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Effects of supplementation with crystalline or coated methionine and lysine in low protein diet on growth performance, intestinal health and muscle quality of gibel carp, *Carassius auratus gibelio*

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ABSTRACT

A 10-week feeding trial was conducted to elucidate the effects of coated amino acids on growth performance, intestinal health, and muscle quality of gibel carp (*Carassius auratus gibelio*) fed with low protein diet. A total of 450 carps (initial body weight of 15.67 g ± 0.04) were selected and randomly assigned into 6 groups, with each group consisting of three replicates and 25 fish per replicate. Six experimental diets were: control group (NP, 31.44% CP), low protein group (LP, 28.69% CP), low protein supplemented with crystalline methionine (CrM), crystalline methionine and crystalline lysine (CrML), coated methionine (CoM) or coated methionine and coated lysine (CoML). The results indicated that compared to the NP group, the LP group showed no significant difference in weight gain, specific growth rate, feed conversion ratio, viscerosomatic index, and condition factor ($P > 0.05$) but had a significant decrease in whole body crude fat content and increase in moisture content ($P < 0.05$). The hepatosomatic index in the CoML group was significantly lower than the LP group ($P < 0.05$), and moisture content in the CrM and CoM groups was significantly lower than LP group ($P < 0.05$). Serum alanine transaminase of the CrML group was significantly lower than the LP group. Methane dicarboxylic aldehyde content and superoxide dismutase activity in the CrML group were significantly lower than those in the LP group ($P < 0.05$), and hardness, firmness, and chewiness in the CoM group were significantly lower ($P < 0.05$) than the LP group. Compared to crystalline amino acids, coated amino acid groups showed a significant increase in crude fat content ($P < 0.05$) and significant enhancements in the activities of superoxide dismutase and catalase ($P < 0.05$). This results indicate that low protein feed supplemented coated amino acids can significantly improve the antioxidant capacity, fat deposition, intestinal health, and muscle quality of gibel carp.

1. Introduction

Protein is an important nutrient for life, playing a key role in body composition and cellular metabolism (Macelline et al., 2021). Reducing protein in fish feed has various effects, as studies show largemouth bass on a 52% protein diet experienced significantly higher weight gain (380.65%) and crude protein content (66.32%) compared to those on a 48% protein diet, also achieving the lowest feed conversion ratio (0.96) (Liu et al., 2023). Reducing dietary protein level from 40% to 30%, the weight gain and specific growth rate of loach (*Paramisgurnus dabryanus*) decreased significantly (Wang et al., 2023). In the gibel carp, whole-body crude lipid content decreased and moisture content increased with reduced dietary protein levels fed to the fish (Tu et al., 2015). The decrease in protein level results in a deficiency of certain limiting amino acids, with methionine and lysine being the main limiting amino acids in plant protein sources (Moyo Ngonidzashe and RapatsaMalatji Mmaditshaba, 2023). The lack of methionine and lysine will affect the growth and health of fishes (Ji et al., 2022; Huang et al., 2022). Supplementation of methionine and lysine can effectively balance the amino acids in feed (Moyo Ngonidzashe and RapatsaMalatji

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Mmaditshaba, 2023).

In rainbow trout and marine fish, the utilization of crystalline amino acids is more efficient (Takeshi et al., 2005; Helena and Aires, 2005); whereas, it is less effective in shrimp (*Penaeus vannamei*) and mirror carp (*Cyprinus carpio* Songpu) (Zhao et al., 2022). A general view is that the absorption of crystalline amino acids is faster than the absorption of feed proteins, because the protein synthesis needs to be in accordance with a certain proportion, the crystalline amino acids have not yet be used to synthesize protein and have been excreted (Zhao et al., 2022; Alberto et al., 2014). Another view is that this phenomenon may be the result of the rapid flow of short peptides and free amino acids through the intestinal wall, which cannot be handled by a gastric fish. As a result, most of these metabolites are flushed out of the digestive system (Sagiv and Amos, 2001). Coating technology is to encapsulate amino acids and, gradually release them in the intestine, and improve the absorption and utilization efficiency of amino acids in fish. Previous studies have demonstrated the positive effects of coated methionine and lysine on the growth performance, digestion and absorption, and immunity of various fish species. For example, coated amino acids slow down the rate of excretion in the intestine of tilapia (*Oreochromis niloticus*) and increase the absorption rate of amino acids (Segovia-Quintero and Reigh, 2004). However, compared to other species, research focusing on gibel carp is significantly scarce (*Carassius auratus gibelio*).

Gibel carp is an important economic crucian carp widely distributed in freshwater systems in China. Moreover, its fast growth rate and flavorful meat made it as one of the important varieties in aquaculture. Therefore, the purpose of this study was to investigate the effects of a low protein diet supplemented with crystalline and coated methionine and lysine on the growth performance, intestinal health and muscle quality of carp, and to provide scientific basis and technical support for its breeding.

2. Materials and methods

2.1. Diet formulation and preparation

Using fish meal, soybean meal, and rapeseed meal as the main protein sources, six diets were prepared (Table 1). The optimum dietary protein level of gibel carp was established at 32.4%, and reducing dietary protein level below 29% will have a negative impact on gibel carp growth performance (Ye et al., 2017). Therefore, this study the protein level was set at 31.44% in the control group (NP, control group), and the low dietary protein level was formulated at 28.69% for other dietary treatment (low protein group, LP). Crystal methionine was supplemented to the low protein feed (CrM group), crystal methionine and crystal lysine were supplemented to the low protein feed (CrML group), coated methionine was supplemented to the low protein feed (CoM group), and coated methionine and coated lysine was supplemented to the low protein feed (CoML group). The fully mixed raw materials were extruded into pellets by a twin screw extruder (diameter 2.5 mm, length 2 mm). The pellets were air-dried at 40 °C to about 10% moisture content and stored in a refrigerator at 20 °C until it was used for feeding experiments.

2.2. Rearing conditions

The experiment was carried out in the recirculating aquaculture system. Carp were disinfected with potassium permanganate before the experiment and acclimated for 2 weeks. 450 carps with an average weight of (15.67±0.04) g were selected and randomly divided into 6 groups, with 3 replicates in each group and 25 fish in each replicate. Initial body weight, final body weight, weight gain, specific growth rate, and feed conversion ratio were counted on the average value of 25 fish per tank, and the remaining indicators were randomly selected from 3 fish per tank for detection. The breeding test was carried out for 10 weeks. These carps were hand-fed at 8:00, and 17:00 every day, each diet was fed at 2.5–3% body weight/day, and the daily ration was adjusted according to the fish weight every two weeks. The experiment was conducted in natural light, and the photoperiod imposed during the experimentation was from 8: 00 a.m. to 8: 00 p.m. The water quality was regularly checked, and replaced to ensure good water quality. During feeding and testing, the water temperature was 24.6–30.4℃, dissolved oxygen ≥6 mg/L, ammonia nitrogen ≤0.3 mg/L, and nitrite <0.05 mg/L.

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

Ingredients	Diet Groups					
	NP	LP	CrM	CrML	CoM	CoML
Fish meal	5	5	5	5	5	5
Soybean meal	26.8	18.1	18.1	18.1	18.1	18.1
Rapeseed meal	12	12	12	12	12	12
Cottonseed meal	10	10	10	10	10	10
Wheat-middling	31.6	38.8	38.8	38.8	38.8	38.8
Soybean lecithin	1.5	1.5	1.5	1.5	1.5	1.5
Soybean oil	1	1	1	1	1	1
Fish oil	1	1	1	1	1	1
Lysine	0	0	0	0.28	0	0
Methionine	0.495	0	0.52	0.52	0	0
Coated lysine (10%)	0	0	0	0	0	2.25
Coated methionine (10%)	0	0	0	0	5.25	5.25
Extruded corn	5.505	7.5	6.98	6.7	2.25	0
$Ca(H_2PO_4)_2$	2	2	2	2	2	2
Vitamin premix[a]	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix[b]	0.2	0.2	0.2	0.2	0.2	0.2
Sodium carboxymethyl cellulose	2	2	2	2	2	2
Choline chloride (50%)	0.2	0.2	0.2	0.2	0.2	0.2
Magnesium sulphate	0.2	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100	100
Nutrient level						
Crude protein	31.44	28.69	29.56	28.49	28.63	28.88
Crude fat	5.80	5.78	5.77	5.76	5.77	5.76
Methionine	0.90	0.38	0.77	0.75	0.79	0.79
Lysine	1.67	1.41	1.43	1.60	1.43	1.54

[a] The premix of Vitamin provided the following per kg of diets: Vitamin A 8000 IU, Vitamin C 500 mg, Vitamin D 3000 IU, Vitamin E 60 mg, Vitamin K 35 mg, Vitamin B_1 15 mg, Vitamin B_2 30 mg, Vitamin B_6 15 mg, Vitamin B_{12} 0.5 mg.

[b] The premix of mineral provided the following per kg of diets: $FeSO_4 \bullet 7\ H_2O$ 754.56 mg, $CuSO_4 \bullet 5\ H_2O$ 23.81 mg, $MnSO_4 \bullet H_2O$ 168.29 mg, $ZnSO_4 \bullet 7\ H_2O$ 444 mg, Na_2SeO_3 2.26 mg, KI 0.79 mg, $CoCl_2$ 2.21 mg, Zeolite meal 604.08 mg.

2.3. Sample collection

Before the experiment, the initial weight of the experimental fish was measured. At the end of the experiment, each group was weighed, and three fish were randomly selected for body composition determination. The growth performance indices were measured as follows:

Survival rate (SR, %) = 100 × The number of final fish/The number of initial fish ;

Weight gain (WG, %) = 100 × [Final body weight (g)-initial body weight (g)]/initial body weight (g) ;

Specific growth rate (SGR, %/d) = 100 × {Ln[final body weight (g)]-Ln[initial body weight (g)]}/Number of days (d) ;

Feed conversion ratio (FCR) = feed intake(g)/[final body weight(g)-initial body weight(g)]

Condition factor (CF, g/cm^3) = 100 × [final body weight (g)/final body length (cm)^3] ;

Hepatosomatic index (HSI, %) =100 × [hepatopancreas weight (g)/

weight of this fish (g)] ;

Viscerosomatic index (VSI, %) = 100 × [viscera weight (g)/weight of this fish (g)].

Additionally, three fish were randomly selected from each group and anesthetized with MS-222 (100 mg/L). Blood was collected from the tail vein and 4 °C, 4000 rpm, centrifuged for 10 min. Then, the upper serum was stored at −80 °C for serum biochemical analysis. Then the hepatopancreas and viscera were dissected and weighed. The hepatopancreas was taken for the determination of antioxidant enzymes, and the hindgut was taken for the preparation of intestinal sections. The white muscles above the lateral line in front of the left dorsal fin of three fish in each group were used to measure muscle texture. These samples were stored at − 80 °C for subsequent analysis.

2.4. Proximate composition of whole fish and diets

Analyzed for proximate composition according to standard methods (AOAC, 2000). The whole body composition and the nutritional composition of the feed was determined by the analysis methods are as follows: 105 °C drying method to determine moisture content, Dumas nitrogen method to determine crude protein content, Soxhlet extraction method to determine crude fat content, Muffle furnace 550 °C high temperature burning to determine crude ash content.

2.5. Determination of serum and hepatopancreas transaminase parameters

The activities of aspartate transaminase (AST) and alanine transaminase (ALT) in serum, and the AST, ALT, superoxide dismutase (SOD), catalase (CAT), Methane dicarboxylic aldehyde (MDA) and glutathione (GSH) in the hepatopancreas were determined by the kit of Nanjing Jiancheng Bioengineering Institute followed the instructions.

2.6. Preparation and observation of hematoxylin-eosin stained gut and muscle sections

The hindgut samples and the white muscle above the lateral line in front of the left dorsal fin was taken and trimmed into 1.0 cm × 1.0 cm × 0.5 cm blocks. Each was placed in a fixed preservation solution (4.0% polymethyl methacrylate), and sliced and stained with HE by Hangzhou Haoke Biotechnology Co., Ltd. Intestinal sections with complete villi and straight vision were selected on each tissue. The images were collected by digital microscope and the villus height, crypt depth, and muscle thickness were analyzed. Muscle samples were cut longitudinally, and 3 sections with a clear vision and complete muscle fibers were selected in each group. Digital microscope was used to collect images and analyze muscle fiber diameter and density.

2.7. Fillet texture analysis

The white muscle above the lateral line in front of the left dorsal fin was trimmed into a block of 1.0 cm × 1.0 cm × 0.5 cm. The texture profile analysis (TPA) was performed by TA.XT plus texture analyzer (SMS, UK) to detect the springiness, hardness, toughness, firmness, chewiness, and compactness of the muscle.

The white muscle above the lateral line in front of the left dorsal fin was taken and trimmed into a 1.0 cm × 1.0 cm × 0.5 cm block. The block was bathed in a water bath for 5 minutes at 95 °C and then cooled with running water. The springiness, hardness, toughness, firmness, chewiness, and compactness of the muscle were measured.

2.8. Statistical analysis

One-way ANOVA was performed on experimental data using SSPS 27.0. Tukey's test was used for multiple comparisons if there were significant differences between groups. The experimental data were expressed as mean ± standard error (mean ±SE). General linear model was used for Two-way ANOVA analysis with 2×2 factors. Independent sample t-test was used to analyze the significant differences between different amino acid additions in coated amino acids or crystalline amino acids. The same amino acid was coated or not also used independent sample t test.

3. Results

3.1. Growth performance

Compared with the control group, there was no significant difference in the weight gain, specific growth rate, feed conversion ratio, viscerosomatic index and condition factor among all dietary groups ($P>0.05$) (Table 2); hepatosomatic index in LP group was significantly higher than that in NP group, and hepatosomatic index in CrM, CrML and CoML groups was significantly lower than that in LP group ($P<0.05$).

Amino acid supplementation (coated or uncoated) did not significantly influence the weight gain, specific growth rate, feed conversion ratio, hepatosomatic index, viscerosomatic index, and condition factor in carp. Furthermore, there was no interaction effect observed between the coated or uncoated amino acids and type of amino acid (lysine or methionine) on these growth metrics ($P>0.05$). Additionally, dietary group CrML significantly reduced hepatosomatic index compared with the addition of CrM, and have significant interaction effets between type of amino acids and coated or uncoated amino acids ($P<0.05$). The results of t test showed that HSI in CoM, CrML group was significantly higher than that in CoML group ($P<0.05$) (Fig. 1).

3.2. Proximate composition of whole fish

Compared with the control group, there was no significant difference in the crude protein and ash content of each group of carp ($P>0.05$). The moisture content of the LP group was significantly higher than that of the NP group, and the moisture content of CrM and CoM groups was significantly lower than that of LP group ($P<0.05$). Compared with the control group, the crude fat content of LP group was significantly lower than that of NP group ($P<0.05$), and there was no significant difference between the other groups and the LP group ($P>0.05$) (Table 3).

Coated amino acids can significantly increase the crude fat content of carp ($P<0.05$), and there is no interaction effect with types of amino acid ($P<0.05$), while the addition of lysine and methionine can significantly increase moisture content compared with the addition of methionine ($P<0.05$), and there is no interaction effect with coated and uncoated amnio acids ($P>0.05$). The results of t test showed that moisture in CoM group was significantly lower than that in CoML group, crude fat in CoML group was significantly higher than that in CrML group, ash in CoML group was significantly lower than that in CrML group ($P<0.05$) (Fig. 2).

3.3. Effects of low protein diet supplemented with lysine and methionine on serum and hepatopancreas transaminases in carp

Compared with the control group, there was no significant difference in the aspartate transaminase and alanine transaminase of hepatopancreas among all dietary groups ($P>0.05$); Serum alanine transaminase in LP group was significantly higher than that in NP group, and alanine transaminase in CrML group was significantly lower than that in LP group ($P<0.05$) (Table 4).

The coated amino acids can significantly increase the serum aspartate transaminase activity of carp, and have no interaction effect with types of amino acids ($P<0.05$). The coated amino acids can significantly increase the serum alanine transaminase activity of carp ($P<0.05$) and have no interaction effect with types of amino acids ($P>0.05$). The addition of lysine and methionine significantly reduced serum aspartate transaminase activity compared with the addition of methionine and no

Table 2
Effects of supplementation of lysine and methionine to low protein diet on growth performance of carp.

Diet groups	IBW (g)	FBW (g)	WG (%)	SGR (%/d)	FCR	HSI (%)	VSI (%)	CF (g/cm^3)
NP	15.68±0.02	28.52±0.29	81.86±2.00	0.85±0.02	2.75±0.07	2.95±0.10^{ab}	8.74±0.42	2.66±0.31
LP	15.65±0.02	27.44±0.77	75.35±5.04	0.80±0.04	3.01±0.19	4.42±0.10^{d}	10.95±0.78	2.64±0.09
CrM	15.68±0.02	29.09±0.77	85.48±5.09	0.88±0.04	2.65±0.16	3.60±0.17^{bc}	10.96±0.52	2.37±0.09
CrML	15.69±0.03	27.20±0.35	73.40±2.42	0.79±0.02	3.06±0.09	3.33±0.09^{ab}	10.06±0.64	2.63±0.05
CoM	15.64±0.02	28.17±0.57	80.09±3.52	0.84±0.03	2.82±0.13	4.13±0.26 ^{cd}	10.47±0.80	2.71±0.20
CoML	15.66±0.04	28.05±0.41	79.20±2.43	0.83±0.02	2.84±0.09	2.89±0.12^{a}	10.19±1.07	2.76±0.10

Note: data are expressed as mean ± SE, and values with different superscript letters in the same row are statistically significant ($P < 0.05$). IBW, initial body weight; FBW, final body weight; WG, weight gain rate; SGR, specific growth rate; FCR, feed conversion ratio; HSI, hepatosomatic index; VSI, viscerosomatic index; CF, condition factor.

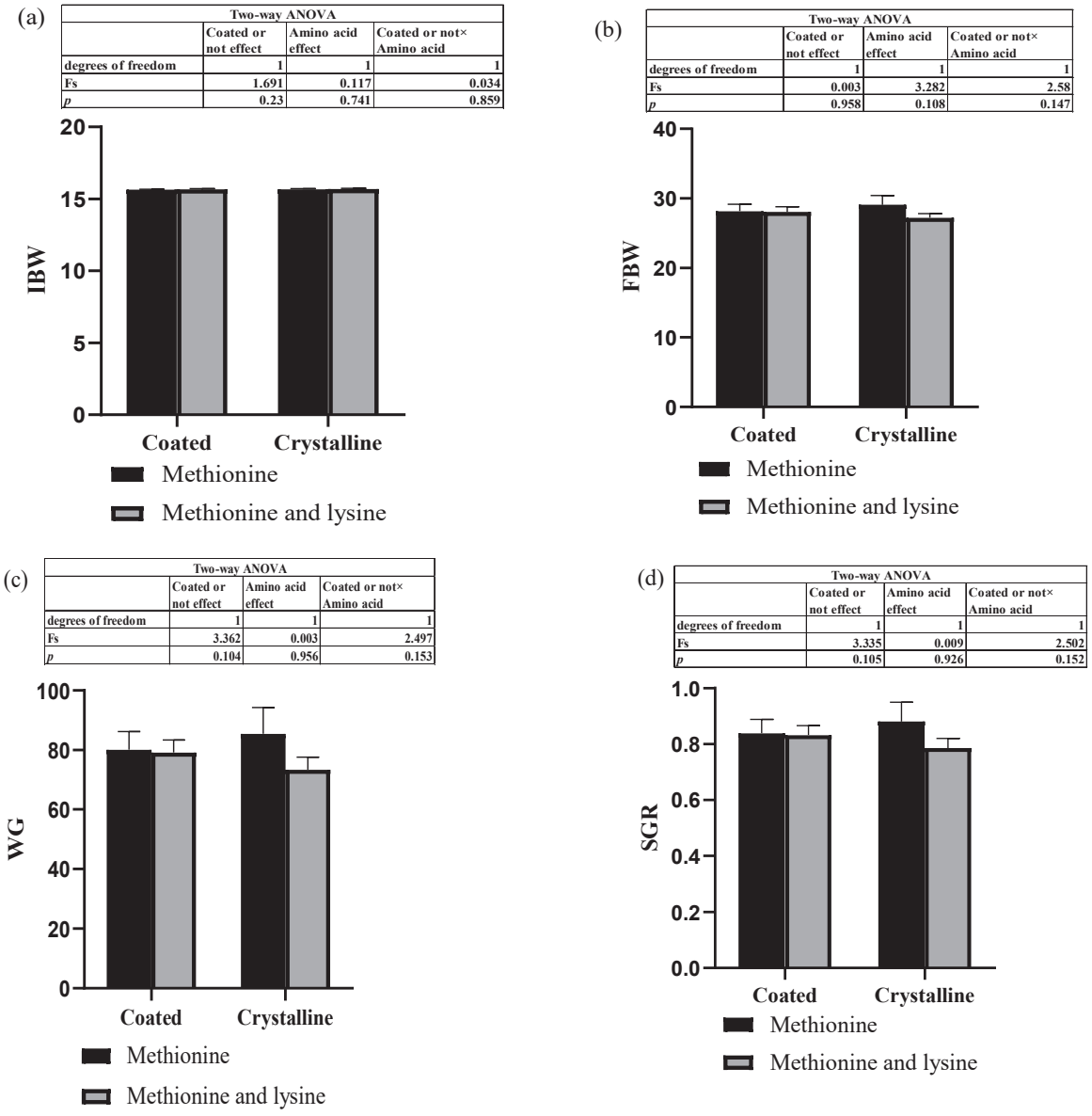


Fig. 1. Effects of supplementation of lysine and methionine to low protein diet on growth performance of carp. initial body weight(IBW) (a); final body weight(FBW) (b); weight gain rate(WG) (c); specific growth rate(SGR) (d);feed conversion ratio(FCR) (e);hepatosomatic index(HSI) (f);viscerosomatic index(VSI) (g);condition factor(CF) (h).* indicates significant differences ($P < 0.05$).

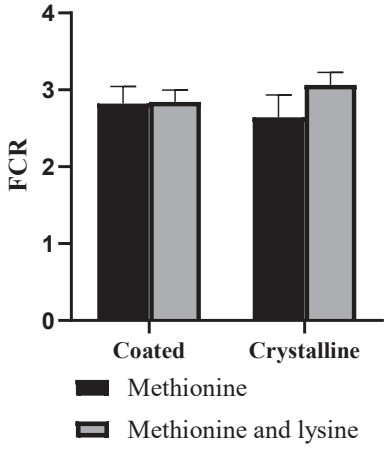
significant interactions between type and coated and uncoated amnio acids ($P < 0.05$).The t test results illustrates that the serum ALT in the CoML group was significantly higher than that in the CrML group ($P < 0.05$) (Fig. 3).

3.4. Effects of low protein diet supplemented with lysine and methionine on antioxidant capacity of carp

Compared with the control group, there was no significant difference

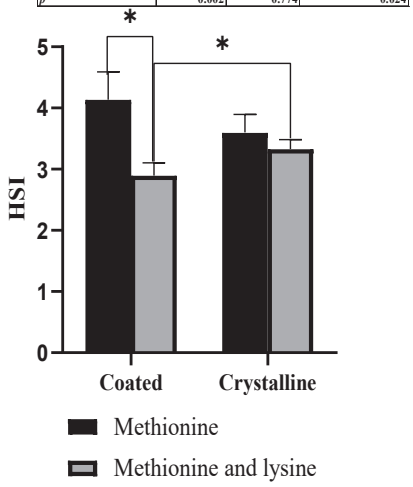
(e)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	3.224	0.031	2.58
p	0.11	0.865	0.147



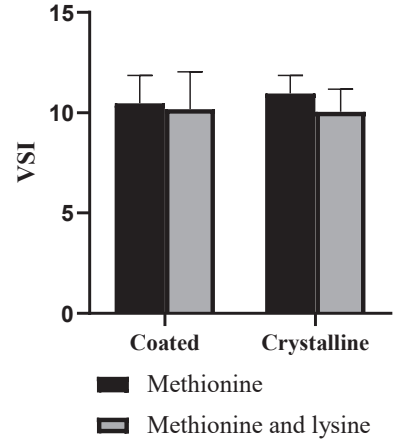
(f)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	18.953	0.088	7.754
p	0.002	0.774	0.024



(g)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.57	0.054	0.158
p	0.472	0.822	0.701



(h)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	1.459	3.671	0.685
p	0.262	0.092	0.432

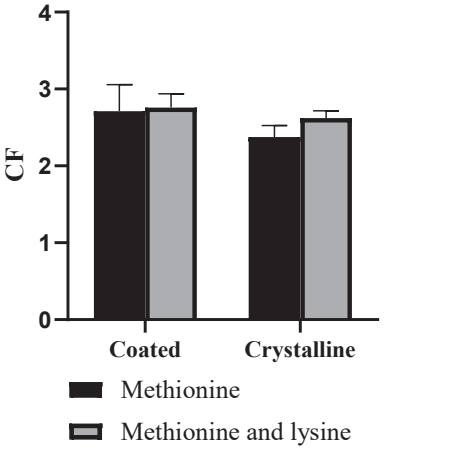


Fig. 1. (*continued*).

Table 3
Effects of supplementation of lysine and methionine to low protein diet on the nutritional composition of carp **%.**

Diet groups	Moisture	Crude protein	Crude fat	Ash
NP	66.82±1.22^{a}	16.21±0.36	10.72±0.59^{c}	4.28±0.48
LP	73.07±0.58^{c}	16.20±0.29	7.22±0.68^{ab}	3.93±0.23
CrM	67.80±1.41^{ab}	17.07±0.22	7.51±0.79^{ab}	4.11±0.05
CrML	71.57±1.17^{bc}	16.32±0.34	5.89±0.71^{a}	4.09±0.06
CoM	68.01±0.36^{ab}	16.00±0.43	8.16±0.36^{abc}	4.03±0.16
CoML	70.53±0.24^{abc}	15.86±0.83	9.06±0.46^{bc}	3.72±0.11

Note: data are expressed as mean ± SE, and values with different superscript letters in the same row are statistically significant ($P<0.05$).

in the glutathione and catalase among all groups ($P>0.05$); Methane dicarboxylic aldehyde content in CrML and CoML groups was significantly lower than that in LP group, and superoxide dismutase activity in CrML group was significantly lower than that in LP group ($P<0.05$) (Table 5).

Coated amino acids can significantly increase the superoxide dismutase activity of hepatopancreas ($P<0.05$), and have no significant interaction with types of amino acids ($P>0.05$). The addition of lysine and methionine significantly reduced Methane dicarboxylic aldehyde content compared with the addition of methionine and had no interaction with coated and uncoated amino acids ($P<0.05$) (Fig. 4).

3.5. Effects of low protein diet supplemented with lysine and methionine on intestinal morphology of carp

Compared with the control group, there was no significant difference in the Crypt depth、Muscular thickness and Villus height/Crypt depth among all groups of carp ($P>0.05$); the villus length of CoM group was significantly lower than that of NP group ($P<0.05$) (Table 6).

Coated amino acids had no significant effect on villus height, muscle thickness and crypt depth of carp, and had no interaction effects with types of amino acids ($P>0.05$). The addition of lysine and methionine significantly increased the villus length and their ratio compared with the addition of methionine and had no interaction with coated and uncoated amino acids ($P<0.05$). The results of t test showed that the intestinal villus height of CoML group was significantly higher than that

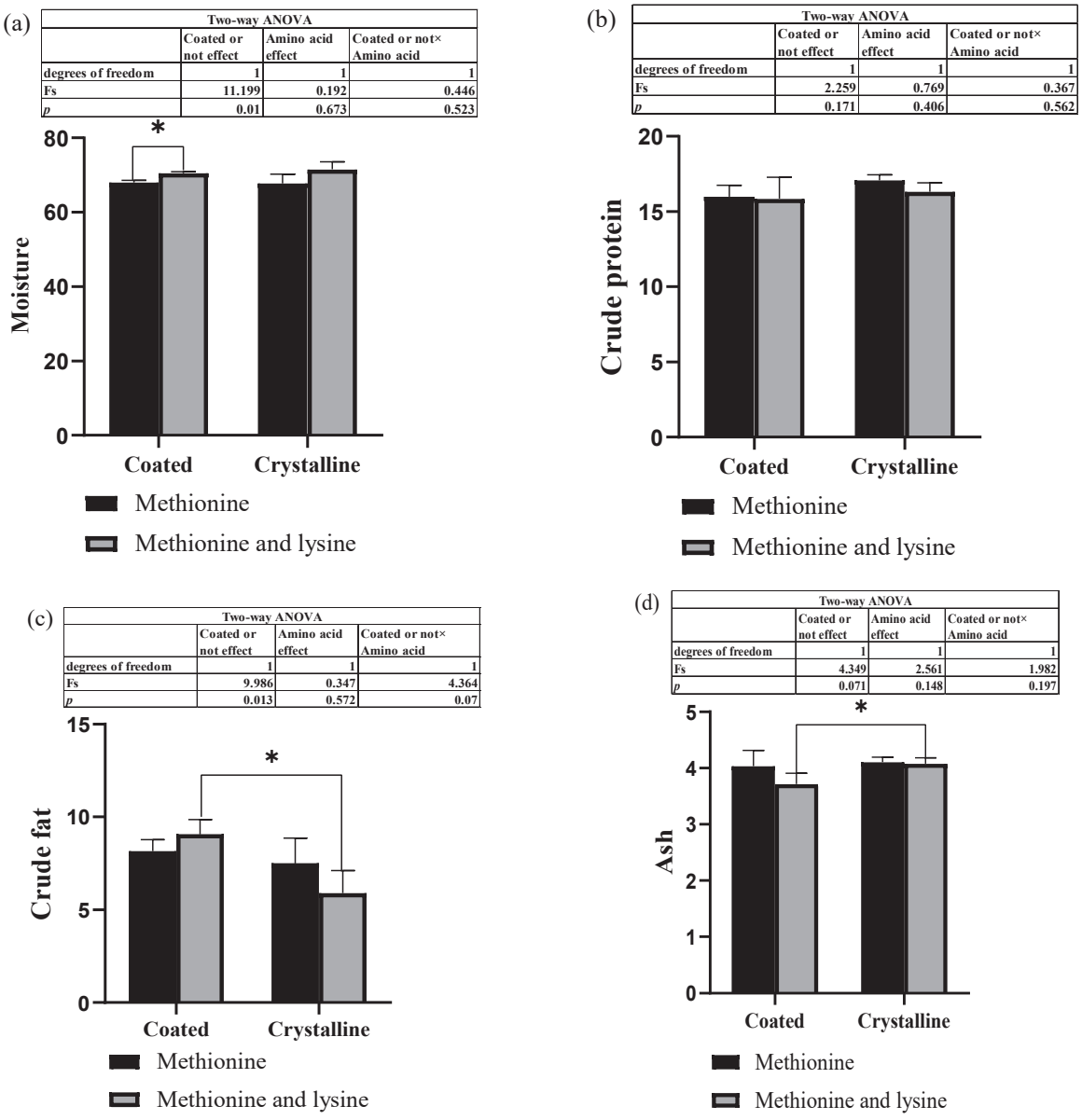


Fig. 2. Effects of supplementation of lysine and methionine to low protein diet on the nutritional composition of carp. Moisture (a); crude protein (b); crude fat (c); ash (d).* indicates significant differences ($P<0.05$).

Table 4
Effect of supplementation of lysine and methionine to low protein diet on transaminase in serum and hepatopancreas of carp.

Diet groups	AST		ALT	
	Serum (U/mL)	hepatopancreas [U/(g prot)]	Serum (U/mL)	hepatopancreas [U/(g prot)]
NP	32.28±2.04^{a}	288.06±22.82	7.71±0.92^{ab}	82.12±20.91
LP	37.50±1.76^{ab}	319.85±8.04	13.90±0.75^{c}	94.65±11.54
CrM	35.78±2.80^{ab}	319.32±22.76	8.77±1.63^{bc}	95.68±25.02
CrML	30.37±1.57^{a}	342.40±19.41	3.69±1.43^{a}	91.62±13.31
CoM	47.18±4.79^{b}	390.01±20.22	10.25±1.37^{bc}	147.84±27.65
CoML	36.97±1.98^{ab}	330.51±42.19	9.61±0.54^{bc}	90.58±14.44

Note: data are expressed as mean ± SE, and values with different superscript letters in the same row are statistically significant ($P<0.05$). AST, aspartate transaminase; ALT, alanine transaminase.

of CoM group, the crypt depth of CoML group was significantly lower than that of CoM group, and the VH / CD of CoML group was significantly higher than that of CoM group ($P<0.05$) (Fig. 5).

3.6. Effects of low protein diet supplemented with lysine and methionine on muscle texture and muscle tissue morphology characteristics of carp

Compared with the control group, there was no significant difference in the Springiness, Toughness, Compactness of raw fillet and Toughness,

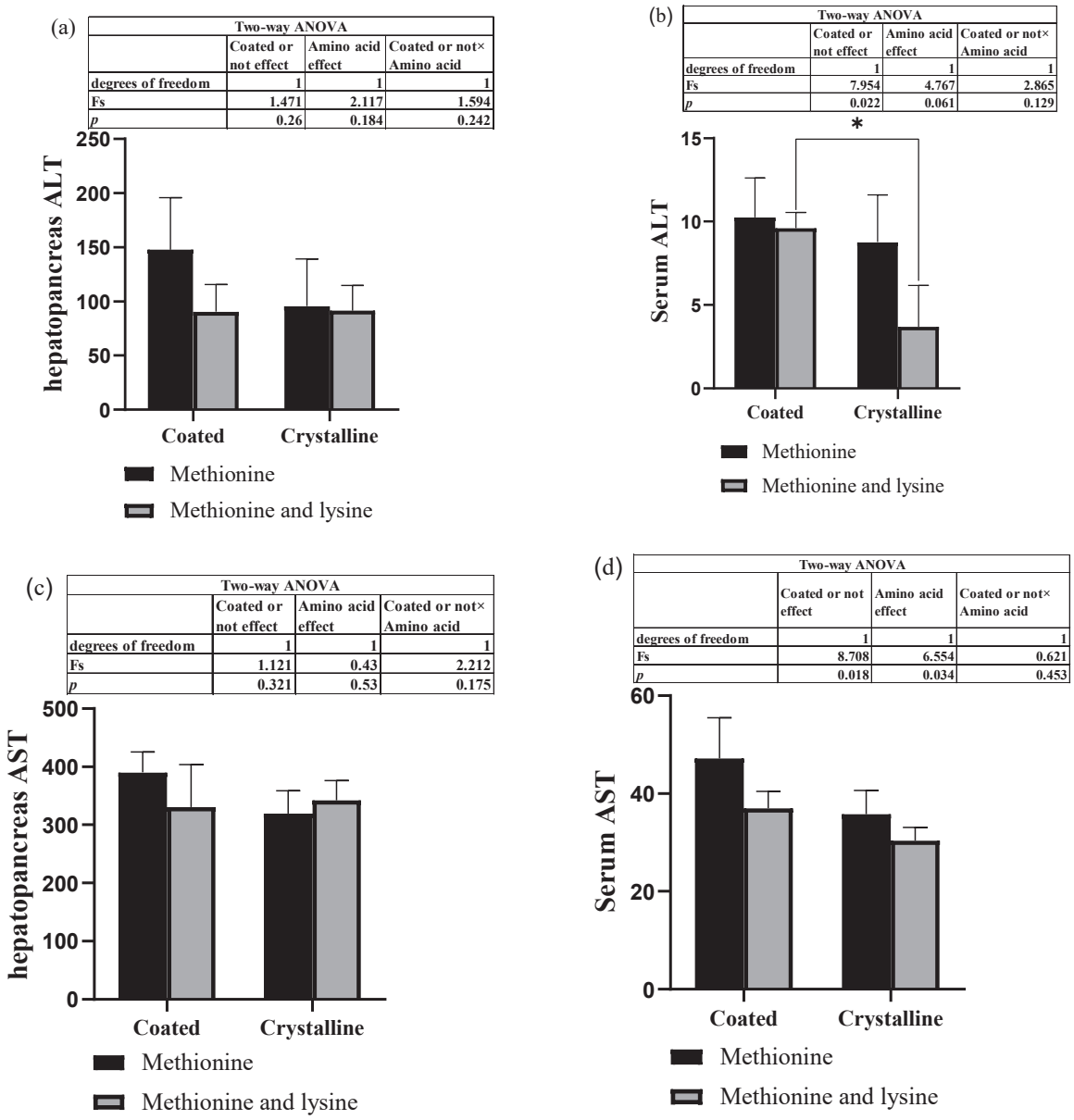


Fig. 3. Effect of supplementation of lysine and methionine to low protein diet on transaminase in serum and hepatopancreas of carp. alanine transaminase (ALT) activity in the hepatopancreas (a); alanine transaminase (ALT) activity in the serum (b); aspartate transaminase (AST) activity in the hepatopancreas (c); aspartate transaminase (AST) activity in the serum (d).* indicates significant differences ($P<0.05$).

Table 5
Effect of supplementation of lysine and methionine to low protein diet on the antioxidant capacity of hepatopancreas of carp.

Diet groups	GSH (U/mg prot)	MDA (U/mg prot)	SOD (U/mg prot)	CAT (U/mg prot)
NP	0.05±0.00	2.67±0.06^{ab}	1.08±0.11^{ab}	0.35±0.03
LP	0.05±0.01	4.15±0.43^{b}	1.38±0.03^{b}	0.50±0.06
CrM	0.12±0.07	3.57±0.56^{ab}	1.09±0.04^{ab}	0.26±0.07
CrML	0.04±0.01	1.78±0.60^{a}	1.02±0.07^{a}	0.25±0.09
CoM	0.19±0.08	2.54±0.53^{ab}	1.23±0.08^{ab}	0.35±0.06
CoML	0.12±0.07	1.78±0.39^{a}	1.22±0.07^{ab}	0.29±0.09

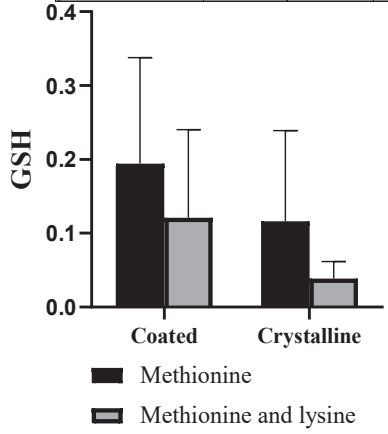
Note: data are expressed as mean ± SE, and values with different superscript letters in the same row are statistically significant ($P<0.05$). GSH, glutathione; CAT, catalase; SOD, superoxide dismutase; MDA, methane dicarboxylic aldehyde.

Firmness, Compactness, Chewiness of cooked fillet in each group ($P>0.05$); Raw fillet Hardness, Firmness and Chewiness in the CoM group were significantly lower than those in LP group ($P<0.05$). The Cooked fillet Springiness of the CoML and CrML groups was significantly lower than that of the NP group, and the Cooked fillet Hardness of the CrML group was significantly lower than that of the NP group ($P<0.05$). Compared with the control group, there was no significant difference in the myofiber density of each group of carp ($P>0.05$); Myofiber diameter in CrM and CrML groups was significantly lower than that in NP group ($P<0.05$) (Table 7).

Coated amino acids had no significant effects on Raw fillet, Cooked fillet Springiness, Hardness, Toughness, Firmness, Compactness and Chewiness of carp ($P>0.05$). Besides that, it was noted that significant interaction effets between type and coated and uncoated amino acids on Cooked fillet Springiness and Hardness ($P<0.05$). The addition of two amino acids significantly increased Raw fillet Hardness, Firmness and Chewiness compared with the addition of one amino acid ($P<0.05$), and had no interaction with coated and uncoated amino acids ($P>0.05$).

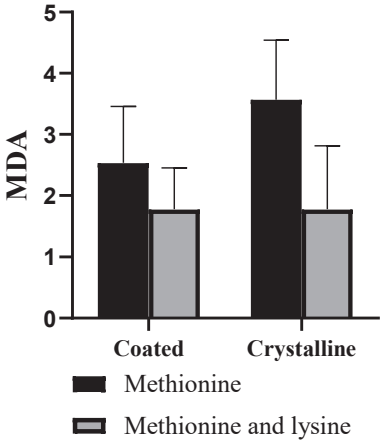
(a)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	1.542	1.353	0.001
p	0.249	0.278	0.973



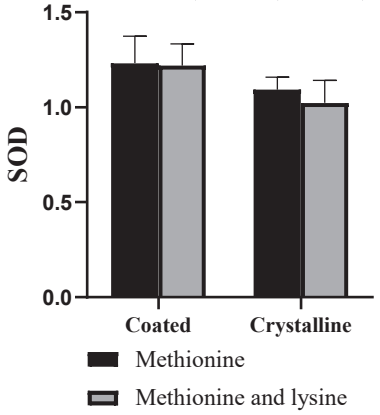
(b)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.95	5.821	0.949
p	0.358	0.042	0.358



(c)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	6.593	0.421	0.195
p	0.033	0.535	0.67



(d)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.745	0.155	0.107
p	0.413	0.704	0.752

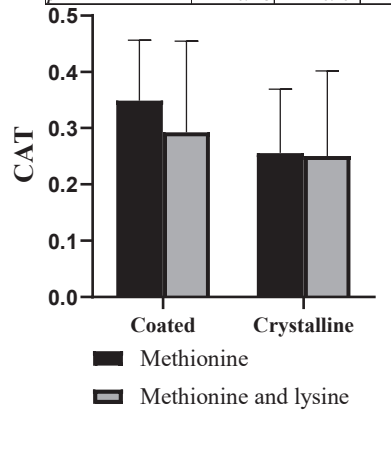


Fig. 4. Effect of supplementation of lysine and methionine to low protein diet on the antioxidant capacity of hepatopancreas of carp. glutathione (GSH) activity(a); methane dicarboxylic aldehyde (MDA) activity(b); superoxide dismutase (SOD) activity (c); catalase (CAT) activity in the hepatopancreas (d).* indicates significant differences ($P<0.05$).

Coated amino acids had no significant effect on Myofiber density and myofiber diameter of carp, and had no interaction effect with types of amino acid ($P>0.05$). The addition of lysine and methionine significantly increased the Myofiber diameter of carp compared with the addition of methionine and had no interaction with coated and uncoated amino acids ($P<0.05$). The results of t test showed that Springness CF in CrM group was significantly higher than that in CrML group, Harderness RF in CrM group was significantly lower than that in CrML group, Harderness CF in CoML, CrM group was significantly higher than that in CrML group, Chewiness RF in CrM group was significantly lower than that in CrML group, Chewiness CF in CrM group was significantly higher than that in CrML group, Compactness RF in CoM group was significantly lower than that in CoML group, MDI in CrM group was significantly lower than that in CrML group ($P<0.05$) (Fig. 6).

4. Discussion

4.1. Growth performance

Protein is mainly used for fish growth and energy metabolism and decreasing its content in the diet will have influence on the growth and energy metabolism of fish (Luo et al., 2022). The weight gain, specific growth rate, PER (protein efficiency rate) and NPU (net protein utilization rate) of loach fed a 30% protein diet were significantly lower than those of a 40% protein diet (Wang et al., 2023). Maintaining the fish meal content in the feed while decreasing the protein content from 42% to 37% did not significantly affect the growth and feed conversion rate of rainbow trout (*Oncorhynchus mykiss*) (Cheng et al., 2003). In gibel carp, crude protein and ash in whole-body, condition factor, vicerosomatic index showed no significant differences when the dietary protein level was reduced by 20% (Tu et al., 2015). Similar findings were noted in this study suggesting that various fish species have differing tolerances to reduced feed protein levels. Gibel carp, in

Table 6
Effects of low protein diet supplemented with lysine and methionine on intestinal morphology of carp.

Diet groups	Villus height/ (μm)	Crypt depth/ (μm)	Muscular thickness / (μm)	VH/CD
NP	387.37 ±37.77^{b}	132.80 ±22.77	39.79±2.30	3.00 ±0.30
LP	333.22 ±13.91^{ab}	105.90 ±20.55	38.43±1.08	3.41 ±0.67
CrM	303.36 ±13.27^{ab}	85.44±8.53	44.57±5.18	3.66 ±0.55
CrML	356.10 ±15.52^{ab}	87.05±13.77	36.18±2.21	4.23 ±0.43
CoM	264.73 ±14.31^{a}	100.53±6.15	38.22±6.43	2.67 ±0.30
CoML	351.48 ±24.40^{ab}	74.71±2.41	34.06±2.20	4.73 ±0.46

Note: data are expressed as mean ± SE, and values with different superscript letters in the same row are statistically significant (P<0.05).VH, Villus height; CD, Crypt depth; MT, Muscular thickness.

particular can tolerate a large degree of feed protein reduction. The degree of protein reduction in this study is not sufficient to significant reduce the growth indices of gibel carp.

The hepatosomatic index refers to the ratio of hepatopancreas weight to fish body weight, which is one of the important indicators to evaluate the growth and metabolic status of fish (Fountoulaki et al., 2009). This study found that in the low protein group, the hepatosomatic index of carp had an increasing trend. This may be due to the lack of amino acid supply in fish caused by low protein diet, which promotes the metabolism of fat and other non-protein substances in hepatopancreas. It shows that reducing dietary protein levels will lead to increased hepatopancreas fat deposition and cause hepatopancreas burden. While reducing dietary protein, the addition of lysine and methionine reduced the hepatosomatic index of rainbow trout (Gaylord and Barrows, 2008). Adding lysine to the diet significantly reduced the hepatosomatic index and viscerosomatic index of juvenile golden pompano (*Trachinotus ovatus*) (Niu et al., 2016). Likewise, this study observed that methionine and lysine are important protein synthesis precursors that can promote the balance of protein synthesis and degradation, thereby reducing the

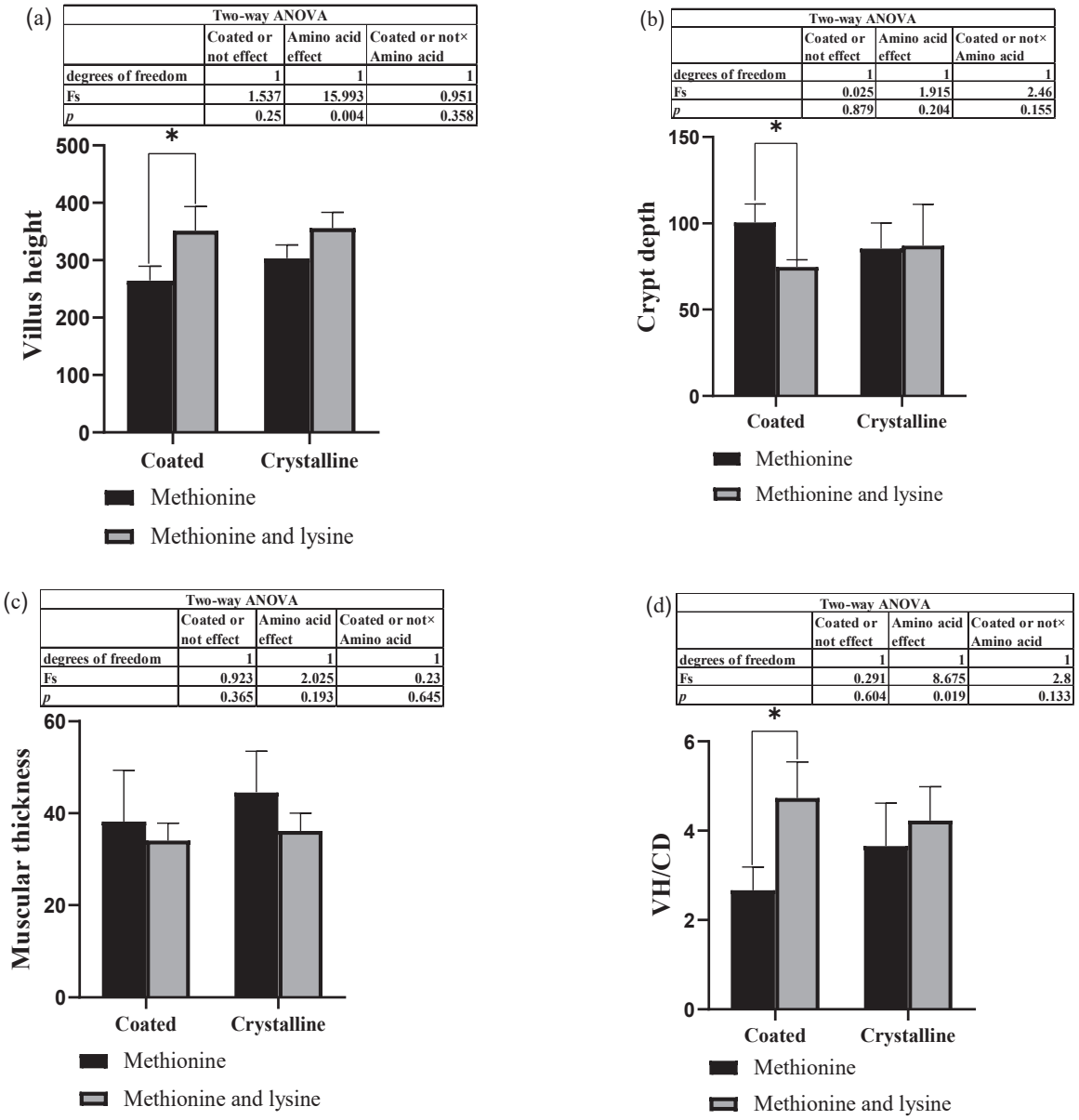


Fig. 5. Effects of low protein diet supplemented with lysine and methionine on intestinal morphology of carp. villus height (VH) (a); crypt depth (CD)(b); muscular thickness (c); villus height/crypt depth(VH/CD) (d).* indicates significant differences ($P<0.05$).

Table 7
Effect of lysine and methionine added to low protein diet on muscle texture and muscle tissue morphology of carp.

Diet groups		NP	LP	CrM	CrML	CoM	CoML
MDI (μm)		42.71±6.19^{b}	28.93±5.08^{ab}	23.05±1.02^{a}	29.71±1.18^{a}	22.90±2.98^{ab}	28.14±2.20^{ab}
MDE (per/mm^2)		240.00±18.00	264.00±90.80	304.00±71.02	288.00±93.02	256.00±61.22	210.00±44.23
Compactness (g.sec)	CF	-0.56±0.04	-0.43±0.04	-0.41±0.17	-0.41±0.05	-0.28±0.08	-0.45±0.12
	RF	-0.55±0.05	-0.87±0.30	-0.37±0.19	-0.45±0.23	-0.15±0.12	-0.70±0.03
Chewiness (g.sec)	CF	45.67±0.46	46.15±2.27	42.81±1.15	31.96±3.16	33.77±7.25	39.20±1.97
	RF	124.28±1.73^{ab}	133.00±14.66^{b}	92.04±5.73^{ab}	116.63±3.41^{ab}	75.69±22.80^{a}	130.27±7.82^{ab}
Firmness (g)	CF	65.43±3.02	66.75±4.83	57.00±2.99	44.02±4.16	45.04±11.43	54.22±3.80
	RF	198.59±0.53^{b}	196.88±17.53^{b}	152.52±9.38^{ab}	178.33±4.26^{ab}	118.92±31.98^{a}	208.68±16.96^{b}
Toughness (mm)	CF	-	-	-	-	-	-
	RF	1.12±0.01	1.40±0.10	1.33±0.06	1.11±0.01	1.19±0.10	1.30±0.22
Hardness (g/sec)	CF	61.07±0.32^{b}	47.47±2.73^{ab}	58.06±3.79^{ab}	40.37±3.11^{a}	44.83±7.22^{ab}	53.18±2.33^{ab}
	RF	125.51±1.85^{ab}	148.13±19.21^{b}	93.70±7.80^{ab}	123.95±0.76^{ab}	73.68±27.91^{a}	136.30±7.55^{ab}
Springiness (%)	CF	53.09±1.39^{b}	47.90±0.91^{ab}	52.64±2.90^{b}	42.75±0.77^{a}	45.76±1.73^{ab}	48.40±2.19^{a}
	RF	49.89±5.19	46.11±3.16	37.13±5.58	48.40±2.25	40.38±2.96	22.90±12.32

Note: data are expressed as mean ± SE, and values with different superscript letters in the same row are statistically significant (P<0.05). RF, Raw fillet; CF, Cooked fillet: MDI, Myofiber diameter; MDE, Myofiber density.

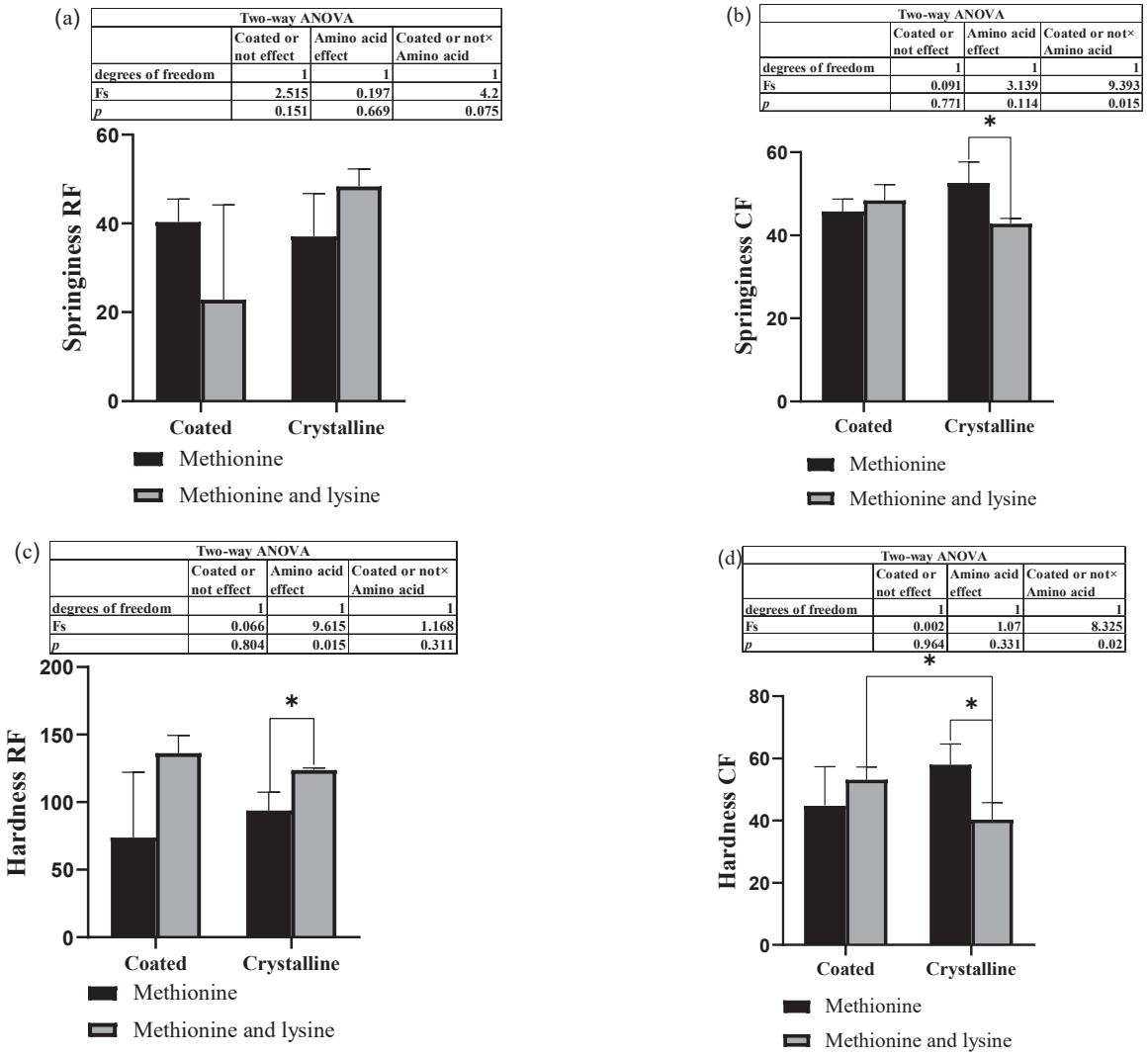
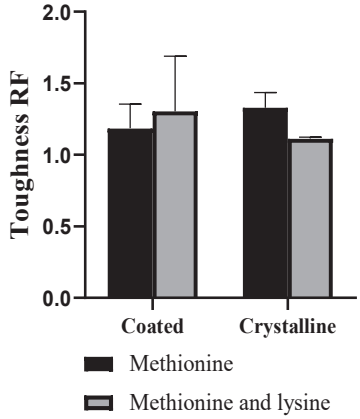


Fig. 6. Effect of lysine and methionine added to low protein diet on muscle texture and muscle tissue morphology of carp. springiness in raw fillet(a); springiness in cooked fillet(b); hardness in raw fillet(c); hardness in cooked fillet(d);toughness in raw fillet(e);firmness in raw fillet(f); firmness in cooked fillet(g); chewiness in raw fillet(h); chewiness in cooked fillet(i); compactness in raw fillet(j); compactness in cooked fillet(k); myofiber density (MDE) (l); myofiber diameter (MDI)(m).* indicates significant differences (P<0.05).

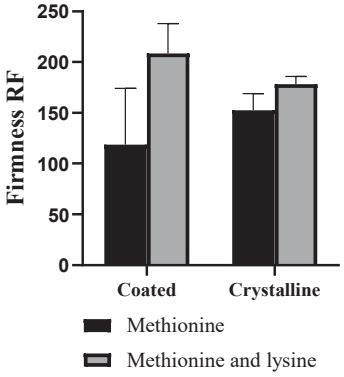
(e)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.034	0.151	1.795
p	0.858	0.708	0.217



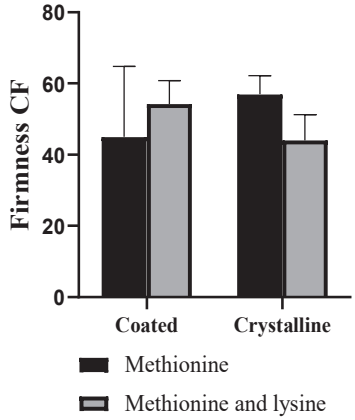
(f)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.007	9.43	2.887
p	0.933	0.015	0.128



(g)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.018	0.084	2.866
p	0.896	0.78	0.129



(h)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.012	10.021	1.437
p	0.916	0.013	0.265

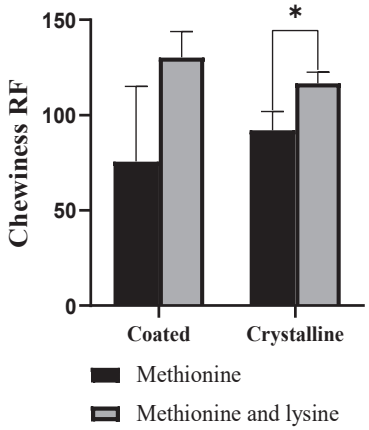


Fig. 6. (*continued*).

formation of hepatopancreas tissue, and resulting in a decrease in hepatosomatic index.

4.2. Proximate composition of whole fish

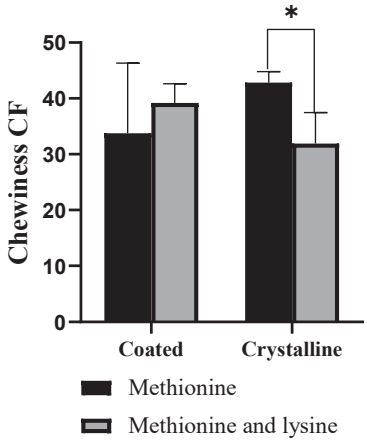
Previous study reported that reducing the dietary protein level did not significantly affect the crude protein of whole fish, such as juvenile *Barbodes altus* (Elangovan and Shim, 1997) and brown trout (*Salmo trutta*) (Arzel et al., 1995), and similar findings were noted in this study. The fat deposition level of brown trout in low protein diet was the highest comparable with high levels of dietary protein (Arzel et al., 1995). In this experiment, the crude fat content of carp with low protein decreased significantly and the moisture content increased significantly, indicating that reducing feed protein will affect the fat deposition of carp. Methionine can participate in regulating the TOR signaling pathway to promote protein synthesis (Flora et al., 2017; Rolland et al., 2015; Kong et al., 2020), while lysine can alter the proportion of amino acids in fish muscle and enhance its flavor (Cai et al., 2018; Jiang et al., 2017). In this experiment, the moisture content of the CrM and CoM groups was significantly lower than that of the LP group, and the crude fat content of the CoM and CoML groups was higher than that of the LP group. It may be because amino acids can regulate the synthesis of protein and fat, resulting in changes in body composition (Jiang et al., 2017). Adding coated lysine and methionine to the diet of juvenile black sea bream has a significant effect on the crude protein of dorsal muscle (Lu et al., 2014). In this experiment, compared with crystalline amino acids, the crude fat content of carp increased significantly and it may be that coated methionine regulates the energy metabolism pathway of carp and affects the synthesis of fat. Coated technology can improve the absorption and distribution of amino acids in animals. Due to the special properties of the coating material, it can control the release rate and location of amino acids in the gastrointestinal tract, so that it can better contact with the intestinal mucosa and be absorbed. Therefore, it promotes protein deposition (Vanjiappan et al., 2021).

4.3. Effects of low protein diet supplemented with lysine and methionine on serum and hepatopancreas transaminases in carp

Aspartate transaminase and alanine transaminase mainly exist in the heart and hepatopancreas of animals. When animals are healthy, there is only a small amount of aspartate transaminase and alanine transaminase in the serum (Thyagarajan et al., 2015; Lin et al., 2004). Therefore,

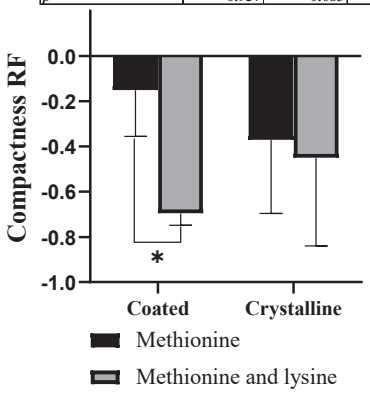
(i)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.048	0.434	3.922
p	0.832	0.528	0.083



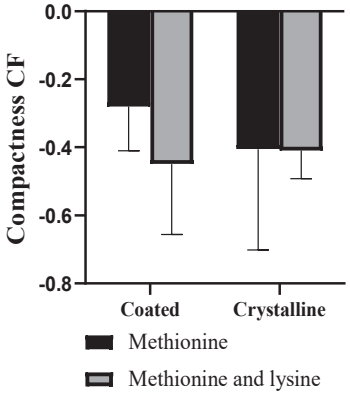
(j)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.007	3.858	2.175
p	0.937	0.085	0.179



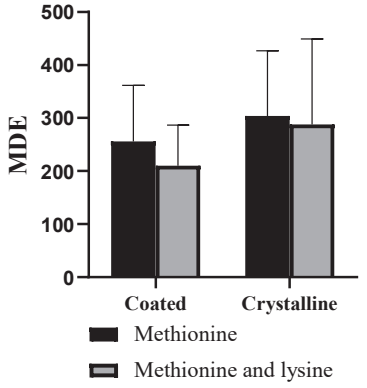
(k)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.143	0.587	0.53
p	0.715	0.466	0.488



(l)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.818	0.198	0.046
p	0.392	0.668	0.835



(m)

Two-way ANOVA	Coated or not effect	Amino acid effect	Coated or not× Amino acid
degrees of freedom	1	1	1
Fs	0.184	8.791	0.125
p	0.68	0.018	0.733

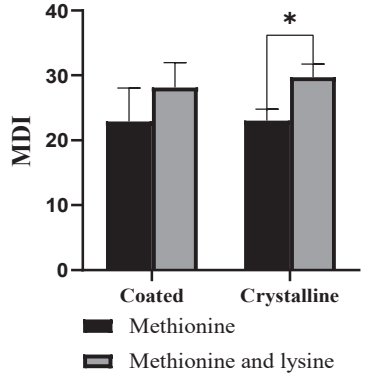


Fig. 6. (*continued*).

serum transaminase can be used as an important indicator of animal health. At the same time, transaminase, as an enzyme catalyzing amino acid transport, can also reflect amino acid metabolism. In this experiment, the serum aspartate transaminase of LP was on the rise compared with NP, and alanine transaminase was significantly increased. The activities of alanine transaminase and aspartate transaminase in the serum of low protein group were significantly higher than those of control group (Zhu et al., 2020). The methionine diet decreased serum

alanine transaminase and aspartate transaminase activities (Li et al., 2021). Present study shows that the addition of two amino acids significantly reduced serum aspartate transaminase compared with the addition of one amino acid. This could be due to methionine and lysine can provide essential amino acid supply to maintain normal metabolism and function of the hepatopancreas. Coated amino acids are widely used to improve the growth performance and immune function of animals, but their effects on serum transaminases in fish are still unclear. Several studies have shown that coated amino acids can improve the immunity of fish, and reduce inflammation and cell damage, thereby reducing transaminase levels (Zhou et al., 2007). On the contrary, present study demonstrated that coated amino acid dietary groups had a significant increase in serum transaminase of carp. These findings also illustrate that coated amino acids have influence on the hepatopancreas function of carp. However, further studies are warranted to determine the specific mechanism of coated amino acids on fish hepatopancreas function. This study also demonstrated that lowering dietary protein level would increase the metabolic demand of hepatopancreas. The addition of methionine and lysine can significantly reduce the activity of serum transaminase and reduce the hepatopancreas metabolic demand.

4.4. *Effects of low protein diet supplemented with lysine and methionine on antioxidant capacity of carp*

Superoxide dismutase and catalase are important enzymes in the antioxidant system, which are very important for maintaining the balance between oxidation and anti-oxidation. The principle of action is to remove oxygen free radical in the body and reduce lipid peroxidation damage (Toshiki and Geert, 2020; Kalinowski et al., 2019). When the activity of superoxide dismutase and catalase increases, it indicates that the body is being attacked by free radicals. Superoxide dismutase and catalase increase their activity to clear these substances. The free radicals produced by the body will attack the cell membrane and make polyunsaturated fatty acid (PUFA) produce Methane dicarboxylic aldehyde and other peroxides. Therefore, the amount of Methane dicarboxylic aldehyde can reflect the oxidation status of the body (Brogden et al., 2012). Glutathione is an important small molecule scavenger in living organisms, which can remove oxides from the body. Lack of glutathione can cause oxidative damage to cells (Martínez-álvarez et al., 2005; Ming et al., 2015). In this study, superoxide dismutase activity and Methane dicarboxylic aldehyde content of carp showed an upward trend after reducing dietary protein level. This could be due to the reduction in protein content leading to a decreased intake of amino acids and proteins in the diet, which elevates the oxidative stress response and consequently diminishes the antioxidant capacity of the hepatopancreas. Methionine could significantly increase the activities of superoxide dismutase, catalase and glutathione-Px in the hepatopancreas and intestine of juvenile Jian carp (Feng et al., 2011). In this study, methane dicarboxylic aldehyde content in the CrML group was significantly lower than those in the LP group. This may be because the addition of methionine and lysine can provide limiting amino acids and promote the synthesis and activity of antioxidant enzymes in the hepatopancreas. This finding elucidates that addition of methionine and lysine can further reduce oxidative stress, reduce the formation of lipid peroxidation products, and enhance the antioxidant capacity of the hepatopancreas. The findings indicated that supplementing amino acids significantly enhanced the antioxidant capacity of carp and mitigated oxidative damage. Introducing two amino acids markedly lowered serum methane dicarboxylic aldehyde levels compared to the introduction of a single amino acid, suggesting that a deficiency in lysine may lead to oxidative damage in carp. The effects of coated amino acids and crystalline amino acids on hepatopancreas antioxidant capacity has not been reported. Several studies paid attention to the effects of coated amino acids on growth but do not on the immune indicators (Alam et al., 2002). Present study reported that the activities of superoxide dismutase and catalase increased significantly between coated amino acids and crystalline amino acids. Moreover, coated amino acids can provide a more stable amino acid supply, thereby promote the synthesis and activity of hepatopancreas antioxidant enzymes. This demonstrates that coated amino acids can better enhance the antioxidant capacity of carp. Low protein feed increased the proportion of starch, non-starch polysaccharides and other feed ingredients in diet. Due to the weak digestion and absorption of carbohydrates such as starch, undigested carbohydrates are fermented and utilized by microorganisms in the hindgut. The production of harmful metabolites such as short-chain fatty acids, ammonia, and biogenic amines endangers the health of fish, resulting in excessive free radicals, cell damage, and increased MDA content (Nicholson et al., 2005). Methionine can scavenge free radicals, and the coating enables amino acids to be fully absorbed, thereby scavenging free radicals and enhancing antioxidant capacity (Kong et al., 2023).

4.5. *Effects of low protein diet supplemented with lysine and methionine on intestinal morphology of carp*

The structural changes in its intestinal tissue can directly reflect the health status of carp. The intestinal villi can greatly increase the absorption area of the intestine, and having longer villi also means faster absorption (Zhou et al., 2007). The crypt is the place where stem cells grow in the gut, reflecting the vitality of the gut. The ratio of villus height to crypt depth is directly proportional to the growth rate of animals (Soares et al., 2020). The thickness of the muscle layer reflects the contractile ability of the intestinal muscles in animals, and animals with contractile ability will also digest and absorb nutrients faster (Liu et al., 2020). Present study shows that low protein dietary group had a decreasing trend on the villus length of carp. This may be due to insufficient protein supply: Reducing dietary protein content may lead to a lack of essential amino acid supply for carp. Amino acids are the basic components of protein synthesis in cells and are essential for the synthesis and maintenance of intestinal villi. Insufficient protein supply may lead to a decrease in the synthesis ability of intestinal villi cells, thus affect the length of intestinal villi. The villus height of jejunum and ileum in the low protein group decreased significantly (Wu et al., 2022). Similarly, the villus length of the CrML and CoML groups have an upward trend compared with the LP group, indicating that the addition of amino acids could alleviate intestinal damage, but it was still not enough to offset the damage caused by reducing protein. Compared with adding one amino acid, adding two amino acids significantly increased intestinal villi and Villus height/Crypt depth ratio. This indicates that the addition of methionine and lysine can promote intestinal peristalsis and digestion and absorption, thereby improving the utilization of nutrients in fish. Coated amino acids have a positive effect on the height of intestinal folds (Cheng et al., 2003), which is different from the results of this experiment.

4.6. *Effects of low protein diet supplemented with lysine and methionine on muscle texture and muscle tissue morphology characteristics of carp*

Texture is an important indicator used to evaluate the quality of meat, with springiness, hardness, toughness, firmness, chewiness, and compactness are the main parameters of texture. The indicators can be used to evaluate the improvement of low protein diets with amino acids on the muscles of carp. The size of hardness affects the bite force of teeth, and the greater the hardness, the greater the springiness. Chewability is obtained by multiplying hardness, cohesiveness, and springiness (Hurling et al., 1996; Song et al., 2022). In this experiment, Hardness, Chewiness had an increasing trend in the low protein dietary group. Protein is an important component of muscle, which plays an important role in the formation and development of muscle. Reducing protein content may lead to a decrease in the number and size of muscle cells, thus affecting the tightness and taste of muscle texture. The effect of dietary protein level on the texture characteristics of fish has not been reported. In this study, reducing protein tends to reduce the cooking

Springiness and Hardness of carp. It is hypothesized that the muscle protein fibers in the low protein feed group are more fragile and more likely to destroy the muscle structure at high temperatures, indicating that reducing protein will change the composition of muscle fibers. The effect of reducing dietary protein levels on the cooking texture of fish muscle has not been reported. In this study, the Hardness, Firmness and Chewiness of low protein added amino acids were significantly reduced: Methionine and lysine play an important role in regulating muscle protein synthesis. In muscle tissue, methionine and lysine can promote protein synthesis, and increase the number and size of muscle cells, thereby improving meat texture. In addition, methionine and lysine can also improve the strength and elasticity of muscle fibers, and increase muscle tightness and taste (Peng et al., 2013). The addition of methionine and lysine to low-protein diets can reduce the level of oxidation and inflammation in muscle tissue, reduce changes in muscle texture, and improve muscle quality and taste. Dietary methionine level regulates the expression of genes involved in the specific transition point of myogenesis and affects the expression of muscle structure genes and growth factors involved in satellite cell activation and muscle growth (Alami--Durante et al., 2018). Similar findings were noted in this study. Compared with adding one amino acid, adding two amino acids significantly increased Hardness, Firmness and Chewiness, indicating that the lack of lysine would affect muscle growth. Moreover, cooking Springiness and Hardness of the low protein added amino acid CrM group were higher than those of the LP group, indicating that the cooking performance of the low protein added amino acid group was better, and the muscle fiber was not easily destroyed at high temperature. Besides that, the effect of reducing dietary protein levels and adding amino acids on the cooking texture of fish has not been reported. After heat treatment, one of the reasons may be the combination of myofibrillar components and sarcoplasmic protein denaturation, coagulation and contraction, which increases the mechanical strength of the muscle, while the muscle fiber diameter and muscle mechanical strength of the amino acid group are larger (Song et al., 2020). Several studies have shown that coated amino acids can promote muscle growth and development (Du et al., 2023). However, several studies have found that coated amino acids have no significant effect on muscle texture (Zhao and Xu, 2022). This discrepancy may arise from variations in fish species and growth stages leading to different responses to coated amino acids and subsequently varying results. Nevertheless, present study shows that coated amino acids and crystalline amino acids have the same effect on muscle texture.

However, since the amino acids provided in the feed still meet the basic needs of muscle cells, the effect of reducing protein content on muscle fiber diameter is not significant. In the experiment on large-mouth bass, reducing protein did not significantly affect the muscle fiber diameter and muscle fiber density, this is similar to the conclusion of this experiment (Wang et al., 2022). Present study indicated that the muscle fiber diameter of the CrML group has a trend of increase compared with the LP group. It shows that the addition of amino acids to low protein feed can promote the growth of muscle fibers. Compared with adding methionine only, adding methionine and lysine significantly increased muscle fiber diameter, indicating that the lack of lysine would affect muscle growth. Methionine increased fish muscle protein, lipid and free amino acid content, improved fish muscle fatty acid composition, and increased protein content, the frequency distribution of muscle fibers with diameter > 50 μm was increased (Fang et al., 2020). This is similar to the conclusion of this experiment. Coated amino acids and crystalline amino acids had the same effect on muscle growth.

5. Conclusions

Feeding gibel carp with lower dietary protein content resulted increased fat deposition in the hepatopancreas of carp, reduced muscle quality and intestinal damage. Supplementing a combination of methionine and lysine amino acids to low protein feed can improve the antioxidant capacity, muscle quality and facilitates the repair of intestinal injuries. Compared with crystalline amino acids, coated amino acids significantly enhance the crude fat content and superoxide dismutase activity in hepatopancreas of gibel carp, indicating that coated amino acids play a significant role in promoting antioxidant capacity and fat deposition of gibel carp.

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CRediT authorship contribution statement

Qiyou Xu: Supervision. **Xianping Shao:** Supervision. **Clement de Cruz:** Writing – review & editing. **xiaowen lin:** Writing – original draft. **Jianhua Zhao:** Conceptualization. **Yingying Du:** 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.

Data availability

Data will be made available on request.

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Author Contributions

Xiaowen Lin: Experimental operation, data analysis, article writing. Yingying Du: Experimental operation, data collation. Clement de Cruz. Manuscript writing. Jianhua Zhao: Data analysis. Xianping Shao: Consumables support, method guidance, manuscript modification. Qiyou Xu: Experimental design and guidance, supervision, manuscript modification.

Institutional Review Board Statement

This study was approved by the Animal Experimental Ethics Committee of Huzhou University (20180306) and conducted in strict accordance with the guidelines for the Care and Use of Laboratory Animals in China.

Informed Consent Statement

Not applicable.

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