Effects of Early Weaning on Glycogen Content and Hepatic Gluconeogenesis and Glycolysis-Related Gene Expression in Piglets: Postprint

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Abstract

This study aimed to investigate the effects of early weaning on glucose metabolism in piglets. Four litters of healthy 18-day-old piglets were selected; four piglets from each litter (totaling 16) were weighed, with four immediately slaughtered for sampling (blood, liver, longissimus dorsi muscle) and the remaining 12 subjected to weaning treatment (weaned group); the remaining piglets in each litter continued to be suckled by the sow (suckling group). On days 1, 3, and 7 post-weaning, four piglets from both the weaned and suckling groups were selected to determine average daily gain, serum glucose and lactate concentrations, hepatic and muscle glycogen content, hepatic pyruvate kinase activity, and relative expression levels of glucose metabolism-related genes. The results showed that compared with the suckling group, the weaned group exhibited significantly decreased average daily gain during the early weaning period (1–7 days post-weaning) ($P<0.05$); on days 1, 3, and 7 post-weaning, serum glucose concentration in the weaned group decreased by 8.33% ($P>0.05$), 17.81% ($P<0.05$), and 20.99% ($P<0.05$), respectively, serum lactate concentration increased by 16.83% ($P<0.05$), 22.75% ($P<0.05$), and 12.06% ($P>0.05$), respectively, hepatic glycogen content decreased by 24.94% ($P<0.05$), 48.99% ($P<0.05$), and 36.51% ($P<0.05$), respectively, and muscle glycogen content decreased by 44.49% ($P<0.05$), 39.68% ($P<0.05$), and 25.52% ($P<0.05$), respectively; hepatic pyruvate kinase activity and its relative gene expression level in the weaned group significantly decreased on days 3 and 7 post-weaning ($P<0.05$), whereas the relative expression levels of genes encoding hepatic gluconeogenesis-related enzymes, including mitochondrial phosphoenolpyruvate carboxykinase (on day 1 post-weaning), cytosolic phosphoenolpyruvate carboxykinase (on days 1, 3, and 7 post-weaning), and glucose-6-phosphatase (on days 1 and 3 post-weaning), significantly increased ($P<0.05$). These results indicate that early weaning reduced serum glucose

concentration and hepatic and muscle glycogen content during the early stage post-weaning, while activating hepatic gluconeogenesis and suppressing glycolysis to maintain glucose metabolic homeostasis.

Full Text

Effects of Early Weaning on Glycogen Content and Expression of Hepatic Genes Associated with Gluconeogenesis and Glycolysis in Piglets

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Abstract: This study investigated the effects of early weaning on glucose metabolism in piglets. Sixteen healthy 18-day-old piglets were selected from four litters (four piglets per litter). After weighing, four piglets (one from each litter) were immediately slaughtered for sample collection (blood, liver, and longissimus dorsi muscle), while the remaining twelve piglets were subjected to weaning (weaned group). The remaining piglets in each litter continued to be suckled by sows (suckling group). On days 1, 3, and 7 post-weaning, four piglets from each group were used to determine average daily gain, serum glucose and lactate concentrations, hepatic and muscular glycogen contents, hepatic pyruvate kinase activity, and relative expression levels of genes related to glucose metabolism. The results demonstrated that during the early weaning period (days 1–7 post-weaning), average daily gain was significantly reduced in the weaned group compared with the suckling group ($P<0.05$). Serum glucose concentrations in weaned piglets decreased by 8.33% ($P>0.05$), 17.81% ($P<0.05$), and 20.99% ($P<0.05$) on days 1, 3, and 7 post-weaning, respectively, while serum lactate concentrations increased by 16.83% ($P<0.05$), 22.75% ($P<0.05$), and 12.06% ($P>0.05$), respectively. Hepatic glycogen content decreased by 24.94% ($P<0.05$), 48.99% ($P<0.05$), and 36.51% ($P<0.05$), and muscle glycogen content decreased by 44.49% ($P<0.05$), 39.68% ($P<0.05$), and 25.52% ($P<0.05$) on days 1, 3, and 7 post-weaning, respectively. Hepatic pyruvate kinase activity and its gene expression level were significantly reduced in weaned piglets on days 3 and 7 post-weaning ($P<0.05$). In contrast, the relative expression levels of gluconeogenic enzymes—mitochondrial phosphoenolpyruvate carboxykinase (on day 1), cytoplasmic phosphoenolpyruvate carboxykinase (on days 1, 3, and 7), and glucose-6-phosphatase (on days 1 and 3)—were significantly elevated ($P<0.05$). These findings indicate that early weaning reduces serum glucose, hepatic glycogen, and muscle glycogen contents in piglets, while activating hepatic gluconeogenesis and suppressing glycolysis to maintain glucose

homeostasis.

Keywords: piglets; early weaning; glycogen; gluconeogenesis; glycolysis

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Modern swine production commonly employs early weaning to improve production efficiency [1]. However, piglets at this developmental stage have immature digestive tracts, insufficient secretion of digestive juices and enzymes, unstable gastrointestinal microflora, and underdeveloped intestinal immune function. Additionally, the weaning process subjects piglets to environmental, psychological, and nutritional stressors, all of which can alter energy metabolism [2].

Previous studies have shown that early weaning reduces feed intake and triggers stress responses, forcing piglets to mobilize body fat, carbohydrate, and protein reserves to meet metabolic demands [3]. When animals experience stress, activation of the stress system induces behavioral changes and alters peripheral organ function to enhance environmental adaptation and survival [1]. The liver serves as a critical effector organ of the stress system and plays a central role in energy supply and carbohydrate metabolism regulation, making it essential for growth, metabolism, and stress resistance. Nafikov et al. [4] reported that hepatic gluconeogenesis accounts for 70% of total blood glucose in newborn piglets, highlighting the vital importance of gluconeogenic function for glucose homeostasis. Key enzymes regulating hepatic glucose metabolism include pyruvate kinase (PK), mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M), cytoplasmic phosphoenolpyruvate carboxykinase (PEPCK-C), and glucose-6-phosphatase (G-6-P). Among these, PK is the rate-limiting enzyme for glycolysis, while PEPCK-M, PEPCK-C, and G-6-P are key gluconeogenic enzymes [5-6]. Although metabolic status necessarily changes during early weaning, the alterations in carbohydrate content and hepatic expression of glucose metabolism-related genes remain unclear. Therefore, this study aimed to investigate the effects of early weaning on carbohydrate content and hepatic gene expression related to glucose metabolism, thereby elucidating metabolic adaptations and providing a theoretical foundation for nutritional regulation during early weaning.

1.1 Experimental Design

Four multiparous sows were selected, each nursing 12 healthy piglets (Duroc × Landrace × Yorkshire). The composition and nutrient levels of the lactation diet are presented in Table 1 . At 18 days of age, four piglets were randomly selected from each litter (16 piglets total) and assigned to the following treatments: (1) four piglets (one per litter) were weighed and immediately slaughtered to obtain baseline data; (2) the remaining 12 piglets were weaned and housed in pens with environmental conditions identical to the sow pens, receiving a starter diet (composition and nutrient levels shown in Table 2). The remaining piglets in each litter continued nursing as the suckling group. On days 1, 3, and 7 post-weaning (corresponding to piglet ages of 19, 21, and 25 days), four piglets from

each group were weighed, bled, and slaughtered for sample collection. Piglets had ad libitum access to feed and water throughout the experiment.

1.2 Sample Collection

Following a 10-hour overnight fast, blood samples were collected from the anterior vena cava of piglets in both groups at 0, 1, 3, and 7 days post-treatment. Serum was obtained by centrifugation at 3,000 × g for 10 minutes at 4°C. Liver and longissimus dorsi muscle samples were collected at slaughter and stored at -80°C [7-8].

1.3 Measurements

1.3.1 Growth Performance Piglets were weighed at each time point, and average daily gain was calculated based on body weight changes during the experimental period.

1.3.2 Determination of Glycometabolism-Related Metabolites and Pyruvate Kinase Activity Serum glucose and lactate concentrations, hepatic and muscular glycogen contents, and hepatic pyruvate kinase activity were measured using commercial kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s protocols.

1.3.3 Analysis of Hepatic Gene Expression Related to Glucose Metabolism Total RNA was extracted from liver tissue using a total RNA extraction kit. RNA purity was assessed using a NanoDrop microspectrophotometer (OD /OD ratio), and integrity was verified by agarose gel electrophoresis. Complementary DNA was synthesized using the Invitrogen Superscript™ II RTase reverse transcription kit. Primers for target genes and the 18S reference gene were designed using Premier 6.0 and Beacon Designer software (sequences shown in Table 3). Real-time quantitative PCR was performed using the TaKaRa SYBR® Premix Ex Taq™ kit in a 25- L reaction mixture containing 10.5 L ddH O, 12.5 L SYBR Premix Ex Taq™ (2×), 1 L each of forward and reverse primers, and 1.0 L cDNA template. Cycling conditions were: 95°C for 1 min, followed by 45 cycles of 95°C for 10 s and 62°C for 25 s, with a melting curve generated from 55°C to 95°C at 0.5°C increments every 5 s. Relative gene expression levels were calculated using the 2^(-ΔΔCt) method [7], with three technical replicates per sample and Ct values averaged arithmetically.

1.4 Data Processing and Analysis

Data were analyzed using SPSS 16.0 software with t-tests. Results are expressed as means ± standard error, with $P<0.05$ considered statistically significant.

2 Results

2.1 Effects of Early Weaning on Growth Performance

As shown in Figure 1 [Figure 1: see original paper], early weaning adversely affected growth performance, particularly on days 1 and 3 post-weaning when piglets exhibited negative weight gain. Body weight of weaned piglets was 11.89% ($P>0.05$) and 16.16% ($P<0.05$) lower than that of suckling piglets on days 3 and 7 post-weaning, respectively. Average daily gain in the weaned group was -70.01 g/d and 40.07 g/d during days 0–1 and 2–3 post-weaning, respectively, which was significantly lower than in the suckling group ($P<0.05$). During days 4–7 post-weaning, average daily gain in weaned piglets was 145.09 g/d, representing a 52.85% reduction compared with suckling piglets ($P<0.05$).

2.2 Effects of Early Weaning on Glycometabolism-Related Metabolites

As presented in Table 3 , serum glucose concentrations in weaned piglets were reduced by 8.33% ($P>0.05$), 17.81% ($P<0.05$), and 20.99% ($P<0.05$) on days 1, 3, and 7 post-weaning, respectively, compared with suckling piglets. Conversely, serum lactate concentrations increased by 16.83% ($P<0.05$) and 22.75% ($P<0.05$) on days 1 and 3 post-weaning, respectively, with a 12.06% increase observed on day 7 ($P>0.05$). Both hepatic and muscular glycogen contents showed similar declining trends. Hepatic glycogen content decreased by 24.94% ($P<0.05$), 48.99% ($P<0.05$), and 36.51% ($P<0.05$) on days 1, 3, and 7 post-weaning, respectively, while muscle glycogen content decreased by 44.49% ($P<0.05$), 39.68% ($P<0.05$), and 25.52% ($P<0.05$), respectively.

2.3 Effects of Early Weaning on Hepatic Enzyme Activity and Gene Expression

As illustrated in Figures 2 and 3 [Figure 2: see original paper][Figure 3: see original paper], hepatic pyruvate kinase activity in weaned piglets decreased by 31.33% ($P<0.05$) and 32.63% ($P<0.05$) on days 3 and 7 post-weaning, respectively, compared with suckling piglets. Similarly, the relative expression level of the PK gene was reduced by 35.71% ($P<0.05$) and 40.91% ($P<0.05$) on days 3 and 7 post-weaning, respectively.

As shown in Table 4 , the relative expression level of hepatic PEPCK-M gene in weaned piglets increased by 57.75% on day 1 post-weaning ($P<0.05$), while PEPCK-C gene expression increased by 65.57%, 97.74%, and 68.60% on days 1, 3, and 7 post-weaning, respectively ($P<0.05$). Hepatic G-6-P gene expression was also significantly elevated in weaned piglets on days 1 and 3 post-weaning, increasing by 67.57% and 128.43%, respectively ($P<0.05$).

3 Discussion

Early-weaned piglets often exhibit a range of adverse symptoms due to environmental, psychological, and nutritional stressors, resulting in growth retardation and metabolic disturbances [9]. This study demonstrated that early weaning impairs growth performance, particularly during the first three days when piglets experience negative growth. Our previous research also found that 21-day weaning reduced body weight and average daily gain by 16.53% and 127.98%, respectively, compared with suckled piglets during days 21–28 [8]. This outcome likely reflects the transition from easily digestible liquid milk rich in lactose, milk protein, and milk fat to a corn-soybean meal-based solid diet with lower digestibility. Sow milk contains high lactose concentrations (5.77 g/dL on day 20 of lactation) and abundant glutamine, arginine, lysine, valine, and polyamines (spermine, spermidine, putrescine) that are readily absorbed and crucial for intestinal development and mucosal integrity [10-12]. In contrast, corn-soybean meal diets contain substantially lower levels of these nutrients. Additionally, weaning stress itself reduces feed intake, damages intestinal morphology, hinders immune and enzyme system development [1-2], and disrupts the balance of gastrointestinal microflora [13], collectively impairing digestive and absorptive capacity.

During the immediate post-weaning period, intestinal structure—including small intestine length, weight, villus height, crypt depth, and mucosal barrier function—undergoes significant changes, while brush border enzyme activity and absorptive capacity decline, affecting amino acid and carbohydrate absorption [8]. Our previous studies revealed that early weaning significantly reduces free lysine concentrations in serum, muscle, and liver, as well as serum essential amino acids and threonine [7-8]. Hampson et al. [14] reported that lactase and sucrase activities in piglet small intestine decreased by at least 50% on days 4–5 post-weaning, with lactase activity continuing to decline thereafter and sucrase activity only beginning to recover on day 11. Pié et al. [15] observed that sucrase activity in the proximal small intestine of 28-day-weaned piglets decreased by 85% on day 1 post-weaning, with distal small intestine sucrase activity declining an additional 30% on day 2. The current study found that 18-day early weaning reduced serum glucose concentrations and decreased hepatic glycogen content by 24.94%, 48.99%, and 36.51% on days 1, 3, and 7 post-weaning, respectively, while muscle glycogen content decreased by 44.49%, 39.68%, and 25.52%. Correspondingly, lactate, a glycogen metabolite, increased. These changes likely reflect the abrupt reduction in dietary carbohydrate and lipid intake combined with increased physical activity due to agitation, leading to glycogenolysis for energy production.

Hepatic glucose metabolism regulation is crucial during starvation, primarily through glycogenolysis and gluconeogenesis to produce endogenous glucose [17]. Since neonatal glycogen stores are rapidly depleted [15], hepatic gluconeogenesis becomes exceptionally important as the main energy source during weaning stress. Phosphoenolpyruvate carboxykinase (PEPCK), present in liver, kidney,

and adipose tissue, catalyzes the conversion of oxaloacetate to phosphoenolpyruvate and CO . Two isoforms exist: mitochondrial PEPCK-M and cytoplasmic PEPCK-C, both critical for glucose homeostasis [5]. In this study, early weaning increased the expression of PEPCK-M, PEPCK-C, and G-6-P genes, reflecting enhanced gluconeogenesis to increase glucose availability and meet metabolic demands [6]. Pyruvate kinase, the final rate-limiting enzyme in glycolysis that catalyzes the second ATP-generating reaction [18], showed significantly reduced activity and gene expression in weaned piglets, indicating suppressed glycolysis. This suppression likely serves to preserve glucose and maintain glycometabolic balance when glucose availability is limited.

4 Conclusion

During early weaning, piglets experience severe energy intake deficiency, resulting in decreased serum glucose concentrations and mobilization of hepatic and muscular glycogen stores, which increases serum lactate levels. Concurrently, expression of gluconeogenic enzymes (PEPCK and G-6-P) is upregulated while the glycolytic key enzyme pyruvate kinase shows reduced expression and activity. These metabolic adjustments reflect the piglet' s strategy of enhancing hepatic gluconeogenesis and suppressing glycolysis to maintain glycometabolic homeostasis during the weaning transition.

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