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Keywords:
Flesh quality; Flavour; Glutamate; Myofibre; Texture

Abbreviations:
Glu, glutamate; IMP, inosine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; CWL, centrifugal weight loss; FBW, final body weight; FR, feeding rate; SGR, specific growth rate; SR, survival rate; FE, feed efficiency; HSI, hepatosomatic index; FAA, flavour amino acids; MRFs, myogenic regulatory factors; MyoD, myoblast determination protein; Myf, myogenic factor; Myog, myogenin; IGF-1insulin-like growth factor-1; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; TOR, target of rapamycin; S6, ribosomal protein s6; *gcn2*, general control nonderepressible 2; *s6k1*, ribosomal protein S6 kinase 1; *eif4e*, eukaryotic translation initiation factor 4E; *4e-bp2*, eukaryotic translation factor 4E-binding protein 2; *mef*, myocyte-specific enhancer factor; *cast*, calpastatin; *mstn*, myostatin; *samd*, SMAD family member; *mlc*, myosin light chain; *tpi*, troponin I; *tpt*, troponin T; *pax*, paired box; *ampd*, AMP deaminase 2; *ppat*, phosphoribosyl pyrophosphate amidotransferase; *pfas*, phosphoribosylformylglycinamidine synthase; *adsl*, adenylosuccinate lyase; *atic*, bifunctional purine biosynthesis protein; *adss*, adenylosuccinate synthetase; *nt5c3*, 5'-nucleotidase III; *impdh1*, inosine5'-monophosphate dehydrogenase 1; *pnp*, purine nucleoside phosphorylase; *gmps*, GMP synthase.

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Dietary supplementation with glutamate improves the flesh quality of gibel carp (*Carassius gibelio*) by altering muscle texture characteristics and increasing the deposition of flavour substances

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Abstract

Nutrition intervention is an effective way to improve flesh qualities of fish. The effect of feed supplementation with glutamate (Glu) on flesh quality of gibel carp (*Carassius gibelio*) was investigated. In trial 1, the fish (initial weight: 37.49 ± 0.08 g) were fed two practical diets with 0 and 2% Glu supplementation. In trial 2, the fish (37.26 ± 0.04 g) were fed two purified diets with 0 and 3% Glu supplementation. The results after feeding trials showed that dietary Glu supplementation increased the hardness and springiness of muscle, whether using practical or purified diets. Glu-supplemented diets increased the thickness and density of myofibres and collagen content between myofibres. Furthermore, Glu promoted muscle protein deposition by regulating the IGF-1-AKT-mTOR signalling pathway, and enhanced the myofibre hypertrophy by upregulating genes related to myofibre growth and development (*mef2a*, *mef2d*, *myod*, *myf5*, *mlc*, *tpi* and *pax7α*). The protein deposition and myofibre hypertrophy in turn improved the flesh texture. In addition, IMP content in flesh increased when supplementing Glu whether to practical or to purified diet. Metabolomics confirmed that Glu promoted the deposition of muscle-flavoured substances and purine metabolic pathway most functioned, echoed by the upregulation of key genes (*ampd*, *ppat* and *adsl*) in purine metabolism. The sensory test also clarified that dietary Glu improved the flesh quality by enhancing the muscle texture and flavour. Conclusively, dietary Glu supplementation can improve the flesh quality in this fish, which can further support evidence from other studies more generally that improve flesh quality of cultured fish.

Introduction

Fish have long been considered a pivotal protein source in the human diet due to their high nutritional value and appetising taste. Currently, over 3.2 billion people receive 20% of their average animal protein intake from aquatic animal foods.[1] Recently, a rapid increase in the demand for fish flesh has led to significant advancement of the aquaculture industry, and consumers are increasingly demanding higher flesh quality.[2] Flesh texture is an important factor for fish quality, directly affecting the acceptability to consumers.[3] Fish with more firm and springy flesh are more acceptable to consumers.[4] Compared with wild fish, farmed fish tend to bear a softer texture.[5] Thus, there is an urgent need to find a viable solution to enhance the flesh firmness of farmed fish. Flavour is another important factor in evaluating the nutrition and freshness of fish.[6] Free amino acids and flavour nucleotides directly contribute to the flavour of fish. Free amino acids can be classified into four categories: sweet, umami, bitter and tasteless amino acids.[7] These compounds can effectively influence food taste and purine metabolites are indirectly involved in flavour development in cuisines.[8] Flavour nucleotides include inosine monophosphate (IMP), adenosine monophosphate (AMP) and guanosine monophosphate (GMP). AMP renders food sweetness, while IMP enhances the umami taste.[9] In particular, because IMP contributes the majority of umami flavour to fish flesh, it is widely regarded as an indicator of flesh freshness. To date, many studies have focused on the level of IMP in meat but barely explored the mechanisms of its metabolic regulation.

Skeletal muscle is the main edible part of fish and makes up approximately 30–80% of the total body mass of fish.[10] Skeletal muscle growth in fish is mainly determined by a combination of the hyperplasia of myofibres and hypertrophy of existing myofibres. Myogenic regulatory factors (MRFs) play an important role in the proliferation of myofibres. Myoblast determination

protein (MyoD), myogenic factor 5 (Myf5), myogenic regulatory factor 6 (Mrf6) and myogenin (Myog) not only control the differentiation of myoblasts into skeletal myofibres but also provide a reserve population of cells for muscle growth during development.[11] Myocyte enhancers can directly bind to muscle promoters or enhancers to regulate muscle growth and development. For instance, methionine promotes muscle growth in rice field eel (*Monopterus albus*) by upregulating myocyte enhancer factors.[10] Protein deposition in muscle is one of the most important factors causing hypertrophy of the myofibres.[12] It has been proofed that insulin-like growth factors (IGFs) can promote muscle hypertrophy by mediating the phosphoinositide 3-kinase (PI3K)- protein kinase B (AKT) pathway, and the activated AKT increases protein synthesis through activation of the target of rapamycin (TOR) signalling pathway, resulting in protein deposition.[13,14] Glutamate (Glu) makes up approximately 5–15% of dietary protein, and is considered a conditionally essential amino acid.[15,16] As a strong activator of the TOR pathway, the report of its contributions to fish muscle growth and flesh quality traits needs further improvement.[17,18]

Gibel carp (*Carassius gibelio*) is one of the major economically important freshwater-cultured fish species in China. Our group has researched the effects of Glu supplementation on the skeletal muscle growth and flavour of triploid crucian carp (*C. auratus* triploid).[18] This study was conducted to investigate the effects of dietary Glu on gibel carp from practical and purified diet perspectives, aiming to enrich the theoretical territory and actual application value of flesh quality effects derived from Glu supplementation.

Materials and methods

Animal ethics

All care conducts for subject animals corresponded to the Guiding Principles for Care and Use of Laboratory Animals, and the fish study was permitted by the ethics committee of the Institute of Hydrobiology, Chinese Academy of Sciences (IHB, CAS, Protocol No. 2016-018).

Experimental diets

Trials 1 and 2 were carried out using practical and purified diets, respectively. In trial 1, Con1 diet and 2%Glu diet were formulated by supplementation with 0% and 2% crystalline L-glutamate acid (Glu) to the practical diet (Table 1). In trial 2, Con2 diet and 3%Glu diet were formulated by supplementation with 0% and 3% Glu to the basal purified diet (Table 2). The different amounts of addition in practical and purified diets were determined by referring to previous studies.[18,19] The main amino acid compositions of the diets were listed in Table 3, which showed that the actual amounts of Glu in the Con1, 2%Glu, Con2 and 3%Glu were 54.08, 64.99, 58.86 and 87.91 g/kg, respectively.

All feeds for both experiments were prepared and stored in accordance with standard laboratory procedures, as described in published papers.[20] All ingredients were superfine grinded, weighed and absolutely mixed, followed by homogenisation after the addition of water. Then, the batter was toppled into a granulator machine, and after making it granular, the pellets were dried at 65 °C for 4 h and stored at 4 °C.

Table 1. Formulation and proximate composition of the practical diets in trial 1 (dry matter)

Ingredients (%)	Con1	2%Glu
Fish meal	10.00	10.00
Soybean meal	20.00	18.00
Rapeseed meal	22.00	20.00
Cottonseed protein concentrate	21.00	21.00
Corn starch	10.00	10.00
Vitamin premix[1]	0.39	0.39
Mineral premix[2]	5.00	5.00
Fish oil	3.00	3.00
Soybean oil	3.00	3.00
Choline chloride[3]	0.11	0.11
Carboxymethyl cellulose	3.00	3.00
L-methionine[4]	0.32	0.35
L-lysine[4]	1.45	1.55
L-threonine[4]	0.48	0.55
L-glutamic acid[4]	0.00	2.00
Cellulose	0.25	2.06
Proximate composition		
Moisture (%)	7.63	8.90
Crude protein (%)	33.84	34.17
Crude lipid (%)	11.66	11.49
Ash (%)	8.67	8.86

[1]Vitamin premix (mg/kg diet): Vitamin B_1, 20; Vitamin B_2, 20; Vitamin B_6, 20; Vitamin B_{12}, 0.020; Folic acid, 5; Calcium pantothenate, 50; Inositol, 100; Niacin, 100; Biotin, 0.1; Cellulose, 3412; Vitamin C, 100; Vitamin A, 11; Vitamin D, 2; Vitamin E, 50; Vitamin K, 10.
[2]Mineral premix (mg/kg diet): NaCl, 500; $MgSO_4{\cdot}7H_2O$, 8155.6; $NaH_2PO_4{\cdot}2H_2O$, 12,500.0; KH_2PO_4, 16,000.0; $CaHPO_4{\cdot}H_2O$, 7650.6; $FeSO_4{\cdot}7H_2O$, 2286.2; $C_6H_{10}CaO_6{\cdot}5H_2O$, 1750.0; $ZnSO_4{\cdot}7H_2O$, 178.0; $MgSO4{\cdot}H_2O$, 61.4; $CuSO4{\cdot}5H_2O$, 15.5; $CoSO4{\cdot}7H_2O$, 0.5; KI, 1.5; Corn starch, 753.7.
[3]Choline chloride was composed of 50% choline chloride and 50% silicon dioxide.
[4]L-methionine, L-lysine, L-threonine and L-glutamate acid were purchased from Yuanye Bio-Technology Co., Ltd., Shanghai, China.

Fish and rearing condition

The experimental fish in this study, bred by the Guanqiao Hatchery of the Institute of Hydrobiology, Chinese Academy of Sciences, acclimated in an indoor recirculating water system and fed the two control diets for two weeks. After a 24-hour fast, 240 fish in trial 1 with apparent health and similar sizes (37.49 ± 0.08 g) were randomly selected and equally released into 6 tanks (40 fish per tank, water volume: 980 L). 120 fish (initial weight: 37.26 ± 0.04 g) in trial 2 with similar size were randomly distributed into 6 tanks (20 fish per tank, water volume: 167 L). Triplicate tanks were randomly assigned to each diet.

Fish were manually fed to apparent satiation at daily 8:30 and 16:30. The feeding period for trial 1 and 2 were lasting for 60 and 50 days respectively. The water Celsius degree was maintained at 30.62 ± 5.04 °C, the pH was 7.39 ± 0.13, and the dissolved oxygen and ammonia nitrogen concentrations remained beyond 8.3 mg/l and less than 0.1 mg/l.

Table 2. Formulation and proximate composition of the purified diets in trial 2 (dry matter)

Ingredients (%)	Con2	3%Glu
Casein[1]	22.00	20.30
Wheat protein concentrate[2]	22.00	20.30
Corn starch[3]	28.30	28.30
Fish oil	4.00	4.00
Soybean oil	4.00	4.00
Mineral premix	5.00	5.00
Vitamin premix	0.39	0.39
Choline chloride	0.11	0.11
Carboxymethyl cellulose	3.00	3.00
Monocalcium phosphate	2.00	2.00
L-arginine[4]	0.76	0.76
L-lysine[4]	0.60	0.60
L-glutamic acid[4]	0.00	3.00
Cellulose	7.84	8.24
Proximate composition		
Moisture (%)	8.60	8.65
Crude protein (%)	39.05	38.32
Crude lipid (%)	8.02	7.93
Ash (%)	5.48	5.38

[1]Casein was purchased from Gansu Hualing Casein Co., Ltd., Gansu, China.
[2]Wheat protein concentrate was purchased from Qufeng Food Technology Co., Ltd., Shandong, China.
[3]Corn starch was purchased from Yufeng Industrial Group Co., Ltd., Hebei, China.
[4]L-arginine, L-lysine and L-glutamic acid were purchased from Yuanye Bio-Technology Co., Ltd., Shanghai, China.

Table 3. Amino acids composition of the experimental diets (g/kg dry matter)

	Trial 1		Trial 2	
Amino acids	Con1	2%Glu	Con2	3%Glu
Lysine	28.79	28.29	28.53	28.74
Methionine	7.74	8.31	8.33	8.22
Threonine	15.74	16.88	20.73	21.71
Arginine	17.97	17.10	17.36	16.69
Leucine	24.56	24.98	27.11	24.45
Histidine	7.45	7.59	8.02	7.18
Isoleucine	10.88	11.11	15.13	13.83
Phenylalanine	12.93	12.24	17.41	15.91
Valine	12.64	13.23	16.82	15.62
Aspartic acid	25.54	26.03	26.05	23.59
Serine	13.06	12.55	15.01	13.54
Glycine	14.46	13.15	21.41	20.54
Glutamate	54.08	64.99	58.86	87.91
Alanine	16.18	16.84	11.46	10.47
Cystine	0.61	0.81	0.90	0.88
Tyrosine	9.70	9.04	12.80	11.83
Proline	17.92	19.25	35.01	33.02
EAA[1]	138.70	139.73	150.19	142.98
NEAA[2]	151.55	162.66	181.5	201.78

[1]EAA: Essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine and arginine).
[2]NEAA: Non-essential amino acids (aspartic acid, serine, glutamate, glycine, alanine, cystine, tyrosine and proline).

Sample collection

After the rearing period finished and 24-hour starvation, fish were anaesthetized by 100 mg/l MS-222 (tricaine methanesulfonate, Argent Chemical Laboratories Inc., Redmond, WA, USA) and bulk weighed. Under random selection, two fish in each tank were weighed and stored at −20 °C for determining the whole-body composition. Three fish were selected for texture analysis, and three fish in trial 1 were selected for sensory analysis. Two weighed fish were dissected, and their livers were isolated and weighed to calculate the hepatosomatic index (HSI). Subsequently, unilateral dorsal muscles of these two bodies were sampled and frozen at −20 °C to analyse the muscle composition and amino acid profile, and dorsal muscles from the other side were sampled and frozen at −80 °C to analyse gene expression, Western blotting or metabolite profiling.

Chemical analysis

The moisture, crude protein, crude lipid and ash contents of the whole body and experimental diets were measured by referencing official methods of Analysis of Official Analytical Chemists International.[21] Moisture was determined by the weight reduction before and after oven drying at 105 °C. The determination of crude protein content (N × 6.25), complying with the Kjeldahl method, was measured by an Auto-Kjeldahl apparatus (Kjeltec-8400, FOSS Tecator, Haganas, Sweden) after samples were digested by concentrated sulfuric acid. A Soxtec system (Soxtec System HT Tecator, Extraction Unit, Hoganas, Sweden) was appointed to measure crude lipid content by aether extraction. Ash content was quantitated by a muffle furnace (Muffle furnace, Yingshan, Hubei, China), in which samples were incinerated at 550 °C for 12 h.

Determination of amino acid compositions of diets and taste substances in muscle

The pretreatment of determination of amino acids compositions of experimental diets and free amino acids profiles of dorsal muscles were preformed according to the method detailed by Tu *et al.*[22] and Xu *et al.*,[23] respectively. Then an amino acid analyser (A300, MembraPure GmbH, Germany) was used to analyse the contents of different amino acids. Part kinds of nucleotides and purine metabolites in dorsal muscles were measured by a method described in our previous study.[20]

Histological analysis

To observe myofibres in dorsal muscle, the Servicebio Company (Wuhan, China) was entrusted to make paraffin sections with Masson and haematoxylin and eosin staining, of which images were harvested by scanning from a fully automatic digital slide scanner (Aperio VERSA 8, Leica, Germany). Measuring diameters of myofibres in every slide with Aperio ImageScope to quantify the frequency of myofibres with different diameters. Quantifying the area of colours of myofibres and collagen in every slide with Image-Pro Plus 6.0 to determine the density of myofibres and collagen content.

Table 4. Effect of Glu supplementation on growth performance, feed utilisation and morphological parameters of gibel carp

	Trial 1		Trial 2	
Parameters	Con1	2%Glu	Con2	3%Glu
IBW, g[1]	37.51 ± 0.13	37.46 ± 0.11	37.25 ± 0.07	37.24 ± 0.08
FBW, g[2]	61.56 ± 0.60	60.41 ± 0.73	62.82 ± 0.54	63.60 ± 0.17
FR, %BW/d[3]	2.41 ± 0.06	2.41 ± 0.05	3.37 ± 0.05	3.50 ± 0.04
SGR, %/d[4]	0.83 ± 0.01	0.80 ± 0.02	1.05 ± 0.02	1.07 ± 0.01
SR, %[5]	98.33 ± 1.67	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
FE, %[6]	26.74 ± 1.24	26.63 ± 1.64	59.38 ± 0.85	57.18 ± 0.73
HSI, %[7]	1.51 ± 0.14[a]	2.01 ± 0.06[b]	2.25 ± 0.08	2.38 ± 0.13

Data are presented as the Means ± SEM ($n \geq 3$). Values in the same row within the same trial with different letters are significantly different ($P < 0.05$).
[1]IBW: initial body weight.
[2]FBW: final body weight.
[3]FR: feeding rate (%BW/d) = 100 × dry feed intake/[days × (initial body weight + final body weight + dead fish body weight)/2].
[4]SGR: specific growth rate (%/d) = 100 × [ln (final body weight) − ln (initial body weight)]/days.
[5]SR: survival rate (%) = 100 × the final fish number/the initial fish number.
[6]FE: feed efficiency (%) = 100 × (final body weight − initial body weight)/feed intake.
[7]HSI: hepatosomatic index = 100 × liver weight/body weight.

Texture analysis

Two fillets were cut from each reserved fish intended for texture analysis. The hardness, springiness, toughness, stringiness, flexibility, fracturability, stickiness and adhesiveness of flesh were quantitated by a texture analyser (Stable Micro systems, Ltd., UK), which contained a 0.5-centimetre-diameter spherical probe. First, the probe pressed the fillet downwards at a 30 mm/min rate until a 10-mm depression was generated and 32 s duration. Second, the probe pressed the fillet with 2 N pressure for 10 s, and then the flesh was detached by the probe at a 600 mm/min rate. The maximal pressure put by the flesh on the probe represented the hardness of the flesh. The ability of the sample to recover its initial form after the deforming force was removed reflected the springiness. The integration below the pressure curve denoted the toughness. The distance of the detachment expressed the stringiness, and the integration below the curve of the detachment typified the adhesiveness. The deformation of the flesh when the pressure reached 2 N denoted its flexibility. The slope between the start and the end of the pressure curve indicated the fracturability. The maximal pressure put by the flesh on the probe during detachment represented the stickiness. Fresh muscle samples (1 g) were centrifuged in plastic pipe at 1000 × g at 4 °C for 30 min. After drying the surface moisture using common qualitative filter paper, the centrifugal weight loss (CWL, %) was calculated as 100 × (the weight before centrifuge – the weight after centrifuge)/the weight before centrifuge.

Sensory analysis

In trial 1, appraisal of the flesh of gibel carp from the sensory organ perspective was carried out. After training by stimuli with single sensory property, we selected 10 evaluators with high sensitivity and discrimination, good stability and repeatability, and high sensory language description ability to form the final assessment panel. Each member stayed in a compartment with the same light and temperature, and the steamed samples, laid in petri dishes, were numbered and provided for the panel at room temperature. Moreover, mineral water was used to clean participants' oral cavities to ensure sensory consistency between samples.[24] All appraisals were conducted on the same day, and the appearance, fragrance, odour, flavour, firmness, greasiness and juiciness of flesh were graded by a 5-point scale, in which 1 point indicated the lowest and 5 point indicated the highest strength of sensory stimulation. As every fish was cut out three fleshes, each sample was repeatedly evaluated three times.

qPCR analysis

To compare the expression of a series of crucial genes, polymerase chain reaction (PCR) was used, and the primer sequences are shown in Supplemental Table 1. TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) was involved in the extraction of total RNA from dorsal muscle, and the concentration was detected by a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, United States). A small part containing 1 μg of total RNA was removed from the solution of total RNA for reverse transcription, after which RNA was turned into cDNA with the M-MLV First-Strand Synthesis Kit (Invitrogen, Shanghai, China). Then, the cDNA was mixed with LightCycle 480 SYBR Green I Master Mix for quantitative real-time PCR, which was implemented on a LightCycle 480 II system (Roche, Switzerland), as detailed by Su *et al.*[25] Each treatment involved six samples, each of which was measured in duplicate. The calculation of the results was on the foundation of the method described by Vandesompele *et al.*,[26] and *gapdh* was set as the internal reference gene.

Western blot analysis

Western blotting was implemented on the basis of the steps detailed by Yang *et al.*[27] In brief, muscle crumbs were lysed by lysate, a mixture blended with RIPA lysis buffer (Beyotime Biotechnology, China), protease inhibitor cocktail and phosphatase inhibitor cocktail (Roche, Basel, Switzerland). Twenty micrograms of total protein were removed and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Afterwards, the separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes, which were blocked for 1 hour with 5% milk in TBST buffer. Then, the membranes were incubated in anti-phospho-AKT antibodies (1:1000, #9272; CST, Danvers, MA, United States), anti-AKT antibodies (1:1000, #4060; CST, Danvers, MA, United States), anti-phospho-ribosomal protein s6 (S6) antibodies (1:1000, #4858;

Table 5. Free amino acid composition of the muscle (mg/100 g fresh weight)

	Trial 1		Trial 2	
Amino acids	Con1	2%Glu	Con2	3%Glu
Lysine	16.09 ± 3.94	20.01 ± 1.54	41.33 ± 5.82	40.32 ± 7.70
Methionine	1.10 ± 0.07	1.02 ± 0.08	1.28 ± 0.12	1.25 ± 0.08
Threonine	19.60 ± 1.62	20.93 ± 0.63	21.04 ± 1.68	24.25 ± 1.37
Arginine	19.59 ± 0.54	19.69 ± 0.42	17.30 ± 0.82	17.59 ± 4.29
Leucine	3.79 ± 0.20	3.56 ± 0.05	3.84 ± 0.28	4.11 ± 0.27
Histidine	174.01 ± 24.01	205.59 ± 3.40	239.61 ± 18.96	231.67 ± 17.53
Isoleucine	1.58 ± 0.09	1.44 ± 0.02	1.70 ± 0.12	1.95 ± 0.07
Phenylalanine	2.30 ± 0.41	2.17 ± 0.13	3.10 ± 0.70	3.04 ± 0.59
Valine	2.65 ± 0.12	2.60 ± 0.08	2.18 ± 0.07	2.53 ± 0.16
Aspartic acid	1.42 ± 0.23	1.60 ± 0.26	2.31 ± 0.38	2.07 ± 0.19
Serine	3.88 ± 0.40	4.53 ± 0.05	3.15 ± 0.36^{b}	2.17 ± 0.29^{a}
Glycine	33.45 ± 0.36	32.56 ± 1.15	20.90 ± 3.03	18.98 ± 1.49
Glutamate	15.64 ± 1.65	14.22 ± 1.06	11.00 ± 0.72^{a}	22.14 ± 1.56^{b}
Alanine	13.42 ± 1.83	16.36 ± 0.87	32.96 ± 3.09	40.52 ± 2.46
Cystine	0.22 ± 0.04	0.16 ± 0.02	0.36 ± 0.06	0.49 ± 0.08
Tyrosine	0.86 ± 0.13	0.91 ± 0.06	1.00 ± 0.12	1.22 ± 0.15
Proline	4.63 ± 1.32^{a}	8.57 ± 0.76^{b}	55.59 ± 3.89^{a}	71.05 ± 3.19^{b}
ΣEAA[1]	240.71 ± 2.96	277.01 ± 0.71	331.38 ± 2.28	326.71 ± 2.28
ΣNEAA[2]	73.52 ± 6.52	78.91 ± 5.24	127.27 ± 9.73	158.64 ± 8.84

Data are presented as the Means ± SEM ($n \geq 3$). Values in the same row within the same trial with different letters are significantly different ($P < 0.05$).
[1]EAA: Essential amino acids (lysine, methionine, threonine, arginine, leucine, histidine, isoleucine, phenylalanine and valine).
[2]NEAA: Non-essential amino acids (aspartic acid, serine, glycine, glutamic acid, alanine, cystine, tyrosine and proline).

CST, Danvers, MA, United States), anti-S6 antibodies (1:1000, #2217; CST, Danvers, MA, United States), and anti-GAPDH antibodies (1:1000, ab8245; Abcam).

Metabolite profiling analysis

The dorsal muscle samples of purified diet trial were weighted into a 2 ml EP tube with 500 µl of precooled extractant (70% methanol in water). The solution was homogenised at 30 Hz for 30 s (4 times). Then the samples were centrifuged at 4 °C for 10 min with a rotation speed of 13680 × *g*. Finally, 200 µl of supernatant was placed into a sample bottle to ultrahigh-performance liquid chromatography coupled with high-resolution mass spectrometry (LC–MS/MS) analysis. The data acquisition instrumentation system consists mainly of ultra-performance liquid chromatography (HPLC) (Shimpack UFLC SHIMADZU CBM30A, https://www.shimadzu.com/) and tandem mass spectrometry (MS/MS) (QTRAP, https://sciex.com/) at Wuhan Metware Biotechnology Company (Wuhan, China). All data were processed and normalised by MetaboAnalyst 5.0 and then used to analyse the differences in the levels of metabolites in the gibel carp dorsal muscle between the experimental group and the control group.

Statistical analysis

All data was conducted to independent samples *t*-test for analysing whether there was a significant difference between the control and experimental groups. When $P < 0.05$, it was considered to indicate a significant difference.

Results

Growth performance, feed utilisation and morphological parameters

Table 4 shows the effect of Glu supplementation on the growth performance, feed utilisation and morphological parameters of gibel carp. In trial 1, the hepatosomatic index (HSI) of 2%Glu group was significantly higher than that of Con1 group ($P < 0.05$), but that in trial 2 showed no significant difference ($P > 0.05$). And no significant difference of the final body weight (FBW), feeding rate (FR), specific growth rate (SGR), survival rate (SR) and feed efficiency (FE) in trial 1 and trial 2 was observed between the control and experimental groups ($P > 0.05$).

Whole body and muscle composition

The effects of Glu supplementation on the whole body and muscle composition of gibel carp are shown in Supplemental Table 2. The moisture, crude protein, crude lipid and ash levels of the whole body in trial 1 and trial 2 showed no significant difference between the control and experimental groups ($P > 0.05$). Similarly, the moisture and crude lipid contents in dorsal muscle also showed no significant difference ($P > 0.05$), whereas the crude protein content significantly increased in Glu-supplemented groups in trial 1 and trial 2 ($P < 0.05$).

Free amino acid profiles of muscle

The free amino acid composition of muscle is presented in Table 5. In trial 1, the Pro content was significantly higher in 2%Glu group

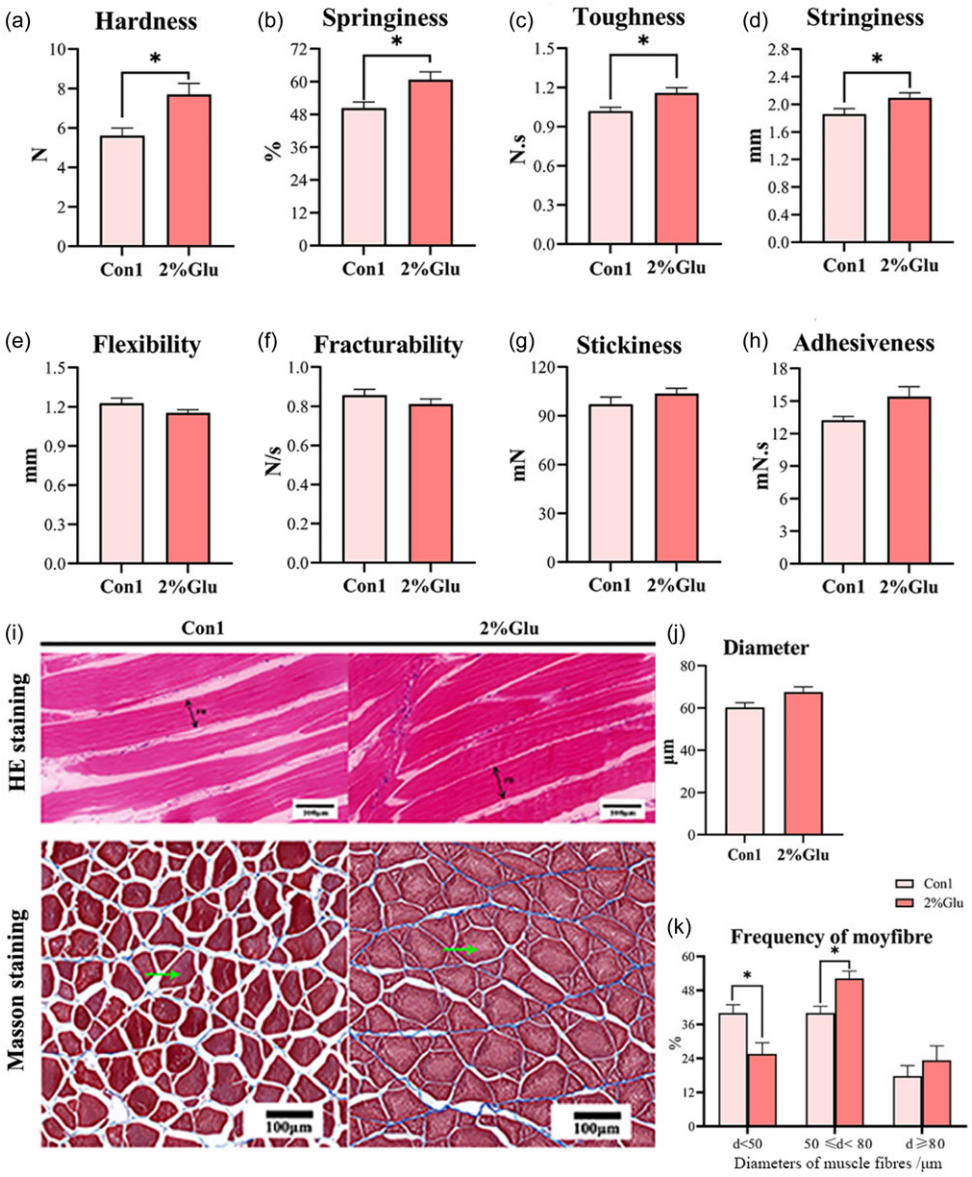

Fig. 1. Effects of Glu supplementation in the practical diets on texture and microstructure of dorsal muscle of gibel carp. (a–h) Muscle textural properties (n = 9). (i) Histological observations of the dorsal muscle after Hematoxylin-Eosin and Masson staining (magnification 400×). Black arrows represent myofibre diameter. Green arrows point to myofibres. (j) The quantitative graph of myofibre diameter. (k) The quantitative graph of frequency of myofibres with different diameters. For each index, '*' showed significantly differences between groups ($P < 0.05$).

than that in the Con1 group ($P < 0.05$). In trial 2, the Ser, Glu and Pro contents were significantly higher in 3%Glu group than that in the Con2 group ($P < 0.05$). The other amino acids and total free amino acids presented no significant difference between the control and experimental groups ($P > 0.05$).

Textural properties and microstructure of dorsal muscle

In trial 1, the effects of 2%Glu supplementation on the texture and microstructure of dorsal muscle are shown in Fig. 1. It demonstrated that 2%Glu supplementation significantly improved the hardness, springiness, toughness and stringiness compared with the Con1 group ($P < 0.05$) (Fig. 1a–d). In contrast, 2%Glu supplementation did not have a significant effect on muscle flexibility, fracturability, stickiness and adhesiveness ($P > 0.05$) (Fig. 1e–h). The transverse and longitudinal sections of dorsal muscle and the quantitative graphs showed that there was no significant difference in the diameter of myofibres between the two groups ($P > 0.05$) (Fig. 1j), but in the 2%Glu group, the percentage of myofibres with diameters less than 50 μm was markedly reduced and the percentage of myofibres with diameters more than 50 μm but less than 80 μm was significantly enhanced compared with the Con1 group (Fig. 1k) ($P < 0.05$). The percentage of myofibres more than 80 μm showed no statistical difference between the two groups ($P < 0.05$) (Fig. 1k).

In trial 2, the effects of 3%Glu supplementation on the texture and microstructure of dorsal muscle are shown in Fig. 2. It demonstrated that 3%Glu supplementation significantly improved the hardness, springiness and stringiness, decreased the centrifugal weight loss (CWL) compared with the Con2 group (Fig. 2a, b, d and i) ($P < 0.05$). In contrast, 3%Glu supplementation did not have a significant effect on muscle toughness, flexibility, fracturability, stickiness and adhesiveness (Fig. 2c, e–h) ($P > 0.05$). The muscle sections with HE and Masson staining and the quantitative graphs showed that the myofibre density and collagen content of dorsal muscle in the 3%Glu group was significantly higher than that in the Con2 group (Fig. 2j–l) ($P < 0.05$).

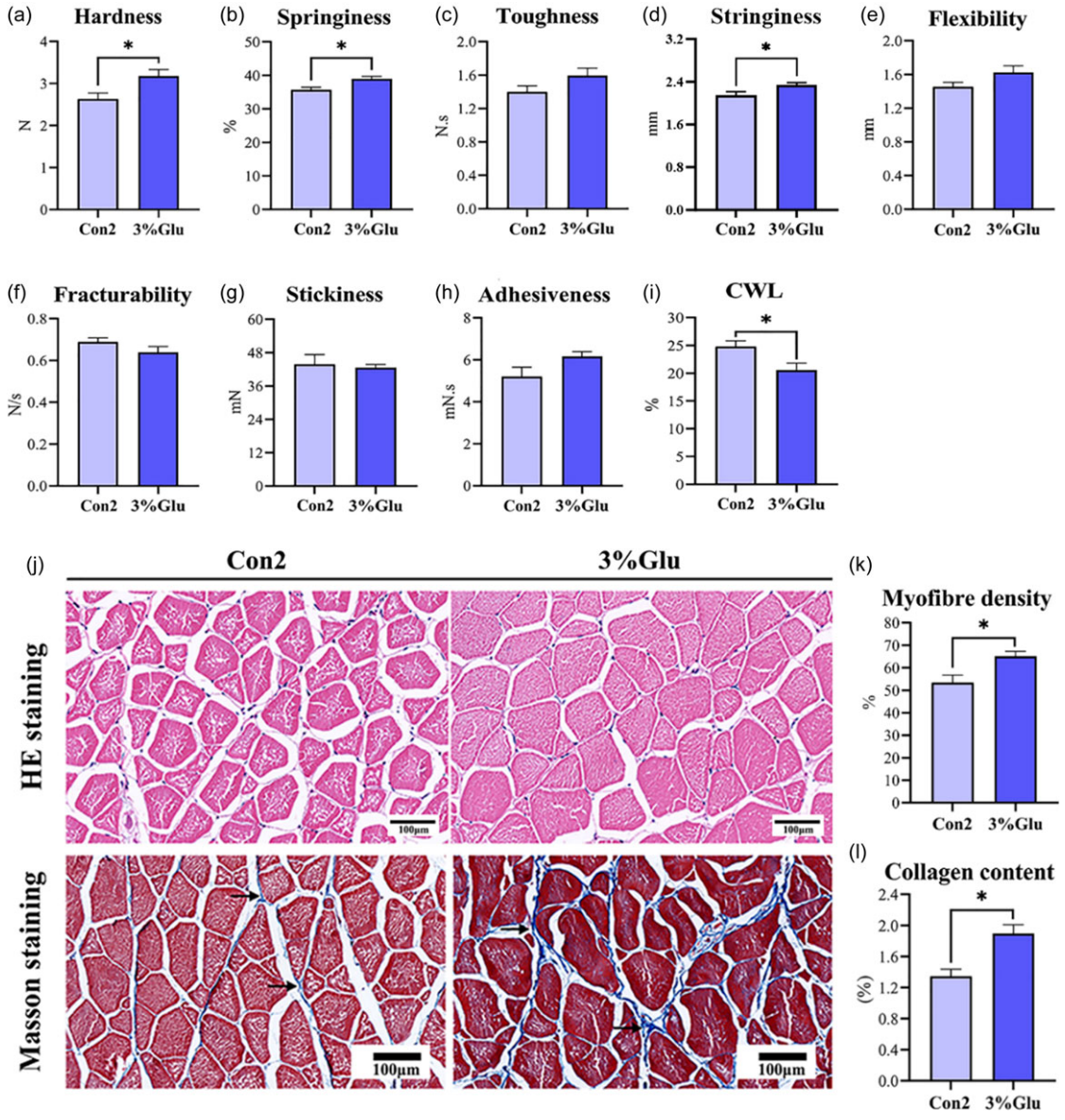

Fig. 2. Effects of Glu supplementation in the purified diets on texture, microstructure and collagen content of dorsal muscle of gibel carp. (a–h) Muscle textural properties ($n = 9$). (i) Centrifugal weight loss. (j) Histological observations of the dorsal muscle after Hematoxylin-Eosin and Masson staining. Black arrows represent collagen. (k) The quantitative graph of myofibre density. (l) The quantitative graph of collagen content. For each index, '*' showed significantly differences between groups ($P < 0.05$).

Muscle protein synthesis-related protein and mRNA expression

The effects of Glu supplementation on muscle protein synthesis-related protein and mRNA expression of gibel carp in trial 1 are depicted in Fig. 3. Compared with the Con1 group, the 2%Glu group significantly facilitated AKT and S6 protein phosphorylation in dorsal muscle ($P < 0.05$) (Fig. 3a–c). *Igf-1, tor, s6k1* and *eif4e* mRNA expressions were significantly upregulated under 2% dietary Glu supplementation ($P < 0.05$), and no significant difference of *gcn2* and *4ebp2* mRNA expressions were observed between the two groups ($P > 0.05$) (Fig. 3d).

Regulation of myofibre growth and development-related mRNA expression

The effect of Glu supplementation on myocyte enhancer factor mRNA expression of gibel carp in trial 1 is depicted in Fig. 4a. Compared with the Con1 group, *mef2a* and *mef2d* mRNA expressions were significantly elevated under 2% dietary Glu supplementation ($P < 0.05$). There was no significant difference in *mef2c* mRNA expression between the two groups ($P > 0.05$).

The effect of Glu supplementation on myofibre growth-related mRNA expression of gibel carp in trial 1 is depicted in Fig. 4b. Compared with the Con1 group, *cast, mstn* and *smad1* mRNA expression was significantly upregulated under 2% dietary Glu supplementation ($P < 0.05$). No significant difference in *smad3* mRNA expression was observed between the two groups ($P > 0.05$).

The effect of Glu supplementation on myogenic regulatory factor mRNA expression of gibel carp in trial 1 is depicted in Fig. 4c. Compared with the Con1 group, *myod* and *myf5* mRNA expressions were significantly upregulated when supplementing 2% dietary Glu ($P < 0.05$). There was no significant difference in *myf6* and *myog* mRNA expressions between the two groups ($P > 0.05$).

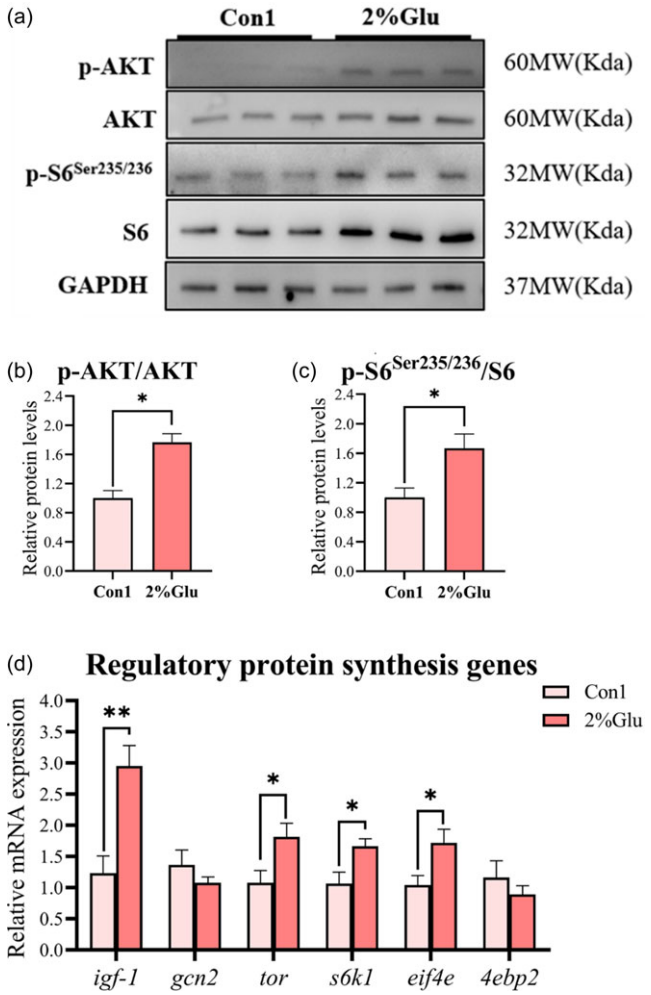

Fig. 3. Effects of Glu supplementation in the practical diets on protein and mRNA expression related to protein synthesis in dorsal muscle of gibel carp. (a) AKT and S6 protein phosphorylation in dorsal muscle were measured by western blot. GAPDH was used as a loading control ($n = 3$). (b) The relative protein expression of p-AKT/AKT. (c) The relative protein expression of p-S6/S6. (d) The relative mRNA expression of protein synthesis in dorsal muscle. '*' showed significantly differences between groups ($P < 0.05$).

Effect of Glu supplementation on myofibre development-related mRNA expression of gibel carp in trial 1 is depicted in Fig. 4d. Compared with the Con1 group, *mlc, tpi* and *pax7a* mRNA expressions were significantly upregulated when supplementing 2% dietary Glu ($P < 0.05$). There was no significant difference in *tpt* and *pax3* mRNA expressions between the two groups ($P > 0.05$).

Muscle flavour substances and related mRNA expression

In trial 1, effects of 2%Glu supplementation on flavour nucleotide, flavour amino acids (FAA) and IMP metabolism in dorsal muscle are shown in Fig. 5. Glu supplementation significantly increased the IMP level in dorsal muscle ($P < 0.05$), but the contents of AMP and umami FAA (Asp, Glu), sweet FAA (Gly, Ala, Leu, Met, Asp, Arg and Pro) and bitter FAA (Val, Lys, Leu, Ile, Phe, His and Tyr) were not significantly different ($P > 0.05$) (Fig. 5a and b). Concerning the IMP anabolism, 2%Glu supplementation significantly upregulated *ampd, ppat* and *adsl* mRNA expression ($P < 0.05$), but there was no significant difference in *pfas* and *atic* mRNA expression between the control and experimental groups ($P > 0.05$) (Fig. 5c). In terms of the IMP catabolism, 2%Glu supplementation significantly upregulated *adss* and *nt5c3* mRNA expression ($P < 0.05$), but there was no significant difference in *impdh1*, *pnp* and *gmps* mRNA expression between the two groups ($P > 0.05$) (Fig. 5d).

In trial 2, effects of 3%Glu supplementation on flavour nucleotide, flavour amino acids and IMP metabolism in dorsal muscle are shown in Fig. 6. Glu supplementation significantly increased the IMP, AMP and umami FAA levels in dorsal muscle ($P < 0.05$), but the levels of GMP and sweet and bitter FAA were not significantly different between the Con2 and 3%Glu groups ($P > 0.05$) (Fig. 6a and b). As for the IMP anabolism, 3%Glu supplementation significantly upregulated *ampd, ppat, pfas* and *adsl* mRNA expression ($P < 0.05$), but there was no significant difference in *atic* mRNA expression between the two groups ($P > 0.05$) (Fig. 6c). Concerning IMP catabolism, 3%Glu supplementation significantly upregulated *nt5c3, pnp and gmps* mRNA expression ($P < 0.05$), but the *adss* and *impdh1* mRNA expressions in the Con2 group were not significantly different from those in the 3%Glu group ($P > 0.05$) (Fig. 6d).

Metabolomics analysis

In trial 2, dorsal muscle samples were subjected to metabolomics analysis. The results of metabolomics analysis are presented in Fig. 7. As shown in the principal component analysis (PCA) score plot (Fig. 7a), the circle of 3%Glu group was completely separated from circle of Con2 group. A heap map was performed to identify the impacts of dietary Glu supplementation on muscular metabolite profiles. The relative peak intensities of 75 metabolites in two groups were visualised in Fig. 7b. The metabolite levels are shown ranging in colour from red (highest) to blue (lowest). The relative peak intensities of cytarabine to L-methionine were lower and the relative peak intensities of glutamine to dihydroxyacetone in 3%Glu group were higher compared with the Con2 group. The results of summary analyses of the dietary Glu supplementation were shown as the bubble plots (Fig. 7c). The pyrimidine metabolism, glutamine and glutamate metabolism, glyoxylate and dicarboxylate metabolism, glutathione metabolism, arginine and proline metabolism and purine metabolism with the high value of –lg (P) was identified as the main impacted pathway. The specific regulatory pathways that induced by dietary Glu supplementation were summarised and presented in Fig. 7d.

Purine metabolites

The effects of 3%Glu supplementation on the levels of purine metabolites in dorsal muscle of gibel carp in trial 2 are displayed in Fig. 8. The contents of inosine and hypoxanthine were lower while adenine was higher in the 3%Glu group than those in the Con2 group ($P < 0.05$) (Fig. 8a, b and e). No significant difference of the contents of guanosine and guanine was observed between the two groups ($P > 0.05$) (Fig. 8c and d).

Sensory evaluation of flesh

The result of sensory evaluation in practical diet trail is described in Fig. 9. It showed that the flavour and firmness of flesh were graded higher in the 2%Glu group those in the Con1 group ($P < 0.05$), but the scores of the appearance, fragrance (smell), fishy (smell) and fishy (taste) showed no significant difference ($P > 0.05$).

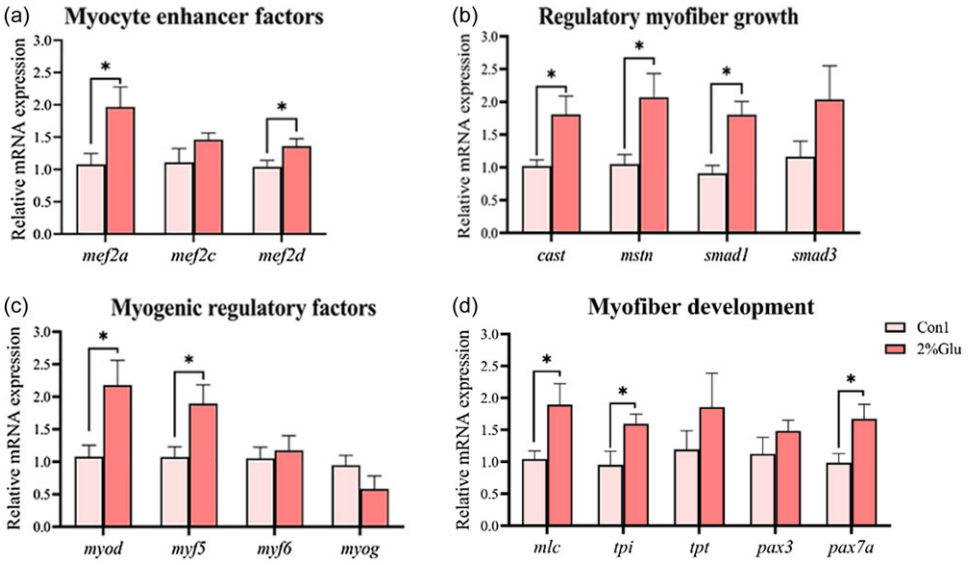

Fig. 4. Effects of Glu supplementation in the practical diets on muscle growth and development of gibel carp. (a) The relative mRNA expression of myocyte enhancer factors in dorsal muscle. (b) The relative mRNA expression of myofibre growth in dorsal muscle. (c) The relative mRNA expression of myogenic regulatory factors in dorsal muscle. (d) The relative mRNA expression of myofibre development in dorsal muscle (n = 3). '*' showed significantly differences between groups ($P < 0.05$).

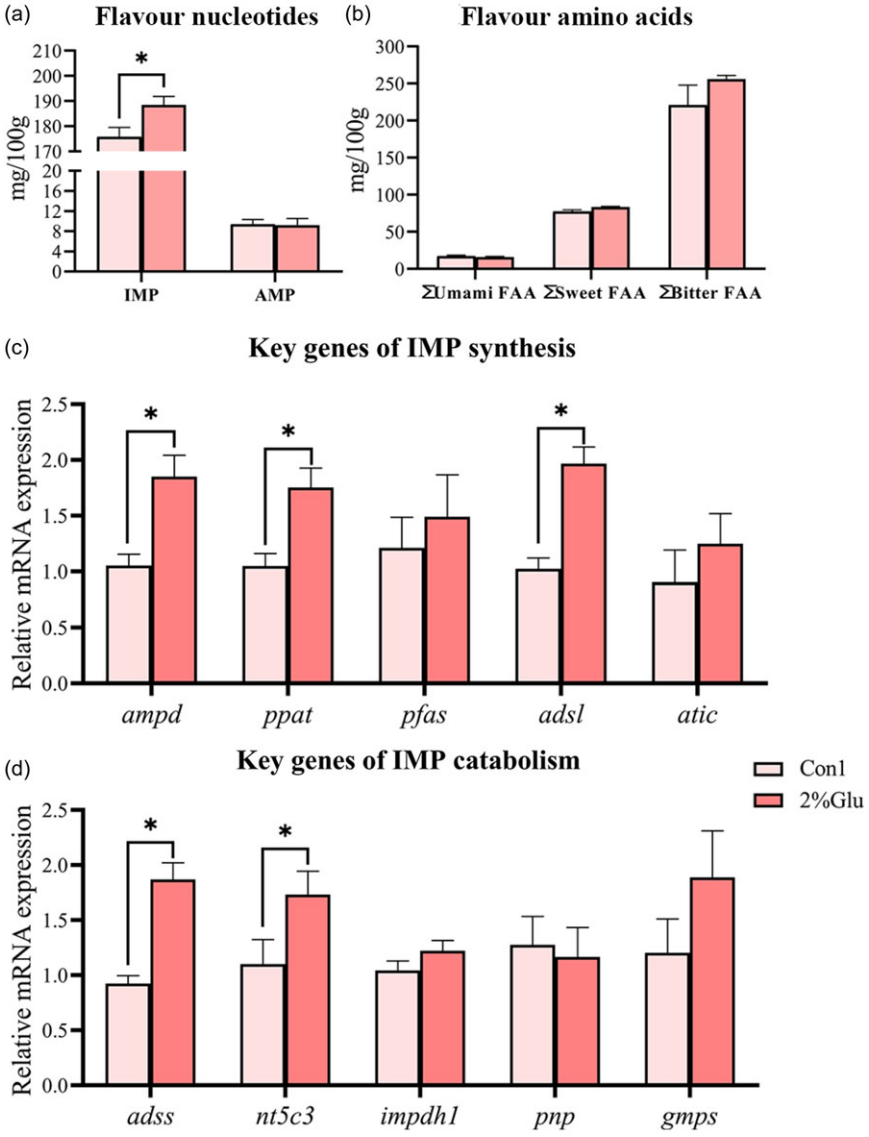

Fig. 5. Effects of Glu supplementation in the practical diets on flavour nucleotide, flavour amino acids and IMP metabolism in dorsal muscle of gibel carp. (a–b) Flavour nucleotides (IMP and AMP) content and flavour amino acids (Umami: aspartic acid and glutamic acid: sweet: aspartic acid, glycine, alanine, methionine, leucine, arginine, and proline; bitter: valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, and lysine) content in muscle (n = 3). (c) The relative mRNA expression of IMP synthesis in muscle (n = 6). (d) The relative mRNA expression of IMP catabolism in muscle (n = 6). For each index, '*' showed significantly differences between groups ($P < 0.05$).

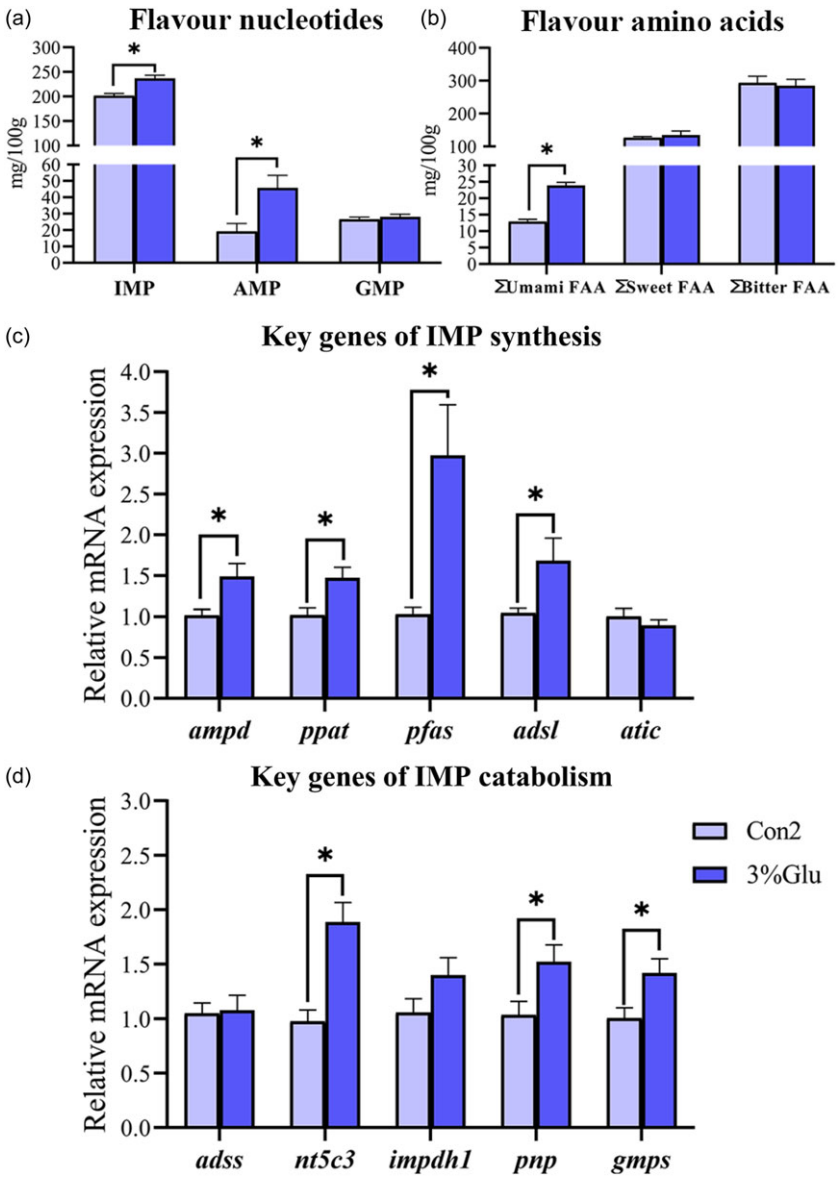

Fig. 6. Effects of Glu supplementation in the purified diets on flavour nucleotide, flavour amino acids and IMP metabolism in dorsal muscle of gibel carp. (a–b) Flavour nucleotides (IMP, AMP and GMP) content and flavour amino acids (Umami: aspartic acid and glutamic acid: sweet: aspartic acid, glycine, alanine, methionine, leucine, arginine, and proline; bitter: valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, and lysine) content in muscle ($n = 3$). (c) The relative mRNA expression of IMP synthesis in muscle ($n = 3$). (d) The relative mRNA expression of IMP catabolism in muscle ($n = 3$). For each index, '*' showed significantly differences between groups ($P < 0.05$).

Discussion

In this study, exogenous Glu was supplemented to practical diet to investigate the effects of Glu on flesh quality of gibel carp from both phenotypic and mechanistic perspectives, then it was supplemented to purified diet again to verify the effects. Above all, there were no significant differences in final body weight, specific growth rate and feed efficiency between the control and experimental groups in the two experiments, indicating that dietary Glu supplementation had no negative effects on the growth performance of gibel carp. A similar result in Atlantic salmon (*Salmo salar* L.) also showed that Glu supplementation did no harm to growth.[28] It is speculated that the mode of amino acids in control diets has already fulfilled the optimal growth of the fish, which will not be further promoted by extra amino acids.

Present study showed that supplementing Glu to the practical and purified diet significantly increased the hardness and springiness of muscle. Other than that, Glu supplementation in purified diet significantly diminished the CWL of muscle, and a bigger CWL indicated poorer water-holding capacity.[29] Similar report showed that dietary Pro supplementation decreased the water loss of muscle in large yellow croaker (*Larimichthys crocea*).[30] As proofed, muscle hardness and springiness were positively associated with the density and diameter of myofibres as well as the amount of collagen between myofibres in muscle.[31–33] In this study, Glu-supplemented diet increased the thick and decreased the thin myofibres, and increased the density of myofibres.

Glu is a pivotal substrate that widely involves in the synthesis of Pro, a material devoting to collagen anabolism.[17,34] In this study, dietary Glu supplementation increased the content of that amino acid in muscle, further promoted the content of collagen in muscle. Moreover, collagen is the most copious protein in connective tissue, whose hydroxyl group can easily bond water molecular to prevent water loss.[32] Therefore, this study found that elevated collagen content increased the CWL in muscle. However, in the study of triploid crucian carp, the hardness was decreased and was attributed to the increased sarcomere length; the CWL was not affected although hydroxyproline (main component of collagen)

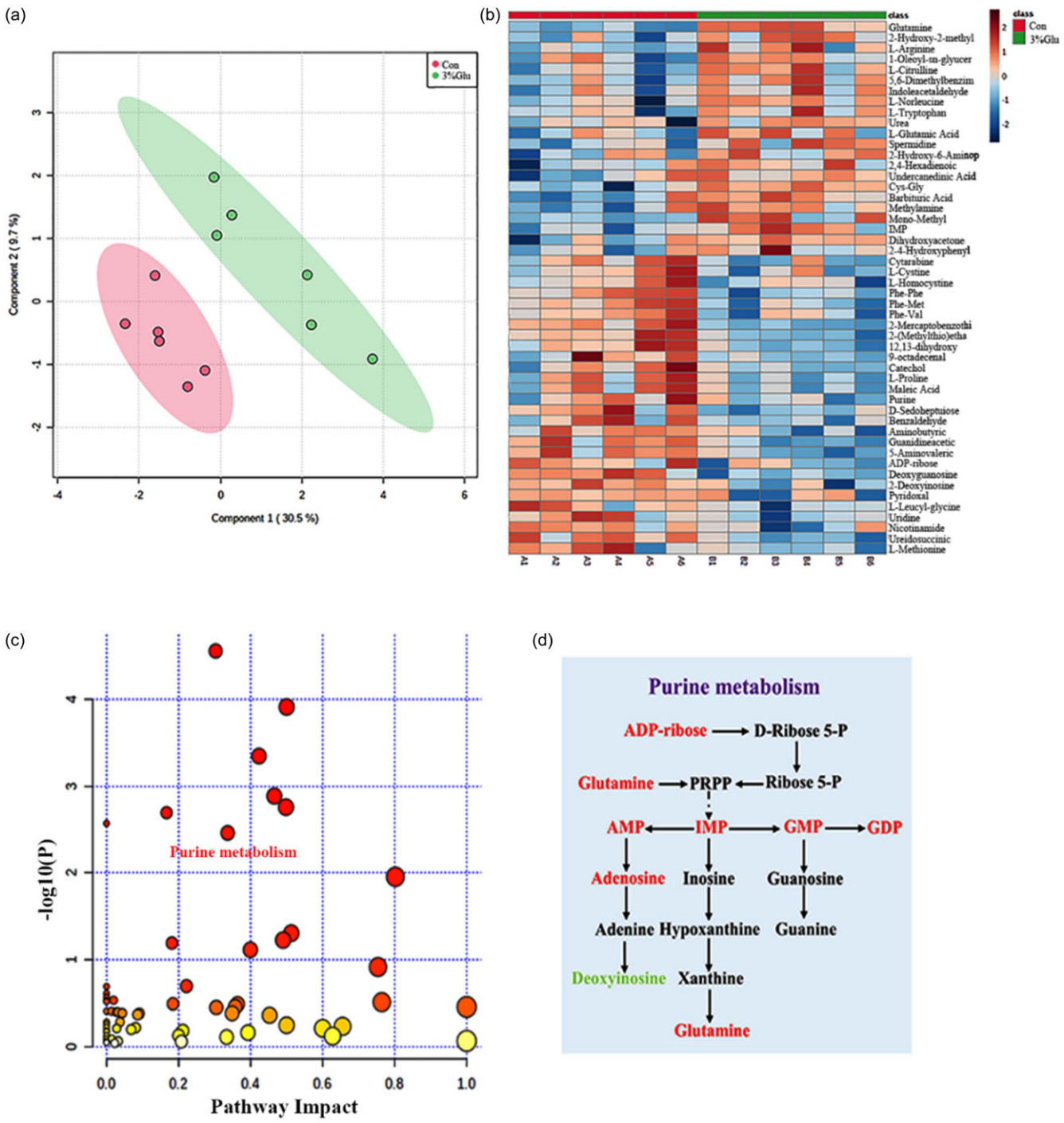

Fig. 7. Effects of Glu supplementation in the purified diets on the metabolomic features in flesh. (a) The PCA score plots for metabolomic profiles of muscle. Con2 group in red, 3% Glu group in green. (b) Heatmap visualisation of differential and overlapping metabolites in Con2 group VS 3%Glu group. Red indicates high abundance, whereas the relatively low-abundance metabolites are shown in blue. (c) Summary of pathway analyses of the effects of dietary 3%Glu supplementation for flesh with MetaboAnalyst 5.0, as visualised by bubble plots. The colour and size of each circle is based on the *P* value and the pathways impact value, respectively. (d) Metabolic pathways affected induced by Glu supplementation in muscle; Red indicated increase; green indicated decrease.

was increased.[18] Previous research revealed that the sarcomere length positively correlated with flesh hardness.[35] As for the same parameters, different studies showed different even contradictory results, inferring that the muscle texture characteristics could relate to more complex microstructures which remained to be excavated.

Previous studies confirmed that the growth of myofibres and the increase of collagen were commonly attendant on the increasing crude protein content of muscle.[32,36] Present study verified this point, and dietary 2%Glu addition significantly increased the expression of *igf-1*, *tor*, *s6k1* and *eif4e* in muscle, meanwhile, it significantly increased phosphorylation of AKT and S6 protein, firstly unveiling that Glu is an activating factor of the IGF-1/AKT/mTOR pathway. Studies in hybrid bagrid catfish (*Pelteobagrus vachelli*♀ × *Leiocassis longirostris*♂) and Atlantic salmon similarly reported that dietary isoleucine and methionine promoted the muscle protein deposition by affecting the signalling

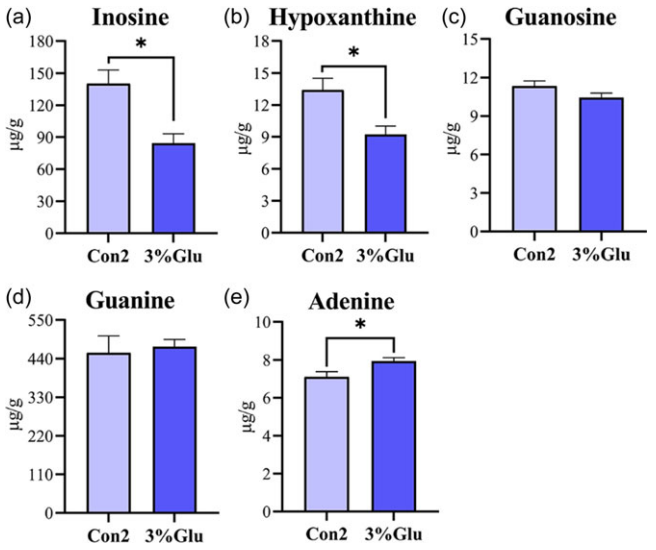

Fig. 8. Effects of Glu supplementation in the purified diets on the levels of purine metabolites in dorsal muscle of gibel carp. Data are shown as mean ± SEM ($n = 3$). For each index, '*' showed significantly differences between groups ($P < 0.05$).

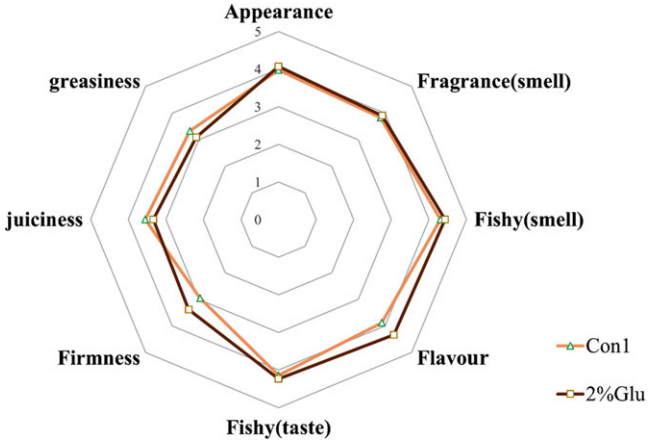

Fig. 9. Effects of Glu supplementation in the practical diets on the appearance, smell and taste of flesh in gibel carp.

pathway.[37,38] Besides, internal Glu can provide energy through transamination and tricarboxylic acid cycle, that could protect some amino acids from catabolism, and let them take more part in body protein synthesis,[39] so the free glutamate was not increased by Glu supplementation in trial 1, but the contents of some other amino acids (such as lysine, threonine and alanine) in muscle were improved. However, the free glutamate content was raised in the 3%Glu group in trial 2, possibly due to the higher dosage of the supplement.[19] The study in triploid crucian carp additionally found that the body protein retention rate was promoted and the key signals of muscle protein degradation involving Adenosine monophosphate-activated protein kinase, muscle atrophy F-box (MAFbx) and muscle ring finger 1 (MuRF1) were inhibited after Glu supplementation.[18] Combining the results of the two studies we could determine that dietary Glu promoted protein deposition in muscle by reinforcing the protein synthesis and suppressing the protein degradation.

The growth and development of myofibres is also regulated by a number of regulatory factors. The upregulation of *mef2a* and *mef2d* expressions in fish fed the diets supplemented with Glu suggested that Glu can regulate muscle growth and development by mediating the myocyte enhancer family genes.[40] And the upregulation of *cast* expression promoted the protein synthesis and accelerated the growth of myofibre.[41] Glu supplementation concomitantly increased the expression of *mstn* and *smad1*, meaning a negatively regulatory system of muscle growth was activated. The reason may connect with a negative feedback regulation of myofibre proliferation and hypertrophy. MRFs regulate the process of satellite cell activation during myogenesis and muscle regeneration.[42] In present study, dietary Glu supplementation significantly increased the mRNA expression level of *myod* and *myf5*. Myosin light chain (*mlc*) is the main component of myofilament, while troponin T (*tpt*) and troponin I (*tpi*) are regulators of muscle activity.[43] The *pax7α* and *pax3* genes mutually regulate and involve in the development of skeletal muscle and are targets for the treatment of sarcopenia and muscular dystrophy.[44] Dietary Glu significantly increased the mRNA expression of *mlc*, *tpi* and *pax7α*. Previous study indicated that the *pax7α* gene induced the expression of *myod* and *myf5* genes,[45] and this study reached a consensus. Herein, Glu exerted an extensive effect in the regulatory factors of myofibres growth, further altering muscle texture characteristics.

Flavour is another important indicator of the flesh quality, mainly including taste.[46,47] The taste characteristics depend on the levels of nucleotides especially IMP and AMP, and FAA such as umami, sweet and bitter FAA in food. As a biosynthetic nutrient and an important flavour enhancer,[48] IMP was increased in flesh when diets were supplemented with Glu. The result was consistent with the study in triploid crucian carp, in which the transcriptomics and qPCR results revealed that dietary Glu promoted the generation of IMP while inhibited the oxidative decomposition of IMP.[18] To complement, we conducted a metabolomic analysis in trial 2 and it displayed that Glu supplementation promoted the deposition of IMP in flesh and mostly affected the purine metabolic pathway. Further determinations of gene expression showed that Glu supplementation not only upregulated the expression levels of IMP synthesis-related genes, *ampd*, *ppat* and *adsl*, but also upregulated the expression level of IMP catabolism-related gene, *nt5c3*, suggesting that Glu upregulated synthetic metabolism rate of the IMP, thus of which an accumulation was induced.[49] Furthermore, it is worth noting that the flavour nucleotides and amino acids in fish fed with practical diets were lower than those with purified diets, and the muscle textural properties in the two trials also diversified. The hardness, springiness, fracturability, stickiness and adhesiveness of fish in practical diet-based groups were higher. These interesting differences may not be attributed simply to single amino acid, as the composition of dietary ingredient was intricate. Systematic comparative studies that focused on the effects of the practical and purified diets on flesh qualities were needed in the future. This will help to achieve pinpoint regulation of flesh qualities in aquaculture.

Besides, purine metabolites make great contribution to flesh quality. For instance, inosine, hypoxanthine, guanosine, guanine and adenine, they are aftermaths of degradation of IMP or AMP, so their concentrations are positively associated with the reduction of freshness.[50,51] So purine metabolites in muscle were also detected by high-performance liquid chromatography (HPLC) in trial 2, as major substances active in purine metabolic pathway, the contents of adenine, inosine and hypoxanthine changed significantly with Glu supplementation. Although Glu supplementation in purified

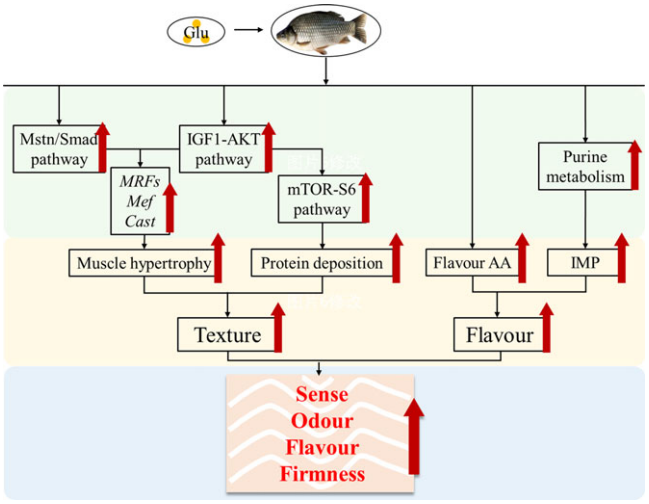

Fig. 10. A schematic diagram showing the effects of Glu supplementation on the flesh quality in gibel carp.

diet slightly increased the content of the adenine, it highly decreased that of the inosine and hypoxanthine. Interestingly, the hypoxanthine and inosine in muscle brings about an unpleasant bitter taste for meats, so we can reason that dietary Glu can improved the flesh flavour by restraining the accumulation of those two items.[52] According to the results of flavour substances, purine metabolites, gene expression and metabolomic analysis, we noted that dietary Glu truly improved the flesh flavour through functioning in purine metabolism, whereby that was a theorical basis of flesh quality improvement of cultured fish.

Based on the human senses, sensory evaluation is the most intuitive and effective method to describe and judge the flesh quality.[53] Therefore, the sensory evaluation was carried out to summarise the effects of Glu on the flesh quality of gibel carp. After sensory examination by professionals, it was found that the flavour and firmness scored higher in the Glu-addition group than those in the control group, consistent with the results on flavour substances and muscle texture characteristics above. Sensory evaluation could directly reflect consumers' preferences for foods and account for subtle differences that are not detectable by instruments. The results of the sensory evaluation visually demonstrated that the dietary Glu supplementation could improve the flesh quality of gibel carp by altering the muscle texture characteristics and increasing the flavour substances content.

Conclusion

In conclusion, this study firstly revealed that dietary Glu promoted the muscle protein deposition as well as the growth and development of myofibres by regulating the IGF1-AKT-mTOR signalling pathway in gibel carp. The deposition of muscle protein and the hypertrophy of myofibres further increased the hardness and springiness of the muscle. On the other hand, dietary Glu supplementation promoted the deposition of flavour substances by regulating purine metabolic pathway. The improvements of the texture characteristics and flavour ultimately enhanced the flesh quality of gibel carp, and the results of the sensory analysis further verified it (Fig. 10). This study provides a reference for providing quality aquatic products to consumers and ensuring the sustainable development of the aquaculture industry.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/jns.2025.10009

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Author contributions. Wanjie Cai and Xing Wang: Investigation, Formal analysis, Data curation, Writing- Original draft preparation. Haokun Liu: Conceptualisation, Data curation, Project administration, Writing - review & editing. Dong Han: Investigation. Zhimin Zhang and Xiaoming Zhu: Methodology. Junyan Jin and Yunxia Yang: Resources. Shouqi Xie: Supervision, Funding acquisition.

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Competing interests. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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