Effects of spermine supplementation on the morphology, digestive enzyme activities, and antioxidant capacity of intestine in weaning rats

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The main objective of this study was to investigate the effects of different doses of spermine and its extended supplementation on the morphology, digestive enzyme activities, and intestinal antioxidant capacity in weaning rats. Nineteen-day-old male rats received intragastric spermine at doses of 0.2 and 0.4 μmol/g BW for 3 or 7 d, whereas control rats received similar doses of saline. The results are as follows: 1) In the jejunum, the seven-day supplementation with both doses of spermine significantly increased crypt depth ($P < 0.05$) compared with the control group; the supplementation extension of the high spermine dose increased villus height and crypt depth ($P < 0.05$); in the ileum, the low spermine dose significantly increased villus height and crypt depth compared with the control group for 7 days ($P < 0.05$). 2) The 3-day supplementation with high spermine dose increased alkaline phosphatase activity in the jejunum ($P < 0.05$). 3) In the jejunum, the anti-hydroxyl radical (AHR), total superoxide dismutase (T-SOD), catalase (CAT), and total antioxidant capacity (T-AOC) activities were increased ($P < 0.05$); however, the malondialdehyde (MDA) content was reduced ($P < 0.05$) in groups supplemented with the high spermine dose relative to those in the control groups after 3 and 7 d; moreover, the anti-superoxide anion (ASA) and glutathione (GSH) contents increased with the high spermine dose that lasted for 3 days ($P < 0.05$). Furthermore, the T-SOD and CAT activities (after 3 and 7 d), ASA (after 3 d), and AHR (after 7 d) increased with the high spermine dose compared with those of the low spermine dose ($P < 0.05$). Extending the supplementation duration (7 d) of the high spermine dose decreased the MDA content and ASA and T-AOC activities ($P < 0.05$). These results suggested that spermine supplementation can modulate gut development and enhance the antioxidant status of the jejunum in weaning rats, and a dosage of 0.4 μmol spermine/g BW had better effects than the dosage of 0.2 μmol spermine/g BW on accelerating gut development and increasing antioxidant capacity.

1. Introduction

The intestine plays a crucial role in digesting and absorbing nutrients, balancing microbiota, protecting immunological functions in young animals and serves as a barrier against harmful pathogens and antigens (Lalles et al., 2007; Barszcz and Skomiał, 2011). Weaning is one of the most complex and stressful periods that may lead to intestinal dysfunction, resulting in increased susceptibility to diseases, dyspepsia, and diarrhea, all of which can degrade animal health and growth after weaning (Lalles et al., 2007; Barszcz and Skomiał, 2011).
2.1. Materials

Spermine (S3256-1G) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sprague–Dawley rats with a body weight (BW) from 38 to 45 g were provided by Dossy Experimental Animals Co., Ltd (Chengdu, China). All enzyme assay and antioxidative reagent kits used in the present study were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animal experiment and sample collection

Animal experimental procedures of this work were approved by the Care and Use of Laboratory Animals of Sichuan Agricultural University of China. All protocols were conducted according to the Guide for the Care and Use of Laboratory Animals of the National Research Council (1997). Thirty six 19-day-old weaning male Sprague–Dawley rats were placed in individual metabolic cages and acclimatized for 1 d. All rats were randomly divided into 6 experimental groups (i.e., C-3, C-7, 50.2-3, 50.2-7, 50.4-3, and 50.4-7) with 6 replicates per group and ad libitum access to diet and water. The rats were intragastrically administered with either spermine (0.2 or 0.4 μmol/g BW) or physiological saline once a day for 3 or 7 d. Room temperature and humidity were set to 25°C and 60%, respectively, and a cycle of 12-h light and 12-h dark was maintained throughout the experiment. Rats were anesthetized by ether 48 h after the last spermine ingestion. The jejunum and ileum were separated and immediately flushed with ice-cold saline. The isolated jejunal and ileal segments of approximately 3 cm in length were stored in 4% paraformaldehyde solution for morphological analyses. The remaining jejunum and ileum samples (approximately 5 cm) were snap-frozen in liquid nitrogen and then stored at –80°C before analysis. The dosages and times of spermine supplementation were selected following a previous experiment (Deloyer et al., 2005).

2.3. Histomorphological study of the jejunum and ileum

The preserved tissues were sectioned and stained with hematoxylin and eosin, and applied with standard paraffin-embedding procedures. At least 10 intact, well-oriented crypt-villus units were selected in triplicate as sources of each rat intestinal cross section. Villus height, villus width, and crypt depth were measured using image processing and analysis systems (Image Pro Plus, Media Cybernetics, Bethesda, MD, USA). The villus surface areas were computed using the following formula: Villus surface area (mm²) = 2π (villus height) × (villus width/2).

2.4. Enzyme assays of the jejunum and ileum

The jejunal and ileal samples were thawed, weighed, and homogenized (1:10, wt/vol) in 9 volumes of ice-cold physiologic saline. The homogenates were centrifuged at 3,000 x g for 10 min at 4°C, the supernatants collected and enzyme activities analyzed. Total protein contents were assayed using the method described by Bradford (1976); maltase, sucrase, and lactase activities were determined as described by Dahlqvist (1964); AKP activity was determined according to the method of Rosalki and Foo (1984).

2.5. Determination of antioxidant index in the jejunum

To evaluate the prooxidant-antioxidant balance in the jejunum, we determined the malondialdehyde (MDA) and glutathione (GSH) contents, as well as the anti-superoxide anion (ASA), anti-hydroxyl radical (AHR), total superoxide dismutase (T-SOD), catalase (CAT), and total antioxidant capacity (T-AOC) activities. The methods of preparing supernatants of jejunal homogenates were described as above (enzyme assay procedure). The MDA content was detected as described by Livingstone et al. (1990); ASA and AHR activities were determined followed the protocol of Jiang et al. (2009). Total superoxide dismutase activity was determined using the method of Zhang et al. (2010), and CAT activity was assayed according to Aebi
(1984). GSH content was measured according to Vardi et al. (2008), and T-AOC activity was estimated according to Miller et al. (1993).

2.6. Statistical analysis

All data were subjected to two-way analysis of ANOVA using the GLM procedure of SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) and presented as means ± standard errors. The main factorial of the model included spermine level (0, 0.2, 0.4 μmol/g BW) and extension time (3 or 7 d). Statistical differences between means were determined by ANOVA and Duncan’s multiple range was used to compare data among treatments. Statistically significant differences were considered as P < 0.05.

3. Results

3.1. Morphological observations of the jejunum and ileum

Tables 1 and 2 present the morphological indices of the jejunum and ileum. In the jejunum, the 7-day supplementation with spermine of both doses significantly increased crypt depth, i.e., C-7 vs. S0.2-7 and C-7 vs. S0.4-7, (P < 0.05), whereas the 7-day supplementation with spermine of high dose significantly increased villus height, i.e., C-7 vs. S0.4-7, (P < 0.05). The supplementation extension of high spermine dose increased villus height and crypt depth, i.e., S0.4-3 vs. S0.4-7, (P < 0.05). Moreover, no difference was observed between C-3 and C-7 groups. In the ileum, the low spermine dose significantly increased both villus height and crypt depth compared with those in the control group after 7 days (P < 0.05).

3.2. Enzyme activities in the jejunum and ileum

Enzyme activities in the jejunum and ileum are presented in Tables 3 and 4. In the jejunum, the 3-day supplementation of high spermine dose increased alkaline phosphatase activity (C-3 vs. S0.4-3). However, the obtained value did not differ between C-7 and S0.4-7 groups. Spermine also had no effect on digestive enzyme activities in the jejunum and ileum regardless of dose and supplementation duration.

3.3. Antioxidant indicators in the jejunum

Table 5 presents the antioxidant indicators in the jejunum. Jejunum ASA, AHR, T-SOD, CAT, and T-AOC activities and GSH content were increased; however, MDA content was reduced in animals will experience profound modifications (Barszcz and Skomiał, 2011). These modifications are achieved by villus proliferation and crypt hyperplasia, which results from both the faster rate of cell division, leading to increased villus length and width, and increased rate of cell renewal, resulting in deeper crypts (Cummins and Thompson, 2002; Madara, 2011; Noah et al., 2011). Increases in villus height and crypt depth significantly contribute to enlargement of the absorptive area of intestinal mucosa. Our results showed that the extended supplementation of the high spermine dose increased villus height and crypt depth. These data suggested that extending spermine supplementation can promote jejunal development by regulating morphological structures, which were consistent with the results of previous studies, wherein spermine accelerated jejunal maturation (Fang et al., 2016). However, no differences were observed in villus width, villus height-to-crypt depth ratio, and villus surface area of jejunum in spermine-supplemented rats, which were consistent with some results, wherein spermine did not significantly affect the jejunal indices in suckling rats (Cao et al., 2015). The low spermine dose significantly increased villus height-to-crypt depth ratio of ileum compared with that in the control groups. This situation implied that the effects of spermine administration on ileum development were influenced by spermine dosage. Evidence confirmed that excess spermine has adverse effects in promoting cells growth; however, low or optimum doses of spermine have beneficial effects on intestinal development (Cheng et al., 2006; Larqué et al., 2007). Collectively, the effects of different spermine doses and its extended supplementation can modulate intestinal morphological changes, especially those administered with 0.4 μmol/g BW.

Morphological changes in the intestine after weaning are accompanied by changes in the activities of brush border enzymes, such as lactase, sucrase, and maltase, which serve as important digestive functions. Hence, we probed the effects of spermine and its extended administration on intestinal enzymatic activity.

4. Discussion

4.1. Effects of spermine dose and extended spermine supplementation on jejunal and ileal morphological structures

During weaning, the intestine structure and morphology of animals will experience profound modifications (Barszcz and Skomiał, 2011). These modifications are achieved by villus proliferation and crypt hyperplasia, which results from both the faster rate of cell division, leading to increased villus length and width, and increased rate of cell renewal, resulting in deeper crypts (Cummins and Thompson, 2002; Madara, 2011; Noah et al., 2011). Increases in villus height and crypt depth significantly contribute to enlargement of the absorptive area of intestinal mucosa. Our results showed that the extended supplementation of the high spermine dose increased villus height and crypt depth. These data suggested that extending spermine supplementation can promote jejunal development by regulating morphological structures, which were consistent with the results of previous studies, wherein spermine accelerated jejunal maturation (Fang et al., 2016). However, no differences were observed in villus width, villus height-to-crypt depth ratio, and villus surface area of jejunum in spermine-supplemented rats, which were consistent with some results, wherein spermine did not significantly affect the jejunal indices in suckling rats (Cao et al., 2015). The low spermine dose significantly increased villus height-to-crypt depth ratio of ileum compared with that in the control groups. This situation implied that the effects of spermine administration on ileum development were influenced by spermine dosage. Evidence confirmed that excess spermine has adverse effects in promoting cells growth; however, low or optimum doses of spermine have beneficial effects on intestinal development (Cheng et al., 2006; Larqué et al., 2007). Collectively, the effects of different spermine doses and its extended supplementation can modulate intestinal morphological changes, especially those administered with 0.4 μmol/g BW.

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4.2. Effects of spermine dose and its extended supplementation on the enzymatic activities in rat jejunum and ileum

After weaning, the intestinal environment changes drastically because of the replacement of highly digestible sow milk with solid food. The intestine has to adapt to the new type of food, leading to changes in enzymatic secretion and activity. The decrease in lactase
and increase in maltase and sucrase activities are believed to be signs of small intestinal maturity in young animals. However, our study found that spermine had no effect on lactase, maltase, and sucrase activities in the jejunum and ileum, which was consistent with the results of Peulen et al. (2004), who reported that spermine did not affect lactase or sucrase activities in the jejunum, maltase or sucrase activities of the ileum in 21-day-old weaning rats. These changes might be associated with age. The intestine might become unresponsive to spermine with age; similarly, spermine could not be recommended to facilitate changes in intestinal enzymatic patterns in typical weaning rats. Furthermore, the other fact is that intestinal enterocytes could become insensitive to spermine supplementation when the intestine becomes capable of obtaining higher contents of exogenous spermine from a solid diet after weaning (Larqué et al., 2007). Nevertheless, the higher spermine dose increased the specific activity of alkaline phosphatase in the jejunum in the 3-day spermine treatment. These results suggested that supplementing 0.4 μmol spermine/g BW for 3 days could

### Table 2
Effects of graded levels of spermine and its time extension on morphology of ileum in weaned rats.³

| Item                                             | 3-d Treatments | 7-d Treatments | SEM | P-value |
|--------------------------------------------------|----------------|----------------|-----|---------|
|                                                   | C    | S0.2 | S0.4 | C    | S0.2 | S0.4 | S   | S   | S x TS |
| Villus height, μm                                | 247.15 | 220.78 | 269.88 | 237.74 | 249.99 | 257.21 | 5.97 | 0.138 | 0.840 | 0.277 |
| Villus width, μm                                 | 83.35 | 85.35 | 96.49 | 78.5 | 89.81 | 90.36 | 2.06 | 0.051 | 0.660 | 0.361 |
| Crypt depth, μm                                  | 135.00 | 127.31 | 142.86 | 155.37 | 132.00 | 149.14 | 3.60 | 0.110 | 0.140 | 0.598 |
| Villus height/crypt depth                         | 1.88b | 1.73ab | 1.89b | 1.54a | 1.9b | 1.73b | 0.04 | 0.388 | 0.133 | 0.017 |
| Villus surface area, mm²                           | 0.06 | 0.06 | 0.08 | 0.06 | 0.07 | 0.07 | 0.00 | 0.035 | 0.770 | 0.178 |

C = control; S0.2 = 0.2 μmol spermine/g BW; S0.4 = 0.4 μmol spermine/g BW; S = spermine; TS = treatments.

³ Within a row, means with different superscript letters significantly differ (P < 0.05) for comparison between C, S0.2, S0.4.

### Table 3
Effects of graded levels of spermine and its duration extension on enzymes activities of jejunum in weaned rats.³

| Item                                             | 3-d Treatments | 7-d Treatments | SEM | P-value |
|--------------------------------------------------|----------------|----------------|-----|---------|
|                                                   | C    | S0.2 | S0.4 | C    | S0.2 | S0.4 | S   | S   | S x TS |
| Protein content, mg/g tissue                      | 124.88 | 126.29 | 131.39 | 126.34 | 123.77 | 128.81 | 2.01 | 0.569 | 0.778 | 0.910 |
| Lactase activity, U/mg protein                    | 8.42 | 7.39 | 5.68 | 10.59 | 8.32 | 7.70 | 0.53 | 0.931 | 0.100 | 0.859 |
| Maltase activity, U/mg protein                    | 186.64 | 172.06 | 159.04 | 167.91 | 140.29 | 101.41 | 9.03 | 0.090 | 0.039 | 0.633 |
| Sucrase activity, U/mg protein                    | 58.69 | 65.35 | 63.51 | 80.09 | 74.22 | 63.14 | 3.48 | 0.701 | 0.168 | 0.465 |
| Alkaline phosphatase activity, U/g protein        | 310.90a | 473.28ab | 503.07b | 572.15b | 554.09b | 596.60b | 23.91 | 0.197 | 0.005 | 0.257 |

C = control; S0.2 = 0.2 μmol spermine/g BW; S0.4 = 0.4 μmol spermine/g BW; S = spermine; TS = treatments.

³ Within a row, means with different superscript letters significantly differ (P < 0.05) for comparison between C, S0.2, S0.4.

### Table 4
Effects of graded levels of spermine and its duration extension on enzymes activities of ileum in weaned rats.³

| Item                                             | 3-d Treatments | 7-d Treatments | SEM | P-value |
|--------------------------------------------------|----------------|----------------|-----|---------|
|                                                   | C    | S0.2 | S0.4 | C    | S0.2 | S0.4 | S   | S   | S x TS |
| Protein content, mg/g tissue                      | 91.60 | 89.64 | 87.96 | 101.78 | 98.11 | 112.63 | 2.79 | 0.602 | 0.009 | 0.386 |
| Lactase activity, U/mg protein                    | 11.37 | 6.74 | 5.48 | 5.04 | 7.62 | 6.01 | 0.86 | 0.499 | 0.339 | 0.163 |
| Maltase activity, U/mg protein                    | 269.54 | 233.15 | 214.8 | 209.98 | 243.83 | 172.88 | 14.86 | 0.379 | 0.324 | 0.618 |
| Sucrase activity, U/mg protein                    | 89.74 | 71.12 | 65.96 | 72.61 | 88.63 | 64.86 | 6.49 | 0.578 | 0.986 | 0.584 |
| Alkaline phosphatase activity, U/g protein        | 845.81 | 1212.38 | 1599.93 | 685.36 | 1432.87 | 826.33 | 114.75 | 0.098 | 0.277 | 0.180 |

C = control; S0.2 = 0.2 μmol spermine/g BW; S0.4 = 0.4 μmol spermine/g BW; S = spermine; TS = treatments.

³ Data are presented as means ± SEM, n = 7.

### Table 5
Effects of graded levels of spermine and its duration on the antioxidant status of jejunum in weaned rats.³

| Item                                             | 3-d Treatments | 7-d Treatments | SEM | P-value |
|--------------------------------------------------|----------------|----------------|-----|---------|
|                                                   | C    | S0.2 | S0.4 | C    | S0.2 | S0.4 | S   | S   | S x TS |
| MDA, nmol/mg protein                              | 0.31c | 0.31c | 0.25bc | 0.26bc | 0.18a | 0.17a | 0.01 | 0.001 | 0.000 | 0.094 |
| ASA, U/mg protein                                  | 150.13e | 157.54e | 212.95e | 163.13ab | 168.78ab | 178.16b | 4.41 | 0.000 | 0.562 | 0.003 |
| AHR, U/mg protein                                  | 170.58e | 225.22e | 259.37e | 205.11ab | 201.19ab | 246.80c | 6.81 | 0.000 | 0.945 | 0.053 |
| T-SOD, U/mg protein                                | 80.99e | 89.63e | 97.60e | 71.59a | 72.97ab | 89.81c | 1.96 | 0.000 | 0.000 | 0.307 |
| CAT, U/mg protein                                  | 3.57e | 5.12e | 6.24e | 3.49e | 5.13a | 6.22c | 0.21 | 0.000 | 0.086 | 0.981 |
| GSH, mg/g protein                                  | 4.05c | 8.99b | 9.8a | 8.8b | 9.41b | 10.58b | 0.43 | 0.000 | 0.000 | 0.001 |
| T-AOC, U/mg protein                                | 1.58ab | 1.90d | 2.05d | 1.50e | 1.69bc | 1.81bc | 0.05 | 0.000 | 0.017 | 0.536 |

C = control; S0.2 = 0.2 μmol spermine/g BW; S0.4 = 0.4 μmol spermine/g BW; S = spermine; TS = treatments; MDA = malondialdehyde; ASA = anti-superoxide anion; AHR = anti-hydroxyl radical; T-SOD = total superoxide dismutase; CAT = catalase; GSH = glutathione; T-AOC = total antioxidant capacity.

³ Within a row, means with different superscript letters significantly differ (P < 0.05) for comparison between C, S0.2, S0.4.

³ Data are presented as means ± SEM, n = 7.
maintain the adult enzymatic pattern of alkaline phosphatase in the jejunum. Our results demonstrated that spermine intake and extended supplementation only have limited effects on enhancing the digestive and absorptive enzymatic activities of the intestine in weaning animals.

4.3. Effects of spermine dose and time extension of spermine administration on the antioxidant status of jejunum

During weaning, animals suffer grievously from oxidative stresses induced by numerous environmental, social, physical, and psychological stressors, such as different temperature and humidity, different food source, and abrupt displacement (Campbell et al., 2013; Yin et al., 2013). Oxidative stress can lead to overproduction of reactive oxygen species (ROS), which can react at a high rate with most of the molecules in the cells and damage proteins, amino acids, and nucleic acids (Srividattav et al., 2006). Spermine has a potential effect against oxidative stress, and can better promote jejunum development in our study. However, no research on the effects of spermine and its extended administration on the antioxidant defense system of organs in weaning animals is available. Therefore, our study further explored the protective effects of spermine dose and its extended supplementation on the antioxidant status of jejunum in weaning rats. The jejunal antioxidant indices include MDA content and ASA, AHR, CAT, S-TOD, GSH, and T-AOC activities.

Excessive amounts of ROS can lead to lipid peroxidation in organisms. Malondialdehyde (MDA) is the most familiar breakdown product of lipid peroxidation, and its level is frequently used as a direct marker of lipid oxidative damage caused by ROS (Feng et al., 2014). The results of MDA in this study indicated that spermine significantly reduced MDA content in the jejunum, thereby suggesting that spermine could depress lipid peroxidation in weaning rats. These findings were similar to previous findings in suckling animals (Cao et al., 2015; Fang et al., 2016). Both doses of spermine could also decrease MDA content regardless of treatment duration. Moreover, lower MDA contents were observed with prolonged spermine supplementation for both doses, indicating that extending spermine administration can improve the capacity of anti-lipid peroxidation in the rat jejunum. Based on the results of spermine preventing lipid peroxidation in the jejunum, we further determined the scavenging ability of spermine on superoxide anion (O2-) and hydroxyl radical (·OH). The superoxide anion and hydroxyl radical are 2 agents strongly involved in lipid peroxidation and oxidative damage in cells (Kohen and Nyska, 2002). In the present study, spermine significantly enhanced ASA and AHR activities compared with the control group, indicating that spermine administration can improve the free radical scavenging ability in the jejunum. Our results also showed that ASA and AHR activities in jejunum increased with higher spermine dose compared with those of lower spermine dose. High spermine dose appears to enhance the function of radical scavenging in the jejunum. Such observations are in accordance with those in other studies that confirmed spermine to be mostly a free radical scavenger only at very high doses in vitro (Kafy et al., 1986).

Scavenging capacity for free radicals is related to enzymatic and non-enzymatic antioxidant defense systems, and these defense systems were measured to further identify the manner of spermine-induced inhibition of oxidative damage in the jejunum. Total superoxide dismutase and CAT are representative enzymatic antioxidants in the body. Superoxide dismutase is one of the most important endogenous antioxidants and the first enzyme to respond to oxygen radicals, as well as offer protection against oxidative stresses (Winston and Di Giulio, 1991). Superoxide dismutase can also transform hydroperoxide and superoxide anions into H2O2 and O2 through enzymatic degradation (Winston and Di Giulio, 1991). Catalase has been labeled as an essential H2O2 defense that eliminates the toxicity of hydroxyl radicals and decomposes H2O2 into O2 and H2O (David et al., 2008). In this study, we found that jejunal T-SOD and CAT activities were significantly increased by spermine administration. These results suggested that spermine supplementation can enhance antioxidative capacities. Significant enhancements of T-SOD and CAT were also observed following the high spermine dose, which suggested that spermine supplementation has dose-dependent effect on enhancing T-SOD and CAT activities in weaning rats. Our results revealed that spermine can enhance antioxidant status through enzymatic systems in weaning rats.

Glutathione is a major non-enzymatic antioxidant, and is commonly used to evaluate organ antioxidative capability. Glutathione is the most abundant intracellular thiol-based antioxidant scavenger and acts as the first line of defense against oxidative stresses (Yang et al., 2013). Total antioxidant capacity (T-AOC) is often adopted as an important index to reflect the total antioxidant capacity of the body and comprehensive indicator of the redox status in the host system (Ren et al., 2012). In the current study, spermine supplementation improved T-AOC activity and GSH contents in rat jejunum. These findings suggested that spermine can protect jejunum from oxidative stress. However, the underlying mechanism of the spermine mediates non-enzymatic antioxidant system requires further investigation. Spermine supplementation can improve the antioxidant status through non-enzymatic antioxidant systems in weaning rats.

The results proved that spermine can inhibit lipid peroxidation, improve free radical scavenging abilities, and enhance the activities of enzymes and non-antioxidant enzymes of jejunum in weaning rats. Particularly, the 0.4 μmol spermine/g BW exerted better effect than 0.2 μmol spermine/g BW on increasing antioxidant capacity.

5. Conclusions

The results of this study suggested that extending spermine supplementation can promote the growth of the jejunum because spermine affects its morphology. Spermine can affect the morphology of the ileum as well as the alkaline phosphatase activity of the jejunum in weaning rats. Spermine administration and its extended supplementation can also promote antioxidant defenses in weaning rats in dose- and time-dependent manners. Weanling rats supplemented with 0.4 μmol spermine/g BW exhibited better intestinal development and increased antioxidant capacity than 0.2 μmol spermine/g BW. These findings provide a new framework for elucidating the effects of spermine ingestion and extended spermine supplementation to advance intestinal development and enhance antioxidant capacity in weaning rats.

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