

Genetic Make-up, Milk Production, and Infrared Predicted Milk Constituents in a Commercial Dairy Sheep Flock of Variable Breed Composition¹

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Summary

The effect of genetic contributions from East Friesian and Lacaune dairy sheep on daily milk and total lactation yield, lactation persistency, and milk component yield and concentration were quantified in a commercial US dairy sheep flock with no pedigree records and variable breed composition. A set of randomly selected ewes ($n = 20$) was genotyped for 54,241 SNPs on the Illumina Ovine 50K SNP beadchip. Breed composition was determined using a model containing four reference breeds (East Friesian, Lacaune, Finnsheep, and Dorset), analyzed for breed admixture, and the model returned a range of 37.4 to 67.8% dairy breed percentage (East Friesian and/or Lacaune) among the 20 ewes. Milk weights and milk samples were collected twice per month through the 171-day lactation.

No model with sensical Wood lactation curve parameters could be fitted to data for three ewes, reducing the dataset to 17 ewes for milk production and milk component analysis with an average dairy breed percentage of 54.1%. Regression on dairy breed percentage only affected ($P = 0.023$) the estimate for the c parameter of the lactation curve, indicating a greater lactation persistence for ewes with greater dairy breed percentage. In this exploratory on-farm trial, genetic dairy breed percentage predicted differences in the shape or duration of the lactation curve. However, there was no effect on milk, fat, or protein yield, nor on milk fatty acids, which differed from published findings in US sheep dairy research flocks.

Key Words: Sheep, Milk Yield, Dairy Breed, Milk Components

Introduction

Traditional European dairy sheep breeds available in the US are East Friesian and Lacaune. East Friesian cross rams were first imported via Canada in 1993; Lacaune genetics were first imported as semen from three rams in the UK and two rams via Canada in 1998 (Thomas et al., 2014). Due to the limited availability of purebred dairy sheep genetics, crossbreeding with domestic meat breeds like the Dorset or Finnsheep is a strategy that US dairy sheep producers have employed for many years (Kochendoerfer and Thoney, 2019).

Commercial US dairy sheep flocks have an estimated average lactation yield of 178 kg of milk per ewe (National Research Council, 2008) compared to commercial East Friesian and Lacaune flocks in Europe producing up to 504 kg in 200-day lactations (Thomas and Haenlein, 2017) and 240 kg in 165-day lactations, respectively (Barillet et al., 2001).

More recently, efforts led by the Dairy Sheep Association of North America (DSANA), in collaboration with the Centre d'Expertise en Production Ovine du Quebec and GenOvis, two Canadian organizations concerned with genetic improvement of North American dairy sheep, are aiming to estimate breeding values to increase productivity. The DSANA coordinated the importation of Lacaune semen in 2019 from government controlled and regulated flocks in France. Yet the increase in genetic merit of US dairy sheep flocks may be slowed due to stringent import restrictions of genetic materials, costly artificial insemination services, and low artificial insemination conception rates (Alvarez et al., 2019).

Earlier investigations into the utilization of traditional US meat sheep breeds in dairy production returned poor suitability due to very low milk yields in lactations of up to 130 days (Sakul and Boylan, 1992a, b). Thomas et al. (2000) reported much greater yields for East Friesian-meat breed crossbreds than for meat breed crossbreds. However, some of the meat breeds utilized in these earlier investigations have a significant advantage in a trait other than milk yield over purebred dairy breeds (especially Dorset, Finnsheep, and their crosses) because

they are aseasonally polyestrous and could be used for year-round sheep dairy systems. Dairy sheep globally and in the US are seasonally polyestrous, and European data suggests that these breeds would likely be seasonal in the US, leading to seasonal supply of fresh milk (Pulina et al., 2007). The utilization of breeds with higher out of season conception rates could be an opportunity for the US dairy sheep industry to produce milk year-round. This would lead to a consistent supply of fresh milk for processing without relying on frozen milk. Increasing milk production per ewe in year-round milking systems could decrease the dependency on costly imported genetics.

Greater peak milk yields were found for meat ewes in weigh-suckle-weigh studies (Ramsey et al., 1998; Cardellino and Benson, 2002) and earlier findings show that even low genetic contributions from dairy breeds lead to greater milk production and persistency (Berger, 2004). The objective of this exploratory trial was to illuminate the influence of dairy genetics on the shape of lactation curves, lactation yield, persistency, and milk composition in a small set of commercial crossbred dairy ewes with no pedigree records and variable breed composition.

Materials and Methods

Animals

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee (protocol 2016-0069). Data were collected on a 600 ewe dairy sheep farm between the months of February and August 2017. A random subset of 25 ewes, second parity and older, within a 2-d period of a lambing group of 360 ewes was selected for the experiment. Of the 25 ewes enrolled in the trial, one died within the first week and her data were excluded from further analyses. On the day of blood sampling, four ewes could not be located due to a pen mix-up and were removed from subsequent analyses. A total of 20 ewes were genotyped, and their records constituted the experimental dataset. The 20 ewes were on days 1 and 2 of lactation and were assigned an individual identification number and received leg bands and

painted numbers on their backs for identification throughout the trial.

This commercial sheep dairy operates with a 60-stanchion, low-line, Greenoak Dairy Equipment, pit parlor. Milking equipment was operated at 40.6 kPa vacuum pressure, a pulsation rate of 160 ppm, and a pulsation ratio of 50:50. Ewes were pre-dipped and stripped, wiped, milked, and dipped. The 6-hour milking shifts began at 500 and 1700. Parlor times for the trial ewes were 1000 and 2200. The ewes were housed in groups of 180, and all trial ewes were housed in the same group throughout the experiment.

Feed

The ewes were fed a total mixed ration consisting of corn and grass-silage, soyhull pellet, soybean meal, and Cornell Sheep Mineral-Vitamin Premix (50% salt, 45.9% corn gluten feed as carrier, 0.5% feed grade oil, 2,500 ppm Manganese, 4,250 IU/lb Vitamin E, 30 ppm Selenium, 2,000 ppm Zinc, 160 ppm Iodine, 120,000 IU/lb Vitamin A, 15,000 IU/lb Vitamin D, 20 ppm Cobalt, and 70 ppm Molybdenum) once daily. The feed was pushed up twice per day after feeding. Feed was sampled on the same days that milk yields were recorded, and milk samples were collected. The feed samples were analyzed with near-infrared spectrometry for total mixed rations by the Dairy One Forage Laboratory, Ithaca, NY, and contained 48.0% dry matter, 17.2% crude protein, 39.3% amylase and ash corrected neutral detergent fiber, 30.7% non-fiber carbohydrate, and 3.4% ether extract.

Breed Composition

Based on the owner's information, the suspected breed composition was predominantly East Friesian, Lacaune, Finnsheep, and Polled Dorset. Whole blood was drawn via jugular venipuncture from each ewe into a vacutainer containing K₂EDTA anti-coagulant. DNA was extracted from whole blood following the Qiagen Puregene Protocol (Gentra Systems, Inc. Minneapolis, MN, USA). The ewes were genotyped for 54,241 Single Nucleotide Polymorphisms (SNPs) on the Illumina Ovine 50K SNP beadchip (Kijas et al., 2014).

Additional genotypes (49,034 SNPs) from East Friesian, Lacaune, and

Finnsheep were obtained from the International Sheep Genome Consortium HapMap project (Kijas et al., 2014) and Polled Dorsets (606,006 SNPs) from a previous study (Posbergh et al., 2019). Twenty random individuals from each of the breeds were selected as reference individuals for subsequent ADMIXTURE analysis (Alvarez et al., 2004). Genotypes were merged and quality control filtered using Golden Helix SNP & Variation Suite software (v8.7.2 win64; Golden Helix, Bozeman, MT, USA www.goldenhelix.com). SNPs were excluded from the analysis if the SNP call rate was less than 0.90, had more than two alleles, had a minor allele frequency less than 0.05, or was located on the sex chromosomes. After filtering, 40,307 autosomal SNPs were left for subsequent analysis. The ADMIXTURE software version 1.3.0 (Alexander et al., 2009) was utilized to examine admixture between the twenty ewes and eighty reference animals using the filtered genotypes. Cross-validation error was used to determine the most probable number of K populations within the dataset (Alexander et al., 2009). The genetic composition of the ewes was expressed in dairy breed (East Friesian and Lacaune) percentage.

Milk Yield And Analyses

Milk yields were collected at 13 timepoints throughout lactation, once weekly for the first 2 wk of lactation and then every 2 wk until the end of the trial at days in milk (DIM) 171. Milk yields were collected in the morning and multiplied by 2 for an estimate of daily milk yield. The milking parlor was not equipped with milk meters. The trial ewes were machine milked into tared buckets that were connected to the parlor low-line, and the milk was weighed and recorded for each ewe.

Milk samples for component analyses were collected on the same days of daily milk yield was estimated. Samples were collected into 57 ml vials, cooled to 4°C, and analyzed fresh with a Fourier transform mid-infrared spectrophotometer (Lactoscope FTA, Delta Instruments, Drachten, the Netherlands). Fat content was validated with Mojonnier ether extract reference chemistry according to AOAC method No. 989.05 (AOAC International, 2019), true protein by

Kjeldahl analysis according to AOAC method No. 991.22 (AOAC International, 2019), and milk urea nitrogen (MUN) reference chemistry (Megazyme, catalogue No. K-URAMR). Milk fatty acids were validated by gas chromatography as described by Wojciechowski and Barbano (2016). Values predicted from infrared (IR) by cow milk calibrations (Wojciechowski and Barbano, 2016) were subsequently adjusted by the mean difference between IR predicted values and reference chemistry values of -0.065% for total fat; 0.343, -0.069, and -0.230 g/100 g milk for de novo, mixed origin, and preformed fatty acids, respectively; 0.269% for true protein, and -4.522 mg/100 g milk for MUN. Somatic cell counts (SCC) were measured with a fluorometric flow cytometer (Delta Instruments).

Statistical Analyses

All response variables were assumed normally distributed, except for SCC, which were converted to natural log values for analysis and then back transformed for presentation. A total of 245 daily milk yield records (5 to 13 records per ewe) and 238 daily milk composition records (4 to 13 records per ewe) were available for analyses. Wood's equation (Wood, 1967) [Eq. 1] was fitted with the *nls* package (Pinheiro et al., 2018), implemented in the R software (R Development Core Team, 2019), to daily milk yields for each ewe to estimate individual lactation curves. The equation parameters x , a , b , and c describe the DIM of each daily milk yield record, milk yield at parturition (i.e., $x = 0$), ascent of milk yield to peak yield, and the rate of decline of the lactation curve, respectively (Portolano et al., 1997). Total lactation milk yield was estimated by integration [Eq.2]. Peak daily milk yield [Eq.3] and DIM at peak daily milk yield [Eq.4] were also calculated.

$$\text{Eq. 1} \quad Y = ax^b \exp(-cx)$$

$$\text{Eq. 2} \quad Y = \frac{a}{c^{b+1}} \Gamma(b + 1)$$

$$\text{Eq. 3} \quad Y_{(\max)} = a \left(\frac{b}{c}\right)^b \exp(-b)$$

$$\text{Eq. 4} \quad x = \frac{b}{c}$$

Step-down polynomial regression with deletion of higher order terms at

$P > 0.05$ was used to select either linear, quadratic, or cubic equations to be fitted to daily milk component yields and concentrations for each individual ewe. Cubic equations were selected for true protein, fat, lactose, and preformed fatty acid concentrations, quadratic equations were selected for de novo fatty acid concentration, MUN, and SCC, and linear equations were selected for true protein, fat, lactose, de novo and performed fatty acid yield, as well as for mixed fatty acid concentrations and yields. The fitted curves were integrated and mean daily component yield and percentage were established through division by lactation length. Then, the effect of dairy breed percentage on the estimates for Wood's equation parameters, daily and total lactation milk yield, peak day and peak yield, lactation length, as well as daily milk component yields and concentrations, were analyzed with a linear model using *lm* in R (R Development Core Team, 2019). Survival analysis of lactation length in relation to dairy breed percentage was performed on the actual record of each individual ewe, using a Cox Proportional Hazard model with the survival package in R (Therneau, 2021). The statistical significance of dairy breed percentage was tested using a log-rank test (Therneau and Grambsch, 2000).

Results and Discussion

Breed Composition

Dairy breed percentage was determined by admixture analysis. The analysis was tested using K values from two through eight. Cross-validation error was lowest for $K = 5$ (0.57467). The cross-validation error difference was only 0.00128 between $K = 5$ (0.57467) and $K = 4$ (0.57595). Because our interest was in the breed composition of the crossbred group, we chose to use the model with $K = 4$ populations. Every purebred population (East Friesian, Lacaune, Finnsheep, and Polled Dorset) showed little within breed admixture, indicating purebred reference populations (Figure 1).

Based on the $K = 4$ admixture analysis, the crossbred ewes averaged 55.3% dairy breed composition, the sum of an average of 21.1% East Friesian and

Figure 1. ADMIXTURE analysis plot showing population assignments for $K = 4$. Each bar represents an individual animal for each breed, and each color represents a different K population generally reflecting the purebred reference breeds of East Friesian (yellow), Finnsheep (red), Lacaune (green), and Polled Dorset (blue).



34.2% Lacaune (Figure 1). The median dairy composition was 57.2%. The lowest dairy percentage ewe was 37.4% while the greatest had 67.8%. The lowest Lacaune percentage ewe was 20.6% while the lowest East Friesian ewe was only 12.9%. The greatest Lacaune percentage ewe was 46.5% while it was only 29.2% for the greatest East Friesian ewe.

The dairy compositions of these ewes were likely consistent with many commercial dairy ewes in the US, given the East Friesian and Lacaune breed importation and development history (Thomas et al., 2014). Utilizing SNPs likely yielded more accurate predictions of breed composition than would be expected from pedigree, even if known, as pedigree estimates assume progeny breed composition is equal to parental average breed composition (Sölkner et al., 2010).

Milk yield and composition

Not all ewes completed the 171 days of lactation. Dairy breed percentage did not significantly affect lactation length in the log-rank test for time to event analysis ($P = 0.35$). No model with senescent lactation curve parameters (infinite milk yield increase predicted) could be fitted to data for three ewes. Two of these ewes had lactations length less than 45

days, and 2 ewes expressed average SCC above 900,000 cells/mL. These ewes were excluded as outliers from the subsequent statistical analysis, reducing the dataset to 17 ewes for milk production and milk component analysis and an average dairy breed percentage of 54.1%. Five of the ewes did not express a peak after DIM 1. For these ewes, peak days and yields were assumed to be at DIM 1. Due to the small sample size ($n=17$) the following results should be interpreted with caution and regarded only as exploratory findings in a commercial sheep dairy flock.

The linear effect of percentage dairy breeding on the c parameter of Wood's equation was significant and negative ($P = 0.023$, Table 1), indicating an inverse relationship between dairy breed composition and lactation persistency. No effect was detected for the a and b parameters of the lactation curve. Peak milk yield was not affected by breed composition and is comparable to yields observed in winter lambing Comisana ewes (1.77 kg/day; Portolano et al., 1997) and Araucana Creole ewes (1.40 \pm 0.3 L/day; Inostroza et al. 2020). Days in milk at peak yield was not affected by breed composition and occurred later than previously observed in dairy ewes (Cannas et al., 2002). Lactation length

was similar to those reported by Thomas et al. (2000) where East-Friesian \times Dorset crossbred ewes achieved lactation lengths of 126 days and no statistical effect of breed composition was detected.

Sakul and Boylan (1992b) reported much lower daily milk yields with purebred Finnsheep (526 \pm 70 ml) and Dorset ewes (584 \pm 51 ml) with up to 122 DIM, allowing our assumption that even low dairy breed percentage could lead to a meaningful increase in persistency in crossbred dairy ewes. Lactation yields were lower than reported for European dairy sheep flocks (Barillet et al., 2001; Hamann et al., 2004; González-García et al., 2015), even though reported lactation length was comparable. Milk yields were also lower than observed for US East Friesian and Lacaune crossbred dairy ewes (Murphy et al., 2017a) but higher than reported averages for US commercial sheep dairy flocks (National Research Council, 2008). Neither was affected by dairy breed percentage, which also had no effect on milk composition. Milk protein and fat concentrations were similar to values previously reported (Nudda et al., 2002; Padilla et al., 2018). The relationship between relative percentages of de novo synthesized, mixed origin, and preformed fatty acids was quadratic, with lowest contributions from mixed origin fatty acids (Table 1), similar to previous findings (Hampel et al., 2004; Kondyli et al., 2012; Mayer and Fiechter, 2012). The relative percentages of de novo synthesized, mixed origin, and preformed fatty acids were not affected by dairy breed percentage suggesting that the relative contribution of fatty acids is influenced more by nutrition than breed composition, similar to results reported by Tsiplakou et al. (2006), who came to a similar conclusion for the contribution to sheep milk fatty acid composition. The SCC were well below the highest allowable level for interstate shipment for sheep milk in the US (FDA, 2017).

This exploratory trial is limited by the lack of environmental and pedigree data for this small subset of commercial dairy ewes. Still, results may point towards opportunities of including meat sheep genetics in commercial flocks to achieve year-round lactation and producers should be advised to collect and record pedigree data as well as genotypic

Table 1. Fit of linear regression of lactation parameters and milk components on dairy breed percentage (East Friesian and Lacaune).

Item	Predicted mean ¹	Residual standard error	r ²	P-value of slope
Estimated lactation parameters from Wood's equation²				
a	1.33	1.406	0.007	0.749
b	0.184	0.253	0.000	0.972
c (slope = -0.000576 ± 0.000228)	0.0099	0.0087	0.299	0.023
Peak yield, kg	1.81	1.395	0.022	0.566
Peak day	31	43.5	0.130	0.156
DIM at dry off	122	57.0	0.018	0.606
Lactation yield, kg/lactation	180.0	159.80	0.000	0.989
Daily milk yield, kg/d	1.32	0.822	0.003	0.847
Daily milk components				
True protein, %	5.10	0.488	0.006	0.770
True protein, g/day	68.2	41.99	0.004	0.818
Fat, %	6.33	1.878	0.043	0.424
Fat yield, g/d	83.7	56.49	0.001	0.909
De novo fatty acids³				
g/100 g milk	2.39	0.352	0.143	0.134
g/d	32.3	20.8	0.001	0.929
g/100 g fatty acid	40.0	9.212	0.002	0.864
Mixed origin fatty acids⁴				
g/100 g milk	1.68	0.373	0.001	0.906
g/d	22.7	15.63	0.006	0.763
g/100 g fatty acid	28.7	3.532	< 0.001	0.995
Preformed fatty acids⁵				
g/100 g milk	1.92	1.256	0.004	0.816
g/d	25.3	18.03	0.001	0.924
g/100 g fatty acid	30.1	11.28	0.002	0.859
Anhydrous lactose, %	4.37	0.606	0.186	0.084
Anhydrous lactose, g/day	62.5	37.64	0.004	0.814
MUN, mg/100 g	13.8	4.71	0.002	0.873
SCC 10 ³ (geometric means)	151.4	1.231	0.075	0.289

¹ At mean dairy breed percentage = 54.1%

² $Y = ax^b \exp(-cx)$

³ C4 to C14

⁴ C16, C16:1, C17

⁵ ≥ C18

data to allow for future genetics research into the effect of breed composition on sheep milk production.

Conclusions

No effect of dairy breed percentage on milk yield or composition was detected in this sample of ewes from a US flock of commercial dairy sheep.

However, the sample of ewes in this study (n =17) was very small and sampled in a single year with no knowledge of age or pedigree structure. The results differ with a much larger US research flock data set (Murphy et al., 2017a, b) that showed significant positive effects of both East Friesian and Lacaune breeding on milk, fat, and protein yields in dairy-meat breed crosses. However, there

may be an opportunity for year-round, high producing dairy sheep systems that utilize optimum combinations of dairy and meat breeds when using meat breeds that have the ability to breed out of season. Including aseasonally polyestrous meat sheep breeds in dairy sheep flocks provides the opportunity to market fresh sheep milk products year-round.

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