ABSTRACT: Improving reproductive efficiency increases the profitability of livestock production. This study utilized a unique population of Rambouillet sheep that have been selected for high and low reproductive rate for over 40 years. Data were collected on reproductive performance, nutrient intake, and genetics. These high and low reproductive rate lines are genetically different as shown by PCA of 54K SNP genotypes. Reproductive performance differed between the lines in both litter size ($P = 0.02$) and kg lamb produced per ewe ($P = 0.02$). Intake was similar in the two lines, but the high line had a higher reproductive efficiency than the low line expressed as kg of TDN consumed per total kg of lamb produced ($P = 0.007$). This makes these populations ideally suited for investigating the genetic basis of reproductive efficiency.

Key words: reproduction; genetics; reproductive efficiency

INTRODUCTION
Reproduction is essential to the continuation and economic sustainability of any livestock enterprise. In 1968, two lines of Rambouillet ewes were formed using a selection index for reproductive rate. This selection index has continued to guide the lines selected for high rate (HL) or low rate (LL) of reproduction to this day. Schoenian and Burfening (1990) found that the HL had higher ovulation rate, lambs born per ewe exposed, and litter size. Burfening et al. (1993) confirmed that selection index values for the lines were diverging. Nutrition as well as genetics is known to affect reproduction in livestock. Nutrition affects reproduction not only by providing energy to develop and sustain the fetus or embryo directly, but also through regulation of hormones that control reproduction and impact development of the neonate (Robinson et al., 2006; Boland et al., 2001). However, no studies have been conducted to investigate the intake and metabolism of nutrients between these two divergent lines to determine if this affects the ability of some ewes to produce more lambs. In addition, nutrition has been shown to interact with progesterone (P4) and pregnancy rates (Parr et al., 1987), and P4 concentrations through gestation have not been measured in these lines. Thus, the objective of this study was to determine if the lines differ in intake or P4 concentrations during gestation, lamb production, or are genetically distinct after approximately 44 yr of selection for high or low reproductive rate.

MATERIALS AND METHODS

Animals
The Montana State University Animal Care and Use Committee approved the use and care of animals for this study. Forty-four yr ago two lines were established for either high (HL) or low (LL) reproductive rate using the selection index, $SI = \text{total number of lambs born in lifetime/ewe's age} - 1$. Details of the selection, breeding, and management of the lines have previously been described (Sochenian and Burfening, 1990; Burfening et al., 1993). Genotype analysis was performed on 50 high line and 46 low line individuals randomly selected to represent line.

This intake trial was conducted with 30 HL and 27 LL ewes, respectively, at the Bozeman Area Research and Teaching (BART) Facility, Bozeman, MT. Nulli-, primi-, and multiparous ewes of both lines ranged from 1 to 4 yr in age.

Genotype Analysis
DNA was extracted from whole blood collected via jugular venipuncture using a Maxwell 16 semi-automated LEV DNA Blood kit from Promega (Madison, WI) utilizing manufacturer protocols. Extracted DNA was sent to GeneSeek (Lincoln, NE) for use with the Ovine 54K Illumina BeadChip. Genotype results were imported into the Golden Helix statistical software (Bozeman, MT). Initial quality control included filtering based on sex inconsistencies, to check for proper sample identification. Further filtering was done to include sample genotyping efficiency > 98% and marker genotyping efficiency > 98%. An incomplete pedigree was included in the analysis and a full pedigree is under construction. Principal component analysis was performed within Golden Helix to evaluate if long-term selection had impacted the genetic structure of the population.

Intake, Nutrition, and Management
Individual ewe intakes were measured using GrowSafe bunks (GrowSafe Systems, Ltd., Airdrie, AB, Canada). During maintenance, 40 ewes were fed a chopped-grass hay diet (7.4% CP, 54% TDN). Intake was recorded for 32 d. Breeding commenced with the introduction of 4 Rambouillet rams. All ewes were single-sire mated in groups of 12 to 15. Rambouillet rams remained with ewes for 18 d. This period ewes were fed grass-hay for 7 d, and then chopped-alfalfa hay (19.4% CP, 54% TDN). Ewes were exposed to 1 of 2 Suffolk rams for an additional 18 d. Intake was recorded for 29 d on the chopped-alfalfa hay diet. Ewes were fed chopped-alfalfa hay through mid-gestation, and intake was recorded for 76 d. During late gestation the diet was changed to an alfalfa, barley, and molasses pellet (18.1% CP, 55% TDN) designed to meet the nutritional requirements of ewes carrying twins, and intake was recorded for 30 d. Individual lamb weights were taken at lambing (digital scale, Premier 1, Washington, IA) prior to first suckling to remove variation in colostrum intake.
**Progesterone Assays**

Blood samples were collected from each ewe by jugular venipuncture 22 d and 8 d before introduction of the rams. Samples were taken again within 24 to 48 hours of mating, and at approximately 30, 60, 90, and 120 d thereafter. Sera were stored at -20°C until assayed for P4. Progesterone concentrations were determined in duplicate by solid-phase RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA), validated for sheep serum in our laboratory. The intra- and inter assay CV were 8.2 and 14.8% for serum that contained 2.4 ng/mL; and 2.0 and 7.4%, respectively, for serum that contained 11.5 ng/mL.

**Statistical Analysis**

All ewes were used for lambing metrics. Gestation of 35 ewes (HL = 20, LL=15) that were bred within an estimated 2-wk period was analyzed for variables of intake and P4 concentrations. Statistical analyses was performed with SAS (SAS Inst. Inc., Cary, NC) with a significance of \( P = 0.05 \). Lambing rate and litter size were analyzed by contingency chi-square analyses. Starting BWs, variables of intake, and lamb weights were analyzed by PROC GLM, with line as class. Temporal patterns of P4 concentration were analyzed by ANOVA using the PROC MIXED model for repeated measures. The model included line by day of gestation, with animal as subject and d of gestation as repeated measure. Means were separated using Bonferroni’s multiple comparison adjustment.

**RESULTS AND DISCUSSION**

Principle component analysis of the 54K SNP genotypes revealed clear separation of the two lines. Figure 1 shows a plot of the first two eigenvectors demonstrating clear genetic separation of the two lines similar to what is seen in the comparison of cattle breeds shown in Lewis et al. (2011). Further analysis is needed to show the genetic signatures of selection in these two unique populations.

Reproduction metrics are presented in Table 1. Pregnancy rate (excluding ewes that died during pregnancy), did not differ \( (P = 0.13) \) between the lines. Schoenian and Burfening (1990) found the number lambing per ewe exposed for all services to have least squares means of 0.85 for both lines. However, litter size was higher \( (P = 0.02) \) for the HL ewes (Table 1). Schoenian and Burfening (1990) reported similar results for litter size of 1.13 and 1.45 for LL and HL ewes at all services, respectively. The LL lambs had greater individual birth weights \( (P = 0.05) \). However, total birth weight of lambs per dam was greater \( (P = 0.02) \) for HL ewes than for LL ewes. This could be explained by the greater proportion of HL ewes that had twins, since twinning is known to decrease individual birth weight and increase total weight of lamb per ewe lambing (Gardner et al., 2007).

There was a line by day of gestation interaction \( (P = 0.04) \) for patterns of P4 concentrations (Figure 2). Progesterone concentrations did not differ between lines from d 30 to d 60. However, P4 in HL ewes increased to a greater concentration from d 60 to d 120 than P4 concentration in LL ewes. Berardinelli et al. (1995) reported that P4 concentrations were higher in HL than LL ewes from breeding through gestation divided by total kg of lamb per ewe lambing.

![Figure 1. Plot of Eigenvectors 1 and 2 of the 54K SNP genotypes of Rambouillet sheep from lines selected for high and low reproductive rates demonstrating the difference in the genetic structure of the two populations.](image-url)

**Table 1. Reproductive and intake measures of Rambouillet ewe lines selected for high (HL) and low (LL) reproductive rate**

<table>
<thead>
<tr>
<th>Item</th>
<th>HL (n)</th>
<th>LL (n)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reproductive measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate,(^1) %</td>
<td>92.6 (27)</td>
<td>77.3 (22)</td>
<td>0.13</td>
</tr>
<tr>
<td>Litter size,(^2) no lambs</td>
<td>1.52 (25)</td>
<td>1.18 (17)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lamb weight,(^3) kg</td>
<td>4.21 (32)</td>
<td>4.62 (17)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total weight lambs ewe,(^4) kg</td>
<td>6.74 (20)</td>
<td>5.22 (15)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total intake,(^5) kg</td>
<td>322.192 (17)</td>
<td>332.417 (15)</td>
<td>0.67</td>
</tr>
<tr>
<td>Total TDN,(^6) kg</td>
<td>174.8 (17)</td>
<td>180.3 (15)</td>
<td>0.67</td>
</tr>
<tr>
<td>Kg TDN/kg lamb per ewe lambing,(^7) kg</td>
<td>26.7 (17)</td>
<td>35.0 (15)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

\(^1\) Percentage of ewes lambing.

\(^2\) Average number of lambs per ewe lambing.

\(^3\) Least squares means of individual lamb weights of lambs born to standardized ewes.

\(^4\) Least squares means of total lamb birth weight per ewe lambing of standardized ewes.

\(^5\) Least squares means of intake of standardized ewes from breeding through gestation.

\(^6\) Least squares means of total TDN intake of standardized ewes from breeding through gestation.

\(^7\) Least squares means of total TDN intake of standardized ewes from breeding through gestation divided by total kg of lamb per ewe lambing.
ewes between d 2 and d 10 of the estrous cycle. They speculated that perhaps clearance rate of P4 in HL ewes was less than the clearance rate in LL ewes. The increase in P4 concentrations of both lines at d 90 is in agreement with Sarda et al. (1973) who reported that P4 concentrations in pregnant ewes have a major increase around d 80 to 90 of gestation.

At the beginning of the intake trial, BW of HL and LL ewe were 52.6 and 49.6 kg, respectively (SEM = 7.3; P = 0.24). Intakes did not differ (P > 0.10) between HL and LL ewes during the breeding season or gestation (Table 1). Total TDN consumed did not differ (P > 0.10) between the lines. However, kg of TDN consumed per total kg of lamb produced per ewe lambing was greater (P = 0.01) for LL ewes than HL ewes.

CONCLUSIONS

The lines of sheep selected for high and low reproductive rate for over 40 years clearly demonstrated divergent reproductive phenotypes including litter size and kg of lamb born per ewe. Temporal patterns of P4 concentrations differed between ewes of these lines, and there was a 25% difference between lines in kg of TDN intake during gestation per kg of lamb born per ewe. This represents a dramatic difference in reproductive efficiency with important implications for increasing profitability of sheep production. Thus these populations provide a powerful tool to study the genetic basis of, and interaction among reproduction, fertility, and efficiency. Perhaps, this work can be extrapolated to other economically relevant livestock species.

Figure 2. Least squares means of progesterone (P4) concentrations during gestation in Rambouillet ewes selected for high (HL; n = 20) and low (LL; n = 15) reproductive rate. Error bars represent ± SE of each mean. Line by d of gestation interaction; P = 0.04. Means with different superscripts differ (P < 0.05).

LITERATURE CITED


