Supplementary methods:

2.1 Animals and diet preparation

The hybrid grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂) juveniles (before sex differentiation) were purchased from a local fish farm (Zhanjiang, China) and acclimated for 7 days by feeding with a commercial diet (50% crude protein, 10% crude lipid). Feeding was performed every day at 8:00 and 17:00 until the apparent satiation (about 3.0–5.0% of the fish body weight). All feed ingredients were crushed into powder through a 380 mm mesh and combined. Fish oil, soy lecithin, and water were then added and mixed thoroughly. After pelletization and air drying for 2–3 days at room temperature, all prepared feed was kept in a refrigerator (-20°C) until use.

2.2 Animals experiment and sample collection

2.2.1 Feeding experiment (8 weeks)

The CD (control diet, 8.27% lipid) and HD diet (15.32%, added soybean oil to the CD diet) were formulated according to the previous findings that 7–13% is an optimal dietary lipid level for grouper [1]. Five BAs diets (BD) were prepared by adding taurocholic acid sodium (TCA, CAS: 345909-26-4, T4009, Sigma Aldrich) levels at 300 (B300D), 600 (B600D), 900 (B900D), 1200 (B1200D), and 1500 (B1500D) mg kg^{-1} to the HD diet. These tanks were randomly assigned to seven groups (CD, HD, B300D, B600D, B900D, B1200D, and B1500D), ensuring four replicates per group (7 × 4 = 28).

Blood was obtained from the caudal vein using sterile syringes and then centrifuged (3000×g) at 4°C for 10 min to obtain the serum, which was quickly frozen in liquid nitrogen and stored at -80°C. The serum samples were used for biochemical indicators. Each liver was divided into four parts: one was placed in 4% paraformaldehyde solution for histological examination, and the remainder was quickly frozen in liquid nitrogen and stored at -80°C. The latter three liver samples were used for testing biochemical indicators, determining gene and protein expression. Each distal intestine sample (from the anus to the first twist of the intestine) was divided into two parts and then quickly frozen in liquid nitrogen and stored at -80°C. These samples were used for testing biochemical indicators and determining gene expression. The distal intestinal contents

were carefully collected, frozen in liquid nitrogen, and stored at -80°C till the bacterial composition and BAs analysis.

2.2.2 Antibiotics experiment (6 days)

Three common antibiotics were purchased from the Aladdin Company (China): vancomycin (CAS: 1404-93-9, V105495), neomycin sulfate (CAS: 1405-10-3, N109017), and metronidazole (CAS: 443-48-1, M109874) [2, 3]. An ASB900D diet was formulated from the B900D diet by supplementing with a mixture of antibiotics (metronidazole 4 g kg^{-1}, neomycin sulfate 4 g kg^{-1}, and vancomycin 2 g kg^{-1}). These antibiotics concentrations were selected based on a marked decrease in the total bacterial quantity in the intestinal content of zebrafish [3]. The short-term (6 days) antibiotics treatment was selected according to our pilot trials and other studies [4]. Ninety fish (body weight 21.31 ± 0.12 g) were randomly distributed into 6 plastic tanks (15 fish per tank, 500L). These tanks were randomly assigned to two groups: a short-term B900D diet group (SB900D) and an ASB900D diet group, ensuring three replicates per group (2 × 3 = 6). After 6 days of the feeding trial, six specimens from each tank were selected randomly to obtain the blood (2 fish), liver (2 fish) and distal intestine (2 fish).

2.2.3 Injection experiment (6 days)

Two kinds of BAs, two inhibitors, and two activators, were used: TCA, taurodeoxycholic acid (TDCA, CAS: 207737-97-1, S168485, Aladdin), obeticholic acid (FXR agonist, CAS: 459789-99-2, HY-12222, MCE) [5], guggulsterone (FXR antagonist, CAS: 95975-55-6, HY-107738, MCE) [6], SBI-115 (TGR5 antagonist, CAS: 882366-16-7, HY-111534, MCE), and INT-777 (TGR5 agonist, CAS: 1199796-29-6, HY-15677, MCE) [5]. Six injection groups were designed as follows: 1) I-TCA group: 50 mg of TCA per kg of fish body weight (injection three times: days 1, 3, and 5); 2) I-TDCA group: 50 mg kg^{-1} of TDCA (injection on days 1, 3, and 5); 3) I-T747 group: I-TCA group + 1 mg kg^{-1} of obeticholic acid (one injection on day 5); 4) I-TGU group: I-TCA group + 25 mg kg^{-1} of guggulsterone (one injection on day 5); 5) I-TSBI group: I-TCA group + 1 mg kg^{-1} of SBI-115 (one injection on day 5); 6) I-T777 group: I-TCA group + 1 mg kg^{-1} of INT-777 (one injection on day 5). The concentrations and periods

of each treatment were selected according to our pilot trials and other studies [7].

2.3.6 The concentrations of BAs in the content of hindgut

Preparation of standard and sample solutions

37 standards were weighed accurately and the stock solutions were prepared using the solvent described in Table 1. Working solutions were prepared through serial dilution using methanol. The information and final concentration of each standard is shown in Table 1 and 2. The standard solutions were stored below -20 ℃. About 10 mg of the hindgut content sample was collected and 300 μL methanol was added to precipitate protein, vortexed for 1min and then centrifuged at 4℃ for 10 min (12, 000 g). The supernatant was concentrated and dried in a vacuum. The residue was dissolved with 100 μL methanol and the supernatant was ready for the LC-MS analysis.

LC/MS method

The UPLC separation was performed on an Acquity UPLC system (Waters, U.K.) equipped with an Acquity UPLC® BEH C18 (1.7um, 2.1x100mm, Waters) column. The temperature of the column was set at 40 ℃. The sample injection volume was 5μL. Eluents consisted of 0.01% formic acid in water (eluent A) and ACN (eluent B). The flow rate was set at 0.25 mL/min. A 38-min elution gradient was performed as follows: 0–4min, 25% B; 4–9min, 25-30% B; 9–14min, 30-36% B; 14–18min, 36-38% B; 18–24min, 38-50% B; 24–32min, 50–75% B; 32–35min, 75–100% B; 35–38min, 100–25% B. The MS analysis was performed using an AB 4000 mass spectrometer (AB, USA) equipped with an ESI source in the negative-ion mode working in the multiple reaction monitoring mode. An ion source voltage of 4.5 kV, a source temperature of 500 ℃ and a desolvation temperature of 380℃ were used. Collision gas and the curtain gas were set at 6 psi and 30 psi, respectively, while the atomization gas and auxiliary gas were both at 50 psi. The parameters for each BA are shown in Table 3.

Calibration curves, validation and samples analyses

The calibration graphs were constructed by plotting the peak area versus concentration for each individual BA. The results are shown in Table 4. The intra- and inter-day precision was determined using a standard mixture of BAs (different final concentrations). The intra- and inter-day precision was 1.52%–10.14% and 2.18%–

23.44%, respectively. The stability of the method was evaluated by quality control (QC) in each batch. The relative standard deviations of all QCs were calculated based on the peak area. The results showed that the stability of each substance was less than 15%, which indicated that this method was stable and reliable, and could be applied to the detection of samples. Samples were analyzed and quantified using the method described above.

References

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Table 1 The detailed information for bile acids

Bile acid	Full name	CAS	Solvent
alloLCA	Allolithocholic acid	2276-93-9	methanol
LCA	Lithocholic acid	434-13-9	methanol
isoLCA	Isolithocholic acid	1534-35-6	methanol
NorDCA	23-Nordeoxycholic acid	53608-86-9	methanol
12-ketoLCA	12-ketolithocholic acid	5130-29-0	methanol
7-ketoLCA	7-ketolithocholic acid	4651-67-6	methanol
β-UDCA	3β-Ursodeoxycholic acid	78919-26-3	methanol
DCA	Deoxycholic acid	83-44-3	methanol
CDCA	Chenodeoxycholic acid	474-25-9	methanol
UDCA	Ursodeoxycholic acid	128-13-2	methanol
HDCA	Hyodeoxycholic acid	83-49-8	methanol
NorCA	Norcholic acid	60692-62-0	methanol
DHCA	Dehydrocholic acid	81-23-2	methanol
7,12-diketoLCA	7,12-diketolithocholic acid	517-33-9	methanol
α-MCA	α-Muricholic acid	2393-58-0	methanol
UCA	Ursocholic acid	2955-27-3	methanol
β-MCA	β-Muricholic acid	2393-59-1	methanol
CA	Cholic acid	81-25-4	methanol
ACA	Allocholic acid	2464-18-8	methanol
βCA	3β-Cholic acid	3338-16-7	methanol
			50%
GLCA	Glycolithocholic acid Sodium Salt	24404-83-9	methanol
GHDCA	Glycohyodeoxycholic acid	13042-33-6	methanol
GCDCA	Glycochenodeoxycholic acid Sodium Salt	16564-43-5	methanol
GUDCA	Glycoursodeoxycholic acid	64480-66-6	methanol
GDCA	Glycodeoxycholic acid Sodium Salt	16409-34-0	methanol
			50%
LCA-3S	Lithocholic acid 3-sulfate Sodium Salt	34669-57-3	methanol
GCA	Sodium Glycocholate Hydrate	863-57-0	methanol
TLCA	Taurolithocholic acid Sodium Salt	6042-32-6	methanol
THDCA	Taurohyodeoxycholic acid Sodium Salt	38411-85-7	methanol
TUDCA	Tauroursodeoxycholic acid Sodium Salt	35807-85-3	methanol
TDCA	Taurodeoxycholic acid Sodium Salt	1180-95-6	methanol
TCDCA	Taurochenodeoxycholic acid	6009-98-9	methanol
TCA	Taurocholic acid Sodium Salt	145-42-6	methanol

T-α-MCA	Tauro-α-muricholic acid Sodium Salt	25696-60-0	methanol
T-β-MCA	Tauro-β-muricholic acid Sodium Salt	145022-92-0	methanol
CDCA-3G	Chenodeoxycholic acid-3-β-D-glucuronide	58814-71-4	methanol
CDCA-24G	Chenodeoxycholic acid 24-Acyl-β-D-glucuronide	208038-27-1	methanol

Table 2 The final concentration of each standard solution (ng/mL)

Name	No.												
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
alloLCA	/	/	/	2	4	10	20	40	100	200	400	1000	2000
LCA	/	/	5	10	20	50	100	200	500	1000	/	/	/
isoLCA	/	/	5	10	20	50	100	200	500	1000	2000	/	/
NorDCA	/	/	1	2	4	10	20	40	100	200	400	/	/
12-ketoLCA	1	2	5	10	20	50	100	200	500	1000	2000	/	/
7-ketoLCA	/	/	1	2	4	10	20	40	100	200	400	1000	2000
β-UDCA	/	/	5	10	20	50	100	200	500	1000	2000	/	/
DCA	/	/	5	10	20	50	100	200	500	1000	/	/	/
CDCA	/	/	5	10	20	50	100	200	500	1000	2000	/	/
UDCA	/	/	1	2	4	10	20	40	100	200	400	/	/
HDCA	/	/	5	10	20	50	100	200	500	1000	2000	/	/
NorCA	/	/	/	2	4	10	20	40	100	200	400	1000	2000
DHCA	/	/	/	/	/	10	20	40	100	200	400	1000	2000
7,12-diketoLCA	/	/	/	/	4	10	20	40	100	200	400	1000	2000
6,7-diketoLCA	/	/	/	/	4	10	20	40	100	200	400	1000	2000
α-MCA	/	/	5	10	20	50	100	200	500	1000	2000	/	/
UCA	/	/	1	2	4	10	20	40	100	200	400	1000	2000
β-MCA	/	/	5	10	20	50	100	200	500	1000	2000	5000	/
CA	/	/	5	10	20	50	100	200	500	1000	2000	/	/
ACA	/	/	1	2	4	10	20	40	100	200	400	1000	/
β-CA	/	/	/	2	4	10	20	40	100	200	400	1000	2000
GUCA	/	/	/	2	4	10	20	40	/	/	/	/	/
GLCA	/	/	1	2	4	10	20	40	100	200	400	1000	2000
GHDCA	/	0.4	1	2	4	10	20	40	100	200	400	1000	2000
GCDCA	/	/	5	10	20	50	100	200	500	1000	2000	/	/
GUDCA	/	/	1	2	4	10	20	40	100	200	400	1000	2000
GDCA	/	/	1	2	4	10	20	40	100	200	400	1000	2000
LCA-3S	/	/	1	2	4	10	20	40	100	200	400	1000	2000

GCA	/	/	5	10	20	50	100	200	500	1000	2000	5000	/
TLCA	/	/	1	2	4	10	20	40	100	200	400	1000	2000
TDCA	/	/	5	10	20	50	100	200	500	1000	2000	5000	10000
TCDCA	/	/	/	10	20	50	100	200	500	1000	2000	5000	10000
TCA	/	/	5	10	20	50	100	200	500	1000	2000	5000	10000
T-α-MCA	/	/	5	10	20	50	100	200	500	1000	2000	5000	10000
THCA	/	/	1	2	4	10	20	40	100	200	400	1000	2000
T-β-MCA	/	/	/	10	20	50	100	200	500	1000	2000	5000	10000
CDCA-G	/	/	2	4	8	20	40	80	200	400	800	2000	4000

Table 3 The parameters of each bile acid

Name	Q1 (m/z)	Q3 (m/z)	DP (v)	EP (v)	CE (v)	CXP (v)
alloLCA	375.145	375.145	-40	-10	-32	-1
LCA	375.3	375.3	-150	-10	-18	-9
isoLCA	375.301	375.301	-150	-10	-18	-9
NorDCA	377.3	377.3	-145	-10	-18	-9
12-ketoLCA	389.301	389.301	-145	-10	-18	-9
7-ketoLCA	389.302	389.302	-150	-10	-18	-9
β-UDCA	391.254	391.254	-150	-10	-26	-9
DCA	391.3	391.3	-145	-10	-18	-9
CDCA	391.301	391.301	-145	-10	-18	-9
UDCA	391.302	391.302	-150	-10	-18	-9
HDCA	391.303	391.303	-150	-10	-18	-9
NorCA	393.211	329.1	-130	-10	-46	-3
DHCA	401.2	401.2	-138	-10	-18	-9
7,12-diketoLCA	403.14	403.14	-125	-10	-38	-10
6,7-diketoLCA	403.3	403.3	-140	-10	-18	-9
α-MCA	407.3	407.3	-166	-10	-18	-9
UCA	407.301	407.301	-140	-10	-18	-9
β-MCA	407.302	407.302	-155	-10	-18	-5
CA	407.303	407.303	-140	-10	-18	-5
ACA	407.304	407.304	-140	-10	-18	-9
β-CA	407.362	407.362	-155	-10	-44	-9
GUCA	416.2	73.9	-120	-10	-58	-3
GLCA	432.401	73.9	-90	-10	-62	-5

GHDCA	448.2	74	-105	-10	-44	-13
GCDCA	448.276	73.9	-135	-10	-68	-5
GUDCA	448.277	73.9	-130	-10	-58	-5
GDCA	448.279	73.9	-90	-10	-68	-5
LCA-3S	455.196	96.9	-140	-10	-72	-1
GCA	464.281	73.9	-115	-10	-64	-5
TLCA	482.223	80	-115	-10	-100	-1
TDCA	498.35	79.8	-175	-10	-102	-5
TCDCA	498.357	79.8	-40	-10	-100	-5
TCA	514.332	79.8	-155	-10	-102	-7
T-α-MCA	514.337	79.9	-145	-10	-100	-5
THCA	514.343	79.9	-185	-10	-98	-7
T-β-MCA	514.346	79.9	-125	-10	-100	-5
CDCA-G	567.525	391.1	-100	-10	-48	-5

Q1: precursor ion; Q3: fragment ion; DP: distribution potential; EP: entrance potential; CE: collision energy; CXP: collision cell exit potential.

Table 4 Calibration curves and LOQ of each bile acid

Name	RT (min)	Linear equation	Correlation coefficient (r)	Linear Range (ng/mL)	LOQ (ng/mL)
alloLCA	30.08	Y=1530X+68	0.9984	2-2000	2
LCA	32.09	Y=11700X+7670	0.9972	5-1000	5
isoLCA	30.49	Y=6320X+3940	0.9968	5-2000	5
NorDCA	24.59	Y=12900X+5790	0.9963	1-400	1
12-ketoLCA	24.68	Y=5280X+4140	0.9968	1-2000	1
7-ketoLCA	24.08	Y=6740X+2070	0.9947	1-2000	1
β-UDCA	20.02	Y=6650X+5560	0.9973	5-2000	5
DCA	27.49	Y=12300X+12000	0.9973	5-1000	5
CDCA	26.89	Y=9950X+17400	0.9931	5-2000	5
UDCA	21.72	Y=15500X+7490	0.9962	1-400	1
HDCA	22.12	Y=6950X+9130	0.9968	5-2000	5
NorCA	16.21	Y=350X+198	0.9986	2-2000	2
DHCA	14.07	Y=4920X+3220	0.9985	10-2000	10
7,12-diketoLCA	13.44	Y=1260X+232	0.998	4-2000	4
6,7-diketoLCA	24.24	Y=3350X+886	0.996	4-2000	4

α-MCA	15.93	Y=5560X+3750	0.9972	5-2000	5
UCA	12.88	Y=9920X+1630	0.9985	1-2000	1
β-MCA	16.75	Y=4810X+7710	0.9962	5-5000	5
CA	21.11	Y=13700X+26400	0.9966	5-2000	5
ACA	20.64	Y=9490X+105	0.9985	1-1000	1
β-CA	15.39	Y=1470X+1030	0.9968	2-2000	2
GUCA	36.72	Y=681X+23.8	0.9984	2-40	2
GLCA	27.98	Y=3180X+267	0.9983	1-2000	1
GHDCA	15.53	Y=1040X+13.2	0.9965	0.4-2000	0.4
GCDCA	21.88	Y=2180X+1500	0.9974	5-2000	5
GUDCA	15.23	Y=2450X+1890	0.996	1-2000	1
GDCA	22.87	Y=2430X+101	0.9987	1-2000	1
LCA-3S	26.44	Y=5700X+59	0.9991	1-2000	1
GCA	15.51	Y=1730X+1430	0.9974	5-5000	5
TLCA	24.41	Y=2220X+3630	0.9982	1-2000	1
TDCA	18.87	Y=2520X+1800	0.9975	5-10000	5
TCDCA	17.53	Y=1280X-550	0.999	10-10000	10
TCA	12.86	Y=1750X+951	0.9987	5-10000	5
T-α-MCA	7.45	Y=564X+368	0.9991	5-10000	5
THCA	10.27	Y=1900X-57.8	0.9992	10-10000	10
T-β-MCA	7.77	Y=707X+184	0.9989	10-10000	10
CDCA-G	20.62	Y=569X+350	0.999	2-4000	2

LOQ is determined by the signal-to-noise ratio (S/N), which is calculated by comparing the signals of known samples with blank samples. Generally, the corresponding concentration is defined as LOQ when the S/N is 10:1 (S/N=10).