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Research Article

Lipotoxic Effects of Gossypol Acetate Supplementation on Hepatopancreas Fat Accumulation and Fatty Acid Profile in *Cyprinus carpio*

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Cottonseed meal is an excellent protein source with low price and abundant nutrients, but the existence of free gossypol restricts its wide application in animal feeds. To explore the toxic effects of free gossypol, the effects of gossypol acetate on the growth performance, hepatopancreatic fat accumulation, and muscle fatty acid composition of the common carp (*Cyprinus carpio*) were investigated in the present study. Carp were randomly divided into four groups, which were fed a diet supplemented with 0, 170, 340, and 680 mg/kg gossypol acetate, respectively. After 60 days of feeding, the gossypol residue in the tissue, growth performance, blood biochemical indices, hepatopancreas histology, fatty acid components, and expression of key genes involved in lipid metabolism pathways were measured. It was found that the concentrations of free gossypol were increased significantly in the serum, bile, and hepatopancreas of carp fed 340 mg/kg and 680 mg/kg gossypol acetate ($P < 0.05$). Dietary gossypol acetate had a limited effect on growth performance, and high concentrations of gossypol acetate induced excessive hepatopancreatic fat deposition and hepatocyte injury in *C. carpio*. In addition, gossypol acetate decreased total polyunsaturated fatty acids (PUFAs) and docosahexaenoic acid in muscle. In the hepatopancreas, decreased *HSL*, *PPARα*, and *CD36* may result in fat accumulation. In the muscle, decreased *FADS2* may be the key reason for the decrease in PUFAs. Taken together, these findings indicated that dietary gossypol acetate can cause lipid metabolism disorders, such as hepatopancreatic damage and significant changes in tissue fatty acid profile, which are regulated by the key lipid metabolic genes in common carp.

1. Introduction

Cottonseed meal has been considered as an alternative protein in terrestrial animals and fish diets [1–3] because it is low cost and rich in nutrients, such as proteins, carbohydrates, and minerals. However, because cottonseed meal contains toxic free gossypol, improper use can cause animal health problems, which restricts its widespread application in animal feed ([4–6] [7, 8]).

Previous animal research has suggested that dietary gossypol produces adverse effects, including growth depression, reduced fertility, lipid metabolic disorder, and pathological changes in the liver and other tissues [9, 10]. For example, when dairy cows were fed with a diet supplemented with 717 mg/kg and 951 mg/kg of free gossypol for 170 days, the pregnancy rate decreased and the incidence of abortion increased [11]. Dietary gossypol can significantly reduce body weight in sheep and cause liver cell swelling, nuclear lysis, and kidney cell necrosis [12]. Dietary gossypol also decreases blood glucose and insulin levels, increases insulin sensitivity, and improves lipid metabolism in rats with type 2 diabetes [13]. Furthermore, the acute toxicity of gossypol can cause liver cell damage and increase fat accumulation in the liver of laying hens [14]. Unlike terrestrial animals,

fish have a strong tolerance to gossypol [2], but high levels of gossypol can cause various problems, such as slow growth, decreased appetite, and reduced reproductive performance [10, 15, 16]. For instance, the growth and survival of adult rainbow trout were not affected by consuming cottonseed meal at gossypol supplementation levels of up to 619 mg/kg over 10 months, but there was reduced reproductive performance in females [17]. In addition, gossypol can reduce growth and cause hepatopancreatic fibrosis and hepatopancreatic injuries in turbot (*Scophthalmus maximus* L.) [18]. At supplementation levels of 1200 mg/kg of dietary gossypol acetate, the hepatopancreatic function can potentially be damaged in tilapia (*Oreochromis niloticus*×*O. aureus*) [19].

The above studies showed that free gossypol in the feed can affect lipid metabolism in various ways in livestock, poultry, mammals, and fish. However, the specific roles of gossypol in lipid metabolism in common carp remain unclear. Thus, in the present study, the diet of common carp was supplemented with different concentrations of gossypol acetate (0, 170, 340, and 680 mg/kg) to analyse the effects of gossypol on carp growth performance; fat accumulation in the hepatopancreas; fatty acid (FA) composition in the brain, hepatopancreas, intestine, and muscle; and mRNA expression of key genes involved in lipid metabolism. The results of this study will provide further insights into the optimal proportions of cottonseed meal in common carp feed and clarify the effects of gossypol on lipid mentalism.

2. Material and Methods

2.1. Feed Preparation. The basal feed, which did not contain free gossypol, was purchased from Tongwei Group Co., Ltd. (China). The content of crude protein, crude fat, ash, and moisture in the basal feed was 39.81%, 5.87%, 9.17%, and 6.68%, respectively. Gossypol acetate (CAS NO.12542-36-8) was purchased from Dalian Meilun Biotechnology Co., Ltd. (China), and the purity of gossypol was greater than 97.5%. Following the methodology previously published by our team [20], the basal feed was mixed thoroughly with gossypol acetate to obtain four different experimental feeds containing 0, 170, 340, and 680 mg/kg gossypol acetate. The diets were converted into 3 mm pellets using a granulator (F-26, Guangzhou Huagong Optical Mechanical Electrical Technology). After natural air drying, the diets were stored at 4°C until required. Finally, the free gossypol concentration in the experimental diets was determined as 0, 161.70 ± 3.56, 342.07 ± 15.67, and 670.35 ± 34.03 mg/kg, respectively (Table S1), which was basically consistent with the theoretical values.

2.2. Feeding Trial and Samples Collection. Healthy common carp (*Cyprinus carpio*) (60 ± 2.5 g) were purchased from Zhongmu National Agricultural Park (Zhengzhou, Henan, China) and cultured in an indoor recirculating aquaculture system of Henan Normal University and acclimatised for two weeks. Then, 360 healthy fish were randomly divided into 12 plastic tanks (four groups) with 30 carp in each tank (200 L). The duration of the breeding experiment was eight weeks. The fish were fed three times a day (9 : 00, 12 : 30, and 17 : 00). One-third of the feeding water was replaced every two days, and the cotton filter was cleaned every day. Water quality was monitored weekly using a multifunctional water quality detector (Shandong Gelanpu Technology Co., Ltd., China). Throughout the experiment, the average water temperature was 25 ± 1°C, the pH was 7.0–7.5, the dissolved oxygen was above 5 mg/L, the ammonia nitrogen was less than 0.01 mg/L, and the light cycle was 12 L : 12D.

After the breeding experiment, all carp were anaesthetized with MS-222 (100 mg/L) (Aladdin, Shanghai, China) prior to sampling. Five fish were randomly selected from each tank for tail vein blood collection. The blood was maintained at 4°C for 12 h, after which it was centrifuged at 3000 rpm for 15 min, and the supernatant obtained was used to determine the serum enzyme activity. Meanwhile, 12–14 fish were randomly chosen from each tank to detect the FA content (3–5 fish/tank) and gene expression levels (9 fish/tank). The tissues, including the brain, intestine, hepatopancreas, and muscle, were dissected and frozen using liquid nitrogen and then stored at −80°C for detecting the FA content and gene expression levels.

All the procedures involving fish followed the NIH Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, revised 1996) and were approved by the Laboratory Animal Care and Use Committee of Henan Normal University.

2.3. Growth Performance and Morphological Index Determination. The growth performance and morphological index were calculated using the following formulas:

$$\begin{aligned}
\text{WGR (weight gain rate)} &= (W_t - W_0)/W_0 \times 100,\\
\text{SGR (specific growth rate)} &= (\ln W_t - \ln W_0)/t \times 100,\\
\text{FCR (feed conversation ratio)} &= F/(W_t - W_0),\\
\text{SR (survival rate)} &= \text{FN/IN} \times 100,\\
\text{CF (condition factor)} &= W_t/L^3 \times 100,\\
\text{VSI (visceral index)} &= W_v/W_t \times 100,\\
\text{HSI (hepatosomatic index)} &= W_h/W_t \times 100,
\end{aligned} \tag{1}$$

where W_t is the final body weight (g), W_0 is the initial body weight (g), F is the food intake (g), FN is the survival quantity (tail), IN is the initial quantity (tail), W_v is the wet weight of the visceral mass (g), W_h is the wet weight of the hepatopancreas (g), L is the body length (cm), and t is the culture time (d).

2.4. Analysis of Gossypol Concentration in Tissues. Following previous research procedures to extract gossypol present in serum, bile, and the hepatopancreas, acetone was used to extract the gossypol [21–23]. High-performance liquid chromatography (HPLC) was used to detect gossypol content. An HPLC system (Agilent 1260, Germany) equipped with a TC-C 18 column (250 mm × 4.6 mm) was used. The samples were detected at 235 nm, with a flow rate of 1.0 ml/min. Using gossypol-acetic acid (Sigma) as a standard, the

external standard curves of each enantiomer were generated for the analyses.

2.5. Analysis of Serum Biochemistry Index. Serum triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in the serum were determined using a test kit purchased from Nanjing Jiancheng Biological Reagent Company, China.

2.6. Histological Analysis of Hepatopancreas. Three carp were randomly selected from each tank, and their hepatopancreas was placed in 4% paraformaldehyde solution for 24 h. The paraffin and frozen sections were prepared following standard histological procedures for staining with HE and oil red O, respectively. Histological images were obtained using a digital slice scanning system (3DHIES-TECH, Panoramic MIDI, Hungary).

2.7. Crude Fat and FA Content Analyses. Three to five fish were randomly selected from each tank. Exactly 2.0 g of hepatopancreas from each fish was dried to a constant weight in a vacuum freeze dryer. The crude fat content of the hepatopancreas was determined using the Soxhlet method [24].

Fatty acids were extracted from the brain, intestinal tract, hepatopancreas, and muscle of *C. carpio* using chloroform-methanol (2 : 1 volume ratio) [25]. The FAs were analysed by gas chromatography (GC) using an Agilent 7890B series gas chromatographer equipped with a 100 m × 250 μm × 0.2 μm HP-88 column (Agilent) with helium as the carrier gas at 1.4 mL/min and a flame ionization detector. The GC had an initial temperature of 120°C for 1 min, followed by an increase of 10°C/min to 190°C for 19 min, and then an increase of 2°C/min to 200°C for 28 min. The injector temperature was 250°C, pressure was 41.8 psi, flow rate was 3 mL/min, and split ratio was 3 : 1.

2.8. Gene Expression Analysis. The total RNA was extracted from the brain, intestine, hepatopancreas, and muscle using a TaKaRa RNAiso PLUS kit. RNA reverse transcription was performed using the PrimeScript™ RT reagent kit (Perfect Real Time) from Takara. Specific primers for real-time PCR amplification were designed using primer 5.0 using the existing gene sequences of carp (Table 1). PCR amplification was performed using the TB Green® Premix Ex Taq™ II kit (Tli RNaseH Plus) from Takara, and the relative expression level was calculated using the $2^{-\triangle\triangle Ct}$ method.

2.9. Statistical Analysis. Not all our data were balanced, for unbalanced data, including FAs content and gene expression, a least-squares analysis was used to provide the LSMEAN ± SEM, and the data were processed using SAS 9.0 (SAS Institute, Cary, NC, USA). For the balanced data, the significant differences in the growth performance, gossypol concentration, and serum biochemistry index were analysed using one-way ANOVA and LSD multiple comparisons using SPSS 22.0. Data are presented as the mean ± SD, unless specifically indicated. The results were considered statistically significant if $P < 0.05$. The regression curve was analysed using Excel. All figures were created using GraphPad Prism 6.02 (GraphPad Software, La Jolla, CA, USA) and Adobe Photoshop CC 2017 (Adobe Systems Incorporated, USA).

3. Results

3.1. Effects of Gossypol Acetate on the Growth Performance and Morphometric Parameters of C. carpio. The WGR (157.26–162.16%), SGR (1.57–1.60%), FCR (1.57–1.61), VSI (7.78–8.62%), HSI (1.87–1.95%), and CF (2.21–2.36) did not vary significantly among the treatment groups ($P > 0.05$) (Table 2). The SR in all groups was 100%, meaning there was no mortality throughout the experiment.

3.2. Gossypol Acetate Deposition in Tissues of C. carpio. No free gossypol was found in the serum, bile, and hepatopancreas of fish from the control and 170 mg/kg groups. The higher levels of dietary gossypol acetate caused the deposition of gossypol acetate in the serum, bile, and hepatopancreas of common carp and increased with increasing levels in the diet (Table 3). In the 680 mg/kg group, the concentration of free gossypol was 197.62 μg/g (hepatopancreas), 0.47 μg/mL (bile), and 0.22 μg/mL (serum), which were significantly higher than those in other groups ($P < 0.05$). The deposition order of free gossypol in the fish was hepatopancreas > bile > serum.

3.3. Effects of Gossypol Acetate on Serum Biochemical Parameters of C. carpio. With increasing gossypol acetate concentration, the serum ALT content showed an increasing trend, and the level in the 680 mg/kg group was significantly higher than that in other groups ($P < 0.05$). Similarly, the serum AST level in the 340 mg/kg and 680 mg/kg groups was significantly higher than that in the control group ($P < 0.05$). In contrast, the serum levels of LDL-C, HDL-C, and TG showed a decreasing trend. In the 680 mg/kg group, the serum LDL-C level was significantly lower than that in the other three groups ($P < 0.05$). The HDL-C and TG content in the 680 mg/kg gossypol acetate supplementation group was significantly lower than that in the control group ($P < 0.05$) (Table 4).

3.4. Effects of Gossypol Acetate on Hepatopancreas Lipids Content and Hepatopancreas Morphology in Common Carp. Our analysis of the lipid content in the hepatopancreas showed that the crude fat content in the hepatopancreas of the 680 mg/kg group was significantly higher than that of the other groups ($P < 0.05$) (Table 4). Quadratic regression analysis showed that crude fat content in the hepatopancreas was the lowest when the gossypol acetate supplemental level was 198 mg/kg (Figure 1(a)). The HE staining of the hepatopancreas (Figure 1(b)) showed that an increase in gossypol acetate concentration had a corresponding significant increase in the fat vacuoles in the hepatopancreas cells. In the control group, the hepatocyte nucleus was located near the center of the cell. In the 170 mg/kg group, the hepatopancreas cells in the carp showed lipid vacuolation. In the 340 mg/kg group, the carp hepatocyte nucleus was significantly shifted with increased lipid vacuolization. In the 680 mg/kg group, the hepatopancreas cell nucleus was squeezed to one side with hyperaemia in the hepatic sinus. Similarly, the oil red

Table 1: Specific primers used in the real-time PCR amplification.

Metabolism function	Gene name	Primer(5′-3′)	Length/bp	Gene ID (accession number)
Transcription factor	*PPARα*	F GCATGAAGCCTACCTCAGACACTT R ACCGAGGCGTACTGGCAGAA	231	FJ849065.1
	PPARβ	F GCAGCACAGCCAGTCCTGAA R AACGCCGTAGTGGAATCCTGAC	128	XM_019110518.1
	PPARγ	F GCAAGGCAGTGGAGGACAAGAAC R ACGCAACACAGCACCATAAGAGG	121	LOC109050306
Lipogenesis	*SREBP-1*	F CACGGCTCTGCTCAACGACAT R TGCGGAGGAGACTGCTGGAA	294	XM_019073316.1
	SREBP-2	F CAGATGAGTGTGAGTCCGCAGAAG R CCATAGGCTGGTCCTCAGAGTCA	174	XM_019064582.1
	FASN	F TCTGTGCTGTGCGGACTGGAA R GCAACATCGGCTGGATATTGAGGAG	253	KY378913.1
	ACACβ	F ACTGGCTGGCTAGATCACCTTATTG R GCACCGCATACCACACCTAACAT	207	XM_019106587.1
Lipolysis	*ATGL*	F CACCAACACCTCCATTCAGTTCACA R ACTCTTCATCCTCCTCACCGTCAG	227	KY906167.1
	HSL	F TTGATGCCTATGCTGGTACGAGTTG R TGATGTGGTTGGAGAGGATGATGCT	182	MF061228.2
PUFA biosynthesis	*FADS2a*	F CTGTAACATTGAGCAATCCGCCTTC R GGTACGCATCCAGCCAGAGTTC	236	MK852165.1
	FADS2b	F AAGTAAGCAGCAGTCAGTCAGAGTT R CCGTGGCATCTTCTCCAGCATAG	242	MK852166.1
Lipid transport	*CD36*	F CCGTAGGCACAGAGGAGGACATAT R GAGTGTGGAATTGGAGCGTTGGA	116	KM030422.1
	FABP1b	F ATCGAATCCCTGACCGGAGA R CCTCTTGTAGACGAGGCTGC	156	XM_019124255.1
	FABP10a	F AACCCCTGGAAAAACCGTCA R GGGTCTCCACCATCTCTCCT	176	EU363800.1
	FABP10b	F AACTCCTTCACCATCGGCAA R CTCCTACCGTCAGGGTCTCC	166	EU363801.1
	FABP11	F TTGAGCTGTTGCTGGAGTGAC R GTTTTGAAGGTGCTCTGCGA	235	XM_019119618.1
Control	*18S*	F CTGAGAAACGGCTACCACATC R CAGACCTGTTATTGCTCCATCT	101	FJ710827.1

staining showed that the fat content in the hepatopancreas gradually increased with increasing gossypol acetate supplementation in the diet (Figure 1(b)).

3.5. Effects of Gossypol Acetate on FA Profile in Different Tissues of Common Carp. Total saturated FA (SA) decreased at the lower concentrations of gossypol acetate (170 mg/kg and 340 mg/kg) and increased when higher levels were provided (680 mg/kg) in both the brain and intestine. In contrast, the total SA content was significantly higher in the hepatopancreas of the 170 mg/kg group and significantly lower in that of the 340 mg/kg and 680 mg/kg groups ($P < 0.05$). The total SA content was significantly lower in the muscle of all carp from the groups fed gossypol acetate. The variations in the stearic acid (C18:0) content in the four tissues of the different groups were consistent with the results of total SA (Figure 2(a) and Table S2). Conversely, the total n6 polyunsaturated FA (PUFA) in the brain was increased in carp fed with low doses of gossypol acetate (170 mg/kg and 340 mg/kg) and decreased in carp fed with the highest dose (680 mg/kg). The results were the opposite in the hepatopancreas. The n6-PUFA contents were significantly lower in the intestinal and muscular tissue of the gossypol acetate treatment groups ($P < 0.05$) (Figure 2(b) and Table S2). The contents of docosahexaenoic acid (DHA, C22:6n3) and total n3-PUFA in the hepatopancreas of the 170 mg/kg and 680 mg/kg groups were significantly higher. Conversely, the contents of DHA and n3-PUFA in the muscle were significantly lower in all gossypol acetate supplement groups (Figure 2(c) and Table S2). The total

Table 2: Effect of gossypol acetate on growth performance of *Cyprinus carpio*.

Group	Gossypol acetate supplementation (mg/kg)			
Parameter	0	170	340	680
Initial body weight (g)	57.02 ± 0.96	57.47 ± 0.43	57.64 ± 0.6	57.84 ± 0.45
Final body weight (g)	149.42 ± 7.9	147.87 ± 12.62	148.23 ± 12.49	151.29 ± 14.71
WGR (%)	162.16 ± 15.80	157.26 ± 21.29	157.32 ± 24.00	161.59 ± 25.53
SGR (%)	1.60 ± 0.10	1.57 ± 0.14	1.57 ± 0.15	1.60 ± 0.16
FCR	1.57 ± 0.11	1.59 ± 0.18	1.61 ± 0.17	1.58 ± 0.17
SR(%)	100	100	100	100
VSI (%)	8.62 ± 0.38	8.32 ± 0.67	7.78 ± 0.48	7.80 ± 0.35
HSI (%)	1.95 ± 0.26	1.89 ± 0.03	1.90 ± 0.14	1.87 ± 0.17
CF(g/cm^3)	2.31 ± 0.08	2.35 ± 0.05	2.21 ± 0.11	2.36 ± 0.06

Data are shown as means ± SD ($n = 3$). Dietary gossypol acetate had little effect on the contents of WGR, SGR, VSI, HSI, and FCR in *C. carpio* ($P > 0.05$). WGR: weight gain rate; SGR: specific growth rate; SR: survival rate; VSI: visceral index; HSI: hepatosomatic index; FCR: feed conversation ratio.

Table 3: Free gossypol in the tissues of *Cyprinus carpio*.

Group	Gossypol acetate supplementation (mg/kg)			
Tissue	0	170	340	680
Serum (μg/mL)	0.00 ± 0.00^b	0.00 ± 0.00^b	0.21 ± 0.02^a	0.22 ± 0.03^a
Bile (μg/ml)	0.00 ± 0.00^c	0.00 ± 0.00^c	0.35 ± 0.03^b	0.47 ± 0.06^a
Hepatopancreas (μg/g)	0.00 ± 0.00^c	0.00 ± 0.00^c	102.01 ± 24.09^b	197.62 ± 23.22^a

Data are shown as means ± SD ($n = 3$). Lowercase letters in the same row indicate significant differences from the control group ($P < 0.05$). Increasing the dietary gossypol acetate content also increased the deposition of gossypol acetate in the hepatopancreas (F), bile (G), and serum (H) in common carp. The free gossypol content was the highest in the hepatopancreas.

Table 4: Effects of gossypol acetate on serum biochemical parameters and liver crude fat.

Group	Gossypol acetate supplementation (mg/kg)			
Parameter	0	170	340	680
ALT (U/mL)	4.91 ± 0.65^b	5.08 ± 0.61^b	5.87 ± 0.29^b	10.79 ± 1.38^a
AST ((U/mL)	34.74 ± 0.65^b	35.56 ± 1.14^b	39.69 ± 1.25^a	38.18 ± 1.33^a
TG (mmol/L)	1.59 ± 0.03^a	1.13 ± 0.07^c	1.26 ± 0.02^b	1.09 ± 0.02^c
LDL-C (mmol/L)	3.58 ± 0.09^a	3.57 ± 0.17^a	3.49 ± 0.17^a	2.99 ± 0.07^b
HDL-C (mmol/L)	2.57 ± 0.19^a	2.57 ± 0.06^a	2.35 ± 0.26^{ab}	2.32 ± 0.06^b
Crude fat in hepatopancreas (%)	13.17 ± 0.74^b	12.71 ± 0.95^{bc}	11.61 ± 0.86^c	22.6 ± 1.11^a

In the serum, dietary gossypol acetate increased the ALT and AST but decreased the LDL-C, HDL-C, and TG content and increased crude fat within the hepatopancreas. Data are shown as means ± SD ($n = 3$). Different superscript letters denote significant differences between the groups fed the different diets as determined by a one-way ANOVA with Tukey and Duncan's comparison tests ($P < 0.05$).

PUFA content in the muscle was significantly lower in the gossypol acetate treatment groups (170, 340, and 680 mg/kg) ($P < 0.05$) (Figure 2(d) and Table S2).

In addition, regardless of the effect of gossypol acetate on FA composition, using the brain tissue as a control, the FA content in four tissues within the control group (0 mg/kg) was compared. Brain tissues were found to contain the highest total SA and the lowest total PUFAs and total n6-PUFA ($P < 0.001$) (Figure 2 and Table S2).

3.6. Effect of Gossypol Acetate on the Expression of Key Genes Involved in the Lipid Metabolism Pathway. In the brain, the expression of *ACACβ*, *PPARγ*, *SREBP-2*, *FADS2a*, and *FADS2b* in the gossypol acetate treatment groups was significantly higher than that in the control group ($P < 0.05$) (Figure 3(a)). The expression of *HSL* and *PPARβ* increased at low dosages (170 mg/kg and 340 mg/kg) and then decreased to the control levels in the high-dose group (680 mg/kg) (Figure 3(b)). The expression of *CD36*, *FABP1b*, *FABP10a*, and *FABP10b* showed a similar trend to that of the catabolic genes ($P < 0.05$) (Figure 3(c)).

In the intestine, the expression of *FASN* and *SREBP-1* in the gossypol acetate groups was significantly lower than that in the control group. The expression of *ACACβ* in the 170 mg/kg group was significantly higher than that in the other groups (Figure 3(d)). The expression of *FADS2a*

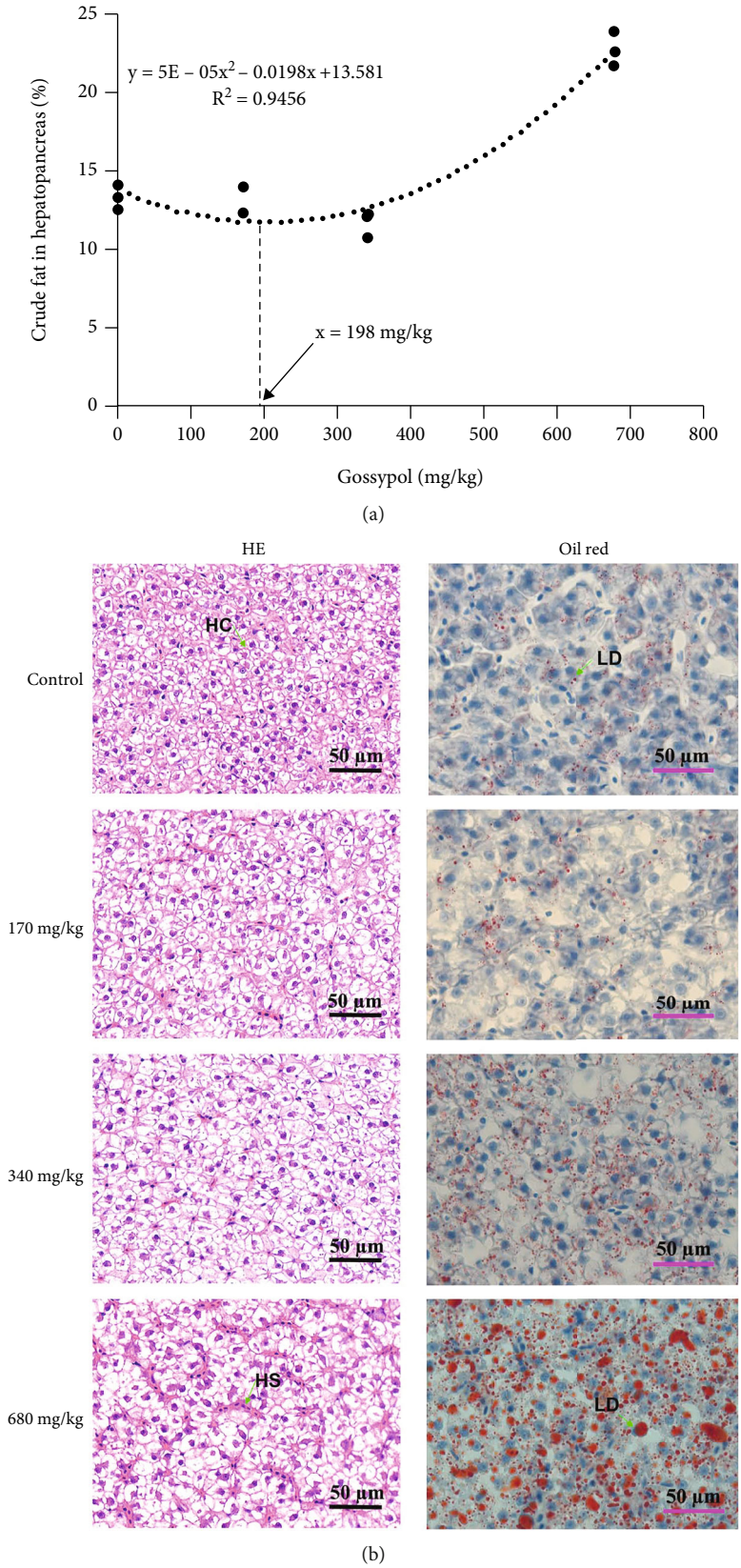


Figure 1: Dietary gossypol acetate increased the lipid accumulation in the hepatopancreas and caused hepatocellular injury in *Cyprinus carpio*. (a) Quadratic regression analysis showing the value of dietary gossypol acetate for increased hepatopancreas crude fat in carp for eight weeks. (b) Hepatopancreas tissue sections stained by HE and oil red. HC: hepatocytes; HS: hepatic sinusoids; LD: lipid droplet. Bar: 50 μm.

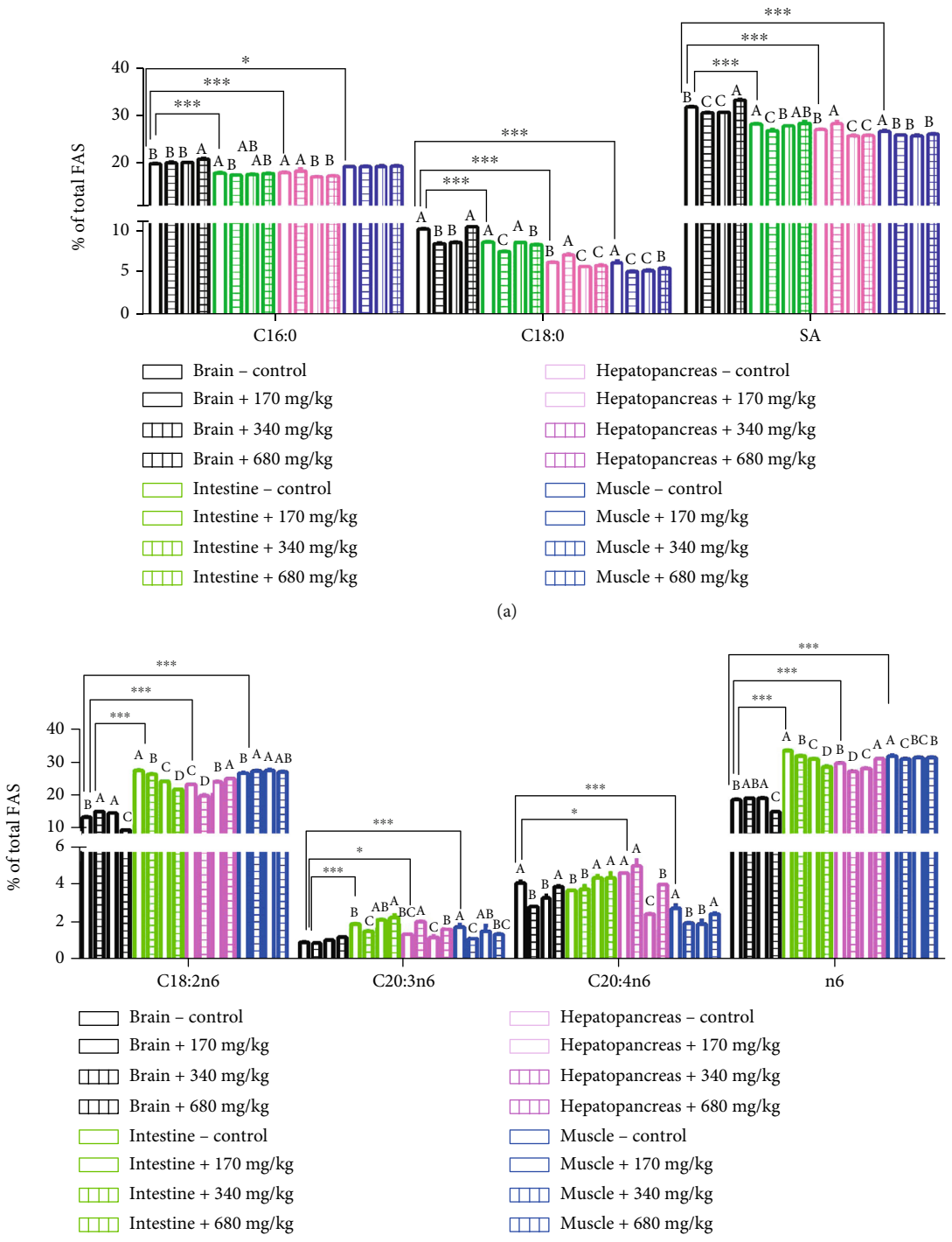
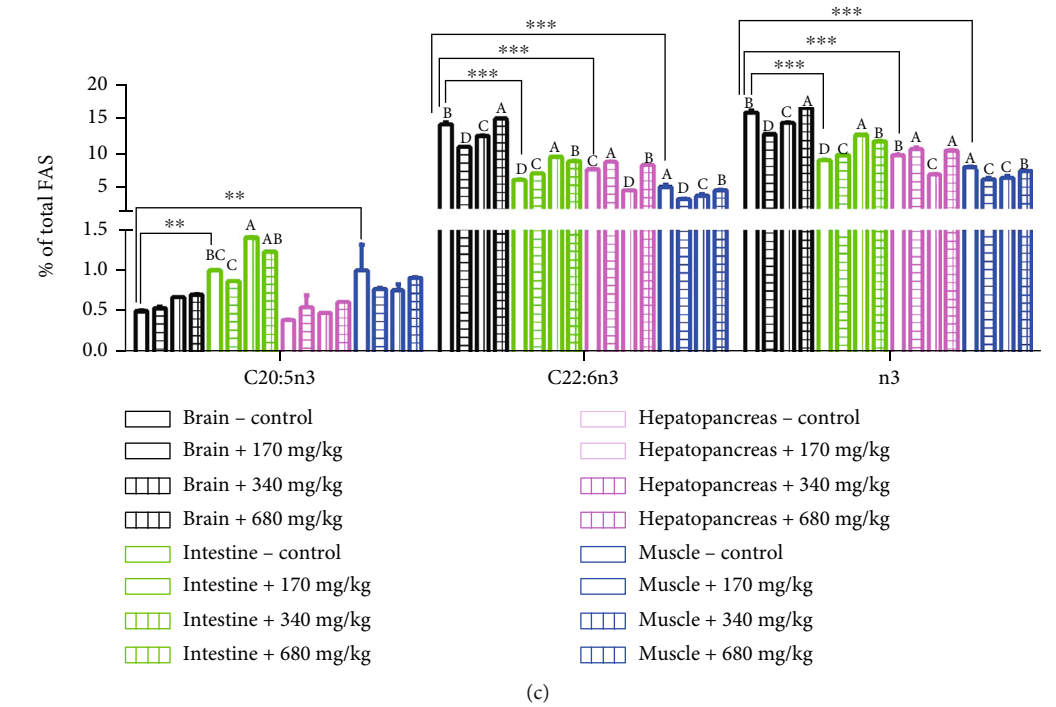
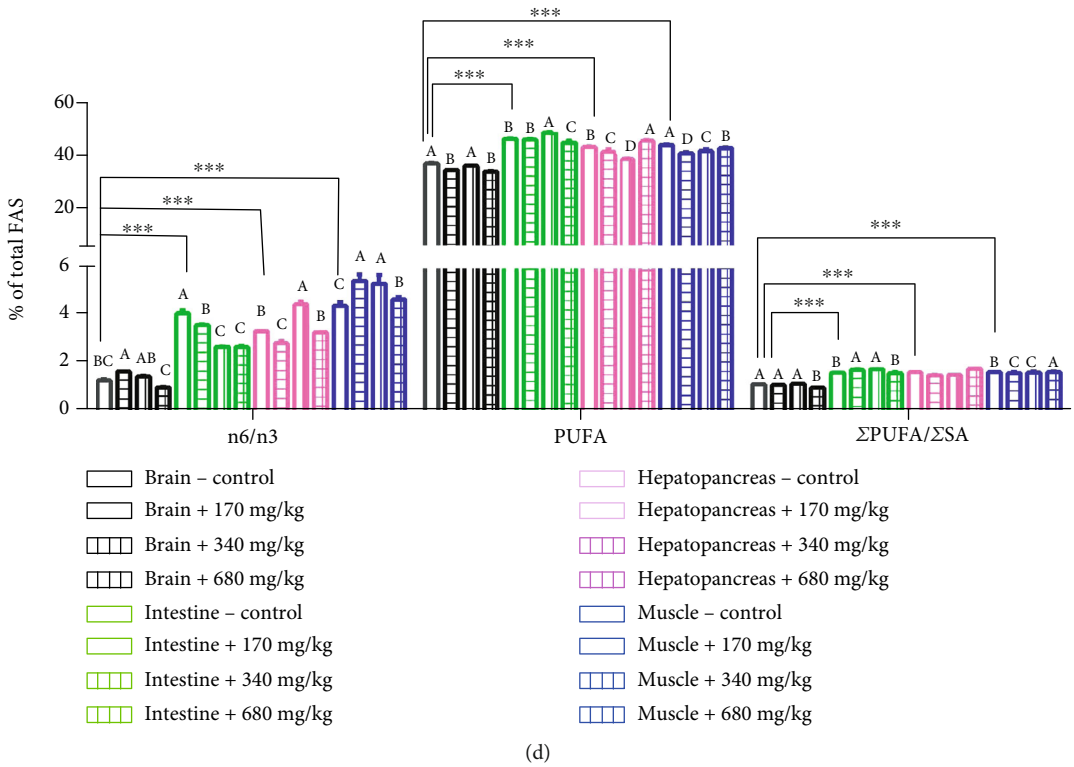


Figure 2: Continued.



(c)



(d)

Figure 2: Gossypol acetate altered the SA (a), n3-PUFA (b), n6-PUFA (c), and PUFA (d) contents in the brain, intestine, hepatopancreas, and muscle of *Cyprinus carpio*. Data are presented as LSMEAN ± SE using at least three independent replicates. Letters indicate significant differences in the FAs between groups treated with different gossypol acetate concentrations in the same tissue ($P < 0.05$)*. When gossypol acetate was not added to the dietary feed (0 mg/kg gossypol acetate), the content of specific FA in the different tissues was significantly different from the brain tissues. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. SA = C14:0 + C16:0 + C18:0 + C20:0; PUFA = C18:2n6 + C18:3n6 + C18:3n3 + C20:2 + C20:3n6 + C20:4n6 + C20:5n3 + C22:6; n3 = 18:3n3 + C20:5n3 + C22:6n3; n6 = C18:2n6 + C18:3n6 + C20:3n6 + C20:4n6.

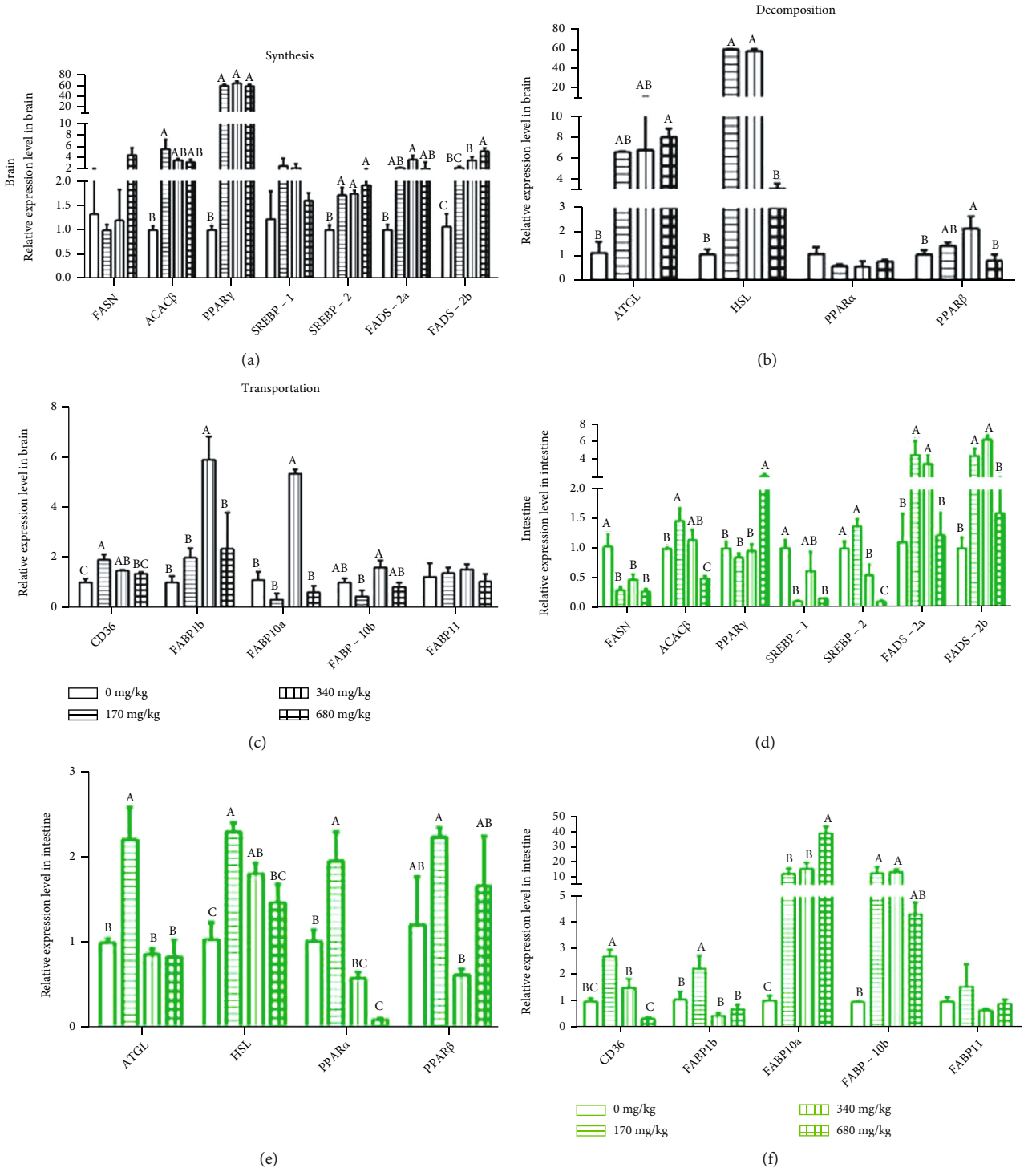


Figure 3: Continued.

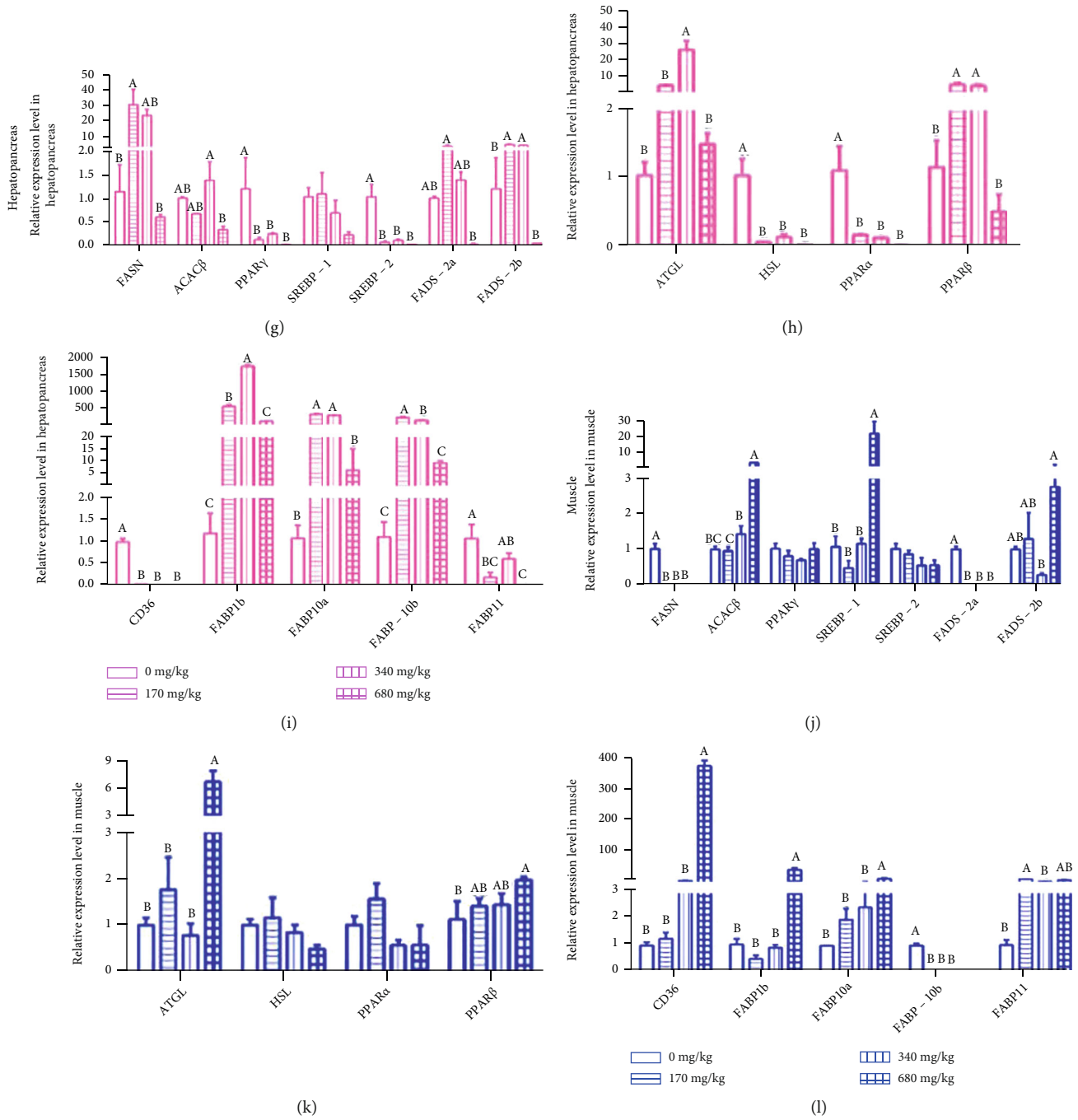


Figure 3: Effects of gossypol acetate on the expression of genes related to lipid metabolism in the brain (a–c), intestine (d–f), hepatopancreas (g–i), and muscle (j–l). Data are presented as LSMEAN ± SE using a minimum of three independent replicates. Different superscript letters denote significant differences between fish fed the different diets ($P < 0.05$).

and *FADS2b* was significantly higher in the 170 mg/kg and 340 mg/kg groups, but the highest dose (680 mg/kg) resulted in *FADS2a* and *FADS2b* expression levels as low as those in the control group (Figure 3(d)). The expression of *ATGL*, *HSL*, *PPARα*, and *PPARβ* showed a trend of increased levels at low doses and decreased levels at high doses of gossypol acetate (Figure 3(e)). The expression of *CD36* and *FABP1b* was higher in carp fed 170 mg/kg of gossypol acetate and lower in those fed 340 mg/kg and 680 mg/kg of gossypol acetate. The expression of *FABP10a* and *FABP10b* was significantly higher in the gossypol acetate groups compared with that in the control group ($P < 0.05$) (Figure 3(f)).

In the hepatopancreas, the expression of *FASN*, *FADS2a*, and *FADS2b* showed a trend of higher levels in carp fed lower doses of gossypol acetate and lower levels in those fed higher doses (Figure 3(g)). The expression of *HSL* and *PPARα* in the gossypol acetate-treated groups was

significantly lower than that in the control group ($P < 0.05$) (Figure 3(h)). The expression of *FABP1b*, *FABP10a*, and *FABP10b* in each experimental group was higher at low doses but lower as the dose of gossypol acetate increased. However, the expression of *CD36* and *FABP11* was significantly lower in the gossypol acetate-treated groups compared with that in the control group ($P < 0.05$) (Figure 3(i)).

In the muscle, the expression of *ACACβ*, *SREBP1*, *CD36*, *FABP1b*, *FABP10a*, *ATGL*, and *PPARβ* was significantly higher in the 680 mg/kg group than those in the control group ($P < 0.05$) (Figures 3(j)–3(l)). However, the expression of *FASN*, *FADS2a*, and *FABP10b* was significantly lower in carp in the gossypol acetate treatment groups compared with that in the control group ($P < 0.05$) (Figures 3(j) and 3(l)).

4. Discussion

4.1. Dietary Gossypol Acetate Has Limited Effects on the Growth Performance of C. carpio. Previous research has shown that gossypol can inhibit growth in poultry [26] and aquatic animals [2]. Fish are more tolerant of gossypol than terrestrial animals [2]. For example, *O. aureus* could tolerate dosages of gossypol up to 1,800 mg/kg in their diet [27]. Conversely, another study found that 900 mg/kg of dietary gossypol acetate could significantly reduce the growth performance of tilapia (*O. niloticus*×*O. aureus*) [19], whereas 198.14 mg/kg and 267.02 mg/kg of dietary gossypol caused no significant difference in the WGR and SGR of Nile tilapia (*O. niloticus*) [9]. Turbot fed with diets supplemented with 600 mg/kg gossypol showed no significant change in their growth performance when compared with fish fed the same diet without gossypol, but growth significantly decreased when the fish were fed diets supplemented with 1200 mg/kg gossypol [18]. The growth of rainbow trout was not affected by 200–600 mg/kg gossypol content in their diet, but growth was impeded when the gossypol content was increased to 1000 mg/kg [28]. In channel catfish (*Ictalurus punctatus*), slow growth was observed when gossypol was higher than 1400 mg/kg in the diet [15], but another study found that 300 mg/kg gossypol in the diet slowed catfish growth [29].

Our results are in agreement with the previous research findings. When compared with the control group, there were no significant differences in growth performance among all gossypol acetate supplement groups (170, 340, and 680 mg/kg), indicating that the common carp can tolerate gossypol acetate. According to the Agricultural Industry Standard of the People's Republic of China (NY 5072-200), the gossypol content in fish compound feed should be no more than 300 mg/kg. In the present study, the concentration of gossypol acetate did not significantly exceed the safe range, which may be within the tolerance range of carp. Therefore, there were no significant differences in WGR, SGR, FCR, SR, VSI, HSI, and CF among the different groups. Moreover, the species, diet composition, and environmental conditions in the different studies may account for the variations in the results.

4.2. Hepatopancreas as the Primary Gossypol Storage Tissue. Previous studies have suggested that the liver is the preferred storage site for gossypol. For example, residues of gossypol were found in chicken organs in the following order: liver > kidney > spleen > heart > muscle [11]. In lambs fed cottonseed, the residual levels of gossypol in the tissues were in the following order: liver > heart > muscle > spleen [30]. In rainbow trout, the gossypol concentration was highest in the hepatopancreas, followed by the kidney, intestine, testis, blood plasma, stomach, and muscle [31]. Similarly, the present study found that free gossypol mainly resided in the hepatopancreas.

4.3. Dietary Gossypol Acetate Increased the Content of ALT and AST but Decreased the Content of LDL-C, HDL-C, and TG in Serum. Studies in rats showed that the serum transaminase content (ALT and AST) significantly increased after gossypol acetate treatment [32]. In addition, a study conducted on tilapia found that the ALT content in fish fed with diets supplemented with 1200 mg/kg gossypol was significantly higher than that of the control group ($P < 0.05$) [19]. In the present study, the levels of ALT and AST increased significantly in carp treated with 680 mg/kg gossypol acetate, which supports the previous findings. The increase in serum ALT and AST levels indicated that gossypol acetate may induce hepatic cell destruction. In addition, the levels of LDL-C, HDL-C, and TG in the 680 mg/kg gossypol acetate-treated group decreased significantly ($P < 0.05$), which was consistent with the results of the previous studies [33]. These results suggest that the ability of lipids accumulated in the hepatopancreas to be transported to the peripheral tissues was blocked, restricting the transfer of excessive fat in the hepatopancreas, resulting in excessive fat accumulation in the hepatopancreas.

4.4. Dietary Gossypol Acetate Caused Excessive Lipid Accumulation and Cell Injury in the Hepatopancreas, Which Is Regulated by Genes Related to Lipid Metabolism. Studies on livestock, poultry, and mammals have found that gossypol can cause pathological changes, such as oxidative stress, hepatopancreas lipid accumulation, hepatopancreas tissue lesions, and other pathological changes in terrestrial animals [33, 34]. In addition, gossypol can inhibit FA desaturation and lead to an increase in cholesterol levels in the body [7]. Studies on fish also found that supplementing the diet with gossypol resulted in nuclear migration, loose arrangements, necrosis, and other damage in the hepatopancreas [6, 18]. In the present study, an increase in gossypol acetate concentration caused hepatopancreas tissue lesions to be aggravated and fat content to increase. Hepatopancreas sinusoid hyperaemia was observed in the 680 mg/kg group, and the hepatopancreatic nuclei were squeezed to the edge of the cell. Additionally, the crude lipid content in the hepatopancreas of the high gossypol acetate treatment group (680 mg/kg) was significantly higher than that in the control group. These results are supported by the previous studies and suggest that gossypol exposure could increase fat accumulation in the hepatopancreas and cause hepatopancreatic tissue damage in common carp.

Lipid metabolism is a complex process involving synthesis, decomposition, and transport and is regulated by many

genes and tissues. To elucidate the reasons for the increased hepatopancreatic fat accumulation after gossypol treatment, the expression of key genes was detected in the lipid metabolism pathways. Fat synthesis genes, including *FASN*, *ACACβ*, *PPARγ*, *SREBP-1*, and *SREBP-2*; lipolysis genes, including *ATGL*, *HSL*, *PPARα*, and *PPARβ*; and FA-binding and transporter genes, including *CD36*, *FABP1b*, *FABP10a*, *FABP10b*, and *FABP11* were further analysed. To understand the causes of the differences in the composition and PUFAs content, including DHA (C22:6n3) in the different tissues, the expression of the FA desaturase genes (*FADS2a* and *FADS2b*) was tested.

In this study, the expression of most synthesis and decomposition genes showed similar trends in the intestine and muscle but displayed the opposite trends in the brain. For example, the gossypol acetate increased the expression of genes both in synthesis and catabolism in the brain but decreased the expression levels in the intestine, hepatopancreas, and muscle. The differences in gene expression among various tissues may account for metabolic physiological differences. However, the specific gene regulatory network across different tissues requires further research.

In the present study, gossypol acetate inhibited the mRNA expression of genes involved with triglyceride decomposition (*HSL* and *PPARα*) in the hepatopancreas of carp. Low expression of these genes may lead to liver fat accumulation.

FA translocase (*CD36*) is an integral membrane glycoprotein that mediates long-chain FA transport [35–37]. Numerous studies have shown that *CD36* can enhance FA uptake in various tissues, including the skeletal muscle, heart, liver, and intestine [38–40]. In the present study, the expression of *CD36* was significantly decreased in the hepatopancreas of the gossypol acetate-treated groups (170 mg/kg, 340 mg/kg, and 680 mg/kg). In contrast, the expression of *CD36* was increased in the muscle with the increase of gossypol acetate. This result indicates that dietary gossypol acetate increases the uptake of FAs in the muscle, which may have been transported from the liver and intestine. However, the transport and balance of FAs between different tissues require further research.

In vivo, free FAs are cytotoxic and need to bind to FA-binding proteins (FABPs) [41], such as the hepatopancreas-type binding protein (FABP1) and the liver-based FA-binding protein (FABP10), which are mainly expressed in the hepatopancreas [42–44]. In the present study, the expression of *FABP1b*, *FABP10a*, and *FABP10a* in the hepatopancreas of the gossypol acetate-treated groups was significantly higher than that in the control group, indicating that an increase in hepatopancreas lipid synthesis requires more FABPs to bind with the free FAs, thereby reducing the damage caused by hepatopancreas fat accumulation.

4.5. Dietary Gossypol Acetate Altered FA Composition. The abundant PUFAs in fish muscle improve the nutritional value. However, the synthesis and accumulation of PUFAs in muscle is a complex process that requires multiple tissues to regulate the uptake, absorption, transport, and metabolism of PUFAs in fish. However, the specific mechanisms remain unclear. Therefore, the composition and content of FAs in different tissues were analysed to provide a theoretical basis for further research on the deposition of PUFAs and other FAs in muscles.

Studies investigating the effects of gossypol acetate on FA composition remain limited. Previous research has identified that supplementation with cottonseed oil can alter FA composition in hens. For example, dietary gossypol acetate decreased the content of C18:0 in the liver of laying hens [3]. Similarly, in the present study, gossypol acetate decreased the content of C18:0 in the hepatopancreas and muscle tissue. Gossypol acetate could regulate the metabolism of FAs to varying degrees in different tissues, and the SA and PUFA contents showed opposite trends in the brain and hepatopancreas. Additionally, dietary gossypol acetate significantly decreased the content of DHA, n3-PUFAs, n6-PUFAs, and total PUFAs in the muscle. These findings indicate that adding gossypol acetate to the diet reduced the muscle quality of common carp.

Fatty acid desaturases (*FADS2a* and *FADS2b*) are key enzymes in the synthesis of PUFAs. In the present study, the contents of key PUFAs in the intestine and muscle were at levels consistent with those of the tissue expression of *FADS2a* and *FADS2b*. In particular, the expression of *FADS2a* was significantly decreased in the muscle of all gossypol acetate-treated groups, which may be the main cause for the decrease in PUFAs.

5. Conclusions

In conclusion, the findings indicate that dietary gossypol acetate has little effect on the growth performance of carp. However, high concentrations of gossypol acetate (680 mg/kg) induce hepatopancreatic injury and excessive lipid accumulation in hepatopancreas and decrease PUFAs in the muscle, which are regulated by the genes involved in lipid metabolism, including synthesis, decomposition, transport, and binding. The present study details the novel approach of using a product with toxic components as a food source in animal production. Furthermore, this product has previously been tested and will continue to be developed as a useful fish feed that will have long-term effects on the industry and environment.

Abbreviations

FAs: Fatty acids
WGR: Weight gain rate
SGR: Specific growth rate
FCR: Feed conversation ratio
SR: Survival rate
CF: Condition factor
VSI: Visceral index
HIS: Hepatosomatic index
TG: Triglyceride
HDL-C: High-density lipoprotein cholesterol
LDL-C: Low-density lipoprotein cholesterol
AST: Aspartate aminotransferase

ALT: Alanine aminotransferase
HPLC: High-performance liquid chromatography
GC: Gas chromatography
PPARα: Peroxisome proliferator activated receptor α
PPARβ: Peroxisome proliferator activated receptor β
PPARγ: Peroxisome proliferator activated receptor γ
SREBP-1: Sterol regulatory element binding transcription factor 1
SREBP-2: Sterol regulatory element binding transcription factor 2
FASN: Fatty acid synthase; *ACACβ*, acetyl-CoA carboxylase β
ATGL: Adipose triglyceride lipase
HSL: Hormone-sensitive lipase
FADS2a: Fatty acid desaturase 2a
FADS2b: Fatty acid desaturase 2b
FABP1b: Fatty acid binding protein 1b
FABP10a: Fatty acid binding protein 10a
FABP11: Fatty acid binding protein 11
SA: Saturated fatty acid
PUFA: Polyunsaturated fatty acid
DHA: Docosahexaenoic acid.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Yuru Zhang wrote the original draft, conceptualized the study, and was responsible for the data curation, funding acquisition, and project administration; Junmei Zhang was responsible for the data curation, visualization, and software; Yanhua Ren was responsible for the investigation and data curation; Mengdan Zhang was responsible for the investigation; Junping Liang was responsible for the resources, data curation, and project administration.

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Supplementary Materials

Table S1: gossypol concentration in diets. Data are shown as mean ± SD ($n = 3$). Table S2: effects of gossypol acetate on the fatty acid composition in different tissues of *Cyprinus carpio*. Data are presented as LSMEAN ± SE using a minimum of three independent replicates. Letters indicate significant differences in the FAs between groups treated with different gossypol acetate concentrations in the same tissue ($P < 0.05$). * When gossypol acetate was not added (0 mg/kg), the content of specific FA in the different tissues was significantly different from that seen in the brain tissues: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. SA = C14:0 + C16:0 + C18:0 + C20:0; PUFA = C18:2n6 + C18:3n6 + C18:3n3 + C20:2 + C20:3n6 + C20:4n6 + C20:5n3 + C22:6; n3 = 18:3n3 + C20:5n3 + C22:6n3; n6 = C18:2n6 + C18:3n6 + C20:3n6 + C20:4n6. Figure S1: graphic abstract. *(Supplementary Materials)*

References

[1] I. C. N. Gadelha, N. B. S. Fonseca, S. C. S. Oloris, M. M. Melo, and S. B. Benito, "Gossypol toxicity from cottonseed products," *Scientific World Journal*, vol. 2014, article 231635, 2014.

[2] M. H. Li and E. H. Robinson, "Use of cottonseed meal in aquatic animal diets: a review," *North American Journal of Aquaculture*, vol. 68, no. 1, pp. 14–22, 2006.

[3] A. Yang, C. Zhang, B. Y. Zhang et al., "Effects of dietary cottonseed oil and cottonseed meal supplementation on liver lipid content, fatty acid profile and hepatic function in laying hens," *Animals (Basel)*, vol. 11, no. 1, p. 78, 2021.

[4] A. Ciereszko and K. Dabrowski, "In vitro effect of gossypol acetate on yellow perch (Perca flavescens) spermatozoa," *Aquatic Toxicology*, vol. 49, no. 3, pp. 181–187, 2000.

[5] K. Dabrowski, J. Rinchard, K. J. Lee, J. H. Blom, A. Ciereszko, and J. Ottobre, "Effects of diets containing gossypol on reproductive capacity of rainbow trout (Oncorhynchus mykiss)," *Biology of Reproduction*, vol. 62, no. 2, pp. 227–234, 2000.

[6] L. H. Roger, "Effects of gossypol on rainbow trout Salmo guirdneri Richardson," *Journal of Fish Biology*, vol. 2, pp. 293–303, 1970.

[7] B. Semon, "Dietary intake of cottonseed toxins is hypothesized to be a partial cause of Alzheimer's disorder," *Medical Hypotheses*, vol. 78, no. 2, pp. 293–298, 2012.

[8] K. Z. Wang, L. Feng, W. D. Jiang et al., "Dietary gossypol reduced intestinal immunity and aggravated inflammation in on-growing grass carp (Ctenopharyngodon idella)," *Fish & Shellfish Immunology*, vol. 86, pp. 814–831, 2019.

[9] M. Jiang, W. W. Zhong, J. Tian et al., "Effect of detoxification cottonseed on growth performance, body composition and serum aminotransferase activities of juvenile Nile tilapia (Oreochromis niloticus) (in Chinese)," *Journal of Northwest Sci-tech University of Agriculture and Forestry*, vol. 39, no. 6, pp. 37–43, 2011.

[10] J. Rinchard, A. Ciereszko, K. Dabrowski, and J. Ottobre, "Effects of gossypol on sperm viability and plasma sex steroid hormones in male sea lamprey," *Petromyzon marinus. Toxicology Letters*, vol. 111, no. 3, pp. 189–198, 2000.

[11] J. E. P. Santos, M. Villasenor, P. H. Robinson, E. J. Depeters, and C. A. Holmberg, "Type of cottonseed and level of gossypol in diets of lactating dairy cows: plasma gossypol, health, and

reproductive performance," *Journal of Dairy Science*, vol. 86, no. 3, pp. 892–905, 2003.

[12] H. Rehemujiang, A. Yimamu, and Y. Wang, "Effect of dietary cotton stalk on nitrogen and free gossypol metabolism in sheep," *Asian-Australasian Journal of Animal Science*, vol. 32, no. 2, pp. 233–240, 2019.

[13] R. R. Wang, *The Effects and Funtional Mechanism of Gossypol on Glucose and Lipid Metabolism and Liver Fibrosis in Type 2 Diabetic Rats (in Chinese)*, Wenzhou Medical College, 2007.

[14] N. N. Hou, *Effects of Gossypol on the Hepatic Metabolism and Antioxidant Function of Laying Hens (in Chinese)*, Shandong Agricultural University, 2014.

[15] W. J. Dorsa, H. R. Robinette, E. H. Robinson, and W. E. Poe, "Effects of dietary cottonseed meal and gossypol on growth of young channel catfish," *Transactions of the American Fisheries Society*, vol. 111, no. 5, pp. 651–655, 1982.

[16] H. Fu, "The toxicity of gossypol to fishes and the prosective utilization of cottonseed in aquafeed (in Chinese)," *Journal of fishery Sciences of China*, vol. 3, no. 4, pp. 114–117, 1996.

[17] J. H. Blom, K. J. Lee, J. Rinchard, K. Dabrowski, and J. Ottobre, "Reproductive efficiency and maternal-offspring transfer of gossypol in rainbow trout (Oncorhynchus mykiss) fed diets containing cottonseed meal," *Journal of Animal Science*, vol. 79, no. 6, pp. 1533–1539, 2001.

[18] F. Y. Bian, H. W. Jiang, M. S. Man et al., "Dietary gossypol suppressed postprandial TOR signaling and elevated ER stress pathways in turbot (Scophthalmus maximus L.)," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 312, no. 1, pp. 37–47, 2017.

[19] L. Chen, *Quantitative and Qualitative Research on the Free Gossypol and Its Effects on Growth Performance of Tilapia, Oreochromis Niloticus×O.Aureus(in Chinese)*, Shanghai Ocean University, 2012.

[20] M. D. Zhang, *The Toxicity and Mechanism of Hepatotoxicity Induced by Gossypol Acetate in Cyprinus Carpio (in Chinese)*, Henan Normal University, 2019.

[21] L. E. Castillon and A. M. Altschul, "Preparation of water-soluble combination products of gossypol and their toxicity to aquarium fish," *Proceedings of Society for Experimental Biology and Medicine*, vol. 74, no. 3, pp. 623–626, 1950.

[22] K. J. Lee and K. Dabrowski, "High-performance liquid chromatographic determination of gossypol and gossypolone enantiomers in fish tissues using simultaneous electrochemical and ultraviolet detectors," *Journal of Chromatography*, vol. 779, no. 2, pp. 313–319, 2002.

[23] K. J. Lee, K. Dabrowski, J. H. Blom, S. C. Bai, and P. C. Stromberg, "A mixture of cottonseed meal, soybean meal and animal byproduct mixture as a fish meal substitute: growth and tissue gossypol enantiomer in juvenile rainbow trout (Oncorhynchus mykiss)," *Journal of Animal Physiology and Animal Nutrition*, vol. 86, no. 7-8, pp. 201–213, 2002.

[24] C. Carpenter, "Determination of fat content," in *Food Analysis Laboratory Manual. Food Science Texts Series*, S. S. Nielsen, Ed., pp. 29–37, Springer, Boston, MA, 2010.

[25] J. Folch, M. Lees, and G. H. Sloane Stanley, "A simple method for the isolation and purification of total lipides from animal tissues," *Journal of Biological Chemical*, vol. 226, no. 1, pp. 497–509, 1957.

[26] N. Devanaboyina, S. Rao, A. K. Panda, and V. Sastry, "Cottonseed meal in poultry diets: a review," *The Journal of Poultry Science*, vol. 44, pp. 119–134, 2007.

[27] E. H. Robinson, S. D. Rawles, P. W. Oldenburg, and R. R. Stickney, "Effects of feeding glandless or glanded cottonseed products and gossypol to Tilapia aurea," *Aquaculture*, vol. 38, no. 2, pp. 145–154, 1984.

[28] L. Wang and K. L. Wang, "A review of the studies on gossypol toxicosis in animals (in Chinese).," *Livestock and Poultry Industry*, no. 5, pp. 26–28, 2002.

[29] M. Yildirim, C. Lim, P. J. Wan, and P. H. Klesius, "Growth performance and immune response of channel catfish (Ictalurus puctatus) fed diets containing graded levels of gossypol–acetic acid," *Aquaculture*, vol. 219, no. 1-4, pp. 751–768, 2003.

[30] H. L. Kim, M. C. Calhoun, and R. D. Stipanovic, "Accumulation of gossypol enantiomers in ovine tissues," *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, vol. 113, no. 2, pp. 417–420, 1996.

[31] K. J. Lee and K. Dabrowski, "Gossypol and gossypolone enantiomers in tissues of rainbow trout fed low and high levels of dietary cottonseed meal," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 10, pp. 3056–3061, 2002.

[32] A. S. El-Sharaky, A. A. Newairy, N. M. Elguindy, and A. A. Elwafa, "Spermatotoxicity, biochemical changes and histological alteration induced by gossypol in testicular and hepatic tissues of male rats," *Food and Chemical Toxicology*, vol. 48, no. 12, pp. 3354–3361, 2010.

[33] C. H. Tang, K. Zhang, T. F. Zhan, Q. Y. Zhao, and J. M. Zhang, "Metabolic characterization of dairy cows treated with gossypol by blood biochemistry and body fluid untargeted metabolome analyses," *Journal of Agricultural and Food Chemistry*, vol. 65, no. 42, pp. 9369–9378, 2017.

[34] C. L. Skutches and F. H. Smith, "Effect of phenobarbital on the level of gossypol in the liver and the effect of gossypol and phenobarbital on liver microsomal O-demethylation and lipid peroxidation activities in the rat," *Journal of Nutrition.*, vol. 104, no. 12, pp. 1567–1575, 1974.

[35] I. R. Fink, E. L. Benard, T. Hermsen, A. H. Meijer, M. Forlenza, and G. F. Wiegertjes, "Molecular and functional characterization of the scavenger receptor CD36 in zebrafish and common carp," *Journal of Molecular Immunology*, vol. 63, no. 2, pp. 381–393, 2015.

[36] T. T. Hao, J. Q. Li, Q. D. Liu et al., "Fatty acid translocase (FAT/CD36) in large yellow croaker (Larimichthys crocea): molecular cloning, characterization and the response to dietary fatty acids," *Aqua*, vol. 528, article 735557, 2020.

[37] J. Tian, W. Liu, W. H. Gao et al., "Molecular cloning and gene/protein expression of FAT/CD36 from grass carp (Ctenopharyngodon idella) and the regulation of its expression by dietary energy," *Fish Physiology and Biochemistry*, vol. 43, no. 3, pp. 875–888, 2017.

[38] A. Chabowski, G. J. Luiken, J. F. Glatz, and A. Bonen, "Evidence for concerted action of FAT/CD36 and FABPpm to increase fatty acid transport across the plasma membrane," *Postaglandins, Leukotrienes, and Essential Fatty Acids*, vol. 77, no. 5-6, pp. 345–353, 2007.

[39] A. G. Jay and J. A. Hamilton, "The enigmatic membrane fatty acid transporter CD36: new insights into fatty acid binding and their effects on uptake of oxidized LDL," *Postaglandins, Leukotrienes, and Essential Fatty Acids*, vol. 138, pp. 64–70, 2016.

[40] B. K. Smith, S. S. Jain, S. Rimbaud et al., "FAT/CD36 is located on the outer mitochondrial membrane, upstream of long-chain acyl-CoA synthetase, and regulates palmitate oxidation," *The Biochemical Journal*, vol. 437, no. 1, pp. 125–134, 2011.

[41] A. Chmurzynska, "The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism.," *Journal of applied genetics*, vol. 47, no. 1, pp. 39–48, 2006.

[42] R. B. Laprairie, E. M. Denovan-Wright, and J. M. Wright, "Subfunctionalization of peroxisome proliferator response elements accounts for retention of duplicated fabp 1 genes in zebrafish," *BMC Evolutionary Biology*, vol. 16, no. 1, p. 147, 2016.

[43] A. B. Venkatachalam, M. B. Parmar, and J. M. Wright, "Evolution of the duplicated intracellular lipid-binding protein genes of teleost fishes," *Molecular Genetics and Genomics*, vol. 292, no. 4, pp. 699–727, 2017.

[44] A. B. Venkatachalam, Q. Fontenot, A. Farrara, and J. M. Wright, "Fatty acid-binding protein genes of the ancient, air-breathing, ray-finned fish, spotted gar (Lepisosteus oculatus). Comparative Biochemistry and Physiology," *Part D, Genomics & Proteomics*, vol. 25, pp. 19–25, 2018.