# Effects of Dietary Cellulase Supplementation in Peanut Vine-Based Diets on Growth Performance, Apparent Nutrient Utilization, and Digestive Enzyme Activity in 5- to 8-Week-Old Zhedong White Geese (Postprint)

**Authors:** Wang Huiying, Guo Baodi, He Daqian, Gong Shaoming, Liu Yi, Li Guangquan, Xu Qi, Chen Guohong

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## Abstract

This study aimed to investigate the effects of dietary cellulase supplementation in peanut vine-based diets on growth performance, apparent nutrient utilization, and digestive enzyme activities in meat geese. A total of 120 healthy 4-week-old Zhedong white geese with similar body weight were randomly allocated into three groups, which were fed experimental diets containing 15% (Group A), 23% (Group B), and 23% (Group C) peanut vine powder supplemented to a basal diet, respectively, with Group C additionally receiving 3‰ cellulase. Each group consisted of 5 replicates with 8 geese per replicate (half male and half female). Measured parameters included body weight, feed intake, apparent nutrient utilization, and endogenous digestive enzyme activities in the pancreas, gizzard, and duodenum. The results demonstrated that, compared with Group B, supplementation of 3‰ cellulase in the peanut vine diet (crude fiber level of 9.76%) 1) significantly increased the average daily gain of meat geese ($P<0.05$), while exerting no significant effects on average daily feed intake or feed-to-gain ratio ($P>0.05$); 2) significantly or highly significantly improved the apparent utilization of crude ash, crude protein, crude fiber, calcium, total phosphorus, glycine, cysteine, and methionine in the diet ($P<0.05$ or $P<0.01$); and 3) significantly or highly significantly enhanced pancreatic protease and amylase activities, as well as gizzard protease and duodenal lipase activities ($P<0.05$ or $P<0.01$). In summary, supplementation of 3‰ cellulase in high-crude-fiber peanut vine diets can enhance digestive enzyme activities in the gastrointestinal tract of meat geese and improve their apparent utilization of nutrients from high-fiber diets, representing an effective approach for the development and utilization of peanut vine feed resources.

## Full Text

# Effects of Cellulase Added to Peanut Seedling Diets on Growth Performance, Nutrient Apparent Utilization, and Digestive Enzyme Activity in 5–8-Week-Old Zhedong White Geese

**WANG Huiying[1], GUO Baodi[1], HE Daqian[2], GONG Shaoming[2], LIU Yi[2], LI Guangquan[2], XU Qi[1], CHEN Guohong[1]***

[1]Animal Science and Technology College, Yangzhou University, Yangzhou 225009, China
[2]Institute of Animal Husbandry and Veterinary Medicine, Shanghai Academy of Agricultural Sciences, Shanghai 201106, China

**Abstract:** This study investigated the effects of dietary cellulase supplementation on growth performance, nutrient apparent utilization, and digestive enzyme activity in meat geese fed peanut seedling diets. A total of 120 healthy four-week-old Zhedong white geese with similar body weights were randomly allocated into three groups. The geese were fed experimental diets containing 15% (Group A), 23% (Group B), or 23% (Group C) peanut seedling powder, with Group C receiving an additional 3‰ cellulase. Each group comprised five replicates of eight geese each, with equal numbers of males and females. Measured parameters included body weight, feed intake, nutrient apparent utilization, and endogenous digestive enzyme activities in the pancreas, muscular stomach, and duodenum. Compared with Group B, the addition of 3‰ cellulase to peanut seedling diets (9.76% crude fiber level) significantly increased average daily gain ($P<0.05$) without significantly affecting average daily feed intake or feed-to-gain ratio ($P>0.05$). Cellulase supplementation also significantly or highly significantly improved apparent utilization of crude ash, crude protein, crude fiber, calcium, total phosphorus, glycine, cysteine, and methionine ($P<0.05$ or $P<0.01$). Furthermore, it significantly or highly significantly enhanced pancreatic protease and amylase activities, muscular stomach protease activity, and duodenal lipase activity ($P<0.05$ or $P<0.01$). In conclusion, adding 3‰ cellulase to high-fiber peanut seedling diets can enhance digestive enzyme activity, improve nutrient apparent utilization, and represents an effective approach for developing and utilizing peanut seedling feed resources.

**Keywords:** peanut seedling; cellulase; Zhedong white geese; body weight; nutrient utilization; digestive enzyme activity

Peanut seedlings are characterized by soft texture and are rich in crude protein, crude fat, various minerals, and vitamins, with good palatability and high yield reaching 27–30 million tons annually. As a byproduct of peanut production, they can be used as roughage for geese, meeting the high dietary fiber requirements of these birds. Effective development of peanut seedling feed resources can not only alleviate the prominent contradiction of competition for grain between humans and livestock but also provide an effective solution to the shortage of

green forage sources for meat geese during autumn and winter.

Cellulase refers to a multi-enzyme complex that effectively degrades cellulose, including endoglucanase, exoglucanase or cellobiase, and -glucosidase. These three enzymes work synergistically to hydrolyze cellulose crystals, thereby improving animal utilization of high-fiber feed and reducing feeding costs. Research and application of cellulase have attracted increasing attention, achieving certain results in animal husbandry. Zhang et al. reported that compared with untreated roughage, cellulase treatment improved dry matter digestibility and reduced crude fiber, crude protein, crude fat, and crude ash content in six types of roughage fed to sheep. Wang et al. and Yuan et al. found that dietary cellulase supplementation significantly improved feed utilization and daily weight gain in growing pigs, demonstrating that cellulase can address the problem of low cellulose utilization. Additionally, numerous studies have shown that cellulase can significantly improve apparent diet digestibility, crude protein utilization, energy utilization, and metabolizable energy values in poultry. Although geese can consume large amounts of green forage, few studies have examined how to reduce the impact of anti-nutritional factors such as cellulose and improve the digestibility of nutrients in roughage. Therefore, this study used Zhedong white geese to investigate the effects of cellulase supplementation in peanut seedling diets on growth performance, nutrient apparent utilization, and endogenous digestive enzyme activities in the pancreas, muscular stomach, and duodenum, aiming to provide valuable reference data for developing peanut seedling feed resources, improving nutrient digestibility in roughage, reducing feeding costs, and enhancing economic benefits.

### 1.1 Experimental Materials

**Peanut seedling powder:** Naturally air-dried peanut seedlings were processed using a peanut seedling film-removing and kneading machine at the Rizhao Yongjie Biomass Resource Development Co., Ltd. base. The product contained 9.5 MJ/kg digestible energy, 11.00% crude protein, 2.96% crude fat, and 19.80% crude fiber.

**Cellulase:** Produced by Shanghai Mengjiu Industrial Co., Ltd., with enzyme activity of 20,000 U/g.

### 1.2 Experimental Animals and Design

The experiment utilized 120 healthy four-week-old Zhedong white geese with similar body weights, randomly divided into three groups. The geese were fed experimental diets containing 15% (Group A), 23% (Group B), or 23% (Group C) peanut seedling powder, with Group C receiving an additional 3‰ cellulase. Each group consisted of five replicates with eight geese each, with equal numbers of males and females. The experiment included a one-week pre-trial period and a four-week formal trial period. The composition and nutrient levels of the basal diet are shown in Table 1 .

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

| Items | Groups |
|---|---|
| **Ingredients** | |
| Corn | |
| Soybean meal | |
| Bran | |
| Soybean germ meal | |
| Peanut vine powder | |
| Cellulase | |
| CaHPO | |
| NaCl | |
| Premix[1] | |
| **Total** | |
| **Nutrient levels[2]** | |
| ME (MJ/kg) | |
| Crude protein CP | |
| Crude fat EE | |
| Crude fiber CF | |
| Total phosphorus TP | |

[1]One kilogram of the premix contained the following: Fe 100 mg, Cu 8 mg, Mn 120 mg, Zn 100 mg, Se 0.4 mg, Co 1.0 mg, I 0.4 mg, VA 8,330 IU, VB 2.0 mg, VB 8 mg, VB 1.2 mg, VB 0.03 mg, VD 1,440 IU, VE 30 IU, biotin 0.2 mg, folic acid 2.0 mg, pantothenic acid 20 mg, niacin acid 40 mg.

[2]ME was a calculated value, while others were measured values.

### 1.3 Feeding Management

Geese were housed indoors throughout the experimental period, raised on net floors with ad libitum access to feed and water, and vaccinated according to routine immunization programs.

### 1.4 Growth Performance Measurement

Body weight of meat geese was measured using an electronic scale at the beginning and end of the trial, feed addition was recorded, and feed-to-gain ratio was calculated.

### 1.5 Nutrient Apparent Utilization Measurement

Before the end of the feeding trial, two healthy geese per replicate were selected based on average body weight. Using acid-insoluble ash (AIA) as an indicator, excreta were collected individually for three consecutive days. Feathers and skin debris were removed from feces with tweezers, nitrogen was fixed with

10% hydrochloric acid, samples were mixed, dried in a 65°C oven, rehydrated naturally for 24 hours, then crushed and prepared for analysis. Crude protein content was determined by the Kjeldahl method (GB/T 6432-1994); crude fat by ether extraction (GB/T 6433-2006); crude fiber by acid-alkali digestion (GB/T 6434-1994); calcium by potassium permanganate titration (GB/T 6436-2002); phosphorus by molybdenum yellow colorimetry (GB/T 6437-2002); and amino acid content by Hitachi L-8800 automatic amino acid analyzer (GB/T 18246-2000). Apparent utilization of a nutrient was calculated using the following formula:

Apparent utilization of a nutrient (%) = 100 - 100 × (b × c) / (a × d)

Where: a = nutrient content in diet (%); b = nutrient content in feces (%); c = hydrochloric acid-insoluble ash content in diet (%); d = hydrochloric acid-insoluble ash content in feces (%).

### 1.6 Digestive Enzyme Activity Measurement

From each replicate, one male and one female eight-week-old goose with body weight close to the group average were selected (30 geese total). After rapid euthanasia, the abdominal cavity was immediately opened to isolate the muscular stomach (with contents), duodenum (with chyme), and pancreas. Samples were snap-frozen in liquid nitrogen and stored at -80°C. Protease, amylase, and lipase activities in the pancreas, muscular stomach, and duodenum were determined according to kit instructions from Nanjing Jiancheng Bioengineering Institute.

### 1.7 Data Processing and Analysis

Excel 2007 was used for data entry and organization. SPSS 20.0 Descriptive module was used to calculate means ± standard deviations. One-way ANOVA was used to test for significant differences among treatment groups. $P<0.05$ was considered significant, and $P<0.01$ was considered highly significant.

### 2.1 Effects of Cellulase on Growth Performance of Meat Geese

As shown in Table 2 , compared with Group A, Group B showed significantly lower average daily gain ($P<0.05$). Cellulase supplementation significantly increased average daily gain ($P<0.05$) but had no significant effect on average daily feed intake or feed-to-gain ratio ($P>0.05$).

**Table 2 Effects of cellulase added to peanut seedling diets on growth performance of geese at 5–8 weeks of age**

| Groups | Average daily gain ADG (g/d) | Average daily feed intake ADFI (g/d) | Feed-to-gain ratio |
|---|---|---|---|
| A | 88.70±7.42b | 371.66±32.63a | 4.19±0.84a |
| B | 78.69±6.28a | 397.38±27.12ab | 5.05±0.57b |

| Groups | Average daily gain ADG (g/d) | Average daily feed intake ADFI (g/d) | Feed-to-gain ratio |
|---|---|---|---|
| C | 85.04±9.51b | 436.31±23.49b | 5.13±0.69b |

Values with different small letter superscripts in the same column indicate significant difference (P<0.05), and different capital letter superscripts indicate highly significant difference (P<0.01). The same applies below.

### 2.2.1 Effects of Cellulase on Conventional Nutrient Apparent Utilization

As shown in Table 3 , compared with Group A, Group B showed significantly or highly significantly reduced apparent utilization of crude protein, crude ash, total phosphorus, and crude fiber (P<0.05 or P<0.01). Cellulase supplementation significantly or highly significantly improved apparent utilization of crude protein, crude ash, calcium, total phosphorus, and crude fiber (P<0.05 or P<0.01), but had minimal effect on crude fat utilization (P>0.05).

**Table 3 Effects of cellulase added to peanut seedling diets on nutrient apparent utilization of geese**

| Groups | Crude protein | Crude fat | Calcium | Total phosphorus | Crude fiber |
|---|---|---|---|---|---|
| A | 25.27±3.56BCb | 38.09±3.70a | 19.52±1.74b | 52.91±5.79a | 31.52±3.31Bb |
| B | 17.66±2.91Bb | 17.88±2.92Aa | 41.52±3.85a | 33.56±1.75a | 51.56±5.00a |
| C | 13.54±1.63Aa | 7.22±0.78Aa | 36.02±3.42ab | 46.06±4.73b | 20.03±2.28b |

### 2.2.2 Effects of Cellulase on Amino Acid Apparent Utilization

As shown in Table 4 , compared with Group A, Group B showed significantly reduced apparent utilization of glycine, cysteine, and methionine (P<0.05). Cellulase supplementation significantly improved apparent utilization of glycine and cysteine (P<0.05) but had no significant effect on other amino acids (P>0.05).

**Table 4 Effects of cellulase added to peanut seedling diets on amino acid apparent utilization of geese**

| Groups | Asp | Cys | Gly | Met | Others |
|---|---|---|---|---|---|
| A | 73.63±3.86b | 67.7±5.97b | 71.89±1.96a | 81.45±4.79a | ··· |
| B | 66.79±4.44a | 64.61±4.79a | 67.71±4.95ab | 77.95±71.62± | ··· |
| C | 73.55±2.85b | 68.61±5.27b | 72.44±4.95ab | 81.86±32.75± | ··· |

### 2.3.1 Effects of Cellulase on Pancreatic Digestive Enzyme Activity

As shown in Table 5 , compared with Group A, Group B showed significantly reduced pancreatic amylase activity (P<0.05). Cellulase supplementation significantly increased this enzyme activity (P<0.05) and highly significantly enhanced pancreatic protease activity (P<0.01), but had no significant effect on pancreatic lipase activity (P>0.05).

**Table 5 Effects of cellulase added to peanut seedling diets on digestive enzyme activity in the pancreas of geese**

| Groups | Protease PRO | Amylase AMS | Lipase LPS |
|---|---|---|---|
| A | 96.61±9.53Aa | 940.15±81.35b | 160.65±10.73 |
| B | 114.18±7.06Bb | 782.08±71.75a | 154.19±7.65 |
| C | 157.62±9.42Cc | 1,010.77±109.14b | 179.28±17.02 |

### 2.3.2 Effects of Cellulase on Muscular Stomach Digestive Enzyme Activity

As shown in Table 6 , dietary cellulase supplementation had no significant effect on muscular stomach amylase or lipase activities (P>0.05) but showed a tendency to increase muscular stomach protease activity (P>0.05).

**Table 6 Effects of cellulase added to peanut seedling diets on digestive enzyme activity in the muscular stomach of geese**

| Groups | Protease PRO | Amylase AMS | Lipase LPS |
|---|---|---|---|
| A | 17.80±1.73a | 13.75±1.20 | 110.94±10.77 |
| B | 20.31±2.87ab | 14.41±1.85 | 107.08±9.26 |
| C | 25.32±1.16b | 13.71±1.92 | 111.54±12.67 |

### 2.3.3 Effects of Cellulase on Duodenal Digestive Enzyme Activity

As shown in Table 7 , compared with Group A, Group B showed highly significantly increased duodenal amylase activity (P<0.01) and significantly decreased lipase activity (P<0.05). Cellulase supplementation significantly increased duodenal lipase activity (P<0.05) and highly significantly decreased amylase activity (P<0.01).

**Table 7 Effects of cellulase added to peanut seedling diets on digestive enzyme activity in the duodenum of geese**

| Groups | Amylase AMS | Lipase LPS |
|---|---|---|
| A | 28.25±1.4Aa | 11.20±1.96b |
| B | 42.63±4.68Bb | 8.97±0.94a |

| Groups | Amylase AMS | Lipase LPS |
|---|---|---|
| C | 31.97±4.42Aa | 12.31±1.57b |

## 3 Discussion

### 3.1 Effects of Cellulase on Growth Performance

Cellulose generally refers to the indigestible plant cell wall components that cannot be broken down by gastrointestinal enzymes. Animals utilize crude fiber primarily through microbial enzyme decomposition products or microbial metabolites. Due to the specificity of microbial enzymes in decomposing crude fiber, the breakdown is often incomplete, representing a major limiting factor in fiber utilization. Cellulose degradation is a complex process requiring multiple types of cellulases working together. As a multi-component protein mixture produced by organisms, cellulase can hydrolyze insoluble cellulose into soluble sugars under appropriate conditions, thereby improving feed utilization value. Li et al. reported that adding 0.2% cellulase to corn-soybean meal + 15% wheat bran diets for Duroc × Landrace × Yorkshire crossbred pigs increased average daily gain by 7.3% and improved feed conversion efficiency. Yang et al. found that 0.1% cellulase supplementation in diets for 15 kg growing pigs increased daily gain by 5.64% compared with the control group, with highly significant differences. Other studies have shown that cellulase supplementation in high-fiber diets significantly improves growth performance in geese and increases daily weight gain while reducing feed consumption in broilers. In this experiment, when dietary crude fiber reached 9.75%, growth performance decreased, but cellulase supplementation increased body weight without significantly changing the feed-to-gain ratio, indicating that cellulase benefits meat goose development. This may occur because cellulase reduces cellulose binding to digestive enzymes, thereby improving nutrient utilization, increasing growth rate, reducing feed-to-gain ratio, and improving growth uniformity. Additionally, some cellulose may be degraded into digestible reducing sugars, enhancing dietary nutritional value and promoting goose growth.

### 3.2 Effects of Cellulase on Nutrient Apparent Utilization

Numerous studies have investigated the effects of cellulase-supplemented diets on nutrient utilization in livestock and poultry. Saleh et al. reported that a mixture of cellulase, hemicellulase, and pectinase added to broiler corn-soybean meal diets significantly improved organic matter and crude protein digestibility compared with the control group. Zhang et al. demonstrated that cellulase supplementation in high-cassava residue diets significantly improved crude protein and crude fiber digestibility in growing pigs, with some improvement in dry matter and energy apparent digestibility. Olkowski reported that a pectinase- and hemicellulase-based enzyme complex significantly improved organic matter and crude protein digestibility in chicks fed lupin diets. Other studies have shown

that different types and levels of fiber can affect total nitrogen and amino acid excretion in pig ileal digesta. This experiment demonstrated that increasing dietary crude fiber levels significantly reduced apparent utilization of crude ash and crude protein, and highly significantly reduced utilization of total phosphorus and crude fiber. After cellulase supplementation, apparent utilization of crude protein, total phosphorus, and crude fiber increased highly significantly compared with the unsupplemented group, even reaching or exceeding levels observed with lower fiber diets. Apparent utilization of crude ash and calcium increased significantly, while crude fat utilization showed minimal improvement. These results indicate that exogenous cellulase can hydrolyze cellulose crystal structures in cell walls, effectively degrading cellulose, hemicellulose, and pectin components in peanut seedlings. This removes barriers to nutrient utilization by the goose digestive system, releasing protein, starch, and minerals surrounded by cell walls for digestion and absorption, thereby reducing the anti-nutritional effects of dietary fiber.

### 3.3 Effects of Cellulase on Digestive Enzyme Activity

Numerous studies have demonstrated that the goose muscular stomach can facilitate cellulose digestion by disrupting cell walls. The pancreas is the most important digestive gland, and the intestine is the primary site for nutrient digestion and absorption. Therefore, enzymes such as amylase, protease, and lipase in these organs constitute the most important and powerful digestive enzyme systems and serve as important indicators of digestive function, with enzyme activity representing the combined effect of various factors. Huang et al. noted that cellulase supplementation in high-fiber diets can affect pancreatic digestive enzyme secretion by altering the physicochemical properties of chyme, enhancing nutrient digestion and absorption, and improving feed conversion efficiency. This experiment showed that cellulase supplementation significantly enhanced pancreatic protease and amylase activities while also increasing muscular stomach protease activity and duodenal lipase activity. Dietary cellulase supplementation may compensate for endogenous enzyme deficiencies, stimulate endogenous enzyme secretion, and reduce or eliminate allergic reactions and damage to the intestine and pancreas caused by anti-nutritional factors, thereby increasing digestive enzyme activities in gastrointestinal chyme. However, the interaction mechanisms between exogenous and endogenous enzymes are highly complex and require further investigation through extensive research.

## 4 Conclusion

Supplementation of 3‰ cellulase in peanut seedling diets (9.75% crude fiber level) significantly increased average daily gain in geese, significantly or highly significantly improved apparent utilization of crude ash, crude protein, crude fiber, calcium, total phosphorus, glycine, cysteine, and methionine, and enhanced pancreatic protease and amylase activities as well as muscular stomach protease and duodenal lipase activities. This represents an effective approach

for developing and utilizing peanut seedling feed resources.

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