Effects of Nisin on Cecal Microbiota Structure and Lipid Metabolism in Diarrheal Mice: Post-print

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Abstract

This experiment aimed to investigate the effects of nisin (Nisin) on cecal microbiota structure and lipid metabolism in diarrheal mice. Fifty 7–9-week-old specific pathogen-free (SPF) mice (half male and half female) were selected and randomly divided into five groups: blank control group, negative control group, ciprofloxacin group, ampicillin group, and Nisin group. Except for the blank control group, which received intraperitoneal injection of an equal volume of sterile physiological saline, mice in the other groups were intraperitoneally injected with pathogenic Escherichia coli (E. coli) O1 suspension (2.50×1011 CFU/mL) at 0.2 mL per mouse for 3 consecutive days to establish a diarrheal mouse model. After 3 consecutive days of injection, the blank control and negative control groups were gavaged with sterile physiological saline, while the other groups were gavaged with the corresponding substances, twice daily at 0.3 mL each time for 15 consecutive days. Samples were collected 2 h after gavage on day 15. High-throughput sequencing technology was used to analyze the bacterial structure in mouse cecal contents, and enzyme-linked immunosorbent assay (ELISA) was used to detect the contents of a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), total cholesterol (TC), and insulin (INS) in mouse serum, jejunum, ileum, and brain tissues. The results showed that compared with the negative control group, Nisin significantly reduced ADAMTS1 content in mouse serum, ileum, and brain tissues and INS content in the ileum ($P<0.05$), significantly increased TC content in serum, jejunum, and brain tissues ($P<0.05$), and significantly increased mouse body weight at all stages ($P<0.05$). The Nisin group exhibited the highest cecal microbiota richness (ACE index = 2 469.54, Chao1 index = 3 340.29) and diversity (Shannon index = 7.56), while the negative control group showed the lowest. In the Nisin group, Verrucomicrobia, Cyanobacteria, Bacteroidetes, and

Firmicutes were the dominant phyla. In the negative control group, Actinobacteria, Gemmatimonadetes, Proteobacteria, and Bacteroidetes were the dominant phyla. It was concluded that Nisin can increase cecal microbiota diversity, reduce ADAMTS1 content in serum, ileum, and brain tissues and INS content in the ileum, increase TC content in serum, jejunum, and brain tissues, thereby affecting cecal microbiota structure and lipid metabolism in diarrheal mice.

Full Text

Effects of Nisin on Caecal Microflora Structure and Lipid Metabolism of Mice with Diarrhea

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Abstract

This study aimed to investigate the effects of nisin on the cecal microflora structure and lipid metabolism in mice with diarrhea. Fifty specific-pathogen-free (SPF) mice aged 7–9 weeks (half male and half female) were randomly divided into five groups: blank control group, negative control group, ciprofloxacin group, ampicillin group, and nisin group. Except for the blank control group, which received intraperitoneal injections of sterile normal saline, all other groups were intraperitoneally injected with pathogenic *Escherichia coli* O1 suspension (2.50×10^{11} CFU/mL) at 0.2 mL per mouse for three consecutive days to establish a diarrheal model. After three days of injection, the blank control and negative control groups were administered sterile normal saline by gavage, while the other groups received their respective treatments. All administrations were given twice daily at a dose of 0.3 mL per mouse for 15 consecutive days.

Samples were collected 2 hours after the final administration on day 15. The bacterial structure in mouse cecal contents was analyzed using high-throughput sequencing technology, while the contents of a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), total cholesterol (TC), and insulin (INS) in serum, jejunum, ileum, and brain tissue were measured by enzyme-linked immunosorbent assay (ELISA).

The results showed that compared with the negative control group, nisin significantly decreased ADAMTS1 content in serum, ileum, and brain tissue, as well

as INS content in ileum ($P<0.05$). Nisin also significantly increased TC content in serum, jejunum, and brain tissue ($P<0.05$) and significantly increased body weight at all stages ($P<0.05$). The nisin group exhibited the highest cecal microflora richness (ACE index = 2,469.54; Chao1 index = 3,340.29) and diversity (Shannon index = 7.56), while the negative control group showed the lowest values. The dominant phyla in the nisin group were *Verrucomicrobia*, *Cyanobacteria*, *Bacteroidetes*, and *Firmicutes*, whereas the negative control group was dominated by *Actinobacteria*, *Gemmatimonadetes*, *Proteobacteria*, and *Bacteroidetes*.

In conclusion, nisin can enhance cecal microflora diversity, decrease ADAMTS1 content in serum, ileum, and brain tissue and INS content in ileum, and increase TC content in serum, jejunum, and brain tissue, thereby affecting the cecal microflora structure and lipid metabolism in mice with diarrhea.

Keywords: nisin; intestinal microbiota; brain-gut axis; ADAMTS1; high-throughput sequencing

Introduction

The intake of probiotics can balance the intestinal microflora structure in animals. Investigating the effects of probiotic metabolites such as nisin on intestinal microbial diversity and lipid metabolism can help deepen our understanding of how these metabolites influence gut microbiota and obesity. Obesity is a chronic disease caused by an imbalance between energy intake and metabolism, which consistently affects animal carcass quality. Recent studies have revealed a close correlation between gut microbiota and obesity, while lipid metabolism disorders profoundly impact the structure of intestinal microflora. Research has demonstrated that intestinal microbial communities are essential for processing dietary polysaccharides, and conventional rearing increases mouse body fat by 60% compared with germ-free rearing, while decreasing insulin content.

ADAMTS1 is a key factor regulating the differentiation of adipose stem cells into adipocytes. High-fat diets and glucocorticoid drugs downregulate ADAMTS1 secretion, ultimately leading to increased adiposity. *Lactococcus lactis* can be used to treat inflammatory bowel disease, ulcerative colitis, diarrhea, celiac disease, allergic reactions, and oral mucositis. Pathogenic *Escherichia coli* can cause various diseases, including diarrhea and sepsis.

Pathogenic *E. coli* O1 is one of the most common pathogens causing calf diarrhea. Currently, antibiotics are widely used to treat diarrhea in humans and livestock, but their abuse has led to increased bacterial resistance. Studies have shown that nisin has the potential to replace antibiotics in treating mouse diarrhea and plays an important role in livestock lipid metabolism. This study established a mouse diarrhea model using pathogenic *E. coli* O1 and then administered nisin to the diarrheal mice to investigate its effects on cecal microflora structure and the contents of ADAMTS1, TC, and INS in serum, jejunum, ileum,

and brain tissue, thereby laying a foundation for understanding how probiotic metabolites like nisin affect intestinal microflora and lipid metabolism.

Materials and Methods

1.1 Test Materials

Pathogenic *E. coli* O1 and nisin were both isolated, extracted, and purified from dairy cow rectal fecal samples by the Animal Production Laboratory of Inner Mongolia Agricultural University. Ampicillin was purchased from Sunflower Pharmaceutical Group Hubei Wudang Co., Ltd., and ciprofloxacin hydrochloride was purchased from Jilin Province Bainian Liufutang Pharmaceutical Co., Ltd.

1.2 Experimental Animals

Specific-pathogen-free (SPF) mice aged 7–9 weeks (half male and half female) weighing 20±2 g were purchased from the Laboratory Animal Center of Inner Mongolia Medical University. The mice were housed in an environment with constant temperature (22±2°C) and humidity (55±5%) and fed SPF-grade feed.

1.3.1 Experimental Grouping and Dosing Regimen

Fifty SPF mice aged 7–9 weeks (half male and half female) were randomly divided into five groups: blank control group, negative control group, ciprofloxacin group, ampicillin group, and nisin group. Except for the blank control group, which received intraperitoneal injections of sterile normal saline (0.2 mL per mouse), all other groups were intraperitoneally injected with pathogenic *E. coli* O1 suspension (2.50×10^{11} CFU/mL) at 0.2 mL per mouse for three consecutive days to establish a diarrheal model. After three days of injection, the blank control and negative control groups were administered sterile normal saline by gavage, while the other groups received their respective test substances (see Table 1 for dosing regimen) for 15 consecutive days, twice daily (at 08:00 and 16:00) at 0.3 mL per mouse. Samples were collected 2 hours after the final gavage on day 15.

Note: The dose for each animal was administered in combination with 0.3 mL distilled water via gavage.

1.3.2 Sample Collection

During the experimental period, mouse body weight was measured daily. On day 15, 2 hours after the final gavage, seven mice from each group were euthanized by cervical dislocation. Blood was collected via retro-orbital bleeding, and serum was separated and stored. Under aseptic conditions, each mouse was disinfected, the abdominal cavity was opened, and 0.5 g of cecal contents, jejunum, ileum, and brain tissue were quickly placed into sterile, enzyme-free 2.5 mL centrifuge tubes and stored at -80°C for later use.

1.3.3 Index Determination

The jejunum, ileum, and brain tissues were homogenized. For each sample, 0.1 g of tissue was mixed with 900 L of phosphate-buffered saline (PBS) to prepare tissue homogenates, which were then centrifuged and the supernatants were retained and stored at -80°C. The contents of ADAMTS1, TC, and INS in serum, jejunum, ileum, and brain tissue were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer' s instructions; the kits were purchased from Beijing Chenglin Biological Technology Co., Ltd.

Centrifuge tubes containing cecal contents were placed in dry ice and sent to Beijing Novogene Bioinformatics Technology Co., Ltd. for amplification and sequencing of the bacterial 16S rRNA V3–V4 region using primers 341F (5' -CCTACGGGNGGCWGCAG-3') and 805R (5' -GACTACHVGGGTATCTAATCC-3'). Detailed procedures are described in reference [17]. Sample sequences were distinguished using barcodes. After filtering the sequence data from each group, alpha diversity and microflora structure analyses were performed. Alpha diversity indices included richness indices (Chao1 and ACE indices), diversity indices (Simpson and Shannon indices), and coverage index (Coverage). Operational taxonomic units (OTUs) for each group were clustered using Mothur software to represent inter-group sample abundance [18].

1.4 Statistical Analysis

Data were processed using Excel 2016 and analyzed by one-way analysis of variance (ANOVA) using SAS 9.0 statistical software. $P<0.05$ was considered statistically significant.

Results

2.1 Effects of Nisin on Mouse Body Weight

As shown in Table 2 , during days 1–5, the body weight of mice in the nisin group was significantly higher than that in the blank control and negative control groups ($P<0.05$). During days 6–10, the nisin group showed significantly higher body weight compared with all other groups ($P<0.05$), while no significant differences were observed among the other groups ($P>0.05$). During days 11–15, the nisin group exhibited significantly higher body weight than the negative control, ciprofloxacin, and ampicillin groups ($P<0.05$), with no significant difference compared with the blank control group ($P>0.05$). These results indicate that nisin can effectively alleviate the effects of *E. coli* O1 infection on mouse body weight.

2.2 Effects of Nisin on ADAMTS1 Content in Serum, Jejunum, Ileum, and Brain Tissue

As shown in Table 3 , in serum, ADAMTS1 content in the nisin group was significantly lower than in the blank control and negative control groups ($P<0.05$). In the jejunum, ADAMTS1 content in the nisin group was significantly lower than in the negative control group ($P<0.05$) but significantly higher than in the ciprofloxacin and ampicillin groups ($P<0.05$). In the ileum, ADAMTS1 content in the nisin group was significantly lower than in all other groups ($P<0.05$). In brain tissue, ADAMTS1 content in the nisin group was significantly lower than in the blank control and negative control groups ($P<0.05$) but significantly higher than in the ciprofloxacin group ($P<0.05$).

2.3 Effects of Nisin on TC Content in Serum, Jejunum, Ileum, and Brain Tissue

As shown in Table 4 , in serum, TC content in the nisin group was higher than in the negative control, ciprofloxacin, and ampicillin groups but lower than in the blank control group; however, these differences were not statistically significant ($P>0.05$). In the jejunum, TC content in the nisin group did not differ significantly from the blank control group ($P>0.05$) but was significantly higher than in all other groups ($P<0.05$). In the ileum, TC content in the nisin group was significantly higher than in the ciprofloxacin group ($P<0.05$). In brain tissue, TC content in the nisin group was significantly lower than in the ampicillin group ($P<0.05$) but significantly higher than in all other groups ($P<0.05$).

2.4 Effects of Nisin on INS Content in Serum, Jejunum, Ileum, and Brain Tissue

As shown in Table 5 , in serum, INS content in the nisin and blank control groups was significantly higher than in all other groups ($P<0.05$), with the nisin group showing the highest value. In the jejunum, INS content in the ampicillin group was significantly lower than in all other groups ($P<0.05$), while the negative control group showed significantly higher INS content than all other groups ($P<0.05$). In the ileum, INS content in the blank control group was significantly higher than in all other groups ($P<0.05$), whereas the nisin group showed significantly lower INS content than all other groups ($P<0.05$). In brain tissue, INS content in the blank control group was significantly higher than in all other groups ($P<0.05$), while the ciprofloxacin group showed significantly lower INS content than all other groups ($P<0.05$).

2.5 Effects of Nisin on Caecal Microflora Diversity

As shown in Table 6 , among the cecal content samples from all groups, the blank control group had the highest number of microbial OTUs (1,652.75), followed by the nisin group (1,507.75), while the ampicillin group had the lowest

(930.0). This indicates that both the nisin and blank control groups had high microflora abundance in the cecum, with differences observed among groups. In cecal content samples, the nisin group showed significantly higher ACE index (1,417.25), Chao1 index (1,378.45), and Shannon index (7.56) compared with the negative control group (ACE index = 969.54, Chao1 index = 340.29, Shannon index = 6.63), and a significantly lower Simpson index (0.0033) compared with the negative control group (0.0174). These findings indicate that cecal microflora diversity was higher in the nisin group than in the negative control group. The nisin group had the highest Shannon index and a relatively low Simpson index among the five groups, suggesting that cecal microflora diversity in the nisin group was at a relatively high level. The Coverage index for cecal microflora in all groups was above 9.7, indicating high sequencing quality and minimal probability of undetected sequences.

Mothur software was used to annotate the samples from each group. Based on the 16S rRNA V3–V4 region sequencing of mouse cecal contents and species annotation results, the relative abundance of species at the phylum level for each group is shown in Figure 1 [Figure 1: see original paper]. The dominant phyla in the nisin group were *Verrucomicrobia*, *Cyanobacteria*, *Bacteroidetes*, and *Firmicutes*. The negative control group was dominated by *Actinobacteria*, *Gemmatimonadetes*, *Proteobacteria*, and *Bacteroidetes*. The ciprofloxacin group showed *Firmicutes*, *Actinobacteria*, *Ktedonobacteria* (unclassified phylum), and *Coriobacteriia* (unclassified phylum) as dominant phyla. The ampicillin group was dominated by *Deferribacteres* and *Firmicutes*. The blank control group had *Bacteroidetes*, *Firmicutes*, and *Saccharibacteria* as dominant phyla.

AMPG: ampicillin group; BCG: blank control group; CIPG: ciprofloxacin group; NCG: negative control group; NIG: nisin group.

Figure 1. Cluster of relative abundances of species in groups

The proportions of the top four phyla in cecal content samples at the phylum level are shown in Table 7 . The proportion of *Firmicutes* in the nisin group was significantly higher than in all other groups ($P<0.05$), while the proportion of *Bacteroidetes* was significantly lower than in all other groups ($P<0.05$). These results suggest that nisin promotes the proliferation of *Firmicutes* and reduces *Bacteroidetes* in the mouse cecum.

Discussion

The animal intestine harbors a large number of bacteria. Once the microecological balance in the intestinal biological barrier is disrupted, numerous pathogenic bacteria (such as *E. coli*) can invade and colonize the gut [19]. Additionally, the intestine is an important site for nutrient absorption in animals; excessive food intake leads to fat accumulation [20]. Intestinal microflora plays a crucial role in regulating energy metabolism, and correcting microbial imbalance can significantly reduce body weight, serum total protein content, and fasting blood glucose levels [21]. After colonizing germ-free mice with intestinal microflora, their

consumption of high-fat and high-sugar foods increased significantly, demonstrating that intestinal microflora can substantially enhance digestive capacity and improve energy acquisition from food [22]. Changes in the numbers of pathogenic and probiotic bacteria (such as *Bifidobacterium* and *Lactobacillus*) in the intestine are important indicators of gut health [23]. Yassour et al. [24] suggest that while the mechanism of antibiotic effects on intestinal microflora remains unclear, antibiotics can significantly impact the diversity of human and animal gut microbiota. Meanwhile, the large number of bacteria present in the human and animal intestines helps break down indigestible food components. Transplanting intestinal microflora from normal mice to germ-free mice significantly increased body fat content without increasing food intake, concurrently altering the levels of several lipid metabolism-related factors, possibly due to the influence of gut microbiota on host energy absorption from food [25].

ADAMTS1 is a key molecule controlling adipocyte differentiation and regulating fat balance [5]. Studies have shown that mature adipocytes normally secrete ADAMTS1 [26,27]. Current research on obesity in mice primarily focuses on detecting signaling pathways and protein expression of relevant factors in blood, as well as high-throughput analysis of gut microbiota structure [28]. Mice with high ADAMTS1 levels store less fat than wild-type mice [29,30].

ADAMTS1 can prevent glucocorticoid-induced adipocyte differentiation [31]. Research demonstrates that reducing ADAMTS1 levels in adipose tissue leads to increased adipose tissue weight, decreased INS sensitivity, and lipid metabolism disorders. This finding aligns with the observation that ADAMTS1 expression is downregulated in obese mice and that ADAMTS1 expression negatively correlates with human body mass index (BMI) [32]. Obesity often causes lipid metabolism disorders through mechanisms involving visceral fat accumulation, promotion of gluconeogenesis, and increased appetite due to high INS levels, which exacerbates cholesterol metabolism disorders and worsens obesity [33]. Studies have found that consumption of foods containing *Lactococcus* significantly increases high-density lipoprotein levels in human blood [34], whereas consumption of foods containing *Lactobacillus* significantly decreases high-density lipoprotein levels [35]. Adding microbial strains to feed can improve growth performance and immunity in pigs, with significant effects on nutrient metabolism [36]. *Lactococcus lactis* from lactic acid fermentation has recently been evaluated as a potential probiotic [37].

In this study, the probiotic metabolite nisin alleviated weight gain and reduced serum TC content in mice, thereby improving intestinal microflora structure and lipid metabolism disorders. This suggests that nisin may reduce the risk of cardiovascular and cerebrovascular diseases [38,39]. Additionally, nisin decreased INS content in the ileum, which is important for regulating lipid metabolism synthesis—a result consistent with findings by Xu et al. [40]. Research has shown that obesity is associated with altered relative abundances of *Bacteroidetes* and *Firmicutes*. Before dietary treatment, obese individuals have significantly fewer *Bacteroidetes* but more *Firmicutes* in their gut compared with lean individu-

als. After dietary treatment, obese individuals show increased *Bacteroidetes* and decreased *Firmicutes* [25].

In this study, compared with the negative control group, nisin significantly reduced ADAMTS1 content in serum, jejunum, ileum, and brain tissue, increased TC content in serum, jejunum, and brain tissue, and decreased INS content in the ileum, leading to increased body weight. Nisin also increased *Firmicutes* and decreased *Bacteroidetes* in mouse cecal contents, demonstrating its ability to modulate cecal microflora composition. Furthermore, nisin significantly increased ACE, Chao1, and Shannon indices while significantly decreasing the Simpson index, indicating enhanced cecal microflora diversity. Additionally, the negative control group showed the lowest ACE, Chao1, and Shannon indices and a significantly higher Simpson index compared with the blank control group, suggesting that *E. coli* O1 disrupts the dynamic balance of intestinal microorganisms and significantly reduces cecal microflora diversity—a result similar to findings by Li et al. [41].

In summary, on one hand, nisin increases mouse body weight by reducing ADAMTS1 content in serum, jejunum, ileum, and brain tissue and INS content in the ileum, while increasing TC content in serum, jejunum, and brain tissue. On the other hand, nisin enhances microflora diversity in intestinal contents, increases the abundance of *Firmicutes* and *Cyanobacteria*, and decreases *Bacteroidetes*, thereby adjusting cecal microflora structure. These findings suggest that nisin likely influences mouse body weight and hormone secretion by altering the structure of the inherent gut microbiota.

Conclusion

Nisin can enhance cecal microflora diversity, decrease ADAMTS1 content in serum, ileum, and brain tissue and INS content in the ileum, and increase TC content in serum, jejunum, and brain tissue, thereby affecting the cecal microflora structure and lipid metabolism in mice with diarrhea.

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Note: Figure translations are in progress. See original paper for figures.