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Maternal metabolizable protein restriction during late gestation on uterine and umbilical blood flows and maternal and fetal amino acid concentrations near term in sheep

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ABSTRACT

To examine the effects of maternal metabolizable protein (**MP**) restriction during late gestation on uterine and umbilical blood flows, conceptus size, and amino acid concentrations in the uterine and umbilical vessels, 11 ewes with singleton pregnancies were assigned to one of three isocaloric diets formulated to provide 60% of MP (**MP60**), 80% of MP (**MP80**), or 100% of MP (**MP100**) requirements from days 100 to 130 of gestation. On day 130 of gestation, intraoperative uterine and umbilical blood flows were obtained as well as serum samples from the uterine artery, uterine vein, umbilical artery, and umbilical vein. Ewes on the MP60 diet had lighter ($P = 0.04$) and smaller ($P \leq 0.05$) fetuses, but increased ($P = 0.02$) uterine blood flow relative to fetal weight compared with MP100 ewes, with MP80 being intermediate. Umbilical blood flow was similar ($P = 0.70$) across treatments. Glutamine, glycine, isoleucine, leucine, ornithine, serine, and valine concentrations were impacted ($P \leq 0.02$) by maternal treatment. While uterine flux of total serum nitrites was greater ($P = 0.03$) in MP60 and MP80 ewes compared with MP100 ewes, fetal flux did not differ. Decreased maternal protein intake resulted in less ($P < 0.01$) maternal cytochrome P450 1A enzyme activity. There were minimal impacts of maternal diet on steroid concentrations. Maternal dietary protein may alter fetal growth by impacting placental vasculature function and nutrient absorptive capabilities.

1. Introduction

Our laboratory has previously reported maternal nutrient restriction during mid to late gestation decreases umbilical and uterine blood flow near term in sheep (Lemley et al., 2012). Moreover, nutrient restriction decreases the amino acids (**AA**) concentrations in fetal circulation (Kwon et al., 2004; Satterfield et al., 2010; Lekatz et al., 2011; Lemley et al., 2013). The question remains if these alterations to blood flow and AA concentrations are due to an overall global restriction of nutrients or if there is a specific nutrient, or nutrients, driving these changes.

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Evidence from rodent models of maternal protein restriction demonstrates reduced offspring birth weight, elevated blood pressure in the offspring, and decreased vascular responses of the uterine artery (Langley and Jackson, 1994; Langley-Evans et al., 1996; Itoh et al., 2002). While much has been learned about the effects of maternal protein restriction in rodents, these findings cannot directly be applied to a ruminant, and data regarding the effects of protein restriction on uteroplacental function during gestation in ruminants is lacking.

Therefore, for the purpose of this study, isocaloric diets with varied amounts of metabolizable protein (**MP**) were evaluated. Metabolizable protein is defined by the NRC (2007) as the true protein, derived from dietary and microbial protein digested post-ruminally and from which the constituent AA are absorbed from the intestine. Therefore, providing adequate MP during gestation may serve as a more appropriate indicator of how protein intake affects ruminant dams and their offspring.

We hypothesized isocaloric diets provided to dams with lower amounts of MP would decrease fetal growth by reducing uteroplacental blood flow, potentially mediated by sex steroids and/or nitric oxide, and nutrient delivery to the conceptus. Further, we hypothesized MP restriction would reduce AA concentrations in uterine and umbilical vessels. Therefore, the objectives of this study were to evaluate the effects of isocaloric diets with varied levels of MP during late gestation (days 100–130) on conceptus development, uterine and umbilical blood flow, as well as sex steroid and AA concentrations in the uterine and umbilical vessels at day 130 of gestation.

2. Materials and methods

Animal care and use was conducted according to protocols approved by the North Dakota State University (**NDSU**) Animal Care and Use Committee (#A0921).

2.1. Animals and experimental design

On ~day 90 of gestation, 45 pregnant multiparous ewes were transported from the Hettinger Research Extension Center (Hettinger, ND, USA) to the Animal Nutrition and Physiology Center at NDSU (Fargo, ND, USA) where they were housed in individual pens (0.91 m × 1.2 m) in an indoor facility until necropsy (130 ± 2 days of gestation). Within the facility, the temperature was held constant at 12 °C, and lighting was controlled automatically (12:12-hour light-dark cycle with lights on at 0700 and off at 1900).

Ewes were acclimated to low-quality hay (Table 1) and the MP100 supplement (100% of the MP requirements, as determined by NRC, 2007; Table 2; Van Emon et al., 2014) for 10 days prior to starting dietary treatments. Ewes were weighed on two consecutive days (days 99 and 100 of gestation) prior to the initiation of treatments. On day 100 ± 2 of gestation ewes were randomly assigned to one of three isocaloric dietary treatments (Table 2): **MP60**: 60% of MP requirements, **MP80**: 80% of MP requirements, and **MP100**: 100% of the MP requirements on a DM basis during the last 4 weeks of gestation (NRC, 2007; Van Emon et al., 2014). Dietary treatments were fed once daily at 0700 from 100 to 130 days of gestation. Ewes were given 1 h to consume the supplement, which was always completely consumed, then low-quality forage (Table 1) was offered. There were no orts to collect throughout the study. Body weights were determined every 7 days throughout the dietary treatment period, and the amount of supplement and low-quality forage offered was adjusted for changes in body weight. All ewes had ad libitum access to fresh water and trace mineralized salt (4000 ppm Zn, 1600 ppm Fe, 1200 ppm Mn, 325 ppm Cu, 100 ppm I, 40 ppm Co; American Stockman, Overland Park, KS).

Table 1
Nutrient composition of fescue straw used in the experiment.[a]

Item	
Diet (% DM)	
DM (%)	96.24
NEm (Mcal/kg)	2.22
CP (% of DM)	2.76
MP (% of DM)	1.95
NDF (% of DM)	80.17
ADF (% of DM)	48.66
Ash (% of DM)	6.00

[a] Ewes were fed fescue straw to limit MP intake.

Table 2
Ingredient and nutrient composition of dietary supplements fed to ewes from day 100 to day 130 of gestation that provided differing levels of metabolizable protein (MP), but were similar in energy.

Item	Treatment[a]		
	MP60	MP80	MP100
Ingredient, % DM			
Corn	18.50	15.00	5.00
DDGS[b]	7.00	20.00	30.00
Soyhulls	9.50	–	–
Nutrient composition			
DM (%)	95.51	95.89	95.90
NEm (Mcal/kg)	2.00	2.22	2.14
CP (% of DM)	13.45	20.53	25.03
MP (% of DM)	8.41	13.01	16.31
NDF (% of DM)	33.61	32.11	40.79
ADF (% of DM)	15.71	8.33	11.61
Ash (% of DM)	3.17	3.50	4.38

[a] Maternal diets (DM basis) were balanced for mature ewes during the last 4 weeks of gestation according to NRC (2007). Treatments included 60% of MP requirements (MP60), 80% of MP requirements (MP80), and 100% of MP requirements (MP100).
[b] Dried distillers grains plus solubles.

2.2. Gestational day 130 intraoperative blood flow measurements

At 130 ± 2 days of gestation, a subset (n = 12; 67.0 ± 2.5 kg) of the 45 ewes carrying singletons (MP60: n = 4; MP80: n = 4; MP100: n = 3 [of this subset, one MP100 ewe was carrying twins and removed from the analysis]) underwent surgery to obtain intraoperative uterine blood flow via transonic blood flow probes and umbilical blood flow via Doppler ultrasonography as previously described by Lemley et al. (2012). Dams were weighed and anesthetized with 3 mg/kg of body weight sodium pentobarbital. Anesthesia was maintained via a jugular catheter. A catheter was placed into the maternal saphenous artery