

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

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Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of diets with different nitrogen-to-energy ratios on the biochemical parameters of amniotic fluid and allantoic fluid in pregnant Huanjiang Xiang pigs. Forty-eight primiparous pregnant 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 diets (digestible energy 14.73 MJ/kg, crude protein content 13.11%, nitrogen-to-energy ratio 0.89) and low nitrogen-to-energy ratio diets (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 biochemical parameter determination. The results showed that under the same gestational age and standardized body weight 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, the 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, the urea and uric acid contents in amniotic fluid and uric acid content in allantoic fluid of fetal pigs in both diet groups gradually increased. These results indicate that high nitrogen-to-energy ratio diets can promote fetal growth and development by regulating biochemical parameters of amniotic fluid and allantoic fluid; moreover, maximum body weight fetuses exhibit higher protein utilization efficiency compared with intermediate and minimum body weight fetuses.

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 amniotic fluid and allantoic fluid of pregnant Huanjiang mini-pigs. Forty-eight primiparous Huanjiang mini-pigs were randomly assigned to two groups with eight pens per group (three pigs per pen). After breeding, 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 sow per pen was slaughtered, and amniotic fluid and allantoic fluid were collected from fetuses with maximal, middle, and minimal body weight within each litter for biochemical parameter analysis. The results showed that under the same gestational stage and fetal weight category, compared with the low nitrogen/energy diet group, the high nitrogen/energy diet group exhibited significantly increased alkaline phosphatase activity in allantoic fluid of minimal-weight fetuses at day 75 ($P < 0.05$), while showing significantly decreased aspartate aminotransferase activity in allantoic fluid of middle- and minimal-weight fetuses at day 75 and minimal-weight fetuses at day 110, uric acid content in maximal-weight fetuses at days 45 and 75, and creatine kinase activity in middle- and minimal-weight fetuses at day 45 and maximal-weight fetuses at day 75 ($P < 0.05$). Compared with

minimal-weight fetuses, albumin content in amniotic fluid of maximal-weight fetuses was significantly higher at days 45 and 75 in the high nitrogen/energy diet group and at days 45 and 110 in the low nitrogen/energy diet group ($P < 0.05$). As gestation progressed, urea and uric acid contents in amniotic fluid and uric acid content in allantoic fluid gradually increased in both diet groups. These findings suggest that a high nitrogen/energy diet can promote fetal growth and development by regulating biochemical parameters in amniotic and allantoic fluids, and that maximal-weight fetuses demonstrate higher protein utilization efficiency than middle- and minimal-weight fetuses.

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

Huanjiang mini-pig is a renowned local breed in China. However, extensive management practices have resulted in low reproductive performance and piglet survival rates. Nutritional levels before and after breeding affect embryo survival rates in primiparous sows during early pregnancy, while both maternal undernutrition and overnutrition can reduce maternal blood supply, nutrient delivery, and oxygen transport to fetuses, thereby impeding fetal growth. Therefore, improving dietary nutrition 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. Except for slightly lower protein content, its composition is similar to maternal plasma. During mid-to-late gestation, fetuses can ingest nutrients, active factors, and precursors for antimicrobial substances by swallowing amniotic fluid, which supports and promotes their growth and intestinal maturation. Allantoic fluid primarily originates 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.

Previous studies have shown that compared with a low nitrogen/energy diet, feeding a high nitrogen/energy diet increased sow backfat thickness at days 45 and 75 and fetal weight at days 45, 75, and 110 of gestation. Other research demonstrated that high nitrogen/energy diets benefit umbilical cord vascular development, while fetal weight in early pregnancy 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 fetal-maternal nutrient transport and fetal developmental status. Improving dietary composition to enhance material exchange between maternal blood and fetal fluids represents an important approach to promote fetal growth and increase 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 to provide nutritional regulation

strategies for improving reproductive performance.

1.1 Experimental Animals, Grouping, and Management

Forty-eight primiparous Huanjiang mini-pigs were randomly divided into two groups (24 pigs per group) and housed in eight pens per group (three pigs per pen). After breeding, 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 Feeding Standard for Swine (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 shown in Table 1. Throughout the trial, each pen received daily feed amounting to 2.5% of the total body weight of the three sows, fed 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 sow per pen was randomly selected, slaughtered after cardiac exsanguination, and all fetuses were weighed. Amniotic fluid and allantoic fluid were collected from fetuses with maximal, middle, and minimal body weight within each litter, placed 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 Biochemistry Technology Co., Ltd.

1.4 Data Processing and Analysis

Data were analyzed using SPSS 17.0 software. Independent samples t-tests were performed for comparisons between diet groups at the same gestational stage and fetal weight category, while one-way ANOVA (LSD) was used for comparisons among different fetal weight categories within the same diet group and gestational stage. Data are presented as “mean ± standard error.” $P < 0.05$ was considered statistically significant.

2.1 Changes in Amniotic Fluid Biochemical Parameters of Pregnant Huanjiang Mini-Pigs

The biochemical parameters in amniotic fluid of pregnant Huanjiang mini-pigs fed different nitrogen/energy diets are presented in Table 2. Under the same ges-

tational stage and fetal weight category, compared with the low nitrogen/energy diet, the high nitrogen/energy diet significantly increased ALB, ALT, and AST activities in maximal-weight fetuses and ALP activity in middle-weight fetuses at day 45, as well as TP content in minimal-weight fetuses. At day 75, it increased ALB activity and UREA content in maximal-weight fetuses and ALT activity in minimal-weight fetuses. At day 110, it increased ALP and CK activities and TP, UREA, and GLU contents in maximal-weight fetuses and AST and CK activities in middle-weight fetuses ($P < 0.05$). Conversely, the high nitrogen/energy diet significantly decreased CK activity in maximal- and middle-weight fetuses and ALT activity in minimal-weight fetuses at day 45; AST activity in maximal-weight fetuses, TP and GLU contents in middle-weight fetuses, and ALB, TP, and GLU contents in minimal-weight fetuses at day 75; and ALT and CK activities in maximal-weight fetuses, CK activity in middle-weight fetuses, and ALT and CK activities and GLU content in minimal-weight fetuses at day 110 ($P < 0.05$).

Within the same diet group and gestational stage, maximal-weight fetuses in the high nitrogen/energy diet group showed significantly higher UREA and UA contents and ALT activity at day 45, and higher ALB, UREA, and UA contents and ALT activity at day 75 compared with middle-weight fetuses ($P < 0.05$). Minimal-weight fetuses showed significantly lower UA content at day 45 and ALB content at day 75 ($P < 0.05$). In the low nitrogen/energy diet group, maximal-weight fetuses exhibited significantly higher TP and UA contents and ALP activity at day 45, UA content and ALP, ALT, and AST activities at day 75, and UREA and UA contents and ALP, ALT, and CK activities at day 110 compared with middle-weight fetuses ($P < 0.05$). Minimal-weight fetuses showed significantly higher UREA and GLU contents and ALT activity at day 110, but lower AST activity at day 45 and CK activity at day 110 ($P < 0.05$).

As gestation progressed, TP content in middle-weight fetuses and ALP activity in all three weight categories increased in the low nitrogen/energy diet group. ALT activity in middle-weight fetuses and CK activity in maximal-weight fetuses increased in the high nitrogen/energy diet group. UREA and UA contents in amniotic fluid increased across both diet groups, as did GLU content and AST activity in most fetal weight categories except minimal-weight fetuses in the high nitrogen/energy diet group.

2.2 Changes in Allantoic Fluid Biochemical Parameters of Pregnant Huanjiang Mini-Pigs

The biochemical parameters in allantoic fluid of pregnant Huanjiang mini-pigs fed different nitrogen/energy diets are presented in Table 3. Under the same gestational stage and fetal weight category, compared with the low nitrogen/energy diet, the high nitrogen/energy diet significantly increased ALT activity in maximal-weight fetuses and ALB, UA contents, and AST activity in minimal-weight fetuses at day 45. At day 75, it increased UREA and ALB contents in maximal-weight fetuses; TP, UREA, UA, and GLU contents in

middle-weight fetuses; and UREA, UA, and GLU contents and ALP, ALT, and CK activities in minimal-weight fetuses. At day 110, it increased GLU content and ALT activity in maximal-weight fetuses and GLU content in middle-weight fetuses ($P < 0.05$). Meanwhile, the high nitrogen/energy diet significantly decreased UA content and AST activity in maximal-weight fetuses, ALT and CK activities in middle-weight fetuses, and CK activity in minimal-weight fetuses at day 45; UA content and CK activity in maximal-weight fetuses and AST activity in middle- and minimal-weight fetuses at day 75; and AST activity in minimal-weight fetuses at day 110 ($P < 0.05$).

Within the same diet group and gestational stage, maximal-weight fetuses in the high nitrogen/energy diet group showed significantly higher ALT activity at day 45, UREA content and AST activity at day 75, and UA content and ALT activity at day 110 compared with middle-weight fetuses ($P < 0.05$). Minimal-weight fetuses exhibited significantly higher ALB content and ALT and AST activities at day 45, UREA content and ALP, AST, and CK activities at day 75, and TP, UREA, and GLU contents and AST activity at day 110 ($P < 0.05$), but lower UA content and AST activity at day 45, CK activity at day 75, and UA content and ALT activity at day 110 ($P < 0.05$). In the low nitrogen/energy diet group, maximal-weight fetuses had significantly higher UA content at day 45, TP and UA contents and ALP, AST, and CK activities at day 75, and minimal-weight fetuses showed higher ALB content and CK activity at day 110 ($P < 0.05$). Minimal-weight fetuses also had significantly higher UA and GLU contents and CK activity at day 45 and AST activity at day 75 ($P < 0.05$), while maximal-weight fetuses showed lower ALT and CK activities at day 45, ALB content at day 75, and ALT, AST, and CK activities at day 110, and minimal-weight fetuses had lower ALT activity at day 110 ($P < 0.05$).

As gestation progressed, ALB activity in minimal-weight fetuses and ALT activity in maximal-weight fetuses increased in the low nitrogen/energy diet group. GLU content in minimal-weight fetuses and ALT activity in middle-weight fetuses increased in the high nitrogen/energy diet group. UA content increased across both diet groups, and UREA content increased in most fetal weight categories except maximal-weight fetuses in the high nitrogen/energy diet group.

3 Discussion

The biochemical components in amniotic and allantoic fluids constitute the material basis for fetal life activities, and changes in their contents or activities can reflect fetal tissue cell permeability and metabolic status. Alkaline phosphatase activity reflects osteoblast activity, bone formation, and calcium-phosphorus metabolism. In this study, the high nitrogen/energy diet significantly increased ALP 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, compared with the low nitrogen/energy diet. This suggests enhanced bone growth and development in these fetuses.

Elevated AST and ALT activities in blood can indicate liver damage. The high nitrogen/energy diet significantly decreased AST activity in allantoic fluid of middle- and minimal-weight fetuses at day 75 and minimal-weight fetuses at day 110, suggesting a protective effect on fetal liver. Uric acid, the end product of purine metabolism primarily excreted by kidneys, can cause kidney and cardiovascular diseases when chronically elevated in blood. The high nitrogen/energy diet significantly decreased UA 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 CK leakage across cell membranes. The high nitrogen/energy diet significantly decreased CK 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 indicates that the high nitrogen/energy diet may protect fetal muscle cell integrity, possibly through functional amino acids acting as signaling molecules to regulate key metabolic pathways and improve fetal health. Glucose is an important indicator of energy balance in animals. The high nitrogen/energy diet significantly decreased GLU content in amniotic fluid of middle- and minimal-weight fetuses at day 75 and minimal-weight fetuses at day 110, suggesting improved glucose utilization efficiency in fetuses of sows fed the high nitrogen/energy diet.

Fetal growth and development is a complex process, and different-weight fetuses exhibit varying efficiencies in nutrient absorption and utilization. Total protein and albumin contents reflect both protein absorption and metabolism status and immune status. In this study, albumin content in amniotic fluid of maximal-weight fetuses was significantly higher than in minimal-weight fetuses at days 45 and 75 in the high nitrogen/energy diet group and at days 45 and 110 in the low nitrogen/energy diet group. This may provide more nitrogen-containing nutrients to larger fetuses, potentially explaining their faster development. Conversely, insufficient nutrient supply in amniotic fluid of small fetuses may cause growth retardation.

Compared with 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 increased maternal protein intake elevates amniotic fluid TP content, thereby alleviating intrauterine growth retardation. 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 nitrogen-containing nutrient utilization, leading to increased albumin excretion. Additionally, uric acid content in amniotic fluid of maximal-weight fetuses was generally significantly higher than in middle- and minimal-weight fetuses, indicating that larger fetuses have greater nutrient requirements, which increases hepatic metabolic burden.

Fetuses at different gestational stages exhibit varying nutrient utilization effi-

ciencies. Urea is the end product of protein and amino acid catabolism, and lower content indicates higher nitrogen utilization efficiency. In this study, urea content in both amniotic and allantoic fluids generally increased with gestational stage, suggesting decreased protein utilization efficiency in late pregnancy. This supports the recommendation to reduce dietary protein levels in late gestation, consistent with previous research. Furthermore, ALP activity in amniotic fluid at day 110 was higher than at other stages, indicating accelerated bone growth in late gestation.

4 Conclusion

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

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