

Dietary pectin caused greatly changes in bile acid profiles of *Pelteobagrus fulvidraco*

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Research Article

Keywords: bile acid profiles, dietary fiber, pectin, yellow catfish

Posted Date: May 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-534668/v1>

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Abstract

To reveal the impact of dietary fiber (DF) on the bile acid (BA) profiles of fish, yellow catfish (*Pelteobagrus fulvidraco*) were fed diet containing 300 g kg⁻¹ dextrin (CON diet, control) or pectin (a type of soluble DF, PEC diet) for 7 days, and then the BA profiles were analyzed by UHPLC-MS/MS. A total of 26 individuals of BAs were detected in the fish body, with 8, 10, 14, and 22 individuals of BAs were detected in the liver, serum, bile and hindgut digesta, respectively. The conjugated BAs (CBAs) of fish were dominated by taurine-CBAs (TCBAs). The concentrations of free BAs (FBAs) and the values of FBAs/CBAs in the bile of fish fed the PEC diet were nearly 5 and 7 times higher, respectively, than those in fish fed the CON diet. The values of glycine-CBAs/TCBAs in the liver, serum and bile of fish fed the PEC diet were significantly lower, and in the hindgut digesta was higher than that of fish fed the CON diet ($P < 0.05$). These results suggested that dietary pectin greatly changed the BA profiles of *Pelteobagrus fulvidraco*, attributed to inhibition of reabsorption of BAs. Therefore, attention should be paid to the impact to BA homeostasis when replacing fishmeal with DF-rich plant ingredients in the fish diet.

1. Introduction

Bile acids (BAs) are synthesized in liver hepatocytes and stored in the gall bladder. The gallbladder contracts and empties bile into the intestines after eating. Approximately 95% of BAs in the intestine are reabsorbed and return to the liver via the portal vein (Chiang, 2004). BAs are reused through this enterohepatic circulation. The fecal excretion of BAs is compensated by synthesis in the liver, therefore, the BA homeostasis is tightly regulated under normal conditions. As hormones or nutrient signaling molecules, BAs involved in regulating glucose, lipid, lipoprotein, and energy metabolism as well as inflammatory responses (Li et al. 2020b; Li et al. 2017; Nguyen and Bouscarel 2008; Wan and Sheng 2018). Even a slight difference in BA hydroxylation patterns may exert drastically different biological responses (Li and Chiang 2009; Song et al., 2015). Therefore, BA profile assessments are garnering increasing interest to screen potential biomarkers for the early diagnosis of human liver and intestinal diseases (Fu et al. 2020; Slopianka et al. 2017), malnutrition (Lin et al. 2020), and healing processes (Roda et al. 2019). It was noticed that the BA profiles of fish could be influenced by diet (Zhang et al. 2017), however, it was unclear about the agent in diet that exerted the effect.

With the increasing shortage of fish meal resource, the inclusion of plant feedstuffs is enhanced in aquatic feed, which means that dietary fibers (DFs) are higher in the fish diet (Flis et al. 2017). Although DFs can not be digested and absorbed by monogastric animals, it can be fermented and utilized by intestinal flora (Flis et al. 2017; Singh et al. 2019). As a result of which, the structure and function of intestinal flora are changed (Cai et al, 2020b; Singh et al. 2019), and further influence the BA profile (Ghaffarzadegan et al. 2018; Li et al. 2020a; Singh et al. 2019). Moreover, DFs can bind to BAs (Gunness et al. 2016) and therefore inhibit their biological function, including working as an activator of the farnesoid X receptor (FXR) (Wang et al. 1999). It was reported that the inhibition of FXR resulted in BA hypersynthesis (Aguilar-Olivos et al. 2014). It can be inferred from above reasons that high load of DFs would result in a change of BA profiles.

It was well known that soluble DFs caused stronger physiological response than insoluble DFs (Ren et al. 2020; Singh et al. 2018). Pectin is one type of soluble DF. In this study, the effects of pectin on the BA profiles of yellow catfish (*Pelteobagrus fulvidraco*) were studied. The research results will provide a reference for better utilization of plant feed resources in aquaculture, and also provide a new perspective to understand and control fish diseases.

2. Materials And Methods

2.1 Diet

Pectin was purchased from Zhengzhou Mingrui Chemical Products Co., Ltd. (Zhengzhou, China). The other feed ingredients were provided by Xinyu Feed Co., Ltd. (Suzhou, China), and their nutrition composition was the same as in our previous report (Ren et al. 2020). A diet containing 300 g kg⁻¹ dextrin was used as a control (CON diet), while the test diet contained 300 g kg⁻¹ pectin (PEC diet). The formulation of diets is shown in Table 1, and diets were pelleted and stored as described by Ren et al. (2020).

2.2 Fish and feeding

Juvenile yellow catfish purchased from Nanxun Commercial Nursery, Huzhou City, Zhejiang Province, were reared in indoor cement ponds for 2 months to acclimate to the experimental conditions. Feeding trials were conducted with polyethylene tanks (400 L). Fish (initial mean weight 20.3 ± 0.2 g) were randomly stocked into tanks with 20 fish per tank. Six tanks of fish were randomly assigned to feed the CON diet and PEC diet in triplicate twice a day at 7:00 am and 17:00 pm. The lab light intensity was lower than 3,000 lux at 14:00 pm with a natural photoperiod. Water was introduced from Yangcheng Lake, deposited for 24 hours and filtered by sand before it was used. The water temperature was 25~30 °C, pH 7.5~7.8, DO > 6.0 mg L⁻¹, and ammonia-nitrogen concentration < 0.01 mg L⁻¹. Fish were fed for 7 days before sampling.

2.3 Sampling and sample preparation

After 7 days of feeding, sampling was conducted as in previous studies (Ren et al. 2020). Briefly, after 12 h of fasting (sampling should not be done after 24 h of fasting because hindgut digesta needed to be collected), fish were rapidly netted and anesthetized with 200 mg L⁻¹ MS-222. Six fish taken from each tank were used to draw blood. Blood was naturally solidified and centrifuged at 3000 rpm to separate serum. Other fish were dissected and then collected the liver, bile and hindgut digesta, frozen in liquid nitrogen and stored at -80 °C.

2.4 Analysis

The BA profiles of liver, serum, bile and hindgut digesta of yellow catfish were determined by UHPLC-MS/MS, which was performed by Shanghai Baitree Biotech Co., Ltd. The sample was processed and

measured according to the method of Han et al. (2015). An Agilent 1290 Infinity series (Agilent Technologies) UHPLC was used, and the chromatographic column was a Waters ACQUITY UPLC Beh C18 (150 × 2.1 mm, 1.7 μm, Waters). Phase A was a 0.01% formic acid aqueous solution, and phase B was acetonitrile. The chromatographic gradient is shown in Table 2. The temperature of the column incubator was 40 °C, the sample tray was 4 °C, and the volume of injection was 3 μL. Q Exactive Focus was used for mass spectrometry analysis. The ion source parameters were as follows: spray voltage = +3500/-3100 V, sheath gas (N₂) flow rate = 40, aux gas (N₂) flow rate = 15, sweep gas (N₂) flow rate = 0, aux gas (N₂) temperature = 350 °C, and capillary temperature = 320 °C. Standards of 38 types of bile acids were used in this study, and their abbreviations are listed in Table 3. Cholic acid (CA), chenodeoxycholic acid (CDCA) and their conjugates with taurine or glycine were regarded as primary BAs (PBAs), while others were regarded as secondary BAs (SBAs) (Yang and Duan 2016).

2.5 Statistics

Data are expressed as the mean ± standard deviation. Statistical differences were examined by t-test using SPSS (version 19.0 SPSS, Chicago, IL, USA), and the significance level was set at $P < 0.05$.

3. Results

After 7 days of feeding, the livers of yellow catfish fed the CON diet were red, while fish fed the PEC diet exhibited typical green liver syndrome (Fig. 1).

A total of 26 individuals of BAs were detected in yellow catfish (Table 3). Eight individuals of BAs were detected in the liver (Fig. 2), including 3 individuals of PBAs and 5 individuals of SBAs. Compared with fish fed the CON diet, a significant increase was observed in tauroolithocholic acid (TLCA) and a decrease was observed in the glycine-conjugated BAs (GCBAs)/taurine-conjugated BAs (TCBAs) ($P < 0.05$, Fig. 2). The mean values of PBAs, SBAs, free BAs (FBAs), conjugated BAs (CBAs,) and TCBAs increased 86%, 129%, 131%, 62% and 62%, respectively, while the GCBAs and the FBAs/CBAs decreased 70% and 33%, respectively. However, the inter-group variations were also large and there was no statistical significance ($P > 0.05$, Fig. 2).

In the serum of yellow catfish, 10 individuals of BAs, including 4 individuals of PBAs and 6 individuals of SBAs, were detected. Compared with the fish fed the CON diet, the taurochenodeoxycholic acid (TDCA) significantly increased in fish fed the PEC diet ($P < 0.05$, Fig. 3). No significant change was observed for other individuals ($P > 0.05$, Fig. 3). The GCBAs/TCBAs significantly decreased ($P < 0.05$, Fig. 3). The mean values of PBAs, CBAs, TCBAs, TBA increased 84%, 98%, 101%, 42% respectively, while the GCBAs and the FBAs/CBAs decreased 54% and 69% respectively, although no statistical significance ($P > 0.05$).

Five individuals of PBAs and 14 individuals of SBAs were detected in bile (Fig. 4). Compared with fish fed the CON diet, PBAs such as GCA and TCDCA considerably decreased ($P < 0.05$, Fig. 4), while CA and CDCA significantly increased in fish fed the PEC diet ($P < 0.05$, Fig. 4). SBAs such as ursolic acid (UCA), 7,12-diketolithocholic acid (7,12-DLCA), glycodehydrocholic acid (GHDC), 7-ketodeoxycholic acid

(7-KDCA), 12-dehydrocholic acid (12-DHCA), 3-dehydrocholic acid (3-DHCA), allocholic acid (ACA), 7-ketolithocholic acid (7-KLCA), 12-ketolithocholic acid (12-KLCA) and deoxycholic acid (DCA) dramatically increased ($P < 0.05$, Fig. 4), while glycodeoxycholic acid (GDCA) sharply decreased ($P < 0.05$, Fig. 4). In addition, PBAs, CBAs, GCBAs, TCBA, TBA, PBAs/SBAs, and GCBAs/TCBAs in fish fed the PEC diet decreased noticeably ($P < 0.05$, Fig. 4), while SBAs, FBAs and FBAs/CBAs increased significantly ($P < 0.05$, Fig. 4). In particular, the FBAs and FBAs/CBAs in the bile of PEC diet fish were approximately 5 and 7 times higher, respectively, than those in the CON diet group (Fig. 4).

A total of 22 individuals of BAs were detected in the hindgut digesta (Fig. 5), including 5 individuals of PBAs and 17 individuals of SBAs. Among PBAs, the CA in fish fed the PEC diet increased notably ($P < 0.05$, Fig. 5), while CDCA, GCDCA, and TCDCA significantly decreased ($P < 0.05$, Fig. 5) compared with fish fed the CON diet. Meanwhile, among SBAs, UCA, 7,12-DLCA, 7-KDCA, 12-DHCA, 3-DHCA, ursodeoxycholic acid (UDCA), 7-KLCA, 12-KLCA, apocholic acid (APOCA), and DCA showed an upward trend ($P < 0.05$, Fig. 5), while allocholic acid (ACA) and hyodeoxycholic acid (HDCA) decreased significantly ($P < 0.05$, Fig. 5). Moreover, PBAs, PBAs/SBAs, FBAs/CBAs, and GCBAs/TCBAs increased significantly, while CBAs, TCBA, and GCBAs decreased significantly ($P < 0.05$, Fig. 5).

4. Discussion

Due to the wide-ranging and important functions of BAs, BA profile analysis is garnering increasing attention in medicine and zootechnics (Fu et al. 2020; Lin et al. 2020). However, neither the reason for nor the effect of changes in BA profiles has received sufficient attention in fish. The results of this study clearly showed that pectin lead to significant changes in BA profiles in the liver, serum, bile, and hindgut digesta of yellow catfish (Figs. 2-5). To our knowledge, this was the first report of the effect of DF on fish BA profiles.

In this study, 26 individuals of BAs were detected in total, and the BA diversity varied in different parts of yellow catfish. There were more BA individuals in the hindgut digesta (22 individuals) than in the liver (8 individuals), serum (10 individuals) and bile (14 individuals). BAs are synthesized in liver hepatocytes, stored in the gall bladder, secreted into intestine after eating. In the intestine, CBAs dissociate into FBAs by intestinal microbiota and further transform into various SBAs through 7 α -dehydroxylation (Jia et al. 2018; Ridlon et al. 2018), while some SBAs are secreted with feces, therefore, the individuals of BAs are the most diverse in hindgut digesta (Li et al. 2019; Li et al. 2020a).

BAs are amphipathic molecules with powerful detergent properties. Most PBAs are conjugated to either glycine or taurine (named bile salt) to increase their solubility before being secreted into biliary bile (Takahashi et al. 2016). The contents of GCBAs and TCBA vary among species. GCBAs are dominant in human BAs (Johnson et al. 1991), while TCBA are the main ingredients in rodents (Killenberg and Jordan 1978). The results of the present study showed that the concentrations of TCBA in the bile of yellow catfish ($1461 \pm 192 \text{ mmol L}^{-1}$, Fig. 4) were nearly one hundred times higher than those of GCBAs ($15 \pm 1.5 \text{ mmol L}^{-1}$, Fig. 4) and were close to the TBA content ($1543 \pm 196 \text{ mmol L}^{-1}$, Fig. 4). The value of

GCBAs/TCBAs was 0.01, 0.02, 0.01 and 0.16 in liver, serum, bile and hindgut digesta, respectively. These results were consistent with the findings in medaka (*Oryzias latipes*) (Hagey et al. 2010), Atlantic salmon (*Salmo salar* L.) (Kortner et al. 2016), Korean rockfish (*Sebastes schlegeli* Hilgendorf) (Kim et al. 2014) and Japanese flounder (*Paralichthys olivaceus*) (Kim et al. 2005), indicating that TCBAs were dominant in the CBAs of fish species.

BAs synthesized in the liver are PBAs. Compared with fish fed the CON diet, the PBA concentration increased 86% in the liver of fish fed the PEC diet, which confirmed that BA synthesis was hyperactive under short-term pectin stress (Cai et al. 2020a). In rats it was also observed that DFs induced hypersynthesis of BAs and enlargement of the BA pool size (Ghaffarzadegan et al. 2018). However, the concentration of PBAs in the bile of fish fed the PEC diet decreased noticeably compared with that in the CON diet group ($P < 0.05$, Fig. 4). This might be due to the following three reasons: (1) only a few of BAs in bile are newly synthesized by liver, most are reabsorbed BAs from intestine (Chiang, 2004). Therefore, the BA profile of bile are mainly affected by the type and concentration of reabsorbed BAs. (2) the PBAs in serum of fish fed the PEC diet was nearly 2 times higher than that of fish fed CON diet (Fig. 3), suggested that the uptake ratio of PBAs from plasma into hepatocytes was low in fish fed PEC diet, which also contributed to the low PBAs in bile. (3) DFs have a capacity to bind BAs, which varied among BAs (Ni et al., 2021; Suharoschi et al. 2019). By binding BAs, DFs increased BA excretion with feces (Gunnness et al. 2016; Gunnness and Gidley 2010; Thandapilly et al. 2018). In this study, the PBA concentrations in the hindgut digesta of fish fed PEC diet were higher than those of fish fed the CON diet ($P < 0.05$, Fig. 5), suggested that the excretion of PBAs increased, which was also the reason for the low PBAs in bile. It could also be inferred that when the loss of BAs with feces was not sufficiently compensated by synthesis, the size of the BA pool would shrink. A reduction in the BA pool size induced by long-term DF stress was observed in our previous study (Cai et al. 2020).

Pectin not only caused changes in the contents and ratios of PBAs and SBAs but also led to an enhancement in FBAs and/or a reduction in CBAs ($P < 0.05$, Fig. 4-5), resulting in an approximately 7-fold increase in the FBAs/CBAs in bile (Fig. 4) and a 4-fold increase in hindgut digesta (Fig. 5). FBAs were more toxic (Penman et al. 2019). This might be one of the reasons for the observation of tissue damage to the hindgut in fish fed the PEC diet in our previous study (Ren et al. 2020). In addition, increases in DCA in digesta (Fig. 5), which is thought to induce gut dysbiosis and promote intestinal inflammation (Xu et al. 2020), might also contribute to intestinal tissue damage. In order to against the hydrophobicity increased with FBAs concentration, more water would be absorbed into bile, which might be the reason for the low TBA concentration in bile of fish fed PEC diet (Fig. 4).

The GCBAs/TCBAs in the digesta of fish fed the PEC diet was higher than that of the CON diet group ($P < 0.05$, Fig. 5). This result indicated that the binding capacity of pectin to GCBAs might be stronger than that to TCBAs and then cause higher loss of GCBAs with feces (Ni et al. 2021). Consistent with the higher value of GCBAs/TCBAs in the digesta, there was a significant decrease in the value of GCBAs/TCBAs in the liver, serum, and bile ($P < 0.05$, Fig. 2-4). These results also implied that pectin might lead to a shortage of glycine in the fish body. It was reported that dietary glycine supplementation relieved

inflammation to alleviate intestinal injury in LPS-challenged piglets (Tian et al. 2018; Xu et al. 2018) and markedly mitigated liver histopathological changes and collagen deposition in cholestatic mice (Heidari et al. 2018). Therefore, shortage of glycine might be another reason for the histopathologic changes induced by high DFs diet (Cai et al. 2020; Ren et al. 2020; Singh et al. 2018), which is valuable to further study.

Despite the decreases in the value of GCBAs/TCBAs in bile, the concentration of TCBAs was also decreased significantly in fish fed the PEC diet compare with fish fed the CON diet ($P < 0.05$, Fig. 4). TCBA is derived from PBAs and taurine. Taurine was found to improve fish growth (Kotzamanis et al. 2020; Martins et al. 2021) and reduce intestine inflammation (Martins et al., 2021) in fish fed plant feedstuff-based diets. The mechanism of improving effect may be involved in the increasing in concentration of TCBAs and then reduction in BA toxicity (Penman et al. 2019).

It is widely recognized that the incidence of chronic diseases could be reduced by high DFs diet. Zhu et al. (2017) reported that pectin penta-oligogalacturonide reduces cholesterol accumulation by promoting bile acid biosynthesis and excretion in high-cholesterol-fed mice. Cheng et al. (2019) reported that xyloglucan affects gut-liver circulating bile acid metabolism to improve liver damage in mice fed with high-fat diet. However, Singh et al. (2018) have shown that feeding innate immune-deficient mice a diet enriched in DFs (including pectin, inulin, and fructo-oligosaccharides) could induce liver cancer, which are initiated with cholestasis. In our previous study, we observed that pectin induced cholestasis and green liver syndrome in yellow catfish (Cai et al. 2020a). In this study, in addition to the dramatic change in BA profiles, green liver syndrome was observed again in fish fed PEC diet (Fig. 1). As mentioned earlier, BAs are hormones or nutrient signaling molecules, their slight fluctuation may exert drastically different biological responses (Li and Chiang 2009; Marica et al. 2017; Song et al. 2015). Results of this study together with above reference suggested that high DFs intake would cause great risk on health, starting from the disruption of BA homeostasis. People often explore the etiology of fish diseases such as bleeding, rotten gills, enteritis, etc. from the perspective of pathogenic organisms, results of this study indicated that the BA homeostasis should be paid enough attention in diseases prevention.

5. Conclusion

A total of 26 individuals of BAs were detected in yellow catfish, with 8, 10, 14, and 22 individuals of BAs detected in the liver, serum, bile and hindgut digesta, respectively. The TCBAs were dominant in the CBAs. Dietary pectin changed the BA profiles greatly, might partly through binding and then inhibition of reabsorption of BAs. It was suggested that attention should be paid to the changes in the BA profiles when fish intake high DFs diet.

Declarations

Funding

This work is supported by the Fishery Science and Technology Projects of Jiangsu Province [Y2018-20], the Major Project of the Natural Science Foundation of the Jiangsu Higher Education Institutions of China [20KJA240001], and the Science and Technology Plan Project of Suzhou [SNG2020060].

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Availability of data

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

Xiamin Cao organized the experiment and was responsible for histological analysis and manuscript writing. Shengjie Ren was responsible for fish feeding, biochemical analysis, and took part in all experimental procedures. Chunfang Cai supervised the research project, verified the data and supervised the writing of the manuscript. Qin Ni took part in data analysis. Xinyue Li, Zijing Meng and Yunhe Meng took part in gene expression analysis. Ye Shi took part in sampling and biochemical analysis. Huang Chen and Rong Jiang took part in preliminary investigation and field sample collection for bile acid profiles analysis. Ping Wu designed of Primer Specific for genes and experimental designing, data analysis. Yuantu Ye took part in experimental designing, data analysis and writing of the manuscript.

Ethics approval

Study protocol was approved in advance by the Ethics Committee of Soochow University.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

This work is supported by the Fishery Science and Technology Projects of Jiangsu Province [Y2018-20], the Major Project of the Natural Science Foundation of the Jiangsu Higher Education Institutions of China [20KJA240001], and the Science and Technology Plan Project of Suzhou [SNG2020060].

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Tables

Table 1 Composition and nutrient levels of experimental diets (g kg⁻¹, air-dry basis)

Ingredients ¹	CON diet	PEC diet
White fish meal	300	300
Squid paste	20	20
Spirulina powder	30	30
Corn protein powder	80	80
Dextrin	300	0
Pectin	0	300
Glycocholic acid	0	0
Mixed plant meal	200	200
Vitamin and mineral premix	10	10
Yeast extract	0.5	0.5
Calcium dihydrogen phosphate	5	5
Zeolite power	24.5	24.5
Fish oil	10	10
Soybean oil	10	10
Soybean Phospholipid	10	10
Total	1000	1000
Proximate composition		
Moisture	124	97
Crude protein	360	367
Crude lipid	44	47
Ash	97	121

¹ Feed ingredients was the same as that used in previous study (Ren et al., 2020).

Table 2 Chromatographic gradient parameters

Number	Time	A (H ₂ O)	B (ACN)	Flow
1	0.0 min	75.0%	25.0%	0.40 mL min ⁻¹
2	5.0 min	74.2%	25.8%	0.40 mL min ⁻¹
3	5.5 min	71.5%	28.5%	0.40 mL min ⁻¹
4	10.0 min	71.0%	29.0%	0.40 mL min ⁻¹
5	12.0 min	64.0%	36.0%	0.40 mL min ⁻¹
6	26.0 min	32.5%	67.5%	0.40 mL min ⁻¹
7	26.2 min	1.0%	99.0%	0.40 mL min ⁻¹
8	28.2 min	1.0%	99.0%	0.40 mL min ⁻¹
9	28.4 min	75.0%	25.0%	0.40 mL min ⁻¹
10	32.0 min	75.0%	25.0%	0.40 mL min ⁻¹

Table 3 Standards of 38 individuals of bile acids (BAs) and detected BAs in liver, serum, bile and hindgut digesta

No.	BA Standards	Abbreviation	Detected BAs (marked \checkmark)			
			Liver	Serum	Bile	Hindgut digesta
1	Dehydrolithocholic acid	DHLCA				
2	Allolithocholic acid	ALCA				\checkmark
3	Isolithocholic acid	ILCA				
4	Lithocholic acid	LCA				\checkmark
5	23-Nordeoxycholic acid	23-NDCA				
6	7-Ketolithocholic acid	7-KLCA		\checkmark	\checkmark	\checkmark
7	12-Ketolithocholic acid	12-KLCA			\checkmark	\checkmark
8	Apochoolic acid	APOCA				\checkmark
9	Ursodeoxycholic acid	UDCA				\checkmark
10	Hyodeoxycholic acid	HDCA				\checkmark
11	Chenodeoxycholic acid	CDCA		\checkmark	\checkmark	\checkmark
12	Deoxycholic acid	DCA			\checkmark	\checkmark
13	Isoodeoxycholic acid	IDCA				
14	Dehydrocholic acid	DHCA				
15	7,12-Diketolithocholic acid	7,12-DLCA			\checkmark	\checkmark
16	6,7-Diketolithocholic acid	6,7-DLCA				
17	7-Ketodeoxycholic acid	7-KDCA		\checkmark	\checkmark	\checkmark
18	7-Dehydrocholic acid	7-DHCA	\checkmark			
19	12-Dehydrocholic acid	12-DHCA	\checkmark	\checkmark	\checkmark	\checkmark
20	3-Dehydrocholic acid	3-DHCA			\checkmark	\checkmark
21	Ursocholic acid	UCA			\checkmark	\checkmark
22	α -Muricholic acid	α -MCA				\checkmark
23	β -Muricholic acid	β -MCA				
24	λ -Muricholic acid	λ -MCA			\checkmark	
25	Allocholic acid	ACA	\checkmark	\checkmark	\checkmark	\checkmark
26	Cholic acid	CA	\checkmark	\checkmark	\checkmark	\checkmark
27	Glycolithocholic acid	GLCA				

28	Glycoursodeoxycholic acid	GUDCA				
29	Glycohyodeoxycholic acid	GHDCA				
30	Glycochenodeoxycholic acid	GCDCA			√	√
31	Glycodeoxycholic acid	GDCA			√	
32	Glycodehydrocholic acid	GHDCA			√	
33	Glycocholic acid	GCA	√	√	√	√
34	Taurolithocholic acid	TLCA	√	√	√	√
35	Tauroursodeoxycholic acid	TUDCA				
36	Taurohyodeoxycholic acid	THDCA				
37	Taurochenodeoxycholic acid	TCDCA	√	√	√	√
38	Taurodeoxycholic acid	TDCA	√	√	√	√

Figures

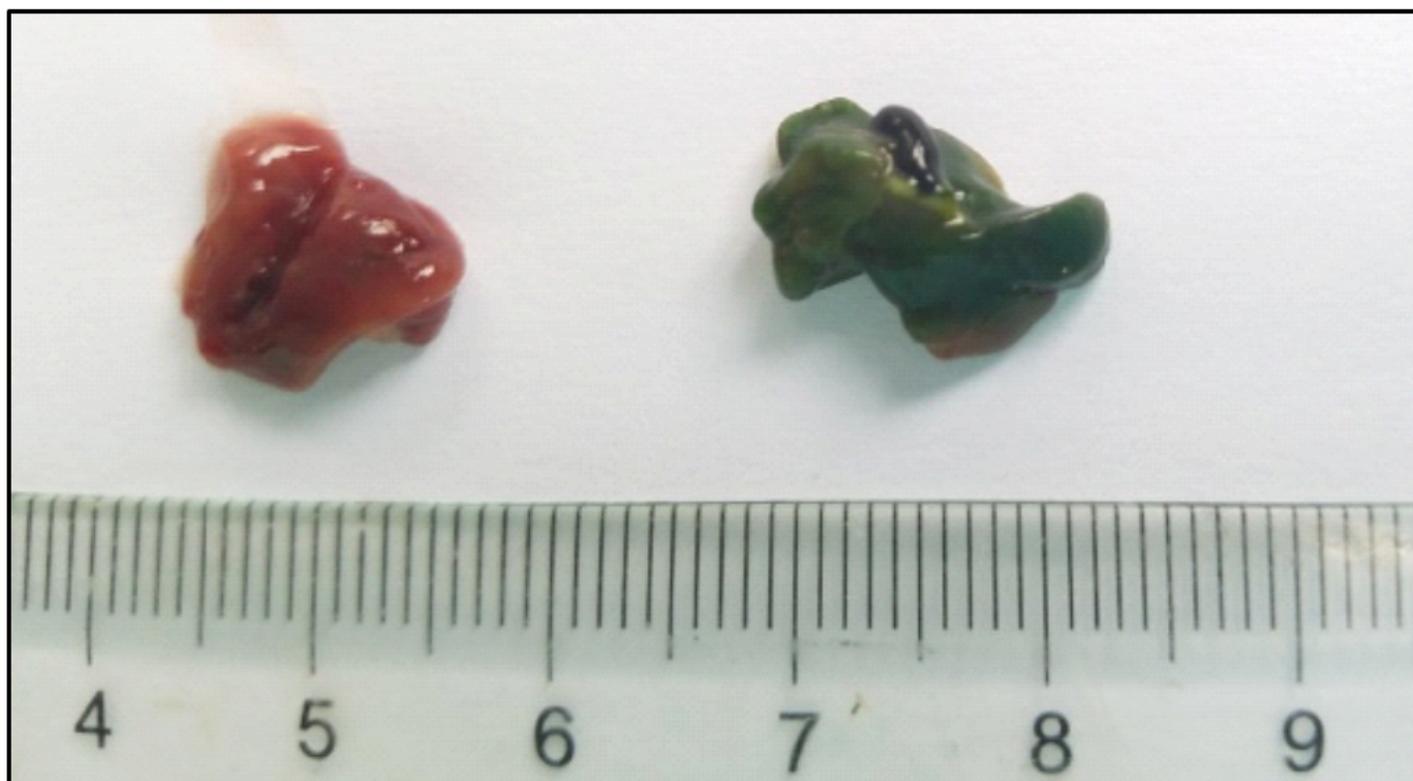


Figure 1

The color of liver of fish fed the CON diet (left) was red and fed the PEC diet (right) was green

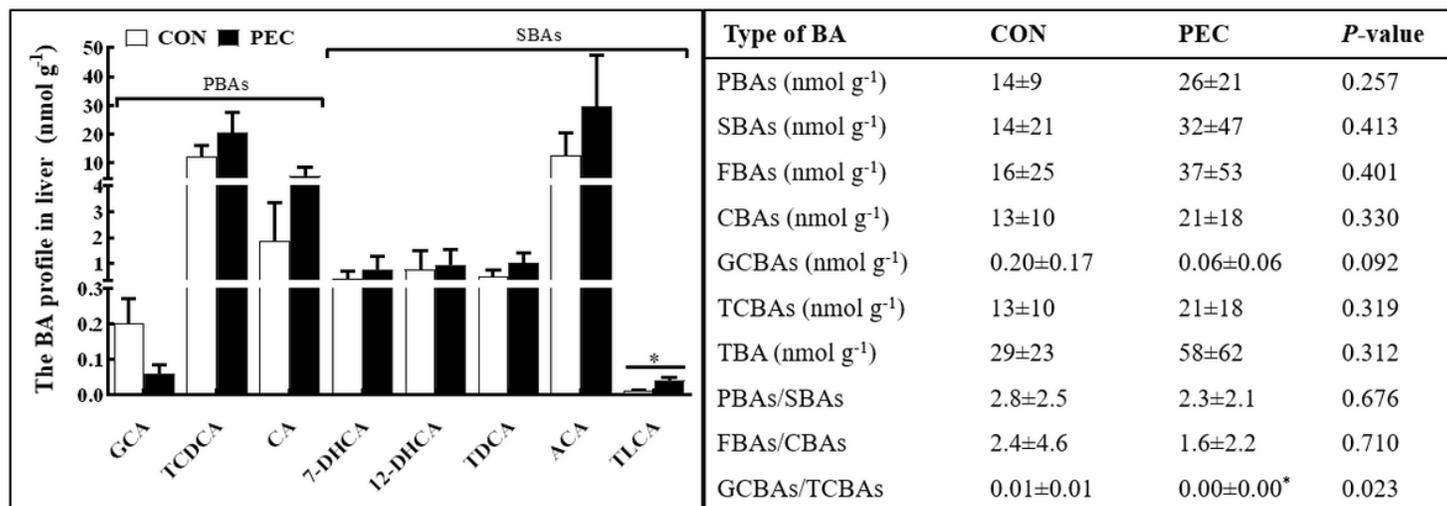


Figure 2

BA profile (nmol g⁻¹) in the liver of yellow catfish, *Pelteobagrus fulvidraco*, fed on the CON diet and PEC diet for 7 days

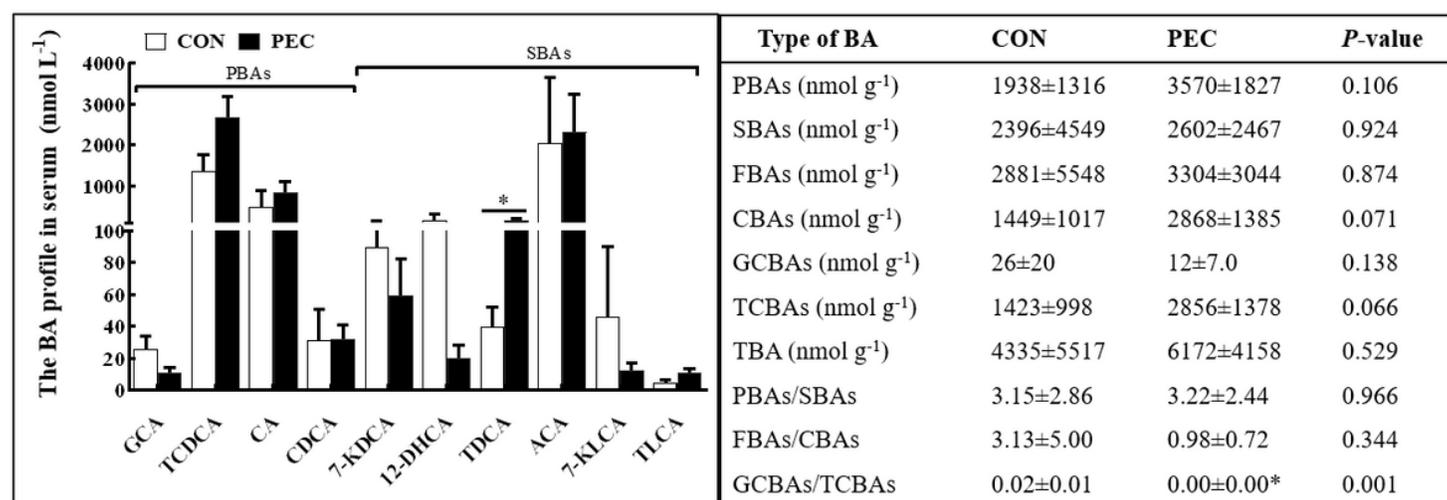


Figure 3

BA profile (nmol L⁻¹) in the serum of yellow catfish, *Pelteobagrus fulvidraco*, fed on the CON diet and PEC diet for 7 days

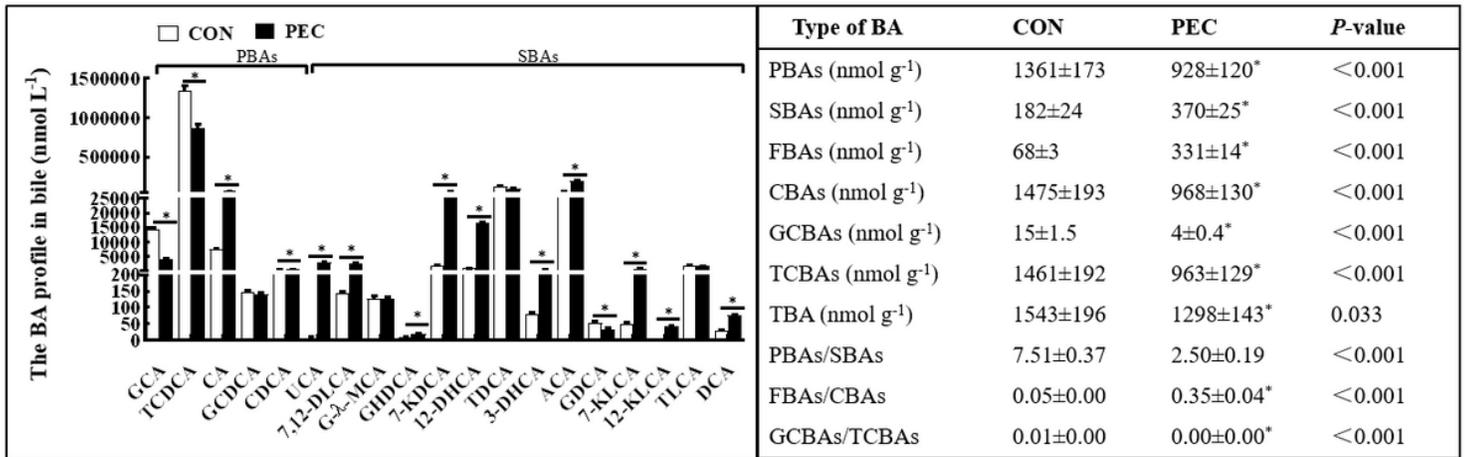


Figure 4

BA profile (nmol L⁻¹) in the bile of yellow catfish, *Pelteobagrus fulvidraco*, fed on the FM and PEC30 diet for 7 days

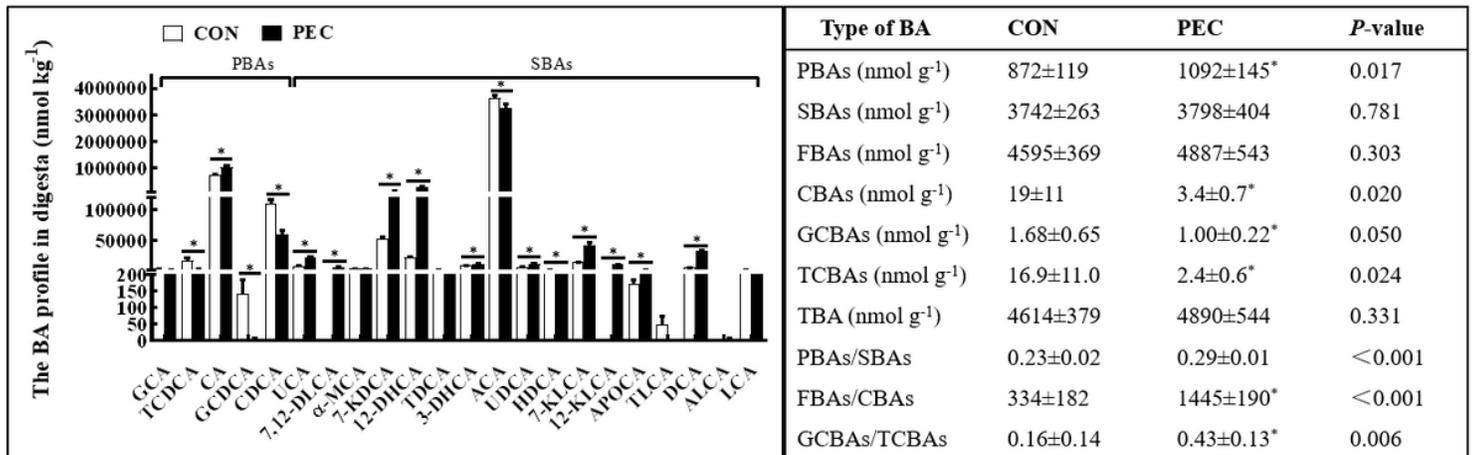


Figure 5

BA profile (nmol kg⁻¹) in the digestion of yellow catfish, *Pelteobagrus fulvidraco*, fed on the CON diet and PEC30 diet for 7 days