Effects of Daheishan Coix Grass Powder Replacing Alfalfa Grass Powder on Intestinal Morphology, Digestive Enzyme Activity, and Cecal Fermentation Parameters in Growing Meat Rabbits (Postprint)

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Date: 2018-12-25T00:00:00+00:00

Abstract

The present study aimed to investigate the effects of different dietary replacement levels of Dahheishan coix seed straw powder for alfalfa meal on intestinal morphology, digestive enzyme activity, and cecal fermentation parameters in growing meat rabbits. One hundred 35-day-old weaned New Zealand White rabbits with similar body weight were randomly assigned to five groups with 10 replicates per group and two rabbits per replicate. The control group (Group C) and four substitution groups (Groups S25, S50, S75, and S100) were fed experimental diets containing five different replacement ratios (0, 25%, 50%, 75%, and 100%) of Dahheishan coix seed straw powder for alfalfa meal. The experimental period lasted 15 days. On the morning of day 15, after overnight fasting and weighing, four rabbits with body weight close to the group mean were selected from different replicates within each group, slaughtered, and samples of small intestine and cecum tissues, mucosa, and chyme were collected for analysis. The results showed that no significant differences were observed in average daily feed intake, average daily gain, and feed conversion ratio among all groups ($P>0.05$). No significant differences were found in villus height, crypt depth, villus height to crypt depth ratio across all small intestinal segments, or cecal crypt depth among all groups ($P>0.05$). The sucrase activity in the duodenum of Group S50 was significantly higher than that of Groups S75 and S100 ($P < 0.05$), while no significant differences were detected in other digestive enzyme activities across all intestinal segments among all groups ($P>0.05$), although Group S50 generally exhibited higher digestive enzyme activities. No significant differences were observed in cecal pH, ammonia nitrogen, total volatile fatty acids, acetate, propionate, butyrate contents, or acetate/(propionate+butyrate) ratio among all

groups (P>0.05). Based on 16S rRNA next-generation sequencing results, no significant differences were found in cecal microbial composition at the phylum and genus levels between Group C and Group S100. In conclusion, replacement of alfalfa meal with Dahheishan coix seed straw powder in the diet does not affect intestinal morphology, digestive enzyme activity, or cecal fermentation parameters in growing meat rabbits, and Dahheishan coix seed straw powder can completely replace alfalfa meal in diets for growing meat rabbits, with a recommended replacement level of 50%.

Full Text

Effects of Dietary Replacement of Alfalfa Meal by Coix lacryma-jobi cv. Daheishan Meal on Intestinal Morphology, Digestive Enzyme Activity and Cecal Fermentation Parameters of Growing Meat Rabbits

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Abstract: This experiment was conducted to investigate the effects of replacing alfalfa meal with different proportions of Coix lacryma-jobi cv. Daheishan meal on intestinal morphology, digestive enzyme activity, and cecal fermentation parameters in growing meat rabbits. A total of 100 weaned New Zealand white rabbits (35 days old) with similar body weights were randomly assigned to 5 groups, each consisting of 10 replicates with 2 rabbits per replicate. The control group (C group) and four replacement groups (S25, S50, S75, and S100 groups) were fed five experimental diets in which alfalfa meal was replaced by Coix lacryma-jobi cv. Daheishan meal at 0%, 25%, 50%, 75%, and 100%, respectively. The experiment lasted for 15 days. On the morning of day 15, after overnight fasting and weighing, 4 rabbits with body weights close to the group mean were selected from different replicates of each group and slaughtered to collect small intestine and cecum tissues, mucosa, and digesta samples for analysis.

The results showed no significant differences in average daily feed intake, average daily gain, or feed-to-gain ratio among all groups (P>0.05). No significant differences were observed in villus height, crypt depth, or the ratio of villus height to crypt depth in any segment of the small intestine, nor in cecal crypt depth (P>0.05). The sucrase activity in the duodenum of the S50 group was significantly higher than that in the S75 and S100 groups (P<0.05), while no significant differences were found in other digestive enzyme activities across intestinal segments (P>0.05), although the S50 group generally exhibited higher enzyme activities. No significant differences were detected in cecal pH or in

the concentrations of ammonia nitrogen, total volatile fatty acids, acetic acid, propionic acid, butyric acid, or the ratio of acetic acid to (propionic acid + butyric acid) among groups (P>0.05). Based on 16S rRNA sequencing results, no obvious differences were observed in cecal microbial composition at either the phylum or genus level between the C group and the S100 group. These findings indicate that replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal does not adversely affect intestinal morphology, digestive enzyme activity, or cecal fermentation parameters in growing meat rabbits. Alfalfa meal can be completely replaced by Coix lacryma-jobi cv. Daheishan meal in diets for growing meat rabbits, with a recommended substitution ratio of 50%.

Keywords: Coix lacryma-jobi cv. Daheishan meal; growing meat rabbits; intestinal morphology; intestinal enzyme activity; cecal fermentation

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As a small herbivorous animal, the rabbit' s health is closely related to the quality and content of dietary fiber [1]. Currently, high-quality alfalfa meal provides partial fiber for rabbits, but its limited resources and high cost affect the economic efficiency of rabbit production [2]. Therefore, developing novel roughage sources is of great significance. Coix is a gramineous plant whose seeds are rich in protein, carbohydrates, dietary fiber, minerals, and vitamins. Studies have shown that its roots, stems, and leaves also contain abundant amino acids, minerals, and other nutrients, indicating high feeding value [3-4]. Additionally, Coix demonstrates significant effects on gastrointestinal health [3-6]. Ethanol extracts from Coix endosperm, bran, and dehulled seeds effectively protect gastrointestinal health and regulate intestinal microbiota [6-8]. Coix seed can improve intestinal barrier function, alleviate diarrhea, increase Lactobacillus populations, and elevate volatile fatty acid content [8-11]. Raw Coix seed powder promotes gastrointestinal motility in mice, while bran-fried Coix seed powder prepared through traditional Chinese medicine processing methods exhibits antidiarrheal effects [12]. Fermented dehulled Coix can balance cecal microbial populations in hamsters [13]. Resistant starch from Coix seed reduces pathogenic bacteria and increases Bifidobacterium populations while improving intestinal morphology in mice [14]. Coix seed oil emulsions enhance intestinal absorption [15], and silaged Coix straw promotes fiber-decomposing bacteria proliferation [16]. Collectively, these findings demonstrate that Coix seed and related products regulate and protect intestinal health while improving growth performance in animal trials [17-19]. However, no studies have reported the effects of Coix application in rabbit diets on intestinal health.

Our previous research found that Coix lacryma-jobi cv. Daheishan meal is nutritionally rich and provides high nutritional value for rabbits, yielding satisfactory growth performance and nutrient digestibility [17,19]. Building upon these findings, this experiment further investigated the effects of replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal on intestinal morphology, digestive enzyme activity, and cecal fermentation parameters in rabbits to provide stronger reference value for rabbit feed development.

1.1 Experimental Design

This experiment employed a single-factor design. A total of 100 purebred New Zealand white rabbits (35 days old) were randomly divided into 5 groups according to similar body weight, with 10 replicates per group and 2 rabbits per replicate. The control group (C group) and four replacement groups (S25, S50, S75, and S100 groups) were fed five experimental diets in which alfalfa meal was replaced by Coix lacryma-jobi cv. Daheishan meal at 0%, 25%, 50%, 75%, and 100%, respectively. The experimental period lasted 15 days. The control diet contained 11.28 MJ/kg digestible energy, 17.50% crude protein, and 17.14% crude fiber. Coix lacryma-jobi cv. Daheishan meal contained 5.69 MJ/kg digestible energy, 13.46% crude protein, and 29.58% crude fiber, while alfalfa meal contained 7.40 MJ/kg digestible energy, 15.30% crude protein, and 26.10% crude fiber. The composition and nutrient levels of experimental diets are presented in Table 1.

1.2.1 Growth Performance

Feed intake, residual feed, and wasted feed were recorded daily on a replicate basis. Individual body weights were measured at the beginning and end of the experiment before morning feeding to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G).

1.2.2 Intestinal Morphology

On the morning of day 15, after overnight fasting and weighing, 4 rabbits with body weights close to the group mean were selected from different replicates of each group (regardless of sex) and slaughtered following the method of Blasco et al. [20]. After slaughter, intestinal segments were isolated and gently rinsed with physiological saline to remove contents. Segments (2-3 cm) were collected from each section and preserved in 4% paraformaldehyde solution for slide preparation. Samples from each group were sent to Chengdu Lilai Biotechnology Co., Ltd. for hematoxylin-eosin (HE) staining to measure villus height (VH), crypt depth (CD), VH/CD ratio in the duodenum, jejunum, and ileum, and CD in the cecum.

1.2.3 Intestinal Digestive Enzyme Activity

Considering the high fiber and crude protein content in both Coix lacryma-jobi cv. Daheishan meal and the experimental diets, the activities of trypsin, sucrase, pectinase, and cellulase were selectively measured.

After slaughter, the small intestine was removed and segmented. Contents were rinsed with physiological saline, and the intestine was longitudinally opened. Mucosa was scraped with a glass slide to determine trypsin and sucrase activities in each small intestinal segment. Simultaneously, cecal contents were collected in cryovials for pectinase and cellulase activity determination. All samples were

temporarily stored in liquid nitrogen. Enzyme activities were measured using assay kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer' s instructions.

1.2.4 Cecal Fermentation Parameters

Immediately after slaughter, the cecum was removed and cecal content pH was measured using a pH-3B pH meter (at 3 different locations). Total volatile fatty acid (TVFA) content in cecal digesta samples was determined using a Varian CP-3800 gas chromatograph after pretreatment [21]. Ammonia nitrogen (NH3-N) content was measured by colorimetry at 700 nm [22]. Additionally, cecal content samples were collected from the C and S100 groups in sterilized cryovials and sent to Chengdu Institute of Biology, Chinese Academy of Sciences for 16S rRNA sequencing to determine microbial composition at the phylum and genus levels.

1.3 Data Processing and Statistical Analysis

Data were initially processed using Excel 2013. One-way ANOVA and Duncan' s multiple comparison tests were performed using SAS 9.20. $P<0.05$ was considered statistically significant, while $P>0.05$ indicated no significant difference.

2.1 Effects of Different Replacement Ratios of Coix lacryma-jobi cv. Daheishan Meal on Growth Performance of Growing Meat Rabbits

As shown in Table 2, no significant differences were observed in ADFI, ADG, or F/G among all groups ($P>0.05$).

2.2 Effects of Different Replacement Ratios of Coix lacryma-jobi cv. Daheishan Meal on Intestinal Morphology of Growing Meat Rabbits

As shown in Table 3, no significant differences were found in VH, CD, or V/C ratio in the duodenum, jejunum, and ileum, nor in cecal CD among all groups ($P>0.05$). The V/C ratio generally tended to be better in the S50 group.

2.3 Effects of Different Replacement Ratios of Coix lacryma-jobi cv. Daheishan Meal on Intestinal Digestive Enzyme Activity of Growing Meat Rabbits

As shown in Table 4, except for duodenal sucrase activity, no significant differences were observed in other digestive enzyme activities across intestinal segments ($P>0.05$), with the S50 group showing relatively higher activities. Specifically, duodenal sucrase activity in the S50 group was significantly higher than that in the S75 and S100 groups ($P<0.05$), but did not differ significantly from other groups ($P>0.05$).

2.4 Effects of Different Replacement Ratios of Coix lacryma-jobi cv. Daheishan Meal on Cecal Fermentation Parameters and Microbial Composition of Growing Meat Rabbits

As shown in Table 5, no significant differences were detected in cecal pH or in the concentrations of NH3-N, TVFA, acetic acid, propionic acid, butyric acid, or the ratio of acetic acid to (propionic acid + butyric acid) among groups ($P>0.05$). Cecal NH3-N content was relatively higher in the S50 group. Meanwhile, no obvious differences were observed in cecal microbial composition at either the phylum or genus level between the C and S100 groups (Figure 1 and Figure 2).

Figure 1 Cecal microbial distribution at the phylum level
Figure 2 Cecal microbial distribution at the genus level

In the figures, T represents the control group (C group) and Hm represents the S100 group.

3.1 Effects of Different Replacement Ratios of Coix lacryma-jobi cv. Daheishan Meal on Intestinal Morphology of Growing Meat Rabbits

The small intestine is the primary site for nutrient digestion and absorption in rabbits. The physical function of villi and chemical action of crypts work synergistically to promote nutrient digestion and absorption [23]. Therefore, VH, CD, and V/C ratio in the small intestine can reflect intestinal development status and nutrient absorption capacity. Studies have shown that fiber source affects rabbit intestinal morphology [24-25]. Active components in Chinese herbal medicine additives (organic acids, flavonoids, alkaloids, etc.) can promote intestinal mucosal integrity and improve intestinal structure to some extent [26]. Our results showed that VH and V/C ratio in the small intestine of replacement groups increased to some degree, while CD remained essentially similar. This indicates that replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal can promote nutrient digestion and absorption in rabbits without adversely affecting intestinal development, which is similar to results from Coix seed trials in mice [27]. These findings align with previously reported results on nutrient digestibility and animal growth performance [19]. The underlying mechanism may be that Coix lacryma-jobi cv. Daheishan meal contains resistant starch similar to that in Coix seed, which improves intestinal morphology and increases beneficial bacteria proliferation, thereby enhancing rabbit digestive capacity [9-11,14].

3.2 Effects of Different Replacement Ratios of Coix lacryma-jobi cv. Daheishan Meal on Intestinal Digestive Enzyme Activity of Growing Meat Rabbits

In animal digestion and metabolism, digestive enzymes in small intestinal mucosa play a primary role [28]. Sucrase, derived from shed intestinal epithelial cells, breaks down dietary disaccharides into monosaccharides for absorption [29]. Trypsin, mainly secreted by the pancreas, reflects the animal' s ability to digest dietary crude protein. Cellulase and pectinase are primarily produced by

microorganisms in the rabbit cecum, and their activities are affected by dietary fiber level and source [30-32]. Studies have shown that Chinese herbal medicine components can activate sucrase activity in the small intestine to some extent [33]. Additionally, with animal growth and development, disaccharidase activity in adult animals is higher in the jejunum than in the duodenum and ileum [34]. In this experiment, sucrase activity was highest in the jejunum, followed by the duodenum and ileum, consistent with Siddons' findings [35] and with animal developmental patterns. Trypsin activity was highest in the ileum, followed by the jejunum, and lowest in the duodenum, indicating that protein digestion occurs primarily in the posterior small intestine. Furthermore, our results showed that enzyme activities in all small intestinal segments (except duodenal sucrase) were higher in replacement groups than in the control group, with the S50 group showing the highest values. This demonstrates that replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal had no negative effects on small intestinal digestive enzyme activities and even improved them to some extent. These results are consistent with findings on nutrient digestibility [19] and may be related to functional substances in Coix lacryma-jobi cv. Daheishan meal [3,6].

3.3 Effects of Different Replacement Ratios of Coix lacryma-jobi cv. Daheishan Meal on Cecal Fermentation Parameters and Microbial Composition of Growing Meat Rabbits

The cecum is one of the most important digestive organs in rabbits. Its internal environment affects microbial growth and reproduction, thereby influencing rabbit health [32]. Cecal pH and concentrations of ammonia nitrogen and volatile fatty acids are important indicators for evaluating rabbit intestinal health. NH3-N is the main product of protein degradation and an important substrate for microbial protein synthesis [36]. Additionally, short-chain fatty acids among volatile fatty acids in the cecum play important roles in rabbit nutrient metabolism and energy supply [37]. Studies have shown that increased cecal pH raises diarrhea incidence in rabbits [38]. In this experiment, cecal pH in the control group was slightly higher than in replacement groups, matching previous results on disease incidence (primarily diarrhea) [19]. This may be because Coix lacryma-jobi cv. Daheishan meal contains the same antidiarrheal active ingredients as Coix seed, indirectly affecting cecal pH [3-4,6,10-11]. Furthermore, cecal NH3-N content was higher in all replacement groups than in the control group, possibly because replacement improved protein utilization in meat rabbits, increasing degradation products and thus NH3-N content. This may be related to the crude protein digestibility observed in this experiment [21]. Additionally, Gidenne et al. [32] reported that dietary fiber level positively correlates with volatile fatty acid content, increasing the acetic acid proportion while decreasing butyric acid proportion. Moreover, fiber lignification negatively affects volatile fatty acids [39]. In this experiment, volatile fatty acid concentrations in replacement groups were higher than in the control group to varying degrees, possibly related to the fiber composition and type in Coix lacryma-jobi cv. Daheishan meal. These results are similar to findings with dehulled Coix

in mice [11,16]. Meanwhile, microbial changes can alter the cecal environment, and comparison of cecal microorganisms between the C and S100 groups further supports the cecal parameter results, demonstrating that Coix lacryma-jobi cv. Daheishan meal can completely replace alfalfa meal.

4 Conclusion

In summary, replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal in diets for growing meat rabbits has no adverse effects on intestinal morphology, digestive enzyme activity, cecal fermentation parameters, or microbial composition. These results further demonstrate that Coix lacryma-jobi cv. Daheishan meal can replace alfalfa meal in commercial meat rabbit diets, with a recommended substitution ratio of 50%.

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