Biol Trace Elem Res (2016) 173:492–500
DOI 10.1007/s12011-016-0673-8

Selenium Deficiency Activates Heat Shock Protein Expression in Chicken Spleen and Thymus

Pervez Ahmed Khoso[1] · **Ci Liu**[1] · **Chunpeng liu**[1] · **Mir Hassan Khoso**[1] · **Shu Li**[1]

Received: 20 December 2015 / Accepted: 14 March 2016 / Published online: 23 March 2016
© Springer Science+Business Media New York 2016

Abstract Heat shock proteins (Hsps) are protective proteins present in nearly all species; they are used as biomarkers of various stress conditions in humans, animals, and birds. Selenium (Se) deficiency, which can depress the production of Hsps, can cause chicken tissue injuries. To investigate Hsp production, mRNA, and protein levels in Se-deficient chicken spleens and thymuses, a total of 180 1-day-old sea blue white laying hens (90 chickens/group) were harvested in two groups (the control group and the Se-deficient group) in 15, 25, 35, 45, and 55 days, respectively. The results showed that mRNA levels of Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 were significantly increased in the spleens and thymuses of the Se-deficient group compared to the control group. Further protein levels of Hsp60, Hsp70, and Hsp90 were also significantly increased in the spleen and thymus of the Se-deficient group compared to the control group. Meanwhile, the spleen expression ratio of Hsp40 mRNA level and Hsp70 protein level were higher in the Se-deficient group than other proteins. In the thymus, the Hsp90 mRNA level and Hsp60 protein expression level were the highest level in the Se-deficient group among other proteins. Based on these results, we concluded that Se deficiency could induce a protective stress response in chicken by means of promoting the mRNA and protein expression of Hsps, thus easing the effects of Se deficiency to some extent.

✉ Shu Li
lishu@neau.edu.cn

[1] College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, People's Republic of China

Keywords Selenium deficiency · Heat shock proteins · Spleen · Thymus · Chicken

Introduction

Selenium (Se) is a biologically important trace element for organs providing immunity. It plays a role in reducing oxidative stress; regulating the function of neutrophils, NK cells, B lymphocytes, and T cells [1]; and improving the immune status and anti-inflammatory action [2]. Se-deficient diets inhibit bursal and thymic growth [3] and suppress immune function [4]. Further, Se deficiency weakens body resistance by lowering antibody production and inhibiting immune response. The spleen and thymus are main immune organs in chickens. Se deficiency caused oxidative stress and showed negative effects on the spleen and thymus of chickens [5]. In addition, studies in mice showed that Se deficiency caused dysfunction of the adaptive immune response and caused moderate to severe atrophy in the spleen and thymus [6].

Heat shock proteins (Hsps) are typical types of highly conserved, stress-related proteins, generated by various factors such as physiological disturbance, chemical factor, free radicals, and environmental stress in the bodies of living species. Hsps are complex networks of proteins that take part in the assembly and disassembly, translocation, and folding and unfolding of proteins [7]. Hsps are also major factors for enhancing various signal proteins such as Cdk4, cell cycle kinase, and steroid hormones receptors for immune defense reaction. Hsps protect cytoplasm contents, enhance immune response, and act further as danger signal proteins in immune cells [8]. Hsps rapidly activate in the tissues in any unfavorable conditions. The Lockwood study indicated that Hsp90, Hsp70, and Hsp60 showed protective effects in cells of blue muscle under heat stress [9]. Hsps play important roles in heat

stress, antioxidant defense system, and inflammatory injury in the livers of Pekin and Muscovy ducks through increased expression of Hsp40, Hsp60, and Hsp70 [10]. Studies in *Sphincterochila* species revealed that different types of Hsp expression produce protection so the species can adapt to conditions unfavorable for their survival in that environment [11]. Liu found that in pigeons, avermectin caused damage in the spleen; high expression of Hsps provided protective effects for the spleen [12]. Hsp27 enhanced the organization of cytoskeleton and took part in stabilizing intracellular actin filaments. Expression of Hsp27 enhanced the ability of cells to prevent injury in stress conditions, and lung cancer risk increased with low levels of Hsp27 expression in lymphocytes [2]. In cultured cells, organs, and whole animal models, Hsp27 and Hsp70 showed cytoprotective effects [13]. Hsp40 and Hsp70 mRNA expression increased in oxidative stress conditions under cold stress in the hearts of chickens [14]. Mitochondria-specific cell stress directly related to expression of Hsp60. Heat stress-produced oxidative damages caused increased expression of Hsp60 in the livers of Channa striatus [3]. Hsp70 protected stress cells and organs; it reduced oxygen radical toxicity and increased synthesis of inducible proteins. Experiments on the liver, brain, kidney, and gills of common carp showed that Hsp70 was increased with treatment of atrazine or chlorpyrifos [15]. In the human bladder, lungs, skin, and increased levels of lymphocytes, Hsp70 was associated with cancer in dose-dependent manure [16]. Previous study has found that Se could decrease the levels of Hsp70 in poultry [17]. Hsp90 has an important role in innate immune functions and cellular response and maintains oxidation reduction homeostasis, protein folding, protein degradation, and signal transduction. High expression of Hsp90 was recorded in heavy-metal oxidative stress. The mRNA expression of Hsp90 was increased with treatment of zinc in insects [18]. All of the previous study shows that Hsps are sensitive to different supplements and unfavorable conditions. However, no comprehensive study exists on effects of Se deficiency on Hsps in the chicken spleen and thymus. In this study, we checked the mRNA and proteins of Hsps in the spleen and thymus of chickens because of chickens stress exposure nature. We found that Hsp levels increased in the spleens and thymuses of chickens with Se deficiency. This increase of Hsps in Se deficiency showed a stressful hierarchy in the chicken spleens and thymuses.

Materials and Methods

Animal Diet and Experimental Design

This study was carried out at the College of Veterinary Medicine, Northeast Agriculture University, China; the Institution of Animal Care and Use Committee approved this experiment. A total of 180 1-day-old sea blue white laying hens (90 chickens per group) were divided into two groups. The control group was fed a diet containing 0.15 mg/kg (sodium selenite) of Se with a basal diet, and the Se-deficient group was fed a diet containing 0.033 mg/kg of Se with a basal diet. The basal diet was produced from corn and soy from the Se-deficient area of Heilongjiang Province, China (Table 1). The birds were provided a neat and clean environment according to standard management practice of laying chickens. Before scarification of the birds, they were first euthanized with sodium barbital to avoid stress. Spleen and thymus tissues were collected on days 15, 25, 35, 45, and 55, and the tissues were immediately processed and stored at −80 °C until use.

Method for Quantitative Real-Time PCR

According to the manufacturer's instructions, TRIzol reagent (TaKaRa, Dalian, China) was used to extract total RNA from the spleen and thymus tissues. Total RNA purity and concentration were checked through spectrophotometric technique at 260/280 nm. Primer Premier Software (PREMIER Biosoft International, USA) was used to design specific primers for Hsp27, Hsp40, Hsp60, Hsp70, Hsp90, and GADPH (Table 2). General PCRs were first performed to confirm the specificity of the primers. First-strand complementary DNA (cDNA) was synthesized from 5 μg of total RNA, using oligo(dT)18 primers and SuperScript II reverse transcriptase according to the manufacturer's instructions (Invitrogen, China). The reaction mixtures for quantitative real-time PCR (qPCR) consisted of the following: 10 μl of 2× SYBR Green I PCR Master Mix (TaKaRa, Dalian, China), 2 μl of diluted cDNA, 0.4 μl of each primer (10 μM), 0.4 μl of 50× ROX reference dye II, and 6.8 μl of PCR-grade water. The PCR program was managed as 1 cycle at 95 °C for 30 s, 40 cycles at 95 °C for 15 s, and at 60 °C for 30 s. Quantitative real-time

Table 1 Basal diet composition

Se deficiency diet regime	Total %
Feedstock	64.73
Mountain flour	1.3
Salt	0.27
Lysine	0.4
Chlorine chloride	0.07
Vitamins	0.04
Soybean	28
Calcium hydrophosphate	2.3
Soybean oil	2.5
DL-methonine	0.19
Complex minerals	0.2
Total	100

Table 2 Gene-specific primers used in the qPCR

Gene	Serial number	Primer sequence (5,→3)
Hsp90	NM_001109785.1	Forward: TCCTGTCCTGGCTTTAGTTT
		Reverse: AGGTGGCATCTCCTCGGT
Hsp70	NM_001006685.1	Forward: CGGGCAAGTTTGACCTAA
		Reverse: TTGGCTCCCACCCTATCTCT
Hsp60	NM_001012916.1	Forward: AGCCAAAGGGCAGAAATG
		Reverse: TACAGCAACAACCTGAAGACC
Hsp40	NM_001199325.1	Forward: GGGCATTCAACAGCATAGA
		Reverse: TTCACATCCCCAAGTTTAGG
Hsp27	NM_205290.1	Forward: ACACGAGGAGAAACAGGATGAG
		Reverse: ACTGGATGGCTGGCTTGG
β-actin	L08165	Forward: CCGCTCTATGAAGGCTACGC
		Reverse: CTCTCGGCTGTGGTGGTGAA

PCR (qPCR) was performed on an ABI PRISM 95 7500 Detection System (Applied Biosystems, Foster City, CA). A dissociation curve was run for each plate to confirm the production of a single product. The amplification efficiency for each gene was determined using the DART-PCR program. The mRNA relative abundance was calculated according to the $2^{-\Delta\Delta CT}$ method, accounting for gene-specific efficiencies, and normalized to the mean expression of GADPH.

Western Blot Analysis

Spleen and thymus tissues were first centrifuged, and supernatants were collected. The protein was then subjected to SDS-PAGEs on 12 % gels on electrophoresis and transferred to polyvinylidene difluoride (PVDF) membrane. The membranes were incubated with 5 % skim milk overnight at 4 °C. After that, they were incubated 1 h at 37 °C with primary antibodies. After washing, they were incubated in peroxidase-conjugated secondary antibodies against rabbit IgG at 37 °C for 1 h and washed. The signal was detected by ChemiScope 5300 (Clinx Science Instruments, Shanghai, China). The optical density (OD) of each band was determined by Image VCD gel imaging system. Hsp90, Hsp70, and Hsp60 expression levels were detected as the ratio of OD of Hsp90, Hsp70, Hsp60, and OD of β-actin, respectively.

Statistical Analysis

All data were statistically analyzed using the SPSS statistical software for Windows (version 13; SPSS, Chicago, IL, USA). A significant value ($P < 0.05$) was obtained by *t* test analysis of variance. Data are expressed as the mean ± SE. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results

Health Status of Birds

Birds showed normal drinking and feeding, bright feathers, good physical growth, and normal excreta in the control group. No mortality or other diseases were found in either group during the exposure of Se deficiency. Near the end of the experiment, some abnormalities, such as reduced movement, growth retardation, and green-bluish or brown color spots on the face, chest, and wing regions were observed in the Se-deficient group.

Hsp mRNA Levels in the Spleen and Thymus of Chickens

To evaluate the effects of Se deficiency on Hsps mRNA levels, we used quantitative real-time PCR. Results showed that (Fig. 1a–e) Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 mRNA levels were significantly increased ($P < 0.05$) in the spleen of the Se-deficient group compared to the control group. Further, the Hsp40 mRNA level was observed higher than other proteins in the spleen. Thymus results showed that (Fig. 2a–e) Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 mRNA levels were significantly increased ($P < 0.05$) in the Se-deficient group compared to the control group. The Hsp90 mRNA level was highly increased with Se deficiency among other proteins in the thymus.

Hsps Protein Levels in the Spleen and Thymus of Chickens

To determine the effects of Se deficiency on Hsps protein levels, we performed the Western blot technique. Results for Hsp protein levels showed that (Fig. 3a–c) Hsp60, Hsp70, and

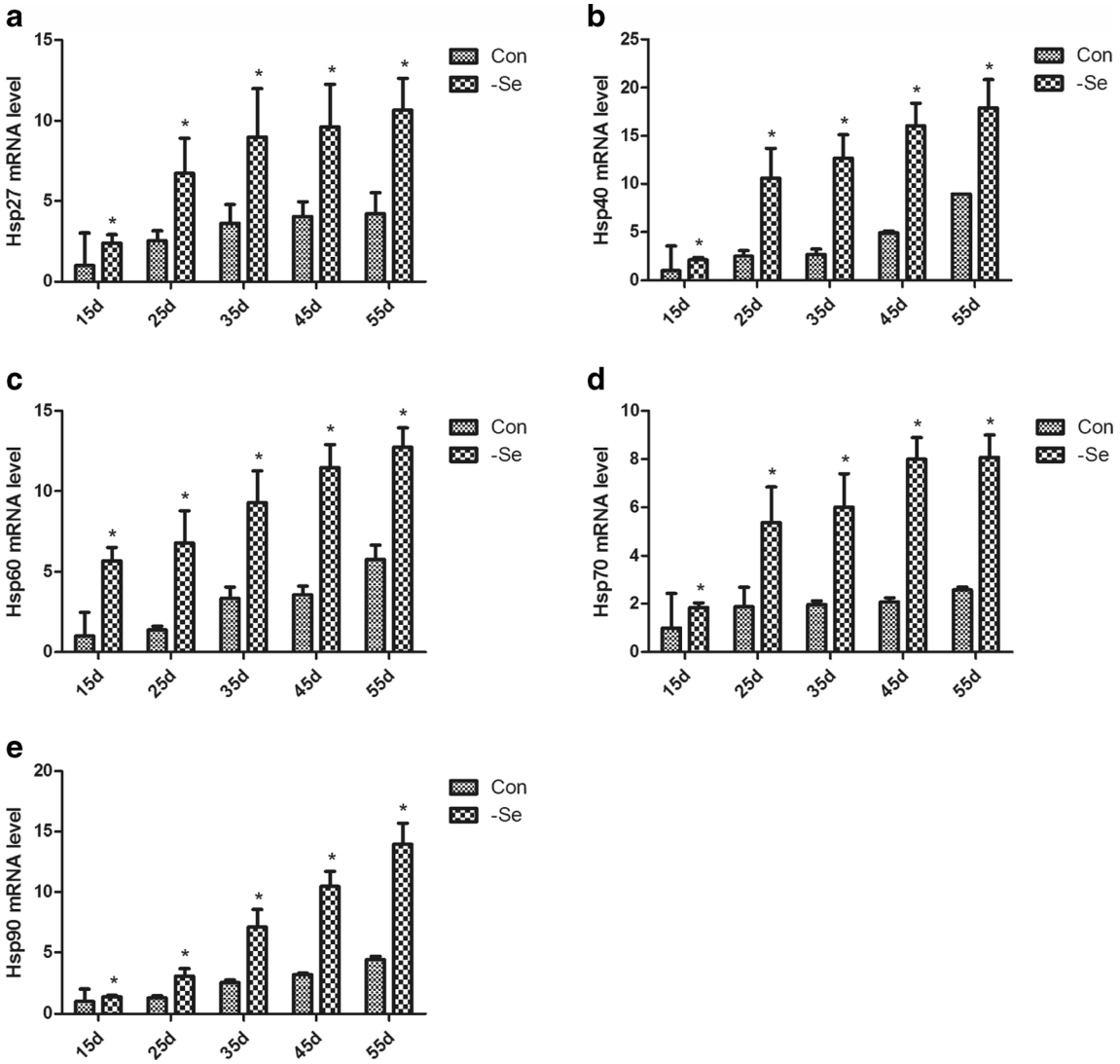

Fig. 1 Se deficiency effects on the Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 mRNA expressions in the spleens of chickens. **a**–**e** The mRNA expression of Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90. *$P < 0.05$ indicates that there are significant differences between the control group and the Se-deficient group at the same time point

Hsp90 were significantly increased ($P < 0.05$) in the Se-deficient group compared to the control group in the spleens of the chickens. The chicken thymus results showed that (Fig. 4a–c) Hsp60, Hsp70, and Hsp90 protein levels were significantly increased ($P < 0.05$) in the Se-deficient group compared to the control group. Moreover, protein levels of Hsp70 increased greatly in the spleen and thymus; Hsp60 protein levels increased more than the other proteins.

Discussion

Se is an important element for normal function of the immune system and protects the host immune system through its antioxidant properties. Previous study in heat-stressed broiler chickens showed that Se influenced the immune function and acted as an antioxidant element [19]. Zhang found that Se-deficient diets induced oxidative stress in chickens' immune organs [20]. Hsps are major factors for enhancing various signal proteins such as Cdk4, cell cycle kinase, steroid hormones receptors, and immune defense reaction [21]. Hsps associated synthesis of misfold and polypeptide folding under stress conditions. Hsps are concerned with immune functions in immune systems, and Hsps are associated with adaptive and innate immune systems. In different stress conditions, Hsps and other physiological elements enhance immune functions.

There are different types of Hsps, known with different names and their molecular weight, such as Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90. Free radicals, oxidized low-density lipoprotein, ischemia, heat, oxidants, and cytokine stimulation stress conditions increased artery wall cells' Hsp levels [22]. There is enough evidence to show that Hsps play

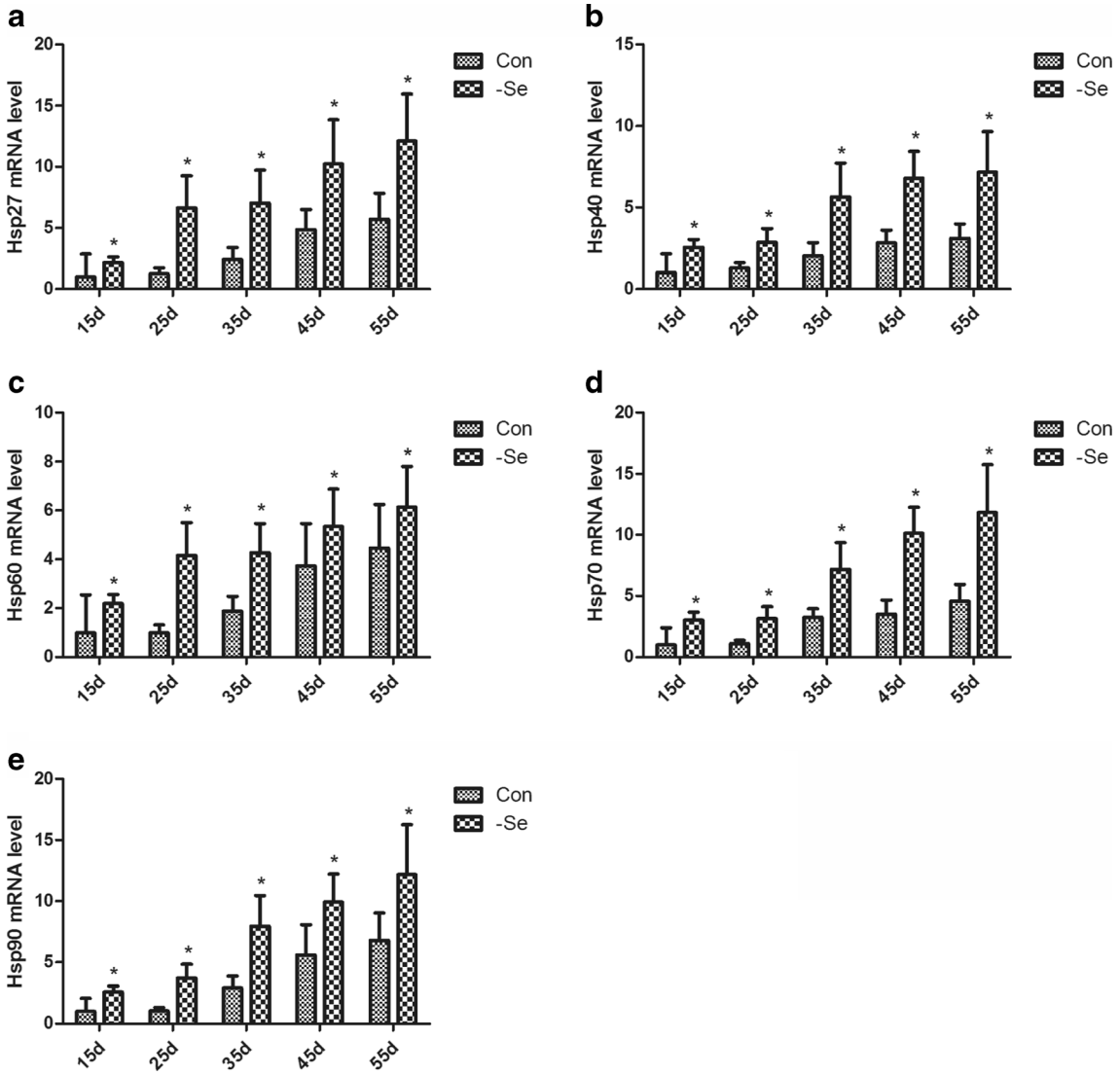

Fig. 2 Se deficiency effects on the Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 mRNA expressions in the thymus of chickens. **a**–**e** The mRNA expression of Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90. *$P < 0.05$ indicates that there are significant differences between the control group and the Se-deficient group at the same time point

protective roles. We found that Hsp27 levels increased in the spleen and thymus of Se-deficient groups of chickens. This increase in the Hsp27 level indicates the protective mode of action during Se deficiency in these organs. Similar to our results, Hollander showed that ischemia–reperfusion injury of the heart decreased with increased expression of Hsp27 in animals [23]. Zhao found that Hsp27 and other Hsps also played special roles in chicken erythrocyte injury from Se deficiency in chickens [24]. Hsp40 found different domains of life and work for degradation, translocation, protein translation, and folding and unfolding of proteins [25]. We showed that the Hsp40 level was increased in the spleen and thymus under Se deficiency. Increase in Hsp40 in the spleen and thymus of chickens may be involved in the some critical function of degradation and translocation of the injured cells observed during Se deficiency. Park showed that Hsp40 was increased in *Chironomus riparius* with exposure of fenbendazole [26]. Hsp60 increased in bovine urinary bladder mucosa during neoplastic transformation, and this was an early mark of this disease in bovines [27]. In the present investigation, we also found that Hsp60 was increased in the spleen and thymus of chickens with Se deficiency. This increase in Hsp60 in both organs is also indication of the formation of disease during Se deficiency. Chen found that Se deficiency particularly increased Hsp40 and Hsp60 during Se deficiency in neutrophils. Increases in Hsp40 have protective roles, and Hsp60 exerted an important function in inhibiting the production of NO in neutrophils [28]. We found that Se deficiency also increased Hsp40, which protected the spleen

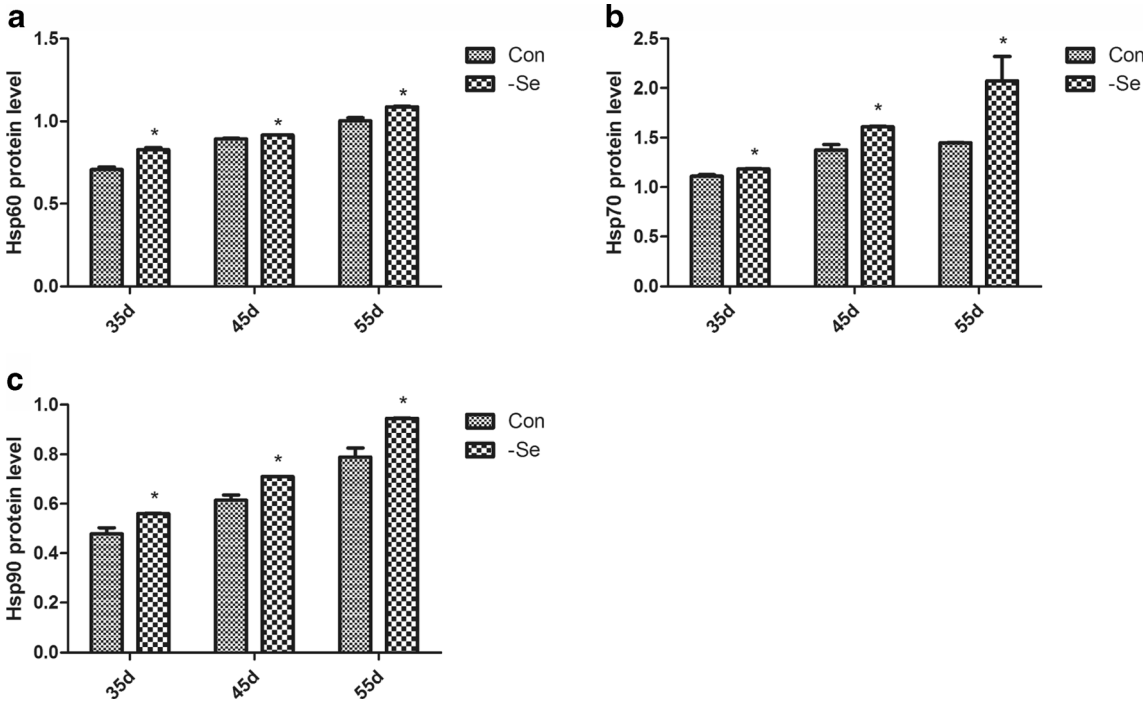

Fig. 3 Se deficiency effects on the Hsp60, Hsp70, and Hsp90 protein expressions in spleens of chickens. **a**–**c** The protein expression of Hsp60, Hsp70, and Hsp90. *$P < 0.05$ indicates that there are significant differences between the control group and the Se-deficient group at the same time point

from damage, and increases in Hsp60 might have inhibited the process of inflammation in the thymus of chickens. Monari found Hsp70 response to physical and chemical stress in *Chamelea gallina* with overexpressed of Hsp70 during this condition [29]. We found that Hsp70 was also increased in the spleen and thymus of chickens, which leads to stress from Se deficiency diet in these tissues. Zhang investigated the possibility that intracellular Hsp70 was increased during the exposure of LPS in fish [30]. Further study in the heart and

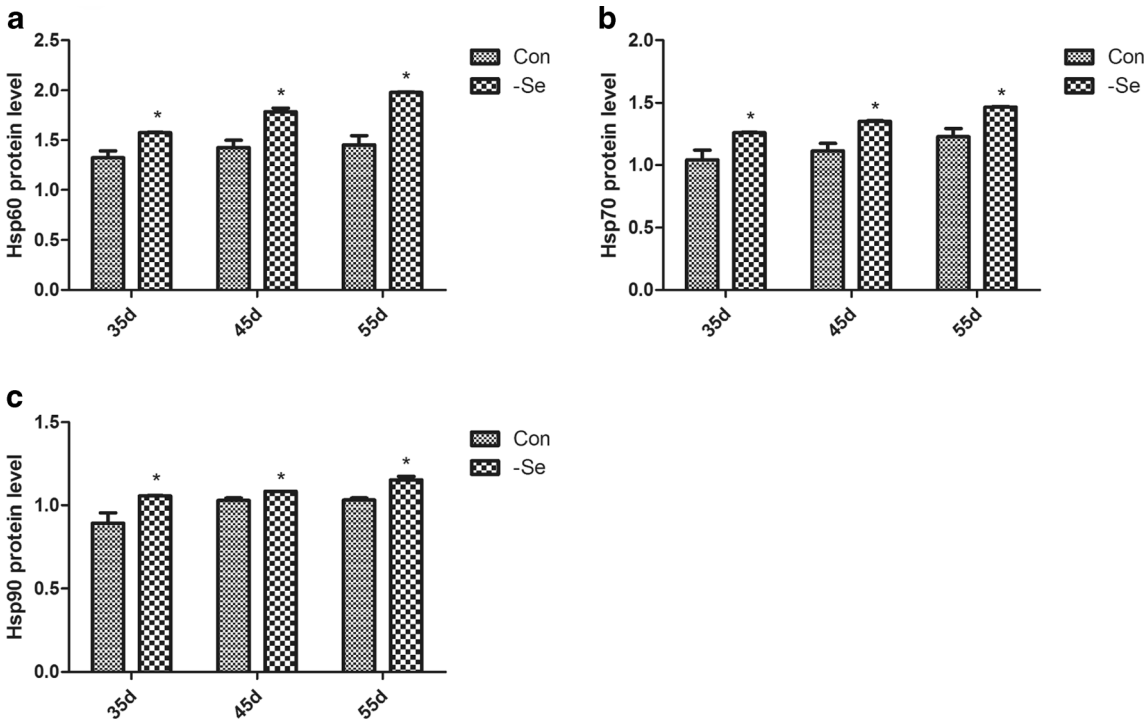

Fig. 4 Se deficiency effects on the Hsp60, Hsp70, and Hsp90 protein expressions in the thymus of the chicken. **a**–**c** The protein expression of Hsp60, Hsp70, and Hsp90. *$P < 0.05$ indicates that there are significant differences between the control group and the Se-deficient group at the same time point

blood vessels showed that mRNA levels of Hsp40, Hsp60, and Hsp70 were increased in heat-stressed broilers [31]. Hsp90 was crucial in stresses and heat shock conditions [32]. We determined that Hsp90 increased in the spleen and thymus of chickens with Se deficiency, and increased Hsp90 in chicken spleen and thymus expresses in stressful atmospheres with Se deficiency. A study in the Pacific oyster, *Crassostrea gigas*, showed that levels of Hsp90 were increased in concentration high in Cd [33]. During treatment with di(2-ethylhexyl) phthalate) concentration in *C. riparius* larvae, the Hsp90 level was elevated [34]. In chicken spleen lymphocytes, manganese-induced cytotoxicity caused increased mRNA levels of Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 [35]. In the liver of chickens, Se caused injuries and oxidative stress, which leads to increased expression of Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 mRNA and protein levels, and increased expression of Hsps have protected liver tissues from damage [36]. We determined that Se deficiency caused increases in Hsp60, Hsp70, and Hsp90 in chicken spleen and thymus (Figs. 5 and 6). Further, Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 were significantly elevated in the immune organs of chickens and showed protective effect in oxidative stress conditions during cold stress exposure [37]. Our previous study showed that Hsps play important roles in immunosuppression in the bursa of Fabricius of chickens with Se deficiency, which enhances the Hsp60, Hsp70, and Hsp90 mRNA and protein levels [38]. Li found that expression of Hsps increased in the brain with AVM treatment, which prevented neurotoxic effect and protected neuron tissues in the brain of pigeons [39]. Further, Liu found that in the bursa and spleen of the black-bone chicken, resveratrol caused overexpression of Hsp27, Hsp70, and Hsp90 levels in heat-stress environments [40]. Studies

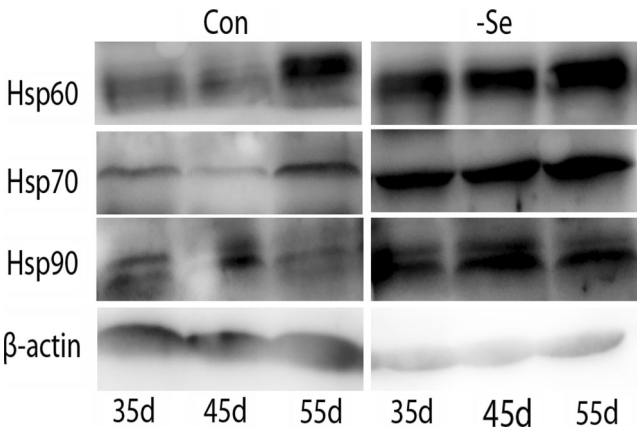

Fig. 5 The protein expression of Hsp60, Hsp70, Hsp90, and β-actin in the spleens of chickens

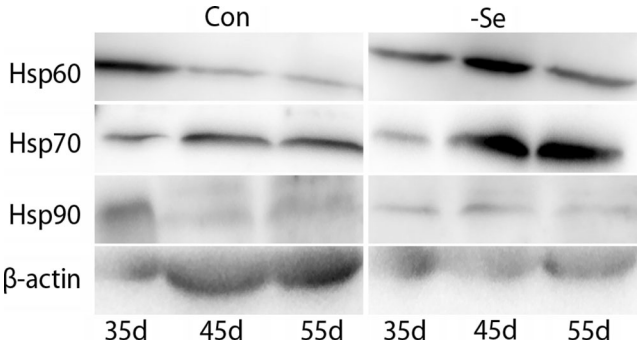

Fig. 6 Hsp60, Hsp70, Hsp90, and β-actin protein expression in the thymus of chickens

in the heart of chickens showed that Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 were increased under cold exposure [14]. Moreover, increased Hsp90, Hsp70, and Hsp60 amounts were observed after treatment with cis-bifenthrin in rat adrenal pheochromocytoma cells [41]. In addition, Hsps also increased in cold (hot) temperature, mild restraint, isolation in the dark, loud noise, stressful social conditions, and inescapable stress situations.

As mentioned earlier, a wide range of causes triggers protective mechanisms that are mediated by Hsps. It has been reported that Hsp inductions were generally correlated with early intracellular events and are secondary consequences of damage that affects cellular integrity. The lipophilic nature of the compounds easily allows them to pass plasma membranes and alter vital cellular functions before interacting with cellular proteins, denaturing them and triggering stress protein induction. A possibility that Se deficiency could evoke Hsps induction in the spleen and thymus of chickens in a similar way cannot be ruled out.

Conclusion

The present results showed that Se deficiency caused increase in Hsps expression in chicken spleen and thymus. This increase in Hsps may be an indication of the protective effects of Hsps in the spleen and thymus under Se deficiency.

Acknowledgments The authors thank the members of the Veterinary Internal Medicine Laboratory, College of Veterinary Medicine, Northeast Agriculture University, for the meaningful help in collecting the chicken spleen and thymus samples and providing lab facilities during this study trial.

Compliance with Ethical Standards The Institution of Animal Care and Use Committee of the Northeast Agricultural University approved this experiment.

Funding This study was supported by the National Natural Science Foundation of China (Grant No. 31472161).

References

1. Hawkes WC, Kelley DS, Taylor PC (2001) The effects of dietary selenium on the immune system in healthy men. Biol Trace Elem Res 81(3):189–213
2. Wang F, Feng M, Xu P, Xiao H, Niu P, Yang X, Bai Y, Peng Y, Yao P, Tan H, Tanguay RM, Wu T (2009) The level of Hsp27 in lymphocytes is negatively associated with a higher risk of lung cancer. Cell Stress Chaperones 14(3):245–251
3. Purohit GK, Mahanty A, Suar M, Sharma AP, Mohanty BP, Mohanty S (2014) Investigating hsp gene expression in liver of Channa striatus under heat stress for understanding the upper thermal acclimation. BioMed Research International 2014:381719
4. Khoso PA, Yang Z, Liu C, Li S (2015) Selenium deficiency downregulates selenoproteins and suppresses immune function in chicken thymus. Biol Trace Elem Res. doi:10.1007/s12011-015-0282-y
5. You L, Liu C, Yang ZJ, Li M, Li S (2014) Prediction of selenoprotein T structure and its response to selenium deficiency in chicken immune organs. Biol Trace Elem Res 160(2):222–231
6. Shrimali RK, Irons RD, Carlson BA, Sano Y, Gladyshev VN, Park JM, Hatfield DL (2008) Selenoproteins mediate T cell immunity through an antioxidant mechanism. J Biol Chem 283(29):20181–20185
7. Bernabo P, Rebecchi L, Jousson O, Martinez-Guitarte JL, Lencioni V (2011) Thermotolerance and Hsp70 heat shock response in the cold-stenothermal chironomid *Pseudodiamesa branickii* (NE Italy). Cell Stress Chaperones 16(4):403–410
8. Wang Z, Wu Z, Jian J, Lu Y (2009) Cloning and expression of heat shock protein 70 gene in the haemocytes of pearl oyster (Pinctada fucata, Gould 1850) responding to bacterial challenge. Fish Shellfish Immunol 26(4):639–645
9. Lockwood BL, Sanders JG, Somero GN (2010) Transcriptomic responses to heat stress in invasive and native blue mussels (genus Mytilus): molecular correlates of invasive success. J Exp Biol 213(Pt 20):3548–3558
10. Zeng T, Li JJ, Wang DQ, Li GQ, Wang GL, Lu LZ (2014) Effects of heat stress on antioxidant defense system, inflammatory injury, and heat shock proteins of Muscovy and Pekin ducks: evidence for differential thermal sensitivities. Cell Stress Chaperones 19(6):895–901
11. Mizrahi T, Heller J, Goldenberg S, Arad Z (2012) The heat shock response in congeneric land snails (Sphincterochila) from different habitats. Cell Stress Chaperones 17(5):639–645
12. Liu C, Li M, Cao Y, Qu JP, Zhang ZW, Xu SW, Li S (2014) Effects of avermectin on immune function and oxidative stress in the pigeon spleen. Chem Biol Interact 210:43–50
13. Garrido C, Brunet M, Didelot C, Zermati Y, Schmitt E, Kroemer G (2006) Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties. Cell Cycle 5(22):2592–2601
14. Zhao FQ, Zhang ZW, Wang C, Zhang B, Yao HD, Li S, Xu SW (2013) The role of heat shock proteins in inflammatory injury induced by cold stress in chicken hearts. Cell Stress Chaperones 18(6):773–783
15. Xing H, Li S, Wang X, Gao X, Xu S, Wang X (2013) Effects of atrazine and chlorpyrifos on the mRNA levels of Hsp70 and Hsc70 in the liver, brain, kidney and gill of common carp (*Cyprinus carpio* L.). Chemosphere 90(3):910–916
16. Yang XB, Zheng JP, Bai Y, Tian FJ, Yuan J, Sun JY, Liang HS, Guo L, Tan H, Chen WH, Tanguay RM, Wu TC (2007) Using lymphocyte and plasma Hsp70 as biomarkers for assessing coke oven exposure among steel workers. Environ Health Persp 115(11):1573–1577
17. Mahmoud KZ, Edens FW (2005) Influence of organic selenium on Hsp70 response of heat-stressed and enteropathogenic Escherichia coli-challenged broiler chickens (*Gallus gallus*). Comp Biochem Physiol Toxicol Pharmacol: CBP 141(1):69–75
18. Shu Y, Du Y, Wang J (2011) Molecular characterization and expression patterns of Spodoptera litura heat shock protein 70/90, and their response to zinc stress. Comp Biochem Physiol Part A, Mol Integr Physiol 158(1):102–110
19. Liao XD, Lu L, Li SF, Liu SB, Zhang LY, Wang GY, Li A, Luo XG (2012) Effects of selenium source and level on growth performance, tissue selenium concentrations, antioxidation, and immune functions of heat-stressed broilers. Biol Trace Elem Res 150(1–3):158–165
20. Zhang ZW, Wang QH, Zhang JL, Li S, Wang XL, Xu SW (2012) Effects of oxidative stress on immunosuppression induced by selenium deficiency in chickens. Biol Trace Elem Res 149(3):352–361
21. Fu D, Chen J, Zhang Y, Yu Z (2011) Cloning and expression of a heat shock protein (Hsp) 90 gene in the haemocytes of Crassostrea hongkongensis under osmotic stress and bacterial challenge. Fish Shellfish Immunol 31(1):118–125
22. Shams S, Shafi S, Bodman-Smith K, Williams P, Mehta S, Ferns GA (2008) Anti-heat shock protein-27 (Hsp-27) antibody levels in patients with chest pain: association with established cardiovascular risk factors. Clin Chim Acta; Int J Clin Chem 395(1–2):42–46
23. Hollander JM, Martin JL, Belke DD, Scott BT, Swanson E, Krishnamoorthy V, Dillmann WH (2004) Overexpression of wild-type heat shock protein 27 and a nonphosphorylatable heat shock protein 27 mutant protects against ischemia/reperfusion injury in a transgenic mouse model. Circulation 110(23):3544–3552
24. Zhao J, Xing H, Liu C, Zhang Z, Xu S (2015) Effect of selenium deficiency on nitric oxide and heat shock proteins in chicken erythrocytes. Biol Trace Elem Res. doi:10.1007/s12011-015-0527-9
25. Zhu Y, Zhu J, Wan X, Zhu Y, Zhang T (2010) Gene expression of sHsps, Hsp40 and Hsp60 families in normal and abnormal embryonic development of mouse forelimbs. Toxicol Lett 193(3):242–251
26. Park K, Bang HW, Park J, Kwak IS (2009) Ecotoxicological multilevel-evaluation of the effects of fenbendazole exposure to *Chironomus riparius* larvae. Chemosphere 77(3):359–367
27. Romanucci M, Malatesta D, Ciccarelli A, Bongiovanni L, Palmieri C, Borzacchiello G, Roperto F, Altamura G, Della Salda L (2012) Expression of heat shock proteins in premalignant and malignant urothelial lesions of bovine urinary bladder. Cell Stress Chaperones 17(6):683–692
28. Chen X, Yao H, Yao L, Zhao J, Luan Y, Zhang Z, Xu S (2014) Selenium deficiency influences the gene expressions of heat shock proteins and nitric oxide levels in neutrophils of broilers. Biol Trace Elem Res 161(3):334–340
29. Monari M, Foschi J, Rosmini R, Marin MG, Serrazanetti GP (2011) Heat shock protein 70 response to physical and chemical stress in *Chamelea gallina*. J Exp Mar Biol Ecol 397(2):71–78
30. Zhang A, Zhou X, Wang X, Zhou H (2011) Characterization of two heat shock proteins (Hsp70/Hsc70) from grass carp (*Ctenopharyngodon idella*): evidence for their differential gene expression, protein synthesis and secretion in LPS-challenged peripheral blood lymphocytes. Comp Biochem Physiol Part B, Biochem Mol Biol 159(2):109–114
31. Yu J, Bao E, Yan J, Lei L (2008) Expression and localization of Hsps in the heart and blood vessel of heat-stressed broilers. Cell Stress Chaperones 13(3):327–335
32. Workman P (2004) Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone. Cancer Lett 206(2):149–157
33. Choi YK, Jo PG, Choi CY (2008) Cadmium affects the expression of heat shock protein 90 and metallothionein mRNA in the Pacific oyster, *Crassostrea gigas*. Comp Biochem Physiol Toxicol Pharmacol: CBP 147(3):286–292

34. Park K, Kwak IS (2008) Characterization of heat shock protein 40 and 90 in *Chironomus riparius* larvae: effects of di(2-ethylhexyl) phthalate exposure on gene expressions and mouthpart deformities. Chemosphere 74(1):89–95
35. Zhu Y, Lu X, Wu D, Cai S, Li S, Teng X (2013) The effect of manganese-induced cytotoxicity on mRNA expressions of HSP27, HSP40, HSP60, HSP70 and HSP90 in chicken spleen lymphocytes in vitro. Biol Trace Elem Res 156(1–3):144–152
36. Liu CP, Fu J, Xu FP, Wang XS, Li S (2015) The role of heat shock proteins in oxidative stress damage induced by Se deficiency in chicken livers. Biometals: Int J Role Metal Ions Biol, Biochem, Med 28(1):163–173
37. Zhao FQ, Zhang ZW, Qu JP, Yao HD, Li M, Li S, Xu SW (2014) Cold stress induces antioxidants and Hsps in chicken immune organs. Cell Stress Chaperones 19(5):635–648
38. Khoso PA, Yang Z, Liu C, Li S (2015) Selenoproteins and heat shock proteins play important roles in immunosuppression in the bursa of Fabricius of chickens with selenium deficiency. Cell Stress Chaperones 20(6):967–978
39. Li M, Wang XS, Xu FP, Liu S, Xu SW, Li S (2014) The change in heat shock protein expression in avermectin induced neurotoxicity of the pigeon (*Columba livia*) both in vivo and in vitro. Ecotoxicol Environ Saf 110:95–102
40. Liu LL, He JH, Xie HB, Yang YS, Li JC, Zou Y (2014) Resveratrol induces antioxidant and heat shock protein mRNA expression in response to heat stress in black-boned chickens. Poult Sci 93(1):54–62
41. Lu X (2013) Enantioselective effect of bifenthrin on antioxidant enzyme gene expression and stress protein response in PC12 cells. J Appl Toxicol: JAT 33(7):586–592