Effects of Dietary Crude Protein Level on Hepatic Amino Acid Metabolic Enzyme Activity and Transporter mRNA Expression in Weaned Piglets (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary crude protein (CP) level on hepatic amino acid metabolic enzyme activities and transporter mRNA expression in weaned piglets. A total of 54 healthy Duroc × Landrace × Yorkshire crossbred weaned piglets at 28 days of age with similar body weight [(7.0±0.5) kg] (half male and half female) were randomly divided into 3 groups [20% CP group (control), 17% CP group, and 14% CP group], with 18 replicates per group and 1 pig per replicate. The experiment consisted of a 7-day pre-trial period and a 45-day formal trial period. On days 10, 25, and 45 of the formal trial period, 6 piglets were selected from each group for slaughter. The results showed: 1) On day 10 of the experiment, hepatic aspartate aminotransferase (GOT) activity in the 14% and 17% CP groups was significantly lower than that in the control group ($P<0.05$); hepatic glutamine synthetase (GS) activity in the 14% CP group was significantly lower than that in the other two groups ($P<0.05$). On day 25, hepatic GOT and GS activities in the 14% CP group were significantly lower than those in the control group ($P<0.05$); hepatic alanine aminotransferase (GPT) and glutamate dehydrogenase (GDH) activities in the 14% and 17% CP groups were significantly lower than those in the control group ($P<0.05$). On day 45, hepatic GPT and GS activities in the 14% CP group were significantly lower than those in the other two groups ($P<0.05$). 2) On day 25 of the experiment, the relative mRNA expression levels of hepatic solute carrier family 6 member 15 (SLC6A15) and solute carrier family 38 member 2 (SLC38A2) in the 14% CP group were significantly lower than those in the control group ($P<0.05$); the relative mRNA expression level of hepatic solute carrier family 36 member 1 (SLC36A1) in the 14% and 17% CP groups was significantly lower than that in the control group ($P<0.05$). On day 45,

the relative mRNA expression levels of hepatic solute carrier family 6 member 20 (SLC6A20) and SLC38A2 in the 14% and 17% CP groups were significantly lower than those in the control group (P<0.05); the relative mRNA expression level of hepatic SLC6A15 in the 14% CP group was significantly lower than that in the other two groups (P<0.05). These results indicate that reducing dietary CP level by 3% and 6% can decrease hepatic amino acid metabolic enzyme activities and transporter mRNA expression levels in weaned piglets.

Full Text

Effects of Dietary Crude Protein Level on Activities of Amino Acid Metabolic Enzymes and mRNA Expression of Amino Acid Transporters in Liver of Weaned Piglets

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Abstract

This study investigated the effects of dietary crude protein (CP) level on activities of amino acid metabolic enzymes and mRNA expression of amino acid transporters in the liver of weaned piglets. Fifty-four healthy 28-day-old "Duroc × Landrace × Yorkshire"hybrid weaned piglets with similar body weight [(7.0±0.5) kg], half male and half female, were randomly allocated to three groups: 20% CP (control), 17% CP, and 14% CP, with 18 replicates per group and one pig per replicate. The pre-trial period lasted 7 days, followed by a 45-day formal trial period. On days 10, 25, and 45 of the trial, six piglets were selected from each group for slaughter. The results showed: 1) On day 10, liver glutamic-oxaloacetic transaminase (GOT) activity in the 14% and 17% CP groups was significantly lower than in the control group (P<0.05), while liver glutamine synthetase (GS) activity in the 14% CP group was significantly lower than in the other two groups (P<0.05). On day 25, liver GOT and GS activities in the 14% CP group were significantly lower than in the control group (P<0.05), and liver glutamic-pyruvic transaminase (GPT) and glutamic acid dehydrogenase (GDH) activities in both the 14% and 17% CP groups were significantly lower than in the control group (P<0.05). On day 45, liver GPT and GS activities in the 14% CP group were significantly lower than in the other two groups (P<0.05). 2) On day 25, the mRNA relative expression levels of solute carrier family 6 member 15 (SLC6A15) and solute carrier family 38 member 2 (SLC38A2) in the 14% CP group were significantly lower than in the control group (P<0.05), while the mRNA relative expression of solute carrier family 36

member 1 (SLC36A1) in both the 14% and 17% CP groups was significantly lower than in the control group (P<0.05). On day 45, the mRNA relative expression levels of solute carrier family 6 member 20 (SLC6A20) and SLC38A2 in the 14% and 17% CP groups were significantly lower than in the control group (P<0.05), and the mRNA relative expression of SLC6A15 in the 14% CP group was significantly lower than in the other two groups (P<0.05). These findings indicate that reducing dietary CP level by 3% and 6% can decrease the activities of amino acid metabolic enzymes and the mRNA relative expression levels of amino acid transporters in the liver of weaned piglets.

Keywords: low-protein diets; piglets; liver; amino acid metabolic enzyme; amino acid transporter

Introduction

With increasing intensification of livestock production, nitrogen pollution from animal farming has become increasingly severe. In China, annual nitrogen emissions from pig production amount to approximately 1,800 tons. Therefore, improving protein utilization efficiency and reducing nitrogen emissions in pigs is of significant scientific and social importance. Currently, low-protein diets represent a universal technology for reducing nitrogen emissions in swine. Research has shown that reducing dietary crude protein (CP) level by 1% can decrease nitrogen emissions by approximately 8% [1]. When essential amino acids are supplemented, dietary CP level can be reduced by 2% to 4% without affecting pig growth and development [2]. Our previous research found that reducing dietary CP level while balancing only key essential amino acids (EAAs) [lysine (Lys), methionine (Met), tryptophan (Trp), and threonine (Thr)] significantly increased EAA consumption in liver tissue [3]. This experiment was designed to investigate the effects of dietary CP level on activities of amino acid metabolic enzymes and mRNA expression of amino acid transporters in the liver of weaned piglets, providing a scientific basis for elucidating the mechanisms by which low-protein diets increase EAA consumption in pig liver, reduce nitrogen emissions, and improve the efficiency of amino acid metabolic conversion in weaned piglets.

1.1 Experimental Design and Diets

Fifty-four 28-day-old healthy "Duroc × Landrace × Yorkshire" hybrid weaned piglets with similar body weight [(7.0±0.5) kg], half male and half female, were randomly allocated to three groups: 20% CP (control), 17% CP, and 14% CP, with 18 replicates per group and one pig per replicate. The 17% and 14% CP groups were supplemented with Lys, Met, Thr, and Trp to match the levels in the control group. The basal diets were formulated according to NRC (2012). The composition and nutrient levels of experimental diets are shown in Table 1 .

1.2 Animal Management

The trial was conducted at the Southwest University Animal Research Facility. The pre-trial period lasted 7 days, followed by a 45-day formal trial period. Experimental piglets were housed individually in stainless steel cages (1.50 m × 0.68 m × 0.75 m). The room temperature was maintained at (25±1) °C. All piglets had ad libitum access to feed and water, with feeding at 08:00 and 18:00 daily. Pens were kept clean and dry throughout the trial period.

1.3 Sample Collection

During the trial, feed samples from each group were collected three times using the quartering method, mixed, ground to pass through a 40-mesh sieve, and stored at room temperature. Dietary dry matter, CP, calcium, phosphorus, crude fiber, and amino acid contents were determined according to *Feed Analysis and Feed Quality Detection Technology* [4]. On days 10, 25, and 45 of the formal trial period, six piglets closest to the average body weight were selected from each group for slaughter. Liver samples were collected, snap-frozen in liquid nitrogen, and stored at -80 °C.

1.4.1 Liver Amino Acid Metabolic Enzyme Activities

Approximately 0.6–0.9 g of liver tissue was weighed and placed in a 10 mL centrifuge tube containing 0.9% chilled physiological saline at a tissue-to-saline ratio of 1:9 (w/v), then homogenized on ice. The homogenate was centrifuged at 3,000 r/min for 10 min at 4 °C, and the supernatant was collected and stored at -20 °C for subsequent enzyme activity assays. Activities of glutamic-pyruvic transaminase (GPT) (C009-2), glutamic-oxaloacetic transaminase (GOT) (C010-2), glutamine synthetase (GS) (A047), and glutamic acid dehydrogenase (GDH) (A125) in liver tissue were measured using colorimetric methods. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, and measurements were performed strictly according to the manufacturer' s instructions.

1.4.2 Liver Amino Acid Transporter mRNA Expression

1.4.2.1 RNA Extraction and cDNA Reverse Transcription Frozen liver tissue stored at -80 °C was placed on ice and minced with sterile scissors. The tissue was thoroughly ground in liquid nitrogen to prevent temperature-induced degradation. After uniform grinding, the powdered liver tissue was transferred to a centrifuge tube. Total RNA was extracted using Total RNA Extractor (Shanghai Bioengineering Co., Ltd.), and reverse transcription to cDNA was performed using MMLV First Strand cDNA Synthesis Kit (Shanghai Bioengineering Co., Ltd.).

1.4.2.2 Primer Design Primers were designed using Primer Premier 5.0 software and synthesized by Shanghai Bioengineering Co., Ltd. Primer sequences

for liver amino acid transporters solute carrier family 6 member 15 (SLC6A15), solute carrier family 6 member 20 (SLC6A20), solute carrier family 36 member 1 (SLC36A1), solute carrier family 38 member 2 (SLC38A2), and the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are shown in Table 2 .

1.4.2.3 Fluorescent Quantitative PCR The fluorescent quantitative PCR reaction system (50 L) contained 24 L Hotstart Fluo-PCR mix, 2 L each of forward and reverse primers (25 mol/L), 2 L cDNA, and 20 L ddH O. The PCR conditions were: pre-denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60–63 °C for 30 s (optimized for each primer), and extension at 72 °C for 30 s. All reagents were purchased from Shanghai Bioengineering Co., Ltd. Relative quantitative expression differences were calculated using the comparative Ct method, where relative expression of the target gene = 2^−ΔΔCt = [(Ct_target − Ct_reference)_treatment − (Ct_target − Ct_reference)_control]. ΔΔCt represents the fold change in target gene expression in the treatment group relative to the control group.

1.5 Statistical Analysis

Raw data were organized using Excel 2007 and analyzed using SAS 8.2 software. One-way ANOVA was performed, followed by LSD multiple comparisons. Results are expressed as "mean ± standard error." Differences were considered significant at $P<0.05$.

Results

2.1 Effects of Dietary CP Level on Liver Amino Acid Metabolic Enzyme Activities

As shown in Figure 1 [Figure 1: see original paper], on day 10, liver GOT activity in the 14% and 17% CP groups was significantly lower than in the control group ($P<0.05$). On day 25, liver GOT activity in the 14% CP group was significantly lower than in the other two groups ($P<0.05$). On day 25, GPT activity in the 14% and 17% CP groups was significantly lower than in the control group ($P<0.05$), while on day 45, liver GPT activity in the 14% CP group was significantly lower than in the other two groups ($P<0.05$). Liver GS activity in the 14% CP group was significantly lower than in the other two groups on days 10 and 45 ($P<0.05$), and significantly lower than the control group on day 25 ($P<0.05$). On day 25, liver GDH activity in the 14% and 17% CP groups was significantly lower than in the control group ($P<0.05$).

2.2.1 Fluorescent Quantitative PCR Amplification and Melting Curve Analysis

The amplification curves for GAPDH, SLC6A15, SLC6A20, SLC36A1, and SLC38A2 showed stable baselines, indicating minimal interfering signals. Addi-

tionally, the no-template control (NTC) remained a flat line throughout amplification, confirming absence of contamination and primer dimers. The melting curves for all genes displayed single sharp peaks, demonstrating high primer specificity without non-specific product formation.

2.2.2 Effects of Dietary CP Level on mRNA Expression of Liver Amino Acid Transporters

As shown in Table 3 , on day 25, the mRNA relative expression of SLC6A15 in the 14% CP group was significantly lower than in the other two groups ($P<0.05$), while SLC36A1 mRNA expression in both the 14% and 17% CP groups was significantly lower than in the control group ($P<0.05$). Additionally, SLC38A2 mRNA expression in the 14% CP group was significantly lower than in the control group ($P<0.05$). On day 45, the mRNA relative expression levels of SLC6A20 and SLC38A2 in the 14% and 17% CP groups were significantly lower than in the control group ($P<0.05$), and SLC6A15 mRNA expression in the 14% CP group was significantly lower than in the other two groups ($P<0.05$).

Discussion

3.1 Effects of Dietary CP Level on Liver Amino Acid Metabolic Enzyme Activities

The results demonstrated that liver GOT and GPT activities in weaned piglets increased with elevated dietary CP levels. GOT, also known as aspartate aminotransferase, catalyzes the conversion of -ketoglutarate (-KG) and aspartate (Asp) to glutamate (Glu) and oxaloacetate. GPT, or alanine aminotransferase, catalyzes the conversion of -KG and alanine (Ala) to Glu and pyruvate. Both GOT and GPT are important enzymes in amino acid metabolic conversion and exhibit high activity in the liver. Primarily intracellular, GOT and GPT are released into the bloodstream when tissue cells are damaged, making them important indicators of liver function [5]. Reports on the effects of dietary CP level on porcine liver GOT and GPT activities have been inconsistent. Luo [6] found that insufficient protein nutrition supply impaired hepatic protein synthesis capacity, leading to significantly increased plasma GOT and GPT activities. Luo [7] reported that plasma GOT and GPT activities in piglets showed a trend of initial increase, followed by decrease, then increase again with increasing dietary CP levels. These findings suggest that evaluating CP level effects on GOT and GPT activities requires clarification of the dietary CP status (normal, overnutrition, or deficiency), as the impact of reducing or increasing CP levels differs across these states. The present study indicates that reducing dietary CP level may decrease Glu synthesis in piglet liver.

This study also revealed that reducing dietary CP level decreased liver GS and GDH activities in weaned piglets. GS is a key enzyme involved in ammonia metabolism, present in all organisms, catalyzing the conversion of L-Glu to glutamine (Gln) [8]. GS plays crucial roles in nitrogen transport between tissues,

detoxification of high ammonia concentrations, and maintenance of acid-base balance. GDH is widely distributed in liver tissue and plays an important role in oxidative deamination of amino acids. Typically, GDH catalyzes the synthesis of Glu from -iminoglutarate. Additionally, during amino acid deamination, GDH can work with transaminases to form Gln and asparagine, which are converted to urea [9]. Luo [6] reported that different dietary amino acid compositions had no significant effect on porcine plasma GDH activity. Combined with our findings that reduced dietary CP level decreased liver GDH and GS activities, we speculate that dietary amino acid quantity has an important influence on liver GDH and GS activities.

3.2 Effects of Dietary CP Level on mRNA Expression of Liver Amino Acid Transporters

The results showed that mRNA relative expression levels of all four detected amino acid transporters decreased to varying degrees with reduced dietary CP level. SLC6A15 is a member of the solute carrier family 6 (SLC6), functioning as a Na - and Cl -dependent neutral amino acid transporter. Uhl et al. [10] discovered SLC6A15 in 1992 and named it SLC6A15, BoAT2, SBAT1, or V7-3 based on its function in SLC6. Takanaga et al. [11] confirmed that SLC6A15 regulates metabolic conversion of proline (Pro), Met, leucine (Leu), valine (Val), and isoleucine (Ile). Hägglund et al. [12] found that SLC6A15 could alter Leu concentration, thereby regulating energy metabolism in body organs. Drgonova et al. [13] demonstrated that knockout of the SLC6A15 gene in mice decreased Leu and Pro uptake by 40% and 15%, respectively. Hägglund et al. [14] reported that SLC6A15 mRNA expression was primarily detected in the brain, with partial expression also found in muscle, intestine, liver, and eyes.

SLC6A20, also known as SIT1, is a Na - and Cl -dependent amino acid transporter. Predominantly distributed in the intestine and kidney of mammals, SLC6A20 is an important component in Pro metabolism, influencing glucose and energy homeostasis by binding Glu, arginine (Arg), and other amino acids [15-16]. Studies have shown that SLC6A20 in the kidney regulates type II diabetes [17], and iminoglycinuria is associated with SLC6A20 mutations [18]. Due to its relatively recent discovery, few reports exist on its regulatory mechanisms in the liver.

SLC36A1, also known as PAT1, encodes a proton-coupled amino acid transporter primarily distributed in the intestine and kidney, with low mRNA expression also detected in the liver [15]. SLC36A1 transports small amino acids such as Ala, Pro, and glycine (Gly) within the body. Under certain H concentrations, SLC36A1 selectively activates the Na /H exchanger III to produce imino acids, thereby maintaining normal H concentration in the body [19]. Chen et al. [20] found that SLC36A1 was expressed in human small intestine, brain, liver, testis, and kidney tissues.

SLC38A2 is a member of the SLC6 family and functions as a Na -dependent

neutral amino acid transporter present in mammalian tissues. SLC38A2 is expressed in nearly all cell types [21]. Ortiz et al. [22] found that feeding rats a high-protein diet increased SLC38A2 expression, suggesting that SLC38A2 can oxidatively decompose excess protein in the body in the form of amino acids. Conti et al. [23] demonstrated that SLC38A2 binds Gln to enter cells and participates in the Glu-Gln metabolic cycle. Our study found that the mRNA relative expression of SLC38A2 in weaned piglet liver increased with dietary CP level, consistent with the findings of Ortiz et al. [22].

The trend of mRNA relative expression of amino acid transporters in piglet liver with dietary CP level was consistent with the trend of amino acid metabolic enzyme activities. Overall, reducing dietary CP level by 3% and 6% decreased the quantity of certain amino acids (such as Glu, Pro, Arg, Leu, etc.) entering hepatocytes, thereby reducing the metabolic rate of these amino acids in the liver.

Conclusions

1. Reducing dietary CP level by 3% and 6% significantly decreased the activities of liver amino acid metabolic enzymes (GOT, GPT, GS, GDH) in weaned piglets.
2. Reducing dietary CP level by 3% and 6% significantly decreased the mRNA relative expression levels of liver amino acid transporters (SLC6A15, SLC6A20, SLC36A1, SLC38A2) in weaned piglets.

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