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Dietary Grape-Seed Procyanidins Decreased Postweaning Diarrhea by Modulating Intestinal Permeability and Suppressing Oxidative Stress in Rats

Peixia Song,[†,||] Ruoji Zhang,[‡,||] Xiaoxiao Wang,[†] Pingli He,[†] Lulin Tan,[†] and Xi Ma*[,†]

[†]State Key Lab of Animal Nutrition, Ministry of Agriculture Feed Industry Centre, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing 100193, People's Republic of China

[‡]The Experimental High School Attached to Beijing Normal University, No. 14 Erlong Road, Beijing 100032, People's Republic of China

ABSTRACT: This study was conducted to evaluate the effects of grape-seed procyanidins in controlling weaning diarrhea using a rat model. Weaned rats were fed either the basal diet or basal diet supplemented with either 250 mg/kg grape-seed procyanidins or 2000 mg/kg ZnO. Treated rats had better performance with a reduced incidence of diarrhea ($P < 0.05$). Both ZnO and grape-seed procyanidins significantly reduced urinary lactulose to mannitol ratios ($P < 0.05$) and enhanced the mRNA and protein expression of the intestinal mucosal tight junction proteins Ocln/ZO-1 ($P < 0.05$). Grape-seed procyanidins increased the activities of SOD, GSH-Px, and GSH while decreasing the level of MDA in the intestinal mucosa ($P < 0.05$). Furthermore, an in vitro investigation revealed that supplementation with grape-seed procyanidins in IEC-6 intestinal epithelial cells significantly enhanced the expression of Ocln/ZO-1 under H_2O_2-induced oxidative stress. Collectively, these results indicate that grape-seed procyanidins have the potential to prevent weaning diarrhea by reducing intestinal permeability and improving antioxidant indices.

KEYWORDS: grape-seed procyanidins, intestinal permeability, oxidative stress, occludin (Ocln), zonula occludens protein-1 (ZO-1)

INTRODUCTION

Weaning is a critical stage for infants and young animals because of alterations in the gastrointestinal tract architecture and function, as well as adaption to enteric microbiota and immune responses.[1,2] Infants and young animals are often challenged by postweaning stresses including diarrhea, low feed intake, and body weight loss, and these stresses can adversely affect intestinal health and function.[3] Antibiotics, as effective medicines to decrease the susceptibility of infection, have been widely applied in the fields of medicine and animal production to solve postweaning problems.[4] However, accumulated research has revealed the severe adverse side effects of antibiotics, such as bacterial resistance, and this has motivated many researchers to attempt to find alternatives to antibiotics.[5,6]

It is well-known that oxidative stress plays an important role during the induction and progression of weaning diarrhea.[1,7] The gastrointestinal tract is a complex and dynamic balanced ecosystem comprising an alliance among the epithelial barrier, immune mediators, and a myriad of microbial species.[8] Newborn infants and young animals generally have not established an appropriate balance in the gastrointestinal tract and therefore often suffer with oxidative stress.[1,8] Oxidative stress is caused by excessive oxidative radicals including reactive oxygen species (ROS) or reactive nitrogen species (RNS), which damage DNA, biomembrane lipids, proteins, and other macromolecules.[9,10]

The addition of natural antioxidant extracts to foods and feed has been put into practice to control oxidative stress in industry.[11,12] Grape-seed procyanidins, consisting of flavan-3-ols, (+)-catechin, and (−)-epicatechin, their gallated derivatives, and their polymeric forms (Figure 1), have been shown to be bioactive micronutrients with potential benefits for human and livestock health as they may

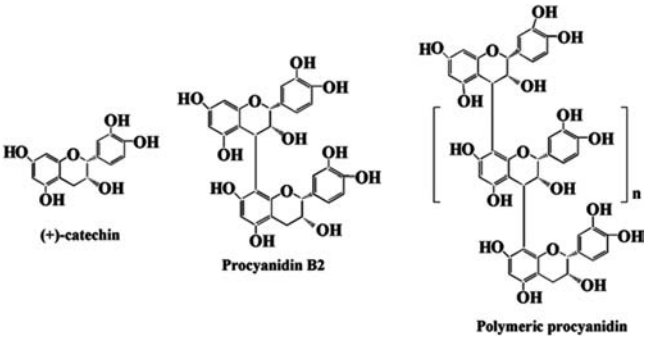

Figure 1. Chemical structures of grape-seed procyanidins.

reduce the risk of metabolic disorders such as hypertriglyceridemia and diabetes.[13,14] Most of the beneficial effects of procyanidins have been related to their antioxidant activity, as they have been shown to act as powerful antioxidants by directly scavenging free radicals and terminating oxidative reactions,[15] as well as by indirectly inhibiting redox-sensitive transcription factors and pro-oxidant enzymes.[16,17] Beyond their antioxidant actions, procyanidins modulate multiple cell signaling pathways and, ultimately, gene transcription and metabolic fluxes.[18]

Recent studies have revealed potent anti-inflammatory properties of procyanidins on experimental inflammation in rats and mice.[19] However, to our knowledge, no research has been

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performed to reveal the effects of grape-seed procyanidins on weaning diarrhea and the intestinal barrier, as well as to establish its potential mechanism of action. In the present study, we evaluated the effects of grape-seed procyanidins in controlling weaning-induced diarrhea and explored the relationship between intestinal permeability and oxidative stress activation using the Sprague−Dawley rat to establish a weaning diarrhea model as well as rat IEC-6 intestinal epithelial cells treated with H_2O_2 as an in vitro oxidative stress model.

MATERIALS AND METHODS

All animals used in this experiment were maintained according to the guidelines of the China Agricultural University Animal Care and Use Ethics Committee.

Chemicals and Reagents. Grape-seed procyanidin extracts were provided by the Jianfeng Co. (Tianjin, China). According to the methods of the manufacturer, polymerization degree of grape-seed procyanidin extract was characterized by HPLC analysis. The procyanidin extract contained 18.4% monomeric, 20.4% dimeric, 15.2% trimeric, 14.1% tetrameric, and 28.5% oligomeric (5−13 units) procyanidins and 4.3% phenolic acids. Rat intestinal jejunal crypt cells (IEC-6, passages 8−14) were purchased from the American Type Culture Collection (Rockville, MD). Dulbecco's Modified Eagle's Medium (DMEM), RNA extraction reagent TRIzol, ThermoSCRIPT RT-PCR System, and fetal bovine serum were procured from Invitrogen Life Technologies (Carlsbad, CA). Rabbit antibodies against β-actin, zonula occludens protein-1 (ZO-1), and occludin (Ocln) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Protease inhibitor cocktail tablets were obtained from Roche Applied Science (Rotkreuz, Switzerland). IRDye 800-conjugated secondary antibodies against rabbit IgG were purchased from LI-COR Bioscience (Lincoln, NE).

Animals, Diets, and Experimental Protocol. Seventy-two, recently weaned, male, Sprague−Dawley rats (21 days old and weighing an average of 28.8 ± 1.7 g) were individually housed in polycarbonate cages with soft wood granulate floors, with self-feeders and automatic stainless nipple waterers. The rats were assigned to one of three treatments (N = 24) on the basis of similar body weight and fed an unsupplemented diet (group I) or the basal diet with either 250 mg/kg grape-seed procyanidins (Ggroup II) or 2000 mg/kg ZnO (group III). The basal diet, which contained approximately 105 mg/kg Zn, was formulated to meet or exceed the nutrient requirements recommended by the American Institute of Nutrition (Table 1). Water and feed were available ad libitum throughout the 29 day growth trial. Rats and feeders were individually weighed after an overnight fast at the start and end of the trial (days 0 and 29) to calculate weight gain, feed intake, and feed conversion.

Diarrhea incidence was determined by a visual assessment of fecal consistency conducted each morning at 8:00 a.m.[20] Briefly, fecal consistency was graded as 0 = solid, 1 = semisolid, 2 = semiliquid, and 3 = liquid. The occurrence of diarrhea was defined as the production of feces at level 2 or 3 for two continuous days. Diarrhea incidence (%) was defined as the number of rats on a treatment with diarrhea × diarrhea days ÷ (total number of rats × 29 days) × 100%.

Intestinal Permeability. Intestinal permeability was assessed on day 28 using the lactulose to mannitol differential absorption test. It is nontoxic, noninvasive, simple to perform, relatively inexpensive, and reproducible. It is commonly accepted as a reliable method for assessing small intestinal permeability.[21] Briefly, a pretest sample of urine was collected after 6 h of fasting for baseline urinary sugar measurement. After the pretest sample was obtained, rats were given 200 mg of lactulose (Sigma, St. Louis, MO) and 100 mg of mannitol (Sigma) dissolved in 2 mL of water by means of intragastric gavage. Rats were fasted for the 6 h study period, but they were allowed to drink water after 30 min. Urine was collected for 6 h, aliquoted, and stored at −80 °C until assayed. Prior to analysis, the urine was centrifuged at 3000 rpm for 10 min. Urinary lactulose and mannitol concentrations were determined by an enzymatic technique.[22] Mannitol excretion was corrected by subtraction of baseline values determined in the pretest samples, and the lactulose to mannitol excretion ratios (L/M ratio) were calculated as an index of intestinal permeability.

Table 1. Dietary Composition and Nutrient Content of the Basal Diet

ingredient	g/kg	nutrient level[a]	%
cornstarch	464.0	gross energy (MJ/kg)	16.22 ± 0.24
casein	140.0	dry matter	96.54 ± 0.61
dextrinzed cornstarch	155.0	ash	3.49 ± 0.11
sucrose	100.0	crude protein	17.55 ± 0.15
soybean oil	40.0	calcium	1.42 ± 0.03
fiber[b]	50.0	total phosphorus	0.40 ± 0.01
mineral mix[c]	35.0	lysine	1.50 ± 0.06
vitamin mix[d]	10.0	methionine + cystine	0.48 ± 0.02
L-methionine	1.8	threonine	0.67 ± 0.04
L-cystine	1.8	tryptophan	0.20 ± 0.01
choline bitartrate	2.4		
tert-butylhydroquinone	0.01		

[a] Each value is the mean ± standard deviation (the mean of three analyzed values). [b] Fiber: cellulose acetate (Cellulose Fibers Co., Nantong, China). [c] Mineral mixture was prepared as AIN-93G (mg/100 g of mixture): $CaCO_3$, 37000; KH_2PO_4, 19600; $K_3C_6H_5O_7 \cdot H_2O$, 7078; NaCl, 7400; K_2SO_4, 4660; MgO, 2400; $FeC_6H_5O_7 \cdot H_2O$, 606; $ZnCO_3$, 165; $MnCO_3$, 63; $CuCO_3Cu(OH)_2 \cdot H_2O$, 32.4; $NaSiO_3 \cdot 9H_2O$, 145; $CrK(SO_4)_2 \cdot 12H_2O$, 27.5; LiCl, 1.74; H_3BO_3, 8.15; NaF, 6.35; $NiCO_3 \cdot 2Ni(OH)_2 \cdot 4H_2O$, 3.06; NH_4VO_3, 0.66; sucrose ad 100 g. [d] Vitamin mixture was prepared as AIN-93 (mg/100 g of mixture): nicotinic, 300; calcium pantothenate, 160; pyridoxine hydrochloride, 70; thiamin hydrochloride, 60; riboflavin, 60; folic acid, 20; D-biotin, 2.0; cyanocobalamin, 250; α-tocopherol, 1500; cholecalciferol, 25; phylloquinone, 7.5; sucrose ad 100 g.

Tissue Sample Collection. On day 29, all rats were sacrificed with an intraperitoneal injection of pentobarbital sodium after a 12 h fast. The abdominal cavity was opened, and the ileum (1 cm anterior to the ileocecal junction) of each rat was quickly isolated, flushed with ice-cold saline to remove the digesta, packed with surgical gauze, frozen by immersion in liquid N_2, and then stored at −80 °C until needed for analysis.

Determination of Antioxidant Indices in Intestinal Samples. The antioxidant index and protein content of the small intestine mucosa samples were determined using assay kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).[23] Briefly, tissues were minced and homogenized (10% w/v) in ice-cold sodium−potassium phosphate buffer (0.01 M, pH 7.4) containing 0.86% NaCl. The homogenate was centrifuged at 3000g for 10 min at 4 °C, and the resultant supernatants were analyzed for their activities of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione (GSH) as well as malondialdehyde (MDA) as an indicator of lipid peroxidation. SOD activity was detected by monitoring the inhibition of nitro blue tetrazolium reduction, whereas GSH-Px activity was measured with 5,5′-dithiobis(p-nitrobenzoic acid), and the change in absorbance at 412 nm was recorded. The MDA level was analyzed with 2-thiobarbituric acid, and the change in absorbance was read at 532 nm. GSH can interact with DTNB to produce oxidized glutathione GSSG and chromophore TNB, which can be measured at 412 nm. The total protein concentration of the supernatants was measured using the BCA protein assay kit

(Pierce, Rockford, IL). All absorbance levels were measured using a Synergy4 Multifunction Microplate Reader (Bio-Tek Instruments, Winooski, VT).

Cell Culture and Treatment. Rat intestinal jejunal crypt cells (IEC-6, passages 8−14) were cultured at 37 °C in a 5% CO_2 incubator. The maintenance cell medium was DMEM, which was supplemented with 10% fetal bovine serum, 5 mg of bovine insulin, 50 μg/mL of penicillin/streptomycin, and a final concentration of 1 mM sodium pyruvate. The medium was changed three times a week according to standard culture protocols. The cultural cells were trypsinized with 0.05% EDTA trypsin when 90−95% confluence was achieved.

Before treatment, approximately 5.0×10^4 IEC-6 cells/well were seeded into 24-well culture plates and fed with fresh culture medium either unsupplemented (control) or supplemented with 10 μg/mL grape-seed procyanidins. The dose was referred to previous study.[16,17] Cells were treated 24 h later with 500 μM hydrogen peroxide (H_2O_2) for 4 h according to a previous paper.[24]

Quantitative Real-Time PCR Analysis. Intestine samples or cells were collected on day 29. Total RNA was prepared using TRIzol reagent, and reverse transcription (RT) was performed with the ThermoSCRIPT RT-PCR System according to the manufacturer's protocol (Invitrogen Life Technologies, Carlsbad, CA). Quantitative real-time PCR analysis of Occludin and ZO-1 was carried out using the TaqMan Sequence Detection System and the DNA double-strand specific SYBR Green I dye (Roche, Basel, Switzerland) according to the manufacturer's instructions. The gene-specific primers for Ocln and ZO-1 were as follows: forward, 5′-ATT ATG CAC CAA GCA ATG-3′, and reverse, 5′-ATG CAC ATC ACA ATA ATG-3′, for Ocln (169 bp); forward, 5′-ACG CTT CAC AGG GCT CCT-3′, and reverse, 5′-CAT TGC AAC TCG GTC ATT-3′, for ZO-1 (159 bp). Results were normalized to RS9 mRNA levels. Each experiment was performed in triplicate with each treatment placed into three PCR tubes.

Protein Extraction and Immunoblot Analysis. The total protein contained in the small intestine samples or IEC-6 cells was extracted using a ProteoJET Total Protein Extraction Kit (Fermentas, Glen Burnie, MD) according to the manufacturer's protocol. The protein concentration of the supernatants was measured using the BCA Protein Assay Kit. Equal amounts of protein were separated by 10.0% SDS-PAGE and transferred onto nitrocellulose membranes (Hybond, ECL, GE Healthcare, Sunnyvale, CA). After blocking in Tris-buffered saline containing 0.05% Tween-20 (TBS-T) and 5% nonfat milk for 2 h, membranes were incubated with corresponding primary antibodies overnight at 4 °C. Membranes were then washed three times and incubated with the appropriate IRDye 800-conjugated secondary antibodies in the dark for 1 h at room temperature. Following another three washes, signals were detected using the LI-COR Infrared Imaging System (Odyssey, Lincoln, NE) and quantified with Odyssey software.

Statistical Analysis. The difference in the occurrence of diarrhea among the three groups was tested by chi-square contingency test. All other data were analyzed using the analysis of variance (ANOVA) procedure of SAS system (version 8.2, SAS Institute, Inc., Cary, NC). A P value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Effects on Performance and Diarrhea. As shown in Table 2, supplementation with grape-seed procyanidins or ZnO in the diet significantly increased weight gain ($P < 0.05$), but had little influence on feed intake ($P > 0.05$), resulting in an improved feed conversion ratio ($P < 0.05$). The occurrence of diarrhea was significantly decreased in the rats fed grape-seed procyanidins compared with unsupplemented rats ($P < 0.05$). The performance in grape-seed procyanidin fed rats was similar to that of rats fed ZnO ($P > 0.05$), whereas the diarrhea occurrence was higher ($P < 0.05$).

Table 2. Effect of 250 mg/kg Grape-Seed Procyanidins on Growth Performance in Weaning Rats[a]

item	control diet	grape-seed procyanidins	ZnO
weight gain[b] (g/day)	3.14 ± 0.46 a	4.12 ± 0.50 b	4.66 ± 0.81 b
feed intake[b] (g/day)	18.24 ± 1.67	17.17 ± 1.50	16.95 ± 1.33
feed conversion[b]	5.81 ± 0.60 a	4.17 ± 0.54 b	3.64 ± 0.56 b
diarrhea incidence[c] (%)	37.5 a	20.8 b	8.3 c

[a] Values within the same row not sharing a common letter are significantly different ($P < 0.05$) from each other. [b] Values are the mean ± standard deviation ($n = 24$). Significantly different by analysis of variance (ANOVA), followed by Duncan's test. [c] Significantly different by chi-square contingency test.

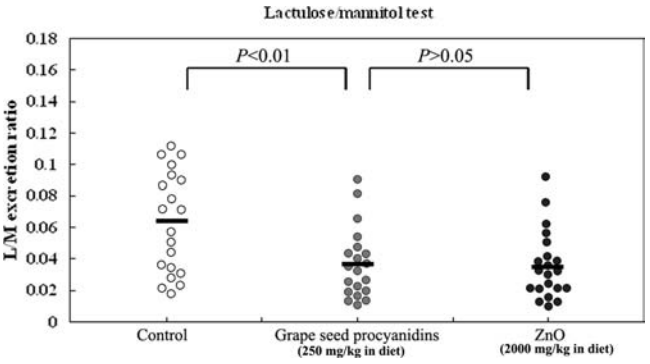

Figure 2. Intestinal permeability measured by lactulose/mannitol excretion ratio (L/M ratio). The weaning rats were assigned to one of three groups fed an unsupplemented (group I) or the basal diet with either 250 mg/kg grape-seed procyanidins (group II) or 2000 mg/kg ZnO (group III). On day 28 intestinal permeability was assessed using the lactulose to mannitol differential absorption test.

These results indicates that supplementation with a low dose of grape-seed procyanidins in diets can increase the average daily weight gain while leading to improved growth performance and diarrhea incidence, without side effects being observed. Other research has provided similar evidence about the effects of grape-seed procyanidins on local and systemic inflammation both in vitro and in vivo.[17,19,25,26] Given the increasing reports of severe adverse side effects of antibiotics,[4−6] our experiments revealed an alternative effective antidiarrhea method to solve postweaning problems.

Effect on Intestinal Permeability. To reveal the reason for improved growth performance and reduced diarrhea occurrence, the intestinal mucosal barrier function was monitored using the lactulose/mannitol test. As shown in Figure 2, inclusion of grape-seed procyanidins or ZnO in the diet significantly reduced urinary lactulose to mannitol ratios of weaning rats compared with the control ($P < 0.01$). The urinary lactulose to mannitol ratios in the grape-seed procyanidins fed rats were comparable to those of rats fed ZnO ($P > 0.05$).

The intestinal permeability and mucosal tight junction proteins provide an important epithelial barrier and have been shown to play a critical role in the pathogenesis of diarrhea.[27,28] Using the urinary lactulose/manmitol test, we revealed that grape-seed procyanidins can reduce intestinal permeability, which may lead to the reduced diarrhea incidence and then

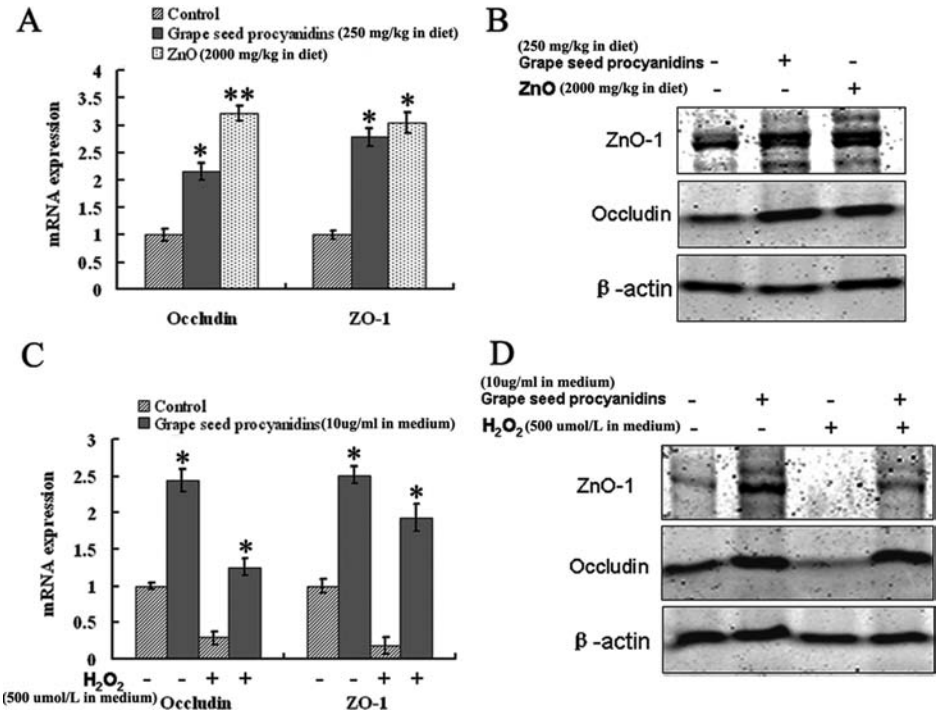

Figure 3. Expression of mucosal tight junction protein was measured at the RNA and protein levels. Q-PCR analysis was performed to analyze mRNA expression of the mucosal tight junction markers Ocln and ZO-1 in the intestinal samples (A) or the cultural IEC-6 intestinal epithelial cells (C). Results were normalized to the expression of RS9 mRNA and expressed as the mean ± SD from three independent experiments. *, $P < 0.05$ the control versus grape-seed procyanidins or ZnO. **, $P < 0.05$ ZnO versus grape-seed procyanidins. Protein levels of Ocln and ZO-1 in the intestinal samples (B) or the cultural IEC-6 cells (D) were determined by Western blot, with β-actin as an internal control.

increased weight gain and feed conversion in weaning rats. Given that the effects of grape-seed procyanidins were similar to ZnO, grape-seed procyanidins may be used as an alternative of ZnO to control diarrhea incidence.

Effect on Expression of Intestinal Mucosal Tight Junction Proteins. To reveal the effects of grape-seed procyanidin supplementation on intestinal mucosal tight junction, the expressions of Ocln and ZO-1 were measured at both mRNA and protein levels. As shown in Figure 3A, the mRNA levels of both Ocln and ZO-1 were moderately higher in rats fed grape-seed procyanidins than in rats fed the control diet ($P < 0.05$), whereas even higher levels were detected in rats fed ZnO. Supplementation with either grape-seed procyanidins or ZnO enhanced protein expression of Ocln and ZO-1 in the small intestine mucosa (Figure 3B). ZnO supplementation was slightly more effective than grape-seed procyanidins in increasing the expressions of Ocln and ZO-1.

In addition, procyanidins significantly increased the expression of Ocln and ZO-1 in the intestinal mucosa at both the mRNA and protein levels. These data suggest that grape-seed procyanidins may inhibit diarrhea through a reduction in intestinal permeability and increasing the expression of mucosal tight junction proteins, in a manner similar to the effects of ZnO on the intestinal mucosa.[24] Recent studies revealed that dietary catechins and procyanidins could modulate Cu/Zn-superoxide dismutase and zinc homeostasis.[29,30] However, whether or not grape-seed procyanidins perform this function on mucosal tight junction proteins via the same mechanism and signal pathway of ZnO is still unknown.

Effect on Intestinal Antioxidant Indices. As shown in Table 3, in the small intestinal samples, SOD ($P < 0.01$) and GSH-Px ($P < 0.01$), the two main antioxidant enzymes, as well as GSH ($P < 0.05$), one of the nonenzymatic antioxidant components, increased in the grape-seed procyanidin group, whereas MDA, a source of free radical mediated lipid peroxidation injury, decreased compared with the control group ($P < 0.05$). In contrast, SOD significantly increased in the ZnO group ($P < 0.05$), whereas the three antioxidant indices, GSH, GSH-Px, and MDA, were comparable to those of the control group ($P > 0.05$).

Table 3. Effect of 250 mg/kg Grape-Seed Procyanidins on Antioxidant Indices in Tissue Samples of the Rats[a]

item	control diet	grape-seed procyanidins	ZnO
SOD (U/mg protein)	7.7 ± 1.4 a	20.3 ± 2.1 b	13.2 ± 1.8 c
GSH-Px (U/mg protein)	32.4 ± 3.7 a	55.1 ± 4.5 b	29.4 ± 4.2 a
GSH (mg/g protein)	4.0 ± 1.9 a	9.7 ± 1.6 b	4.5 ± 1.5 a
MDA (nmol/mg protein)	2.2 ± 0.7 a	1.0 ± 0.3 b	2.3 ± 0.4 a

[a] Values are the mean ± standard deviation ($n = 24$). Values within the same row not sharing a common letter are significantly different ($P < 0.05$) from each other analyzed by analysis of variance (ANOVA), followed by Duncan's test. Data are one representation of three separate experiments.

Taken together, the observed alteration in antioxidant indices by grape-seed procyanidin supplementation, including amount of MDA, GSH, and antioxidative enzymes, leads to improved oxidative stress in mucosa, which may lead to the reduced diarrhea incidence. In contrast, ZnO supplementation increased only the level of GSH while having little effect on SOD, GSH-Px, and MDA, which suggests that the mechanism by which

grape-seed procyanidins control diarrhea may be different from that of ZnO.[21,31]

Effect on Expression of Tight Junction Protein in Oxidation-Induced Cells. To reveal the relationship between increased mucosal tight junction proteins and the improved antioxidative stress activities caused by grape-seed procyanidin supplementation, IEC-6 intestinal epithelial cells were cultured and oxidative stress was induced by H_2O_2. As shown in Figure 3C,D, both the mRNA and protein levels of Ocln and ZO-1 were significantly increased in the IEC-6 cells after 10 μg/mL grape-seed procyanidin incubation compared with the control ($P < 0.05$). More importantly, when the intestinal epithelial cells were treated with H_2O_2 to induce oxidative stress, grape-seed procyanidin incubation significantly overcame the reduction in mRNA and protein levels of Ocln and ZO-1 induced by oxidative stress ($P < 0.05$).

Weaning rats were used as a weaning stress model in the present study. Accumulated studies revealed that the rat model of weaning diarrhea is comparable with that of infants and young animals and has many merits including low cost and a short period of reproduction, and there are high physiological similarities between rats, farm animals (such as pigs, chickens, turkeys, and calves) and infants.[20,23] Therefore, the rat model has been used for the study of weaning diarrhea, as well as its potential mechanism.

In our study, the grape-seed procyanidin extracts contained monomeric, dimeric, trimeric, tetrameric, and oligomeric (5−13 units) procyanidins, as well as 4.3% phenolic acids. Although in the extract there is a little content of phenolic acids, which are known to be readily absorbed in the intestine of mammals, procyanidins can be considered as the main active components of the extract that are responsible for the observed effects according to the previous studies.[13,25] Besides, the extracts may contain a little content of other kinds of phenolic compounds except for procyanidins. Some previous studies revealed that polyphenols can suppress oxidative and inflammatory stress response to high-fat, high-carbohydrate meals, thereby reducing the risk of diabetes.[32] Further research based on procyanidin extracts purified by chromatographic separation is needed to confirm the antidiarrhea effects.

Because procyanidins are stable to gastric and enteric digestion, only being heavily metabolized by colonic micro flora, a much larger concentration than 10 μg/mL might occur in the intestine in our animal experiment. Nevertheless, these data in vitro suggested that grape-seed procyanidins improve the intestinal tight junction and permeability by improving antioxidant ability, a mechanism distinct from the mechanism of ZnO.[21,31]

It has been confirmed that grape-seed procyanidins can attenuate oxidative damage in vivo.[33,34] In the present study, the extent of antioxidative stress in the intestinal mucosa was evaluated by monitoring these antioxidant indices. The data illustrated that supplementation with grape-seed procyanidins in the diet significantly increased the activities of SOD and GSH-Px and the level of GSH while decreasing the level of MDA in the mucosa of the small intestine, in agreement with previous studies.[18,35] Excessive oxidative radicals can be eliminated by the antioxidant system including nonenzymatic components and a series of antioxidant enzymes.[16] Nonenzymatic components include GSH, Se, and some vitamins such as vitamins C and E. The antioxidant enzymes include SOD and GSH-P_X, which are the major antioxidant enzymes capable of minimizing oxidative stress.[36] The degree of lipid peroxidation is often used as an indicator of ROS mediated damage,[37] and the concentrations of MDA in blood and tissues are generally used as biomarkers of radical-induced damage and endogenous lipid peroxidation.[35]

Our study demonstrated evidence about the relationship between weaning diarrhea and oxidative stress in mucosa. Mucosa oxidative stress has been suggested to be sufficient to induce diarrhea and play an important role during the progression of weaning diarrhea.[1,38,39] GSH and GSH-P_X have been proved to be important to protect mucosa cells in the gastrointestinal tract against damage from insults such as radiation, endotoxins, and ROS.[40] In addition, procyanidins modulated inflammatory response in activated macrophages by the inhibition of NO and PGE2 production, suppression of iNOS expression, and NFkB translocation,[17] which might subsequently reduce inflammatory diarrhea. Finally, when levels of oxidative stress are low, proteins are seldom oxidized and GST expression is not induced.[36−38] A decrease in oxidative stress may lead to the increased tight junction proteins such as Ocln and ZO-1, which subsequently lead to reduced intestinal permeability.

In summary, the present study demonstrated that supplementation with a low dose of grape-seed procyanidins in the diet can significantly increase weight gain and feed conversion while reducing diarrhea incidence in weaning rats, without adverse side effects. These changes were accompanied by improved antioxidative stress ability of intestinal mucosa, as well as reduced intestinal permeability and increased mucosal tight junction proteins. With previous studies,[17−19,25,26] grape-seed procyanidins might act as antioxidants and immunomodulators and have potential application in attenuating weaning-induced diarrhea.

AUTHOR INFORMATION

Corresponding Author

*E-mail: maxi@cau.edu.cn. Phone: +86-10-62733588-1112. Fax: +86-10-62733688.

Author Contributions

‖ These authors contributed equally to this work.

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ABBREVIATIONS USED

ROS, reactive oxygen species; RNS, reactive nitrogen species; ZO-1, zonula occludens protein-1; SOD, superoxide dismutase; Ocln, occludin; GSH-Px, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; H_2O_2, hydrogen peroxide.

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