Effects of Bile Acids Supplement in High Plant Protein Diet on Common Carp (Cyprinus Carpio) Bile Acids Profile and Hepatopancreas Health

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Effects of bile acids supplement in high plant protein diet on Common Carp (*Cyprinus carpio*) bile acids profile and hepatopancreas health

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Abstract

Background

Bile acids (BAs) have considerable importance in the metabolism of glycolipid and cholesterol. BAs profile in mammals has been widely reported, but lacking in fishes. The purpose of the present study is to clarify BAs profiles of common carp and how exogenous additions of BAs could alleviate hepatopancreas injured of common carp under a high plant protein diet. A 11-week feeding trial was conducted with high plant protein diet (18%) (HP) and high plant protein diet (18%) added 600 mg/kg BAs (HP+BAs) for Common carp, and then the UHPLC-MS/MS technology was used to analyze the BAs in the bile and plasma of two groups.

Results

HP could induce vacuolation of hepatocytes and accumulation of glycogen in Common carp, while these phenotypes were significantly improved in HP+BAs group. In addition, BAs profile of HP group and HP+BAs group were described in detail, for Common carp bile with treatment by exogenous BAs, TCA, CA, TβMCA and TωMCA were the main components. Furthermore, in HP+BAs group plasma, CDCA, CA, LCA, and GCDCA increased significantly, they could activate TGR5, the activation of hepatopancreas TGR5 might regulate glucose metabolism to relieve hepatopancreas glycogen accumulation.

Conclusions

HP could induce glycogen accumulation in common carp hepatopancreas while supplemented BAs to HP could mitigate this symptom. And we determined that the reduction of common carp hepatopancreas glycogen accumulation in the HP+BAs group is importantly related to the change in the endogenous BAs profile after the addition of BAs in HP through an integrated bile acids profile determination by UHPLC-MS/MS. This study has important guiding significance for aquaculture.

Keywords: Bile acids; Common Carp; High plant protein diet; Hepatopancreas; Glycogen accumulation; UHPLC-MS/MS

1. Introduction

Bile acids (BAs) are series of amphipathic molecules, that synthesized in the liver from cholesterol and stored in the gallbladder, which synthesized in liver from cholesterol [1]. Most of BAs conjugated with taurine or glycine in liver[2], and then hydrolyzed, dehydroxylated, deconjugated and functioned in gut[3, 4]. BAs secret into duodenum to promote lipid digestion and absorption in small intestine and then were reabsorbed in ileum by liver via BAs transporters and portal vein, which is defined as metabolism enterohepatic circulation[5]. BAs have been known to accelerate digestion and absorption of lipids in the gut[6], and regulate cholesterol homeostasis[7]. Moreover, recent years, scientists have found that BAs also acted as various signals receptors to participate in regulations of homeostasis of glucose and energy metabolism[8], as well as in signaling pathways[9, 10]. LCA and DCA activate TGR5 to regulate glucose metabolism and anti-inflammatory response[11, 12]. TβMCA and CDCA act together on FXR to regulate BA synthesis and glycolipid metabolism[13, 14]. These new founding of BAs' functions helped to solve various diseases caused by metabolic disorders.

In order to meet the needs of environmental protection and reduce the cost of feed, plant protein is more and more widely used in aquatic feed. However, the application of plant protein could bring negative

effects like liver and intestine damage simultaneously, finally induced BAs enterohepatic circulation disorder and hepatic lesions, together with remarkable reduction of growth performance but increased, inflammatory reaction in Amur sturgeon and Japanse seabass[15, 16]. Bile acids were widely used in aquaculture of China, it has a positive effect on fish growth performance, nutrient digestibility and immunity[17, 18]. But not all BAs have a positive effect, some BAs would bring negative effects, for example, both TCA and bovine bile salt supplementary in a low fishmeal diet to the Atlantic salmon could cause slight or moderate inflammation of the distal intestine.[19]. At present, in mammals, profile of BAs has got a lot of results and interesting discoveries have been found[20, 21], while there were few reports about concentrated on BAs in fish, since most of them focused on roles of BAs in fish pheromone systems and identification of some new BAs in saltwater fish[22-25]. In general, BAs in fish have less study, and which is relatively one-sided[26-28].Previous studies of our team suggested that high plant protein induced common carp liver injury, while BAs supplementary could help to alleviate [29], but which BA played the leading role or how to affect liver health? These are still unknown and BAs profiles offish still kept uncovered.

Common carp [30] is a kind of omnivorous fish, an important economical freshwater fish in China. It needs a certain amount of animal

protein, and a high proportion of plant protein may cause intestinal and liver diseases, thereby reducing the benefit of breeding. Therefore, the present study combined UHPLC-MS/MS technology to explore the BAs profile of Common Carp comprehensively and the effect of BAs supplement in high plant protein feed on Common Carp BAs profile and hepatopancreas health.

2. Methods

During the feeding period, the experimental fishes were maintained in compliance with the Laboratory Animal Welfare Guidelines of China (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of China, GB/T 35,892–2018).

2.1 Chemicals and reagents

Reference standards of unconjugated and conjugated BAs (list in table 1) including cholic acid-d4 (CA-d4), chenodeoxycholic acid-d4 (CDCA-d4), lithocholic acid-d4 (LCA-d4) and glycocholic acid -d4 (GCA-d4) were purchased from Steraloids Inc (Newport, RI, USA). taurocholic acid-d5 sodium salt (TCA-d5), tauro-*β*-muricholic acid-d4 sodium salt (T*β*MCA-d4) and tauroursodeoxycholic acid-d5 (TUDCA-d5) were obtained from Toronto Research Chemicals (North York, Ontario, Canada). *β*-muricholic acid-d5 *(β*MCA-d5) was bought from IsoSciences (Ambler, PA, USA). Seven deuterium-labeled BAs containing

deoxycholic acid-d4 (DCA-d4), glycolithocholic acid-d4 (GLCA-d4), glycoursodeoxycholic acid-d4 (GUDCA-d4), taurodeoxycholic acid-d4 sodium salt (TDCA-d4), glycochenodeoxycholic acid-d4 (GCDCA-d4), glycodeoxycholic acid-d4 (GDCA-d4) and ursodeoxycholic acid-d4 (UDCA-d4) were the products of Cambridge Isotope Laboratories Inc (Tewksbury, MA, USA). LC-MS grade methanol, acetonitrile and formic acid were the products of Fisher Scientific. Other materials were obtained from Shanghai Anpel Laboratory Technologies (Shanghai, China). Bile acids suppmentary products were supplied by Shandong Longchang Animal Health Care Co., Ltd., Dezhou, China (8.0% hyocholic acid (HCA), 70.9% hyodeoxycholic acid (HDCA), and 20.2% chenodeoxycholic (CDCA))

The ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) utilized in the project was an Agilent 1290 Infinity II UHPLC coupled to an Agilent 6470A TripleQQQ (TQQQ) and AB SCIEX TripleTOF6600 (QTOF). The UPLC BEH C_{18} column (100mm×2.1mm, 1.7μm) (Waters, USA) C18-Aq GracePure TM (500 mg/3 mL), was the products of Grace Davison Discovery Sciences TM (Waukegan Rd, USA). The refrigerated centrifuge, Type 5430R, was bought from Eppendorf Inc. The Tissue Gnostics Fluorescence Imaging System (TissueGnostics, Vienna, Austria)

2.2 Bile and plasma sampling

As described in Yao et al.[29], high plant (Cottonseed concentrate protein) protein diet (18%) (HP) and high plant protein diet (18%) added 600mg/kg BAs (HP+BAs) for Common carp, respectively. The fishes were fed to apparent satiation four times daily (8: 00, 11:00, 14:00 17:00) for 11 weeks. And then, plasma, liver and bile were collected after empty stomach 24 hours for analysis.

2.3 Plasma biochemical parameters

Plasma ALT (alanine aminotransferase), AST (aspartate aminotransferase), glucose and total cholesterol (TC) were measured by Reagent kits (Nanjing Jiancheng Co., Nanjing, China) following the given protocols.

2.4 Histopathological detections of hepatic tissues

The hepatopancreas tissue fixation, dehydration, embedding, hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) staining procedures are conducted as described by Yu et al.[31]. Then the pictures were visualized using TissueGnostics Fluorescence Imaging System (TissueGnostics, Vienna, Austria) and analyzed the glycogen granules and effective nucleus by StrataQuest Analysis Software (TissueGnostics, Vienna, Austria). BAs were extracted and analyzed for the corresponding plasma and bile samples with obvious hepatopancreas damage observed in HP group and no obvious abnormalities in the hepatopancreas observed in HP+BAs group. The graph abstract is shown in Figure 3.

2.5 Bile acids quantitative analysis

Plasma and bile samples were prepared following by previous report[32]. The eluted substances of UHPLC-TQqQ-MS/MS were ionized in an electro spray ionization source in negative mode (ESI^-). Both temperatures of ESI^- source drying gas and sheath gas were 300°C. The flow rate of ESI- source drying gas and sheath gas were 5 and 11 L/min, respectively. The pressure of nebulizer was 45 psi, and capillary voltage was 4000 V. The dynamic multiple reaction monitoring (dMRM) was used to acquire data in optimized MRM transition (precursor -> product), fragmentor, and collision energy (CE) as Table 1. The total scan time of per cycle was 300 ms. Chromatographic separation was operated on an UPLC BEH C_{18} column (100mm×2.1mm, 1.7μm). The column temperature was 40°C, and the flow rate was 0.45 mL/min. The mobile phase consisted of water in 0.1% formic acid (A) and acetonitrile in 0.1% formic acid (B). The chromatographic separation was conducted by a gradient elution program as follows: 0.5 min, 15% B; 1 min, 25% B; 3min, 25% B; 5 min, 34% B; 8 min, 40% B; 9 min, 52% B; 10.2 min, 58% B; 10.21 min, 100% B; 11.2 min, 100% B; 11.21 min, 15% B; 12.5 min, 15% B. The gradient elution was applied and MS detection proceeded in negative mode. Standards for all BAs were used to identify the different BA metabolites detected by UHPLC-MS/MS. The Agilent Mass Hunter software (version B.08.00) was used to control instruments and acquire

data. The raw data were processed by Agilent Mass Hunter Workstation Software (version B.08.00) by using the default parameters and assisting manual inspection to ensure the qualitative and quantitative accuracies of each compound. The peak areas of target compounds were integrated and output for quantitative calculation.

2.6 TβMCA and TωMCA qualitative analysis

TβMCA and TωMCA were qualitied by UHPLC (Agilent 1290)-Q-TOF (AB SCIEX| 6600)-MS/MS with an ESI source. The main parameters of ESI-MS /MS are as follow, declustering potential (DP): -100v, collision energy (CE): -60v, ion source gas1 (GS1): 50 arb, ion source gas2 (GS2): 60 arb, curtain gas (CUR):30arb, temperature: 600℃.

Chromatographic separation was operated as 2.4. A mass range of m/z 50 to 1000 was acquired. PeakView 2.1 Software of AB SCIEX was used to analyze the ion fragment information of TβMCA and TωMCA standards and samples.

2.7 Statistical analyses

Independent-samples T test of variance by the software SPSS Statistics 20 was used to analyze all data. Homogeneity test of variance (F-test) was also performed for the data between the two groups, log transformation analysis will be executed on the data when variance was irregular. Data were presented as mean ± SEM. Statistically significant

results were indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Graphics were dawn using GraphPad Prism 8.0 (GraphPad Software Inc. USA). All BAs unit conversion calculated from ng/mL to mM, and summated individual BAs concentration as total BAs concentration (TBA). The average concentration of individual BAs and the total BAs concentration were normalized to calculate the ratio of individual BAs to the total BAs.

3. Results

3.1 Growth performance

Compared with HP group, growth performance of HP+BAs group improved significantly ($p<0.01$) (Figure 2), the final body weight (BW) increased significantly ($p<0.01$), and hepatosomatic index (HSI) significantly decreased in HP group. The mean of HSI in HP and HP+BAs groups were 0.07 and 0.03, respectively, while the HSI of bony fish is generally 0.007-0.02[33].

3.2 Hepatopancreas histopathological examination

Fish hepatopancreas sections were examined after PAS staining and H & E staining, and 8 samples were selected to be observed and quantified the glycogen granules and effective nucleus in each group. Two typical phenotypes are shown in Figure 1A. Phenotypes I: Hepatopancreas have obvious glycogen accumulation, vacuolization, blurred cell membrane boundaries, and cell nuclei aggregation.

Phenotypes II: No significant accumulation of glycogen, and cell morphology showed no obvious abnormality, and effective nuclei also increased. Glycogen granules in HP group was significant more than HP+BAs group (p<0.001), while HP+BAs group has more active nucleus.

3.3 Plasma biochemical parameters and hepatic glycogen

Plasma biochemical parameters of ALT, AST, TC were listed in Table2. ALT and AST in HP group were apparently higher than HP+BAs (p<0.05). Supplement BAs to high plant protein diet not affected the content of TC in plasma. Plasma glucose and liver glycogen in the HP+BAs group were significantly lower (p<0.05) than those in the HP group (Figure 1D).

3.4 Bile acids profile of in common carp bile and plasma

Ten compounds, including TCA, TβMCA, TωMCA, CA,GLCA, GHCA, GCDCA, HDCA, CDCA and 7,12-KLCA were found quantified in bile samples, whose EIC was show in Figure 4. BAs profiles of common carp bile were summarized in Table 3 and Figure 6, which suggested TCA was the main bile acids in Common Carp, and followed by CA which accounted for 88-92% and 6-7% respectively, CA ranged from 140-170 μM, however, no TCDCA existed.

Moreover, eight BAs, including TCDCA, CDCA, CA, LCA, HDCA, GLCA, GCDCA and DCA were detected and quantified from the plasma

samples. Table 4showed the detailed BAs in bile and plasma.

This was the first time that MCA was found to be exist in fish. So, we confirmed this point. The confirmations of MCA detected were verified by the retention time (RT) and the abundance ratio of MS/MS. The RT and abundance ratio of MS/MS of TβMCA and TωMCA standard were 2.79 min and 2.37 min, m/z 124.0017: 106.9758: 80.9615: 79.9523 = 6: 3: 2: 4 and m/z 124.0018: 106.9759: 80.9611: 79.9536 = 40: 19: 13: 40, respectively, that in sample was consistent (Figure 5).

3.5 Supplement BAs to high plant protein feed altered the BAs Profile

The ratio of GCDCA, TβMCA, GLCA, HDCA increased significantly in HP+BAs group, while TCA and CDCA decreased (Figure6). An increase of BAs diversity in HP+BAs group could be observed in both bile and plasma. Supplementary BAs to high plant protein diet increased the proportion of G-BAs, which accounted for 0.6% and 1.5% in HP group and HP+BAs group, respectively.

4. Discussion

4.1 BAs profile changes caused by Supplementary of BAs

TCA was the main BA in common carp bile, which was consistent with the results of the only previously reported about the BAs profile of fish (angelfish (*Pterophyllumeimekei*)) bile [23]. In this study G-BAs such as GCDCA, GLCA, GHCA and GCA were detected. However, this

result is different from previous results pointed out that all animals, except for the placental mammals, conjugate their BAs exclusively with taurine[34, 35]. the reason for this difference is the insufficient coverage of non-mammals in previous studies, only chickens and croaker fish. That conclusion has always been continued use to cause little attention to G-BAs in subsequent studies on fish BAs. The food source of croaker fish in the early stage was mainly fish and shrimp. In this experiment, common carp was an omnivorous fish, and there was a relatively high proportion of plant-based feed in the diet. An animal-based diet significantly increases both BAs tauro-conjugation, and a plant-based diet promotes G-BAs[36]. Therefore, the detection of G-BAs in common carp can be reasonably explained. In addition, compared with the HP group, the percentage of G-BA in the HP+BA group increased while the percentage of T-BA decreased that consistent with G-BA and T-BA have a mutual inhibition relationship in previous studies[36, 37].

Supplementary BAs (mainly HCA, HDCA, CDCA) increased the contents of TβMCA, GLCA, GCDCA, and HDCA in common carp bile, we suppose the following could take responsibility with the help of intestinal microorganisms. HCA transformed into βMCA by 6b-epimerization and further 7b-epimerization [38], then βMCA was reabsorbed back to liver and combined with taurine into TβMCA, while HDCA was directly reabsorbed to the liver. CDCA is dehydroxylated into

LCA (Based on KEGG secondary bile acid biosynthesis, map00121), which is partly excreted from the body, and part is reabsorbed to the liver and combined with glycine to form GLCA. The other part of CDCA is reabsorbed to the liver to combine with glycine.

The discovery of TβMCA and TωMCA in Common Carp was a break though since they were thought to be a rodent specific bile acid[38-42], and it not indeed be found in birds and monogastric animal BAs analysis[43, 44], but which has also been found in humans by some reports[20, 45]. Rodents branch off from fish in the evolutionary tree, and humans and rodents are on a small branch, we considered that MCA maybe a common specie of bile acids existed in fish, rodents, and primates. More work should be done in future.

4.2 Supplement BAs affected common carp BAs profile to reduced hepatopancreas glycogen accumulation and alleviated hepatopancreas damage with a high plant protein diet

Supplement BAs reduced liver glycogen accumulation and alleviated liver damage in common Carp with a high plant protein diet. High plant protein could induce fish intestinal and liver damage that has been confirmed in the previous research through our laboratory[15, 29, 46, 47]. Intestinal is the organ to digest and absorb nutrients, while damage and functional barriers would lead to nutritional metabolism disorders, especially proteins[48, 49],thus affecting the synthesis of key enzymes of

other nutrients. In this study, the reason that a high-plant-protein diet cause liver glycogen accumulation and hyperglycemia is the high-plant-protein diet injures the Common carp's intestines and leading to protein digestion and absorption disorders, and then resulting in a lack of phosphorylase (the key enzymes in liver glycogen decomposition) synthesis. Disorders of glucose metabolism can stimulate cell inflammation and apoptosis, which caused liver damage[31, 50]. However, supplementary BAs to high plant protein feed could alleviate intestinal and liver damage[29]. The saponins of plant protein feed may be the main cause of Common carp intestinal damage[51]. BAs can be combined with non-starch polysaccharides and excreted from the body[52]. The structure of saponins is similar to that of non-starch polysaccharides. After BAs supplementary could be combined with saponins, thereby reduce the damage of saponins to the intestinal, and then improved protein digestion and absorption, reduced liver glycogen accumulation.

Common carp hepatopancreatic inflammation and glucose metabolism may be regulated by three purposes that were LCA, CDCA and CA to activate liver TGR, increased liver glycine concentration and TβMCA inhibits intestinal FXR. TGR5 could be activated by some BAs, in which LCA is the most potent agonist for TGR5, DCA and the conjugations, CDCA and the conjugations, and CA and the conjugations

activate TGR5 effectively simultaneously [53]. TGR5 plays an important role in anti-inflammatory and glucose metabolism[54]. TGR5 restrains the activated B cells (NF-κB) to control the proinflammatory factors secretion by mediation of the interaction between IκBα and β-arrestin2 and thus exert anti-inflammatory effects[55-57]. Activating liver TGR5 could reduce blood glucose in mice with a high-fat die [53]. That suggested the potential Hypoglycemic function of TGR5. In present study, plasma CDCA, CA, LCA and GCDCA increased significantly in HP+BAs group, with the assistant of enterohepatic circulation of BAs[58], they will enter the liver and activate TGR5, especially LCA, that cannot be synthesized directly in the liver while can only be recovered from intestinal BAs by blood circulation, thus enhancing the anti-inflammatory ability of the body, regulating glucose metabolism and reducing blood glucose, and decreased the liver inflammation[59]. The expression of TGR5 gene in the liver increase after supplement BAs to HP have been confirmed by Yao et al[29].

In the results of T. IDE et al., it can be found that the content of G-BAs in bile increases with the increase of liver glycine concentration[60]. Kupffer cells in an activated state could release a variety of inflammatory mediators and play a leading role when the liver is invaded, Glycine inactivates Kupffer cells and can protect the liver from inflammation[61]. TβMCA varied quite distinctly in bile between

HP group and HP+BAs group. TβMCA is an farnesoid X receptor (FXR) nuclear receptor antagonist[14].FXR is a member of the nuclear receptor superfamily that is primarily expressed in the liver, kidney, and intestine[62].In FXR gene knockout mice, intestinal glucose absorption was delayed, together with blood glucose decreased[63]. TβMCA suppressed the enterohepatic FXR-FGF15 signaling could affect glucose metabolism, reduce blood glucose and treat diabetes [64]. Therefore, the increase of TβMCA in bile after the addition of BAs also plays a certain role in the reduction of plasma glucose.

5. Conclusion

In summary, HP could induce glycogen accumulation in common carp hepatopancreas while supplemented BAs to HP could mitigate this symptom. BAs supplementary in a high plant protein diet could change the BAs profile of common carp, among them, plasma LCA, CDCA, CA increased significantly, TβMCA and the proportion of G-BAs in bile increased significantly, which might play a leading role in it that reduced accumulation of hepatopancreas glycogen and maintained hepatopancreas health. This study proceeded an integrated bile acids profile determination by UHPLC-MS/MS to identify the effect of exogenous BAs supplementary on the endogenous BAs profile and hepatopancreas health of common carp and discussed how the BAs supplementary is transformed in the body, providing a theoretical basis for the application

of BAs products in aquatic animals and a data basis for revealing the mystery of fish BAs.

6. Availability of data and materials

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

7. Abbreviations

BAs: Bile acids

HP: High plant protein diet (18%)

HP+BAs: High plant protein diet (18%) added 600 mg/kg BAs

UHPLC-MS/MS: Ultra-high performance liquid chromatography-tandem mass spectrometry

FXR: Farnesoid X receptor

TGR5: G protein coupled bile acid receptor 1

CA-d4: Cholic acid-d4

CDCA-d4: Chenodeoxycholic acid-d4

LCA-d4: Lithocholic acid-d4

GCA-d4: Glycocholic acid -d4

TCA-d5: Taurocholic acid-d5 sodium salt

TβMCA-d4: Tauro-β-muricholic acid-d4 sodium salt

TUDCA-d5: Tauroursodeoxycholic acid-d5

βMCA-d5: β-muricholic acid-d5

DCA-d4: Deoxycholic acid-d4

GLCA-d4: Glycolithocholic acid-d4

GUDCA-d4: Glycoursodeoxycholic acid-d4

TDCA-d4: Taurodeoxycholic acid-d4 sodium salt

GCDCA-d4: Glycochenodeoxycholic acid-d4

GDCA-d4: Glycodeoxycholic acid-d4

UDCA-d4: Ursodeoxycholic acid-d4

HCA: Hyocholic acid

HDCA: Hyodeoxycholic acid

TQQQ: Agilent 6470A TripleQQQ

QTOF: AB SCIEX TripleTOF6600

CA: Cholic acid

CDCA: Chenodeoxycholic acid

LCA: Lithocholic acid

GCA: Glycocholic acid

TCA: Taurocholic acid

TβMCA: Tauro-β-muricholic acid

TUDCA: Tauroursodeoxycholic acid

βMCA: β-muricholic acid

DCA: Deoxycholic acid

GLCA: Glycolithocholic acid

GUDCA: Glycoursodeoxycholic acid

TDCA: Taurodeoxycholic acid

GCDCA: Glycochenodeoxycholic acid

GDCA: Glycodeoxycholic acid

UDCA: Ursodeoxycholic acid

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

TC: Total cholesterol

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Contributions

Xian Wei, Ting Yao and Fatou Ndoye FALL conceived the experiment. Xian Wei analyzed all data. Xian Wei and Ting Yao wrote the manuscript. Xian Wei, Ting Yao, Fatou Ndoye FALL, Xiaofang Liang, Jie Wang, Wenlong Du, Xu Gu and Min Xue reviewed the manuscript and read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

Not applicable.

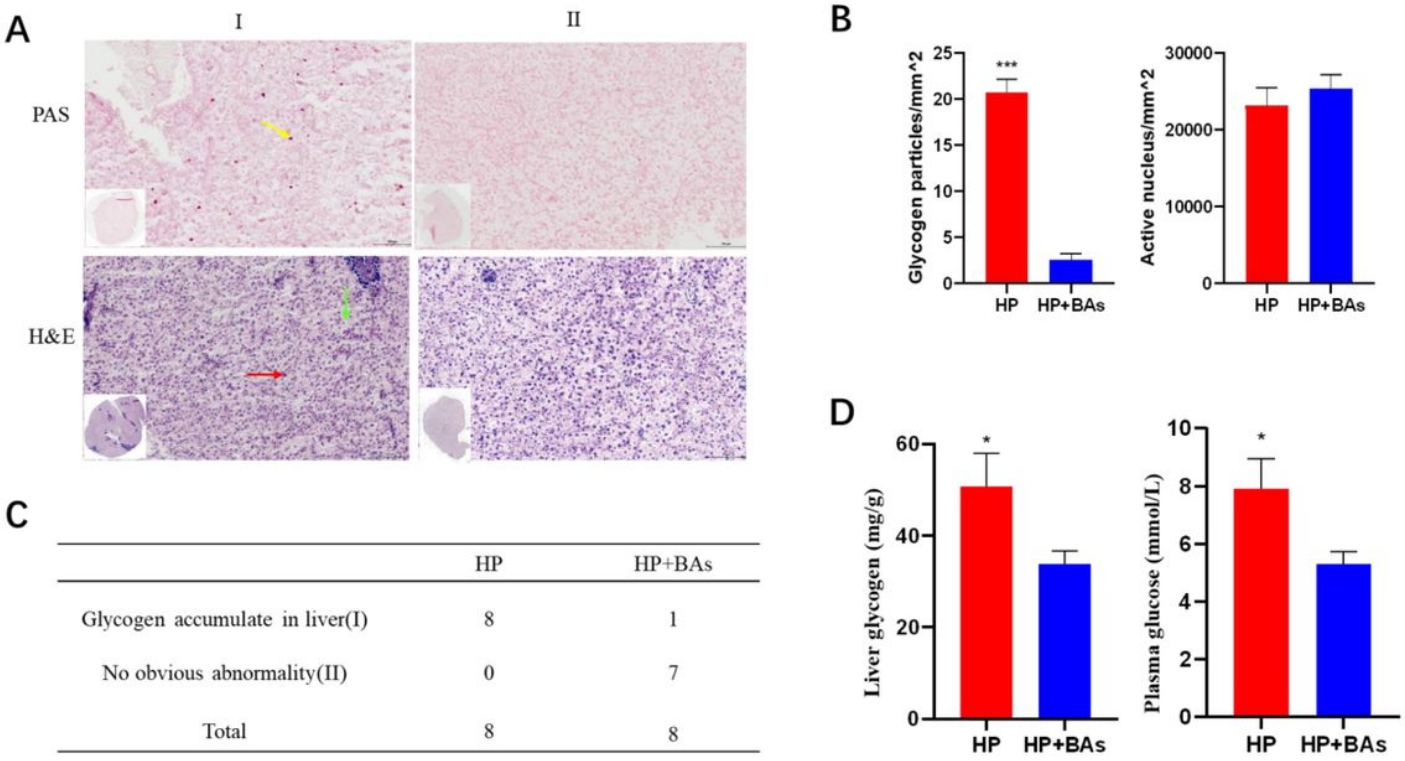
Consent for publication

All the authors read and agree to the content of this paper and its publication.

Competing interests

The authors declare no competing interest.

Figures



C

	HP	HP+BAs
Glycogen accumulate in liver(I)	8	1
No obvious abnormality(II)	0	7
Total	8	8

Figure 1

Supplement BAs to high plant protein feed reduced liver histological lesions (Statistically significant results were indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)): (A) PAS and H&E staining of liver sections with bar = 100 μm, intracellular accumulation of glycogen (marked with yellow arrow), deformed cells (marked with green arrow) and Nuclear gathered (marked with yellow arrow) were clearly observed in the damaged liver. (B) Quantification of glycogenosome and active nucleus. (C) Phenotype of liver histopathological examination in HP group and HP+BAs group. (D) Supplement BAs to high plant protein feed reduced liver glycogen and plasma glucose (Statistically significant results were indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$), n=7).

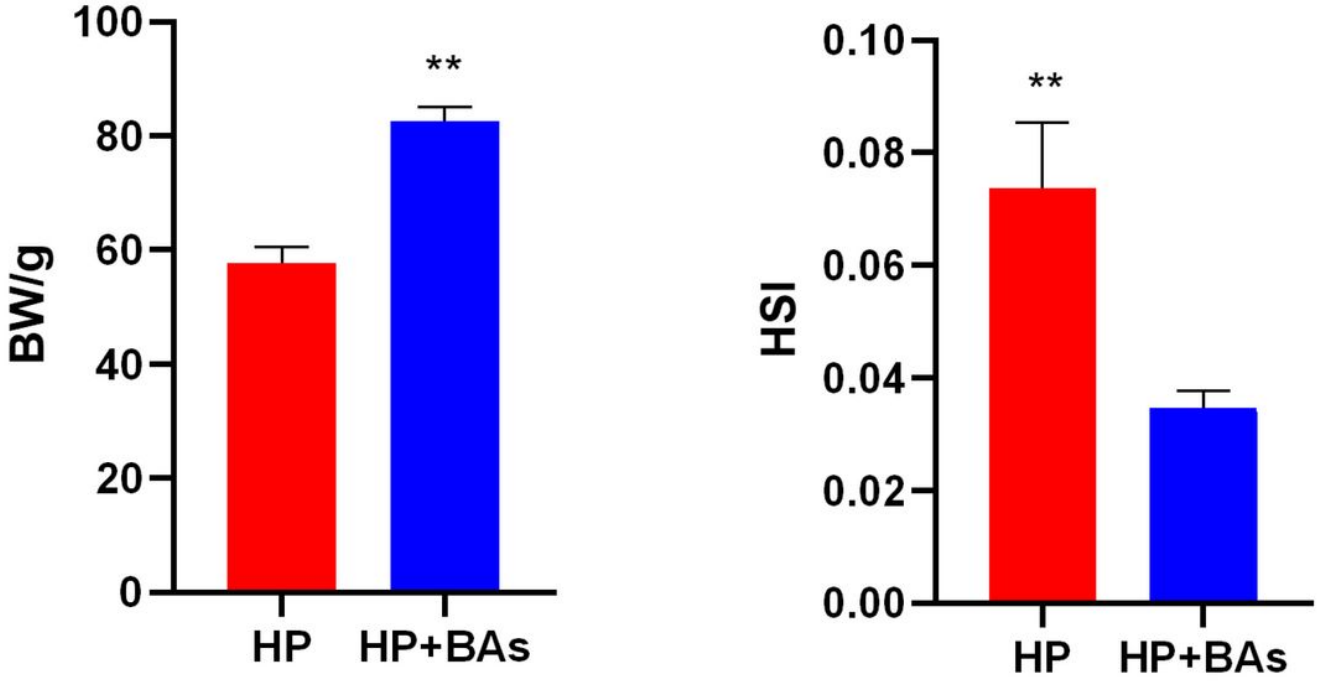


Figure 2

Supplement BAs to high plant protein feed improved the growth performant and reduced the hepatosomatic index (HSI) in Common carp (Statistically significant results were indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$), n=7).

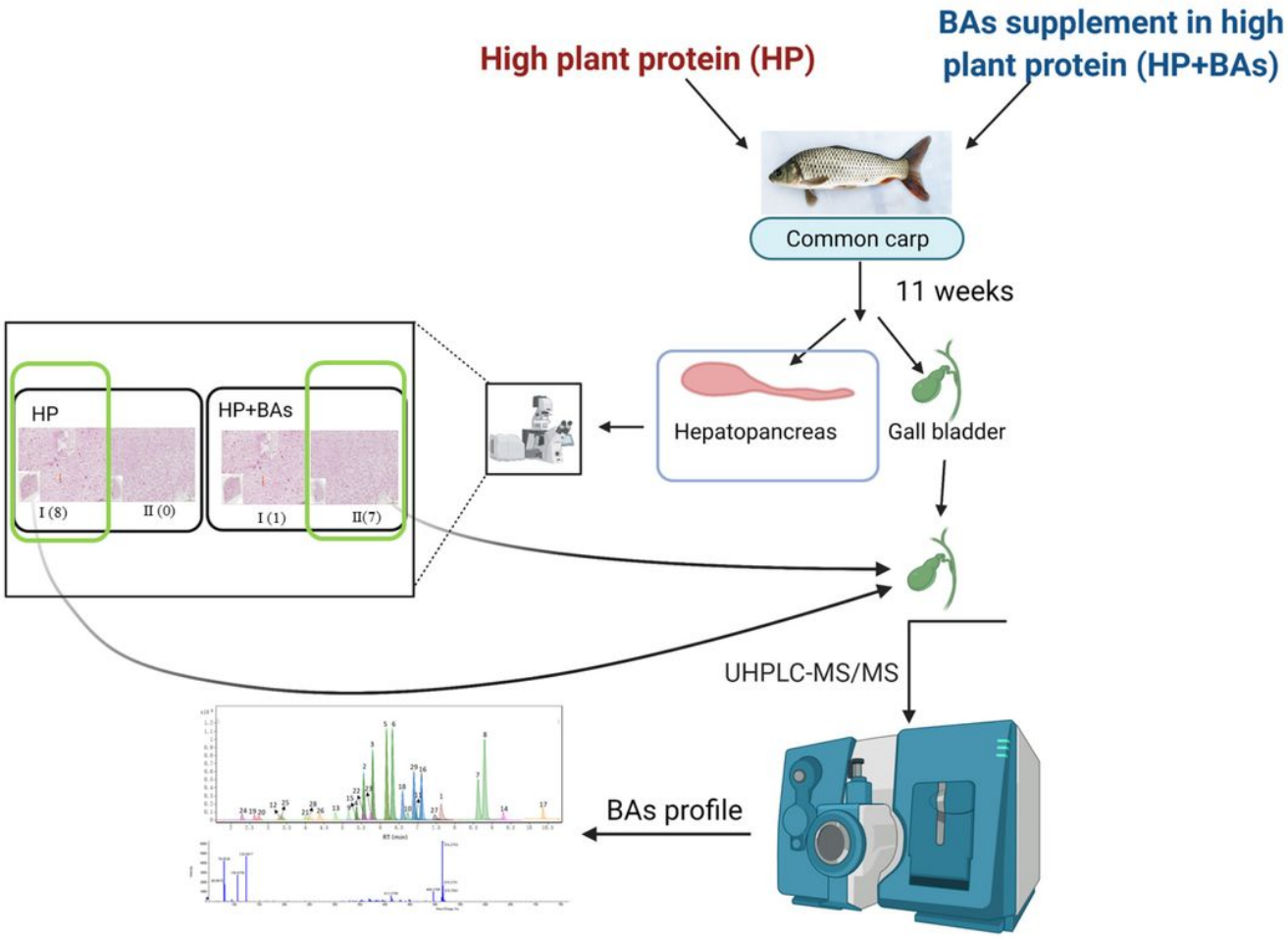


Figure 3

The workflow of the effect in bile acids supplement to high plant protein diet on Common Carp bile acids profile and hepatopancreas health.

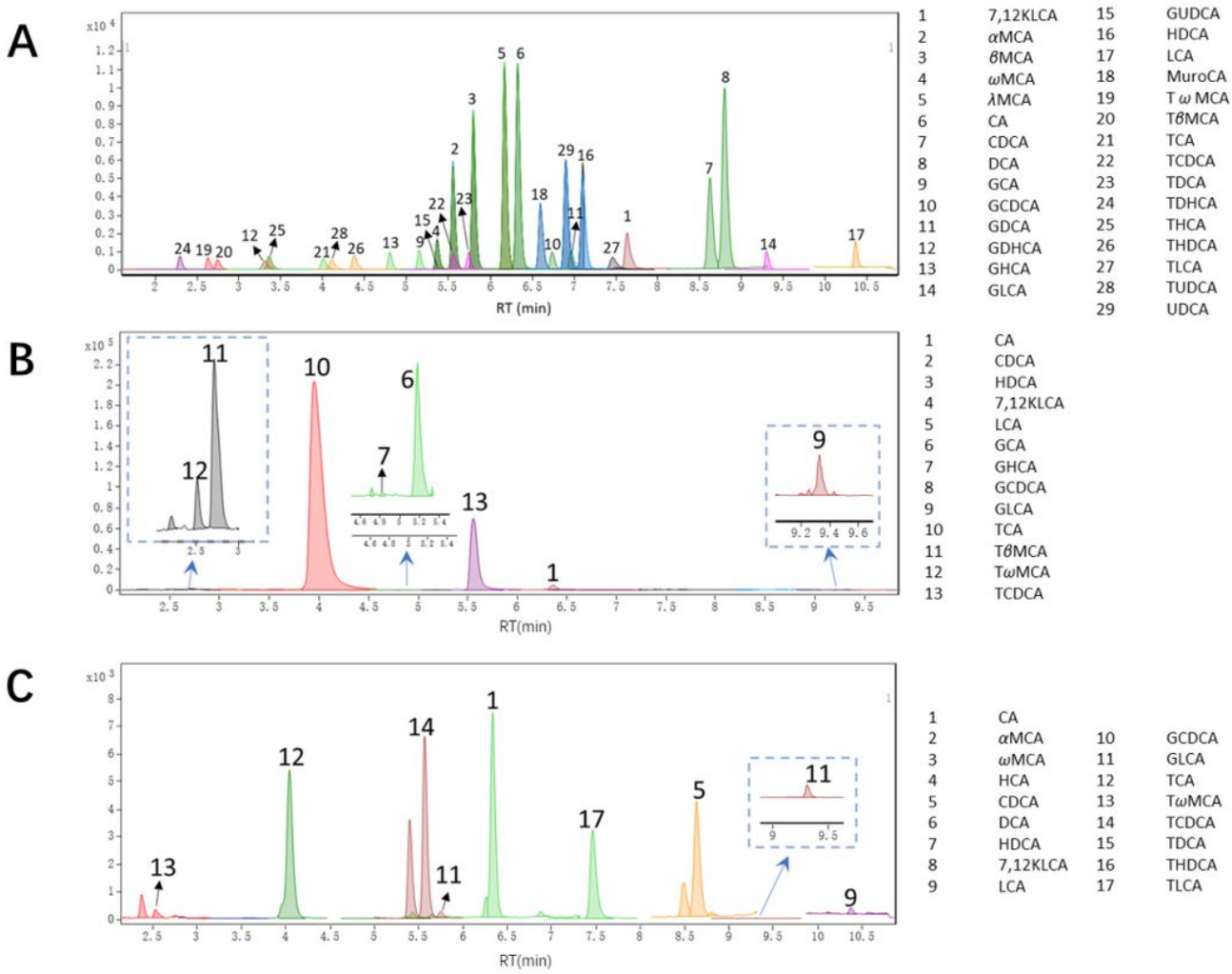


Figure 4

Bile acids Extracted Ion Chromatogram (EIC) in sample and standard. A is standard EIC, B for bile sample, C for plasma sample.

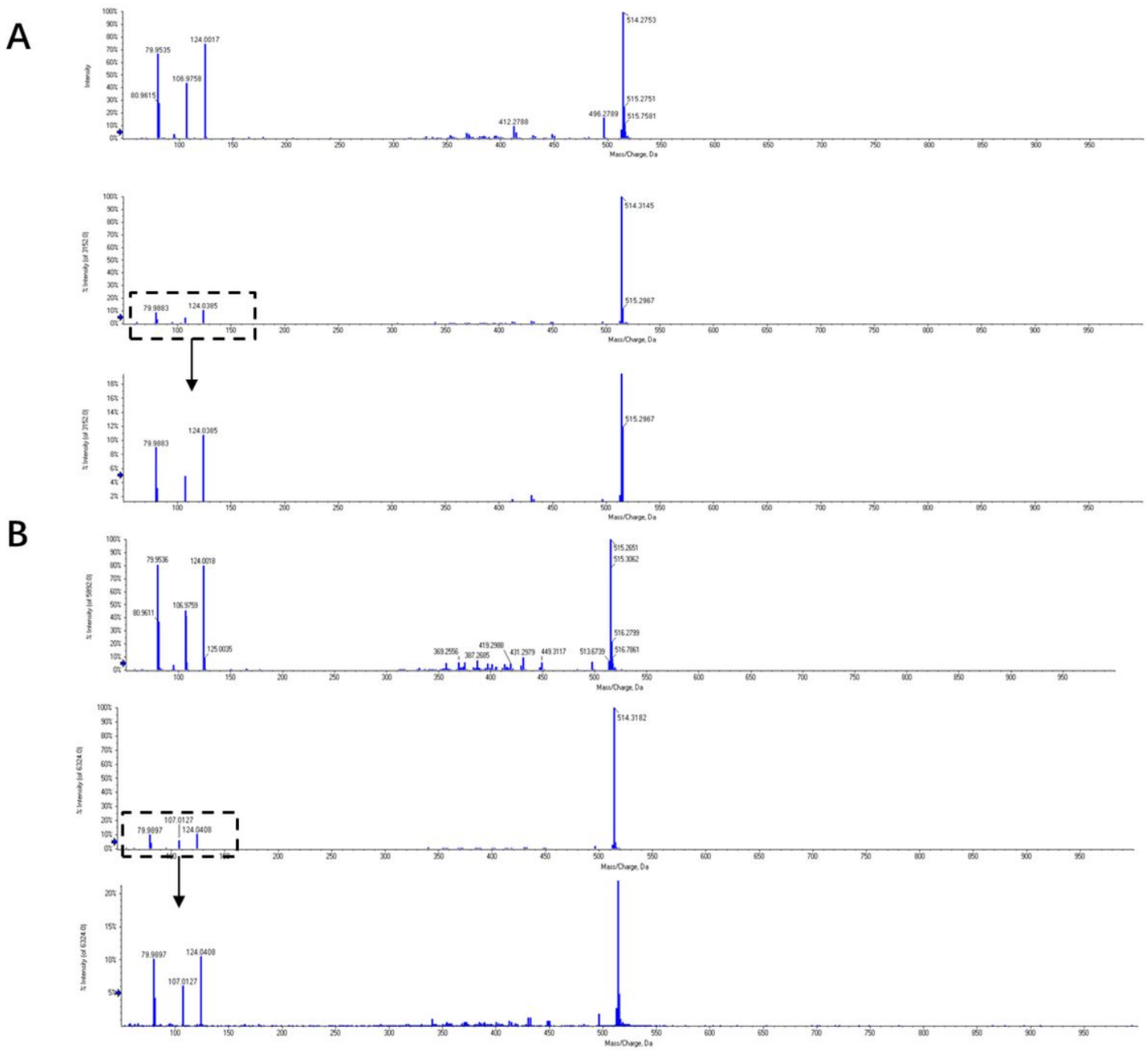


Figure 5

Tandem mass spectrometry (MS/MS) of TβMCA and TωMCA in sample and standard. A for TβMCA, B for TωMCA.

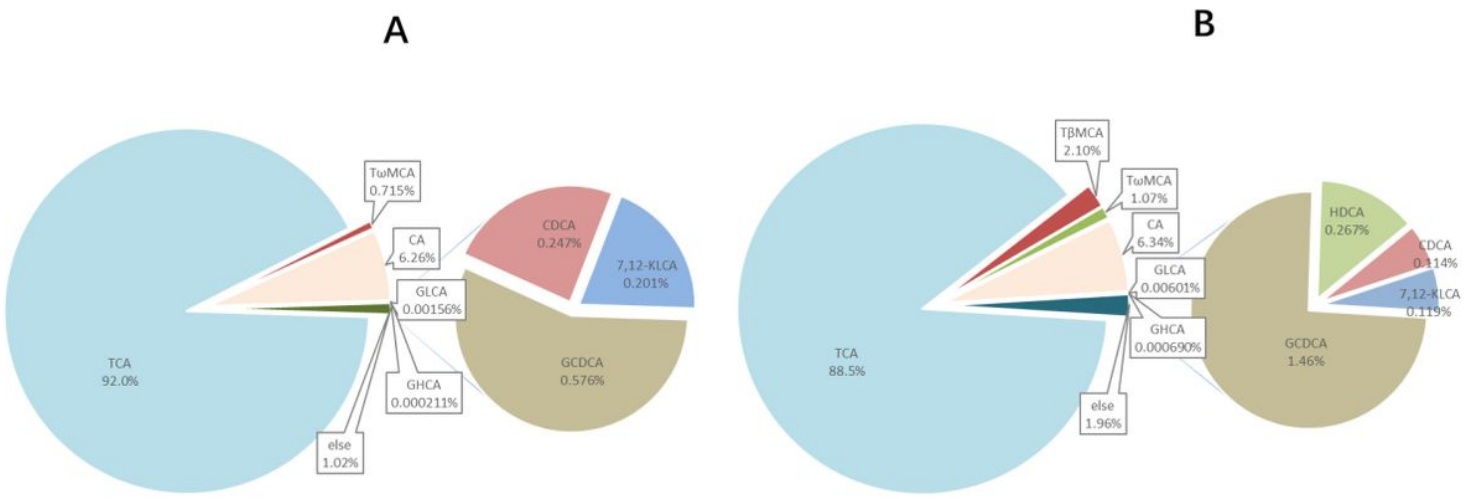


Figure 6

BAs species composition in bile, A and B for HP and HP+BAs, respectively (n=7).

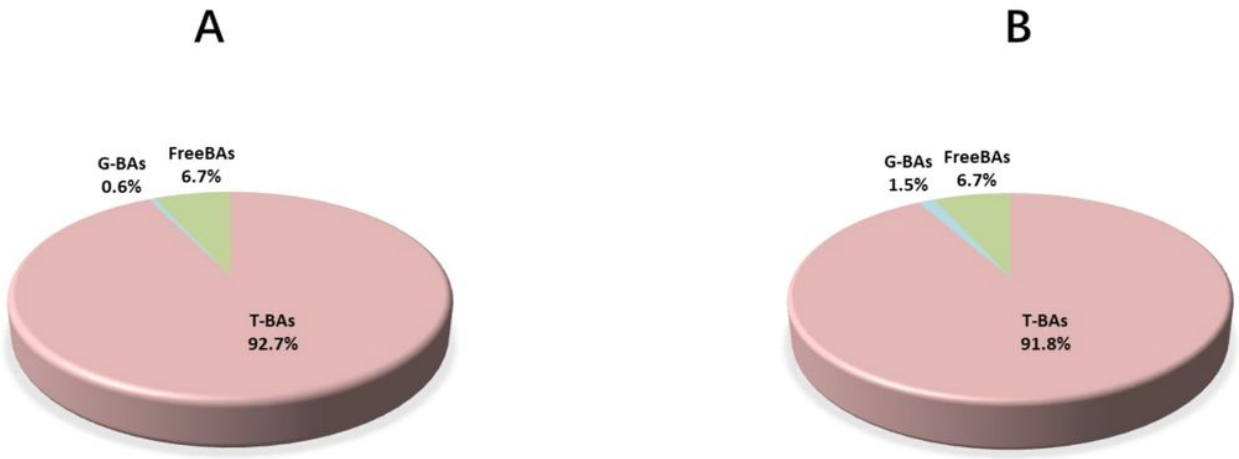


Figure 7

Supplement BAs to high plant protein feed increases the proportion of Glycine-conjugation BAs, A and B for HP and HP+BAs, respectively (n=7).