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The response of glandular gastric transcriptome to T-2 toxin in chicks

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ABSTRACT

This study was conducted to determine the effect of T-2 toxin on the transcriptome of the glandular stomach in chicks using RNA-sequencing (RNA-Seq). Four groups of 1-day-old Cobb male broilers (n = 4 cages/group, 6 chicks/cage) were fed a corn-soybean-based diet (control) and control supplemented with T-2 toxin at 1.0, 3.0, and 6.0 mg/kg, respectively, for 2 weeks. The histological results showed that dietary supplementation of T-2 toxin at 3.0 and 6.0 mg/kg induced glandular gastric injury including serious inflammation, increased inflammatory cells, mucosal edema, and necrosis and desquamation of the epithelial cells in the glandular stomach of chicks. RNA-Seq analysis revealed that there were 671, 1393, and 1394 genes displayed ≥2 ($P < 0.05$) differential expression in the dietary supplemental T-2 toxin at 1.0, 3.0, and 6.0 mg/kg, respectively, compared with the control group. Notably, 204 differently expressed genes had shared similar changes among these three doses of T-2 toxin. GO and KEGG pathway analysis results showed that many genes involved in oxidation-reduction process, inflammation, wound healing/bleeding, and apoptosis/carcinogenesis were affected by T-2 toxin exposure. In conclusion, this study systematically elucidated toxic mechanisms of T-2 toxin on the glandular stomach, which might provide novel ideas to prevent adverse effects of T-2 toxin in chicks.

1. Introduction

Trichothecene mycotoxins, containing four types (A, B, C, and D), are secondary metabolites produced mainly by various Fusarium species (Desjardins et al., 1993; He et al., 2012). T-2 toxin and deoxynivalenol are type A and B trichothecenes, respectively, with high toxicity for humans and animals (Wu et al., 2017). However, T-2 toxin is the most toxic of all, not only causes fatal alimentary toxic aleukia in humans (Joffe, 1983), induces anorexia, reduces nutritional efficiency, impairs immune and gastrointestinal function, but also inhibits growth in experimental animals and livestock (Makowska et al., 2018; Sheng et al., 2019; Wei et al., 2019). All over the world, various types of cereals, such as maize, wheat, barley, oat, and rice are widely contaminated by T-2 toxin (Morcia et al., 2016; Pinotti et al., 2016; Ma et al., 2018; Park et al., 2018). Such great contamination caused by T-2 toxin on food and feed consumption poses a threat to the health of humans and the productivity of livestock, thus further causes significant economic losses. Therefore, numerous studies over the past decades have focused on the toxic molecular mechanism of T-2 toxin that might be helpful for the development of antidotes and countermeasures.

T-2 toxin is a well-known ribotoxin and capable of inducing a "ribotoxic stress response" (He et al., 2012). It binds with peptidyl transferase, an integral part of the 60S ribosomal subunit, thus results in inhibition of protein synthesis (He et al., 2012; Wu et al., 2017). That is to say, it activates the mitogen-activated protein kinases, aberrant gene expression, single-strand breaks in DNA, and ribosomal RNA cleavage that induces apoptosis (He et al., 2012; Agrawal et al., 2015). Apart from that, several studies have used transcriptional and proteomic analysis to elucidate that genes are mainly associated with transcriptional or translational regulation, cell redox homeostasis, cell proliferation and cell cycle, stress response, apoptosis, transport, drug metabolism, lipid metabolism, carbohydrate metabolism, and(or) protein degradation involved in T-2 toxin induced-toxicity in livers and placentas of rats (Sehata et al., 2005), chickens (Mu et al., 2013) and porcine primary hepatocytes (Wang et al., 2011), and GH3 cell line (Wan et al., 2015).

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Broiler chicks are relatively sensitive to the toxicity of T-2 toxin, especially in the gastrointestinal tissues (Chi et al., 1977; Osselaere et al., 2013). The glandular stomach, with functions of secretion of hydrochloric acid and enzymes, plays important roles in the digestion of nutrients. However, the toxic mechanism of T-2 toxin on the glandular stomach of chicks remains unknown. Therefore, the objective of the present study was designed to use an RNA-sequencing (RNA-Seq) method to systematically analyze the altered response of the glandular stomach transcriptome to T-2 toxin exposure in broiler chicks. These findings will allow a better understanding of the underlying mechanisms of T-2 toxin-induced glandular stomach injury in chicks, hence contribute to developing antidotes for T-2 toxin.

2. Materials and methods

2.1. Chickens, treatments, and sample collection

The animal protocol was approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural University, China. In total, 96 1-day-old male Cobb broilers were randomly allocated to 4 groups, each group was assigned to 4 cages of 6 birds/cage. Birds were allowed free access to water and a corn-soybean-based diet (**control**) formulated to meet the nutritional requirements for broilers (Supplemental Table 1) (Sun et al., 2016), and the control supplemented with T-2 toxin (Pribolab Pte. Ltd., Singapore) at doses of 1.0, 3.0, and 6.0 mg/kg, respectively. The tested doses were chosen based on previous studies which showed that dietary consumption of 2.5–6.0 mg/kg T-2 toxin-induced toxic effects in chicks (Edrington et al., 1997; Diaz et al., 2016). This experiment lasted for 2 weeks. At the end of the experiment, eight birds (2 birds/cage) from each group were chosen randomly to be euthanized and the glandular stomach was collected for gastric histologic examinations and transcriptome analysis (Gao et al., 2018). The remaining chicks were euthanized followed the ethical requirements.

2.2. Transcriptome analysis

RNA-Seq analyses of the glandular stomach samples were performed as previously described (Wang et al., 2019). Briefly, 3 pooled glandular stomach samples (2 samples were pooled by 3 individual tissues and 1 sample was pooled by 2 individual tissues in equal amounts) from each treatment were prepared and submitted to Shanghai Personal Biotechnology Corporation (Shanghai, China) for total RNA isolation, mRNA purification, library preparation, and sequencing. Following that, the total RNA was extracted from the glandular stomach of broilers using Trizol (Takara, Japan) and purified with DNase (Qiagen, Germany) according to the manufacturer's instructions. Quality and quantity control were held by Agilent 2100 Bioanalyzer and RNA 6000 Labchip kit (Agilent Technologies, Santa Clara, CA, US), which revealed that all RNA samples were found with RNA integrity number over 8.5. Moreover, the poly (A) mRNA was purified by poly-T oligo-attached magnetic beads (Invitrogen); the libraries were sequenced by the Illumina Hiseq platform (Shanghai Personal Biotechnology Corporation, Shanghai, China). After filtering the raw data and trimming the low-quality reads with the Cutadapt software, the selected clean reads in FASTQ format were aligned to the reference genomic sequence through Tophat2 method and calculated transcripts expression using HTSeq. Differential expression analysis of gene (**DEG**) was conducted using the DESeq method, in such a transcript was considered to have significant DE if the P value < 0.05. All DEGs were analyzed by gene ontology (**GO**) enrichment and Kyoto Genes and Genomes (**KEGG**) enrichment.

2.3. Real-time q-PCR analysis

Total RNA from 8 individual glandular stomach samples in each treatment were isolated, as well as quality and quantity of RNA were analyzed as described previously (Zhou et al., 2009). To estimate the accuracy of RNA-Seq results, 5 DEGs, alpha-tropomyosin 1 (*Tpm1*), hephaestin like 1 (*Hephl1*), leukocyte cell derived chemotaxin 2 (*Lect2*), collagen type IV alpha 2 chain (*Col4α2*), and apoptosis enhancing nuclease (*Aen*) were randomly selected and further examined using real-time q-PCR (CFX384, Bio-Rad) as described in our previous study (Zhou et al., 2009). The primer sequences used for each gene were presented in Supplemental file 1. The 2^{-ddCt} method was used for the quantification with glyceraldehyde 3-phosphate dehydrogenase as a reference gene, and the relative abundance was normalized to the control (as 1).

2.4. Statistical analysis

One-way ANOVA was used to test the main effects of administration T-2 toxin on real-time q-PCR data. The Bonferroni t-test was followed for multiple mean comparisons if there was a main effect. Data were presented as means ± standard error (SE) and the significance level was set at $P < 0.05$. The analyses were conducted using SAS 8.2 (SAS Institute, USA).

3. Results

3.1. Glandular gastric histology

The histological results showed that dietary supplementation of T-2 toxin induced injury in the glandular stomach (Fig. 1). Specifically, compared to the control group, dietary supplemental T-2 toxin at 3.0 mg/kg induced severe inflammation, mucosal edema and increased inflammatory cells, including heterophilic granulocytes, lymphocytes, and macrophages, in the submucosal layer and interstitial tissue of glandular stomach of chicks. Furthermore, T-2 toxin at 6 mg/kg additionally increased the glandular gastric damage including necrosis and desquamation of the epithelial cells compared with 3.0 mg/kg T-2 toxin. However, the glandular gastric histology was not significantly affected by dietary supplementation of T-2 toxin at 1.0 mg/kg for 2 weeks.

3.2. Sequencing, de novo assembly and annotation

A total of 40,709,910 to 46,468,666 raw reads of 150 bp-paired

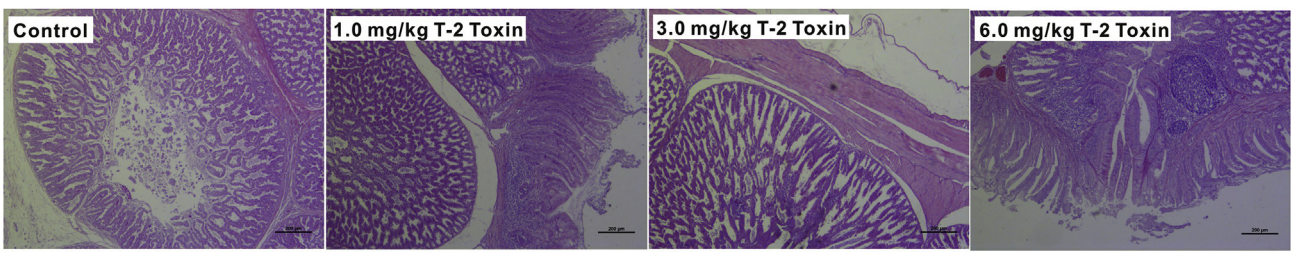

Fig. 1. Photomicrographs of sections of the glandular stomach stained with hematoxylin and eosin (200 magnification) of chicks from different dose of T-2 toxin groups on week 2.

Table 1
Statistical summary of the glandular stomach RNA-seq datasets[a].

Samples	Raw Reads number	Q20 value[b]	Clean Reads number	Clean Reads percentage	Total mapped reads percentage	Uniquely mapped reads percentage
Control-1	42,062,992	95.91	41,718,956	99.18	81.83	78.31
Control-2	46,468,666	95.84	45,903,044	98.78	83.47	80.36
Control-3	43,749,472	95.76	43,285,576	98.93	82.50	79.59
T-2 toxin-1.0-1	43,627,354	95.96	43,283,846	99.21	82.10	77.71
T-2 toxin-1.0-2	41,048,248	95.64	40,727,758	99.21	78.00	74.73
T-2 toxin-1.0-3	43,056,464	95.79	42,568,376	98.86	83.24	80.04
T-2 toxin-3.0-1	44,288,432	95.77	43,917,124	99.16	81.48	77.58
T-2 toxin-3.0-2	42,881,166	95.79	42,546,916	99.22	80.76	76.72
T-2 toxin-3.0-3	40,709,910	95.75	40,360,452	99.14	81.91	78.82
T-2 toxin-6.0-1	43,943,698	95.64	43,573,298	99.15	79.94	76.81
T-2 toxin-6.0-2	42,596,924	95.76	42,232,028	99.14	80.74	77.36
T-2 toxin-6.0-3	44,685,016	95.30	44,083,724	98.65	81.62	78.20

[a] Control, T-2 toxin-1.0, T-2 toxin-3.0 and T-2 toxin-6.0 means the glandular stomach samples from the diets supplemental T-2 toxin at the doses of 0, 1.0, 3.0, and 6.0 mg/kg, respectively.
[b] Q20 value means the sequencing quality values that correspond to 1% chance of error.

ends, with Q20 values range from 95.30 to 95.96% were collected for 12 libraries from the control and T-2 toxin treatment groups, respectively (Table 1). After filtering adapters and trimming ambiguous and low-quality reads, an average of 42,850,092 high-quality and clean reads were obtained, accounting for an average of 99.05% of total raw reads respectively (Table 1). Moreover, the high-quality clean reads were further mapped onto the *Gallus gallus* genome (Ensembl Database) using the Bowtie and TopHat tools. The average ratio of high-quality reads in comparison with the reference genome was 81.47% (Table 1) and 18346 genes were aligned to the database (Supplemental file 2).

3.3. Differential expression and functional analysis

Differential expression (**DE**) analysis results revealed that various doses of T-2 toxin treatment responsive genes existed in the glandular stomach of chicks (Fig. 2). Compared to the control, there were 671, 1393, and 1394 transcripts showing 2-fold or greater ($P < 0.05$) DE in dietary supplementation of T-2 toxin at 1.0, 3.0, and 6.0 mg/kg, respectively (Fig. 2A). Notably, there were 204 transcripts shared similar changes among the different doses of T-2 toxin treatments. Specifically, compared to the control, 37 genes were upregulated and 167 genes were downregulated by the dietary supplementation of T-2 toxin at all three doses (Fig. 2B–D; Supplemental file 3). The full list of DE transcripts can be found in Supplemental file 3.

Moreover, the GO analysis results showed that 158 DEGs, accounting for 77.45% of all significant DEGs, were associated (FDR < 0.05) with biological process, cellular compound and molecular functions (Supplemental file 4). The top 10 groups in the three main categories were shown in Fig. 3. Within the biological process category, the most abundant groups including the cellular process, biological regulation, regulation of biological process, and regulation of cellular process. In the cellular components category, the cell, cell part, and intracellular were the most highly GO terms. Likewise, binding, protein binding, and ion binding accounted for the most enriched position in the category of the molecular function. Meanwhile, 60 DEGs fell into the KEGG pathway (Supplemental file 5). The Top 10 pathways of the DEGs were shown in Fig. 4, the most abundant groups were focal adhesion, regulation of actin cytoskeleton, pathways in cancer. Furthermore, the DEGs related to the GO and KEGG pathway results involved in the oxidation-reduction process, inflammation, wound healing/bleeding, and carcinogenesis/apoptosis were summarized in Table 2. Real-time q-PCR result showed that glandular gastric mRNA levels of *Tpm1*, *Hephl1*, *Lect2*, and *Col4a2* were decreased ($P < 0.05$) and *Aen* was increased ($P < 0.05$) by the T-2 toxin supplementation at 1.0, 3.0, and(or) 6.0 mg/kg (Fig. 5), which were similar with the RNA-Seq results.

4. Discussion

The glandular gastric injury was replicated in chicks through feeding of T-2 toxin diets at concentrations of 3.0 and 6.0 mg/kg, respectively. Broilers consumed diets with T-2 toxin at the doses of 3.0 and 6.0 mg/kg manifested clinical signs of glandular stomach injury including severe inflammation, increased inflammatory cells, mucosal

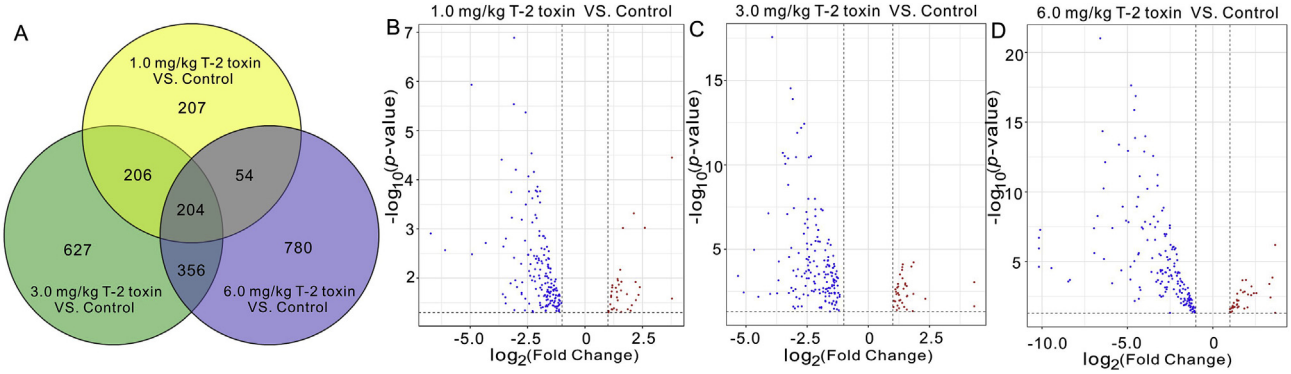

Fig. 2. Transcriptome data profile generated by Illumina Hiseq platform and differential expression analysis. Venn diagram showed unique and co-differentially expressed genes in response to dietary T-2 toxin supplementation at 1.0, 3.0, and 6.0 mg/kg (A); The visualization of volcano plot showed the transcript expression profiles between control and dietary T-2 toxin supplementation at 1.0 (B), 3.0 (C) and 6.0 (D) mg/kg to the identified 204 co-differentially expressed transcripts among three doses of T-2 toxin treatments. The red dot and green dot defines the genes upregulated and downregulated, respectively. The y-axis indicates = $-\log_{10}{}^{(p\text{-value})}$, and the x-axis indicates the $\log_2{}^{(\text{Fold Change})}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

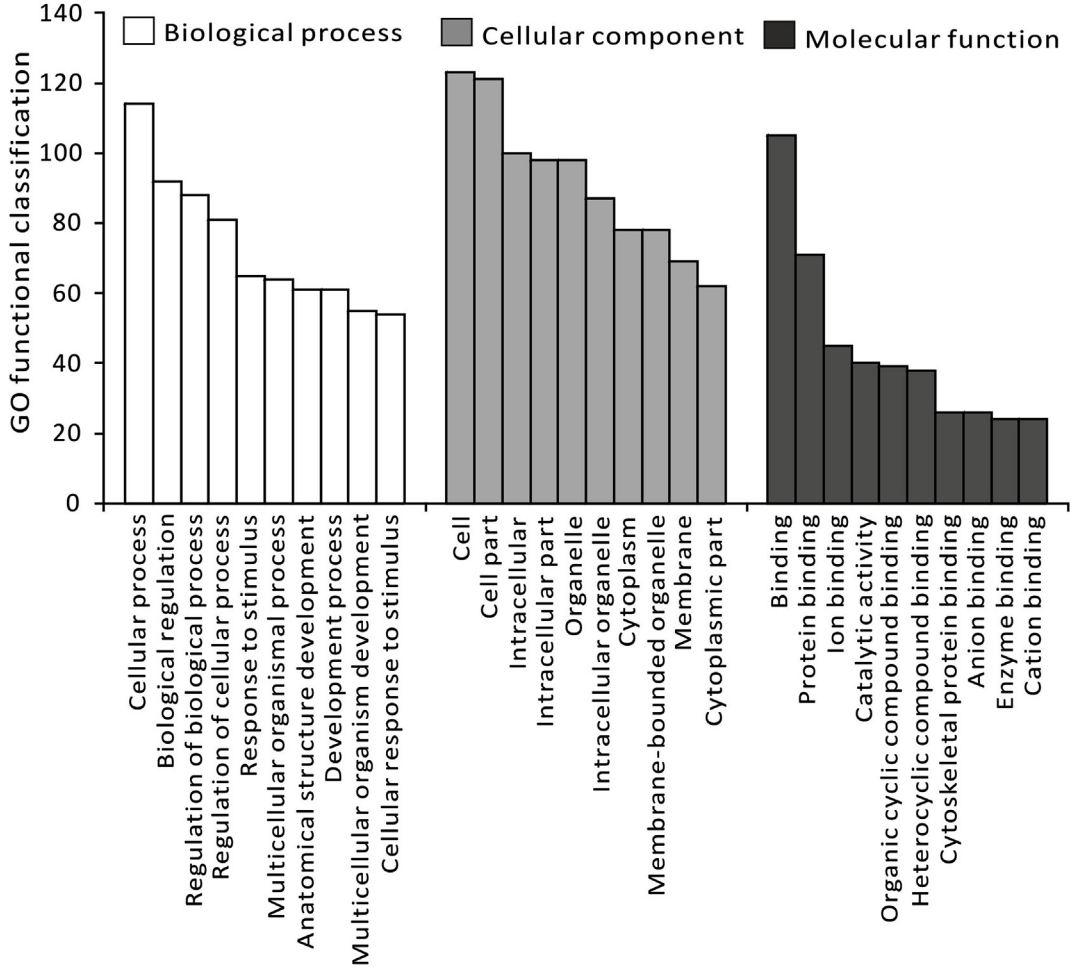

Fig. 3. GO functional enrichment analysis of DEGs in the glandular stomach of chicks. The top 10 groups in the three main categories: cellular component, biological process, and molecular function, were summarized. The x-axis indicates the subcategories, and the y-axis indicates the number of genes in the same category.

edema, and necrosis and desquamation of the epithelial cells. These outcomes were similar with previous studies, which provided evidence that gastrointestinal injury was induced by T-2 toxin in chicks (Chi et al., 1977), ducklings (Rafai et al., 2000), pigs (Pang et al., 1987), and mice (Hayes et al., 1980).

This is the first study, to our knowledge, in analyzing the transcriptome response of the glandular stomach to T-2 toxin in chicks. In the current study, the RNA-Seq produced an average of 43.3 M raw data reads (6.5 Gb) for each treatment and 18346 transcripts were assembled after filtering and trimming the raw data. Although dietary supplementation of T-2 toxin at 1.0 mg/kg did not induce significant injury in the glandular stomach by pathohistological analysis, it induced 671 DEGs compared with the control. Moreover, dietary supplementation of T-2 toxin at 3.0 or 6.0 mg/kg induced 1393 and 1394 DEGs in the glandular stomach, respectively. The 204 DGEs shared similar changes among the three doses of T-2 toxin treatments, which might have higher possibility involved in the T-2 toxin-induced glandular gastric injury, thus were selected to be further analyzed. The mRNA levels of five randomly selected DEGs detected by real-time q-PCR method were similar with RNA-Seq technology which indicated that the RNA-Seq results were reliable (Zhang et al., 2016).

According to the GO and KEGG pathway analysis, the chosen DEGs related to the glandular stomach injury including oxidation-reduction process, inflammation, wound healing/bleeding, and apoptosis/carcinogenesis were summarized in Table 2. These profiles have enabled us for the first time to systematic elucidates the toxic mechanisms of T-2 toxin on the glandular stomach in chicks.

T-2 toxin can induce reactive oxygen species generation, causing oxidative stress and subsequently inducing organ damage (Chaudhary and Rao, 2010; Wu et al., 2012). In this regard, 10 DEGs involved in the oxidation-reduction process were affected by T-2 toxin treatment (Table 2). Notably, polyamine oxidase (*Paox*) code for oxidase enzyme (Bjelakovic et al., 2010) was upregulated, while forkhead box O3 (*Foxo3*) (Marinkovic et al., 2007), *Tpm1* (Gagat et al., 2016) and ABL proto-oncogene 1 (*Abl1*) (Sourbier et al., 2014) genes code for proteins that possess anti-oxidant stress were downregulated. Upregulation of the oxidase enzyme gene, along with downregulation of the anti-oxidant stress genes in the glandular stomach of chicks exposed to T-2 toxin, could attribute to the oxidative stress induced by T-2 toxin (Chaudhary and Rao, 2010; Wu et al., 2012). Strikingly, retinol saturase (*Retsat*) code for proteins possess reductase activity (Moise et al., 2004) was upregulated, but gamma-butyrobetaine hydroxylase 1 (*Bbox1*) (Galland et al., 1999), peroxidasin (*Pxdn*) (Péterfi et al., 2009), *Hephl1*(Hudson et al., 2010), molecule interacting with casl protein 3 (*Mical3*) (Lundquist et al., 2014), and lysyl oxidase (*Lox*) (Guo et al., 2016) code for oxidase enzyme or oxygenase were downregulated by T-2 toxin treatments. The changes in these genes expression could result in mitigating the oxidative stress induced by T-2 toxin, which may be interpreted by a compensatory mechanism to help broilers to cope with T-2 toxin-induced oxidative stress. However, the potential roles of these genes in T-2 toxin-impaired redox homeostasis need to be explored in the future.

Consistent with previous studies, T-2 toxin induced inflammation in the glandular stomach of chicks (Agrawal et al., 2012; Wu et al., 2017).

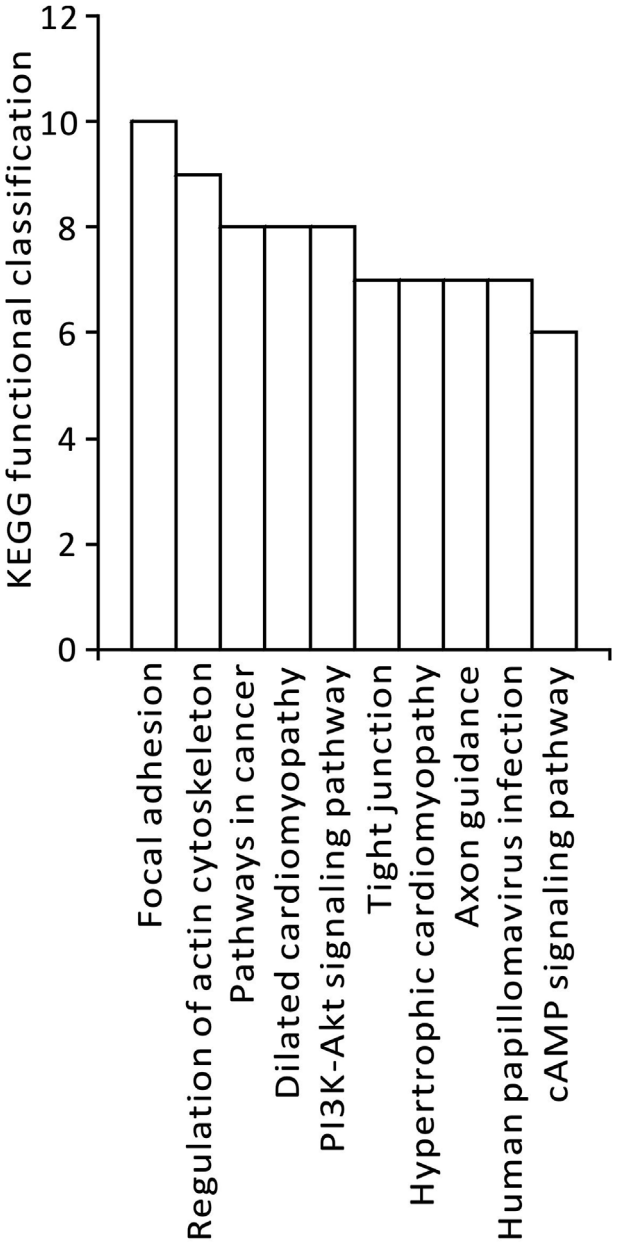

Fig. 4. KEGG pathway enrichment analysis of DEGs in the glandular stomach of chicks. The DEGs fell into the top 10 pathways were summarized. The x-axis indicates functional pathways, and the y-axis indicates the number of genes in the same pathway.

In the current study, 9 DEGs related to inflammation were affected by T-2 toxin exposure (Table 2). Specifically, T-2 toxin exposure downregulated the expression of erythroferrone (*Erfe*) (Kautz et al., 2014), junctional adhesion molecule 3 (*Jam3*) (Immenschuh et al., 2009), calcium/calmodulin-dependent protein kinase II gamma (*Camk2g*) (Proietti et al., 2018), and *Lect2* (Yamagoe et al., 1998) genes code for proteins that contributed to recovery from inflammation, reduce inflammatory response or defense against invading pathogens in the glandular stomach. Strikingly, E74 like ETS transcription factor 3 (*Elf3*) (Otero et al., 2012), innate immunity activator (*Inava*) (Yan et al., 2017), and nuclear factor kappa B subunit 2 (*Nfkb2*) (de Wit et al., 1998) genes code for proteins that contribute as pro-catabolic factor in inflammatory stress or maintain immune homeostasis were upregulated, while frizzled class receptor (*Fzd*) *1* and *7* genes code for proteins which may initiate/augment inflammation (Sen and Ghosh, 2008) were downregulated by T-2 toxin administration. This may be explained by a complex feedback mechanism in regulating the inflammation induced by T-2 toxin exposure (Zhang et al., 2016).

Numerous studies have shown that the gastrointestinal bleeding is a typical clinical sign for T-2 toxin exposure (Edrington et al., 1997; Hemmati et al., 2012). However, T-2 toxin did not induce significant bleeding in the glandular stomach in the current study, which might be due to the differences in the experimental conditions, including exposure doses, duration, and animal species. Notably, it was still interesting to find that 12 DEGs related to wound healing/bleeding were affected by T-2 toxin exposure (Table 2), which might help us to understand the potential underlying mechanism of T-2 toxin-induced bleeding. Interestingly, T-2 toxin administration downregulated the expression of 8 genes, integrin subunit alpha 1 (*Itga1*) (Enenstein and Kramer, 1994), integrin subunit beta 1 (*Itgb1*) (Shi et al., 2013), WAP four-disulfide core domain 1 (*Wfdc1*) (McAlhany et al., 2003), yes associated protein 1 (*Yap1*) (Wang et al., 2014), neuropilin 2 (*Nrp2*) (Takashima et al., 2002), parvin alpha (*Parva*) (Fraccaroli et al., 2015), protein kinase C epsilon (*Prkce*) (Sharma et al., 2007), and CD109 molecule (*Cd109*) (Kenneth et al., 2006), which play roles in angiogenesis, blood vessel remodeling or wound healing. On the other hand, CGMP-Dependent 1 (*Prkg1*) (Antl et al., 2007), bos taurus caldesmon 1 (*Cald1*) (Kordowska et al., 2006), KH domain containing RNA binding (*Qki*) (van der Veer et al., 2013), and calponin 1 (*Cnn1*) (Gu et al., 2010) genes code for proteins that play roles in inhibiting vascular smooth muscle cell contraction and preventing platelet aggregation were downregulated by T-2 toxin administration. The alternations in these genes expression might help broilers to counteract T-2 toxin-induced gastrointestinal bleeding.

Oxidative stress and inflammation induced by T-2 toxin can cause DNA damage thus triggers apoptosis and even carcinogenesis (Schoental et al., 1979; Bartsch and Nair, 2006; Chaudhari et al., 2009). In this regard, 9 DEGs associated with apoptosis and carcinogenesis were affected by T-2 toxin administration (Table 2). Early studies had shown upregulation of *Aen* (Lee et al., 2005) and downregulation of fibronectin 1 (*Fn1*) (Cai et al., 2018), alpha-ctinin 1 (*Actn1*) (Glück and Ben-Ze'ev, 1994), vinculin (*Vcl*) (Magro et al., 2007), histone deacetylase 9 (*Hdac9*) (Inoue et al., 2007), and AKT serine/threonine kinase 3 (*Akt3*) (Paul-Samojedny et al., 2014), which could increase apoptosis or tumorigenicity. Upregulation of *Aen* and downregulation of *Fn1*, *Actn1*, *Vcl*, *Hdac9*, and *Akt3* in the glandular stomach of chicks exposed to T-2 toxin might attribute to the potential apoptosis and carcinogenesis caused by T-2 toxin. Strikingly, three genes code for proteins with functions of promoting cancer, ABL proto-oncogene 1 (*Abl1*) (Daley and Baltimore, 1988), adenylate cyclase 5 (Adcy5) (Yan et al., 2007), and *Col4a2* (Huang et al., 2018), were downregulated by T-2 toxin. These changes may be interpreted as an adaptation to the potential apoptosis and carcinogenesis induced by T-2 toxin.

In conclusion, the present study showed that dietary supplementation of T-2 toxin at 3.0 and 6.0 mg/kg induced glandular gastric injury in broiler chicks. Furthermore, RNA-Seq analysis results showed that broilers exposed to T-2 toxin-induced serious inflammation, increased inflammatory cells, mucosal edema, and necrosis and desquamation of the epithelial cells in the glandular stomach, which mainly associated with dysregulated expression of pivotal genes involved in oxidation-reduction process, inflammation, wound healing/bleeding, and apoptosis/carcinogenesis. However, further validation of the exact functions and mechanisms of these key DEGs in T-2 toxin metabolism will be in need and would be potentially beneficial to prevent the toxic processes.

Declaration of interests

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

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Table 2
The differentially expressed genes grouped by GO and KEGG pathways of interest[a].

Accession	Gene name	Symbol	FC[b]	*P*-value
Oxidation-reduction process				
ENSGALG00000016377	Peroxidasin	PXDN	0.35	≤1.79E-03
ENSGALG00000015297	Forkhead box O3	FOXO3	0.41	≤3.22E-02
ENSGALG00000003521	Alpha-tropomyosin 1	TPM1	0.16	≤4.33E-03
ENSGALG00000003875	ABL proto-oncogene 1	ABL1	0.41	≤1.78E-02
ENSGALG00000034358	Retinol saturase	RETSAT	2.37	≤4.32E-02
ENSGALG00000003557	Polyamine oxidase	PAOX	2.21	≤4.97E-02
ENSGALG00000013297	Gamma-butyrobetaine hydroxylase 1	BBOX1	0.17	≤3.97E-03
ENSGALG00000013042	Molecule interacting with CasL protein 3	MICAL3	0.34	≤2.92E-02
ENSGALG00000028063	Lysyl oxidase	LOX	0.22	≤1.57E-03
ENSGALG00000043027	Hephaestin like 1	HEPHL1	0.11	≤1.11E-02
Inflammation				
ENSGALG00000000616	E74 like ETS transcription factor 3	ELF3	2.99	≤2.82E-02
ENSGALG00000044612	Innate immunity activator	INAVA	2.62	≤1.19E-02
ENSGALG00000005653	Nuclear factor kappa B subunit 2	NFKB2	2.17	≤2.15E-02
ENSGALG00000001472	Junctional adhesion molecule 3	JAM3	0.30	≤9.94E-03
ENSGALG00000005088	Calcium/Calmodulin dependent protein kinase II gamma	CAMK2G	0.35	≤2.58E-02
ENSGALG00000009064	Frizzled class receptor 1	FZD1	0.28	≤1.88E-02
ENSGALG00000034973	Frizzled class receptor 7	FZD7	0.37	≤1.74E-02
ENSGALG00000001857	Erythroferrone	ERFE	0.19	≤4.07E-03
ENSGALG00000006323	Leukocyte cell derived chemotaxin 2	LECT2	0.09	≤2.64E-04
Wound healing/bleeding				
ENSGALG00000003805	Protein kinase CGMP-dependent 1	PRKG1	0.16	≤3.53E-03
ENSGALG00000010000	Protein kinase C epsilon	PRKCE	0.35	≤4.05E-02
ENSGALG00000033471	Bos taurus caldesmon 1	CALD1	0.16	≤4.02E-02
ENSGALG00000014891	Integrin subunit alpha 1	ITGA1	0.07	≤1.30E-07
ENSGALG00000005503	WAP four-disulfide core domain 1	WFDC1	0.22	≤1.07E-02
ENSGALG00000011555	KH domain containing RNA binding	QKI	0.35	≤4.68E-03
ENSGALG00000038154	Yes associated protein 1	YAP1	0.35	≤1.65E-02
ENSGALG00000041266	Calponin 1	CNN1	0.14	≤2.30E-02
ENSGALG00000015910	CD109 molecule	CD109	0.17	≤1.73E-02
ENSGALG00000008621	Neuropilin 2	NRP2	0.29	≤3.77E-02
ENSGALG00000005438	Parvin alpha	PARVA	0.21	≤5.42E-04
ENSGALG00000007145	Integrin subunit beta 1	ITGB1	0.34	≤3.61E-02
Apoptosis/carcinogenesis				
ENSGALG00000044204	Apoptosis enhancing nuclease	AEN	3.30	≤4.08E-02
ENSGALG00000031244	Adenylate cyclase 5	ADCY5	0.32	≤1.80E-02
ENSGALG00000003875	ABL proto-oncogene 1	ABL1	0.41	≤1.78E-02
ENSGALG00000034081	AKT serine/threonine kinase 3	AKT3	0.29	≤2.73E-02
ENSGALG00000016843	Collagen type IV alpha 2 chain	COL4A2	0.18	≤2.89E-04
ENSGALG00000003578	Fibronectin 1	FN1	0.17	≤2.16E-04
ENSGALG00000010854	Histone deacetylase 9	HDAC9	0.25	≤2.04E-02
ENSGALG00000042458	Alpha-ctinin 1	ACTN1	0.22	≤1.24E-02
ENSGALG00000005079	Vinculin	VCL	0.29	≤1.04E-02

[a] Putative functions were identified for DEGs using GO, KEGG pathway or primary literature; FC, fold change.
[b] FC was determined as the mean value of mRNA abundance of the dietary supplementation of T-2 toxin at 1.0, 3.0, and 6.0 mg/kg VS. control.

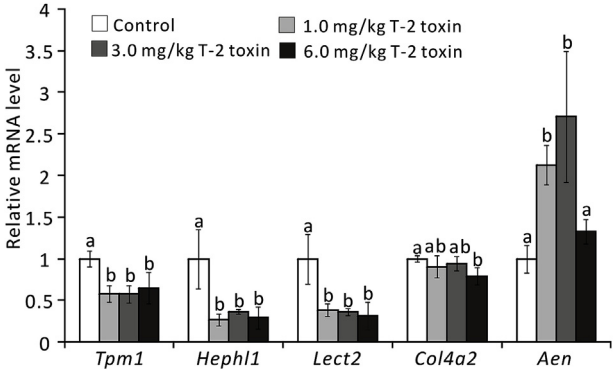

Fig. 5. Effects of T-2 toxin on relative mRNA abundance of *Tpm1*, *Hephl1*, *Lect2*, *Col4a2*, and *Aen*. Values are means ± SE, n = 8. Means without a common letter differ, $P < 0.05$.

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

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Transparency document

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