

Effect of Concentrate Supplementation Level on Postpartum Uterine Involution in Tibetan Sheep: Postprint

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Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effect of concentrate supplementation level during lactation on uterine involution in Tibetan ewes. A total of 160 lactating Tibetan ewes, aged 3 years, in their second parity, with similar body condition were selected. Ewes in groups I, II, and III were housed and fed concentrates at 0.50, 0.75, and 1.00 kg/(d · head), respectively, with 40 ewes per group. Group IV served as the control group with 40 ewes raised under traditional grazing management. The pre-trial period lasted 6 days, and the formal trial period lasted 35 days. Measurements included ewe body weight, uterine weight, sum of three diameters, cotyledon area, cotyledon height, and uterine wall thickness. The results showed that weight gain at 34 days postpartum in groups II and III was extremely significantly higher than that in groups I and IV ($P < 0.01$), and group I was extremely significantly higher than group IV ($P < 0.01$). With increasing concentrate feeding level during lactation, uterine weight, sum of three diameters, cotyledon area, and cotyledon height decreased, while uterine wall thickness increased. Ewes in groups II and III had completed uterine involution by 34 days postpartum. The results suggest that concentrate supplementation at 0.75 kg/(d · head) can promote uterine involution within 34 days postpartum in Tibetan ewes.

Full Text

Effect of Concentrate Supplemental Level on Uterine Recovery of Tibetan Sheep after Parturition

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Abstract

This study investigated the effects of concentrate supplementation level during lactation on uterine recovery in Tibetan ewes. A total of 160 three-year-old Tibetan ewes at their second parity with similar body condition were selected and randomly divided into four groups (40 ewes per group). Groups I, II, and III were housed indoors and fed concentrates at 0.50, 0.75, and 1.00 kg/(d · head), respectively, while Group IV served as the control with traditional grazing management. The pre-trial period lasted 6 days, followed by a 35-day formal trial period. Measurements included ewe body weight, uterine weight, sum of three diameters, cotyledon area, cotyledon height, and uterine wall thickness. The results showed that weight gain in Groups II and III was significantly higher than in Groups I and IV ($P < 0.01$), with Group I significantly higher than Group IV ($P < 0.01$). As concentrate supplementation increased during lactation, uterine weight, sum of three diameters, cotyledon area, and cotyledon height decreased, while uterine wall thickness increased. Groups II and III completed uterine recovery by 34 days postpartum. These findings indicate that concentrate supplementation at 0.75 kg/(d · head) promotes uterine recovery in Tibetan ewes within 34 days after parturition.

Keywords: Tibetan ewes; uterine involution; cotyledon; B-ultrasound

Introduction

Uterine involution refers to the physiological process by which the uterus returns to its non-pregnant state and function after parturition. This process includes the expulsion of uterine lochia and bacteria (i.e., uterine cleansing), reduction of uterine size, and histological restoration of the endometrium [1]. The completion time for uterine involution varies from 3 to 8 weeks postpartum in cattle [2-3] and from 17 to 60 days in sheep [4-5]. These substantial variations primarily result from differences in research methodologies and inconsistent criteria for determining uterine involution completion.

Nutritional level during the postpartum period is critical for maternal body condition recovery, uterine involution, and follicular development. Increasing dietary nutrient content and supplementing energy and protein levels beyond maintenance requirements are fundamental to ensuring and improving reproductive performance in livestock [6]. In the alpine pastoral regions of Qinghai, the withered grass period lasts 8-9 months, coinciding with the critical breeding period of Tibetan ewes (late gestation and lactation). During winter and spring, grazing Tibetan sheep consume only 1,077 g/d of dry matter. After parturition, ewes have urgent nutritional demands to maintain body condition, support lactation, facilitate uterine recovery, and restore reproductive function. The severe deficiency of nutrients during this period directly impacts the reproductive and productive performance of Tibetan ewes [7-12]. Therefore, implementing standardized feeding practices, early lamb weaning, and barn or semi-barn feeding

during critical breeding periods can enhance ewe body condition and effectively prevent reproductive diseases (such as ovarian quiescence and uterine atony) caused by nutritional deficiencies [13]. This study investigated the effects of different nutritional levels during lactation on postpartum uterine recovery in Tibetan ewes during the natural grassland withered season, providing a theoretical basis for shortening the reproductive interval and improving reproductive performance.

Materials and Methods

1.1 Experimental Animals and Grouping

The experiment selected 160 three-year-old lactating Tibetan ewes at their second parity with similar body condition, randomly divided into four groups of 40 ewes each. Groups I, II, and III were experimental groups managed under indoor housing, while Group IV served as the control group with traditional grazing production methods.

1.2 Experimental Design

The experimental diets were formulated based on ewe body weight and nutritional requirements, referencing the *Feeding Standard of Meat-producing Sheep* (NYT 816-2004). Ewes in Groups I, II, and III were grazed during gestation and then housed indoors during lactation (34 days). During days 1-5 of lactation, concentrate supplementation levels were 0.3, 0.4, and 0.5 kg/(d · head) for Groups I, II, and III, respectively. During days 6-34, these levels were increased to 0.50, 0.75, and 1.00 kg/(d · head), respectively. Group IV ewes remained on natural pasture throughout gestation and lactation. The concentrate composition and nutrient levels are presented in Table 1, while diet composition and feeding amounts are shown in Table 2.

The pre-trial period was from January 7 to January 12, 2015, and the formal trial period was from January 13 to February 16, 2015. The experiment was conducted at the Haibei Prefecture Experimental Base in Qinghai Province.

1.3 Feeding Management

Ewes in the three experimental groups were fed at 09:00 and 17:30 daily. Water was provided twice per day, morning and evening. During the day, ewes were active in the barn exercise area, and at night they were managed in warm sheds. Group IV ewes were managed under traditional nomadic production on natural grassland and kept in warm sheds after returning from grazing. Each morning before feeding, the pens, feed troughs, and water troughs were cleaned. All experimental ewes were injected with a sheep quadrivalent vaccine (1 mL/head) 30 days before parturition. At the start of the experiment, all ewes were ear-tagged and numbered, with routine observations and records maintained.

1.4 Measurement Indicators

1.4.1 Body Weight Body weight was measured on the day of parturition and at 34 days postpartum for each group.

1.4.2 Uterine Position and Width Five ewes with similar body weight and condition were randomly selected from each group. Uterine position and width were observed at 10 and 17 days postpartum using a new SS-9 notebook computer platform B-ultrasound machine. All B-ultrasound examinations were performed at 08:00 (when ewes were fasting) in a darkened room to facilitate image acquisition and observation. Ewes were placed in left lateral recumbency, and the uterus was examined via B-ultrasound probe on the right side of the udder and inguinal region. Uterine involution was assessed based on uterine position (abdominal or pelvic cavity), uterine width, amount of intrauterine fluid, and endometrial color.

1.4.3 Uterine Weight, Sum of Three Diameters, Cotyledon Area, Cotyledon Height, and Uterine Wall Thickness At 34 days postpartum, five ewes with similar body weight and condition from each group were slaughtered in the early morning while fasting (excluding ewes that had undergone B-ultrasound examination). After exsanguination via carotid artery, the uterus and its appendages were removed, placed in plastic containers, and immediately transported to the laboratory for measurement. After weighing, the uterus was dissected and photographed. Uterine wall thickness was measured using vernier calipers, and the sum of three diameters (cervical diameter and left and right uterine horn diameters), cotyledon height, and cotyledon area were determined using ImageJ 2.1.4.7 software.

1.5 Data Processing

Experimental data were initially processed using Excel 2003 and then analyzed statistically using SAS 9.1 software. Analysis of variance was performed, and Duncan's multiple comparison test was used for pairwise comparisons. Results are expressed as mean \pm standard deviation (\pm SD).

Results

2.1 Effect of Concentrate Supplementation on Body Weight

As shown in Table 3, body weight at 34 days postpartum and weight gain during this period did not differ significantly between Groups II and III ($P > 0.05$), but both were significantly higher than Groups I and IV ($P < 0.01$). Group I was significantly higher than Group IV ($P < 0.01$).

2.2 Effect of Concentrate Supplementation on Uterine Position and Width

As shown in Table 4 , uterine width at 10 days postpartum did not differ significantly among Groups I, II, and III ($P > 0.05$), but Groups II and III were significantly lower than Group IV ($P < 0.05$). At 17 days postpartum, uterine width did not differ significantly between Groups II and III ($P > 0.05$), but both were significantly lower than Groups I and IV ($P < 0.01$). At 10 days postpartum, the uterus was located in the abdominal cavity in all four groups, with some fluid accumulation present (Figure 1 [Figure 1: see original paper]). By 17 days postpartum, the uterus had returned to the pelvic cavity in all groups, with uterine horns clearly visible (Figure 2 [Figure 2: see original paper]).

2.3 Effect of Concentrate Supplementation on Uterine Weight, Sum of Three Diameters, and Uterine Wall Thickness

As shown in Table 5 , uterine weight in Groups I, II, and III was significantly lower than in Group IV ($P < 0.05$), with Groups II and III significantly lower than Group I ($P < 0.05$). The sum of three diameters was significantly lower in Groups I, II, and III compared to Group IV ($P < 0.01$), with Group III significantly lower than Group I ($P < 0.05$) but not significantly different from Group II ($P > 0.05$). Uterine wall thickness did not differ significantly among the three experimental groups ($P > 0.05$), but all were significantly higher than Group IV ($P < 0.01$).

2.4 Effect of Concentrate Supplementation on Cotyledon Area, Cotyledon Height, and Cotyledon Number

As shown in Table 6 , cotyledon area was negatively correlated with nutritional level, though differences among the four groups were not significant ($P > 0.05$). Cotyledon height did not differ significantly between Groups II and III ($P > 0.05$), but both were lower than Group I and significantly lower than Group IV ($P < 0.05$). Cotyledon number was positively correlated with nutritional level, with Groups I and II significantly higher than Group IV ($P < 0.01$), though no significant difference existed between Groups I and II ($P > 0.05$).

With increasing concentrate supplementation during lactation, uterine weight, sum of three diameters, cotyledon area, and cotyledon height decreased, while cotyledon number, arrangement density, uniformity, and uterine wall thickness increased. Observation of uteri from ewes slaughtered at 34 days postpartum revealed that Group I had clean endometrium without fluid accumulation; Groups II and III had clear, clean endometrium without fluid; Group IV showed slight fluid accumulation that could not be collected with a medical syringe, with dull or brown-colored endometrium. Based on uterine position, width, internal structure, endometrial color, and fluid accumulation, Groups II and III completed uterine involution by 34 days postpartum, Group I had substantially completed involution, and Group IV had not completed involution.

Discussion

The primary factors affecting postpartum reproductive function recovery and shortening the reproductive interval in ewes are uterine involution and ovarian follicular development, both of which are essential. Uterine involution speed is positively correlated with body condition during lactation. As lambs grow and their nutritional demands increase, combined with the decline in the lactation peak around 6 weeks postpartum, ewes experience increased weight loss and deteriorating body condition, which severely impacts uterine involution, follicular development, and estrus synchronization. Hou et al. [14] reported that due to severe nutritional deficiency during the natural grassland withered period in alpine pastoral regions, Tibetan ewes lost 13-17 kg of body weight during lactation (60 days), resulting in poor reproductive performance. Rational supplementation during gestation and lactation can reduce maternal energy reserve depletion and enhance the body condition of lactating ewes. This study demonstrated that body condition recovery in experimental ewes during the 34-day lactation period was positively correlated with nutritional supply level. As dietary nutritional level increased, weight loss during lactation decreased significantly. Groups II and III did not differ significantly but were both significantly higher than Groups I and IV; Group I was significantly higher than Group IV.

Uterine involution refers to the physiological process by which the uterus returns to its non-pregnant state and function after parturition. Research methods for studying uterine involution have primarily focused on histomorphology and laparoscopic observation [15-17]. More reports exist on humans, dairy cows, and mice, with fewer studies on sheep uterine involution. The speed and extent of uterine involution in livestock are closely related to reproductive efficiency [18-27]. Studies using B-ultrasound probes to examine dairy cow uteri and ovaries can rapidly, clearly, intuitively, and accurately reflect physiological morphological changes in the postpartum uterus. Postpartum body condition recovery and uterine involution in sheep are influenced by genetics, nutrition, age, parity, and lactation, with nutritional level directly affecting the duration of body condition recovery and uterine involution [28-29]. Cao [30] reported that uterine walls in Small-tailed Han sheep first thinned and then thickened after parturition, completing uterine involution by 25 days postpartum. The present study showed that Tibetan ewe uteri returned to the pelvic cavity by 17 days postpartum. With increasing nutritional levels during lactation, uterine weight, sum of three diameters, cotyledon area, and cotyledon height decreased by 34 days postpartum, while cotyledon number and uterine wall thickness increased. In Groups II and III, cotyledons were tightly and uniformly arranged in three rows per uterine horn, whereas Group I showed two or three irregular, loosely arranged rows, and Group IV had scattered, sparse cotyledons. Groups II and III had clear, clean uterine interiors; Group I had some uteri with darker, less clear interiors; Group IV had darkened uteri, some with blackened interiors and

small amounts of brown mucus. Based on uterine structure, endometrial color, and fluid accumulation, Groups II and III completed uterine involution by 34 days postpartum, providing a theoretical basis for shortening the reproductive interval and achieving three lambings in two years for Tibetan ewes.

Conclusion

Concentrate supplementation at 0.75 kg/(d · head) promotes uterine involution in Tibetan ewes within 34 days after parturition.

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