Effects of Gracilaria Residue Fermented Culture on Growth Performance and Rectal Microbiota of Weaned Piglets (Postprint)

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Abstract

This experiment aimed to investigate the effects of fermented Gracilaria residue culture on growth performance and rectal microbiota in weaned piglets. A total of 210 healthy 28-day-old weaned piglets were selected and randomly divided into 7 groups, with 3 replicates per group and 10 piglets per replicate. The control group was fed a basal diet, while the experimental groups were fed experimental diets supplemented with 0.10%, 0.25%, and 0.50% Gracilaria residue or fermented Gracilaria residue culture on top of the basal diet. The results showed that, compared with the control group, Gracilaria residue had no significant effect on the growth performance of piglets ($P>0.05$); whereas fermented Gracilaria residue culture could significantly increase the average daily gain of piglets ($P<0.05$), significantly decrease the feed conversion ratio and diarrhea rate ($P<0.05$), and significantly reduce the moisture content of feces and Escherichia coli count ($P<0.05$). Among them, the 0.10% experimental group showed better effects, with the feed conversion ratio and diarrhea rate decreasing by 7.34% and 52.86%, respectively, compared with the control group ($P<0.05$); the number of Lactobacillus in the rectum increased by 45.73% ($P<0.05$), and the number of Escherichia coli decreased by 16.48% ($P<0.05$); the moisture content and Escherichia coli count in feces also decreased by 12.63% and 25.68%, respectively ($P<0.05$). Therefore, dietary supplementation with 0.10% fermented Gracilaria residue culture can effectively improve the growth performance of piglets, reduce the feed conversion ratio and diarrhea rate, and improve the microbial flora in the rectum.

Full Text

Effects of Gracilari Residue Fermentation Culture on Growth Performance and Rectal Microflora of Weaned Piglets

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Abstract: This experiment was conducted to investigate the effects of Gracilari residue fermentation culture on the growth performance and rectal microflora of weaned piglets. A total of 210 healthy 28-day-old weaned piglets were randomly allocated into 7 groups with 3 replicates per group and 10 piglets per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 0.10%, 0.25%, and 0.50% Gracilari residue or Gracilari residue fermentation culture, respectively. The results showed that compared with the control group, Gracilari residue had no significant effect on piglet growth performance ($P>0.05$). However, Gracilari residue fermentation culture significantly improved average daily gain ($P<0.05$), significantly reduced feed to gain ratio and diarrhea rate ($P<0.05$), and significantly decreased fecal water content and *Escherichia coli* count ($P<0.05$). Among all treatments, the 0.10% supplementation group showed the best results: compared with the control group, feed to gain ratio and diarrhea rate decreased by 7.34% and 52.86%, respectively ($P<0.05$); rectal *Lactobacillus* count increased by 45.73% ($P<0.05$) while *E. coli* count decreased by 16.48% ($P<0.05$); fecal water content and *E. coli* count also decreased by 12.63% and 25.68%, respectively ($P<0.05$). These findings indicate that dietary supplementation with 0.10% Gracilari residue fermentation culture can effectively improve growth performance, reduce feed to gain ratio and diarrhea rate, and enhance rectal microflora in weaned piglets.

Keywords: Gracilari residue; fermentation culture; weaned piglet; growth performance; rectal microflora

China is the world's largest producer of seaweed, with Gracilaria being an important large economic marine algae. Due to its large thallus, strong adaptability, and rapid growth, Gracilaria serves as the primary raw material for agar extraction [1-2]. The main components of Gracilaria include polysaccharides and crude fiber, with considerable amounts of protein and fat, and it is also rich in minerals and vitamins [3]. Currently, Gracilaria is primarily used for agar extraction, with some applications in food and abalone feed, and reports of its use in aquaculture water purification and health product development [4-8]. Gracilari residue is the waste remaining after agar extraction. It is estimated that producing 1 ton of agar generates approximately 2.5 tons of residue (dry basis). With Fujian Province's annual agar production of about 4,000 tons, over 10,000 tons of residue are not effectively utilized each year, resulting in resource

waste and environmental pollution [9].

Gracilari residue consists mainly of crude cellulose (content exceeding 50%), followed by protein (10%-20%), with residual agar also present, making it a relatively nutritious substrate. Current utilization of Gracilari residue primarily involves chemical or enzymatic modification of cellulose for dietary fiber and carboxymethyl cellulose preparation [9-12], while the protein and residual agar are not rationally utilized. Therefore, this study utilized the cellulose, protein, and residual agar in Gracilari residue as primary substrates, supplemented with appropriate amounts of starch, soybean meal, and trace elements as microbial fermentation media. Through synergistic fermentation using composite microbial inoculants including *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and *Lactobacillus acidophilus*, the product value of Gracilari residue was enhanced. The optimal supplementation level and effects in weaned piglets were investigated to develop a functional feed product containing probiotics.

1.1 Experimental Materials

Gracilari residue was provided by Fujian Jinyan Marine Biology Science and Technology Co., Ltd., and Gracilari residue fermentation culture was prepared in our laboratory.

1.2 Experimental Design

Two hundred and ten healthy 28-day-old "Duroc × Landrace × Yorkshire" crossbred weaned piglets with an initial average body weight of (8.11$±$0.36) kg were randomly divided into 7 groups with 3 replicates per group and 10 piglets per replicate. The experimental design is shown in Table 1 . Gracilari residue and Gracilari residue fermentation culture were directly added to the basal diet at the specified rates per ton. The composition and nutrient levels of the basal diet are presented in Table 2 . The experiment was conducted at a pig farm in Minqing, Fujian, with a duration of 28 days.

1.3 Measurement Indicators and Methods

1.3.1 Main Components of Gracilari Residue and Fermentation Culture Crude protein content was determined by the Kjeldahl method according to GB/T 6432–1994. Crude fiber content was measured using a Shanghai Fiber Determination Instrument SLQ-6. Crude ash content was determined according to GB/T 6438–2007. Peptide content was measured by the trichloroacetic acid soluble nitrogen (TCA-NSI) method as described in reference [13]. Probiotic counts were determined as follows: yeast according to GB/T 22547–2008, *Bacillus licheniformis* according to NY/T 1461–2007, and *Lactobacillus* using MRS agar plate counting.

1.3.2 Growth Performance Indicators Piglets were weighed at the beginning and end of the experiment, with feed consumption recorded by weight.

Average daily feed intake (ADFI), average daily gain (ADG), and feed to gain ratio (F/G) were calculated. From the start of the experiment, fecal conditions were observed daily, with diarrheic individuals and episodes recorded, and diarrhea rate calculated per group.

1.3.3 Microbial Flora Counts One piglet with body weight close to the replicate average was selected from each replicate. Fresh rectal contents were collected aseptically using a sterile spoon into sterilized centrifuge tubes for pH measurement and enumeration of *Lactobacillus* and *E. coli* per gram of content using MRS agar and MacConkey agar, respectively. Additionally, one fresh fecal sample from a healthy piglet was collected per replicate to determine fecal water content and *E. coli* count.

1.4 Statistical Analysis Experimental data were analyzed using SPSS 18.0 software, with Duncan' s multiple range test used for pairwise comparisons. Differences were considered significant at $P<0.05$. Data are expressed as mean±standard deviation.

2.1 Comparison of Main Components Between Gracilari Residue and Fermentation Culture

Gracilari residue fermentation culture was prepared using Gracilari residue as the primary substrate, supplemented with 1% corn starch, 3% wheat bran, 5% soybean meal, and 0.1% trace elements. Liquid fermentation was used to prepare individual cultures of *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and *Lactobacillus acidophilus*, which were mixed at a 2:1:1 ratio and inoculated into the solid substrate at 10% inoculation rate. Water was added to achieve 45% moisture content, followed by solid-state fermentation at 32°C for 24 h aerobic fermentation and 48 h anaerobic fermentation. The product was dried by air flow to below 10% moisture content. A comparison of main components before and after fermentation is shown in Table 3 .

As shown in Table 3, fermentation significantly increased crude protein content by 17.55%, primarily due to added soybean meal in the solid substrate and microbial protein synthesis. Crude fiber and ash contents decreased significantly by 11.93% and 24.62%, respectively. Peptide content increased from 2.6% to 12.9%, mainly derived from protein degradation in both Gracilari residue and soybean meal. The fermentation product also contained probiotics at >1.0\times10^{9}$ CFU/g, predominantly *Bacillus licheniformis*. These results demonstrate that using Gracilari residue as the primary solid substrate, supplemented with appropriate carbon and nitrogen sources such as starch, soybean meal, and trace elements, effectively enhanced microbial biodegradation, reduced crude fiber content, and increased protein, peptide, and bacterial counts. Both Gracilari residue and its fermentation culture were subsequently used in weaned piglet feeding trials.

2.2 Effects of Gracilari Residue and Fermentation Culture on Growth Performance of Weaned Piglets

As shown in Table 4 , there were no significant differences in ADFI among groups supplemented with either Gracilari residue or its fermentation culture compared with the control group (P>0.05). For ADG, the three Gracilari residue groups showed slightly higher values than the control group, but without significant differences (P>0.05). In contrast, the three fermentation culture groups exhibited significantly higher ADG compared with the control group (P<0.05), with Group D showing the best improvement at 8.92% higher than control, followed by Groups E and F at 5.52% and 4.69% higher, respectively.

Regarding feed to gain ratio, no significant differences were observed among the three Gracilari residue groups compared with the control (P>0.05). However, the three fermentation culture groups showed significantly reduced F/G (P<0.05), with Group D achieving the lowest ratio of 1.64, representing a 7.34% reduction from control. For diarrhea rate, the three Gracilari residue groups showed increased rates with higher supplementation levels, though not significantly (P>0.05). Conversely, all three fermentation culture groups exhibited significantly lower diarrhea rates than control (P<0.05), with Group F showing the greatest reduction at 57.14%, followed by Groups D and E at 52.86% and 54.28% lower, respectively.

2.3 Effects of Gracilari Residue and Fermentation Culture on Rectal Microflora of Weaned Piglets

As shown in Table 5 , the three Gracilari residue groups showed no significant differences in rectal content pH compared with control (P>0.05), whereas all three fermentation culture groups exhibited significantly lower pH values (P<0.05), with Groups E and F both reducing pH by 2.36% relative to control. Analysis of rectal microbial flora revealed no significant differences in *Lactobacillus* counts among the three Gracilari residue groups compared with control (P>0.05). However, the fermentation culture groups significantly increased rectal *Lactobacillus* counts (P<0.05) in a dose-dependent manner, with Group F showing the highest increase at 60.98%, and Groups D and E showing increases of 45.73% and 49.39%, respectively.

Similarly, no significant differences in rectal *E. coli* counts were observed among the three Gracilari residue groups compared with control (P>0.05). In contrast, the fermentation culture groups significantly reduced *E. coli* counts (P<0.05) in a dose-dependent manner, with Group F showing the greatest reduction at 24.72%, and Groups D and E reducing counts by 16.48% and 18.68%, respectively.

2.4 Effects of Gracilari Residue and Fermentation Culture on Fecal Characteristics of Weaned Piglets

As shown in Table 6 , the three Gracilari residue groups showed lower fecal water content than control, but without significant differences (P>0.05). All three fermentation culture groups exhibited significantly reduced fecal water content (P<0.05) in a dose-dependent manner, with Groups D, E, and F showing reductions of 12.63%, 16.22%, and 17.95%, respectively.

For fecal *E. coli* counts, no significant differences were observed among the three Gracilari residue groups compared with control (P>0.05). However, all three fermentation culture groups showed significantly lower *E. coli* counts than control (P<0.05), with reductions of 25.68%, 39.63%, and 59.97% for Groups D, E, and F, respectively. These results demonstrate that Gracilari residue fermentation culture is more effective than raw residue in reducing fecal water content, improving fecal consistency, and decreasing fecal *E. coli* counts.

In this study, Gracilari residue alone failed to improve piglet growth performance or rectal microflora and even increased diarrhea rate and rectal *E. coli* counts at the 0.50% supplementation level, though not significantly. This limited efficacy as a direct feed product likely relates to its composition, as Gracilari residue is primarily cellulose with certain amounts of protein and residual agar that are difficult to degrade, along with large molecular weight proteins that limit utilization by piglets. Similar findings have been reported for other seaweed residues in livestock feeding. For example, Wang et al. [14] reported that replacing 5% wheat bran with kelp residue in piglet diets had no effect on growth performance. Wang et al. [15] found that 5% kelp residue supplementation in laying hen diets did not significantly affect feed intake, laying rate, or feed to egg ratio, though it improved average egg weight. In aquaculture, moderate seaweed residue can improve feed utilization. Gan et al. [16] reported that 5% kelp residue supplementation in tilapia diets improved weight gain and growth ratio compared with control, but 30% supplementation severely deteriorated growth performance, indicating that excessive dietary crude fiber impairs nutrient absorption and utilization. Thus, direct use of seaweed residues as feed products shows limited benefits, primarily due to high cellulose content, though they may serve as cost-effective substitutes for ingredients like wheat bran and may be more suitable for growing pigs and sows.

In contrast, Gracilari residue fermentation culture effectively improved piglet growth performance and rectal microflora. All three supplementation levels reduced feed to gain ratio, with the 0.10% level showing optimal results at 1.64. All fermentation culture groups increased *Lactobacillus* counts and decreased *E. coli* counts, thereby improving microbial balance and reducing diarrhea rate. Using cellulose, protein, and residual agar in Gracilari residue supplemented with starch, soybean meal, and trace elements as carbon and nitrogen sources, synergistic fermentation with *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and *Lactobacillus acidophilus* effectively modified the composition. The fer-

mented product showed increased protein and peptide contents and added viable probiotics, significantly improving product quality. Zhao [17] reported similar findings, showing that fermented kelp residue fed to sea cucumbers was superior to unfermented residue, improving meat quality, body color, and overall quality. These results demonstrate that microbial fermentation technology can effectively enhance the value and utilization of seaweed processing waste.

However, it should be noted that the three microbial strains used in this study (*Bacillus licheniformis*, *Saccharomyces cerevisiae*, and *Lactobacillus acidophilus*) had limited cellulose degradation capability, reducing crude fiber content by only 11.93%. Therefore, incorporating cellulase-producing *Aspergillus* species (such as *Aspergillus oryzae*) for synergistic fermentation could more effectively degrade cellulose in Gracilari residue and improve its utilization.

Currently, other seaweed residues (such as kelp residue) have been developed into feed products and applied in livestock and aquaculture with certain success. Therefore, rational development and utilization of seaweed processing by-products and industrial waste can effectively prevent potential environmental pollution, increase the added value of seaweed products, and promote healthy development of the marine processing industry.

Conclusions: 1. Gracilari residue can serve as a primary substrate for fermentation by *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and *Lactobacillus acidophilus*. The fermented culture exhibits reduced crude fiber content and increased protein, peptide, and microbial counts.

2. Dietary supplementation with 0.10% Gracilari residue fermentation culture improves piglet intestinal microflora, reduces diarrhea, and promotes growth, increasing ADG by 8.92% and decreasing F/G by 7.34%. These results demonstrate that microbial fermentation can improve Gracilari residue quality, suggesting potential for development as a novel microecological preparation product.

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