

Effects of *Eucommia ulmoides* Leaves on Growth Performance, Carcass Traits, Antioxidant Capacity, and Gut Microbiota of Finishing Pigs: Post-print

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Abstract

This experiment aimed to investigate the effects of dietary *Eucommia* leaf supplementation on growth performance, carcass traits, antioxidant capacity, and gut microbiota in finishing pigs, and to explore its application efficacy. Sixty Landrace × Large White pigs with similar birth age, good health status, and no significant difference in body weight [(27±\$1) kg] were randomly divided into 3 groups with 20 pigs per group. The control group was fed a basal diet (without any antibiotics), while the treatment groups were fed diets in which 3% and 4% *Eucommia* leaf powder replaced the same proportion of wheat middlings in the basal diet, respectively. The experimental period lasted for 102 (52+50) days. At the end of the experiment, 4 pigs (half male and half female) were randomly selected from each group for slaughter to determine carcass traits and meat quality indicators. The results showed that: during days 1-52, compared with the control group, the 4% *Eucommia* leaf supplementation group exhibited significantly improved average daily gain ($P<0.05$) and extremely significantly reduced diarrhea rate ($P<0.01$); during days 53-102, the treatment groups showed extremely significantly decreased feed conversion ratio ($P<0.01$). Compared with the control group, the treatment groups had increased loin eye area ($P>0.05$), extremely significantly reduced drip loss ($P<0.05$), extremely significantly elevated glutathione peroxidase activity in the liver ($P<0.01$), and extremely significantly decreased malondialdehyde content in the liver ($P<0.01$). The 4% *Eucommia* leaf supplementation group had significantly lower numbers of *Escherichia coli* and *Salmonella* than the control group ($P<0.05$), while the numbers of *Bifidobacterium* and *Lactobacillus* showed no significant difference ($P>0.05$). The results suggest that dietary supplementation with 4% *Eucommia*

mia leaf can significantly reduce the diarrhea rate of finishing pigs, improve their growth performance, enhance carcass traits and meat quality, increase hepatic antioxidant capacity, and exert certain regulatory effects on gut microbiota numbers.

Full Text

Effects of *Eucommia ulmoides* Leaves on Growth Performance, Carcass Characters, Antioxidant Capacity and Intestinal Microbial Flora of Finishing Pigs

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Abstract

This experiment was conducted to investigate the effects of dietary *Eucommia ulmoides* leaves (EUL) on growth performance, carcass characters, antioxidant capacity, and intestinal microbial flora of finishing pigs, and to explore its application potential in pig production. Sixty healthy crossbred pigs (Landrace × Yorkshire) with similar birth dates and initial body weight [(27±\$1) kg] were randomly allocated into three groups (n=20 per group). The control group was fed a basal diet without antibiotics, while the experimental groups were fed the basal diet in which 3% or 4% wheat middlings were replaced by EUL powder. The trial lasted for 102 days (52 days + 50 days). At the end of the experiment, four pigs (half barrows and half gilts) were randomly selected from each group for slaughter to determine carcass traits and meat quality parameters. The results showed that during days 1-52, the 4% EUL group exhibited significantly higher average daily gain (P<0.05) and extremely significantly lower diarrhea rate (P<0.01) compared with the control group. During days 53-102, the feed-to-gain ratio was extremely significantly reduced in both experimental groups (P<0.01). Compared with the control group, the experimental groups showed increased loin-eye area (P>0.05), extremely significantly decreased drip loss (P<0.01), extremely significantly elevated glutathione peroxidase activity in liver (P<0.01), and extremely significantly reduced malondialdehyde content in liver (P<0.01). The 4% EUL group had significantly lower counts of *Escherichia coli* and *Salmonella* than the control group (P<0.05), while no significant differences were observed in *Bifidobacterium* and *Lactobacillus* counts (P>0.05). These findings indicate that dietary supplementation with 4% *Eucommia ulmoides* leaves can significantly reduce diarrhea rate, improve growth

performance, enhance carcass characters and meat quality, increase antioxidant capacity, and modulate intestinal microbial flora in finishing pigs.

Keywords: Eucommia ulmoides leaves; growth performance; carcass characters; antioxidant capacity; intestinal microbial flora

Introduction

With the continuous improvement of living standards, consumers increasingly demand higher quality of livestock and poultry products. In recent years, developing bioactive Chinese herbal additives has become a hotspot for improving meat quality. According to the *Shennong Bencao Jing*, *Eucommia ulmoides* is warm in nature, sweet in taste, non-toxic, and possesses a clear aroma. Previous studies have demonstrated that *Eucommia* leaves serve as a safe, residue-free green additive, rich in amino acids, trace elements, vitamins, and other nutrients, while also exhibiting pharmacological and physiological functions, particularly in improving meat quality, enhancing immunity, and increasing antioxidant capacity. However, post-extraction *Eucommia* leaves have not been effectively utilized, resulting in resource waste and environmental pollution. Therefore, using *Eucommia* leaves as a feed additive can not only improve meat quality traits but also increase economic returns for medicinal farmers.

Numerous scholars have investigated the supplementation of *Eucommia* leaf extracts, such as chlorogenic acid, to enhance animal growth performance, carcass traits, and meat quality indices. Leng et al. reported that dietary chlorogenic acid from *Eucommia* leaves effectively reduced muscle water loss rate and decreased muscle fiber diameter, thereby improving muscle quality in grass carp. Mo et al. found that *Eucommia* extract significantly increased average daily gain, reduced feed-to-gain ratio and mortality rate, and decreased shoulder fat thickness in finishing pigs. Yang et al. demonstrated that flavonoid glycosides and other polyphenols in *Eucommia* leaves could reduce malondialdehyde (MDA) content in liver and enhance superoxide dismutase (SOD) activity in mice. Jia observed that *Eucommia* could promote collagen synthesis in mouse tissues and delay aging. Additionally, dietary *Eucommia* leaves have been shown to regulate the dynamic balance of intestinal microbial populations.

Addressing the issues of expensive wheat middlings, abundant *Eucommia* resources in Shaanxi, and the complex, costly extraction processes for chlorogenic acid and other compounds, this study utilized whole leaf powder without extracted chlorogenic acid to replace wheat middlings in basal diets. The objective was to investigate the effects of *Eucommia* leaves on growth performance, carcass traits, meat quality, antioxidant capacity, and intestinal microbial flora in finishing pigs, providing a basis for using *Eucommia* leaves as a feed additive for high-quality pork production.

1.1 Experimental Animals and Design

This experiment employed a single-factor design. Sixty crossbred pigs (Landrace × Yorkshire) with similar age, healthy status, and initial body weight [(27±1) kg] showing no significant differences ($P>0.05$) were randomly divided into three groups (n=20 per group). The control group received a basal diet without any antibiotics, while experimental groups 1 and 2 were fed basal diets in which 3% and 4% wheat middlings were replaced by Eucommia leaf powder, respectively. The trial lasted for 102 days (52 days + 50 days). At the end of the experiment, four pigs per group (half barrows and half gilts) were randomly selected for slaughter (12 pigs total) to determine carcass traits and meat quality parameters.

The experiment was conducted from August to December 2015 at the Qinyangchen National Core Breeding Farm in Ankang, Shaanxi Province. Prior to the trial, all pig houses, pens, floors, and feeding equipment were thoroughly cleaned and disinfected, with a 7-day vacancy period. Pigs had ad libitum access to feed and water throughout the experiment, with three daily feedings. To eliminate confounding factors, no antibiotics were administered during the trial; only basic disinfection procedures were implemented.

The experimental diets were formulated according to NRC (2012) standards to meet the nutritional requirements of finishing pigs. Diet composition and nutrient levels are presented in Table 1. Eucommia leaves were provided by Ankang Hanyin Huaye Plant Pharmaceutical Co., Ltd. in Shaanxi Province as whole leaf powder without extracted chlorogenic acid. The nutrient composition of Eucommia leaves is shown in Table 2.

1.2 Growth Performance Measurement

At the beginning and end of the experiment, body weight was measured for each group. Total feed intake, diarrhea incidence, and disease occurrence were recorded to calculate average daily feed intake, average daily gain, and feed-to-gain ratio.

1.3 Sample Collection and Processing

Fecal samples were collected every 10 days throughout the trial, properly labeled, and analyzed using microbial plate culture methods for isolation, purification, classification, identification, and quantitative analysis of different microorganisms.

At the conclusion of the experiment, slaughtered pigs were processed, and liver samples were collected. Liver tissues were washed with phosphate buffer saline

(PBS), and approximately 1 g samples were excised and homogenized with physiological saline. The homogenate was centrifuged at 3,000 r/min for 15 minutes, and the supernatant was stored at -80°C for antioxidant capacity analysis. Assay kits for glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (T-AOC) were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4 Carcass Index Determination

1.4.1 Carcass Trait Measurement Measurement methods followed the procedures described in *Swine Production Science*. Prior to slaughter, live weight was recorded. After conventional slaughtering procedures (removing head, feet, tail, and internal organs while retaining leaf fat and kidneys), carcass weight, dressing percentage, carcass straight length, carcass oblique length, fore-carcass width, hind-carcass width, loin-eye area, backfat thickness at thoracolumbar and lumbosacral junctions, and skin thickness were measured.

1.4.2 Meat Quality Determination Muscle pH, water-holding capacity, tenderness, and meat color were measured using a pH meter, compression apparatus, digital muscle tenderness meter, and colorimeter, respectively. Meat color and marbling scores were evaluated according to the 5-point scale of the National Pork Producers Council (NPPC) color panel (1991 edition). Approximately 100 g of longissimus dorsi muscle sample was collected within 2 hours post-slaughter, weighed before steaming, steamed in a boiling water bath for 30 minutes, then cooled in a shaded indoor area for 15–20 minutes before reweighing to calculate cooking loss percentage.

1.4.3 Crude Protein Content Determination Crude protein content was determined using the Kjeldahl method according to literature procedures and GB/T 6432–1994 protocols. Samples were digested with concentrated sulfuric acid to convert organic nitrogen to inorganic ammonium salts, which were then converted to ammonia under alkaline conditions, distilled with steam, absorbed by excess boric acid solution, and titrated with standard hydrochloric acid to calculate nitrogen content.

1.4.4 Intramuscular Fat Content Determination Intramuscular fat content was determined using Soxhlet extraction according to literature methods and GB/T 14772–2008 *Determination of Crude Fat in Foods*. The method involved ether extraction of fat from samples, with three consecutive weighings after drying until constant mass was achieved.

1.4.5 Shear Force Determination Longissimus dorsi muscle samples (minimum dimensions 6 cm × 3 cm × 3 cm) were collected within 2 hours post-slaughter, placed in clean plastic bags, and aged at 0–4°C for 96 hours. After

removing from refrigeration and equilibrating at room temperature for 15 minutes, a 0.5 cm diameter glass thermometer was inserted into the muscle center. The bagged sample was heated in a water bath until the core temperature reached 70°C, then rapidly removed and cooled to room temperature. A 1.50 cm thick slice was cut perpendicular to muscle fiber direction, and a 1.27 cm diameter corer was used to extract samples parallel to fiber direction. Samples were positioned perpendicular to muscle fibers on the shear force apparatus and sheared at 1 mm/s to record shear force values.

1.4.6 Crude Ash Content Determination Clean crucibles were calcined in a muffle furnace at 550°C for 30 minutes, cooled in air for 1 minute, transferred to a desiccator for 30 minutes, and weighed. This process was repeated until consecutive weighings differed by less than 0.0005 g to achieve constant weight. Approximately 5 g of dried meat sample (accurate to 0.0001 g) was placed in a crucible with the lid slightly ajar, carbonized at low temperature until smokeless, then transferred to a preheated 550°C muffle furnace for 3 hours. After cooling in air for 1 minute and desiccating for 30 minutes, samples were weighed repeatedly until consecutive measurements differed by less than 0.001 g.

1.5 Statistical Analysis

Data were processed using Excel 2013 and analyzed using SPSS 22.0 software via one-way ANOVA. Multiple comparisons were performed using LSD method. $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered extremely significant. Results are expressed as “mean \pm standard error.”

2.1 Effects of *Eucommia ulmoides* Leaves on Growth Performance of Finishing Pigs

As shown in Table 3, during days 1–52, the 3% EUL group showed no significant difference in average daily gain compared with the control group ($P > 0.05$), while the 4% EUL group exhibited significantly increased average daily gain ($P < 0.05$). Both experimental groups demonstrated reduced feed-to-gain ratio ($P > 0.05$) and extremely significantly decreased diarrhea rate ($P < 0.01$) compared with the control group. During days 53–102, no significant differences in average daily gain were observed between experimental and control groups ($P > 0.05$), while the feed-to-gain ratio was extremely significantly reduced in experimental groups ($P < 0.01$).

2.2 Effects of *Eucommia ulmoides* Leaves on Carcass Characters of Finishing Pigs

As presented in Table 4 , compared with the control group, experimental groups showed no significant differences in pre-slaughter live weight ($P>0.05$), while dressing percentage and loin-eye area were increased ($P>0.05$). The 4% EUL group exhibited significantly decreased carcass oblique length ($P<0.05$), and reduced rib fat thickness, rib skin thickness, and average skin thickness ($P>0.05$).

2.3 Effects of *Eucommia ulmoides* Leaves on Meat Quality of Finishing Pigs

According to Table 5 , experimental groups demonstrated increased cooking percentage ($P>0.05$) and extremely significantly decreased drip loss ($P<0.01$) compared with the control group. The 3% EUL group showed significantly reduced shear force ($P<0.05$), while experimental groups exhibited significantly decreased crude ash content in muscle ($P<0.05$). The 4% EUL group had significantly reduced intramuscular fat content ($P<0.05$).

2.4 Effects of *Eucommia ulmoides* Leaves on Antioxidant Capacity of Finishing Pigs

As indicated in Table 6 , dietary EUL supplementation extremely significantly enhanced liver GSH-Px activity ($P<0.01$) and extremely significantly reduced liver MDA content ($P<0.01$) compared with the control group, while other antioxidant indices remained unaffected ($P>0.05$).

2.5 Effects of *Eucommia ulmoides* Leaves on Intestinal Microbial Flora of Finishing Pigs

Table 7 reveals that the 4% EUL group significantly reduced *Escherichia coli* and *Salmonella* counts ($P<0.05$) compared with the control group, while *Bifidobacterium* and *Lactobacillus* counts showed no significant differences ($P>0.05$) but exhibited an increasing trend. The 3% EUL group demonstrated no significant differences in counts of *Escherichia coli*, *Bifidobacterium*, *Salmonella*, or *Lactobacillus* ($P>0.05$).

3.1 Effects of *Eucommia ulmoides* Leaves on Growth Performance of Finishing Pigs

Growth performance directly determines meat production capacity and affects farm economic efficiency. As a green additive with pharmacological and physiological functions, *Eucommia* leaves can improve dietary nutrient utilization and play an important role in intestinal health. Li et al. reported that compared with antibiotic supplementation, 250 mg/kg *Eucommia* leaf extract increased daily gain by 5% and reduced feed-to-gain ratio by 5% in weaned piglets. In this study, using whole *Eucommia* leaf powder without extracted chlorogenic acid to replace wheat middlings reduced feed-to-gain ratio and increased average daily gain, possibly due to the abundant vitamins and trace elements in *Eucommia* leaves that stimulate central nervous system excitation and thereby influence growth performance. Wang et al. observed that 1,500 mg/kg *Eucommia* leaf extract significantly reduced daily diarrhea scores, increased average daily gain, and decreased feed-to-gain ratio, potentially because chlorogenic acid and flavonoid glycosides in *Eucommia* leaves inhibit nuclear factor- κ B (NF- κ B) signaling pathway activation, thereby suppressing release of interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)- α in small intestinal epithelial cells, which are associated with diarrhea. Additionally, the high arginine content in *Eucommia* leaves can improve immune system health and enhance disease defense capabilities.

3.2 Effects of *Eucommia ulmoides* Leaves on Carcass Characters and Meat Quality of Finishing Pigs

Meat quality is primarily manifested through tenderness, color, water-holding capacity, and intramuscular fat content. Research indicates that *Eucommia* extract can reduce carcass skin thickness and drip loss while increasing loin-eye area, meat color scores, and marbling scores. Cao et al. found that Chinese herbal feed additives containing *Eucommia* leaf extract decreased backfat thickness by 20.35%, increased loin-eye area by 6.7%, and reduced fresh meat water loss rate by 12.02% in finishing pigs. Wang et al. reported that 1,500 and 2,000 mg/kg *Eucommia* leaf extract significantly increased intramuscular fat content in finishing pigs. Zhou et al. demonstrated that dietary *Eucommia* leaf powder significantly improved meat quality traits and reduced pork fiber structure, resulting in better texture. These effects may be related to threonine and methionine in *Eucommia* leaves, which accelerate fat metabolism and improve meat quality. Furthermore, geniposide and aucubin in *Eucommia* leaves can slow lipid peroxidation, inhibit ferrous ion (Fe^{2+})-induced MDA formation, and prevent rapid muscle oxidation, thereby maintaining stable carcass traits and meat quality.

Post-slaughter muscle pH decreases due to anaerobic glycolysis of muscle glycogen producing lactic acid. Since pH is closely related to meat color, marbling,

drip loss, and shelf life, it serves as an important reference for meat quality evaluation. Muscle chemical composition is a primary factor affecting meat quality, with moisture content and water-holding capacity directly influencing quality. Low moisture content indicates high crude protein and dry matter content, while water-holding capacity correlates with tenderness. Sun et al. reported that the degree of pH decline 24 hours post-slaughter positively correlates with drip loss and water-holding capacity and negatively correlates with shear force, consistent with our findings. Marbling, the intermingling of fat within muscle tissue, is associated with juiciness, flavor, and shear force. Lee et al. reported that dietary supplementation with 3% and 5% *Eucommia* extract in finishing pigs significantly increased daily gain, reduced white blood cell counts, increased hemoglobin and hematocrit, and significantly improved meat color, drip loss, and marbling. In this study, experimental groups showed increased cooking percentage, extremely significantly decreased drip loss, and significantly reduced shear force and crude ash content. The L^* value represents meat lightness; chlorogenic acid's antioxidant capacity protects cell membrane lipid layers from peroxidation interference, reducing membrane carrier channels and thereby decreasing muscle water seepage and light reflection. The a^* value indicates myoglobin conversion to metmyoglobin, while the b^* value relates to oxidation. In this trial, *Eucommia* leaf supplementation improved meat color and maintained pork color stability at both 45 minutes and 24 hours post-slaughter, demonstrating that the antioxidant capacity of *Eucommia* leaves can delay oxidative processes.

3.3 Effects of *Eucommia ulmoides* Leaves on Antioxidant Capacity of Finishing Pigs

Living organisms continuously generate free radicals through metabolic processes. Free radicals are highly oxidative and associated with aging and antioxidant capacity. Organisms primarily rely on their complete antioxidant defense system to eliminate free radicals. Chen et al. observed that adding different levels of *Eucommia* leaf extract to Arbor Acres broiler diets produced a quadratic trend in muscle GSH-Px activity, with 0.05%, 0.15%, and 0.20% supplementation levels increasing activity by 20.50%, 14.91%, and 12.89%, respectively, compared with the control group. Zhou et al. found that *Eucommia* leaf water extract enhanced GSH-Px and SOD activities in lung tissue and erythrocytes while inhibiting MDA production in aging mice. During exercise, organisms produce highly reactive oxidative species (ROS) that modify proteins, lipids, and DNA, causing tissue damage, cellular activity impairment, and antioxidant imbalance. The strong antioxidant capacity of *Eucommia* leaves can accelerate ROS elimination and reduce tissue damage. Chlorogenic acid, flavonoid glycosides, and lignans in *Eucommia* leaves exert antioxidant effects by scavenging free radicals and protecting normal cellular metabolism. In this study, replacing partial wheat middlings with 3% and 4% *Eucommia* leaf powder extremely sig-

nificantly increased liver GSH-Px activity and extremely significantly reduced liver MDA content, indicating that *Eucommia* leaf supplementation accelerated free radical elimination and enhanced antioxidant capacity.

3.4 Effects of *Eucommia ulmoides* Leaves on Intestinal Microbial Flora of Finishing Pigs

Intestinal microbial flora participates in numerous physiological and metabolic activities, affecting animal growth performance and intestinal diseases such as diarrhea. Wen et al. found that adding Chinese herbal polysaccharides (including *Eucommia*) to weaned piglet diets at 400 and 600 mg/kg significantly reduced *Escherichia coli* counts by 10.63% and 13.30% while extremely significantly increasing *Lactobacillus* and *Bifidobacterium* populations. Cao et al. reported that dietary supplementation with 30, 5, and 3 g/kg Chinese herbal additives (containing *Eucommia*) significantly reduced *Escherichia coli* counts and promoted proliferation of *Bifidobacterium* and *Lactobacillus* in weaned piglets. Chlorogenic acid, a key bioactive component of *Eucommia*, promotes proliferation of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium*, producing antimicrobial substances including vitamin B and lactic acid that alter intestinal pH and inhibit pathogenic bacteria like *Escherichia coli* and *Salmonella*, thereby enhancing immunity and growth performance. Additionally, intestinal flora can regulate lipid metabolism, with volatile fatty acids from microbial fermentation serving as important regulators. In this study, replacing wheat middlings with 3% and 4% *Eucommia* leaf powder increased *Bifidobacterium* and *Lactobacillus* counts while reducing *Escherichia coli* and *Salmonella* populations, consistent with previous research, though the relationship and mechanism between *Eucommia* leaf supplementation and *Salmonella* require further investigation.

Conclusion

Dietary supplementation with *Eucommia ulmoides* leaves can improve carcass characters and meat quality indices, enhance antioxidant capacity, and exert regulatory effects on intestinal microbial populations in finishing pigs.

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