Effects of Dietary Supplementation with Fermented Chinese Herbal Residues on Fecal Microbiota and Their Metabolites in Periparturient Sows: Postprint

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Abstract

This study aimed to compare the effects of traditional Chinese medicine residue and fermented traditional Chinese medicine residue on fecal microbiota and their metabolites in sows, providing a basis for their application in sow diets. Sixty multiparous sows (parity 2-4) with similar expected farrowing dates were randomly allocated into three groups (n=20 per group). The three groups were fed basal diets supplemented with 2 kg/t rice bran (control group), 2 kg/t traditional Chinese medicine residue, and 2 kg/t fermented traditional Chinese medicine residue, respectively. Feeding commenced on day 85 of gestation and continued until day 21 postpartum. Fresh fecal samples were randomly collected from 8 sows per group on day 110 of gestation and day 21 postpartum to determine microbial counts and the contents of short-chain fatty acids (SCFA) and biogenic amines. The results showed: Compared with the control group, the traditional Chinese medicine residue group exhibited significantly increased propionate content in prepartum feces ($P<0.05$), with increasing trends observed for butyrate ($P=0.086$), straight-chain fatty acids ($P=0.068$), and total SCFA ($P=0.089$) contents, and significantly reduced phenylethylamine content ($P<0.05$); postpartum feces showed significantly decreased 1,7-heptanediamine and tyramine contents ($P<0.05$). The fermented traditional Chinese medicine residue group demonstrated significantly reduced contents of 1,7-heptanediamine, spermidine, and spermine in prepartum feces ($P<0.05$), with increasing trends for acetate ($P=0.068$), butyrate ($P=0.082$), straight-chain fatty acids ($P=0.058$), and total SCFA ($P=0.064$) contents; postpartum feces exhibited significantly increased Lactobacillus counts ($P<0.05$); both prepartum and postpartum feces showed significantly reduced Escherichia coli counts and tyramine content ($P<0.05$). In conclusion, dietary supplementa-

tion with traditional Chinese medicine residue or fermented traditional Chinese medicine residue can improve hindgut microbiota balance in perinatal sows, increase SCFA content, and reduce biogenic amine content, which is beneficial for energy supply and health of the organism.

Full Text

Effects of Dietary Fermented Herb Residue on Fecal Microbes and Their Metabolites in Peripartum Sows

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Abstract

This study was conducted to compare the effects of herb residue (HR) and fermented herb residue on fecal microorganisms and their metabolites in sows, providing a basis for their application in sow diets. Sixty crossbred sows (parities 2-4) with similar expected delivery dates were randomly allocated to three groups (n=20). The groups were fed basal diets supplemented with 2 kg/t rice bran (control), 2 kg/t HR, or 2 kg/t fermented HR from day 85 of gestation to day 21 postpartum. Fresh fecal samples were randomly collected from eight sows per group on day 110 of gestation and day 21 postpartum to determine microbial populations and contents of short-chain fatty acids (SCFA) and bioamines. Compared with the control group, the HR group exhibited significantly increased fecal propionate content prepartum ($P<0.05$), with trends toward higher butyrate ($P=0.086$), straight-chain fatty acids ($P=0.068$), and total SCFA ($P=0.089$), along with significantly reduced phenylethylamine ($P<0.05$). Postpartum fecal 1,7-heptanediamine and tyramine contents were also significantly decreased ($P<0.05$). The fermented HR group showed significantly reduced prepartum fecal contents of 1,7-heptanediamine, spermidine, and spermine ($P<0.05$), with trends toward increased acetate ($P=0.068$), butyrate ($P=0.082$), straight-chain fatty acids ($P=0.058$), and total SCFA ($P=0.064$). Additionally, postpartum fecal *Lactobacillus* populations increased significantly ($P<0.05$), while *Escherichia coli* populations and tyramine content decreased significantly both prepartum and postpartum ($P<0.05$). In conclusion, dietary supplementation with HR or fermented HR can improve hindgut microbial balance, increase SCFA production, and reduce bioamine contents in peripartum sows, thereby benefiting energy supply and overall health.

Keywords: pregnant sow; fermented herb residue; feces; microorganism; metabolites

Introduction

Traditional Chinese herbs serve as functional feed additives with abundant resources, environmental friendliness, and prominent biological functions. Due to limitations in extraction methods and efficiency, herb residues generated by processing plants retain various bioactive compounds and nutrients. For instance, 49.8% of volatile oils remain in Banxia Houpu decoction residues, and 72.1% of astragaloside persists in astragalus residues. Previous research indicates that herb residues are rich in crude fiber (11.82%-44.86%), minerals (4.72%-12.56%), and crude protein (10.56%-16.46%), demonstrating their potential value for development and utilization. Furthermore, modern fermentation processes can reduce crude fiber content while increasing protein levels in herb residues, producing functional secondary metabolites such as oligosaccharides that enhance their value. Recent studies have shown that fermented herb residue preparations can promote growth and health in livestock. For example, fermented herb residues composed of Rehmannia glutinosa, hawthorn, tangerine peel, malt, and licorice can increase intestinal absorption area and nutrient digestibility in weaned piglets, regulate blood lipid concentrations, and improve antioxidant capacity, thereby enhancing growth performance. Similarly, supplementation with fermented herb residues containing astragalus, angelica, motherwort, and honeysuckle during the peripartum period can increase the number of live-born piglets and litter weight gain at weaning. Our previous research found that dietary supplementation with fermented herb residues composed of astragalus, angelica, Rehmannia glutinosa, and white peony significantly improved litter weight gain at weaning. The colon harbors a large microbial community involved in various metabolic processes, and microbial metabolites such as short-chain fatty acids (SCFA), indole, skatole, ammonia nitrogen, and bioamines affect host metabolism and health. Intestinal dysbiosis can cause constipation and other intestinal diseases in sows, yet few studies have investigated the effects of herb residues on fecal microbes and their metabolites in sows. Therefore, this study further investigated the effects of fermented herb residues composed of astragalus, angelica, Rehmannia glutinosa, and white peony on fecal microbial populations and metabolites in peripartum sows to provide a basis for their dietary application.

Materials and Methods

1.1 Herb Residue Preparation and Composition

The herb residues of astragalus, angelica, Rehmannia glutinosa, and white peony used in this study were provided by Hunan Shengyakai Biotechnology Co., Ltd. Water-extracted herb residues were sterilized at 121 °C for 30 min, then mixed at a ratio of 4:2:2:2 (dry weight basis). Moisture content was controlled at 40%-60%. A composite microbial inoculum (containing *Bacillus subtilis*, yeast, lactic acid bacteria, and *Clostridium butyricum*, viable count 2×10^1 CFU/g) was added at 0.4%, and the mixture was fermented at 25 °C for one week with 1-2 daily turnings. After fermentation, the material was dried under vacuum, pulverized, and yielded a brown powder. Analysis showed that the HR and fermented HR preparations contained 17.12 and 16.42 MJ/kg gross energy, 95.82% and 96.81% dry matter, 11.57% and 16.91% crude protein, 7.71% and 5.67% crude fiber, and 5.60% and 3.96% crude fat, respectively.

1.2 Experimental Animals, Groups, and Management

The animal feeding trial was conducted from September to November 2015 at the Animal Experimental Base of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, located at Yong' an Branch of Hunan New Wufeng Co., Ltd. Sixty crossbred sows (parities 2-4) at day 85 of gestation with similar expected delivery dates were randomly divided into three groups (n=20) and housed individually. The groups received basal diets supplemented with 2 kg/t rice bran (control), 2 kg/t HR preparation (HR group), or 2 kg/t fermented HR preparation (fermented HR group), which replaced equivalent amounts of the basal diet. The supplementation levels were determined based on preliminary trials by the manufacturer. Sows were fed gestation diets from day 85 of gestation, switched to lactation diets on day 100, and the trial continued until day 21 postpartum (piglet weaning). During the trial, feeding, watering, and vaccination followed the routine management procedures of the farm. The composition and nutrient levels of the basal diets are shown in Table 1 .

1.3 Determination of Fecal Microbial Populations

Fresh fecal samples were randomly collected from eight sows per group on day 110 of gestation and day 21 postpartum and stored at -80 °C. Microbial DNA was extracted using the QIAamp DNA Stool Mini Kit (QIAgen, Germany) for quantitative PCR analysis. Microbial quantification followed the method of Jiao et al., with results expressed as log (CFU/g) of feces. The group-specific primers used for absolute quantitative PCR are listed in Table 2 and were synthesized by Shanghai Sangon Gene Technology Co., Ltd.

1.4 Determination of Fecal Microbial Metabolite Contents

Straight-chain fatty acids (including acetate, propionate, butyrate, and valerate), branched-chain fatty acids (BCFA, including isobutyrate and isovalerate), and total SCFA (straight-chain fatty acids + BCFA) were determined by gas chromatography. Bioamine contents were detected by high-performance liquid chromatography.

1.5 Statistical Analysis

Data were initially processed using Excel 2010 and then analyzed using SPSS 22.0 software for ANOVA and t-tests. Results are expressed as "mean ± standard error." P<0.05 was considered statistically significant, and 0.05 P<0.10 indicated a trend toward significance.

Results

2.1 Effects of Dietary Herb Residue Preparation on Fecal Microbial Populations in Sows

As shown in Table 3 , compared with the control group, the fermented HR group exhibited significantly increased *Lactobacillus* populations in postpartum feces (P<0.05) and significantly reduced *Escherichia coli* populations in both prepartum and postpartum feces (P<0.05). Compared with the HR group, the fermented HR group showed significantly reduced postpartum *E. coli* populations (P<0.05) and a trend toward increased *Lactobacillus* populations (P=0.090). Relative to prepartum values, all groups displayed significantly reduced *Bifidobacterium* populations in postpartum feces (P<0.05), while the HR group showed a trend toward increased *E. coli* populations postpartum (P=0.096).

2.2 Effects of Dietary Herb Residue Preparation on Fecal SCFA Contents in Sows

As shown in Table 4 , the HR group exhibited significantly increased prepartum fecal propionate content (P<0.05), with trends toward higher butyrate (P=0.086), straight-chain fatty acids (P=0.068), and total SCFA (P=0.089) compared with the control group. The fermented HR group showed trends toward increased prepartum fecal acetate (P=0.068), butyrate (P=0.082), straight-chain fatty acids (P=0.058), and total SCFA (P=0.064). Relative to prepartum values, all groups displayed significantly increased postpartum fecal contents of acetate, propionate, butyrate, straight-chain fatty acids, isobutyrate, branched-chain fatty acids, and total SCFA (P<0.05), while the control and fermented HR groups also showed significantly increased valerate content (P<0.05).

2.3 Effects of Dietary Herb Residue Preparation on Fecal Bioamine Contents in Sows

As shown in Table 5 , the HR group exhibited significantly reduced prepartum fecal phenylethylamine and postpartum 1,7-heptanediamine and tyramine contents (P<0.05) compared with the control group. The fermented HR group showed trends toward reduced prepartum fecal phenylethylamine (P=0.054), putrescine (P=0.063), and cadaverine (P=0.069), with significantly reduced 1,7-heptanediamine, tyramine, spermidine, and spermine ($P<0.05$), and significantly reduced postpartum tyramine content ($P<0.05$). Compared with prepartum values, the control group showed significantly increased postpartum spermidine and spermine ($P<0.05$), the HR group displayed trends toward increased cadaverine (P=0.072) and spermine (P=0.085), and the fermented HR group exhibited significantly increased cadaverine, 1,7-heptanediamine, and tyramine postpartum ($P<0.05$).

Discussion

Lactobacillus is an important beneficial bacterium in the animal gut that produces SCFA, reduces intestinal pH, and inhibits pathogen proliferation, playing a crucial role in improving gastrointestinal function and maintaining microbial balance. Studies have shown that *Lactococcus lactis* possesses superoxide dismutase activity and can reduce cellular damage caused by oxygen free radicals, while excessive oxidative stress can decrease litter size and growth rate of nursing piglets. Our findings indicate that dietary fermented HR significantly increased fecal *Lactobacillus* populations and reduced *E. coli* populations, possibly due to the abundant lactic acid bacteria and prebiotics present in fermented HR. Previous research demonstrated that dietary fermented HR supplementation significantly increased average daily weight gain of weaned piglets, which may be related to improved gut microbiota in sows.

SCFA are primarily produced through fermentation of dietary fiber, resistant starch, and oligosaccharides by beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* in the colon, maintaining intestinal electrolyte balance, regulating microbial equilibrium, and improving gut function. Acetate serves as an energy source for various epithelial cells and gut bacteria, propionate participates in the tricarboxylic acid cycle to provide energy, and butyrate is the main energy source for colonic epithelial cells, maintaining intestinal integrity and promoting colonic growth. Branched-chain fatty acids like isobutyrate and isovalerate originate from metabolism of branched-chain amino acids and serve as ideal markers of protein catabolism in the intestinal lumen. Our results demonstrate that dietary HR or fermented HR promoted microbial fermentation and increased SCFA content in the hindgut of peripartum sows. Beyond serving as an energy source, SCFA regulate proliferation, differentiation, and gene expression in colonocytes and act as signaling molecules to enhance nutrient absorption. Increased intestinal SCFA benefits maternal health and fetal development, likely because dietary fiber in HR serves as a substrate for anaerobic fermentation to produce SCFA, while fermented HR contains abundant lactic acid bacteria and prebiotics that increase SCFA production. Compared with prepartum values, the increased nutrient levels in postpartum diets (gestation diets contained 14.17% crude protein, 5.16% crude fat, 0.98% lysine, and 0.68% threonine, whereas lactation diets contained 19.78% crude protein, 6.02% crude fat, 1.53% lysine, and 0.99% threonine) substantially enhanced microbial fermentation, as evidenced by total SCFA contents of 5.67, 7.71, and 8.06 mg/g prepartum versus 13.06, 12.22, and 11.13 mg/g postpartum in the control, HR, and fermented HR groups, respectively, which may explain why fermented HR supplementation did not significantly affect postpartum SCFA content.

Bioamines participate in the development and maintenance of the intestinal mucosal barrier, thereby affecting host health. Their production depends on both metabolic substrates and colonic microorganisms. For example, amino acids such as ornithine, arginine, lysine, and tyrosine are metabolized by *E. coli*, *Bacteroides*, and *Bifidobacterium* to produce putrescine, cadaverine, and tyramine. Our study found that dietary HR or fermented HR reduced prepartum fecal bioamine content, possibly related to decreased *E. coli* populations. Research indicates that low concentrations of spermine significantly promote cell proliferation, whereas higher concentrations of spermidine and putrescine are required for significant proliferation, suggesting that fermented HR supplementation benefits colonic epithelial cell proliferation. Compared with prepartum values, increased postpartum fecal bioamine content may be associated with higher crude protein levels in lactation diets, as previous studies have also shown that high-nutrient diets increase colonic bioamine content in sows.

In summary, dietary supplementation with herb residue or fermented herb residue improves intestinal microbial balance, increases SCFA content, and reduces bioamine content in peripartum sows, thereby benefiting energy supply and overall health.

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