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Original Research Article

New insights into the role of spermine in enhancing the antioxidant capacity of rat spleen and liver under oxidative stress

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ABSTRACT

Oxidative stress can damage cellular antioxidant defense and reduce livestock production efficiency. Spermine is a ubiquitous cellular component that plays important roles in stabilizing nucleic acids, modulating cell growth and differentiation, and regulating ion channel activities. Spermine has the potential to alleviate the effects of oxidative stress. However, to date no information is available about the effect of spermine administration on antioxidant property of the liver and spleen in any mammalian *in vivo* system. This study aims to investigate the protective effect of spermine on rat liver and spleen under oxidative stress. Rats received intragastric administration of either 0.4 μmol/g body weight of spermine or saline once a day for 3 days. The rats in each treatment were then injected with either diquat or sterile saline at 12 mg/kg body weight. Liver and spleen samples were collected 48 h after the last spermine ingestion. Results showed that regardless of diquat treatment, spermine administration significantly reduced the malondialdehyde (MDA) content by 23.78% in the liver and by 5.75% in the spleen, respectively ($P < 0.05$). Spermine administration also enhanced the catalase (CAT) activity, anti-hydroxyl radical (AHR) capacity and glutathione (GSH) content by 38.68%, 15.53% and 1.32% in the spleen, respectively ($P < 0.05$). There were interactions between spermine administration and diquat injection about anti-superoxide anion (ASA), AHR capacity, CAT activity, GSH content, and total antioxidant capacity (T-AOC) in the liver and about ASA capacity and T-AOC in the spleen of weaned rats ($P < 0.05$). Compared with the control group, spermine administration significantly increased the AHR capacity, CAT activity, GSH content, and T-AOC by 40.23%, 31.15%, 30.25%, 35.37% in the liver, respectively ($P < 0.05$) and increased the T-AOC by 8% in the spleen of weaned rats ($P < 0.05$). Compared with the diquat group, spermine + diquat group significantly increased ASA capacity by 15.63% in the liver and by 73.41% in the spleen of weaned rats, respectively ($P < 0.05$). Results demonstrate that spermine administration can increase the antioxidant capacity in the liver and spleen and can enhance the antioxidant status in the spleen and liver under oxidative stress.

1. Introduction

Reactive oxygen species (ROS) are generated during physiological processes and xenobiotic exposure in living organisms. Reactive oxygen species can be considered beneficial or harmful to organisms depending on their concentration. At physiologically low levels, ROS functions as a “secondary messenger” in intracellular signaling and regulation; however, excess ROS can result in oxidative stress (Circu and Aw, 2010). Oxidative stress can cause adverse damage to cellular macromolecules such as nucleic acids, proteins and lipids (Brieger et al., 2012), and intestine cells (Kim et al., 2012). Moreover, oxidative stress can affect the normal

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function of the immune system (Wang et al., 2010), reduce nutrient absorption and metabolism, and depress growth performance (Yuan et al., 2007). Oxidative stress is also related to a number of health disorders, including inflammatory disease, cancer (Reuter et al., 2010), cardiovascular, diabetes (Jomova and Valko, 2011), neurological (Jomova et al., 2010), and many other diseases. A previous study has suggested that substances such as vitamin C, vitamin E, and carbohydrates can suppress oxidative stress (Ryan et al., 2010; Kadian and Garg, 2012). Thus, supplementing components or food with antioxidant abilities to animals can be an effective approach to reduce oxidative stress (Devasagayam et al., 2004).

Spermine, a novel small molecule substance, is distributed in many living organisms such as animals, plants, some fungi, and some bacteria (Pegg and Michael, 2010) and plays important roles in myriad mechanism, including cellular proliferation and differentiation, gene transcription and translation (Pegg, 2014), modulation of ion channel function, cellular signal (Rao et al., 2012), and macromolecular synthesis (Igarashi and Kashiwagi, 2010). Previous experiments have shown that spermine administration can induce the maturation of villus and crypt cell function in jejunum and ileum (Buts et al., 1993). Further studies have revealed that spermine administration can significantly increase the specific activities of disaccharidase (e.g., maltase) in jejunum and enhance the intestinal absorption of macromolecules (Sugita et al., 2007; Cao et al., 2015). Therefore, spermine has been receiving considerable attention as a nutritional substance for accelerated functional maturation of the small intestines (Ramani et al., 2014). Furthermore, spermine acts as a free radical scavenger (Ha et al., 1998), a biologically important antioxidant *in vitro* (LØvaas and Carlin, 1991; Guerin et al., 2001; Shoji et al., 2005; Rider et al., 2007; Toro-Funes et al., 2013), and an anti-inflammatory agent (LØvaas and Carlin, 1991). Spermine administration can enhance the jejunum antioxidant properties of suckling rats (Cao et al., 2015) and serum antioxidant capacity of suckling piglets (Fang et al., 2016a), and alleviate serum oxidative stress in weaned rats (Liu et al., 2014). Therefore, spermine has potential functions against oxidative stress.

The liver is the main detoxifying organ in the body; this organ possesses a high metabolism rate and is prone to much damage potentially caused by oxidative stress. Thus, a correct status of the hepatic antioxidant defense system is significantly important for health maintenance. The spleen is one of the most important immune organs in the body and is mainly responsible for making antibodies, differentiating B cells, regulating immune responses, filtering aging erythrocytes, storing blood, and initiating immune reactions to blood-borne antigens. The normal structure and function of immune organs are connected with animal immunity. Oxidative damage caused by oxidative stress often leads to alteration in the structure and function of numerous organs (Azadzoi et al., 2005). Therefore, maintaining balance in the antioxidant defense system of the liver and spleen is very important for livestock breeding. However, to date no information is available about the effect of spermine administration on antioxidant property of the liver and spleen in any mammalian *in vivo* system. Diquat is a common herbicide, whose toxicity is related to disturbance of the total antioxidant capability of the body, and is widely used to cause oxidative stress in animal models such as rats and piglets (Abdollahi et al., 2004; Mao et al., 2014; Liu et al., 2016). Therefore, diquat was intraperitoneally injected to induce oxidative stress in the present study.

This study is part of a larger study that involved determining the metabolic profiles of spermine against oxidative stress (Liu et al., 2014). This study aims to explore the effects of spermine on the antioxidant status in rat liver and spleen under oxidative stress. The results can provide scientific evidence of the capacity of spermine to modulate antioxidant status and may pave the way for spermine development as a functional feed additive.

2. Material and methods

2.1. Experimental material

Weaned male Sprague–Dawley (SD) rats and their food were provided by Dossy Experimental Animals Co., Ltd. (Chengdu, China). Spermine (S3256-1G) and diquat (45422-250 mg-R) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Catalase (CAT), anti-superoxide anion (ASA), glutathione (GSH), malondialdehyde (MDA), total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC), anti-hydroxyl radical (AHR) and protein detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All antioxidant parameters were measured by colorimetric analysis at the corresponding wavelength by multifunctional microplate reader SpectraMax M5 (San Francisco, USA) according to the reagent specification.

2.2. Experimental design and feeding management

The animal procedures for this study were approved by the Care and Use of Laboratory Animals of Sichuan Agricultural University, and followed the Guide for the Care and Use of Laboratory Animals established by the National Research Council. All rats were placed in individual metabolic cages and acclimatized to experimental conditions 1 day before starting the experiment. Forty 21-day-old weaned male SD rats weighing 38 to 45 g were randomly assigned to 4 treatments (10 rats per treatment): control, spermine, diquat, and spermine + diquat. The rats received intragastric administration of either 0.4 μmol/g body weight of spermine (spermine was dissolved in physiological saline) or sterile saline per day for 3 days. Subsequently, half of the saline-received rats were intraperitoneally injected with diquat at 12 mg/kg body weight, whereas the other half was injected with the same volume of sterile saline. The spermine-received rats were also divided into 2 groups (diquat-injection or sterile saline injection). The liver and spleen were immediately removed after ether anesthesia 24 h after the diquat injection. The tissues were washed in cold saline (0.9% NaCl; 4 °C), frozen in liquid nitrogen, and then transferred to storage at −80 °C until analysis. Rats had access to food and water *ad libitum*. The experimental conditions throughout the experiment were maintained at a temperature ranging from 22 to 25 °C, a humidity between 50% and 70%, and a cycle of 12 h light/12 h dark.

2.3. Biochemical assays

2.3.1. Sample preparation

The sample was prepared using the method of Zhang et al. (2008). Approximately 0.1 g of sample (liver or spleen) was quickly weighed, thawed, and homogenized in 10 volumes (wt/vol) of ice-cold normal saline (0.7 g/mL). The homogenates of sample were centrifuged at 6,000 × *g* for 10 min at 4 °C. The supernatant was acquired and stored at −20 °C for biochemical analysis.

2.3.2. Protein content assay

The protein content of spleen and liver was determined using the method described by Georgiou et al. (2008) using a protein analysis kit (Coomassie Brilliant Blue), and bovine serum albumin as the protein standard. The sample preparation of protein is in accordance with the previously described in sample preparation section. Protein concentrations were calculated by the absorbance at 595 nm because of the binding of Coomassie brilliant blue G-250 to protein.

2.3.3. Catalase activity assay

The catalase activities of spleen and liver were measured according to the method described by Beers and Sizer (1952). The

enzyme participates in scavenging hydrogen peroxide and converts it to water and molecular oxygen. The enzyme activity was measured by monitoring the disappearance of hydrogen peroxide at 240 nm for 1 min at 25 °C. The CAT activity was expressed as units per milligram of protein, and 1 unit of CAT activity was defined as the amount of enzyme required to decompose 1 mmol/L H_2O_2 within 1 s per milligram of tissue protein at 37 °C.

2.3.4. Superoxide dismutase (SOD) activity assay

The SOD activity was evaluated spectrophotometrically at 550 nm according to the method of Zhang et al. (2008). This method contains the reduction of the outcome (superoxide ions) in the xanthine/xanthine oxidase system and the generated red formazan by reacting with 2-(4-iodophenyl)3-(4-nitrophenol)-5-phenyltetrazolium chloride. The result was expressed as U/mg protein. One U SOD was defined as the amount of enzyme needed to suppress superoxide ion production in the reaction by 50%.

2.3.5. GSH measurement

The GSH content was measured in terms of 5-thio-2-nitrobenzoate formation spectrophotometrically detected at 412 nm, as described by Akerboom and Sies (1980). The GSH content in the extract was represented as milligram per gram of protein by utilizing commercial GSH to act as a standard.

2.3.6. ASA and AHR assay

The ASA and AHR activities in the liver and spleen were determined following the method described by Jiang et al. (2010), and based on the operating instructions of the corresponding experiment kits. O_2^- was generated by the xanthine and xanthine oxidase reaction. After adding the electron acceptor, a coloration reaction was developed using the Griess reagent (Kelley et al., 2010). The coloration degree was directly proportional to the amount of superoxide anion in the reaction. The ASA activity was expressed as units per milligram of protein for the assay. One ASA unit is defined as the quantity of superoxide anion free radicals required to scavenge 1 mg of tissue protein for 40 min at 37 °C, which is equal to each gram of vitamin C-scavenging under the same condition. OH^- was generated based on the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$). After adding the electron acceptor, a coloration reaction was developed using the Griess reagent. The coloration degree was directly proportional to the quantity of hydroxyl radicals in the reaction (Fu et al., 2010). The tissue AHR capacity is defined as units per milligram of protein for the assay, and one unit is defined as the amount that decreases 1 mmol/L of H_2O_2 within 1 min per milligram of tissue protein.

2.3.7. T-AOC assay

Total antioxidant capacity was estimated using the colorimetric technique described by Miller et al. (1993). The integral cellular endogenous antioxidative ability that includes enzymatic and non-enzymatic antioxidants is reflected by T-AOC. All antioxidants were able to reduce Fe^{3+} to Fe^{2+}, and the latter can develop colored and stable chelates when combined with phenanthroline. The T-AOC is expressed as milligram per gram of protein for the assay, and one unit of T-AOC is defined as the absorbance value that increases by 0.01 within 1 min per milligram of tissue protein.

2.3.8. MDA assay

Malonaldehyde is the end product of lipid per-oxidation. Its content can directly reflect the degree of oxidation of the cell membrane, and therefore it was used to measure the degree of oxidative stress damage. Lipid per-oxidation was measured according to Cynamon et al. by using thiobarbituric acid reactive substances (TBARS) (Cynamon et al., 1985). For the assay, tissue homogenates was measured dilution concentration of 10%, a 500 aliquot of homogenate was mixed with 1 mL of 30% (wt/vol) TCA and centrifuged for 10 min at 4,000 × *g*. After that, 20 μL of supernatant was mixed with 600 μL of thiobarbituric acid (120 Mmol/L) and 100 μL of HCl (0.6 Mmol/L), and the mixture was heated at 95 °C for 40 min. Malonaldehyde content was calculated based on the absorption at 535 nm. Results are presented as nanomoles per milligram of protein.

2.4. Statistical analysis

Data were expressed as the means ± standard errors. All the data were analyzed as a two-way ANOVA (a 2 × 2 factorial arrangement) using the general linear model procedure, and the model included the main effects of spermine levels (0, 0.4 μmol per g BW) and diquat levels (0, 12 mg per kg BW) as well as their interaction. If a significant treatment effect of their interaction was observed, the significance between the treatment differences was identified separately by the least significant difference test. Results were considered significant at $P < 0.05$.

3. Results

3.1. Antioxidant parameters under oxidative stress

The antioxidant parameters of spleen and liver are displayed in Tables 1–4. As shown in Tables 1 and 2, regardless of spermine treatment, diquat injection significantly increased MDA content by

Table 1
Effects of spermine on MDA content and on ASA and AHR capacities of rat liver.[1]

Item	Spermine, μmol/g BW	Diquat, mg/kg BW	MDA, nmol/mg protein	ASA, U/g protein	AHR, U/mg protein
Group 1	0	0	10.31 ± 0.31	169.01 ± 4.17[a]	266 ± 20.1[b]
Group 2	0	12	11.48 ± 0.17	130.21 ± 1.26[c]	262 ± 17.9[b]
Group 3	0.4	0	7.93 ± 0.22	173.81 ± 3.40[a]	373 ± 7.77[a]
Group 4	0.4	12	8.67 ± 0.24	150.56 ± 3.62[b]	265 ± 14.7[b]
Main effects					
Spermine	0		10.89 ± 0.17[a]	149.61 ± 2.33	264 ± 11.3
	0.4		8.30 ± 0.17[b]	162.19 ± 2.33	319 ± 11.8
Diquat		0	9.12 ± 0.17[b]	171.41 ± 2.33	319 ± 11.8
		12	10.08 ± 0.17[a]	140.39 ± 2.33	263 ± 11.3
P-value					
Spermine			0.000	0.001	0.003
Diquat			0.001	0.000	0.002
Spermine × diquat			0.385	0.027	0.004

MDA = malondialdehyde; ASA = anti-superoxide anion; AHR = anti-hydroxyl radical.
[a–c]Within a column, means with different superscript letters significantly differ ($P < 0.05$) for comparison between groups (Group 1 = control, Group 2 = diquat, Group 3 = spermine, Group 4 = spermine + diquat), between 2 doses of spermine (0 and 0.4 μmol/g BW, main effects), and between 2 doses of diquat injection (0 and 12 mg/kg BW, main effects) for one of all parameters (MDA, ASA, AHR), respectively.
[1] Data are expressed as means ± SEM.

Table 2
Effects of spermine on CAT, T-AOC, and T-SOD activities and on GSH content of rat liver.[1]

Item	Spermine, μmol/g BW	Diquat, mg/kg BW	CAT, U/mg protein	GSH, mg/g protein	T-SOD, U/mg protein	T-AOC, U/mg protein
Group 1	0	0	122 ± 6.75^{b}	5.19 ± 0.30^{b}	37.8 ± 1.52	0.82 ± 0.07^{b}
Group 2	0	12	104 ± 8.75^{bc}	4.25 ± 0.28^{b}	34.3 ± 0.17	0.83 ± 0.03^{b}
Group 3	0.4	0	160 ± 6.38^{a}	6.76 ± 0.42^{a}	38.1 ± 0.91	1.11 ± 0.04^{a}
Group 4	0.4	12	97.5 ± 8.58^{c}	4.34 ± 0.28^{b}	34.3 ± 1.12	0.80 ± 0.05^{b}
Main effects						
Spermine	0		113 ± 5.44	4.72 ± 0.23	36.0 ± 0.79	0.83 ± 0.04
	0.4		129 ± 5.44	5.55 ± 0.23	36.2 ± 0.76	0.96 ± 0.04
Diquat		0	141 ± 5.44	5.97 ± 0.23	37.9 ± 0.76^{a}	0.97 ± 0.04
		12	101 ± 5.44	4.30 ± 0.23	34.3 ± 0.79^{b}	0.82 ± 0.04
P-value						
Spermine			0.051	0.017	0.893	0.014
Diquat			0.000	0.000	0.003	0.007
Spermine × diquat			0.008	0.031	0.872	0.003

CAT = catalase; T-AOC = total antioxidant capacity; T-SOD = total superoxide dismutase; GSH = glutathione.
[a–c]Within a column, means with different superscript letters significantly differ ($P < 0.05$) for comparison between groups (Group 1 = control, Group 2 = diquat, Group 3 = spermine, Group 4 = spermine + diquat), between 2 doses of spermine (0 and 0.4 μmol/g BW, main effects), and between 2 doses of diquat injection (0 and 12 mg/kg BW, main effects) for one of all parameters (CAT, T-AOC, T-SOD, GSH), respectively.
[1] Data are expressed as means ± SEM.

Table 3
Effects of spermine on MDA content and on ASA and AHR capacities of rat spleen.[1]

Item	Spermine, μmol/g BW	Diquat, mg/kg BW	MDA, nmol/mg protein	ASA, U/g protein	AHR, U/mg protein
Group 1	0	0	0.85 ± 0.02	81.61 ± 1.87^{a}	110 ± 4.43
Group 2	0	12	0.88 ± 0.02	39.86 ± 1.63^{c}	95.5 ± 3.07
Group 3	0.4	0	0.80 ± 0.01	86.21 ± 1.93^{a}	125 ± 3.33
Group 4	0.4	12	0.84 ± 0.01	69.12 ± 0.95^{b}	114 ± 4.14
Main effects					
Spermine	0		0.87 ± 0.01^{a}	60.73 ± 1.16	103 ± 2.68^{b}
	0.4		0.82 ± 0.01^{b}	77.66 ± 1.16	119 ± 2.68^{a}
Diquat		0	0.82 ± 0.01^{b}	83.91 ± 1.16	118 ± 2.68^{a}
		12	0.86 ± 0.01^{a}	54.49 ± 1.16	105 ± 2.68^{b}
P-value					
Spermine			0.010	0.000	0.000
Diquat			0.019	0.000	0.002
Spermine × diquat			0.838	0.000	0.621

MDA = malondialdehyde; ASA = anti-superoxide anion; AHR = anti-hydroxyl radical.
[a–c] Within a column, means with different superscript letters significantly differ ($P < 0.05$) for comparison between groups (Group 1 = control, Group 2 = diquat, Group 3 = spermine, Group 4 = spermine + diquat), between 2 doses of spermine (0 and 0.4 μmol/g BW, main effects), and between 2 doses of diquat injection (0 and 12 mg/kg BW, main effects) for one of all parameters (MDA, ASA, AHR), respectively.
[1] Data are expressed as means ± SEM.

Table 4
Effects of spermine on CAT, T-AOC, and T-SOD activities and on GSH content of rat spleen.[1]

Item	Spermine, μmol/g BW	Diquat, mg/kg BW	CAT, U/mg protein	GSH, mg/g protein	T-SOD, U/mg protein	T-AOC, U/mg protein
Group 1	0	0	11.8 ± 0.58	22.49 ± 0.52	13.69 ± 0.28	1.00 ± 0.02^{b}
Group 2	0	12	9.39 ± 0.64	19.96 ± 0.51	11.89 ± 0.26	0.98 ± 0.02^{b}
Group 3	0.4	0	16.4 ± 0.92	23.65 ± 0.52	13.46 ± 0.25	1.08 ± 0.02^{a}
Group 4	0.4	12	13.0 ± 0.69	19.37 ± 0.53	12.41 ± 0.34	0.96 ± 0.02^{b}
Main effects						
Spermine	0		10.6 ± 0.51^{b}	21.23 ± 0.38^{b}	12.79 ± 0.20	0.99 ± 0.01
	0.4		14.7 ± 0.51^{a}	21.51 ± 0.36^{a}	12.93 ± 0.20	1.02 ± 0.01
Diquat		0	14.1 ± 0.51^{a}	23.07 ± 0.36	13.58 ± 0.20^{a}	1.04 ± 0.01
		12	11.2 ± 0.51^{b}	19.67 ± 0.38	12.15 ± 0.20^{b}	0.97 ± 0.01
P-value						
Spermine			0.000	0.000	0.623	0.217
Diquat			0.000	0.597	0.000	0.001
Spermine × diquat			0.504	0.108	0.200	0.021

CAT = catalase; GSH = glutathione; T-SOD = total superoxide dismutase; T-AOC = total antioxidant capacity.
[a–b]Within a column, means with different superscript letters significantly differ ($P < 0.05$) for comparison between groups (Group 1 = control, Group 2 = diquat, Group 3 = spermine, Group 4 = spermine + diquat), between 2 doses of spermine (0 and 0.4 μmol/g BW, main effects), and between 2 doses of diquat injection (0 and 12 mg/kg BW, main effects) for one of all parameters (CAT, T-AOC, T-SOD, GSH), respectively.
[1] Data are expressed as means ± SEM.

10.53% ($P < 0.05$), and decreased T-SOD activity by 9.5% in the liver ($P < 0.05$). There were interactions between spermine administration and diquat injection with regard to ASA capacity in the liver of weaned rats ($P < 0.05$). Compared with the control group, diquat decreased ASA capacity by 22.96% ($P < 0.05$). As shown in Tables 3 and 4, regardless of spermine treatment, diquat injection reduced the T-SOD, CAT activities and AHR capacity by 10.53%, 20.57% and 11.02%, respectively ($P < 0.05$), but significantly increased MDA

content by 4.88% in the spleen ($P < 0.05$). There were interactions between spermine administration and diquat injection with regard to ASA capacity in the spleen of weaned rats ($P < 0.05$). Compared with the control group, diquat decreased ASA capacity by 51.16% in the spleen of weaned rats ($P < 0.05$).

3.2. Effect of spermine on antioxidant parameters

As shown in Tables 1 and 2, regardless of diquat treatment, spermine administration significantly reduced the MDA content by 23.78% in the liver ($P < 0.05$). There were interactions between spermine administration and diquat injection about ASA, AHR capacity, CAT activity, GSH content, and T-AOC in the liver of weaned rats ($P < 0.05$). Compared with the control group, spermine administration significantly increased the AHR capacity, CAT activity, GSH content, and T-AOC by 40.23%, 31.15%, 30.25%, 35.37% in the liver of weaned rats, respectively ($P < 0.05$). Compared with the diquat group, spermine + diquat group significantly increased ASA capacity by 15.63% in the liver of weaned rats ($P < 0.05$). Irrespective of the diquat treatment, spermine administration also enhanced the CAT activity, AHR capacity and GSH content by 38.68%, 15.53% and 1.32%, respectively ($P < 0.05$), but reduced the MDA content by 5.74% in the spleen (Tables 3 and 4, $P < 0.05$). There were interactions about ASA capacity and T-AOC in the spleen of weaned rats ($P < 0.05$). Compared with the control group, spermine administration significantly increased the T-AOC by 8% in the spleen of weaned rats ($P < 0.05$). Compared with the diquat group, spermine + diquat group significantly increased ASA capacity by 73.41% in the spleen of weaned rats ($P < 0.05$).

4. Discussion

4.1. Effects of spermine on the antioxidant status of the liver and spleen

4.1.1. Effects of spermine on free radical scavenging ability and MDA content

Spermine can enhance antioxidant status. We first investigated the effects of spermine supplement on the radical scavenging ability of animal liver and spleen. Reactive oxygen species, such as superoxide anion and hydroxyl radical, can induce oxidative stress in cells (Gallagher et al., 1995). In this study, AHR capacity in the liver and spleen were higher by spermine supplementation. Results suggest that spermine administration can enhance the ability of free radical inhibition in the liver and spleen. This result does not agree with that of our previous study indicating that spermine supplementation had no effect on the AHR activities of suckling rats' jejunum. The possible reason for this may be the different organ systems and growth stages involved (Cao et al., 2015). Lipid peroxidation damage is primarily caused by superoxide anion and hydroxyl radical (Abdollahi et al., 2004). Thus, we next investigated the effect of spermine supplement on MDA content. Malonaldehyde is the end-product of lipid peroxidation, and MDA content can be used as a marker of oxidative damage in the body (Cynamon et al., 1985). In this study, spermine administration significantly reduced MDA content in the liver and spleen. The possible reason is that spermine can bind to membranes and inhibit lipid peroxidation by forming a compound with the phospholipid polar head (Cao et al., 2015). With all these findings taken together, spermine administration can enhance antioxidant status. Free radical scavenging abilities benefit from enzymatic (e.g., SOD and CAT) and non-enzymatic (e.g., GSH) antioxidant defense systems. A complex system of enzymatic antioxidants and non-enzymatic antioxidants in mammals can protect these organisms against oxidative stress (Bhor et al., 2004). We therefore next investigated the effect of spermine on enzymatic and nonenzymatic antioxidant systems.

4.1.2. Effects of spermine on enzymatic antioxidant systems

Catalases, heme-containing enzymes that can catalyze hydrogen peroxide breakdown to water and molecular oxygen, are widely regarded as essential antioxidants against hydroxyl radical toxicity (Bagnyukova et al., 2005). In the current study, spermine enhanced CAT activity in the liver and spleen. Our previous study found that spermine could enhance the mRNA levels of CAT in liver and spleen of suckling piglet (data not shown), so the improvement of CAT activity in the liver and spleen may be related to the expression level. These results indicate that spermine supplementation can improve antioxidative status through enzymatic antioxidant systems in rat liver and spleen.

4.1.3. Effects of spermine on nonenzymatic antioxidant systems

Aside from enzymatic antioxidant defense function, nonenzymatic antioxidant defense also plays an important role in protecting organisms against oxidative damage. Glutathione is the most important and ubiquitous endogenous antioxidant agent, and plays a protective role in fighting against oxidative stress by scavenging hydroxyl radicals and singlet oxygen molecules (Meister and Anderson, 1983). In the present study, the GSH content of the liver and spleen was increased by spermine supplementation. This result is consistent with that of a previous research indicating that spermine administration significantly increased the GSH content of sulking rats' ileum (Liu et al., 2015). The value of T-AOC can reflect the total antioxidant capacity of an organism (Ren et al., 2012). In the current study, the T-AOC activity of the liver and spleen was increased by spermine supplementation. This finding is consistent with that of a previous study in our laboratory stating that spermine increased T-AOC in rats' jejunum (Cao et al., 2015; Fang et al., 2016b). This information suggests that spermine supplementation can improve antioxidative status through nonenzymatic antioxidant systems in the liver and spleen.

4.2. Effects of diquat on the antioxidant status of the liver and spleen

Diquat can form free radicals (superoxide radicals and Hydroxyl free radicals) which can induce lipid peroxidation (Abdollahi et al., 2004). In the present study, ASA activities were decreased, and MDA content was increased in rat liver and spleen, and AHR activity was decreased in the spleen by diquat supplementation. This result suggests that the total amount of radicals exceeded the ability of the body to eliminate these radicals from both organs, and diquat injection caused oxidative damage in the liver and spleen. A previous study indicated that oxidative damage was accompanied by a decrease in antioxidant defense (Jiang et al., 2015). In this study, the SOD activities in the liver and spleen, and the CAT activity in the spleen were significantly decreased under oxidative stress. This information suggests that diquat can decrease the enzymatic antioxidant defense function of rat liver and spleen. Taken together, diquat injection damaged both enzymatic and nonenzymatic antioxidant defenses.

4.3. Effects of spermine on the antioxidant status of the liver and spleen under oxidative stress

Spermine possesses the potential to alleviate oxidative stress. In the present study, spermine administration can enhance the activities of ASA in the liver and spleen of weaned rats under oxidative stress, which suggest that spermine can suppress the generation of free radicals in the liver and spleen, and this could be

regarded as the protective effects of the spermine when encountering the changes of environment factors. In summary, spermine administration can counteract oxidative stress in the liver and spleen.

5. Conclusions

We report 2 primary, novel, and interesting results: spermine administration can improve the antioxidant status of the liver and spleen, including enzymatic antioxidant and non-enzymatic antioxidant activities, and spermine can also enhance antioxidant capacity of spleen and liver under oxidative stress. The results obtained from this study reveal the potential ability of spermine as a stress-resistant component. In addition, spermine can promote the animal gastrointestinal development and maturation, and reduce piglet early weaning stress, so the research of spermine will pave a new way for the animal husbandry production. However, the underlying molecular mechanism by which spermine supplementation increases the antioxidant status needs further investigation in the future.

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