Effects of Resistant Starch on Defecation Status and Gut Microbiota in Diet-Induced Obese Rats: Postprint

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Abstract

This study aimed to investigate the effects of resistant starch (RS) on defecation status and gut microbiota in diet-induced obese (DIO) rats, providing a basis for the rational utilization of RS. One hundred healthy male Sprague-Dawley (SD) rats were randomly divided into two groups: a control group (n=10) fed a basal diet and a high-fat group (HF group, n=90) fed a high-fat diet. After 7 weeks, 27 DIO rats were selected from the HF group based on body weight and randomly divided into three groups (n=9 each): the HF group (fed a high-fat diet), the high-fat diet plus resistant starch group (HFRS group, fed a high-fat diet containing 10% RS), and the high-fat diet plus resistant starch plus dextran sulfate group (HFRS+DS group, fed a high-fat diet containing 10% RS). During the experimental period, the HFRS+DS group received daily gavage of 1 mL of 5% DS, while the other groups received 1 mL of distilled water. The trial lasted for 5 weeks. Fresh formed feces (1 g) were collected at weeks 7 and 12, diluted, and then tested using selective media to detect and enumerate Enterococcus, Enterobacter, Bifidobacterium, Lactobacillus, and Bacteroides, as well as total colony counts. The results showed: 1) At week 7, compared with the control group, the HF group exhibited significantly decreased fecal wet weight, fecal moisture content, and counts of Enterococcus, Bifidobacterium, Lactobacillus, and Bacteroides in fecal samples ($P<0.05$), while fecal pellet count and Enterobacter count in fecal samples were significantly increased ($P<0.05$). 2) At week 12, compared with the control group, DIO rats in the HF group showed significantly decreased fecal wet weight, fecal moisture content, and counts of Enterococcus, Bifidobacterium, Lactobacillus, and Bacteroides in fecal samples ($P<0.05$), whereas fecal pellet count and Enterobacter count in fecal samples were significantly increased ($P<0.05$). Compared with the HF group, DIO rats in the HFRS group demonstrated significantly increased fecal wet weight, fecal dry weight, fecal moisture content, and counts of Enterococcus, Bifidobacterium, Lactobacillus, and Bacteroides in fecal samples ($P<0.05$), while

fecal pellet count and Enterobacter count in fecal samples were significantly decreased (P<0.05). Compared with the HFRS group, DIO rats in the HFRS+DS group exhibited significantly decreased fecal wet weight, fecal moisture content, and counts of Bifidobacterium, Lactobacillus, and Bacteroides in fecal samples (P<0.05), while counts of Enterobacter and Enterococcus in fecal samples were significantly increased (P<0.05). These findings indicate that under the conditions of this experiment, RS can improve defecation status and gut microbiota dysbiosis induced by high-fat diet in DIO rats.

Full Text

Effects of Resistant Starch on Defecation and Intestinal Microflora in Diet-Induced Obese Rats

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Abstract

This study investigated the effects of resistant starch (RS) on defecation and intestinal microflora in diet-induced obese (DIO) rats to provide a basis for the rational utilization of RS. One hundred healthy male Sprague-Dawley (SD) rats were randomly divided into two groups: a control group (n=10) fed a basal diet and a high-fat group (HF group, n=90) fed a high-fat diet. After seven weeks, 27 DIO rats were selected from the HF group based on body weight and randomly allocated into three groups of nine animals each: the HF group (continued on high-fat diet), the high-fat resistant starch group (HFRS group, fed a high-fat diet containing 10% RS), and the high-fat resistant starch plus dextran sulfate group (HFRS+DS group, fed a high-fat diet containing 10% RS). The experimental period lasted five weeks. During this time, the HFRS+DS group received daily gavage of 1 mL 5% dextran sulfate solution, while the other groups received 1 mL distilled water. Fresh formed feces (1 g) were collected during weeks 7 and 12, diluted, and plated on selective media to enumerate *Enterococcus*, *Enterobacter*, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides*, with total colony counts recorded. The results showed: (1) At week 7, compared with the control group, the HF group exhibited significantly decreased fecal wet weight, fecal water content, and counts of *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* (P<0.05), while fecal pellet number and *Enterobacter* counts increased significantly (P<0.05). (2) At week 12, compared with the control group, DIO rats in the HF group showed significantly reduced fecal wet weight, water content, and counts of *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* (P<0.05), with significantly increased fecal pellet number and *Enterobacter* counts (P<0.05). Compared with the HF group, HFRS DIO rats demonstrated significantly elevated fecal wet weight, dry weight, water content, and counts of *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* (P<0.05), while fecal pellet number and *Enterobacter* counts decreased

significantly (P<0.05). Compared with the HFRS group, the HFRS+DS group showed significantly lower fecal wet weight, water content, and counts of *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* (P<0.05), but significantly higher *Enterobacter* and *Enterococcus* counts (P<0.05). These findings indicate that under the conditions of this experiment, RS can improve defecation status and ameliorate intestinal microflora disturbances in DIO rats induced by high-fat diets.

Keywords: resistant starch; high-fat diet; diet-induced obesity; intestinal microflora

Introduction

The animal intestine harbors a vast and diverse microbial community that exists in a state of mutual constraint and balanced growth. These microorganisms participate in numerous host functions including physiology, metabolism, and immunity, making the maintenance of intestinal microecological stability crucial for host health [1-2]. While genetics, growth, and environmental factors can all influence intestinal microflora structure, diet represents the most important environmental factor [3]. Because different intestinal microorganisms exhibit substrate selectivity during fermentation, alterations in the proportion and quantity of dietary energy-yielding nutrients—such as protein, fat, and carbohydrates—can induce corresponding changes in the intestinal microecology [4]. Previous studies have demonstrated that increased dietary protein and lipid content shifts the intestinal microflora toward reduced beneficial bacteria and increased pathogenic bacteria, while simultaneously exacerbating metabolic disorders [5-6]. High-fat diets have become a major focus of research [7], and investigating dietary modulation of intestinal microecology has emerged as a primary topic in nutrition science.

Some studies suggest that appropriately reducing digestible carbohydrates while increasing indigestible carbohydrates can decrease the rate of food digestion and absorption, increase the amount of carbohydrates reaching the colon, provide sufficient carbon sources for colonic probiotic growth, and induce beneficial shifts in intestinal microflora [8-9]. Resistant starch (RS) is a type of starch that cannot be digested and absorbed in the small intestine. Research has confirmed that RS exhibits fiber-like effects, and that appropriate RS intake can improve glucose and lipid metabolism, promote *Bifidobacterium* proliferation, and inhibit *Escherichia coli* growth [10-12]. However, whether RS can regulate intestinal microflora disturbances caused by high-fat diets requires further investigation. This study established intestinal microflora disturbances in diet-induced obese (DIO) rats and then fed them a high-fat diet containing 10% RS to explore the regulatory effects of RS on intestinal microflora disorders in DIO rats, providing a basis for dietary modulation of intestinal microflora structure.

Materials and Methods

Experimental Animals and Diets One hundred specific-pathogen-free (SPF) male SD rats weighing 70–90 g were obtained from the Animal Center of Gansu University of Chinese Medicine [SCXK (Gan) 2011-0001]. Experimental diets included a basal diet (control group), high-fat diet (HF group), and high-fat resistant starch diet (HFRS group), all formulated by Beijing Keao Xieli Feed Co., Ltd. Diet composition and nutrient levels are presented in Table 1 . The RS4 used was HI-MAIZE 260 [origin: USA, purchased from National Starch Industry (Shanghai) Co., Ltd., purity 67.7%, batch number HAIO380], with an actual addition rate of 14.8% to achieve an effective concentration of 10%.

Dextran sulfate (DS, Watson Bio) was dissolved in distilled water to prepare a 5% solution for later use.

Animal Grouping The 100 SD rats were randomly divided into two groups: a control group (n=10) with initial body weight of (73.40$±2.59)$gfedthebasaldiet, andanHFgroup$(n = 90)$withinitialbodyweightof$(74.20±$2.89) g fed the high-fat diet. No statistically significant difference in body weight existed between the two groups (P>0.05). After a one-week acclimation period without abnormalities, the formal experiment began. Rats were housed in groups with *ad libitum* access to food and water. Environmental conditions were maintained at 21–24 °C with relative humidity $ 60±$15.10) g, confirming successful model establishment.

Experimental Design The control group (n=10) continued on the basal diet. From the 55 DIO rats, 27 were randomly selected and divided into three groups (n=9 each): the HF group (high-fat diet), the HFRS group (high-fat diet with 10% RS), and the HFRS+DS group (high-fat diet with 10% RS). During the five-week experimental period, the HFRS+DS group received daily gavage of 1 mL 5% DS solution, while the other groups received 1 mL distilled water.

Fecal Weight Measurement Every Saturday at 08:00, rats were placed individually in metabolic cages for 24-hour fecal collection before returning to their original cages. Fecal wet weight was measured, then samples were blotted dry with qualitative filter paper, wrapped, and dried in an oven at 80 °C for 72 hours before measuring dry weight. Water content percentage was calculated as: Water content (%) = [(wet weight - dry weight) / wet weight] × 100. Fecal pellet number per gram of dry feces was also counted and expressed as pellets/g.

Intestinal Microflora Detection At weeks 7 and 12, 1 g of fresh formed feces was collected from each group using sterile forceps and placed in a 10 mL sterile EP tube. Saline was added to reach 10 mL, and the mixture was vortexed thoroughly until uniformly dispersed. The fecal suspension was then serially diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. Appropriate dilutions were

selected, and 50 L aliquots (three replicates per dilution) were plated on selective media: *Enterococcus* on enterococcus chromogenic medium, *Enterobacter* on eosin methylene blue (EMB) agar, *Lactobacillus* on LBS medium, *Bifidobacterium* on BS medium, and *Bacteroides* on BDS medium. *Enterococcus* and *Enterobacter* were incubated aerobically at 37 °C for 36 hours, while *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* were incubated anaerobically at 37 °C for 36 hours. Colonies were counted as colony-forming units per gram (CFU/g) and expressed as $\log_{10}$(CFU/g) [13].

Statistical Analysis Data were analyzed using one-way ANOVA with LSD and Duncan's multiple comparison tests in SPSS 11.0 software. All data are presented as means ± standard deviation (±SD), with $P<0.05$ considered statistically significant.

Results

Defecation Status of Experimental Rats As shown in Table 2 , after seven weeks of high-fat feeding, the HF group exhibited significantly decreased fecal wet weight and water content compared with the control group ($P<0.05$), while fecal pellet number increased significantly ($P<0.05$). Fecal dry weight showed a decreasing trend but did not differ significantly from the control group ($P>0.05$). After 12 weeks of high-fat feeding, DIO rats in the HF group showed significantly reduced fecal wet weight and water content ($P<0.05$) with significantly increased fecal pellet number ($P<0.05$) compared with controls. Although fecal dry weight tended to decrease, no significant difference was observed ($P>0.05$). Compared with the HF group, HFRS DIO rats demonstrated significantly increased fecal wet weight, dry weight, and water content ($P<0.05$) with significantly decreased fecal pellet number ($P<0.05$). Compared with the HFRS group, the HFRS+DS group showed significantly reduced fecal wet weight and water content ($P<0.05$).

Fecal Microflora Counts in Experimental Rats As presented in Table 3 , at week 7, the HF group showed significantly elevated *Enterobacter* counts compared with the control group ($P<0.05$), while *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* counts decreased significantly ($P<0.05$). At week 12, compared with controls, DIO rats in the HF group exhibited significantly increased *Enterobacter* counts ($P<0.05$) and significantly decreased counts of *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* ($P<0.05$). Compared with the HF group, HFRS DIO rats showed significantly reduced *Enterobacter* counts ($P<0.05$) and significantly increased counts of *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* ($P<0.05$). Compared with the HFRS group, the HFRS+DS group demonstrated significantly increased *Enterobacter* and *Enterococcus* counts ($P<0.05$) but significantly decreased *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* counts ($P<0.05$).

Discussion

Resistant starch is a type of starch that escapes digestion in the human small intestine and exerts fiber-like effects in the colon [14-15]. Dietary fiber cannot be degraded by human digestive enzymes and can be fermented by microorganisms upon reaching the colon, thereby regulating intestinal microflora. Based on this principle, RS likely shares similar functions, though research on RS effects on intestinal microflora remains limited, and mechanistic studies are particularly scarce. In preliminary experiments, we administered 5%, 10%, and 15% RS to DIO rats and found that 10% RS provided optimal weight gain control [16], consistent with reports by Yang et al. [17] and Zhang et al. [18]. Therefore, this study utilized 10% RS as the intervention dose to investigate its effects on rat intestinal microflora. Since intestinal microflora is strongly influenced by dietary composition, we standardized crude protein (20% casein), vitamin, and mineral contents across all diets to isolate the effects of RS. The control diet contained 5% lard, while HF and HFRS diets contained 15% lard. The sole difference between HF and HFRS diets was starch composition, with HFRS substituting 10% digestible starch with 10% RS. Given that digestible starch has a digestion and absorption rate exceeding 99% in the small intestine, with a glycemic index reaching (99$±$9)% at 120 minutes post-ingestion in rats [18-19], minimal digestible starch reaches the colon. Thus, differences between HF and HFRS groups can be attributed primarily to RS.

Our results demonstrate that high-fat diets significantly decreased fecal wet weight and water content while increasing fecal pellet number in SD rats, without significantly affecting dry weight. This indicates that high-fat diets substantially impact defecation status, primarily through reduced fecal water content and altered fecal morphology. The mechanism likely involves excessive dietary fat intake, where large amounts of unabsorbed fat enter the colon and increase fecal hydrophobicity, resulting in drier, smaller, and more numerous fecal pellets. RS supplementation significantly increased fecal wet weight, dry weight, and water content while decreasing pellet number, indicating that RS possesses favorable water-holding capacity and can ameliorate intestinal dysfunction induced by high-fat diets. The increased dry weight suggests that RS can enhance total fecal mass, likely due to its stool-promoting effects.

Intestinal microflora plays a crucial role in animal health, contributing to intestinal barrier function and nutrient metabolism [2]. Among various influencing factors, dietary structure is predominant [3,20-21]. Previous studies have shown that reduced dietary fiber decreases fecal water content and stool output while prolonging intestinal transit time, significantly altering defecation patterns [22]. Additionally, different microbial species require distinct substrates for growth and reproduction. Variations in dietary composition lead to differences in food residues reaching the colon, which selectively stimulates the growth of specific bacterial populations and consequently influences intestinal microflora. Excessive protein intake or malabsorption allows undigested protein to undergo prolonged fermentation in the colon, inducing proliferation of

putrefactive bacteria, increasing intestinal pH, and suppressing beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* [6,23]. High-fat diets can also disrupt the microbial environment by reducing available carbohydrates in the colon, increasing oxidative stress, and generating secondary metabolites that cause dysbiosis [24-26].

In this study, high-fat feeding first induced intestinal microflora disturbances in rats. Subsequent administration of a 10% RS diet increased probiotic populations (*Bifidobacterium* and *Lactobacillus*) while decreasing conditional pathogens (*Enterobacter*), demonstrating that RS can improve high-fat diet-induced microflora imbalances in DIO rats, consistent with prebiotic characteristics. Dextran sulfate is both a colitis inducer and a butyrate inhibitor. Butyrate, the primary fermentation product of RS in the colon, serves as the main energy source for intestinal epithelial cells. DS gavage induced colitis in DIO rats, resulting in abnormal defecation with decreased fecal wet weight, dry weight, and water content, along with disrupted microflora characterized by increased *Enterobacter* and *Enterococcus* and decreased probiotics. This indicates that DS blocked the ameliorative effects of RS on intestinal dysfunction, likely by inhibiting butyrate production. These findings suggest that dietary modulation through appropriate intake of indigestible carbohydrates like RS can effectively maintain intestinal microflora stability.

The mechanisms by which RS improves high-fat diet-induced microflora disturbances likely involve several factors. First, undigested protein and fat entering the colon inhibit anaerobic bacteria while promoting putrefactive bacteria proliferation. Through its fiber-like effects, RS improves defecation and reduces intestinal transit time, thereby decreasing fermentation time for undigested residues, particularly protein and fat, and consequently reducing putrefactive bacterial populations [16,18]. Second, RS fermentation by colonic microorganisms produces short-chain fatty acids that lower intestinal pH and improve the microenvironment [16]. Third, chronic high-fat feeding induces intestinal oxidative stress [26], causing inflammatory damage to the intestinal wall and resulting in dysbiosis, often manifested as *E. coli* overgrowth. RS can modulate lipid metabolism to improve oxidative stress status. Fourth, RS alters dietary digestion and absorption characteristics; being indigestible in the small intestine, it provides abundant carbon sources for probiotics like *Bifidobacterium* and *Lactobacillus* in the colon, thereby promoting their growth.

In conclusion, dietary supplementation with appropriate RS levels can increase fecal wet weight, dry weight, and water content while decreasing pellet number, thereby improving defecation status. Additionally, RS can increase *Bifidobacterium* and *Lactobacillus* populations while decreasing *E. coli* counts in DIO rats.

References

[1] ZHU Weiyun, YU Kaifan, MU Chunlong, et al. Intestinal microbiota and

host nutrient metabolism in pigs[J]. Chinese Journal of Animal Nutrition, 2014, 26(10): 3046–3051.

[2] CAESAR R, FÅK F, BÄCKHED F. Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism[J]. Journal of Internal Medicine, 2010, 268(4): 320–328.

[3] FUKUDA S, OHNO H. Gut microbiome and metabolic diseases[J]. Seminars in Immunopathology, 2014, 36(1): 103–114.

[4] LEY R E, TURNBAUGH P J, KLEIN S, et al. Microbial ecology: human gut microbes associated with obesity[J]. Nature, 2006; 444(7122): 1022–1023.

[5] CHEN Xingyun, ZENG Benhua, WEI Hong, et al. Effects of high-fat diet on gut microbiota structure in human flora-associated mice[J]. Food Science, 2013, 34(17): 278–283.

[6] ZHOU Zhongkai, YANG Yan, ZHENG Paiyun, et al. Relationship between intestinal microbial protein fermentation and intestinal health[J]. Food Science, 2014, 35(1): 303–309.

[7] SU Chang, WANG Huijun, ZHANG Bing, et al. Dietary nutrient intake status and trends among Chinese adults aged 18–49 years from 1989 to 2009 (V): Dietary fat and cholesterol intake status and trends[J]. Acta Nutrimenta Sinica, 2011, 33(6): 546–550.

[8] XIANG Zhentian. Effects of different dietary starch sources on intestinal function and health of weaned piglets and their mechanisms[D]. PhD Thesis. Ya'an: Sichuan Agricultural University, 2011.

[9] WALKER A W, INCE J, DUNCAN S H, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota[J]. The ISME Journal, 2011, 5(2): 220–230.

[10] FANG Jiandong. Study on the effects of resistant starch on intestinal microflora in mice and its mechanism[D]. Master's Thesis. Hangzhou: Zhejiang Gongshang University, 2014.

[11] AZORÍN-ORTUÑO M, URBÁN C, CERÓN J J. Effect of low inulin doses with different polymerisation degree on lipid metabolism, mineral absorption, and intestinal microbiota in rats with fat-supplemented diet[J]. Food Chemistry, 2009, 113(4): 1058–1065.

[12] HIGGINS J A, BROWN M A, STORLIEN L H. Consumption of resistant starch decreases postprandial lipogenesis in white adipose tissue of the rat[J]. Nutrition Journal, 2006, 5(1): 25–26.

[13] WU Yinwei. Effects of Bifid Triple Viable Capsule on intestinal microflora and therapeutic efficacy in patients with diarrhea-predominant irritable bowel syndrome[J]. Chinese Journal of Microecology, 2014, 26(4): 438–440.

[14] WANG Zhu, YANG Yuexin, ZHOU Ruihua, et al. Metabolism of resistant starch and its regulation on blood glucose[J]. Acta Nutrimenta Sinica, 2003, 25(2): 190–195.

[15] BAI Yongliang, PENG Zhenfu, CHEN Qingfa, et al. Regulatory effects of banana powder on intestinal microflora in diabetic rats[J]. Modern Food Science and Technology, 2013, 29(9): 2110–2114.

[16] WANG Zhifan, CHEN Wangsheng, ZHANG Yinxia. Improvement effect of resistant starch on intestinal fermentation status in diet-induced obese rats[J]. Journal of Jilin University (Medicine Edition), 2013, 39(1): 42–45.

[17] YANG Yuexin, WANG Zhu, HONG Jie, et al. Effect of resistant starch fermentation in colon on intestinal health in rats[J]. World Chinese Journal of Digestology, 2004, 12(11): 2618–2622.

[18] ZHANG Zhenzhen, YIN Fugui, HUANG Ju, et al. Study on digestibility of coarse grain starch in piglets[J]. Food Science, 2010, 31(3): 217–219.

[19] ZHANG Hui, HONG Yan, GU Zhengbiao, et al. Digestibility of starch from three whole grain flours and its influencing factors[J]. Food and Fermentation Industries, 2012, 38(11): 26–31.

[20] NATIVIDAD J M, HUANG X X, SLACK E, et al. Host responses to intestinal microbial antigens in gluten-sensitive mice[J]. PLoS One, 2009, 4(7): e6472-e6473.

[21] DIBAISE J K, ZHANG H S, CROWELL M D, et al. Gut microbiota and its possible relationship with obesity[J]. Mayo Clinic Proceedings, 2008, 83(4): 460–469.

[22] WANG Zhifan, MA Hui, CHEN Wangsheng, et al. Effects and significance of high-fat diet on defecation and fecal microflora in SD rats[J]. Journal of Jilin University (Medicine Edition), 2014, 40(4): 734–738.

[23] Welloek IJ. Effect of weaning age, protein nutrition and enterotoxigenic *Escherichia coli* challenge on the health of newly weaned piglets[J]. Livestock Science, 2007, 108(1): 102–105.

[24] RESTA S C. Effects of probiotics and commensals on intestinal epithelial physiology: implications for nutrient handling[J]. Journal of Physiology, 2009, 587(Pt 17): 4169–4174.

[25] ATTENE-RAMOS M S, NAVA G M, MUELLNER M G, et al. DNA damage and toxicogenomic analyses of hydrogen sulfide in human intestinal epithelial FHs 74 Int Cells[J]. Environmental and Molecular Mutagenesis, 2010, 51(4): 304–314.

[26] ZHANG Rong, SUN Jin, LI Yaxin, et al. Effects of lipoic acid on intestinal redox status and microbial flora in high-fat diet-fed mice[J]. Science and Technology of Food Industry, 2010, 31(9): 321–326, 369.