

## Effects of Different Starch Sources on Endogenous Nitrogen and Energy Losses in Rex Rabbits (Postprint)

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### Abstract

This experiment was conducted to investigate the effects of different starch sources on endogenous nitrogen and energy losses in rex rabbits. Thirty healthy rex rabbits at approximately 1.5 years of age were selected and randomly allocated into three groups (n=10 per group) with a 1:1 sex ratio. The three groups were fed nitrogen-free diets based on corn starch, potato starch, and cassava starch, respectively. Both the preliminary and formal experimental periods lasted 7 days each. The results showed that regarding endogenous nitrogen loss, there were no significant differences in metabolic fecal nitrogen (MFN) and endogenous urinary nitrogen (EUN) among the groups ( $P>0.05$ ); overall, the cassava starch group exhibited the highest endogenous nitrogen loss, the potato starch group the lowest, and the corn starch group was intermediate. Regarding endogenous energy loss, there were no significant differences in metabolic fecal energy (FmE) and endogenous urinary energy (UeE) among the groups ( $P>0.05$ ); overall, the cassava starch group exhibited the highest endogenous energy loss, the potato starch group the lowest, and the corn starch group was intermediate. It was concluded that although different starch sources had no significant effects on MFN, EUN, FmE, and UeE in rex rabbits, the cassava starch group demonstrated higher endogenous nitrogen and energy losses compared to the corn starch and potato starch groups.

### Full Text

## Effects of Different Starch Sources on Endogenous Nitrogen and Endogenous Energy Losses in Rex Rabbits

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## Abstract

This study investigated the effects of different starch sources on endogenous nitrogen and endogenous energy losses in Rex rabbits. Thirty healthy Rex rabbits approximately 1.5 years old were selected and randomly divided into three groups (n=10 per group, half male and half female). The three groups were fed nitrogen-free diets based on corn starch, potato starch, and cassava starch, respectively. Both the pretrial and formal trial periods lasted 7 days. The results showed that for endogenous nitrogen loss, no significant differences were observed among groups in metabolic fecal nitrogen (MFN) or endogenous urinary nitrogen (EUN) ( $P>0.05$ ). Overall, the cassava starch group exhibited the highest endogenous nitrogen loss, followed by the corn starch group, with the potato starch group showing the lowest. For endogenous energy loss, no significant differences were detected among groups in fecal energy from metabolic origin (FmE) or urinary energy from endogenous origin (UeE) ( $P>0.05$ ). Overall, the cassava starch group demonstrated the highest endogenous energy loss, the potato starch group the lowest, and the corn starch group intermediate levels. In conclusion, although different starch sources did not significantly affect MFN, EUN, FmE, or UeE in Rex rabbits, the cassava starch group generally exhibited higher endogenous nitrogen and energy losses compared to the corn and potato starch groups.

**Keywords:** Rex rabbits; different starch sources; endogenous nitrogen loss; endogenous energy loss

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## Introduction

In recent years, the determination of endogenous losses has gradually become a focus of attention. By correcting apparent digestibility and digestible energy using endogenous losses, the adverse effects of individual variation in endogenous losses on feed digestibility can be eliminated, enabling more accurate evaluation of feed nutritional value. Endogenous losses include both endogenous nitrogen and endogenous energy losses. Endogenous nitrogen loss serves as the foundation for evaluating animals' maintenance protein requirements and feed protein biology, while endogenous energy loss forms the basis for studying true digestible energy and metabolizable energy. Numerous factors influence animal endogenous losses, including dietary composition, environmental conditions, and animal body weight [1]. Starch is an important dietary component that provides nearly half of the energy required by animals [2]. However, recent research indicates that different starch sources vary in structural composition, which can affect digestive performance. For instance, different starches exhibit varying digestion rates and extents, resulting in different energy supply efficiencies [3-5]. Nevertheless, whether different starch sources affect endogenous losses has not been reported in rabbits. This study used white Rex rabbits as experimental animals and selected corn starch, potato starch, and cassava

starch as starch sources to formulate corn starch-type, potato starch-type, and cassava starch-type nitrogen-free diets, aiming to investigate the effects of different starch sources on endogenous nitrogen and energy losses in Rex rabbits and provide references for accurately estimating endogenous losses and evaluating feed utilization efficiency.

## Materials and Methods

### 1.1 Experimental Materials

ARBOCEL®, Opticell, cassava starch, and palm fat powder (yellow granular, content 98%) were obtained from J. Rettenmaier & Söhne, Agromed Austria, Guangxi Hongfeng Starch Co., Ltd., and Tai'an Feed Company, respectively. Potato starch, corn starch, and sucrose were also used. The experimental animals consisted of 30 healthy white Rex rabbits approximately 1.5 years old with similar body weight (around 3 kg), half male and half female, provided by the Rex Rabbit Breeding Base of Hebei Agricultural University.

### 1.2 Experimental Diets

Referring to the nutritional requirements for Rex rabbits recommended by Gu Zilin [6], three nitrogen-free diets were formulated using corn starch, potato starch, and cassava starch as starch sources, respectively. The composition and nutrient levels are presented in Table 1. Feed ingredients were weighed and thoroughly mixed according to proportions. To accommodate the feeding habits of rabbits, the diets were processed into pellet feed with a diameter of approximately 4 mm and length of about 10 mm. The crude protein content of the diets was <0.5%.

#### Table 1 Composition and Nutrient Levels of Nitrogen-Free Diets (Air-Dry Basis)

*Note: The rabbit premix provided the following per kg of diets: Fe (as ferric sulfate) 70 mg, Cu (as copper sulfate) 20 mg, Zn (as zinc sulfate) 70 mg, Mn (as manganese sulfate) 10 mg, Se (as sodium selenate) 0.25 mg, Co 0.15 mg, I 0.2 mg, VA 10,000 IU, VD 900 IU, VE 50 mg, VK 2 mg, thiamine 2 mg, riboflavin 6 mg, pantothenic acid 50 mg, pyridoxine 2 mg, VB12 0.02 mg, niacin 50 mg, choline 1,000 mg, biotin 0.2 mg. DE was a calculated value, while the others were measured values.*

### 1.3 Experimental Time and Location

The experiment was conducted from October 2017 to January 2018 at the Teaching and Experimental Base of Hebei Agricultural University.

#### 1.4 Digestion Trial

Thirty healthy Rex rabbits approximately 1.5 years old were selected and randomly divided into three groups (n=10 per group, half male and half female). The three groups were fed nitrogen-free diets based on corn starch, potato starch, and cassava starch, respectively. The rabbits were individually housed in digestion-metabolism cages under specialized management. They were fed twice daily with ad libitum access to feed and water, under natural ventilation and lighting. Both the pretrial and formal trial periods lasted 7 days. During the formal trial period, body weight and daily feed intake were recorded. The total feces collection method was employed: feces were collected and weighed daily each morning, and urine volume was also collected and recorded. Fresh feces and urine were each divided into two portions. For one fecal portion, 10 mL of 10% hydrochloric acid solution was added per 100 g of feces for nitrogen fixation, thoroughly mixed, and used for determining fecal nitrogen content. The other fecal portion was stored at -20°C without acid addition for energy determination. For one urine portion, 5 mL of 10% hydrochloric acid solution was added per 100 mL of urine for nitrogen content determination. The other urine portion was stored at 4°C without acid addition for energy determination. After the formal trial period, feces were thoroughly mixed and dried in an oven at approximately 65°C for 48 hours to constant weight, then equilibrated in air for 24 hours, ground into air-dry samples, and sealed in sample bags for subsequent analysis.

#### 1.5 Measurement Indicators and Methods

**Nitrogen content determination:** The Kjeldahl method was used with a K9860 automatic Kjeldahl nitrogen analyzer.

**Energy determination:** The oxygen bomb calorimetry method was employed using a Changsha Youxin YX-ZR9302 automatic calorimeter.

**Urinary energy determination:** The filter paper method was used. Three milliliters of urine sample were pipetted and dropped multiple times onto a 7 cm diameter quantitative filter paper, dried in a 65°C forced-air oven, and the filter paper with dried urine was folded and placed in a crucible for energy value determination. The energy value of blank filter paper was also determined, and the difference between the two values represented the urinary energy.

Endogenous nitrogen loss includes endogenous urinary nitrogen (EUN), metabolic fecal nitrogen (MFN), and surface loss nitrogen (SLN). In this experiment, EUN was expressed on the basis of metabolic body weight (BW<sup>0.75</sup>), MFN was expressed on the basis of dry matter intake (DMI), and SLN in Rex rabbits was relatively small and often negligible.

Endogenous energy loss includes fecal energy from metabolic origin (FmE) and urinary energy from endogenous origin (UeE).

## 1.7 Data Processing and Analysis

Data were processed and analyzed using Excel 2010 and SPSS 20.0 statistical software. Results are expressed as mean  $\pm$  standard deviation.

## Results

### 2.1 Endogenous Nitrogen Loss in Rex Rabbits Fed Nitrogen-Free Diets with Different Starch Sources

As shown in Table 2, no significant differences were observed among groups in daily dry matter intake or daily fecal excretion ( $P>0.05$ ). Both parameters were highest in the potato starch group, which were 16.23% and 4.80% higher than the corn starch group, and 33.87% and 14.70% higher than the cassava starch group, respectively. Daily urinary excretion was highest in the cassava starch group, being 24.91% and 27.63% higher than the corn and potato starch groups, respectively, with significant differences ( $P<0.05$ ). No significant differences were detected among the three groups in fecal nitrogen content ( $P>0.05$ ). Urinary nitrogen content in the potato starch group was significantly higher than in the cassava starch group ( $P<0.05$ ), showing a 34.50% increase compared to the cassava starch group, while the corn starch group showed no significant difference from the other two starch groups ( $P>0.05$ ). No significant differences were found among groups in MFN or EUN ( $P>0.05$ ). MFN was highest in the cassava starch group, being 2.32% and 12.82% higher than the corn and potato starch groups, respectively. EUN was highest in the corn starch group, being 0.94% and 2.08% higher than the potato and cassava starch groups, respectively.

#### Table 2 Endogenous Nitrogen Loss of Rex Rabbits in Each Group

*Note: In the same row, values with the same or no letter superscripts indicate no significant difference ( $P>0.05$ ), while different lowercase letter superscripts indicate significant difference ( $P<0.05$ ). The same applies below.*

### 2.2 Endogenous Energy Loss in Rex Rabbits Fed Nitrogen-Free Diets with Different Starch Sources

As shown in Table 3, no significant differences were observed among groups in fecal energy or urinary energy ( $P>0.05$ ). No significant differences were detected among groups in FmE or UeE ( $P>0.05$ ). However, FmE in the cassava starch group was 10.00% higher than both the corn and potato starch groups, while UeE in the cassava starch group was 8.70% and 19.05% higher than the corn and potato starch groups, respectively.

#### Table 3 Endogenous Energy Loss of Rex Rabbits in Each Group

## Discussion

### 3.1 Effects of Different Starch Sources on Endogenous Nitrogen Loss in Rex Rabbits

Starch is the main component of energy feed and can provide energy for body nitrogen synthesis, thereby improving nitrogen utilization efficiency. The digestion rate of starch [7], the ratio of amylose to amylopectin [8], and the content of resistant starch (RS) [9] can affect animal digestive and metabolic processes, consequently influencing endogenous nitrogen loss. Bin Shiyu et al. [10] formulated diets using different starch sources and found that nutrient digestibility was negatively correlated with dietary amylose content, with RS reducing both protein and energy digestibility. Dai Qiuzhong et al. [11] reported that the amylose/amylopectin ratio in starch significantly affected amino acid digestibility and energy metabolism rate in broilers, with the highest amino acid digestibility and energy metabolism rate observed at a ratio of 0.23. Xiang Zhentian [12] fed weaned piglets four semi-purified diets formulated with different starch sources to study their digestibility, finding that the cassava starch group (with a lower amylose/amylopectin ratio) showed the fastest digestion rate and highest digestibility, followed by the corn starch group (with a balanced amylose/amylopectin ratio), while the pea starch group (with a higher amylose/amylopectin ratio) showed the slowest digestion and lowest digestibility.

The potato starch used in this experiment contained 8.3% rapidly digestible starch (RDS) and 74.8% RS [13], corn starch contained 26.6% RDS and 21.6% RS [14], and cassava starch contained 80.62% RDS and 4.18% RS [15]. RDS is rapidly degraded through chemical digestion in the small intestine with high digestibility. RS, with properties similar to soluble fiber, decomposes slowly and cannot be digested in the small intestine, being primarily fermented by microorganisms in the large intestine into fatty acids with low energy supply efficiency and digestibility, and can affect the digestibility of other nutrients [16-17]. Both potato and cassava starches are low in amylose/amylopectin ratio [18], but potato starch contains substantial RS. Corn starch has a higher amylose/amylopectin ratio than the other two starches but lower RS content than potato starch. Cassava starch has both a low amylose/amylopectin ratio and low RS content. The results of this experiment indicated that when Rex rabbits were fed nitrogen-free diets with different starch sources, MFN was highest in the cassava starch group, lowest in the potato starch group, and intermediate in the corn starch group. After correction with apparent digestibility, the cassava starch group showed higher true digestibility, while the potato starch group showed lower true digestibility. These findings demonstrate that the amylose/amylopectin ratio and RS content in starch indeed affect nutrient digestibility.

### 3.2 Effects of Different Starch Sources on Endogenous Energy Loss in Rex Rabbits

Different starches exhibit certain differences in molecular composition and spatial structure. Han Rui et al. [19] demonstrated that different starches have varying energy supply efficiencies due to differences in internal structure, with the amylose/amylopectin ratio affecting animal metabolizable energy—diets with higher amylose proportions had lower metabolizable energy. Zhang Aoran et al. [20] reported that feeding piglets with corn ingredients higher in amylopectin content significantly improved digestible energy and energy digestibility. Dai Qiuzhong et al. [21] showed that starch is primarily absorbed by the body in the forms of glucose and volatile fatty acids, with the latter having lower effective energy values and energy utilization efficiency. Bin Shiyu et al. [22] fed piglets diets formulated with corn, glutinous rice, and RS, finding that the RS group had lower metabolizable energy than the other two groups. It has been reported that RS can significantly reduce starch digestibility and energy utilization efficiency [23], while starch digestibility shows high correlation with metabolizable energy [24].

Cassava starch is high in RDS and low in RS, with most being absorbed in the small intestine and showing high digestibility. Potato starch is high in RS and low in RDS, being barely digested in the small intestine and entering the large intestine for microbial fermentation and degradation, resulting in low digestibility, and RS can directly affect metabolizable energy. Corn starch has relatively high amylose content, with higher RDS content and lower RS content compared to potato starch. The results of this experiment revealed that endogenous energy losses differed when Rex rabbits consumed nitrogen-free diets with different starch sources, with the cassava starch group showing the highest endogenous energy loss, the potato starch group the lowest, and the corn starch group intermediate levels. These differences may be related to variations in the nutritional composition and digestibility of the starches.

### Conclusion

Although different starch sources did not significantly affect MFN, EUN, FmE, or UeE in Rex rabbits, they still exerted certain influences on endogenous nitrogen and energy losses. These factors should be fully considered in practice to improve the accuracy of endogenous loss determination.

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