



Breed and shearing effects on milk composition and rennet-induced coagulation properties in dairy ewes

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ARTICLE INFO

Keywords:

Dairy sheep
Breed
Shearing
Milk curd
Milk coagulation

ABSTRACT

Shearing during late-pregnancy and mid-lactation has been proposed as improving welfare practices in dairy sheep. With this aim, and as a follow up of a previous experiment, milk composition and milk rennet-induced coagulation properties were assessed in 2 breeds of dairy ewes (Manchega, MN, $n = 43$; Lacaune, LC, $n = 28$) which were shorn at different moments of their production cycle: i) unshorn (CO), ii) shorn before breeding (SBB), or iii) shorn at d 100 of pregnancy (S100). Individual milk samples were collected at d 160 and 161 of lactation and composited for milk analyses and milk batches produced by breed and shearing treatment for assessing milk rennet-induced coagulation properties with an Optigraph laboratory instrument (rennet coagulation time, RCT; Curd firmness at 45 min, F45). Milk composition and cheese rennet-induced coagulation properties varied markedly by breed, the MN showing greater values than the LC ewes, but no differences in major milk components nor in rennet-induced coagulation properties were detected by effect of shearing in both breeds. Nevertheless, opposite differences by shearing treatment were detected in RCT, which was longer in MN (9 %) and shorter in LC (– 8 %), when S100 and CO treatments were compared. The obtained results showed marked differences in cheese extract and yield in favor to the richer MN milk, with economic consequences on milk price, whereas shearing moment only modified RCT, the sense of variation depending on the breed and not being associated to changes in milk composition; therefore, assessment of additional factors is required for in-depth understanding.

1. Introduction

Since sheep milk is mainly devoted for cheese production (Pulina et al., 2018) the balance among milk yield, composition and milk coagulation properties are key for the cheese industry. In this sense, milk clotting properties are widely used to assess milk efficiency in cheese making owing to they determine the quantity and quality of cheese (Bencini and Pulina, 1997; Caballero-Villalobos et al., 2018). It has been reported that greater concentration of milk components, faster renneting time and high curd firmness are strongly associated with high cheese yield (De Marchi et al., 2008). Nevertheless, the relationships among clotting properties, milk components, and cheese making have most been studied in cow's milk but has not been fully elucidated in dairy ewes (Vacca et al., 2019; Garzón et al., 2023) and scarcely compared by breed.

The factors influencing milk coagulation properties are those related to milk composition changes and cheese making procedure itself (Caballero-Villalobos et al., 2018; Pazzola, 2019). Milk composition is determined by many interrelated factors such as genetics, season, physiology, milking techniques and husbandry practices. The last include nutrition, reproduction and shearing (Bencini and Pulina, 1997).

With this regard, Assaf ewes shorn in late pregnancy under summer conditions and with barn cooling, increased intake and milk performances (Leibovich et al., 2011). Similarly, Elhadi et al. (2019) reported that shearing lactating during winter increased intake, milk yield and milk protein in Lacaune ewes. Moreover, summer shearing in late pregnancy improved the thermal comfort and milk composition in Manchega and Lacaune ewes in the next lactation (González-Luna et al., 2023). Yet to our knowledge, no study has assessed the effect of shearing

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ewes on milk rennet-induced coagulation properties and on cheese yield.

As a follow up of the previously cited research (González-Luna et al., 2023), our hypothesis was that shearing could modify milk composition with consequences on milk coagulation properties. To test this hypothesis, the milk of 2 breeds of dairy ewes shorn at different moments of their productive cycle, were compared in late lactation under similar conditions.

2. Materials and methods

The experimental procedures and management practices here reported were in accordance to the Guidance on the operation of the Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63 and they did not require approval of the Ethics Committee on Animal and Human Experimentation (CEEAH-2013) of the UAB.

2.1. Animals, feeding and management conditions

As a follow up of a previous experiment, a total of 71 multiparous ewes of 2 dairy breeds (Manchega, MN, $n = 43$; Lacaune, LC, $n = 28$) from the experimental farm of the Servei de Granges i Camps Experimentals (SGCE) of the UAB were used during a whole cycle of pregnancy and the following lactation. Details of the animals, diets and management may be consulted in González-Luna et al. (2023). In brief, the ewes grazed 6-h daily throughout the year (autumn to spring) and were fed with green chopped sorghum in the barn during summer. Additionally, they were complemented with alfalfa hay *ad libitum* and concentrate according to their requirements (INRA, 2018) on the barn. The ewes were mated in spring (mid-April), after ram effect, for autumn lambing and the lambs were weaned at 28 d of age. Afterwards the ewes were milked twice daily (0700 and 1700 h) in a 2×12 stalls milking parlor with a parallel arrangement (Amarre Azul I; DeLaval, Alcobendas, Spain) from autumn to spring until d 210 after the weaning of the lambs or when milk yield was lower than 0.4 kg/d.

2.2. Experimental treatments

Ewes were blocked in 3 balanced groups, based on previous lactation, to which the experimental treatments were randomly assigned. They were: i) control (CO) ewes not shorn from the previous year (MN, $n = 14$; LC, $n = 9$); ii) shorn before breeding (SBB), ewes shorn 15 d before introducing the rams on mid-May (MN, $n = 13$; LC, $n = 10$); and, iii) shorn at late pregnancy (S100), ewes shorn at d 100 of pregnancy on mid-August (MN, $n = 16$; LC, $n = 9$).

2.3. Milk sampling

Individual milk samples per ewe were collected at d 160 and 161 of lactation and composited according to milking interval (a.m., 60 %; p.m., 40 %) and in duplicate. One sample (100 mL) was preserved with 1 tab of Bronopol broad spectrum micro-tablets II (D&F Control Systems, San Ramon, CA) for composition analysis. The other (100 mL), without preserver, was composited in batches according to breed (2), day (2) and shearing treatment (3) and in duplicate (24 batches), for assessing the milk rennet-induced coagulation properties. All samples were stored at 4 °C until analysis. Milk sampling was done in late lactation (April), before the next mating according to the reproductive cycle followed in the UAB experimental farm. Values of daily mean temperature and relative humidity on the sampling days averaged 10.9 ± 0.7 °C and 68 ± 6 %, corresponding to a mean temperature-humidity index (Mader et al., 2006) of THI = 53 (thermoneutral). Milk composition was standardized by fat and protein according to INRA (2018).

2.4. Milk composition analysis

Preserved milk samples were analyzed for fat, total protein, lactose, and total solids using a Milkoscan FT2 (Foss, Hillerød, DK) in the Dairy Herd Improvement Laboratory of Catalonia (ALLIC, Cabrils, Barcelona, ES). Protein/fat ratio, cheese-extract (fat + protein) and estimated cheese yield according to Van Slyke equation (Mullan, 2008) were calculated.

2.5. Milk rennet-induced coagulation properties

Milk batches for renneting ability were processed after 24 h storage at 4 °C. Coagulating traits were assessed in an Optigraph laboratory instrument (Ysebaert, Frepillon, FR), consisting of 10 wells (10 mL each) operating simultaneously and interfaced with a computer. Samples were previously conditioned at 34 °C for 15 min in a water bath. Coagulation temperature was set at 34 °C, and the coagulation test lasted for 60 min. Coagulation properties of milk samples (RCT, rennet coagulation time; F45, firmness at 45 min) were determined in quadruplicate after addition of rennet enzyme (43 µL). Diluted rennet enzyme (1:10) was prepared by mixing 1 mL of calf rennet, containing 780 mg/L of chymosin (Larbus, Madrid, ES), with 9 mL of distilled water. Fresh diluted rennet enzyme was prepared and kept at 4 °C until use. Moreover, milk coagulation efficiency (F45/RCT) was calculated according to Caballero-Villalobos et al. (2018).

2.6. Statistical analyses

Data analyses were performed using SAS v. 9.4 (SAS Inst. Inc.; Cary, North Carolina, USA). Milk composition and rennet-induced coagulation properties data were analyzed using the MIXED procedure containing the fixed effects of breed and shearing treatment. Pearson correlation coefficients (r) were calculated using the CORR procedure of SAS. Differences between least square means were determined with the PDIF option of SAS and significance declared at $P < 0.05$.

3. Results and discussion

The average milk yield in late lactation (160 d in milk) was 0.90 ± 0.06 kg in MN and 1.53 ± 0.11 kg in LC ewes (data not shown). Milk composition and rennet-induced coagulation properties of the ewes are shown in Table 1. Both breeds markedly differed on main milk components, the MN ewes showing richer milk composition than LC ewes ($P < 0.001$) in total solids, fat, protein and cheese extract (3.12 % units), on average, whereas did not differ in lactose content ($P = 0.20$). Similar differences between milk composition of MN and LC ewes throughout lactation were reported by Rovai et al. (2008), Elhadi et al. (2019) and González-Luna et al. (2023). According to these data and using the current milk quality price for sheep's milk in Spain (0.1088 €/cheese extract unit) an averaged milk price of 1.545 €/kg was obtained for the mean cheese extract of both breeds (14.2 ± 0.4 %, data not shown), with a difference of 0.348 €/kg (25.4 %) in favor of MN ewes milk (1.719 €/kg) compared to LC ewes milk (1.371 €/kg) according to their milk composition. This result shows evidence of the economic advantage of producing a richer milk in fat and protein, as is the case of MN ewes, for the current milk quality payment policy.

In line with the above discussed differences in milk composition, predicted cheese yield was also greater in MN than LC ewes (23.69 ± 0.34 % vs. 18.88 ± 0.38 %, on average; $P < 0.001$; Table 1) indicating that 4.2 and 5.3 kg of milk/kg of cheese were needed, respectively, for the milk of the ewes of each breed. As a result, milk costs for cheese production with MN ewe's milk ($4.2 \text{ kg milk} \times 1.719 \text{ €/kg} = 7.22 \text{ €/kg}$) and LC ewe's milk ($5.3 \text{ milk} \times 1.371 \text{ €/kg} = 7.26 \text{ €/kg}$), were similar between breeds.

Values of F45 of coagulation milk tended to be higher ($P = 0.09$) in MN (45.99 ± 1.36 mm) compared with LC (42.56 ± 1.29 mm). Rennet

Table 1

Effects of breed (MN = Manchega; LC = Lacauene) and shearing treatment (CO, control unshorn; SBB, shorn before breeding; S100, shorn at d 100 of pregnancy) on milk composition and milk rennet-induced coagulation properties at late lactation in dairy ewes (data are LSM).

Item	MN			Mean ± SEM	LC			Mean ± SEM	Effect (P-value)		
	CO	SBB	S100		CO	SBB	S100		Breed	Shearing	B × S
Milk composition											
Total solids, %	21.0	21.4	21.6	21.3 ± 0.2	18.5	17.8	18.2	18.2 ± 0.2	0.001	0.81	0.37
Fat%	8.37	8.61	8.70	8.56 ± 0.15	6.93	6.31	6.43	6.56 ± 0.16	0.001	0.77	0.18
Protein, %	7.25	7.28	7.19	7.24 ± 0.13	6.20	5.79	6.46	6.15 ± 0.14	0.001	0.46	0.27
Lactose, %	4.61	4.62	4.72	4.65 ± 0.04	4.72	4.82	4.65	4.73 ± 0.04	0.20	0.76	0.17
Protein/Fat ratio	0.85	0.85	0.83	0.84 ± 0.02	0.90	0.93	0.97	0.93 ± 0.02	0.003	0.72	0.25
SM factor ^a	1.12	1.15	1.15	1.14 ± 0.01	0.98	0.92	0.95	0.95 ± 0.01	0.001	0.73	0.22
Rennet-induced coagulation properties											
Cheese extract ^b , %	15.5	15.9	15.9	15.8 ± 0.2	13.1	12.1	12.7	12.6 ± 0.3	0.001	0.70	0.23
Cheese yield ^c , %	23.3	23.9	23.9	23.7 ± 0.3	19.7	18.1	18.9	18.88 ± 0.38	0.001	0.71	0.22
F45 ^d , mm	46.6	44.6	46.7	46.0 ± 1.4	42.5	45.4	39.8	42.6 ± 1.3	0.09	0.73	0.28
RCT ^e , min	9.23 ^d	9.56 ^d	10.02 ^c	9.60 ± 0.08	11.27 ^a	11.03 ^a	10.41 ^b	10.90 ± 0.08	0.001	0.84	0.001

^a Standardized milk = 0.071 · Fat (%) + 0.043 · Protein (%) + 0.2224, according to [INRA \(2018\)](#).

^b Fat + total protein contents used for milk payment and cheese production prediction.

^c According to Van Slyke equation ([Mullan, 2008](#)).

^d Curd firmness at 45 min (Optigraph).

^e Rennet coagulation time (Optigraph).

coagulation time (RCT) slightly varied according to breed ([Table 1](#)) where milk of MN was coagulated faster than LC ewes (9.6 vs. 10.9 min $P = 0.001$). Interestingly, a significant interaction showed that in the case of the MN ewes, the S100 treatment slightly delayed the formation of curd (-9% ; $P = 0.001$), whereas in LC it was faster (8% ; $P = 0.001$) when compared to their respective CO ewes. Coagulation efficiency at 45 min was greater ($P = 0.001$) in MN milk (4.80 ± 0.12 mm/min) than LC milk (3.96 ± 0.12 mm/min) which supported that high concentrated milk led to firmer clot and greater cheese yield.

When assessing only the shearing treatment, no effects on milk composition nor milk rennet-induced coagulation properties were detected ([Table 1](#); $P = 0.46$ – 0.84) suggesting no relevant metabolic adjustments or at least, detectable in major milk components as we hypothesized. This result differs to milk fat, protein and total solids boosting reported in dairy ewes at early ([González-Luna et al., 2023](#)) or mid lactation ([Leibovich et al., 2011](#)) and which had been shorn at late pregnancy. This lack of effects on milk composition also differs to [Knight et al. \(1993\)](#) who stated an increase of milk fat, protein and total solids in Dorset ewes shorn at late pregnancy and early lactation.

Cold stress imposed to shorn pregnant ewes (winter) evoked complex endocrine changes probably aimed to increase nutrient supply towards mammary gland in early lactation ([Symonds et al., 1990](#)). In the present study, the environmental conditions in late lactation were mild (i.e., THI = 53) enabling effective ewe's thermoregulation.

Negative and no significant correlation between RCT and F45 was found in both breeds (-0.12 , $P = 0.58$) which agreed with the values reported by [Pazzola et al. \(2014\)](#) in dairy sheep and showed that curd firmness of sheep milk is nearly independent from coagulation time ([Pazzola, 2019](#)).

The reported results show noticeable differences between breeds in almost all the evaluated variables (except in lactose and F45) and determined the rennet coagulation time according to shearing treatment irrespective of milk composition. This interaction indicates it would be useful the measurement of Ca concentration, SCC, initial pH, titratable acidity, plasmin activity or casein micelle characteristics of milk since these factors have been associated to variations in renneting properties ([Pellegrini et al., 1997](#); [Caballero-Villalobos et al., 2018](#)). In this regard, milk pH has a relevant impact on κ -casein hydrolysis process and therefore, on milk clotting time. A lower pH allows solubilization of micellar calcium phosphate, decrease casein net charge and casein dissociation from micelles ([Nájera et al., 2003](#)). Although, the percentage of non-coagulating milk samples is very low in sheep species

([Pazzola, 2019](#)) a higher milk pH, lower titratable acidity, lower values of micellar Ca were observed in non-coagulating milk samples in comparison with optimal milk, in terms of rennet coagulation indicators in cows ([Malacarne et al., 2014](#)). According to [Yildirim and Erdem \(2015\)](#) the micellar structure seems to be more compact in sheep milk due to higher casein content and lower number surface hydrophobic sites that may have an impact on renneting properties, and which leads to consider milk collision distance of proteins, micelle size and integrity.

As far as subclinical mastitis is concerned, [Bentayeb et al. \(2023\)](#) reported a longer rennet clotting time and lower clotting activity in milk samples from cows infected with *Staphylococci* compared to those obtained from uninfected quarters. These results were associated with an increase in conductivity (most probably owing to ion balance changes) and milk pH.

Since the several factors that determine milk clotting properties have been mainly studied in cow but less in sheep ([Nájera et al., 2003](#)) the measurements of physico-chemical characteristics and know how they are interrelated could shed some light on potential effects of breed characteristics (e.g., wool insulation, milk yield and composition) according to shearing moment on milk coagulation time at late lactation.

4. Conclusions

The present study provides the first evidence, to the best of our knowledge, that milk coagulation time at late lactation varied in shorn pregnant ewes regardless of milk composition. The coagulation of MN ewe's milk was delayed by the S100 treatment, whereas that of LC ewes was faster in late lactation when compared to CO ewes. Nevertheless, although all ewes had apparently healthy udders, somatic cell count and other microbial status indicators were not measured in our ewes. The observed differences at laboratory level are difficult to extrapolate at industrial cheese manufacturing and additional measurements (e.g., physico-chemical characteristics) are further required. The results also emphasize the higher values in most milk components, cheese extract and cheese yield in MN when compared to LC dairy ewes.

CRedit authorship contribution statement

A. A. K. Salama: Visualization, Resources, Methodology, Investigation, Data curation. **A. Contreras-Jodar:** Methodology, Data curation. **G. Caja:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **S. González-Luna:** Writing – original draft,

Investigation, Formal analysis, Data curation. **L. Cordón:** Investigation, Data curation.

Funding

This study was supported by a grant of the Plan Nacional (Project AGL2013-44061-R) of the Spanish Ministry of Economy and Competitiveness (Madrid, Spain).

Declaration of Competing Interest

The authors declare no conflict of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to CONAHCYT (Mexico City, Mexico) for the PhD scholarship to Sandra González Luna, and to the team of the Servei de Granges i Camps Experimentals of the Universitat Autònoma de Barcelona (Bellaterra, Spain) for taking care of the animals.

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