Effects of Dietary Supplementation of Creatine on Fetal Muscle and Placental Development in Mid-and-Late Gestating Gilts

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The creatine-creatine kinase-phosphocreatine (Cr-CK-PCr) system plays a role in interorgan metabolism among the kidneys, liver, and muscle to yield adenosine triphosphate (ATP) for cellular bioenergetics. The Cr-CK-PCr system has been hypothesized to have a role in fetal-placental development in pigs as energy demands increase during gestation. This study tested the hypothesis that dietary supplementation of creatine from Day 10 to either Day 60 or 90 of gestation would enhance Cr-PCr metabolism in the placenta to improve placental and fetal muscle development. Gilts were synchronized to estrus during the spring using synthetic progesterone, Matrix, and observed for estrus (Day 0). Gilts were then artificially inseminated at 12 h and 24 h after first insemination following observed estrus. Gilts were assigned randomly to one of 4 groups: Day 60 CON, Day 60 Cr, Day 90 CON, or Day 90 Cr (n=10 gilts/treatment). All gilts were fed a diet of 2lbs containing 14% crude protein twice daily and gilts assigned to the Cr treatment groups were supplemented with 30 g/day Cr from Day 10 to either Day 60 or 90 of gestation when tissues were collected by hysterectomy. This study had one replicate where gilts were synchronized in spring and tissues collected in the summer and another where gilts were synchronized in fall and tissues collected in the winter. Following euthanasia, individual placentas were separated from the endometrium and their lengths and weights were measured. Samples from the endometrium, chorioallantois, and amnion and were snap frozen in liquid nitrogen for analysis of gene and protein expression and fixed in 4% paraformaldehyde for histological analysis. Additionally, biceps femoris muscle from 3 male and 3 female fetuses closest to the mean weight were snap frozen in liquid nitrogen, frozen in OCT gel or fixed in 4% paraformaldehyde. Statistical analyses revealed that placental weights from gilts supplemented with Cr were lighter than those from CON gilts at Day 60 (P=0.02) of gestation. However, there were no significant treatment effects on placental weights due to dietary supplementation at Day 90 of gestation. Additionally, no significant effects on placental weights were detected between seasonal replicates. For placental length, there were no treatment effects; however, there were statistically significant differences between replicates from both Day 60 (P=0.01) and Day 90 (P=0.02). D60 and D90 tissues collected in the summer had greater mean placental weights than tissues collected in the winter. Results of this study suggest that dietary supplementation of Cr had limited effects on placental weight and length. Currently, mRNAs for key enzymes in the pathway for synthesis of PCr and ATP and transporters for Cr and guanidinoacetate are being quantified. Further, placental expression of creatine, phosphocreatine, and guanidinoacetate proteins is being determined. This research was supported by **Agriculture and Food Research Initiative Competitive Grant no. 2022-67015-36376** from the *USDA National Institute of Food and Agriculture.*