# Dynamic Changes in Plasma Amino Acid Metabolic Profile of Early-Weaned Piglets: Postprint

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## Abstract

This study aimed to investigate the dynamic changes in plasma amino acid metabolic profiles in piglets during early weaning. Eight 21-day-old 'Duroc × Landrace × Large White' weaned piglets were selected, and blood samples were collected at 0, 1, 3, 5, 7, 10, 15, and 30 days post-weaning for amino acid analysis. The results showed that plasma arginine, citrulline, and ornithine concentrations decreased significantly within 1–5 days post-weaning ($P<0.05$) and increased from 7–30 days post-weaning. Leucine, proline, tyrosine, and taurine concentrations decreased significantly within 1–3 days post-weaning ($P<0.05$) and then increased. Isoleucine, lysine, glycine, and serine concentrations at 5 days post-weaning were significantly lower than those at other time points ($P<0.05$). Histidine, phenylalanine, valine, aspartic acid, and cysteine concentrations were lowest at 10 days post-weaning. Glutamic acid concentration was significantly lower at 0, 1, and 10 days post-weaning than during other periods ($P<0.05$). Total amino acid content decreased gradually during 1–5 days post-weaning ($P<0.05$), increased from 7 days post-weaning, and showed no significant change up to 30 days post-weaning ($P>0.05$). Principal component analysis revealed that as time post-weaning increased, the difference between the piglet plasma amino acid metabolic profile and that at 0 days post-weaning gradually increased, then gradually approached the position of the metabolic profile at 0 days post-weaning and stabilized, with the most substantial changes in the plasma amino acid metabolic profile occurring at 3–5 days post-weaning. Partial least squares discriminant analysis models indicated that glutamic acid was the most important amino acid for distinguishing plasma metabolic profiles at various time points post-weaning under the NRC (1998) feeding standards. Thus, significant changes occur in protein or amino acid metabolism in piglets during the early weaning stage, with the most pronounced changes occurring at 3–5 days post-weaning.

## Full Text

# Dynamic Changes of Plasma Amino Acid Metabolic Profiles in Early-Weaned Piglets

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**Abstract:** This study investigated the dynamic changes in plasma amino acid (AA) metabolic profiles during the early weaning period in piglets. Eight 21-day-old "Duroc × Landrace × Large Yorkshire" weaned piglets were selected, and blood samples were collected on days 0, 1, 3, 5, 7, 10, 15, and 30 post-weaning for amino acid analysis. The results showed that plasma concentrations of arginine, citrulline, and ornithine decreased significantly ($P<0.05$) from days 1 to 5 post-weaning, then increased from days 7 to 30. Leucine, proline, tyrosine, and taurine concentrations decreased significantly ($P<0.05$) during days 1-3, then subsequently increased. Isoleucine, lysine, glycine, and serine concentrations were significantly lower on day 5 compared to other time points ($P<0.05$). Histidine, phenylalanine, valine, aspartic acid, and cysteine reached their lowest concentrations on day 10. Glutamic acid concentrations were significantly lower on days 0, 1, and 10 compared to other periods ($P<0.05$). Total amino acid concentrations decreased gradually from days 1 to 5 ($P<0.05$), then increased from day 7 onward, with no significant changes observed up to day 30 ($P>0.05$). Principal component analysis revealed that as time progressed post-weaning, the plasma amino acid metabolic profiles gradually diverged from the day 0 profile, then slowly converged back toward it and stabilized, with the most pronounced changes occurring on days 3–5. Partial least squares discriminant analysis modeling indicated that glutamic acid was the most important amino acid for distinguishing plasma metabolic profiles at different post-weaning time points under NRC (1998) feeding standards. These findings demonstrate that significant changes in protein and amino acid metabolism occur in piglets during early weaning, with the most dramatic alterations taking place on days 3–5 post-weaning.

**Keywords:** piglets; early weaning; plasma; amino acids; metabolic profiles

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Early-weaned piglets often exhibit a range of adverse symptoms due to environmental, psychological, and nutritional stress, including anorexia or feed refusal, growth retardation, digestive disorders, diarrhea, edema, and low feed utilization efficiency, which severely impact metabolic homeostasis. Amino acids, as products of protein metabolism and other metabolic processes, play regula-

tory roles in critical metabolic pathways governing animal growth, maintenance, and health. The amino acid pattern—comprising various amino acids and their concentrations in blood—contains important biochemical information reflecting metabolic and functional status. However, due to limitations in data analysis techniques, the biological information embedded in plasma amino acid patterns has not been fully utilized, with analytical applications long restricted to simple Fischer molar ratios or branch-chain amino acid to tyrosine molar ratios.

Metabolomics, an emerging branch of systems biology that quantitatively measures small-molecule metabolites in biological samples, is playing an increasingly important role in nutritional analysis and disease diagnosis. Based on fingerprint analysis of metabolite changes and mathematical modeling, metabolomics identifies biomarkers closely associated with physiological processes, providing new insights for studying the effects of dietary nutritional components. Plasma amino acids, as important metabolites in metabolic networks, reflect the status of tissue protein and energy metabolism. This study employed metabolomic approaches to systematically investigate plasma amino acid metabolic profiles during early weaning in piglets, exploring their dynamic changes and identifying key amino acids.

### 1.1 Experimental Animals

Four third-parity sows were selected, each nursing 10 "Duroc × Landrace × Large Yorkshire" piglets.

### 1.2 Experimental Design

Piglets were weaned at 21 days of age. Eight healthy piglets with uniform body weight (half male and half female) were selected from the four sows for the experiment. Experimental piglets were housed individually and, following the method of Flynn et al. [4], were used for body weight measurement and blood sample collection on days 0, 1, 3, 5, 7, 10, 15, and 30 post-weaning. During the experiment, piglets were housed in an environmentally controlled nursery at (25±2)°C with 65%–75% relative humidity and continuous lighting. Piglets had free access to water and feed throughout the trial. The experimental diet was provided by Zhejiang Guomao Feed Co., Ltd., with the basal diet formulated according to NRC (1998) nutrient requirements for weaned piglets. Diet composition and nutrient levels are presented in Table 1 .

### 1.3 Serum Sample Collection and Preservation

On days 0, 1, 3, 5, 7, 10, 15, and 30 post-weaning, blood samples (4 mL) were collected from the anterior vena cava 8 hours after nursing or feeding. Serum was obtained by centrifugation at 3,000 rpm for 10 min at 4°C and stored at -20°C for amino acid analysis.

### 1.4 Plasma Free Amino Acid Content Determination

One milliliter of serum was mixed with 2.5 mL of 7.5% trichloroacetic acid by vortexing and centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was used for amino acid analysis (ion-exchange amino acid analyzer, Hitachi L-8900 Auto-Analyzer, Tokyo, Japan). The injection volume was 20 L per sample with an analysis cycle of 150 min. Column equilibration time was 35 min, and detection wavelength was 570 nm (440 nm for proline) [5].

### 1.5 Statistical Analysis

Piglet body weight and plasma amino acid concentrations were analyzed using one-way ANOVA and LSD multiple comparisons with SPSS 16.0 software. Data are expressed as mean±SEM.

Data for the 20 amino acids were imported into SIMCA-P (Version 12.0) software. Unsupervised principal component analysis (PCA) was first performed to observe sample clustering, dispersion, and changing trends at different post-weaning time points. To further differentiate amino acid metabolism among post-weaning time points, supervised partial least-squares discriminant analysis (PLS-DA) was employed to identify major differential variables causing clustering or dispersion of plasma samples at different time points, thereby identifying amino acids most relevant to changes in post-weaning metabolic profiles. PLS-DA modeling was performed using post-weaning time points (days) as the Y matrix and amino acid concentrations as the X matrix. Orthogonal partial least-square discriminant analysis (O-PLS-DA) was then used to validate the selected important differential variables [6].

### 2.1 Body Weight of Weaned Piglets

Piglet body weight changed minimally during days 1–3 post-weaning. From day 5 onward, body weight increased gradually, reaching 14.75 kg by day 30 (Table 2 ).

### 2.2 Plasma Amino Acid Content in Weaned Piglets

As shown in Table 3 , plasma concentrations of arginine and its direct precursors citrulline and ornithine decreased significantly ($P<0.05$) during days 1-5 post-weaning, then increased during days 7–30. Leucine, proline, tyrosine, and taurine concentrations decreased significantly ($P<0.05$) during days 1–3, then increased thereafter. Isoleucine, lysine, glycine, and serine concentrations were significantly lower on day 5 compared to other time points ($P<0.05$), then increased during days 7–30. Methionine concentrations changed minimally during days 0–3 ($P>0.05$), but on day 5 increased by 41.61% ($P<0.05$), 43.51% ($P<0.05$), and 41.11% ($P<0.05$) compared to days 0, 1, and 3, respectively, remaining stable thereafter. Plasma threonine concentration on day 3 increased by 31.67% ($P<0.05$) and 79.06% ($P<0.05$) compared to days 0 and 1, respectively, reaching maximum on day 10, then decreasing significantly on days 15

and 30. Glutamic acid content (which includes glutamine as the two cannot be separated by the amino acid analyzer) was significantly lower on days 0, 1, and 10 compared to other time points ($P<0.05$). Alanine concentration decreased significantly on day 1 ($P<0.05$), increased during days 3–10, then decreased during days 15–30. Total amino acid concentration was significantly higher on day 0 compared to all post-weaning time points ($P<0.05$), decreased gradually from day 1 to day 5, then increased from day 7 onward with no significant changes up to day 30 ($P>0.05$).

### 2.3 Principal Component Analysis

To make the analysis of multiple samples more intuitive, visual, and statistically meaningful, this study first employed unsupervised PCA to group piglet plasma samples. The PCA score plot (Figure 1 [Figure 1: see original paper]) showed that day 0 plasma samples were clearly separated from all other time points (each point on the PCA score plot represents a corresponding sample). Day 1 samples clustered with days 15 and 30; days 3 and 5 clustered together and showed the greatest difference from day 0; days 7 and 10 were separated from other time points. PCA results reflected substantial dynamic changes in plasma amino acids post-weaning, with profiles beginning to stabilize from day 15 onward.

Variables in the loading plot reflect their contribution to sample discrimination at different time points and the correlation between variables—the farther from the origin, the greater the contribution to grouping. As shown in Figure 2 [Figure 2: see original paper], glutamic acid, threonine, proline, and citrulline were located relatively far from the origin, indicating their substantial contribution to discriminating samples at different time points and their adequate explanation by these two principal components.

Additionally, this study used mean values of the 20 amino acids to depict temporal trends in plasma amino acid metabolic profiles of weaned piglets through PCA modeling (Figures 3 [Figure 3: see original paper] and 4 [Figure 4: see original paper]). Both figures clearly revealed the pattern of plasma amino acids transitioning from large variations to stabilization after weaning. From day 1 post-weaning, profiles differed from day 0; days 3 and 5 showed the greatest changes, deviating from all other time points; from day 7 onward, metabolic profile spatial coordinates began moving gradually toward day 1, and by day 30 had approached day 1 and were relatively close to the day 0 position.

### 2.4 Partial Least Squares Discriminant Analysis

PLS-DA is a supervised analytical method based on PCA modeling that establishes mathematical models between categories to achieve maximum separation among sample groups and predict unknown samples using the established multi-parameter model. In this experiment, to further investigate the effects of early weaning on piglet plasma amino acid metabolic profiles, PLS-DA was used to

remodel plasma samples from different post-weaning time points to identify amino acids with greater model contributions, facilitating deeper understanding of weaning stress effects on piglet protein and amino acid metabolism from a metabolite perspective. Variable importance in projection (VIP) values in PLS-DA models reflect the correlation between X variables and Y, and are used to select important metabolites. Typically, variables with VIP values greater than 1 show good correlation with Y. Figure 5 [Figure 5: see original paper] shows that in the amino acid metabolic profile PLS-DA model, glutamic acid+glutamine, glycine, alanine, proline, lysine, and threonine had VIP values greater than 1, indicating they were important amino acids, with glutamic acid showing the highest VIP value of 1.74.

### 2.5 Orthogonal Partial Least Squares Discriminant Analysis

The orthogonal partial least squares score plot reflects important variables in the PLS-DA model from another perspective—variables farther from the origin are more important. As shown in Figure 6 [Figure 6: see original paper], glutamic acid+glutamine, glycine, alanine, proline, and lysine were located relatively far from the origin, consistent with the VIP value ranking results.

Amino acids are fundamental components of proteins and important substances in life activities. They are converted into carbohydrates or lipids through a series of biochemical metabolic pathways and synthesize various essential bioactive substances such as hormones, enzymes, nucleic acids, polyamines, and neurotransmitters. Under normal conditions, free amino acid levels are relatively stable. Essential amino acids that cannot be synthesized in the body are primarily obtained from food, while non-essential amino acids are regulated through synthesis, release, and utilization. Amino acid imbalances typically impair normal growth and metabolism and may even cause pathological changes. More than 20 amino acids constitute the amino acid metabolic pool in mammals, which comprises exogenous amino acids (absorbed from the gastrointestinal tract) and endogenous amino acids (from protein degradation or de novo synthesis), participating in various metabolic processes. Calculated by total free amino acids, muscle accounts for 50%–80% of the amino acid metabolic pool, liver for 10%, kidney for approximately 4%, and blood for 1%–6%. Although blood amino acid content is not high, its rapid metabolism plays an important role in transporting and exchanging various amino acids in the pool, and its concentration normally remains constant. Plasma amino acid content is influenced not only by growth stage, diet, and environmental factors but also by protein digestion, absorption, synthesis and degradation, gluconeogenesis and oxidative decomposition, and amino acid transport. However, through certain metabolic regulatory mechanisms, concentrations remain relatively constant, forming a specific ratio known as the amino acid profile.

Although amino acid composition generally remains relatively constant, amino acid concentrations in physiological fluids such as plasma and milk can undergo corresponding dynamic changes at different stages. Flynn et al. [4] found that

in suckling piglets, plasma concentrations of arginine and its direct precursors citrulline and ornithine decreased gradually from 3 to 14 days after birth; glutamine content decreased continuously within 7 days after birth; and branched-chain amino acids, valine, and alanine were significantly lower at 14 and 21 days compared to 1 and 3 days after birth. During the 1- to 21-day lactation period, free glutamine concentration in sow milk increased from 0.1 mmol/L to 4 mmol/L [8], corresponding to a decrease of over 50% in glutamine content in lactating sow muscle [9]. This study systematically investigated dynamic changes in plasma amino acids after early weaning in piglets and found that 21-day-old weaned piglets showed substantial changes in amino acid content during days 3-7 post-weaning compared to day 0, with most amino acids showing a decreasing trend. From days 10–30 post-weaning, plasma free amino acid concentrations began to maintain relatively stable and higher levels. Total plasma amino acid content was lowest on day 5, at 73.61% of day 0 and 86.16% of day 30 values.

Weaning is typically accompanied by dramatic changes in living environment, food type, and intake patterns [10]. After weaning, piglets consume complete compound feeds rich in nitrogen compounds but low in easily digestible fats, proteins, and carbohydrates, with virtually no free amino acids [11-12]. These dietary changes cause substantial alterations in intestinal structure, with feed intake and nutrient digestion/absorption capacity decreasing sharply [13]. Pié et al. [14] found that piglets consumed only 11 g of feed within 24 h after weaning; proximal small intestinal villus circumference decreased by 29% within 24 h post-weaning with no significant changes in the subsequent 8 days; mid and distal small intestinal villus circumference decreased significantly by ~16% on days 1 and 2; proximal small intestinal sucrase activity decreased by 85% on day 1; and distal small intestinal sucrase activity decreased by 30% on day 2 compared to day 1; intestinal interleukin-1 (IL-1 ), interleukin-6 (IL-6), and tumor necrosis factor- (TNF- ) mRNA levels increased. These findings reflect rapid reduction in amino acid absorption from the small intestine after weaning, leading to decreased amino acid entry into blood and partially explaining the reduced amino acid content during days 3-7 post-weaning. As time progressed after weaning, piglets adapted to their new environment and solid feed, while their gastrointestinal tracts continued developing with increased digestive enzyme activity, restoring pre-weaning levels by two weeks post-weaning [15-16]. In this study, plasma amino acid concentrations gradually returned to normal levels from day 10 post-weaning, likely due to recovery of gastrointestinal function, enhanced nutrient digestion and absorption, increased amino acid entry through intestinal epithelial cells, and elevated plasma free amino acid concentrations.

Glutamine directly or indirectly affects numerous physiological activities including protein synthesis and degradation, extracellular matrix synthesis, glycogenesis, lipid metabolism, cell proliferation and apoptosis, redox potential, respiratory burst, and insulin secretion and resistance [7,17]. This study found that plasma glutamic acid and glutamine concentrations in weaned piglets showed a decreasing→increasing→decreasing→increasing pattern. Glutamic acid+glutamine concentrations decreased significantly on day 1, by 17.50%

compared to day 0. Glutamine is the most abundant free amino acid in sow milk [8]; after weaning, piglets no longer receive milk intake, while glutamine production from muscle and other tissues is weak on day 1, resulting in decreased plasma glutamine. During days 3–5 post-weaning, due to continuously low feed intake and digestion/absorption rates, reduced exogenous amino acid intake led to accelerated muscle protein breakdown to meet amino acid metabolic needs, releasing large amounts of glutamine and alanine into blood [18], increasing plasma glutamic acid+glutamine concentrations by 23.42% and 20.90% on days 3 and 5 compared to weaning day, respectively, with alanine also increasing to varying degrees. From a nutritional perspective, accelerated muscle protein breakdown reduces essential amino acid utilization in muscle [18], correspondingly decreasing lysine content during days 3–5. During days 7–10, glutamic acid and glutamine concentrations began decreasing, with day 10 concentrations 23.34% lower than weaning day, likely due to substantial glutamine depletion in muscle and still insufficient glutamine from digestion/absorption. After 14 days post-weaning, piglet intestinal structure developed substantially with enhanced digestion/absorption capacity [16], allowing large-scale amino acid absorption from the intestine and maintaining balance in the amino acid pool. Therefore, during days 15–30, glutamic acid+glutamine concentrations increased to higher levels, reaching 1.12–1.19 times the weaning day values.

Plasma amino acid metabolic profiles in early-weaned piglets were in dynamic flux. Total amino acid content was significantly higher on weaning day than at all post-weaning time points, decreasing gradually from days 1 to 5, then increasing from day 7 with no significant changes up to day 30. PCA score plots showed that as time progressed post-weaning, plasma amino acid metabolic profiles first diverged from the day 0 profile, then gradually converged back toward it and stabilized, with the most pronounced changes occurring on days 3–5. Glutamic acid was the most important amino acid for distinguishing plasma metabolic profiles at different post-weaning time points in this PLS-DA model under NRC (1998) feeding standards.

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