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Effects of Selenium-Enriched Probiotics on Heat Shock Protein mRNA Levels in Piglet under Heat Stress Conditions

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ABSTRACT: The effects of selenium-enriched probiotics (SP) on tissue selenium (Se) deposition, glutathione peroxidase-1 (GPx1) activity and mRNA level, and heat shock protein (Hsp) mRNA levels of piglets under heat stress conditions were investigated. A total of 48 crossbred ([Landrace × Yorkshire] × Duroc) piglets were randomly divided into 4 groups and fed a basal diet (Con, 0.16 mg Se/kg) or basal diets with added probiotics (P, 0.16 mg Se/kg), sodium selenite (SS, 0.46 mg Se/kg), or SP (0.46 mg Se/kg), respectively, for 42 days. Three piglets were randomly selected from each group for blood sample collection at days 0, 14, 28, and 42 and for liver, kidney, and spleen sample collection at day 42. The results showed that P, SS, and SP could significantly down-regulate the average mRNA levels of Hsp70 (17.3, 23.7, and 40.1%) and Hsp27 (22.4, 24.4, and 44.7%) of the tissues, respectively ($P < 0.05$), whereas SS and SP could significantly elevate Se concentration, GPx1 activity and mRNA level ($P < 0.05$). The maximal effects of these parameters were observed in SP. It was concluded that SP is a feasible dietary supplementation of piglets under heat stress conditions during the summer season.

KEYWORDS: *selenium-enriched probiotics, GPx1, Hsp70, heat stress, piglet*

INTRODUCTION

Present global warming has resulted in heat stress being considered as a primary factor influencing animal health and growth, particularly during the summer.[1] Across the United States, heat stress is estimated to be responsible for a loss of between $1.69 and $2.36 billion to livestock industries.[2] Heat stress can cause numerous negative influences on pigs, resulting in enormous economic losses.[3,4]

The heat shock proteins (Hsps) are a family of stress-responsive proteins found in all species. On the basis of their molecular weight, Hsps are classified into four major groups, namely, small Hsps, Hsp60, Hsp70, and Hsp90.[5] Heat and other stressors rapidly induce the synthesis of Hsps.[2,6,7] Stress-inducible heat shock proteins such as Hsp70 and Hsp27 are molecular chaperones that protect cells and organisms from stress damage by keeping cellular proteins in a folding competent state and preventing them from irreversible aggregation.[8] A recent study showed that exposure of mammals to heat stress over a period of time enhances Hsp70 and Hsp27 expressions.[2,9]

Selenium (Se) is an essential trace element for all mammalian species, which is known to play an important role in both antioxidant defense[10] and immune function,[11,12] and a better effect of organic Se than inorganic Se has already been reported.[13] There is an inverse relationship of selenium concentration and stress response during the capture that has been found in eider ducks.[14] The relationship between stress response and selenium may be applied to stress response under heat stress conditions in piglets. Previous studies in poultry using organic selenium supplements such as selenium yeast have found the relationship between selenium and decreasing level of Hsp70;[15−17] the effect of selenium on Hsp70 and Hsp27 response in piglets under heat stress conditions was investigated in the present study.

It has also been proven that probiotics (P) have effects on improving growth performance and beneficial gut microflora.[18,19] Probiotic bacteria (lactobacilli) have the ability to decrease the Hsp70 mRNA level of heat-shocked enterocyte-like Caco-2 cells compared with no starter lactobacilli.[20] Because there is limited information about probiotics and Hsp70 and Hsp27 mRNA levels in piglets under heat stress conditions, further experiments are required to assist in the justification of the effect of probiotics on the mRNA levels of Hsp70 and Hsp27.

Our laboratory has developed a Se-enriched probiotics (SP) that has the combination effect of Se and P as a new feed additive product for promoting animal industries. To produce this SP, two strains of microorganisms, *Lactobacillus acidophilus (L. acidophilus)* and *Saccharomyces cerevisiae (S. cerevisiae)*, were cultured under appropriate microenvironmental conditions with sodium selenite (SS, inorganic Se) being added into the culture media. The conversion of inorganic Se into organic Se in the production of SP has been proved to be very efficient,[21,22] with >90% being organic Se and >75% being selenomethionine (Se-Met) in SP. Up until now, there have not been any reports of research on SP in relation to piglets under heat stress conditions.

The objectives of this paper were to investigate the effects of SP on tissue Se deposition, glutathione peroxidase-1 (GPx1)

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activity and mRNA level, and Hsp mRNA levels of piglets under heat stress conditions.

■ MATERIALS AND METHODS

Chemicals. The GPx assay kit and the total protein assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and Biyuntian Institute (Nanjing, China), respectively. All reagents used for Se assay were of analytical grade. Se stock standard solution of sodium selenite [GBW(E)080215] and the certified Se reference material, pork liver (GBW 08551), were provided by the National Research Center for Standard Materials (Beijing, China) and the Food Detection Science Institute of the Ministry of Commerce (Beijing, China), respectively. Reagents used for real-time PCR were purchased from TaKaRa (Dalian, China).

Selenium-Enriched Probiotics, Sodium Selenite, and Probiotics. Both the SP and P products were provided by the Institute of Nutritional and Metabolic Disorders of Domestic Animals and Fowls, Nanjing Agricultural University (Jiangsu, China). Both SP and P products contain two probiotic strains, *L. acidophilus* and *S. cerevisiae*. The colony-forming units (CFU) of *L. acidophilus* and *S. cerevisiae* in both products were approximately 10^{11}/mL and 10^{9}/mL, respectively. The total Se content in the SP is 10.0 mg/L, with >90% being organic Se and >75% being selenomethionine. The SS used in this study is the SS stock solution, and the total Se content is 100 mg/L.

Animals and Diets. All experimental protocols were approved by the Committee for the Care and Use of Experimental Animals at Agriculture University of Nanjing, and the Animal Ethical Number is SYXK (Su) 2011-0036.

According to a completely randomized experimental design, 48 (Landrace × Yorkshire) × Duroc weanling piglets (4 weeks old, body mass = 7.9 ± 0.5 kg) were divided into four groups of 12 piglets in each. Each group had three replicates, with four piglets per replicate. All experimental piglets in four groups had been fed the same diet for 2 weeks and had similar Se status before the experiment began (as shown in Table 4). The composition of basal diets (Table 1) used in this study satisfies the requirement of National Research Council (NRC) of China, and the basal diet contains 0.16 mg Se/kg.

SP and SS that supply Se at rates of 10 and 100 mg/L, respectively, were mixed into the basal diet, and P product was mixed into the basal diet at a level at which the probiotics level was equal to that of the SP diet. Briefly, stock volumes of 30 mL SP, 3 mL SS, and 30 mL P stock liquids were diluted separately to three 250 mL working solutions and further mixed into 1 kg of basal diet, and the final calculated Se levels for the four different groups are listed in Table 2. Basal diet with no added selenium and probiotics served as a blank control (Con group), whereas the basal diet with added probiotics served as a probiotics control (P group) with a level of probiotics equal to that of the SP group; the basal diet with added sodium selenite (SS group) was used as an inorganic selenium with a selenium level equal to that of the SP group, whereas SP contained an organic selenium.

Table 1. Composition of Basal Diets

ingredient[a]	content, g/kg
corn	620
extruded full-fat soybean	280
whey	50.0
lysine	10.0
methionine	3.80
tryptophan	2.90
threonine	8.40
salt	4.00
$CaHPO_4$	6.40
$CaCO_3$	4.00
trace mineral premix[b]	10.0
vitamin premix[c]	0.50

[a]Analyzed Se content in the basal diets was 0.16 mg/kg. [b]Trace mineral premix provided per kg diet: iron, 100 mg; copper, 10.0 mg; manganese, 40.0 mg; zinc, 100 mg; selenium, 0.10 mg; iodine, 0.50 mg. [c]Vitamin premix provided per kg diet: retinyl acetate, 3.03 mg; α-tocopheryl acetate, 32.0 mg; menadione, 2.00 mg; thiamin, 4.00 mg; riboflavin, 14.0 mg; calcium pantothenate, 40.0 mg; niacin, 60.0 mg; pyridoxal, 6.00 mg; D-biotin, 0.20 mg; folacin, 1.20 mg; cobalamin, 0.07 mg.

Table 2. Selenium Levels in the Different Experimental Diets

group	diet	supplemental Se level, mg/kg	final total Se level, mg/kg
Con	basal diet	0.0	0.16
P	basal diet + P	0.0	0.16
SS	basal diet + SS	0.3	0.46
SP	basal diet + SP	0.3	0.46

The experiment, which lasted for 42 days (from June 19, 2011, to July 30, 2011), was conducted at Bangcheng pig farm located in Xinghua city (32° 93′ N latitude and 119° 82′ E longitude), Jiangsu province, China. During the first 33 animal experiment days, the temperature in the pig shed ranged between 25 and 38 °C from 12:00 a.m. to 8:00 p.m., and during the last 9 animal experiment days, the temperature in the pig shed ranged between 31 and 40 °C from 12:00 a.m. to 8:00 p.m. The daily average temperature from 12:00 a.m. to 8:00 p.m. over the 42 day experiment is shown in Figure 1. All 48 piglets were housed in the pig shed and raised with unlimited access to food and water throughout the 42 day experiment period. Food intake was recorded daily, and the average daily food intake (ADFI) of a piglet in each group was calculated.

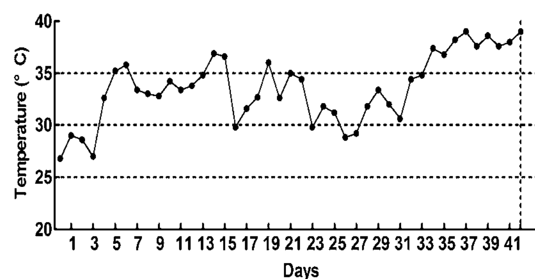

Figure 1. Daily average temperature from 12:00 a.m. to 8:00 p.m. over the 42 day experiment.

Sample Collection. Three piglets were randomly selected from each group at days 0, 14, 28, and 42 between 2:00 and 4:00 p.m., and the average temperatures at the times of blood collection were 27.6, 37.7, 33.0, and 40.0 °C, respectively. About 5 mL of blood sample was collected in a 10 mL syringe primed with EDTA through precaval vein. To prepare erythrocyte lysates, eppendorf tubes containing 1 mL of blood samples were centrifuged at 3000 rpm for 10 min, and the remaining red blood cells were washed three times with physiological saline and resuspended in 4 mL of ice-cold distilled water, shaken vigorously to force hemolysis, and then centrifuged at 3000 rpm for 10 min; the supernatant (erythrocyte lysate) was stored at −20 °C for GPx1 activity. The rest of the blood samples were stored at 4 °C for <1 week for Se analysis.

At the end of the study, three piglets from each group were randomly selected and euthanized; one-fourth of the liver, kidney, and spleen was rapidly excised and perfused with ice-cold isotonic saline, then snap-frozen in liquid nitrogen, and stored at −70 °C for analysis of GPx1, Hsp70, and Hsp27 mRNA levels. One-fourth of liver, kidney, spleen, and muscle were rapidly excised and stored at −20 °C for Se analysis.

Selenium Assay. Se concentration in whole blood and tissue was analyzed according to the method described by Ran et al.[23] using an AFS-930A atomic fluorescence spectrometer (Jitian Analysis Instrument Co., Beijing, China). Briefly, the homogenized sample aliquots

Table 3. Primers Used for Real-Time PCR

gene	accession no.	primer sequence (5′−3′)	product, bp
Actb	DQ845171.1	forward: CTGCGGCATCCACGAAACT reverse: AGGGCCGTGATCTCCTTCTG	147
GPx1	NM_214201	forward: TGGGGAGATCCTGAATTG reverse: GATAAACTTGGGGTCGGT	172
Hsp27	NM_001007518	forward: TCTCGGAGATCCAGCAGACT reverse: GCAGCGTGTATTTTCGAGTG	180
Hsp70	M29506	forward: GTGGCTCTACCCGCATCCC reverse: GCACAGCAGCACCATAGGC	114

for blood (1.0 mL) and tissues (1.0 g, wet weight) were dissolved with 10 mL of 36% HNO_3 and 70% $HClO_4$ ($HNO_3/HClO_4$ = 1:1) transferred to a Kjeldahl flask. After 12 h of predigestion at room temperature, the mixture in the Kjeldahl flask was heated on a galvanothermy board to observe the appearance of white fumes; 5 mL of 219 g/L HCl was added to the mixture and further heated on the galvanothermy board to observe the appearance of white fumes. Ultrapure water and a certified reference material for pork liver, digested using the same method, served as the blank and the standard control, respectively. Before the samples were injected into the 5% HCl (v/v) carrier, 2 mL of the prepared sample was transferred to a volumetric tube with 1 mL of 100 g/L $k_3Fe(CN)_6$ and made up to a final volume of 25 mL with 5% HCl (v/v). The treated sample was then injected into the HCl carrier. After it was merged with the KBH_4 stream, the volatile hydride was formed and swept out of the gas−liquid separator by an argon stream into a chemically generated hydrogen diffusion flame. The flame was maintained by the excess of hydrogen produced in the reaction between KBH_4 and HCl. The hydride was atomized in the flame, and the atoms were detected by fluorescence spectrometry.

Under the optimal concentrations of HCl (109.5 g/L) and KBH_4 (10 g/L, dissolved in 5 g/L NaOH solution) and flow rate (800 mL/min) of argon, quantitative determinations were performed by plotting calibration curves against Se standard solutions of SS. Five Se concentration levels ranging from 1 to 50 μg/L Se were used for plotting the standard curve.

GPx1 Activity Assay. The activity of GPx1 in erythrocytes and tissues was measured according to the method described by Lei,[24] with *tert*-butyl hydroperoxide (t-Bu-OOH) as the peroxide substrate. Briefly, 50 μL of 10% tissue homogenate or erythrocyte lysate was transferred to a 3 mL quartz cuvette containing 1900 μL of the reaction mixture [50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM GSH, 1 mM NaN_3, 0.1 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), and 0.9 U of GSH reductase (Sigma, USA)]. The reaction mixture then was preincubated for 3 min at 25 °C, and 50 μL of t-Bu-OOH (8 mM) was finally added. The oxidation rate of NADPH was monitored in a spectrophotometer at 340 nm at 25 °C for 5 min. The nonenzymatic reaction rate was determined by substituting water (serving as the blank) for the erythrocyte lysate and tissue homogenate and recording the decrease in NADPH absorbance. One unit of enzyme activity was defined as 1 μmol of NADPH oxidized per minute under these conditions. The activity of GPx1 in erythrocyte and tissue was expressed as unit per gram of protein.

Real-Time Polymerase Chain Reaction (Real-Time PCR). Levels of GPx1, Hsp70, and Hsp27 mRNA were quantitatively determined by real-time PCR. Total RNA was isolated from the frozen tissues using the RNAiso Plus reagent (TaKaRa) according to the manufacturer's instruction. Briefly, isolated RNA pellets were resuspended in 30 μL of diethyl- pyrocarbonate-treated water, quantified by the measurement of the absorbamce at 260/280 nm, and stored at −70 °C prior to cDNA synthesis. First-strand cDNA was synthesized from 1 μg of total RNA using Oligo dT primers and RNase M-MLV according to the manufacturer's instructions. Primer Premier Software (Premier Biosoft International, Palo Alto, CA, USA) was used to design specific primers for β-actin, GPx1, and Hsp27 on the basis of known pig sequences (Table 3), and the primer sequence for Hsp70 used was obtained from a published paper.[25] Real-time PCR was carried out as previously described[26] with some modifications. Reactions were performed in a 25 μL reaction mixture containing 12.5 μL of 2× SYBR Green I PCR Master Mix (TaKaRa), 10 μL of cDNA, 1 μL of each primer (10 μM), and 0.5 μL of PCR-grade water. Reactions were followed in an ABI Prism 7300 Detection System (Applied Biosystems, USA) consisting of a 95 °C step for 30 s followed by 40 cycles consisting of 95 °C for 5 s and 60 °C for 31 s. A dissociation curve was performed for each plate to confirm the production of a single product, whereas the no-template control served as the negative control.

Relative mRNA levels of the GPx1, Hsp70, and Hsp27 in the three tissue samples were determined using the Δ cycle threshold (ΔCt) method as outlined in the protocol of Applied Biosystems.[27] The result was applied to each gene by calculating the expression $2^{-\Delta\Delta CT}$.

Statistical Analysis. All of the data were analyzed statistically using SPSS 17.0 for Windows statistical software package (Statistical Product and Service Solutions, Inc., USA). All results are presented as the mean ± SE. A one-way ANOVA was performed to statistical analysis. Treatment differences were determined by Duncan[a] contrasts, and a P value was considered to be significant at 0.05.

RESULTS

ADFI in Different Groups. ADFI values in different groups are shown in Figure 2. ADFI values in P, SS, and SP groups show no significant difference with that in the Con group.

Se Concentrations of Blood in Different Groups. Se concentrations in blood are shown in Table 4. On days 14, 28, and 42 post feeding with P, SS, and SP diets, blood Se concentrations of the P group shows no significant difference

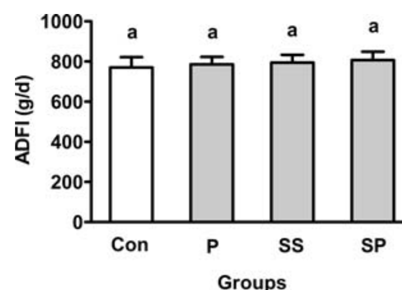

Figure 2. ADFI of a piglet in groups treated with basal diets without any added as a blank (Con) and with added probiotics (P), sodium selenite (SS), or selenium-enriched probiotics (SP). Data are the mean ± SE (n = 3), and statistical difference between treatment groups was set at P < 0.05.

Table 4. Selenium Concentrations (Micrograms per Liter) in Piglet Blood[a]

group	day 0	day 14	day 28	day 42
Con	166 ± 3.18a	169 ± 4.42a	170 ± 3.32a	167 ± 3.44a
P	170 ± 5.11a	174 ± 4.29a	169 ± 4.62a	173 ± 4.32a
SS	169 ± 6.18a	182 ± 3.95b	187 ± 4.50b	190 ± 3.30b
SP	169 ± 3.96a	199 ± 3.54c	203 ± 4.37c	208 ± 4.17c

[a]Selenium concentration of piglets in whole blood treated with basal diets without any added as a blank (Con) and with added probiotics (P), sodium selenite (SS), or selenium-enriched probiotics (SP). Data are the mean ± SE (n = 3), and statistical difference between treatment groups was set at $P < 0.05$.

with those in Con group. A significant increase in blood Se concentrations of SP and SS groups was observed ($P < 0.05$) compared to that of the Con or P group, and the blood Se concentrations of the SP group were significantly higher ($P < 0.05$) than that of SS group.

Se Concentrations of Tissues in Different Groups. Se concentrations in tissues are shown in Table 5. Se concentrations in liver, kidney, spleen, and muscle in the P group show no significant difference with those in the Con group, respectively, however, a significant increase in Se concentrations in these tissues was observed in SP and SS groups ($P < 0.05$) compared to that of Con or P group. Tissue Se concentrations of SP group were significantly higher ($P < 0.05$) than that of SS group.

Table 5. Selenium Concentrations (Nanograms per Gram) in Tissues (Wet Weight) at Day 42[a]

group	liver	kidney	muscle	spleen
Con	240 ± 5.38a	745 ± 2.79a	158 ± 3.15a	327 ± 4.65a
P	244 ± 4.58a	737 ± 3.64a	165 ± 2.85a	333 ± 4.24a
SS	365 ± 5.11b	830 ± 5.14b	199 ± 4.04b	385 ± 5.33b
SP	569 ± 5.50c	1140 ± 4.21c	301 ± 4.31c	435 ± 4.85c

[a]Selenium concentration of piglets in liver, kidney, spleen, and muscle treated with basal diets without any added as a blank (Con) and with added probiotics (P), sodium selenite (SS), or selenium-enriched probiotics (SP). Data are the mean ± SE (n = 3), and statistical difference between treatment groups was set at $P < 0.05$.

GPx1 Activities of Erythrocytes in Different Groups. Erythrocytes GPx1 activities are shown in Table 6. On days 14, 28, and 42 post feeding with P, SS, and SP diets, the erythrocyte GPx1 activities in the P group were not significantly different from those in the Con group. A significant increase in erythrocyte GPx1 activities was observed in the SP and SS groups ($P < 0.05$) relative to those of the Con or P group, and the erythrocyte GPx1 activities in the SP group were significantly higher ($P < 0.05$) than those of the SS group.

Table 6. GPx1 Activities (Units per Gram Protein) in Erythrocytes[a]

group	day 0	day 14	day 28	day 42
Con	19.7 ± 1.48a	21.5 ± 1.51a	19.6 ± 1.50a	19.2 ± 1.75a
P	20.6 ± 1.75a	22.5 ± 2.09a	21.6 ± 2.03a	21.0 ± 2.45a
SS	19.7 ± 1.90a	32.9 ± 1.96b	34.9 ± 2.09b	35.2 ± 1.09b
SP	22.3 ± 1.87a	55.8 ± 2.04c	56.5 ± 1.79c	58.6 ± 2.43c

[a]GPx1 activity of piglets in erythrocytes treated with basal diets without any added as a blank (Con) and with added probiotics (P), sodium selenite (SS), or selenium-enriched probiotics (SP). Data are the mean ± SE (n = 3), and statistical difference between treatment groups was set at $P < 0.05$.

GPx1 Activities of Tissues in Different Groups. GPx1 activities in tissues are shown in Figure 3. GPx1 activities in liver, kidney, and spleen in the P group show no significant difference from those in the Con group. A significant increase in GPx1 activities was observed in the tissues of the SP and SS group ($P < 0.05$) relative to those of the Con or P group, and the SP group also showed a significant elevation of tissue GPx1 activities ($P < 0.05$) relative to those of the SS group.

GPx1 mRNA Levels of Tissues in Different Groups. Levels of GPx1 mRNA in tissues measured by real-time PCR are shown in Figure 4. When compared with the Con group, no significant differences of GPx1 mRNA levels were observed in all tissues of the P group; however, significant increases of GPx1 mRNA levels were observed in all tissues in the SP and SS groups ($P < 0.05$). The SP group showed a significant increase in GPx1 mRNA level relative to that of the SS or P group.

Hsp70 mRNA Levels of Tissues in Different Groups. Levels of Hsp70 mRNA in tissues measured by real-time PCR are shown in Figure 5. When compared with the control group, significant decreases in Hsp70 mRNA levels were observed in all tissues in the P, SS, and SP groups ($P < 0.05$). However, no significant difference in Hsp70 mRNA level between the P and SS groups was observed. A significant difference in decreasing Hsp70 mRNA level between the SP group and the SS or P group ($P < 0.05$) was observed.

Hsp27 mRNA Levels of Tissues in Different Groups. Levels of Hsp27 mRNA in tissues measured by real-time PCR are shown in Figure 6. When compared with control, significant decreases in Hsp27 mRNA levels were observed in all tissues in the P, SS, and SP groups ($P < 0.05$). However, there was no significant difference in decreasing Hsp27 mRNA level between the P and SS groups. A significant difference in decreasing Hsp27 mRNA level between the SP group and the SS or P group was observed ($P < 0.05$).

■ DISCUSSION

Until now, previous studies of the effects of selenium supplementation on tissue Se concentration, activity, and mRNA level of GPx1 have focused on animals under normal environment conditions. Evidence indicated that organic selenium had the advantage in elevating Se concentration[28−31] and the ability to increase the activity and mRNA level of GPx1 in pigs.[32] Studies of the effects of selenium or probiotics on Hsp70 mRNA level have been reported in poultry or enterocyte-like Caco-2 cells under heat stress.[15−17,20] The developed SP feed supplement for animals in our laboratory has combined effects of organic Se and probiotics.[21] This experiment is the first to investigate the effects of SP on food intake, tissue Se deposition, GPx1 activity and mRNA level, and Hsp mRNA levels of piglets under heat stress conditions.

In this study, the basal diets contain 0.16 mg/kg Se. We added 0.3 mg/kg Se from SS and SP to the basal diets as the experimental diets, respectively. Se concentration in the experimental diets is 0.46 mg/kg Se, which is a little higher than that in previous studies[33,34] under normal conditions. However, piglets in the current experiment are under heat stress conditions, and a little higher dose of selenium was used. The dose is <0.50 mg/kg Se, which accords with the NRC criteria of China.

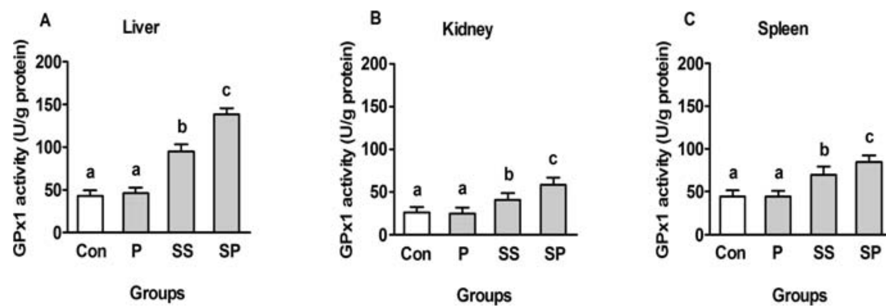

Figure 3. GPx1 activity (U/g protein) of piglets in (A) liver, (B) kidney, and (C) spleen treated with basal diets without any added as a blank (Con) and with added probiotics (P), sodium selenite (SS), or selenium-enriched probiotics (SP). Data are the mean ± SE ($n = 3$), and statistical difference between treatment groups was set at $P < 0.05$.

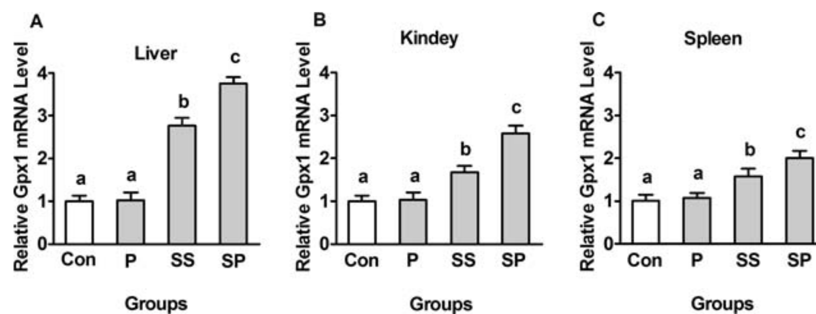

Figure 4. Tissue GPx1 mRNA level of piglets in liver (A), kidney (B), and spleen (C) treated with basal diets without any added as a blank (Con) and with added probiotics (P), sodium selenite (SS), or selenium-enriched probiotics (SP). Data are the mean ± SE ($n = 3$), and statistical difference between treatment groups was set at $P < 0.05$.

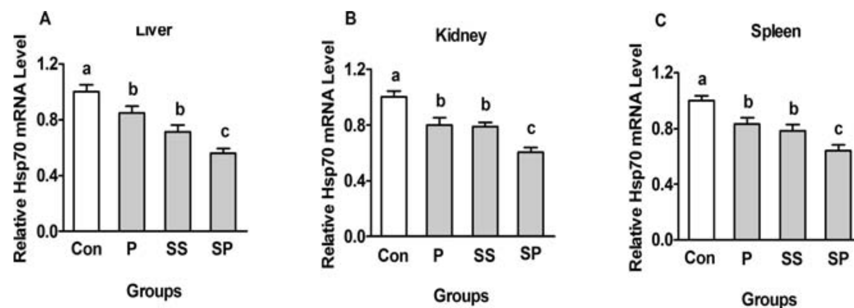

Figure 5. Effects of P, SS, and SP on Hsp70 mRNA level of piglets in liver (A), kidney (B), and spleen (C) treated with basal diets without any added as a blank (Con) and with added probiotics (P), sodium selenite (SS), or selenium-enriched probiotics (SP). Data are the mean ± SE ($n = 3$), and statistical difference between treatment groups was set at $P < 0.05$.

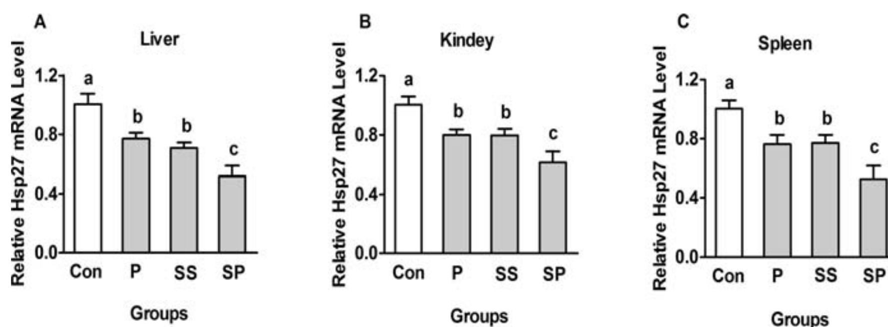

Figure 6. Tissue Hsp27 mRNA level of piglets in liver (A), kidney (B), and spleen (C) treated with added probiotics (P), sodium selenite (SS), or selenium-enriched probiotics (SP). Basal diets without any added were used as control (Con). Data are the mean ± SE ($n = 3$), and statistical difference between treatment groups was set at $P < 0.05$.

In this study, we have observed a significant increase in tissue Se concentration, GPx1 activity, and GPx1 mRNA level when piglets were supplemented with SS and SP, but there are no effects on these indices in piglets fed P under heat stress conditions compared with the control. However, the observed increase percentage of Se concentration and GPx1 activity of

liver in piglets fed Se diets under heat stress conditions is lower than that under normal conditions in a previous study.[35] It has been reported that heat stress increases metabolic activity[36] and causes a resultant oxidative stress, which results in increasing levels of ROS along with large increases in lipid peroxidation products.[37] Therefore, heat stress may require more antioxidants such as GPx in which selenium plays an essential role and causes reduction in Se concentration and GPx1 activity.

In this study, we observed that SP could significantly increase tissue Se concentration, GPx1 activity, and GPx1 mRNA level of piglets under heat stress conditions when compared with SS. The better effects of SP than SS may be due to the better bioavailability of Se-Met in SP. It has been reported that the bioavailability of Se depends on the conversion of absorbed Se into a biologically active form and tissue retention.[38,39] In mammals, ingested organic Se is absorbed in the small intestine[40] through the Na^+-dependent neutral amino acid transport system, and it can substitute for methionine in proteins or be converted to selenocysteine (Se-Cys).[41] However, when animals ingested Se as selenite, they were unable to synthesize Se-Met, and only Se-Cys was detected.[38,42,43] Thus, it is reasonable that tissue Se concentrations, activity and mRNA level of GPx1 of pigs fed SP were significantly higher than those of pigs fed SS in heat-stressed piglets in the present study.

In this study, significant down-regulations of Hsp70 and Hsp27 mRNA levels in the liver, kidney, and spleen of piglets in each experimental group were observed under heat stress conditions compared with Con. The effects of P on decreasing Hsps mRNA levels are consistent with the previous study in enterocyte-like Caco-2 cells,[20] but the mechanism of probiotics (*L. acidophilus*) in down-regulating the Hsps mRNA levels is still unknown. The effects of SS on decreasing Hsps mRNA levels in piglets under heat stress conditions have not been reported until now. Previous studies had demonstrated that Se might play an important role in the regulation of the body's glutathione peroxidase and improve antioxidant status,[44,45] which was very efficient in clearing free radicals. The free radicals induced by heat stress were involved in the induction of Hsps of chickens,[15,46] so we think that effects of SS on down-regulating Hsp70 and Hsp27 mRNA levels of heat-stressed piglets in the current experiment may be due to the increasing tissue Se concentration and GPx1 activity and mRNA levels. SP supplementation in piglet diets has maximum effects on decresing Hsp70 and Hsp27 mRNA levels, suggesting that SP is more beneficial to piglets under heat stress conditions than SS or P. Obviously, the maximum effects on decreasing Hsp70 and Hsp27 mRNA levels result from the combined effects of Se and P in SP. In this study, it is concluded that SP is a feasible dietary supplementation of piglets under heat stress conditions during the summer season.

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