Dietary exposure of deoxynivalenol affected cytochrome P450 and growth related-gene expression via DNA methylation in piglet liver

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Abstract

Background

Deoxynivalenol (DON) is an inevitable contaminant in animal feed and human food and can lead to decreased appetite and growth retardation in children and piglets, which are often used as models for children. Hepatotoxicity induced by DON is closely related to growth inhibition. Although many molecular mechanisms are related to the liver damage caused by DON, few studies have been done on cytochrome P450 (CYP450s) and DNA methylation, and the role of DNA methylation in growth inhibition of piglets was also unclear.

Results

In the present study, piglets were randomly assigned to the following three different dietary treatments for 4 weeks: control diet, diet containing 1 mg DON/kg feed, and diet containing 3 mg DON/kg feed. Compared to the control group, elevated alanine aminotransferase activity and globulin level were observed in DON groups; however, aspartate aminotransferase activity was decreased in 3 mg/kg DON group. DON exposure increased the mRNA expression of CYP450s including CYP1A1, CYP1A2, CYP2B22, CYP2C33, CYP2D25, CYP2E1, CYP3A22 and CYP3A39, in which DNA methylation was involved in the regulation of the expression of these enzymes. By estimating the benchmark dose value of the metabolic enzymes, CYP1A1 was found to be the most sensitive metabolic enzyme to evaluate the clinical liver injury caused by DON. DON upregulated the expression of DNA methyltransferases (DNMT1 and DNMT3B) in a dose-dependent manner, thus increasing the level of 5-mC in the whole genome. Notably, DON downregulated the expression of nicotinamide n-methyltransferase, possibly reducing the weight of the piglets. Additionally, 1 mg/kg DON decreased the expression of galanin-like peptide (GALP), while 3 mg/kg DON significantly increased the level of GALP through DNA methylation, thus affecting the appetite of the piglets. DON can significantly reduce the methylation level of insulin-like growth factor 1 (IGF-1) promoter and thus increase its expression.

Conclusions

Taken together, increased CYP450 expression and DNA methylation are important mechanisms of liver injury and growth inhibition induced by DON, which provide future reference values determination of antagonistic toxicity.

Background

Deoxynivalenol (DON), as a serious natural source of global contaminant, is one of the most common mycotoxins produced by several pathogenic fungi, such as *Fusarium graminearum* [1]. DON is generally stable and therefore not easily destroyed or removed from food even after processing [2]. DON contamination is very widespread, which not only brings great loss to feed enterprises and breeders, but also threatens human health. The contamination of grain by DON is associated with the strain, temperature, humidity, ventilation, sunshine and other factors [3]. DON is mainly present in wheat, barley, oats, corn and other cereal crops [4], bread, biscuits, wheat snacks and other food products [5], animal milk, eggs [6] and fruit [7]. DON can also cause

pollution in fresh and waste water treatment plants [1]. It was reported that DON was omnipresent in the primary effluent samples of three Swiss waste water treatment plants in concentrations of 32–118 ng/L [8]. The detection rate of DON in cracker biscuits produced in southern Brazil was 99% [9].

Infants and young children are at risk of a high level of DON exposure because of their high cereal intake relative to body weight [10]. In China, the average DON exposure is the highest at ages of 3–6 years. In one report, DON exposure was 2.12 μg/kg/day in the children of northern areas of China and 0.73 μg/kg/day in the children of southern areas [4]. Estimation of dietary DON revealed that children in the UK were frequently exposed to levels exceeding the tolerable daily intake, with 52% of cases for DON [11]. The levels of the estimated dietary DON intake in Henan province were high, especially for children [12]. In Norway, the mean exposure to DON was in the range of up to twice the tolerable daily intake in 1-year-old infants and 2-year-old children [13]. Worryingly, long-term intake of food containing DON leads to loss of appetite, growth retardation and weight loss in children [4]. It is noteworthy that the liver injury caused by DON is closely related to growth inhibition. For instance, DON induces strong G_2/M cell cycle arrest in HepG2 cells by highly upregulating the cell cycle inhibitory protein p21 [14]. Therefore, DON is a potential threat to children's health and has aroused wide concern.

Cytochrome P450s (CYP450s) in the liver do not directly metabolise DON, but these enzymes may be closely involved in hepatotoxicity. Previous studies have found many mechanisms of liver injury caused by DON, such as lipid peroxidation, oxidative stress [15], apoptosis [1], inflammatory response [16] and autophagy [17], but this is all phenotypic. CYP450s may be important sources of toxic liver injury under DON exposure. For example, CYP450 1A1 (CYP1A1), CYP450 1B1 (CYP1B1) and CYP450 2E1 (CYP2E1) can induce oxidative stress in hepatocytes [18]. Although DON is metabolised by the liver endoplasmic reticulum UDP-glucuronosyltransferases (UGTs), recent studies have found that DON can upregulate the expression of CYP450 1A4 (CYP1A4) in the jejunum [19]. However, it is still not clear whether DON affects the expression of CYP450s in the liver.

Recent studies have shown that DNA methylation is closely associated with toxin-induced liver damage. Lu et al. found that T-2 toxin significantly increased the levels of DNA methyltransferase 1 (DNMT1) and DNA methyltransferase 3A (DNMT3A), which were mainly concentrated at the site of liver injury [20]. The 5-methylcytosine (5-mC) level of genomic DNA was also raised in T-2 toxin-treated rat livers [20]. Additionally, DNA methylation regulated the expression of Ras association domain family member 4 (RASSF4) under T-2 toxin, along with activation of its downstream pathways, resulting in apoptosis in the rat liver [21]. However, to the best of our knowledge, the potential role of DNA methylation in DON-induced CYP450 expression has not been identified.

The toxicity of DON-induced retardation of human growth has become a burning topic. Studies have found that DON can cause growth retardation and weight loss through protein synthesis inhibition and other phenotypic mechanisms [22]. Recently, it was found that DNA methylation was closely related to growth inhibition [23, 24]. The expression of growth hormone (GH), growth hormone receptor (GHR) and insulin-like growth factor 1 (IGF-1) were influenced by DNA methylation [25, 26]. Another study revealed that 8 mg/kg of DON in the diet was responsible for significant decreases in the mRNA expression of nicotinamide N-methyltransferase (NNMT), galanin-like peptide (GALP) and IGF-1, but significantly increased the expression of insulin-like growth factor-

binding protein 2 (IGFBP2) [16]. However, how DNA methylation affects the expression of these genes under DON exposure is still unknown.

On the basis of previous studies, we used piglets as models to study the role of CYP450s and DNA methylation in DON-induced growth inhibition in children, because piglets are used as models of human paediatric surrogacy and show similarities to observations in humans in studies of the expression of CYPs [27, 28]. The dose used in this study was selected on the basis of previous research reports on DON in piglets [29–31], and piglets were exposed to chronic toxic levels of 1 mg/kg and 3 mg/kg for 4 weeks of treatment. The effect of DON on liver function was determined by analysing the changes of liver function indices in the piglets' blood. To check whether DON affected liver metabolising enzymes and DNA methylation, the expression of CYP450 and growth- and DNA methylation-related genes was detected by qPCR. Furthermore, the level of 5-mC in the whole genome was detected by colorimetry. The methylation level of CYP450 genes and growth-related gene promoter in the liver was detected by methylation-specific PCR (MSP) to further study DNA methylation in liver injury caused by DON.

Methods

Reagents and chemicals

DON toxin (CAS NO. 514810–8), glutamine and dimethylsulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA). M-MLV RTase, dNTP Mixture (10 mM), 5 × M-MLV Buffer, Oligo d(T)18, RNAiso Plus and SYBR Premix Ex Taq™ (Perfect Real Time) were from TaKaRa Biotechnology Co., Ltd. (Dalian, Liaoning, China). The universal genomic DNA kit was procured from CWBIO (Beijing, China). The MethylFlash methylated DNA quantification kit (Colorimetric) was purchased from Epigentek (Farmingdale, NY, USA). The EZ DNA Methylation-Gold™ Kit and Zymo Taq™ PreMix were purchased from Zymo Research Corp (Orange, CA, USA). All the materials used in this study were of analytical grade.

Experimental animals and design

A total of 15 crossbred newly weaned piglets (3 weeks of age) were selected and allotted into three dietary treatment groups and each group contained five piglets. All diets were formulated to meet the amino acid requirements of NRC (1994). Treatment Group 1 received the basal diet (BD, NRC 2012, control), Group 2 received BD + 1.0 mg/kg DON and Group 3 received BD + 3.0 mg/kg DON. The piglets were immunised with the OVA (0.5 mg + Quil A adjuvant) pseudorabies (PS) vaccine, swine fever (SF) vaccine and porcine circoviruses (PC) vaccine at 4 (1 week into the feeding trial) and 5 weeks of age (2 weeks into the feeding trial). After 4 weeks of experiment, the piglets were humanely killed and the blood and liver collected for further assays.

Measurement of hepatic biochemical indices

Serum aspartate aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activity, albumin, total protein (TP), globulin (GLO), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), alkaline phosphatase (ALP), glutamyl transpeptidase (GGT), creatinine (UREA), creatinine (CREA) and lactate dehydrogenase (LDH) were determined by laboratory equipment as described previously [32–34].

Quantitative real-time PCR (qPCR) examination

Total RNA from the liver was isolated using the TRIzol extraction method according to the manufacturer's instructions (Invitrogen Inc., Carlsbad, CA). The quality of RNA was verified by evaluating the optical density at 260 nm and 280 nm. The extracted RNA was reverse-transcribed into cDNA using a Prime Script reverse transcription-PCR kit (TaKara, Dalian, P.R. China) for qPCR.

The Primer Express software was applied to design the rat-specific primers according to the software guidelines (Table 1). Each 10 μL reaction mixture consisted of 5 μL SYBR® Premix Ex Taq™, 0.4 μL of each primer (10 μM), 1 μL of cDNA and 3.2 μL RNase-free H_2O. For all genes, the cycling conditions were as follows: step 1: 30 s at 95 °C; step 2: 45 cycles at 95 °C for 5 s, 50–60 °C for 30 s; step 3: dissociation stage. In this study, the housekeeping gene β-actin was used as an internal calibrator reference gene for expression profiling of genes. Data were analysed and quantified using the $2^{-\Delta\Delta Ct}$ methods.

Table 1
Piglet primers used for qPCR analysis

Genes	Type	Primer sequence (5'to 3')	Product size (bp)	Annealing temperature (°C)
CYP1A1	Forward	GCCATATGCTTTGGTCAGCG	136	59
	Reverse	TGGGCAGGTAACGGAGGATA		
CYP1A2	Forward	TGAATTTGTGGAGACCGCCT	199	59
	Reverse	GTGCTTGAATAGGGCGCTTG		
CYP2B22	Forward	CCGAAAGGGCTTTCTCAAGT	174	59
	Reverse	TCCACCACAGCGATTTTCCC		
CYP2D25	Forward	TCCTGGTGGACTTGATGCAC	121	59
	Reverse	AAGCTGAGACGTGGGTCTTG		
CYP2C33	Forward	TGTGCTCCCTGCAATGTGAT	123	59
	Reverse	CCAGGGAGAGCTTATTTGACGA		
CYP2E1	Forward	CCGGTGTTCACTGTGTACCT	151	59
	Reverse	AAATGACCCCTTTGTCCTTGTG		
CYP3A29	Forward	CACTTACCTGCCCTTTGGGA	165	59
	Reverse	GGTTGTGTAAGCCCTTGCGT		
CYP3A22	Forward	TGTTCCCCATCATTGCCCAG	128	59
	Reverse	TGTGCTAGTGATCACATCCATGC		
IGFBP2	Forward	CGAGGGCACTTGCGAAAAG	114	60
	Reverse	TTCCCATCCACGTGGTTCTC		
IGF-1	Forward	TGCTTGCTCTCCTTCACCAG	124	60
	Reverse	ACCCTGTGGGCTTGTTGAAA		
NNMT	Forward	ACCGACTACACGGACCAGAA	118	60
	Reverse	TGACTCTGTTCCCTTCGAGC		
GALP	Forward	CAGTGCTGGTTACCTCCTGG	148	60
	Reverse	CCTCTTGGAGGCCAACTGAG		
DNMT1	Forward	GGTTGTTCGGCAACATCCTG	152	60
	Reverse	CGTAGTCTCTTCCTCCTTGACT		
DNMT3A	Forward	ATCAGTACGACGATGACGGC	88	60
	Reverse	CAGCAATTGTTGTTCCCGCA		

Genes	Type	Primer sequence (5'to 3')	Product size (bp)	Annealing temperature (°C)
DNMT3B	Forward	ATCAGAGGCCGCAGATCAAG	212	60
	Reverse	CGGCTGGAGGTACTGTTGTT		
β-actin	Forward	GCTGTCCCTGTACGCCTCTG	123	60
	Reverse	GCTCGTTGCCGATGGTGAT		

Calculation of benchmark dose (BMD)

According to the expression of different metabolising enzymes in the liver of piglets, the BMD curves of gene expression were calculated and the corresponding BMD and lower confidence limit of the BMD (BMDL) values obtained using BMD software (https://benchmarkdose.org/). The BMD and BMDL values of metabolic enzymes are shown in Table 2.

Table 2
BMD and BMDL (mg/kg) of gene expression in the dose-response model of DON

Index	Model	BMD (mg/kg)	BMDL (mg/kg)
CYP1A1	Linear	0.0476	0.00556
	Power	1.60	0.609
	Hill	7.05	0.525
	Michaelis Menten	0.165	0.0157
CYP1A2	Linear	6.20	1.52
	Power	3.35	2.89
	Hill	17.4	3.45
	Michaelis Menten	-5.34	-234
CYP2B22	Linear	3.57	0.546
	Power	2.99	2.20
	Hill	22.4	3.84
	Michaelis Menten	8.41	-145
CYP2C33	Linear	3.42	0.545
	Power	2.98	2.29
	Hill	13.3	2.24
	Michaelis Menten	4.64	-129
CYP2D25	Linear	3.57	0.571
	Power	3.00	2.15
	Hill	22.2	3.63
	Michaelis Menten	8.28	-141
CYP2E1	Linear	3.18	0.489
	Power	2.87	2.03
	Hill	22.3	3
	Michaelis Menten	7.09	-136
CYP3A22	Linear	5.88	1.44
	Power	3.33	2.70
	Hill	26.1	3.36
	Michaelis Menten	-4.59	-235

Index	Model	BMD (mg/kg)	BMDL (mg/kg)
CYP3A39	Linear	2.83	0.382
	Power	2.8	2.06
	Hill	9.55	1.59
	Michaelis Menten	3.80	-122
NNMT	Linear	4.62	2.94
	Power	3.23	2.99
	Hill	16.1	3.87
	Michaelis Menten	4.80	-33.6
GALP	Linear	4.37	2.87
	Power	3.21	2.98
	Hill	20.6	4.16
	Michaelis Menten	7.27	-72.5
IGF-1	Linear	4.26	1.01
	Power	3.18	2.45
	Hill	26.3	2.95
	Michaelis Menten	4.04	-175
IGFBP2	Linear	1.64	0.213
	Power	2.77	1.53
	Hill	16.8	2.70
	Michaelis Menten	3.52	-41.7

Genome-wide DNA methylation analysis

To test whether methylation of genomic DNA was affected by DON, DNA in the liver tissue was extracted using a universal genomic DNA kit following treatment with DON toxin. A MethylFlash methylated DNA quantification kit was used to detect DNA methylation in the genome, according to the manufacturer's instructions. Finally, the absorbance (OD) was measured and read at 450 nm within 2–15 min. The following formula was used to calculate 5-mC level:

5-mC = [(Sample OD-ME3 OD) ÷ S]/[(ME4 OD-ME3 OD) × 2 ÷ P]

where ME3 = Negative control; ME4 = Positive control; S = Input of DNA sample; P = Quantity of ME4 DNA.

MSP analysis

To determine the methylation level of gene promoter regions related to the metabolism and growth in liver tissue, the EZ DNA Methylation-Gold™ kit was used for bisulfite conversion of GC-rich DNA according to the manufacturer's directions. Methylated and unmethylated primers for CYP450s and NNMT, GALP, IGF-1 and IGFBP2 genes were synthesised by Nanjing Genescript Co. Ltd., Nanjing, P.R.China (Table 3). MSP used the following cycle parameters: 95 °C for 5 min, followed by 20, 30 and 40 cycles, respectively, at 95 °C for 30 s, 50–60 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 7 min. The PCR product (5 μl) was subjected to electrophoresis on 3% agarose gel and stained with 0.5 μg/ml ethidium bromide. Optical density values were measured using Quantity One 4.6.2 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The following formula was used to calculate the results of methylation or unmethylation:

Table 3
Primers used for Methylation-specific PCR (MSP)

Genes		Type	Primer sequence (5'to 3')	Product size (bp)	Annealing temperature (°C)
CYP1A1	m	Forward	TTTTTTTTATTTTGGGAAGATCGT	102	48
		Reverse	AAACAACTTTTACGTATAACCACGC		
	u	Forward	TTTTTTTTATTTTGGGAAGATTGT	101	48
		Reverse	AACAACTTTTACATATAACCACACC		
CYP1A2	m	Forward	AATAAATTATATAATTTTTTTGCGT	136	48.5
		Reverse	TATAAACCCCATATTATTATCCGAA		
	u	Forward	ATTTATAATAAATTATATAATTTTTTTGTG	142	48.5
		Reverse	TATAAACCCCATATTATTATCCAAA		
CYP2B22	m	Forward	TTTGGTGGGTTTTATTTTTATTTTC	143	47.3
		Reverse	CCTCAACTACTACCTCATTTATCGAC		
	u	Forward	TTTGGTGGGTTTTATTTTTATTTTT	142	47.2
		Reverse	CTCAACTACTACCTCATTTATCAAC		
CYP2D25	m	Forward	ATTGATTTAAGGGTATTGTGAGACG	195	49.3
		Reverse	CTACAAAAAAAATAAAACCCACGAA		
	u	Forward	TTGATTTAAGGGTATTGTGAGATGA	194	49.5
		Reverse	CTACAAAAAAAATAAAACCCACAAA		
CYP2C33	m	Forward	GGGGTATAGCGTAAATTTAAGTAGC	132	50.3
		Reverse	AAAAAATCAAAACAAAAAAATCGAT		
	u	Forward	TTGGGGTATAGTGTAAATTTAAGTAGTGT	134	50.2
		Reverse	AAAAAATCAAAACAAAAAAATCAAT		
CYP2E1	m	Forward	GTATATGAGGTATAAAACGGGTTCG	139	51.4
		Reverse	TTTAACAATATAACAAACCCTCGAT		
	u	Forward	TATATGAGGTATAAAATGGGTTTGA	132	51
		Reverse	TTTAACAATATAACAAACCCTCAAT		
CYP3A29	m	Forward	TTTTATTTAGGAAAGGGTTTTTCGT	105	52
		Reverse	AACCTCTCTATCCGTCTAAATCGAT		
	u	Forward	TTTTATTTAGGAAAGGGTTTTTTGT	105	52

m = methylated, u = unmethylated

Genes		Type	Primer sequence (5'to 3')	Product size (bp)	Annealing temperature (°C)
		Reverse	AACCTCTCTATCCATCTAAATCAAT		
IGFBP2	m	Forward	TAGGTAGTAGTGAGTGAAAATTCGA	100	50
		Reverse	CCATATAACCGACAAAAAACGTC		
	u	Forward	TAGGTAGTAGTGAGTGAAAATTTGA	103	50
		Reverse	TTCCCATATAACCAACAAAAAACAT		
IGF-1	m	Forward	TTATTGAAATGGTAAAATTTCGGAC	207	50
		Reverse	CAAAAATCTACTAAAAACTCTCGTC		
	u	Forward	ATTGAAATGGTAAAATTTTGGATGT	205	50
		Reverse	CAAAAATCTACTAAAAACTCTCATC		
NNMT	m	Forward	GAGTAAGGTTAGGGATCGAAT	93	53
		Reverse	AAAAAACTCAAATTAAAATTCCCG		
	u	Forward	TTGAGTAAGGTTAGGGATTGAA	95	42
		Reverse	AAAAAACTCAAATTAAAATTCCCATC		
GALP	m	Forward	AAAGTTAGATATAGGAAGATAAATATTACG	102	53.2
		Reverse	ATCGAATTTATTAAACCACGACG		
	u	Forward	GATATAGGAAGATAAATATTATGTGA	93	54.2
		Reverse	GATATAGGAAGATAAATATTATGTGA		
m = methylated, u = unmethylated					

Methylation rate (%) = [ODm]/[ODm + ODu]

where m indicates methylated and u indicates unmethylated.

Bisulfite sequencing (BSP) analysis

DNA extracted from piglet liver was treated with bisulfite modification and examined for the methylation status of CpG dinucleotides within the promoter region of NNMT, GALP, IGF-1 and IGFBP2. These gene promoter regions were amplified from the bisulfite-modified sequence by BSP primers synthesised by Nanjing Genescript Co. Ltd. (Table 4). For the primer amplification system, each 20 μL reaction mixture consisted of 10 μL Zymo Taq™ Premix, 0.5 μL of each primer (10 μM), 2 μL template DNA and 7 μL RNase-free H_2O. For all genes, the cycling conditions were as follows: initial denaturation for 10 min at 95 °C; denaturation at 30 s at 95 °C;

annealing at 50–55 °C for 30 s; extending at 72 °C for 30 s; and final extension at 72 °C for 7 min. The PCR products were recovered and purified by agarose electrophoresis. The PCR product was joined to T vector and then cloned and the blue colonies of sequence selected. The bisulfite sequence data on CpG site methylation analysis were aligned, visualised, and quantified by quantitative tool for methylation analysis (QUMA) software.

Table 4
Primers used for Bisulfite sequencing PCR (BSP)

Genes		Type	Primer sequence (5'to 3')	Product size (bp)	Annealing temperature (°C)	CpG
IGF-1	BSP	Forward	TTATGGGGTTTTGATTGTGATATG	126	53.2	6
		Reverse	TTACTTTCAAAAAAAACAAATTAAAC			
IGFBP2	BSP	Forward	AAATTTATTTGAAGGTTATGTTTGA	226	54	13
		Reverse	AAAATCCCCTAACTCCCTCC			
NNMT	BSP1	Forward	GGTATATGGAGGTTTTTAGG	368	52	17
		Reverse	AAACACACAATAAATATTCAAAA			
	BSP2	Forward	TATTTTAGGGGTTGAGAAATGG	369	54	10
		Reverse	CACTCAAAACATATAAAAATTCCCAA			
GALP	BSP1	Forward	ATTTTAGAAAATTTGGGAGTA	396	52	20
		Reverse	ATTCTCAAACTAAAAATAAAATTAAAACTA			
	BSP2	Forward	GTTGTGATTTAGTGTAGAAGGGAA	400	52	12
		Reverse	AAAATATTTATAACAATAAAACAAAAA			

Statistical analysis

Statistical analysis was performed using SPSS, version 13 (Chicago, IL, USA). Data were presented as mean ± SD. Difference between groups was determined by a one -way ANOVA using a significance level of $P < 0.05$. The BSP data were assessed by the Mann–Whitney non-parametric test for multiple comparisons.

Results

DON affected serum liver biochemical indices, causing liver damage

To observe the effect of DON toxin on piglet liver function, serum chemical parameters including AST, ALT, ALP, TP, GLO, ALB, TBIL, DBIL, IBIL, GGT, UREA and LDH were measured. According to the data presented in Fig. 1, serum ALT activity and GLO levels in the DON group significantly increased after DON administration (1 or 3 mg/kg) ($p < 0.5$). However, AST and LDH activity in the DON-treated group at 3 mg/kg exhibited lower levels compared to the control group. Additionally, a sharp increase was observed in GGT activity following DON

treatment at 1 mg/kg when compared to the control group ($p < 0.01$). These results suggested that DON leads to liver dysfunction in piglets.

DON exposure caused changes in CYP450s expression in piglet liver

To check whether DON exposure cause abnormal expression of CYP450s, the mRNA level was examined. As shown in Fig. 2, the mRNA expression levels of CYP450s, including CYP1A1, CYP1A2, CYP450 2B22 (CYP2B22), CYP450 2C33 (CYP2C33), CYP450 3A22 (CYP3A22), CYP450 2D25 (CYP2D25), CYP2E1 and CYP450 3A39 (CYP3A39), were significantly increased after DON administration at 3 mg/kg ($p < 0.05$). However, the expression of CYP2D25 and CYP2E1 in the DON toxin group at 1 mg/kg exhibited a significant decrease compared to the control group ($p < 0.05$). Through BMD online software, we estimated the BMD and BMDL with the expression of metabolic enzyme genes. Table 2 demonstrates that the BMD and BMDL values of CYP1A1 were the lowest compared to those of other metabolic enzymes. These results indicated that CYP1A1 was the most sensitive index in evaluating liver injury caused by DON.

DON exposure caused abnormal expression of DNA methyltransferases and affected the genomic 5-mC level in piglet liver

In DNA methylation analysis, we focused primarily on level of 5-mC, which is regulated by DNA methyltransferases (DNMT1, DNMT3A, DNMT3B). As shown in Fig. 3, the mRNA expression levels of DNMT1 and DNMT3B were significantly increased after DON toxin exposure compared to the control group ($p < 0.05$). However, the DNMT3B expression of DON groups showed no significant difference compared to the control group. We tested the genomic DNA methylation levels in pig livers, where our results revealed that 5-mC global levels were significantly higher in livers treated with DON toxin (1 or 3 mg/kg) than in the control ($p < 0.05$).

DON exposure caused DNA methylation changes in promoters of metabolic enzyme genes in piglet liver

The MSP was used to measure the methylation levels of CpG sites in the CYP450 gene promoter region. As shown in Fig. 4, in one DON exposure group (3 mg/kg), the methylation levels of CYP1A1, CYP2D25, CYP2E1, and CYP3A39 promoters were significantly decreased compared to the control ($p < 0.05$), thus inducing the expression of these genes. Additionally, the methylation level of CYP1A2 promoter in DON-treated groups was lower than the control group ($p < 0.05$), which possibly promoted the transcription of the CYP1A2 gene. Although the methylation level of CYP2B22 promoter was increased, the expression of CYP2B22 gene was raised. The possible reason was that other regulated factors also influence the expression of CYP2B22.

DON exposure caused changes of growth-regulating gene expression in piglet liver

To investigate the effect of DON exposure on piglet growth, we also examined the mRNA expression of growth-related genes in the liver (Fig. 5). The results demonstrated that the mRNA expression levels of NNMT were markedly decreased after DON toxin administration (1 or 3 mg/kg; $p < 0.05$) compared to the control group.

DON exposure at 1 mg/kg reduced the expression of GALP, while DON exposure at 3 mg/kg sharply elevated GALP expression. In addition, sharp increases in IGF-1 and IGFBP2 expression in DON-treated livers were observed when compared to the control group ($p < 0.05$), which might be the compensatory effect of DON-induced growth inhibition toxicity.

DON exposure caused DNA methylation changes in promoters of growth-regulated genes in piglet liver

To further determine whether DNA methylation was involved in the expression of NNMT, GALP, IGF-1 and IGFBP2 after exposure to DON toxin in pig liver, MSP and BS were applied. For these genes, the CpG sites of MSP assay were included in the sequence of BSP analysis. As shown in Fig. 6A–B, although only methylated primers amplified the bands of all groups, the methylated bands of the DON treatment group were stronger, suggesting an increased methylation level of NNMT to some extent. BSP results of NNMT gene also revealed that some CpG sites in DON-exposed groups were completely methylated compared to the control group, thus promoting NNMT transcription. For the GALP gene in Fig. 6C–D, the methylation level in one DON group (3 mg/kg) was lower than the control group and induced GALP expression. In Fig. 6E–F, the results showed that DON treatment could decrease the promoter methylation level of IGF-1, promoting IGF-1 expression. However, the IGFBP2 methylation level in DON groups exhibited no significant difference compared to the control group (Fig. 6G–H).

Discussion

Due to its high resistance to temperature, high toxicity levels and widespread occurrence in food, DON is considered an unavoidable contaminant in nature and to pose a serious threat to public health [35]. Although the liver toxicity of DON has received much attention, the role of DNA methylation and CYP450s on its deleterious toxicity is still poorly understood. In this study, DON exposure increased the serum ALT and GLO levels. Moreover, the present study proved for the first time that DNA methylation regulated the expression of CYP450s in DON-treated piglet livers. DON exposure reduced the expression of NNMT and GALP, with decreases in feed intake and weight of piglets. It was worthy of note that DON could regulate the expression of NNMT, GALP and IGF-1 through DNA methylation and thus affect the growth of piglets.

Feed levels of 1 mg/kg and 3 mg/kg of DON are possible daily doses for children. In Portugal, by using HPLC to analyse 307 samples of plant crops, the highest levels of DON concentration was found to be 17.9 mg/kg [36]. It was reported that the average content of 23,980 samples contaminated by DON was as follows: wheat, 9900 mg/kg; corn, 4772 mg/kg; rice, 183 mg/kg; barley, 6349 mg/kg; oats, 537 mg/kg; and rye, 190 mg/kg [37]. DON was also detected in pasta with the highest level in the European Union of 3200 μg/kg [38]. Therefore, the dose used in this study was within the exposure dose range of children to DON.

Although CYP450 does not participate in the direct metabolism of DON, abnormal changes in CYP450s were the mechanism for DON-induced hepatotoxicity. Recent literature suggested that CYP450s are involved in oxidative stress, apoptosis and inflammatory response against foreign particles [39, 40]. Different metabolic enzyme patterns also accompanied the pathological lesions. For example, the activities of liver microsomal mixed-function oxidase, ethoxyresorufin-O-deethylase and methoxyresorufin-O-demethylase were unaffected,

whereas pentoxyresorufin-O-dependentylase activity was increased. Protein levels of glutathione S-transferase α and π were increased, whereas CYP1A protein level was unchanged [41, 42]. Another study reported that DON had no effect on the mRNA expression of different CYP450s (CYP1A4 and CYP3A37) in duodenum and liver [43]. However, in the present study, we found that 3 mg/kg DON could significantly increase the mRNA expression level of CYP450s (CYP1A1, CYP1A2, CYP2B22, CYP2C33, CYP2D25, CYP2E1, CYP3A22 and CYP3A39). The differences in the result may be related to the DON dose, the animal species or environmental conditions. Aflatoxin B1 (AFB1)-induced generation of reactive oxygen species can lead to oxidative stress, potentially requiring the activation of CYP450s [44]. Similarly, the increase in CYP450s might be an important mechanism of liver injury under DON exposure. Importantly, increased CYP1A1 expression was the most sensitive metabolic enzyme in the assessment of DON-induced liver injury.

Piglets are one of the most sensitive species with regard to their response to DON-contaminated feed and are the best models for studying the toxic effect of DON on children [22, 45]. The liver plays a key role in the metabolism and detoxification of DON [46]. However, various investigations into DON in piglet liver generated inconsistent results, with some showing hepatotoxicity of DON, and others not. For example, DON at 3.1 mg/kg feed for 37 days had no impact on pig liver [47]. Similar results were reported by Van Le Thanh et al. (2016), who found that the activity of other antioxidant enzymes or glutathione concentrations were not affected by DON (0.8 and 3.1 mg/kg feed) over 17 days of exposure [30]. Renner et al. (2017) did not find the liver histology activity index (HAI) in young pigs fed DON at 4.59 mg/kg feed for 27 days [48]. However, pigs given DON-contaminated feed (4 mg/kg for 15, 30 and 37 days) showed oxidative stress and lipid peroxidation [49]. Gerez et al. (2015b) found that pigs given DON-contaminated feed (1.5, 2 and 3 mg/kg for 28 days) showed significant histological changes in the liver [50]. The study by Pierron et al. (2018) found that piglets exposed by gavage to 1 and 0.5 nM DON/kg b.w/day for 3 weeks revealed a slight decrease in weight gain [51]. A diet containing 8 mg/kg DON fed for 4 weeks disrupted the immune-related processes in the liver of piglets [52]. Based on previous literature [53], in the present study we selected the administration dose of DON of 1 and 3 mg/kg feed and fed piglets for 4 weeks. Herein, DON at 1 mg/kg elevated the ALT and GGT levels, suggesting that DON may destroy the liver cell membrane, leading toleakage of enzymes from injured hepatocytes [54, 55]. However, compared with the 1 mg/kg DON group, DON at 3 mg/kg decreased serum levels of ALT, AST and LDH, especially AST and LDH, suggesting that 3 mg/kg DON may lead to massive necrosis of liver cells or acute hepatitis, basically resulting in depletion of transaminase in the liver tissue [56, 57]. In addition, DON significantly increased the level of serum GLO, suggesting that DON may cause an inflammatory response and immune system disorders in piglet liver [58].

DNA methylation could be used as a sensitive molecular indicator of DON-induced liver damage. Regarding DNA methylation, DNMT1, DNMT3A and DNMT3B, maintain synergistically the stability of DNA methylation [20]. Most of the changes in DNA methylation are due to chemicals, including mycotoxins, in food and in the environment [20, 21]. It was found that 10 mM DON increased the percentage of 5-methylcytosine in DNA from 4.5–9% in Caco-2 cells [59]. However, another study reported that DON at 3 mg/kg decreased the expression of methyltransferases and upregulated methyl-CpG-binding domain 2 (MBD2) expression in porcine splenic lymphocytes [60]. Abnormal changes in DNA methylation are common in tumorigenesis [61]. In the current study, DON exposure resulted in the increased expression of the DNMT1 and DNMT3B genes, and raised the genomic 5-mC level in piglet livers. We also observed that with the higher concentration of DON the effect was

also greater. This might indicate that DON has a liver-cancer-promoting effect [62], and the content of genomic 5-mC may be a potential epigenetic biomarker for the hepatotoxicity of DON [63].

DNA methylation is a molecular switch that regulates CYP450 expression of DON-exposed piglet liver. Previous studies have shown that the expression of the CYP450 gene is related to the methylation of its promoter region, such as CYP1A1 [64], CYP1B1, CYP2E1 [65], CYP450 3A4 (CYP3A4) and CYP450 2D6 (CYP2D6) [66]. In the present study, it was found that DON at 3 mg/kg could reduce the methylation level of the promoter region of enzymes, including CYP1A1, CYP1A2, CYP2D25, CYP2E1 and CYP450 3A29 (CYP3A29), and thus increase their mRNA expression levels. Strangely, the results found that DON at3 mg/kg significantly increased the methylation level of the CYP2B22 gene promoter, but the expression of CYP2B22 was significantly increased, which suggested that DON also affected CYP2B22 expression through other transcriptional factors [67, 68]. There were differences in the regulation of DNA methylation on the expression of different CYP450s, which may be related to the polymorphism of the CYP450 gene [69].

DNA methylation affects the expression of genes related to animal feeding and growth. As a key cytosolic methyltransferase in the liver, NNMT is classified as a phase II metabolising enzyme [70, 71]. NNMT is essential for the biotransformation and detoxification of some heterogenous compounds, and plays a role in catalysing N-methylation of nicotinamide, pyridine and other structural analogues [72, 73]. The abnormal expression of NNMT has been found in many diseases and pathophysiological processes, such as cancer, obesity and cirrhotic liver [72]. It was found that the inhibition of NNMT increases the level of S-adenosylmethionine (SAM) and nicotinamide adenine dinucleotide (NAD) in fat and consequently produces the effect of weight loss [72]. In addition, NNMT is closely related to ALT release and liver inflammation [74]. In our study, DON significantly reduced the expression of NNMT, which may be an important factor in the weight loss of piglets. The methylation level of several CpG sites in the NNMT promoter increased slightly, which may have partially reduced the expression of NNMT.

GALP, as a protein-coding gene, is involved in regulating appetite and inflammation, energy metabolism and reproduction [75–77]. The expression of GALP can be detected in pituitary, brain, liver and testis tissue [52, 75, 78–80]. After fasting, the expression of GALP gene decreases significantly [81]. It has been proved that acute GALP treatment can change the food intake of primates, mice and rats. For example, GALP (1–10 ptg) was infused into the ventricles of rats with satiety and starvation at the same time and the feeding increased at 1 h after injection, but the feeding and weight decreased significantly 24 h after injection. The short-term and long-term effects of GALP on food intake may be achieved through different neural pathways [82]. In addition, the expression of GALP gene is controlled by a leptin signal [78]. Our study found that DON at 1 mg/kg can significantly reduce the expression of GALP in the liver, which may lead to decreased appetite and weight loss in piglets, as found in the previous study [83]. However, a higher dose of DON (3 mg/kg) significantly increased the expression of GALP in the liver, which may be due to the fact that the high dose of DON fed to piglets for a long time resulted in a sharp decrease in weight, malnutrition and an increase in the secretion of leptin in the animals, resulting in an increase in the expression of GALP [84]. Meanwhile, DON at 3 mg/kg could demethylate CpG sites in the promoter region of the GALP gene, leading to a sharp increase in the expression of GALP, which is the first time it has been discovered.

IGF1 is the key mediator of GH. GH is synthesised in the anterior pituitary and then released into the blood, stimulating the liver to produce IGF1. In turn, IGF1 stimulates whole-body growth and plays a growth-promoting role in many cell types [85]. In addition, IGFBP2 affects the animal's immune response and cell proliferation [52]. In our study, it was found that DON could significantly increase the expression of IGF-1 and IGFBP2, which may be a compensatory response or negative feedback regulation of the GH reduction caused by DON [46]. Besides, it was found that DNA methylation of IGF-1 promoter decreased in DON-exposed piglet liver, which is consistent with existing research [86].

Conclusion

In this work, we found that DON could induce hepatotoxicity, increase the expression of CYP450s and disturb the expression of growth-related genes NNMT, GALP, IGF-1 and IGFBP2, in which DNA methylation regulated the expression of these genes. In addition, DON increased the expression of DNMTs and the level of genomic 5-mC. Therefore, the liver damage caused by DON was related to the high expression of metabolic enzymes and DNA methylation. CYP450s may be a therapeutic target to antagonise the hepatotoxicity of DON, and CYP1A1 is the most sensitive metabolic enzyme in the liver injury induced by DON. Moreover, 5-mC might be used as a biomarker of DON-induced liver damage. Further studies are still required to explore the effect of CYP450s in the toxicity of DON.

Abbreviations

AFB1: Aflatoxin B1; ALT: Alanine aminotransferase; ALP: Alkaline phosphatas; AST: Aspartate aminotransferase; BMD: Benchmark dose; BMDL: Lower confidence limit of the BMD; BSP: Bisulfite sequencing; CREA: Creatinine; CYP450: Cytochrome P450; CYP1A1: CYP450 1A1; CYP1A2: CYP450 1A2; CYP1A4: CYP450 1A4; CYP3A22: CYP450 3A22; CYP3A29: CYP450 3A29; CYP3A39: CYP450 3A39; CYP3A37: CYP450 3A37; CYP3A4: CYP450 3A4; CYP1B1: CYP450 1B1; CYP2B22: CYP450 2B22; CYP2C33: CYP450 2C33; CYP2D25: CYP450 2D25; CYP2D6: CYP450 2D6; CYP2E1: CYP450 2E1; DBIL: Direct Bilirubin; DON: Deoxynivalenol; DNMT1: DNA methyltransferases 1; DNMT3A: DNA methyltransferases 3A; DNMT3B: DNA methyltransferases 3B; GALP: Galanin-like peptide; GGT: Glutamyl transpeptidase; GH: Growth hormone; GHR: Growth hormone receptor; GLO: Globulin; HAI: Histology activity index; IBIL: Indirect bilirubin; IGF-1: Insulin-like growth factor 1; IGFBP2: Insulin-like growth factor-binding protein 2; LDH: Lactate dehydrogenase; 5-mC: 5-methylcytosine; MSP: Methylation specific PCR; NAD: Nicotinamide adenine dinucleotide; NNMT: Nicotinamide N-methyltransferase; SAM: S-adenosylmethionine; TBIL: Total bilirubin; TP: Total protein; UGTs: UDP-glucuronosyltransferases; UREA: Creatinine; RASSF4: Ras association domain family member 4.

Declarations

Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The animal experiment was conducted according to the individual license (Aimei Liu, w20180312) obtained by the training base of experimental animal practitioners in Hubei Province, and animals were followed by proper veterinary surveillance throughout the experiment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conception and design: Aimei Liu; Financial support and designed research: Aimei Liu, Xu Wang and Lvhui Sun; Animal feeding: Aimei Liu; Performed research and analysed data: Aimei Liu, Siyi Hu, Xiaohui Zhu, Awais Ihsand, Xu Wang, Lvhui Sun, Zonghui Yuan. All authors have read and approved the final manuscript.

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References

1. Zhou Y, Wu S, Wang F, Li Q, He C, Duan N, et al. Assessing the toxicity in vitro of degradation products from deoxynivalenol photocatalytic degradation by using upconversion nanoparticles@TiO2 composite. Chemosphere. 2020;238:124648.
2. Stadler D, Lambertini F, Bueschl C, Wiesenberger G, Hametner C, Schwartz-Zimmermann H, et al. Untargeted LC-MS based (13)C labelling provides a full mass balance of deoxynivalenol and its degradation products formed during baking of crackers, biscuits and bread. Food Chem. 2019;279:303–11.
3. Martinez M, Ramirez Albuquerque L, Arata AF, Biganzoli F, Fernandez Pinto V, Stenglein SA. Effects of Fusarium graminearum and Fusarium poae on disease parameters, grain quality and mycotoxins contamination in bread wheat (Part I). J Sci Food Agric. 2020;100(2):863–73.
4. Wang XD, Yang X, Xu HB, Cao P, Gao P, Liang J. [Exposure status and health risk assessment of deoxynivalenol from cereals in Chinese population in different regions]. Chinese J preventive med. 2019;53(4):394–7.

5. Suman M, Generotti S, Cirlini M, Dall'Asta C. Acrylamide Reduction Strategy in Combination with Deoxynivalenol Mitigation in Industrial Biscuits Production. Toxins (Basel). 2019;11(9):499.
6. Molina A, Chavarria G, Alfaro-Cascante M, Leiva A, Granados-Chinchilla F. Mycotoxins at the Start of the Food Chain in Costa Rica: Analysis of Six Fusarium Toxins and Ochratoxin A between 2013 and 2017 in Animal Feed and Aflatoxin M-1 in Dairy Products. Toxins. 2019;11(6):312.
7. Pallares N, Carballo D, Ferrer E, Fernandez-Franzon M, Berrada H. Mycotoxin Dietary Exposure Assessment through Fruit Juices Consumption in Children and Adult Population. Toxins (Basel). 2019;11(12):684.
8. Wettstein FE, Bucheli TD. Poor elimination rates in waste water treatment plants lead to continuous emission of deoxynivalenol into the aquatic environment. Water Res. 2010;44(14):4137–42.
9. de Souza TD, Caldas SS, Primel EG, Furlong EB. Exposure to deoxynivalenol, Ht-2 and T-2 toxins by consumption of wheat-based product in southern Brazil. Food Control. 2015;50:789–93.
10. Postupolski J, Starski A, Ledzion E, Kurpinska-Jaworska J, Szczesna M. Exposure assessment of infants and young children on selected Fusarium toxins. Roczniki PZH. 2019;70(1):5–14.
11. Gratz SW, Currie V, Duncan G, Jackson D. Multimycotoxin Exposure Assessment in UK Children Using Urinary Biomarkers-A Pilot Survey. J Agric Food Chem. 2020;68:351–7.
12. Wang X, Liang J, Cao P, Zhou S, Wu A, Gao P, et al. Biomonitoring Study of Deoxynivalenol Exposure in Chinese Inhabitants. Int J Env Res Pub He. 2019;16(12):2169.
13. Sundheim L, Lillegaard IT, Faeste CK, Brantsaeter AL, Brodal G, Eriksen GS. Deoxynivalenol Exposure in Norway, Risk Assessments for Different Human Age Groups. Toxins (Basel). 2017;9(2):46.
14. Yuan L, Mu P, Huang B, Li H, Mu H, Deng Y. EGR1 is essential for deoxynivalenol-induced G2/M cell cycle arrest in HepG2 cells via the ATF3DeltaZip2a/2b-EGR1-p21 pathway. Toxicol Lett. 2018;299:95–103.
15. Yu M, Peng Z, Liao YX, Wang LL, Li D, Qin CY, et al. Deoxynivalenol-induced oxidative stress and Nrf2 translocation in maternal liver on gestation day 12.5 d and 18.5 d. Toxicon. 2019;161:17–22.
16. Reddy KE, Jeong JY, Lee Y, Lee HJ, Kim MS, Kim DW, et al. Deoxynivalenol- and zearalenone-contaminated feeds alter gene expression profiles in the livers of piglets. Asian-Australas J Anim Sci. 2018;31(4):595–606.
17. Peng Z, Liao Y, Wang X, Chen L, Wang L, Qin C, et al. Heme oxygenase-1 regulates autophagy through carbon-oxygen to alleviate deoxynivalenol-induced hepatic damage. Arch Toxicol. 2020;94(2):573–88.
18. Korashy HM, Ansari MA, Maayah ZH, Imam F, Raish M, Attafi IM, et al. Differential Effects of Sunitinib on the Expression Profiles of Xenobiotic-Metabolizing Enzymes and Transporters in Rat Liver and Kidneys. Basic Clin Pharmacol. 2016;119(2):173–83.
19. Peng Z, Chen L, Nussler AK, Liu L, Yang W. Current sights for mechanisms of deoxynivalenol-induced hepatotoxicity and prospective views for future scientific research: A mini review. J Appl Toxicol. 2017;37(5):518–29.
20. Liu A, Sun Y, Wang X, Ihsan A, Tao Y, Chen D, et al. DNA methylation is involved in pro-inflammatory cytokines expression in T-2 toxin-induced liver injury. Food Chem Toxicol. 2019;132:110661.
21. Liu A, Xu X, Hou R, Badawy S, Tao Y, Chen D, et al. DNA methylation and RASSF4 expression are involved in T-2 toxin-induced hepatotoxicity. Toxicology. 2019;425:152246.

22. Pestka JJ. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch Toxicol. 2010;84(9):663–79.
23. Yang S, Zhao N, Yang Y, Hu Y, Dong H, Zhao R. Mitotically Stable Modification of DNA Methylation in IGF2/H19 Imprinting Control Region Is Associated with Activated Hepatic IGF2 Expression in Offspring Rats from Betaine-Supplemented Dams. J Agric Food Chem. 2018;66(11):2704–13.
24. Reynolds CM, Perry JK, Vickers MH. Manipulation of the Growth Hormone-Insulin-Like Growth Factor (GH-IGF) Axis: A Treatment Strategy to Reverse the Effects of Early Life Developmental Programming. Int J Mol Sci. 2017;18(8):1729.
25. Ouni M, Castell AL, Linglart A, Bougneres P. Genetic and Epigenetic Modulation of Growth Hormone Sensitivity Studied With the IGF-1 Generation Test. J Clin Endocr Metab. 2015;100(6):E919–25.
26. Strobl JS, Dannies PS, Thompson EB. Rat growth hormone gene expression is correlated with an unmethylated CGCG sequence near the transcription initiation site. Biochemistry. 1986;25(12):3640–8.
27. Walters EM, Wolf E, Whyte JJ, Mao J, Renner S, Nagashima H, et al. Completion of the swine genome will simplify the production of swine as a large animal biomedical model. Bmc Med Genomics. 2012;5:55.
28. Rasmussen MK, Theil PK, Oksbjerg N. Constitutive expression of cytochrome P450 in foetal and adult porcine livers-Effects of body weight. Toxicol Lett. 2016;258:87–92.
29. Gerez JR, Pinton P, Callu P, Grosjean F, Oswald IP, Bracarense APFL. Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs. Exp Toxicol Pathol. 2015;67(2):89–98.
30. Van Le Thanh B, Lemay M, Bastien A, Lapointe J, Lessard M, Chorfi Y, et al. The potential effects of antioxidant feed additives in mitigating the adverse effects of corn naturally contaminated with Fusarium mycotoxins on antioxidant systems in the intestinal mucosa, plasma, and liver in weaned pigs. Mycotoxin Res. 2016;32(2):99–116.
31. Zhang L, Ma R, Zhu MX, Zhang NY, Liu XL, Wang YW, et al. Effect of deoxynivalenol on the porcine acquired immune response and potential remediation by a novel modified HSCAS adsorbent. Food Chem Toxicol. 2020;138:111187.
32. Peng Z, Liao Y, Chen L, Liu S, Shan Z, Nussler AK, et al. Heme oxygenase-1 attenuates low-dose of deoxynivalenol-induced liver inflammation potentially associating with microbiota. Toxicol Appl Pharm. 2019;374:20–31.
33. Wang S, Liang C, Zhao L, Meng Z, Zhang C, Jia Q, et al. Influence of radioactive iodine therapy on liver function in patients with differentiated thyroid cancer. Nucl Med Commun. 2018;39(12):1113–20.
34. Santana AM, Silva DG, Clemente V, Pizauro LJL, Bernardes PA, Santana CH, et al. Blood gas and serum biochemical RIs for healthy newborn Murrah buffaloes (Bubalus bubalis). Vet Clin Path. 2018;47(1):94–9.
35. Wang X, Liu Q, Ihsan A, Huang L, Dai M, Hao H, et al. JAK/STAT pathway plays a critical role in the proinflammatory gene expression and apoptosis of RAW264.7 cells induced by trichothecenes as DON and T-2 toxin. Toxicol Sci. 2012;127(2):412–24.
36. Marques MF, Martins HM, Costa JM, Bernardo F. Co-occurrence Of Deoxynivalenol And Zearalenone In Crops Marketed In Portugal. Food Addit Contam B. 2008;1(2):130–3.
37. Wang L, Liao Y, Peng Z, Chen L, Zhang W, Nüssler AK, et al. Food Raw Materials and Food Production Occurrences of Deoxynivalenol in Different Regions. Trends Food Sci Tech. 2019;83:41–52.

38. Marin S, Ramos AJ, Cano-Sancho G, Sanchis V. Mycotoxins: Occurrence, toxicology, and exposure Assessment. Food Chem Toxicol. 2013;60.
39. Cong YM, Chi QR, Teng XH, Li S. The Protection of Selenium Against Cadmium-Induced Mitochondrial Damage via the Cytochrome P450 in the Livers of Chicken. Biol Trace Elem Res. 2019;190(2):484–92.
40. Xie JB, Dong WY, Liu R, Wang YM, Li YB. Research on the hepatotoxicity mechanism of citrate-modified silver nanoparticles based on metabolomics and proteomics. Nanotoxicology. 2018;12(1):18–31.
41. Gouze ME, Laffitte J. and, et al. Effect of various doses of deoxynivalenol on liver xenobiotic metabolizing enzymes in mice. Food Chem Toxicol. 2006.
42. Odeku OA. Assessment of Albizia zygia gum as a binding agent in tablet formulations. Acta Pharmaceutica. 2005;55(3):263–76.
43. Antonissen G, Devreese M, De Baere S, Martel A, Van Immerseel F, Croubels S. Impact of Fusarium mycotoxins on hepatic and intestinal mRNA expression of cytochrome P450 enzymes and drug transporters, and on the pharmacokinetics of oral enrofloxacin in broiler chickens. Food Chem Toxicol. 2017;101:75–83.
44. Sun L-H, Zhang N-Y, Zhu M-K, Zhao L, Zhou J-C, Qi D-S. Prevention of Aflatoxin B1 Hepatoxicity by Dietary Selenium Is Associated with Inhibition of Cytochrome P450 Isozymes and Up-Regulation of 6 Selenoprotein Genes in Chick Liver. J Nutr.146(4):655–661.
45. Amuzie CJ, Pestka JJ. Suppression of insulin-like growth factor acid-labile subunit expression–a novel mechanism for deoxynivalenol-induced growth retardation. Toxicological Sci. 2010;113(2):412–21.
46. Wu L, Wang W, Yao K, Zhou T, Yin J, Li T, et al. Effects of dietary arginine and glutamine on alleviating the impairment induced by deoxynivalenol stress and immune relevant cytokines in growing pigs. Plos One. 2013;8(7):e69502.
47. Stanek C, Reinhardt N, Diesing AK, Nossol C, Kahlert S, Panther P, et al. A chronic oral exposure of pigs with deoxynivalenol partially prevents the acute effects of lipopolysaccharides on hepatic histopathology and blood clinical chemistry. Toxicol Lett. 2012;215(3):193–200.
48. Renner L, Kahlert S, Tesch T, Bannert E, Frahm J, Barta-Boszormenyi A, et al. Chronic DON exposure and acute LPS challenge: effects on porcine liver morphology and function. Mycotoxin Res. 2017;33(3):207–18.
49. Wu M, Xiao H, Ren W, Yin J, Tan B, Liu G, et al. Therapeutic Effects of Glutamic Acid in Piglets Challenged with Deoxynivalenol. Plos One. 2014;9.
50. Gerez JR, Pinton P, Callu P, Grosjean F, Oswald IP, Bracarense APFL. Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs. Exp Toxicol Pathol. 2015;67(2):89–98.
51. Pierron A, Bracarense APFL, Cossalter AM, Laffitte J, Schwartz-Zimmermann HE, Schatzmayr G, et al. Deepoxy-deoxynivalenol retains some immune-modulatory properties of the parent molecule deoxynivalenol in piglets. Arch Toxicol. 2018;92(11):3381–9.
52. Reddy KE, Jeong JY, Lee Y, Lee HJ, Kim MS, Kim DW, et al. Deoxynivalenol- and zearalenone-contaminated feeds alter gene expression profiles in the livers of piglets. Asian Austral J Anim. 2018;31(4):595–606.
53. Peng Z, Chen LK, Nussler AK, Liu LG, Yang W. Current sights for mechanisms of deoxynivalenol-induced hepatotoxicity and prospective views for future scientific research: A mini review. J Appl Toxicol.

2017;37(5):518–29.

54. Li WY, Guo FX, Jiang XY, Li Y, Li XH, Yu ZG. Compound ammonium glycyrrhizin protects hepatocytes from injury induced by lipopolysaccharide/florfenicol through oxidative stress and a MAPK pathway. Comp Biochem Phys C. 2019;225:108585.
55. Woolbright BL, Williams CD, Ni HM, Kumer SC, Schmitt T, Kane B, et al. Microcystin-LR induced liver injury in mice and in primary human hepatocytes is caused by oncotic necrosis. Toxicon. 2017;125:99–109.
56. Gonzalez LT, Minsky NW, Espinosa LE, Aranda RS, Meseguer JP, Perez PC. In vitro assessment of hepatoprotective agents against damage induced by acetaminophen and CCl4. BMC Complem Altern M. 2017;17(1):39.
57. Waterfield CJ, Westmoreland C, Asker DS, Murdock JC, George E, Timbrell JA. Ethionine toxicity in vitro: the correlation of data from rat hepatocyte suspensions and monolayers with in vivo observations. Arch Toxicol. 1998;72(9):588–96.
58. Sherif AH, Mahfouz ME. Immune status of Oreochromis niloticus experimentally infected with Aeromonas hydrophila following feeding with 1, 3 beta-glucan and levamisole immunostimulants. Aquaculture. 2019;509:40–6.
59. Kouadio JH, Dano SD, Moukha S, Mobio TA, Creppy EE. Effects of combinations of Fusarium mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. Toxicon. 2007;49(3):306–17.
60. Ren Z, Chen C, Fan Y, Chen C, He H, Wang X, et al. Toxicity of DON on GPx1-Overexpressed or Knockdown Porcine Splenic Lymphocytes In Vitro and Protective Effects of Sodium Selenite. Oxid Med Cell Longev. 2019;2019:5769752.
61. Cui L, Hu J, Wang M, Li CC, Zhang CY. Label-Free and Immobilization-Free Electrochemical Magnetobiosensor for Sensitive Detection of 5-Hydroxymethylcytosine in Genomic DNA. Anal Chem. 2019;91(2):1232–6.
62. Schiefer HB, Rousseaux CG, Hancock DS, Blakley BR. Effects of low-level long-term oral exposure to T-2 toxin in CD-1 mice. Food Chem Toxicol. 1987;25(8):593–601.
63. Ye C, Tao R, Cao Q, Zhu D, Wang Y, Wang J, et al. Whole-genome DNA methylation and hydroxymethylation profiling for HBV-related hepatocellular carcinoma. Int J Oncol. 2016;49(2):589–602.
64. Liu Y, Li X, Zhang B, Fu Y, Yang A, Zhang H, et al. CYP1A1 methylation mediates the effect of smoking and occupational polycyclic aromatic hydrocarbons co-exposure on oxidative DNA damage among Chinese coke-oven workers. Environ health. 2019;18(1):69.
65. Patel SAA, Bhambra U, Charalambous MP, David RM, Edwards RJ, Lightfoot T, et al. Interleukin-6 mediated upregulation of CYP1B1 and CYP2E1 in colorectal cancer involves DNA methylation, miR27b and STAT3. Brit J Cancer. 2014;111(12):2287–96.
66. Shi Y, Li M, Song C, Xu Q, Huo R, Shen L, et al. Combined study of genetic and epigenetic biomarker risperidone treatment efficacy in Chinese Han schizophrenia patients. Transl Psychiat. 2017;7(7):e1170.
67. Gray MA, Squires EJ. Effects of nuclear receptor transactivation on boar taint metabolism and gene expression in porcine hepatocytes. J steroid biochem. 2013;133:110–9.
68. Messina A, Nannelli A, Fiorio R, Longo V, Gervasi PG. Expression and inducibility of CYP1A1, 1A2, 1B1 by beta-naphthoflavone and CYP2B22, 3A22, 3A29, 3A46 by rifampicin in the respiratory and olfactory

mucosa of pig. Toxicology. 2009;260(1–3):47–52.

69. Tang X, Chen S. Epigenetic Regulation of Cytochrome P450 Enzymes and Clinical Implication. Curr drug metabo. 2015;16(2):86–96.
70. Aksoy S, Szumlanski CL, Weinshilboum RM. Human liver nicotinamide N-methyltransferase. cDNA cloning, expression, and biochemical characterization. J Biol Chem. 1994;269(20):14835.
71. Human NicotinamideN-Methyltransferase Gene. Molecular Cloning, Structural Characterization and Chromosomal Localization. Genomics.29(3):0-561.
72. Kraus D, Yang Q, Kong D, Banks AS, Zhang L, Rodgers JT, et al. Nicotinamide N-methyltransferase knockdown protects against diet-induced obesity. Nature. 2014;508(7495):258–62.
73. Tomida M, Ohtake H, Yokota T, Kobayashi Y, Kurosumi M. Stat3 up-regulates expression of nicotinamideN-methyltransferase in human cancer cells. J cancer res clin oncol. 2008;134(5):551–9.
74. Sternak M, Khomich TI, Jakubowski A, Szafarz M, Szczepanski W, Bialas M, et al. Nicotinamide N-methyltransferase (NNMT) and 1-methylnicotinamide (MNA) in experimental hepatitis induced by concanavalin A in the mouse. Pharmacol Rep. 2010;62(3):483–93.
75. Man PS, Lawrence CB. Galanin-like peptide: a role in the homeostatic regulation of energy balance? Neuropharmacology. 2008;55(1):1–7.
76. Dungan Lemko HM, Clifton DK, Steiner RA, Fraley GS. Altered response to metabolic challenges in mice with genetically targeted deletions of galanin-like peptide. Am J Physiol Endocrinol Metab. 2008;295(3):E605–12.
77. Kageyama H, Takenoya F, Kita T, Hori T, Guan JL, Shioda S. Galanin-like peptide in the brain: effects on feeding, energy metabolism and reproduction. Regul peptides. 2005;126(1–2):21–6.
78. Takatsu Y, Matsumoto H, Ohtaki T, Kumano S, Kitada C, Onda H, et al. Distribution of galanin-like peptide in the rat brain. Endocrinology. 2001;142(4):1626–34.
79. Hirako S, Wada N, Kageyama H, Takenoya F, Kim H, Iizuka Y, et al. Effect of Intranasal Administration of Galanin-like Peptide (GALP) on Body Weight and Hepatic Lipids Accumulation in Mice with Diet-induced Obesity. Curr pharm design. 2017;23(25):3751–6.
80. Kowalyk S, Veith R, Boyle M, Taborsky GJ. Jr. Liver releases galanin during sympathetic nerve stimulation. Am J Physiol. 1992;262(5 Pt 1):E671–8.
81. Sergeant L, Rodriguez-Dimitrescu C, Barney CC, Fraley GS. Injections of Galanin-Like Peptide directly into the nucleus of the tractus solitarius (NTS) reduces food intake and body weight but increases metabolic rate and plasma leptin. Neuropeptides. 2017;62:37–43.
82. Lawrence CB, Baudoin FM, Luckman SM. Centrally administered galanin-like peptide modifies food intake in the rat: a comparison with galanin. J neuroendocrinol. 2002;14(11):853–60.
83. Liu M, Zhang L, Chu XH, Ma R, Wang YW, Liu Q, et al. Effects of deoxynivalenol on the porcine growth performance and intestinal microbiota and potential remediation by a modified HSCAS binder. Food Chem Toxicol. 2020;141:111373.
84. Lawrence C, Fraley GS. Galanin-like peptide (GALP) is a hypothalamic regulator of energy homeostasis and reproduction. Front neuroendocrin. 2011;32(1):1–9.

85. Dosouto C, Calaf J, Polo A, Haahr T, Humaidan P. Growth Hormone and Reproduction: Lessons Learned From Animal Models and Clinical Trials. Front endocrin. 2019;10:404.

86. Jin C, Zhuo Y, Wang J, Zhao Y, Xuan Y, Mou D, et al. Methyl donors dietary supplementation to gestating sows diet improves the growth rate of offspring and is associating with changes in expression and DNA methylation of insulin-like growth factor-1 gene. J Anim Physiol Anim Nutr (Berl). 2018;102(5):1340–50.

Figures

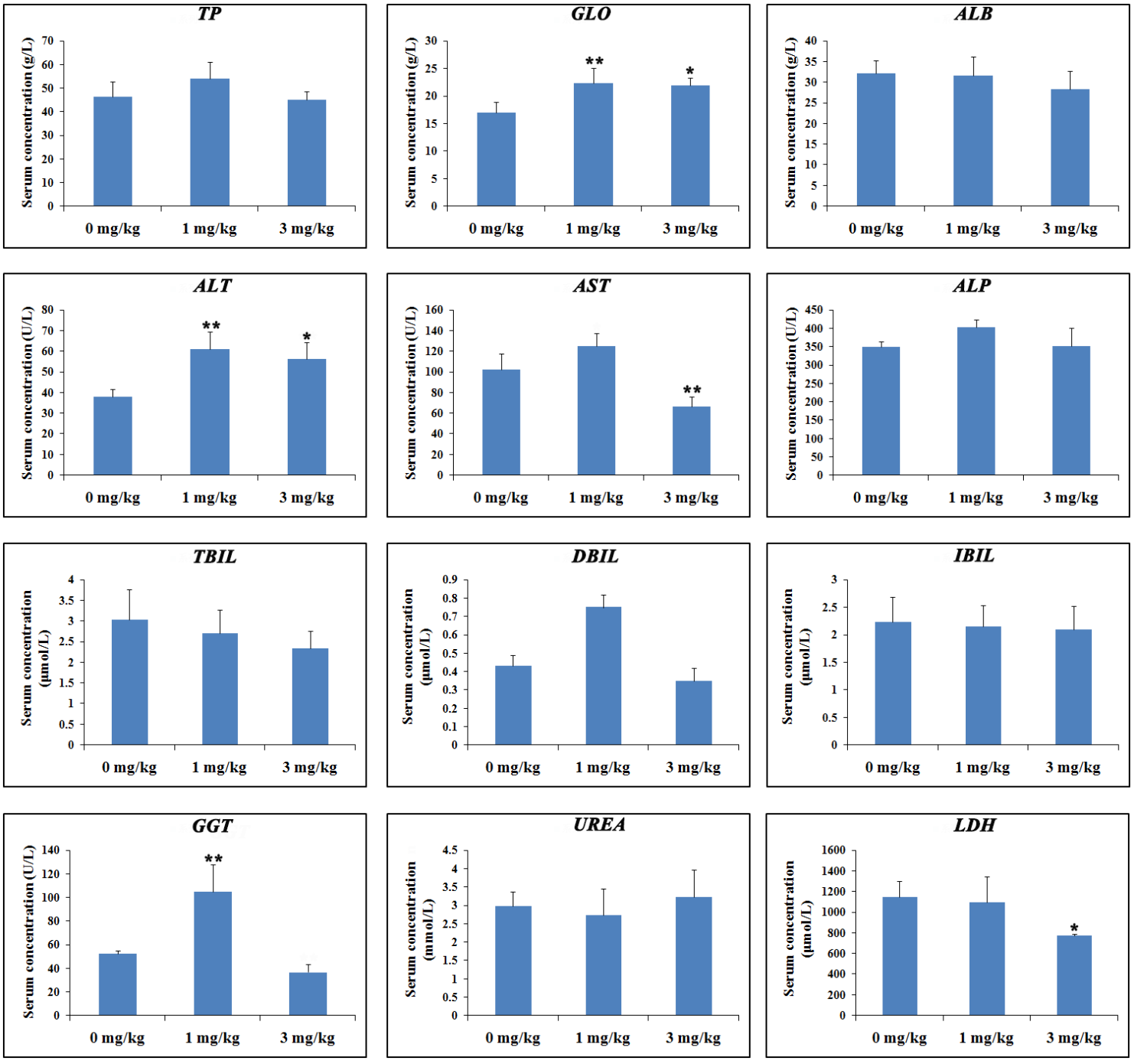

Figure 1

Serum chemical parameters of liver function in piglets fed diets containing DON-contaminated corn (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001 versus 0 mg/kg group. Note: ALT, alanine aminotransferase, AST, aspartate aminotransferase, TBIL, total bilirubin; TP, total protein; GLO, globulin; TBIL, total bilirubin; DBIL, direct Bilirubin; IBIL, indirect bilirubin; ALP, alkaline phosphatas; GGT, glutamyl transpeptidase; CREA, creatinine; LDH, lactate dehydrogenase.

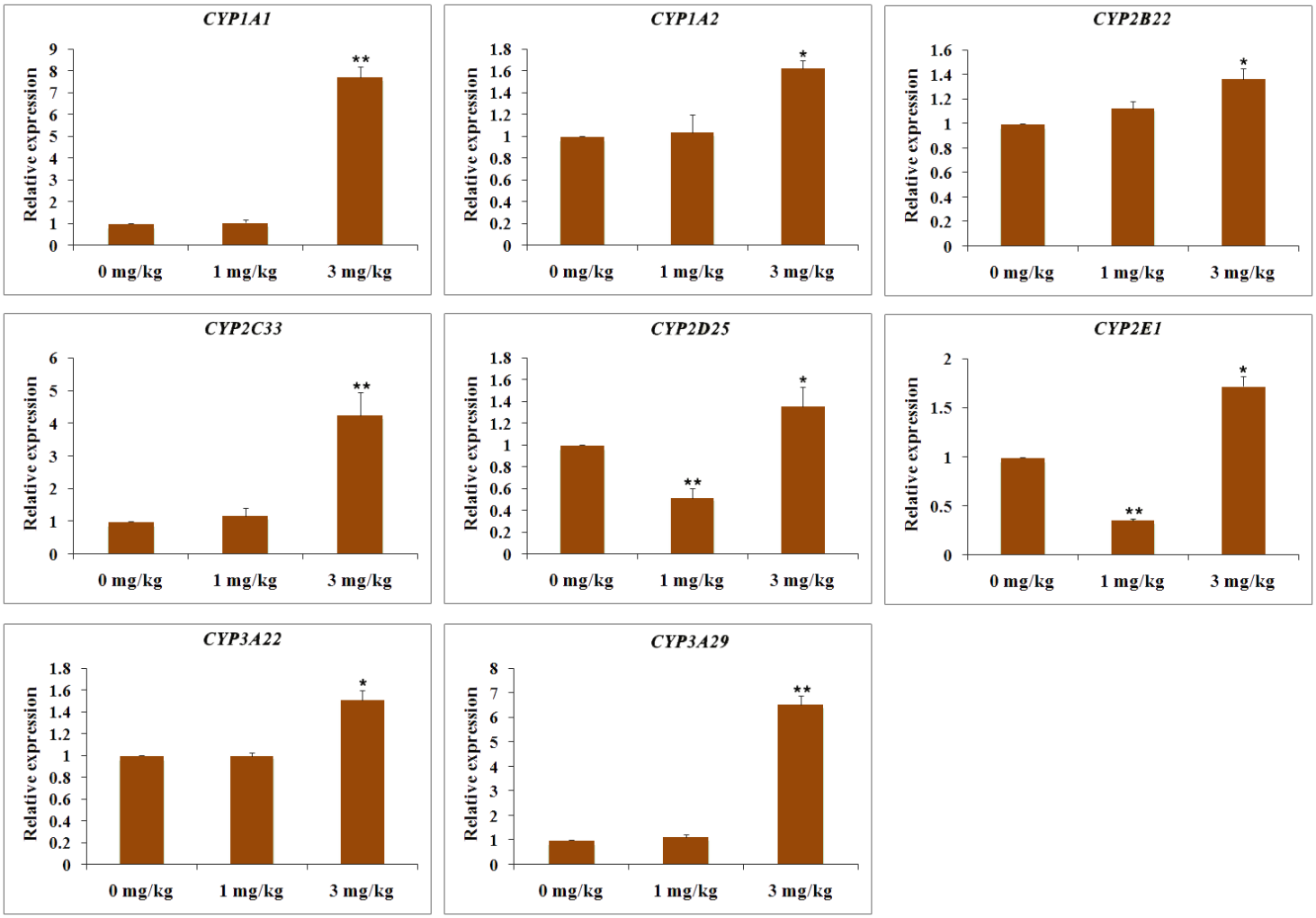

Figure 2

CYP450 gene expression in the liver of piglets fed diets containing DON-contaminated corn (n = 5). The mRNA levels of CYP1A1, CYP1A2, CYP2B22, CYP2C33, CYP2D25, CYP2E1, CYP3A22 and CYP3A39 detected by qPCR. *p < 0.05, **p < 0.01, ***p < 0.001 versus 0 mg/kg group.

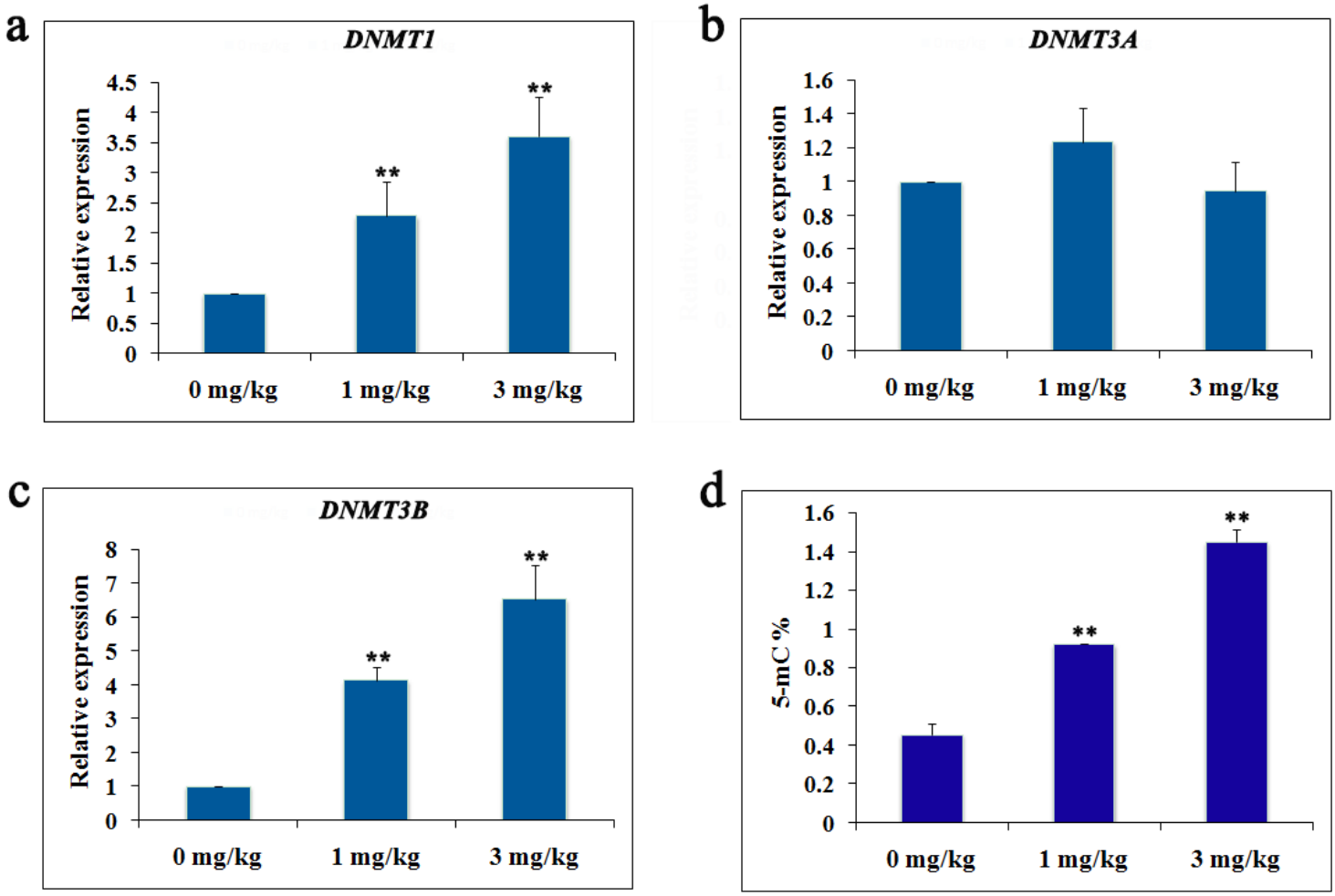

Figure 3

DNA methyltransferase expression and genome-wide 5-mC level in the liver of piglets fed diets containing DON-contaminated corn (n = 5). The mRNA levels of DNMT1, DNMT3A and DNMT3B detected by qPCR (a, b, c). The 5-mC level of genomic genes was assessed using the MethylFlash Methylated DNA Quantification Kit (colorimetric) (d). *p < 0.05, **p < 0.01, ***p < 0.001 versus 0 mg/kg group.

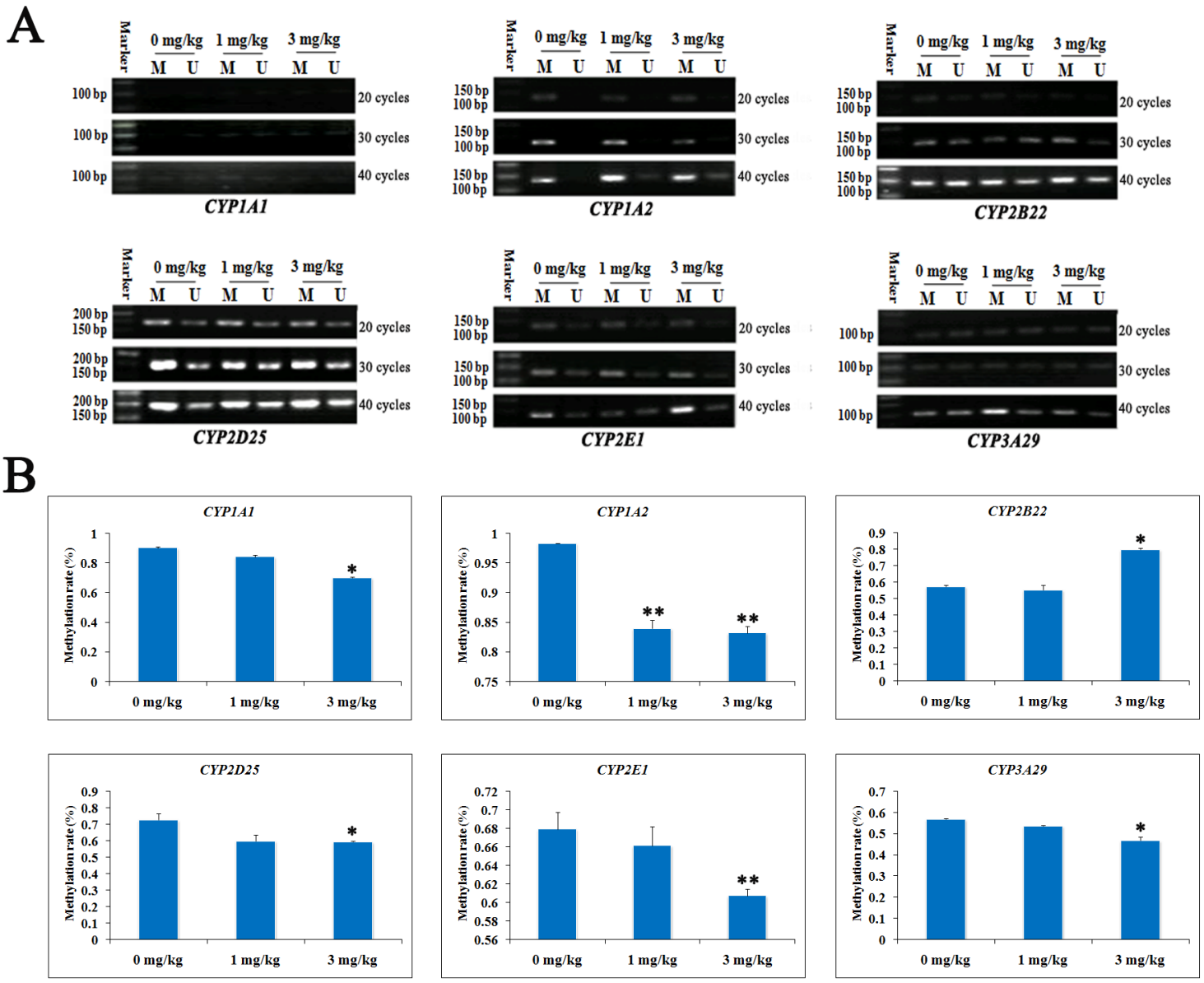

Figure 4

The methylation levels of CYP450 genes in the liver ofpiglets fed diets containing DON-contaminated corn (n = 5). Genomic DNA extracted from the liver was treated with bisulfite and then subjected to methylation-specific PCR (MSP) using the methylated DNA (m)-and unmethylated DNA (u)-specific primer sets. In the figure, M represents methylation, U represents unmethylation. *p < 0.05, **p < 0.01, ***p < 0.001 versus 0 mg/kg group.

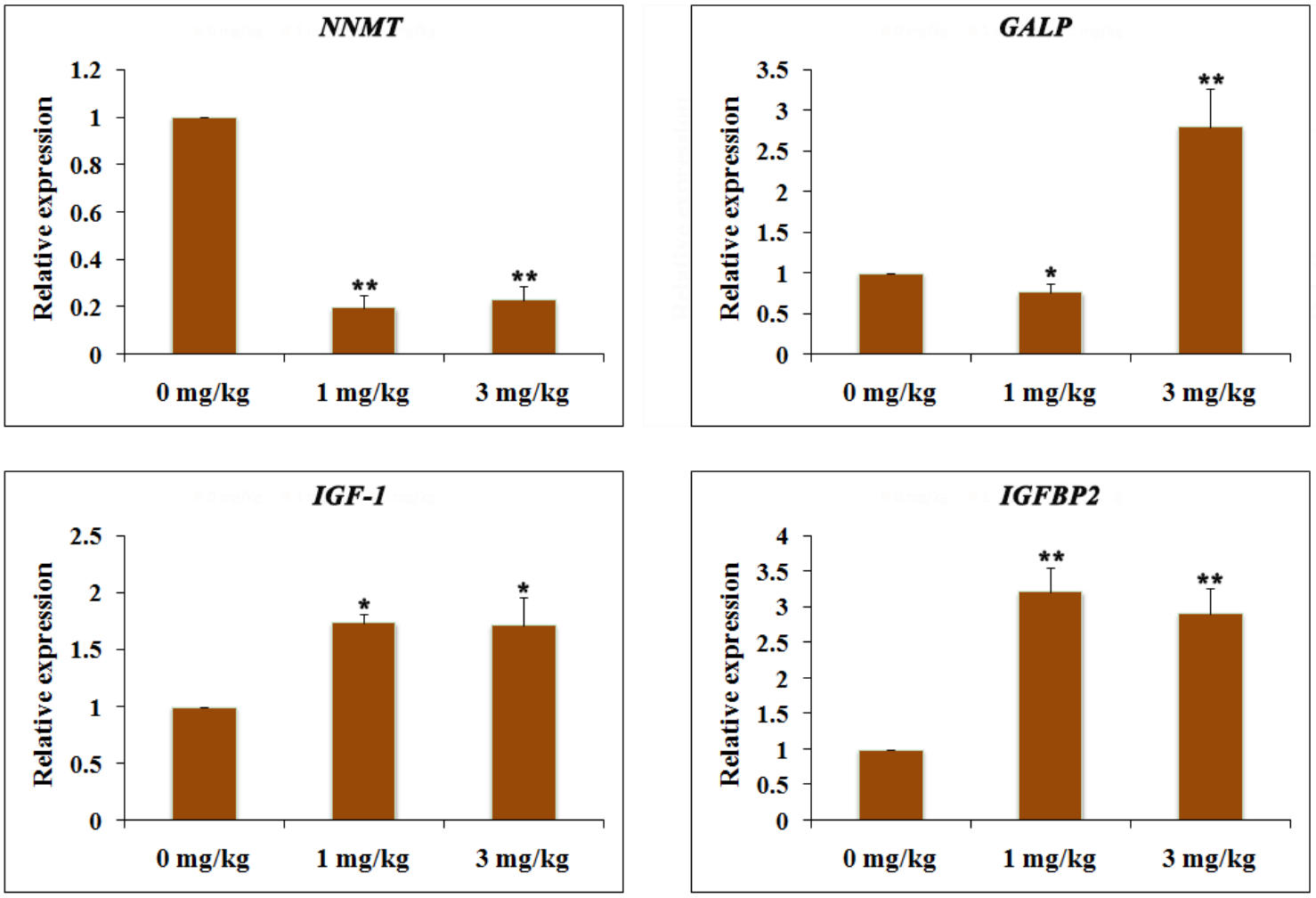

Figure 5

Gene expression closely related to growth (NNMT, GALP, IGF-1, IGFBP2) in the liver of piglets fed diets containing DON-contaminated corn (n = 5). The mRNA levels of NNMT, GALP, IGF-1 and IGFBP2 detected by qPCR. *p < 0.05, **p < 0.01, ***p < 0.001 versus 0 mg/kg group.

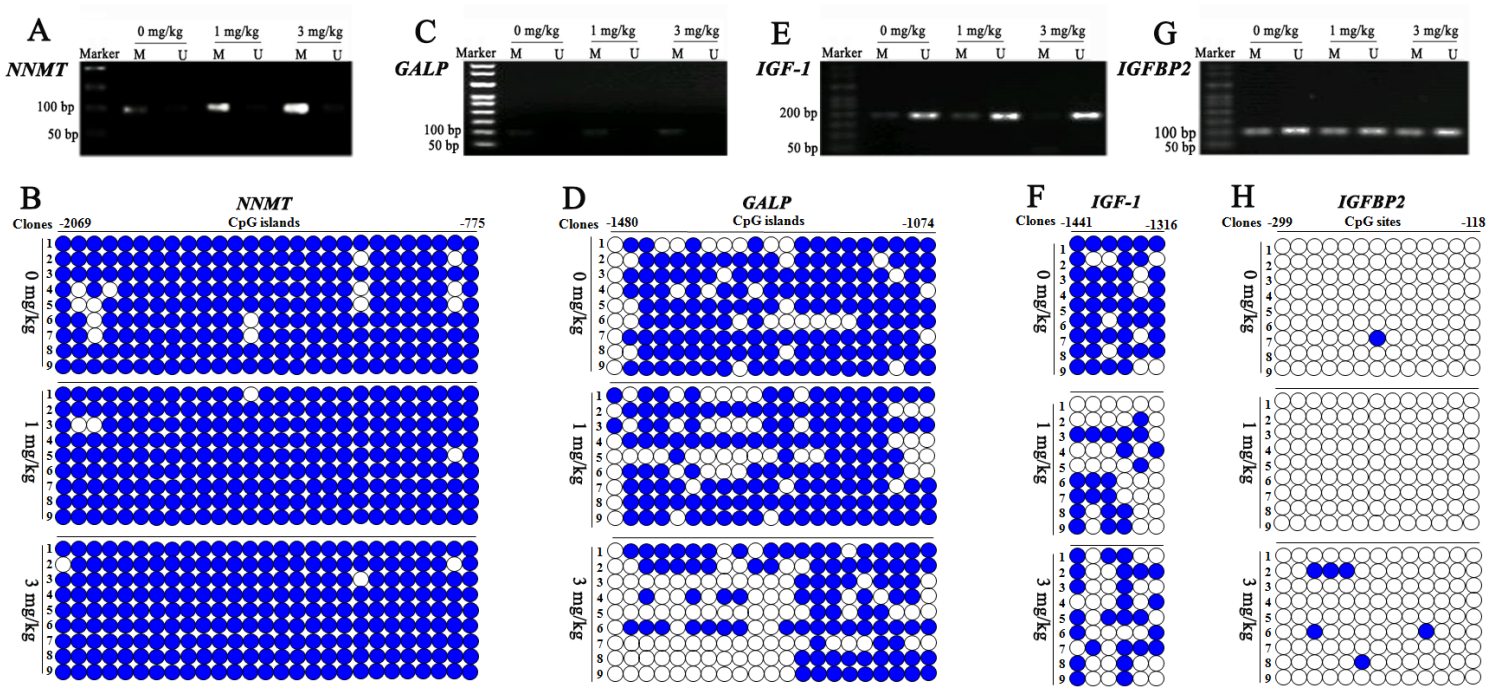

Figure 6

Methylation levels of genes closely related to growth (NNMT, GALP, IGF-1, IGFBP2) in the liver of piglets fed diets containing DON-contaminated corn (n = 5). Genomic DNA extracted from the liver was treated with bisulfite and then subjected to MSP using the methylated DNA (m)-and DNA (u)-specific primer sets. In the figure, M represents methylation, U represents unmethylation (A, C, E, G). The methylation levels in the promoter regions of gene promoter regions was detected by BSP, which indicates bisulfate sequencing by PCR; the blue dots represent the CpG sites of methylation, and the white dots represent the unmethylated CpG sites (B, D, F, H).