

Effects of Different Dietary Nitrogen-to-Energy Ratios on Biochemical Parameters of Amniotic and Allantoic Fluids in Pregnant Huanjiang Xiang Pigs (Postprint)

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Abstract

This experiment aimed to investigate the effects of different nitrogen-to-energy ratio diets on biochemical parameters of amniotic fluid and allantoic fluid in pregnant Huanjiang Xiang pigs. Forty-eight primiparous Huanjiang Xiang pigs were selected and randomly divided into 2 groups, with 8 pens (replicates) per group and 3 pigs per pen. After mating, sows in the two groups were fed high nitrogen-to-energy ratio diet (digestible energy 14.73 MJ/kg, crude protein content 13.11%, nitrogen-to-energy ratio 0.89) and low nitrogen-to-energy ratio diet (digestible energy 12.24 MJ/kg, crude protein content 9.77%, nitrogen-to-energy ratio 0.80), respectively. On days 45, 75, and 110 of gestation, one sow per pen was slaughtered, and amniotic fluid and allantoic fluid corresponding to the maximum, intermediate, and minimum body weight fetuses in each litter were collected for determination of biochemical parameters. The results showed that under the same gestational age and weight category conditions, compared with the low nitrogen-to-energy ratio diet group, the high nitrogen-to-energy ratio diet group exhibited significantly increased alkaline phosphatase activity in allantoic fluid of minimum body weight fetuses on gestation day 75 ($P < 0.05$), and significantly decreased aspartate aminotransferase activity in intermediate and minimum body weight fetuses on gestation day 75 and minimum body weight fetuses on gestation day 110, uric acid content in maximum body weight fetuses on gestation days 45 and 75, and creatine kinase activity in intermediate and minimum body weight fetuses on gestation day 45 and maximum body weight fetuses on gestation day 75 ($P < 0.05$). Compared with minimum body weight fetuses, albumin content in amniotic fluid of maximum body weight fetuses was significantly increased in the high nitrogen-to-energy ratio diet group on gestation days 45 and 75 and in the low nitrogen-to-energy ratio diet group on

gestation days 45 and 110 ($P < 0.05$). With the extension of gestation time, urea and uric acid contents in fetal amniotic fluid and uric acid content in allantoic fluid gradually increased in both diet groups. In conclusion, high nitrogen-to-energy ratio diet can promote fetal growth and development by regulating biochemical parameters of amniotic fluid and allantoic fluid; compared with intermediate and minimum body weight fetuses, maximum body weight fetuses have higher protein utilization efficiency.

Full Text

Effects of Different Nitrogen/Energy Diets on Biochemical Parameters in Amniotic Fluid and Allantoic Fluid of Pregnant Huanjiang Mini-Pigs

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Abstract

This study investigated the effects of different nitrogen/energy diets on biochemical parameters in the amniotic fluid and allantoic fluid of pregnant Huanjiang mini-pigs. Forty-eight primiparous Huanjiang mini-pigs were randomly assigned to two groups, each consisting of eight pens (replicates) with three pigs per pen. Following mating, the two groups were fed either a high nitrogen/energy diet (digestible energy: 14.73 MJ/kg, crude protein: 13.11%, nitrogen/energy ratio: 0.89) or a low nitrogen/energy diet (digestible energy: 12.24 MJ/kg, crude protein: 9.77%, nitrogen/energy ratio: 0.80). At days 45, 75, and 110 of gestation, one pig from each pen was slaughtered, and the amniotic fluid and allantoic fluid were collected from fetuses with maximal, middle, and minimal body weight within each litter for biochemical analysis.

The results demonstrated that under identical gestational stages and fetal weight categories, the high nitrogen/energy diet significantly increased alkaline phosphatase activity in allantoic fluid of minimal-weight fetuses at day 75 ($P < 0.05$) compared to the low nitrogen/energy diet. Conversely, it significantly decreased aspartate aminotransferase activity in allantoic fluid of middle- and minimal-weight fetuses at day 75 and minimal-weight fetuses at day 110, reduced uric acid content in maximal-weight fetuses at days 45 and 75, and lowered creatine

kinase activity in middle- and minimal-weight fetuses at day 45 and maximal-weight fetuses at day 75 ($P < 0.05$).

Compared to minimal-weight fetuses, maximal-weight fetuses exhibited significantly higher albumin content in amniotic fluid at days 45 and 75 in the high nitrogen/energy group, and at days 45 and 110 in the low nitrogen/energy group ($P < 0.05$). As gestation progressed, both dietary groups showed gradual increases in urea and uric acid contents in amniotic fluid, as well as uric acid content in allantoic fluid. These findings indicate that high nitrogen/energy diets promote fetal growth and development by modulating biochemical parameters in fetal fluids, and that maximal-weight fetuses demonstrate higher protein utilization efficiency compared to their middle- and minimal-weight littermates.

Keywords: Huanjiang mini-pigs; gestational stage; dietary nitrogen/energy ratio; amniotic fluid; allantoic fluid; biochemical parameters

Introduction

Huanjiang mini-pig is a renowned local breed in China. However, extensive management practices have resulted in low reproductive rates and poor piglet survival [1]. Nutritional levels in diets before and after mating affect embryo survival rates in primiparous sows during early pregnancy [2], while both maternal undernutrition and overnutrition can reduce the maternal supply of blood, nutrients, and oxygen to the fetus, thereby impeding fetal growth and development [3-6]. Therefore, improving dietary nutritional supply is crucial for enhancing sow reproductive performance and fetal development.

Amniotic fluid is a dialysate derived from maternal plasma that passes through fetal membranes into the amniotic cavity, along with fluid produced by the amnion covering the umbilical cord and placental surface. Its composition is similar to maternal plasma except for slightly lower protein content [7]. During mid-to-late gestation, fetuses can ingest amniotic fluid to obtain nutrients, active factors, and precursors for antimicrobial substances, thereby supporting and promoting their own growth, development, and intestinal maturation [8]. Allantoic fluid originates primarily from urine and allantoic epithelial secretions, containing various nutrients such as amino acids and proteins that can be absorbed by the allantois, enter the fetal-placental circulation, and ultimately be utilized by the fetus [9-10].

Previous research has shown that compared to low nitrogen/energy diets, high nitrogen/energy diets increase sow backfat thickness (27.2 mm vs. 26.9 mm at day 45; 36.6 mm vs. 28.1 mm at day 75) and fetal weight (16.1 g vs. 13.6 g at day 45; 184.6 g vs. 155.7 g at day 75; 591.5 g vs. 418.0 g at day 110) [11]. Zhu et al. [12] reported that high nitrogen/energy diets benefit umbilical cord vascular development in fetuses. Liu et al. [3] found that fetal weight in early gestation is closely correlated with placental weight, uterine weight,

amniotic fluid plus allantoic fluid volume, and biochemical parameters. Thus, changes in the chemical composition of amniotic and allantoic fluids can reflect the status of nutrient transfer between mother and fetus and fetal growth and development [8]. Therefore, improving maternal-fetal fluid exchange through dietary modification represents an important strategy for promoting fetal pig development and increasing live litter size. This study aimed to investigate the effects of different nitrogen/energy diets on biochemical parameters in amniotic and allantoic fluids of pregnant sows, providing a nutritional basis for improving sow reproductive performance.

1.1 Experimental Animals, Grouping, and Management

Forty-eight primiparous Huanjiang mini-pigs were randomly allocated to two groups, each comprising 24 pigs housed in eight pens (replicates) with three pigs per pen. After mating, the groups were fed either a high nitrogen/energy diet (formulated according to NRC [2012] nutrient requirements; digestible energy: 14.73 MJ/kg, crude protein: 13.11%, nitrogen/energy ratio: 0.89) or a low nitrogen/energy diet (formulated according to Chinese “Swine Feeding Standards” [NY/T 65-2004] for local breeds; digestible energy: 12.24 MJ/kg, crude protein: 9.77%, nitrogen/energy ratio: 0.80). The composition and nutrient levels of the two experimental diets are presented in Table 1. Throughout the trial, daily feed allowance per pen was set at 2.5% of the total body weight of the three sows. Pigs were fed twice daily at 08:30 and 17:00 with free access to water.

1.2 Sample Collection

At days 45 (early), 75 (mid), and 110 (late) of gestation, one pig from each pen was randomly selected, slaughtered following cardiac exsanguination, and the fetuses were collected and weighed. The amniotic fluid and allantoic fluid corresponding to fetuses with maximal, middle, and minimal body weight within each litter [11] were collected in 10 mL centrifuge tubes, snap-frozen in liquid nitrogen, and stored at -80°C.

1.3 Biochemical Parameter Determination

After thawing at 4°C, samples were analyzed using a CX4 automatic biochemical analyzer (Beckman) to determine total protein (TP), albumin (ALB), urea (UREA), uric acid (UA), and glucose (GLU) contents, as well as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase (CK) activities. Biochemical assay kits were purchased from Beijing Leadman Biochemical Technology Co., Ltd.

1.4 Data Processing and Analysis

Data were analyzed using SPSS 17.0 software. Independent samples t-tests were performed to compare data between dietary groups at the same gestational stage and fetal weight category. One-way ANOVA (LSD) was used to analyze data across different fetal weight categories within the same dietary group and gestational stage. Data are expressed as “mean \pm standard error.” $P < 0.05$ was considered statistically significant.

2.1 Changes in Amniotic Fluid Biochemical Parameters

The biochemical parameters in amniotic fluid of pregnant Huanjiang mini-pigs fed different nitrogen/energy diets are presented in Table 2. Under identical gestational stages and fetal weight categories, the high nitrogen/energy diet significantly increased albumin content, alanine aminotransferase activity, and aspartate aminotransferase activity in maximal-weight fetuses at day 45, alkaline phosphatase activity in middle-weight fetuses at day 45, and total protein content in minimal-weight fetuses at day 45 compared to the low nitrogen/energy diet ($P < 0.05$). At day 75, the high nitrogen/energy diet significantly elevated albumin content and urea content in maximal-weight fetuses and alanine aminotransferase activity in minimal-weight fetuses ($P < 0.05$). At day 110, it significantly increased alkaline phosphatase and creatine kinase activities and total protein, urea, and glucose contents in maximal-weight fetuses, as well as aspartate aminotransferase and creatine kinase activities in middle-weight fetuses ($P < 0.05$).

Conversely, the high nitrogen/energy diet significantly reduced creatine kinase activity in maximal- and middle-weight fetuses and alanine aminotransferase activity in minimal-weight fetuses at day 45 ($P < 0.05$). At day 75, it decreased aspartate aminotransferase activity in maximal-weight fetuses, total protein and glucose contents in middle-weight fetuses, and albumin, total protein, and glucose contents in minimal-weight fetuses ($P < 0.05$). At day 110, it lowered alanine aminotransferase activity in maximal-weight fetuses, creatine kinase activity in middle- and minimal-weight fetuses, and alanine aminotransferase activity and glucose content in minimal-weight fetuses ($P < 0.05$).

Within the same dietary group and gestational stage, maximal-weight fetuses in the high nitrogen/energy group showed significantly higher urea and uric acid contents and alanine aminotransferase activity at day 45, and higher alanine aminotransferase activity and albumin, urea, and uric acid contents at day 75 compared to middle-weight fetuses ($P < 0.05$). Minimal-weight fetuses exhibited significantly lower uric acid content at day 45 and lower albumin content at day 75 ($P < 0.05$). In the low nitrogen/energy group, maximal-weight fetuses had significantly higher total protein, uric acid, and alkaline phosphatase activity at day 45, higher uric acid and alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities at day 75, and higher urea and

uric acid contents and alkaline phosphatase, alanine aminotransferase, and creatine kinase activities at day 110 compared to middle-weight fetuses ($P < 0.05$). Minimal-weight fetuses showed significantly higher urea and glucose contents and alanine aminotransferase activity at day 110 ($P < 0.05$), while maximal- and minimal-weight fetuses had lower aspartate aminotransferase activity at day 45 and minimal-weight fetuses had lower creatine kinase activity at day 110 ($P < 0.05$).

As gestation progressed, the low nitrogen/energy group showed gradual increases in total protein content in middle-weight fetuses, alkaline phosphatase activity across all weight categories, alanine aminotransferase activity in maximal-weight fetuses, urea and uric acid contents across all groups, and glucose content and aspartate aminotransferase activity in most weight categories. The high nitrogen/energy group exhibited similar increasing trends for alanine aminotransferase activity in middle-weight fetuses, creatine kinase activity in maximal-weight fetuses, urea and uric acid contents, and glucose content and aspartate aminotransferase activity in most categories.

2.2 Changes in Allantoic Fluid Biochemical Parameters

The biochemical parameters in allantoic fluid of pregnant Huanjiang mini-pigs fed different nitrogen/energy diets are presented in Table 3. Under identical gestational stages and fetal weight categories, the high nitrogen/energy diet significantly increased alanine aminotransferase activity in maximal-weight fetuses and albumin, uric acid contents, and aspartate aminotransferase activity in minimal-weight fetuses at day 45 ($P < 0.05$). At day 75, it elevated urea and albumin contents in maximal-weight fetuses, total protein, urea, uric acid, and glucose contents in middle-weight fetuses, and urea, uric acid, and glucose contents and alkaline phosphatase, alanine aminotransferase, and creatine kinase activities in minimal-weight fetuses ($P < 0.05$). At day 110, it increased glucose content and alanine aminotransferase activity in maximal-weight fetuses and glucose content in middle-weight fetuses ($P < 0.05$).

The high nitrogen/energy diet also significantly reduced uric acid content and aspartate aminotransferase activity in maximal-weight fetuses, alanine aminotransferase and creatine kinase activities in middle-weight fetuses, and creatine kinase activity in minimal-weight fetuses at day 45 ($P < 0.05$). At day 75, it decreased uric acid content and creatine kinase activity in maximal-weight fetuses and aspartate aminotransferase activity in middle- and minimal-weight fetuses ($P < 0.05$). At day 110, it lowered aspartate aminotransferase activity in minimal-weight fetuses ($P < 0.05$).

Within the same dietary group and gestational stage, maximal-weight fetuses in the high nitrogen/energy group exhibited significantly higher alanine aminotransferase activity at day 45, urea content and aspartate aminotransferase activity at day 75, and uric acid content and alanine aminotransferase activity at

day 110 compared to middle-weight fetuses ($P < 0.05$). Minimal-weight fetuses showed significantly higher albumin content and alanine aminotransferase and aspartate aminotransferase activities at day 45, urea content and alkaline phosphatase, aspartate aminotransferase, and creatine kinase activities at day 75, and total protein, urea, and glucose contents and aspartate aminotransferase activity at day 110 ($P < 0.05$). Maximal-weight fetuses had lower uric acid content and aspartate aminotransferase activity at day 45, creatine kinase activity at day 75, and uric acid content and alanine aminotransferase activity at day 110 ($P < 0.05$).

In the low nitrogen/energy group, maximal-weight fetuses showed significantly higher uric acid content at day 45, total protein and uric acid contents and alkaline phosphatase, aspartate aminotransferase, and creatine kinase activities at day 75, and minimal-weight fetuses had higher albumin content and creatine kinase activity at day 110 compared to middle-weight fetuses ($P < 0.05$). Minimal-weight fetuses exhibited significantly higher uric acid and glucose contents and creatine kinase activity at day 45, and aspartate aminotransferase activity at day 75 ($P < 0.05$). Maximal-weight fetuses had lower alanine aminotransferase and creatine kinase activities at day 45, albumin content at day 75, and alanine aminotransferase, aspartate aminotransferase, and creatine kinase activities at day 110, while minimal-weight fetuses showed lower alanine aminotransferase activity at day 110 ($P < 0.05$).

As gestation progressed, the low nitrogen/energy group showed increasing albumin activity in minimal-weight fetuses and alanine aminotransferase activity in maximal-weight fetuses. The high nitrogen/energy group exhibited increasing glucose content in minimal-weight fetuses and alanine aminotransferase activity in middle-weight fetuses. Both dietary groups demonstrated gradual increases in uric acid content across all weight categories and urea content in most fetal weight categories.

Discussion

The biochemical components in amniotic and allantoic fluids constitute the material basis for fetal life activities, and changes in their concentrations or activities can reflect fetal tissue cell permeability and metabolic status. Alkaline phosphatase activity reflects osteoblast activity, bone formation, and calcium-phosphorus metabolism [13]. In this study, the high nitrogen/energy diet significantly increased alkaline phosphatase activity in amniotic fluid of middle-weight fetuses at day 45 and maximal-weight fetuses at day 110, as well as in allantoic fluid of minimal-weight fetuses at day 75, which would facilitate bone growth and development in fetuses.

Elevated aspartate aminotransferase and alanine aminotransferase activities in blood can indicate liver damage [14]. The high nitrogen/energy diet significantly reduced aspartate aminotransferase activity in allantoic fluid of middle-

and minimal-weight fetuses at day 75 and minimal-weight fetuses at day 110, suggesting that high nitrogen/energy diets may protect fetal liver function. Uric acid, the end product of purine metabolism, is primarily excreted through the kidneys, and chronically elevated blood uric acid can cause kidney and cardiovascular diseases [15]. The high nitrogen/energy diet significantly reduced uric acid content in allantoic fluid of maximal-weight fetuses at days 45 and 75, which may promote fetal kidney development and enhance renal function.

Structurally intact and functionally normal muscle cells allow minimal creatine kinase leakage across cell membranes [16]. The high nitrogen/energy diet significantly decreased creatine kinase activity in amniotic fluid of maximal- and middle-weight fetuses at day 45 and middle-weight fetuses at day 110, as well as in allantoic fluid of middle- and minimal-weight fetuses at day 45 and maximal-weight fetuses at day 75. This suggests that high nitrogen/energy diets can protect the integrity of fetal muscle cells, possibly because certain functional amino acids in these diets act as signaling molecules to regulate key metabolic pathways and improve fetal health status [17]. Glucose is an important indicator of energy balance in animals [18]. The high nitrogen/energy diet significantly reduced glucose content in amniotic fluid of middle- and minimal-weight fetuses at day 75 and minimal-weight fetuses at day 110, indicating that high nitrogen/energy diets can improve fetal glucose utilization efficiency.

Fetal growth and development is a complex process, and fetuses of different weights have different nutrient absorption and utilization efficiencies [19]. Total protein and albumin contents reflect both protein absorption and metabolism status and immune status [20]. In this study, albumin content in amniotic fluid of maximal-weight fetuses was significantly higher than that of minimal-weight fetuses at days 45 and 75 in the high nitrogen/energy group and at days 45 and 110 in the low nitrogen/energy group. This could supply more nitrogen-containing nutrients to larger fetuses, potentially explaining their faster development. It is also speculated that insufficient nutrient supply in amniotic fluid of small fetuses causes their growth retardation [21]. Compared to the low nitrogen/energy diet, the high nitrogen/energy diet significantly increased total protein content in amniotic fluid of minimal-weight fetuses at day 45, suggesting that high nitrogen/energy diets can increase maternal protein intake and amniotic fluid total protein content, thereby alleviating intrauterine growth retardation [22]. The high nitrogen/energy diet also significantly increased albumin content in allantoic fluid of minimal-weight fetuses at day 45, suggesting that intrauterine growth-retarded fetuses may have impaired utilization of nitrogen-containing nutrients, leading to increased albumin excretion. Additionally, uric acid content in amniotic fluid of maximal-weight fetuses was generally significantly higher than that of middle- and minimal-weight fetuses, indicating that larger fetuses have correspondingly higher nutrient requirements, which increases the metabolic burden on the liver [23].

Fetuses at different gestational stages have different nutrient utilization efficiencies [24]. Urea is the end product of protein and amino acid catabolism, and

lower content indicates higher nitrogen utilization efficiency [25]. In this study, urea content in both amniotic and allantoic fluids generally increased with gestational stage, suggesting that protein utilization efficiency decreases in late gestation and that dietary protein levels could be appropriately reduced. This finding is consistent with Guo et al. [2]. Furthermore, alkaline phosphatase activity in amniotic fluid was higher at day 110 than at other stages, indicating accelerated bone growth in late gestation.

Conclusion

In summary, feeding high nitrogen/energy diets can promote fetal growth and development by regulating biochemical parameters in amniotic and allantoic fluids. Maximal-weight fetuses demonstrate higher protein utilization efficiency compared to middle- and minimal-weight fetuses.

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