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Effects of Selenium Deficiency on Principal Indexes of Chicken Kidney Function

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Abstract Selenium (Se) deficiency leads to many pathological changes in animals. However, there have been very few reports regarding chicken tissue injury in the kidney caused by Se deficiency. In this study, a chicken Se-deficient disease model has been constructed, and two renal function indexes [including creatinine (CREA) and uric acid (URIC)], seven renal antioxidative function indexes [including glutathione peroxidase (GPx), anti-hydroxyl radical (AHR), catalase (CAT), hydrogen peroxide (H_2O_2), nitrogen monoxide (NO), glutathione (GSH), and malonyldialdehyde (MDA)], and two organ/tissue injury-related indexes [including inducible nitric oxide synthase (iNOS) and inducible heme oxygenase (HO)-1] were detected and analyzed to investigate the effects of Se deficiency on chicken kidney tissue. The results showed that Se deficiency caused a significant increase in CREA and URIC levels and a decrease in renal antioxidative capacity. Meanwhile, Se deficiency upregulated the expression of organ/tissue injury-related genes, such as the messenger RNA (mRNA) of HO-1 and iNOS as well as their protein expression levels, in the chicken kidney tissue. These data suggest that Se deficiency in birds triggers renal function regression and oxidative stress in the kidney tissue.

Keywords Selenium deficiency · Kidney tissue · Injury

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Introduction

Selenium (Se) is a crucial microelement that performs important biological functions through different selenoproteins in human and animal bodies [1–4]. Se deficiency can cause regression and disease in various tissues and organs, including "white muscle disease," cardiovascular disease, intestinal disease, hepatonecrosis, weakened immunity, and reduced reproductive ability [5–11]. The kidneys contain the highest Se level among all organs, and Se is shown to protect the kidneys by reducing renal toxicity damage, increasing the glomerular filtration rate, and easing inflammation [7, 12]. However, there has been little reported research regarding Se deficiency-induced renal injury mechanisms in birds.

Inducible heme oxygenase (HO)-1, a stress protein, is plentiful in the microsomes of mammals and is involved in antioxidative stress, anti-inflammatory injury, anti-cellular proliferation, and anti-apoptosis processes [13–15]. Inducible nitric oxide synthase (iNOS) is one of the three enzymes in the nitric oxide synthase (NOS) superfamily. It is highly expressed in pathological conditions, catalyzing the production of a large amount of nitrogen monoxide (NO) in the body and causing DNA damage and mitochondrial respiratory depression [16–22]. Therefore, HO-1 and iNOS are new target molecules used to evaluate tissue and organ injury in the body. Studies have shown that hydrogen peroxide (H_2O_2), catalase (CAT), nitrogen monoxide (NO), glutathione (GSH), glutathione peroxidase (GPx), anti-hydroxyl radical (AHR), and malonyldialdehyde (MDA) are primary antioxidative indexes that are highly correlated to Se deficiency. In addition, creatinine (CREA) and uric acid (URIC) are important indexes of kidney function [23]. To summarize, routine antioxidative indexes, HO-1 and iNOS, as well as renal function indexes are suitable for the study of molecular mechanisms in Se deficiency-related renal injury.

In this study, a Se-deficient model was constructed by serving chickens a Se-deficient diet and measuring the effects

of Se deficiency on renal function indexes (creatinine and uric acid) and routine antioxidative indexes (H_2O_2, CAT, NO, GSH, GPx, AHR, and MDA) and the expression of HO-1 and iNOS. This study provided a theoretical foundation for clarifying the molecular mechanism of Se deficiency-induced kidney injury.

Materials and Methods

Se-Deficient Animal Model and Sample Preparation

One hundred and eighty healthy 1-day-old chicks were selected, randomly divided into two groups with 90 chicks per group, and provided ad libitum access to food and water. In the control group, the normal group, the chickens were fed a diet supplemented with Se at 0.15 mg/kg; in the Se-deficient (SeD) group, the chickens were maintained on a Se-deficient diet containing Se at 0.033 mg/kg. During a feeding period of 65 days, heart blood samples were collected at days 15, 25, 35, 45, 55, and 65, followed by serum separation procedures and tissue sample acquisition. All the collected samples were stored at −80 °C.

Measurement of Renal Function Indexes and Antioxidative Indexes in Kidney Tissue

Biochemical index test kits were used according to the manufacturer's instructions to determine chicken kidney function indexes, including serum CREA and URIC levels, using a Hitachi 7150 automatic biochemical analyzer. Relevant antioxidative indexes including GPx, AHR, CAT, H_2O_2, NO, GSH, and MDA were also detected according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of HO-1 and iNOS Messenger RNA by qRT-PCR

The specific primers for β-actin, HO-1, and iNOS were designed on basis of the nucleotide sequences published by GenBank (Table 1). Quantitative PCR reactions were performed along with the SYBR Green I fluorescent dye assay on the ABI 7500 Real-Time PCR System. PCR reactions were performed in a 20-μL reaction system that included 10 μL of 2× TransStart™ Green qPCR SuperMix (+ dye), 0.6 μL (10 pmol) of forward primers, 0.6 μL (10 pmol) of reverse primers, 2 μL of template DNA, and 6.8 μL of sterile water. Reaction conditions were set as follows: denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s, and a final dissociation stage. All experiments were performed in triplicate, and the standard curve of gene expansion was constructed using a fivefold serial dilution for all the samples. The changes in HO-1 and iNOS messenger RNA (mRNA) relative to the control group were calculated as $2^{-\Delta\Delta Ct}$ using β-actin as the internal reference.

Determination of HO-1 and iNOS Protein Expression in Kidney Tissue

Tissue proteins were extracted from the kidney tissue using a tissue protein extraction kit according to the manufacturer's instructions (BestBio Company, Shanghai, China), and then, Western blot experiment was carried out. Briefly, the extracted protein from the kidney tissue was separated by 12 % SDS-PAGE. Then, the separated kidney tissue proteins were transferred to nitrocellulose (NC) membrane using a semidry transfer apparatus (Bio-Rad). The NC membrane was blocked with 5 % (*w*/*v*) nonfat dried milk in phosphate-buffered saline (PBS) at 4 °C for 12 h and then incubated with polyclonal antibodies against the recombinant HO-1 protein (1:300 dilution in PBS) or polyclonal antibodies against the recombinant iNOS protein (1:500 dilution in PBS) at 37 °C for 1 h. After washing three times with PBS, the NC membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:5000 dilution in PBST) at 37 °C for 1 h. After the X-ray film was exposed, imaged, and scanned, the grayscale values of the protein bands were determined, using β-actin as the internal reference, by grayscale analysis software. The protein expression levels were represented as the grayscale values of the HO-1 and iNOS bands and the internal reference (β-actin) bands.

Statistical Analysis

Statistical analysis of all data was performed using GraphPad Prism 5.0. One-way ANOVA and the *t* test were used for data analysis and graphic production. All data are presented as

Table 1 Primers for the real-time quantitative reverse transcription PCR

Gene name	GenBank accession no.	Primer sequences	Amplified length (bp)
β-Actin	L08165	F: 5′CCGCTCTATGAAGGCTACGC3′ R: 5′CTCTCGGCTGTGGTGGTGAA3′	128
HO-1	NM_205344.1	F: 5′ACTTCTATGGCAGCAACT3′ R: 5′AATAGCGGGTGTAGGC3′	129
iNOS	NM_204961.1	F: 5′CCTGGAGGTCCTGGAAGAGT3′ R: 5′CCTGGGTTTCAGAAGTGGC3′	81

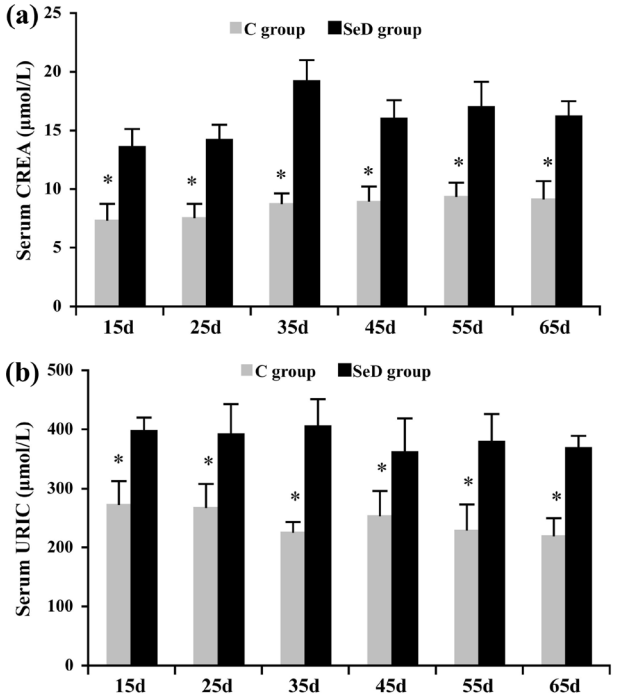

Fig. 1 Effects of selenium deficiency on creatinine (*CREA*) and uric acid (*URIC*) in the serum of chicken. **a** CREA (n=5). **b** URIC (n=5)

"average value±standard deviation." t tests were performed on the experimental results. The asterisk represents significant difference between the SeD group and the control group ($p<0.05$).

Results

Effect of Se Deficiency on Chicken Renal Function Indexes Creatinine and Uric Acid

The low-Se chick model was successfully established using the low-Se diet. The renal function indexes, including CREA and URIC, were measured. The results showed that the CREA level significantly increased in the SeD group compared to the control group ($p<0.05$), and the CREA level in the SeD showed an increasing trend from 15 to 65 days (Fig. 1a). The URIC level significantly increased in the SeD group compared to the control group ($p<0.05$), and the URIC level in the control group showed an decreasing trend from 15 to 65 days (Fig. 1b).

Effect of Se Deficiency on Chicken Kidney Antioxidative Indexes

The results of the major kidney antioxidative indexes showed that the enzymatic activity of GPx, AHR, and CAT

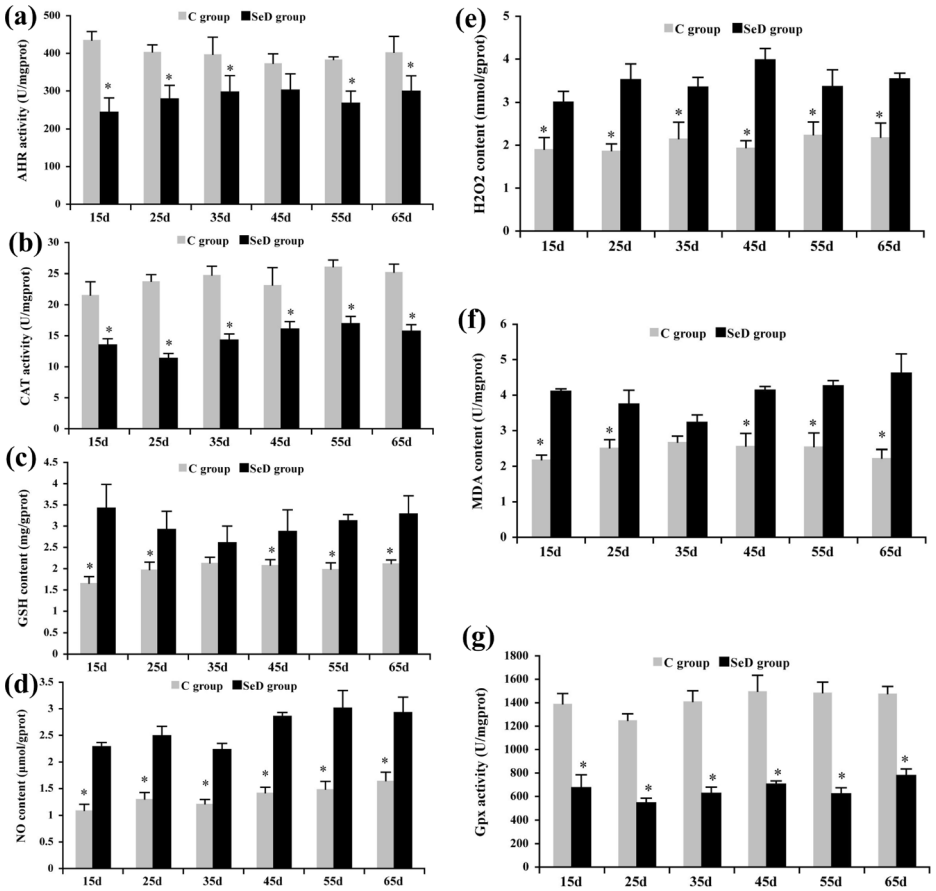

Fig. 2 Effects of selenium deficiency on antioxidative indices in kidney tissues of chicken. **a** Anti-hydroxyl radical (*AHR*) activity (n=5). **b** Catalase (*CAT*) activity (n=5). **c** Glutathione (*GSH*) (n=5). **d** Nitric oxide (*NO*) content (n=5). **e** Hydrogen peroxide (H_2O_2) content (n=5). **f** Malonaldehyde (*MDA*) content (n=5). **g** Glutathione peroxidase (*GPx*) activity (n=5)

significantly decreased in the SeD group compared to the control group ($p<0.05$), while the content of H_2O_2, NO, GSH, and MDA increased in the SeD group compared to the control group ($p<0.05$) (Fig. 2). The results reveal that Se deficiency decreased the antioxidative capacity of the kidneys.

Determination of Enzymatic Activity, mRNA, and Protein Expression Levels of iNOS in Chicken Kidney Tissue

To investigate the effect of Se deficiency on iNOS, the enzymatic activity, mRNA, and protein expression levels of the iNOS were tested in the chicken kidney tissue. The result indicated that compared to the normal group, the SeD group showed significantly increased enzymatic activity ($p<0.05$) of iNOS in the kidney tissue at the 35th, 45th, and 55th days. In addition, both the mRNA and protein expression levels of iNOS increased ($p<0.05$) in the SeD group compared to the control group (Fig. 3).

Determination of mRNA and Protein Expression Levels of HO-1 in Chicken Kidney Tissue

To investigate the effect of Se deficiency on HO-1, the mRNA and protein expression levels of HO-1 were tested using qPCR and Western blot, respectively. The result revealed that both mRNA and protein expression levels of HO-1 in the chicken kidneys increased in the SeD group compared to the normal group at the 35th, 45th, and 55th days (Fig. 4).

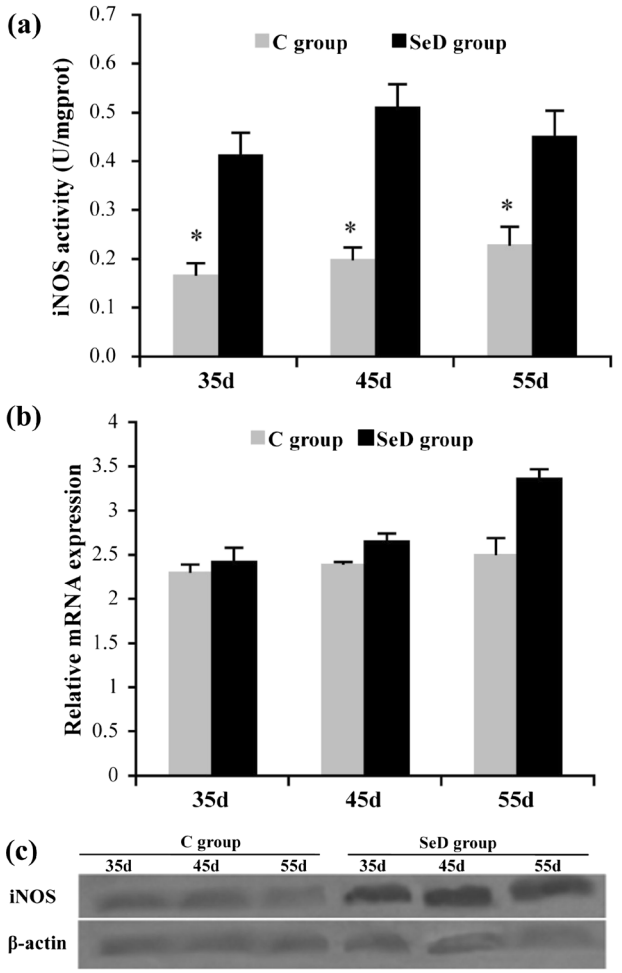

Fig. 3 Effects of selenium deficiency on inducible nitric oxide synthase (*iNOS*) activity and expression level of mRNA and protein of the iNOS gene in kidney tissues of chicken. **a** iNOS activity test ($n=5$). **b** Analysis of the mRNA expression level of the iNOS gene by qPCR ($n=5$). **c** Analysis of the protein expression level of the iNOS gene by Western blot

Discussion

The concentration of serum creatinine (CREA) depends on the glomerular filtration capacity. Therefore, when the kidney tissue is injured, the decreased glomerular filtration rate results in a surge in serum CREA concentration [24, 25]. Meanwhile, the concentration of nitrogen metabolites, such as serum uric acid (URIC), also increases due to its retention in the blood. The serum URIC is a purine metabolite that is produced in the bone marrow, muscles, and liver, and among other tissues, 75 % of which should be excreted with urine after glomerular filtration and the rest of which is excreted through the intestines with bile. URIC has a strong antioxidative capacity, playing a highly important role in preserving normal cell functions [23]. Therefore, when renal function regresses, the serum CREA and URIC levels will increase. This study showed that Se deficiency causes an increase in serum CREA and URIC levels.

H_2O_2, CAT, NO, GSH, GPx, AHR, and MDA are routine indexes used for the study of oxidative stress in the body. The results of this study showed that Se deficiency caused the increased accumulation of oxidative substances in the kidney tissue, including H_2O_2, NO, MDA, and GSH. The enzyme activity of antioxidative stress enzymes, including AHR, CAT, and GPx, decreased, indicating that Se deficiency hindered the synthesis of antioxidative Se proteins (Gpxs, Txnrd, and Selw), hence reducing the antioxidative capacity of the kidney tissue. This reduction led to the accumulation of a large amount of oxidative substances, such as free radical oxygen and NO, thus causing oxidative injury to the kidneys. These data are in agreement with the abnormal renal function indexes.

HO-1 is a stress protein that serves as an initial enzyme and a rate-limiting enzyme during the degradation of heme. HO-1 can catalyze the degradation of heme into bilirubin, CO, and Fe^{2+}. The degradation product, bilirubin, has a very strong antioxidative capacity, which protects cells from oxidative stress, clears excessive free radicals, and prevents the extensive damage caused by free radical-initiated free radical chain

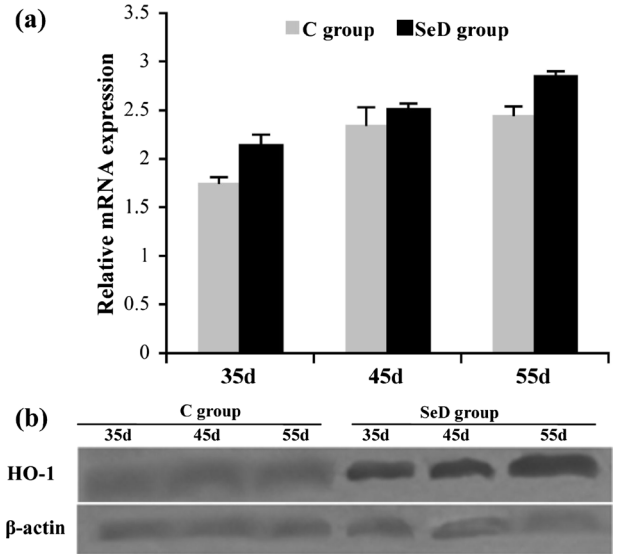

Fig. 4 Effects of selenium deficiency on the expression level of mRNA and protein of the includible heme oxygenase (*HO*)-1 gene in kidney tissues of chicken. **a** Analysis of the mRNA expression level of the HO-1 gene by qPCR (n=5). **b** Analysis of the protein expression level of the HO-1 gene by Western blot

reactions (e.g., lipid peroxidation injury). HO-1 plays an important role in maintaining the oxidation-reduction equilibrium in the body [13, 14]. Its biological properties resemble HSP70. Many stimulants can induce the expression of HO-1, such as inflammation, bilirubin, shock, radiation, high temperature, heavy metal, anoxia, and chemical toxins [26]. This study showed that the mRNA and protein expression levels of HO-1 increased in the Se-deficient chicken kidneys. In addition, there was a trend of increasing mRNA and protein expression levels of HO-1 with increased duration of Se deficiency. Thus, we speculate that Se deficiency is a harmful stimulus to the body and that the overexpression of HO-1 can alleviate pathological processes such as inflammatory damage, protein oxidation, and lipid peroxidation.

NO, as a messenger molecule, is widely distributed in various tissues and is involved in inflammatory response, signal transduction, immune regulation, and vasodilation, mediating a series of physiological and pathological process [27, 28]. Many stress conditions, such as cold stress, heavy metal, anoxia, and inflammation, can stimulate the expression of iNOS and produce a large amount of NO, which is harmful to the body tissue [20]. This study showed that compared to the normal group, the SeD group exhibited increased iNOS enzyme activity and upregulated mRNA and protein expression levels. The results suggest that Se deficiency damaged the antioxidative defense system of the bird kidney tissue. The high expression of iNOS resulted in the substantial production of NO and free radicals, causing oxidative stress in chicken kidney cells, damaging the cell membrane, and disturbing the normal metabolism of substances and energy. These events disrupted the biochemical equilibrium in the body and resulted in a series of pathological changes. This result is in agreement with the abnormal routine kidney function indexes and the changes in body antioxidative indexes.

Se, which shows high-level expression in the kidney tissue, plays an important role in the maintenance of renal function. The present study revealed that Se deficiency induced the overexpression of HO-1 and iNOS, promoted serum creatinine and uric acid levels, and impaired the antioxidative defense capacity in the kidney tissue. These data regarding renal function indexes, antioxidant index, HO-1, and iNOS support the potential fact that Se deficiency resulted in reduced biosynthesis of antioxidative selenoproteins, weakened antioxidative capacity of kidney tissue, and strengthened oxidative injury to the kidneys. To the best of our knowledge, these data will provide a valuable insight into the effects of selenium deficiency on chicken kidney function. However, the exact mechanisms needed to be further studied.

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Conflict of Interest The authors declare that there were no conflicts of interest.

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