

## Effects of Dietary Fiber Source and Crude Fiber Level on Nutrient Digestibility, Intestinal Digestive Enzyme Activity, and Cecal Microbiota Diversity in Meat Rabbits (Postprint)

**Authors:** Yang Guiqin, Sun Jiayi, Dongxin Guo, Tian He, Li Jiantao, Pan Xiudong

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### Abstract

This experiment aimed to investigate the effects of dietary fiber source and crude fiber (CF) level on nutrient digestibility, intestinal digestive enzyme activity, and cecal microbial diversity in meat rabbits. A two-factor experimental design was adopted, and based on the isocaloric and isonitrogenous principle, 2 fiber sources (alfalfa meal, rice hull meal) and 3 CF levels (12%, 14%, 16%) were set, resulting in 6 experimental diets. One hundred forty-four healthy Ira growing meat rabbits aged 70–80 days with an average body weight of  $(2,002 \pm 77)$  g were selected and randomly divided into 6 groups with 4 replicates per group and 6 rabbits per replicate, housed individually in cages. At the end of the feeding trial, a digestion trial was conducted using the total feces collection method, followed by slaughter to collect intestinal chyme for determination of digestive enzyme activity and cecal microbial diversity. The results showed: 1) The digestibility of dry matter (DM), CF, acid detergent fiber (ADF), and lignin (ADL) in the alfalfa meal group was significantly or extremely significantly higher than that in the rice hull meal group ( $P < 0.05$  or  $P < 0.01$ ), while the crude protein digestibility was extremely significantly lower than that in the rice hull meal group ( $P < 0.01$ ); the 12% CF level group had significantly higher digestibility of DM, neutral detergent fiber (NDF), and ADF than the 14% CF level group ( $P < 0.05$ ), and simultaneously, the energy digestibility and apparent digestible energy (ADE) of this group were extremely significantly higher than those of the 14% and 16% CF level groups ( $P < 0.01$ ). 2) Duodenal trypsin activity in the rice hull meal group was extremely significantly higher than that in the alfalfa meal group ( $P < 0.01$ ). Compared with the 14% and 16% CF level groups, the 12% CF level group had the lowest jejunal amylase ( $P > 0.05$ ), trypsin activity ( $P < 0.05$ ), and cecal cellulase activity ( $P < 0.01$ ). 3) Fiber source had

no significant effect on cecal microbial richness and Shannon index ( $P>0.05$ ), but the cecal microbial evenness index in the alfalfa meal group was extremely significantly higher than that in the rice hull meal group ( $P<0.01$ ). CF level had a significant effect on cecal microbial richness and Shannon index ( $P<0.05$ ), with the 16% CF level group showing the highest values. In conclusion, under the conditions of this experiment: 1) The DM, CF, ADF, and ADL digestibility and cecal microbial evenness index in the alfalfa meal group were significantly or extremely significantly higher than those in the rice hull meal group, but duodenal trypsin activity was extremely significantly lower than that in the rice hull meal group. 2) When dietary CF level was 12%, meat rabbits had the highest ADE and digestibility of major nutrients; higher CF levels resulted in higher jejunal amylase, trypsin activity, and cecal cellulase activity in meat rabbits; dietary CF level of 16% resulted in higher cecal microbial diversity in meat rabbits.

## Full Text

### Effects of Dietary Fiber Source and Crude Fiber Level on Nutrient Digestibility, Intestinal Digestive Enzyme Activities and Cecal Microbial Diversity of Meat Rabbits

\*\*YANG Guiqin<sup>1</sup>, SUN Jiayi<sup>1</sup>, GUO Dongxin<sup>1\*</sup>, TIAN He<sup>1</sup>, LI Jiantao<sup>1</sup>, PAN Xiudong<sup>2\*\*</sup> (1. College of Animal Husbandry and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110866, China; 2. Shenyang Aidi Biotechnology Co., Ltd., Shenyang 110161, China)

**Abstract:** This experiment was conducted to investigate the effects of dietary fiber source and crude fiber (CF) level on nutrient digestibility, intestinal digestive enzyme activities, and cecal microbial diversity in meat rabbits. A two-factor experimental design was employed, with two fiber sources (alfalfa hay meal and rice husk meal) and three CF levels (12%, 14%, and 16%), resulting in six experimental diets formulated according to isonitrogenous and isocaloric principles. A total of 144 healthy Hyla growing meat rabbits aged 70-80 days with an average body weight of  $(2,002\pm\$77)$  g were randomly allocated to six groups, each consisting of four replicates with six rabbits per replicate and housed individually. At the end of the feeding trial, a digestion experiment was conducted using the total fecal collection method, followed by slaughter to collect intestinal chyme for determination of digestive enzyme activities and cecal microbial diversity. The results showed: 1) The digestibility of dry matter (DM), CF, acid detergent fiber (ADF), and acid detergent lignin (ADL) in the alfalfa hay meal group was significantly or extremely significantly higher than that in the rice husk meal group ( $P<0.05$  or  $P<0.01$ ), whereas crude protein digestibility was extremely significantly lower ( $P<0.01$ ). The 12% CF level group exhibited significantly higher DM, neutral detergent fiber (NDF), and ADF digestibility compared with the 14% CF level group ( $P<0.05$ ), and extremely significantly higher energy digestibility and apparent digestible energy

(ADE) compared with both the 14% and 16% CF level groups ( $P < 0.01$ ). 2) Duodenal trypsin activity was extremely significantly higher in the rice husk meal group than in the alfalfa hay meal group ( $P < 0.01$ ). Compared with the 14% and 16% CF level groups, the 12% CF level group showed the lowest jejunal amylase ( $P > 0.05$ ), jejunal trypsin ( $P < 0.05$ ), and cecal cellulase activities ( $P < 0.01$ ). 3) Fiber source had no significant effect on cecal microbial richness or Shannon index ( $P > 0.05$ ), but the species evenness index was extremely significantly higher in the alfalfa hay meal group than in the rice husk meal group ( $P < 0.01$ ). CF level significantly affected cecal microbial richness and Shannon index ( $P < 0.05$ ), with the highest values observed in the 16% CF level group. In conclusion, under the conditions of this experiment: 1) The alfalfa hay meal group showed significantly or extremely significantly higher DM, CF, ADF, and ADL digestibility and cecal microbial species evenness compared with the rice husk meal group, but extremely significantly lower duodenal trypsin activity. 2) A dietary CF level of 12% resulted in the highest ADE and digestibility of major nutrients in meat rabbits; higher CF levels increased jejunal amylase, trypsin, and cecal cellulase activities; and a dietary CF level of 16% promoted higher cecal microbial diversity.

**Keywords:** meat rabbits; fiber source; crude fiber level; digestibility; digestive enzyme activity; cecal microbial diversity

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Rabbits are monogastric herbivores with a well-developed cecum containing abundant microorganisms capable of fermenting poorly digestible fiber into low-molecular-weight organic acids and other nutrients for host absorption. Therefore, investigating the effects of dietary fiber source and crude fiber (CF) level on digestive physiology and cecal microflora in meat rabbits holds important practical significance for the rational utilization of roughage resources and determination of appropriate dietary CF levels. Depending on analytical methods, the fiber content in complete diets for growing rabbits generally ranges from 15% to 50%, with CF levels typically between 14% and 18% and a broader range of 12.2% to 24.4%. Rabbits' capacity to digest and utilize fibrous substances from forages and feeds is generally 17%-25%, significantly lower than that of ruminants such as cattle, sheep, and horses. Nevertheless, dietary fiber plays a crucial role in rabbit growth and health. Research indicates that low-fiber diets (lignin/cellulose  $< 0.4$ ) can cause reduced growth rates and prolonged retention time of digesta in the gastrointestinal tract, and may lead to feeding and digestive disorders and even diarrhea in the absence of specific pathogen infections. However, excessively high dietary CF levels can also compromise rabbit health. Moreover, fiber utilization in rabbits is influenced by multiple factors including fiber source (quality), feed form, breed, and age. It is generally accepted that rabbits benefit from microbial fermentation of dietary fiber in the cecum. Therefore, this experiment utilized two types of roughage as primary fiber sources—high-quality (alfalfa hay meal) and low-quality (rice husk meal)—each at three CF levels to formulate six experimental diets, aiming to investigate the effects

of different fiber sources and CF levels on nutrient digestibility and intestinal digestive enzyme activities in meat rabbits, while qualitatively exploring the relationship between intestinal microbial diversity and fiber source/CF level, thereby providing a theoretical basis for further research on the nutritional and physiological roles of dietary fiber in meat rabbits.

### 1.1 Experimental Animals and Diets

A total of 144 healthy Hyla commercial growing meat rabbits aged 70–80 days with an initial average body weight of  $(2,002 \pm 77)$  g were selected and randomly divided into six groups (Groups I, II, III, IV, V, and VI) based on sex and body weight. Each group comprised four replicates with six rabbits per replicate, housed individually in the same rabbitry and fed different experimental diets. The trial employed a two-factor factorial design with two fiber sources (alfalfa hay meal and rice husk meal) and three CF levels (12%, 14%, and 16%) under isonitrogenous and isocaloric conditions. The inclusion rates of alfalfa hay meal and rice husk meal in the formulations were 24.50%, 31.50%, 37.50% and 14.00%, 17.50%, 22.00%, respectively. Based on chemical composition data for common rabbit feed ingredients from the literature, six experimental diets were formulated with composition and nutrient levels shown in Table 1. All diets were pelleted to approximately 4 mm in diameter.

**Table 1** Composition and nutrient levels of experimental diets (air-dry basis)

**Table 2** Composition and nutrient levels of experimental diets (air-dry basis) (continued)

*Notes: 1) Mineral premix provided per kg of diet: Zn 50.0 mg, Mn 80.0 mg, Fe 25.0 mg, Cu 8.0 mg, I 2.0 mg, Se 0.1 mg, Mg 133.4 mg. 2) Vitamin premix provided per kg of diet: VA 12,020 IU, VD<sub>3</sub> 2,100 IU, VE 12 IU, VK 2.0 mg, VB<sub>1</sub> 1.1 mg, VB<sub>2</sub> 3.0 mg, VB<sub>6</sub> 1.5 mg, VB<sub>12</sub> 0.005 mg, pantothenic acid 9 mg, nicotinic acid 10.0 mg, biotin 0.15 mg, folic acid 0.44 mg, choline 350 mg. 3) DE is a calculated value; all others are measured values.*

### 1.2 Experimental Procedure and Sample Collection

At the conclusion of the feeding trial, one rabbit per replicate (ensuring two males and two females per group) was selected for a digestion experiment using the total fecal collection method. Subsequently, these rabbits were slaughtered, and chyme from the jejunum, duodenum, and cecum (two portions) was rapidly collected in 10 mL capped centrifuge tubes, immediately snap-frozen in liquid nitrogen for 20 minutes, and then stored at  $-80^{\circ}\text{C}$  until analysis.

#### 1.3.1 Nutrient Apparent Digestibility

The digestion experiment consisted of a 1-week preliminary period followed by a 4-day collection period, during which daily feed intake was recorded and all fresh feces were collected and weighed. Fecal samples were collected in two portions:

one fixed with 10% hydrochloric acid solution for volatile nitrogen determination of crude protein (CP) content, and the other left unfixed for other nutrient analyses. Fecal samples were dried to constant weight in a 65–70°C oven, removed, equilibrated in air for 24 hours, and weighed to determine initial moisture content. Air-dried samples were ground using a mill and passed through a 40-mesh sieve (0.45 mm aperture). Fecal samples from the 4-day collection period were pooled by replicate, and subsamples were sealed in bags for subsequent analysis. Dry matter (DM) content was determined according to GB/T 6435-2006. CP content was measured using a KjelFlex K-360 automatic Kjeldahl analyzer (Büchi, Switzerland). Crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were analyzed using an ANKOM A2000i automated fiber analyzer (Ankom, USA). Gross energy was determined using an IKA C2000 automatic bomb calorimeter (IKA, Germany).

### 1.3.2 Intestinal Digestive Enzyme Activities

Activities of amylase and trypsin in jejunal and duodenal chyme, and cellulase activity in cecal chyme were determined using enzyme-linked immunosorbent assay (ELISA) kits purchased from Nanjing Jiancheng Bioengineering Institute, following the manufacturer's instructions.

### 1.3.3 Cecal Microbial Diversity

**1.3.3.1 Genomic DNA Extraction** Genomic DNA was extracted from samples using the CTAB manual extraction method.

**1.3.3.2 Bacterial 16S rDNA Fragment PCR Amplification** Sample genomic DNA was used as template to amplify the V3 region of 16S rDNA using bacterial universal primers GC-338F (5'-CGCCCGGGGCGCGCCCCGGGGCGGGGCGGGGGCGCGGGGG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3'). The PCR reaction system (50  $\mu$ L) contained: 10 $\times$ PCR Buffer 5  $\mu$ L, dNTP (2.5 mmol/L) 3.2  $\mu$ L, rTaq (5 U/ $\mu$ L) 0.4  $\mu$ L, GC-338F (20  $\mu$ mol/L) 1  $\mu$ L, 518R (20  $\mu$ mol/L) 1  $\mu$ L, template DNA 50 ng, with ddH<sub>2</sub>O added to 50  $\mu$ L. The PCR program was: 94°C pre-denaturation for 5 min; 30 cycles of 94°C denaturation for 1 min, 55°C annealing for 45 s, 72°C extension for 1 min; and final extension at 72°C for 10 min.

PCR products were purified and recovered using Omega's DNA Gel Extraction Kit. The PCR instrument was a T-gradient from Biometra, and gel imaging was performed using a Gel-Doc2000 system from Bio-Rad.

**1.3.3.3 Denaturing Gradient Gel Electrophoresis (DGGE) Analysis of PCR Products** Ten microliters of PCR product were subjected to DGGE analysis using 7% polyacrylamide gel with a denaturing gradient of 35%–55% at 150 V and 60°C for 5 h in 1 $\times$ TAE buffer. After electrophoresis, gels were silver-stained.

PCR-DGGE profiles were digitally analyzed using Bio-Rad' s Quantity One software package to determine band number and density for each sample.

- (1) **Dice' s coefficient (Cs)** was calculated for pairwise comparison of microbial community differences between samples:

$$Cs = \frac{2j}{N_x + N_y}$$

where: Cs is Dice' s coefficient; Nx is the number of bands in lane x; Ny is the number of bands in lane y; and j is the number of common bands between the two lanes.

A phylogenetic tree was constructed using MEGA 4.1 software for cluster analysis.

- (2) **Shannon index (H), richness (S), and evenness index (E)** were calculated as follows:

$$E = \frac{H}{H_{\max}} = \frac{H}{\ln S}$$

where: H is the Shannon index; pi is the proportion of intensity of a single band relative to the total intensity of all bands in the sample; N is the total density of all bands in a single lane; Ni is the density of the ith band; S is richness, defined as the total number of bands in a sample; E is the evenness index; and Hmax is the maximum H value (diversity under maximum uniformity conditions).

#### 1.4 Statistical Analysis

Data were processed and analyzed on a replicate basis using the GLM procedure in IBM SPSS Statistics version 22, with fiber source and CF level as fixed factors. Duncan' s multiple range test was used for post-hoc comparisons, with P<0.05 considered significant and P<0.01 considered highly significant.

#### 2.1 Dietary Nutrient Apparent Digestibility

As shown in Table 2, during the digestion trial, meat rabbits fed rice husk meal diets had extremely significantly higher intake of NDF, ADF, and ADL compared with those fed alfalfa hay meal diets (P<0.01). The 16% CF level group exhibited extremely significantly higher intake of CF, NDF, and ADF compared with the 12% and 14% CF level groups (P<0.01). The interaction between fiber source and CF level had an extremely significant effect on nutrient intake (P<0.01). Fiber source significantly affected the digestibility of DM, CP, CF, ADF, and ADL (P<0.05). When alfalfa hay meal served as the fiber source, all nutrient digestibility coefficients were significantly higher than in the rice husk meal group (P<0.05), except for CP digestibility, which was extremely

significantly lower ( $P < 0.01$ ). CF level significantly affected DM, NDF, and ADF digestibility ( $P < 0.05$ ). The 12% CF level group showed significantly higher DM apparent digestibility than the 14% CF level group ( $P < 0.05$ ), while NDF and ADF digestibility exhibited a “high at both ends (12% and 16% CF), low in the middle (14% CF)” pattern.

The 14% CF rice husk meal group (Group V) had significantly lower DM digestibility than all other groups except Group VI ( $P < 0.05$ ). The 16% CF alfalfa hay meal group showed significantly higher CF digestibility than the 12% and 14% CF rice husk meal groups ( $P < 0.05$ ). The 16% CF rice husk meal group had significantly higher NDF digestibility than the 14% CF alfalfa hay meal group ( $P < 0.05$ ). All three alfalfa hay meal groups exhibited higher ADF and ADL digestibility than the three rice husk meal groups.

Fiber source had no significant effect on apparent digestible energy (ADE) ( $P > 0.05$ ), while CF level and the interaction between fiber source and CF level had extremely significant effects on ADE ( $P < 0.01$ ). The 12% CF level group showed extremely significantly higher ADE than the 14% and 16% CF level groups ( $P < 0.01$ ), with the 12% CF alfalfa hay meal group achieving the highest ADE (12.99 MJ/kg). Neither fiber source nor its interaction with CF level significantly affected energy digestibility ( $P > 0.05$ ), but CF level had an extremely significant effect ( $P < 0.01$ ).

**Table 2** Effects of dietary fiber source and CF level on nutrient apparent digestibility of meat rabbits

*Note: In the same column, values within the same item with the same or no letter superscripts indicate no significant difference ( $P > 0.05$ ), different lowercase letters indicate significant difference ( $P < 0.05$ ), and different uppercase letters indicate highly significant difference ( $P < 0.01$ ). The same applies below.*

## 2.2 Intestinal Digestive Enzyme Activities

As shown in Table 3, fiber source had an extremely significant effect on duodenal trypsin activity ( $P < 0.01$ ). CF level significantly affected jejunal trypsin activity ( $P < 0.05$ ) and extremely significantly affected cecal cellulase activity ( $P < 0.01$ ). The interaction between fiber source and CF level extremely significantly affected duodenal amylase activity ( $P < 0.01$ ) and significantly affected cecal cellulase activity ( $P < 0.05$ ).

Meat rabbits fed rice husk meal diets exhibited extremely significantly higher duodenal trypsin activity than those fed alfalfa hay meal diets ( $P < 0.01$ ). The 12% CF level group showed the lowest activities for jejunal amylase (1,053.96 U/g), jejunal trypsin (35.54 U/mg), and cecal cellulase (213.87 g/g). The 16% CF rice husk meal group had significantly higher jejunal trypsin activity than both the 12% CF alfalfa hay meal and rice husk meal groups ( $P < 0.05$ ). The 12% CF rice husk meal group exhibited extremely significantly higher duodenal amylase activity (1,628.16 U/g) than the 16% CF rice husk meal group ( $P < 0.01$ ) and

significantly higher activity than the 12% and 14% CF alfalfa hay meal groups ( $P < 0.05$ ). The 12% and 14% CF alfalfa hay meal groups showed significantly lower duodenal trypsin activity than all other groups ( $P < 0.05$ ). The 12% CF alfalfa hay meal group had extremely significantly lower cecal cellulase activity than all other groups ( $P < 0.01$ ).

**Table 3** Effects of dietary fiber source and CF level on intestinal digestive enzyme activities of meat rabbits

### 2.3.1 Microbial Similarity Analysis

Figures 1 [Figure 1: see original paper] and 2 [Figure 2: see original paper] present the genomic DNA extraction results and DGGE analysis of 16S rDNA PCR products from 24 cecal microbial samples, respectively. As shown in Figure 2, rabbit cecal total bacteria samples contained numerous microbial species, reflected by abundant bands. Sample 6-1 from the 16% CF rice husk meal group exhibited the most bands (52), while samples 5-1, 5-2, and 5-4 from the 14% CF rice husk meal group showed the fewest bands (34 each). Based on band number and position in each lane, Dice' s coefficient (Cs) was calculated and a phylogenetic tree was constructed (Figure 3 [Figure 3: see original paper]).

As shown in Figure 3, the 24 cecal samples from six groups clearly clustered into two major groups: samples 4-2 to 5-2 formed one cluster, and samples 2-4 to 3-3 formed another. However, each major cluster contained samples from different groups. Four pairs showed high similarity ( $C_s > 0.5$ ): samples 1-2 and 6-1, samples 6-2 and 5-2, samples 5-4 and 6-3, and samples 4-3 and 3-3. Overall, no clear clustering pattern was observed between or within groups. For example, samples 4-1, 4-2, 4-3, and 4-4 represented cecal microbial samples from different rabbits within the same group, yet they failed to cluster together.

**Figure 1** Genomic DNA extraction results of 24 cecal microbial samples (M: DL2000)

*Note: Samples 1-1, 1-2, 1-3, and 1-4 are from Group I; samples 2-1, 2-2, 2-3, and 2-4 are from Group II; samples 3-1, 3-2, 3-3, and 3-4 are from Group III; samples 4-1, 4-2, 4-3, and 4-4 are from Group IV; samples 5-1, 5-2, 5-3, and 5-4 are from Group V; and samples 6-1, 6-2, 6-3, and 6-4 are from Group VI. The same applies below.*

**Figure 2** DGGE fingerprints of 16S rDNA PCR products

**Figure 3** Cluster analysis of phylogenetic tree based on Dice' s coefficient ( $C_s$ )

### 2.3.2 Microbial Diversity Analysis

As shown in Table 4 , fiber source had no significant effect on cecal microbial richness (band number) or Shannon-Wiener index ( $P > 0.05$ ). However, the species evenness index was extremely significantly higher in meat rabbits fed alfalfa hay meal diets compared with those fed rice husk meal diets ( $P < 0.01$ ).

CF level significantly affected cecal microbial richness and Shannon-Wiener index ( $P < 0.05$ ), with the highest values observed in the 16% CF level group. The interaction between fiber source and CF level had no significant effect on cecal microbial diversity ( $P > 0.05$ ).

**Table 4** Effects of dietary fiber source and CF level on cecal microbial diversity of meat rabbits

### 3.1 Effects of Dietary Fiber Source and CF Level on Nutrient Digestibility in Meat Rabbits

Fiber digestion in rabbits primarily depends on cecal microbial activity, digesta retention time in the cecum, and the chemical composition and structure of fiber. Li et al. reported that Rex rabbits exhibited significantly higher digestibility of CF and CP (28.77% and 82.94%, respectively) in diets containing alfalfa hay meal as the main fiber source compared with diets containing corn stalk and soybean straw, though no significant differences were observed in ether extract (EE), NDF, or ADF digestibility among the three fiber sources. The present study found that, except for CP, all major nutrient digestibility coefficients were significantly higher in the alfalfa hay meal group than in the rice husk meal group, while CP digestibility was extremely significantly higher in the rice husk meal group. These results partially contradict previous reports, possibly due to higher trypsin activities in the jejunum and duodenum of rabbits fed rice husk meal diets. CF level significantly affected DM, NDF, and ADF digestibility. The low CF level (12%) group showed significantly higher DM and energy digestibility and ADE compared with medium and high CF level (14% and 16%) groups, while NDF and ADF digestibility exhibited a “high at both ends (12% and 16% CF), low in the middle (14% CF)” pattern. This differs from He et al., who reported a “low at both ends, high in the middle” pattern for 2-month-old meat rabbits, with 12% CF showing significantly higher CF and energy digestibility than 10% and 14% CF levels. This discrepancy is related to rabbit age and the chemical composition of fiber sources. In the present study, the 14% CF diets contained higher ADL content than the 12% and 16% CF diets (Table 1), and elevated ADL content affected digesta passage rate and fiber fermentation. Thus, different fiber sources with varying fiber composition can produce different digestibility outcomes even at the same CF level, indicating that nutrient digestibility, particularly fiber digestibility, does not change linearly with dietary CF level. Therefore, further research on fiber nutrition in meat rabbits is needed to determine how the basic constituents of cellulose and hemicellulose affect digestibility. The present study demonstrated that increasing dietary CF level reduced energy digestibility, consistent with Qiao et al., primarily because higher CF levels increase gastrointestinal burden and promote greater production of acetic acid and less propionic and butyric acid in the cecum. Reduced butyric acid decreases its inhibitory effect on intestinal motility, enhancing intestinal excitability and accelerating peristalsis, which shortens retention time and reduces nutrient digestibility. Additionally,

soluble fiber in diets can increase digesta viscosity, reduce contact between digesta and digestive enzymes, and hinder nutrient absorption. Therefore, dietary CF level should be adjusted according to rabbit breed, physiological stage, and fiber quality.

### **3.2 Effects of Dietary Fiber Source and CF Level on Intestinal Digestive Enzyme Activities in Meat Rabbits**

Nutrient digestion in rabbits primarily relies on endogenous digestive enzymes. Rabbits possess an enzyme profile similar to other monogastric animals, secreting amylase, protease, and lipase, but not cellulase. Dietary cellulose can only be digested by cellulase produced by intestinal microorganisms, with fibrous materials fermented by cecal bacteria into volatile fatty acids (VFAs) that are absorbed through the intestinal wall as an energy source.

Trypsin activity in intestinal contents is considered a good indicator of protein digestive capacity. Cui reported that activities of amylase, trypsin, and chymotrypsin in young rabbit intestinal contents decreased with increasing dietary CF levels (10%, 12%, 14%). Chao et al. found that trypsin and chymotrypsin activities in 3-month-old meat rabbits increased initially then decreased with increasing ADF levels. Additionally, cecal cellulase activity in meat rabbits increased with dietary CF level. The present study demonstrated that 16% CF level significantly increased jejunal trypsin activity and extremely significantly increased cecal cellulase activity, while the 12% CF level group showed significantly lower jejunal amylase, trypsin, and cecal cellulase activities compared with the 14% and 16% CF level groups. These consistent findings regarding CF level effects on cecal cellulase activity occur because fiber reaching the cecum and colon is decomposed into VFAs by intestinal microorganisms, providing energy for cellulolytic bacteria and thereby increasing their population and activity. Therefore, when formulating meat rabbit diets, appropriately increasing dietary CF level or using higher-fiber feed ingredients may reduce feeding costs. Yang et al.'s research on the effects of fiber source and CF level on growth performance in meat rabbits supports this conclusion. However, reports on CF level effects on intestinal amylase and protease activities are inconsistent, primarily due to variations in fiber sources and CF levels used across studies, resulting in different ratios of soluble to insoluble fiber. The present study found that rice husk meal as a fiber source extremely significantly increased duodenal trypsin activity compared with alfalfa hay meal, possibly due to higher insoluble fiber content promoting digestive juice secretion or representing an "adaptive secretion" of intestinal digestive enzymes in response to dietary fiber, which requires further investigation.

### **3.3 Effects of Dietary Fiber Source and CF Level on Cecal Microbial Diversity in Meat Rabbits**

The cecum serves as the primary site for dietary fiber digestion, utilizing microbial enzymes or microbial metabolites to degrade CF. Under normal conditions,

beneficial bacteria dominate, forming a relatively stable and complex ecosystem. The species, quantity, and balance of cecal bacteria are important indicators of digestive function health and reflect normal gastrointestinal structure and function.

PCR-DGGE technology is a rapid and reliable method for comparing microbial diversity and community changes. Phylogenetic trees constructed based on Dice' s coefficient can intuitively reflect the similarity of microbial communities between different samples and indicate the specificity of community composition relative to other samples. In this study, low similarity was observed among cecal microbial communities across samples, with no clear clustering pattern, indicating substantial variation in cecal microbial community structure among individual meat rabbits both between and within groups. This aligns with Zhu' s findings of large individual differences in intestinal microbial community structure in growing meat rabbits, suggesting that future rabbit microbiota studies should increase sample size to enhance representativeness.

Michelland et al. reported that reducing dietary ADF level (20.4% vs. 10.7%) altered cecal microbial composition and relative abundance of bacterial genera in growing meat rabbits without changing diversity. The present study found that high CF level (16%) significantly increased cecal microbial richness and diversity, which partially contradicts Michelland' s findings but is related to fiber source composition. This result is consistent with Liu' s report that increasing fiber level increased microbial species diversity in goose ceca.

Awati et al. demonstrated that different diets resulted in different rabbit intestinal microbiota compositions, with dietary fiber playing an important role. De Filippo et al. reported that African children consuming fiber-rich diets had higher proportions of Bacteroidetes and lower proportions of Firmicutes in fecal microbiota compared with European children consuming high-protein, low-fiber diets lacking Bacteroidetes. Matsui et al. suggested that ceca rich in Bacteroidetes exhibited stronger fermentation capacity. Gu et al. found that dietary CF level (7%, 9%, 12%, 14%) significantly affected cecal microbial numbers in weaned Rex rabbits, with beneficial bacteria dominating at 12% CF level, while excessively high or low CF levels, particularly low levels, substantially reduced beneficial bacteria and increased pathogenic bacteria (*E. coli*, *Salmonella*). Therefore, in practical production, dietary CF level can be gradually increased to improve rabbit utilization of fibrous feeds through microbial adaptation to substrates.

## Conclusions

1. Meat rabbits fed alfalfa hay meal diets exhibited significantly or extremely significantly higher DM, CF, ADF, and ADL digestibility and cecal microbial species evenness compared with those fed rice husk meal diets, but extremely significantly lower duodenal trypsin activity.
2. A dietary CF level of 12% resulted in the highest ADE and major nutrient

digestibility in meat rabbits. Higher CF levels increased jejunal amylase, trypsin, and cecal cellulase activities. A dietary CF level of 16% enhanced cecal microbial richness and diversity.

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