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Animal factors affecting the cheese-making properties and the heat coagulation time of milk from dairy sheep in a New Zealand flock

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ABSTRACT

The objective of this study was to evaluate the effect of animal factors on the cheese-making properties and on the heat coagulation time of milk from individual dairy sheep in a New Zealand flock. A total of 521 individual records were obtained from a seasonal pasture-based flock of 169 ewes milked once-a-day, from 50 to 182 days in milk. A statistical model was used to quantify the effects of animal factors (coat colour variety, age, litter size and stage of lactation) on the studied traits. Stage of lactation, confounded with seasonality, strongly influenced all properties of milk investigated. With the advancement of lactation, the milk took longer to coagulate after rennet addition, and the curd was softer. Higher relative cheese yield was achieved towards the end of lactation. The milk was also less stable to high-temperature treatment in late lactation. Coefficient of variation for processability traits was high and ranged from 20.2% to 58%, which can be largely attributed to stage of lactation but could also indicate room for genetic improvement of traits. Further genetic studies are underway to define animal genetic variance, heritability, and the phenotypic and genetic correlations between these processability and milk composition traits.

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Milk coagulation; cheese yield; heat coagulation time; milk composition; dairy sheep; stage of lactation; seasonal

Introduction

The New Zealand sheep dairy industry, initiated in the 1990s, is still in its early stages and can gain insights from global counterparts, and from the robust New Zealand cow dairy industry, which stands as one of the world's strongest (Lees and Lees 2018). Despite few studies having investigated milk production of dairy sheep (Scholtens 2016; Marshall

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et al. 2023), there is a notable gap in research regarding the milk coagulation patterns and cheese-making efficiency of individual dairy sheep within New Zealand's pasture-based farming systems.

Several dairy sheep farms in New Zealand produce cheeses that are sold in the domestic market. Sheep milk is higher in total solids than cow milk and generates more cheese per litre (Park et al. 2007). The quantity and the quality of cheese produced will depend on several factors, which include milk composition (percentages of fat, protein, casein, mineral content) and pH. These are known to vary with factors related to animal physiology (stage of lactation, age, litter size, health), animal genetics (breed, genetic variety and genotype) and to the farming system (feed management, milking practices) (Bencini and Pulina 1997).

In cheese-making, chymosin hydrolyses the κ -casein proteins at the surface of the casein micelle, thereby removing the electrostatic stabilisation that keeps micelles as a colloidal suspension in milk. This leads to aggregation into flocs, precipitation and formation of cheese curd. Cutting of the curd leads to the expulsion of liquid whey and firming of the protein gel. It is well known that the coagulation speed and strength of the gel formed are higher if the temperature is increased, pH is reduced, or Ca^{2+} concentration is increased (Lucey 2011). Milk coagulation properties (MCP) reflect the milk's suitability for cheese-making and are measured using a Formagraph (Foss Analytics). Additionally, micro-manufactured cheese yield from milk samples of individual animals can be measured to estimate the percentage of milk that is converted into a fresh soft curd (Othmane et al. 2002).

Although sheep milk is excellent for cheese-making, it is known to have lower stability under heat treatments when compared with cow milk. This is due to its high concentration of solids, and other factors such as casein micelle properties (size, mineralisation and composition), and denaturation of whey proteins (Raynal-Ljutovac et al. 2007). Stability of milk to ultra-high temperature treatment (135–140°C, for 2–3 s) is relevant for processing into other dairy products such as infant formula and beverages. Heat stability refers to the ability of milk to withstand a heat treatment without coagulation, i.e. the formation of proteinaceous flocs that become visible particles in the milk (Huppertz 2016). Heating milk above 100°C causes several simultaneous reactions including dissociation of κ -casein from micelles, acidification, dephosphorylation of proteins and proteolysis, which collectively drive protein coagulation (Fox et al. 1998). It is known that larger proportions of κ -casein, higher calcium, lower citrate and phosphate content, and lower proportions of urea reduce heat stability (Timlin et al. 2021).

Fluctuations in milk composition due to ewe physiology or due to seasonal changes can affect processability, especially in the context of grass-based seasonal milk production, where dietary patterns are influenced by weather conditions. Understanding variations in coagulation behaviour among individual samples is valuable for identifying animals that produce milk with superior cheese-making ability. Therefore, the objective of this study was to evaluate the effects of animal factors (coat colour variety, age or parity, litter size and stage of lactation) on milk rennet coagulation properties, relative cheese-yield, and heat coagulation time of milk from individual dairy sheep in a New Zealand flock.

Materials and methods

Data were collected from 169 ewes from a commercial flock in Masterton, Wairarapa, New Zealand. Ethics approval for this study was obtained (MUAEC Protocol 21/45). The farm operates on a pasture-based system with low supplementation. Pasture is composed of white clover and lucerne. During the production season, the metabolisable energy in the pasture decreased from 12.0 to 8.2 MJ/kg DM, crude protein declined from 26.7% to 14.1% DM throughout the milk production season, and NDF increased from 36.5% to 60.6% DM. The breed is mainly composed of East-Friesian genetics, and two varieties are observable: black or white ewes (Marshall et al. 2023). A minimum of 2 milk tests were obtained from each ewe from 50 to 182 days in milk. Test-day milk yield of individual animals was manually recorded, and a representative milk sample was taken. The milk samples were immediately refrigerated for transportation to Palmerston North. Sodium azide (at final concentration of 0.025%) was added to milk samples on arrival in Palmerston North and kept refrigerated. All analyses were performed within 3 days after milk collection.

Milk composition

An aliquot was analysed by Milk Test NZ Ltd. (Hamilton, NZ) using a Combi FOSS instrument (Foss Analytics). The composition analysis included fat (%), protein (%), lactose (%) and somatic cell count (SCC, cells/mL). The analyses for casein (%), urea (mg/100 mL) and citric acid (mg/100 mL) were performed using a Fourier-transform Infrared (FTIR) milk-analyser MilkoScan FT6000 (Foss Analytics). Milk samples were also submitted to a contract laboratory (Massey Nutrition Lab) for analysis of total calcium content (mg/100 mL). Ratio of casein to calcium was calculated as casein (%) divided by total calcium content (mg/100 mL), multiplied by 100. Ratio of casein to protein was calculated as casein (%) divided by protein (%), multiplied by 100, likewise for ratio of casein to fat. Somatic cell score (SCS) was calculated as $SCS = \text{Log}_2(\text{SCC})$.

Milk coagulation

Measures of traditional milk coagulation properties were obtained using a Formagraph (Foss Analytics). For each individual milk sample, 10 mL was heated to 31°C. Rennet solution was added to reach final concentration of 0.0513 IMCU/mL of milk (Cipolat-Gotet et al. 2016). After rennet addition, analysis was continued for 30 min. The curd firmness (mm) of each sample was measured every 7.5 s. The traditional milk coagulation parameters (MCPs) obtained from the Formagraph included rennet coagulation time (RCT, min), time to reach curd firmness of 20 mm (K20, min) and curd firmness at 30 min (A30, mm) (McMahon and Brown 1982).

Individual milk coagulation curves were fitted with the 2nd order plus dead time (SOPDT) model (Seborg et al. 2016) expressed as follows:

$$CF_t = CF_p \left(1 - \frac{\tau_1}{\tau_1 - \tau_2} e^{\left(\frac{-(t-RCT_{eq})}{\tau_1} \right)} + \frac{\tau_2}{\tau_1 - \tau_2} e^{\left(\frac{-(t-RCT_{eq})}{\tau_2} \right)} \right)$$

where CF_t is the curd firmness at time t (mm); CF_p is the asymptotical potential value of

curd firmness at an infinite time (mm); τ_1 and τ_2 are the time constants; RCTeq is the rennet coagulation time (dead time) equivalent to the RCT in traditional Formagraph (Sanjayaranj et al. 2023). The parameters of this equation were estimated using the Generalized Reduced Gradient algorithm implemented in the Solver module of Microsoft Excel for Microsoft 365 MSO (Version 2311 Build 16.0.17029.20140) setting up as the objective function to minimise the sum squares of errors (differences between predicted and actual values of the curd firmness curve).

Milk pH

Milk pH was measured at 31°C, using a pH meter (EcoScan Model pH5) on the same day as milk coagulation properties were obtained.

Individual laboratory cheese yield

A smaller set of a total of 376 samples, due to feasibility reasons, was processed for measurement of individual laboratory cheese yield (ILCY), as per Othmane et al. (2002). Raw milk was warmed to ambient temperature and 10 g weighed into test tubes (15-mm internal diameter) and then equilibrated at 31°C for 10 min in a water bath. Rennet solution (40 μ L) was added to the milk samples in the tubes, reaching a final dose of 0.060 IMCU/g. The tubes were closed and quickly inverted, to ensure uniform distribution of the rennet, and kept at 31°C for 1 h in a water bath. The formed coagulum was cut (inside the tube) and centrifuged at 4000 rpm for 15 min, to separate curd from whey. The whey was removed by draining with the test tube facing downwards. ILCY was expressed in % (w/w) of the relative weight of the centrifuge residue on the initial weighed milk.

Heat coagulation time

The heat coagulation time (HCT) of whole raw milk was defined as the time at which the sample coagulated after heating to 140°C in an oil bath. An aliquot of 4 mL of milk was pipetted into a 10 mL screw-capped glass tube. The tube was then inserted into a rocking apparatus and submerged into the oil bath, as described by Cole and Tarassuk (1946).

Statistical analysis

Descriptive statistics (mean, standard deviation, minimum and maximum values, and coefficient of variation) for MCPs and composition traits were obtained in SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). Analyses of variances were performed using the MIXED procedure with a linear model that included the fixed effects of ewe coat colour as an indicator of variety within the breed (categorical variable with two levels: black or white), litter size (categorical variable with two levels: 1 lamb or 2 lambs and greater), ewe age (categorical variable with four levels: 1, 2, 3 and 4 years and older) and stage of lactation, which is a categorical variable with three levels: 1, 2 and 3, representing the different ranges of days in milk (date of test – date of lambing) from ≥ 50 and < 95 , ≥ 95 and < 140 , ≥ 140 and ≤ 182 , respectively, and deviation from median lambing

date as covariate. Random effects included effect of ewe and random residual error:

$$y_{ijklmn} = \mu + \text{coat colour}_i + \text{litter size}_j + \text{age}_k + \text{sol}_l + \text{dml}_m + \text{ewe}_n + e_{ijklmn}$$

where y_{ijklmn} represents the dependent variable which include MCPs, ILCY, HCT, pH, composition traits (percentages of fat, protein, lactose and casein), total calcium, citric acid, urea and SCS. The least squares means of traits were plotted across the different stages of lactation (mid- to late-lactation). The same linear model was used to obtain the least squares means and standard error of parameters obtained from the CFt equation and used to obtain the curd firmness curves.

Results

Descriptive statistics and analysis of variance

The means, standard deviations, and the F-values and associated probabilities for each dependent variable from the analyses of variance are shown in Table 1. Significant ewe effect ($p < 0.01$) was observed for all dependent traits. Effect of stage of lactation was

Table 1. Means, standard deviation (SD), coefficient of variation (CV) and F-value for effects of animal factors on milk yield and composition, SCS, pH, milk coagulation properties, cheese yield and heat coagulation time for dairy sheep milked once-a-day in mid- and late-lactation during the 2021–2022 production season.

Trait ^a	N	Mean	SD	CV (%)	F-value				dml ^c
					Coat colour	Age	Litter size	Stage of lactation ^b	
Milk yield (L/day)	521	0.57	0.25	43.9	0.83	8.5***	0.63	157.8***	5.1*
Fat (%)	521	6.30	1.27	20.3	1.0	0.4	3.9*	146.3***	5.3*
Protein (%)	521	5.52	0.68	12.3	1.1	4.9**	6.1*	269.4***	4.1*
Lactose (%)	521	4.72	0.27	5.7	0.0	0.6	0.1	326.8***	2.7
Total solids (%)	521	17.18	1.69	9.8	0.1	2.9*	0.0	190.0***	6.4**
Solids non-fat (%)	512	11.04	0.60	5.4	0.6	6.5***	6.0*	111.5***	1.6
Casein (%)	512	4.20	0.49	11.7	0.1	4.2**	5.3*	239.6***	1.0
Casein:Protein	521	76.5	2.1	2.7	27.8***	1.0	0.4	8.0***	6.0**
Casein:Fat	512	69.1	14.2	20.5	4.1*	1.2	15.8***	24.4***	0.1
Calcium (mg/100 mL)	521	185.7	25.3	13.6	1.0	4.5**	0.0	52.1***	1.7
Casein:Calcium	512	2.29	0.31	13.5	0.3	1.1	2.9	38.3***	4.0*
Citric acid(mg/100 mL)	512	83.9	29.3	34	3.5	1.6	0.9	48.8***	0.5
Urea (mg/100 mL)	512	39.82	7.58	19.0	2.9	0.1	0.0	53.6***	4 ^{3*}
SCS (log ₂ SCC)	521	16.23	2.05	12.6	0.6	1.8	0.8	32.4***	10.6**
pH	521	6.59	0.09	1.4	4.2*	5.9***	0.4	155.3***	2.06
RCT (min)	494	13.51	3.41	25.2	10.6**	0.9	6.3**	20.4***	0.5
K20 (min)	487	2.75	1.27	46.2	0.24	2.1	4.5*	7.2***	4.7*
A30 (mm)	494	52.64	10.64	20.2	2.0	1.3	4.4*	11.9***	1.32
ILCY (%)	372	44.7	9.4	21.0	5.2*	7.9***	0.3	10.5***	0.6
HCT (min)	344	1.43	0.83	58.0	6.3**	1.2	1.3	19.4***	6.6**

^aCasein:Protein = ratio of casein to protein. Casein:Fat = ratio of casein to fat. Casein:Calcium = ratio of casein to total calcium. SCS = somatic cell score; RCT = rennet coagulation time after rennet addition; K20 = time to reach curd firmness of 20 mm after coagulation; A30 = curd firmness at 30 min after rennet addition; ILCY = individual laboratory cheese yield; HCT = heat coagulation time. ^bStage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing). ^cdml: deviation from median lambing date of the flock (ewe lambing date – median lambing date of the flock). Statistical significance is given as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

significant on all dependent traits ($p < 0.001$). Effects of age of ewe at lambing, coat colour, litter size and dmld were significant for some of the dependent traits.

The flock produced an average of 0.57 L of milk/day per ewe and 17.18% of total solids in milk. Coefficient of variation for gross milk composition traits ranged from 5.4% to 20.3%. Average SCS calculated as $\text{Log}_2(\text{SCC})$ was 16, and coefficient of variation was 12.6%. When calculated as $3 + \text{Log}_2(\text{SCC}/100)$ (Wiggans and Shook 1987) this average was 12.6. The actual average SCC was 390,000 cells/mL. In this flock, only 8% of the milk samples had SCC above 500,000 cells/mL.

Average RCT, K20 and A30 were 13.51, 2.75 min and 52.64 mm, respectively. Only 5% of the samples did not coagulate within 30 min of rennet addition, and only 6.8% of the samples did not reach curd firmness of 20 mm. Non-coagulating samples and samples that did not reach 20 mm occurred, on average, at 132 days in milk, and had an average SCS of 18.5. Average cheese yield of the flock was 44.7%. Coefficient of variation for traits related to cheese-making ranged from 20.2% to 46.2%.

The individual milk samples from this flock took, on average, 1.43 min (1 min and 26 s) to coagulate in an oil bath set at 140°C, at natural milk pH. There was large variation in this flock for this trait (58%).

Tables 2–5 present least squares means and standard errors of all dependent traits for the different animal factors considered in this study. Figure 1 depicts the least squares means of cheese-making properties, heat coagulation time, ILCY, gross milk composition, calcium content, milk urea and citric acid at three stages of lactation. The patterns of milk curd firmness over time after rennet addition are presented in Figure 2, for different lactation stages, ewe coat-colours, ewe ages and litter sizes.

Discussion

Flock performance for composition traits

The level of milk production per ewe in the season was previously discussed (Marshall et al. 2023). Average gross milk composition was within the expected range for dairy sheep (Scholtens 2016; McCoard et al. 2023). Amongst the traditional milk components, fat was the most variable. This is expected, as fat percentage is largely affected by changes in NDF content of the diet (Nudda et al. 2020).

Average SCS was within the wide physiological range reported for other populations of sheep. However, there is still no agreement on the acceptance threshold of SCC for sheep milk destined for cheesemaking, and there is still no regulation in New Zealand for bulk sheep milk. Moderate variation of SCS was found. Physiological variation in SCC occurs, being high at the beginning and at the end of lactation (Kaskous et al. 2023).

Average calcium content was similar to the literature for sheep milk, which is about 60%–70% higher than average calcium content of cow milk (Park et al. 2007; McCoard et al. 2023). Moderate variation was observed for calcium content.

Average MU was also within the physiological range for dairy sheep (Cannas 2004). However, MU had large variation, with minimum and maximum values surpassing thresholds linked to impaired reproduction (>40–50 mg/dl) and to insufficient dietary protein and low milk production (<25–30 mg/dl) (Cannas 2004). Milk urea is an

Table 2. Least squares means and standard error of milk coagulation properties, cheese yield, heat coagulation time and pH for different animal factors of dairy sheep milked once-a-day in mid- and late-lactation during the 2021–2022 production season.

Effect		Trait ¹					
		RCT (min)	K20 (min)	A30 (mm)	ILCY (%)	HCT (min)	pH
Coat colour	Black	15.18 ^a ± 0.50	2.84 ± 0.19	50.41 ± 1.52	47.37 ^a ± 1.25	1.79 ^a ± 0.12	6.61 ^a ± 0.01
	White	13.38 ^b ± 0.24	2.74 ± 0.09	52.76 ± 0.72	44.19 ^b ± 0.63	1.43 ^b ± 0.06	6.58 ^b ± 0.01
Age	1	14.95 ± 0.73	2.39 ± 0.27	53.11 ± 2.21	49.82 ^a ± 1.70	1.62 ± 0.16	6.63 ^a ± 0.01
	2	14.06 ± 0.44	2.69 ± 0.16	52.88 ± 1.33	47.89 ^a ± 1.17	1.69 ± 0.11	6.59 ^a ± 0.01
	3	14.34 ± 0.44	3.02 ± 0.17	50.03 ± 1.35	42.46 ^b ± 1.20	1.67 ± 0.11	6.60 ^a ± 0.01
	≥4	13.75 ± 0.37	3.04 ± 0.14	50.31 ± 1.14	42.93 ^b ± 0.98	1.46 ± 0.09	6.56 ^b ± 0.01
Litter size	1	13.72 ^b ± 0.37	2.61 ^b ± 0.14	52.98 ^a ± 1.12	46.09 ± 0.93	1.67 ± 0.09	6.59 ± 0.01
	2	14.83 ^a ± 0.35	2.96 ^a ± 0.13	50.19 ^b ± 1.05	45.46 ± 0.89	1.55 ± 0.08	6.60 ± 0.01
Stage of lactation ²	1 (≥50 and <95)	13.26 ^c ± 0.27	2.57 ^b ± 0.12	53.79 ^a ± 0.97	43.44 ^b ± 1.29	2.00 ^a ± 0.13	6.65 ^a ± 0.01
	2 (≥95 and <140)	14.34 ^b ± 0.31	2.74 ^b ± 0.12	52.38 ^a ± 1.00	44.66 ^b ± 0.84	1.69 ^b ± 0.08	6.60 ^b ± 0.01
	3 (≥140 and ≤182)	15.23 ^a ± 0.32	3.06 ^a ± 0.14	48.58 ^b ± 1.14	49.22 ^a ± 0.98	1.14 ^c ± 0.09	6.53 ^c ± 0.01

¹RCT = rennet coagulation time after rennet addition; K20 = time to reach curd firmness of 20 mm; A30 = curd firmness at 30 min after rennet addition; ILCY = individual laboratory cheese yield; HCT = heat coagulation time.

²Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).

^{a,b,c}Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Table 3. Least squares means and standard error of milk yield, gross composition traits, and ratio of casein to fat, for different animal factors of dairy sheep milked once-a-day in mid- and late-lactation during the 2021–2022 production season.

Effect	Trait						
	MY ² (L/day)	Fat (%)	Protein (%)	Lactose (%)	Casein (%)	Casein:Fat ³	
Coat colour	Black	0.50 ± 0.03	6.39 ± 0.12	5.68 ± 0.08	4.67 ± 0.03	4.25 ± 0.06	70.5 ^a ± 1.6
	White	0.53 ± 0.01	6.52 ± 0.06	5.58 ± 0.04	4.68 ± 0.01	4.27 ± 0.03	66.9 ^b ± 0.8
Age	1	0.33 ^b ± 0.04	6.54 ± 0.18	5.81 ^a ± 0.12	4.68 ± 0.04	4.39 ^a ± 0.09	69.2 ± 2.4
	2	0.53 ^a ± 0.02	6.50 ± 0.11	5.76 ^a ± 0.07	4.70 ± 0.02	4.34 ^a ± 0.05	70.6 ± 1.4
	3	0.59 ^a ± 0.02	6.35 ± 0.11	5.46 ^b ± 0.07	4.66 ± 0.03	4.16 ^b ± 0.05	67.4 ± 1.4
	≥4	0.59 ^a ± 0.02	6.43 ± 0.09	5.48 ^b ± 0.06	4.66 ± 0.02	4.15 ^b ± 0.04	67.6 ± 1.2
Litter size	1	0.52 ± 0.02	6.57 ^a ± 0.09	5.53 ^b ± 0.06	4.67 ± 0.02	4.20 ^b ± 0.04	65.9 ^b ± 1.2
	2	0.50 ± 0.02	6.34 ^b ± 0.08	5.72 ^a ± 0.06	4.68 ± 0.02	4.32 ^a ± 0.04	71.6 ^a ± 1.1
Stage of lactation ¹	1 (≥50 and <95)	0.68 ^a ± 0.02	5.52 ^c ± 0.09	5.14 ^c ± 0.05	4.88 ^a ± 0.02	3.92 ^c ± 0.03	74.2 ^a ± 1.2
	2 (≥95 and <140)	0.50 ^b ± 0.02	6.30 ^b ± 0.09	5.52 ^b ± 0.05	4.74 ^b ± 0.02	4.18 ^b ± 0.03	68.2 ^b ± 1.2
	3 (≥140 and ≤182)	0.36 ^c ± 0.02	7.55 ^a ± 0.10	6.22 ^a ± 0.05	4.41 ^c ± 0.02	4.69 ^a ± 0.04	63.8 ^c ± 1.4

¹Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).

²MY = daily milk yield.

³Casein:Fat = ratio of casein to fat.

^{a,b,c}Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Table 4. Least squares means and standard error of ratio of casein to protein, ratio of casein to calcium, contents of calcium, citric acid, and urea, and somatic cell score, for different animal factors of dairy sheep milked once-a-day in mid- and late-lactation during the 2021–2022 production season.

Effect		Trait					
		Casein:Protein ²	Casein:Calcium ³	Calcium (mg/100mL)	Citric acid (mg/100mL)	Urea (mg/100mL)	SCS ⁴ (log ₂ SCC)
Coat colour	Black	75.2 ^b ± 0.3	2.30 ± 0.04	185.9 ± 3.3	75.9 ± 4.6	40.71 ± 0.96	16.5 ± 0.2
	White	76.8 ^a ± 0.1	2.28 ± 0.02	189.5 ± 1.6	85.2 ± 2.2	38.93 ± 0.46	16.3 ± 0.1
Age	1	74.1 ± 1.6	2.23 ± 0.06	197.7 ^a ± 4.9	87.1 ± 6.8	40.11 ± 1.42	16.7 ± 0.3
	2	74.1 ± 1.0	2.30 ± 0.04	189.8 ^{ab} ± 2.9	75.5 ± 4.1	39.80 ± 0.85	16.1 ± 0.2
	3	75.9 ± 1.0	2.30 ± 0.04	182.9 ^{bc} ± 3.0	76.3 ± 4.1	39.89 ± 0.85	16.2 ± 0.2
	≥4	74.9 ± 0.8	2.34 ± 0.03	180.4 ^c ± 2.5	83.6 ± 3.4	39.48 ± 0.71	16.6 ± 0.2
	Litter size	1	74.8 ± 0.8	2.26 ± 0.03	187.7 ± 2.4	82.6 ± 3.4	39.87 ± 0.71
Stage of lactation ¹	2	74.7 ± 0.7	2.32 ± 0.03	187.7 ± 2.3	78.6 ± 3.2	39.77 ± 0.67	16.5 ± 0.2
	1 (≥50 and <95)	76.3 ^a ± 0.2	2.15 ^b ± 0.03	182.5 ^b ± 2.1	92.1 ^a ± 2.8	43.55 ^a ± 0.64	15.6 ^c ± 0.1
	2 (≥95 and <140)	76.0 ^b ± 0.2	2.35 ^a ± 0.03	179.0 ^b ± 2.2	78.0 ^b ± 2.8	39.98 ^b ± 0.66	16.6 ^b ± 0.1
	3 (≥140 and ≤182)	75.6 ^c ± 0.2	2.37 ^a ± 0.03	201.6 ^a ± 2.4	71.7 ^c ± 3.0	35.93 ^c ± 0.74	17.1 ^a ± 0.2

¹Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).

²Casein:Protein = ratio of casein to protein.

³Casein:Calcium = ratio of casein to total calcium.

⁴SCS = somatic cell score.

^{a,b,c}Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Table 5. Least squares means and standard error of parameters describing the curves of curd firmness for ewe coat-colour varieties, ages, litter sizes and stages of lactation of dairy sheep milked once-a-day in mid- and late-lactation during the 2021–2022 production season.

Effect		CF _t parameter ²			
		CF _p	τ ₁	τ ₂	RCT _{eq}
Coat colour	Black	51.75 ± 1.48 ^b	0.29 ± 0.03	6.68 ± 0.34	14.80 ± 0.39 ^a
	White	54.99 ± 0.75 ^a	0.25 ± 0.01	6.49 ± 0.17	13.36 ± 0.20 ^b
Age	1	52.80 ± 1.87 ^b	0.27 ± 0.03	6.43 ± 0.43	14.60 ± 0.49
	2	57.19 ± 1.36 ^a	0.29 ± 0.02	6.28 ± 0.31	13.91 ± 0.36
	3	52.71 ± 1.33 ^b	0.26 ± 0.02	6.67 ± 0.31	14.15 ± 0.35
	≥4	50.78 ± 1.18 ^b	0.28 ± 0.02	6.98 ± 0.27	13.64 ± 0.31
Litter size	1	53.98 ± 1.12	0.29 ± 0.02	6.27 ± 0.26 ^b	13.49 ± 0.29 ^b
	2	52.76 ± 1.06	0.26 ± 0.02	6.90 ± 0.24 ^a	14.67 ± 0.28 ^a
Stage of lactation ¹	1 (≥50 and <95)	55.65 ± 1.08 ^a	0.32 ± 0.02 ^a	5.96 ± 0.25 ^b	12.80 ± 0.28 ^b
	2 (≥95 and <140)	54.66 ± 1.18 ^a	0.22 ± 0.02 ^{bc}	6.67 ± 0.27 ^a	14.31 ± 0.31 ^a
	3 (≥140 and ≤182)	49.81 ± 1.44 ^b	0.28 ± 0.03 ^{ac}	7.13 ± 0.33 ^a	15.13 ± 0.38 ^a

¹Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).
²CF_p: asymptote or maximum potential curd firmness at infinite time. T1 and T2: time constants. RCT_{eq}: coagulation time (dead time) equivalent to the RCT in traditional Formagraph.

^{a,b,c}Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

indicator of nitrogen intake and utilisation, and a negative correlation is reported with milk protein. Milk urea is largely influenced by the balance between level of crude protein and energy content of the diet (Nudda et al. 2020).

Flock performance for processability traits

Similar to the findings of the present study, Manca et al. (2016), have reported a low percentage of non-coagulating milk samples among dairy sheep. However, it is worth noting

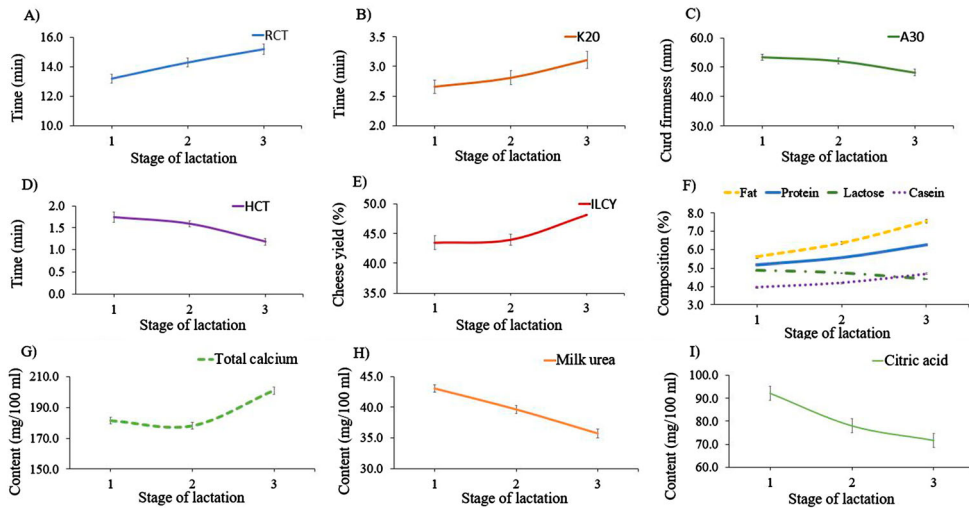


Figure 1. Milk coagulation properties (RCT = rennet coagulation time, K20 = time to reach curd firmness of 20 mm, A30 = curd firmness at 30 min after rennet addition, in A, B and C, respectively), heat coagulation time of milk (HCT, in D), cheese yield (ILCY, in E), milk composition percentage (in F), total calcium in milk (in G), milk urea (in H) and citric acid (in I) of dairy sheep milked once-a-day from mid-lactation, during the production season of 2021–2022.

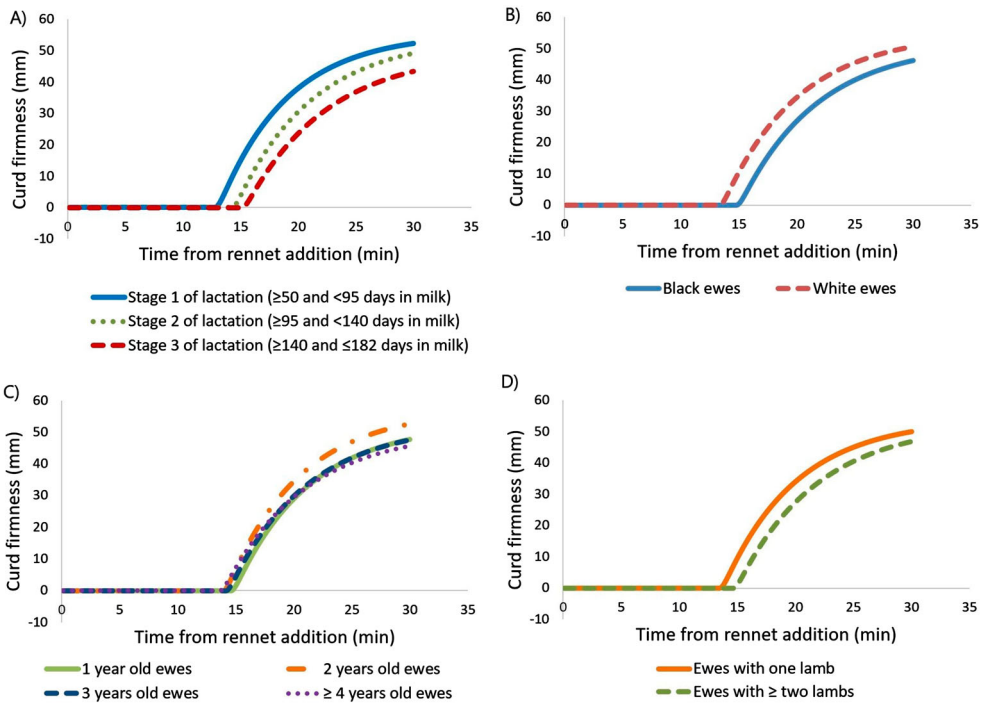


Figure 2. The pattern of curd firming of milk from ewes at different stages of lactation (1, 2 and 3), of different coat-colours (black vs white ewes), age (ewes of 1, 2, 3, ≥ 4 years old) and litter sizes (ewes with one vs two or more lambs), in A, B, C and D respectively.

that another study reported a higher percentage, reaching 19.42% (Garzón et al. 2021). Average RCT was similar to 13.39 min reported for Sarda ewes (Manca et al. 2016). Longer RCT values have also been reported, ranging from 17 to 31 min (Pelayo et al. 2021; Jiménez et al. 2023). Average K20 was longer than the range of 0.40–1.75 min (Manca et al. 2016; Pelayo et al. 2021), but shorter than the range of 4.47–8.11 min observed by others (Sánchez-Mayor et al. 2019; Jiménez et al. 2023). Lower values for A30 of 12–28 mm have been reported (Sánchez-Mayor et al. 2019; Jiménez et al. 2023). It is important to note that differences in some of these reported results are not only attributed to different breeds and farming systems, but also to final dosages of IMCU and different temperatures used in the analyses of MCPs in these studies. This flock of dairy sheep performed, on average, better than dairy cows for MCPs (Bittante et al. 2015; Sanjayanjan et al. 2023).

Relative cheese yield (ILCY) obtained from this flock was high compared to other reports (Vacca et al. 2019; Pelayo et al. 2021). Methods like the one used in the present study, however, tend to overestimate industrial cheese yield due to increased moisture retention, consequence of the lower temperature and small volume of milk used, and increased fat retention due to centrifugation step (Cipolat-Gotet et al. 2016). Average milk pH was 6.58, which is within the pH range of 6.51–6.85 for sheep milk (Park et al. 2007).

There are no other reports on HCT of individual raw sheep milk samples for comparison, only for bulk skim sheep milk (Pan et al. 2023), later discussed.

Effect of stage of lactation

Stage of lactation, naturally confounded with seasonality in grazing systems, had a strong significant effect and explained most of the variation for milk composition, consequently impacting MCPs and HCT. Average MY decreased by nearly half from mid- to late-lactation. This reduction in MY was previously discussed (Marshall et al. 2023). Whereas FP and PP increased, due to the concentration effect. Fat percentage increased in late season also probably due to the increase in fibre content of pasture throughout the season. In the opposite direction, lactose percentage decreased in late season. Lactose is an osmotic regulator and its secretion usually remains constant, however, a decrease in late lactation has also been reported (Auldust et al. 1996) and this is suggested to be due to a parallel increase of milk salts diffusion when the mammary epithelial cells become damaged (Timlin et al. 2021).

The RCT and K20 increased by 2 and 0.5 min, respectively, and A30 decreased by 5 mm, in agreement with previous findings for ewe milk (Sevi et al. 2004; Manca et al. 2016). On the other hand, ILCY increased by 3.2% in late lactation, indicating a higher moisture content in the curd due to the deterioration of fresh curd quality. Heat coagulation time significantly reduced, in the latest stage of lactation (stage 3), the milk coagulated 52 s faster, consistent with findings observed in cows (Loveday et al. 2021).

The casein content is crucial for cheese production, with a significant emphasis on its ratios with other components like calcium, total protein and fat, which may have greatly impacted MCPs. Although protein and calcium contents increased in late lactation, the higher ratio of casein to total calcium in late lactation suggests a greater proportion of available calcium being bound to the micelle rather than in the soluble phase, which could have significantly impaired rennet coagulation (Lucey 2011). Although seasonal variations in pasture calcium content have been observed (Aston et al. 1931), ewes are able to compensate for nutritional deficits in calcium due to bone demineralisation. The increasing concentration of major soluble minerals in milk (including calcium) in late lactation is suggested to be a result from the decreasing effectiveness of tight junctions between mammary epithelial cells as lactation progresses (Hettinga 2019).

Additionally, the reduction in the ratio of casein to protein indicates an elevation in whey protein content which may have impaired MCPs in late lactation. This increase in whey concentration in late lactation is also associated with heightened permeability of the mammary epithelium (Auldust et al. 1996). Whereas the impact of the ratio of casein to fat on rennet coagulation properties is contradictory, and is likely to be dependent on the size of casein micelles and of milk fat globules (Logan et al. 2015), these are determined by the protein and lipid compositions, respectively, which were not evaluated in the present study.

The decrease in milk pH from mid-to-late lactation agrees with another study (Albenzio et al. 2009), but different trends were also noted (Kuchtík et al. 2008). The decrease in milk pH may elucidate the decrease in heat stability, a phenomenon highly dependent on pH levels (Huppertz 2016). The pH of milk reflects the quantity of protons present, and the partitioning of minerals in the soluble phase should be investigated for a better understanding of natural variation in milk pH. Studies on bulk skim sheep milk samples showed maximum HCT of sheep skim milk at pH of 6.9, with heat stability

reducing at higher or lower pH values (Pan et al. 2023). Measuring titratable acidity could also provide information on the buffering capacity of milk and the hygienic quality of it.

The reduction in MU with the progress of lactation may have impacted both HCT and MCPs. Milk urea can have a beneficial impact on milk heat stability because of its buffering effect (Huppertz 2016). The decrease in citric acid might also have contributed to lower HCT in late lactation, but to a lesser extent than MU (Fox et al. 1998). Regarding the impact of MU on MCPs, previous studies have associated it with firmer A30 (Bland et al. 2015) and shorter RCT (Poulsen et al. 2015) in cow's milk. It is possible that certain concentration of MU can reduce the second phase of rennet coagulation, but the opposite is also true in cases of very high MU (McGann and Fox 1974). The reduction in MU with the progress of lactation is likely to be due to the decrease in the content of crude protein (CP) in the pasture dry matter from Spring (early lactation) until late Summer (Marshall et al. 2023), and a more favourable balance between CP and energy content of the ewes' diet.

The worsening of MCPs and HCT might have been magnified by the physiological increase in SCC at the end of lactation. The presence of SCC in milk per se impairs whey drainage from the curd, resulting in retained moisture content (Albenzio et al. 2004). Also, in cases of drastic MY reduction, the mammary epithelium may be compromised during mammary involution in late lactation, causing an influx of blood components into the milk in a similar way to mastitis (Auld et al. 1996). Differential cell count and microbiological analyses offer additional insights into milk quality for processing and help distinguish milk with high SCC due to mastitis.

Effect of age

Age (or parity) did not show any significant effect on any of MCPs but did impact CF_p , which is consistent with findings reported for cows (Bittante et al. 2015). CF_p was notably higher in the second parity, decreasing thereafter, possibly due to the lower calcium concentration in the milk of older ewes. Other studies have reported a significant effect of parity on MCPs, showing a deterioration of MCPs with the aging of ewes (Jaramillo et al. 2008; Pazzola et al. 2014), which aligns with the findings of the present study.

Mature ewes yielded more milk than one-year-old ewes (Marshall et al. 2023), and produced milk with lower PP, while FP remained unaffected by age, in line with findings by Bittante et al. (2015). Additionally, mature ewes exhibited lower calcium levels in their milk. The lower milk production of first parity ewes may be due to the still developing mammary glands and nutrient allocation for body growth rather than milk production.

The significant lower PP in milk of older ewes could be due to the dilution effect. The lower calcium concentration in the milk of older ewes could be due to the limited capacity to absorb calcium in the intestine and to mobilise calcium from bone reserves (Braithwaite and Riazuddin 1971). Consequently, older ewes (three- and four-years-old) also produced significantly less ILCY than the younger ewes (one- and two-years-old).

Effect of litter size

Ewes with twin lambs typically yield more milk, resulting in less concentrated milk compared to those with single lambs, due to increased secretion of placental lactogen and

mammary gland development of twin bearing ewes (Abecia and Palacios 2017). In this study, however, litter size did not impact MY, probably because these differences are pronounced in earlier stages of lactation.

Despite this, litter size significantly affected major milk composition (fat, protein and casein percentage). The milk from twin-bearing ewes had 0.19% more protein and 0.23% less fat, compared to milk from single-bearing ewes, in agreement with Fuertes et al. (1998). For Bittante et al. (2015), twin-bearing ewes also produced milk with greater protein and non-fat solids contents. There was no difference in MU between single- and twin-bearing ewes and therefore no difference in energy balance status.

Litter size also significantly affected all MCPs and RCT_{eq} . The milk from single-bearing ewes coagulated 1 min faster, and had slightly firmer curd, compared to milk from twin-bearing ewes. This agrees with other studies that found low protein to be correlated to short coagulation time (Vacca et al. 2019), which at constant total calcium content can be explained by the partitioning of more calcium in the serum, which strengthens ionic shielding and salt-bridging effects that accelerate coagulation. Bittante et al. (2015) noted no differences in CF_p in milk samples from twin or single-bearing ewes, but there was more rapid syneresis of milk from twin-bearing ewes. In the present study, syneresis was not observed during the Formagraph analyses.

Effect of coat-colour variety

Artificial selection based on adaptation to an extensive production system, rather than for pure white wool, allows for the retention of variations in coat colour in a flock, which some farmers link to production traits. A few studies with quantitative analyses have been made to support this (Pascual-Alonso et al. 2014). Furthermore, different levels of tolerance to heat-stress between white and black dairy animals can result in different levels of milk production (Arenas-Báez et al. 2023). Bernabucci et al. (2015) demonstrated how heat stress (and compositional changes to pasture in hot temperatures) affected coagulating parameters, with reduction in casein concentration, and changes in protein fractions.

In the present study, coat-colour did not significantly affect milk production or major milk composition, except for the ratio of casein to protein, and casein to fat. On the other hand, coat colour was a significant effect on processability traits, including RCT, HCT ($p < 0.01$), ILCY, pH, CFP and RCT_{eq} ($p < 0.05$).

Milk from white ewes had a greater proportion of casein in protein, lower ratio of casein to fat, coagulated 1.8 min earlier by rennet (RCT) and produced curd that was 3.24 mm firmer (CFP). Milk from white sheep, however, resulted in less ILCY, pointing out again to an unfavourable correlation between fresh curd quality and quantity. This is especially true for the method used for estimation of cheese yield, which tends to retain high moisture content. Also, milk from white ewes coagulated 0.36 min (21 s) earlier by heat.

Conclusion

Several ewe physiological and environmental factors are known to affect milk production and composition, consequently affecting the processability of milk, and should not be

overlooked by the sheep dairy industry. In this study, stage of lactation, naturally confounded with seasonality, strongly impacted the properties of milk for cheese making and milk heat stability. Key mechanisms that may be influencing the variation observed in the processability of milk include variations in milk pH (which is highly determined by minerals in milk soluble phase), and factors contributing to the buffering capacity of milk. The ratios of casein to calcium and to total protein are of notable importance. Somatic cell count could be associated with impaired milk processability. Furthermore, protein composition, lipid and mineral profiles should be investigated. Further studies are underway to investigate milk protein profile, and to define animal genetic variance, heritability, and genetic and phenotypic correlations between the production, composition and technological traits of this flock of dairy sheep.

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