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Arginine supplementation between 41 and 146 days of pregnancy reduces uterine blood flow in dairy heifers

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ABSTRACT

We hypothesized that arginine (Arg) supplementation during early pregnancy could foster placental vascularization. The objective of this study was to evaluate the effects of Arg supplementation on uterine artery hemodynamics between 40 and 140 days of gestation. At 41 days of gestation, 17 Holstein heifers (448 ± 73.9 kg) were surgically fitted with a catheter in the peritoneal cavity. After surgery, nine heifers received a daily intraperitoneal infusion of 40 mg of Arg/kg of body weight (BW; ARG), whereas the remaining eight heifers received a daily intraperitoneal infusion of saline solution at equivalent volumes as ARG heifers relative to BW (CTRL). Daily infusions took place every 12 hours until heifers reached 146 days of pregnancy. At 41, 62, 83, 104, 125, and 146 days of pregnancy, all heifers were body weighed, bled, and uterine blood flow volume (FV) and other hemodynamics were determined using Doppler ultrasonography. The measurements included heart rate, FV, pulsatility index, and resistance index. Plasma concentrations of amino acids (AAs), nitric oxide, glucose, insulin growth factor, progesterone (P4), growth hormone, and prolactin were analyzed. In a second experiment, we evaluated Arg metabolism when infused either intravenously or intraperitoneally. Overall, FV did not differ between the treatments, but it increased ($P < 0.001$) with pregnancy day. Uterine blood FV evolved differently between the treatments, with CTRL heifers having a greater ($P < 0.05$) FV (1104 ± 65.42 mL/min) at 146 days of pregnancy than in ARG heifers (806 ± 65.32 mL/min). Pulsatility index and resistance index decreased throughout pregnancy but did not differ between the treatments. Heart rate was decreased ($P < 0.05$) in ARG (74 ± 1.4 beats/min) compared with CTRL heifers (81 ± 1.5 beats/min). Plasma concentrations of Arg tended ($P < 0.09$) to be greater in ARG than those in CTRL heifers, but carnitine, valine, isoleucine, leucine, phenylalanine, and tryptophan were lower ($P < 0.05$) in ARG than those in CTRL heifers. The proportion of essential AAs (with respect to total AAs) in plasma was greater ($P < 0.05$) in CTRL (73 ± 2.4%) than that in ARG heifers (65 ± 2.3%). No differences between the treatments were found in plasma prolactin ($P = 0.43$), insulin growth factor ($P = 0.97$), glucose ($P = 0.45$), growth hormone ($P = 0.39$), or nitric oxide ($P = 0.89$) concentrations. However, plasma P4 concentration was greater ($P < 0.05$) in ARG than in

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CTRL heifers. Contrary to our hypothesis, Arg supplementation did not increase blood flow to the uterus but did change other parameters that could influence placental and fetal growth such as heart rate, maternal concentration of plasma AAs, or P4 synthesis. Furthermore, infusion sites (blood vs. peritoneum) of Arg render different metabolic responses.

1. Introduction

Nowadays, it is known that fetal organs and system are sensitive to the environment of the uterus and that the influence of this environment can lead to long-term consequences [1], such as changes in performance and metabolic function of the offspring [2,3]. These processes are usually called developmental programming. Thus, it is critical to provide the uterus with sufficient nutrients during pregnancy, and this can only be reached by a proper maternal nutrition that permits an optimal placental growth and development. However, according to Reynolds and Caton [4], poor nutritional environment is quite common in livestock, mainly among other things, because of precocious inseminations and competition between nutrient needs for milk production and fetal development. Current nutritional models for dairy cattle ignore the needs during early pregnancy [5]. This problem is aggravated in heifers when requirements for growth and development compete with pregnancy. In the early stage of pregnancy, when nutrient requirements do not seem to have an important influence on the future offspring, maximal placental growth, vascularization, and fetal organogenesis take place [6,7]. Proper development of the placenta is essential for fetal growth because it supplies nutrients and oxygen to the calf [8]. To solve this dilemma, the first thought is an accurate and adequate dietary nutrient (such as amino acid [AA]) that may target specific processes [4].

Arginine (Arg) is considered to be an essential AA for dairy cattle that participates in an array of body functions [9], such as lactation, growth, development, tissue remodeling, and response to several hormones and signaling molecules [10]. Arginine stimulates the synthesis of growth hormone (GH), prolactin (PRL), and insulin [11]. Moreover, Arg is an essential substrate for nitric oxide (NO) synthesis [12] and polyamines [13,14]. Nitric oxide is a vasoactive compound, which participates in placental growth and angiogenesis, and thus, it is necessary for increasing uterine and placental–fetal blood flow [14] and to secure the fulfillment of fetal nutrient needs [15].

In addition, differences due to site of administration of Arg should be studied. Few studies can be found describing this objective; however, the effect in some blood metabolites is not the same if the administration takes place *via* the jugular vein or *via* abomasal infusion. For example, Vicini et al. [16] reported an increase of somatotropin and insulin when performing an injection of Arg into the jugular vein but not when the infusion was *via* the abomasum.

We hypothesized that supplying Arg to early pregnant heifers would lead to an increased uterine blood flow, which in turn would enhance placental and uterine development helping the calf to optimally develop in the uterus. Thus, the objectives of the study were to (1) evaluate the effects of Arg supplementation on uterine artery hemodynamics between 40 and 140 days of gestation and (2) investigate the different effects of Arg after a single peritoneal infusion (PrI) compared with a single jugular infusion (JI) in pregnant dairy heifers.

2. Materials and methods

All experimental procedures were approved by the Institutional Animal Care and Use of North Dakota State University (#A12043).

In experiment 1, Holstein heifers ($N = 17$) averaging 448 ± 73.9 kg of body weight (BW) and 552 ± 146.8 days of life were bred at Day 0 to the same bull by artificial insemination. At Day 30, after checking that all heifers were pregnant using a transrectal ultrasonography, animals were moved to a tie stall facility, and at 41 days of gestation, heifers were surgically fitted with a catheter in the peritoneal cavity. These catheters were later used to conduct infusions of treatments throughout the study. After surgery, nine heifers that were randomly selected received a daily intra-PrI of 40 mg of Arg/kg of BW (ARG), whereas the remaining eight heifers received a daily intra-PrI of saline solution at equivalent volumes as ARG heifers relative to BW (CTRL). Arginine was 100% L-Arg (Evonik industries, Hanau, Germany). The dose of Arg was based on the National Research Council [5] model. Using this guideline, we calculated the Arg supply of the diet would be about 48 g of metabolizable L-Arg/day. We aimed to provide a 40% supplementation over the regular Arg intake of a heifer and that corresponded to a daily dose 40 mg of Arg/kg of BW. Arginine was dissolved in 1 L of distilled water, and 35 mL of 2N HCl was added to achieve a final pH of 7. Infusions of either CTRL or ARG took place every 12 hours until heifers reached 146 days of pregnancy. The BW of each heifer was determined every 3 weeks, and the quantity of Arg or saline solutions to be infused into each heifer was adjusted accordingly.

Animals had free access to water and were fed *ad libitum* exactly the same total mixed ration (corn silage: 60%, distillers' dried grains: 30%, and grass hay: 10%) that met National Research Council (2001) [5] requirements. The ration was sampled once a week and analyzed for dry matter (DM), crude protein, neutral detergent fiber, nonfiber carbohydrates, and ether extract. Feed intake was recorded daily for each heifer. Three times a week, all the animals were walked for about 10 minutes.

At days 41, 62, 83, 104, 125, and 146 of gestation, all heifers were weighed, a blood sample was collected by venipuncture of the jugular vein, and the uterine artery blood hemodynamics was determined. Blood samples were