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The effects of diet and arginine treatment on serum metabolites and selected hormones during the estrous cycle in sheep

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ABSTRACT

The aim of this study was to determine the effects of diet and arginine (Arg) treatment on serum concentrations of selected metabolites and metabolic and reproductive hormones in nonpregnant ewes. Sixty days before the onset of estrus (Day 0), Rambouillet ewes were randomly assigned to one of three dietary groups: maintenance control (C; N = 16; 100% National Research Council requirements), overfed (O; N = 16; 2 × C), or underfed (U; N = 16, 0.6 × C) to achieve and maintain three different body conditions during their estrous cycle(s). At Day 0, ewes from each nutritional group were randomly assigned to receive one of two treatments: saline (Sal) or Arg (L-Arg-HCl; 155 μmol Arg per kg of body weight [BW]; intravenous), which was administered three times per day for 21 or 26 days. Blood samples were collected on Days 0, 6, 10, 12, 16, 21, and 26 of Sal or Arg treatment for evaluation of Arg, nitric oxide metabolite, cholesterol, glucose, insulin, insulin-like growth factor 1, leptin, and progesterone. For a time-response trial, blood samples were collected at 0, 1, 2, 4, and 7 hours after Sal or Arg treatment at the mid-luteal phase to determine serum Arg concentrations. During the 11-week study, C maintained body weight, O gained 9.6 ± 0.7 kg, and U lost 13.9 ± 0.1 kg. Overall, serum concentrations of Arg, glucose, insulin, insulin-like growth factor 1, leptin, and progesterone were greater ($P < 0.05$) in O ewes than C and/or U ewes and were not affected by Arg treatment. Serum Arg concentration increased at 1 and 2 hours and decreased to basal level at 4 and 7 hours after Arg treatment. These data reinforce the importance of diet in regulation of metabolic and endocrine functions, and demonstrated that the dose and duration of Arg treatment used in this study does not alter serum metabolites or hormones in nonpregnant ewes of various nutritional planes.

1. Introduction

Extrinsic and intrinsic factors, such as dietary intake and body condition, can have a profound effect on the endocrine system and may affect fertility [1–5]. For ruminants, it has been reported that inadequate nutrition (e.g., restricted or excess diets) results in delayed puberty attainment, aberrant estrous cycles, low conception and pregnancy rates, and/or reduced offspring birth weights [6,7]. Furthermore, plane of nutrition can affect peripheral concentrations of various metabolic and reproductive hormones, such as insulin, insulin-like growth factor 1

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(IGF1), leptin, estradiol 17β (E2), and progesterone (P4) in nonpregnant sheep and cows [8–14]. Subsequently, altering specific hormone concentrations can cause changes in ovarian and reproductive functions including endocrine activities [2,9], embryo development and fertilization [6], oocyte growth and quality [15], and fertility [16].

In ruminants, overfeeding caused an increase in ovulation rates and insulin, IGF1, and leptin concentrations [3,6,7,17], whereas underfeeding decreased insulin and leptin serum concentrations [13,18,19]. Furthermore, circulating P4 concentration in ruminants can be affected by diet [8,20]. For example, Rhind et al. [21] and McEvoy et al. [22] reported that when dietary intake increased systemic P4 concentrations decreased in nonpregnant sheep overfed for 9 to 25 days. However, in heifers that received a high-energy diet, P4 concentrations in plasma were increased [6]. Therefore, plane of nutrition has a direct effect on metabolic and reproductive hormones in nonpregnant ruminants.

Not only different energy levels but also some components of the diet, such as protein or urea concentrations, or the addition of supplements (e.g., lupin grain and barley, and/or citrus-beet pulp concentrate) may affect reproductive function of livestock species [3,11,20,23,24]. Arginine (Arg), an amino acid and a possible supplement, has been recently reported to improve reproductive performance and the uterine environment for the maintenance of pregnancy in sheep [25–28]. For example, Arg treatment during late gestation increased transport of nutrients to the unborn lamb [29], enhanced fetal protein accretion, ultimately increasing lamb birth weight [30], enhanced percentage of lambs born alive [27], and prevented fetal growth restrictions in underfed dams [26]. Because Arg is a precursor for nitric oxide (NO), it has the potential to affect ovarian function by acting through the NO system [28,31]. The NO system is involved in numerous processes including the regulation of angiogenesis and vascular function, steroidogenesis, hypothalamic-pituitary-gonadal axis, oocyte development, ovulation, and luteolysis in several species [31–33]. However, the effects of body condition and nutritional plane in combination with Arg treatment on endocrine and ovarian function in the nonpregnant animals have not been studied in detail.

We hypothesized that Arg treatment will affect serum concentrations of selected metabolites and hormones in nonpregnant ewes fed inadequate diets compared with ewes fed maintenance control diet. Our objective was to determine if *in vivo* Arg treatment impacts serum concentrations of Arg, NO metabolite, cholesterol, glucose, insulin, IGF1, leptin and P4 in nonpregnant ewes with different body conditions as a result of different planes of nutrition (e.g., maintenance control, overfeeding, or underfeeding).

2. Materials and methods

All animal procedures performed were approved by the North Dakota State University Institutional Animal Care and Use Committee (#A12013).

2.1. Animals and experimental design

The experimental design is presented in Figure 1. Nonpregnant, nonlactating Rambouillet ewes (N = 48) aged between 3 and 5 years and of similar genetic background were individually penned at the Animal Nutrition and Physiology Center on the North Dakota State University campus. Ewes were stratified by weight and randomly assigned into one of three dietary groups: maintenance control (C; N = 16; 100% National Research Council [NRC] requirements; 2.4 Mcal of metabolizable energy per kilogram BW), overfed (O; N = 16; 200% NRC requirements), or underfed (U; N = 16; 60% NRC requirements) as described [14]. Diets were initiated 60 days before the onset of estrus (Day 0). Ewes were fed their individual diets twice daily at 8 AM and 3 PM for the duration of the experiment, and every week ewes were weighed. Individual diets were adjusted weekly to ensure the proper body weight (e.g., C, O, and U) was achieved at Day 0 and maintained throughout the estrous cycle(s) and until completion of the experiment on Day 21 or 26 of saline (Sal) or Arg treatment. Estrus was synchronized by insertion of a controlled internal drug release device for 14 days. On the basis of the previous results (Grazul-Bilska et al., 2010, unpublished data), approximately 36 hours after removal of the controlled internal drug release device, ewes were considered in estrus, which was treated as Day 0 of the estrous cycle. At Day 0, ewes were randomly assigned into Sal or Arg treatment groups (N = 24 per group), and blood collection and treatment injections were initiated at 7 AM.

2.2. Jugular vein cannulation

Jugular vein cannulations were conducted 5 to 7 days before Day 0 of the estrous cycle. Wool on the neck of the sheep was removed by shearing, then disinfected with betadine scrub, and wiped with sterile gauze. Approximately 10 minutes before the cannulation process, a local anesthetic (lidocaine HCl, 2%, sterile; Phoenix Scientific Inc., St. Joseph, MO, USA) was injected subcutaneously into two sites (approximately 1 mL per site) along the jugular vein. The sheep was restrained manually and a small incision was made into the neck. Then, a sterile cannulation needle (12 ga; Popper & Sons Inc., New Hyde Park, NY, USA) was inserted

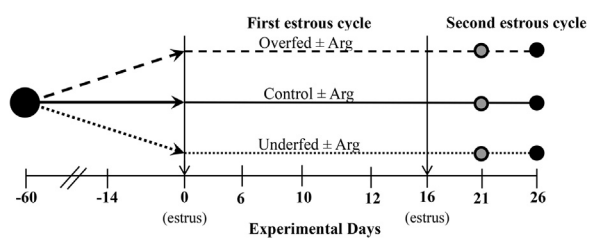

Fig. 1. Experimental design: nutritional groups were initiated 60 days before the onset of estrus (Day 0) to obtain control, overweight, and underweight models. A controlled internal drug release device was inserted vaginally into each ewe for 14 days. At estrus (Day 0), nutritional groups were continued to maintain the three different body weights, and ewes were randomly assigned to either a saline (Sal) or arginine (Arg) treatment that lasted for 21 (gray circle) or 26 days (black circle). Blood was collected on Days 0, 6, 10, 12, 16, 21, and 26 of Sal or Arg treatment.