

The Role of Nitric Oxide and Oxidative Stress in Intestinal Damage Induced by Selenium Deficiency in Chickens

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Abstract Nitric oxide (NO) is an essential messenger molecule and is associated with inflammation and oxidative stress. Although NO has important biological functions in mammals, its role in the mechanism that occurs after intestinal injuries in chickens remains unknown. The objective of the present study was to investigate the real role of NO and oxidative stress in the intestinal injuries of chickens induced by selenium (Se) deficiency. A total 150 chickens were randomly divided into the following two groups: a low-Se group (L group, fed a Se-deficient diet containing 0.020 mg/kg Se) and a control group (C group, fed a commercial diet containing 0.2 mg/kg Se). The activities and mRNA levels of glutathione peroxidase (GSH-Px), the production of glutathione (GSH) and NO, and the protein and mRNA levels of inducible nitric oxide synthase (iNOS) were examined in the intestinal tissues (duodenum, jejunum, and rectum) at 15, 25, 35, 45, and 55 days. Methane dicarboxylic aldehyde (MDA) levels were also detected by assay kits. Then, the morphologies of the tissues were observed under the microscope after hematoxylin and eosin staining (H&E staining). The results showed that Se deficiency induced higher inflammatory damage and MDA levels ($P < 0.05$), which were accompanied by higher levels of iNOS and NO but lower levels of GSH and GSH-Px ($P < 0.05$). Our results indicated that Se deficiency induced oxidative damage in the intestinal tracts of chickens and that low levels of GSH-Px and high contents of NO may exert a major role in the injury of the intestinal tract induced by Se deficiency.

Keywords Selenium deficiency · Nitric oxide · GSH-Px · Intestine · Chicken

Introduction

Selenium (Se), an essential trace element, reserves kinds of functions in both human beings and domestic animals [1]. Se mainly incorporates into a selenoprotein that possesses several types of biological functions [2, 3]. In a previous study, an area with soil Se content below 0.5 mg/kg was considered a low-Se region [4]. Generally, the appropriate Se content in feed is 0.1 mg/kg, and when it is less than 0.05 mg/kg, the low Se level could lead to a disease [5]. Se deficiency could induce oxidative stress, inflammation, and apoptosis in many tissues [6]. The intestinal tract, as one main organ absorbing Se, is the tissue targeted by Se deficiency. This disruption of the intestinal tract function may influence the exertion of the biological function of Se and affect the normal function of other organs. Therefore, studying the effects of Se deficiency on the intestinal tract is an important field. It has been indicated that Se deficiency reduced the intestinal dilation of rats [7] and increased the intestinal motility in the chronic phase of parasite infection [8]. Se deficiency influenced the level of intestinal glutathione peroxidase (GSH-Px) and induced inflammation and oxidative stress in the rat intestine. The GSH-Px activity and mRNA level were significantly depressed in the ileum of Se-deficient mice [9]. In addition, it is known that Se deficiency can induce intestinal inflammation in chickens [10]. However, the exact mechanism of the influence of the intestinal mucosal immunization induced by a low-Se diet remains unclear. Moreover, the concentration of previous studies has been on mammals, but the real role of Se in the intestinal mucosal function of chickens has been reported to a lesser extent. As mentioned above, in mammals, GSH-Px is one crucial target of Se in the intestinal tract, but studies that

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evaluated the effects of Se on the intestinal function of chickens are rare. The change in GSH-Px due to Se deficiency may be the major cause of injury in the intestinal tract. Therefore, it is reasonable to hypothesize that Se deficiency may influence the function of intestinal tracts of chickens by decreasing the activity of GSH-Px.

Nitric oxide (NO), as one type of active messenger molecule, has broad biological functions. NO is produced by nitric oxide synthases including constitutive NO synthases (cNOS) and inducible NO synthases (iNOS) that are mainly expressed in the intestinal tract [11]. It was shown that NO could regulate the intestinal mucosal blood flow and vascular integrity [12]. In research about intestinal injury in mammals due to chronic alcohol use, Bagyanszki et al. confirmed that NO protected the integrity of the intestinal mucosa and enhanced its motivation [13]. In addition, the NO produced by iNOS aggravated gastrointestinal injury by a mechanism of oxidative stress that was induced by NO and ROS in mice [14]. Thus, we can see that NO always exists in conjunction with an intestinal tract injury. However, whether Se deficiency also induces NO production in the intestinal tract is unknown. In the present study, through the detection of the antioxidative selenoprotein, GSH-Px, and the NO level in the intestinal tract of 150 sea blue white laying hens, we discuss the potential mechanism of intestinal damage induced by Se deficiency over 55 days.

Materials and Methods

Animals and Diet

All of the procedures used in the present study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. A total of 300 1-day-old sea blue white laying chicks were divided into two groups. Each treatment group consisted of 150 chickens, and each of those groups was randomly divided into groups so that the trials were conducted in triplicate. The chickens were maintained either on a Se-deficient diet (L group, Se-deficient granulated diet including corn, soybean meal, and wheat bran from Longjiang County, a typical Se-deficient region of the Heilongjiang Province in China and blends material that did not add Se (Weiwei Co. Ltd., Harbin, China)) or a normal-Se-content diet (control group, Weiwei Co. Ltd., Harbin, China) for 55 days. According to the different nutritional needs during the growth stages of sea blue white chickens (0–21, 22–42, and 43–55 days), different types of feed were prepared for the control and L groups. Se was added in the form of sodium selenite, and the final content of Se was 0.2 mg/kg in the control group and 0.02 mg/kg in the L group, as determined by fluorescence spectrophotometry using GB/T 13,883-2008 (PONY TEST Co., Beijing, China). Over the entire experimental period, the chickens were allowed ad

libitum consumption of feed and water. Clinical symptoms and mortality were also recorded. Then, 15 chickens in each group were killed with sodium pentobarbital at 15, 25, 35, 45, and 55 days old, respectively. The intestinal tissues (duodenum, jejunum, and rectum) were quickly removed, minced, and stored at -80°C in order to determine the index of oxidative stress and isolate the RNA. In this study, 15 chickens were killed per group at the five sampling events, and five chickens per group were used in the official test ($n=5$), which was repeated in triplicate. The remaining tissues were used in the preliminary experiment and served as standby tissues.

Morphologies of the Intestinal Mucosa

The tissues of each segment of the intestine were collected, cut to a size approximately 0.5 cm^3 , placed in 10 % paraformaldehyde ($\text{pH}=7.4$) for 24 h at 25°C , and embedded in paraffin. The fixed well tissues were H&E-stained and mounted after the processes of dehydration, transparent, dipping wax, embedded, sectioned, and affixed. Then, the paraffin sections were placed under a microscope (Leica DM-2500, Germany) with a high-resolution digital camera to observe and record the relevant morphologies.

Gene Expression Analysis

The total RNA was isolated from each of the tissue samples ($n=5$ /diet group) using TRIzol reagent according to the instructions of the manufacturer (Invitrogen, Carlsbad, CA, USA). The concentrations of the total RNA were determined using GeneQuant 1,300 (GE Healthcare Biosciences, Piscataway, NJ, USA). The reverse transcription reaction (20 μL) consisted of the following: 5 μg of total RNA, 0.5 μL of Moloney Murine Leukemia Virus reverse transcriptase (200 U/ μL), 0.5 μL of RNase inhibitor (40 U/ μL), 2 μL of deoxynucleoside triphosphate (10 mM), 1 μL of Oligo dT (50 μM), 2 μL of dithiothreitol (0.1 M), and 4 μL of 5 \times reverse transcriptase buffer. The procedure of the reverse transcription was according to the instructions of the manufacturer (Invitrogen). The reverse transcription products (cDNA) were then stored at -20°C for PCR.

Primer Premier Software (PREMIER Biosoft International, USA) was used to design specific primers for iNOS (GenBank accession no. NM_204961), GSH-Px (GenBank accession no. NM_001277854.1), and GADPH (GenBank accession no. K01458) based on known chicken sequences. GADPH, a housekeeping gene, was used as an internal reference. Primers were synthesized by Invitrogen Bionotechnology Co. Ltd. in Shanghai, China. The following set of primers were used: for iNOS, forward 5'- CCT GGA GGT CCT GGA AGA GT -3' and reverse 5'- CCT GGG TTT CAG AAG TGG C -3'; for GSH-Px, forward 5'- ATC GCC AAG TCC TTC TAC GA -3'

and reverse 5'- ACG TTC TCG ATG AGG ACC AC-3'; and for GADPH, forward 5'- AGA ACA TCA TCC CAG CGT -3' and reverse 5'- AGC CTT CAC TAC CCT CTT G -3'. The PCR products were subjected to electrophoresis on 2 % agarose gels, extracted, cloned into the pMD18-T vector (Takara, Ohtsu, Japan), and sequenced.

Real-time PCR was used to detect the expression of the iNOS gene in the intestines by using SYBR Premix Ex Taq (Takara), and real-time PCR was performed on an ABI PRISM 7,500 detection system (Applied Biosystems, USA). The program was 1 cycle at 95 °C for 30 s, 40 cycles at 95 °C for 5 s, and 40 cycles 61 °C for 34 s. The melting curve analysis showed only one peak for each PCR product. Electrophoresis was performed on the PCR products to verify the specificity of the primer and the product purity. The amplification efficiency for each gene was determined using the Data Analysis for Real-Time PCR (DART-PCR) program [15]. The relative abundance of the mRNA was calculated according to the method of Pfaffl [16] and, to account for gene-specific efficiencies, was then normalized to the mean expression of GADPH.

NO and iNOS Activity Assay

The homogenized intestinal tissues of each treatment group were used for the NO and iNOS activity assays. The NO and iNOS activities were determined using NO and iNOS activity assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The method used in the present study was according to the procedure previously published by our group [17] and used an ELX800 Microplate reader (BioTek Instruments, USA) to detect the OD at 550 and 530 nm, respectively.

MDA, GSH, and GSH-Px Activity Assay

According to the manufacturer's instructions, MDA, GSH, and GSH-Px activity assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used to determine the MDA and GSH contents as well as the activity of GSH-Px in the intestines using an ELX800 Microplate reader (BioTek Instruments, USA) to detect the OD at 532, 420, and 412 nm, respectively.

Western Blotting Analysis

An equivalent amount of tissue (depending on the tissue examined, between 50 and 150 mg) was homogenized in 800 μ L of ice-cold grind buffer (20 mM Tris-HCl, pH=7.4, 2 mM EDTA, 2 mM EGTA, 1 mM PMSF, 30 mM NaF, 30 mM sodium pyrophosphate, 0.1 % SDS, 1 % Triton X-100, and protease inhibitor cocktail). The sample was then centrifuged for 10 min at 10,000 g and 4 °C, and the

supernatant was collected. The protein content was measured according to Bradford's procedure [18]. Equal amounts of the total protein (40 μ g/condition) were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions on 10 % gels. The separated proteins were then transferred to nitrocellulose membranes using a tank transfer for 2 h at 200 mA in Tris-glycine buffer containing 20 % methanol. Membranes were blocked with 5 % skim milk for 16–24 h and incubated overnight with diluted primary rabbit antibody against iNOS (1:200, Abcam, USA), followed by a horse-radish peroxidase (HRP) conjugated secondary antibody against rabbit IgG (1:1000, Santa Cruz, USA). To verify the equal loading of the samples, the membrane was incubated with a monoclonal β -actin antibody (1:1000, Santa Cruz, USA), followed by incubation with a HRP conjugated goat anti-mouse IgG (1:1000). The protein bands were visualized by enhanced chemiluminescence detection reagents (Applygen Technologies Inc., Beijing, China). The signal was detected by X-ray films (TransGen Biotech Co., Beijing, China). The optical density (OD) of each band was determined by an Image VCD gel imaging system (Beijing Sage Creation Science And Technology Co. Ltd., Beijing, China), and the iNOS expression was expressed as the ratio of the OD of iNOS and the OD of β -actin.

Statistical Analysis

Statistical analysis of all of the data was performed using the SPSS statistical software for Windows (version 13; SPSS Inc., Chicago, IL, USA). The data are expressed as mean \pm SD. When a significant value ($P < 0.05$) was obtained by one-way analysis of variance, further analysis was performed. All of the data showed a normal distribution and passed equal variance testing. Differences between means were assessed by Tukey's honestly significant difference test for post hoc multiple comparisons. Differences were considered to be significant when $P < 0.05$.

Results

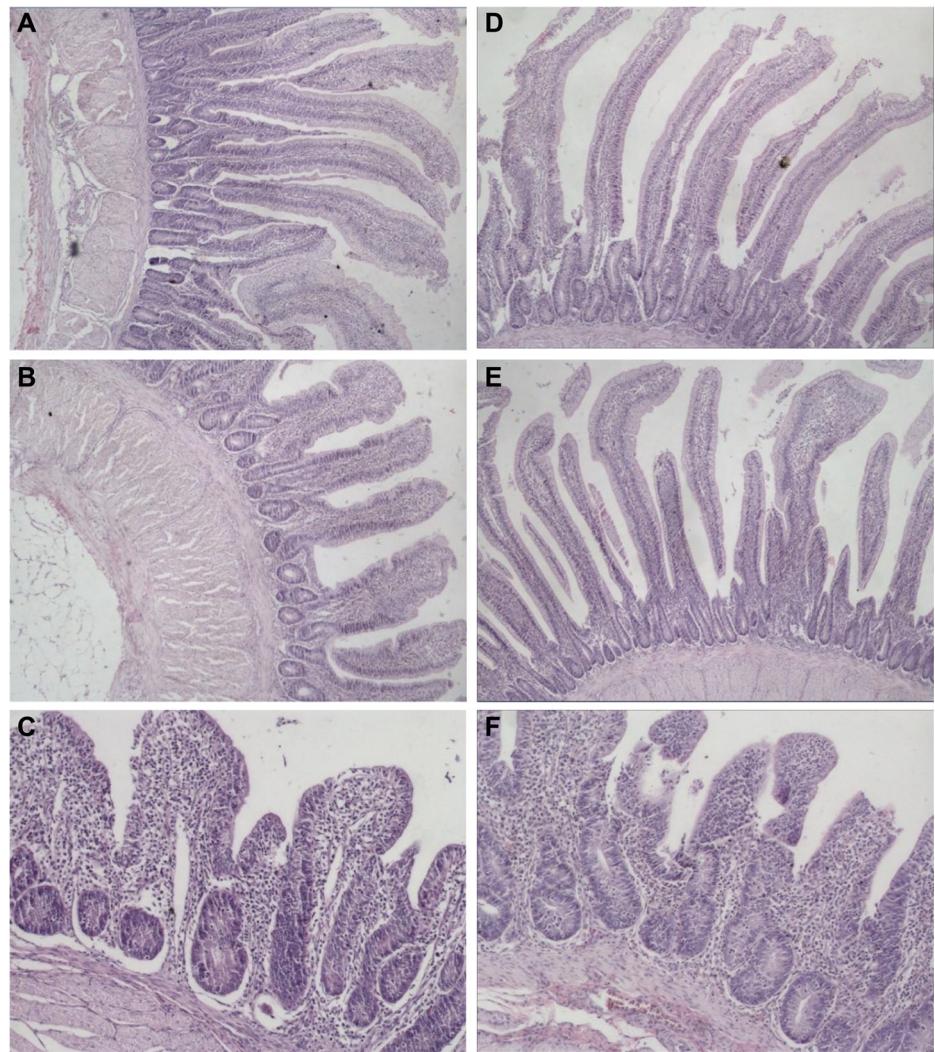
Mortality Rate of the Chickens

During the experiment, at 25 days of age, disease and death occurred in the chickens (L group). The dead chickens showed poor feathering, pancreatic degeneration, subcutaneous exudation, and necrotic lesions in different tissues. The mortality rate in the L group at 15–35 days was 2.75 %; between 45 and 55 days, no chicken death occurred.

Histopathological Observations of the Intestinal Tissues

During the entire experimental period, no ultrastructural damage was observed in the control group (Fig. 1a–c). Compared

Fig. 1 Effects of Se deficiency on ultrastructural damage in the intestinal tissues ($\times 400$). **a** Duodenum of the control group. **b** Jejunum of the control group. **c** Rectum of the control group. **d** Duodenum of the L group. **e** Jejunum of the L group. **f** Rectum of the L group



to the control group, a large number of inflammatory cells were infiltrated, the mucosa of the duodenum was shed, the structure of the jejunum was loosely arranged, the number of lymphocytes increased, and a large number of macrophages emerged in the L group at each time point (Fig. 1d–f).

Effects of Se Deficiency on the mRNA Level of GSH-Px in the Intestinal Tissues of Chickens

The expression of GSH-Px mRNA in the intestinal tissues of chickens is shown in Fig. 2. Compared with the control group, at each time point there was a significant decrease ($P < 0.05$) that could be observed in the L group between 15 and 55 days of age. As the experiment progressed, a significant decrease of GSH-Px mRNA expression was observed from 25 to 55 days in all three tissues for chickens that were fed a Se-deficient basal diet.

Effects of Se Deficiency on GSH Production and GSH-Px Activity in the Intestinal Tissues of Chickens

Figures 3 and 4 summarize the changes in GSH production and GSH-Px activity in the intestinal tissues of chickens, and both were significantly lower ($P < 0.05$) in L the group than in the control group at each time point. Compared to the control group at each of the five time points in the three tissues, the production of GSH was reduced beginning at 15 or 25 days (Fig. 3a–c). GSH-Px activity demonstrated the same behavior and decreased beginning at 15 days (Fig. 4a–c).

Effects of Se Deficiency on the MDA in the Intestinal Tissues of Chickens

From Fig. 5, compared with the control group at each time point, a significant increase ($P < 0.05$) was observed in the L group between 15 and 55 days of age. We can observe that the increases in the total MDA began at different time points in each of the three tissues. In both the duodenum and rectum,

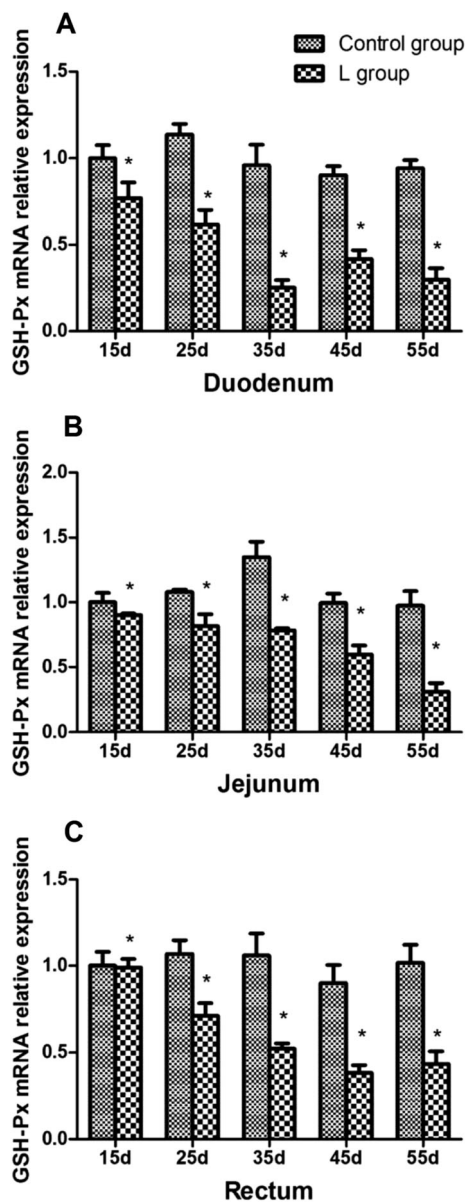


Fig. 2 Effects of Se deficiency on the mRNA expression of the GSH-Px gene in intestinal tissues of chickens. **a** Effects of Se deficiency on the mRNA level of GSH-Px in the duodenum. **b** Effects of Se deficiency on the mRNA level of GSH-Px in the jejunum. **c** Effects of Se deficiency on the mRNA level of GSH-Px in the rectum. Each value is represented as the mean \pm SD of five individuals ($n=5$). The *asterisk* indicates that there are significant differences ($P < 0.05$) between the control group and the L group at the same time point

the MDA increased at 15 days (Fig. 5a, c), but in the jejunum, the increase in the total MDA began at 25 days (Fig. 5b).

Effects of Se Deficiency on the mRNA and Protein Levels of iNOS in the Intestinal Tissues of Chickens

The expression of iNOS mRNA and protein levels in the intestinal tissues of chickens is shown in Fig. 6. Compared with the control group, a significant increase ($P < 0.05$) in the

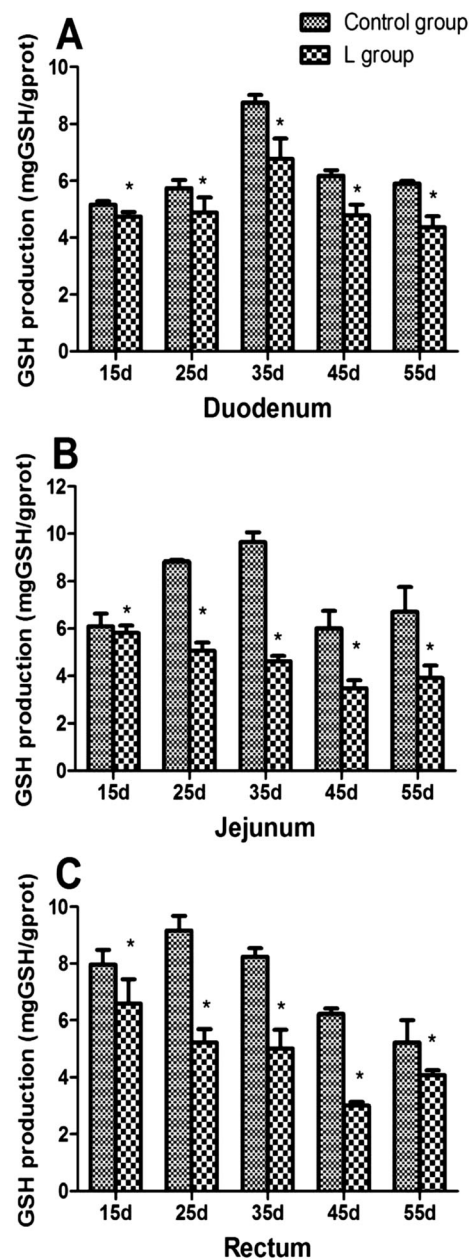


Fig. 3 Effects of Se deficiency on the GSH production in the intestinal tissues of chickens. **a** Effects of Se deficiency on the GSH production in the duodenum. **b** Effects of Se deficiency on the GSH production in the jejunum. **c** Effects of Se deficiency on the GSH production in the rectum. Each value is represented by the mean \pm SD of five individuals ($n=5$). The *asterisk* indicates that there are significant differences ($P < 0.05$) between the control group and the L group at the same time point

expression of the iNOS gene was observed in the L group from 15 to 55 days of age. However, in the rectum, the significant increase ($P < 0.05$) of the iNOS expression began at 25 days (Fig. 6c). Compared with the control group, a significant increase ($P < 0.05$) in the expressions of the iNOS protein level in the L group was observed in both the duodenum and jejunum from 15 to 55 days of age (Fig. 6d, e). Interestingly, there was no significant difference at 15 days in the rectum (Fig. 6f)

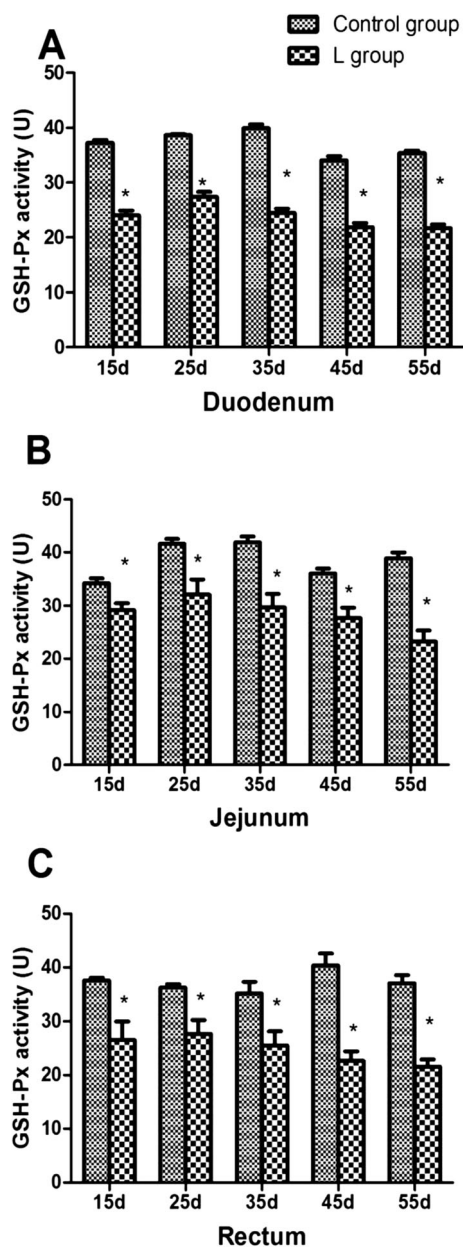


Fig. 4 Effects of Se deficiency on the GSH-Px activity in the intestinal tissues of chickens. **a** Effects of Se deficiency on the GSH-Px activity in the duodenum. **b** Effects of Se deficiency on the GSH-Px activity in the jejunum. **c** Effects of Se deficiency on the GSH-Px activity in rectum. Each value is represented by the mean \pm SD of five individuals ($n=5$). The *asterisk* indicates that there are significant differences ($P<0.05$) between the control group and the L group at the same time point

compared with the control group. The conclusion that can be drawn from Fig. 6 is that the protein levels of iNOS were consistent with the mRNA expression levels.

iNOS Activity and NO Production

The effects of Se deficiency on the iNOS activity in intestinal tissues are shown in Fig. 7. The activity of iNOS was significantly higher ($P<0.05$) in the L group than in the control

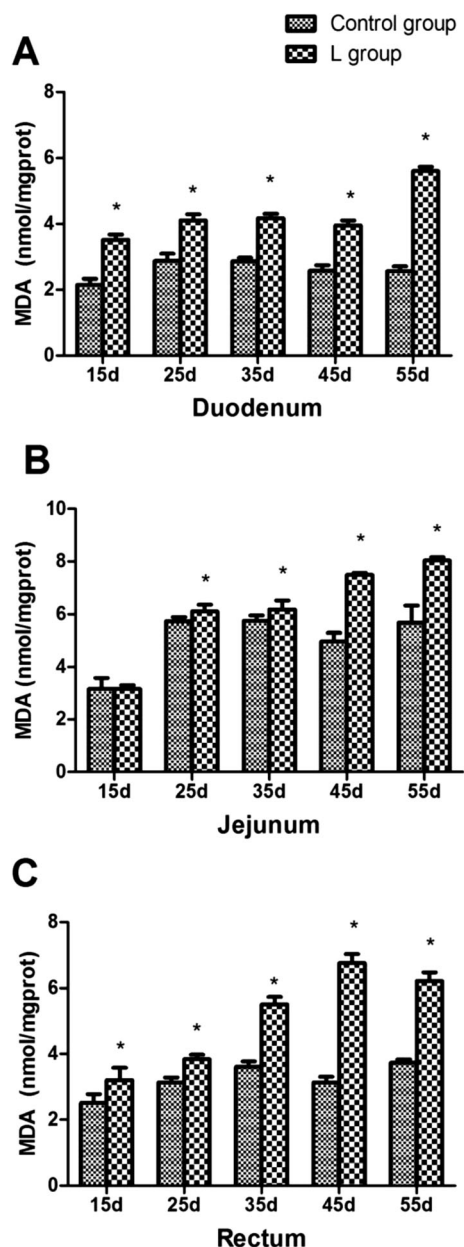
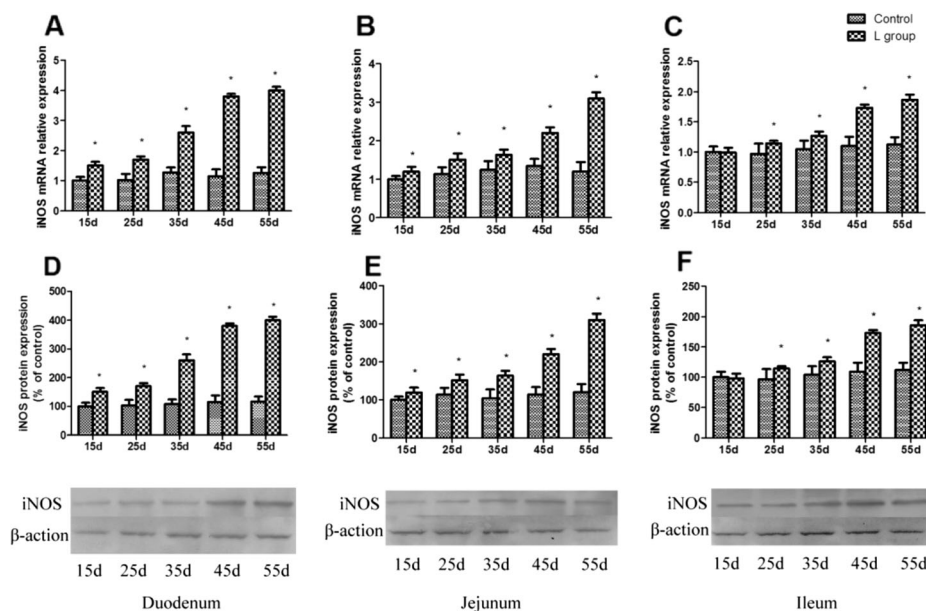


Fig. 5 Effects of Se deficiency on the MDA production in the intestinal tissues of chickens. **a** Effects of Se deficiency on the MDA production in the duodenum. **b** Effects of Se deficiency on the MDA production in the jejunum. **c** Effects of Se deficiency on the MDA production in the rectum. Each value is represented by the mean \pm SD of five individuals ($n=5$). The *asterisk* indicates that there are significant differences ($P<0.05$) between the control group and the L group at the same time point

group at each time point. NO production in the intestinal tissues of chickens was measured by calculating the production of nitrite oxide.

The effect of Se deficiency on the production of NO in intestinal tissues is summarized in Fig. 8, and compared with control group, NO production was significantly higher ($P<0.05$) in the L group duodenum and jejunum from 15 to 55 days. In the rectum, the significant upward trend ($P<0.05$) was begun at 25 days.

Fig. 6 Effects of Se deficiency on the mRNA expression of the iNOS gene in intestinal tissues of chickens. **a, d** Effects of Se deficiency on the mRNA and protein levels of iNOS in the duodenum. **b, e** Effects of Se deficiency on the mRNA and protein levels of iNOS in the jejunum. **c, f** Effects of Se deficiency on the mRNA and protein levels of iNOS in the rectum. The mRNA and protein expressions from the solvent control group were used as the reference values in **a–f**. Each value is represented by the mean \pm SD of five individuals ($n=5$). The *asterisk* indicates that there are significant differences ($P<0.05$) between the control group and the L group at the same time point



Discussion

As one important antioxidant selenoprotein, GSH-Px exists in several types of organs, is especially expressed in intestinal tissues, and has a certain physiological significance on the protection of integrity of the intestinal mucosa [19]. A decrease of GSH-Px or other antioxidative selenoproteins induced by Se deficiency has been shown to cause oxidative damage in some types of cells and intestinal tissues in lambs [20–22]. A low-Se-content diet could induce an ulcer through the down-regulation of the level of GSH-Px in the intestinal tract of rats [23]. The intestinal tissues of chickens were also affected by Se deficiency [3], but whether Se deficiency also induced the change of the GSH-Px level and caused oxidative damage in intestinal tissues of chickens was still unclear. In the present study, we examined the effect of Se deficiency on the levels of GSH-Px and the GSH in intestines of chickens. The relative data may provide us some clues as to the mechanism of Se deficiency-related diseases in intestinal tissues while also revealing the potential role of Se in intestinal damage. As one important antioxidative selenoprotein in the intestinal tract, GSH-Px is one crucial target of Se deficiency. Similarly, a decreased level of GSH-Px induced by Se deficiency has been shown to be the cause of damage to intestinal tissues. Pawłowicz et al. indicated that Se deficiency decreased the level of GSH-Px in the colons of humans and also caused oxidative stress and bowel cancer [24]. Rao reported that Se deficiency reduced GSH-Px and other antioxidant enzymes in rat intestines and led to DNA damage and oxidative stress [25]. Bolkent et al. also confirmed that a concentration of Se below 0.2 mg/kg in the diet caused increased lipid peroxidation levels and decreased glutathione levels, thus exacerbating the intestinal mucosal injury in the

intestines of rats [26]. At the same time, previous studies confirmed that not only can the GSH-Px level be reduced by oxidative stress but histological changes, such as lymphocyte infiltration and cell structure disorder in the liver of drake, were induced [27]. An investigation on a model of cerebral ischemia/reperfusion also revealed that acute oxidative injury caused histomorphology changes and even apoptosis in rats [28]. In our study, we found that Se deficiency reduced the GSH-Px activity, the GSH-Px mRNA level, and the GSH content at each time point. As the experiment progressed, the GSH-Px mRNA level decreased continuously with duration of the Se deficiency. The duodenum and jejunum showed sensitive reactions in 15 days, but in the rectum, the mRNA expression began to be reduced at 25 days. Morphological observations also revealed the obvious infiltration of inflammatory cells, intestinal mucosal lesion, and intestinal villi fractures in the duodenum and jejunum. Those results suggested that duodenum and jejunum were the primary organs targeted by Se deficiency, and the rectum has a certain tolerance for the lack of Se. The decrease of these antioxidant enzymes further caused oxidative damage and higher MDA contents, which is consistent with the research of Aw. T. Y. et al. [29]. The reduction of GSH-Px and GSH in the intestine by Se deficiency may decrease the antioxidant capacity of the intestine itself, influencing the normal functions of the intestine and thus causing further damage to the intestinal tract [30]. Because GSH-Px is the main resident selenoprotein in the intestine [31], it was reasonable to speculate that a decrease in GSH-Px may be the main pathway of Se deficiency-related intestinal disease.

NO is a small signaling molecule with important regulatory effects in many tissues. The reaction of NO with molecular oxygen allows it to function as a rapidly reversible, specific,

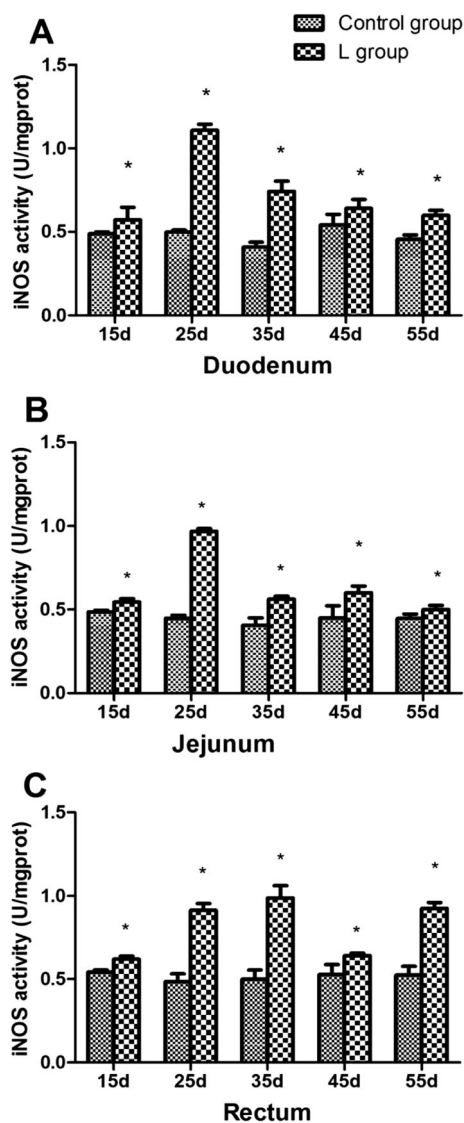


Fig. 7 Effects of Se deficiency on the iNOS activity in the intestinal tissues of chickens. **a** Effects of Se deficiency on the iNOS activity in the duodenum. **b** Effects of Se deficiency on the iNOS activity in the jejunum. **c** Effects of Se deficiency on the iNOS activity in the rectum. Each value is represented by the mean \pm SD of five individuals ($n=5$). The *asterisk* indicates that there are significant differences ($P<0.05$) between the control group and the L group at the same time point

and local signal transduction molecule in tissue damage [32]. Under physiological conditions, the sustainability and low-level expression of NO contribute to maintaining the integrity of the gastrointestinal mucosa. However, a large amount of NO leads to damage of the intestinal barrier and causes cell death [33]. The main mechanism behind this damage is that NO can combine with superoxide anions and form peroxynitrite, which is decomposed into more toxic free radicals, such as the hydroxyl free radical (-OH) and nitrogen dioxide (NO₂), causing damage to proteins, nucleic acids, and lipid membranes [34]. Prior studies indicated that Se can

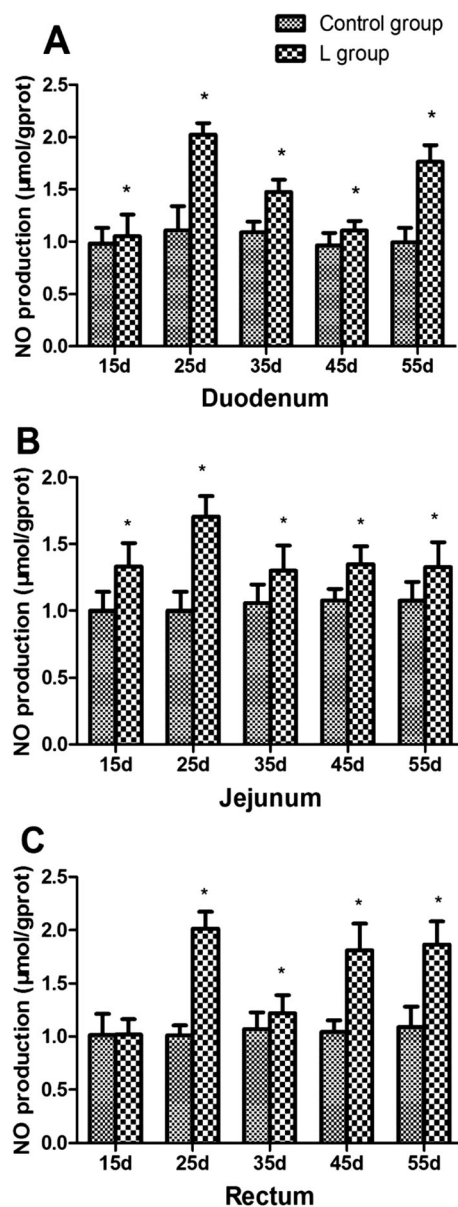


Fig. 8 Effects of Se deficiency on the NO production in the intestinal tissues of chickens. **a** Effects of Se deficiency on the NO production in the duodenum. **b** Effects of Se deficiency on the NO production in the jejunum. **c** Effects of Se deficiency on the NO production in the rectum. Each value is represented by the mean \pm SD of five individuals ($n=5$). The *asterisk* indicates that there are significant differences ($P<0.05$) between the control group and the L group at the same time point

influence the generation of NO in intestinal tissues and further regulate the normal function of intestinal tissues. Ozturk showed that Se protected the intestine against free radical damage by inhibiting the expression of iNOS in the ischemia/reperfusion (I/R) model of rats and helped to effectively maintain the integrity of the intestinal mucosal [35]. However, in a study of the small intestines of humans, it was proven that Se deficiency up-regulated the concentration of NO and led to lesions in the mucosa [36]. In addition, Se

deficiency was associated with oxidative stress through activating the pathway of the nuclear transcription factor of kappa B (NF- κ B), which further stimulated the activity of iNOS, leading to the generation of NO [37, 38]. So, the production of NO may be one important mediator for the Se deficiency-induced intestinal damage in chickens. Consistent with the studies described above, we found that Se deficiency induced high levels of iNOS and NO in all of the samples, and the iNOS mRNA expression levels and protein levels were consistent in the L group. The activity of iNOS was significantly increased at 25 days in all three of the tissues. The study of endothelial dysfunction showed that NO deprivation would reduce the cellular ROS content [39]. Considering the decreased antioxidative selenoproteins and the increased NO level in the intestine, we observed that oxidative damage is the main injury induced by Se deficiency. The results indicated that under the condition of Se deficiency, the antioxidant defense system was undermined in vivo, which led to the accumulation of oxygen free radicals and then the release of a large number of inflammatory mediators while stimulating an increase in the expression of iNOS and the excessive release of NO.

In summary, the present study showed that Se deficiency caused oxidative damage in chicken intestinal tissues by decreasing the GSH-Px production and increasing the NO level, which is released by iNOS. The results suggested that NO and oxidative stress played a major role in the injury of the intestinal tract induced by Se deficiency.

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Conflicts of Interest There are no conflicts of interest.

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