Effects of selenide chitosan sulfate on glutathione system in hepatocytes and specific pathogen-free chickens

Fu Chen ,*,[1] Lele Hou,*,[1] Lianqin Zhu,* ChengboYang,† Fenghua Zhu,* Huiling Qiu,‡,[2] and Shunyi Qin§,[2]

*Institute of Animal Nutritional Metabolic Disease and Poisoning Disease, College of Veterinary Medicine, Qingdao Agricultural University, Qingdao, 266109, China; †Department of Animal Science, University of Manitoba, Winnipeg Manitoba, R3T 2N2, Canada; ‡Haidu College, Qingdao Agricultural University, Laiyang, 265200, China; and §College of Animal Science and Veterinary Medicine, Tianjin Agricultural University, Tianjin, 300384, China

ABSTRACT This study aimed to investigate the effects of selenide chitosan sulfate (**Se-CTS-S**) on glutathione (**GSH**) system in hepatocytes and chickens. Chitosan, sodium selenite ($\mathbf{Na_2SeO_3}$), selenide chitosan, chitosan sulfate (**CTS-S**), and Se-CTS-S were added to the culture medium and the basal diets; glutathione peroxidase (**GSH-Px**) activity, GSH content, total antioxidant capacity (**T-AOC**), and mRNA levels of cellular GPx (**GPx-1**) and phospholipid hydroperoxide GPx (**GPx-4**) in vivo and in vitro were determined. The results showed that Se-CTS-S increased ($P < 0.05$) GPx-1 and GPx-4 mRNA levels in hepatocytes and livers, and GSH-Px activity, GSH content, and T-AOC in the medium, hepatocytes, plasma, and livers compared with the control and chitosan treatments. Compared with CTS-S, Se-CTS-S treatments increased ($P < 0.05$) GPx-1 and GPx-4 mRNA levels in hepatocytes and livers, and GSH-Px activity, GSH content, and T-AOC capacity in the medium, hepatocytes, and livers. Compared with Na_2SeO_3 and CTS-Se, Se-CTS-S increased ($P < 0.05$) GPx-1 mRNA levels in hepatocytes and livers, GPx-4 mRNA levels in hepatocytes and livers, GSH-Px activity in the medium, hepatocytes, and livers, GSH contents in plasma and livers, and T-AOC in the medium, plasma, and livers. Thus, Se-CTS-S showed better biological activity that mainly benefited from the synergistic effects of Se and sulfate on GSH system.

Key words: selenide chitosan sulfate, glutathione, glutathione peroxidase, hepatocyte, chicken

2020 Poultry Science 99:3979–3986
https://doi.org/10.1016/j.psj.2020.04.024

INTRODUCTION

Chitosan (**CTS**), the generic term for a family of linear amino polysaccharides, is an N-acetylated product of chitin with properties of nontoxicity, biocompatibility, biodegradability, being environmentally friendly, and involving low cost (Sahariah and Másson, 2017; Adhikari and Yadav, 2018). CTS is involved in a variety of biological functions including immune enhancement, anti-aging, anti-oxidation, anti-cancer, anti-rheumatism, and antimicrobial functions (Jan et al., 2012; Smith et al., 2014; Younes and Rinaudo, 2015). Therefore, it is highly suitable for use in food, animal production, textile, environment, and medical and pharmaceutical applications (Suh et al., 2016; Wang et al., 2018).

In a CTS molecule, there are 2 active groups: hydroxyl and amidogen, which are an ideal carrier of a variety of trace elements and chemical groups. Chemical modification of CTS can improve its solubility and enhance its biological effects (Fan et al., 2012; Qin et al., 2015). Especially in solution, the hydroxyl and amidogen groups of CTS are protonated, the resultant soluble polysaccharide is positively charged (cationic), and CTS molecules are easy to combine or chelate with sulfonic acid groups and multiple trace element ions (Patel et al., 2012; Pires et al., 2013; Xing et al., 2015). When CTS binds to them, these CTS complexes show better bioavailability, biological activity, and lower toxicity (Battin and Brumaghim, 2009; Wang et al., 2012; Qin et al., 2015; Zhai et al., 2017).

Selenide chitosan sulfate (**Se-CTS-S**) is a product of CTS modified by selenization and sulfation. We believe that Se-CTS-S might have excellent biological activity. To date, there are no studies that indicate the biological

Received October 10, 2019.
Accepted April 25, 2020.

[1]These authors contributed equally to this work and should be considered co-first authors.

[2]Corresponding authors: hlqiu_njau@163.com (HQ); qinshunyi@163.com (SQ)

activity of Se-CTS-S, and we do not know whether it can play the synergistic role of Se and sulfate. Glutathione peroxidase (**GSH-Px**), an important peroxide-degrading enzyme, can catalyze the conversion of reduced glutathione (**GSH**) to oxidized GSH, reduce toxic peroxides to non-toxic hydroxyl compounds, and thereby protect cell membrane structure and function from peroxide interference and damage (Wu et al., 2010; Gan et al., 2014; Bakhshalinejad et al., 2018). Nowadays, GSH system is one of the most important indicators commonly used for evaluating the biological activity of Se compounds and polysaccharide sulfates (Chen et al., 2017; Wang et al., 2017; Bakhshalinejad et al., 2018). Therefore, the purpose of this study was to investigate the effects of Se-CTS-S on GSH system and compare them with those of CTS, sodium selenite (**Na_2SeO_3**), selenide chitosan (**Se-CTS**), and chitosan sulfate (**CTS-S**) in vitro and in vivo.

MATERIALS AND METHODS

CTS-S, Se-CTS, and Se-CTS-S Preparation

CTS-S, Se-CTS, and Se-CTS-S were synthesized with CTS (molecular weight $\leq$ 5,000 g/mol) with a deacetylation degree of 90.5% (Qingdao BZ Oligo Co., Ltd., Qingdao, China), a sulfonated reagent (Chengdu Laibao Technology Co., Ltd., Chengdu, China), and (or) Na_2SeO_3 (analytical grade >98.5%) solution. Se content was detected by hydride generation atomic fluorescence spectrometry (Pires et al., 2013) and sulfate content was detected by the gelatin–$BaCl_2$ method (Dodgson and Price, 1962). Se content was 30 to 40 mg/g and sulfate content was 200 to 250 mg/g in Se-CTS-S.

In Vitro Experiment

White Leghorn hens (aged 60 D) were used to obtain hepatocytes by the collagenase perfusion method and cultured by serum-free culture method (Wu et al., 2010). Hepatocyte monolayers were treated with CTS, CTS-S, Na_2SeO_3, Se-CTS, and Se-CTS-S in 6-well plates, respectively. Group 1 served as a control that was treated without Se, sulfate, and CTS; group 2 was treated with 10 mg/L of CTS; groups 3 and 4 were treated with 1.5 μmol/L of Se as Na_2SeO_3 and Se-CTS, respectively; group 5 was treated with 3 mg/L of sulfate as CTS-S; and group 6 was treated with 1.5 μmol/L of Se and 3 mg/L of sulfate as Se-CTS-S. Se, sulfate, and CTS content in the culture medium are shown in Table 1. Each treatment was done in 6 wells. After 24 h of incubation, the culture medium was collected for assay of GSH-Px activity, GSH content, and total antioxidant capacity (**T-AOC**); hepatocytes were harvested and stored in liquid nitrogen for assay of GSH-Px activity, GSH content, T-AOC, and the mRNA levels of cellular GPx (**GPx-1**) and phospholipid hydroperoxide GPx (**GPx-4**).

In Vivo Experiment

Feeding experiment was carried out at the Laboratory Animal Center, and all experimental protocols were approved by the Institutional Animal Care and Use Committee of Qingdao Agricultural University. A total of 2 hundred and forty 7-day-old specific pathogen-free male White Leghorns chickens with initial body weight of 43.0 ± 2.0 g were purchased from the Institute of Animal Husbandry and Veterinary Medicine, Shandong Academy of Agricultural Sciences and randomly allocated to 6 experimental treatments with 4 replicate pens, with each replication containing 10 chickens. Group 1 served as a control and was fed a basal diet; group 2 was fed the basal diet supplemented with 30 mg CTS per kg; groups 3 and 4 were fed the basal diet supplemented with 0.3 mg Se from Na_2SeO_3 (analytical grade >98.5%) and Se-CTS per kg, respectively; group 5 was fed the basal diet supplemented with 6.0 mg sulfate from CTS-S per kg; and group 6 was fed the basal diet supplemented with 36.3 mg Se-CTS-S per kg. The basal diet was a corn-soybean meal-based diet, which was formulated to approximately meet the nutrient requirements for chickens. The basal diet formulation and approximate composition are shown in Table 2. Over the entire experimental period of 28 D, chickens were housed in a closed and ventilated building and provided with continuous light; water and feed were provided ad libitum. On days 14 and 28, blood samples were drawn from the heart, and plasma was collected and stored at −20°C for assay of GSH-Px activity, GSH content, and T-AOC; 12 chickens from each group (3 chickens per pen) were selected and euthanized with dimethyl ether, and livers were removed and stored in liquid nitrogen for assay of GSH-Px activity, GSH content, T-AOC, and the mRNA levels of GPx-1 and GPx-4.

Laboratory Assay

GSH-Px activity, GSH content, and T-AOC in the medium, hepatocytes, plasma, and livers were determined according to the instructions of GPx assay kit (colorimetric method), GSH assay kit (microenzyme labeling method), and T-AOC assay kit (ABTS method) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Table 1. Se, sulfate, and CTS content in the culture medium.

Items	Control	CTS	Na_2SeO_3	CTS-Se	CTS-S	Se-CTS-S
Se content (μmol/L)	0	0	1.5	1.5	0	1.5
Sulfate content (mg/L)	0	0	0	0	3	3
CTS content (mg/L)	0	10	0	10	10	10

Abbreviations: CTS, chitosan; CTS-S, chitosan sulfate; CTS-Se, selenide chitosan; Na_2SeO_3, sodium selenite; Se-CTS-S, selenide chitosan sulfate.

Table 2. Formulation and approximate composition of experimental diets.

Ingredient (%)	
Maize	66.15
Soybean meal	27.0
Fish meal	3.30
Dicalcium phosphate	1.20
Limestone	1.00
Salt (NaCl)	0.25
DL-Methionine	0.10
Mineral premix[1]	0.50
Vitamin premix[2]	0.50
Total	100
Chemical composition	
Gross energy (kcal/kg)	2,843
CP (%) (DM)	19.0
Calcium (%)	0.91
Available phosphorus (%)	0.41
Lysine (%)	1.03
Methionine (%)	0.44
Methionine + cystine (%)	0.78

[1]Mineral premix supplied the following per kilogram of complete feed: $ZnSO_4$, 80 mg; $MnSO_4$, 100 mg; $FeSO_4$, 80 mg; $CuSO_4$, 6 mg; KI, 0.35 mg; and $CoCl_2$, 0.4 mg.

[2]Vitamin premix supplied the following per kilogram of complete feed: vitamin A: 4,000 IU; vitamin B_1: 1.8 mg; vitamin B_2: 3.6 mg; vitamin B_{12}: 0.01 mg; vitamin D_3: 800 IU; vitamin E: 10 IU; vitamin K: 0.5 mg; biotin: 0.15 mg; choline: 1,300 mg; folacin: 0.55 mg/kg; niacin: 30 mg; and pantothenic acid: 10 mg.

The mRNA levels of GPx-1 and GPx-4 in hepatocytes and livers were determined by quantitative real-time reverse transcription PCR (Chen et al., 2017). Total RNA was extracted from the hepatocytes and livers with TRIzol reagent (Sangon Biotech [Shanghai] Co., Ltd., China), and then reversely transcribed in 40 μL of reaction mixture according to the manufacturer's instructions (Sangon Biotech [Shanghai] Co., Ltd.), and stored in liquid nitrogen. Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, CA) was used to design specific primers for GPx-1, GPx-4, and β-actin (Chen et al., 2018; Su et al., 2018; Zhang et al., 2018) (Table 3). Quantitative real-time reverse transcription PCR was performed using SYBR Premix Ex Taq II (Sangon Biotech [Shanghai] Co., Ltd.) with 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The ratio of GPx-1 and GPx-4 mRNA levels to that of the β-actin internal control was used for statistical comparison of the different treatments.

Statistical Analyses

Data were expressed as means ± SD analyzed by using one-way ANOVA to compare means and the multivariate analysis of the GLM procedure of IBM SPSS 22.0 for Windows statistical software package (IBM Corporation, Armonk, NY). The least significant difference and Dunnett's T3 tests were used to determine differences between means. The culture well and the pen were respectively defined as the experimental unit for statistical analysis and all calculations were generated based on well and pen averages. Differences were considered significant at $P < 0.05$ for all tests.

RESULTS

GSH Content, GSH-Px Activity, and T-AOC in Culture Medium and Hepatocytes

The effects of CTS, sulfate, and Se on GSH content in vitro are shown in Figure 1A. Compared with the control, CTS, and CTS-S groups, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH content in the culture medium and hepatocytes ($P < 0.05$). No significant effects on GSH content in the culture medium and hepatocytes were observed among the Se treatments (Na_2SeO_3, CTS-Se, and Se-CTS-S) and among the control, CTS, and CTS-S groups ($P > 0.05$).

The effects of CTS, sulfate, and Se on GSH-Px activity and T-AOC in vitro are shown in Figures 1B and 1C. Compared with the control, CTS, and CTS-S groups, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH-Px activity and T-AOC in the culture medium and hepatocytes ($P < 0.05$). Compared with the control group, CTS supplementation increased GSH-Px activity and T-AOC in hepatocytes, and CTS-S supplementation increased GSH-Px activity and T-AOC in the culture medium and hepatocytes ($P < 0.05$). Compared with the CTS group, CTS-S supplementation increased GSH-Px activity and T-AOC in the culture medium and hepatocytes ($P < 0.05$). Compared with the Na_2SeO_3 and CTS-Se groups, Se-CTS-S supplementation increased GSH-Px activity and T-AOC in the culture medium and hepatocytes ($P < 0.05$).

GSH Content, GSH-Px Activity, and T-AOC in Plasma

The effects of dietary CTS, sulfate, and Se supplementation on GSH content in plasma are shown in Figure 2A. Compared with the control, CTS, and CTS-S groups, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH content in plasma ($P < 0.05$). Compared with the control group, dietary CTS

Table 3. Primers used for real-time PCR.

Target gene	Primer sequences (5′–3′)	Annealing temperature (°C)	Fragment length (bp)	GenBank accession number
β-Actin	F: agt gtc ttt ttg tat ctt ccg cc	59.7	147	NM_205518
	R: cca cat act ggc act tta ctc cta	58.5		
GPx-1	F: tct ac ctg gta act ttc gag caa	59.0	147	AB371709.1
	R: cct tta ttg cag agc ctc ctt	58.3		
GPx-4	F: gcc acc tcc atc tac gac ttc	58.6	92	NM_204220
	R: ttg gtg atg atg cag acg aag	59.2		

Abbreviations: GPx-1, cellular glutathione peroxidase; GPx-4, phospholipid hydroperoxide glutathione peroxidase.

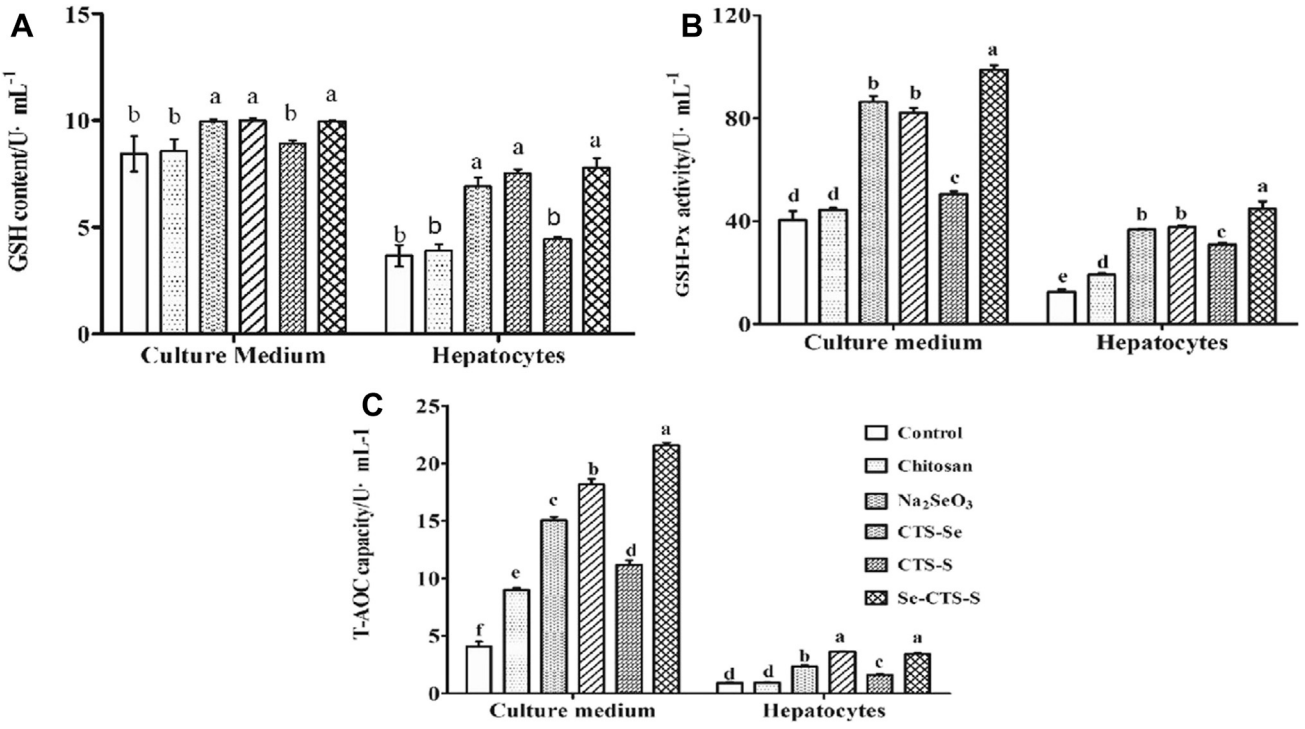

Figure 1. Effects of CTS, Se, and sulfate on glutathione content, GSH-Px activity, and T-AOC in the culture medium and hepatocytes in vitro. A: GSH content, B: GSH-Px activity, and C: T-AOC activity. a–f: Means in the same row with different superscripts significantly differ ($P < 0.05$). Data are presented as means ± SD (n = 3). Abbreviations: CTS, chitosan; CTS-S, chitosan sulfate; CTS-Se, selenide chitosan; GSH, glutathione; GSH-Px, glutathione peroxidase; Na_2SeO_3, sodium selenite; Se-CTS-S, selenide chitosan sulfate; T-AOC, total antioxidant capacity.

supplementation increased GSH content in plasma (on day 14) ($P < 0.05$), and dietary CTS-S supplementation increased GSH content in plasma (on days 14 and 28) ($P < 0.05$). Compared with the Na_2SeO_3 and CTS-Se groups, dietary Se-CTS-S supplementation increased GSH content in plasma (on days 14 and 28) ($P < 0.05$).

The effects of dietary CTS, sulfate, and Se supplementation on GSH-Px activity in plasma are shown in Figure 2B. Compared with the control group, dietary CTS supplementation increased GSH-Px activity in plasma (on day 14) ($P < 0.05$), and dietary CTS-S, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH-Px activity in plasma (on days 14 and 28) ($P < 0.05$). Compared with the CTS group, dietary CTS-S, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH-Px activity in plasma (on days 14 and 28) ($P < 0.05$). Compared with the CTS-S group, dietary CTS-Se and Se-CTS-S supplementation increased GSH-Px activity in plasma (on day 14) ($P < 0.05$). Compared with the Na_2SeO_3 group, dietary CTS-Se supplementation decreased GSH-Px activity in livers (on day 14) ($P < 0.05$), and dietary Se-CTS-S supplementation increased GSH-Px activity in livers (on day 28) ($P < 0.05$). Compared with the CTS-Se group, dietary Se-CTS-S supplementation increased GSH-Px activity in livers (on day 28) ($P < 0.05$).

The effects of dietary CTS, sulfate, and Se supplementation on T-AOC in plasma are shown in Figure 2C. Dietary Na_2SeO_3 and Se-CTS-S supplementation increased T-AOC in plasma (on day 14) compared to the CTS and CTS-S groups ($P < 0.05$), dietary CTS-S, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased T-AOC in plasma (on day 28) compared to the control group ($P < 0.05$), dietary CTS-Se supplementation increased T-AOC in plasma (on day 28) compared with the CTS and CTS-S group ($P < 0.05$), and dietary Se-CTS-S supplementation increased T-AOC in plasma (on day 28) compared with the CTS, Na_2SeO_3, and CTS-S group ($P < 0.05$).

GSH Content, GSH-Px Activity, and T-AOC in Livers

The effects of dietary CTS, sulfate, and Se supplementation on GSH content in livers are shown in Figure 3A. Compared with the control group, dietary CTS supplementation increased GSH content in livers (on day 14) ($P < 0.05$), and dietary CTS-S, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH content in livers (on days 14 and 28) ($P < 0.05$). Compared with the CTS group, dietary Na_2SeO_3 and CTS-S supplementation increased GSH content in livers (on day 28) ($P < 0.05$), and dietary CTS-Se and Se-CTS-S supplementation increased GSH content in livers (on days 14 and 28) ($P < 0.05$). Compared with the CTS-S group, dietary Na_2SeO_3 supplementation increased GSH content in livers (on day 28) ($P < 0.05$), and dietary CTS-Se and Se-CTS-S supplementation increased GSH content in livers (on days 14 and 28) ($P < 0.05$). Compared with the Na_2SeO_3 group, dietary CTS-Se

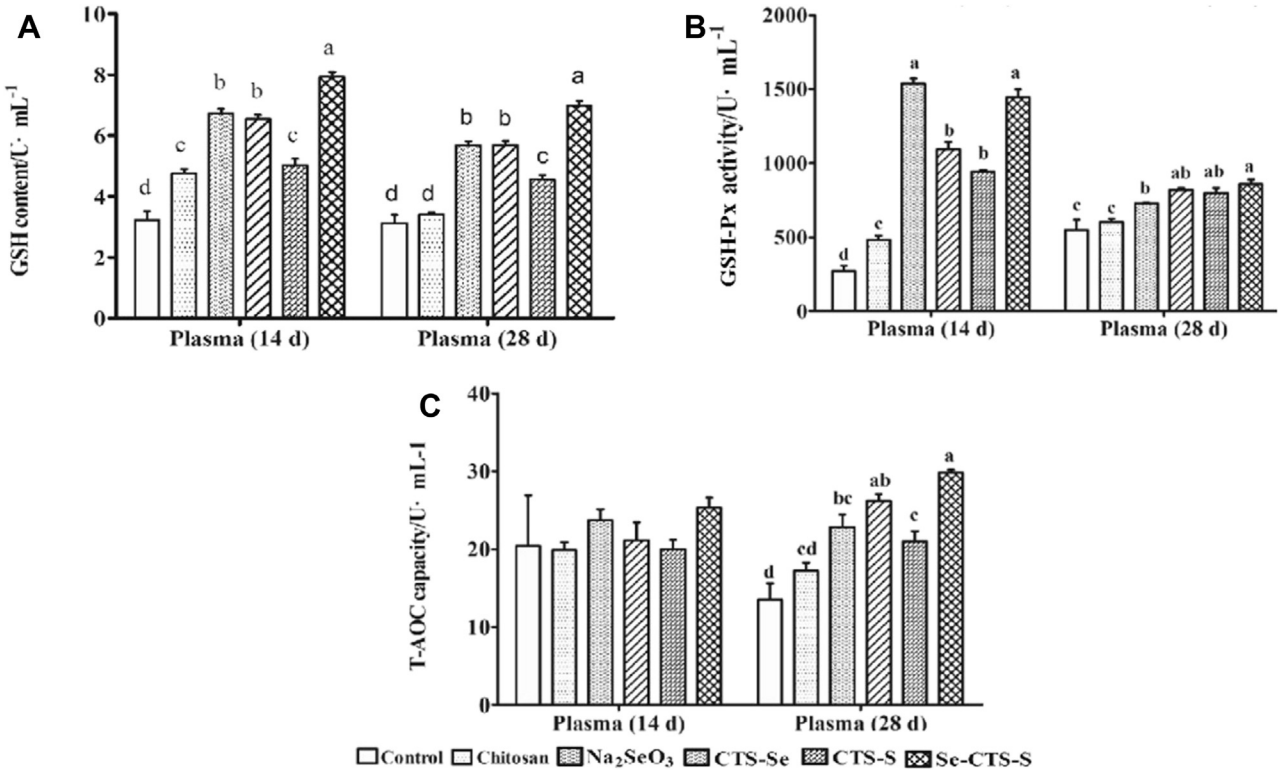

Figure 2. Effects of CTS, Se, and sulfate on glutathione content, GSH-Px activity, and T-AOC in plasma in vivo. A: GSH content, B: GSH-Px activity, and C: T-AOC activity. a–d: Means in the same row with different superscripts significantly differ ($P < 0.05$). Data are presented as means ± SD (n = 4). Abbreviations: CTS, chitosan; CTS-S, chitosan sulfate; CTS-Se, selenide chitosan; GSH, glutathione; GSH-Px, glutathione peroxidase; Na_2SeO_3, sodium selenite; Se-CTS-S, selenide chitosan sulfate; T-AOC, total antioxidant capacity.

supplementation increased GSH content in livers (on day 28) ($P < 0.05$), and dietary Se-CTS-S supplementation increased GSH content in livers (on days 14 and 28) ($P < 0.05$). Compared with the CTS-Se group, dietary Se-CTS-S supplementation increased GSH content in livers (on day 28) ($P < 0.05$).

The effects of dietary CTS, sulfate, and Se supplementation on GSH-Px activity in livers are shown in Figure 3B. Compared with the control group, dietary CTS supplementation increased GSH-Px activity in livers (on day 14) ($P < 0.05$), and dietary CTS-S, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH-Px activity in livers (on days 14 and 28) ($P < 0.05$). Compared with the CTS group, dietary CTS-S, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH-Px activity in livers (on days 14 and 28) ($P < 0.05$). Compared with the CTS-S group, dietary Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased GSH-Px activity in livers (on day 14) ($P < 0.05$). Compared with the Na_2SeO_3 and CTS-Se groups, dietary Se-CTS-S supplementation increased GSH-Px activity in livers (on days 14 and 28) ($P < 0.05$).

The effects of dietary CTS, sulfate, and Se supplementation on T-AOC in livers are shown in Figure 3C. Compared with the control group, dietary CTS-S, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased T-AOC in livers (on days 14 and 28) ($P < 0.05$). Compared with the CTS group, dietary CTS-Se and Se-CTS-S supplementation increased T-AOC in livers (on day 14) ($P < 0.05$), and dietary CTS-S, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased T-AOC in livers (on day 28) ($P < 0.05$). Compared with the CTS-S group, dietary CTS-Se and Se-CTS-S supplementation increased T-AOC in livers (on days 14 and 28) ($P < 0.05$). Compared with the Na_2SeO_3 and CTS-Se group, dietary Se-CTS-S supplementation increased T-AOC in livers (on day 28) ($P < 0.05$).

mRNA Levels of GPx-1 and GPx-4 in Hepatocytes and Livers

The effects of CTS, sulfate, and Se supplementation on the mRNA levels of GPx-1 in hepatocytes and livers are shown in Figure 4A. Compared with the control group, CTS and CTS-S supplementation increased the mRNA levels of GPx-1 in hepatocytes ($P < 0.05$). Compared with the control, CTS, and CTS-S groups, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased the mRNA levels of GPx-1 in hepatocytes and livers (on days 14 and 28) ($P < 0.05$). Compared with the Na_2SeO_3 group, CTS-Se supplementation decreased the mRNA levels of GPx-1 in livers (on

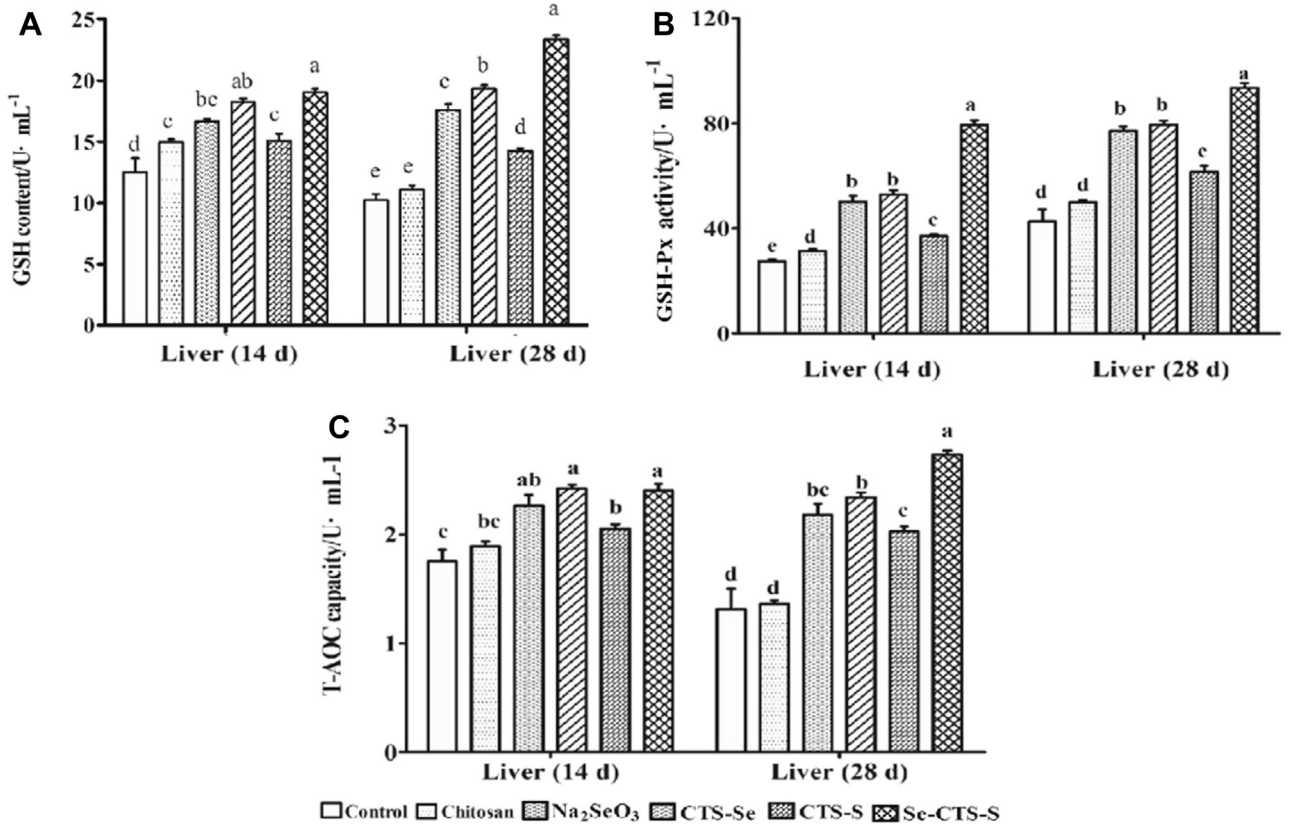

Figure 3. Effects of CTS, Se, and sulfate on glutathione content, GSH-Px activity, and T-AOC in livers in vivo. A: GSH content, B: GSH-Px activity, and C: T-AOC activity. a–d: Means in the same row with different superscripts significantly differ ($P < 0.05$). Data are presented as means ± SD (n = 4). Abbreviations: CTS, chitosan; CTS-S, chitosan sulfate; CTS-Se, selenide chitosan; GSH, glutathione; GSH-Px, glutathione peroxidase; Na_2SeO_3, sodium selenite; Se-CTS-S, selenide chitosan sulfate; T-AOC, total antioxidant capacity.

day 14) ($P < 0.05$), and Se-CTS-S supplementation increased the mRNA levels of GPx-1 in hepatocytes and livers (on days 14 and 28) ($P < 0.05$). Compared with the CTS-Se group, Se-CTS-S supplementation increased the mRNA levels of GPx-1 in hepatocytes and livers (on days 14 and 28) ($P < 0.05$).

The effects of CTS, sulfate, and Se supplementation on the mRNA levels of GPx-4 in hepatocytes and livers are shown in Figure 4B. Compared with the control, CTS, and CTS-S groups, Na_2SeO_3, CTS-Se, and Se-CTS-S supplementation increased the mRNA levels of GPx-4 in hepatocytes and livers (on days 14 and 28). Compared with the Na_2SeO_3 group, Se-CTS-S supplementation increased the mRNA levels of GPx-4 in hepatocytes and livers (on day 28) ($P < 0.05$). Compared with the CTS-Se group, Se-CTS-S supplementation increased the mRNA levels of GPx-4 in hepatocytes ($P < 0.05$).

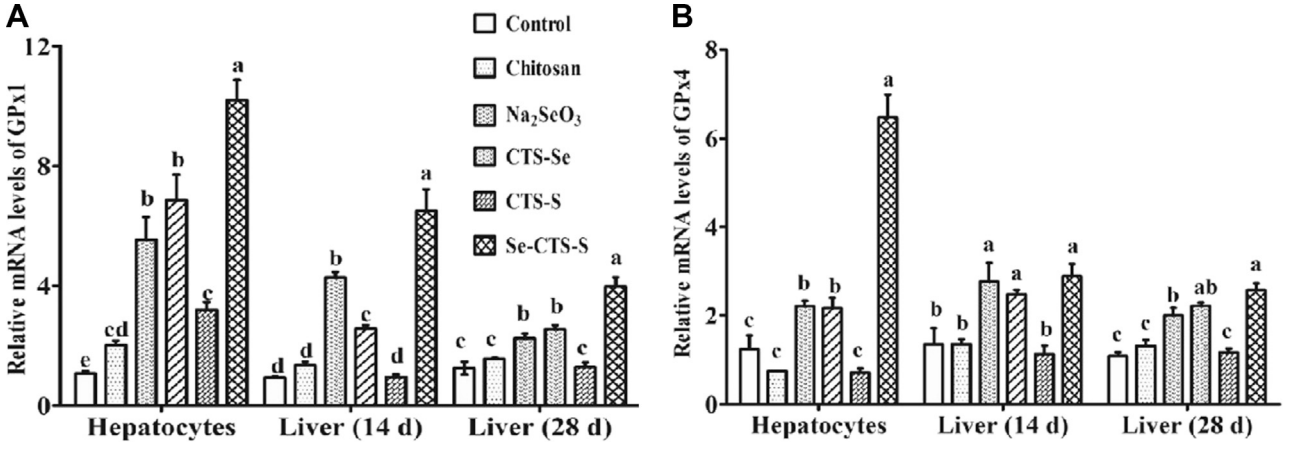

Figure 4. Effects of CTS, Se, and sulfate on GPx-1 and GPx-4 mRNA levels in hepatocytes and livers. A: GPx-1 mRNA levels and B: GPx-4 mRNA levels. a–e: Means in the same row with different superscripts significantly differ ($P < 0.05$). Data are presented as means ± SD (n = 4). Abbreviations: CTS, chitosan; CTS-S, chitosan sulfate; CTS-Se, selenide chitosan; GPx-1, cellular glutathione peroxidase; GPx-4, phospholipid hydroperoxide glutathione peroxidase; Na_2SeO_3, sodium selenite; Se-CTS-S, selenide chitosan sulfate.

DISCUSSION

The scavenging effects of 3 kinds of CTS with different molecular weights and their sulfate derivatives on O_2^- and -OH were studied. The results showed that the scavenging ability of CTS-S to O_2^- was significantly enhanced after sulfation, and CTS-S could achieve the effect of scavenging O_2^- at a very low mass concentration (0.1 g/L) (≥90%). This shows that the sulfated product of CTS is a highly active scavenger of O_2^- (Xing et al., 2015). The antioxidant activity of CTS-S may be the reason for increasing the relative mRNA level of GPx-1 in hepatocytes. Accumulating evidence indicated the antioxidant effects of CTS on indices of oxidative stress by scavenging free radicals or preventing oxidative damage (Ngo and Kim, 2014; Ngo et al., 2015; Wang et al., 2016; Kim et al., 2018), and the protective and repair effects of sulfate (sulfated polysaccharides) on damaged cells caused by oxidative stress (Liu et al., 2014; Shao et al., 2014; Deng et al., 2015; Bai et al., 2017). Similarly, the present study confirmed the antioxidant effects of CTS and CTS-S in vivo and in vitro. Moreover, we found that sulfate supplementation from CTS-S treatment enabled better antioxidant activity than CTS treatments both in vivo and in vitro. These results suggested that sulfate in the CTS-S molecule played an important role in enhancing the antioxidant function in vitro and in vivo, and CTS became a more efficient compound after modification of sulfation (Battin and Brumaghim, 2009; Wang et al., 2012).

It is noteworthy that the antioxidant properties are one of the most important characteristics of Se compounds, especially regulating effects on antioxidant enzymes (Khan et al., 2018; Zoidis et al., 2018; Yang et al., 2019). Compared with the control and CTS treatments, Se supplementation from different Se treatments (Na_2SeO_3, Se-CTS, and Se-CTS-S) indicated higher GSH-Px activity, GSH content, T-AOC, and mRNA levels of GPx-1 and GPx-4 in vitro and in vivo. These results further demonstrated that Se supplementation could improve the antioxidant function in vitro and in vivo (Wu et al., 2010; Fan et al., 2012; Chen et al., 2017; Yang et al., 2019). Moreover, the antioxidant activity of CTS was significantly higher following Se modification and Se-CTS was a more efficient compound (Qin et al., 2015).

The present study showed that Se-CTS treatments exhibited similar antioxidant activity as Na_2SeO_3, except that Se-CTS had higher GSH content in livers (day 28) and T-AOC in the medium, and lower GSH-Px activity in plasma (day 14) and GPx-1 mRNA level in livers (day 14) than those of Na_2SeO_3 treatments. In contrast, Qin et al., (2015) reported that Se-CTS increased blood GSH-Px activity compared to the control and Na_2SeO_3 treatments, and its biological activity was better than Na_2SeO_3 in mice. Bai et al., (2017) reported that Se nanoparticles-loaded CTS microspheres displayed powerful antioxidant activities, and demonstrated similar efficacy in increasing GSH-Px activity in the blood and liver of mice compared with Na_2SeO_3. We believe that this difference may be explained by the statement that different forms of Se-CTS may exhibit different biological activities in different samples and animals.

Previous studies have investigated the chemical mechanisms for antioxidant behavior and demonstrated that sulfate and Se compounds could ameliorate oxidative damage by utilizing multiple complex antioxidant mechanisms (Battin and Brumaghim, 2009). In the present study, Se supplementation from different Se treatments (Na_2SeO_3, Se-CTS, and Se-CTS-S) exhibited better antioxidant activities than CTS-S treatments, especially increasing GSH content, GSH-Px activity, T-AOC, and GPx-1 and GPx-4 mRNA levels in vitro and in vivo. These results suggested that the biological ability of Se, especially the antioxidant activity, was more pronounced than that of sulfate. This could be explained by the theory that the antioxidant activities of sulfate and Se might be quite distinct as a result of utilizing different antioxidant mechanisms; sulfate itself does not primarily exhibit GSH-Px activity; moreover, as Se-dependent antioxidant enzymes, their expressions, activity, and effectors (GSH) of GPx were mainly regulated by Se rather than by sulfate (Battin and Brumaghim, 2009; Wu et al., 2010; Chen et al., 2017; Yang et al., 2019).

The present study suggested that Se-CTS-S treatments exhibited higher antioxidant activity by increasing GSH content, GSH-Px activity, T-AOC, and GPx-1 and GPx-4 mRNA levels than CTS-S and Se-CTS treatments in vivo and in vitro. We considered that both selenite and sulfate may synergistically play an important role in enhancing antioxidant function in vitro and in vivo when CTS became a more efficient compound (Se-CTS-S) after modification of selenization and sulfation.

In summary, Se-CTS-S supplementation could improve antioxidant function by activating the GSH system, increasing GPx-1 and GPx-4 mRNA expressions, GSH-Px activity, and GSH produce, and then enhancing T-AOC of hepatocytes and chickens. Moreover, Se-CTS-S showed better biological activity, which mainly benefits from the synergistic effects of selenite and sulfate on enhancing antioxidant function.

ACKNOWLEDGMENTS

We are grateful to our laboratory technicians for their assistance with the experimental animals. This study was supported by Shandong Major Scientific and Technological Innovation Project (2019JZZY020611), Shandong Province Higher Educational Science and Technology Program (J18KA131 and J18KA143), Chinese National Natural Science Foundation (31001093 and 31101867), Shandong modern agricultural industry technology system poultry innovation team projects (SDAIT-11-07), and one-three-one Program of Innovative Talents Training of Tianjin, Young and Middle

aged Backbone Innovative Talents Training Program of Higher Educational Institutions of Tianjin, and Tianjin Research Program of Application Foundation and Advanced Technology (15JCYBJC30600).

Conflict of Interest Statement: The authors declare that they have no competing interest.

REFERENCES

Adhikari, H. S., and P. N. Yadav. 2018. Anticancer activity of chitosan, chitosan derivatives, and their mechanism of action. Int. J. Biomater. 2018 2952085.

Bai, K., B. Hong, J. He, Z. Hong, and R. Tan. 2017. Preparation and antioxidant properties of selenium nanoparticles-loaded chitosan microspheres. Int. J. Nanomedicine 12:4527–4539.

Bakhshalinejad, R., R. Akbari Moghaddam Kakhki, and E. Zoidis. 2018. Effects of different dietary sources and levels of selenium supplements on growth performance, antioxidant status and immune parameters in ross 308 broiler chickens. Br. Poult. Sci. 59:81–91.

Battin, E. E., and J. L. Brumaghim. 2009. Antioxidant activity of sulfur and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. Cell Biochem. Biophys. 55:1–23.

Chen, F., L. Zhu, H. Qiu, and S. Qin. 2017. Selenium-enriched saccharomyces cerevisiae improves growth, antioxidant status and selenoprotein gene expression in arbor acres broilers. J. Anim. Physiol. Anim. Nutr. (Berl.) 101:259–266.

Chen, R., S. Zhuang, Y. P. Chen, Y. F. Cheng, C. Wen, and Y. M. Zhou. 2018. Betaine improves the growth performance and muscle growth of partridge shank broiler chickens via altering myogenic gene expression and insulin-like growth factor-1 signaling pathway. Poult. Sci. 97:4297–4305.

Deng, C., J. Xu, H. Fu, J. Chen, and X. Xu. 2015. Characterization, antioxidant and cytotoxic activity of sulfated derivatives of a water-insoluble polysaccharides from dictyophora indusiata. Mol. Med. Rep. 11:2991–2998.

Dodgson, K. S., and R. G. Price. 1962. A note on the determination of the ester sulphate content of sulphated polysaccharides. Biochem. J. 84:106–110.

Fan, L., P. Wu, J. Zhang, S. Gao, L. Wang, M. Li, M. Sha, W. Xie, and M. Nie. 2012. Synthesis and anticoagulant activity of the quaternary ammonium chitosan sulfates. Int. J. Biol. Macromol. 50:31–37.

Gan, F., X. Chen, S. F. Liao, C. Lv, F. Ren, G. Ye, C. Pan, D. Huang, J. Shi, X. Shi, H. Zhou, and K. Huang. 2014. Selenium-enriched probiotics improve antioxidant status, immune function, and selenoprotein gene expression of piglets raised under high ambient temperature. J. Agric. Food Chem. 62:4502–4508.

Jan, S.-S., D.-C. Liu, X.-Y. Dong, Y.-M. Hu, and J.-d. Chen. 2012. Effects of chitosan and its derivative added to water on immunological enhancement and disease control. Immunotherapy 4:697–701.

Khan, A. Z., S. Kumbhar, Y. Liu, M. Hamid, C. Pan, S. A. Nido, F. Parveen, and K. Huang. 2018. Dietary supplementation of selenium-enriched probiotics enhances meat quality of broiler chickens (gallus gallus domesticus) raised under high ambient temperature. Biol. Trace Elem. Res. 182:328–338.

Kim, T.-K., J.-Y. Shim, K.-E. Hwang, Y.-B. Kim, J.-M. Sung, H.-D. Paik, and Y.-S. Choi. 2018. Effect of hydrocolloids on the quality of restructured hams with duck skin. Poult. Sci. 97:4442–4449.

Liu, X., T. Chen, Y. Hu, K. Li, and L. Yan. 2014. Catalytic synthesis and antioxidant activity of sulfated polysaccharide from momordica charantia l. Biopolymers 101:210–215.

Ngo, D.-H., and S.-K. Kim. 2014. Antioxidant effects of chitin, chitosan, and their derivatives. Adv. Food Nutr. Res. 73:15–31.

Ngo, D. H., T. S. Vo, D. N. Ngo, K. H. Kang, J. Y. Je, H. N. D. Pham, H. G. Byun, and S. K. Kim. 2015. Biological effects of chitosan and its derivatives. Food Hydrocolloids 51:200–216.

Patel, D. D., V. N. Patel, T. V. Thakkar, and R. T. Gandhi. 2012. Preparation and evaluation of levosalbutamol sulphate chitosan microsphere for the treatment of asthma. J. Pharm. Bioallied Sci. 4:S46–S47.

Pires, N. R., P. L. R. Cunha, J. S. Maciel, A. L. Angelim, V. M. M. Melo, R. C. M. de Paula, and J. P. A. Feitosa. 2013. Sulfated chitosan as tear substitute with no antimicrobial activity. Carbohydr. Polymers 91:92–99.

Qin, S., B. Huang, J. Ma, X. Wang, J. Zhang, L. Li, and F. Chen. 2015. Effects of selenium-chitosan on blood selenium concentration, antioxidation status, and cellular and humoral immunity in mice. Biol. Trace Elem. Res. 165:145–152.

Sahariah, P., and M. Másson. 2017. Antimicrobial chitosan and chitosan derivatives: a review of the structure-activity relationship. Biomacromolecules 18:3846–3868.

Shao, P., X. Chen, and P. Sun. 2014. Chemical characterization, antioxidant and antitumor activity of sulfated polysaccharide from sargassum horneri. Carbohydr. Polym. 105:260–269.

Smith, A., M. Perelman, and M. Hinchcliffe. 2014. Chitosan: a promising safe and immune-enhancing adjuvant for intranasal vaccines. Hum. Vaccin. Immunother. 10:797–807.

Su, Y., X. Zhang, H. Xin, S. Li, J. Li, R. Zhang, X. Li, J. Li, and J. Bao. 2018. Effects of prior cold stimulation on inflammatory and immune regulation in ileum of cold-stressed broilers. Poult. Sci. 97:4228–4237.

Suh, J. W., J. S. Lee, S. Ko, and H. G. Lee. 2016. Preparation and characterization of mucoadhesive buccal nanoparticles using chitosan and dextran sulfate. J. Agric. Food Chem. 64:5384–5388.

Wang, H., J. Qian, and F. Ding. 2018. Emerging chitosan-based films for food packaging applications. J. Agric. Food Chem. 66:395–413.

Wang, Y., H. M. Yang, W. Cao, and Y. B. Li. 2017. Effect of selenium supplementation on pigeon reproductive performance, selenium concentration and antioxidant status. Poult. Sci. 96:3407–3413.

Wang, Z., Y. Yan, X. Yu, W. Li, B. Li, and C. Qin. 2016. Protective effects of chitosan and its water-soluble derivatives against lead-induced oxidative stress in mice. Int. J. Biol. Macromol. 83:442–449.

Wang, T., Y. Zhou, W. Xie, L. Chen, H. Zheng, and L. Fan. 2012. Preparation and anticoagulant activity of n-succinyl chitosan sulfates. Int. J. Biol. Macromol. 51:808–814.

Wu, X., K. Huang, C. Wei, F. Chen, and C. Pan. 2010. Regulation of cellular glutathione peroxidase by different forms and concentrations of selenium in primary cultured bovine hepatocytes. J. Nutr. Biochem. 21:153–161.

Xing, R., X. He, S. Liu, H. Yu, Y. Qin, X. Chen, K. Li, R. Li, and P. Li. 2015. Antidiabetic activity of differently regioselective chitosan sulfates in alloxan-induced diabetic rats. Mar. Drugs 13:3072–3090.

Yang, J., M. Zhang, and Y. Zhou. 2019. Effects of selenium-enriched bacillus sp. Compounds on growth performance, antioxidant status, and lipid parameters breast meat quality of Chinese huainan partridge chicks in winter cold stress. Lipids Health Dis. 18:63.

Younes, I., and M. Rinaudo. 2015. Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar. Drugs 13:1133–1174.

Zhai, X., C. Zhang, G. Zhao, S. Stoll, F. Ren, and X. Leng. 2017. Antioxidant capacities of the selenium nanoparticles stabilized by chitosan. J. Nanobiotechnology 15:4.

Zhang, Y., X. Guan, Z. Chen, D. Cao, Z. Kang, Q. Shen, Q. Lei, F. Li, H. Li, M. F. Leghari, Y. Wang, X. Qi, X. Wang, and Y. Gao. 2018. The high conserved cellular receptors of avian leukosis virus subgroup j in Chinese local chickens contributes to its wide host range. Poult. Sci. 97:4187–4192.

Zoidis, E., I. Seremelis, N. Kontopoulos, and G. P. Danezis. 2018. Selenium-dependent Antioxidant Enzymes: Actions and Properties of Selenoproteins, Vol 7. Antioxidants, Basel, Switzerland).