Effects of Dietary Proline Supplementation on Reproductive Performance and Plasma Biochemical Parameters in Gestating Huanjiang Xiang Pigs (Postprint)

Authors: Zha Wei, Kong Xiangfeng, Tan Minjie, Geng Meimei, Xu Liwei, You Jinming

Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of proline metabolism on reproductive performance in pregnant Huanjiang Xiang pigs and explore its potential physiological and biochemical mechanisms. Forty-eight replacement Huanjiang sows were selected and randomly divided into three groups 15 days after mating, with eight replicates per group and two pigs per replicate. Each group was fed experimental diets supplemented with 0.77% L-alanine (alanine group), 1% L-proline (proline group), or 0.77% L-alanine + 0.0167% difluoromethylornithine (DFMO) (DFMO group). On days 45 and 70 of gestation, one sow per replicate was randomly selected, exsanguinated and euthanized; blood was collected with heparin anticoagulation, centrifuged to separate plasma for biochemical parameter analysis; sows were dissected to record corpus luteum number, fetal number, and fetal weight. The results showed: on day 70 of gestation, litter weight in the proline group was significantly higher than that in the alanine group ($P<0.05$); on day 45 of gestation, plasma alanine aminotransferase activity and albumin concentration in the proline group were significantly lower than those in the alanine group ($P<0.05$). Compared with day 45, plasma total cholesterol concentration in the proline group, and plasma high-density lipoprotein and albumin concentrations in the DFMO group were significantly decreased on day 70 of gestation ($P<0.05$). No significant differences were observed among groups for other parameters ($P>0.05$). These results suggest that dietary proline supplementation may promote fetal growth and development in pregnant Huanjiang Xiang pigs by improving nitrogen metabolism and lipid metabolism.

Full Text

Effects of Dietary Proline Supplementation on Reproductive Performance and Plasma Biochemical Parameters of Pregnant Huanjiang Mini-pigs

ZHA Wei[1,2,3], KONG Xiangfeng[2,3], TAN Minjie[2,3], GENG Meimei[2], XU Liwei[2], YOU Jinming[1]*

[1]. Jiangxi Agricultural University, Key Laboratory of Animal Nutrition in Jiangxi Province, Jiangxi Nutrition Feed Development Engineering Center, Nanchang 330045, China
[2]. Institute of Subtropical Agriculture, Chinese Academy of Sciences, Key Laboratory for Agro-Ecological Processes in Subtropical Region, Changsha 410125, China
[3]. Huanjiang Observation and Research Station for Karst Ecosystems, Chinese Academy of Sciences, Research Center of Mini-Pig, Huanjiang 547100, China

Abstract

This experiment was conducted to investigate the effect of proline metabolism on reproductive performance in pregnant Huanjiang mini-pigs and explore its underlying physiological and biochemical mechanisms. Forty-eight replacement Huanjiang sows were selected and randomly divided into 3 groups 15 days after mating, with 8 replicates per group and 2 pigs per replicate. Each group was fed experimental diets supplemented with 0.77% L-alanine (alanine group), 1% L-proline (proline group), or 0.77% L-alanine + 0.0167% difluoromethylornithine (DFMO) (DFMO group). On days 45 and 70 of gestation, one sow per replicate was randomly selected, slaughtered by exsanguination, and blood was collected with heparin anticoagulation. Plasma was separated by centrifugation for biochemical parameter analysis. The sows were dissected to record the number of corpora lutea, fetus number, and fetal weight. The results showed that on day 70 of gestation, the litter weight in the proline group was significantly higher than that in the alanine group ($P<0.05$). On day 45 of gestation, plasma alanine aminotransferase activity and albumin concentration in the proline group were significantly lower than those in the alanine group ($P<0.05$). Compared with day 45, plasma total cholesterol concentration in the proline group and plasma high-density lipoprotein and albumin concentrations in the DFMO group were significantly decreased on day 70 ($P<0.05$). No significant differences were observed in other parameters among groups ($P>0.05$). These findings indicate that dietary proline supplementation may promote fetal growth and development in pregnant Huanjiang mini-pigs by improving nitrogen metabolism and lipid metabolism.

Keywords: proline; biochemical parameters; reproductive performance; pregnancy; Huanjiang mini-pig

Primiparous sows experience 20%–50% embryonic/fetal loss during gestation,

with early pregnancy being the peak period for embryonic/fetal mortality [1]. This high embryonic loss rate in early pregnancy severely restricts sow reproductive efficiency. Fetal growth and development is a complex biological process influenced by genetics, nutrition, environment, and maternal maturity. Nutritional interventions can improve placental growth and the intrauterine environment, thereby reducing embryonic/fetal mortality during gestation. For example, dietary arginine can be converted to ornithine via arginase catalysis, and then synthesize polyamines (including putrescine, spermidine, and spermine) under ornithine decarboxylase (ODC) catalysis. As important metabolites of arginine, polyamines promote embryogenesis, accelerate placental vascular development, and facilitate the delivery of blood, nutrients, and oxygen to fetuses, thereby promoting fetal growth and development. Our previous research found that treating porcine embryonic trophoblast cells cultured in vitro with putrescine increased protein synthesis by activating the mammalian target of rapamycin (mTOR) signaling pathway, thereby promoting placental cell proliferation [6]. Since porcine placenta lacks arginase activity, arginine cannot be converted to ornithine in porcine placenta, and the ornithine and its metabolite polyamines may originate from other tissues and organs. Arginine can also synthesize nitric oxide (NO) under NO synthase catalysis. As a major relaxation factor produced by endothelial cells, NO plays an important role in promoting vascular development, regulating placental-fetal blood flow, and transporting nutrients from mother to fetus [7]. Therefore, dietary arginine supplementation can promote fetal growth and improve sow reproductive performance through both polyamine and NO pathways. However, porcine placenta contains proline oxidase (POX), ornithine aminotransferase (OAT), and ODC activities. Proline-derived ornithine, produced via POX and OAT, can be further decarboxylated by ODC to form putrescine, which is subsequently converted to spermidine and spermine by spermidine synthase and spermine synthase, respectively [8]. Studies have shown that proline plays an important role in cell differentiation and conceptus development [8-9]. In recent years, extensive research has been conducted on amino acids such as arginine and glutamate in improving sow reproductive performance [10-11], but few studies have focused on proline. Huanjiang mini-pig is a famous local small breed in China, characterized by delicious meat, genetic purity, and strong stress resistance [12]. However, due to long-term consumption of roughage such as green forage and rice bran, it lacks scientific and reasonable nutritional requirements and feeding management standards, resulting in low production efficiency and difficulty in large-scale, intensive production. Based on previous research findings [8,13], this experiment supplemented the diet with 1% L-proline to promote polyamine generation in vivo, and added difluoromethylornithine (DFMO), a specific irreversible inhibitor of ODC [14], to inhibit polyamine generation in vivo, aiming to investigate the effects of polyamine metabolism on reproductive performance in pregnant Huanjiang mini-pigs and explore the underlying physiological and biochemical mechanisms, providing a basis for proline application in pregnant sow diets.

1.1 Experimental Diets

The basal diet was formulated according to NRC (1998) standards and combined with feeding practices from Huanjiang mini-pig farms (Table 1).

1.2 Experimental Animals and Management

Forty-eight healthy replacement Huanjiang mini-pig sows with similar body weight and meeting breeding conditions were selected for the trial. They were naturally estrus and naturally mated (remated once after 12 h). The sows were fed the basal diet for the first 15 days post-mating. On day 16 post-mating, they were weighed and randomly divided into 3 groups according to body weight, with 8 replicates (pens) per group and 2 pigs per replicate. The three groups were fed experimental diets supplemented with 0.77% L-alanine (alanine group, isonitrogenous control), 1% L-proline (proline group), or 0.77% L-alanine + 0.0167% DFMO (DFMO group, which reduces polyamine generation in vivo by inhibiting synthase activity). The DFMO dosage was converted from the optimal dosage obtained in preliminary trials on pregnant mice. Feeding was conducted daily at 09:00 and 18:00, with daily feed amount approximately 3% of body weight, and water was provided ad libitum. The animal trial was conducted from August to December 2013 at the Huanjiang Mini-pig Experimental Base of the Chinese Academy of Sciences in Minglun Town, Huanjiang County. Before the trial, pens were cleaned, washed, and disinfected. Other daily management followed conventional feeding practices.

1.3 Sample Collection and Measurement Indicators

On days 30 and 55 of the trial (corresponding to days 45 and 70 of gestation), sows in each group were weighed, and one sow per pen was randomly selected, slaughtered by exsanguination, and blood was collected into 10 mL heparin-coated tubes. Plasma was separated by centrifugation at 3,000 r/min for 10 min and stored at -20°C. The sows were dissected, the entire uterus was removed and weighed, fetuses were stripped from the uterus one by one, their positions in the uterus were observed and recorded, and fetus number, corpora lutea number, and individual fetal weight were measured. Samples were thawed at 4°C, and plasma concentrations of total protein, albumin, blood ammonia, urea nitrogen, total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein, as well as activities of alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transpeptidase were determined using a CX4 automatic biochemical analyzer (Beckman). All biochemical kits were purchased from Beijing Leadman.

1.4 Data Processing

Data were analyzed using SPSS 19.0 software. One-way ANOVA was used for data comparison among different groups at the same gestational age, and

independent samples t-test was used for data comparison between different gestational ages within the same group. Results are expressed as mean ± standard error. $P < 0.05$ was considered statistically significant.

2.1 Effects of Dietary Proline Supplementation on Reproductive Performance of Pregnant Huanjiang Mini-pigs

As shown in Table 2, on day 70 of gestation, litter weight in the proline group was significantly higher than that in the alanine group ($P<0.05$). No significant differences were observed among groups in other parameters including corpora lutea number, fetus number, conceptus weight, and fetal weight ($P>0.05$).

2.2 Effects of Dietary Proline Supplementation on Plasma Enzyme Activity of Pregnant Huanjiang Mini-pigs

As shown in Table 3, on day 45 of gestation, plasma alanine aminotransferase activity in the proline group was significantly lower than that in the alanine group ($P<0.05$). No significant differences were observed among groups in other parameters ($P>0.05$).

2.3 Effects of Dietary Proline Supplementation on Plasma Metabolite Concentrations of Pregnant Huanjiang Mini-pigs

As shown in Table 4, on day 45 of gestation, plasma albumin concentration in the proline group was significantly lower than that in the alanine and DFMO groups ($P<0.05$). Compared with day 45, plasma total cholesterol and low-density lipoprotein concentrations in the proline group, and plasma albumin and high-density lipoprotein concentrations in the DFMO group were significantly decreased on day 70 ($P<0.05$). No significant differences were observed in other parameters ($P>0.05$).

Maternal nutrition is the primary factor influencing the intrauterine environment. The placenta is the crucial site for material exchange between mother and fetus, and providing appropriate nutrients to the placenta is essential for fetal growth, development, and survival [15]. In normal pregnancies, the concentration of most free amino acids in porcine umbilical vein blood is higher than that in maternal circulation [16]. Furthermore, there is a significant positive correlation between most amino acids in fetal and maternal circulation. Proline is one of the most abundant amino acids in placental tissue and serves as a rich nitrogen carrier in porcine placenta [17]. During gestation days 20–40, proline concentration in porcine placenta significantly increased from 0.41 mmol/L to 0.58 mmol/L, reaching maximum concentration on day 60 of gestation. During this period, placental proline concentration was 3–10 times higher than that in maternal circulation. Additionally, studies have found that on day 60 of gestation, proline concentrations in placenta, amniotic fluid, and allantoic fluid corresponding to intrauterine growth retardation (IUGR) fetuses were significantly lower than those of normal fetuses [18]. In this experiment, dietary

proline supplementation significantly increased litter weight by 32.43% on day 70 of gestation compared with the alanine group, with certain improvements also observed in fetal weight and conceptus weight. This may be because the body utilized proline to synthesize more polyamines, which promoted placental development, enabling better nutrient transfer to fetuses and thus facilitating fetal growth and development [18,19].

Nutrients absorbed and metabolites from various tissues and organs enter the circulatory system, so blood biochemical parameters can reflect the body' s metabolic status. Alanine aminotransferase and aspartate aminotransferase are important transaminases in animals, playing crucial roles in amino acid metabolism and the metabolic conversion of proteins, fats, and carbohydrates [19]. In this experiment, on day 45 of gestation, plasma alanine aminotransferase activity in proline group sows was significantly reduced by 20.31% compared with the alanine group. Reduced blood alanine aminotransferase activity may decrease the risk of pregnancy syndromes such as obesity and hyperlipidemia [20].

Plasma total cholesterol and triglyceride concentrations reflect lipid metabolism status. Low-density lipoprotein and high-density lipoprotein are carriers for cholesterol transport [21]. Low-density lipoprotein transports cholesterol from liver to peripheral tissues, while high-density lipoprotein returns cholesterol from tissues to liver for metabolism. In this experiment, dietary proline supplementation had no significant effect on lipid metabolism in pregnant sows. However, when comparing day 70 with day 45 of gestation, plasma total cholesterol concentration in the proline group decreased by 43.75%, and low-density lipoprotein concentration decreased by 26.80%, suggesting enhanced cholesterol metabolism capacity in Huanjiang mini-pigs during mid-gestation.

In conclusion, dietary supplementation with 1% proline may promote fetal growth and development in pregnant Huanjiang mini-pigs by improving nitrogen utilization and lipid metabolism.

References

[1] NOVAK S, ALMEIDA F R C L, COSGROVE J R, et al. Effect of pre- and postmating nutritional manipulation on plasma progesterone, blastocyst development, and the oviductal environment during early pregnancy gilts[J]. Journal Animal Science, 2003, 81(3): 772–783.
[2] WU G, BAZER F W, WALLACE J M, et al. Board-invited review: intrauterine growth retardation: implications animal sciences[J]. Journal Animal Science, 2006, 84(9): 2316–2337.
[3] BAZER F W, SPENCER T E, JOHNSON G A, et al. Comparative aspects of implantation[J]. Reproduction, 2009, 138(2): 195–209.
[4] WU G Y, BAZER F W, DAVIS T A, et al. Arginine metabolism and nutrition in growth, health and disease[J]. Amino Acids, 2009, 37(1): 153–168.
[5] TAN MJ, KONG XF, LIU QY, et al. Polyamines and conceptus development

in mammals[J]. Chinese Journal of Animal Nutrition, 2015, 27(1): 43-48.
[6] KONG X F, TAN B, YIN Y L, et al. L-Arginine stimulates the mTOR signaling pathway and protein synthesis in porcine trophectoderm cells[J]. The Journal of Nutritional Biochemistry, 2012, 23(9): 1178-1183.
[7] LIU JF, HU H, KONG XF, et al. Research progress on arginine nutrition in sows[J]. Chinese Journal of Animal Nutrition, 2010, 22(4): 840-844.
[8] WU G Y, BAZER F W, DATTA S, et al. Proline metabolism in the conceptus: implications for fetal growth and development[J]. Amino Acids, 2008, 35(4): 691-702.
[9] PHANG J M, LIU W. Proline metabolism cancer[J]. Frontiers Bioscience, 2012, 17(1): 1835-1845.
[10] KONG XF, YIN YL, WU GY. Research progress on functional amino acid nutrition in pigs[J]. Chinese Journal of Animal Nutrition, 2009, 21(1): 1-7.
[11] XIE CY, ZHANG YZ, WU X, et al. Physiological role and regulation of glutamate-glutamine cycle and exchange in the placenta-fetus complex[J]. Chinese Journal of Animal Nutrition, 2014, 26(6): 1430-1434.
[12] YANG XJ. Congjiang Xiang pig[J]. Shanghai Journal of Animal Husbandry and Veterinary Medicine, 2002(2): 28-29.
[13] WANG J, LI G R, TAN B E, et al. Oral administration of putrescine and proline during the suckling period improves epithelial restitution after early weaning in piglets[J]. Journal of Animal Science, 2015, 93(4): 1679-1688.
[14] FOZARD J R, PART M L, PRAKASH N J, et al. Inhibition of murine embryonic development by α-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase[J]. European Journal of Pharmacology, 1980, 65(4): 379-391.
[15] REYNOLDS L P, REDMER D A. Angiogenesis the placenta[J]. Biology of Reproduction, 2001, 64(4): 1033-1040.
[16] WU G Y, POND W G, OTT T, et al. Maternal dietary protein deficiency decreases amino acid concentrations fetal plasma and allantoic fluid of pigs[J]. The Journal of Nutrition, 1998, 128(5): 894-902.
[17] WU G Y, OTT T L, KNABE D A, et al. Amino acid composition of the fetal pig[J]. The Journal of Nutrition, 1999, 129(5): 1031-1038.
[18] WU G Y, BAZER F W, HU J B, et al. Polyamine synthesis from proline in the developing porcine placenta[J]. Biology of Reproduction, 2005, 72(4): 842-850.
[19] LIU Y Y, KONG X F, JIANG G L, et al. Effects of dietary protein/energy ratio on growth performance, carcass trait, meat quality, and plasma metabolites in pigs of different genotypes[J]. Journal of Animal Science and Biotechnology, 2015, 6: 36.
[20] CUI JY, HOU LJ, ZHANG LL. Investigation on the relationship between metabolic syndrome and alanine aminotransferase[J]. Health Medicine Research and Practice, 2011, 8(2): 16-18.
[21] SHARMAN M J, FERNANDEZ M L, ZERN T L, et al. Replacing dietary carbohydrate with protein and fat decreases the concentrations of small LDL and the inflammatory response induced by atherogenic diets the guinea pig[J]. The Journal of Nutritional Biochemistry, 2008, 19(11): 732-738.

Corresponding author: Professor YOU Jinming, E-mail: youjinm@163.com
(Responsible editor: WU Hailong)

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