Effects of Glutamic Acid Supplementation in Low-Protein Diets on Protein Utilization and Production Performance in Finishing Pigs: Post-print

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Abstract

This experiment aimed to investigate the effects of adding glutamic acid (Glu) to low-protein diets on protein utilization and production performance in finishing pigs. Experiment 1 was a nitrogen balance trial, in which 6 three-way crossbred barrows (Duroc × Landrace × Yorkshire) with a body weight of (54.2±1.0) kg were selected and subjected to a 3×3 replicated Latin square design, divided into 3 periods of 7 d each. Three diets with different crude protein (CP) levels were formulated: 14.0% CP (control group), 12.5% CP+Glu, and 11.0% CP+Glu. Experiment 2 was a feeding trial, employing a completely randomized block design, in which 30 three-way crossbred barrows (Duroc × Landrace × Yorkshire) with a body weight of (57.4±0.2) kg were randomly allocated to 3 groups, with 10 replicates per group and 1 pig per replicate. The experimental diets were the same as in Experiment 1. The experimental period for both trials was 35 d. The results showed that: 1) Compared with the control group, adding Glu to low-protein diets significantly reduced total nitrogen intake, urinary nitrogen, and total nitrogen excretion in finishing pigs ($P<0.05$); the nitrogen biological value of the 11.0% CP+Glu group was significantly higher than that of the control group ($P<0.05$). 2) Compared with the control group, adding Glu to low-protein diets had no significant effect on initial body weight, final body weight, and average daily gain in finishing pigs ($P>0.05$), but significantly reduced crude protein intake and average daily crude protein intake/average daily gain ($P<0.05$). 3) Compared with the control group, adding Glu to low-protein diets significantly reduced plasma glucose, urea nitrogen, and free fatty acid concentrations in finishing pigs ($P<0.05$). 4) The jejunal mucosa glucose and pyruvate concentrations in finishing pigs of the 11.0% CP+Glu group were significantly higher than those of the control group ($P<0.05$), while the jejunal mucosa lactate concentrations in the 11.0% CP+Glu and 12.5% CP+Glu groups

were significantly lower than those of the control group (P<0.05). In conclusion, adding Glu to low-protein diets can reduce urinary nitrogen and total nitrogen excretion, improve protein utilization efficiency, and has no significant effect on the production performance of finishing pigs.

Full Text

Effects of Low-Protein Diet Supplemented with Glutamate on Protein Utilization and Performance of Finishing Pigs

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Abstract

This study investigated the effects of low-protein diet supplemented with glutamate (Glu) on protein utilization and performance in finishing pigs. Experiment 1 was a nitrogen balance trial using six "Duroc×Landrace×Yorkshire" three-way hybrid castrated boars with an initial body weight of (54.2±1.0) kg. A 3×3 replicated Latin square design was employed across three periods, each lasting 7 days. Three diets with different crude protein (CP) levels were formulated: 14.0% CP (control), 12.5% CP+Glu, and 11.0% CP+Glu. Experiment 2 was a feeding trial utilizing a completely randomized block design with thirty "Duroc×Landrace×Yorkshire" three-way hybrid castrated boars weighing (57.4±0.2) kg. Pigs were randomly allocated to three groups with ten replicates per group and one pig per replicate. The experimental diets were identical to those used in Experiment 1. Both trials lasted 35 days. The results demonstrated that: (1) Compared with the control group, low-protein diets supplemented with Glu significantly reduced total nitrogen intake, urinary nitrogen, and total nitrogen excretion (P<0.05), while the 11.0% CP+Glu group exhibited significantly higher nitrogen biological value (P<0.05). (2) Dietary Glu supplementation had no significant effects on initial weight, final weight, or average daily gain (ADG) (P>0.05), but significantly decreased crude protein intake and the ratio of average daily CP intake to ADG (P<0.05). (3) Plasma glucose, urea nitrogen, and free fatty acid concentrations were significantly reduced in pigs fed low-protein diets with Glu (P<0.05). (4) The 11.0% CP+Glu group showed significantly higher glucose and pyruvic acid contents in jejunal mucosa compared with the control (P<0.05), while both the 11.0% CP+Glu and 12.5% CP+Glu groups had significantly lower lactate content in jejunal mucosa (P<0.05). In conclusion, supplementing low-protein diets with Glu can reduce urinary and total nitrogen excretion, improve protein utilization efficiency, and maintain production performance in finishing pigs.

Keywords: low-protein diet; finishing pig; nitrogen balance; performance; amino acid

Introduction

The intensification of swine production has led to increasingly severe nitrogen pollution. In China, total nitrogen excretion from livestock and poultry reaches approximately 30 million tons annually, with monogastric animals (primarily pigs) accounting for about 57% of this total. Reducing nitrogen emissions, minimizing nutrient waste, and decreasing environmental impact represent fundamental challenges and urgent priorities for the Chinese swine industry. Lowering dietary protein levels is a widely adopted strategy to reduce nitrogen excretion. However, to maintain pig performance, essential amino acids (EAAs) must be supplemented when dietary protein is reduced. Previous research from our group revealed that simply balancing EAAs (lysine, methionine, threonine, and tryptophan) in low-protein diets leads to non-essential amino acid (NEAA) deficiency, causing substantial EAA catabolism in the liver for NEAA synthesis and resulting in significant EAA waste. Glutamate (Glu) is a crucial NEAA that plays vital roles in promoting animal growth and maintaining health. This study examined the effects of Glu supplementation in low-protein diets (balanced for four EAAs) on protein utilization, performance, and metabolism in finishing pigs, providing scientific guidance for reducing nitrogen emissions and improving protein efficiency.

Materials and Methods

Experimental Design and Diets

Experiment 1 (Nitrogen Balance Trial): Six "Duroc×Landrace×Yorkshire" three-way hybrid castrated boars (54.2±1.0 kg) were used in a 3×3 replicated Latin square design with three 7-day periods. Three diets with different crude protein levels were formulated: 14.0% CP (control), 12.5% CP+Glu, and 11.0% CP+Glu. All diets contained 0.1% titanium dioxide as an indigestible marker. Diet composition and nutrient levels are presented in .

Experiment 2 (Feeding Trial): Thirty "Duroc×Landrace×Yorkshire" three-way hybrid castrated boars (57.4±0.2 kg) were randomly assigned to three groups using a completely randomized block design, with ten replicates per group and one pig per replicate. The experimental diets were identical to those used in Experiment 1. Both trials lasted 35 days.

Animal Management

Both experiments were conducted at the Southwest University Animal Research Facility. One week before Experiment 1, pigs were individually housed in metabolism cages (1.50 m × 0.75 m × 0.68 m) to acclimate to the environment

and diets. In Experiment 2, pigs were housed in stainless steel pens. Room temperature was maintained at (25±1)°C, and pigs had ad libitum access to feed and water. Feed was provided at 08:00 and 18:00 daily. Pens were kept clean and dry throughout the trial. Daily feed allowance and refusals were recorded accurately. Fasted body weight was measured at 08:00 on days 1 and 35.

Sample Collection and Analysis

Nitrogen Balance Trial Diet samples were randomly collected three times per period, pooled, ground through a 40-mesh sieve, and stored at room temperature. Dry matter, CP, calcium, phosphorus, crude fiber, and amino acid contents were analyzed according to *Feed Analysis and Feed Quality Detection Technology*. The last three days of each period were designated for fecal and urine collection. Feces were collected twice daily, mixed thoroughly, and a 10% subsample was stored in sealed bags with 10% sulfuric acid for nitrogen fixation (10 g feces: 1 mL sulfuric acid) at -20°C. At the end of each period, three-day fecal samples from each pig were pooled, dried at 65°C to constant weight, equilibrated at room temperature for 24 hours, weighed, ground through a 40-mesh sieve, and stored at -20°C until analysis. Total urine was collected in containers pre-treated with 10 mL of 10% sulfuric acid. Urine volume was measured accurately (filtered through nylon mesh if feed or fecal contamination occurred), and a 5% aliquot was stored in plastic bottles at -20°C. Three-day urine samples were pooled per pig. Fecal dry matter and nitrogen content and urinary nitrogen were analyzed. Nitrogen intake [(feed offered - refusals) × dietary nitrogen content], fecal nitrogen (fecal output × fecal nitrogen content), urinary nitrogen (urine volume × urinary nitrogen content), total nitrogen excretion (fecal nitrogen + urinary nitrogen), nitrogen retention (nitrogen intake - fecal nitrogen - urinary nitrogen), and nitrogen biological value [100 × (nitrogen intake - fecal nitrogen - urinary nitrogen) / (nitrogen intake - fecal nitrogen)] were calculated.

Feeding Trial Diet samples were collected on days 1, 18, and 35, pooled by group, ground through a 40-mesh sieve, and stored at room temperature. Analytical methods followed those described in the nitrogen balance trial. Average daily gain [(final weight - initial weight) / trial days], crude protein intake (feed intake × dietary CP content), and the ratio of average daily CP intake to ADG were calculated.

At the end of the feeding trial, five pigs per group were randomly selected for blood collection via anterior vena cuncture into 10 mL heparinized tubes. Plasma was separated by centrifugation at 3,500 r/min for 15 min at 4°C and stored at -80°C. Plasma glucose, urea nitrogen, and free fatty acid concentrations were determined using commercial kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

After blood collection, pigs were slaughtered and the gastrointestinal tract was isolated and ligated. Jejunal mucosa samples were collected, snap-frozen in liquid nitrogen, and stored at -80°C. Jejunal mucosal metabolites (glucose, pyruvic

acid, and lactic acid) were measured using kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer' s protocols.

Statistical Analysis

Data from Experiment 1 were analyzed using the MIXED procedure of SAS 9.0, with the statistical model including random effects for pigs and fixed effects for period and diet. Data from Experiment 2 were analyzed using one-way ANOVA in SAS 9.0. Significant differences were further evaluated using LSD multiple comparison tests, with $P<0.05$ considered statistically significant.

Results

Nitrogen Balance

As shown in , low-protein diets supplemented with Glu significantly reduced total nitrogen intake, urinary nitrogen, and total nitrogen excretion compared with the control group ($P<0.05$). The 11.0% CP+Glu group exhibited significantly higher nitrogen biological value than the control group ($P<0.05$).

Growth Performance

presents the growth performance data. No significant differences were observed among the three groups for initial body weight, final body weight, or ADG ($P>0.05$). However, low-protein diets with Glu significantly decreased crude protein intake and the ratio of average daily CP intake to ADG ($P<0.05$).

Plasma Biochemical Indices

Low-protein diets supplemented with Glu significantly reduced plasma glucose, urea nitrogen, and free fatty acid concentrations compared with the control group ($P<0.05$) ().

Jejunal Mucosal Metabolites

The 11.0% CP+Glu group showed significantly higher glucose and pyruvic acid contents in jejunal mucosa than the control group ($P<0.05$), while both the 11.0% CP+Glu and 12.5% CP+Glu groups had significantly lower lactate content in jejunal mucosa ($P<0.05$) ().

Discussion

Effects on Nitrogen Balance

Reducing dietary protein while supplementing key EAAs is a common strategy to decrease nitrogen excretion in pigs, with each 1% reduction in dietary

protein decreasing total nitrogen excretion by approximately 8%. Although previous studies show that reducing dietary protein by 2-3% does not affect nitrogen retention in finishing pigs, low-protein diets have not been widely adopted in intensive production systems. Our previous research indicated that simply balancing EAAs in low-protein diets reduces portal-drained viscera (stomach, intestines, pancreas, spleen, and omental fat) net absorption of NEAAs, forcing the liver to catabolize substantial EAAs for NEAA synthesis and causing EAA waste. We hypothesized that supplementing specific NEAAs (e.g., Glu) to low-protein diets balanced for EAAs would address this limitation. The current results demonstrate that Glu supplementation in diets with 21.4% reduced protein significantly decreased total nitrogen excretion without negatively impacting nitrogen retention. This may occur because Glu, a major NEAA, is extensively metabolized by portal-drained viscera, with only a small fraction entering the mesenteric vein from the intestinal lumen. Supplementing Glu to low-protein diets may increase energy supply to portal-drained viscera, reducing their consumption of other amino acids and allowing a more balanced amino acid pattern to reach the liver. Consequently, EAA catabolism for NEAA synthesis decreases, improving overall amino acid utilization and protein deposition. However, without measurements of ileal amino acid digestibility, portal amino acid net absorption, or hepatic amino acid utilization, these mechanisms require further validation through cannulation studies.

Effects on Growth Performance

Previous research indicates that reducing dietary protein by 2-3% with EAA supplementation does not impair pig performance, but reductions exceeding 3% can inhibit growth, limiting practical application. Our findings show that Glu supplementation maintained performance despite a 21.4% protein reduction, suggesting Glu alleviates growth limitations of conventional low-protein diets. The growth-promoting effects relate to Glu' s metabolic functions: (1) Although not a limiting amino acid, Glu occupies a central position in amino acid metabolism; (2) Glu serves as a major energy source in portal-drained viscera, sparing other potentially growth-limiting amino acids; and (3) As a hub for amino acid metabolism, Glu can transaminate to replenish other amino acids when deficient. Additionally, Glu supplementation affects performance through other physiological functions, including reduced fat deposition, decreased muscle fiber diameter, and improved feed efficiency. Feng et al. reported that dietary monosodium glutamate enhances jejunal amino acid absorption by upregulating amino acid transporter expression. Glu also reduces oxidative stress and alleviates toxin-induced intestinal damage, contributing to intestinal and systemic health.

Effects on Metabolism

Glucose is the primary energy source, while free fatty acids reflect lipid metabolic status. Low-protein diets with Glu reduced plasma glucose and free fatty acid

concentrations, possibly because: (1) Fewer amino acids were used for energy, increasing glucose and fatty acid utilization; or (2) Reduced gluconeogenesis and fatty acid synthesis occurred. Plasma urea nitrogen reflects protein utilization efficiency, with lower values indicating higher efficiency. The significant reduction in plasma urea nitrogen with low-protein Glu supplementation indicates decreased amino acid catabolism, consistent with nitrogen retention and performance data. These results suggest that Glu supplementation increases gastrointestinal glucose and fatty acid utilization, reducing amino acid oxidation and allowing more amino acids to enter circulation for protein synthesis, thereby maintaining performance in low-protein fed pigs.

Conclusion

1. Supplementing low-protein diets with Glu significantly reduced total nitrogen intake, urinary nitrogen, and total nitrogen excretion while improving protein utilization efficiency in finishing pigs.
2. Dietary Glu supplementation maintained finishing pig performance despite a 21.4% reduction in dietary protein content.
3. Low-protein diets with Glu significantly decreased plasma glucose and free fatty acid concentrations while enhancing glucose utilization by gastrointestinal epithelial tissues.

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