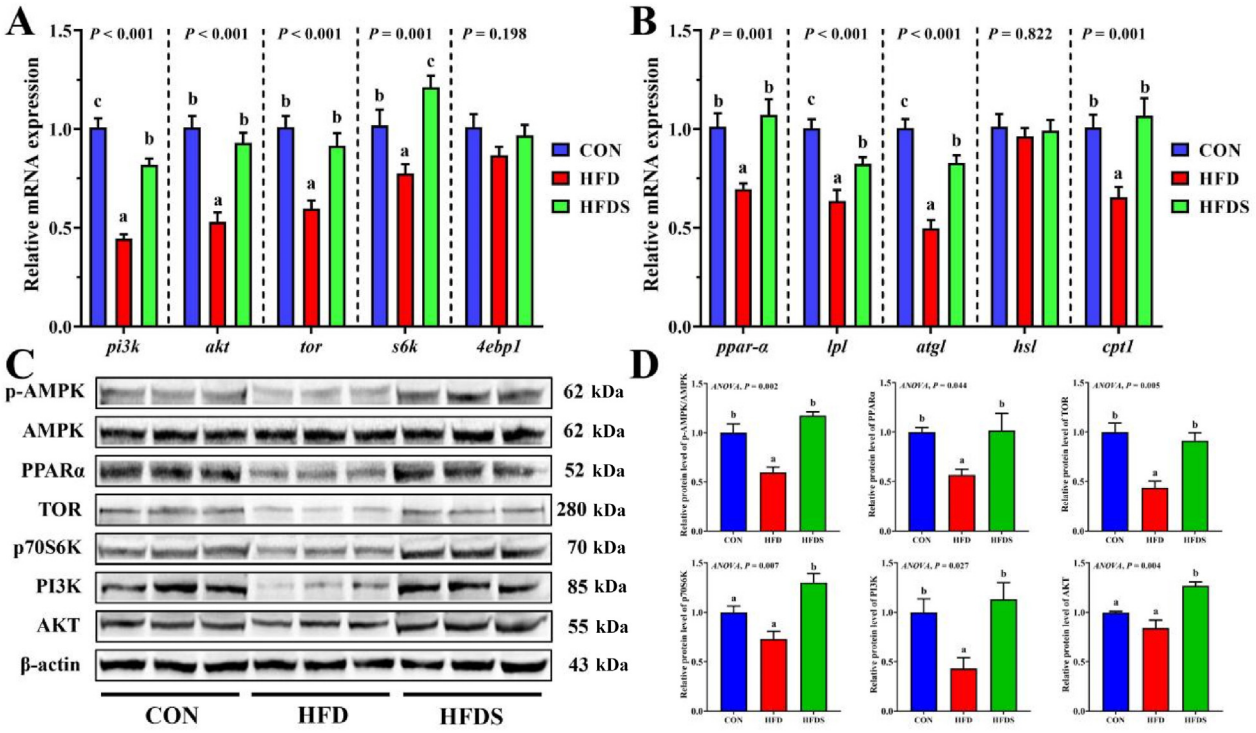

Fig. 5. The effects of different diets on muscle protein synthesis and lipolysis of grass carp. (A) Protein synthesis related gene expression; (B) lipolysis-related gene expression; (C) Western blot analysis of p-AMPK, AMPK, PPARα, TOR, p70S6K, PI3K, and AKT protein expression; (D) relative quantification of p-AMPK, PPARα, TOR, p70S6K, PI3K, and AKT protein expression. The bars indicate the mean ± standard error (SE). Different superscripts denote significant differences ($P < 0.05$). CON = a control group (containing 4.95% crude fat); HFD = a high fat diet (containing 10.27% crude fat); HFDS = supplementing 1200 μg/kg sanguinarine to HFD.

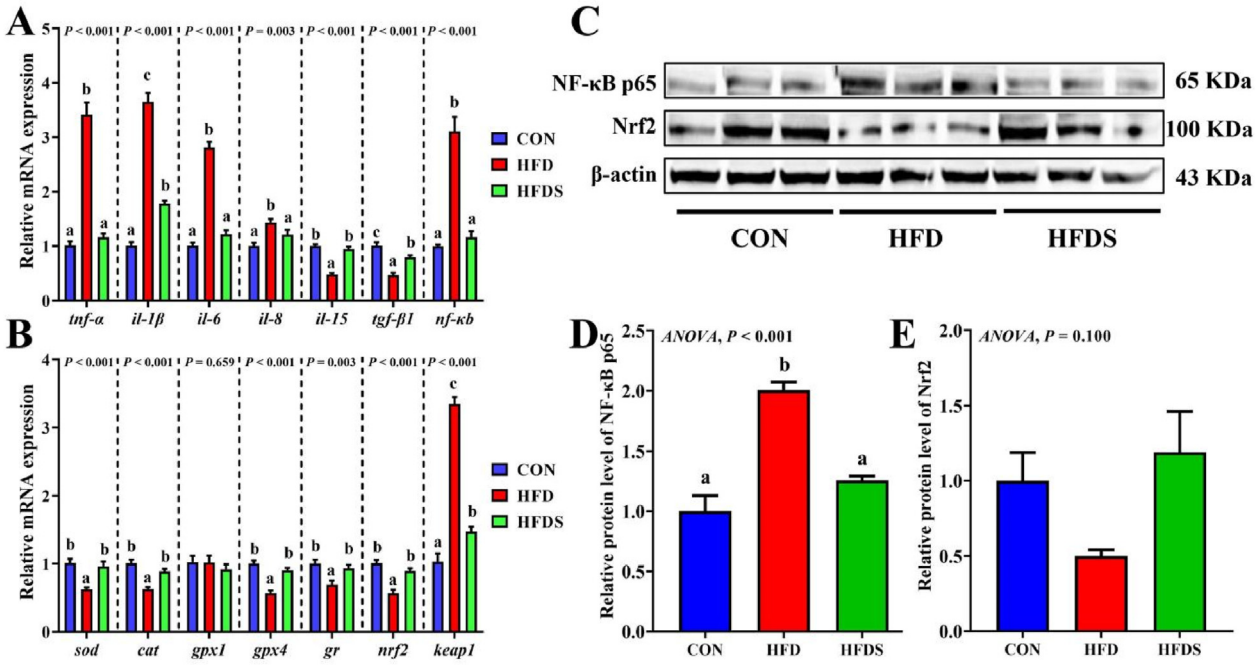

Fig. 6. The effects of different diets on inflammation and antioxidants in the muscle of grass carp. (A) Inflammation related gene expression; (B) antioxidants related gene expression; (C) Western blot analysis of NF-κB p65 and Nrf2 protein expression; (D) relative quantification of NF-κB p65 protein expression; (E) relative quantification of Nrf2 protein expression. The bars indicate the mean ± standard error (SE). Different superscripts denote significant differences ($P < 0.05$). CON = a control group (containing 4.95% crude fat); HFD = a high fat diet (containing 10.27% crude fat); HFDS = supplementing 1200 μg/kg sanguinarine to HFD.

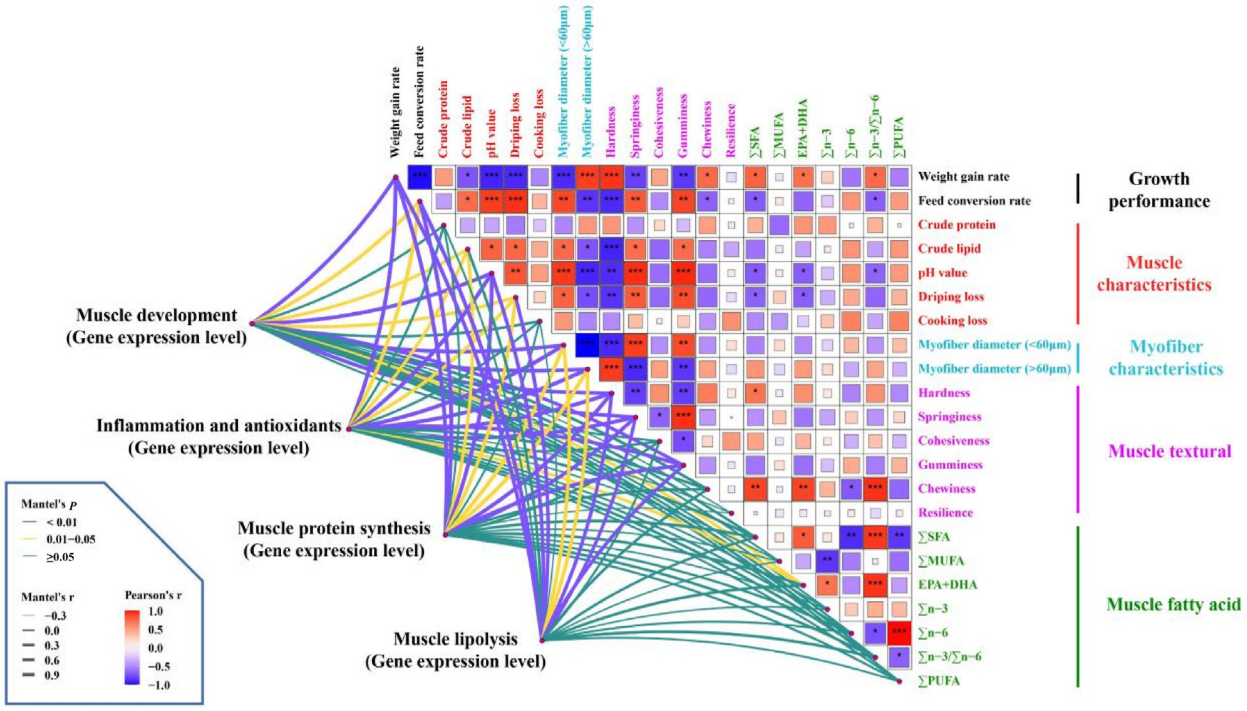

Fig. 7. Correlation analysis of characterization and its mechanism in the muscle of grass carp. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

(AMPK) is recognized as a pivotal controller of fat metabolism, opening fat catabolic pathways and converting fat to adenosine triphosphate enzyme (ATP) for consumption (He et al., 2016). Additionally, peroxisome proliferator-activated receptor α (PPARα) plays a pivotal role in upholding fat metabolism equilibrium. It not only detects and responds to alterations in fatty acid levels by activating the transcription of specific genes, but also governs fatty acid breakdown, along with the gene and protein expression of enzymes associated with bodily fat balance (Reddy and Rao, 2006; Sugden et al., 2002). Within this research endeavor, HFD remarkably inhibited AMPK phosphorylation and PPARα expression and significantly down-regulated the expression of downstream lipolysis-related genes, including *lpl*, *atgl*, *cpt1*, etc. These results may be an important mechanism leading to muscle fat deposition in grass carp that have been fed HFD. Studies have shown that sanguinarine is a new type of AMPK direct activator, which binds at the cleft between the β and γ subunits of AMPK (Choi et al., 2011). Additionally, it has been found that sanguinarine can activate PPARα and enhance its transcriptional activity, thus having the potential to reduce fat content (Tian et al., 2021). In the current investigation, it was also confirmed that supplementing sanguinarine to HFD can activate PPARα and AMPK. Consequently, this activation resulted in an elevation of the transcriptional levels of genes associated with lipolysis. This orchestrated molecular response contributed to the mitigation of muscle fat deposition resulting from the HFD, thereby enhancing the sensory attributes of meat flavor in grass carp.

Beyond the crude fat content in muscle, the fatty acid composition within muscle plays a pivotal role in influencing both the nutritional value and palatability of aquatic animals (Zhang et al., 2021). In this investigation, the inclusion of sanguinarine to HFD was found to promote the accumulation of beneficial fatty acids in grass carp muscle. Moreover, the consumption of the HFD resulted in a conspicuous decrease in the cumulative levels of EPA and DHA within the musculature of grass carp, indicating a decline in the nutritional quality of the fish. Nevertheless, the addition of sanguinarine resulted in a significant increase in the contents of EPA and DHA, thus enhancing the nutritional quality and meat flavor of grass carp nourished with HFD.

4.3. Dietary sanguinarine promoted muscle protein synthesis of grass carp fed HFD

Protein assumes a pivotal role in human nutrition and health, and fish are commonly recognized as a superior reservoir of dietary protein. Therefore, the protein content of fish muscle serves as a critical indicator for evaluating fish quality. Prior research has indicated that a prolonged HFD can result in reduced crude protein deposition in the muscle of Nile tilapia, potentially stemming from a decline in protein synthesis and an increase in protein degradation (Lv et al., 2021). The findings of this study corroborate this notion, demonstrating that the inclusion of sanguinarine in HFD resulted in a significant augmentation of protein synthesis in grass carp muscle, thereby indicating an improvement in the nutritional quality. The possible reason is that sanguinarine can improve protein synthesis in the muscle of grass carp fed HFD, thus increasing protein deposition in muscle. The PI3K/AKT/TOR signaling pathway assumes a critical role in the physiological regulation of protein synthesis (Li et al., 2019). Additionally, increased protein content in muscle was associated with p70S6K signaling molecules (Zhao et al., 2016). This investigation demonstrated that HFD markedly reduced the protein expression of TOR and PI3K in the muscle, along with a decline in the transcriptional activity of *s6k*, *tor*, *akt*, and *pi3k*. However, the addition of sanguinarine significantly improved the protein and mRNA levels of AKT, PI3K, TOR, and P70S6K. These results indicated that sanguinarine can promote protein synthesis in the muscle through the PI3K/AKT/TOR signaling pathway, thereby enhancing protein deposition in the muscle and improving the nutritional quality of grass carp.

4.4. Dietary sanguinarine alleviated the negative effects on the myofiber of grass carp fed HFD

Myofiber characteristics are the key factors affecting meat quality. In this study, when compared with the CON group, the myofiber diameter was significantly reduced by long-term HFD, while the myofiber diameter was significantly increased by the inclusion of sanguinarine in HFD. Previous studies conducted on mice and Nile tilapia have identified a negative correlation between myofiber diameter and dietary fat content (Trovato et al., 2018; Zhang et al., 2022). Therefore, feeding HFD can decrease myofiber diameter, subsequently increasing myofiber density, a phenomenon that can be significantly alleviated by adding sanguinarine to diets. Prior research has demonstrated that myofiber growth is chiefly regulated by the mechanisms of myofiber hypertrophy and hyperplasia, where hypertrophy increases myofiber diameter, and hyperplasia increases myofiber count (White et al., 2018). Muscle growth and differentiation are controlled by specific gene products of myogenic cytokines in myogenic regulatory factors (MRFs) (Wirth-Dzięciołowska et al., 2011), in which Mrf4, Myf5, MyoG, and MyoD are the four major members of MRFs. MyoD and MyoG, pivotal members of the MyoD transcription factor family, play a critical role in orchestrating skeletal muscle maturation. Pax participates in initiating myogenic processes during development by modulating the expression of Myf5 or MyoD (McKinnell et al., 2008). Studies in Turbot showed that the expression levels of *myog*, *myf5*, and *mrf4* in the muscle were up-regulated in HFD (Huang et al., 2022). This study also demonstrated that the HFD led to a substantial elevation in the expression levels of *myog*, *mrf4*, *mrf5*, and *pax* within the muscle. Notably, the incorporation of sanguinarine to HFD exhibited a mitigating effect on the occurrence of this phenomenon to a certain extent. These results suggest that the increase in myofiber density and decrease in myofiber diameter induced by HFD were related to the regulation of MRFs, and that the above phenomena can be significantly alleviated by supplementing sanguinarine to the diets.

4.5. Sanguinarine relieved muscle oxidative damage caused by fat deposition in grass carp fed HFD

Abundant research endeavors have demonstrated that the prolonged consumption of HFD in fish leads to fat deposition, resulting in an increase in fat peroxidation products. This cascade effect consequently disrupts the homeostasis of the antioxidant system (Shi et al., 2022a). Oxidative damage is also recognized as a significant factor contributing to the deterioration of fish meat quality (Jiang et al., 2015). Therefore, enhancing the antioxidative potential of fish muscle plays a pivotal role in the amelioration of fish meat quality. In grass carp, it has been observed that HFD down-regulate the expression of antioxidant enzyme genes (primarily including *cat*, *sod*, and *gpx*) via the Keap1/Nrf2 signaling pathway, consequently diminishing the antioxidant capacity of muscle (He et al., 2012). The results of this experiment align with the findings of the aforementioned study. Additionally, it was discovered that incorporating sanguinarine into HFD can elevate the protein and transcription levels of Nrf2 while reducing the transcription levels of Keap1. This action enhances the transcription levels of *sod*, *gpx4*, *cat*, and *gr*, thereby alleviating the decline in antioxidant capacity triggered by the HFD. Previous research has indicated that introducing an appropriate dose of sanguinarine into diets can enhance antioxidant enzyme activities through the Nrf2/Keap1 pathway, effectively mitigating the oxidative stress induced by hydrogen peroxide in rice field eel (Shi et al., 2022b). Consequently, the present study provides additional confirmation that sanguinarine can enhance the antioxidant capacity of grass carp through the Keap1/Nrf2 pathway, ultimately leading to an improvement in muscle quality.

The occurrence of oxidative stress in the body is commonly associated with the induction of an inflammatory response. Numerous investigations have provided evidence that the consumption of HFD can lead to the up-regulation of pro-inflammatory factors, such as *il-1β* and *tnf-α*, in fish including tilapia (Jia et al., 2020) and black seabream (Jin et al., 2019). Nuclear factor kappa-B (NF-κB) represents a key transcription factor responsible for the regulation of cytokines. It has been demonstrated that sanguinarine is an effective inhibitor of NF-κB, which inhibits the expression of pro-inflammatory factors (Hou and Zeng, 2018). The present investigation revealed that the inclusion of sanguinarine in HFD exerted an obvious alleviating effect on the inflammatory response through inhibiting NF-κB activity. This phenomenon was predominantly characterized by the elevation of anti-inflammatory factors, such as *il-15* and *tgf-β1*, coupled with the reduction of pro-inflammatory factors, including *tnf-α*, *il-8*, *il-6*, and *il-1β*.

5. Conclusion

In summary, long-term feeding of HFD leads to the reduction of growth performance of grass carp, the increase of muscle fat deposition, the reduction of muscle hardness and protein deposition, the shortening of myofiber diameter, and the oxidative damage in muscle caused by fat deposition, thereby diminishing muscle quality and nutrient composition. However, the supplementation of sanguinarine to HFD alleviated the alterations in these abnormal indexes, thereby improving muscle quality and promoting growth performance. Therefore, the appropriate use of sanguinarine as a feed additive has been proven beneficial in ameliorating the muscle quality decline of fish fed HFD.

Data availability

All data are available from the corresponding author by request.

Author contributions

Yong Shi: Data curation, Formal analysis, Software, Project administration, Writing - Original draft. **Lei Zhong:** Funding acquisition, Project administration, Validation. **Yuanxiang Liu:** Data curation, Methodology. **Shude Xu:** Investigation. **Jihong Dai:** Investigation. **Yaozhengtai Zhang:** Data curation. **Yi Hu:** Project administration, Supervision, Writing - Review & editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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