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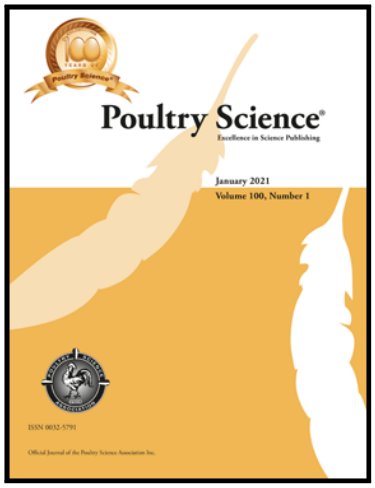

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EFFECT OF HERBAL ON LIVER INJURY

Proteome and transcriptome analysis revealed florfenicol via affected drug metabolism and lipid metabolism induce liver injury of broilers

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Abstract: In order to explore the mechanism of liver injury induced by florfenicol (FFC) in broilers. Sixty broilers were randomly divided into two groups: control group: normal drinking water and feed were given every day; FFC group: tap water containing FFC (0.15g/L) was given every day and feed was taken freely; each group was given 5 days of continuous medication and feed was taken freely. The results showed that compared with the control group, FFC could significantly inhibit the weight gain of broilers (P<0.05), and significantly inhibit the expression of CYP1A1 and CYP2H1 in liver tissue (P<0.05). It was found that the expression of genes related to the effect of cytochrome P450 on the metabolism of exogenous substances, the peroxisome proliferators-activated receptors signal pathway, peroxisome pathway and glutathione metabolic pathway in the liver of broilers. The results of qPCR of UDP glucuronosyltransferase family 2A1 (UGT2A1), glutathione S-transferase-like 2 (GSTAL2), hematopoietic prostaglandin D synthase (HPGDS), glutathione S-transferase theta 1(GSTT1), isocitrate dehydrogenase (NADP(+)) 1 (IDH1), acyl-CoA oxidase 2 (ACOX2), fatty acid binding protein 1 (FABP1), adenylosuccinate lyase (ADSL) and phosphoribosyl aminoim idazolesuccino carboxamide synthase (PAICS) genes which were randomly selected from the most significant genes were consistent with those of RNA-seq. The results showed that FFC can affect the drug metabolism and lipid synthesis in the liver of broiler, thus impairing the normal function of liver and the growth and development of broiler.

Key words: Proteome, Transcriptome, Florfenicol, Broilers, Liver injury

1. Introduction

The abuse of antibiotics is widespread in every country, which causes serious harm to the safety of human and animals(Manyi-Loh, et al., 2018). It has been reported that the health of humans correlates directly with the environment (i.e., their habitat and its components, including plants, animals, microorganisms, and other human beings) and the quality of food that they consume(Ames, 1983; Soto, 2013) . Taking into consideration the growing human population, the changing standard of living conditions, the food shortages, and the greater demands for the intensified production of animal proteins for human consumption across the globe, essential practices to improve on the agricultural and industrial productivity are needed(Anderson, et al., 1998). Of interest is the critical use of antibiotics in agriculture to meet the demands of the rising human population, as the use of antibiotics in this setting has been associated with several benefits. It is therefore anticipated that, in the future, almost all the animals slaughtered and consumed as food must have received a chemotherapeutic or a prophylactic agent of some sort(Lee, et al., 2001) . However, the consumption of these meats, milk, and eggs contaminated with antibiotic residues usually has tremendous impacts on the health of humans. These effects may be direct or indirect, owing to the high dose of the residues, which must have accrued over a prolonged period (Hanekamp and Bast, 2015; Lee, Lee and Ryu, 2001). They can be exhibited as drug hypersensitivity reactions, aplastic anemia, carcinogenic, mutagenic, immunologic and teratogenic effects, nephropathy, hepatotoxicity, disruption of the normal flora of the intestines, a reproductive disorder, as well as the development of antibiotic-resistant bacteria in the gut (Beyene, 2015; Nisha, 2008).

Florfenicol (FFC), a veterinary antibiotic, as analogues of chloramphenicol that are frequently used as substitutes, FFC and thiamphenicol exhibit similar therapeutic benefits(M, et al., 2019).

The use of FFC has become especially prevalent, due to the appearance of fewer side effects(Lai, et al., 2009) . At present, in the practice of broiler breeding, FFC is often added to the feed or drinking water of broiler from 1 day old in a low dose to fight against intestinal bacterial infection to improve the survival rate and growth rate of chickens(Rue, et al., 2011) . However, in this process, there are a lot of abuse of FFC, so the side effects of FFC on broilers are inevitable(Ola, et al., 2014) . In recent years, the toxic and side effects of FFC in normal dosage have been widely reported, which can cause moderate myelodysplasia(Ola, Hassanin, Fatma, Abdallah, Ashraf and Awad, 2014) , and promote the apoptosis of chicken liver cells (Li, et al., 2018). In addition, FFC not only causes serious damage to chicken body, leads to multiple immunosuppressive diseases, affects breeding efficiency, but also leads to drug residues in chicken body, affects the quality and safety of chicken food, and then affects human health(Donoghue, 2003; Nasim, et al., 2016). Previous studies in our laboratory also found that the administration of FFC at the age of 1 day can significantly inhibit the early weight gain of chicks, damage the hemopoietic function of chicks, and damage the liver function of chicks (Han, et al., 2020). However, the mechanism of FFC on liver injury in broilers is not clear. In this project, we intend to use eukaryotic transcriptome sequencing, TMT isotope labeled quantitative proteome sequencing, PCR, and other technologies to study in detail the key targets of liver damage caused by FFC from the perspective of transcriptome and proteomics, so as to provide a basis for the rational use of FFC in broilers.

2. Materials and methods

2.1 Drugs and reagents

chickens (Arbor Acres broilers) were purchased from Hebei Dawu Agricultural Group Poultry Company Ltd (Baoding, China). FFC (purity≥95%) were purchased from Shenniu Biological Technical Co. Ltd (Shandong, China). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assay kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Commercial kits for detecting chicken cytochromeP450 1A1(CYP1A1) and CYP2H1 were purchased from Shanghai hengyuan Biotech CO.,Ltd (Shanghai china). Other chemicals used in these experiments of analytical grade were from commercial sources.

2.3 Experiment design

A total of 60 one-day-old chicks were housed in a clean well ventilated room and kept under good sanitation and hygienic management. Feed and water were available ad libitum. After an acclimatization period of one day, chicks (average body weight = 42.34g ±1.3 g/chick) were randomly allotted into 2 groups (30 chicks per group). FFC was given orally in drinking water at 0.0 g/L for control group, FFC at 0.15 g/L (Recommended dosage of Chinese Veterinary Pharmacopoeia in chick) for FFC group. The FFC treatment was from day 1 to day 5 of age consecutively and chicks in all groups were fed with basal diet (purchased from Hebei Dawu Agricultural Group Poultry Company Ltd). At age of day 5, 21 and 42, chickens were sacrificed following euthanization with sodium pentobarbital. Blood samples were collected from the veinand the liver of the chickens was obtained. In addition, three broiler livers were randomly selected from each group used to transcriptome and proteome sequencing at age of day 5, which was completed by PERSONAL Company (Nanjing, China). All the experimental protocols were approved by the Animal Care and Use Committee of Agricultural University of Hebei prior to the

initiation of the study.

2.4 Amount of body weight gain

Each group of chickens were recorded their initial body weight, and weighed each group again after feeding to 5-day-old , 21-day-old and 42-day-old chickens, and then calculated the amount of weight gain of each group.

2.5 Biochemical parameter measurement

At Day 5, 21 and 42, the blood samples were collected from 10 birds of each group. The blood samples were left to coagulate at room temperature. The serum was separated by centrifugation of coagulated blood at 3000 rpm for 15 min. The clear serum was kept in a freezer (-20 °C) until use to detect the serum total protein, albumin, ALT and AST, superoxide dismutase (SOD), glutathione(GSH), catalase (CAT), lipid peroxidation (MDA) by Biochemical analyzer(Han, et al., 2019) .

2.6 ELISA analysis

Sections of liver tissue were homogenized in 9 volumes of cold Tris buffer (0.01 M Tris-HCl, 0.1 mM EDTA-Na2, 0.01 M sucrose, 0.9% saline [pH 7.4]) and a 10% (wt/vol) tissue homogenate was prepared. The supernatant obtained from 4000 g at 4°C for 15 min of centrifugation. The contents of CYP1A1 and CYP2H1 in the supernatant of liver homogenate were measured by ELISA kit (Shanghai hengyuan Biotech CO.,Ltd. Shanghai china).

2.7 RNA-seq

RNA-seq was performed on livers of chickens using Illumina's sequencing platform Illumina HiSeq. Testicular tissue total RNA was extracted using the Eastep Super Total RNA Extraction Kit (Promega, LS1040, US). Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina®(NEB, USA) following manufacturer's recommendations. The library preparations were sequenced on an Illumina Hiseq platform. Reference genome and gene model annotation files were downloaded from genome website directly (https://www.ncbi.nlm.nih.gov/genome/?term=Mus+musculus). Differential expression analysis was performed using the DEGSeq R package (1.20.0). The P values were adjusted using the Benjamini & Hochberg method.

Corrected P-value of 0.005 and Log2(Fold change) of 1 were set as the threshold for significantly differential expression. Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the GOseq R package. KOBAS software was used to test the statistical enrichment of differential expression genes in KEGG pathways.

2.8 Protein extraction and TMT Labeling

All samples were homogenized in lysis buffer (4% SDS, 1 mM DTT, 150 mM Tris-HCl pH 8.0, protease inhibitor). After 3 min incubation in boiling water, the homogenate was sonicated on ice. The crude extract was then incubated in boiling water again and clarified by centrifugation at 16000 x *g* at 25°C for 10 min. Protein content was determined with the BCA protein assay reagent (Beyotime). 200 μg of proteins for each sample were incorporated into 30 μl SDT buffer (4% SDS,

100 mM DTT, 150 mM Tris-HCl pH 8.0). The detergent, DTT and other low-molecular-weight components were removed using UA buffer (8 M Urea, 150 mM Tris-HCl pH 8.0) by repeated ultrafiltration (Pall units, 10 kD). Then 100 μl 0.05 M iodoacetamide in UA buffer was added to block reduced cysteine residues and the samples were incubated for 20 min in darkness. The filters were washed with 100 μL UA buffer three times and then 100 μl DS buffer (50 mM triethylammoniumbicarbonate at pH 8.5) twice. Finally, the protein suspensions were digested with 2 μg trypsin (Promega) in 40 μl DS buffer overnight at 37 °C, and the resulting peptides were collected as a filtrate. The peptide content was estimated by UV light spectral density at 280 nm using an extinctions coefficient of 1.1 of 0.1% (g/l) solution that was calculated on the basis of the frequency of tryptophan and tyrosine in vertebrate proteins.

2.9 UPLC-QTOF-MS/MS Analysis

TMT labeled peptides were fractionated by SCX chromatography using the AKTA Purifier system (GE Healthcare).The fractioned peptides were collected and combined into 20 final fractions, which were applied to LC-MS/MS. In brief, the prepared sample was loaded on an EASY-nLC 1000 nano HPLC (Termo Scientifc, USA). The peptides were eluted onto an analytical C18 column (10 cm× 75μm). The peptides were subjected to electrospray ionization followed by tandem mass spectrometry (MS/MS) in a Q-Exactive (Termo scientifc, USA). Intact peptides were detected in the Orbitrap. Te electrospray voltage applied was 2.1 kV. Automatic gain control (AGC) was used to optimize the spectra generated by the orbitrap. For MS scans, the m/z scan range was 350–2000.

2.10 Sequence Database Searching and Data Analysis

LC-embedded into Proteome Discoverer 1.4 (Thermo Electron, San Jose, CA.) against uniport Gallus gallus 32641 20190702 (32641 sequences, download at 20190702) and the decoy database. For protein identification, the following options were used. Peptide mass tolerance=20 ppm, MS/MS tolerance=0.1 Da, Enzyme=Trypsin, Missed cleavage=2, Fixed modification: Carbamidomethyl (C), TMT10(K), TMT10(N-term), Variable modification : Oxidation(M), FDR≤0.01.

2.11 Bioinformatics

Functional analysis was conducted using gene ontology (GO) annotations by Blast2GO sofware (v4.5 pipeline 5), and the proteins were categorized according to their biological processes, molecular functions, and cellular localizations. Te diferentially expressed proteins were further assigned to the Kyoto Encyclopedia of genes and genomes (KEGG) database (http://www.genome.jp/kegg/pathway.html). The most important is the integrated analysis of the pathway. According to the results of pathway analysis of different genes and proteins, the genes and proteins involved in the same pathway were screened, and the corresponding data were analyzed comprehensively. In addition, the ID of gene and protein was transformed according to KEGG format by using transformation ID method, and the ID was mapped into KEGG database to obtain the path map after gene and protein integration. Protein-protein interaction networks were analyzed using the publicly available program STRING (http://string-db.org/)and minimum required interaction score set 0.400.

2.12 QRT-PCR detection of mRNA transcription in liver tissue

At age of day 5, 21 and 42, Ten chickens were sacrificed following euthanization with sodium pentobarbital and the liver samples were obtained from each chicken. The total RNA Extraction Kit (Promega, Beijing, China) was used to extract RNA from the liver and reverse transcription to obtain cDNA. The mRNA levels of CYP1A1, CYP2H1, UGT2A1, GSTAL2, HPGDS, GSTT1, IDH1, ACOX2, FABP1, ADSL and PAICS were detected by real-time quantitative PCR. Primers were designed and synthesized by Takara (Dalian, China) (Table 1). The pre denaturation was at 95 ℃ for 120 seconds, and the amplification was carried out in 45 cycles, including 5 seconds at 95 ℃ and 30 seconds at 64 ℃. Then 72 ℃ for 30s. The internal reference gene is β - actin, and the relative transcription level of each gene is calculated by $2^{-\Delta\Delta CT}$(Livak and Schmittgen, 2001).

2.15 Statistical analysis

Using SPSS21.0 software (IBM Corporation, Armonk, NY) to test the normality of the counting data. Then the differences among groups were compared by T test, the data in this experiment were mean ± standard deviation (SD) and significant differences among groups were set at a value of $P < 0.05$, $P < 0.01$.

3 Results

3.1 Effect of FFC on weight gain in Broilers

As shown in Fig1, compared with the control group, 0.15g/L FFC significantly reduced the weight gain of broilers at age of 5 days, 21 days and 42 days ($P < 0.05$).

3.2 Effect of FFC on ALT and AST in serum of chickens

As shown in Figure 2, compared with the control group, FFC significantly increased AST and ALT content in serum of broilers and decreased TP and ALB content in serum of broilers at age of day 5 and 21 ($P < 0.05$); while at age of day42, compared with the control group, FFC only significantly reduced Alb content in serum of broilers ($P < 0.05$), However, FFC did not significantly cause changes of AST, ALT and TP contents ($P > 0.05$).

3.3 Effect of FFC on the content of GSH, MDA and CAT in chicken liver

As shown in Fig 3, compared with the control group, FFC significantly reduce the content of SOD and CAT in the broilers liver tissue at age of day 5 and 21 ($P < 0.05$), and significantly reduce the content of GSH in the broilers liver tissue at age of day 5 and 21 ($P < 0.05$). compared with the control group, FFC significantly increased the content of MDA in broilers liver tissue at age of day 5, 21 and 42 ($P < 0.05$).

3.4 Effect of FFC on the contents of CYP1A1 and CYP2H1 and mRNA transcription in chicken liver

As shown in Figure 4, compared with the control group, FFC induced the CYP1A1 content and mRNA transcription level in the broilers liver tissue decreased significantly at the age of day 5 and 21 ($P < 0.05$); while at the age of day 42, the CYP1A1 content and mRNA transcription level of the broilers liver tissue in the control group and FFC group had no significant difference ($P > 0.05$). At age of day 21 and 42, compared with the control group, FFC also significantly reduced the content of CYP2H1 in chicken liver; but, at age of day 5, 21 and 42, the mRNA transcription level of CYP2H1 of broilers liver tissue in FFC group was significantly lower than that in control

group ($P < 0.05$).

3.5 Expression of differential genes and proteins

Principal component analysis (PCA) showed large variations in transcript and protein (Fig. 5A) abundance between chicken liver in FFC group and liver in control group. The results of transcriptome group showed that compared with the control group, FFC caused 1278 mRNA expression differences, 344 of which were up-regulated genes and 934 of which were down-regulated genes(Fig. 5B), Fig. 5D showed the clustering of these 1278 genes; while the results of proteome group showed that compared with the control group, FFC caused 917 protein expression differences, There are 488 up-regulated proteins and 429 down-regulated proteins of those 917 proteins(Fig.5C), and Fig5E shows the clustering of 917 proteins.

We also explored the expression of proteins and its corresponding mRNA, as shown in Figure 6A and Figure 6B, compared with the control group, the expression of 1106 mRNA and 824 proteins in broiler liver treated by FFC was different. There were 93 differences in the expression of protein and corresponding mRNA. As shown in Table 2, there are 49 up-regulated and 44 down-regulated mRNA and protein expressions in the 93 differentially expressed proteins and mRNA, while there are 47 up-regulated and 42 down-regulated proteins and related mRNA, 2 up-regulated and 2 down regulated proteins and corresponding mRNA. Fig. 6C shows the expression of the top 20 proteins and their corresponding mRNA in the liver tissue of broilers induced by FFC.

3.6 Bioinformatics analysis of differentially expressed genes and proteins

The functions of differential genes are divided into three categories: biological process, cell

composition and molecular function. The results showed that the difference genes in broilers liver were related to the process of biosynthesis and metabolism after FFC exposure. The results of enrichment analysis will give the directed acyclic graphs of three Ontology (cell composition, molecular function and biological process) of GO respectively(Fig7A). Enrichment analysis results will give the directed acyclic graph of three go ontologies (cell composition, molecular function and biological process) respectively. It can be seen from the graph that the genes with significant changes in the liver caused by FFC are mainly enriched to a more detailed level and perform specific biological functions (Fig7B). From the KEGG enrichment result, we also found that the expression of genes related to the effect of cytochrome P450 on the metabolism of exogenous substances (UGT2A1, GSTAL2, HPGDS, GSTT1) was significantly changed by FFC, and the genes related to PPAR signal pathway (ACOX2, ACAA1, FABP1) was also significantly changed. FFC also caused changes in the expression of peroxisome related genes and glutathione metabolic pathway (ACOX2, ACAA1, FABP1) related genes in the liver of broilers (Fig7C, Table 3). To explore the direct and indirect interaction between these expressed significant target proteins, to generate the interaction network and analyze the network, we found that: GSTT1, UGT2A1, HPGDS, ACOX2 and other genes can be used as the target molecules of meat and liver damage caused by FFC (Fig 7D).

3.7 Validation of Differentially Expressed Genes Using qRT-PCR

As shown in Table 2, the qPCR results of UGT2A1, GASTAL2, HPGDS, GSTT1, IDH1, ACOX2, FABP1, ADSL and PAICS genes screened from the transcriptome are consistent with the RNA-seq results and the content of TMT protein (Table4, Fig8A). At the same time, we detected

the expression of UGT2A1 and GSTT1 mRNA and ACOX2 and FABP1 mRNA related to lipid metabolism, and found that at the age of day 5, 21 and 42, compared with the control group, FFC significantly increased the transcription level of UGT2A1, GSTT1 and ACOX2 mRNA ($P < 0.05$); The transcription level of FABP1 mRNA was significantly inhibited ($P < 0.05$) (Fig. 8B).

4. Discussion

Our previous study found that: the normal recommended treatment dose (0.15g / L) of FFC has certain damage effect on growth performance, hematopoietic function and liver function of broilers within 7 days of age . In this study, we further explored the toxic side effects of FFC on 21 day old broilers and 42 day old broilers, the result showed that at age of day 21 and 42, the normal recommended dose (0.15g / L) and higher dose of FFC still significantly inhibited the weight gain of broilers ($P < 0.05$). The results show that during the whole growing period the inhibitory effect of FFC on the growth and development of broilers was always existed. In addition, the results showed that FFC still caused liver damage at age day 21, However, effects of FFC induce broilers liver injure was alleviated when the age of day 42.

In order to further explore the mechanism of FFC on liver injury in broilers, we used transcriptome and proteomics to analyze the key targets of FFC on liver injury in broilers, in order to reveal the mechanism of action of FFC on liver toxicity. The results showed that FFC could induce drug metabolism - cytochrome P450, glutamate metabolism, PPAR signaling pathway, metabolism of xenobiotics by cytochrome P450, steroid biosynthesis, drug metabolism - other enzymes, steroid hormone biosynthesis, peroxisome and other pathways have different expression of related proteins, indicating that FFC can affect the normal liver function of Broilers by causing

abnormal expression of these genes or proteins.

The most common causes of Drug-induce liver injury (DILI) in broilers are antibiotics, aflatoxins, heavy metals, etc. most of their metabolites have hepatotoxic (Leise, et al., 2014). There are two steps of drug reaction in liver, i.e. phase I and phase II(Hellriegel, et al., 1996). Phase I reaction: the drug is hydrolyzed, oxidized and reduced to produce metabolites. The main metabolic enzymes are cytochrome P450 (CYP), CYP1A1 and CYP2H1, which are key enzymes related to drug-induced liver injury (Dohnal, et al., 2014). Therefore, the content of liver drug enzymes in hepatocytes is directly related to the detoxification ability of liver. Our results showed that the contents of CYP1A1 and CYP2H1 in the liver of 21 day old broilers fed with FFC decreased significantly, and showed a certain dose dependence. It is suggested that FFC can increase the concentration of FFC in the liver and cause liver damage by inhibiting the activity of liver drug enzymes. Glutathione S-transferase (GSTs) is a group of enzymes related to the detoxification function of liver(Sun, et al., 2012). Its glutathione and free radicals combine to promote the elimination of toxic substances from the body and reduce the potential toxicity of drugs. GSTs dysfunction will lead to the occurrence of DILI(Roy, et al., 2008). Glutathione S-transferase (GST) is one of the important metabolizing enzymes. GST has high concentration in the solute of hepatocytes. It can reduce toxicity and promote urine excretion by catalyzing the combination of glutathione (GSH) and active metabolites in the liver. When the liver is seriously damaged, GST and GSH will be released from the liver cytoplasm into the plasma, resulting in the decrease of GST activity in the liver cytoplasm(Zhang, et al., 2017). Therefore, the gene regulation of GST plays a decisive role in the metabolism of GSH. The monitoring of GST activity in liver tissue may be a potential strategy for the diagnosis of DILI(Tian, et al., 2019). Our

study found that FFC can significantly improve the expression of GSTT1 mRNA and protein, which indicates that FFC can accelerate the metabolism of drugs in the liver, cause a large number of drug metabolites in the liver, and then damage the liver function. In addition, UDP-glucuronosyl transferase (UGT), as another important transferase in phase II reaction, is a large family of enzymes that can catalyze the combination of glucuronic acid and nucleophilic substrate(Alkharfy, et al., 2017). It mainly exists in the liver and combines with the microparticle membrane to catalyze the glucuronide combination reaction in phase II reaction of liver biotransformation. Several studies have shown that genes UGT1A9 and UGT147 are significantly correlated with DILI (Jiang, et al., 2015; Stickel, 2015; Terelius, et al., 2016). In this study, FFC significantly promoted the expression of ugt2a1, indicating that FFC can affect the phase II response of drug metabolism in liver through ugt2a1, and then affect the function of liver. In addition, a large number of metabolites will be produced due to the excessive metabolism of drugs in the liver. The accumulation of these metabolites may affect the secretion of bile and cause cholestasis. The accumulation of bile acids may cause indirect damage to liver cells and bile duct cells (Zollner and Trauner, 2006).

Peroxisome proliferator-activated receptors (PPARs), as one of the important nuclear transcription factors involved in the regulation of lipid metabolism in vivo, can be activated by binding with ligands, regulate the oxidation of fatty acids, increase insulin sensitivity of the body, regulate the storage of triacylglycerol in adipose tissue(Goto, 2019; Silva and Peixoto, 2018), which is highly expressed in liver, skeletal muscle, heart muscle, renal cortex and other tissues with strong energy metabolism, and regulate glucose and lipid metabolism, energy balance, inhibit inflammation and anti-inflammation It should be closely related(Li and Liu, 2018; Liao, et al.,

2017; Liss and Finck, 2017). In this study, we found that six proteins and their corresponding mRNA expressed differentially enriched in PPAR signaling pathway, they are acyl-coenzyme A oxidase-2 (ACOX2), delta-6 fatty acid destination (FADS2), fatty acid binding protein 7 (FABP7), acyl CoA acyltransferase 1 (ACAA1), long chain fatty acid -- CoA ligase acsbg2 (ACSBG2), fatty acid binding Protein 1 (FABP1) and so on indicate that FFC can damage the normal function of liver by affecting the metabolism of lipid in liver. FABP1 is located downstream of PPAR α in PPAR α signaling pathway, which is closely related to fatty acid transport. Studies have shown that FABP1 plays an important role in free fatty acid transport and metabolism of liver cells(Yi, et al., 2016). A research has showed that overexpression of chicken FABP gene can induce high lipolysis rate and decrease abdominal fat content(Shi, et al., 2010). The results of this study showed that FFC could significantly reduce the expression of FABP1 gene mRNA in the liver of broilers. This may be because the liver of broilers was damaged after exposure to FFC, breaking the normal expression of FABP1, causing oxidative stress in the liver, and further aggravating the liver damage. In addition, PPARs can regulate the transcription of a variety of target genes (such as ACAA1 and ACOX2) that have peroxisome proliferative response elements in the promoter region(Hariya, et al., 2015), these genes encode key enzymes or proteins involved in lipid metabolism(Osumi and Hashimoto, 1978; Wanders, et al., 2001), abnormal expression of these genes can interfere with the synthesis of fat in the liver, and then mediate liver damage. Our results show that florfenicol can significantly increase the expression of ACOX2 mRNA and protein, and then affect the synthesis of fatty acids in the liver. In conclusion, FFC can affect the synthesis and metabolism of lipids in liver, and then damage the function of liver.

5. Conclusion

Feeding young broilers with FFC can inhibit the weight gain and damage the liver function during the whole growth cycle. The mechanism of FFC induced broilers liver injury were that: on the one hand, FFC disrupts the expression of UGT2A1, GSTT1 and their regulated proteins, thus affecting the drug metabolism ability of broiler liver, causing drugs to accumulate in the liver and further damage the liver function; on the other hand, FFC damages broilers by affecting the expression of acox2, FABP1 and other genes and their regulated proteins, and then affecting the lipid metabolism of broiler liver.

Declaration of competing interest The authors have no conflicts of interest.

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Figure 1

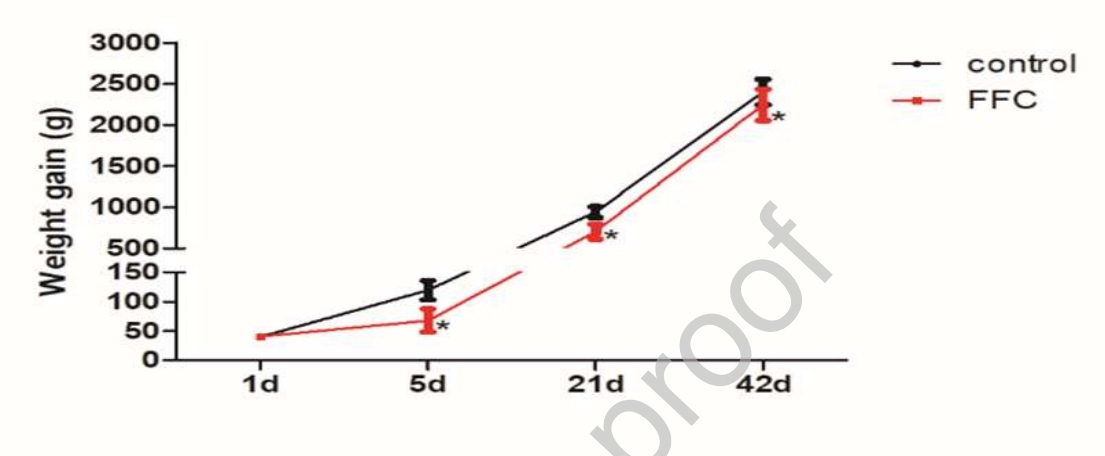

Fig. 1 weight gain of broilers of different ages. *P < 0.05, compared with the control group.

Figure 2

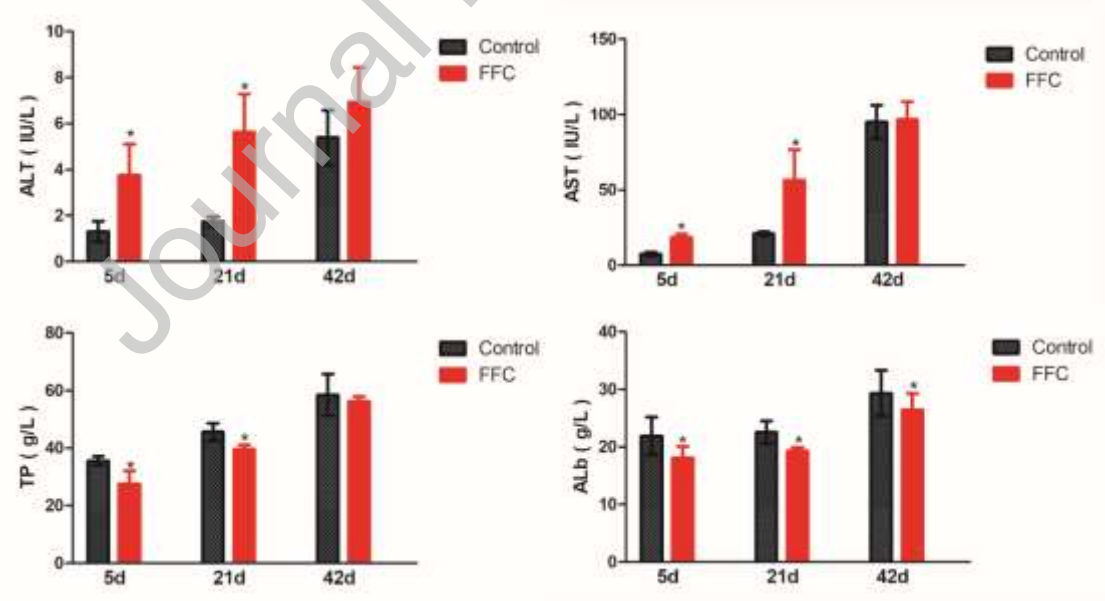

Fig. 2 contents of alt, AST, TP and ALB in broilers serum of different days. *P < 0.05, compared with the control group.

Figure 3

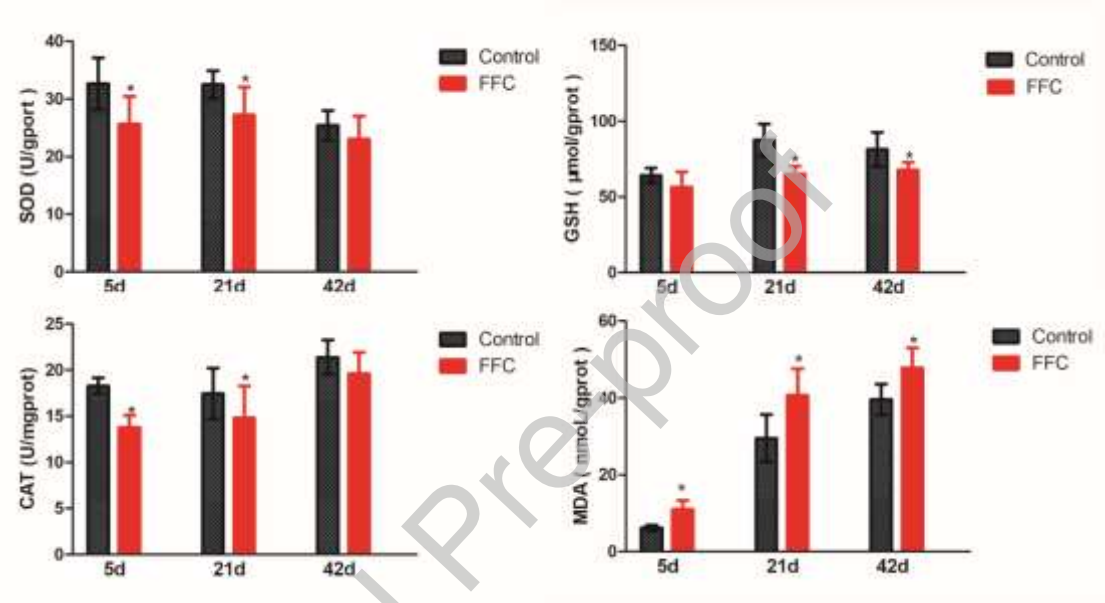

Fig. 3 contents of SOD, GSH, MDA and cat in liver tissues of broilers of different ages. *$P < 0.05$, compared with the control group.

Figure 4

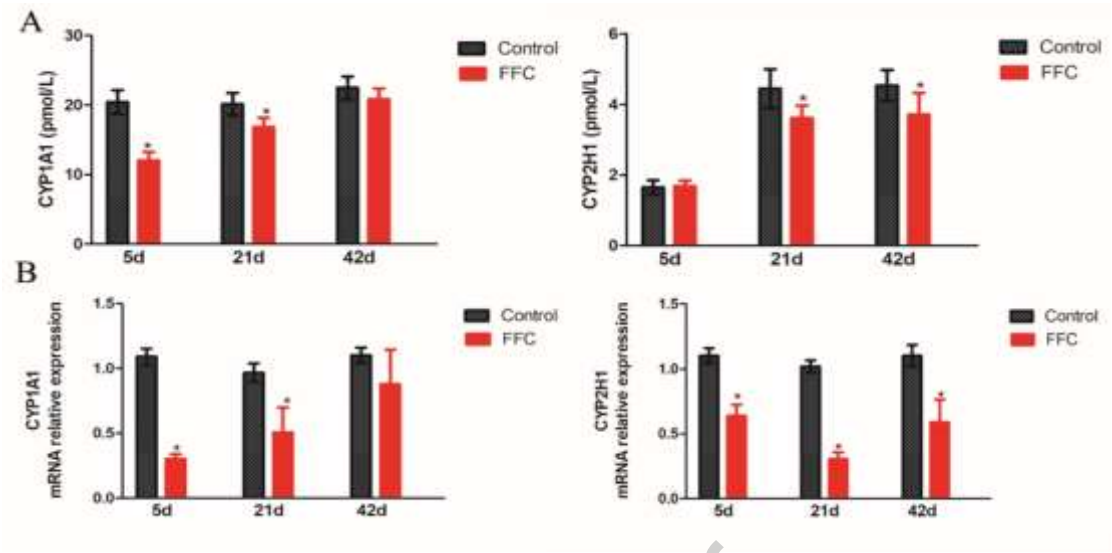

Fig. 4A: the content of CYP1A1 and CYP2H1 in liver tissue of each group; B: the transcription level of CYP1A1 and CYP2H1 mRNA in liver tissue of each group. *P < 0.05, compared with the control group.

Figure 5

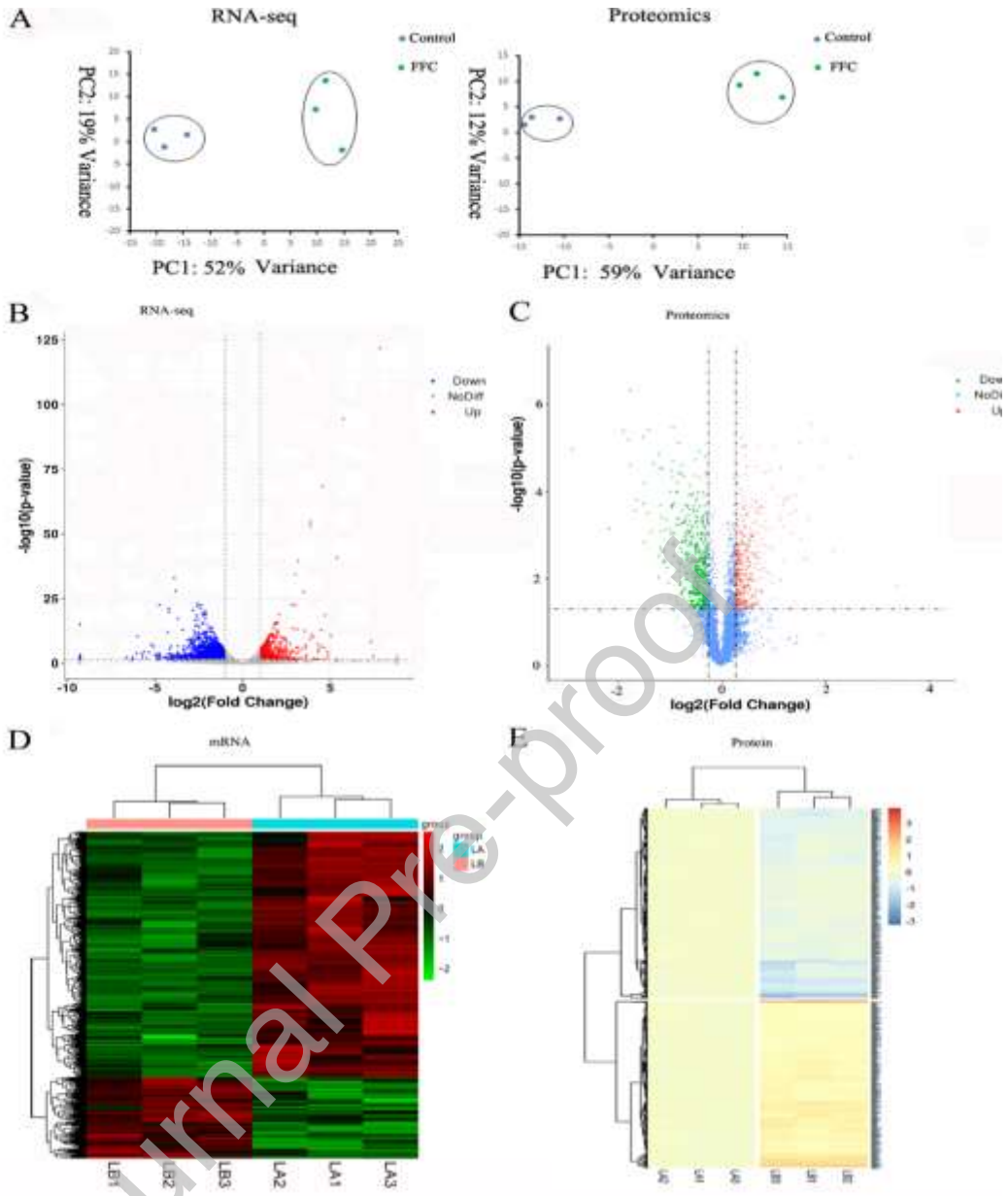

Fig.5 Transcriptomic and proteomic mapping of broilers liver . (A) PCA for RNA-seq or proteomics data, the points represent biological replicates . (B) (B and C) Volcano plots showing the relative abundances of transcripts (B) or proteins (C). Transcripts were considered differentially expressed at FDR < 1% and fold change > 3 between proximal and distal regions, whereas proteins were considered differentially expressed at FDR < 5%. (D and E) Heat maps of 1278 transcripts or 917 proteins.

Figure 6

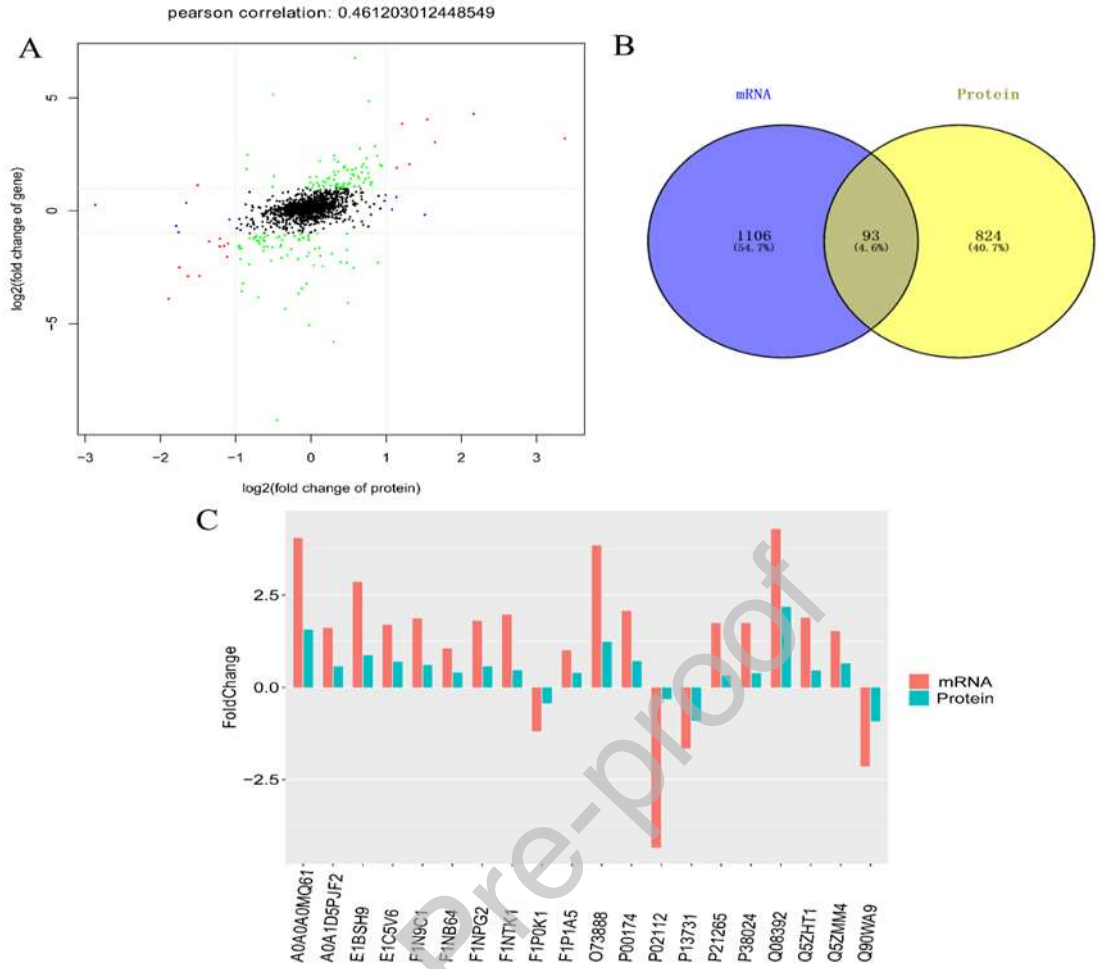

Figure 6A For the correlation analysis of protein and gene expression quantity, the abscissa and ordinate are the multiple of protein and gene expression difference, red represents the result of significant difference expression at transcripts and protein level, blue represents the result of significant difference expression only at protein level, green represents the result of significant difference expression only at transcription level, black represents the result of no significant difference between the two levels Results. B: Venn map of total differential proteins and corresponding differential transcripts; C: Figure 3 result s of differential expression of proteins and related transcripts. Abscissa is the corresponding gene name of related proteins and transcripts, ordinate is the multiple of differential expression, red is the transcripts, and blue is the egg white matter.

Figure 7

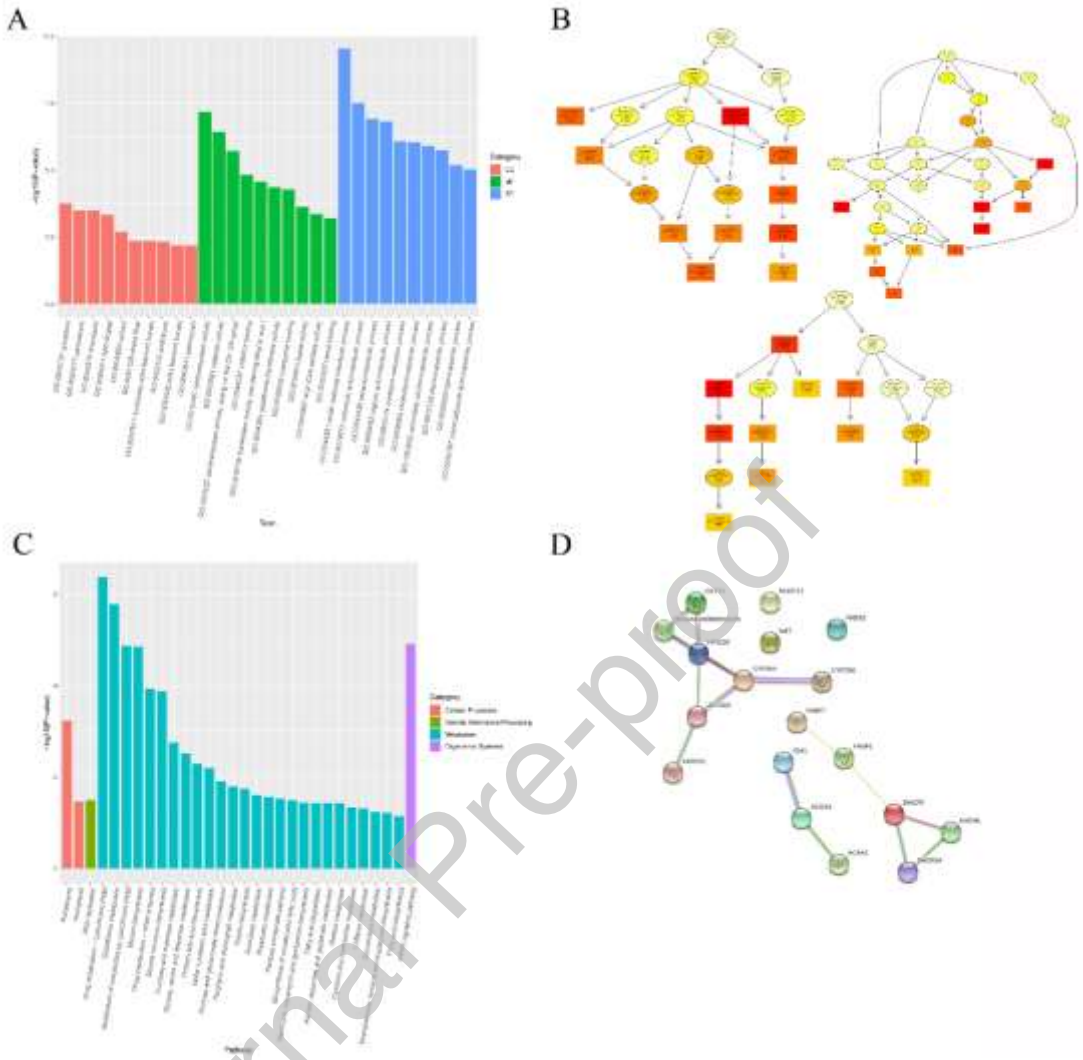

Fig.7A Go enrichment analysis histogram; Fig 6B gene ontology DAG enrichment diagram, each node represents a go term, branch represents inclusion relationship, the functional scope defined from top to bottom is getting smaller and smaller, the box represents the GO term with top 10 enrichment degree, and the darker the color, the higher the enrichment degree; Fig7C KEGG pathway enrichment result histogram, abscissa is pathway The vertical coordinate is the diagram of - log10 (p-value) ; Fig 7D PPI network enriched by each pathway - gene expression.

Figure 8

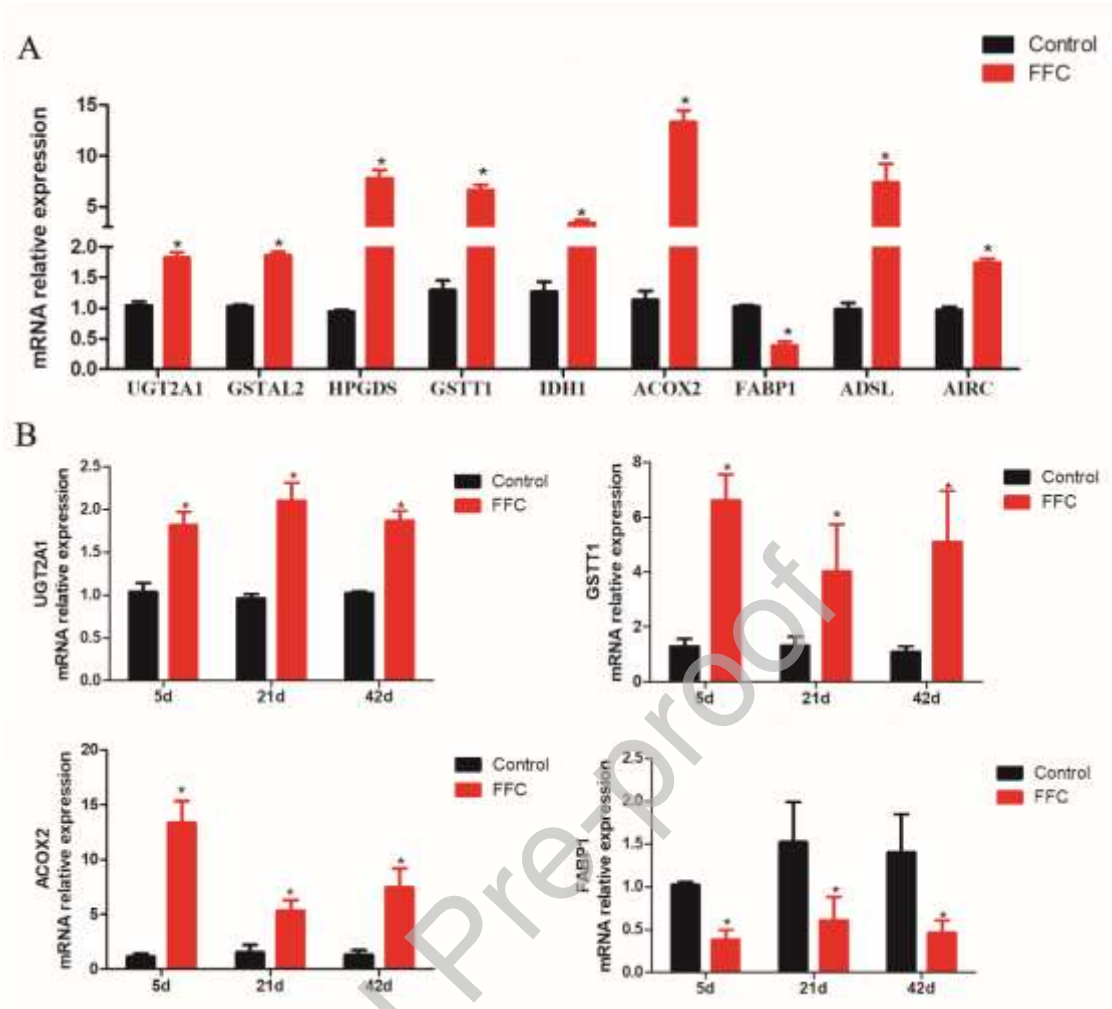

Figure 8 A The mRNA transcription level of the selected key genes in the liver tissue of 5-day-old broilers, B the mRNA transcription level of UGT2A1, GSTT1, ACOX2 and FABP1 in the liver tissue of different day-old broilers. *$P < 0.05$, compared with the control group.

Table1 Primers used for qRT-PCR

Gene	Primer sequence (5'-3')	Product length (bp)	Accession number
CYP1A1	F: AGGACGGAGGCTGACAAGGT	136	NM_205147.1
	R:CAGGATGGTGGTGAGGAAGAG		
CYP2H1	F: ATCCCCATCATTGGAAATGT	148	NM_001001616.1
	R: TCGTAGCCATACAGCACCAC		
UGT2A1	F: CCAGCTTCCACAAAATGTCCTC	150	XM_004941194.3
	R: CCATTGGTCCCACCATGAGTAA		
GSTAL2	F: GGGTGGTTGAAGGATCGAACT	157	XM_025148955.1
	R: CTTCACTGGTTCCATTCGGC		
HPGDS	F: GCAGAAATCTGCCGCTACCT	199	NM_205011.1
	R: TCTGACCTGCCAGACCTGAT		
GSTT1	F: CCATGTGGATCAAGGTGCTGA	158	NM_205365.1
	R: CAAGGAAATCTCGCTCCCGA		
IDH1	F: CTCTGTTGCACAAGGTTATGGC	154	XM_004942682.3
	R: AATGGGGTTCGTGGAGGTTT		
ACOX2	F: TGAATTCTGCACTCGGCAGT	100	XM_015293306.2
	R: TCATAGGCTGGGCTTATCTGC		
FABP1	F: CAGGAGAGAAGGCCAAGTGTAT	100	NM_204192.3
	R: GTGTCTCCGTTGAGTTCGGT		
ADSL	F: TCACTCACTACCAACCTGCAC	131	NM_205529.1

	R: GCCTTTCACACCCCGAAAAC		
PAICS	F: ATGCGTTCTGATGCAGTCCA	141	NM_205524.2
	R: GCCGTTTTGATTCCTGCTTCC		
β-actin	F: ATTGTCCACCGCAAATGCTTC	124	NM_205518.1
	R:AAATAAAGCCATGCCAATCTCGTC		

Table 2 Expression of differential protein and its corresponding differential transcription

terms	mRNA (up)	mRNA (down)	Total
Protein (up)	47	2	49
Protein (down)	2	42	44
Total	49	44	93

Table 3 List of genes involved in the most significant pathway of KEGG enrichment

Pathway ID	Pathway	Gene list
gga00982	Drug metabolism - cytochrome P450	UGT2A1, GSTAL2, HPGDS, GSTT1, DM5L
gga00480	Glutathione metabolism	GSTAL2, IDH1, HPGDS, GSTT1, GCLC
gga03320	PPAR signaling pathway	ACOX2, FADS2, FABP7, ACAA1, ACSBG2, FABP1
gga00980	Metabolism of xenobiotics by cytochrome P450	UGT2A1, GSTAL2, HPGDS, GSTT1,
gga00100	Steroid biosynthesis	LSS, NSDHL, DHCR24, DHCR7

gga00983	Drug metabolism - other enzymes	UGT2A1, GSTAL2, GSTT1, NAT
gga00140	Steroid hormone biosynthesis	UGT2A1, AKR1D1, CYP2D6, CYP3A4
gga04146	Peroxisome	RCJMB04_1j22, IDH1,ACOX2,ACAA1, NUDT12

Table4 Correlated protein and mRNA pair expression comparisons.

Protein ID	Protein name	Gene name	Log2 (fold change)	
			Protein	gene
F1NMB3	UDP-glucuronosyltransferase	UGT2A1	1.158	1.898
A0A0A0MQ61	Uncharacterized protein	GSTAL2	1.566	4.047
O73888	Hematopoietic prostaglandin D synthase	HPGDS	1.232	3.851
P20135	Glutathione S-transferase theta-1	GSTT1	0.785	1.471
F1NPG2	Isocitrate dehydrogenase [NADP]	IDH1	0.568	1.780
E1C5V6	Acyl-coenzyme A oxidase	ACOX2	0.694	1.693
Q90WA9	Fatty acid-binding protein	FABP1	-0.919	-2.148
P21265	Adenylosuccinate lyase	ADSL	0.326	1.739
P38024	Multifunctional protein ADE2	AIRC	0.381	1.740