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Effect of *Flammulina velutipes* (golden needle mushroom, eno-kitake) polysaccharides on constipation

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Abstract: *Flammulina velutipes*, (Curt. ex Fr.) Sing, a popularly edible fungus, has been widely used both as a restorative drug and a tonic food in China. In the current study, the effect of *F. velutipes* polysaccharides was evaluated with a constipated rat model induced by loperamide hydrochloride. The rats were divided into six groups: normal group, model group, positive control group, *F. velutipes* polysaccharides high, moderate and low dose groups. Rats were given 2 mL of Loperamide hydrochloride (3 mg·kg·d), by intragastric administration 2 times per day for 10 days. *F. velutipes* polysaccharides at the doses of 600, 400 and 200 mg/kg (1 mL/100g weight) were administered to treat rats with constipation for 7 days. The gastrointestinal hormones, including motilin (MTL), gastrin (GAS), substance P (SP), somatostatin (SS), and intestinal propulsive rate and feces weight at 24 hours after treatment were used as the indexes to evaluate the effects of *F. velutipes* polysaccharides on constipation. The levels of MTL, GAS and SP in serum significantly increased and the levels of SS in serum of rats significantly decreased after the treatment of rats with *F. velutipes* polysaccharides as compared with those of rats in the model group.

Keywords: Constipation, *Flammulina velutipes* polysaccharides, Loperamide, Hydrochloride

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1 Introduction

Figure 1: *Flammulina velutipes*.

Flammulina velutipes (Curt. ex Fr.) Sing (Figure 1), belonging to Tricholomataceae, is a fruiting body [1], and one of the most popular edible fungi [2]. As a culinary and medicinal mushroom, *F. velutipes* also named golden needle mushroom (China) and enokitake (Japan) has been widely used as a restorative drug and as a tonic food in China [3]. It is a highly nutritious food rich in protein, vitamins, carbohydrates and crude fiber. One of its main groups of active substances are the polysaccharides, which have been reported to possess a wide spectrum of biological functions including anti-oxidation, anti-tumor, anti-virus, anti-fatigue activities and improvement of memory [4]. Using the DEAE Cellulose-52, high gel filtration chromatography (HPGFC) and PMP-HPLC, Li et al. analyzed the composition of the polysaccharides, molecular weight and monosaccharide constituents. Their results showed that the *F. velutipes* polysaccharides might be mainly composed of glucan, which was mixed with some other fractions, such as galactose glycan, mannan, xylan and fucosan [5].

Constipation is a common bowel complaint, which is mainly characterized by difficulty in defecation, prolonged defecation time, dry stools and disorders of

intestine physiological function [6-7]. Constipation has a significant negative impact on the quality of life of patients and can even seriously threaten their lives [8-9]. The pathogenesis of constipation is very complicated. Disorders of gastrointestinal (GI) metabolism, smooth muscle lesions, and disorders of the enteric nervous system are the main causes. GI hormones are one type of hormone discharged by dozens of endocrine cells, which live in the mucous membrane of the GI region. GI hormones are regular peptides and their physiological effects comprise regulating digestive gland secretion, the movements of the digestive tract [8-10] and cholecystokinin (CCK). Motilin (MTL), gastrin (GAS), cholecystokinin (CCK), somatostatin (SS) and Substance P are a group of the GI hormones and can be used as a constipation index.

Studies on the utilization of polysaccharides prepared from other plants in the treatment of patients with constipation have been reported. For instance, Chuncai polysaccharides showed therapeutic effects on treating constipation patients [11]. Plantago crude polysaccharides were evaluated for the treatment of constipation patients, and they presented a better therapeutic effect [12]. However, to date, no studies on the utilization of *F. velutipes* polysaccharides in treating constipation have been reported. Therefore, in this study, we firstly established a constipation model with loperamide hydrochloride and then evaluated the therapeutic effect of *F. velutipes* polysaccharides in the treatment of constipation with this model, aiming to evaluate the therapeutic effect of *F. velutipes* polysaccharides in the treatment of constipation.

2 Materials and Methods

2.1 Drugs and chemicals

F. velutipes powders (Figure 2) were provided by Henan LongFeng Industrial Co., Ltd. Loperamide hydrochloride (batch number: 170421860) was obtained from Xian Janssen Pharmaceutical Ltd. (Xian, Shaanxi, China). Arabian gum powder (batch number: 20170203) was purchased from Tianjin KeMiou Chemical Reagent Co., Ltd. (Tianjin, China). Rat Substance P (SP), Rat Motilin (MTL), Rat Somatostatin (SS), Rat Gastrin (Gas) (Batch numbers: 2017Z0060711, 2017Z060401, 2017Z060702, 2017Z060305, respectively) were obtained from Shenzhen Ziker Biological Technology Co. Ltd. (Shenzhen, Guangdong, China).

Figure 2: *Flammulina velutipes* powders.

2.2 Animals

Male Sprague-Dawley/specific pathogen free (SD/SPF) rats weighting 150-170 g were purchased from the Henan Animal Experiment Center (with a License key of SCXK 2015-0004) (Zhengzhou, Henan, China).

2.3 Extraction and isolation of polysaccharides from *F. velutipes* powders

F. velutipes powders were extracted three times with petroleum ether and 70% ethanol for 24 h each time. The extracts were then filtered and dried. The dried powder was extracted with distilled water of 1:20 (w/v), at 90°C every 3 h three times, then the filtrate was filtered and concentrated under reduced pressure and then precipitated with 95% ethanol (the final concentration was 70%) overnight. The insoluble precipitate was centrifuged after 24 h. The protein present was removed by the Sevage method. Then the refined polysaccharides were dialyzed using a dialysis bag for 24 h against distilled water and another 12 h against ultra-pure water. Finally, the dialyzed polysaccharides solution was dehydrated by freeze-drying to obtain the polysaccharides.

2.4 Acute toxicity test

The maximum dosage test was carried out according to the methods described previously [13-18]. The result showed that after administrating the rats, no excitement, scurry, listlessness, convulsions and other phenomena were observed and no change in skin and respiration, no death and no significant change in weight were found after 4

days of observation. The rats were sacrificed and their hearts, livers and spleens were excised, and no change was observed with the naked eye observation. However, the rats in the drug administrated-group developed flatulence. Therefore, *F. velutipes* caused no visible toxicity.

2.5 Establishment of Constipation Model with loperamide hydrochloride

Male Sprague-Dawley (SD)/SPF rats were kept for a week and offered a standard diet and tap water at 25±2°C and 40-45% relative humidity. According to body weight, all the rats were randomly divided into six groups: normal group, model group, positive control group, *F. velutipes* polysaccharides high dose group, moderate dose group and low dose group, respectively. Expect for the normal group, other groups were modeled with loperamide hydrochloride (3 mg·kg·d·2 mL) according to weight for 10 days [18-19]. During the establishment of the constipation model, the 24 h-feces were collected on days 1, 3, 5, 7, 9 and 10.

2.6 Treatment of Rats with *F. velutipes* Polysaccharides

After successful establishment of the constipation model, the rats in normal group and model group received saline (1 mL/100 g weight). Rats in the positive control group received Maren boluses (3 g/kg, 1 mL/100 g weight). Rats in *F. velutipes* polysaccharides high, moderate and low dose groups received 600, 400, and 100 (mg/kg), 1 mL/100 g weight of *F. velutipes* polysaccharides, respectively. Medication was given consecutively for 7 days.

During the experiment, the feces weight of 6 hours was collected daily. The body weight of the rats was recorded daily.

On the last day of the experiment, all the rats were left with no food for 12 hours. All the rats were given activated carbon suspension by intragastric administration. The rats were sacrificed and their intestinal tissue samples were dissected out and measured. The concentrations of MTL, GAS, SS and substance P were measured by abdominal aorta blood collection according to the methods described previously [10,20-21].

Statistical analysis of experimental results: the results were expressed as the arithmetic mean plus or minus standard deviation. Numerical statistics were performed using SPSS 19.0 software with single factor analysis of variance (ANOVA One-Way) to determine the significant differences.

Table 1: The feces weight of rats at 24 h after treatment.

	The feces weight (g)
Normal group	55.00±3.19
Model group	36.50±4.20***
Positive control group	38.56±5.29***
High dose group	40.40±1.50***
Moderate dose group	40.26±3.00***
Low dose group	37.86±2.23***

(n=8) 0.001<**P<0.01, *P<0.05, ***P<0.001

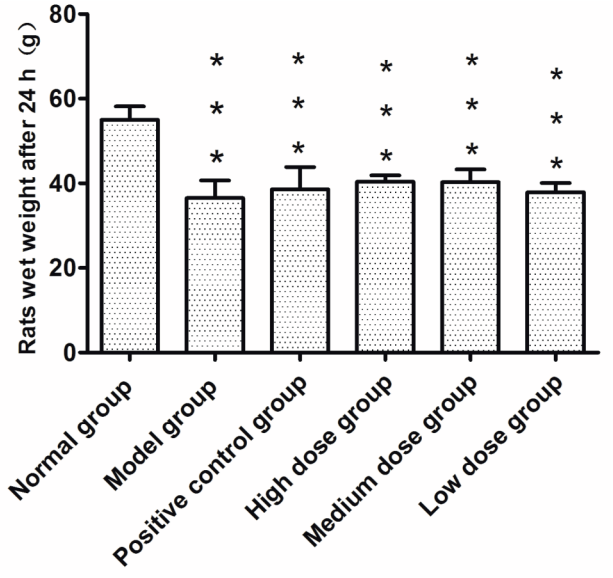

Figure 3: The body weight of rats for 24 h.

Ethics: We declare that the ethical background to this study was approved by the National Ethical Committee.

3 Results

During the course of establishing the model, the diet, activity and feces of rats were observed daily. The rats in the normal groups showed no significant difference. But the rats in model group, positive control group, *F. velutipers* polysaccharides high, moderate and low dose group all took less diet. Their activities and feces weights were lower than those of rats in normal group. The data in all the tables is also shown in Figure 3.

As shown in Table 1 and Figure 3, at 24 h, the feces weights of rats of the model group, positive control group, *F. velutipes* polysaccharides high, moderate and low dose

Table 2: Rat granules and intestinal propulsive rate at 6 h after treatment.

	The feces weight (g)	intestinal propulsive rate
Normal group	22.48±0.61	90.99±5.70
Model group	9.68±1.42***	78.31±5.88***
Positive control group	22.23±3.26###	98.19±3.69###
High dose group	18.20±1.38###&&	96.12±7.26###
Moderate dose group	22.03±1.07###	97.84±3.01###
Low dose group	14.33±0.74###&&&	82.77±8.60

(n=8) 0.001<##P<0.01, *P<0.05, ***P<0.001, ###P<0.001, &&&P<0.001 0.001<&&P<0.01

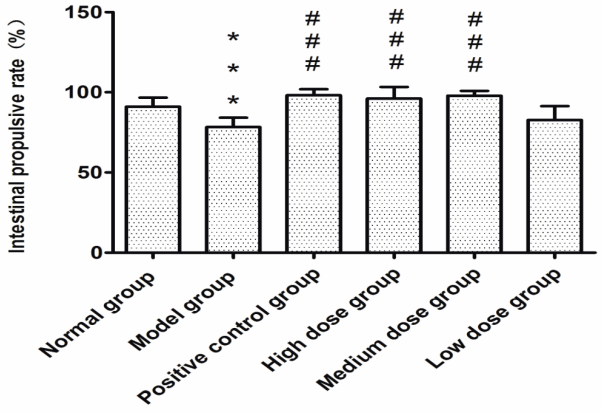

Figure 4a: Intestinal propulsive rate.

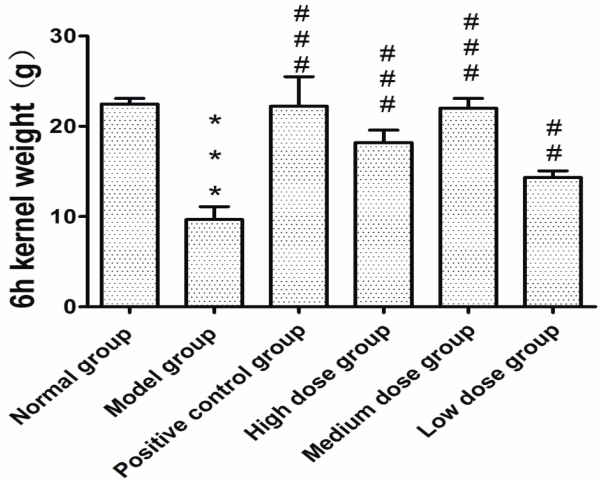

Figure 4b: The body weight of rats for 6 h.

groups significantly decreased compared with that of the normal group (P<0.001), indicating the successfully establishment of constipation model.

It can be seen from Table 2, Figures 4a and 4b that the intestinal propulsive rate and the feces weight

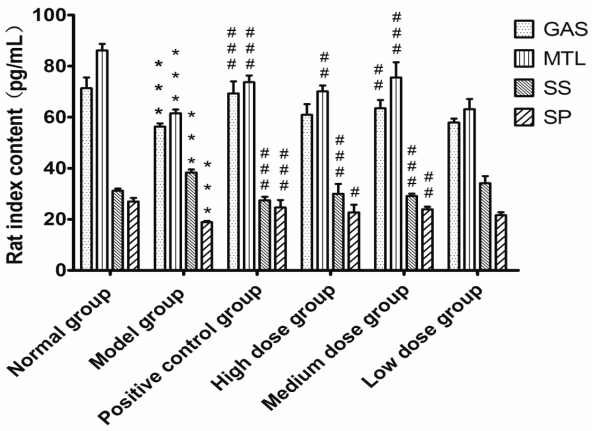

Figure 5: Contents of MTL, GAS, and SS of rats.

of rats in the model group significantly decreased as compared with those of the normal group (P<0.001). The intestinal propulsive rate significantly increased in the rats in the positive control group (P<0.001), *F. velutipes* polysaccharides high, moderate and low dose groups (P<0.001 and P<0.01), compared with that of the model group. The intestinal propulsive rate of *F. velutipes* polysaccharides moderate dose group was relatively similar to that of the positive control group.

The feces weight of rats in the moderate dose group at 6 hours was similar to that of rats in the positive control group. Therefore, *F. velutipes* polysaccharides at moderate level can have better therapeutic effect.

In Table 3 and Figure 5, compared with those in the normal group, the amounts of the GAS (P<0.001), MTL (P<0.001) and substance P (P<0.001) significantly decreased in the rats of the model group while the amount of SS (P<0.001) significantly increased. Compared with that of the model group, the amounts of GAS, MTL, and SP (P<0.001) in rats of the positive control group and *F. velutipes* polysaccharides moderate dose group significantly increased where as the amount of SS was significantly decreased (P<0.001). The levels of MTL and SP in the rats of the high dose group increased but the level of SS was decreased. Thus, *F. velutipes* polysaccharides at high and moderate dose groups can have good therapeutic effect, but the effect of the moderate dose was much better.

The levels of MTL, GAS and SP in rats were lower than those in healthy rats. However, the level of SS was higher than that of healthy rats. From the changes in MTL, GAS, SP and SS, we can clearly observe the changes in the intestinal peristalsis. These results showed that *F. velutipes* polysaccharides at either high or moderate doses could improve intestinal peristalsis and contraction of the

Table 3: Contents of MTL, GAS, and SS of rats.

	GAS	MTL	SS	SP
Normal group	71.31±4.25	86.15±2.57	31.18±0.86	27.00±1.37
Model group	56.36±1.20***	61.54±1.44***	38.33±1.18***	18.90±0.47***
Positive control group	69.32±4.71###	73.68±2.64###	27.46±1.36###	24.60±2.96###
High dose group	60.90±4.22	70.08±2.34##	30.00±3.87###	22.69±3.00#
Moderate dose group	63.47±3.23##	75.54±5.94###	29.09±0.93###	23.89±1.01##
Low dose group	57.88±1.59	63.13±3.99	34.16±2.77	21.63±1.13

(n=8) 0.001<##*P*<0.01, *P<0.05, ***P<0.001, ###P<0.001

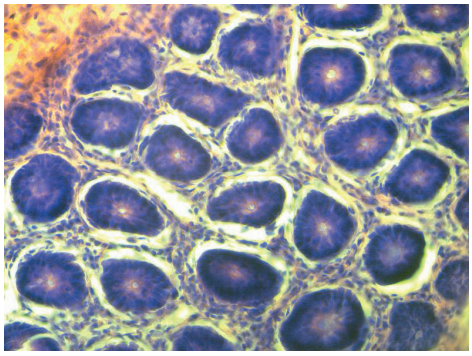

Figure 6a: Normal group.

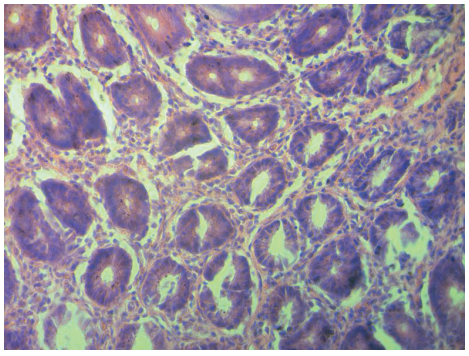

Figure 6b: Model group.

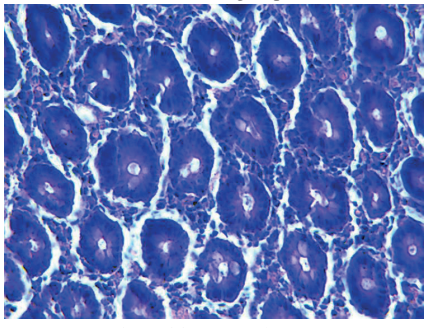

Figure 6c: Positive control group.

intestinal smooth muscle with a moderate dose exhibiting a better therapeutic effect.

4 Histological observations

Colon samples were collected, washed with saline, fixed in 4% para formaldehyde for 24 h, dehydrated and embedded in paraffin, made into 5 μm small sections and stained with hematoxylin-eosin.

According to the cross section shown in Figure 6a, the cells of the normal group were very complete and well organized in great order. As shown in Figure 6b, after being molded, pathological changes in the colon tissue were found, and the colon cells of the model group began to break, and the stroma increased and its shape went irregular. Figure 6c and Figure 6d showed that after being treated, the cells in the positive control group and the moderate dose group began to recover, and the cell membrane and the nucleus also returned to normal without breakage, and the cells were well-organized. As illustrated in Figure 6e, cells in the high dose group, when compared with the model group, showed remarkable improvement, but when compared with the positive control group, the cells showed less obvious improvement and some cells were still damaged. As illustrated in Figure 6f, cells in the low dose group, when compared with the model group, showed no obvious change, had excessive stroma, were arranged disorderly, and some cells were damaged.

5 Discussion

In the current study, the effect of *F. velutipes* polysaccharides on the improvement of constipation was evaluated through measuring the contents of MTL, GAS, SS and Substance P [20-21].

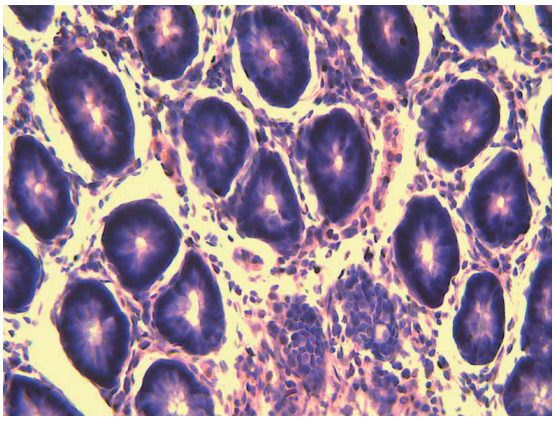

Figure 6d: Moderate dose group.

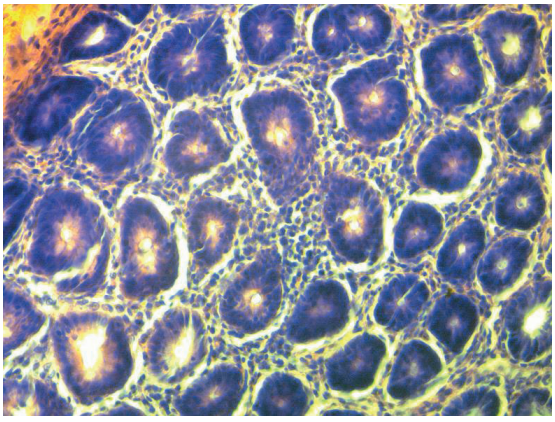

Figure 6e: High dose group.

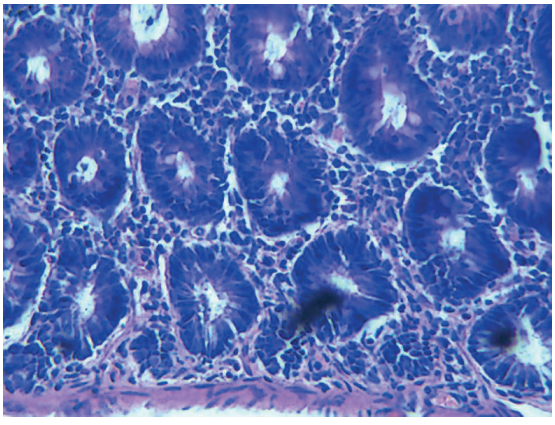

Figure 6f: Low dose group.

It has been demonstrated that MTL, GAS, SS and SP can be evaluated as the indexes for constipation. They are all GI hormones and can regulate the movements and secretions of the alimentary tract. MTL can improve intestinal peristalsis and contraction of the intestinal smooth muscle. Thus, it can be used as an indicator of constipation. GAS has the effect of promoting intestinal peristalsis and intestinal smooth muscle contraction. Thus, it can improve the contraction of the pyloric sphincter. SS can inhibit the secretion of pepsin and reduce the contraction of smooth muscle, and slow down peristalsis. Therefore, the increased content of SS can increase the constipation in rats. Substance P is a type of GI hormone that inhibits the secretion of gastrointestinal mucosa and promotes intestinal peristalsis. Thus, it is one of the main indexes used for evaluation of constipation [10,22-23].

As a kind of edible fungus, *F. velutipes* is rich in nutrients, protein, carbohydrates, vitamins and crude fiber [4]. One of the main active substances of *F. velutipes* is polysaccharides, which has been reported to play important roles in controlling a wide spectrum of biological roles, including anti-oxidation, anti-tumor, anti-virus, anti-fatigue activities and memory-improving function [24-29]. Polysaccharides represent a structurally diverse class of macromolecules and have a variety of biological functions, especially immune-enhancing activity. Studies by Chen et al. proved that the *F. velutipes* polysaccharides stimulate a macrophage whose supernatant has good inhibition on B16F10 and L919 proliferation, most likely through stimulating macrophages to produce cytokines that lead to the apoptosis of tumor cells [30]. Pan et al. used the *F. velutipes* polysaccharides at a dose of 1.5 mg/mL to gavage for 20 days, and then conducted the behavior training for 7 days. When the training was over, according to the original mode of administration, administration was continued for 32 d. The results showed the *F. velutipes* polysaccharides had a positive effect on learning and memory [19]. After *F. velutipes* polysaccharides were separated using various methods, including DEAE52-cellulose ion-exchange column chromatography and Sephadex S-200 gel column chromatography, its antioxidant activity was detected. The results showed that *F. velutipes* polysaccharides did possessed antioxidant activity [31]. At the present, while there have been many reports on the effects of *F. velutipes* polysaccharides such as anti-virus and anti-oxidation, there are no reports about the use of *F. velutipes* polysaccharides to treat constipation [4]. In the current study, experimental results showed that the *F. velutipes* polysaccharides at a moderate dose could significantly improve the contents of MTL, GAS and SP in the constipation of rats and significantly reduce the content of SS, indicating that the *F. velutipes* polysaccharides at a moderate dose can have a beneficial effect.

The *F. velutipes* polysaccharides might be mainly composed of glucan, which is mixed by some fractions, such as galactose glycan, mannan, xylan and fucosan. Galactomannan is composed of mannose and galactose and can promote the proliferation of intestinal bacteria, improving the constipation effect [32]. Schoeni et al. used the manna oligosaccharides to feed small broilers, and found a decrease in *Staphylococcus aureus* in the intestinal tract [33]. The method of PCR-DGGE and with the object of weaned piglets was used to study galactomannan. Galactomannan promotes the propagation of probiotics such as lactobacillus, bifidobacterium and inhibits the growth of *S. aureus* [34]. Yao et al. learned that galactomannan significantly improved intestinal motility, regulated intestinal function and improved constipation [35]. *F. velutipes* polysaccharides can be used to treat constipation. This function is due to the reason that polysaccharides in the composition of intestinal bifidobacteria and other probiotics improve the intestinal microflora, bifidobacteria and other probiotics proliferation of lactic acid, acetic acid and other short-chain fatty acids, stimulate peristalsis, increase stool moisture, improve intestinal motility, and improve constipation [35].

The *F. velutipes* polysaccharides at a moderate dose can improve the GI function and strengthen the GI peristalsis of patients with constipation, thus alleviating constipation and improving patients' living quality. Therefore, the *F. velutipes* polysaccharides at a moderate dose group can have the beneficial function of relaxing the bowel with few toxic side effects.

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