Choline and Bile Acids Mixture Improved Growth Performance and Intestinal Immunity via Altering Gut Microbiota and Bacterial Metabolites in the Colon of Weaned Piglets.

Yueqin Qiu
College of Animal Science, South China Agricultural University https://orcid.org/0000-0002-6989-7026
Kebiao Li
Institute of animal science, Guangdong science academy of agricultural sciences
Shilong Liu
Institute of animal science, Guangdong academy of agricultural sciences
Li Wang
Institute of animal science, Guangdong academy of agricultural sciences
Kaiguo Gao
Institute of animal science, Guangdong academy of agritulture sciences
Xuefen Yang (yangxuefen@gdaas.cn)
Institute of animal science, Guangdong academy of agricultural sciences
Zongyong Jiang (jiangzy@gdaas.cn)
Institute of animal science, Guangdong academy of agricultural sciences

Research

Keywords: Choline, bile acids, growth performance, intestinal immunity, gut microbiota, bacterial metabolites, weaned piglets

Posted Date: June 5th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32927/v1

Abstract

Background: Choline or bile acids has many beneficial roles in physiological function. However, little was known about growth performance, intestinal mucosal function and microbiota-host interactions of weaned piglets in response to choline or bile acids supplementation. This study aimed to investigate the effect of choline and bile acids mixtures (ChB) supplementation on growth performance, intestinal mucosal barrier function, gut microbiota and bacterial metabolites of weaned piglets. One hundred and twenty-eight crossbred (Duroc × Landrace × Large White) weaned piglets (initial body weight: approximately 8 kg; 21 d of age) were randomly allocated to four different dietary treatments(a control diet (Control) and the other three groups were control diet supplemented with 800 mg/kg choline chloride (choline), 500 mg/kg bile acids (bile acids) or 800 mg/kg choline chloride plus 500 mg/kg bile acids (ChB), respectively) and for 28-d feeding trail.

Results: ChB significantly increased average daily gain (ADG) and reduced feed/gain (F/G) ratio, associated with elevation of lipase activity and total bile acids level in ileal digesta compared with control diet. Additionally, ChB altered colonic microbiota by increasing the relative abundance of Lactobacillus and Faecalibacterium , and decreasing the relative abundances of unidentified-Clostridiales , Parabacteroides and Unidentified-Ruminococcaceae , when compared with control diet. Meanwhile, ChB increased the butyrate level and decreased the production of bile acid profiles in the colonic digesta. Besides, feeding ChB improved gut immunity, as reflected by increasing the abundance of IL-10 , FXR and mucin2 transcript, while downregulated expression of TLR4 , MyD88 , NF-κBp65 and TNF-α genes in the intestinal mucosa. Quantitative proteomics of jejunal mucosa further showed that ChB regulated the proteins that were related to inflammatory response. Furthermore, the changes in the ADG and genes expression were associated with alteration of gut microbiota composition and their metabolites.

Conclusions: Collectively, our findings demonstrated that choline and bile acids mixture may improve the growth performance and intestinal immune response of weaned piglets through alteration of gut microbiota composition and bacterial metabolites, which promoted gut health.

Background

It has been demonstrated that weaning stress could cause intestinal inflammation and intestinal dysfunction in piglets, leading to diarrhea, poor growth of weaned piglets [1,2]. Antibiotics that efficiently improved growth performance and reduced the diarrhea of weaned piglets, is no longer feasible due to the increasing bacterial resistance and public health concerns [3]. As a result of antibiotics prohibition recently, an urgent need to find potential alternative for safeguarding general health of weaned piglets.

As a crucial dietary nutrient, choline has many beneficial roles in physiological function. The well-known function of choline was to emulsify fats, which extended the contact surface between lipase and fats, and accelerated lipids metabolism in the small intestine [4]. Besides, several studies recently showed that choline also exerted an anti-inflammatory effect. Dietary choline inhibited the expressions of pro-inflammatory biomarkers including TNF-α, IL-1β and NF-κB, while increased the abundance of anti-

inflammatory cytokine genes in fish models [5,6]. To our knowledge, however, evidence to confirm the effect of dietary choline on the intestinal mucosal barrier function of mammal, especially weaned piglets was scarce.

Bile acids were produced in the liver and then released into small intestine after feed intake to promote emulsification of fats and accelerate lipids digestion and absorption. A majority of bile acids was reuptake in the ileum, while a small amount would reach to the colon and metabolized by resident microbiota [7].The major functions of bile acids has been shown to assist the intestinal absorption of lipids, to maintain homeostasis between gut microbiota and intestinal mucosal barrier [8]. A previous study in fish revealed that bile acid supplementation promoted lipids utilization and improve fish growth performance and feed efficiency [9]. In addition, several studies demonstrated that administration single bile acid (deoxycholic acid or chenodeoxycholic acid) could alleviate gut-associated inflammation and improve gut health, but without effect on the growth performance of weaned piglets [10,11]. Nevertheless, no established study has reported the effects of bile acids mixture on the growth performance and intestinal immune response of weaned piglets. Besides, secreted bile acids by weaned piglets were in small amounts and have limited ability to emulsify dietary fats [12]. Consequently, it may be a potential feeding strategy to supplement bile acids into diet for increasing fats utilization as well as maintaining gut health in weaned piglets.

In recently decade, microbe-host crosstalk is a particularly popular area. Gut microbiota, rapidly affected by feed dietary, play a critical role in nutritional digestion and host metabolism in human and other mammals [13,14]. As a substrate shared by the host and the gut microbiota, diet impacted host physiology through numerous bioactive bacterial metabolites. Previous studies have demonstrated the importance of bacterial metabolites such as short-chain fatty acids and bile acids for regulation of inflammatory [15,16]. Despite the potential of gut microbiota to modulate bioavailability of dietary choline and bile acids [17,18], only few studies have investigated the effect of dietary choline or bile acid supplementation on the gut microbial ecosystem [19,20].

Taken together, the data introduced above showed that little was known about growth performance, intestinal mucosal function and microbiota-host interactions of weaned piglets in response to choline or bile acids supplementation. Here, we focused on determining the effects of dietary supplementation with choline, bile acids or the two conjugates on the growth performance, microbial composition, bacterial metabolite profiles and the expression of proteins/genes related to innate immune response in the intestine.

Materials And Methods

All animal procedures used in this study were approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences (authorization number GAASIAS-2016-017). All efforts were made to minimize any suffering of animals, following the Guidelines for the Care and Use of Animals for Research and Teaching.

Animals and diets.

A total of 128 weaned pigs (Duroc × Landrace × Yorkshire × Pietrain, 21 d of age) with an initial body weight of 8 kg were randomly allocated to 4 dietary treatments with 8 replicate pens per treatment, each pen containing 2 barrows and 2 gifts with a complete randomized experimental design. Control piglets (Control) were fed a basal diet, and the other groups were fed basal diet supplemented with 800 mg/kg choline chloride (choline), basal diet supplemented with 500 mg/kg bile acids (bile acids) or basal diet supplemented with 800 mg/kg choline chloride plus 500 mg/kg bile acids (ChB) for a 28-d feeding trial. The basal diet based on pig body weight was formulated to meet nutrient recommendation of the National Research Council (NRC, 2012), and the compositions of the basal diet and nutrient profile were presented in Table 1. All pigs had free access to feed and water throughout. At the end of study, the ADG, average daily feed intake (ADFI) and F/G ratio were calculated. One pig from each pen with medium bodyweight was selected and sacrificed after anesthesia with sodium pentobarbital (40 mg/kg BW). Digesta of intestine and colon, and craped mucosal samples of the middle duodenum, the middle jejunum, distal ileum and colon were immediately collected and frozen in liquid nitrogen, and then stored at -80°C.

Ingredient composition	Content (%)	Calculated nutrient profile	Content
Corn	28.72	DE□kcal/kg	3700
Expanded corn	10.00	Total CP%	22.9
Fermented soybean meal	10.00	SID CP%	18.82
De-hulled soybean meal	11.00	SID Lys%	1.49
Fishmeal	5.37	SID Met%	0.52
Whey protein concentrate	5.00	SID Met+Cys%	0.8
Low protein whey powder	15.00	SID Thr%	0.87
Sucrose	2.00	SID Trp%	0.25
Yeast extract	2.00	Total P%	0.69
Soybean oil	4.35	STTD P%	0.49
NaCl	0.55	Ca%	0.82
Calcium hydrophosphate	1.10	Na %	0.38
Calcium citrate	0.50	Cl%	0.68
L-Lysine HCl	0.28	SID Lys / DE, g/Mcal	4.03
DL-Methionine	0.16		
L-Threonine	0.10		
Phytase	0.02		
Complex acid	0.50		
Vitamin and mineral premix*	0.28		
Antioxidants	0.02		
Mildew preventive	0.06		
Complex Enzyme	0.10		
Tributyrin	0.10		
ZnO	0.18		
TiO2	0.40		
Vitamin E	0.01		
Limestone	2.20		
Total	100.00		

* Supplied per kilogram of complete diet□Fe 120 mg, Cu 10 mg, Zn 120 mg, Mn 35 mg, I 0.25 mg, Se 0.2 mg, VA 8000 IU, VD3 1000 IU, VE 30 mg, VK3 2 mg, VB1 2 mg, VB2 6 mg, VB6 4.0 mg, VB12 0.02 mg, niacin 25 mg, calcium pantothenate 10 mg, folic acid 1.0 mg, biotin 0.25 mg.

Table 1
Compositions of Basal Diet and Nutrient Profile.

Analysis of biochemical variables in intestinal digesta

The concentrations of lipase activity, total bile acids, non-free fatty acid (NEFA), total cholesterol (T-CHO) and triglyceride (TG) in digesta of duodenum, jejunum and ileum were measured using commercial kits purchased from the Nanjing Jiancheng Institute of Bioengineering, China.

Determination of SCFA concentrations

Colon content samples from weaned piglets were determined the concentrations of acetic acid, propionic acid and butyric acid by gas chromatography using a GCMS-7890B-7000D Ultra instrument (Agilent Technologies Inc.). Briefly, about 0.1 g of colon digesta of each sample was diluted with 2 mL of 25% metaphosphoric acid solution and vortexed for 2 min until the mixture was homogenized, and then 2 mL diethyl ether was added for 10 min extraction. Afterwards, this mixture was centrifuged at 4000g for 10 min at 4 °C. The collected supernatant was filtered through a 0.22-mm membrane and then analyzed using gas chromatography. Besides, an external standard calibration was used to determine the concentration of each SCFA.

Gut microbiome

Total DNA of each sample was extracted using the a QIAamp PowerFecal DNA Kit (QIAGEN, Hilden, Germany) following the manufacturer’s instructions. The DNA concentration and quality of each sample were detected using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, United States). The genes of all bacterial 16S rRNA covering V3–V4 region were amplified with a universal forward primer 338F (50-ACTCCTRCGGGAGGCAGCAG-30) and a reverse primer 806R (50-GGACTACCVGGGTATCTAAT-30). PCR amplicons were purified using the Qiagen Gel Extraction Kit (Qiagen, Germany) in accordance with the manufacturer’s instructions. Sequencing libraries were generated using a TruSeq® DNA PCR-Free sample preparation kit (Illumina, USA) according to the manufacturer's recommendations, and the index codes were added. The quality of library was then evaluated by a Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, Carlsbad, CA, United States) and an Agilent Bioanalyzer 2100 system. The library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated. Bioinformatics analysis was conducted following a recent study [21].

Quasi-targeted metabolomic analysis of bile acid profiles in colonic digesta

100 mg of colonic digesta were grinded with liquid nitrogen and then 500μL prechilled solution containing 80% methanol and with 0.1% formic acid was added. This lysate was well vortexed until the

mixture was homogenized. The homogenate was incubated on ice for 5 min and then were centrifuged for 10 min (at 15000 rpm, 4°C). Then the obtained supernatant was diluted with LC-MS grade water (2:1, v/v)) and centrifuged at 15000 g for 20 min at 4°C. After centrifugation, the supernatant was collected and used for LC-MS analysis. The raw HPLC-MS/MS data were processed using the SCIEX O. After normalization to total peak intensity, the processed data were performed at metaX, where can flexibly and comprehensively processed metabolomics data. The variable importance in the projection (VIP) value of each variable in the OPLS-DA model was calculated to evaluate its contribution to the classification. Univariate analysis (t-test) was applied to calculate the statistical significance (p value). The metabolites with VIP > 1 and P-value< 0.05 and fold change≥2 or FC≤0.5 were considered as significantly differential metabolites. R language package was used to plot clustering heat maps after the data were normalized using z-scores of the intensity areas of bile acids profile.

Quantitative Real-time PCR (qPCR).

Gene expression was determined by qPCR according to the method described in a previous study [22]. Gene-specific primers were listed in Table 2. The mRNA abundance of the target genes, relative to *β-actin* was analyzed using the $2^{-\Delta\Delta Ct}$ method, $\Delta C_t = C_{t\ (target\ gene)} - C_{t\ (\beta\text{-actin})}$ and $\Delta\Delta C_t = \Delta C_{t\ (Treatment)} - \Delta C_{t\ (Control)}$.

Genes	Sequences (5'–3')		Product Size (bp)	GenBank Accession
TLR4	Forward	TGACGCCTTTGTTATCTACTCC	246	NM_001113039
	Reverse	GGTCTGGGCAATCTCATACTC		
Myd88	Forward	CCCCAGCGATACCCAGTTTGT	152	NM_001099923
	Reverse	ATCCGACGGCACCTCTTTTCA		
NF-kappa Bp65	Forward	ACCCCTTCCAAGTTCCC	195	NM_001114281
	Reverse	CCCGAGTTCCGATTCAC		
TNF-α	Forward	CACGCTCTTCTGCCTACTGC	164	NM_214022.1
	Reverse	GTCCCTCGGCTTTGACATT		
β-actin	Forward	CATCGTCCACCGCAAAT	210	NC_010445
	Reverse	TGTCACCTTCACCGTTCC		
Claudin-1	Forward	GATTTACTCCTACGCTGGTGAC	199	NM_001244539.1
	Reverse	CACAAAGATGGCTATTAGTCCC		
Occludin	Forward	GCACCCAGCAACGACAT	144	XM_005672525
	Reverse	CATAGACAGAATCCGAATCAC		
IL-10	Forward	GAAGCGCATCGAGGCCATTC	162	NM_214015.1
	Reverse	GAAGCGCATCGAGGCCATTC		
Mucin2	Forward	CTGCTCCGGGTCCTGTGGGA	100	XM_007465997.1
	Reverse	CCCGCTGGCTGGTGCGATAC		
FXR	Forward	CCGAGAGGCAGTAGAGAA	144	NM_001287412.1
	Reverse	GCGTGGTGATGGTTGAA		
TGR5	Forward	AAGCCCAAGATGACACCCAA	187	XM_013984487.1
	Reverse	CCAGGAGCAGACTCAGGAAGAA		

Table 2
Primer sequences used in this study.

Proteomic analysis

An isobaric tag for relative and absolute quantitation (iTRAQ) analysis was carried out to detect changes in the proteomic profile of jejunal mucosa among the four diet treatments according to the method described in a previous study [23]. A reliable protein was identified accordance to the screening criteria: unused>1.3, unique peptide⩾1. The identified protein was considered as (DEP) when the fold change was greater than 1.2 or less than 0.83, accompanied with t-test p-value less than 0.05.

2.10. Statistical Analysis

Data were expressed as means ± SEM. The data analyses of growth performance, biochemical indexes and genes expression were carried out by SPSS 20.0 software (SPSS v. 20.0, SPSS Inc., Chicago IL, USA). The other traits were performed using GraphPad Prism Version 8 (GraphPad Software, La Jolla, CA). The statistical significance of differences was analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, and difference was considered significant when $p< 0.05$. The correlations between gut microbiota, bacterial metabolites and genes expression were analyzed by Pearson's correlation using SPSS 20.0 software and significant differences were declared when $p < 0.05$.

Results

Diets effect on growth performance of weaned piglets

As shown in Table 3, piglets fed with ChB diet gained a greater body weight and a significantly higher ADG than those fed control diet during the post-weaning period (BW:26.03 ± 0.74 vs 24.51± 0.91 kg; ADG: 638.4± 16.1 vs 580.8 ±22.9 g/d), and a visibly lower F/G (1.176± 0.034 vs 1.27±0.016). Piglets fed the choline diet tended to have a same variable trend as ChB diet for body weight and ADG, but without statistics. Whereas choline group had a significantly lower F/G than those fed control diet. No differences in body weight, ADG and F/G were observed between bile acids group and control group. Furthermore, there was no difference in ADFI among the four groups.

Item	Control	Choline	BAs	ChB
Initial BW kg	8.24± 0.44	8.22 ± 0.43	8.21 ± 0.44	8.17 ± 0.42
Final BW kg	24.51± 0.91	25.88 ± 0.82	24.40 ± 0.69	26.03 ± 0.74
ADG, g	580.8 ±22.9^{b}	630.4 ± 18.2^{ab}	576.9± 27.4^{b}	638.4± 16.1^{a}
ADFI, g	771.4±27.01	739± 20.05	706.0± 19.16	747.3± 11.15
F/G	1.27±0.016^{a}	1.18± 0.038^{b}	1.23± 0.048^{a}	1.17± 0.034^{b}
Diarrhea index %	2.09± 1.03	0	0	0
Data presented as means±SEM, and different superscripts in the same row are significantly different ($p < 0.05$). N=32. BAs, bile acids; ChB, choline and bile acids mixture.				

Table 3

Diet effects on the growth performance in weaned piglets.

Diet effect on biochemical indexes related to fats digestion in intestinal digesta of weaned piglets

As shown in Table 4, piglets fed ChB displayed a notably higher lipase activity in jejunal digesta, and significantly higher level of total bile acids in both jejunal and ileal digesta than those fed control diet. However, there were no significant differences in lipase activity and total bile acids concentration between choline and control group or bile acids and control group. In addition, both choline and ChB supplementation significantly increased the NEFA content in the jejunal digesta compared to control diet. No difference for NEFA content in the jejunal digesta was found between bile acids group and control group. However, bile acids decreased NEFA content in the ileal digesta compared with control diet. Furthermore, choline, bile acids as well as ChB diet significantly decreased TG concentration in the jejunal digesta compared with control diet. Choline group or bile acids group also showed a profound reduction of TG concentration in the ileal digesta, when compared to the control group. Besides, both choline diet and bile acids diet significantly reduced the T-CHO level in the duodenal digesta compared to control diet. T-CHO level in the duodenal digesta of ChB group tend to be lower than the control group, but without statistics. In the jejunal digesta, bile acids diet markedly decreased the level of T-CHO, as compared with control diet.

Items	Control	Choline	BAs	ChB
Lipase activity in intestinal digesta (U/gprot)				
Duodenum	43.14± 12.55	34.23 ± 6.65	31.51±2.51	42.93± 22.06
Jejunum	115.58± 0.18.33^{b}	189.04 ± 50.76^{ab}	139.57 ± 25.94^{ab}	221.13 ± 0.43^{a}
Ileum	31.66± 5.97	52.33± 8.60	35.20 ± 6.22	51.00 ± 3.92
Bile acid content in intestinal digesta (umol/gprot)				
Duodenum	626.58± 93.90	517.62±49.46	528.41±130.05	529.65± 6.60
Jejunum	457.51± 77.44^{b}	506.32±39.40^{ab}	505.09±76.11^{ab}	647.75± 76.11^{a}
Ileum	15.36± 2.43^{b}	29.21±7.95^{b}	29.85±10.43^{b}	175.99± 24.83^{a}
NEFA content in intestinal digesta (umol/gprot)				
Duodenum	0.63± 0.09	0.99±0.25	0.93±0.09	1.03± 0.33
Jejunum	1.12± 0.25^{b}	1.89±0.33^{a}	0.87±0.18^{b}	1.90± 0.16^{a}
Ileum	0.35± 0.12^{a}	0.19±0.027^{ab}	0.13±0.01^{b}	0.38± 0.09^{a}
TG content in intestinal digesta (mmol/gprot)				
Duodenum	2.17± 0.19	2.30±0.31	2.40±0.56	2.31± 0.66
Jejunum	6.34±1.14^{a}	3.38±0.38^{b}	2.60±0.36^{b}	2.97± 0.41^{b}
Ileum	1.52±0.15^{a}	1.09±0.12^{b}	1.01±0.086^{b}	1.29± 0.15^{ab}
T-CHO content in intestinal digesta (mmol/gprot)				
Duodenum	2.08±0.62^{a}	0.94±0.17^{b}	0.95±0.12^{b}	1.23± 0.32^{ab}
Jejunum	2.18±0.30^{a}	1.87±0.29^{a}	0.36±0.17^{b}	1.53± 0.33^{a}
Ileum	1.35±0.29	2.15±0.67	1.54±0.19	1.27± 0.42
Data presented as means±SEM, and different superscripts in the same row are significantly different ($p < 0.05$). N=8. BAs, bile acids; ChB, choline and bile acids mixture.				

Table 4
Biochemical indexes related to fats digestion in intestinal digesta of weaned piglets.

Diets induced changes in the composition of colonic microbiota of weaned piglets

A total of 16704 operational taxonomic units (OTUs) (522 OTUs per sample) from 32 samples were identified and for further analysis. Venn analysis of OTUs showed that 151, 45, 55 and 27 unique OTUs

were identified in control group, choline group, bile acids group and ChB group, respectively, and 666 OTUs were shared among the four groups (Figure 1a). The diversity and richness of the colonic digesta microbiota were present in Table 5. ChB supplementation caused reduction in richness and diversity indices compared with control diet, as reflected by the reduction in Shannon, Simpson and ACE index with statistical differences. Beta-diversity analysis showed clustering of samples according to diets. In particular, the PCA results showed that ChB group and choline group were separated from control group (Figure 1b). Diet-induced changes in bacterial composition were clearly visible at the genus level. *Lactobacillus* and *Faecalibacterium* were considerably more abundant in piglets fed ChB than those in control group. In addition, the relative abundances of *unidentified-Clostridiales*, *Parabacteroides* and *Unidentified-Ruminococcacace* were decreased by ChB, as compared with the control diet. Piglets fed choline showed a decreased abundance of *Parabacteroides* compared to those fed control diet (Figure 1c, d). Bile acids increased the abundance of *Bacteroides* compared with control diet, with a decrease in the abundance of *Parabacteroides*. Besides, choline or bile acids diet can slightly increase the relative abundances of *Lactobacillus*, and decrease the relative abundances of *Unidentified-Ruminococcacace*, but without statistics significant. Furthermore, LEfSe analysis (LDA score >4; Figure1e) showed significant distinguishing bacteria with class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae*, genus *Lactobacillus* in the ChB group, with family u*nidentified Clostridiales* and *Bacteroidaceae*, genera *Bacteroides* and *Actinobacillus* in the bile acid group, and with the family *Muribaculaceae* in the control group when compared with the other three groups (Figure 1e).

Items	Control	Choline	BAs	ChB
Diversity indices				
Shan	6.04± 0.24^{a}	5.41 ± 0.13^{b}	5.66±0.21^{ab}	5.32± 0.17^{b}
Simpson	0.95± 0.0.015^{a}	0.92 ± 0.008^{ab}	0.93 ±0.12^{ab}	0.91 ± 0.012^{b}
Richness				
Chao1	652.30± 44.93	585.22±11.86	665.83±22.42	589.88± 33.56
ACE	666.28±47.45^{a}	590.51±9.91^{ab}	663.41±18.38^{ab}	578.29± 23.71^{b}
Data presented as means± SEM, and different superscripts in the same row are significantly different ($p < 0.05$). N=8. BAs, bile acids; ChB, choline and bile acids mixture.				

Table 5
Summary statistic of colonic digesta bacterial community at the 3% dissimilarity level.

Diet effect on SCFA production in weaned piglets

No significant differences in propionate level among four groups were found (Figure 2). Choline induced an increase in the level of acetate compared with control group. Compared to piglets fed control diet,

those fed ChB diet showed a profound elevation of the concentration of butyrate. However, there was not different in the concentration of butyrate among the other three groups.

Diet effect on bile acid profiles level in weaned piglets

As shown in Figure 3, in the colonic digesta, levels of primary bile acids including chenodeoxycholic acid (CDCA) and 3β-ursodeoxycholic acid (3βUDCA), and secondary bile acid including hyodeoxycholic acid (HDCA) and ursodeoxycholic acid (UDCA) were significantly decreased in ChB group, as compared with the control group. Choline supplementation decreased the relative levels of 23-norcholic acid (23-NCA), 3βUDCA, HDCA and UDCA compared with control group. Piglets fed bile acids displayed significantly higher levels of β- muricholic acid (MCA) and α-MCA compared to those fed control diet.

Diet effect on the proteomic profile related to innate immune response in jejunal mucosa of weaned piglets

As shown in Supplementary figure 1a, b, c and d, volcano plot was used to illustrate the differentially expressed proteins (DEPs) expression patterns, from the volcano plot we found that more DEPs were identified in the jejunal mucosa sample of weaned piglets fed ChB diet. Compared with the control group, there were 35 upregulated proteins and 45 downregulated proteins in the choline group, 84 upregulated proteins and 46 downregulated proteins in bile acid group, 132 upregulated proteins and 84 downregulated proteins in the ChB group, respectively. Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was used to collect the DEPs function information of the jejunal mucosa samples from the four groups. Particularly, DEPs involved in immune system were payed close attention and described utilizing the heatmap analyses. As shown in Figure 4a, b, c and Table 6, there were 11,10 and19 DEPs related to innate immune system in the choline group, bile acid group and ChB group, respectively, as compared with the control group. In addition, the DEPs accumulated to NF-κB, Toll-like receptor and NOD-like receptor signaling pathway, which were highly involved in inflammation, were analyzed. Compared to the control diet, choline diet upregulated Ig-like domain-containing protein and Interleukin-18, while downregulated IgM and tripartite motif-containing protein. Piglets fed bile acids had significant higher levels of Ig-like domain-containing protein and tyrosine-protein kinase, but lower levels of mitogen-activated protein kinase 7, IgG heavy chain and thioredoxin-interacting protein isoform 1 than those fed control diet. The amount of protein kinase domain-containing protein, Ig-like domain-containing protein (FCGRT), PPM-type phosphatase domain-containing protein (PDP2), Integrin beta, ANK_REP_REGION domain-containing protein and NF-κB inhibitor epsilon were significantly increased in ChB group, while IgG heavy chain, IgM, probable ATP-dependent RNA helicase DDX58(DDX58), MyD88 and BCL10 immune signaling adaptor (BCL10) were markedly down-regulated, as compared with the control diet.

	Protein ID	Description	Gene	FC	P value
choline	A0A4X1SUP3	Ig-like domain-containing protein		1.80	0.02
	O77947	MHC class I antigen 2 (Fragment)		1.30	0.01
	A0A287AH22	Membrane cofactor protein	CD46	1.28	0.03
	A0A2C9F3D6	Interleukin-18	IL18	1.21	0.03
	A0A1P8VJR2	Aminopeptidase	APN	1.21	0.03
	A0A480MNY7	Platelet endothelial cell adhesion molecule isoform X1		0.82	0.03
	A0A480Z8U9	Guanine nucleotide exchange factor VAV2 isoform 2 (Fragment)		0.78	0.02
	A0A287ADL4	Polymeric immunoglobulin receptor	PIGR	0.69	0.02
	A0A480K9S0	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase		0.66	0.05
	A0A480QMR6	IgM	IgM	0.68	0.04
	B6ICV0	Tripartite motif-containing protein 15	TRIM15	0.76	0.03
BAs	A0A4X1VTZ6	Protein kinase domain-containing protein	MAP2K2	1.65	0.01
	A0A075B7I5	Ig-like domain-containing protein		1.32	0.03
	G0KXP9	MHC class I antigen	SLA-3	1.30	0.05
	A0A4X1W3V2	Tyrosine-protein kinase	LOC100739325	1.29	0.04
	A0A1P8VJR2	Aminopeptidase	APN	1.25	0.02
	A0A480UWI6	Plasma protease C1 inhibitor		0.69	0.03
	A0A4X1SK84	Neutrophil cytosolic factor 1	NCF1	0.80	0.02
	B0LXP5	Mitogen-activated protein kinase 7	TAK1b	0.82	0.00
	L8AXK8	IgG heavy chain	IGHG	0.78	0.01
	A0A480K2Q0	Thioredoxin-interacting protein isoform 1		0.63	0.03
ChB	A0A4X1VTZ6	Protein kinase domain-containing protein	MAP2K2	1.68	0.01
	A0A4X1VYT4	Ig-like domain-containing protein	FCGRT	1.56	0.04
	A0A4X1ULK6	PPM-type phosphatase domain-containing protein	PDP2	1.28	0.03
	A0A4X1T9Z3	Integrin beta	ITGB3	1.27	0.01

	K7GSL6	ANK_REP_REGION domain-containing protein	BCL3	1.25	0.04
	A0A4X1SNA9	Protein kinase domain-containing protein	MAP2K4	1.22	0.04
	A0A4X1V587	NFKB inhibitor epsilon		1.22	0.05
	A0A286ZZN4	Protein kinase domain-containing protein	GSK3A	0.82	0.05
	A0A480Z8U9	Guanine nucleotide exchange factor VAV2 isoform 2 (Fragment)		0.82	0.03
	A0A286ZT79	RPOLD domain-containing protein	POLR1C	0.78	0.03
	A0A0A0MY60	Signal transducer and activator of transcription	STAT5A	0.77	0.01
	A0A4X1W2Z9	C1q domain-containing protein	C1QB	0.74	0.01
	A0A287ADL4	Polymeric immunoglobulin receptor	PIGR	0.72	0.03
	F1RX35	Fibrinogen C-terminal domain-containing protein	FGG	0.71	0.03
	L8B0R9	IgG heavy chain	IGHG	0.76	0.00
	A0A480QMR6	IgM		0.58	0.02
	A0A4X1UKT2	Probable ATP-dependent RNA helicase DDX58	DDX58	0.70	0.03
	A0A140TAK4	Myeloid differentiation primary response protein MyD88	MYD88	0.79	0.03
	A0A4X1UEV0	BCL10 immune signaling adaptor	BCL10	0.61	0.01
N=8. BAs, bile acids; ChB, choline and bile acids mixture.					

Table 6
Differentially expressed proteins in the pathway of immune system compared with control diet fed weaned piglets.

Diet effect on the intestinal mucosal barrier function of the weaned piglets

As shown in Figure 5, there was no significant effect of diet treatments on the abundance of *claudin-1* and *occludin* transcripts in the intestine. However, ChB supplementation profoundly elevated the abundance of *IL-10* transcript, while significantly decreased *MyD88*, *TLR4* and *TNF-α* genes expression in the duodenal mucosa compared to the control diet, the transcript level of *NF-κBp65* tended to have the same variable trend, but without no statistics. In the jejunal mucosa, ChB diet significantly increased the expression of *mucin2* gene, while markedly decreased abundance of *MyD88*, *TLR4*, *TNF-α* and *NF-κBp65* transcripts. Piglets fed ChB showed a higher amount of ileal *IL-10* gene expression. Choline

supplementation induced a visibly higher abundance of *IL-10* transcript and lower abundance of *TNF-α* transcript in the duodenal mucosa, lower abundance of *TLR4*, *TNF-α* and *NF-κBp65* transcripts in the jejunal mucosa, when compared with those fed control diet. Additionally, bile acids supplementation significantly decreased the gene expressions of *TNF-α* and *MyD88* in the duodenal mucosa, and markedly decreased the levels of *TLR4*, *TNF-α* and *NF-κBp65* transcripts in the jejunal mucosa, but increased the abundance of *IL-10* transcript in the ileal mucosa, as compared with the control diet.

Diet effect on the expression of *FXR* and *TGR5* genes

No difference in *TGR5* gene expression in the ileal and colonic mucosa of weaned piglets was observed among four groups. However, the expression of *FXR* gene in the colonic mucosa was increased in choline group and ChB group compared to control group. However, no difference for this expression in ileal mucosa among the four groups was observed (Figure 6).

Correlation analysis between gut microbiota, bacterial metabolites, ADG and intestinal genes expression

A Pearson correlation analysis was used to investigate the associations between genes expression and the abundance of the main microbial genera and their metabolites (Figure 7). The results revealed that the expression of *TLR4 transcript* was significantly negatively correlated with the abundance of *Lactobacillus*, while positively related to CDCA. *MyD88* genes expression showed positive correlations with HDCA and UDCA, while it was negatively associated with butyrate. The abundance of *TNF-a* gene was positively correlated with level of *UDCA*. The abundance of *NF-κB*p65 transcript had a negative correlation with the relative abundance of *Lactobacillus*. *FXR* gene expression was significantly correlated with the abundance of *Lactobacillus*, while negatively correlated with the level of 23-NCA, HDCA and UDCA as well as the relative abundance of *Parabacteroides* and *Unidentified ruminococcaceae.* The abundance of *IL-10 transcript* showed positive association with acetate level and the relative abundance of *Lactobacillus and Faecalibacterium*, while showed negative correlation with HDCA, UDCA and *Parabacteroides*. In addition, ADG was positively correlated with the relative abundance of *Lactobacillus* and butyrate level.

Discussion

In the present study, bile acids and choline mixture supplementation, but not choline or bile acids alone, improved the growth performance of weaned piglets, reflecting as a marked higher ADG, a lower feed to gain ratio and a more body weight gain than control diet. The increased body weight gain and ADG by ChB was likely due to this mixture supplementation promoted dietary lipids digestion and absorption, reflecting as higher lipase activity in the jejunal digesta and higher concentration of total bile acids in the jejunum and ileum than those in control group [24,25]. Meanwhile, an augment of NEFA level and reduction in TG in the jejunal digesta further confirmed that ChB diet accelerated dietary lipids digestion. Previous studies demonstrated that dietary choline significantly improved the fish specific growth rate [26,27]. To our knowledge, there was limited study on the choline effect on the swine growth performance. As for the effect of bile acids supplementation here, there was no impact on growth performance of

weaned piglets. This finding was consistent with the previous studies that administration of CDA or CDCA had no effect on the growth performance of weaned piglets [10,11].

Evidence has shown that diets induced the changes in body weight gain by altering the composition of gut microbiota. Gut microbiota play an important role in suppression of pathogen infection, regulation of nutrient digestion and absorption, maintenance of intestinal homeostasis and immune regulation [28]. Beneficial effects of functional substances supplementation on the regulation of gut microbial composition and maintenance of host health have been reported. In the present study, choline and bile acids mixture profoundly enhanced the abundance of the genera *Lactobacillus* and *Faecalibacterium* in the colonic digesta, while decreased the abundance of the genera *unidentified-Clostridiales, Parabacteroides* and *unidentified-Ruminococcaceae*. *Lactobacillus* has been shown to enhance the concentrations of bile acids in the intestinal lumen and therefore promote emulsification and absorption of lipids [29]. This finding partly agreed with previously established study that there was a positive correlation between antimicrobial growth promoter induced body weight gain and an increase in abundance of *Lactobacillus* [30]. In agreement with these finding, our present study denoted that ChB increased total bile acids level in the duodenum and illume may be linked to the increased abundance of genus *Lactobacillus*. Moreover, a previous study found that the abundance of *Parabacteroides* had a significantly negative association with body mass index [31]. Similarly, investigators found that the abundance of *Parabacteroides distasonis* was relatively lower in patients with obesity and nonalcoholic fatty liver [32]. Collectively, these finding above suggested that increased ADG and body weight gain may be likely attributed to the increased abundance of *Lactobacillus* and the reduction in abundance of *Parabacteroides* induced by ChB supplementation.

Furthermore, *Lactobacillus* has been repeatedly reported to be crucial for prevention of pathogen infection and alleviation of intestinal inflammation [33]. A previous study demonstrated that a reduction in the abundance of *Faecalibacterium* in active IBD patient [34]. *Prausnitzii*, a specie in the genus *Faecalibacterium*, has been shown to not only produce butyrate, but also display an additional anti-inflammatory effect in mice with colitis [35].The enrichment of *Ruminococcaceae* has been shown to involve in colonic mucosal inflammation, which can trigger colitis upon disruption of the barrier function of colonic epithelial cell [36]. In line with these findings, the results in the present study indicated that the higher abundances of the beneficial bacteria including *Lactobacillu* and *Faecalibacterium* as well as the lower level of *Ruminococcaceae* induced by ChB supplementation might have beneficial effects on the gut health of weaned piglets.

SCFAs including acetate, propionate and butyrate, were mainly produced by bacterial fermentation of non-digestible carbohydrates in the colon [37]. These substances have been shown to play an important role in alleviation of intestinal inflammation and provide energy for the colonic epithelial cells [38]. Changes in the concentrations of SCFA, specifically butyrate may be linked to the altered composition of gut microbiota. Herein we found that choline and bile acid mixture greatly increased butyrate level in the colonic digesta. Thus, the higher level of butyrate induced by ChB diet was supported by the increased

abundance of *Lactobacillus* and *Faecalibacterium,* which were the main butyrate-producing bacteria in the colon [39].

Diet treatments here might have effects on colonic bile acid profiles characterized by a significant reduction in several bile acid profiles in ChB group as compared to control group. These results here were partly in line with a previous result that a lower amount of bile acids was associated with higher butyrate concentration in the porcine colon [40]. Accumulating evidence showed that abnormally higher levels of free bile acids in the colon, would disrupt colonic epithelial barrier integrity and cause epithelial cells oxidative stress and apoptosis, leading to gut dysfunction [41,42]. Previous study has reported that CDCA can induce epithelial permeability, which impaired colonic epithelial barrier integrity [43]. Furthermore, increased levels of secondary bile acids displayed harmful effects on the colonic epithelium function through activation of NF-κB [44]. Collectively, the reduction of primary bile acid (particularly CDCA) and secondary bile acid (UDCA and HDCA) induced by ChB supplementation suggested that this supplementation could reduce the cytotoxicity caused by CDCA and secondary bile acid and therefore enhanced gut barrier and alleviated inflammation.

Changes in bile acids concentrations induced by diet treatments in the colon were possible responsible for FXR activation, as it has been demonstrated that UDCA was the antagonists of FXR [43]. In the current study, ChB supplementation increased the expression of *FXR* gene in the colonic mucous. Increasing evidence repeatedly described FXR activation exhibited anti-inflammatory effects and protected against colitis induced by chemical, whereas FXR knock out mice showed increased susceptibility to chemical injury [45,46]. Together with these results, compelling evidence from proteomic analysis further indicated that ChB supplementation showed a efficiently anti-inflammatory role by increasing the amount of proteins related to anti-inflammatory signaling, such as FCGRT, PDP2 and NF-κB inhibitor epsilon, while significantly down-regulated the proteins expressions that were positively promoted inflammatory signaling, including MyD88, DDX58 and BCL10 in the intestine [47,48]. Furthermore, the decreased abundance of *TNF-α* transcript in duodenal and jejunal mucosa, and the elevated *IL-10* gene expression in ileum in the current study may further indicated the lower inflammatory response of weaned pigs fed ChB [49]. Meanwhile, ChB suppressed TLR4-Myd88-NF-κB signal pathway, which play an important role in inflammatory response [50]. Collectively, these finding suggested that ChB efficiently inhibited the inflammatory response, which might contribute to maintaining the intestinal mucosal immune homeostasis. Furthermore, diet effect on the expression of mucin can affect intestinal function and integrity. In the present study, we found that choline and bile acids mixture significantly increased the abundance of *mucin2*, which negatively modulated inflammatory response and played an important role in maintaining homeostasis in the intestinal epithelium [51].

Conclusion

Collectively, our results in the present study indicated that dietary supplementation with choline and bile acids mixture may efficiently improve the growth performance and the intestinal mucosal immunity of weaned piglets through alteration of gut microbial composition and bacterial metabolite profiles.

Therefore, these finding suggested that choline and bile acids mixture may act as a beneficial antibiotic alternative for weaned piglets' production.

Abbreviations

SCFA: short chain fatty acids; CDCA: chenodeoxycholic acid; 3βUDCA: 3β-ursodeoxycholic acid; HDCA: hyodeoxycholic acid; UDCA: ursodeoxycholic acid; DCA: deoxycholic acid; 23-NCA: 23-norcholic acid; MCA: muricholic acid; MyD88: myeloid differentiation factor 88; NF-κB: nuclear factor kappa B; TLR4: toll like receptor 4; TNF-a: tumor necrosis factor-a; IL-10: interleukin-10; TGR5: G-protein-coupled BA receptor; FXR: farnesoid X receptor; OUT: operational taxonomic units.

Declaration

Ethics approval and consent to participate

All animal procedures used in this study were approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences (authorization number GAASIAS-2016-017).

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed in the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Funding sources

This work was financially supported by the National Key R&D Program of China (No. 2018YFD0500605 and 2018YFD0500403), Guangzhou Science and Technology Project（201906010021）, Special Fund for Scientific Innovation Strategy-construction of High Level Academy of Agriculture Science (R2016PY-QF007), Guangdong Provincial Program of Withdrawal Technology of In-feed Antibiotics, China Agriculture Research System (CARS-35).

Authors' contributions

XFY and ZYJ conceived and designed the whole trial; YQQ, KBL, SLL and KGG conducted the pig trial; YQQ, KBL and LW conducted laboratory analyses. YQQ and XFY wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank the staff at our laboratory for their kindly help.

References

1. Smith F, Clark, JE, Overman BL, Tozel CC, Huang JH, Rivier JE, Blikslager, AT, Moeser AJ. Early weaning stress impairs development of mucosal barrier function in the porcine intestine. Am J Physiol Gastrointest Liver Physiol. 2010;298 (3): 352-363.
2. Hu CH, Xiao K, Luan ZS, Song J. Early weaning increases intestinal permeability, alters expression of cytokine and tight junction proteins, and activates mitogen-activated protein kinases in pigs. J Anim Sci. 2013; 91 (3): 1094-1101.
3. Ashiru-Oredope D, Hopkins S. Antimicrobial resistance: moving from professional engagement to public action. J Antimicrob Chemother. 2015;70 (11): 2927-2930.
4. Leermakers ET, Moreira EM, Kiefte-de JC, Darweesh SK, Visser T, Voortman T, Bautista PK, Chowdhury R, Gorman D, Bramer WM, Felix JF, Franco OH. Effects of choline on health across the life course: A systematic review. Nutr Rev. 2015;73 (8): 500-522.
5. Wu P, Jiang WD, Jiang J, Liu Y, Zhang YA, Zhou XQ, Feng L. Dietary choline deficiency and excess induced intestinal inflammation and alteration of intestinal tight junction protein transcription potentially by modulating NF-κB, STAT and p38 MAPK signaling molecules in juvenile Jian carp. Fish Shellfish Immunol.2016;58:462-473.
6. Zhao HF, Jiang WD, Liu Y, Jiang J, Wu P, Kuang SY, Tang L, Tang WN, Zhang YA, Zhou XQ, Feng L. Dietary choline regulates antibacterial activity, inflammatory response and barrier function in the gills of grass carp (Ctenopharyngodon idella).Fish Shellfish Immunol.2016;52:139-150.
7. Devlin AS, Fischbach A. A biosynthetic pathway for a prominent class of microbiota-derived bile acids. Nat Chem Biol. 2015;11(9):685-690.
8. Begley M, Sleator RD, Gahan CG, Hill C. Contribution of three bile-associated loci, bsh, pva and btlB, to gastrointestinal persistence and bile tolerance of listeria monocy-togenes. Infect Immun. 2005;73(2): 894-904.
9. Ding T, Xu N, Liu YT, Du JL, Xiang XJ, Xu D, Liu QD, Yin ZY, Li JB, Mai KS, Ai QH. Effect of dietary bile acid (BA) on the growth performance, body composition, antioxidant responses and expression of lipid metabolism related genes of juvenile large yellow croaker (Larimichthys crocea) fed high lipid diets. Aquaculture. 2020; 518:734-768.
10. de Diego-Cabero N, Mereu A, Menoyo D, Holst JJ, Ipharraguerre IR. Bile Acid Mediated Effects on Gut Integrity and Performance of Early-Weaned Piglets. BMC Vet Res. 2015; 11:111.
11. van der Meer Y, Gerrits WJJ,van den Bosch M, Holst JJ, Moreto M, Buurman WA, Kulik W, van Kempen TATG. Chenodeoxycholic Acid Reduces Intestinal Permeability in Newly Weaned Piglets. J Anim Sci. 2012,4,302-304.

12. Jones DB, Hancock JD, Walker CE. Effects of soy lecithin and distilled monoglyceride in combination with tallow on nutrient digestibility, serum lipids and growth performance in weanling pigs. Kansas State University Swine Day-Report of Progress.1991; 641(11): 61-65.
13. Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. Nature. 2016;535(7610):56-62.
14. David LA, Maurice CF, Carmod RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, VarmaY, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. Nature, 2014;505 (7484):559-563.
15. den Besten G, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk TH, Oosterveer MH, Jonker JW, Groen AK, Reijngoud DJ, Bakker BM. Short-chain fatty acids protect against high-fat diet-induced obesity via a PPARgamma-dependent switch from lipogenesis to fat oxidation. Diabetes. 2015;64 (7):2398-2408.
16. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kleiner DE, Doo E. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, nonalcoholic steatohepatitis (FLINT): a multicentre, randomised, placebocontrolled trial. Lancet. 2015;385(9972):956-965.
17. Walker AK, Jacobs RL, Watts JL, Rottiers V, Jiang K, Finnegan DM, Shioda T Hansen M, Yang F, Niebergall LJ, Vance DE, Tzoneva M, Hart AC, Näär AM. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. Cell. 2011; 147(4):840-852.
18. Begley M, Gahann CGM, Hill C. The interaction between bacteria and bile. FEMS Microbiol Rev. 2005;29 (4): 625-651.
19. Islam S, Fukiya S, Hagio M, Fujii, N, Ishizuka S, Ooka T, Ogura Y, Hayashi T, Yokota A. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology. 2011;141 (5): 1773-1781.
20. Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA. Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. Gastroenterology. 2011;140 (3): 976-986.
21. Xiong YX, Yi HB, Wu QW, Jiang ZY, Wang L. Effects of acute heat stress on intestinal microbiota in grow-finishing pigs, and associations with feed intake and serum profile. J Appl Microbiol. 2020; 28 (3): 840-852.
22. Qiu YQ, Yang XF, Wang L, Gao KG, Jiang ZY. L-Arginine Inhibited Inflammatory Response and Oxidative Stress Induced by Lipopolysaccharide via Arginase-1 Signaling in IPEC-J2 Cells. Int J Mol Sci. 2019;20 (7): 1800.
23. Yi HB, Yang GD, Xiong YX, Wu QW, Xiao H, Wen X, Yang XF, Wang L, Jiang ZY. Integrated Metabolomic and Proteomics Profiling Reveals the Promotion of Lactobacillus Reuteri LR1 on Amino Acid Metabolism in the Gut-Liver Axis of Weaned Pigs. Food Funct. 2019; 10 (11): 7387-7396.
24. Sandra S, Decker, EA, McClements DJ. Effect of Interfacial Protein Cross-Linking on the in Vitro Digestibility of Emulsified Corn Oil by Pancreatic Lipase. J Agric Food Chem. 2008; 56 (16): 7488-

7494.

25. Sarkar A, Li H, Cray D, Boxall S. Composite Whey Protein-cellulose Nanocrystals at Oil-Water Interface: Towards Delaying Lipid Digestion. Food Hydrocoll. 2018; 77, 436-444.
26. Wu P, Feng L, Kuang SY, Liu Y, Jiang J, Hu K. Effect of dietary choline on growth, intestinal enzyme activities and relative expressions of target of rapamycin and eIF4E-binding protein2 gene in muscle, hepatopancreas and intestine of juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture. 2012; 317(1-4):107-116.
27. Hansen AKG, Kortner TM, Krasnov A, Björkhem I, Penn M, Krogdahl Å. Choline supplementation prevents diet induced gut mucosa lipid accumulation in post-smolt Atlantic salmon (Salmo salar L.). BMC Veterinary Research. 2020; 16(1):32.
28. Song X, Sun X, Oh SF, Wu M, Zhang Y, Zheng W, Geva-Zatorsky N, Jupp R, Mathis D, Benoist C, Kasper DL. Microbial bile acid metabolites modulate gut RORγ+ regulatory T cell homeostasis. Nature. 2020;577 (7790):410-415.
29. Lin J. Antibiotic growth promoters enhance animal production by targeting intestinal bile salt hydrolase and its producers. Front Microbiol. 2014; 5:33.
30. Kim HB, Borewicz K, White BA, Singer RS, Sreevatsan S, Tu ZJ, Isaacson RE. Microbial shifts in the swine distal gut in response to the treatment with antimicrobial growth promoter, tylosin. Proc Natl Acad Sci USA. 2012; 109 (38):15485-15490.
31. Wang K, Liao M, Zhou N, Bao L, Ma K, Zheng Z, Wang Y, Liu C, Wang W, Wang J, Liu SJ, Liu H. Parabacteroides distasonis Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids. Cell Rep. 2019; 26 (1):222-235.
32. Cekanaviciute E, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R, Bencosme Y, Lee YK, Hauser SL. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. Proc Natl Acad Sci USA. 2017; 114 (40): 10713-1071868.
33. Azad M, Sarker M, Li TJ, Yin J. Probiotic species in the modulation of gut microbiota: an overview. Biomed Res Int. 2018; 2018:9478630
34. Lopez-Siles M, Enrich-Capó N, Aldeguer X, Sabat-Mir M, Duncan SH, Garcia-Gil LJ, Martinez-Medina M. Alterations in the Abundance and Co-occurrence of Akkermansia muciniphila and Faecalibacterium prausnitzii in the Colonic Mucosa of Inflammatory Bowel Disease Subjects. Front Cell Infect Microbiol. 2018; 8:281.
35. Breyner NM, Michon C, de Sousa CS, Vilas Boas PB, Chain F, Azevedo VA, Langella P, Chatel JM. Microbial Anti-Inflammatory Molecule (MAM) from Faecalibacterium prausnitzii Shows a Protective Effect on DNBS and DSS-Induced Colitis Model in Mice through Inhibition of NF-κB Pathway. Front Microbiol. 2017; 8:114.
36. Nakanishi Y, Sato T, Ohteki T. Commensal gram-positive bacteria initiates colitis by inducing monocyte/macrophage mobilization. Mucosal Immunol. 2015; 8 (1):152–160.
37. Liu JH, He ZY, Ma N, Chen ZY. Beneficial effects of dietary polyphenols on high-fat diet-induced obesity linking with modulation of gut microbiota. J Agric Food Chem. 2020; 68 (1):33-47.

38. Ohira H, Tsutsui W, Fujioka Y. Are short chain fatty acids in gut microbiota defensive players for 749 inflammation and atherosclerosis. J Atheroscler Thromb. 2017; 24 (7): 660-672.
39. Gao K, Pi Y, Peng Y, Mu CL, Zhu WY. Time-course responses of ileal and fecal microbiota and metabolite profiles to antibiotics in cannulated pigs. Appl. Microbiol. Biot. 2018;102 (5): 2289-2299.
40. Yan S, Zhu C, Yu T, Huang W, Huang J Kong Q, Shi J, Chen Z, Liu Q, Wang S, Jiang Z, Chen Z. Studying the Differences of Bacterial Metabolome and Microbiome in the Colon between Landrace and Meihua Piglets. Front Microbiol. 2017;8,1812.
41. Barrasa JI, Olmo N, Lizarbe MA, Turnay J. Bile acids in the colon, from healthy to cytotoxic molecules. Toxicol In Vitro. 2013, 27 (2): 964-977.
42. Sarathy J, Detloff SJ, Ao M, Khan N, French S, Sirajuddin H, Nair T, Rao MC. The Yin and Yang of bile acid action on tight junctions in a model colonic epithelium. Physiol Rep. 2017;5 (10): e13294.
43. Gupta B, Liu Y, Chopyk DM, Rai RP, Desai C, Kumar P, Farris AB, Nusrat A, Parkos CA, Anania FA, Raeman R. Western diet-induced increase in colonic bile acids compromises epithelial barrier in nonalcoholic steatohepatitis. FASEB J. 2020;34 (5):7089-7102.
44. Hofmann AF, Cravetto C, Molino G, Belforte G, Bona B. Simulation of the metabolism and enterohepatic circulation of endogenous deoxycholic acid in humans using a physiologic pharmacokinetic model for bile acid metabolism. Gastroenterology. 1987;93 (4):693-709
45. Gadaleta RM, van den Brink GR. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut. 2011; 60 (4): 463-472.
46. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. J. Immunol.2009;183 (10):66251-6261.
47. Li Z, Nabel GJ. A new member of the IkappaB protein family, IkappaB epsilon, inhibits RelA (p65)-mediated NF-kappaB transcription. Mol Cell Biol. 1997; 17 (10): 6184-6190.
48. Vaishnava S, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, Ley R, Wakeland EK, Hooper LV. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science. 2011; 334(6053):255-258.
49. Yu M, Li ZM, Chen WD, Rong T, Wang G, Ma XY. Hermetia illucens larvae as a potential dietary protein source altered the microbiota and modulated mucosal immune status in the colon of finishing pigs. J Anim Sci Biotechnol. 2019; 10:50.
50. Kim N, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harbor Perspect Biol.2012; 4(3): a006049.
51. Cornick S, Kumar M, Moreau F, Gaisano H, Chadee K. VAMP8-mediated MUC2 mucin exocytosis from colonic goblet cells maintains innate intestinal homeostasis. Nat Commun. 2019; 10(1):4306.

Figures

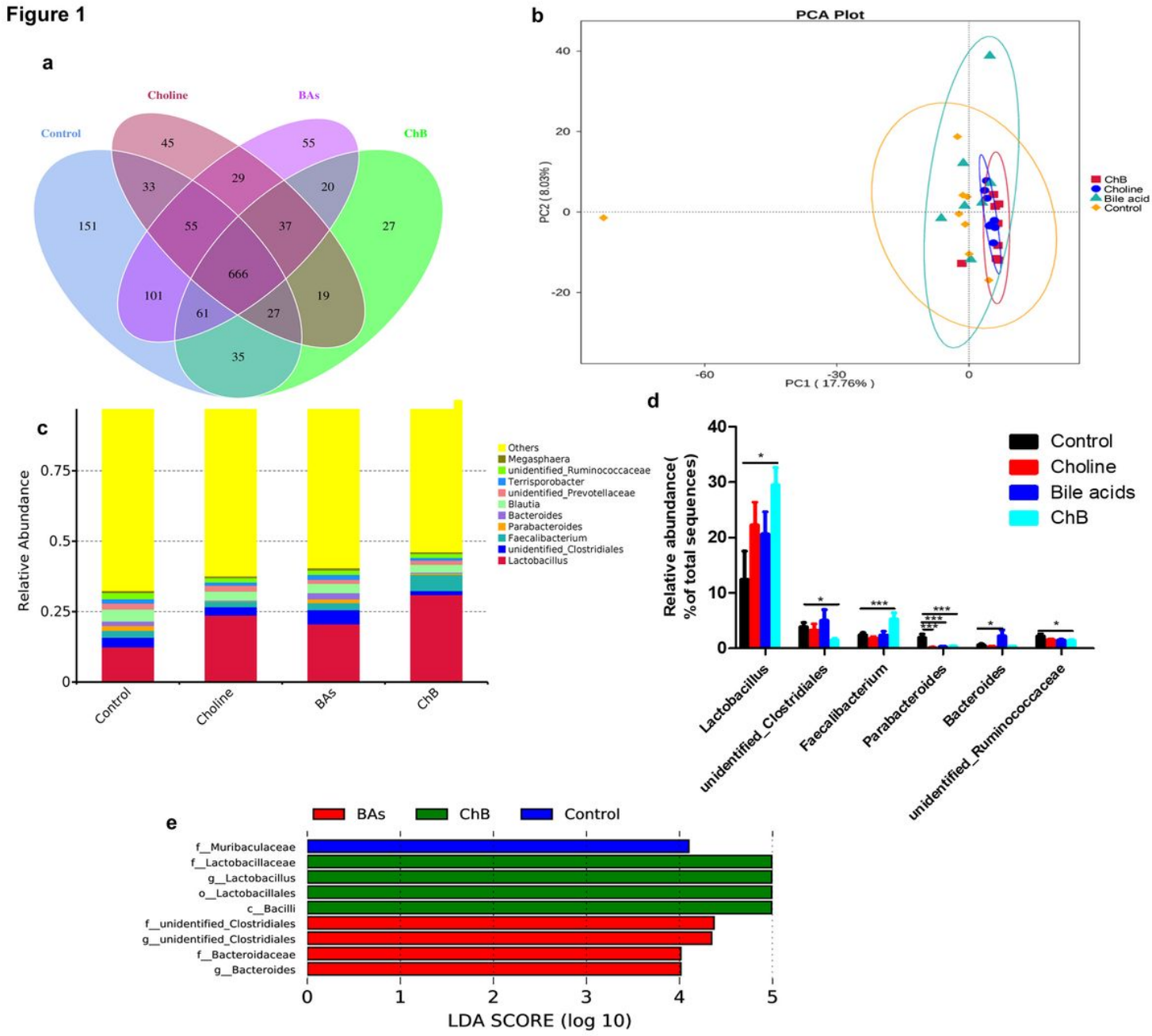

Figure 1

Diet effect on gut microbiota. a, a Venn diagram showing unique and shared OTUs dietary groups. b, Principal Component Analysis. c, Stacked bar chart showing the relative abundance of colonic bacteria, at the genus level (Top 10) in the different dietary groups. d, Bar chart showing significantly altered bacterial at the genus level. e, LDA scores calculated by LEfSe analysis identified the significant biomarker of bacteria in weaned piglets fed control diet, choline, bile acids, choline and bile acids mixture. LDA score is greater than 4. All data are expressed as means ± SEM (n = 8). Differences were analyzed by one-way ANOVA followed by Dunnett' T test. *p < 0.05, ***p < 0.001, compared with control diet. OUT: operational taxonomic units.

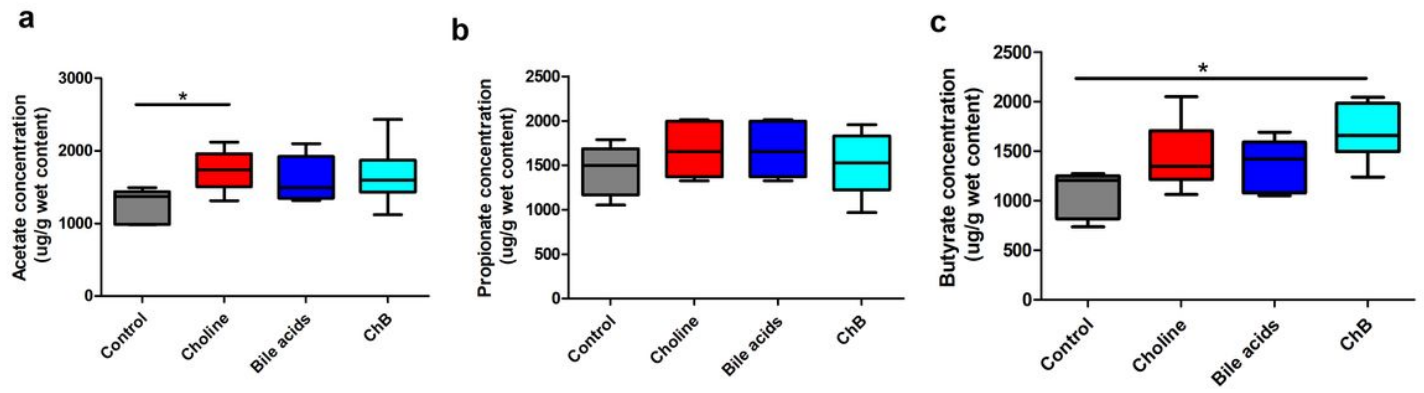

Figure 2

Diet affects short chain fatty acid level in colonic digesta of weaned piglets. Concentrations of (a) acetate, (b)propionate and (c)butyrate in the colonic contents of 28-d weaning piglets. All data are expressed as means ± SEM (n = 8). Differences were analyzed by one-way ANOVA followed by Dunnett' T test. *p < 0.05, compared with control diet.

Figure 3

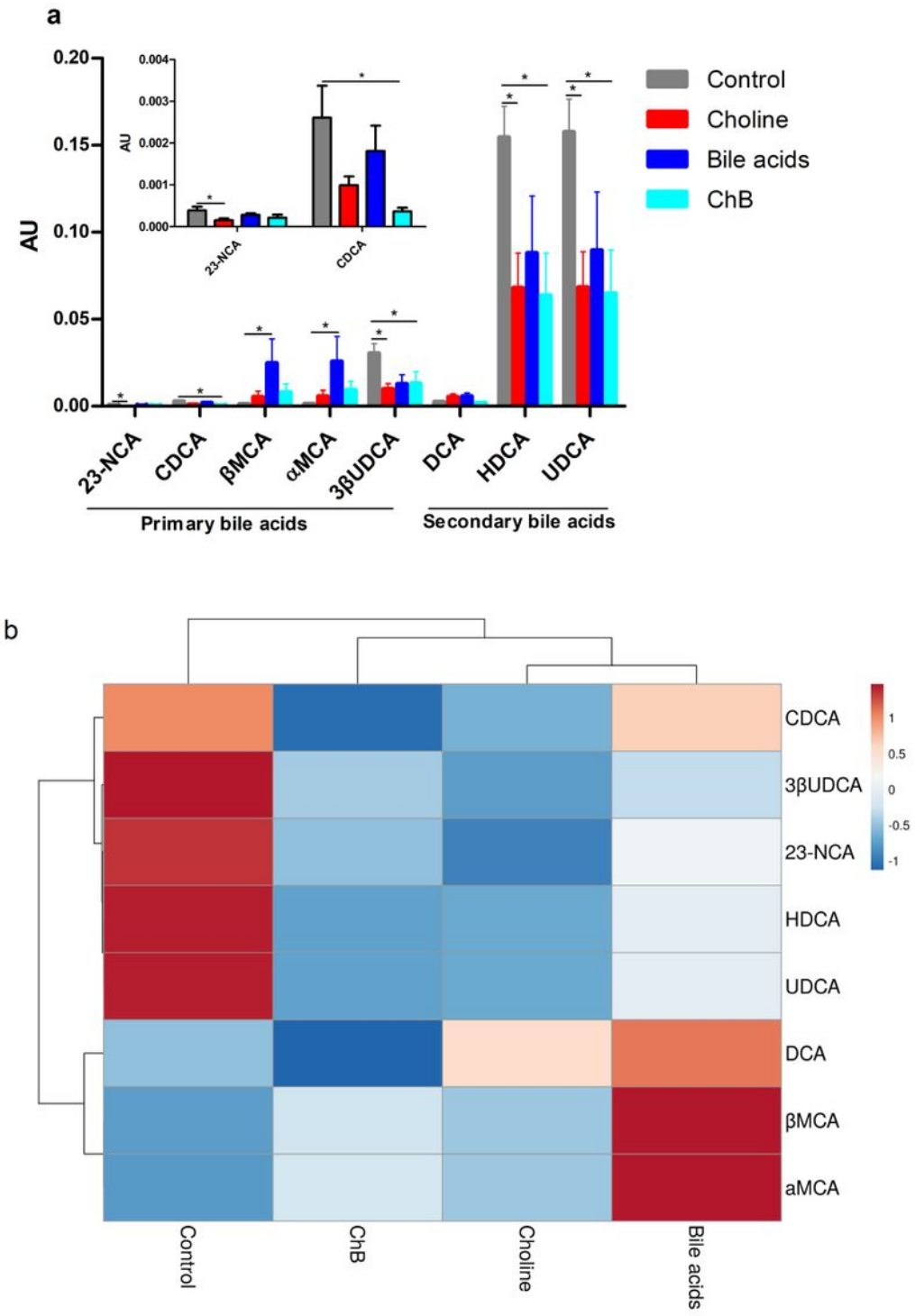

Figure 3

Diet effect on the relative concentrations of bile acids in the colonic digesta of weaned piglets. a, Bar plot showing relative concentrations of bile acids expressed in arbitrary units (AU, normalized peak areas). b, Heatmap of the mean abundance of the bile acids in colonic digesta. Differences were analyzed by one-way ANOVA followed by Dunnett' T test. *p < 0.05, compared with control diet. CDCA: Chenodeoxycholic acid; 3βUDCA: 3β-Ursodeoxycholic acid; HDCA: Hyodeoxycholic acid; and UDCA: Ursodeoxycholic acid;

DCA: Deoxycholic acid; 23-NCA: 23-Norcholic acid; β-MCA: Beta-Muricholic acid; α-MCA: Alpha-Muricholic acid.

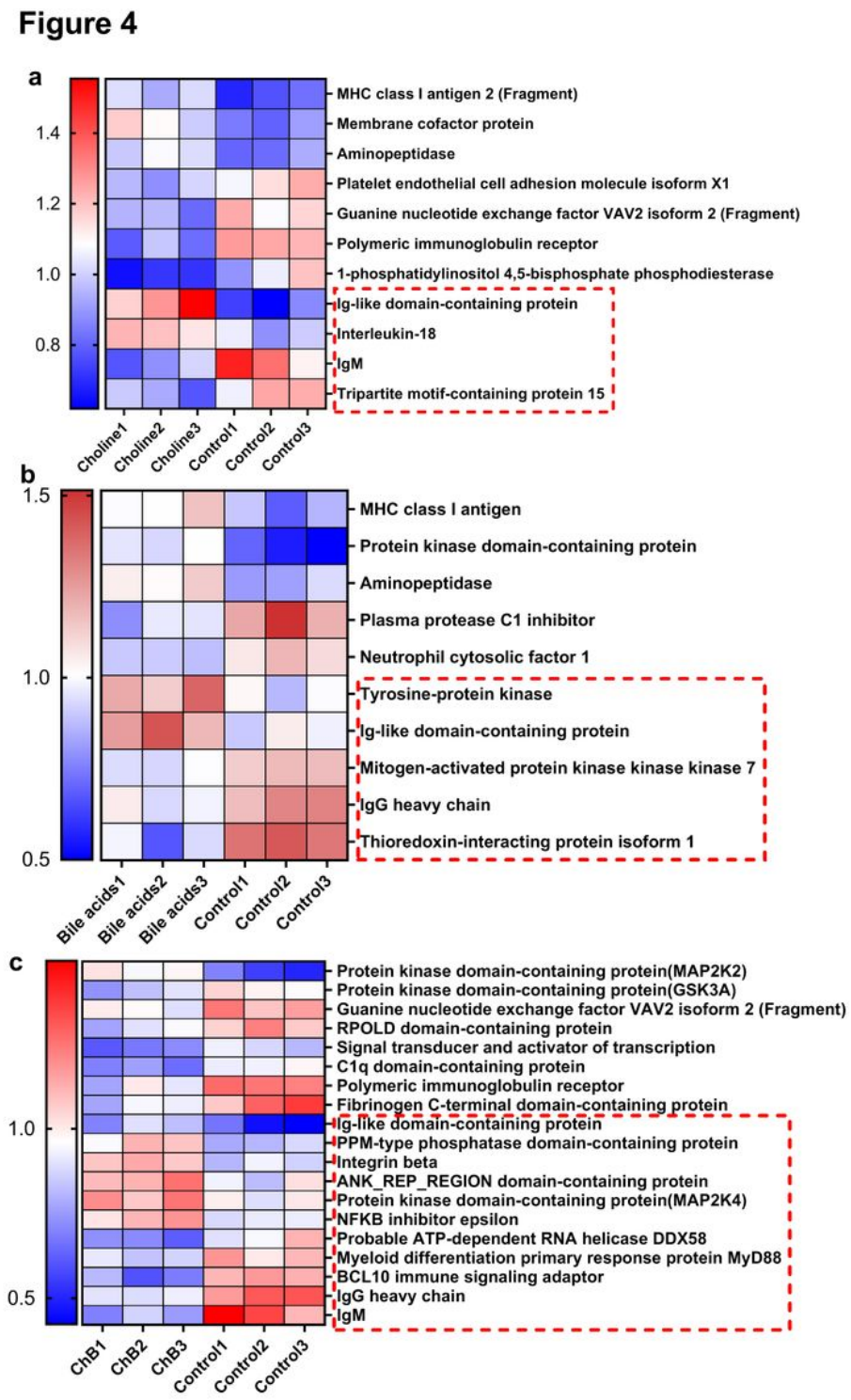

Figure 4

Proteomics analysis of the jejunal mucosa in weaned pigs. Heatmap analysis for the DEPs involved in immune system KEGG pathways of jejunal mucosa sample of weaned piglets fed choline (a), bile acids (b) and ChB (c). The proteins in the red dotted box related to NF-κB signaling pathway, toll-like receptor

signaling pathway or NOD-like receptor signaling pathway. ChB, choline and bile acids mixture; DEP, differentially expressed protein; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 5

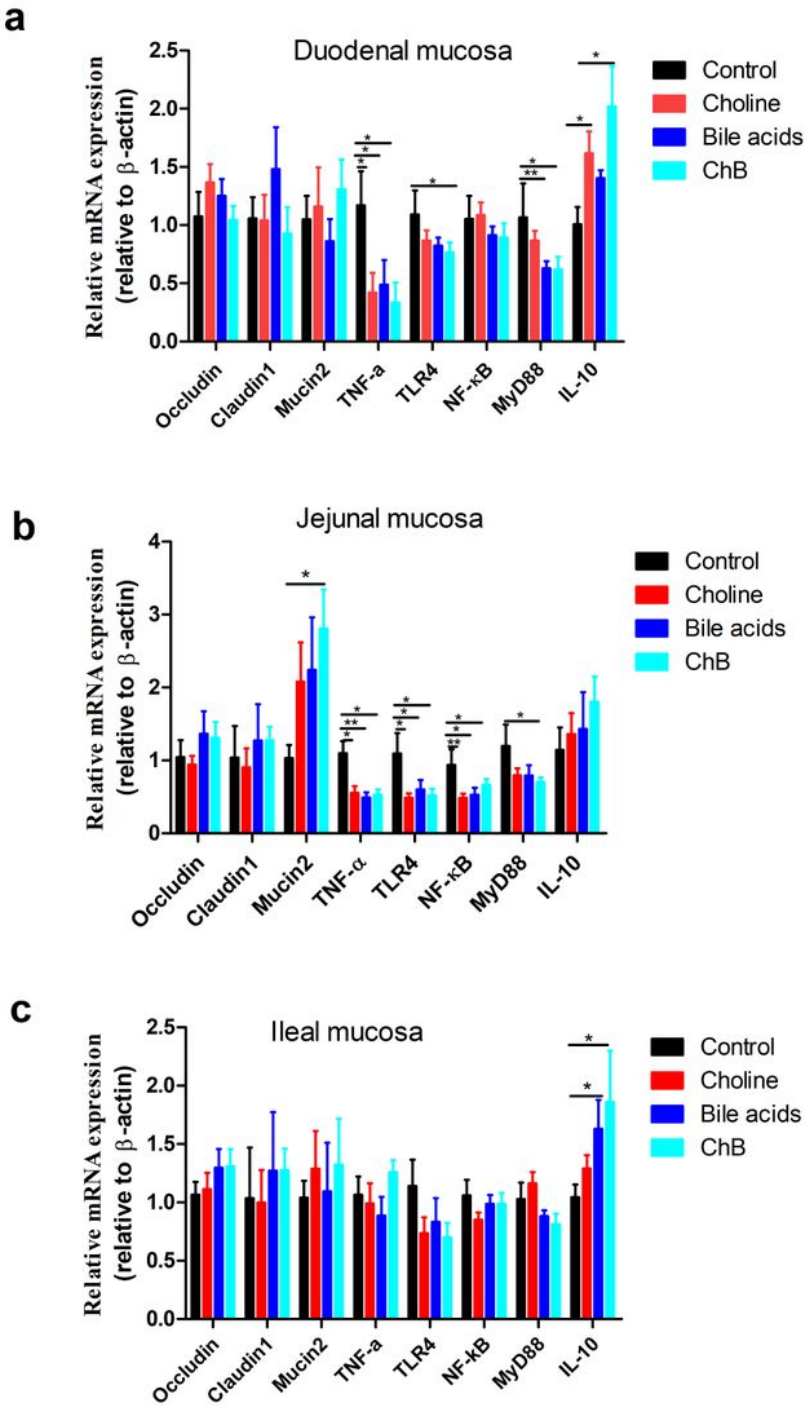

Figure 5

Diets effect on the innate immunity related and tight junction related gene expressions (a) in the duodenal mucosa, (b) in the jejunal mucosa, (c) in the ileal mucosa of weaned piglets. Differences were analyzed

by one-way ANOVA followed by Dunnett' T test. *p < 0.05, **p < 0.01, compared with control diet. ChB, choline and bile acids mixture.

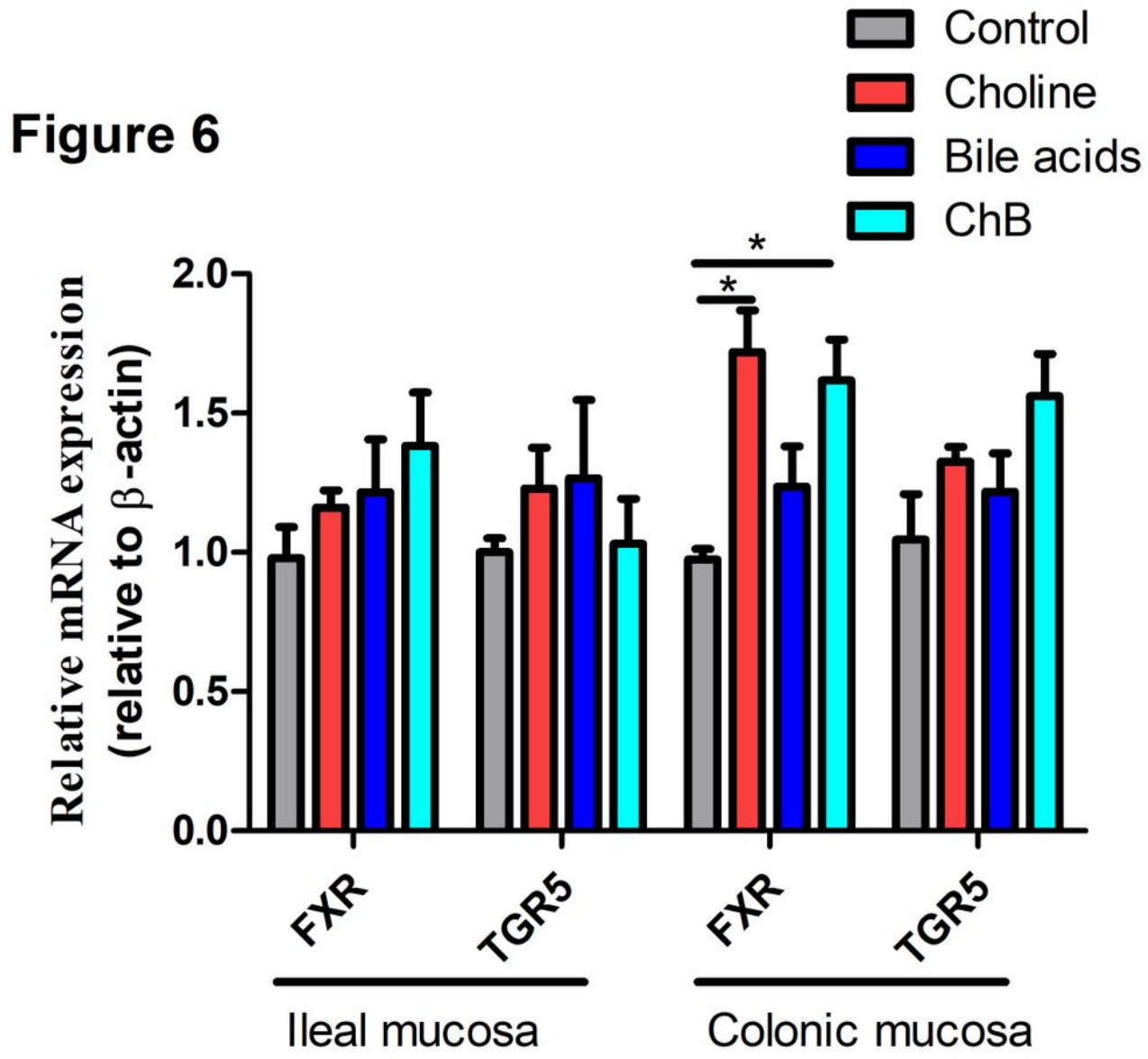

Figure 6

Diets effect on the gene expressions of FXR and TGR5 in the ileal mucosa and colonic mucosa. Differences were analyzed by one-way ANOVA followed by Dunnett' T test. *p < 0.05, compared with

control diet. TLR-4, toll-like receptor 4; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor kappa B; TNF-α, tumour necrosis factor-α; IL-10, interleukin-10; ChB, choline and bile acids mixture.

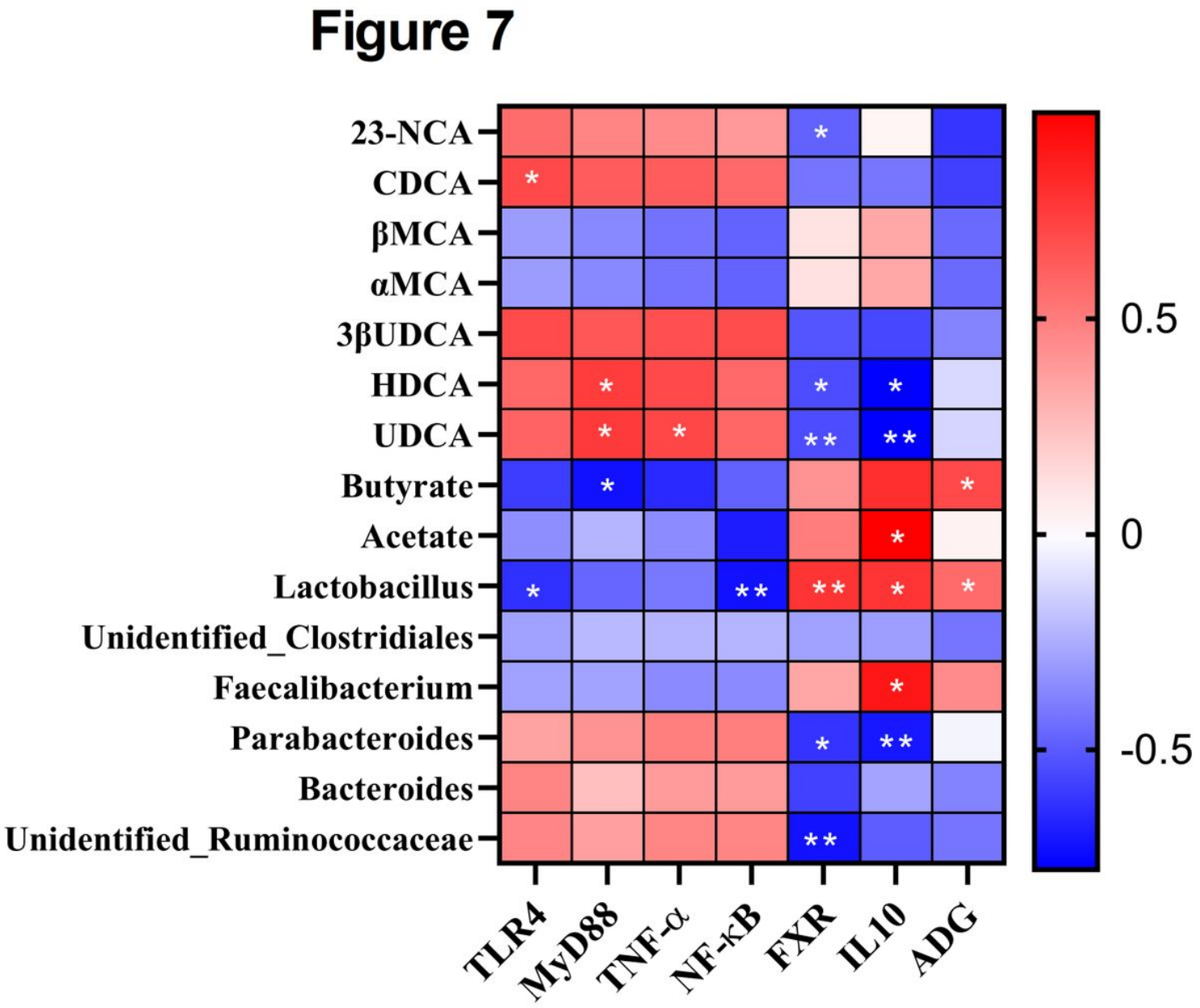

Figure 7

Heatmap of Pearson's rank correlation coefficients between the relative abundance of genera, ADG, bacterial metabolites and intestinal genes expression. In the panels, *p < 0.05 and **p < 0.01. CDCA: chenodeoxycholic acid; 3βUDCA: 3β-ursodeoxycholic acid; HDCA: hyodeoxycholic acid; UDCA: ursodeoxycholic acid; DCA: deoxycholic acid; 23-NCA: 23-norcholic acid; MCA: muricholic acid; MyD88: myeloid differentiation factor 88; NF-κB: nuclear factor kappa B; TLR4: toll like receptor 4; TNF-□: tumor necrosis factor-□; IL-10: interleukin-10; FXR: farnesoid X receptor; ADG: average daily gain.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure1.tif