

## Postprint: Effects of Dietary Crude Protein Level and Amino Acid Balance on Growth Performance, Carcass Traits, and Meat Quality in Finishing Pigs

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### Abstract

This study aimed to investigate the effects of dietary crude protein level and amino acid balance on growth performance, carcass traits, and meat quality of finishing pigs. One hundred twenty-five Duroc × Landrace × Yorkshire barrows with an average initial body weight of  $(69.3 \pm 3.6)$  kg were selected and randomly allocated to 5 groups with 5 replicates per group and 5 pigs per replicate. The five groups were: a high-protein diet group (crude protein level approximately 14%), a low-protein (crude protein level approximately 10%)-amino acid balanced diet group, and three low-protein (crude protein level approximately 10%)-amino acid imbalanced diet groups (with the ratios of sulfur-containing amino acids, threonine, and tryptophan to lysine reduced by 10% respectively based on the low-protein-amino acid balanced diet group). The standardized ileal digestible lysine level was set at 0.71% in all diets, and the ratios of standardized ileal digestible threonine, standardized ileal digestible sulfur-containing amino acids, and standardized ileal digestible tryptophan to standardized ileal digestible lysine in the low-protein-amino acid balanced diet group were 0.67, 0.60, and 0.20, respectively. The experimental period lasted 28 days. The results showed that, compared with the low-protein-amino acid balanced diet group, the average daily feed intake of finishing pigs in the high-protein diet group tended to decrease ( $P=0.05$ ), the average daily gain and average daily feed intake of finishing pigs in the low-protein-low-tryptophan diet group decreased significantly ( $P<0.05$ ), and the growth performance of finishing pigs in the low-protein-low-threonine group and low-protein-low-sulfur-containing amino acids diet group showed no significant differences ( $P>0.10$ ). Dietary crude protein level and amino acid balance had no significant effects on carcass traits and meat quality of finishing pigs ( $P>0.10$ ). Therefore, the low-protein-amino acid balanced diet did not affect growth performance, carcass traits, and meat quality

of finishing pigs, reducing standardized ileal digestible sulfur-containing amino acids, standardized ileal digestible tryptophan, and standardized ileal digestible threonine by 10% respectively had no significant effects on carcass traits and meat quality of finishing pigs, but reducing standardized ileal digestible tryptophan by 10% significantly decreased average daily gain and average daily feed intake of finishing pigs.

## Full Text

### Abstract

This study was conducted to investigate the effects of dietary crude protein level and amino acid balance on growth performance, carcass traits, and meat quality of finishing pigs. A total of 125 Duroc  $\times$  Landrace  $\times$  Large White barrows with an average initial body weight of  $(69.3 \pm 3.6)$  kg were randomly allocated into five groups, each consisting of five replicates with five pigs per replicate. The five dietary treatments were: 1) a high-protein diet group (approximately 14% crude protein), 2) a low-protein balanced amino acid diet group (approximately 10% crude protein), and 3) three low-protein amino acid-imbalanced diet groups (in which the ratios of sulfur-containing amino acids, threonine, and tryptophan to lysine were each reduced by 10% relative to the low-protein balanced amino acid group). The standard ileal digestible lysine (SID Lys) level was set at 0.71% in all diets. In the low-protein balanced amino acid group, the ratios of standard ileal digestible threonine (SID Thr), standard ileal digestible sulfur-containing amino acids (SID SAA), and standard ileal digestible tryptophan (SID Trp) to SID Lys were 0.67, 0.60, and 0.20, respectively. The experimental period lasted 28 days. The results showed that compared with the low-protein balanced amino acid group, the high-protein group exhibited a tendency for decreased average daily feed intake (ADFI) ( $P = 0.05$ ), while the low-protein low-tryptophan group showed significant reductions in both average daily gain (ADG) and ADFI ( $P < 0.05$ ). No significant differences in growth performance were observed in the low-protein low-threonine or low-protein low-sulfur amino acid groups ( $P > 0.10$ ). Dietary crude protein level and amino acid balance had no significant effects on carcass traits or meat quality ( $P > 0.10$ ). These findings indicate that low-protein amino acid-balanced diets do not adversely affect growth performance, carcass traits, or meat quality in finishing pigs. While reducing SID SAA, SID Thr, and SID Trp by 10% each had no significant impact on carcass traits or meat quality, a 10% reduction in SID Trp significantly decreased ADG and ADFI.

**Keywords:** low-protein diet; amino acid balance; growth performance; carcass traits; meat quality

Low-protein diets typically refer to amino acid-balanced diets formulated by reducing dietary crude protein levels by 2-4 percentage points relative to NRC (1998) or Chinese Feeding Standard (NY/T 65-2004) recommendations, supplemented with synthetic amino acids. Such low-protein amino acid-balanced diets

can meet animals' amino acid requirements while reducing protein ingredient usage, alleviating shortages of feed protein resources in China, and serving as a primary technical approach to reducing nitrogen emissions in swine production.

Recent research has demonstrated that reducing dietary crude protein by up to 4 percentage points and supplementing with at least four essential amino acids—lysine (Lys), methionine (Met), threonine (Thr), and tryptophan (Trp)—based on net energy systems and ideal amino acid models does not impair growth performance or carcass traits in growing-finishing pigs. Our previous work indicated that for 75–100 kg finishing pigs under low-nitrogen dietary conditions, the optimal ratios of standard ileal digestible threonine (SID Thr), sulfur-containing amino acids (SID SAA), and tryptophan (SID Trp) to SID Lys for maximum growth performance were 0.67, 0.63, and 0.21, respectively—higher than NRC (2012) recommendations of 0.62, 0.57, and 0.18. Since synthetic amino acid supplementation increases feed costs, this study evaluated growth performance, carcass traits, and meat quality as comprehensive indicators to investigate the effects of reducing SID Thr, SID SAA, and SID Trp ratios by 10% from our previous levels (approximately to NRC 2012 recommendations) under commercial conditions, thereby providing additional technical support for practical application of low-protein diets.

## 1. Materials and Methods

### 1.1 Experimental Animals and Diets

A single-factor completely randomized design was employed. A total of 125 Duroc × Landrace × Large White barrows with an average initial body weight of  $(69.3 \pm 3.6)$  kg were selected and allocated into five groups based on similar body weight and genetic background, with five replicates per group and five pigs per replicate. The five dietary treatments were: 1) high-protein (HP) group with approximately 14% crude protein; 2) low-protein balanced amino acid (LP-BAA) group with approximately 10% crude protein, with SID Thr, SID SAA, and SID Trp to SID Lys ratios of 0.67, 0.60, and 0.20, respectively; 3) low-protein low sulfur amino acid (LP-SAA) group, with SID SAA level reduced by 10% compared to LP-BAA while other nutrients remained unchanged; 4) low-protein low threonine (LP-Thr) group, with SID Thr level reduced by 10% compared to LP-BAA; and 5) low-protein low tryptophan (LP-Trp) group, with SID Trp level reduced by 10% compared to LP-BAA.

Experimental diets were based on corn-soybean meal formulations, with synthetic amino acids added to approximate ideal protein patterns. All diets contained 0.71% SID Lys. The amino acid and crude protein contents of major ingredients (corn, soybean meal, and wheat bran) were analyzed before the experiment. Diet composition and nutrient levels are presented in Table 1 .

## 1.2 Animal Management

The trial was conducted at the breeding farm of Beijing Resources Asia-Pacific Food Co., Ltd. Pigs were housed in fully enclosed facilities with artificial temperature and humidity control maintained at approximately 20°C. Each pen was equipped with a single-sided five-hole feeder and two drinkers. Pigs were randomly assigned to five pens (4 m × 3 m) and allowed ad libitum access to feed and water. Feeding occurred three times daily at 07:00, 14:00, and 21:00. Feed allowance was adjusted every 2–3 days based on consumption, providing slightly more than satiation levels. Daily feed allocation and refusals were accurately measured and recorded per pen. Pigs were weighed per pen after a 12-hour fast at the beginning and end of the experiment. Diet samples (approximately 250 g) were collected weekly, pooled, and stored for subsequent analysis. Pens were cleaned twice daily, with routine disinfection and deworming performed according to standard farm protocols. Surrounding areas were disinfected weekly. The entire experimental procedure strictly followed farm management regulations and animal welfare standards of China Agricultural University. The experimental period lasted 28 days.

## 1.3 Sample Collection

At the end of the experiment, after a 12-hour fast, one pig closest to the average pen weight was selected from each pen (five pigs per group, 25 total) and slaughtered at Beijing Resources Asia-Pacific Food Co., Ltd. Blood samples were collected from the anterior vena cava, allowed to clot for 0.5 h, then centrifuged at  $3,000 \times g$  for 10 min at 4°C. Serum was harvested and stored at -20°C for subsequent analysis. Pigs were electrically stunned, exsanguinated, and processed using conventional slaughter procedures (decapitation, hoof removal, skinning, evisceration). Samples were collected for carcass trait and meat quality evaluation.

## 1.4 Analytical Methods

**1.4.1 Dietary Nutrient Analysis** Dietary dry matter, crude protein, starch, ether extract, crude fiber, calcium, and total phosphorus were determined according to GB/T 6435–1986, GB/T 6432–1994, SN/T 0800.5–1999, GB/T 6433–1994, GB/T 6434–1994, GB/T 6436–2002, and GB/T 6437–2002, respectively.

For amino acid analysis, diet samples were hydrolyzed in 6 mol/L HCl at 110°C for 24 h, and 15 amino acids were quantified using an automatic amino acid analyzer (Hitachi L-8800, Japan). Sulfur-containing amino acids (SAA) were determined after performic acid oxidation at 0°C for 16 h followed by acid hydrolysis for 24 h. Tryptophan content was measured after alkaline hydrolysis in 4 mol/L NaOH at 110°C for 22 h using high-performance liquid chromatography (Shimadzu LC-10A, Japan).

**1.4.2 Serum Free Amino Acid Analysis** Serum free amino acids were determined by the ninhydrin post-column derivatization method using a lithium column system on an amino acid analyzer (S-433D Amino Acid Analyzer, Sykam, Germany). Briefly, 0.5 mL serum was mixed with 1.5 mL (3 volumes) of 4% sulfosalicylic acid in a 2.5 mL tube, vortexed thoroughly, and placed on ice for 20 min. Then 0.175 mL of 2 mol/L lithium hydroxide was added, vortexed again, and 2 mL was transferred to a centrifuge tube. After balancing, samples were centrifuged at  $11,000 \times g$  for 30 min at 4°C. The supernatant was filtered through a 0.1  $\mu$ m membrane and injected onto the analyzer. Free amino acids were separated on a lithium ion-exchange column and derivatized with ninhydrin at 130°C for quantification.

**1.4.3 Growth Performance Measurements** Pigs were individually weighed after an overnight fast at the start and end of each period. Feed consumption was recorded per replicate to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

**1.4.4 Carcass Trait and Meat Quality Measurements** Carcass trait measurements and calculations followed the Performance Testing Regulations for Breeding Pigs (NY/T 822-2004). Meat quality was evaluated according to the methods described by Luo et al. [4].

## 1.5 Statistical Analysis

All data were analyzed using the GLM procedure in SAS (2001) with replicate as the experimental unit. Multiple comparisons were performed using the SNK test, and LSMEANS were calculated for mean values. Contrasts were used for pairwise comparisons of growth performance and serum free amino acid contents between the HP and LP-BAA groups, as well as among the low-protein amino acid balance groups. Carcass traits and meat quality parameters were compared using SNK multiple range tests. Significance was declared at  $P \leq 0.05$ , and trends were noted at  $0.05 < P < 0.10$ .

## 2. Results

### 2.1 Effects of Dietary Crude Protein Level and Amino Acid Balance on Growth Performance

The effects of dietary crude protein level and amino acid balance on growth performance are summarized in Table 2. During days 1-14, no significant differences were observed in ADG, ADFI, or FCR between the LP-BAA and HP groups ( $P > 0.05$ ), though the HP group showed a tendency for reduced ADFI ( $P = 0.06$ ). Compared with the LP-BAA group, the LP-Trp group exhibited significant decreases in ADG and ADFI ( $P < 0.05$ ), while the LP-SAA group showed a tendency for reduced ADG ( $P = 0.09$ ) and the LP-Thr group showed no significant differences in ADG or ADFI ( $P > 0.10$ ). During days 15-28, the

HP group tended to have lower ADG compared to the LP-BAA group ( $P = 0.08$ ), while the LP-Trp group again showed significant reductions in ADG and ADFI ( $P < 0.05$ ). Neither the LP-Thr nor LP-SAA groups differed significantly from the LP-BAA group in growth performance ( $P > 0.10$ ). Over the entire 28-day period, the HP group showed a tendency for lower ADFI compared to the LP-BAA group ( $P = 0.05$ ), with ADG and FCR patterns similar to those observed during days 14-28.

## **2.2 Effects of Dietary Crude Protein Level and Amino Acid Balance on Carcass Traits and Meat Quality**

The effects of dietary crude protein level and amino acid balance on carcass traits and meat quality are presented in Table 3 . Amino acid balance had no significant influence on carcass traits (dressing percentage, tenth rib backfat thickness, and loin eye area) or meat quality parameters (pH, muscle color, and drip loss) ( $P > 0.10$ ).

## **2.3 Effects of Dietary Crude Protein Level and Amino Acid Balance on Serum Free Amino Acid Content**

The effects of dietary crude protein level and amino acid balance on serum free amino acid concentrations are shown in Table 4 . Compared with the LP-BAA group, the HP group exhibited significantly elevated serum free phenylalanine (Phe) content ( $P < 0.05$ ) and tended to have higher serum free isoleucine (Ile) and valine (Val) concentrations ( $P < 0.10$ ), while serum free Lys showed a decreasing trend ( $P < 0.10$ ). The LP-SAA and LP-Trp groups tended to have reduced serum concentrations of their respective limiting amino acids compared to the LP-BAA group ( $P < 0.10$ ). Additionally, the LP-Trp group showed tendencies for increased serum concentrations of several essential amino acids (Ile and Met) and non-essential amino acids [alanine (Ala), aspartic acid (Asp), and glutamic acid (Glu)] ( $P < 0.10$ ).

## **3. Discussion**

### **3.1 Effects of Dietary Crude Protein Level and Amino Acid Balance on Growth Performance**

Numerous recent studies have demonstrated that reducing dietary crude protein by up to 4 percentage points while supplementing synthetic amino acids according to requirements or ideal amino acid models does not impair growth performance in growing-finishing pigs [5-7]. Our findings align with these reports, showing that reducing dietary crude protein by approximately 4 percentage points did not affect ADG or FCR compared to the HP group. However, we observed a tendency for reduced ADFI during both the early and entire experimental periods in the HP group compared to the LP-BAA group, which numerically decreased ADG. Previous studies have reported inconsistent effects

of dietary crude protein level on ADFI. Cui et al. [8] found that ADFI in finishing pigs fed low-protein (11.2%) amino acid-balanced diets was significantly reduced by 5.48% compared to the control group. Conversely, Atakora et al. [9] reported that reducing dietary crude protein from 19% to 12% significantly increased body weight and ADFI, while other studies found no significant effect of crude protein level on ADFI [10]. Overall, dietary crude protein level appears to have minimal impact on feed intake in finishing pigs.

In contrast, reducing dietary Trp level significantly decreased ADFI and consequently ADG. Le Floch [11] reported that Trp and Val imbalance in low-protein diets markedly reduces pig ADFI. Our results further demonstrate that deficiencies in different amino acids (10% reduction) affect growth performance to varying degrees: Trp deficiency significantly reduced ADG by suppressing feed intake, SAA deficiency only affected early-stage weight gain, while Thr deficiency showed no detrimental effects on growth performance.

### 3.2 Effects of Dietary Crude Protein Level and Amino Acid Balance on Carcass Traits and Meat Quality

The effect of low-protein diets on carcass fatness remains controversial. Yi [1] observed that reducing dietary net energy from 10.26 MJ/kg to 10.05 MJ/kg significantly decreased tenth rib backfat thickness in finishing pigs, speculating that this might result from amino acid deficiencies in low-protein diets that reduce protein deposition, while increased carcass fat could be attributed to higher dietary net energy. They recommended net energy and Lys:net energy ratios of 10.05 MJ/kg and 0.84 g/MJ, respectively, for low-protein amino acid-balanced diets. In contrast, Madrid et al. [12] reported significantly increased backfat thickness in finishing pigs fed diets with 10% lower crude protein, consistent with Cui et al. [8], who found lower dressing percentage and significantly higher fat thickness in low-protein diet groups compared to normal protein controls. Kerr et al. [13] reported no significant differences in fat content among high, medium, and low net energy groups, but noted that low-protein-fed finishing pigs in the high net energy group had nearly 2 percentage points higher fat content than medium and low net energy groups (33.16%, 31.05%, and 31.48%, respectively). Other studies have shown limited effects of reduced crude protein on carcass fat content, with Thr [14] and Trp [15] balance in low-protein diets having no significant impact on carcass fat content.

Our results showed no significant differences in tenth rib backfat thickness between the LP-BAA and HP groups or among the amino acid-balanced and imbalanced groups. Therefore, carcass fat deposition appears primarily related to energy content rather than amino acid balance.

Drip loss and meat color are practical concerns regarding low-protein diets. Ruusunen et al. [16] found that low-protein diets reduced pH45 min and increased drip loss in longissimus dorsi muscle, though pH24 h did not differ significantly, suggesting a correlation between drip loss and pH45 min. Similarly, Zhang et

al. [17] reported significantly higher drip loss in longissimus dorsi of low-protein-finished finishing pigs compared to normal protein controls, with no differences in pH45 min or pH24 h. While pH24 h is an important meat quality indicator, it only explains 4% of variation in water-holding capacity, whereas pH within 2 h post-slaughter better predicts drip loss [18]. In our study, although numerical differences in pH45 min, pH24 h, and drip loss were observed among groups, none were statistically significant. To date, few reports have addressed the effects of amino acid balance on meat color and drip loss in finishing pigs, and further research is needed to elucidate how dietary amino acid ratios influence meat quality.

### 3.3 Effects of Dietary Crude Protein Level and Amino Acid Balance on Serum Free Amino Acid Content

Serum free amino acids serve as a common indicator of amino acid metabolism, reflecting the relationship between dietary amino acid composition and whole-body amino acid metabolism. Generally, serum free amino acid concentrations at 2.5 h post-feeding reflect dietary amino acid absorption, while levels at 8 h post-feeding reflect amino acid metabolism status [19,20]. In our study, after a 12-hour fast, the HP group showed elevated serum free Ile and Val compared to the LP-BAA group, reflecting the approximately 20% higher content of these essential amino acids in high-protein diets. The LP-Trp group exhibited higher serum concentrations of most essential amino acids than other low-protein groups, clearly demonstrating Trp limitation—insufficient Trp could not meet protein synthesis requirements, leading to accumulation of other essential amino acids in blood.

## Conclusions

1. Reducing dietary crude protein by 4 percentage points (from 14% to 10%) while supplementing synthetic amino acids to meet requirements does not significantly affect growth performance, carcass traits, or meat quality in 70–100 kg finishing pigs.
2. Under low-protein amino acid-balanced conditions, reducing SID SAA, SID Thr, and SID Trp by 10% each had differential effects on growth performance. Specifically, a 10% reduction in SID Trp significantly decreased ADFI and ADG, but had no significant effects on carcass traits or meat quality.

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