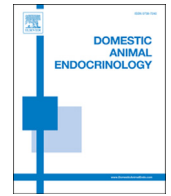




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## Supplementation of metabolizable protein during late gestation and fetal number impact ewe organ mass, maternal serum hormone and metabolite concentrations, and conceptus measurements

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## ABSTRACT

To examine the effects of maternal metabolizable protein (MP) supplementation during late gestation on serum hormone and metabolites and organ masses, multiparous ewes ( $n = 45$ ) carrying singletons or twins were allotted randomly (within pregnancy group) to 1 of 3 treatments: 60% (MP60), 80% (MP80), or 100% (MP100) of MP requirements. Blood samples were drawn before the initiation of diets (day 100) and before slaughter (day 130) for chemistry panel analysis and weekly for hormone analysis including progesterone (P4) and estradiol-17 $\beta$  (E2). At day 130, ewe organ masses were recorded. Despite being fed isocaloric diets, MP60 ewes gained less weight throughout pregnancy compared with MP80 and MP100 ewes which were similar. Although diet did not impact E2 or P4 concentrations, ewes carrying twins had greater ( $P < 0.05$ ) concentrations of both as gestation advanced. Albumin, aspartate aminotransferase, and total protein were reduced ( $P < 0.05$ ) in MP60 compared with MP100 ewes near term. There was a diet by fetal number interaction ( $P = 0.03$ ) for lactate dehydrogenase. Twin-carrying MP80 ewes had greater lactate dehydrogenase compared with all other groups on day 130 of gestation. Ewes that were fed MP80 had greater body weight on day 130 of gestation compared with MP60 ewes. Kidney and heart weights were lighter in MP60 ewes compared with MP80 ewes. There was a maternal diet by fetal number interaction ( $P = 0.05$ ) on fetal weight per unit empty ewe body weight. In ewes carrying singletons, MP60 ewes supported less fetal weight compared with MP100. In contrast, MP60 ewes supported more fetal mass compared with MP100 ewes when carrying twins. The level of protein, and not just total energy, in the diet appears to impact some aspects of the maternal system. Moreover, it appears some measurements of mobilizing maternal body resources are enhanced in ewes carrying twins.

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### 1. Introduction

Adequate protein intake is necessary for maternal maintenance, lactation performance, and rebreeding success [1–3]. In beef cows, Sasser et al [4] reported that diets fed to dams during late gestation and into early

lactation that were equal in energy but deficient in crude protein resulted in decreased prepartum and postpartum weight gains, decreased first service conception rates, and increased interval from parturition to postpartum estrus compared with dams that had adequate protein. In sows that had a decreased protein content in their gestational diets, piglet weights were decreased and growth was reduced compared with sows receiving greater amounts of protein [5]. Inadequate protein supply in lactating sows has been shown to decrease certain maternal visceral organ weights, such as kidney, heart, and liver, although having less effect on carcass measurements such as subcutaneous back fat [6]. However, we have recently reported that decreases in metabolizable protein (MP) intake during late gestation causes a reduction in body condition score, which is primarily determined by subcutaneous back fat [7]. The protein pool of the body is dynamic with protein turnover rates differing greatly between tissues [8]. Tissues with greater metabolic rate (eg, liver, small intestine, pancreas, and so forth) typically have greater protein turnover rates. Skeletal muscle provides the largest protein pool, and although it can be rapidly mobilized when needed, it typically exhibits decreased protein turnover compared with more metabolically active tissues. When animals are in negative energy and/or protein balance, muscle protein turnover is increased to provide energy and/or amino acids for vital functions [9,10]. Whole body and tissue-specific protein turnover rates increased during gestation and lactation as compared with nonpregnant and nonlactating women and ewes [11].

Recently, our laboratory has demonstrated that maternal dietary nutritional plane during gestation impacts the endocrine and metabolic profile of the ewe [12,13]. It was also noted that placental steroids, estradiol-17 $\beta$  (E2), and progesterone (P4) were increased in ewes experiencing a global nutrient restriction during pregnancy. Thyroxine (T4), but not triiodothyronine (T3), concentrations were reduced in nutrient-restricted ewes compared with adequately fed ewes during mid-to-late gestation [13]. It is not known what influence protein specifically has on those hormone concentrations in late pregnant ewes, let alone the impact that multiple fetuses may have on those hormones in a protein-deficient diet.

In a recent study, we concluded that increased maternal MP intake may positively impact the ewe, without altering offspring performance from birth to weaning [7]. Our hypothesis was that isocaloric diets deficient in MP would not hinder conceptus or mammary development as weaning weights were not affected in a sister study [7]. However, we hypothesized that ewes deficient in MP would have increased markers of inflammation and experience more fat, organ mass, and skeletal muscle loss (ie, mobilize their bodily resources) to ensure that their fetuses would have adequate nutrients while in utero. Moreover, hormones associated with maternal metabolism would be decreased with protein-deficient diets. Therefore, the objective of this study was to examine the effects of maternal MP supply on ewe weight and condition, visceral organ weights, serum chemistry panel values, hormone concentrations, and fetal and placental weights.

## 2. Materials and methods

Animal care and use was approved by the Institutional Animal Care and Use Committee (#A0921) at North Dakota State University (NDSU), Fargo.

### 2.1. Animals and diets

On approximately day 90 of gestation, 45 pregnant multiparous Rambouillet ewes were transported from the Hettinger Research Extension Center (Hettinger, ND, USA) to the Animal Nutrition and Physiology Center at North Dakota State University (Fargo, ND, USA). On arrival, ewes were individually housed in 0.91  $\times$  1.2-m pens in a temperature-controlled (12°C) and ventilated facility for the duration of the study. Lighting within the facility was automatically timed to a 12:12-h light-dark cycle with lights on at 7 AM and off at 7 PM.

Ewes were acclimated to low-quality hay (Table 1) and a supplement which met 100% of MP requirements (MP100), as determined by National Research Council (2007), for 10 d before starting dietary treatment [7]. The low-quality hay was used to limit MP intake. Ewes were weighed on 2 consecutive days (day 99 and 100 of gestation) before initiation of treatment.

On day 100  $\pm$  2 of gestation, ewes were randomly assigned to 1 of 3 dietary treatments (Table 2) designed to be isocaloric and provide the following: 60% (MP60), 80% (MP80), or 100% (MP100) of MP requirements on a dry matter basis during the last 4 wk of gestation [7,14]. Nutrient requirements were based on National Research Council [14] recommendations for a 70-kg pregnant mature ewe carrying twins [7]. Thirty-five percent of the total intake was fed as a supplement at 7 AM, ewes were given 1 h to consume the supplement, and then low-quality forage (Table 1) was provided to supply the remaining 65% of total intake. The supplement was always completely consumed. Body weight (BW) was measured every 7 d throughout the treatment period, and the amount of supplement and forage offered was adjusted for changes in BW. Throughout the project ewes had free access to water and a trace mineralized salt block (salt [minimum] 95.5%, salt [maximum] 98.5%, zinc [minimum] 3,500 ppm, iron [minimum] 2,000 ppm, manganese [minimum] 1,800 ppm, copper [minimum] 280 ppm, copper [maximum] 420 ppm,

**Table 1**  
Nutrient composition of fescue straw.<sup>a</sup>

Item	Unit
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

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DM, dry matter; MP, metabolizable protein; NDF, neutral detergent fiber; NEm, Net energy for maintenance.

<sup>a</sup> Ewes were fed fescue straw to limit MP intake.

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