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Dietary effects of fish meal substitution with *Clostridium autoethanogenum* on flesh quality and metabolomics of largemouth bass (*Micropterus salmoides*)

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ABSTRACT

The study investigated dietary effects of replacing fish meal (FM) with *Clostridium autoethanogenum* powder (CAP) on growth, flesh quality and metabolomics of largemouth bass (*Micropterus salmoides*). The control diet contained 350 g/kg FM, and then dietary FM was decreased to 300 g/kg, 250 g/kg, 200 g/kg, 150 g/kg and 100 g/kg by CAP inclusion, respectively (FM-30, FM-25, FM-20, FM-15, FM-10). Then, the six diets were fed to largemouth bass with initial body weight of 110 g for 56 days. The high CAP inclusion (FM-10) decreased specific growth rate, flesh collagen, flesh hardness and increased feed conversion ratio ($P < 0.05$), but no significant difference was found in feed intake among all the groups. FM-25 and FM-10 groups presented higher flavoring amino acids contents and better performance in sensory evaluation than the control group ($P < 0.05$), and FM-10 group also showed better values in electronic tongue evaluation. The differential metabolites in FM-25 and FM-10 groups were found to be involved in lipid, amino acid and protein metabolism. In conclusion, in a basal diet containing 350 g/kg FM, 150 g/kg dietary FM could be successfully replaced by CAP, while higher FM substitution decreased the flesh collagen content and flesh hardness of largemouth bass.

1. Introduction

The largemouth bass (*Micropterus salmoides*) industry has rapidly expanded in many countries in recent years for its fast growth and delicious taste (He et al., 2020a). Generally, the commercial diets for largemouth bass contained high level of fish meal (350–500 g/kg), resulting in a high feed cost. Due to the shortage of fish meal resources, it is necessary to find some new protein sources to meet the increasing demand for the fast-growing aquaculture industry (Henry et al., 2015). In largemouth bass, some alternative proteins have been reported to decrease the dietary fish meal inclusion, including fermented soybean meal (He et al., 2020a), brewer's yeast hydrolysate (Zhou et al., 2018), poultry by-product meal and soybean meal mixtures (Ren et al., 2018). These studies focused on the growth performance (Li et al., 2019), nutrients utilization (He et al., 2020a), intestinal structure and flora (He et al., 2020b), but relatively fewer studies were reported in flesh quality. Cochran et al. (2009) found that dietary replacement of 37% fish meal with plant and animal protein mixture did not significantly affect the body composition of largemouth bass (average 210 g). In the study of He et al. (2020b), the arginine and aspartic acid content in flesh increased, while glycine content decreased when 45% and 60% of dietary fish meal were substituted by fermented soybean meal. Metabolomics is derived from the profiling study of metabolites, reflecting the end products of gene expression. High performance liquid chromatography with tandem mass spectrometry (HPLC-MS) has been used for quantitative determination of meat quality and related metabolites in aquatic products (Gil-Solsona et al., 2019; Wei et al., 2018a; Yu et al., 2020). In recent years, metabonomics was used to analyze the effects of different levels of hydroxyproline on the meat quality of large yellow croaker (*Larimichthys crocea*) (Wei et al., 2018b), and the effects of reactive oxygen species on

Abbreviations: CAP, Clostridium autoethanogenum powder.

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the muscle texture of grass carp (*Ctenopharyngodon idllus*) (Yu et al., 2020).

Clostridium autoethanogenum powder (CAP) is a new microbial protein produced by bacterial fermentation with carbon monoxide from steel-making waste gas as carbon source and ammonia as nitrogen source. CAP has a high protein content up to 85% with plenty of essential amino acid, vitamins and other nutrients, which shows a similar amino acid profile to fish meal (Chen et al., 2020). A relatively full genome sequence of *Clostridium autoethanogenum* has been obtained, and no toxic gene fragment has been reported (Christopher et al., 2015). In aquatic animals, dietary inclusion of CAP has been reported in grass carp (*Ctenopharyngodon idllus*) (Wei et al., 2018b), Jian carp (*Cyprinus carpio* var. Jian) (Li et al., 2021), black sea bream (*Acanthopagrus schlegelii*) (Chen et al., 2020), and Pacific white shrimp (*Litopenaeus vannamei*) (Yao et al., 2022). In juvenile largemouth bass (initial body weight of 17.75 g), the substitution of 63% dietary fish meal with CAP showed no adverse impacts on hepatic and hindgut histology (Zhu et al., 2022). Also in largemouth bass, CAP successfully replaced 150 g/kg fish meal in diet containing 350 g/kg fish meal without adverse effects on growth, feed utilization and intestinal histology (Yang et al., 2021).

Considering the importance of decreasing fish meal inclusion, CAP may be a good candidate of replacing fish meal in the diet of largemouth bass due to its higher crude protein content and lower price than fish meal. Although CAP has been reported to replace partial fish meal in the diets of largemouth bass (Yang et al., 2021; Zhu et al., 2022), these studies focused on the growth, feed utilization, digestive capacity and intestinal morphology, but not on flesh quality. It is not clear that fish meal replacement with CAP will negatively affect the flesh quality or not. Therefore, CAP was used in this study to replace different proportions of dietary fish meal to feed large-size largemouth bass, and then biochemical analysis, flesh quality evaluation and metabolomics were combined to evaluate the impact on the growth performance and flesh quality.

2. Materials and methods

2.1. Experimental design

The basal diet was formulated to contain 350 g/kg fish meal (FM-35, Control), and then six iso-nitrogenous diets were prepared by decreasing fish meal level to 300 g/kg, 250 g/kg, 200 g/kg, 150 g/kg and 100 g/kg with the inclusion of 38 g/kg, 76 g/kg, 114 g/kg, 152 g/kg and 190 g/kg CAP (FM-30, FM-25, FM-20, FM-15, FM-10), respectively. Histidine, tryptophane and arginine (Shanghai Yuanye Bio-Technology Co., Ltd) were added to the diets to maintain the same levels as the control diet. The protein ingredients were ground, screened (60-mesh), and mixed with oil and water (30%). Then the mixture was extruded to form sinking pellets (a diameter of 2.0 mm) using a single-screw extruder (SLP-45; Chinese Fishery Machinery and Instrument Research Institute, Shanghai, China) at an extruding temperature of 85–90 °C. All diets were air-dried naturally and preserved at 4 °C until they were used. The diets formulation and proximate composition were shown in Table 1. The contents of crude protein, crude lipid and ash of the CAP were 835 g/kg, 190 g/kg and 350 g/kg, respectively. The amino acid composition of fish meal, CAP and experimental diets was shown in Table 2.

2.2. Experimental fish and feeding management

Largemouth bass were supplied by a local aquaculture farm in Huzhou, China. All fish were fed with commercial diets (containing 500 g/kg crude protein) and acclimatized to the environment for 2 weeks. Then, a total of 216 fish with initial body weight of 110.0 ± 0.5 g were randomly allocated into 18 cages (1.5 × 1.0 × 1.2 m) with 3 replicates (cage) per treatment and 12 fish per cage. The cages were hung in indoor cement pools without direct sunshine. The six diets were fed to the fish three times daily (08:00, 13:00, 17:00) for 56 days. The daily feeding rate was 2–2.5% of body weight, and the feed intake was adjusted according to the feeding behavior and water temperature to ensure no feed residue left in 10 min after feeding. About one third of the cultured water was renewed with filtrated pond water and the waste in the pools was siphoned out once a week. Water quality was monitored every day,

Table 1
Ingredients and proximate composition of experimental diets (air dry basis, g/kg).

Ingredients[a]	FM-35	FM-30	FM-25	FM-20	FM-15	FM-10
Fishmeal	350.0	300.0	250.0	200.0	150.0	100.0
Clostridium autoethanogenum powder[b]	0.0	38.0	76.0	114.0	152.0	190.0
Wheat flour	201.0	202.7	204.6	206.4	208.2	210.0
Fish oil	25.5	29.0	32.5	36.0	39.5	43.0
Vitamin premix[c]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[d]	5.0	5.0	5.0	5.0	5.0	5.0
$Ca(H_2PO_4)_2$	15.0	20.0	25.0	30.0	35.0	40.0
Arginine	0	0.9	1.7	2.5	3.4	4.2
Histidine	0	0.6	1.1	1.7	2.2	2.8
Tryptophan	0	0.3	0.6	0.9	1.2	1.5
Orhers[e]	402.5	402.5	402.5	402.5	402.5	402.5
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Proximate composition						
Crude protein	468.8	468.8	468.9	470.0	468.2	467.8
Crude lipid	117.1	116.7	116.3	116.9	117.1	115.7
Ash	87.3	79.2	73.4	66.2	59.4	53.3
Moisture	71.4	71.3	70.9	71.1	70.6	70.5

[a] The ingredients were purchased from the Yuehai Feed Company (Zhejiang, China), and the protein contents of ingredients are as follow: fish meal (670.0 g/kg), soybean meal (410.0 g/kg), soybean protein concentrate (650.0 g/kg), wheat flour (800.0 g/kg), wheat middling (136.0 g/kg), corn gluten (630.0 g/kg), beer yeast (524.0 g/kg).

[b] The *Clostridium autoethanogenum* powder was provided by Hebei Shoulang New Energy Technology Co., Ltd, China. CAP has a crude protein, crude lipid and crude ash content of 835 g/kg, 190 g/kg and 350 g/kg, respectively.

[c] Vitatim premix (mg or IU/kg diet): VA, 10 000 IU; VD_3, 3 000 IU; VE 150 IU; VK_3, 12.17 mg; VB_1, 20 mg; VB_2, 20 mg; VB_3, 100 mg; VB_6, 22 mg; VB_{12}, 0.15 mg; VC, 1000 mg; biotin, 0.6 mg; folic acid, 8 mg; inositol, 500 mg.

[d] Mineral premix (mg/kg diet): I, 1.5 mg; Co, 0.6 mg; Cu, 3 mg; Fe, 63 mg; Zn, 89 mg; Mn, 11.45 mg; Se, 0.24 mg; Mg, 180 mg.

[e] Orhers: Soybean protein concentrate 100, Soybean meal 100.5, Wheat middling 35, Corn gluten 70, Beer yeast 40, VC 1, Other: Choline chloride 5, Soybean oil 25.5, Soybean lecithin 25.5.

Table 2
Amino acid composition of fish meal, *Clostridium autoethanogenum* powder and experimental diets (dry matter basis, g/kg).

Amino acid	Diets						Fish meal	*Clostridium Autoethanogenum* powder
	FM-35	FM-30	FM-25	FM-20	FM-15	FM-10		
EAAs								
His	20.1	22.4	21.3	21.4	20.5	19.4	20.1	16.8
Phe	25.0	24.2	25.2	24.8	25.6	23.9	26.1	33.0
Lys	30.5	38.5	37.3	35.7	32.5	30.3	49.7	87.0
Thr	19.5	20.4	20.1	19.1	22.3	18.8	27.4	40.2
Val	20.8	20.5	15.6	16.6	21.5	20.5	31.1	54.4
Met	9.7	13.3	7.6	6.8	12.1	12.1	18.6	22.9
Ile	22.4	21.8	20.3	19.1	21.7	23.4	26.1	52.8
Leu	31.2	30.2	31.9	29.8	29.2	27.9	49.7	63.8
Arg	32.1	34.2	31.1	30.1	31.6	29.1	39.3	34.0
NEAAs								
Asp	34.4	50.6	47.7	53.1	48.1	44.9	40.0	95.4
Ala	29.8	37.4	36.1	29.7	29.4	30.1	25.8	46.3
Glu	70.7	70.5	89.6	85.5	82.7	74.6	67.4	97.8
Tyr	20.0	19.7	19.6	19.1	20.6	19.9	13.0	6.2
Ser	28.4	26.5	29.9	24.9	29.9	22.5	17.5	32.1
Pro	48.5	50.0	50.1	52.1	48.3	40.9	15.8	24.0
Cys	6.2	3.4	4.1	2.5	3.3	7.3	3.2	9.5
Gly	28.9	34.1	30.8	25.9	24.9	26.0	25.8	38.7
TAA	478.5	517.8	518.5	496.2	504.2	471.6	496.6	754.9

EAAs, Essential amino acids; NEAAs, Non-essential amino acids; TAA: total amino acids; Glu, glutamic acid; Asp, aspartic acid; Leu, leucine; Ile, isoleucine; His, histidine; Gly, glycine; Thr, threonine; Ala, alanine; Arg, arginine; Phe, phenylalanine; Lys, lysine; Pro, proline; Tyr, tyrosine; Val, valine; Met, methionine; Ser, serine; Cys, cysteine.

and the water temperature, dissolved oxygen, pH and ammonia nitrogen levels were 25–30 ℃, > 5 mg/L, 7.5–8.0 and < 0.2 mg/L, respectively. In accordance with the laboratory animal welfare regulations set by Chinese Association for Laboratory Animal Science, all experimental fish were approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Ocean University (Shanghai, China).

2.3. Sample collection

Prior to slaughter, all fish were deprived of diets for 24 h, then measured and weighed in bulk. Individual fish was euthanized by an overdose of anesthetic (MS-222) and clinical signs of death were ensured prior to sampling. Survival, specific growth rate (SGR), feed conversion ratio (FCR) and Feed intake (FI) were calculated based on the above information and feed intake record. Three fish per cage were selected for the flesh quality evaluation after the bleeding. Three blocks of dorsal flesh (about 2–3 g) from the left side of the body were sampled to immediately measure water-holding capacity. The forth block of flesh from the same side was used for the immediate measurement of texture properties. Another four blocks of dorsal flesh (2–3 g) from the right side were used for drip loss measurement. The other flesh samples were stored at − 80 ℃ for proximate composition, free amino acids, collagen and electronic tongue evaluation.

FM-35, FM-25 and FM-10 groups were selected for the metabolomic assay. About 0.2 g dorsal flesh from the left side was collected and frozen in liquid N_2, then stored at − 80 ℃ until use. The flesh of three fish per cage was pooled as one sample when performing the metabolomic assay. The other dorsal flesh was used for sensory evaluation immediately.

2.4. Analytical methods

2.4.1. Growth

FI (%/day) = 100 × total amount of the feed consumed / [(initial body weight + final body weight) / 2] / days

SGR (%/day) = 100 × [Ln (final body weight) - Ln (initial body weight)] / days

Survival (%)=100 × final number of fish / initial number of fish

2.4.2. Proximate composition

Crude lipid, crude protein, moisture and ash contents were analyzed following the method of AOAC (2000a). The moisture and ash contents were measured by drying samples to constant weight at 105 °C, and by combusting samples in a muffle furnace at 550 °C for 6 h, respectively. The crude protein content was estimated with the Kjeldahl system method (2300 Auto analyser; FOSS Tecator, AB, Hoganas, Sweden), and the crude lipid was measured gravimetrically after extraction by chloroform–methanol.

2.4.3. Free amino acid composition

The flesh free amino acid composition was determined following the method described by Yang et al. (2020). Fresh flesh (40 mg) was mixed with 1.2 mL mixture of methanol and water (4:1), then homogenized in ice-water bath with ultrasonic for 10 min, stored at − 20 °C for 2 h. After centrifuging at 12,000 r/min for 30 min (4 °C), the supernatant was collected for free amino acid analysis by using a Waters ACQUITY Ultra Performance LC/MS (Waters, USA).

2.4.4. Collagen content

The collagen content was determined following the method described by Yang et al. (2020). Hydroxyproline (Hyp) content in flesh was determined by alkaline hydrolysis method via the kit from Nanjing Biotechnology Institute (Nanjing, China). The principle of the method is that the oxidation products of hydroxyproline and oxidizing agent reacts with dimethylaminobenzaldehyde to present a purple-red color, and the color depth is positively related to the Hyp content. Then, by referring to AOAC method 990.26 (AOAC, 2000b), the content of collagen was calculated by multiplying the Hyp content by 8.

2.4.5. Texture properties

The texture properties of flesh were determined following the method described by Yang et al. (2020). A block of flesh about 1 cm^3 was used for texture profile analysis (TPA) including hardness, chewiness, elasticity, cohesiveness, adhesion and recovery (Universal TA texture analyzer, Tengba, Shanghai, China). In the analysis, a cylindrical probe was used with diameter of 25 mm and speed of 1 mm/s. The contact induction force was 5 gf, and the deformation was 30% of the original

thickness.

2.4.6. Water-holding capacity

The water-holding capacity of flesh was determined following the method described by Zhang et al. (2020). The steaming loss and centrifugal loss were calculated by steaming the flesh sample (W_1) in pot for 5 min, or by centrifuging the flesh sample (W_1) for 10 min at the speed of 3500 r/min, then weighted (W_2) after wiping off the surface liquid. Another block of flesh was weighed (W_1), then stored at − 20 ℃ for 24 h. After thawing at room temperature, the surface liquid was wiped off and the flesh was weighed (W_2). The drip loss was calculated by hanging the flesh sample (W_1) at 4 ℃ in a refrigerator for 2, 4, 6 and 24 h, respectively, then the samples were weighted after wiping off the surface liquid (W_2). The water loss was calculated as follows:

Steaming (centrifugal, thawing, drip) loss (%)= 100 × (W_1-W_2)/W_1.

2.4.7. Sensory evaluation

The flesh sensory evaluation was determined following the method described by Li et al. (2013). Flesh samples were cooked in boiling water for 8 min, then sensory evaluation was conducted by 9 trained judges independently. Flavor, tenderness and taste were scored varying from 1 point (the worst) to 5 points (the best). For flavor, tenderness and taste, one point represents extremely fishy odor, less palatability, less elasticity, and 5 points represents good acceptability good tenderness, good elasticity. The sum of three scores is the overall acceptability of the sample.

2.4.8. Electronic tongue evaluation

Astree electronic tongue system (Instrument type Smart tongue, Alpha. MOS Co., Ltd., France) was used in the present study, which comprises five test sensors, two reference sensors, one reference electrode, electrical signal processors and pattern recognition systems. The three test sensors were expressed as CTS (salty), ANS (acid), and UMS (fresh).

Before the determination, the sensors were activated for 24 h. The flesh sample thawed at 4 °C, then homogenized (2.00 g) with distilled water (10 mL) at 4 °C for 5 min (8000 r/min). The supernatant was used for electronic tongue analysis. Each sample was measured for seven times, and the data obtained from the last four times were used for subsequent chemometrics analyzing. The sampling time was 120 s, and the cleaning time was 10 s. After the measurement, principal component analysis (PCA) and radar map analysis were carried out.

2.4.9. Metabolomic analysis

The control, FM-25 and FM-10 groups were used for the metabolomic analysis. Flesh sample was precisely weighed (50 mg), then extracted with a mixture (400 μL) of methanol and water (4:1, v/v). The sample was treated by tissue crusher (High throughput tissue crusher Wonbio-96c, Wanbo Biotechnology co., LTD, Shanghai, China) at 50 Hz for 6 min, then subjected to cryogenic sonication treatment for 30 min, kept at − 20 °C for 1 h, centrifuged (12,000 g) at 4 °C for 15 min. The supernatant (20 μL) was collected and transferred for LC-MS/MS analysis by Shanghai Majorbio Bio-Pharm Technology Co.,Ltd.

The chromatographic column was ACQUITY UPLC HSS T3 (100 mm × 2.1 mm i.d., 1.8 μm; Waters, Milford, USA). The mobilephases consisted of solvent A (0.1% formic acid) and solvent B (acetonitrile:isopropanol=1:1 (v/v), containing 0.1% formic acid). The sample injection volume was 10 μL, and the flowing rate was 0.4 mL/min with column temperature of 40 ℃. Electrospray positive ion (ESI^+) mode and electrospray negative ion (ESI^-) mode were used to collect mass spectrum signal (Triple TOFTM5600 +, AB Sciex, USA). During the period of analysis, all samples were stored at 4 ℃, and a quality control (QC) sample was inserted every 5–15 samples to evaluate the stability of the analytical system and assess the reliability of the results.

Metabonomics raw data was imported into Progenesis QI (Waters Corporation, Milford, USA) for preprocessing. Statistically significant metabolites among groups were selected with VIP> 1 and $P < 0.05$ for PCA. Partial least squares discriminate analysis (PLS-DA) was used for statistical analysis to determine flesh metabolic changes between comparable groups. The model validity was evaluated from model parameters R^2 and Q^2, which provide information for the interpretability and predictability and avoid the risk of over-fitting (Chen et al., 2012). Differential metabolites between two groups were summarized and mapped into their biochemical pathways through metabolic enrichment and pathway analysis based on database search (http://www.kegg.com) (Song et al., 2018; Xu et al., 2019).

2.5. Statistical analysis

The experimental data were presented as the means and standard deviation (the means ± SD). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 19.0 for Windows (SPSS, Chicago, IL, USA). A one-way analysis of variance (ANOVA) was combined with the LSD method for multiple comparisons. The significance level for the differences among treatments was $P < 0.05$.

3. Results

3.1. Growth performance and flesh composition

In Table 3, only FM-10 group showed significantly lower SGR and higher FCR than the fish meal group ($P < 0.05$) in all fish meal substituted groups. No significant difference was found in feed intake and survival among the all groups ($P > 0.05$).

The flesh moisture contents in FM-30, FM-25 and FM-20 groups were significantly lower ($P < 0.05$), while the crude lipid content in FM-30 and FM-25 groups were higher than those of the control group ($P < 0.05$). There was no significant differences in flesh crude protein content among all the groups ($P > 0.05$). When fish meal inclusion was lower than 200 g/kg, the flesh collagen decreased with the increasing dietary CAP level, and the collagen content in FM-10 group was significantly lower than that in the control group ($P < 0.05$).

Table 3
Effects of *Clostridium autoethanogenum* powder on growth, feed intake and proximate composition in flesh of largemouth bass.

Group	FM-35	FM-30	FM-25	FM-20	FM-15	FM-10
Growth performance						
IBW/g	110.9 ± 0.1	111.1 ± 0.3	110.7 ± 0.3	110.8 ± 0.5	110.8 ± 0.3	110.5 ± 0.3
FBW/g	240.6 ± 14.5^{a}	241.5 ± 9.1^{a}	243.8 ± 12.1^{a}	235.2 ± 12.8^{ab}	224.0 ± 8.4^{ab}	219.0 ± 8.4^{b}
SGR	1.34 ± 0.06^{ab}	1.31 ± 0.15^{ab}	1.41 ± 0.09^{a}	1.35 ± 0.10^{ab}	1.27 ± 0.06^{ab}	1.16 ± 0.03^{b}
FI	1.72 ± 0.11	1.70 ± 0.04	1.69 ± 0.05	1.66 ± 0.07	1.61 ± 0.04	1.68 ± 0.02
FCR	1.28 ± 0.04^{a}	1.29 ± 0.09^{a}	1.26 ± 0.11^{a}	1.29 ± 0.07^{a}	1.33 ± 0.09^{ab}	1.40 ± 0.09^{b}
Survival /%	94.4 ± 9.6	91.6 ± 8.3	94.4 ± 4.8	97.2 ± 4.8	100.0	94.4 ± 4.8
Flesh proximate composition (wet weight, g/kg)						
Moisture	776.2 ± 3.1^{a}	768.7 ± 4.4^{b}	767.8 ± 4.4^{b}	766.3 ± 3.2^{b}	770.4 ± 1.9^{ab}	772.6 ± 3.8^{ab}
Crude protein	203.1 ± 2.5	211.2 ± 2.1	207.6 ± 5.9	209.9 ± 4.0	202.8 ± 1.8	208.1 ± 2.0
Crude lipid	10.9 ± 0.3^{b}	12.5 ± 0.7^{a}	12.4 ± 0.4^{a}	10.8 ± 0.2^{b}	11.0 ± 0.2^{b}	11.4 ± 0.4^{b}
Collagen	3.62 ± 0.29^{a}	3.77 ± 0.27^{a}	3.52 ± 0.32^{ab}	3.70 ± 0.10^{a}	3.40 ± 0.07^{ab}	3.12 ± 0.14^{b}

IBW, initial body weight; FBW, final body weight; SGR, Specific growth rate (%/day); FI, Feed intake (%/day); FCR, feed conversion ratio.
The data of FCR and survival referred to Yang et al. (2021).
Values in the same row with different superscripts alphabets indicate significant differences ($p < 0.05$).

3.2. Water-holding capacity and texture characteristics of flesh

In Table 4, the steaming loss and drop loss$^{6\ h}$ in FM-25, FM-20, FM-15 and FM-10 groups and the drop loss$^{4\ h}$ in FM-20 group were significantly lower than those in control group ($P < 0.05$). In FM-25 and FM-20 groups, the flesh stickiness was significantly increased, and the hardness of FM-10 group was decreased ($P < 0.05$). Drop loss$^{2\ h}$, drop loss$^{24\ h}$, thawing loss, centrifugal loss, springiness, gumminess, cohesiveness and chewiness showed no significant differences among all the treatments ($P > 0.05$).

3.3. Free amino acids in flesh

In Table 5, a total of 17 free amino acids were detected in the flesh, and Gly had the highest level, followed by His. The content of delicious amino acids in FM-25 and FM-10 groups was significantly higher than that in the control group ($P < 0.05$), and Glu in FM-25, FM-20 and FM-15 groups and Gly in FM-10 group were significantly increased ($P < 0.05$). In addition, Phe and Lys in FM-25 and FM-20 groups were significantly higher ($P < 0.05$), while the Cys in FM-30, FM-15 and FM-10 groups and His in FM-10 group were significantly lower than those of the control group ($P < 0.05$).

3.4. Sensory evaluation for the flesh samples

As shown in Table 6, flavor, taste and acceptability in FM-25 group, and acceptability in FM-10 group were significantly higher than those of the control group ($P < 0.05$), while the flavor and taste of FM-10 group were just numerically higher than the control group ($P > 0.05$).

Table 4
Effects of *Clostridium autoethanogenum* powder on flesh water-holding capacity and texture characteristics of largemouth bass.

Group	FM-35	FM-30	FM-25	FM-20	FM-15	FM-10
Water-holding capacity (%)						
Centrifugal loss	12.8 ± 2.1	12.1 ± 2.5	13.8 ± 1.9	13.6 ± 1.4	13.0 ± 1.9	13.7 ± 1.7
Steaming loss	14.5 ± 0.4^{a}	14.3 ± 0.3^{a}	13.5 ± 0.4^{b}	13.2 ± 0.3^{b}	13.5 ± 0.5^{b}	13.3 ± 0.1^{b}
Thawing loss	8.99 ± 0.29	9.04 ± 0.43	9.05 ± 0.18	8.96 ± 0.25	9.12 ± 0.19	9.38 ± 0.29
Drop loss$^{2\ h}$	8.20 ± 0.24	7.93 ± 0.15	8.11 ± 0.75	8.09 ± 0.47	8.13 ± 0.48	8.64 ± 0.08
Drop loss$^{4\ h}$	10.59 ± 0.65^{a}	9.29 ± 0.09^{ab}	9.38 ± 0.50^{ab}	8.98 ± 0.63^{b}	9.57 ± 0.51^{ab}	10.0 ± 1.49^{ab}
Drop loss$^{6\ h}$	11.7 ± 0.9^{a}	11.6 ± 0.7^{a}	10.5 ± 0.2^{b}	10.5 ± 0.4^{b}	10.7 ± 0.2^{b}	10.6 ± 0.7^{b}
Drop loss$^{24\ h}$	33.8 ± 3.8	36.1 ± 4.0	35.5 ± 2.3	35.2 ± 1.8	36.5 ± 2.9	36.0 ± 2.9
Texture parameters						
Springiness	0.55 ± 0.03	0.56 ± 0.01	0.55 ± 0.02	0.52 ± 0.03	0.53 ± 0.01	0.52 ± 0.02
Stickiness	0.88 ± 0.10^{b}	1.10 ± 0.11^{ab}	1.10 ± 0.21^{a}	1.06 ± 0.16^{a}	0.87 ± 0.11^{ab}	0.80 ± 0.08^{b}
Resilience	1.25 ± 0.06^{ab}	1.31 ± 0.11^{a}	1.12 ± 0.04^{b}	1.16 ± 0.05^{b}	1.22 ± 0.03^{ab}	1.20 ± 0.06^{ab}
Hardness /gf	552.5 ± 29.7^{a}	536.2 ± 34.1^{ab}	557.2 ± 28.7^{a}	568.6 ± 21.1^{a}	542.6 ± 15.4^{ab}	507.5 ± 10.4^{b}
Chewiness /gf	167.2 ± 12.4	158.6 ± 10.4	165.2 ± 14.1	169.8 ± 12.7	161.2 ± 13.2	170.8 ± 11.1
Gumminess	285.6 ± 1.6	264.3 ± 18.8	246.2 ± 29.1	245.8 ± 33.1	279.3 ± 65.4	284.0 ± 29.7
Cohesiveness /gf	0.62 ± 0.05	0.63 ± 0.03	0.57 ± 0.05	0.61 ± 0.04	0.58 ± 0.02	0.66 ± 0.01

Values in the same row with different superscripts alphabets indicate significant differences ($p < 0.05$).

Table 5
Effects of *Clostridium autoethanogenum* powder on flesh free amino acid composition of largemouth bass (fresh tissue, mg/kg).

Group	FM-35	FM-30	FM-25	FM-20	FM-15	FM-10
Asp#	0.24 ± 0.07	0.41 ± 0.06	0.34 ± 0.14	0.30 ± 0.06	0.27 ± 0.08	0.31 ± 0.06
Ala#	4.08 ± 0.11	4.24 ± 0.45	4.37 ± 0.27	4.20 ± 0.36	4.36 ± 0.29	4.49 ± 0.63
Glu#	0.37 ± 0.01^{b}	0.51 ± 0.06^{ab}	0.57 ± 0.07^{a}	0.63 ± 0.01^{a}	0.55 ± 0.11^{a}	0.49 ± 0.06^{ab}
Gly#	40.4 ± 1.5^{b}	42.5 ± 0.7^{b}	43.0 + ± 1.1^{b}	42.7 ± 1.5^{b}	43.0 ± 1.2^{b}	45.8 ± 1.0^{a}
Ser	1.93 ± 0.06	2.06 ± 0.05	1.93 ± 0.06	2.05 ± 0.07	1.89 ± 0.04	2.03 ± 0.08
Pro	2.42 ± 0.26	2.15 ± 0.40	2.19 ± 0.33	2.15 ± 0.08	2.05 ± 0.11	2.47 ± 0.26
Cys	1.61 ± 0.08^{a}	1.19 ± 0.02^{b}	1.64 ± 0.12^{a}	1.73 ± 0.12^{a}	1.21 ± 0.14^{b}	1.18 ± 0.36^{b}
Ile	0.88 ± 0.07	0.79 ± 0.06	0.72 ± 0.01	0.79 ± 0.01	0.83 ± 0.02	0.75 ± 0.01
His	27.7 ± 0.7^{ab}	29.1 ± 0.9^{a}	26.9 ± 1.6^{ab}	25.6 ± 1.7^{bc}	25.5 ± 0.9^{bc}	23.7 ± 0.2^{c}
Tyr	1.57 ± 0.12	1.29 ± 0.17	1.45 ± 0.15	1.47 ± 0.21	1.40 ± 0.07	1.69 ± 0.18
Phe	0.88 ± 0.07^{b}	0.98 ± 0.32^{ab}	1.13 ± 0.27^{a}	1.24 ± 0.11^{a}	1.08 ± 0.33^{ab}	0.99 ± 0.01^{ab}
Lys	2.27 ± 0.21^{b}	2.61 ± 0.14^{ab}	2.92 ± 0.15^{a}	3.12 ± 0.26^{a}	2.63 ± 0.11^{ab}	2.76 ± 0.08^{ab}
Thr	4.04 ± 0.12	4.00 ± 0.09	4.71 ± 0.11	4.64 ± 0.46	4.27 ± 0.06	4.71 ± 0.45
Val	1.34 ± 0.71	0.98 ± 0.2	1.12 ± 0.26	1.00 ± 0.12	1.07 ± 0.12	1.08 ± 0.13
Met	0.46 ± 0.01	0.51 ± 0.01	0.55 ± 0.10	0.57 ± 0.06	0.58 ± 0.07	0.47 ± 0.03
Leu	1.20 ± 0.26	1.21 ± 0.13	1.41 ± 0.12	1.25 ± 0.03	1.31 ± 0.11	1.35 ± 0.01
Arg	0.88 ± 0.07	0.70 ± 0.11	0.68 ± 0.07	0.65 ± 0.14	0.68 ± 0.11	0.67 ± 0.06
DAAs	45.2 ± 1.5^{b}	47.7 ± 1.4^{b}	48.4 ± 0.9^{a}	47.3 ± 0.5^{b}	48.2 ± 1.4^{ab}	51.1 ± 1.6^{a}
TFAAs	91.5 ± 0.5	94.5 ± 2.9	95.3 ± 1.9	92.8 ± 0.3	92.6 ± 1.6	95.5 ± 3.6

Values in the same row with different superscripts alphabets indicate significant differences ($p < 0.05$).
DAAs,delicious amino acids (#); TFAAs, total free amino acids.

Table 6
Evaluation of *Clostridium autoethanogenum* powder on the sensory senses of largemouth bass.

Group	FM-35	FM-25	FM-10
Flavor	2.79 ± 0.78^{b}	3.79 ± 0.65^{a}	3.04 ± 0.68^{b}
Taste	2.70 ± 0.82^{b}	3.67 ± 0.52^{a}	3.33 ± 0.49^{ab}
Tenderness	3.25 ± 0.41	3.66 ± 0.51	3.50 ± 0.54
Overall acceptability	8.75 ± 0.88^{c}	11.00 ± 0.63^{a}	10.00 ± 0.63^{b}

Values in the same row with different superscripts alphabets indicate significant differences ($p < 0.05$).

3.5. Analysis of flesh electronic tongue

The main components analysis of the flesh samples in the control and FM-10 groups were shown in Fig. 1. The total PCA contribution of electronic tongue is higher than 85%, which indicates that the experimental method is feasible (Liu et al., 2012). The contribution of the first and the second principal components was 87.92% and 10.93%, respectively. Therefore, the principal components 1 and 2 were used as the principal components in the flesh of largemouth bass. The scatter plot of the same sample gathered together to a group, and each group separated independently. The farther the interval, the more obvious the taste difference is. Radar images showed that the response values of CTS (salty), ANS (acid) and UMS (fresh) were higher in FM-10 group than those in the control group.

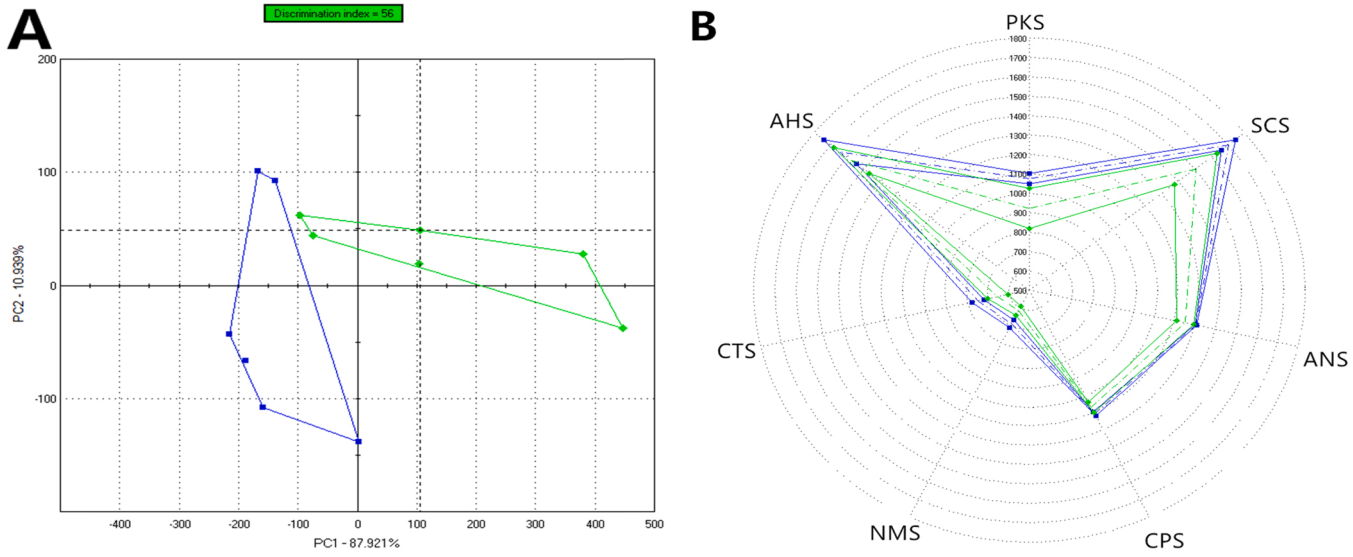

Fig. 1. PCA (A) and radar chart analysis (B) of flesh electronic tongue in the control and FM-10 groups.

3.6. Metabolomics analysis of the control, FM-25 and FM-10 groups using UPLC-MS

The PCA and PLS-DA models of flesh metabolites analyzed by spectrum analysis in the control, FM-25 and FM-10 groups were shown in Fig. 2. It was observed that the unsupervised PCA (Fig. 2, **A**) model could not clearly distinguish the metabolites due to the limits of PCA model (Zhang et al., 2019). Unlike the PCA model, PLS-DA is a supervised model that could reduce system noise and extracts variable information. Therefore, the PLS-DA model has stronger classification ability than the PCA model (Zhao et al., 2019). As shown in Fig. 2 **(**B), the PLS-DA scores plot of the three groups shows strong clustering for flesh metabolism products without any overlap. In the permutation test, the values of R^2 and Q^2 were 0.898 and − 0.1084, respectively, indicating a good repeatability and predictability of the model (Mahadevan et al., 2008).

A total of 916 metabolites were identified by metabolomics analysis, and 757 metabolites were labeled. Compared to the control group, a total of 15 and 46 up-regulated metabolites, 14 and 50 down-regulated metabolites were identified in FM-25 (Fig. 3, **A**) and FM-10 groups (Fig. 3, **B**), respectively. According to a projection (VIP) value (> 1) and *P*-value (< 0.05), some significantly differential metabolites were identified between FM-25 and the control groups, and between FM-10 and the control groups (Table 7), then the associated metabolic pathways were determined by KEGG analysis. There were 8 and 24 KEGG pathways significantly enriched in FM-25 and FM-10 groups ($P < 0.05$), respectively. The major pathways in FM-25 group were involved in lipid metabolism, amino acid metabolism and carbohydrate metabolism. The metabolic pathways in FM-10 group were mainly involved in amino acid metabolism, lipid metabolism, nucleotide metabolism, glycerophospholipid metabolism, linoleic acid metabolism, mineral absorption, purine metabolism and choline metabolism. The KEGG topology analysis of metabolic pathways was shown in Fig. 4 ($P < 0.05$).

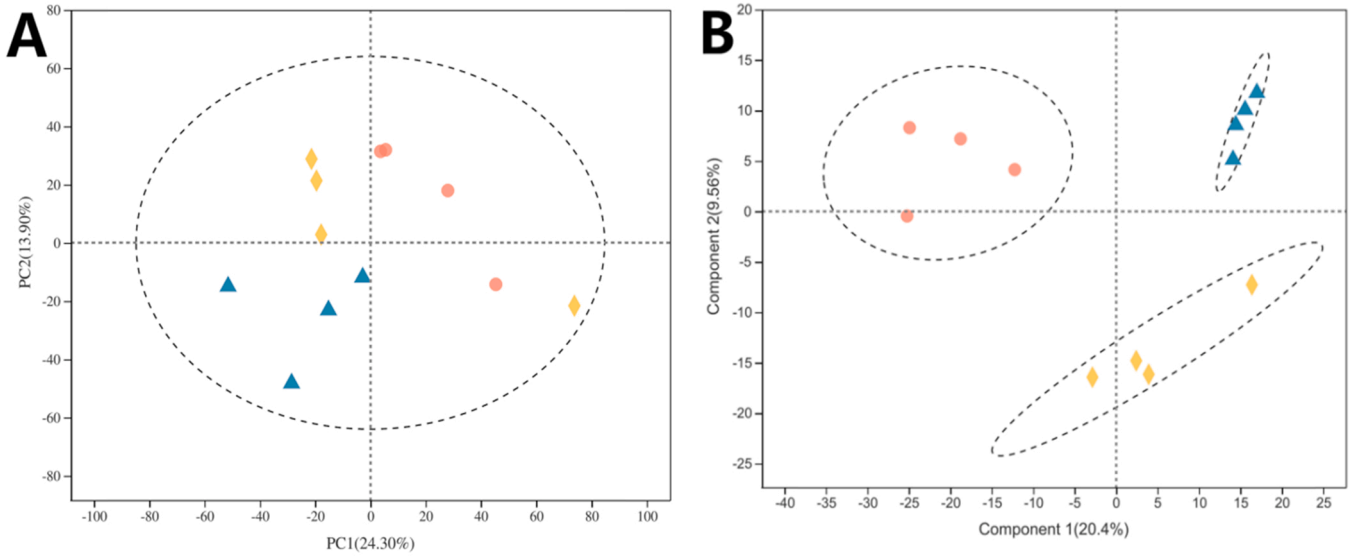

Fig. 2. PCA and PLS-DA model analysis principal of metabolic histology in control, FM-25 and FM-10 groups.

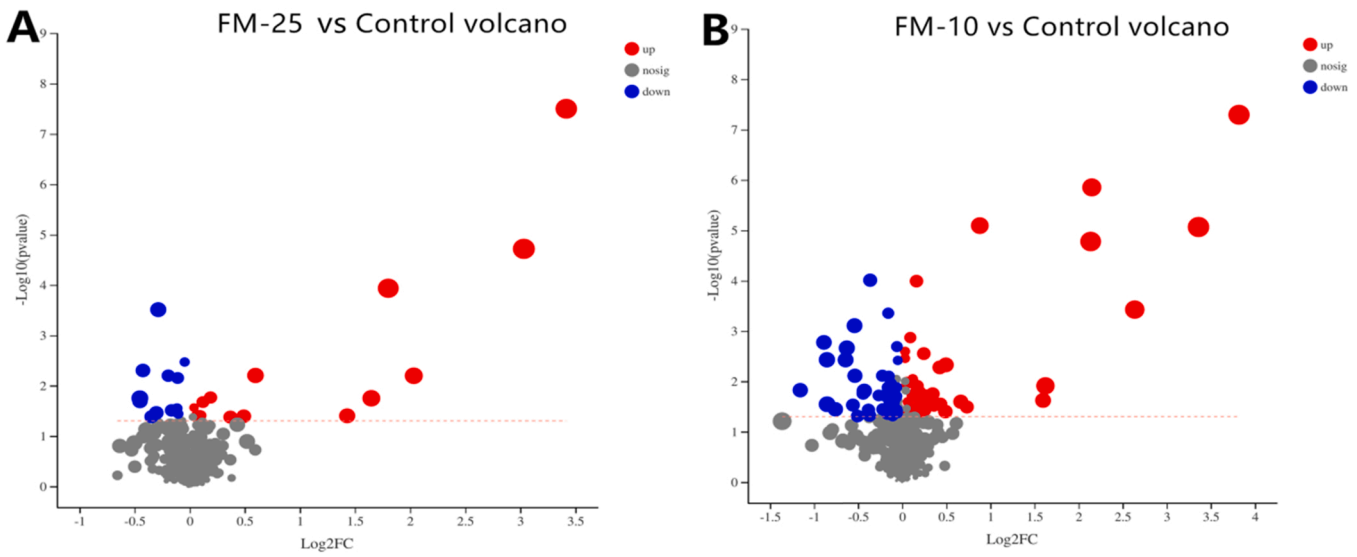

Fig. 3. Volcano plot of differently expressed metabolites between control and FM-25 (A) and FM-10 (B) groups in muscle.

Table 7
Main metabolites and pathways associated with FM-25, FM-10 and the control groups.

Group	Metabolite	VIP	*P*-value	M/Z	Trend	KEGG pathway description
FM-25	Sphingosine	1.62	0.0428	300	pos	Sphingolipid metabolism
	5b-Cyprinol sulfate	2.88	0.0203	550	pos	Primary bile acid biosynthesis
	N-Acetyl-L-phenylalanineq2	3.89	0.0178	208	pos	Phenylalanine metabolism
	Acetone	1.70	0.0388	117	pos	Propanoate metabolism
	17a,21-Dihydroxy-5b-pregnane-3,11,20-trione	2.16	0.0310	407	neg	Steroid hormone biosynthesis
FM-10	Inosine	1.09	0.0026	269	pos	Purine metabolism
	L-Proline	1.46	0.0172	116	pos	Mineral absorption;ABC transporters;Arginine and proline etabolism;Biosynthesis of amino acids;Protein digestion and absorption
	Hypoxanthine	1.06	0.0035	137	pos	Purine metabolism
	PC(15:0/18:1(11Z))	2.31	0.0053	785	pos	Glycerophospholipid metabolism;Arachidonic acid metabolism;Linoleic acid metabolism; alpha-Linolenic acid metabolism
	Phosphocholine	1.45	0.0408	184	pos	Glycerophospholipid metabolism; Choline metabolism in cancer
	N-Acetyl-L-phenylalanine	2.70	0.0230	208	pos	Phenylalanine metabolism
	L-Methionine	1.67	0.0181	150	pos	Cysteine and methionine metabolism;Biosynthesis of amino acids;Protein digestion and absorption
	17a,21-Dihydroxy-5b-pregnane-3,11,20-trione	1.63	0.0132	407	neg	Steroid hormone biosynthesis
	PE(O-16:1(1Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2.15	0.0285	793	neg	Glycerophospholipid metabolism
	LysoPC(18:0)	1.21	0.0387	568	neg	Glycerophospholipid metabolism;Choline metabolism in cancer
	Galabiosylceramide (d18:1/16:0)	2.66	0.0008	899	neg	Sphingolipid metabolism
	13S-hydroxyoctadecadienoic acid	1.93	0.0370	341	neg	Linoleic acid metabolism; PPAR signaling pathway
	Inosinic acid	1.76	0.0360	385	neg	Antifolate resistance;Taste transduction;Purine metabolism

4. Discussion

4.1. Effect of Clostridium autoethanogenum powder on the growth of largemouth bass

In the previous study (Yang et al., 2021), CAP successfully substituted 200 g/kg fish meal in the diet of largemouth bass without significantly adverse effects on growth performance, but higher inclusion resulted in the reduction of growth performance in FM-10 group. In general, the decreased growth performance resulted from the reduced feed palatability and (or) nutritional imbalance. However, the feed intake was not affected by the fish meal replacement with CAP in the current study, thus, the decrease of nutrition quality is the main reason. Although His, Trp and Arg were supplemented in CAP diets, the contents of bioactive factors such as small peptides and taurine in bacterial protein powder were much lower than those in fish meal, which may decreased the growth performance when high proportion of fish meal was replaced with CAP. The detailed information has been discussed in the previous study by Yang et al. (2021).

4.2. Effects of Clostridium autoethanogenum powder on the flesh physical index

The physical index of the flesh include pH, WHC and texture characteristics, such as hardness, springiness and chewiness (Johnston et al., 2006). WHC refers to the ability of flesh to retain moisture when subjected to external forces (pressurization, shredding, heating, freezing, etc.), and it is an important index reflecting the quality of flesh (Otto et al., 2006). Usually, the loss of water in flesh is accompanied with the loss of water-soluble proteins and soluble flavor substances, thus

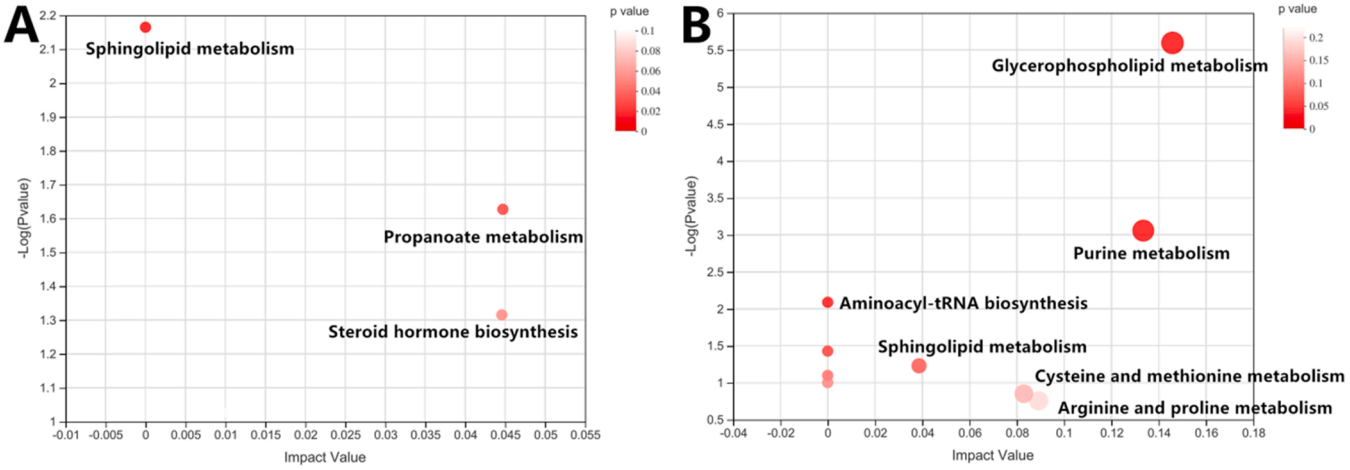

Fig. 4. Bubble diagram of differently expressed metabolites between control and FM-25 (A) and FM-10 (B) groups in muscle.

affecting the flesh quality (Luciano et al., 2009). The present study showed that the steaming loss and drop loss$^{6\,h}$ were significantly decreased when the replacement of fish meal with CAP was higher than 100 g/kg, which may be related to the differences in protein metabolism. Many factors, such as glycolysis, pH variation and oxidation, all affect the WHC of flesh (Zhang et al., 2020).

Flesh stickiness reflects the affinity between muscle cells and hardness, the most direct indicator of taste, affects structural parameters such as chewiness and gumminess in texture analysis (Cheng et al., 2013). Studies have shown that flesh hardness is positively related to the content of collagen, namely, the higher content of collagen often indicate the greater hardness (Johnston et al., 2006). Li et al. (2010) reported a positive correlation between the flesh texture property and the collagen content of *Siniperca chuatsi* (Basilewsky). The study of Periago et al. (2005) indicated that the hardness, masticatory force and elasticity of flesh were positively related to the collagen content of sea bass (*Dicentrarchus labrax*). As an important component in muscle, collagen can form a dense membrane surrounding the muscle fiber bundle and maintain the muscle structure, flexibility and texture. In the present study, the flesh collagen content in FM-10 group was significantly decreased, which may be an important reason to explain the decrease of muscle hardness in this group. In Pacific white shrimp, when CAP substituted 252 g/kg fish meal (45% of dietary fish meal), the flesh hardness, chewiness and collagen content were also significantly decreased (Yao et al., 2022). Compared with fish meal, plant protein and bacterial protein are deficient in Hyp, which might affect the collagen synthesis in muscle when fish meal was replaced by a high proportion of CAP. In the future, Hyp and other functional factors that can promote the formation of collagen will be considered in the low fish meal diet to improve the flesh quality.

4.3. *Effects of Clostridium autoethanogenum powder on flesh flavor of largemouth bass*

Some FAAs are related to the characteristic flavor of fish, such as Asp, Glu, Ala and Gly (Ruiz-Capillas and Moral, 2001). In the present study, the DAAs in FM-25 and FM-10 groups were significantly higher than those in the control group, especially for Glu in FM-25 group and Gly in FM-10 group, which was consistent with the results of tasting.

In sensory evaluation, FM-25 group showed stronger flesh sweetness and freshness, less fishy taste, and more elastic texture. The flesh acceptability of FM-25 and FM-10 groups was also higher than that of the control group. Meanwhile, the electronic tongue measurement of FM-10 group showed that the response values of tastes were higher than that of the control group. The increase in overall sensory acceptability may be related to the changes of metabolic pathways In the amino acid metabolites of FM-10 group, Met, Phe, Pro, Arg and Ile were up-regulated, while Leu was down-regulated. Changes in FM-10 protein metabolic pathway indicated that the high replacement of fish meal with CAP might increase energy consumption in flesh tissue, which probably arise from the enhanced protein synthesis and lipid metabolism (Wang et al., 2016; Kim et al., 2016).

4.4. *Effects of Clostridium autoethanogenum powder on flesh metabolism of largemouth bass*

In the present study, the differential metabolites in FM-25 and FM-10 groups were found to be mainly involved in lipid metabolism, amino acid metabolism and protein metabolism, and glycerol phospholipid metabolism, arachidonic acid metabolism and linoleic acid were upregulated in FM-10 groups. Linoleic acid, arachidonic acid and α-linolenic acidis are the main components of polyunsaturated fatty acids. After a series of chemical reactions, some flavor substances will be produced in the flesh, and polyunsaturated fatty acids are the precursors of these substances (Tian et al., 2013). The study of Jiang et al. (2010) indicated that linoleic acid metabolism regulates lipid deposition and increases intramuscular fat content by affecting the proliferation and differentiation of adipocytes as well as the key genes regulating lipid metabolism. Arachidonic acid can produce aldehydes under the action of lipoxidase, emitting a clear fragrance. The increase in glycerol metabolism suggests that the replacement of fish meal with CAP may accelerate glycolysis and promote lipid synthesis in flesh, which may affect the metabolism in fish and indirectly affect the taste and composition of flesh. The acceptabilities of sensory evaluation of FM-25 and FM-10 groups were higher than that of the control group. Although fat is not a flavor substance, it can increase the flesh tenderness and the flavor richness, and the increase of intramuscular fat in a certain range can improve the flesh flavor (Guo et al., 2011).

Compared with the control group, inosine and hypoxanthine metabolites were up-regulated and the purine metabolic pathway was significantly enriched in FM-10 group. Purine can be catabolized to purine bases by enzyme action (Xanthine, hypoxanthine, guanine and adenine, etc.). Hypoxanthine can enter cells through the cell membrane, improve the activities of a variety of enzymes, and participate in the regulation of energy metabolism of organisms. As an important flavor substance, hypoxanthine can enhance the overall flavor of the flesh, reduce the bitterness and improve the quality of flesh (Jones et al., 2010). In the future, a variety of flavor substances need to be detected to provide direct evidence.

5. Conclusion

In a diet containing 350 g/kg fish meal, CAP could successfully replace 150 g/kg fish meal without negative effects on growth and flesh quality of largemouth bass. Higher fish meal replacement (250 g/kg fish meal) with CAP reduced the flesh collagen content and flesh hardness, although the free amino acids content in flesh was increased.

CRediT authorship contribution statement

Pinxian Yang: Investigation, Data curation, Project administration, Validation, Writing – original draft. **Wenxiang Yao**: Investigation, Data curation, Project administration, Validation, Writing – original draft. **Yuanyuan Wang**: Project administration, Conceptualization, Funding acquisition. **Menglu Li**: Conceptualization, Funding acquisition, Supervision. **Xiaoqin Li**: Funding acquisition, Investigation, Writing – review & editing. **Xiangjun Leng**: Funding acquisition, Investigation, 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

All data generated or analyzed during this study are included in this article.

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