

Effects of taurine on gut microbiota and metabolism in mice

Haining Yu¹ · Zhengzhao Guo¹ · Shengrong Shen² · Weiguang Shan¹

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Abstract As being a necessary amino acid, taurine plays an important role in the regulation of neuroendocrine functions and nutrition. In this study, effects of taurine on mice gut microbes and metabolism were investigated. BALB/C mice were randomly divided into three experimental groups: The first group was administered saline (CK), the second was administered 165 mg/kg natural taurine (NE) and the third one administered 165 mg/kg synthetic taurine (CS). Gut microbiota composition in mice feces was analyzed by metagenomics technology, and the content of short-chain fatty acids (SCFA) in mice feces was detected by gas chromatography (GC), while the concentrations of lipopolysaccharide (LPS) and superoxide dismutase (SOD) were detected by a LPS ELISA kit and a SOD assay kit, respectively. The results showed that the effect of taurine on gut microbiota could reduce the abundance of *Proteobacteria*, especially *Helicobacter*. Moreover, we found that the SCFA content was increased in feces of the NE group while LPS content was decreased in serum of the NE group; the SOD activity in serum and livers of the NE and CS groups were not changed significantly compare to that of the CK group. In conclusion, taurine could regulate the gut micro-ecology, which might be of benefit to health by

inhibiting the growth of harmful bacteria, accelerating the production of SCFA and reducing LPS concentration.

Keywords Taurine · Gut microbiota · Metagenomics technology · SCFA · LPS · SOD activity

Introduction

Taurine is a beta amino acid with a simple structure and mostly exists in organism in free-state. It is a necessary amino acid in the body (Huxtable 1992). A large number of studies have shown that taurine has many important physiological functions to human body. It can improve immunity, resist oxidation, delay senility, reduce blood pressure, promote recovery from acute hepatitis, etc. (Averin 2015; Wang et al. 2013; De Luca et al. 2015; Ito et al. 2012). In addition, taurine can also improve the metabolism of the nutrients and play an important role in the regulation of neuroendocrine (Cuttitta et al. 2013; Camargo et al. 2015). Because taurine has these special physiological and pharmacological effects, it has been widely used in food and pharmaceutical industries.

Trillions of bacteria live in the human gut, the number of them is about 10 times higher than that of human cells. These gut microbes are collectively referred to as a gut microbiota. They are important for metabolism and health and involve in the human's whole life. They can affect a variety of physiological functions of human body (Yano et al. 2015; Dogra et al. 2015; Eckburg et al. 2005; Tremaroli and Backhed 2012). The development of a new generation of gene sequencing technology and the bioinformatics analysis tools can pioneer scientific researchers to study a new area of human gut microbiota's composition and structure. After an overwhelming exploration of

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✉ Shengrong Shen
shrshen@zju.edu.cn

¹ College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China

² Department of Food Science and Nutrition, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, People's Republic of China

taurine's benefits for medical and health values, taurine has drawn much attraction for research subjects all around the world. Though there are numerous studies, the correlations between taurine and gut microbiota remain unclear. Therefore, we decide to investigate these correlations in mice using a combination of macro-genomic research method and bioinformatics analysis technique, which is a new concept of the studies. Moreover, we decide to investigate some activities of biochemical index, including short-chain fatty acids (SCFA), lipopolysaccharide (LPS) and superoxide dismutase (SOD), related to gut microbiota and bioactivities of taurine.

Materials and methods

Materials

BALB/C mice were obtained from a Shanghai Slack Co., Ltd, China. Natural taurine was extracted from octopus viscera (>98 %), while synthetic taurine (>98 %), acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid and other standards were from Aladdin reagent net. QIAamp[®] DNA Stool Mini Kit was ordered from Germany QIAGEN Company and mouse LPS ELISA kit was from a research domain chemical reagent Co., Ltd. SOD and BCA protein concentration assay kits were from Nanjing Jiancheng biological engineering Co., Ltd, China. Concentrated sulfuric acid, ethyl ether, and anhydrous calcium chloride (analytical pure) were purchased from Sinopharm, China.

Experimental animals and sample collection

The experiments were carried out in compliance with the guidelines of the Ethical Committee of Zhejiang University (SYXK(zhe)2012-0178). 30 BALB/C mice, weighing in between 18 and 20 g, were half male and half female and they were maintained under a carefully controlled condition of humidity (40–60 %), temperature (20–24 °C) and light condition (12 h). When the 30 mice were brought into the experimental house, they were first fed normally for a week for adaptation to the environment. Then they were randomly divided into three groups: control group (CK), natural taurine group (NE) and synthetic taurine group (CS); each group consisted of equal numbers of males and females. A NE and a CS group were orally administered with taurine according to the recommended daily intake (165 mg/kg), and a CK group was with saline. They access to fodder from 9 am to 8 pm every day; the nutritional contents of the mice fodder obtained from Zhejiang Academy of Medical Sciences are shown in Table 1. The mice growth, activity, diet and defecation were surveyed

Table 1 Nutrient contents of mice fodder

Nutrient content	Amount
Crude protein	20–25 %
Water	8–10 %
Crude ash	6–8 %
Crude fat	4–5 %
Crude fibre	4–5 %
Calcium	1.2–1.8 %
Lysine	1.20–1.42 %
Methionine+cysteine	0.78–1.10 %
Phosphorus	0.8–1.2 %
Tryptophan	0.27–0.34 %
Vitamin A	12,500–15,000 IU/kg
Vitamin D	1250–1500 IU/kg

daily. Weight of mice was recorded weekly, and mice feces were collected at the day before taurine (or saline) treatment and the 28th day after taurine (or saline) treatment, and feces from each mice were stored at –80 °C separately. Blood samples were collected from all mice at the 28th day after natural taurine (or saline) treatment. At the end of experiment, mice were killed by cervical dislocation and dissected. Liver were removed and stored at –80 °C separately.

DNA extraction

300 mg feces of individuals was collected, and then were mixed together in the same group so that 6 mixed samples were used for DNA extraction and high-throughput sequencing and bioinformatics analysis: feces sample from CK group collected at the day before saline treatment (CK1); feces sample from CK group collected at the 28th day after saline treatment (CK2); feces sample from NE group collected at the day before natural taurine treatment (NE1); feces sample from NE group collected at the 28th day after natural taurine treatment (NE2); feces sample from CS group collected at the day before synthetic taurine treatment (CS1); feces sample from CS group collected at the 28th day after synthetic taurine treatment (CS2). Metagenomic DNA was extracted from the mixed feces by the QIAamp DNA stool mini kit (Qiagen, USA) according to the manufacturer's instructions. The extracted DNA was sub-packaged into four tubes to avoid multi-gelation and stored at –20 °C.

High-throughput sequencing and bioinformatics analyses

Extracted intestinal macro-genomic DNA needs high-throughput Solexa sequencing. Firstly, DNA molecules

were segmented into proper size. The DNA fragments were tied on a joint primer on both ends and connected to a flow cell by covalent bonds to achieve bridge amplification. After that, millions of double-stranded DNA fragments were obtained. Finally, four dNTPs labeled by different fluorescence were added; they simultaneously became a reversible terminator dye. In the process of amplification and extension, a fluorescence signal was collected by sequencer, and converted into a sequence image before obtaining sequencing data. The coarse data from high throughput Solexa sequencing could produce high quality reads through quality control.

The sequences based on the distance between them had a clustering structure, and then divided into an operational taxonomic unit (OTU) according to their similarities. Generally, an OTU was considered as a genus or a species when the threshold was 0.97 or 0.99, respectively. Uclust (v1.1.579) was employed to measure the clustering structures by first screening the longest read from the sequences as seed (Edgar 2010). If they are found similar sequences in threshold; they will be classified as a class. This procedure was executed until all sequences were clustered into each class as an OTU.

Species classification was carried out on the processed sequences by the software RDP classifier (v2.10.1) (Lan et al. 2012), which is based on Bergey's taxonomy, adopting the Naive Bayesian assignment algorithm for each sequence to calculate p values to rank at the genus level; the classification result is usually reliable ($p > 0.8$). Bergey's taxonomy is divided into six layers: phylum, class, order, family, genus and species. The dominant bacteria were analyzed mainly on the phylum, class, and species levels in order to explore the effects of taurine on gut microbiota.

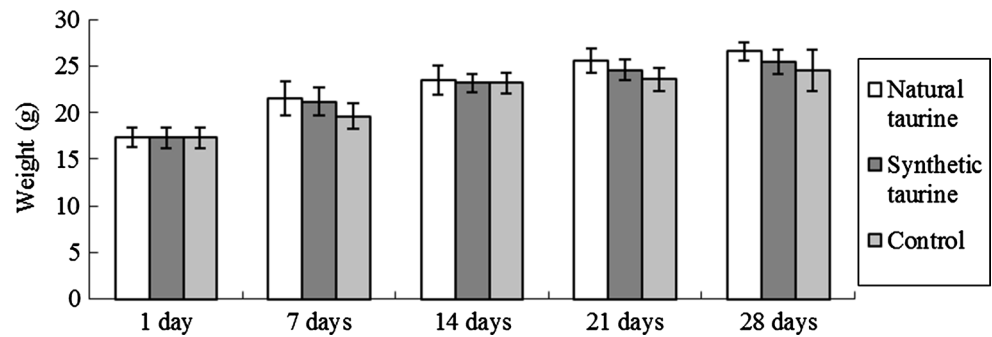
Determination of SCFA in mice feces

30 feces samples collected at the 28th day after taurine (or saline) treatment were used for short-chain fatty acid (SCFA) analysis, and SCFA was extracted from feces as follows: 60 mg of lyophilized stool samples were put into a round-bottomed flask and gently suspended in 1.6 mL of distilled water. Subsequently, 0.4 mL 50 % aqueous H_2SO_4 and 2 mL diethyl ether were added and mixed with an orbital shaker for 45 min, and then centrifuged at 3000g for 5 min at room temperature. Anhydrous $CaCl_2$ was added to the collected supernatant to remove residual water, then 2 μ L supernatant was analyzed by injection in the Gas Chromatography system.

Gas Chromatography analysis was carried out using Agilent 7890A gas chromatograph fitted with a flame ionization detector (FID). GC column (ZB-FFAP, Phenomenex, America) of 30 m \times 0.32 mm \times 0.25 μ m was used. Nitrogen was supplied as the carrier gas at a flow rate of 1.69 mL/min in non-split mode (injector temperature: 250 $^{\circ}C$). The initial oven temperature was 100 $^{\circ}C$ for 2 min, and then rose at a rate of 8 $^{\circ}C$ /min to 240 $^{\circ}C$, upholding there for 10 min. The temperatures of the FID and injection port were 350 $^{\circ}C$. Short-chain fatty acids were quantified by an external standard method using the mix standard solution of acetic, propionic, iso-butyric, butyric, iso-valeric, valeric acids.

Determination of LPS in mice serum

Concentrations of LPS in mice serum was determined by mouse LPS ELISA kit: (1) Standard solution: the kit provides a stock standard (240 μ g/L), allowing the standard to sit for a minimum of 5 min with gentle mixing prior to making dilutions. Pipette 150 μ L of standard dilution into each tube. Using the 150 μ L of stock solution to produce a twofold dilution series (including 120, 60, 30, 15, and 7.5 μ g/L). (2) Sample treatment: setting blank wells separately (blank wells for comparison are treated the same like treated wells except that the sample and ELISA reagent are not added). 50 μ L of standard solution or samples (40 μ L standard dilution + 10 μ L serum) to the appropriate level of well of the antibody pre-coated microtiter plate, and gently mixed prior to incubating for 30 min at 37 $^{\circ}C$. (3) Liquid configuration: 20 times of washed solution diluted 20 times with distilled water and reserved. (4) Washing step: liquid is dried by swinging and washing with buffer solution to every well, upholding for 30 s before removing (repeat 5 times). (5) Biotinylated anti-IgG treatment: adding 50 μ L of diluted biotinylated anti-IgG to all wells, and then incubating for 30 min at 37 $^{\circ}C$. (6) Washing: operation procedure see step (4). (7) Streptavidin-HRP treatment: adding 50 μ L of streptavidin-HRP to all wells, gently mixed and then incubated for 15 min at 37 $^{\circ}C$. (8) Washing: operation procedure see step (4). (9) Chromogen solution adding: 50 μ L of chromogen solution B is added to each well, allowing to incubate for 10 min at 37 $^{\circ}C$. (10) Stop reaction: adding 50 μ L of stop solution to each well to stopping the reaction (the blue color changes to yellow color immediately). (11) Assay: taking blank well as zero, measuring the optical density (OD) at 450 nm after adding stop solution within 15 min.

Fig. 1 Weight changes of mice

Determination of SOD content in mice serum

SOD activity in mice serum was determined by SOD assay kit: (1) Check blood serum, if it is turbid, then centrifuge at 3500 rpm for 10 min, take supernatant to use. (2) Dilute lipid blood serum with physiological saline to different concentrations, do preliminary experiment. (3) Operation table as follows:

	Contrast well (μL)	Contrast blank well (μL)	Sample well (μL)	Sample blank well (μL)
Blood serum	–	–	20	20
Double distilled water	20	20	–	–
Enzyme working solution	20	–	20	–
Enzyme diluent	–	20	–	20
Substrate working solution	200	200	200	200

Mix sufficiently, incubate at 37 °C for 20 min, use ELIASA to measure absorbance at 450 nm

(4) Calculations: ① Definition: If SOD inhibition ratio reaches to 50 % in this reaction system, then correenzyme quantity can be considered as 1 SOD activity unit (U). ② Formulas as follows:

$$(a) \text{ SOD inhibition ratio formula: SOD inhibition ratio (\%)} = \frac{\{(A_{\text{contrast}} - A_{\text{contrast blank}}) - (A_{\text{sample}} - A_{\text{sample blank}})\}}{(A_{\text{contrast}} - A_{\text{contrast blank}})} \times 100 \%$$

$$(b) \text{ SOD activity (U/ml)} = \text{SOD inhibition ratio} / (50 \% \times \text{Reaction system dilute multiple (0.24ml/0.02ml)}) \times \text{Sample dilute multiple before assay.}$$

Determination of SOD content in mice liver

SOD activity in mice liver was determined by SOD assay kit: (1) Weight 0.1 g mice liver tissue accurately, add 9 times volume physiological saline according to mass-volume ratio of 1:9, cut liver tissue to small pieces, make homogenate in ice water bath, centrifuge at 3000 rpm for 10 min, take supernatant as 10 % homogenate to use. (2) After sample preparation, we use BCA kit to measure protein concentration in sample, and it will be used in calculation. (3) Operation table as follows:

	Contrast well (μL)	Contrast blank well (μL)	Sample well (μL)	Sample blank well (μL)
Liver homogenate supernatant	–	–	20	20
Double distilled water	20	20	–	–
Enzyme working solution	20	–	20	–
Enzyme diluent	–	20	–	20
Substrate working solution	200	200	200	200

Mix sufficiently, incubate at 37 °C for 20 min, use ELIASA to measure absorbance at 450 nm

(4) Calculations: ① Definition: If SOD inhibition ratio reaches to 50 % in this reaction system, then correenzyme quantity can be considered as 1 SOD activity unit (U). ② Formulas as follows:

$$(a) \text{ SOD inhibition ratio formula: SOD inhibition ratio (\%)} = \frac{\{(A_{\text{contrast}} - A_{\text{contrast blank}}) - (A_{\text{sample}} - A_{\text{sample blank}})\}}{(A_{\text{contrast}} - A_{\text{contrast blank}})} \times 100 \%$$

Table 2 Ratio of sample's flora abundance value to the total number of bacteria on phylum level

Bacteriophyta	CK1 ratio (%)	CK2 ratio (%)	NE1 ratio (%)	NE2 ratio (%)	CS1 ratio (%)	CS2 ratio (%)
<i>Firmicutes</i>	52.19	51.84	55.55	57.38	62.07	60.17
<i>Bacteroidetes</i>	31.39	36.38	24.07	31.65	24.21	32.28
<i>Proteobacteria</i>	13.59	10.37	18.16	9.79	11.42	5.95
<i>Candidata</i>	1.70	0.37	1.14	0.50	0.87	0.46
<i>Unclassified</i>	0.65	0.61	0.65	0.35	1.00	0.55
<i>Tenericutes</i>	0.33	0.30	0.24	0.21	0.10	0.31
<i>Deferribacteres</i>	0.05	0.07	0.08	0.11	0.17	0.22
<i>Spirochaetae</i>	0.07	0.05	0.01	0.01	0.12	0.04
<i>Verrucomicrobia</i>	0.02	0	0.1	0	0.02	0

CK1 feces of mice in CK group collected at the day before saline treatment, CK2 feces of mice in CK group collected at the 28th day after saline treatment, NE1 feces of mice in NE group collected at the day before natural taurine treatment, NE2 feces of mice in NE group collected at the 28th day after natural taurine treatment, CS1 feces of mice in CS group collected at the day before synthetic taurine treatment, CS2 feces of mice in CS group collected at the 28th day after synthetic taurine treatment

$$(b) \text{ SOD activity (U/mgprot)} = \text{SOD inhibition ratio} / \\ (50\% \times \text{Reaction system dilute multiple (0.24ml/0.02ml)}) \\ \times \text{Protein concentration in sample to assay (mg protein/ml)}$$

Results and discussion

Effects of taurine on mice health

During 1 month of treatment, all mice behaved actively. Their clothing hairs were level and smooth, and their diet had no obvious changes. As shown in Fig. 1, there was no significant difference in weight changes in each group; weight gain was within the normal range, suggesting that mice in three groups were all in good health.

Distribution of gut microbiota on phylum levels

Table 2 showed the ratios of sample's flora abundance value to the total number of bacteria on phylum levels. The dominant intestinal bacteriophyta of mice were *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. The abundance values of the three bacteriophyta accounted for over 97 % of the total number of bacteria. As shown in Fig. 2, after 1 month, the number of *Firmicutes* in three groups had all slightly changed, accounting for more than 50 % of the total amount of bacteria, which was the largest bacteriophyta in the gut. The numbers of *Bacteroidetes* in three groups had all changed. CK, NE and CS had an intestinal bacteroidetes abundance increase of 4.99, 7.58 and 8.07 %, respectively. The ranges of increased bacteroidetes abundance in taurine-treated groups were greater than those of the control group. Bacteroidetes were involved in polysaccharide, bile acid and cholesterol metabolism, carbohydrate fermentation, and the normal physiological intestine maintenance.

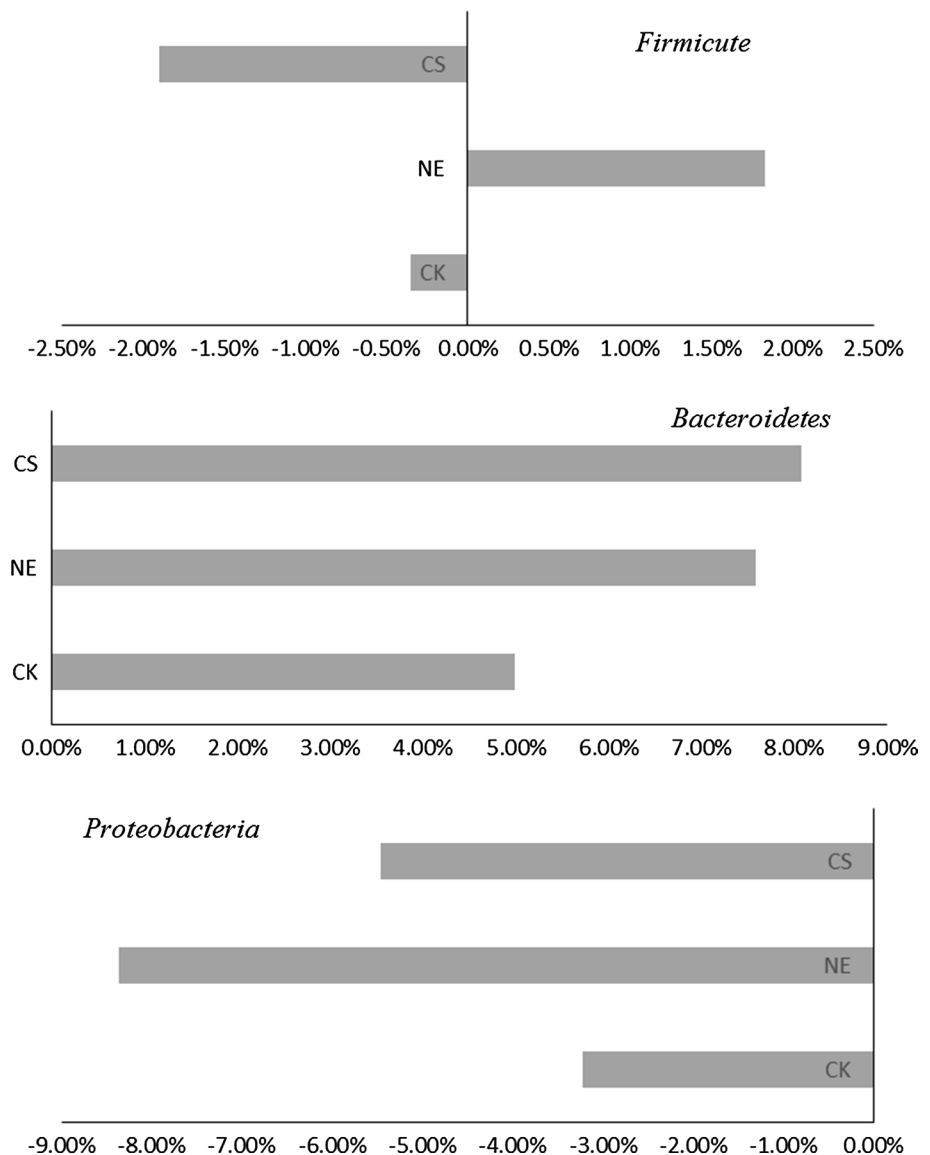
Therefore, taurine had positive effects on improving flora abundance and maintaining health. Meantime, the *Proteobacteria* abundance in CK, NE and CS reduced 3.22, 8.37 and 5.47 %, respectively. The ranges of the decreased *Proteobacteria* abundance in taurine-treated groups were much greater than those of the control group. Most *Proteobacteria* were known as pathogenic bacteria, hence taurine had a role in reducing the number of intestinal pathogenic bacteria and diseases prevention.

Distribution of gut microbiota on class levels

Table 3 showed the ratios of sample's flora abundance value to the total number of bacteria on class levels. The dominant mice intestinal bacteria classes were *Clostridia*, *Bacteroidia*, *Bacilli* and *Epsilonproteobacteria*. As shown in Fig. 3, the *Clostridia* abundance in CK, NE and CS reduced to 0.61, 2.65 and 10.9 %, respectively. Both natural taurine and synthetic taurine had certain effects on reduction of abundance of intestinal bacteria, and the amount of the decreased *Clostridia* in a synthetic taurine group was much greater than that of a natural taurine group. The *Bacteroidia* abundance in CK, NE and CS increased by 5, 7.59 and 8.08 %, respectively, indicating that the intake of taurine can improve the abundance values of *Bacteroidia* abundance and had positive effects on health promotion.

Bacilli, which were closely related to human health, belonged to gram-positive bacillus rod-shaped facultative bacteria. They can form spores, which had strong resistance to heat, dryness, radiation, chemical disinfectants and other physical and chemical factors. Bacilli abundance in a CK group did not change obviously, yet it was improved in a NE group and a CS group. This result proved that taurine had an effect of improving Bacilli abundance values.

Fig. 2 Ratio changes of *Firmicutes*, *Bacteroidetes* and *Proteobacteria* [CK ratio change between CK1 (feces of mice in CK group collected at the day before saline treatment) and CK2 (feces of mice in CK group collected at the 28th day after saline treatment); NE ratio change between NE1 (feces of mice in NE group collected at the day before natural taurine treatment) and NE2 (feces of mice in NE group collected at the 28th day after natural taurine treatment); CS ratio change between CS1 (feces of mice in CS group collected at the day before synthetic taurine treatment) and CS2 (feces of mice in CS group collected at the 28th day after synthetic taurine treatment)]



Epsilonproteobacteria are gram-negative bacteria, including a number of important pathogenic bacteria, such as *Escherichia coli*, probably a cause of food-poisoning bacteria. Bacilli abundance in CK, NE and CS decreased by 2.6, 7.31 and 4.11 %, respectively. Due to this phenomenon, it could be speculated that both natural taurine and synthetic taurine were effective to reduce the Bacilli abundance in intestine, which was consistent with the results above.

Distribution of gut microbiota on order levels

The ratios of sample's flora abundance value to the total number of bacteria on order levels were showed in Table 4. The dominant intestinal bacteria of mice were *Clostridiales*, *Bacteroidales*, *Campylobacterales*, and *Lactobacillales* in orders. As shown in Fig. 4, the *Clostridiales*

abundance in CK, NE and CS groups reduced to 0.61, 2.65 and 10.9 %, respectively. The change of *Clostridiales* in taurine-treated groups was greater than that in a CK group, and a CS group was shown up greater than a NE group. Moreover, the *Bacteroidales* abundance in CK, NE and CS groups increased by 5, 7.59 and 8.08 %, respectively. The increase latitude of *Bacteroidales* in taurine-treated groups was higher than that in the CK group. The *Campylobacterales* abundance in CK, NE and CS groups reduced 2.6, 7.31 and 4.11 %, respectively. The decreased range of *Campylobacterales* in taurine-treated groups was greater than that in the CK group, and the range of NE group was greater than that in the CS group. The *Lactobacillales* abundance in CK, NE and CS group increased 5.7, 7.9 and 12.87 %, respectively. The increased latitude of *Bacteroidales* in taurine-treated groups was greater than that in a CK group.

Table 3 Ratio of sample's flora abundance value to the total number of bacteria on class level

Bacteria class	CK1 ratio (%)	CK2 ratio (%)	NE1 ratio (%)	NE2 ratio (%)	CS1 ratio (%)	CS2 ratio (%)
<i>Clostridia</i>	36.53	35.92	44.17	41.52	53.17	42.27
<i>Bacteroidia</i>	31.38	36.38	24.06	31.65	24.2	32.28
<i>Bacilli</i>	15.53	15.79	11.2	15.78	8.58	17.72
<i>Epsilonproteobacteria</i>	12.58	9.98	16.28	8.97	9.82	5.71
<i>Unknown</i>	1.70	0.37	1.14	0.50	0.87	0.46
<i>Deltaproteobacteria</i>	0.88	0.31	1.66	0.41	1.41	0.19
<i>Erysipelotrichia</i>	0.13	0.13	0.17	0.07	0.32	0.18
<i>Coriobacteriia</i>	0.36	0.48	0.34	0.24	0.62	0.46
<i>Actinobacteria</i>	0.15	0.01	0.13	0.04	0.30	0
<i>Deferribacteres</i>	0.05	0.07	0.08	0.11	0.17	0.22
<i>Gammaproteobacteria</i>	0.06	0.01	0.12	0.35	0.09	0.01
<i>Unclassified</i>	0.14	0.13	0.19	0.07	0.08	0.09
<i>Alphaproteobacteria</i>	0.07	0.06	0.09	0.05	0.06	0.03
<i>Mollicutes</i>	0.33	0.30	0.24	0.21	0.1	0.31
<i>Spirochaetes</i>	0.07	0.05	0.01	0.01	0.12	0.04
<i>Verrucomicrobia</i>	0.02	0	0.10	0	0.02	0

CK1 feces of mice in CK group collected at the day before saline treatment, CK2 feces of mice in CK group collected at the 28th day after saline treatment, NE1 feces of mice in NE group collected at the day before natural taurine treatment, NE2 feces of mice in NE group collected at the 28th day after natural taurine treatment, CS1 feces of mice in CS group collected at the day before synthetic taurine treatment, CS2 feces of mice in CS group collected at the 28th day after synthetic taurine treatment

Distribution of gut microbiota on family levels

Table 5 showed the ratios of sample's flora abundance values to the total number of bacteria on family levels. The dominant mice intestinal bacteria's families were *Lachnospiraceae*, *Rikenellaceae*, *Porphyromonadaceae* and *Helicobacteraceae*. As shown in Fig. 5, the *Lachnospiraceae* abundance in CK and NE groups had little change, and reduced to 4.45 % in the CS group. *Lachnospiraceae* belongs to *Clostridiales*. The *Rikenellaceae* abundance in CK, NE and CS groups decreased to 4.45, 0.56 and 1.26 %, respectively, and the decrease range of *Rikenellaceae* in taurine-administered groups was smaller than that in the control group. *Rikenellaceae* belonged to *Bacteroidales*. The *Porphyromonadaceae* abundance in CK, NE and CS groups increased, and the latitude of increase was consistent. *Porphyromonadaceae* belonged to *Bacteroidales*. The *Helicobacteraceae* abundance in CK, NE and CS groups decreased to 2.6, 7.31 and 4.11 %, respectively, and the decreasing latitude of *Helicobacteraceae* in taurine-fed groups was larger than that in the control group.

Distribution of gut microbiota on genus levels

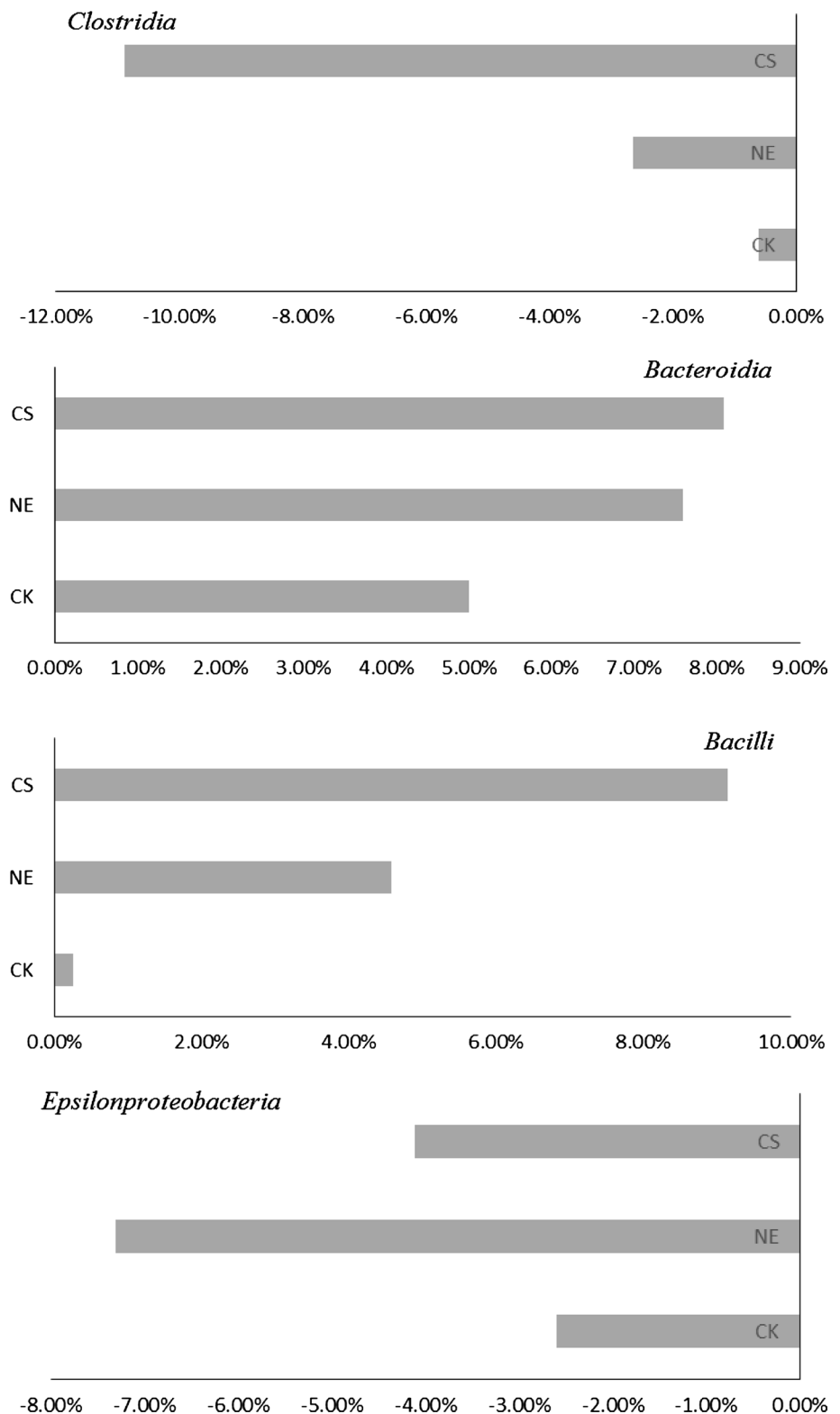
The ratios of sample's flora abundance values to the total number of bacteria on genus levels were showed in Table 6, and the dominant mice intestinal bacteria's genus were

Lachnospiraceae, *Porphyromonadaceae*, *Alistipes* and *Helicobacter*. *Alistipes* was a beneficial bacterium in the body. The *Alistipes* abundance decreased to 2.79 % in the CK group, while it increased to 1.30 and 0.97 % in NE and CS groups. It was demonstrated that taurine could improve the number of *Alistipes* bacteria in mice intestines. *Helicobacter* was intended to be an intestinal pathogenic bacterium. After the experimentation, the *Helicobacter* abundance in CK, NE and CS decreased to 2.6, 7.31 and 4.11 %, respectively, and the range of the decreased latitude of *Helicobacter* in taurine-fed groups was greater than that in the control group.

Distribution of gut microbiota on species levels

The ratio of sample's flora abundance values to the total number of bacteria on species level was shown in Table 7, and the dominant mice intestinal bacteria's genus were *uncultured bacterium* and *Helicobacter*. So, the distribution of *Helicobacter* was analyzed and presented in Fig. 6. The results showed that *Helicobacter* abundance in CK, NE and CS decreased to 2.18, 7.11 and 3.68 %, respectively. The decrease latitude of *Helicobacter* in NE and CS was stronger than that in the control group, and the latitude in the NE was greater than that of the CS group. Furthermore, this suggested that taurine could reduce the intestinal pathogenic bacteria and prevent diseases.

Fig. 3 Ratio changes of *Clostridia*, *Bacteroidia*, *Bacilli* and *Epsilonproteobacteria* [CK ratio change between CK1 (feces of mice in CK group collected at the day before saline treatment) and CK2 (feces of mice in CK group collected at the 28th day after saline treatment); NE ratio change between NE1 (feces of mice in NE group collected at the day before natural taurine treatment) and NE2 (feces of mice in NE group collected at the 28th day after natural taurine treatment); CS ratio change between CS1 (feces of mice in CS group collected at the day before synthetic taurine treatment) and CS2 (feces of mice in CS group collected at the 28th day after synthetic taurine treatment)]



Total content of short-chain fatty acids (SCFAs) in mice feces

SCFAs, also known as volatile fatty acids, mainly included acetic acid, propionic acid, isobutyric acid, butyric acid,

isovaleric acid and valeric acid. These short-chain fatty acids had a variety of physiological functions, such as providing energy, promoting the absorption of sodium, improving intestinal circulation and inhibiting the growth of pathogenic microorganisms (Bhutia and Ganapathy

Table 4 Ratio of sample's flora abundance value to the total number of bacteria on order level

Bacteria order	CK1 ratio (%)	CK2 ratio (%)	NE1 ratio (%)	NE2 ratio (%)	CS1 ratio (%)	CS2 ratio (%)
<i>Clostridiales</i>	36.53	35.92	44.17	41.52	53.17	42.27
<i>Bacteroidales</i>	31.38	36.38	24.06	31.65	24.2	32.28
<i>Campylobacteriales</i>	12.58	9.98	16.28	8.97	9.82	5.71
<i>Lactobacillales</i>	10	15.7	7.75	15.65	4.85	17.72
<i>Bacillales</i>	5.53	0.10	3.45	0.13	3.72	0
<i>Unknown</i>	1.7	0.37	1.14	0.50	0.87	0.48
<i>Desulfovibrionales</i>	0.88	0.31	1.66	0.41	1.41	0.19
<i>Coriobacteriales</i>	0.36	0.48	0.34	0.24	0.62	0.46
<i>Anaeroplasmatales</i>	0.33	0.3	0.24	0.21	0.09	0.3
<i>Erysipelotrichales</i>	0.13	0.13	0.17	0.07	0.32	0.18
<i>Rhizobiales</i>	0.04	0.05	0.08	0.04	0.05	0.03
<i>Verrucomicrobiales</i>	0.02	0	0.10	0	0.02	0
<i>Kineosporiales</i>	0.01	0	0	0	0	0
<i>Desulfovibrionales</i>	0.88	0.31	1.66	0.41	1.41	0.19
<i>Pasteurellales</i>	0.01	0.01	0	0	0.02	0.01
<i>Deferribacteriales</i>	0.05	0.07	0.08	0.11	0.17	0.22
<i>Corynebacteriales</i>	0.15	0	0.12	0.04	0.28	0
<i>Pseudomonadales</i>	0.02	0.01	0.02	0.12	0.01	0
<i>Spirochaetales</i>	0.07	0.05	0.01	0.01	0.12	0.04
<i>Burkholderiales</i>	0	0	0.02	0	0.03	0.01
<i>Rhodospirillales</i>	0.02	0.01	0.01	0.02	0.01	0
<i>Micrococcales</i>	0	0.01	0.01	0	0.01	0
<i>Rhodocyclales</i>	0	0	0	0	0.01	0

CK1 feces of mice in CK group collected at the day before saline treatment, CK2 feces of mice in CK group collected at the 28th day after saline treatment, NE1 feces of mice in NE group collected at the day before natural taurine treatment, NE2 feces of mice in NE group collected at the 28th day after natural taurine treatment, CS1 feces of mice in CS group collected at the day before synthetic taurine treatment, CS2 feces of mice in CS group collected at the 28th day after synthetic taurine treatment

2015; Asarat et al. 2015). The total content of SCFA in each group was shown in Fig. 7. SCFA contents in the NE feces was significantly higher than that in the CK and CS, but the difference was not sizeable among them. The natural taurine was superior to synthetic taurine in promoting the production of SCFAs in mice.

Concentrations of lipopolysaccharide (LPS) in mice serum

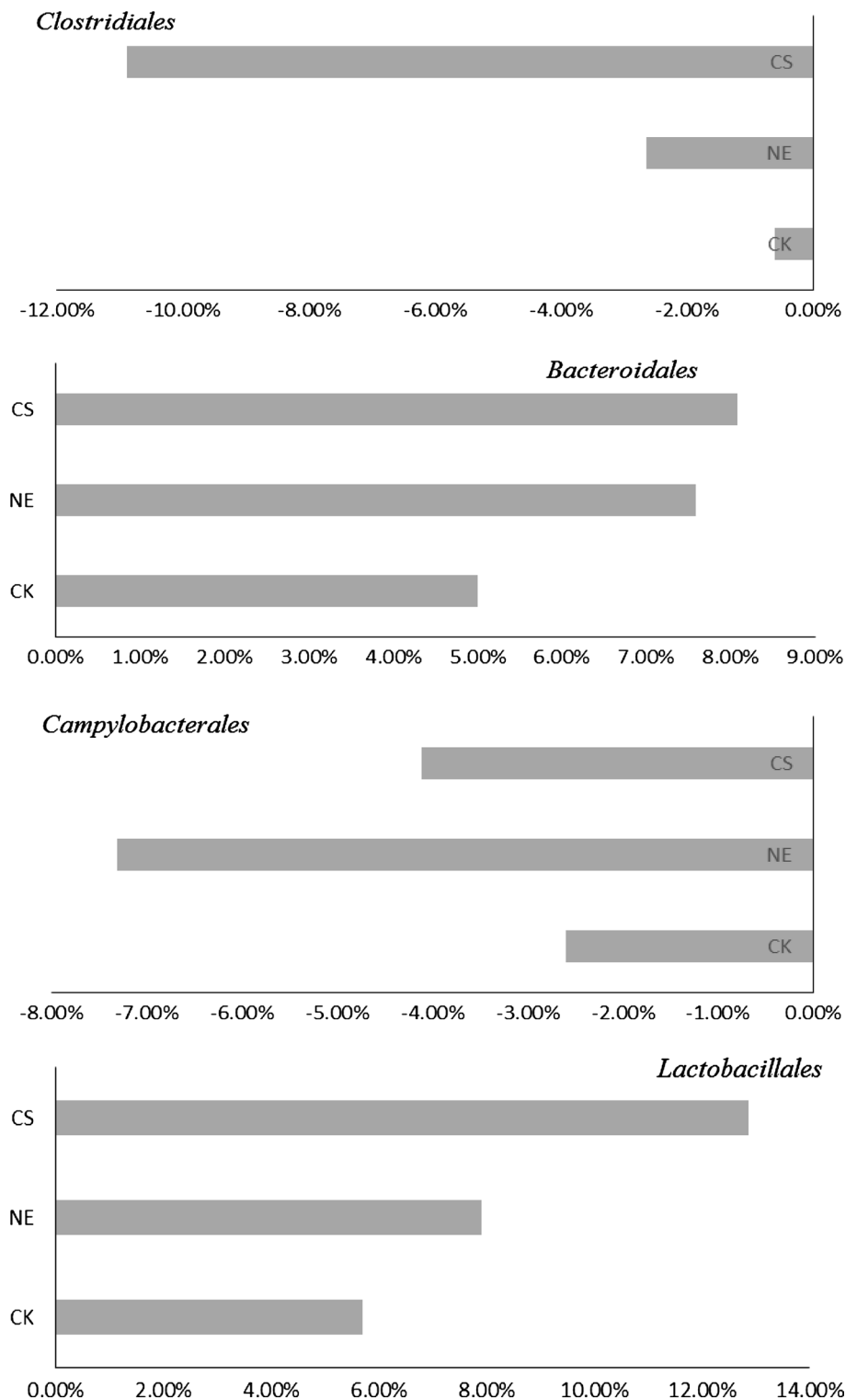
Lipopolysaccharide, also called endotoxin, was one of the main components in cell walls of gram-negative bacteria. The compounds formed from combination of lipopolysaccharide and some biological molecules could cause toxicity reaction (Okawa et al. 2015). Some studies suggested that LPS had harmful effects to liver and can induce liver cell apoptosis (Kim et al. 2015). The results of LPS contents in mice

were shown in Fig. 8. The LPS content in the serum of a NE group was significantly lower than that in CK and CS groups and there was no differences observed between CK and CS. Hence, the natural taurine could reduce the content of LPS in mice and relieve the liver damage while synthetic taurine was not illustrated a clear indication to have this effect.

Activity of superoxide dismutase (SOD) in mice serum and liver

Superoxide dismutase (SOD) was a biological active substance in the body. It had many kinds of physiological functions such as antioxidant, anti-aging, and immunity enhancement. The measured results of SOD activity in serum and liver were shown in Fig. 9. Based on the findings, the natural taurine and synthetic taurine could not exert significant effects on improving SOD activity ($p > 0.05$).

Fig. 4 Ratio changes of *Clostridiales*, *Bacteroidales*, *Campylobacterales* and *Lactobacillales* [CK ratio change between CK1 (feces of mice in CK group collected at the day before saline treatment) and CK2 (feces of mice in CK group collected at the 28th day after saline treatment); NE ratio change between NE1 (feces of mice in NE group collected at the day before natural taurine treatment) and NE2 (feces of mice in NE group collected at the 28th day after natural taurine treatment); CS ratio change between CS1 (feces of mice in CS group collected at the day before synthetic taurine treatment) and CS2 (feces of mice in CS group collected at the 28th day after synthetic taurine treatment)]



Conclusions

The natural taurine and the synthetic taurine could regulate the gut micro-ecology, which might be of benefit to health by inhibiting the growth of harmful bacteria. More

importantly, the natural taurine accelerated the production of SCFA and reducing LPS concentration, while the synthetic taurine was not. On the other hand, the natural taurine and synthetic taurine did not exert significant effects on SOD activities in serum and liver. The taurine might exert

Table 5 Ratio of sample's flora abundance values to the total number of bacteria on family level

Bacteria family	CK1 ratio (%)	CK2 ratio (%)	NE1 ratio (%)	NE2 ratio (%)	CS1 ratio (%)	CS2 ratio (%)
<i>Lachnospiraceae</i>	26.46	27.61	32.88	32.64	36.24	32.25
<i>Rikenellaceae</i>	15.4	10.95	11.2	10.64	12.17	10.91
<i>Porphyromonadaceae</i>	12.07	18.56	7.79	13.7	8.98	15.66
<i>Helicobacteraceae</i>	12.58	9.98	16.28	8.97	9.82	5.71
<i>Ruminococcaceae</i>	8.46	7.18	9.80	7.79	15.19	8.63
<i>Desulfovibrionaceae</i>	0.88	0.31	1.66	0.41	1.41	0.19
<i>Bacteroidaceae</i>	2.09	3.82	3.34	4.92	1.38	3.05
<i>Prevotellaceae</i>	1.82	3.05	1.73	2.39	1.67	2.67
<i>Gracilibacteraceae</i>	0.02	0.06	0.02	0	0.01	0.05
<i>Lactobacillaceae</i>	2.54	15.5	6.82	15.39	3.40	17.64
<i>Streptococcaceae</i>	0.12	0.1	0.41	0.06	0.38	0.07
<i>Aerococcaceae</i>	7.08	0.09	0.50	0.16	0.96	0
<i>Comamonadaceae</i>	0	0	0	0	0.02	0.01
<i>Corynebacteriaceae</i>	0.15	0	0.12	0.04	0.28	0
<i>Enterobacteriaceae</i>	0.02	0	0.1	0.23	0.04	0
<i>Methylobacteriaceae</i>	0.01	0.01	0	0	0	0
<i>Deferribacteraceae</i>	0.05	0.07	0.08	0.11	0.17	0.22
<i>Neisseriaceae</i>	0	0	0	0	0	0
<i>Chitinophagaceae</i>	0	0	0	0	0	0
<i>Bifidobacteriaceae</i>	0	0	0	0	0.01	0
<i>Planctomycetaceae</i>	0	0	0	0	0	0
<i>Deftuviitaleaceae</i>	0.42	0.23	0.62	0.27	0.70	0.30
<i>Leptotrichiaceae</i>	0	0	0	0	0	0
<i>Leuconostocaceae</i>	0	0	0	0	0	0
<i>Alcaligenaceae</i>	0	0	0.01	0	0.01	0
<i>Staphylococcaceae</i>	5.49	0.10	3.45	0.13	3.56	0
<i>Peptococcaceae</i>	0.11	0.07	0.08	0.12	0.16	0.14
<i>Anaeroplasmataceae</i>	0.33	0.30	0.24	0.21	0.09	0.30
<i>Peptostreptococcaceae</i>	0.06	0.05	0.08	0.02	0.09	0.03
<i>Coriobacteriaceae</i>	0.36	0.48	0.34	0.24	0.62	0.46
<i>Christensenellaceae</i>	0.74	0.55	0.46	0.55	0.5	0.65
<i>Methanosaetaceae</i>	0	0	0	0	0	0
<i>Unclassified</i>	0.14	0.13	0.19	0.07	0.08	0.09
<i>Rhodocyclaceae</i>	0	0	0	0	0.01	0
<i>Erysipelotrichaceae</i>	0.13	0.13	0.17	0.07	0.32	0.18
<i>Verrucomicrobiaceae</i>	0.02	0	0.10	0	0.02	0
<i>Rhodobiaceae</i>	0.03	0.04	0.08	0.04	0.03	0.02
<i>Spirochaetaceae</i>	0.07	0.05	0.01	0.01	0.12	0.04
<i>Rhodospirillaceae</i>	0.01	0.01	0.01	0.02	0.01	0
<i>Unknown</i>	1.70	0.37	1.14	0.50	0.88	0.48
<i>Enterococcaceae</i>	0.02	0.01	0	0.02	0.07	0
<i>Pasteurellaceae</i>	0.01	0.01	0	0	0.02	0.01
<i>Hyphomicrobiaceae</i>	0	0	0.01	0	0	0

CK1 feces of mice in CK group collected at the day before saline treatment, CK2 feces of mice in CK group collected at the 28th day after saline treatment, NE1 feces of mice in NE group collected at the day before natural taurine treatment, NE2 feces of mice in NE group collected at the 28th day after natural taurine treatment, CS1 feces of mice in CS group collected at the day before synthetic taurine treatment, CS2 feces of mice in CS group collected at the 28th day after synthetic taurine treatment

Fig. 5 Ratio changes of *Lachnospiraceae*, *Rikenellaceae*, *Porphyromonadaceae* and *Helicobacteraceae* [CK ratio change between CK1 (feces of mice in CK group collected at the day before saline treatment) and CK2 (feces of mice in CK group collected at the 28th day after saline treatment); NE ratio change between NE1 (feces of mice in NE group collected at the day before natural taurine treatment) and NE2 (feces of mice in NE group collected at the 28th day after natural taurine treatment); CS ratio change between CS1 (feces of mice in CS group collected at the day before synthetic taurine treatment) and CS2 (feces of mice in CS group collected at the 28th day after synthetic taurine treatment)]

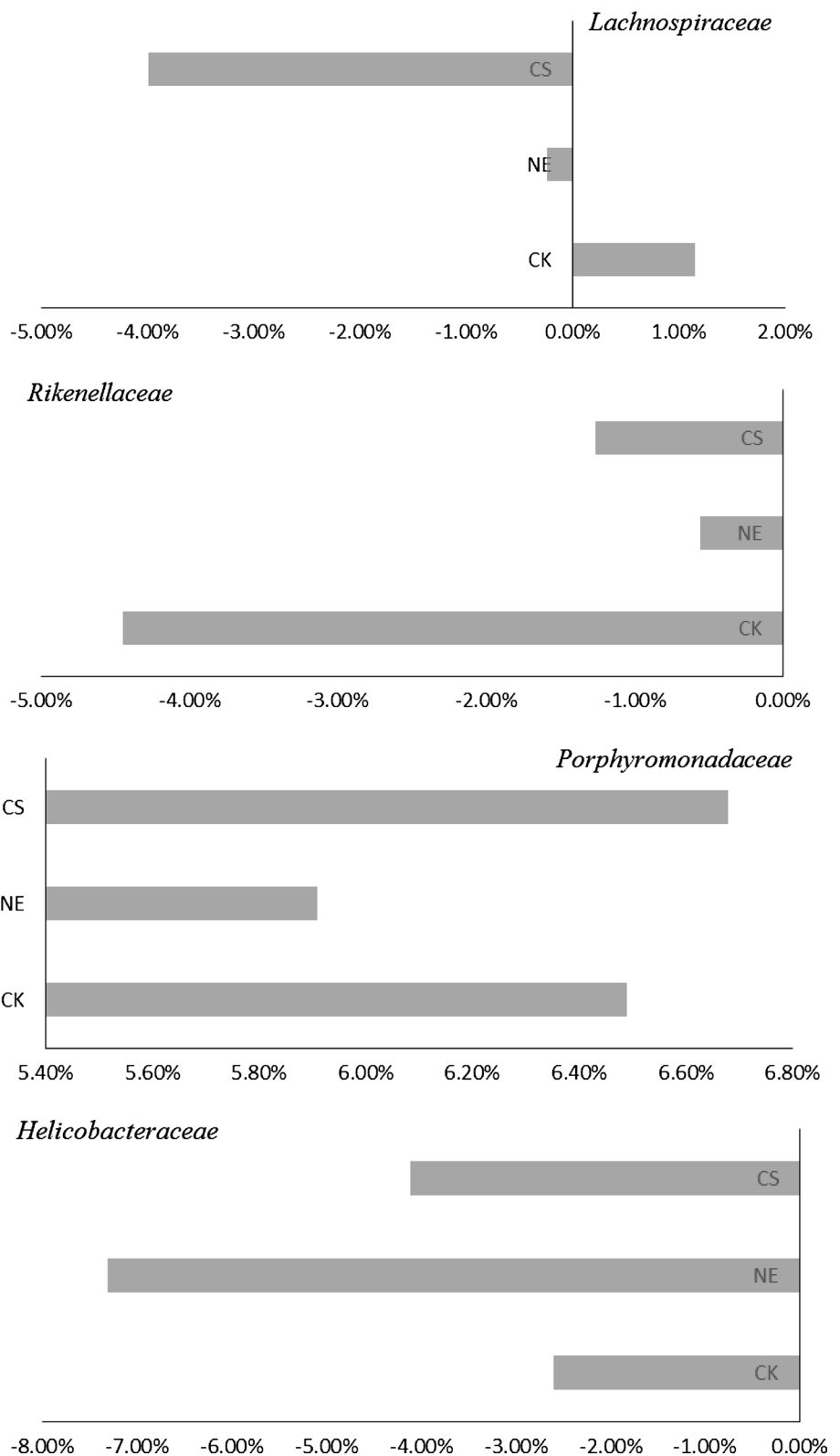


Table 6 Ratio of sample's flora abundance value to the total number of bacteria on genus level

Bacteria genus	CK1 ratio (%)	CK2 ratio (%)	NE1 ratio (%)	NE2 ratio (%)	CS1 ratio (%)	CS2 ratio (%)
<i>Unclassified_Lachnospiraceae</i>	12.48	12.38	13.51	14.99	17.14	14.57
<i>Unclassified_Porphyrmonadaceae</i>	10.31	15.88	5.48	11.33	7.49	14.64
<i>Alistipes</i>	12.31	9.52	8.21	9.51	9.14	10.11
<i>Helicobacter</i>	12.58	9.98	16.28	8.97	9.82	5.71
<i>Blautia</i>	8.29	10.64	12.14	10.53	11.93	11.25
<i>Lactobacillus</i>	2.54	15.5	6.82	15.39	3.4	17.64
<i>Bacteroides</i>	2.09	3.82	3.34	4.92	1.38	3.05
<i>Rikenella</i>	1.6	0.62	1.54	0.69	1.29	0.33
<i>Alloprevotella</i>	1.19	2.22	1.49	1.76	1.34	1.94
<i>Intestinimonas</i>	0.06	0.09	0.07	0.1	0.19	0.09
<i>Parabacteroides</i>	0.72	0.21	1.16	0.14	0.49	0.1
<i>RC9</i>	1.49	0.81	1.45	0.44	1.74	0.47
<i>Anaerotruncus</i>	0.78	0.65	1.23	0.77	1.67	0.52
<i>Paraprevotella</i>	0.05	0.13	0.06	0.06	0.12	0.17
<i>Unclassified_Christense</i>	0.73	0.52	0.45	0.54	0.47	0.65
<i>Ruminococcus</i>	0.37	0.18	0.27	0.11	0.22	0.29
<i>Faecalibacterium</i>	0.03	0.01	0.01	0.04	0.04	0.03
<i>Coprococcus</i>	0.23	0.51	0.63	0.58	0.36	0.41
<i>Defluviitaleaceae_IncertaeSedis</i>	0.07	0.06	0.17	0.07	0.15	0.06
<i>Lactococcus</i>	0.01	0.01	0.11	0	0.04	0.01
<i>Mucispirillum</i>	0.05	0.07	0.08	0.11	0.17	0.22
<i>Acetatifactor</i>	0.11	0.08	0.08	0.06	0.08	0.08
<i>Desulfovibrio</i>	0.84	0.28	1.58	0.37	1.26	0.17
<i>Prevotella</i>	0.02	0.05	0.02	0.05	0	0.05
<i>Roseburia</i>	3.21	1.34	3.74	3.5	3.07	2.54
<i>Staphylococcus</i>	3.05	0.09	2.14	0.05	1.35	0
<i>Unclassified_Family</i>	0.01	0.02	0.03	0.01	0.05	0.03
<i>Bilophila</i>	0.04	0.03	0.07	0.05	0.13	0.01
<i>Enterorhabdus</i>	0.36	0.48	0.34	0.24	0.62	0.46
<i>Sedimentibacter</i>	0.06	0.05	0.04	0.04	0.05	0.06
<i>Unclassified_Ruminococcaceae</i>	4.94	3.86	5.14	4.10	8.46	5.18
<i>Candidatus</i>	1.83	0.42	1.23	0.56	0.99	0.53
<i>Serratia</i>	0.02	0	0.02	0.01	0.01	0
<i>Marvinbryantia</i>	0.09	0.18	0.06	0.2	0.22	0.28
<i>Unclassified</i>	0.14	0.13	0.19	0.07	0.08	0.09
<i>Odoribacter</i>	1.03	2.44	1.15	2.23	1.01	0.91
<i>Ruminococcaceae_Incertae_Sedis</i>	1.75	1.58	2.42	1.86	2.94	1.69
<i>Pedobacter</i>	0.01	0	0.01	0	0	0
<i>Enterococcus</i>	0.02	0.01	0	0.02	0.04	0
<i>Escherichia-Shigella</i>	0	0	0.08	0.19	0.02	0
<i>Treponema</i>	0.07	0.05	0.01	0.01	0.12	0.04
<i>Actinobacillus</i>	0	0.01	0	0	0	0.01

CK1 feces of mice in CK group collected at the day before saline treatment, CK2 feces of mice in CK group collected at the 28th day after saline treatment, NE1 feces of mice in NE group collected at the day before natural taurine treatment, NE2 feces of mice in NE group collected at the 28th day after natural taurine treatment, CS1 feces of mice in CS group collected at the day before synthetic taurine treatment, CS2 feces of mice in CS group collected at the 28th day after synthetic taurine treatment

Table 7 Ratio of sample's flora abundance value to the total number of bacteria on species level

Bacteria species	CK1 ratio (%)	CK2 ratio (%)	NE1 ratio (%)	NE2 ratio (%)	CS1 ratio (%)	CS2 ratio (%)
<i>Uncultured_bacterium</i>	79.6	85.11	77.08	85.56	82.04	91.21
<i>Helicobacter_ganmani</i>	11.50	9.32	15.35	8.42	8.77	5.09
<i>Uncultured_Bacteroidales_bacterium</i>	1.09	1.17	1.37	1.01	0.76	0.73
<i>Firmicutes_bacterium_K4</i>	2.42	0.01	1.28	0.07	2.18	0
<i>Streptococcus_acidominimus</i>	0.02	0	0	0	0.02	0
<i>Uncultured_Acinetobacter_sp.</i>	0.02	0	0.01	0.02	0.01	0
<i>Uncultured_soil_bacterium</i>	0	0	0	0.01	0	0
<i>Clostridium_sp._Culture-1</i>	0.01	0.01	0.03	0.01	0.1	0.01
<i>Aerococcus_urinaeequi</i>	0.11	0	0.02	0	0	0
<i>Desulfovibrio_sp._3_1_syn3</i>	0.05	0.02	0.07	0	0.12	0
<i>Clostridium_carnis</i>	0.01	0	0	0	0	0
<i>Gracilibacter_thermotolerans</i>	0.01	0.05	0.02	0	0.01	0.01
<i>Lactobacillus_hominis</i>	0	0.01	0	0	0	0
<i>Uncultured_prokaryote</i>	0.04	0.02	0.02	0.03	0.01	0.02
<i>Vagococcus_sp._SIX2(2011)</i>	0.01	0	0	0	0.03	0
<i>Uncultured_Firmicutes_bacterium</i>	0.01	0	0	0	0	0
<i>Lachnospiraceae_bacterium_3-1</i>	0.01	0	0.03	0.01	0	0
<i>Clostridium_leptum</i>	0.02	0.04	0.09	0.16	0.13	0.06
<i>Lachnospiraceae_bacterium_A4</i>	0	0.02	0	0.04	0.01	0.01
<i>Uncultured_Helicobacter_sp.</i>	0.47	0.44	0.79	0.43	0.84	0.28
<i>Mucispirillum_sp._69</i>	0.04	0.07	0.07	0.11	0.14	0.20
<i>Uncultured_organism</i>	0.14	0.14	0.29	0.14	0.21	0.04
<i>Corynebacterium_stationis</i>	0	0	0	0.01	0	0
<i>Uncultured_bacterium_CA26</i>	0.09	0.03	0.08	0	0.07	0.03
<i>Sporosarcina_sp._MPI(2011)</i>	0.02	0	0	0	0	0
<i>Pasteurella_pneumotropica</i>	0.01	0	0	0	0.02	0
<i>Swine_fecal_bacterium_RF2B-Xyl3</i>	0	0.01	0	0	0.02	0
<i>Corynebacterium_urealyticum</i>	0.01	0	0	0	0	0
<i>Unidentified</i>	1.67	2.51	1.73	2.35	2.75	1.44
<i>Actinobacillus_muris</i>	0	0.01	0	0	0	0.01
<i>Lachnospiraceae_bacterium_615</i>	0.02	0.07	0.03	0.02	0.05	0.12
<i>Atopostipes_suicloacalis</i>	0.18	0.01	0.01	0	0.03	0
<i>Bacteroides_sartorii</i>	0.01	0.1	0.04	0.19	0.02	0.03
<i>Lachnospiraceae_bacterium_COE1</i>	0.02	0.03	0.01	0.05	0.09	0.05
<i>Uncultured_Rikenellaceae_bacterium</i>	0	0.03	0.01	0	0.04	0
<i>Mouse_gut_metagenome</i>	0.1	0.03	0.16	0.01	0.10	0
<i>Lactobacillus_reuteri_ATCC_53608</i>	0	0	0	0.01	0.01	0
<i>Unclassified</i>	0.14	0.13	0.19	0.07	0.08	0.09
<i>Bacterium_enrichment_culture_clone_Ecwsrb007</i>	0	0	0.01	0	0	0
<i>Uncultured_Clostridiales_bacterium</i>	0.26	0.18	0.59	1.00	0.43	0.07
<i>Eubacterium_plexicaudatum_ASF492</i>	0.01	0.01	0	0	0.04	0
<i>Uncultured_Desulfovibrionales_bacterium</i>	0.02	0	0.08	0	0.05	0.01
<i>Uncultured_Porphyrionadaceae_bacterium</i>	0	0	0.01	0.01	0	0
<i>Alistipes_timonensis_JC136</i>	0.02	0.03	0	0.02	0	0
<i>Uncultured_crenarchaeote</i>	0	0	0	0	0	0.03
<i>Porphyromonas_sp._M110-1288x</i>	0	0.02	0	0	0	0
<i>Uncultured_rumen_bacterium</i>	0.09	0.07	0.03	0.04	0.15	0.05
<i>Clostridium_papyrosolvens</i>	0.10	0	0.08	0.07	0	0

Table 7 continued

Bacteria species	CK1 ratio (%)	CK2 ratio (%)	NE1 ratio (%)	NE2 ratio (%)	CS1 ratio (%)	CS2 ratio (%)
<i>Staphylococcus_sp._Y3</i>	1.02	0.07	0.21	0	0.32	0

CK1 feces of mice in CK group collected at the day before saline treatment, CK2 feces of mice in CK group collected at the 28th day after saline treatment, NE1 feces of mice in NE group collected at the day before natural taurine treatment, NE2 feces of mice in NE group collected at the 28th day after natural taurine treatment, CS1 feces of mice in CS group collected at the day before synthetic taurine treatment, CS2 feces of mice in CS group collected at the 28th day after synthetic taurine treatment

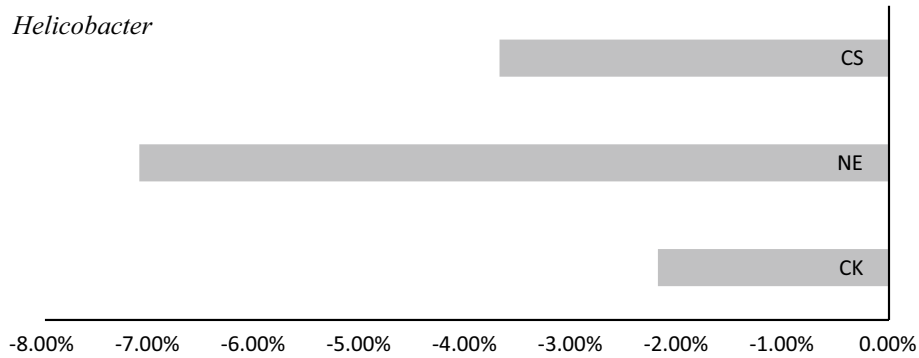


Fig. 6 Ratio change of *Helicobacter* [CK ratio change between CK1 (feces of mice in CK group collected at the day before saline treatment) and CK2 (feces of mice in CK group collected at the 28th day after saline treatment); NE ratio change between NE1 (feces of mice in NE group collected at the day before natural taurine treatment) and

NE2 (feces of mice in NE group collected at the 28th day after natural taurine treatment); CS ratio change between CS1 (feces of mice in CS group collected at the day before synthetic taurine treatment) and CS2 (feces of mice in CS group collected at the 28th day after synthetic taurine treatment)]

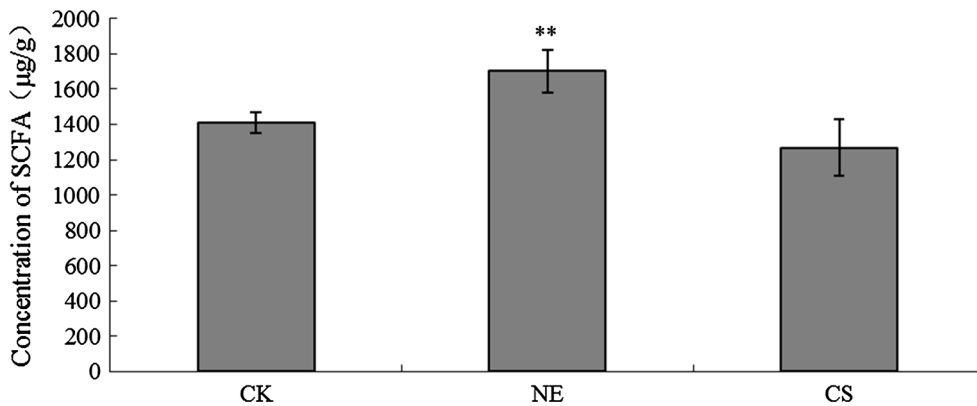


Fig. 7 Concentrations of SCFA in mice feces (CK feces of mice in CK group collected at the 28th day after saline treatment; NE feces of mice in NE group collected at the 28th day after natural taurine treat-

ment; CS feces of mice in CS group collected at the 28th day after synthetic taurine treatment; ** $p < 0.01$; $n = 10$)

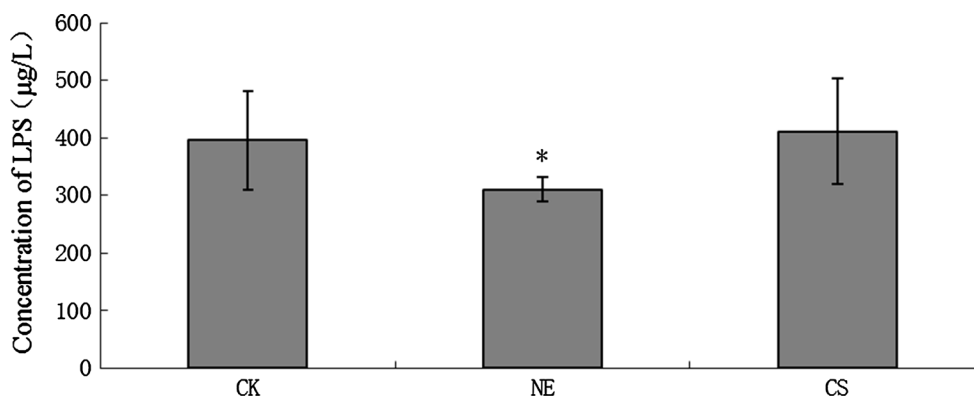
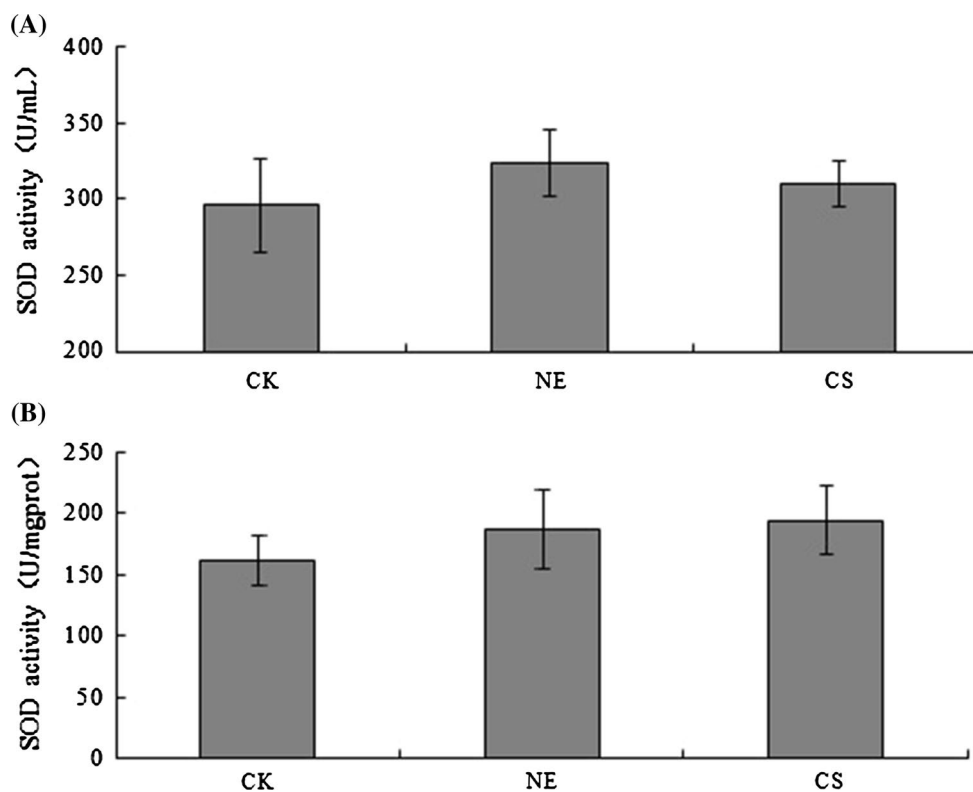


Fig. 8 Concentrations of LPS in mice serum (CK feces of mice in CK group collected at the 28th day after saline treatment; NE feces of mice in NE group collected at the 28th day after natural taurine treat-

ment; CS feces of mice in CS group collected at the 28th day after synthetic taurine treatment; * $p < 0.05$; $n = 10$)

Fig. 9 SOD activity in mice serum and liver (CK feces of mice in CK group collected at the 28th day after saline treatment; NE feces of mice in NE group collected at the 28th day after natural taurine treatment; CS feces of mice in CS group collected at the 28th day after synthetic taurine treatment; **a** SOD activity in mice serum; **b** SOD activity in mice liver; $n = 10$)



effects by changing gut microbiota and relative metabolites such as SCFA and LPS. To sum up, taurine should be considered as a healthy nutrient in body.

Compliance with ethical standards

Funding There is no fund support for this research.

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the guidelines issued by the Ethical Committee of Zhejiang University (SYXK(zhe)2012-0178).

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