

## Dynamic Responses of Serum Amino Acid Content and Biochemical Parameters in Weaned Piglets to Different Dietary Protein Levels: Postprint

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**Date:** 2017-11-07T00:00:00+00:00

### Abstract

This study aimed to investigate the dynamic response of serum amino acid content and biochemical parameters in weaned piglets to diets with different protein levels. Eighteen 35-day-old crossbred (Duroc × Landrace × Yorkshire) weaned barrows with an average body weight of  $(10.0 \pm 1.0)$  kg were selected and randomly divided into 3 groups (6 replicates per group, 1 pig per replicate), and fed diets with protein levels of 14% (LP), 17% (MP), and 20% (HP), respectively. Each experimental pig was fitted with a carotid artery catheter, recovered for 5 days post-surgery, and the formal experiment was conducted on days 6–8. Before feeding and at 30, 60, 120, 180, and 300 min after feeding each day, 10 mL of blood was continuously collected from the carotid artery to determine serum free amino acid content and related biochemical indices. The results showed that dietary protein level had no significant effect on the content of free lysine (Lys), methionine (Met), threonine (Thr), tryptophan (Trp), aspartic acid (Asp), serine (Ser), glycine (Gly), and glutamic acid (Glu) in serum at each time point before and after feeding in piglets ( $P > 0.05$ ). However, the contents of Lys, Met, Thr, and Trp in serum increased with decreasing dietary protein level. The contents of serum free amino acids [valine (Val), isoleucine (Ile), arginine (Arg)] and urea in the HP group were significantly higher than those in the MP and LP groups at each time point before and after feeding ( $P < 0.05$ ). Serum Leu content in the HP group was significantly higher than that in the LP group at 120–300 min after feeding ( $P < 0.05$ ). Serum total protein (TP) and albumin (ALB) contents and alkaline phosphatase (ALP) activity in the LP group were significantly higher than those in the HP and MP groups at each time point after feeding ( $P < 0.05$ ). There was no significant difference in serum free amino acid content and related biochemical indices over time after

feeding among all groups ( $P>0.05$ ). In conclusion, when the diet of weaned piglets was supplemented with 4 amino acids (Lys, Met, Thr, Trp) to maintain balance, reducing the conventional dietary protein level by 3 percentage points significantly decreased serum free Val, Ile, and Arg contents, but had no significant effect on other amino acids and related biochemical indices in serum; while reducing the protein level by 6 percentage points significantly decreased serum free Leu and urea contents, significantly increased TP and ALB contents and ALP activity, but had no significant effect on other amino acids and related biochemical indices in serum.

## Full Text

### Dynamic Responses of Serum Amino Acid Content and Biochemical Parameters in Weaned Piglets to Diets with Different Protein Levels

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## Abstract

This study investigated the dynamic responses of serum amino acid content and biochemical parameters in weaned piglets to diets with different protein levels. Eighteen 35-day-old crossbred (Duroc × Landrace × Large White) castrated male piglets with an average body weight of (10.0±\$1.0) kg were randomly assigned to three groups (6 replicates per group, 1 pig per replicate) and fed diets containing 14% (LP), 17% (MP), and 20% (HP) crude protein, respectively. Each experimental pig was fitted with a carotid artery catheter. After a 5-day postoperative recovery period, the formal trial was conducted on days 6–8. Blood samples (10 mL) were collected from the carotid artery before feeding and at 30, 60, 120, 180, and 300 min postprandially each day to determine serum free amino acid concentrations and related biochemical indices. The results showed that dietary protein level had no significant effect on serum concentrations of free lysine (Lys), methionine (Met), threonine (Thr), tryptophan (Trp),

aspartic acid (Asp), serine (Ser), glycine (Gly), and glutamic acid (Glu) at any time point before or after feeding ( $P>0.05$ ). However, serum concentrations of Lys, Met, Thr, and Trp tended to increase as dietary protein level decreased. Serum concentrations of free amino acids [valine (Val), isoleucine (Ile), arginine (Arg)] and urea in the HP group were significantly higher than those in the MP and LP groups at all time points ( $P<0.05$ ). Serum leucine (Leu) concentration in the HP group was significantly higher than that in the LP group at 120–300 min postprandially ( $P<0.05$ ). Serum total protein (TP), albumin (ALB) concentrations, and alkaline phosphatase (ALP) activity in the LP group were significantly higher than those in the HP and MP groups at all postprandial time points ( $P<0.05$ ). No significant differences were observed in serum free amino acid concentrations or related biochemical indices over time within any group ( $P>0.05$ ). In conclusion, when dietary protein level was reduced by 3 percentage points while balancing four amino acids (Lys, Met, Thr, Trp), serum concentrations of free Val, Ile, and Arg were significantly decreased, but other amino acids and related biochemical parameters were not significantly affected. When dietary protein level was reduced by 6 percentage points, serum concentrations of free Leu and urea were significantly decreased, while TP, ALB concentrations, and ALP activity were significantly increased, though other amino acids and related biochemical parameters remained unaffected.

**Keywords:** protein level; weaned piglets; amino acids; biochemical parameters

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## Introduction

With the intensification of swine production in recent years, problems related to manure discharge and feed ingredient waste have become increasingly severe. More than 50% of nitrogen in pig feed is excreted in feces and urine, most of which is oxidized into nitrates that flow into rivers and lakes, causing widespread environmental pollution [1]. Addressing excessive nitrogen emissions and reducing waste of dietary protein ingredients represents a major challenge for the pig industry. Many scholars believe that dietary protein level can be reduced while supplementing with synthetic amino acids to improve feed utilization and reduce environmental pollution without compromising or even improving production performance [2–3]. Research has shown that reducing dietary protein level increases both the number and degree of limiting amino acids [4]. Therefore, when formulating low-protein diets using an “amino acid balance pattern,” it is necessary to supplement synthetic limiting amino acids such as lysine (Lys), methionine (Met), threonine (Thr), and tryptophan (Trp) to meet animal requirements. This study employed carotid artery catheterization technology to investigate the dynamic effects of different dietary protein levels on serum amino acid concentrations and biochemical parameters in weaned piglets, monitoring changes in blood amino acids and biochemical indices before and after feeding in real time, to provide a scientific reference for reducing dietary protein level, improving feed utilization, and decreasing environmental pollution.

### 1.1 Experimental Design

Eighteen 35-day-old crossbred (Duroc  $\times$  Landrace  $\times$  Large White) weaned castrated male piglets (purchased from Hunan New Wufeng Yong' an Branch) with an average body weight of  $(10.0 \pm 1.0)$  kg were randomly allocated to three groups (6 replicates per group, 1 pig per replicate) and housed individually. They were fed diets containing 14% (LP), 17% (MP), and 20% (HP) crude protein. Diet formulation followed the nutrient requirements for weaned piglets recommended by NRC (2012) [5]. The protein sources were consistent across diets, with balanced levels of Lys, Met, Thr, and Trp. The contents of these four amino acids and energy levels were maintained identical across diets. Diet composition and nutrient levels were consistent with those reported in our previous study [6].

### 1.2 Experimental Materials and Instruments

- Amino acid analyzer (L-8800, Japan)
- XC4 automatic biochemical analyzer (Beckman, USA)
- TG16W micro high-speed centrifuge (China)
- Sulfosalicylic acid (China)

### 1.3 Animal Management and Carotid Artery Catheterization Surgery

Piglets were housed in a clean, well-ventilated barn at  $(23 \pm 1)^\circ\text{C}$  with ad libitum access to feed and water. After 5 days of feeding their respective experimental diets, the piglets were fasted for 12 h (with free access to water) and weighed on day 6. Carotid artery catheterization surgery was then performed on each experimental piglet by group. Prior to surgery, atropine (0.05 mg/kg, Xinchang Pharmaceutical Factory, Zhejiang Pharmaceutical Co., Ltd.) was injected, followed by Zoletil 50 (0.1 mg/kg, Virbac, France) 15 min later. After 10-15 min, when the animal was anesthetized, the right side of the neck was disinfected and shaved. The carotid artery catheterization procedure followed the methods described by Li et al. [7] and Morris et al. [8].

### 1.4 Postoperative Management

The operating room was maintained at approximately  $25^\circ\text{C}$  during surgery. Postoperatively, piglets were individually housed in metabolic cages, fasted for 1 day, and fed only 5% glucose solution. On the second day post-surgery, piglets were fed their respective experimental diets with ad libitum access to feed and water. At 08:00, 16:00, and 23:00 each day, a small amount of fresh blood was drawn from the vascular catheter using a sterile syringe, followed by infusion of 10 mL heparin sodium solution (400 IU/mL) to maintain catheter patency. After a 5-day recovery period (during which piglets resumed normal feed intake), the formal trial commenced.

## 1.5 Sample Collection and Storage

On days 6–8 post-surgery, blood samples were collected continuously before feeding and at 30, 60, 120, 180, and 300 min after feeding. Each time, 10 mL of blood was drawn from each pig using a 10 mL syringe into a 10 mL centrifuge tube, centrifuged at 3,000 r/min for 10 min at 4°C, and the supernatant was aliquoted into 1.5 mL centrifuge tubes and stored at -80°C for subsequent determination of serum amino acid concentrations and biochemical indices.

### 1.6.1 Determination of Serum Free Amino Acid Content

One mL of serum was mixed with 1 mL of 8% sulfosalicylic acid by vortexing, left to stand overnight at 3°C, and centrifuged at 12,000 r/min for 15 min at 4°C. The supernatant was filtered through a 0.22 μm filter, and 500 μL of the filtrate was analyzed using an amino acid analyzer.

### 1.6.2 Analysis of Serum Biochemical Indices

A fully automatic biochemical analyzer was used to determine serum concentrations of urea, total protein (TP), albumin (ALB), immunoglobulin M (IgM), immunoglobulin G (IgG), and alkaline phosphatase (ALP) activity in freshly separated serum.

## 1.7 Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 21.0 software. Duncan's multiple comparison test was used to determine significant differences, with  $P < 0.05$  as the criterion for statistical significance.

## Results

### 2.1 Dynamic Response of Serum Essential Amino Acids Lys, Met, Thr, and Trp to Different Dietary Protein Levels

This study was based on our previous research, which demonstrated that reducing dietary protein level from 20% to 17% for weaned piglets (10–30 kg) while meeting Lys, Met, Thr, and Trp requirements did not affect average daily gain, average daily feed intake, or feed conversion ratio, but reducing protein to 14% significantly impaired growth performance [9]. As shown in [Figure 1: see original paper], reducing dietary protein level while supplementing the four essential amino acids (Lys, Met, Thr, and Trp) resulted in no significant differences in serum free Lys, Met, Thr, and Trp concentrations at any time point before or after feeding ( $P > 0.05$ ). Postprandially, serum concentrations of free Lys, Met, Thr, and Trp in all groups showed a trend of increasing initially, then decreasing, and finally stabilizing, but these temporal changes were not significant ( $P > 0.05$ ).

Letter a indicates significant difference between HP and MP groups at the same time point ( $P < 0.05$ ), letter b indicates significant difference between HP and LP groups ( $P < 0.05$ ), letter c indicates significant difference between MP and LP groups ( $P < 0.05$ ), and no letter indicates no significant difference ( $P > 0.05$ ). The same applies below.

**Fig.1** Effects of diets with different protein levels on contents of free Lys, Met, Thr and Trp in serum of weaned piglets

## 2.2 Dynamic Response of Serum Amino Acids Val, Ile, Leu, and Arg to Different Dietary Protein Levels

As shown in [Figure 2: see original paper], serum concentrations of free branched-chain amino acids (Val, Ile, Leu) and Arg decreased with decreasing dietary protein level. Postprandially, serum concentrations of free Val and Ile in the HP group were significantly higher than those in the MP and LP groups ( $P < 0.05$ ). The MP group showed significantly higher Val concentrations at 120 and 180 min postprandially and higher Ile concentration at 180 min compared to the LP group ( $P < 0.05$ ), with no significant differences between MP and LP groups at other time points ( $P > 0.05$ ). Preprandially, Leu concentration in the HP group was significantly higher than in the MP and LP groups ( $P < 0.05$ ). At 30 and 60 min postprandially, Leu concentrations in the LP and MP groups showed an upward trend, with no significant differences among HP (24.34 and 26.70 g/mL), MP (18.42 and 21.16 g/mL), and LP (22.32 and 23.39 g/mL) groups ( $P > 0.05$ ). From 120 min postprandially onward, Leu concentration in the LP group was significantly lower than in the HP group ( $P < 0.05$ ). At 300 min postprandially, Leu concentration in the HP group was significantly higher than in the MP group ( $P < 0.05$ ), with no significant differences between LP and MP groups at other time points ( $P > 0.05$ ). Preprandially, Arg concentration in the HP group was significantly higher than in the LP group ( $P < 0.05$ ), with no significant difference between HP and MP groups ( $P > 0.05$ ). From 30 to 180 min postprandially, Arg concentration in the HP group was significantly higher than in both MP and LP groups ( $P < 0.05$ ), while at 300 min, no significant differences were observed among the three groups ( $P > 0.05$ ). No significant temporal changes in serum concentrations of free Val, Ile, Leu, and Arg were detected within any group ( $P > 0.05$ ).

**Fig.2** Effects of diets with different protein levels on contents of free Val, Ile, Leu and Arg in serum of weaned piglets

## 2.3 Dynamic Response of Serum Non-Essential Amino Acids Pro, Asp, Ser, Gly, Cys, and Glu to Different Dietary Protein Levels

As shown in [Figure 3: see original paper], except for free Pro and Cys, no significant differences were observed in serum concentrations of free Asp, Ser, Gly, and Glu among groups at any time point ( $P > 0.05$ ). Preprandially, Pro concentration in the HP group (20.34 g/mL) was significantly higher than in

the MP (10.81 g/mL) and LP (13.90 g/mL) groups ( $P < 0.05$ ), and remained significantly higher than in the LP group at 180 min postprandially ( $P < 0.05$ ). No significant differences were observed between HP and MP groups or between MP and LP groups at other time points ( $P > 0.05$ ). Except for Cys and Gly, concentrations of non-essential amino acids Asp, Ser, Pro, and Glu decreased with decreasing dietary protein level, while Cys and Gly concentrations increased. No significant temporal changes in serum concentrations of non-essential amino acids Pro, Asp, Ser, Gly, Cys, and Glu were observed within any group ( $P > 0.05$ ).

**Fig.3** Effects of diets with different protein levels on contents of free Pro, Asp, Ser, Gly, Cys and Glu in serum of weaned piglets

#### 2.4 Dynamic Response of Serum Biochemical Parameters to Different Dietary Protein Levels

As shown in [Figure 4: see original paper]-A, 4-B, and 4-C, TP, ALB concentrations, and ALP activity in the LP group were higher than in the other two groups at all time points. Except for the preprandial time point, TP concentration in the LP group was significantly higher than in the HP and MP groups ( $P < 0.05$ ), with no significant difference between HP and MP groups ( $P > 0.05$ ). Serum ALB concentration in the LP group (25.96, 25.36, and 25.60 g/L) was significantly higher than in the HP (22.52, 22.86, and 22.54 g/L) and MP (22.98, 22.86, and 22.54 g/L) groups at 120, 180, and 300 min postprandially ( $P < 0.05$ ), with no significant differences among groups at other time points ( $P > 0.05$ ). Serum ALP activity in the LP group was significantly higher than in the HP group from 60 min postprandially onward ( $P < 0.05$ ) and significantly higher than in the MP group at 60 and 180 min postprandially ( $P < 0.05$ ), with no significant differences among groups at other time points ( $P > 0.05$ ). ALP activity increased with decreasing dietary protein level. No significant temporal changes in serum TP, ALB concentrations, or ALP activity were observed within any group ( $P > 0.05$ ).

As shown in [Figure 4: see original paper]-D, serum urea concentration in the HP group was significantly higher than in the LP group at all time points ( $P < 0.05$ ) and significantly higher than in the MP group preprandially and at 30 and 300 min postprandially ( $P < 0.05$ ). The MP group showed significantly higher urea concentration than the LP group at 120-300 min postprandially ( $P < 0.05$ ). No significant temporal changes in serum urea concentration were observed within any group ( $P > 0.05$ ). As shown in [Figure 4: see original paper]-E and 4-F, serum IgG and IgM concentrations in the LP group were significantly lower than in the HP group at 120 min postprandially ( $P < 0.05$ ), with no significant differences among groups at other time points ( $P > 0.05$ ). No significant temporal changes in serum IgM and IgG concentrations were observed within any group ( $P > 0.05$ ).

**Fig.4** Effects of diets with different protein levels on contents of TP, ALB, urea,

IgG, IgM and ALP activity in serum of weaned piglets

## Discussion

### 3.1 Dynamic Response of Serum Lys, Met, Thr, and Trp Concentrations to Different Dietary Protein Levels in Weaned Piglets

Previous studies have shown that reducing dietary protein level while supplementing Lys, Met, Thr, and Trp can improve dietary protein utilization and reduce nitrogen excretion in feces and urine [10-11]. In this study, dietary protein level was reduced by 3 and 6 percentage points, and the “amino acid balance pattern” was applied to supplement synthetic Lys, Met, Thr, and Trp to investigate changes in serum free amino acid concentrations. The results showed that reducing dietary protein level by 3 or 6 percentage points had no significant effect on serum Lys, Met, Thr, and Trp concentrations, and the concentrations of these four supplemented amino acids increased with decreasing dietary protein level. However, when dietary protein level was reduced by 6 percentage points, serum concentrations of free Lys, Met, Thr, and Trp were higher than in the other two groups at 0-120 min postprandially, consistent with the findings of Deng et al. [12]. This may be because as dietary protein level decreases, the content of supplemented Lys, Met, Thr, and Trp increases, and these synthetic amino acids can be absorbed directly by the gastrointestinal tract without requiring protease digestion. This suggests that appropriately reducing protein level while supplementing synthetic amino acids to meet piglet requirements can enhance gastrointestinal absorption of Lys, Met, Thr, and Trp to some extent.

### 3.2 Dynamic Response of Serum Val, Ile, Leu, and Arg Concentrations to Different Dietary Protein Levels in Weaned Piglets

Serum free amino acid concentrations can reflect animal amino acid requirements to some extent. When dietary amino acid content is insufficient, serum free amino acid concentrations remain low and accumulate rapidly after feeding [13]. When dietary protein level was reduced by 3 or 6 percentage points, serum concentrations of free branched-chain amino acids (Val, Ile, Leu) and Arg at all time points were significantly lower than in piglets fed the 20% protein diet. This indicates that reducing dietary protein level significantly affects essential amino acid concentrations in weaned piglet serum, likely because the reduced-protein diet cannot provide sufficient Val, Ile, Leu, and Arg to meet piglet growth requirements [12]. Additionally, this suggests that synthetic amino acids differ from those derived from dietary protein, and therefore cannot completely replace amino acids from protein sources. Branched-chain amino acids Val, Leu, and Ile are closely related to protein and energy metabolism. Leu regulates energy and protein metabolism through the mammalian target of rapamycin (mTOR) pathway, promoting animal growth and health [14-15]. Studies have shown that reducing dietary protein level while supplementing branched-chain amino acids can improve piglet performance, increase feed intake, and enhance

muscle growth [16]. In this study, reducing dietary protein level while supplementing only Lys, Met, Thr, and Trp resulted in lower serum concentrations of free branched-chain amino acids, suggesting that diets should also be supplemented with appropriate amounts of branched-chain amino acids to meet piglet growth and health needs.

### **3.3 Dynamic Response of Serum Pro, Asp, Ser, Gly, Cys, and Glu Concentrations to Different Dietary Protein Levels in Weaned Piglets**

Reducing dietary protein level by 3 or 6 percentage points had no significant effect on serum concentrations of free non-essential amino acids (except Pro and Cys) at any postprandial time point. Serum free non-essential amino acid concentrations did not change significantly over time, but concentrations of non-essential amino acids (except Gly and Cys) decreased with decreasing dietary protein level, while Cys and Gly concentrations increased. Research indicates that when dietary non-essential amino acids cannot meet animal requirements, animals can synthesize them through transamination reactions [17-18]. In this study, reducing protein level decreased serum Pro concentration but increased Gly and Cys concentrations. This may be because Pro serves as an important indicator of intestinal stress and protein catabolism, playing a crucial role in maintaining healthy growth of weaned piglets [19-23], and reducing protein level directly causes endogenous Pro deficiency, affecting normal growth and development. Serum Gly and Cys play important roles in fat metabolism and detoxification [24-26], and reducing dietary protein level itself is a stressor that may upregulate serum Gly and Cys concentrations to mitigate the effects of insufficient dietary protein. However, the different response mechanisms of Pro, Gly, and Cys in weaned piglet serum to reduced protein level require further investigation.

### **3.4 Dynamic Response of Serum Biochemical Parameters to Different Dietary Protein Levels in Weaned Piglets**

Changes in serum biochemical indices reflect overall metabolic function and alterations in tissue cell permeability. Serum TP concentration represents dietary protein nutritional level and animal protein digestion and absorption to some extent. ALB, synthesized by liver parenchymal cells, is the most abundant protein in serum (accounting for 40–60% of total serum protein), and its synthesis efficiency is affected by dietary protein level [27]. Elevated TP and ALB concentrations indicate vigorous protein metabolism and improved feed utilization. Studies have shown that reducing dietary protein level by 3 percentage points in chickens and geese significantly increased serum TP concentration without affecting ALB concentration [28-29], similar to our results. When dietary protein level was reduced by 6 percentage points, serum TP and ALB concentrations increased significantly, indicating that this level of protein reduction improved piglets' digestion and absorption of dietary protein and enhanced liver albumin synthesis capacity.

Serum urea, the end product of protein metabolism, reflects protein metabolic status and is inversely related to nitrogen utilization efficiency in the diet [30-31]. Nitrogen metabolism in different tissues and organs is coordinated through key nitrogenous metabolites including glutamine, alanine, and urea [32-35]. In this study, serum urea concentration decreased with decreasing dietary protein level, indicating that appropriate protein reduction can improve nitrogen utilization efficiency and reduce nitrogen emissions. These results are consistent with findings from Zheng et al. [36], Zhang et al. [37], and Toledo et al. [38].

ALP is a key enzyme in digestion and metabolism that generates phosphate to form calcium phosphate precipitates, participating in bone calcification and serving as an important indicator of osteoblast activity [39]. This study found that feeding weaned piglets a 14% protein diet resulted in significantly higher serum ALP activity. This may be because a 6-percentage-point reduction in dietary protein causes abnormal liver function and related diseases, as studies have reported that liver diseases such as obstructive jaundice, primary liver cancer, secondary liver cancer, and cholestatic hepatitis cause excessive ALP production by hepatocytes, leading to elevated serum ALP activity [40].

Humoral immunity is an important mechanism for disease resistance, depending on the types and numbers of immunoglobulins [41]. IgM binds to pathogens first to dissolve them upon infection, while IgG has antibacterial and antiviral effects [42]. This study found that reducing dietary protein level by 6 percentage points significantly decreased serum IgG and IgM concentrations at 120 min postprandially, with no effects at other time points, similar to results from Hou et al. [43] on the effects of 20% and 18% protein diets on serum IgG in early-weaned piglets. This suggests that reducing dietary protein level generally does not affect IgG and IgM concentrations and does not compromise piglet immune capacity, though the transient decrease at 120 min postprandially warrants further investigation.

## Conclusion

Under the conditions of this study, reducing dietary protein by 3 percentage points while supplementing Lys, Met, Thr, and Trp did not affect serum concentrations of these amino acids, Leu, or non-essential amino acids (Asp, Ser, Gly, Cys, Glu), nor did it affect serum IgM, IgG, urea, TP, ALB concentrations, or ALP activity, but significantly decreased serum essential amino acids Val, Ile, and Arg. Reducing dietary protein by 6 percentage points while supplementing Lys, Met, Thr, and Trp significantly decreased serum essential amino acid Leu and urea concentrations while increasing serum TP, ALB concentrations, and ALP activity. Therefore, to maintain growth performance while reducing dietary protein level in weaned piglets (to 17%), requirements for Val, Leu, Ile, and Arg must be considered in addition to Lys, Met, Thr, and Trp to provide scientific guidance for improving feed utilization and reducing nitrogen emissions.

## Acknowledgments

We thank the Public Technology Service Center of the Institute of Subtropical Agriculture, Chinese Academy of Sciences for assistance with serum amino acid and biochemical parameter analyses.

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