Effects of replacing alfalfa meal with Daheishan coix straw meal on serum biochemical parameters, intestinal immunity, and antioxidant function in growing meat rabbits (Postprint)

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Abstract

This study aimed to investigate the effects of replacing dietary alfalfa meal with different proportions of Dahheishan coix seed straw powder on serum biochemical indices, relative organ weights, and intestinal immune and antioxidant functions in growing meat rabbits. One hundred 35-day-old weaned New Zealand White rabbits with similar body weight were randomly and equally divided into 5 groups (10 replicates per group, 2 rabbits per replicate) and fed five isoenergetic, isonitrogenous, and isofibrous diets, including one control diet (C) and four test diets (S25, S50, S75, and S100, in which Dahheishan coix seed straw powder replaced 25%, 50%, 75%, and 100% of alfalfa meal in the control diet, respectively). The experimental period lasted 29 days. On days 15 and 29, 4 rabbits were selected from each group for blood collection and slaughter to collect tissue and organ samples. The results showed that: 1) Except for serum urea nitrogen content on day 15, which differed significantly among groups ($P<0.05$), no significant differences were observed in other measured serum biochemical indices ($P>0.05$); 2) No significant differences were found in measured relative organ weights among groups ($P>0.05$); 3) No significant differences were detected in superoxide dismutase (SOD) and catalase (CAT) activities, as well as secretory immunoglobulin A (SIgA) and mucin 2 (MUC2) contents in duodenal, jejunal, and ileal mucosa among groups ($P>0.05$); however, overall, SOD and CAT activities in the test groups were numerically higher than those in group C, especially in group S50. It can be concluded that replacing dietary alfalfa meal with Dahheishan coix seed straw powder had no negative effects on the physiological functions of growing meat rabbits and slightly improved intestinal antioxidant function, particularly at the 50% replacement ratio.

Full Text

Effects of Replacing Alfalfa Meal with Coix lacryma-jobi cv. Daheishan Meal on Serum Biochemical Parameters, Intestinal Immunity, and Antioxidant Capacity in Growing Rabbits

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Abstract

This experiment aimed to investigate the effects of substituting different proportions of alfalfa meal with Coix lacryma-jobi cv. Daheishan meal in diets on serum biochemical parameters, organ relative weights, and intestinal immune and antioxidant function in growing rabbits. One hundred weaned New Zealand white rabbits (35 days of age) with similar body weight were randomly divided into 5 groups (10 replicates per group, 2 rabbits per replicate) and fed five isoenergetic, isonitrogenous, and isofibrous diets, including one control diet (C) and four test diets (S25, S50, S75, and S100), where Coix lacryma-jobi cv. Daheishan meal replaced 25%, 50%, 75%, and 100% of alfalfa meal in the control diet, respectively. The experimental period lasted 29 days. On days 15 and 29, blood and tissue/organ samples were collected from 4 rabbits per group. The results showed: 1) Except for serum urea nitrogen content on day 15, which differed significantly ($P<0.05$), no significant differences were observed in other measured serum biochemical parameters ($P>0.05$). 2) No significant differences were found in organ relative weights among groups ($P>0.05$). 3) No significant differences were detected in superoxide dismutase (SOD) and catalase (CAT) activities or secretory immunoglobulin A (SIgA) and mucin 2 (MUC2) contents in duodenal, jejunal, and ileal mucosa among groups ($P>0.05$), although SOD and CAT activities in test groups were numerically higher than in group C, particularly in group S50. In conclusion, replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal had no negative effects on the physiological function of growing rabbits and slightly improved intestinal antioxidant capacity, especially at a 50% replacement level.

Keywords: Coix lacryma-jobi cv. Daheishan meal; serum biochemical parameters; intestinal immunity; intestinal antioxidant capacity; growing rabbits

Introduction

Dietary fiber is crucial for rabbits and is primarily supplied by alfalfa meal and agricultural processing by-products [1]. However, the scarcity and high cost of alfalfa in China substantially increase rabbit production costs [2]. Therefore, identifying novel forage resources and systematically evaluating their feeding value is essential for promoting the healthy development of China' s rabbit industry. Coix lacryma-jobi is a medicinal and edible herb with a long cultivation history. Due to its high biomass, tender texture, and good palatability, attempts have been made to develop it as a forage resource both domestically and internationally [3]. Studies have shown that Coix lacryma-jobi (seeds, leaves, stems, and roots) is rich in nutrients and bioactive components such as coixol, coixenolide, polyphenols, flavonoids, and polysaccharides [3-4]. Feeding animals (poultry, pigs, dairy cattle, meat rabbits) with fresh-cut Coix lacryma-jobi, its seeds, or by-products yields favorable production performance [3,5-6]. Dehulled adlay [7] and extracts from adlay seeds [8-10] and bran [11] can reduce blood lipids in rats or hamsters, while dehulled adlay [7] and extracts from adlay seeds [12] and stems [13] can reduce blood glucose in rats or mice. Adlay seeds [7,14], stem [15-16], and leaf extracts [15,17] have protective effects on organs such as liver, kidney, spleen, or thymus in mice. Furthermore, adlay seeds [8-10], testa [18], hull [19], stem [20-23], and leaf extracts [24-27] exhibit antioxidant activity in vivo (rats or hamsters) or in vitro, while adlay seeds [28-30], testa [18,31], bran [32], and hull extracts [33] enhance immune function in vivo (mice) or in vitro. However, no reports have examined the effects of Coix lacryma-jobi meal on physiological functions in rabbits. Our previous research demonstrated that Coix lacryma-jobi cv. Daheishan meal has high nutritional value for growing meat rabbits [34] and can completely replace alfalfa meal in diets for growing meat rabbits based on production performance, health status, nutrient apparent digestibility, and slaughter performance [35]. Therefore, this experiment further investigated whether replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal adversely affects growing rabbits by examining serum biochemical parameters, organ functional status, and intestinal immune and antioxidant function, aiming to accumulate comprehensive evaluation data for Coix lacryma-jobi cv. Daheishan meal as an alfalfa substitute and alleviate pressure on the rabbit industry caused by alfalfa shortage.

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1 Materials and Methods

1.1 Experimental Design

This experiment employed a single-factor design. One hundred weaned New Zealand white rabbits (35 days of age) with similar body weight were randomly divided into 5 groups (10 replicates per group, 2 rabbits per replicate) and fed five isoenergetic [digestible energy (DE) 11.28 MJ/kg], isonitrogenous [crude protein (CP) 175.0 g/kg], and isofibrous [crude fiber (CF) 171.4 g/kg] pelleted

diets, including one control diet (C) and four test diets (S25, S50, S75, and S100), where Coix lacryma-jobi cv. Daheishan meal replaced 25%, 50%, 75%, and 100% of alfalfa meal in the control diet, respectively. The composition and nutrient levels of experimental diets are shown in Table 1 . The experimental period lasted 29 days.

1.2 Sample Collection and Analysis

1.2.1 Serum Biochemical Parameters On days 15 and 29 of the experiment, after fasting weighing, 4 rabbits with body weight close to the group average were selected from different replicates in each group, and approximately 5 mL of blood was collected via cardiac puncture. Whole blood was placed in vacuum tubes without anticoagulant, left at room temperature for 30 min, then centrifuged at 3,000 r/min for 15 min. Serum was collected, aliquoted, and stored at -20°C.

Total protein, albumin, globulin, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein contents, and creatine kinase activity were determined using colorimetric methods. Urea nitrogen (enzymatic method), glucose (hexokinase method), total cholesterol (enzymatic method), alanine aminotransferase (rate method), aspartate aminotransferase (rate method), and alkaline phosphatase activity (rate method) were measured using corresponding methods. All these indicators were analyzed by Ya' an People' s Hospital of Sichuan Province using a Roche cobas8000 c702 biochemical analyzer.

1.2.2 Organ Relative Weight Animals fasted, weighed, and bled on days 15 and 29 were slaughtered according to the method of Blasco et al. [36]. The stomach, liver, kidneys, spleen, thymus, appendix, and sacculus rotundus were dissected and weighed. Organ relative weight was calculated using the following formula:

Organ relative weight (%) = 100 × organ weight (g) / live body weight (g).

1.2.3 Intestinal Immune and Antioxidant Indices From animals slaughtered on day 15, the duodenum, jejunum, and ileum were isolated, and approximately 10 cm segments were taken from each. After washing away intestinal contents with physiological saline, segments were longitudinally opened with surgical scissors, everted, placed on ice packs, excess moisture absorbed with filter paper, and mucosa scraped with glass slides. Mucosa was placed in cryovials, wrapped in tin foil, and stored in liquid nitrogen for determination of the following indices: SOD and CAT activities were measured using kits from Nanjing Jiancheng Bioengineering Institute; SIgA and MUC2 contents were determined using enzyme-linked immunosorbent assay kits from Shanghai Hengyuan Biological Technology Co., Ltd. All procedures were performed according to kit instructions.

1.3 Data Processing and Statistical Analysis

Data were processed using Excel 2013 and subjected to one-way ANOVA and Duncan's multiple comparison tests using SAS 9.2 statistical software. $P < 0.05$ was considered statistically significant, while $P > 0.05$ indicated no significant difference.

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2 Results

2.1 Serum Biochemical Parameters

As shown in Table 2 , no significant differences were observed in serum biochemical parameters among groups ($P > 0.05$), except for serum urea nitrogen content on day 15 ($P < 0.05$). On day 15, serum urea nitrogen content in test groups was higher than in group C to varying degrees, with significant differences in groups S25, S50, and S100 compared to group C ($P < 0.05$).

2.2 Organ Relative Weight

As shown in Table 3 , no significant differences were found in the relative weights of stomach, liver, kidneys, or immune organs among groups on either day 15 or day 29 ($P > 0.05$).

2.3 Intestinal Immune and Antioxidant Function

As shown in Table 4 and Table 5 , no significant differences were detected in SOD and CAT activities or SIgA and MUC2 contents in duodenal, jejunal, and ileal mucosa among groups ($P > 0.05$). However, SOD and CAT activities in test groups were numerically higher than in group C overall, particularly in group S50. Additionally, substantial differences in SOD and CAT activities were observed among different intestinal segments, with SOD activity following the pattern jejunum > duodenum > ileum, and CAT activity showing duodenum > jejunum and ileum.

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3 Discussion

3.1 Serum Biochemical Parameters

Serum biochemical parameters reflect material metabolism and functional status changes of certain tissues and organs in animals [37]. This experiment found no significant differences in serum biochemical parameters reflecting carbohydrate, lipid, and protein metabolism and liver, kidney, and immune function between test and control groups throughout the experimental period, indicating that replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal does not affect the physiological function of growing rabbits. This result is inconsistent with

reports that Coix lacryma-jobi and its extracts can reduce blood lipids and/or glucose in rodents (normal or experimentally diseased mice, rats, or hamsters) [3,7-13], but aligns with results on production performance and health status [35].

In this experiment, serum urea nitrogen content in test groups was higher than in the control group on day 15, possibly related to slightly higher nitrogen absorption in the former (i.e., related to crude protein digestibility). This is because feed intake in test groups was comparable to the control group, while actual dietary crude protein levels and apparent digestibility in the former were slightly higher than in the latter [35]. The specific reason remains unclear, and no similar reports exist for Coix lacryma-jobi. However, based on production performance and health status during the experimental period, no obvious differences were observed among groups [35], indicating that normal physiological function was not adversely affected.

3.2 Organ Relative Weight and Intestinal Immune and Antioxidant Function

Organ relative weight is a major biological characteristic of animals that can reflect their functional status [38]. This experiment found no significant differences in organ relative weight between test and control groups, indicating that replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal does not affect the functional status of growing rabbits. This is not entirely consistent with reports that adlay seeds [8,14,30], stem [16], and leaf extracts [17] affect organ relative weights of liver, kidney, spleen, or thymus in rats or mice, but aligns with reports that adlay stem [15] and leaf extracts [15] or adlay oil -cyclodextrin inclusion compounds [39] showed no significant effects on organ changes in rats or mice. These findings are also consistent with results on production performance and health status [35].

SIgA is an antibody in intestinal mucosa that plays an important role in activating mucosal immunity and maintaining intestinal homeostasis [40]. MUC2 is the main component of the intestinal mucus layer and is crucial for intestinal mucosal barrier protection and assisting intestinal mucosal immune system function [41]. This experiment found no significant differences in SIgA and MUC2 contents in mucosa of three small intestinal segments between test and control groups, indicating that replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal does not affect intestinal mucosal immune function in growing rabbits. This is inconsistent with reports that adlay water extract [28] and polysaccharides [30] have protective effects on immunosuppressed mice, adlay decoction [29] can improve immune status in mice after high-intensity exercise, and adlay testa [18,31], bran [32], and hull extracts [33] can enhance immune cell function in vitro.

The gastrointestinal tract is a major source of reactive oxygen species (ROS) and a site for endogenous antioxidant enzymes, with SOD and CAT playing sig-

nificant roles in scavenging gastrointestinal ROS and preventing mucosal damage and inflammatory responses [42]. This experiment found that SOD and CAT activities in duodenal, jejunal, and ileal mucosa of test groups (especially group S50) were slightly higher than in the control group, indicating that replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal can enhance small intestinal antioxidant function in growing rabbits to some extent. This is consistent with reports on antioxidant activity of various parts of adlay seeds [3,10,19], stem [20-23,43], and leaf extracts in vitro [24-27] and adlay extracts in rats [8-9].

4 Conclusion

Replacing alfalfa meal with Coix lacryma-jobi cv. Daheishan meal in diets had no negative effects on serum biochemical parameters, organ relative weight, or intestinal immune and antioxidant function in growing rabbits, and slightly improved intestinal antioxidant capacity, particularly at a 50% replacement level.

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