Effects of Dietary Apple Pectic Oligosaccharides on Growth Performance, Antioxidant Capacity, and Intestinal Health in Weaned Rats: Postprint

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Date: 2017-10-11T00:00:00+00:00

Abstract

This study aimed to investigate the effects of dietary supplementation with apple pectic oligosaccharide (APOS) on growth performance, antioxidant capacity, and intestinal health in weaned rats. Twenty-four healthy weaned Wistar rats were selected for the experiment and divided into 2 treatments according to the principle of similar body weight, fed a basal diet and a diet supplemented with 800 mg/kg APOS, respectively, with an experimental period of 14 d. The results showed that dietary APOS supplementation significantly increased the average daily gain and average daily feed intake of weaned rats ($P<0.05$), and significantly decreased the feed-to-gain ratio ($P<0.05$); dietary APOS supplementation also significantly increased the serum total antioxidant capacity of weaned rats ($P<0.05$) and decreased the content of malondialdehyde in serum ($P<0.05$); dietary APOS supplementation also significantly increased the villus height-to-crypt depth ratio in jejunal mucosa ($P<0.05$); in addition, dietary APOS supplementation also increased the numbers of Lactobacillus and Bifidobacterium in cecal chyme ($P<0.05$) and decreased the number of Escherichia coli in cecal chyme ($P<0.05$). In conclusion, supplementation of APOS in the diet of weaned rats can improve their growth performance, which may be attributed to its improvement of antioxidant capacity, jejunal mucosal morphology, and intestinal microflora structure in rats.

Full Text

Effect of Dietary Apple Pectic Oligosaccharide Supplementation on the Growth Performance, Antioxidant Capacity, and Intestinal Health of Weaned Rats

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Abstract

This experiment was conducted to determine the effect of dietary apple pectic oligosaccharide (APOS) supplementation on the growth performance, antioxidant capacity, and intestinal health of weaned rats. Twenty-four healthy weaned Wistar rats were randomly assigned to two dietary treatments based on similar body weight: a basal diet and a basal diet supplemented with 800 mg/kg APOS. The experimental period lasted 14 days. The results showed that dietary APOS supplementation significantly increased average daily gain and average daily feed intake ($P<0.05$), while significantly decreasing the feed-to-gain ratio ($P<0.05$). APOS supplementation also significantly enhanced serum total antioxidant capacity ($P<0.05$) and reduced serum malondialdehyde content ($P<0.05$). Furthermore, dietary APOS significantly increased the villus height-to-crypt depth ratio in jejunal mucosa ($P<0.05$). Additionally, APOS supplementation increased the populations of Lactobacillus and Bifidobacterium in cecal digesta ($P<0.05$) and decreased the Escherichia coli population ($P<0.05$). In conclusion, dietary APOS supplementation improved the growth performance of weaned rats, likely by enhancing antioxidant capacity, jejunal mucosal morphology, and intestinal microbiota structure.

Keywords: apple pectic oligosaccharide; weaned rats; growth performance; antioxidant capacity; intestinal health

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Oligosaccharides are carbohydrates composed of 2–10 monosaccharide molecules linked by glycosidic bonds, forming linear or branched structures that play important roles in biological activities. Based on their biological functions, oligosaccharides can be classified as either common or functional. Common oligosaccharides include familiar compounds such as sucrose, maltose, and lactose, which can be digested and absorbed by the body and are thus considered nutritive oligosaccharides. Functional oligosaccharides, by contrast, cannot be

degraded or absorbed by gastric acid or digestive enzymes. However, appropriate doses of functional oligosaccharides can regulate various physiological functions, including glucose and lipid metabolism, immune function, mineral absorption, intestinal microflora structure, antioxidant activity, and anti-tumor effects, thereby promoting growth and maintaining health [1-5].

As a functional oligosaccharide, pectic oligosaccharide is primarily produced through enzymatic hydrolysis of natural pectin from plant fruits, roots, stems, and leaves. Its main components are galacturonic acid and pectin disaccharides and trisaccharides formed with other monosaccharides [6]. Recent studies on its nutritional functions have shown that pectic oligosaccharides from different sources (such as hawthorn and citrus) can regulate lipid metabolism, enhance cellular and systemic antioxidant capacity, and improve the intestinal microenvironment [7-9]. Preliminary experiments by our research group found that dietary supplementation with different levels (200–1,600 mg/kg) of apple pectic oligosaccharide (APOS) could improve the growth performance of weaned rats to varying degrees, with 800 mg/kg showing the optimal effect (unpublished data). However, whether pectic oligosaccharides can regulate intestinal mucosal morphology has not been reported, and whether APOS exhibits similar functions to pectic oligosaccharides from other sources requires further investigation. Therefore, this study aimed to explore the effects of dietary APOS supplementation on antioxidant capacity, jejunal mucosal morphology, and cecal microflora structure in weaned rats, providing experimental support for the application of pectic oligosaccharides in improving the health of piglets or infants in production or clinical settings.

1.1 Experimental Materials

APOS was provided by the Feed Research Institute of the Chinese Academy of Agricultural Sciences. The product contained more than 30% pectic oligosaccharides (primarily pectin disaccharides, pectin trisaccharides, and galacturonic acid monosaccharides), with corn starch as the excipient.

1.2 Experimental Animals and Design

Twenty-four healthy 21-day-old weaned Wistar rats with an average body weight of approximately 46.80 g were obtained from Chengdu Dossy Experimental Animals Co., Ltd. The rats were randomly allocated to two treatment groups (12 replicates per treatment, one rat per replicate) based on similar body weight. The groups were fed either the basal diet or the experimental diet (basal diet supplemented with 800 mg/kg APOS) for 14 days. The experiment was conducted at the teaching and research base of the Institute of Animal Nutrition, Sichuan Agricultural University. Rats were housed individually in cages under conventional management with natural lighting and ventilation, and had free access to feed and water.

The basal diet was formulated according to the AIN-93G purified diet standard

for rats and prepared by Chengdu Dossy Experimental Animals Co., Ltd. The composition and nutrient levels of the basal diet are shown in Table 1 . The experimental diet was formulated by substituting corn starch in the basal diet with 800 mg/kg APOS.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis), %

Item	Content
Ingredients	
Corn starch	
Casein (protein >85%)	
Dextrinized cornstarch	
Sucrose	
Soybean oil (no additives)	
Fiber	
Mineral mixture[1]	
Vitamin mixture[2]	
L-Cys	
Choline bitartrate (41.1% choline)	
Tert-butylhydroquinone	
Total	
Nutrient levels[3]	
Metabolizable energy (MJ/kg)	
Crude protein	
Fiber	
Fat	
Carbohydrate	

[1]Mineral mixture ingredients (g/kg): calcium carbonate 357.000, monopotassium phosphate 196.000, potassium citrate monohydrate 70.780, sodium chloride 74.000, potassium sulfate 46.600, magnesium oxide 24.000, ferric citrate 6.060, zinc carbonate 1.650, manganese carbonate 0.630, copper carbonate 0.300, potassium iodate 0.010, sodium selenite anhydrous 0.010, ammonium paramolybdate 4 hydrate 0.008, sodium metasilicate 9 hydrate 1.450, chromium potassium sulfate 12 hydrate 0.275, lithium chloride 0.017, boric acid 0.082, sodium fluoride 0.064, nickel carbonate 0.032, ammonium vanadate 0.007, powdered sucrose 221.026.

[2]Vitamin mixture ingredients (g/kg): D-calcium pantothenate 1.600, pyridoxine HCl 0.700, nicotinic acid 3.000, thiamine HCl 0.600, riboflavin 0.600, folic acid 0.200, D-biotin 0.020, vitamin B12 2.500, vitamin E (500 IU/g) 15.000, vitamin A (500,000 IU/g) 0.800, vitamin D3 (400,000 IU/g) 0.250, vitamin K 0.075, powdered sucrose 974.655.

[3]Nutrient levels were calculated values.

1.3 Measurement Indicators and Methods

1.3.1 Growth Performance All rats were weighed on an empty stomach on days 1 and 15 of the experiment, and daily feed intake was recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G).

1.3.2 Sample Collection On day 15, after weighing, blood was collected from all rats via eyeball removal into centrifuge tubes, left to stand at low temperature for 30 minutes, and then centrifuged at 3,000 rpm for 15 minutes to prepare serum, which was stored at -20°C for later use. After blood collection, rats were euthanized by cervical dislocation, and intestinal segments were rapidly isolated. A 3 cm segment of jejunum was fixed in 10% neutral formalin solution, and cecal digesta was collected and stored at -80°C.

1.3.3 Serum Antioxidant Capacity Serum malondialdehyde (MDA) content and total antioxidant capacity (T-AOC) were measured using assay kits from Nanjing Jiancheng Bioengineering Institute, with all operations performed according to the manufacturer' s instructions.

1.3.4 Jejunal Mucosal Morphology Jejunal mucosal morphology (villus height, crypt depth, and villus height-to-crypt depth ratio) was determined according to the method described by Mao et al. [10].

1.3.5 Microbial Populations in Cecal Digesta Real-time fluorescence quantitative PCR was used to determine the populations of microorganisms (total bacteria, Lactobacillus, Bifidobacterium, and Escherichia coli) in rat cecal digesta, following the method described by Mao et al. [11].

1.4 Data Processing and Analysis

Experimental data were initially processed using Excel 2003. All measurement results were statistically analyzed with each rat as an experimental unit. Data were analyzed by t-test using SAS 8.1 software, with $P<0.05$ as the criterion for significant difference. Data are expressed as "mean ± standard error."

2.1 Effect of Dietary APOS Supplementation on Growth Performance of Weaned Rats

As shown in Table 2 , dietary APOS supplementation significantly improved the growth performance of weaned rats ($P<0.05$). Compared with the control group fed the basal diet, rats fed the APOS-supplemented diet showed increases of 15.41% and 51.16% in ADFI and ADG, respectively ($P<0.05$), while F/G decreased by 24.05% ($P<0.05$).

Table 2 Effect of dietary APOS supplementation on the growth performance of weaned rats (n=12)

Item	Control group	APOS group
Initial body weight (g)	46.80$±1.08\|46.79±1.07\|\|$ADFI(g)$\|6.49±0.21^b\|7.49±0.24^a\|\|$ADG(g)$\|0.86±0.04^b\|1.30±0.02^a\|\|F$	

In the same row, values with different letter superscripts indicate significant difference (P<0.05). The same applies below.

2.2 Effect of Dietary APOS Supplementation on Serum Antioxidant Capacity of Weaned Rats

As shown in Table 3 , dietary APOS supplementation significantly improved the antioxidant capacity of weaned rats (P<0.05). Compared with the control group, rats fed the APOS-supplemented diet showed a 99.46% increase in serum T-AOC (P<0.05) and a 14.69% decrease in serum MDA content (P<0.05).

Table 3 Effect of dietary APOS supplementation on the serum antioxidant capacity of weaned rats (n=12)

Item	Control group	APOS group
T-AOC (U/mL)	1.85$±0.27^b\|3.69±0.48^a\|\|$MDA(nmol/mL)$|4.63±0.18^a|3.95±$0.13	

2.3 Effect of Dietary APOS Supplementation on Jejunal Mucosal Morphology of Weaned Rats

As shown in Table 4 , dietary APOS supplementation had no significant effect on jejunal villus height or crypt depth (P>0.05) but significantly increased the villus height-to-crypt depth ratio (P<0.05).

Table 4 Effect of dietary APOS supplementation on the jejunal mucosal morphology of weaned rats (n=12)

Item	Control group	APOS group
Villus height (m)	163.31$±10.47\|178.90±8.87\|\|$Cryptdepth(μm)$\|52.32±2.68\|49.37±3.29\|\|$Villusheight/cryptdept	

2.4 Effect of Dietary APOS Supplementation on Microbial Community Structure in Cecal Digesta of Weaned Rats

As shown in Table 5 , dietary APOS supplementation significantly affected the microbial community in cecal digesta of weaned rats (P<0.05). Compared with the control group, rats fed the APOS-supplemented diet showed significantly

increased populations of Lactobacillus and Bifidobacterium (P<0.05) and significantly decreased Escherichia coli population (P<0.05), though total bacterial numbers showed no significant difference (P>0.05).

Table 5 Effect of dietary APOS supplementation on the microbial community structure in the cecal digesta of weaned rats (n=12), lg(CFU/g)

Item	Control group	APOS group
Lactobacillus	5.47$\pm$0.01^b\|5.94$\pm$0.02^a\|\|*Bifidobacterium*\|3.23$\pm$0.01^b\|3.58$\pm$0.01^a\|\|*Escherichiacoli*\|8.51$\pm$0.03

Numerous recent studies have demonstrated that functional oligosaccharides can improve animal antioxidant capacity and intestinal health [5,12-13]. Consequently, dietary supplementation with functional oligosaccharides (such as mannan oligosaccharides, chitosan oligosaccharides, and fructooligosaccharides) can significantly enhance the growth performance of livestock, poultry, and aquatic animals [4,14-15]. Preliminary experiments found that dietary supplementation with different levels (200–1,600 mg/kg) of APOS could improve the growth performance of weaned rats to varying degrees, with 800 mg/kg being the most effective (unpublished data). The present study yielded similar results, showing that dietary supplementation with 800 mg/kg APOS increased ADFI and ADG and significantly decreased F/G in weaned rats, indicating that APOS promoted rat growth. It can therefore be speculated that dietary APOS supplementation may have improved rat growth by enhancing antioxidant capacity and/or intestinal health.

The results of this study indicate that dietary supplementation with 800 mg/kg APOS in weaned rats significantly increased serum T-AOC and decreased serum MDA content, which is consistent with results from studies on pectic oligosaccharides from other sources [7,9]. The dynamic balance between free radical production and antioxidant capacity is closely related to animal health. T-AOC represents the primary system for antagonizing oxygen free radicals, scavenging excess free radicals to maintain normal metabolism and function. Recent studies have shown that T-AOC reflects the compensatory capacity of the antioxidant system in response to external stimuli and the metabolic status of free radicals in the body [16]. MDA is the end product of polyunsaturated fatty acid peroxidation and serves as an important indicator of lipid peroxidation and redox status [17-18]. Therefore, it can be inferred that dietary APOS supplementation improves rat health by enhancing antioxidant capacity and reducing excess free radicals and lipid peroxidation products.

The integrity of intestinal mucosal morphology is an important indicator for evaluating intestinal function. On the one hand, intestinal mucosal morphology constitutes a crucial component of the intestinal epithelial barrier function. On the other hand, villus height, crypt depth, and their ratio can reflect digestive and absorptive capacity to some extent [19-20]. In this study, dietary APOS supplementation did not significantly affect villus height or crypt depth in weaned

rats but significantly increased the villus height-to-crypt depth ratio. These results suggest that dietary APOS supplementation can improve intestinal mucosal structure and integrity to a certain extent, thereby facilitating intestinal function.

Previous studies have shown that pectic oligosaccharides can significantly improve the microbial composition in the intestines of humans and animals [8], which is consistent with the present findings that dietary APOS supplementation significantly increased beneficial bacterial populations and decreased harmful bacterial populations in cecal digesta. The intestinal microecological environment is an essential component of intestinal barrier function and is closely related to human and animal health [21]. Therefore, these results also indicate that the improved growth performance of weaned rats fed APOS-supplemented diets is closely related to improved intestinal microflora.

Dietary supplementation with 800 mg/kg APOS significantly improved the growth performance of weaned rats, which was associated with improved serum antioxidant capacity, intestinal mucosal morphology, and microbial community structure. This study provides an experimental basis for the clinical and swine production applications of pectic oligosaccharides. As a type of functional oligosaccharide, APOS may also regulate various other physiological functions, such as immune function and nutrient metabolism, which are beneficial for animal growth. Therefore, further research on these aspects is warranted.

References

[1] XU Q S, WEI P, DOU J L, et al. Inhibitory effect of chitosan oligosaccharide on proliferation of hepatoma cell line SMMC-7721 and its mechanism [J]. Natural Product Research and Development, 2009, 21(1): 152–154.

[2] LI T P, LI S H, DU L J, et al. Effects of haw pectic oligosaccharide on lipid metabolism and oxidative stress in experimental hyperlipidemia mice induced by high-fat diet [J]. Food Chemistry, 2010, 121(4): 1010–1013.

[3] XIAO D F, TANG Z R, YIN Y L, et al. Effects of chitosan on growth and immunity of piglets challenged with Escherichia coli [J]. Chinese Journal of Animal Nutrition, 2011, 23(10): 1783–1789.

[4] YAN L, KIM I H. Evaluation of dietary supplementation of delta-aminolevulinic acid and chitooligosaccharide on growth performance, nutrient digestibility, blood characteristics, and fecal microbial shedding in weaned pigs [J]. Animal Science Technology, 2011, 169(3/4): 275–280.

[5] YANG X F, XIONG G Y, ZHOU G L, et al. Effects of milk-derived oligosaccharides on piglet intestinal health and biochemical mechanisms of oligosaccharide synthesis in sow mammary gland [J]. Chinese Journal of Animal Nutrition, 2012, 24(6): 991–1000.

[6] SUN L N. Enzymatic preparation of oligogalacturonic acid and its effect on

growth characteristics of mice as a copper chelate [D]. Master' s thesis. Beijing: Chinese Academy of Agricultural Sciences, 2007.

[7] LI T P, ZHU R G, DONG Y P, et al. Effects of pectin pentaoligosaccharide from Hawthorn (Crataegus pinnatifida Bunge.var.Major) on the activity and mRNA levels of enzymes involved in fatty acid oxidation in the liver of mice fed a high-fat diet [J]. Journal of Agricultural and Food Chemistry, 2013, 61(31): 7599-7605.

[8] LEIJDEKKERS A G M, AGUIRRE M, VENEMA K, et al. In vitro fermentability of sugar beet pulp derived oligosaccharides using human and pig fecal inocula [J]. Journal of Agricultural and Food Chemistry, 2014, 62(5): 1079-1087.

[9] LI T P, LIU Y H, DONG Y P, et al. Anti-fat deposition and antioxidant effects of haw pectic oligosaccharide in liver high-fat-fed mice [J]. CyTA-Journal of Food, 2014, 12(1): 27-31.

[10] MAO X B, LIU M H, TANG J, et al. Dietary leucine supplementation improves the mucin production in jejunal mucosa of the weaned pigs challenged by porcine rotavirus [J]. PLoS One, 2015, 10(9): e0137380.

[11] MAO X B, GU C S, HU H Y, et al. Dietary Lactobacillus rhamnosus GG supplementation improves the mucosal barrier function in the intestine of weaned piglets challenged by porcine rotavirus [J]. PLoS One, 2016, 11(1): e0146312.

[12] SHAO L P, ZHOU L J, LI G P, et al. Effects of oral administration of mannan oligosaccharide (MOS) on immune function and blood GSH-Px, SOD in suckling piglets [J]. Chinese Journal of Veterinary Science, 2000, 20(3): 257-260.

[13] HU C H, WANG Y M. Effects of fructooligosaccharide on growth and intestinal microflora of finishing pigs [J]. Journal of Wuxi University of Light Industry, 2001, 20(6): 568-572.

[14] CHEN J, XUE Z W, JIANG Q Y. Biological functions of mannan oligosaccharides and factors affecting their efficacy in piglets [J]. Technical Advisor for Animal Husbandry, 2012(3): 243-245.

[15] MA Q G, XU C L, CHEN X D, et al. Effects of fructooligosaccharide feed additive on performance of weaned piglets [J]. Chinese Journal of Animal Science, 2004, 40(10): 53-55.

[16] LÜ M. Effects of oxidative stress on tryptophan metabolism and requirement characteristics in piglets and their mechanisms [D]. PhD thesis. Ya' an: Sichuan Agricultural University, 2009.

[17] JAIN S K. The accumulation of malonyldialdehyde, a product of fatty acid peroxidation, can disturb aminophospholipid organization in the membrane bilayer of human erythrocytes [J]. The Journal of Biological Chemistry, 1984, 259(6): 3391-3394.

[18] MAO X B, LÜ M, YU B, et al. The effect of dietary tryptophan levels on oxidative stress of liver induced by diquat in weaned piglets [J]. Journal of Animal Science and Biotechnology, 2014, 5(1): 49.

[19] CHEN H, MAO X B, HE J, et al. Dietary fibre affects intestinal mucosal barrier function and regulates intestinal bacteria in weaning piglets [J]. British Journal of Nutrition, 2013, 110(10): 1837–1848.

[20] MAO X B, ZENG X F, QIAO S Y, et al. Specific roles of threonine in intestinal mucosal integrity and barrier function [J]. Frontiers in Bioscience (Elite Edition), 2011, 3: 1192–1200.

[21] SEKIROV I, RUSSELL S L, ANTUNES L C, et al. Gut microbiota in health and disease [J]. Physiological Reviews, 2010, 90(3): 859–904.

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