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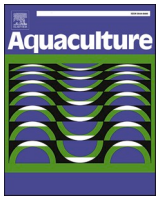

Administration of *Cetobacterium somerae ceto* isolated from the intestine of yellow catfish (*Pelteobagrus fulvidraco*) as potential probiotics against chronic ammonia stress

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ABSTRACT

The objective of this investigation was to examine the viability of utilizing host-associated *Cetobacterium somerae ceto* (*C. somerae ceto*) as a probiotic agent to ameliorate growth impediments induced by persistent ammonia toxicity and enhance ammonia detoxification. The experiment was divided into 4 groups, administered 0 and 1 $\times$ 10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total ammonia nitrogen (T-AN) named CON and CS, respectively. Administration of 0 and 1 $\times$ 10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM and AMCS, respectively. Fish with an average weight of 3.33 $\pm$ 0.01 g were cultivated in a set of 12 buckets featuring three replicates per set. The experiment was conducted over a period of 6 weeks. At the end of the trial, an examination was conducted to assess the probiotic impacts of *C. somerae ceto* on yellow catfish, encompassing evaluations of growth performance, serum antioxidant parameters, intestinal microbiota profile, liver metabolites, and the expression of genes associated with ammonia metabolism in the liver. The study findings demonstrated a notable reduction in the weight gain rate (WGR) and specific growth rate (SGR) of yellow catfish under chronic ammonia stress (CAS) ($P < 0.05$). Moreover, the growth impediment induced by CAS was effectively mitigated by the administration of *C. somerae ceto* under CAS. Additionally, CAS was observed to markedly elevate the levels of total superoxide dismutase (T-SOD), catalase (CAT), and total antioxidant capacity (T-AOC) in the serum ($P < 0.05$). However, these parameters exhibited a significant decline following the administration of *C. somerae ceto* during CAS ($P < 0.05$), suggesting a substantial reduction in the oxidative stress instigated by CAS. The examination of intestinal microbiota revealed a notable rise in the relative abundance and copies of *C. somerae ceto* in the CS and AMCS groups in comparison to the CON and AM ($P < 0.05$). This discovery indicates that the successful colonization of *C. somerae ceto* in the intestine of yellow catfish can be achieved through the exogenous administration of the *C. somerae ceto*. Furthermore, the heightened intestinal colonization of *C. somerae ceto* led to a significant reduction in serum and liver ammonia levels, along with an increase in urea content ($P < 0.05$). This observation suggests that the increases of *C. somerae ceto* in the intestine may play a role in facilitating the conversion of ammonia to urea for detoxification. Meanwhile, liver metabolomic analysis showed that CAS significantly reduced the contents of argininosuccinic acid, ornithine, and urea ($P < 0.05$). Compared with CON and AM, the argininosuccinic acid, ornithine, citrulline, and urea contents in CS and AMCS were significantly increased. These data imply that the heightened presence of *C. somerae ceto* within the intestine may supply the essential intermediary metabolites for hepatic ureagenesis, consequently facilitating the process of ammonia detoxification under CAS.

1. Introduction

Ammonia is predominantly generated in aquaculture systems through the breakdown of feed, excreta, and nitrogen-rich organic substances. In aquatic environments, ammonia exists in two forms: NH_4^+ and NH_3. Together, these forms make up total ammonia nitrogen (T-AN) (Hargreaves, 1998; Parvathy et al., 2023). The NH_3 possesses lipophilic properties, thereby enabling facile permeation through the gill epithelial

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cell membrane and subsequent entry into the bloodstream, and rapid dissemination throughout various tissues. Ammonia toxicity occurs when its concentration exceeds aquatic organisms' metabolic threshold, resulting in issues like developmental retardation, decreased mobility, and potential mass mortality events (Ip and Chew, 2010; Ip et al., 2004; Randall and Tsui, 2002). Recent research has indicated that the growth performance of yellow catfish (Zhang et al., 2016), Japanese sea perch (Zhang et al., 2022), and blunt snout bream (Zhang et al., 2019), were significantly inhibited and the antioxidant system, liver tissue structure, and immune system are severely damaged under chronic ammonia stress (CAS). Losses and diseases resulting from ammonia poisoning are prevalent within the aquaculture sector. This is primarily due to the widespread utilization of high-protein feed and high-density farming modes, leading to elevated levels of ammonia in aquaculture water. For instance, in the intensive culture mode of shrimp, the maximum T-AN can reach 46 mg/L T-AN (Zhao et al., 2020). Furthermore, the escalating temperatures of aquaculture water due to global warming have intensified the toxicity of ammonia to aquatic organisms (Duan et al., 2021). Consequently, enhancing the yellow catfish's resilience to CAS has emerged as a crucial focus in yellow catfish farming and the broader aquaculture industry.

Probiotics can enhance the resilience of fish when faced with acute and chronic ammonia-induced stressors. For instance, incorporated probiotics (*Pediococcus acidilactici*) into water has been shown to notably diminish the levels of T-AN and NH_3 present in the water (Hendam et al., 2023). Supplementing the *Bacillus subtilis DSM 32315* in diet has been shown to improve the resilience to acute ammonia stress in Nile tilapia (*Oreochromis niloticus*) (Liao et al., 2023b). The supplementation of diets with a blend of probiotics such as *Bacillus amyloliquefaciens 54 A* and *Bacillus pumilus 47B* has been shown to notably enhance the tolerance of striped catfish (*Pangasianodon hypophthalmus*) to acute ammonia stress (Thy et al., 2017). Furthermore, in cases of CAS, the inclusion of *Bacillus licheniformis Dahb1* in the diet has been demonstrated to mitigate ammonia toxicity by bolstering antioxidant capacity, acetylcholinesterase activity, and non-specific immune response, resulting in a noteworthy improvement in the survival rate of *Oreochromis mossambicus* (Gopi et al., 2022). The aforementioned studies laid a robust foundation for the current investigation; however, they did not explicitly clarify the underlying mechanism responsible for the ammonia-reducing properties of probiotics.

Cetobacterium somerae (*C. somerae*) is a probiotic commonly found in the intestine of freshwater fish (Larsen et al., 2014). Research indicates that it possesses the ability to enhance fish resistance against pathogens. Notably, the supplementation of *C. somerae* or its fermentation has been shown to bolster the immunity of crucian carp (*Carassius auratus*) (Zhao et al., 2024a), largemouth bass (*Micropterus salmoides*) (Zhang et al., 2023), and tilapia (GIFT, *Oreochromis niloticus*) (Zhou et al., 2022). Mechanistically, *C. somerae* can synthesize VB12 and acetate in the intestine of zebrafish. VB12 plays a role in reinforcing the intestinal microbiota interactions and intestinal barrier integrity to combat pathogenic invasions (Qi et al., 2023). Acetate can enhance immunity by elevating antimicrobial peptide (AMP) and IL-22 levels (Liao et al., 2023a). *C. somerae* has the ability to regulate nutrient metabolism and counteract the negative impacts of a high-fat diet on zebrafish's metabolism, immunity, and intestinal microbiota (Li et al., 2023a). Furthermore, acetate produced by *C. somerae* can enhance the glucose utilization capacity by stimulating the parasympathetic nerves of zebrafish (Wang et al., 2021a). The fermentation byproducts of *C. somerae* have been found to alleviate liver lipid accumulation induced by plant protein in carp (Xie et al., 2021). Studies have also shown that *C. somerae* can alleviate environmental stress, such as reducing trichlorfon-induced liver damage (Zhao et al., 2024b). Nonetheless, there is a current lack of research on the detoxification of ammonia-poisoned fish by using probiotics.

Yellow catfish (*Pelteobagrus fulvidraco*) serves as a vital species in freshwater aquaculture within China. This species exhibits a notable sensitivity to ammonia, yet possesses high ammonia tolerance, making it an ideal candidate for research on complex ammonia stress (CAS). A previous investigation revealed a notable rise in the abundance of intestinal *C. somerae* in response to acute ammonia stress (Wang et al., 2021b), suggesting a potential link to the formation of ammonia tolerance. Consequently, the *Cetobacterium somerae ceto* (*C. somerae ceto*) strain has been isolated from the intestine of yellow catfish for a comprehensive evaluation of its impact on growth, morphology of the intestines and liver, liver ureagenesis-related genes and metabolites, microbiota composition, and its role in promoting ammonia detoxification. The research aims to create a strong theoretical basis for the future use and commercialization of promising probiotic.

2. Materials and methods

2.1. Ethics statement of experimental fish

Animal Experiment Ethics Committee of Ningbo University authorized the fish management and sampling program SYXK (2012−011012).

2.2. Isolation, purification and preservation of Cetobacterium somerae ceto

The *C.somerae ceto* strain was isolated from the intestine of yellow catfish and stored at the Guangdong Microbial Culture Collection Center (GDMCC, NO: 64562). The bacterial freeze-dried powder was dissolved in 600 μL sterile PBS and then coated on four Columbia Blood Plates (Qingdao Hi-Tech Industrial Park Hope Bio-Technology Co., Ltd., HBPM0124–1) for activation. After the bacterial lawn grows on the plate, it is scraped off and its purity is assessed through 16 s rRNA sequencing. Pure bacteria are then transferred to Columbia Blood Plate and cultured in a sterile incubator without oxygen at 37 °C for 24 h to expand culture. The obtained bacteria are moved to sterile PBS, and the bacteria community of live *C.somerae ceto* strain is determined through plate colony counting after shaking, mixing, and dilution with sterile PBS to achieve a fresh bacteria solution with a concentration of 1×10^8 CFU/mL. This solution is then added to the basal commercial diet. The wet mixture was dried at room temperature in a vacuum drying oven, stored at 4 °C, and used within 7 days.

2.3. Experimental design and treatment

Juvenile yellow catfish (3–5 cm in body length) were purchased from a breeding base in Zhejiang Province and examined by veterinarians. Yellow catfish with scratches on the body surface, bleeding in the gills, white spots on the top of the head, pale body colour, and small body size should be avoided due to the associated risks of water mildew, parasites, *Edwardsiella* infection, and hepatobiliary syndrome.

Domestication was performed by feeding commercial feed before the experiment. A total of 360 yellow catfish with an average initial weight of 3.03 ± 0.02 g was evenly placed in 12 buckets containing 30 fish each. The 12 buckets in this experiment were divided into four groups and fed PBS solution containing 0 and 1×10^8 CFU/mL CFU/mL *C. somerae ceto* in water containing 0 mg/L T-AN, which were named CON group and CS group. PBS solutions containing 0 and 1×10^8 CFU/mL *C. somerae ceto* were fed in water containing 25 mg/L T-AN and were designated as AM and AMCS groups, respectively. The experiment lasted for six weeks. They are fed commercial feed twice daily at 08:00 and 19:00, with an amount equivalent to 4 % of the catfish's body weight. Any uneaten feed is gathered, dried, and weighed two hours after feeding. This can reduce the pollution of feed residues, improve water quality, and obtain a more accurate feed efficiency. During the experiment, the YSI ProPlus multiparameter water quality instrument (YSI, USA) is used to continuously measure water quality to determine water temperature, dissolved oxygen, and pH value. The culture conditions

include a temperature of 28 ± 1 °C, dissolved oxygen level at approximately 7 mg/L, and pH value between 7.1 and 7.3. The experiment is conducted with a 12-h light-dark cycle under natural lighting. Chronic ammonia stress (CAS) is induced at a level of 10 % of the 96-h half-lethal concentration in yellow catfish. T-AN concentration in CON and CS groups is kept at 0.1–0.2 mg/L, the AM and AMCS groups maintain a T-AN level of 25 mg/L. YSI is employed to analyze the T-AN level in water. When the T-AN level surpasses 0.2 mg/L, two-thirds of the water in each bucket is replaced, and YSI is utilized for the real-time detection until the T-AN levels decrease below the threshold. When the T-AN level in water is lower than 25 mg/L, the 10 g/L NH_4Cl stock solution is supplemented and YSI is utilized for real-time detection until the T-AN level in water reaches 25 mg/L.

2.4. Growth performance measuring and sampling

At the trial's conclusion, the fish in each tank were weighed and tallied. Metrics such as final weight (FBW), weight gain (WG), weight gain rate (WGR), feed conversion ratio (FCR), feed intake (FI), and specific growth rate (SGR) were determined according to Zhang et al. (2025). The 5 fish (15 in total per group) were randomly chosen from each bucket and anesthetized with 100 mg/L eugenol (Li et al., 2023b), and blood samples were extracted by puncture of caudal blood vessels with a syringe moistened with heparin sodium. Plasma samples were centrifuged for 10 min at 3500 rpm and 4 °C and then stored at −20 °C for the determination of antioxidant indices, ammonia, and urea. Liver and intestinal samples were then collected and immediately frozen in liquid nitrogen and stored at −80 °C for further determination of liver ammonia, liver urea content, expression of ammonia metabolism-related genes, 16 s rRNA sequencing, and liver metabolomics analysis.

2.5. Determination of crude composition of diets and whole fish

The experimental diets and whole fish samples underwent analysis according to the guidelines outlined by the Association of Official Analytical Chemists (AOAC, 2005). Diets and whole fish samples of four groups were dried and detected moisture (105 °C drying methods, GB/T6435–2014), crude protein content (Kjeldahl nitrogen-determination method; GB/T6432–2018), crude lipid content (chloroform-methanol extraction method; GB/T 6433–2006) and crude ash content (Muffle furnace ashing method; GB/T6438–2007).

2.6. Enzyme activity assays

Liver samples from three yellow catfish per bucket were homogenized for analysis. Total superoxide dismutase (T-SOD, A001–1-1), total antioxidant capacity (T-AOC, A015–2-1), catalase (CAT, A007–1-1), serum and liver ammonia content (A086–1-1), and urea content (C0131–1) were measured by kits of Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Absorbance was determined using an automatic biochemical analyzer (BX-3010, Sysmex Corporation, Tokyo, Japan).

2.7. Gene quantification in the liver

Total RNA was extracted from primary hepatocytes using TRIzol reagent following the manufacturer's instructions. The RNA samples were assessed for purity and concentration with a Nano-Drop ND-1000 Spectrophotometer. RNA integrity was confirmed by agarose gel electrophoresis. RT-qPCR analysis was conducted in triplicate using the TB Green RT-qPCR kit on a Roche LightCycler 96 system. Data from cDNA samples were normalized to β-actin and GAPDH mRNA expression utilizing the comparative Ct method and expressed as fold change ($2^{-\triangle\triangle Ct}$). The RT-qPCR protocol involved 40 cycles with specific temperature conditions. Primer sequences are available in Table 1.

2.8. 16S rRNA sequencing and analysis

The isolation, purification, and verification of total intestinal DNA, synthesis of V3-V4 region primers, and raw data processing were conducted by Biozeron Biotechnology Co., Ltd. in Shanghai, China. High-throughput sequencing was done on the MiSeqPE300 platform by the same company. Bioinformatics analysis was performed using the online tool MicrobiomeAnalyst at https://www.microbiomeanalyst.ca/.

2.9. Metabolomics analysis of liver

Sample pretreatment was performed by BIOZERON Biotechnology Company (Shanghai, China). In short, liver samples stored at −80 °C (100 mg) are thawed at 4 °C. Add 1 mL water and 4 mL acetonitrile methanol reagent (1,1, *V*/V), grind for 30 s, vortex, ultrasonic water bath for 1 min, and then centrifuge. After drying with nitrogen, the supernatant was re-dissolved with methanol-water reagent (4,1, V/V), then the supernatant was taken after centrifugation and then tested by Waters Acquity UPLC chromatography system after organic phase filtration membrane. After peak extraction, peak alignment, baseline correction, metabolite identification, and other data preprocessing for the obtained LC-MS raw data by Progenesis QI, metabolites were annotated using the human metabolome database (HMDB). Bioinformatic analysis of metabolomics data was performed using online software MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/).

Table 1
Primers for q-PCR analysis.

Target gene	Primer sequence (5′–3′)	Size (bp)	GenBank no.	Annealing temperature, °C
Carbamyl phosphate synthetase cps1	F: CCTGAAGACGGTGCTGATGA R: TCTGTGGCGATGATGGACTC	267	XM_047814812.1	59.75
Ornithine carbamoyltransferase otc	F: TTCTACTCACCACGCTCACT R: GTCAGTCAGGATACATACAATCAAC	87	XM_047815226.1	58.38
Argininosuccinate synthetase ass	F: AGACTCACCGAATACACCAGAC R: CTCCGACTTCATTGAGGTAGGT	134	XM_027136752.2	59.51
Argininosuccinic acid lyase asl	F: GGCAGCCGTAGAAATCAACA R: GAATGTTGACCCGCTTCCTG	152	XM_027156981.2	59.20
Arginase arg	F: AAGAAGGTTGCTGATGCTGTT R: CTGTTGGACTGGTTAGAGGTG	181	XM_027167958.2	58.41
Cetobacterium 16S rRNA	F: AAGAGAGAGCCTTGCGTTCC R: GTGTCTCAGTCCCCTTGTGG	108	CP143319	60.04
β-actin	F: TACCCTGGCATTGCAGACAG R: GAGTCGGCGTGAAGTGGTAA	160	XM_027148463.2	60.04
GAPDH	F: TCTGGGGTACACAGAACACC R: ACTAGGTCACAGACACGGTT	165	XM_027149217.1	58.95

Reference genes were *β-actin* and GAPDH = *glyceraldehyde phosphate dehydrogenase*, respectively

2.10. Statistical analysis

The statistics reported are based on a minimum of three biological replicates with results presented as mean ± SEM. Statistical analyses were conducted using GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA) with a student *t*-test used for group comparisons, considering $P < 0.05$ as statistically significant.

3. Results

3.1. Growth performance and survival rate

As shown in Table 2, FBW, WG, WGR, SGR, and FI of yellow catfish in AM were significantly lower than those in CON ($P < 0.05$), but FCR was significantly increased compared with CON ($P < 0.05$). The FCR of CS was significantly lower than that of CON ($P < 0.05$), but there were no significant differences in other growth parameters between CS and CON ($P > 0.05$). In addition, compared with AM, FBW, WG, WGR, SGR, and FI in AMCS were significantly increased compared with AM ($P < 0.05$), but FCR was significantly lower than that in AM ($P < 0.05$).

3.2. Whole body composition

As shown in Table 3, compared with CON, crude protein and crude lipid contents of whole fish in AM were significantly lower than those in CON ($P < 0.05$). Compared with CON, the crude protein content of CS was significantly increased compared with CON ($P < 0.05$), but there was no significant difference in crude lipid content compared with CON ($P > 0.05$). Compared with AM, crude protein and crude lipid contents in AMCS were significantly increased ($P < 0.05$).

3.3. Antioxidant enzyme activity

As shown in Table 4, compared with CON, serum T-AOC, serum T-SOD activity, and serum CAT activity in AM were significantly increased ($P < 0.05$). Compared with CON, serum T-AOC in CS had no significant change ($P > 0.05$), but serum SOD and CAT activities in CS were significantly decreased compared with CON ($P < 0.05$). Compared with AM, serum T-AOC, SOD, and CAT activities in AMCS were significantly decreased ($P < 0.05$).

3.4. Intestinal microbiota composition and variation characteristics

As shown in Fig. 1, Principal component analysis (PCA) shows that the three samples of AM are located in the upper left corner of the coordinate system, the three samples of CON are located in the center of the coordinate system, and the six samples of AMCS and AM group are located in the lower right corner of the coordinate system, mixed into clusters. Shannon and Simpson indices in the AM and CS are significantly decrased compared with CON ($P < 0.01$). There were no significant differences in Shannon and Simpson indices between AM and AMCS ($P > 0.05$). As shown in Fig. 1C and D, the Chao1 index and Ace index of AM are significantly lower than those of CON ($P < 0.05$). As shown in Fig. 1E, the absolute abundance (copies) of *C. somerae ceto* in AM and CS were significantly higher than those in CON. The copies of *C. somerae ceto* in AMCS were significantly higher than those in AM ($P < 0.001$).

As shown in Fig. 2A, the intestinal microbiota of yellow catfish mainly consisted of 13 phyla. The figure on the left shows the actual number of fragments of these 13 phyla, and the figure on the right shows the percentage composition of these 13 phyla. The proportion of *Pseudomonadota* in the CON was the highest. *Bacillota* has the highest proportion in AM. *Fusobacteriota* in CS and AMCS is the highest. As shown in Fig. 2B, at the family level, the proportion of *Chitinophagaceae* in CON was the highest. The proportion of *Clostridiaceae* AM was the highest. The proportion of *Fusobacteriaceae* in CS and AMCS is the highest. The *Vibrionimonas* abundance had the highest value in CON group (Fig. 2C). The proportion of *Candidatus_Arthromitus* in AM was the highest. The proportion of Cetobacterium in CS and AM was the highest.

3.5. Comparative analysis of intestinal microbiota

As shown in Fig. 3, the relative abundance of *Cetobacterium* in AM and CS was significantly increased compared with CON ($P < 0.05$). The relative abundance of *Cetobacterium* in AMCS group was increased significantly compared with AM ($P < 0.05$). *Candidatus_Arthromitus* abundance in AM was significantly increased compared with CON ($P < 0.001$), but it decreased in AMCS copmpared with AM ($P < 0.001$). The relative abundance of *Vibrionimonas*, *Mesorhizobium*, *Bradyrhizobiumg*, *Rhodanobacter*, *Methylovirgula*, *Burkholderia*, *Pseudolabrys*, *Variovorax*, *Phyllobacterium*, *Labrys*, *Aquamicrobium*, *Mycobacterium* in AM were decreased compared with CON ($P < 0.05$). *Enterococcus* abundance in AM was significantly decreased compared with CON, but it was significantly increased in CS compared with CON ($P < 0.05$). *Negativibacillus* abundance in AM and CS was significantly decreased compared with CON ($P < 0.05$). The *Negativibacillus* abundance in AMCS was significantly decreased with AM ($P < 0.05$).

As shown in Fig. 4A, linear discriminant analysis effect size (Lefse) analysis shows that *Candidatus_Arthromitus* and *Cetobacterium* in AM have significant differences compared with CON (LDA > 2), and they are significantly enriched in the AM (orange). As shown in Fig. 4B, *Enterococcus* and *Cetobacterium* in CS was significantly increased compared with CON (LDA > 2, orange node). The abundance of *Enterococcus*, *Vibrionimonas*, *Cetobacterium* and *Rhodanobacter* in the AMCS have significant differences (LDA > 2) compared with the AM, and they are significantly enriched in the AMCS (orange) (Fig. 4C).

Table 2
Growth performance of yellow catfish for 6 weeks.

	CON[1]	AM[2]	CS[3]	AMCS[4]	*P*1	*P*2	*P*3
IBW[2]	3.33 ± 0.00	3.33 ± 0.01	3.33 ± 0.00	3.33 ± 0.01	0.853	0.644	0.781
FBW[3]	14.96 ± 0.65	8.28 ± 0.32	15.22 ± 0.58	13.04 ± 0.77	<0.001	0.054	0.004
WG[4]	11.63 ± 0.65	4.94 ± 0.33	11.89 ± 0.57	9.71 ± 0.77	<0.001	0.054	<0.001
WGR[5]	348.87 ± 19.19	148.44 ± 10.01	356.47 ± 16.33	291.59 ± 22.83	<0.001	0.052	<0.001
SGR[6]	5.83 ± 0.14	3.80 ± 0.16	5.89 ± 0.12	5.40 ± 0.20	<0.001	0.079	<0.001
FI[7]	14.30 ± 0.64	8.92 ± 0.38	14.24 ± 0.31	13.92 ± 0.16	<0.001	0.536	<0.001
FCR[8]	1.23 ± 0.02	1.81 ± 0.04	1.20 ± 0.04	1.45 ± 0.11	<0.001	0.043	0.004

IBW = initial mean body weight; FBW = final mean body weight; WG = weight gain; WG = weight gain rate; SGR = specific growth rate; FCR = feed conversion ratio; FI = food intake. Data were presented as mean ± SEM ($n = 3$). "*P1*," "*P2*," and "*P3*" denote the *P*-values from *t*-tests comparing the CON and AM, CON and CS, AM and AMCS groups, respectively.

[1] CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. [2]AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. [3]CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. [4]AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS.

Table 3
Proximate composition yellow catfish.

	CON[1]	AM[2]	CS[3]	AMCS[4]	*P1*	*P2*	*P3*
Protein (% dry matter)	51.68 ± 0.57	44.71 ± 1.49	56.88 ± 0.73	54 0.14 ± 0.93	0.001	0.006	<0.001
Lipid (% dry matter)	22.35 ± 1.98	14.60 ± 0.29	22.14 ± 1.35	22.36 ± 2.25	0.01	0.931	0.01
Ash (% dry matter)	16.09 ± 0.46	17.18 ± 0.05	14.90 ± 0.80	15.71 ± 1.14	0.327	0.283	0.196
Moisture (%)	79.14 ± 0.93	83.15 ± 0.84	80.14 ± 0.83	79.10 ± 1.27	0.184	0.977	0.336

[1] CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. [2]AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. [3]CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. [4]AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. Data were presented as mean ± SEM ($n = 3$). "*P1*," "*P2*," and "*P3*" denote the *P*-values from *t*-tests comparing the CON and AM, CON and CS, AM and AMCS groups, respectively.

Table 4
Serum antioxidant indexes of yellow catfish.

	CON[1]	AM[2]	CS[3]	AMCS[4]	*P1*	*P2*	*P3*
T-AOC (U/mL)	2.75 ± 0.60	12.70 ± 0.19	2.90 ± 0.25	2.10 ± 0.43	<0.001	0.805	<0.001
SOD (U/mL)	54.67 ± 2.48	61.69 ± 0.65	37.60 ± 1.39	52.81 ± 0.48	0.01	<0.001	0.003
CAT (U/mL)	13.97 ± 0.37	16.68 ± 1.19	8.91 ± 1.99	13.67 ± 0.17	0.022	0.009	0.016

[1] CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. [2]AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. [3]CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. [4]AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. Data were presented as mean ± SEM (n = 3). "*P1*," "*P2*," and "*P3*" denote the *P*-values from t-tests comparing the CON and AM, CON and CS, AM and AMCS groups, respectively. T-AOC = total antioxidant capacity; SOD = superoxide dismutase; CAT = catalase.

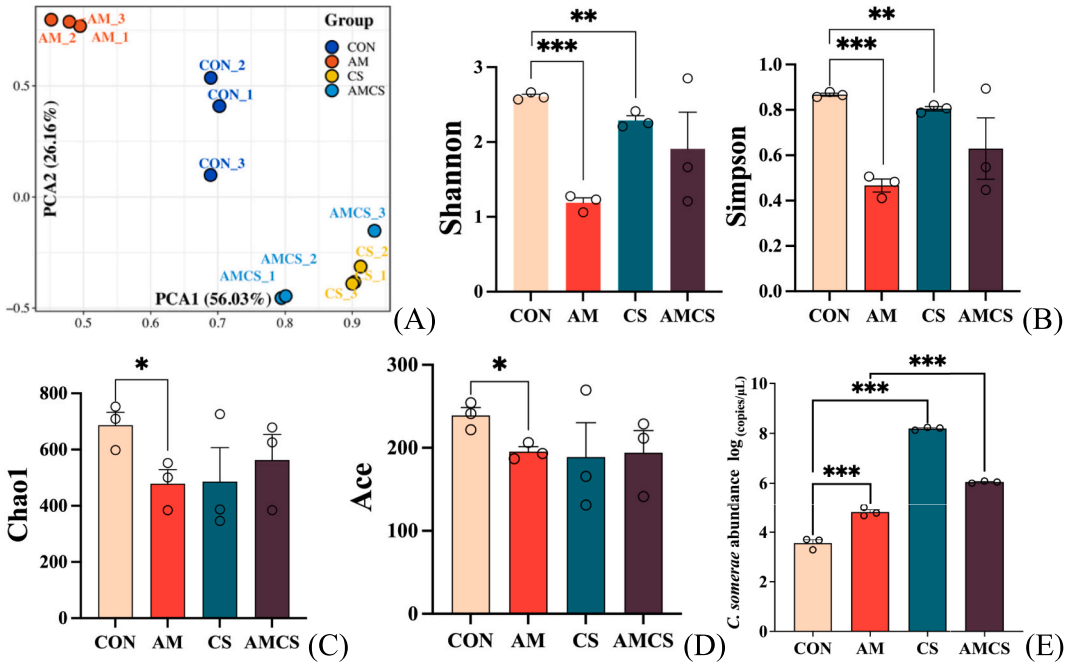

Fig. 1. CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. Data were presented as mean ± SEM (n = 3). Asterisk (*) indicates significant differences. * represents $P<0.05$, ** represents $P<0.01$, and *** represents $P<0.001$ (n = 3, Student t-test). Intestinal microbiota composition of intestine. (A) The Principal Component Analysis (PCA) of intestinal microbiota in yellow catfish fish fed with test diets. (B) The number of sequences analyzed, estimated diversity index (Shannon and Simpson), and OTU richness including Chao1 (C) and Ace (D), and for 16S rRNA libraries of intestine in yellow catfish fed with test diets. (D) Total copies of *C. somerae* detected by absolute quantification q-PCR in the intestinal microbiota of yellow catfish. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

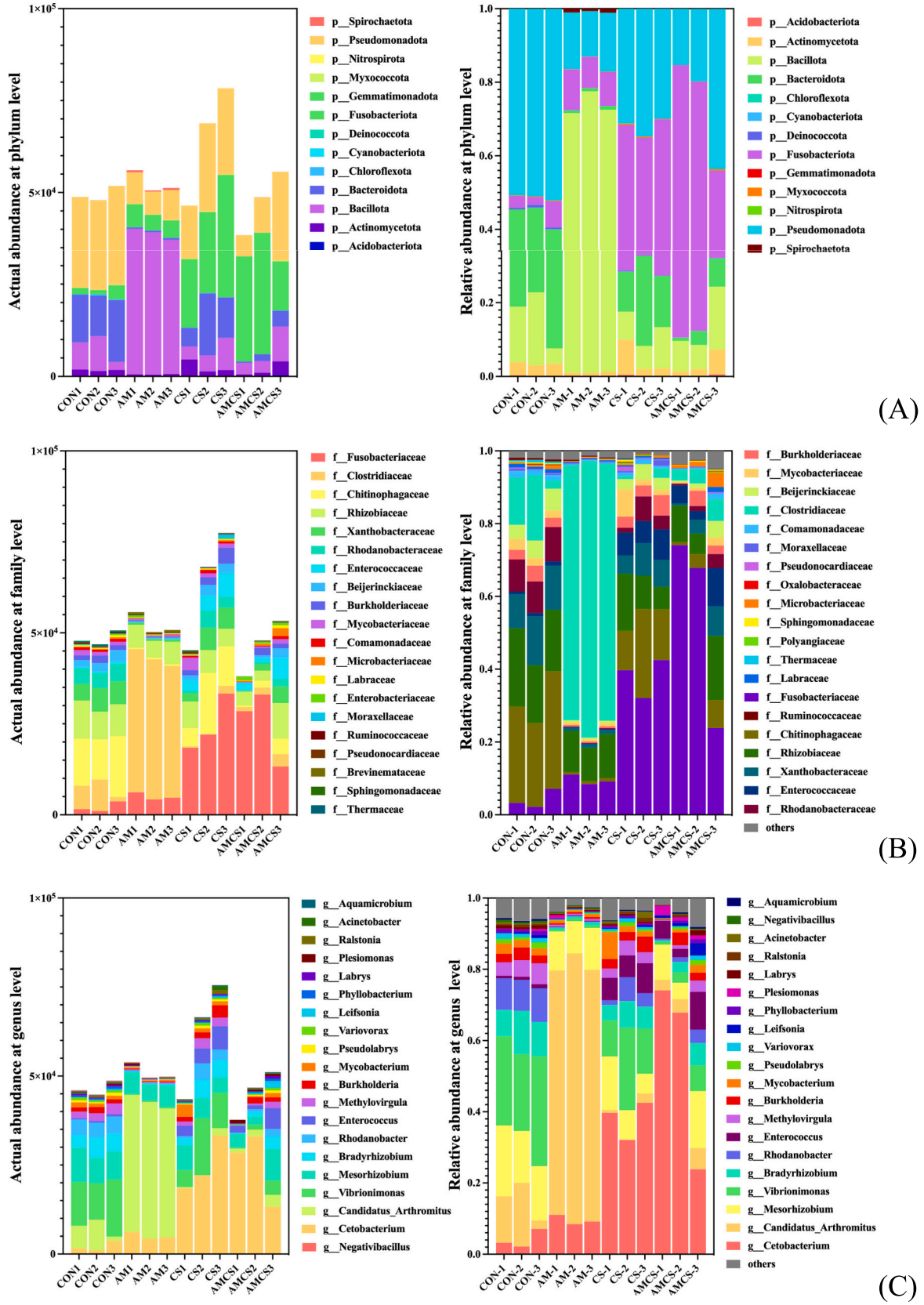

Fig. 2. Actual and relative abundance of intestinal microbiota at the (A) phylum, (B) family, and (C) genus level in yellow catfish fed with test diets. CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

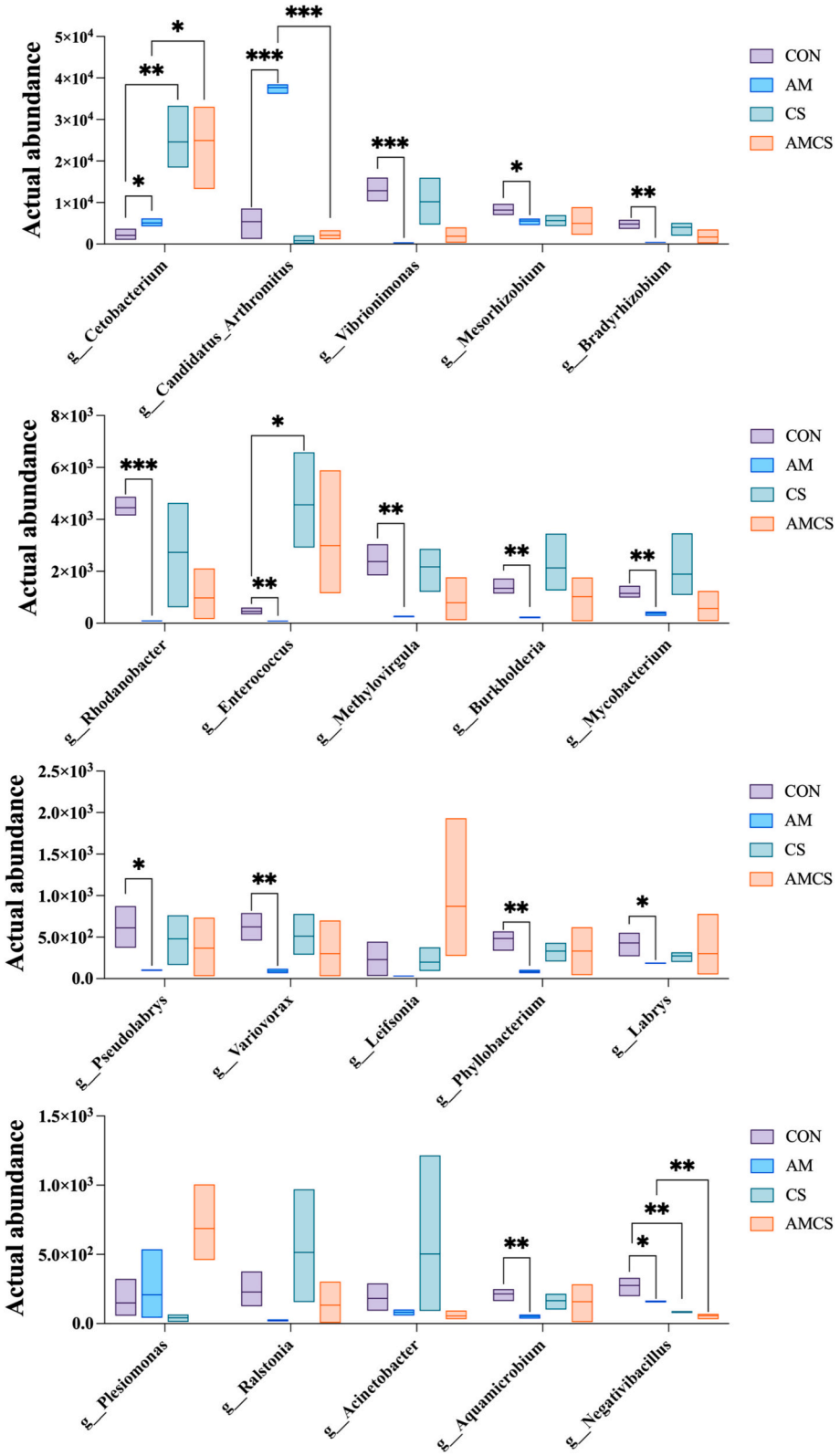

Fig. 3. Comparative analysis of the top 20 genera with the highest number of fragments. CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. Asterisk (*) indicates significant differences. * represents $P<0.05$, ** represents $P<0.01$, and *** represents $P<0.001$ (n = 3, Student t-test).

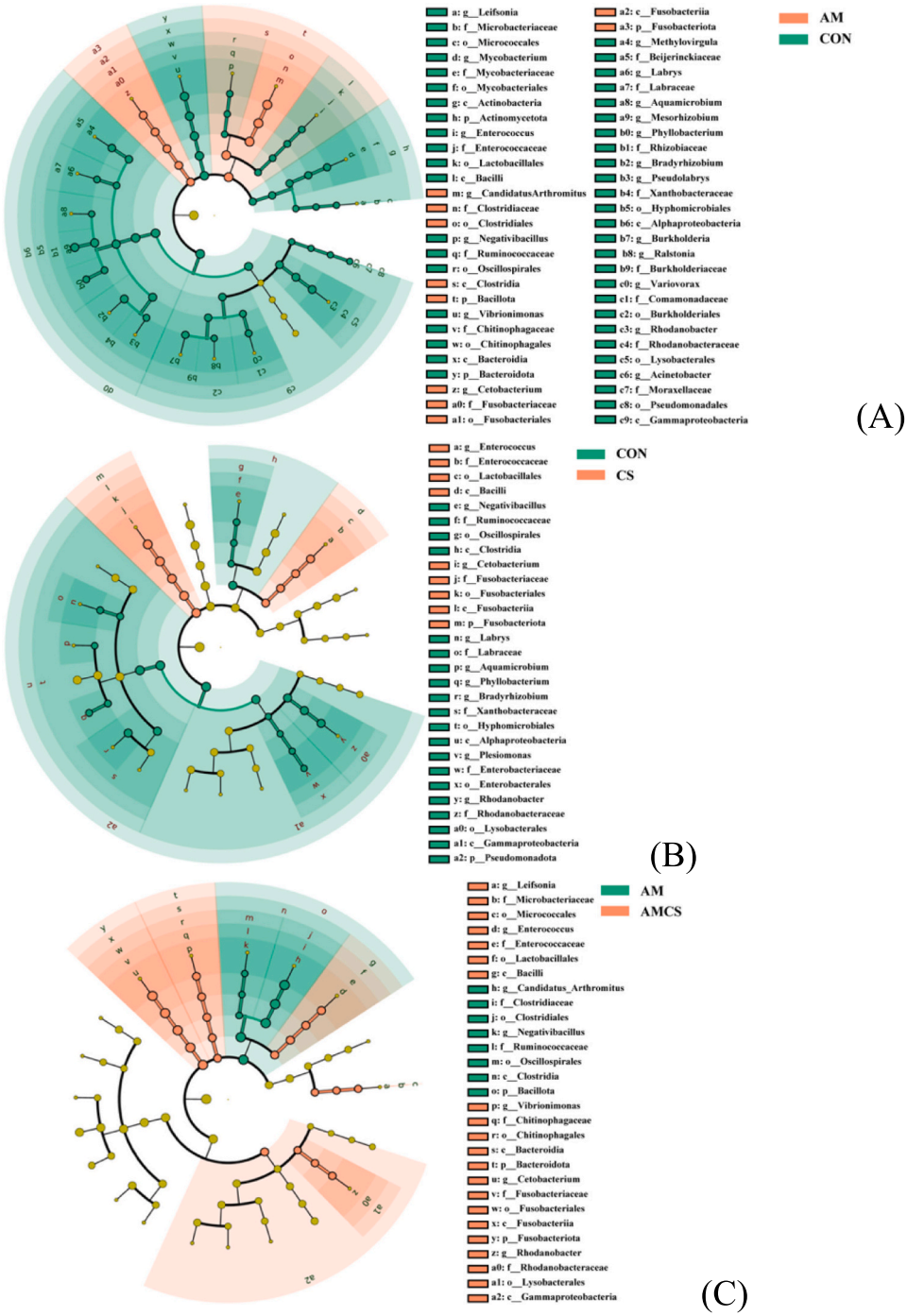

Fig. 4. Liner discriminant analysis effect size (LEfSe) was used to analyze the difference in microbial abundance between CON vs AM, CON vs CS, and AM vs AMCS groups. The LDA value threshold was set at 2.0. CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS.

3.6. Network interaction analysis of intestinal microbiota

As shown in Fig. 5A, the gene-genus association network for the CON/AM comprises 99 nodes and 2436 edges, categorized into four modularity levels. The modules are distributed as follows: 25.25 % for module 0, 28.28 % for module 1, 30.3 % for module 2, and 16.16 % for module 3. Analysis revealed that 86.29 % of correlations were positive, while 13.71 % were negative. As shown in Fig. 5B, the gene-genus association network for the CON/CS comprises 97 nodes and 1142 edges, categorized into five modularity levels. The modules are distributed as follows: 25.77 % for module 0, 19.59 % for module 1, 13.4 % for module 2, 15.46 % for module 3, and 25.77 % for module 4. Analysis revealed that 84.94 % of correlations were positive, while 15.06 % were negative. As shown in Fig. 5C, the gene-genus association network for the AM/AMCS comprises 100 nodes and 1142 edges, categorized into six modularity levels. The modules are distributed as follows: 5 % for module 0, 16 % for module 1, 38 % for module 2, 28 % for module 3, 10 % for module 4 and 3 % for module 5. Analysis revealed that 99.13 % of correlations were positive, while 0.87 % were negative. As shown in Fig. 5D, the gene-genus association network for the CS/AMCS comprises

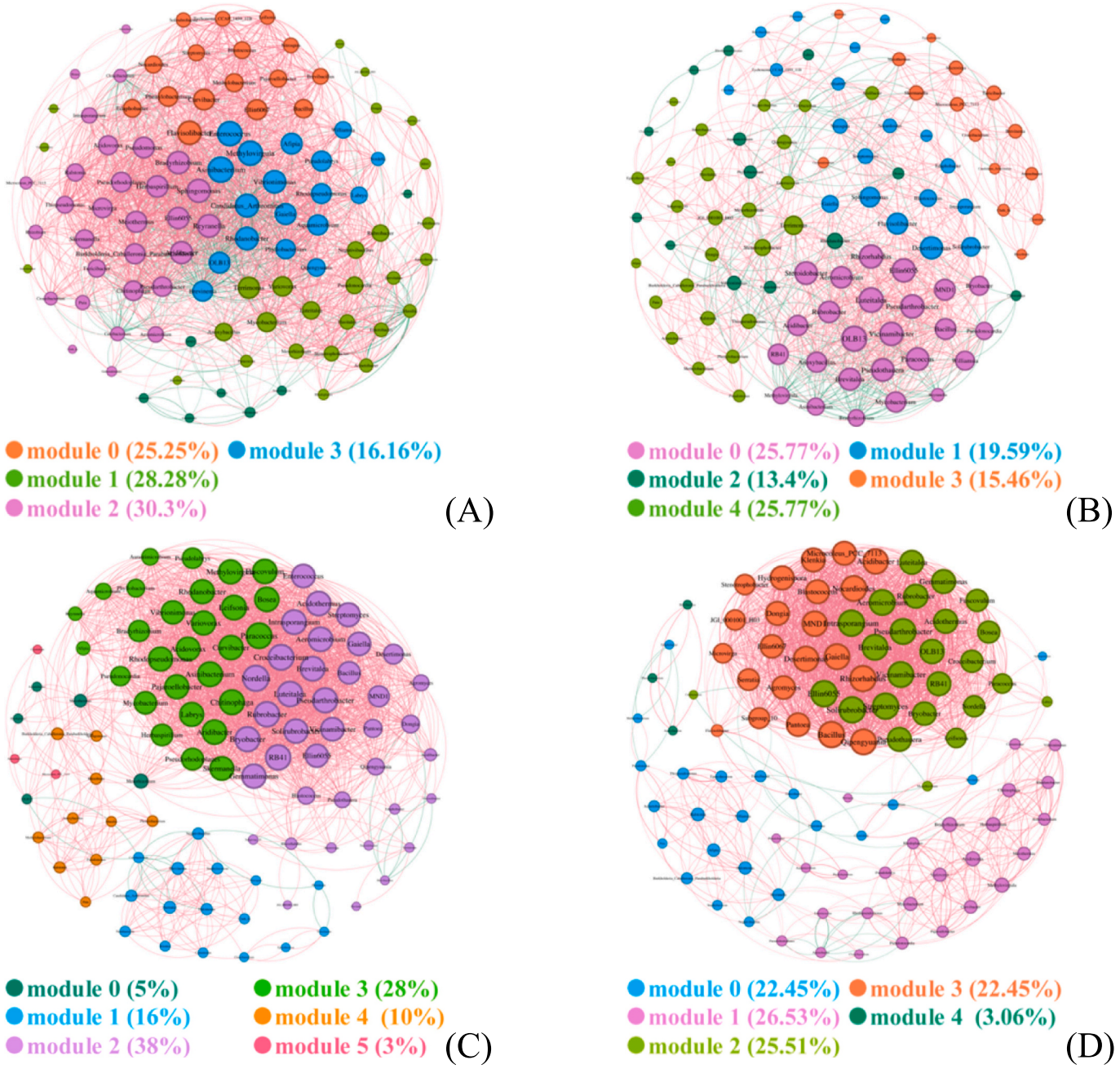

Fig. 5. The network visualizes the genus-genus interactions between (A) CON and AM groups, (B) CON and CS groups, (C) AM and AMCS groups, and (D) CS and AMCS groups. CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. Positive correlations are displayed in red, and negative correlations are shown in green. The nodes are colored according to different types of modularity classes. The size of each node is proportional to the betweenness centrality. The width of the line is indicative of the $-\log_{10}^{P}$ value, with a thicker line denoting a more pronounced correlation. The Pearson's |R| value>0.8, and *P*-value<0.05, and the significant correlation is considered. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

98 nodes and 1938 edges, categorized into five modularity levels. The modules are distributed as follows: 22.45 % for module 0, 26.53 % for module 1, 25.51 % for module 2, 22.45 % for module 3, 3.06 % for module 4 and 3 % for module 5. Analysis revealed that 98.56 % of correlations were positive, while 1.44 % were negative.

3.7. Changes in the levels of serum ammonia and urea

As shown in Fig. 6, compared with CON, serum and liver ammonia content in AM were significantly increased ($P < 0.001$). However, urea content in serum and liver was significantly decreased ($P < 0.001$). Compared with CON, serum and liver ammonia content in CS were significantly decreased ($P < 0.01$), and the urea content in serum and liver was significantly increased ($P < 0.001$). Compared with AM, serum and liver ammonia contents in AMCS were significantly decreased ($P < 0.001$). The content of urea in serum and liver was significantly increased ($P < 0.01$).

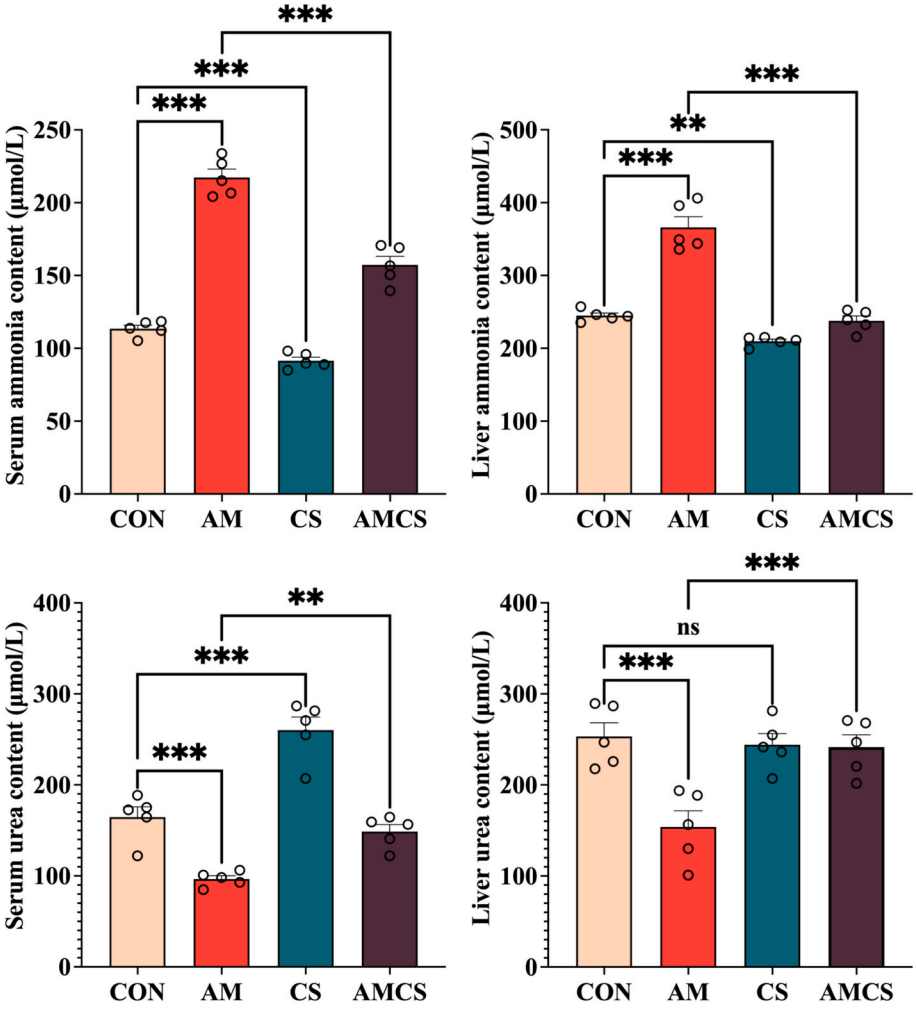

Fig. 6. The ammonia and urea contents of serum and liver of yellow catfish among dietary treatments. CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1 × 10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1 × 10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. Asterisk (*) indicates significant differences. * represents $P<0.05$, ** represents $P<0.01$, and *** represents $P<0.001$ (n = 3, Student t-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.8. Metabolomics analysis of liver

In Fig. 7A, 55 down-regulated metabolites and 75 up-regulated metabolites were found in the liver of AM compared to CON ($P < 0.05$). KEGG analysis revealed enrichment in *Arginine biosynthesis*. Notably, citrulline, ornithine, arginine, and carbamyl phosphate levels increased significantly, while aspartate, fumaric acid, glutamic acid, argininosuccinic acid, and urea decreased significantly in AM ($P < 0.05$). In Fig. 7B, 55 down-regulated metabolites and 42 up-regulated metabolites were found in the liver of CS compared to CON ($P < 0.05$). KEGG analysis revealed enrichment in *Arginine biosynthesis*. Notably, citrulline, ornithine, arginine, and carbamyl phosphate levels increased significantly, while aspartate, fumaric acid, glutamic acid, argininosuccinic acid, and urea decreased significantly in AM ($P < 0.05$). In Fig. 7C, 46 down-regulated metabolites and 130 up-regulated metabolites were found in the liver of AM compared to AMCS ($P < 0.05$). KEGG analysis revealed enrichment in *Arginine biosynthesis* and *Arginine and proline metabolism*. Notably, citrulline, ornithine, arginine, and carbamyl phosphate levels increased significantly, while aspartate, fumaric acid, glutamic acid, argininosuccinic acid, and urea decreased significantly in AM ($P < 0.05$).

3.9. Effects of exogenous C. somerae ceto on expression of ureagenesis-related genes in liver

As shown in Fig. 8, compared with CON, gene expression levels of *cps*, *ass*, and *otc* in AM were significantly increased ($P < 0.05$), but the gene expression levels of *asl* and *arg* were significantly decreased ($P < 0.05$). Compared with CON, gene expressions of *cps* in CS were significantly decreased ($P < 0.05$). The gene expressions of *asl*, *ass,* and *otc* were significantly increased ($P < 0.05$). Compared with AM, the gene expression of *cps* in AMCS was significantly decreased ($P < 0.05$). However, the gene expression of *asl*, *arg*, *ass,* and *otc* were increased significantly ($P < 0.05$).

3.10. Network interaction analysis between liver metabolites and intestinal microbiota

In Fig. 9A, the microbiota and metabolites association network of CON and AM included 26 nodes and 124 edges. The positive correlation accounted for 62.1 %, and the negative correlation accounted for 37.9 %. The network is segmented into three modules: Modules 0, 1, and 2 accounted for 15.38 %, 30.77 %, and 53.85 % respectively. Metabolites within the same module could be impacted by microorganisms in that module. In Fig. 9B, the microbiota and metabolites association network

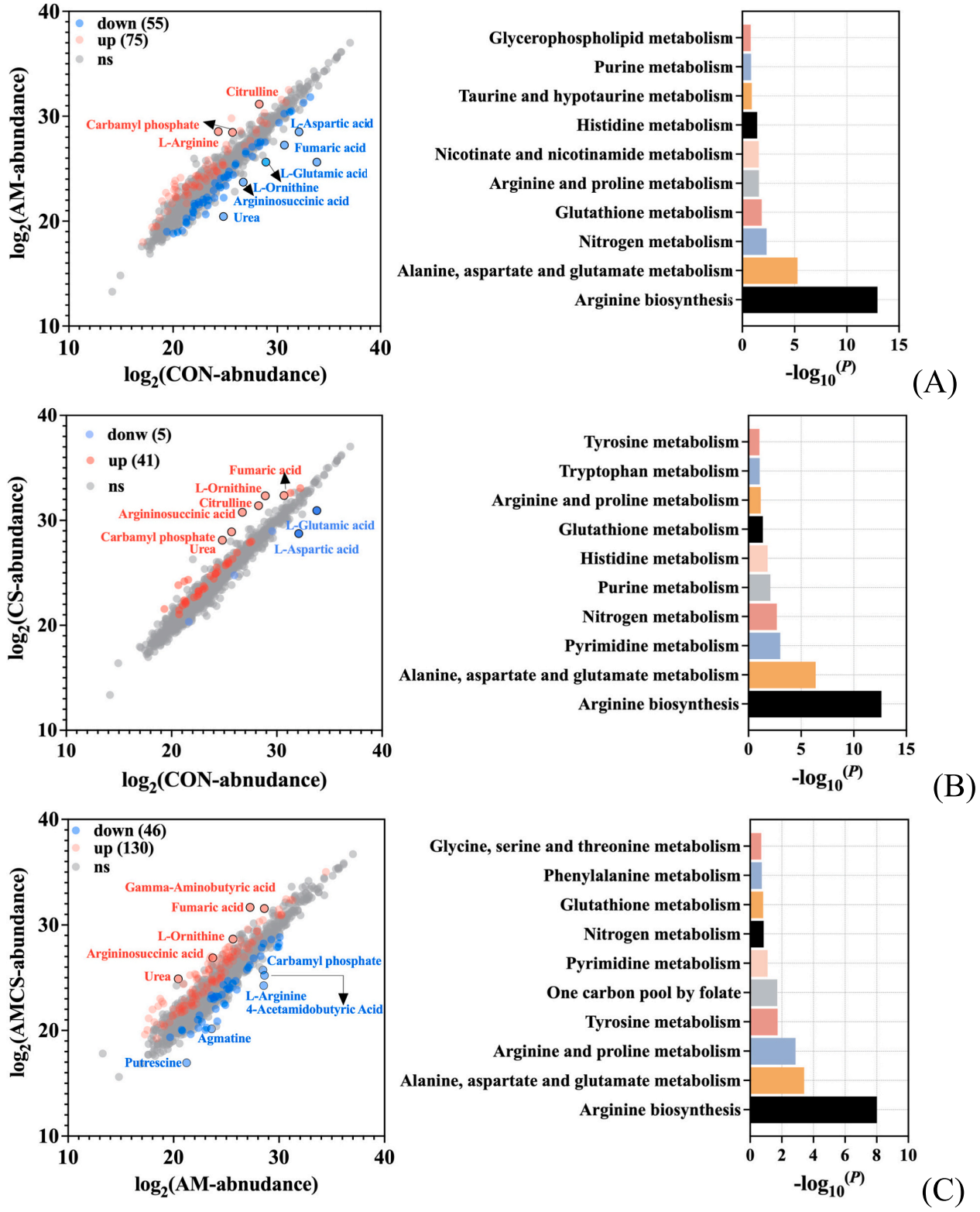

Fig. 7. Liver metabolomics analysis and functional enrichment analysis of differential metabolites of yellow catfish between (A) CON vs AM, (B) CON vs CS, and AM vs AMCS groups. CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. The red circles represent up-regulated metabolites, and the blue circles represent down-regulated metabolites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

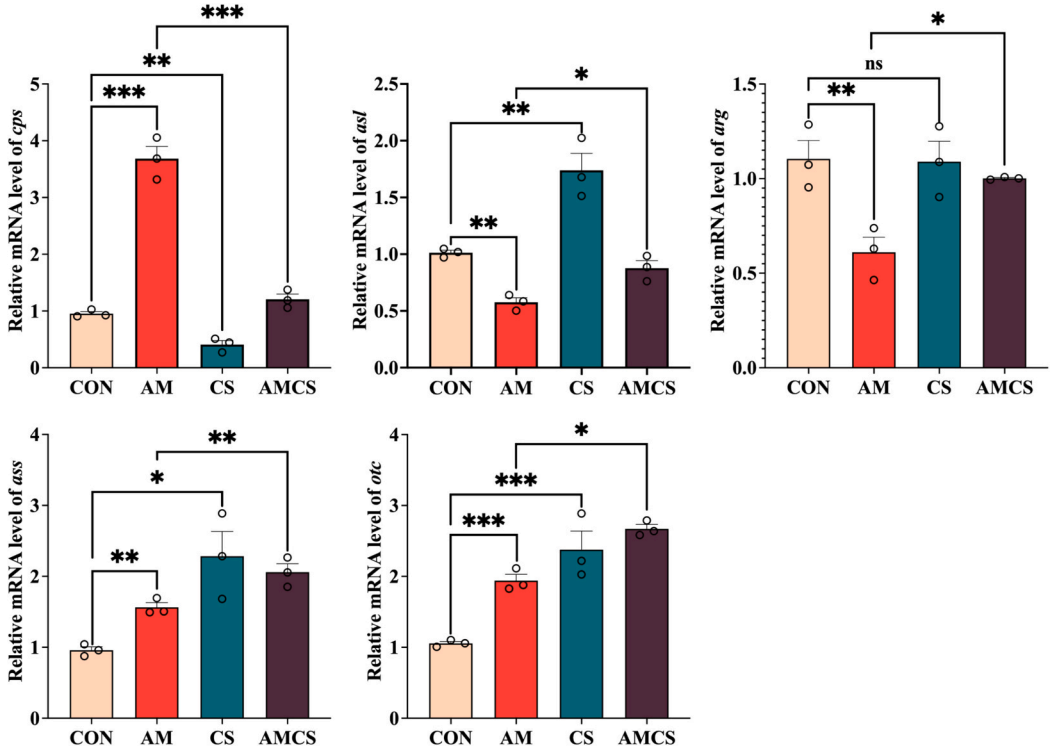

Fig. 8. Effect of *C. somerae ceto* supplementation on the relative mRNA level of liver carbamyl phosphate synthetase (*cps*), argininosuccinic acid lyase (*asl*), arginase (*arg*), argininosuccinate synthetase (*ass*), and ornithine transcarbamylase (*otc*). CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. Asterisk (*) indicates significant differences. * represents $P<0.05$, ** represents $P<0.01$, and *** represents $P<0.001$ (n = 3, Student t-test).

of CON and CS included 19 nodes and 39 edges. The positive correlation accounted for 53.85 %, and the negative correlation accounted for 46.15 %. The network is segmented into three modules: Modules 0, 1, and 2 accounted for 36.84 %, 52.63 %, and 10.53 % respectively. In Fig. 9C, the microbiota and metabolites association network of AM and AMCS included 15 nodes and 30 edges. The positive correlation accounted for 53.33 %, and the negative correlation accounted for 46.67 %. The network is segmented into two modules: Modules 0 and 1 accounted for 53.33 % and 46.67 %, respectively. In Fig. 9D, the microbiota and metabolites association network of CS and AMCS included 13 nodes and 11 edges. The positive correlation accounted for 72.73 %, and the negative correlation accounted for 27.27 %. The network is segmented into four modules: Modules 0, 1, 2, and 3 accounted for 23.08 %, 46.15 %, 15.38 %, and 15.38 %, respectively.

4. Discussion

C. somerae, a native bacterium found in freshwater fish intestines, acts as a probiotic promoting fish health. Notably, it possesses a comprehensive array of genes responsible for synthesizing vitamin B12, contributing to the enhancement of intestinal tight junctions (Qi et al., 2023). Furthermore, it has been observed to elevate the levels of short-chain fatty acids within the zebrafish intestines (Wang et al., 2021a). Moreover, the inclusion of fermented *C. somerae* products in the fish diet has been shown to promote immune system functionality, as well as improve intestinal and hepatic health in zebrafish (Xie et al., 2022a; Xie et al., 2022b). The study found that CAS negatively impacted the growth of yellow catfish, possibly by reducing their food intake. Fish tend to adjust to harsh ammonia-stressed conditions by reducing their food intake to lessen the strain on their digestive system and energy expenditure (Sun et al., 2023). Furthermore, it was also found that the growth parameters of the AMCS were significantly better than AM, and the growth performance of yellow catfish was not negatively affected by the administration of *C. somerae ceto* alone. These data imply that the administration of *C. somerae ceto* can effectively mitigate the growth inhibition induced by chronic ammonia stress. The research revealed that CAS significantly reduced the accumulation of crude protein and crude lipids in yellow catfish. This is attributed to the continuous utilization of lipids and proteins as energy reserves by fish experiencing environmental stress to sustain vital biological functions (Mommsen et al., 1999). Furthermore, the diminished food consumption by yellow catfish led to an impaired ability to effectively accumulate essential nutrients (protein and lipids). The study showed that crude protein and lipid levels in whole fish from the AMCS group were significantly higher than those in the AM group. This suggests that the utilization of *C. somerae ceto* might be efficacious in mitigating the depletion of protein and lipid induced by CAS.

The antioxidant defense system in fish serves as a crucial frontline in preventing oxidative damage to various organs (Atli et al., 2016; Li et al., 2024). Monitoring SOD and CAT activities can evaluate ammonia's impact on yellow catfish (Tan et al., 2017). However, elevated levels of environmental ammonia can inhibit the activities of SOD and CAT, exacerbating oxidative damage in fish (Li et al., 2024). This study reveals a significant increase in SOD, CAT, and T-AOC in the AM group compared to the CON group, indicating that chronic ammonia stress induces oxidative stress, and the increased activities of SOD and CAT aim to eliminate the elevated ROS induced by environmental stressors (Zhang et al., 2023). In the AMCS, the antioxidant capacity is significantly reduced compared to the AM, suggesting that *C. somerae ceto* can effectively alleviate oxidative stress induced by CAS.

In the present study, CAS significantly increased the relative abundance of *C.somerae* and *Candidatus_Arthromitus* in the intestine of yellow catfish, while the relative abundance of other genera decreased markedly. This indicates that CAS can alter the intestinal microbiota structure

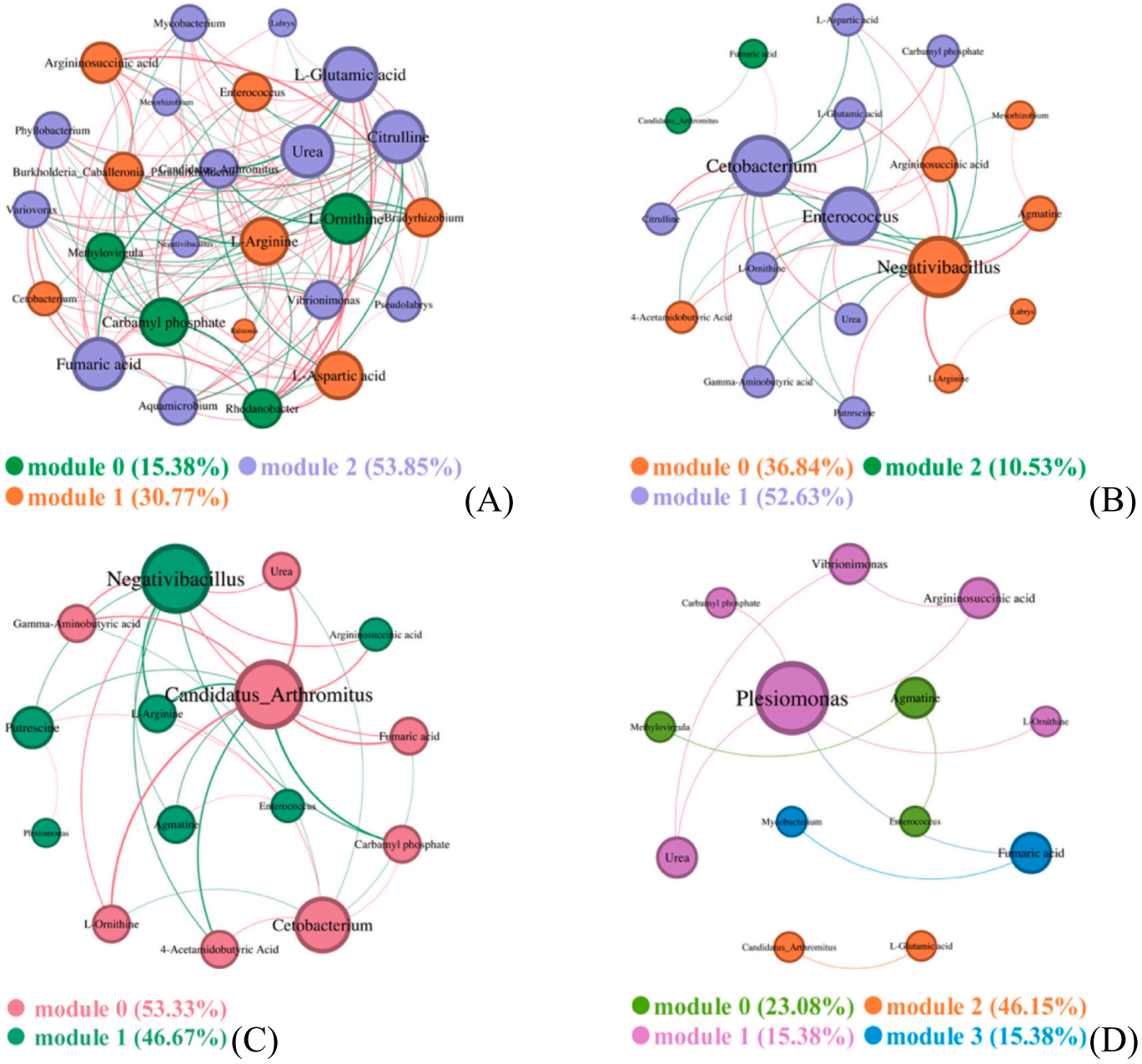

Fig. 9. The network visualizes the genus-differential metabolites interactions between (A) CON and AM groups, (B) CON and CS groups, (C) AM and AMCS groups, and (D) CS and AMCS groups. CON = Administration of 0 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CON. AM = Administration of 0 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AM. CS = Administration of 1×10^8 CFU/mL of *C. somerae ceto* in water with 0 mg/L total T-AN named CS. AMCS = Administration of 1×10^8 CFU/mL *C. somerae ceto* in water with 25 mg/L T-AN, denoted as AMCS. Positive correlations are displayed in red, and negative correlations are shown in green. The nodes are colored according to different types of modularity classes. The size of each node is proportional to the betweenness centrality. The width of the line is indicative of the $-\log_{10}^{P}$ value, with a thicker line denoting a more pronounced correlation. The Pearson's |R| value>0.8, and *P*-value<0.05, and the significant correlation is considered. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and reduce the richness of the intestine in yellow catfish. *C.somerae* is a recognized probiotic that enhances the intestinal integrity and immune capacity of zebrafish by producing VB12 (Qi et al., 2023) and short-chain fatty acids (Liao et al., 2023a). The CsEPS extracellular polysaccharide from *C. somerae* binds to the TLR2 receptor, activating the TLR2-MyD88 pathway to boost resistance against viral infections (Liang et al., 2024). Furthermore, it can alleviate the hepatotoxicity induced by trichlorfon in the environment (Zhao et al., 2024b). Our previous study found that acute ammonia stress significantly increased the relative abundance of *C.somerae* in the intestine (Wang et al., 2021b). The results of this study also indicate that CAS can significantly increase the relative abundance of *C.somerae* in the intestine of yellow catfish. The rise in *C. somerae* levels might be linked to the development of ammonia tolerance in yellow catfish, but the exact mechanism is still unknown. Moreover, *Candidatus_Arthromitus* is a beneficial bacterium for fish immunity (Woo et al., 2021). CAS can increase the susceptibility of the intestine to pathogenic bacteria (Li et al., 2016) Therefore, the possible increase in abundance of *Candidatus_Arthromitus* may represent a potential mechanism for the intestinal defense against pathogenic bacteria invasion in yellow catfish.

Current research has shown that the transplantation of probiotics into the intestine of fish can alleviate the adverse effects caused by heavy

metals (Giri et al., 2024), pesticides (Zhao et al., 2024b), and temperature (Naiel et al., 2022) in the environment. Moreover, probiotic transplantation has emerged as a novel practical strategy to promote the growth and healthy development of fish (Zhang et al., 2024). However, a crucial issue lies in determining whether exogenous probiotics can successfully colonize the intestine of fish. This study used 16S rRNA sequencing and absolute quantitative q-PCR to confirm the presence of *C. somerae ceto* in the intestine and assess its impact on the intestinal microbiota in yellow catfish. The *C. somerae* copies in the CS and AMCS were significantly higher than CON and AM, establishing *C. somerae* as the dominant species. These findings suggest that *C. somerae ceto* can effectively colonize the intestine, albeit at the cost of a significant reduction in the relative abundance of other bacteria. Subsequent colonization of *C. somerae ceto* in the intestine, it was observed that the presence of *C. somerae ceto* significantly reduces serum ammonia levels while increasing serum urea levels. This indicates that *C. somerae ceto* in the intestine may possess the function of detoxifying ammonia into urea. Similarly, a previous study found that by deleting the negative regulatory factor for L-arginine biosynthesis in the *E. coli* Nissle genome and inserting an L-arginine biosynthetic enzyme with feedback resistance, a synthetic strain SYNB1020 was created. This engineered strain is capable of synthesizing arginine, allowing SYNB1020 to produce L-arginine in an extracellular system by consuming NH_3. Consequently, this lowers the blood ammonia levels in ornithine transcarbamylase-deficient mice (Kurtz et al., 2019). This research inspires us that the bacteria in the intestine have the potential to reduce ammonia, much like the liver detoxifies ammonia into urea (Li et al., 2020) and arginine. Furthermore, the study found that administering *C. somerae ceto* can significantly reduce the ammonia content in the liver and increase the urea content. This indicates that the proliferation of *C. somerae ceto* can also detoxify ammonia by promoting ureagenesis in the liver. However, the mechanism behind this is not clearly understood.

To determine how intestinal *C. somerae ceto* promotes the conversion of ammonia to urea in the liver, we performed a metabolomic analysis of the liver. It was found that the content of argininosuccinic acid in liver decreased significantly under CAS, and the content of argininosuccinic acid as a substrate of argininosuccinate lyase inhibited the expression of *asl*. In addition, we found that the content of arginine increased significantly, but the content of urea and ornithine decreased significantly after CAS. Ornithine is one of the products of arginase, and the decrease of its content may be the cause of the decreased expression of *arg*. In conclusion, CAS causes the depletion of argininosuccinic acid and ornithine, as well as the abnormal accumulation of arginine and citrulline in the ureagenesis pathway of the liver, which directly results in the liver's inability to detoxify ammonia directly into urea. Previous studies have also indicated that the depletion of intermediate metabolites in the ureagenesis pathway is one of the reasons for the interruption of liver ammonia detoxification function (Zhang et al., 2025). The present study revealed that administering *C. somerae ceto* significantly increased the levels of argininosuccinic acid, ornithine, citrulline, and urea in the liver. A notable positive correlation was observed between the abundance of these metabolites and *C. somerae ceto*. Therefore, we hypothesize that *C. somerae cet* may possess the ability to directly generate these substances, as well as stimulate the host's liver to produce these metabolites. However, the precise mechanisms remain unclear. At the gene level, gene expression levels of *otc*, *ass*, and *asl* increased significantly after the administration of *C. somerae ceto*, possibly because ornithine and argininosuccinic acid act as substrates for ureagenesis, stimulating the expression of *otc*, *ass*, and *asl*. Increasing the abundance of *C. somerae ceto* was found to promote the ureagenesis pathway in the liver. This may be due to *C. somerae ceto* can provide essential intermediate metabolites (citrulline and arginine) for urea generation. Similarly, previous studies have shown that enhancing autophagy to produce the necessary intermediate metabolites for ureagenesis can facilitate hepatic urea production, thereby alleviating ammonia toxicity (Soria et al., 2018; Soria et al., 2021).

5. Conclusion

In conclusion, exogenously administrated *C. somerae ceto* can successfully colonize it in the intestine of yellow catfish and increase its abundance. Furthermore, increasing the colonization of *C. somerae ceto* in the intestine can significantly reduce the ammonia content in the serum and liver by promoting ureagenesis. Administration of *C. somerae ceto* increases the levels of argininosuccinic acid, ornithine, and citrulline in the liver. This provides essential intermediary metabolites for the ureagenesis pathway, facilitating the conversion of ammonia into urea in the liver, thereby accomplishing ammonia detoxification.

CRediT authorship contribution statement

Shidong Wang: Writing – review & editing, Writing – original draft, Conceptualization. **Xue Li:** Writing – review & editing, Conceptualization. **Muzi Zhang:** Resources, Project administration, Conceptualization. **Ming Li:** Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interest.

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Data availability

No data was used for the research described in the article.

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