

## Effects of Different Dietary Replacement Levels of Silage Ramie on Growth Performance, Intestinal Development, Apparent Nutrient Metabolic Rate, and Serum Biochemical Indices in Landes Geese (Postprint)

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**Date:** 2018-12-24T00:00:00+00:00

### Abstract

This study aimed to investigate the effects of dietary replacement of basal diet with varying proportions of ramie silage on growth performance, intestinal development, nutrient apparent metabolic rate, and serum biochemical parameters in Landes geese. Three hundred sixty healthy 21-day-old Landes geese with similar body weight were randomly assigned to 5 treatments, each comprising 9 replicates of 8 geese. Treatment I (control) received the basal diet, while experimental treatments (Treatments II-V) received diets in which 20%, 30%, 40%, and 50% of the basal diet was replaced by ramie silage, respectively. The trial lasted 43 days. The results indicated: 1) Average daily feed intake of geese in Treatments II-V was significantly higher than that in Treatment I ( $P < 0.05$ ), and feed-to-gain ratio of geese in Treatments III, IV, and V was significantly higher than that in Treatment I ( $P < 0.05$ ); geese in Treatment II exhibited the highest average daily gain, though no significant differences were observed among treatments ( $P > 0.05$ ). 2) No significant differences were detected among treatments in total intestinal length, duodenal length, jejunoileal length, cecal length, or intestinal weight ( $P > 0.05$ ); however, ileal villus height and villus height-to-crypt depth ratio in Treatment II were significantly greater than those in Treatment I ( $P < 0.05$ ). 3) Apparent metabolic rates of gross energy and crude protein in Treatment I were significantly higher than in other treatments ( $P < 0.05$ ); apparent metabolic rate of crude fat in Treatments I and III was significantly higher than in other treatments ( $P < 0.05$ ); and apparent metabolic rate of crude fiber in Treatments III and V was significantly higher than in other treatments ( $P < 0.05$ ). 4) Serum total protein, albumin, globulin, glucose, total cholesterol, and urea

concentrations did not differ significantly among treatments ( $P>0.05$ ). In summary, under the present experimental conditions and based on growth performance, nutrient apparent metabolic rate, intestinal development, and serum biochemical indices as evaluation criteria, dietary inclusion of 20% ramie silage as a replacement for basal diet proved optimal for Landes geese aged 21 to 64 days.

## Full Text

### Effects of Different Proportions of Silage Ramie Instead of Basal Diet on Growth Performance, Intestinal Development, Nutrient Apparent Metabolic Rate and Serum Biochemical Indices of Landes Geese

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## Abstract

This experiment was conducted to investigate the effects of different proportions of silage ramie replacing the basal diet on growth performance, intestinal development, nutrient apparent metabolic rate, and serum biochemical indices of Landes geese. A total of 360 healthy 21-day-old Landes geese with similar body weight were randomly allocated to 5 groups with 9 replicates per group and 8 geese per replicate. Geese in group (control group) were fed a basal diet, while those in test groups (groups - ) were fed experimental diets in which silage ramie replaced 20%, 30%, 40%, and 50% of the basal diet, respectively. The experimental period lasted 43 days. The results showed: (1) The average daily feed intake (ADFI) of geese in groups - was significantly higher than that in group ( $P<0.05$ ), and the feed-to-gain ratio (F/G) of geese in groups , , and was significantly higher than that in group ( $P<0.05$ ). Geese in group achieved the highest average daily gain (ADG), though no significant differences in ADG were observed among all groups ( $P>0.05$ ). (2) No significant differences were found among groups in total intestinal length, duodenal length, jejunum+ileum length, cecum length, or intestinal weight ( $P>0.05$ ). However, the ileal villus height and villus height/crypt depth ratio of geese in group were significantly higher than those in group ( $P<0.05$ ). (3) The apparent metabolic rates of gross energy and crude protein in group were significantly higher than those in other groups ( $P<0.05$ ). The apparent metabolic rate of ether extract in groups and was significantly higher than that in other groups ( $P<0.05$ ), while the apparent metabolic rate of crude fiber in groups and was significantly higher than that

in other groups ( $P < 0.05$ ). (4) No significant differences were observed among groups in serum total protein, albumin, globulin, glucose, total cholesterol, or urea content ( $P > 0.05$ ). In conclusion, under the conditions of this experiment and based on the evaluation of growth performance, nutrient apparent metabolic rate, intestinal development, and serum biochemical indices, replacing 20% of the basal diet with silage ramie was optimal for 21- to 64-day-old Landes geese.

**Keywords:** silage ramie; Landes geese; growth performance; intestinal development; nutrient apparent metabolic rate

## Introduction

The shortage of protein-rich forage resources in southern China has become a major challenge constraining the development of animal husbandry in the region. The Ministry of Agriculture explicitly stated in the *National Planting Structure Adjustment Plan (2016-2020)* that southern China should focus on developing unconventional feed resources such as forage ramie, rapeseed, and mulberry leaves. Ramie is characterized by rapid growth, high biological yield, and nutrient-rich tender stems and leaves with high crude protein content and balanced amino acid composition, demonstrating great potential for development as a protein feed 原料. Geese, as poultry capable of consuming high-fiber forage to meet their growth requirements, have shown certain feeding effects when fed fresh ramie. However, the high content of anti-nutritional factors such as tannins and abundant non-starch polysaccharides like cellulose in ramie negatively affect its palatability and feeding value. Silage technology addresses this by sealing fresh green forage in silos or wrapped bales for anaerobic microbial fermentation, which preserves certain original characteristics of the green forage while degrading anti-nutritional factors such as non-starch polysaccharides, thereby improving palatability and available nutrient levels.

Wu Duanqin et al. found that silage ramie has a soft texture and aromatic smell, with crude protein, ether extract, crude fiber, and crude ash contents all higher than those of alfalfa hay. Their study also revealed that replacing 33%-67% of alfalfa hay with silage ramie in dairy cow diets did not affect production performance, milk composition, or serum biochemical indices, demonstrating good feeding effects. Gao Gang et al. investigated the effects of ramie tender stem and leaf silage on meat quality and fattening performance of Boer goats, finding that adding ramie silage to total mixed rations did not affect normal feed intake. Compared with alfalfa hay, feeding ramie silage significantly improved weight gain, reduced stearic acid content in mutton, increased flavor amino acid content, and improved meat quality. However, overall, no relevant research reports on feeding silage ramie to geese have been published domestically or internationally. Therefore, this experiment used Landes geese as experimental animals to investigate the effects of different proportions of silage ramie replacing the basal diet on growth performance, intestinal development, nutrient apparent metabolic rate, and serum biochemical indices, aiming to comprehensively evaluate the effects of feeding silage ramie to geese and provide a theoretical basis

for its rational application in meat goose production.

## Materials and Methods

### 1.1 Silage Ramie Preparation

Ramie fresh grass at approximately 1.5 m plant height was harvested, chopped to about 3 cm using a rubbing cutter, and then baled and sealed with a film-wrapping machine for ensiling. The nutrient composition of silage ramie was consistent with that reported by Wu Duanqin et al.

### 1.2 Basal Diet

The basal diet was formulated with corn, soybean meal, and other main ingredients according to the NRC (1994) nutrient requirements for geese. The composition and nutrient levels of the basal diet are shown in Table 1 .

### 1.3 Experimental Animals and Design

A total of 360 healthy 21-day-old Landes geese with similar body weight were randomly divided into 5 groups with 9 replicates per group and 8 geese per replicate. No significant differences were observed in average initial body weight among groups ( $P>0.05$ ). Group (control) was fed the basal diet, while test groups (groups - ) were fed experimental diets in which silage ramie replaced 20%, 30%, 40%, and 50% of the basal diet, respectively. The experimental period lasted 43 days.

### 1.4 Management

The experiment was conducted at the experimental goose farm of the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. Geese were raised on net floors with conventional immunization, free access to feed and water, 24-hour lighting, natural ventilation, normal temperature, and relative humidity of  $(60\pm 5)\%$ . Goose houses were cleaned of manure once daily.

### 1.5 Measured Nutrient Levels of Experimental Diets

The measured nutrient levels of the basal diet and experimental diets with different proportions of silage ramie were as follows: basal diet contained 17.88% crude protein and 3.13% crude fiber; group diet contained 18.32% crude protein and 9.60% crude fiber; group diet contained 18.49% crude protein and 12.70% crude fiber; group diet contained 18.68% crude protein and 16.11% crude fiber; and group diet contained 18.83% crude protein and 19.27% crude fiber.

## 1.6 Measurement Indicators

**1.6.1 Growth Performance** At 21 and 64 days of age, geese were weighed at 08:00 after 12 hours of feed deprivation. During the experimental period, daily feed consumption and residual feed were recorded by replicate to calculate average initial weight, average final weight, average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G).

**1.6.2 Intestinal Development and Mucosal Morphology** At 64 days of age, one healthy goose per replicate with body weight close to the group average, normal feed intake, and no abnormal behaviors was selected from each group. After slaughter by neck bleeding, the intestine was rapidly isolated to measure total intestinal length and weight. The duodenum, jejunum, ileum, and cecum were then separated to measure each segment length. Approximately 1.5 cm sections from the middle of the duodenum, jejunum, and ileum were gently cleared of contents with a glass rod, rinsed with saline, dried with filter paper, and fixed in 10% formaldehyde phosphate buffer. The fixed specimens were processed through dehydration, transparency, paraffin embedding, sectioning, and routine hematoxylin-eosin (HE) staining to prepare 4–6  $\mu$ m thick paraffin sections. Qualified sections were observed under a microscope at 40 $\times$  and 100 $\times$  magnification, with multiple non-consecutive fields randomly selected and typical fields photographed. Image analysis software was used to measure villus height, crypt depth, and intestinal wall thickness, and to calculate the villus height/crypt depth ratio.

**1.6.3 Nutrient Apparent Metabolic Rate** At 64 days of age, one healthy goose per replicate with body weight close to the group average, normal feed intake, and no abnormal behaviors was selected from each group and moved to metabolic cages for adaptation and metabolic trials. The metabolic trial used the forced-feeding method after fasting, consisting of four periods: pre-feeding, fasting and emptying, forced-feeding, and excreta collection. The pre-feeding period lasted 7 days, followed by a 4-day formal trial period. The first 2 days constituted the fasting and emptying period, during which each goose received 50 g of glucose daily through drinking water. On day 3, forced-feeding was conducted with 50 g of experimental diet per goose using a force-feeding device, with individual records maintained. After forced-feeding, total excreta collection was performed for 48 h, with multiple collections daily. To prevent sample spoilage and ammonia volatilization during storage, 0.1 mol/L HCl was added and mixed thoroughly after each collection, then immediately stored at -20°C. After complete collection, excreta were dried in a 60–65°C oven with forced air to constant weight, equilibrated at room temperature for 24 h, weighed to obtain air-dry weight, ground, passed through a 40-mesh sieve, mixed thoroughly, and stored in sealed bags for analysis.

Gross energy was determined using an automatic oxygen bomb calorimeter, crude protein by the Kjeldahl method, ether extract by Soxhlet extraction, and

crude fiber by acid-alkali digestion. Nutrient apparent metabolic rate was calculated as follows:

$$\text{Nutrient apparent metabolic rate (\%)} = 100 \times (\text{nutrient intake} - \text{nutrient excretion}) / \text{nutrient intake}$$

**1.6.4 Serum Biochemical Indices** At 64 days of age, one healthy goose per replicate with body weight close to the group average, normal feed intake, and no abnormal behaviors was selected from each group. Blood (8 mL) was collected from the wing vein into 10 mL centrifuge tubes, placed at an angle to allow natural coagulation. After 30 minutes, serum was separated by centrifugation at 3,000 r/min for 15 minutes and stored at -20°C for analysis. Glucose (GLU) was determined by the glucose oxidase method, total cholesterol (TC) by the cholesterol oxidase method, urea (UREA) by the urease method, total protein (TP) by the biuret method, and albumin (ALB) by the bromocresol green method. All indices were measured using corresponding kits from Guilin Urit Medical Electronic Co., Ltd. on a URIT-8000 automatic biochemical analyzer (Guilin Urit Medical Electronic Co., Ltd.). Globulin (GLO) content was calculated as: globulin content = total protein content - albumin content.

## 1.7 Statistical Analysis

Experimental data were preliminarily processed using Excel 2003 software and analyzed by one-way ANOVA using SPSS 13.0 statistical software. If significant differences were detected among groups, Duncan's multiple comparison test was performed with a significance level of  $P < 0.05$ . Results were expressed as "mean  $\pm$  standard deviation."

## Results

### 2.1 Effects on Growth Performance

The effects of different proportions of silage ramie replacing the basal diet on growth performance of Landes geese are shown in Table 2. The ADFI and F/G of geese increased gradually with increasing replacement proportion of silage ramie. Compared with group , ADFI of geese in all silage ramie replacement groups was significantly higher ( $P < 0.05$ ), while F/G of geese in groups , , and was also significantly higher ( $P < 0.05$ ). No significant differences in ADG were observed among groups ( $P > 0.05$ ), though geese in group achieved the optimal ADG, which was 8.77% higher than that in group ( $P > 0.05$ ). No significant differences were found in average initial weight or average final weight among groups ( $P > 0.05$ ).

### 2.2 Effects on Intestinal Development and Mucosal Morphology

The effects of different proportions of silage ramie replacing the basal diet on intestinal development of Landes geese are shown in Table 3. No significant dif-

ferences were observed among groups in total intestinal length, duodenal length, jejunum+ileum length, cecum length, or intestinal weight ( $P>0.05$ ). However, compared with group , intestinal weight, total intestinal length, jejunum+ileum length, and cecum length in all silage ramie replacement groups tended to increase ( $P>0.05$ ).

The effects on intestinal mucosal morphology are shown in Table 4 . Compared with group , the ileal villus height and villus height/crypt depth ratio of geese in group were significantly increased ( $P<0.05$ ). No significant differences were observed among groups in duodenal or jejunal villus height, crypt depth, intestinal wall thickness, or villus height/crypt depth ratio ( $P>0.05$ ).

### 2.3 Effects on Nutrient Apparent Metabolic Rate

The effects of different proportions of silage ramie replacing the basal diet on nutrient apparent metabolic rate of Landes geese are shown in Table 5 . The apparent metabolic rates of gross energy and crude protein in group were significantly higher than those in other groups ( $P<0.05$ ). The apparent metabolic rate of ether extract in groups and was significantly higher than that in other groups ( $P<0.05$ ), while the apparent metabolic rate of crude fiber in groups and was significantly higher than that in other groups ( $P<0.05$ ).

### 2.4 Effects on Serum Biochemical Indices

The effects of different proportions of silage ramie replacing the basal diet on serum biochemical indices of Landes geese are shown in Table 6 . No significant differences were observed among groups in serum total protein, albumin, globulin, glucose, total cholesterol, or urea content ( $P>0.05$ ).

## Discussion

### 3.1 Effects on Growth Performance

Currently, few studies have reported on the application of ramie in meat goose diets. Jiang Guitao et al. investigated the effects of different ramie-to-concentrate ratios in diets on growth performance and intestinal mucosal morphology of Landes geese, finding that based on growth performance and intestinal morphological development, the proportion of ramie in diets should be controlled below 50% during the 2-4 and 5-7 week age periods, with concentrate supplementation in later stages achieving effects comparable to continuous concentrate feeding. Li Chuang et al. studied the effects of different concentrate-to-ramie ratios on growth performance of Xiangbai geese, reporting that a 1:3 concentrate-to-ramie ratio during the 2-4 week age period and a 1:4 ratio during the 5-7 week age period, followed by concentrate supplementation, yielded better production benefits. However, no research reports on the application of silage ramie in meat goose diets have been published. In this experiment, ADFI and F/G of geese increased gradually with increasing silage ramie replacement proportion, while

no significant differences in ADG were observed among groups. These results are consistent with Chen et al.'s study on the effects of different dietary fiber levels on growth performance of Roman geese, which found that as dietary fiber level increased, ADG of 7-10 week-old geese did not change significantly, while ADFI and F/G increased. In this experiment, group with 20% silage ramie replacement achieved the optimal ADG, and its F/G did not differ significantly from group fed the basal diet.

### **3.2 Effects on Intestinal Development and Mucosal Morphology**

Previous studies have reported that dietary fiber level affects digestive tract development in geese, with more pronounced effects on length and weight of various intestinal segments in goslings. Yang Shuming et al. reported that as dietary alfalfa meal content increased, the proportion of small intestine and cecum weight to total digestive tract weight in Huoyan geese increased. Zhou Xiuli et al. demonstrated that dietary alfalfa content significantly affected cecum length and weight in geese. Meanwhile, intestinal villus height, crypt depth, and their ratio are important indicators for evaluating intestinal digestion and absorption function. Increased villus height enhances nutrient absorption capacity, crypt depth reflects intestinal epithelial cell renewal rate, and the villus height/crypt depth ratio comprehensively reflects small intestinal functional status. An increased villus height/crypt depth ratio indicates larger intestinal mucosal area, improved intestinal mucosal structure, increased villus cell number per unit area, and enhanced digestion and absorption function. In this experiment, intestinal weight, total intestinal length, and length of each intestinal segment tended to increase in all silage ramie replacement groups. Additionally, group with 20% silage ramie replacement showed significantly increased ileal villus height and villus height/crypt depth ratio, indicating that appropriate proportions of silage ramie replacing the basal diet can improve intestinal development and mucosal morphology in Landes geese.

### **3.3 Effects on Nutrient Apparent Metabolic Rate and Serum Biochemical Indices**

Many reports have confirmed that as dietary fiber level increases, the metabolic rate of crude fiber in geese increases, while the metabolic rate of other nutrients (such as crude protein) decreases, which is consistent with this experiment's results. In this study, as silage ramie replacement proportion increased, the apparent metabolic rate of crude fiber in geese gradually increased, while the apparent metabolic rates of gross energy and crude protein gradually decreased. This may be attributed to the fact that geese are poultry capable of utilizing large amounts of green roughage, while the reduction in metabolic rate of other nutrients is mainly related to the effect of dietary fiber level on feed retention time and retention rate in the digestive tract. However, in this experiment, feed intake of geese increased with increasing silage ramie replacement level, which offset the negative effects of decreased nutrient apparent metabolic rate

on growth performance. Among all treatments, group with 20% silage ramie replacement not only achieved the best ADG but also showed no significant difference in F/G compared with group fed the basal diet.

Serum physiological and biochemical indices can serve as good indicators of physiological condition and health status in animals. In this experiment, no significant differences were observed among groups in serum total protein, albumin, globulin, glucose, total cholesterol, or urea content, indicating that silage ramie can partially replace the basal diet for feeding Landes geese without affecting their physiological and biochemical status or health.

## Conclusion

Based on the comprehensive evaluation of growth performance, nutrient apparent metabolic rate, intestinal development, and serum biochemical indices under the conditions of this experiment, replacing 20% of the basal diet with silage ramie was optimal for 21- to 64-day-old Landes geese.

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