Small Ruminant Research 206 (2022) 106584

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Milkability traits across milk flow curve types in Sarda sheep

Angela Costa[a,*], Carlo Boselli[b], Massimo De Marchi[a], Giuseppe Todde[c], Maria Caria[c]

[a] *Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020, Legnaro, PD, Italy*
[b] *Experimental Zoophylactic Institute Lazio and Toscana Mariano Aleandri, National Reference Centre for Ovine and Caprine Milk and Dairy Products Quality, Via Appia Nuova 1411, 00178, Rome, RM, Italy*
[c] *Department of Agricultural Sciences, University of Sassari, Viale Italia 39a, 07100, Sassari, SS, Italy*

ARTICLE INFO

Keywords:
Dairy ewe
Milk yield
Overmilking
Milk flow rate
Emission curve

ABSTRACT

A temporary interruption of the milk flow is observed between alveolar and cisternal fraction in ewes for the peculiar anatomy of the mammary gland. Often during milking a physiological delay in milk excretion between the cisternal and the alveolar fraction occurs. However, long delays may indicate suboptimal pre-stimulation of teats and/or insufficient oxytocin release in the bloodstream, resulting in undesired protracted interruptions between the 2 milk peaks. Moreover, a clear distinction between the fractions may be undetectable in high-producing ewes, particularly in early lactation. Based on both milk flow curve shape and duration of different excretion phases during milking, 3 curve types can be identified: single-peak (P_1), double-peak (P_2), and long plateau (P_L). The aim of the present study was to compare milkability traits across the 3 milk flow curve types using data recorded in Sarda sheep breed; for this scope, 10 features were measured with a milkmeter in 568 ewes belonging to different parities, lactation stages, and commercial farms. After editing, data of 544 ewes reared in 17 farms were available for the analysis. The P_1 curves were the most frequent (57.5 %), but ewes with a P_L curve were significantly more productive than those with a P_1 curve (0.61 ± 0.03 *vs* 0.53 ± 0.02 kg of milk/milking). Milk yield of P_2 ewes was intermediate (0.58 ± 0.03 kg of milk/milking) and in all curves the majority of milk was excreted in the first 60 s of milking. The highest milk flow rate and the shortest milk emission time were estimated for the P_1 curves; moreover, the blind time, indicator of overmilking, was the minimum in P_L curves and the maximum in P_1 curves. Based on such findings, farmers are recommended to pay more attention to milk emission curves in their herd in order to avoid overmilking, particularly in the presence of P_1 curves. Considering the large herd size of Sarda breed farms (170.3 ewes per farm in 2020), part of milking routine could be slightly modified and customized according to milk flow curve type and lactation stage. Ideally, this will allow optimal milking practices to be adopted within groups of ewes with similar milkability. Monitoring milk emission curves and adopting less-standard and more adjusted milking practices is useful to meet the ewe's individual milking ability and in the long term would limit mammary gland stress, reduce antimicrobial use due to udder issues, and improve the udder health in Sarda sheep.

1. Introduction

After cattle, sheep is the most important specie for milk and dairy production in Europe. Milk obtained from ewes in Italy is usually intended for cheese manufacturing . According to the Associazione Italiana Allevatori (AIA), 143,952 ewes spread over 1063 herds were officially registered in Italy in 2019 (Associazione Italiana Allevatori (AIA), 2021). Out of these, the 85 % (n = 122,046) belonged to the Sarda breed, which is very popular in the Mediterranean area, delivering milk with favourable composition for cheesemaking and good coagulation characteristics. On average, milk obtained from Sarda ewes is characterized by an average fat and protein content of 6.48 and 5.59 %, respectively (Pulina et al., 2021). For this reason, Italian Sarda sheep has been historically linked to the production of Protected Geographical Indication (PGI) or Protected Designation of Origin (PDO) cheeses, such as the Fiore Sardo PDO and the Pecorino Romano PDO (Pulina et al., 2021). Boosted by the favourable market demand and price (CLAL, 2021), farmers are nowadays more and more motivated in enhancing quality rather than volume of milk. In fact, compared to previous decades, more efforts have been recently put for improving animal health

---

[*] Corresponding author.

E-mail addresses: angela.costa@unipd.it (A. Costa), carlo.boselli@izslt.it (C. Boselli), massimo.demarchi@unipd.it (M. De Marchi), gtodde@uniss.it (G. Todde), mariac@uniss.it (M. Caria).

https://doi.org/10.1016/j.smallrumres.2021.106584
Received 17 June 2021; Received in revised form 4 November 2021; Accepted 7 November 2021

in sheep farms and for maximising both farm and dairy chain productivity and efficiency.

Milking is among the aspects needing improvement in sheep farms. In other species, milkability traits have been used to evaluate the response to milking technology and manipulation (Kulinová et al., 2012). Such traits include a variety of characteristics related to milk flow and can be recorded through a milkmeter. Some milkmeters, such as the Lactocorder® (WMB, Balgach, Switzerland), are portable and are able by default to classify milk flow curves and record the different phases (Marie-Etancelin et al., 2006; Tančin et al., 2011; Mačuhová et al., 2020). For example, lag or latency time (LT) is the first phase identificated by the device during milking, as it represents the time between the attachment of the milking clusters and the moment where a specific milk flow is reached (Steidle et al., 2000; Marie-Etancelin et al., 2006). After LT, the plateau (PPT) takes place. This is the phase where the milk flow is constant and undisturbed. Finally, from the end of PPT to a milk flow drop below 0.20 kg/min, the decreasing phase (DPT) is detected (Steidle et al., 2000; Marie-Etancelin et al., 2006). In presence of overmilking, a fourth stage, known as blind phase (BT), can be recorded. Literature demonstrated that multiple exposure to prolonged BT during the productive life translates into greater mammary tissues stress and risk of mastitis (Herve et al., 2018; Dzidic et al., 2019).

For anatomical reasons, a temporary interruption of the milk flow is frequently observed in ewes. This is due to a delay in the alveolar milk fraction excretion after emptying of the cisternal structure. Researches refer to this delay as bimodality, as 2 separated milk emission peaks occur. However, a unique emission peak can be detected in high-producing animals, particularly around the milk yield lactation curve peak (Dzidic et al., 2019). A long delay between the first and the second emission peak could likely be due to suboptimal pre-stimulation of teats and thus to an insufficient release of oxytocin in the bloodstream (Olechnowicz, 2012). Based on the duration of milking phases, different milk flow curve types (MFC_{TYPE}) can be identified in ewes.

The aims of the present study were to assess the frequency of MFC_{TYPE} in Sarda ewes farmed in the production area of Pecorino Romano PDO (Italy) and to estimate the effect of milk flow curve type on milk yield and milkability traits.

2. Materials and methods

2.1. Design

Data were collected from 2007 to 2021 in 568 Sarda ewes farmed in 17 herds. All farms were visited once, where milk yield (MY, kg), milk flow curve and milkability traits were recorded during the evening milking. The milking machine was under standard dynamic conditions during data collection in all farms, with similar pulsation rate and with vacuum showing variation from a minimum of 28.0 kPa to a maximum of 46.0 kPa across the herds.

Out of all milk flow curves available, 24 (4.22 %) were discarded, as the minimum flow required to return all parameters was not reached. Finally, 544 curves belonging to ewes in different parities and lactation stages were considered.

2.2. Milk flow traits

The portable milkmeter Lactocorder® (WMB, Balgach, Switzerland) was used for collection of the milkability features and was installed in each farm based on Boselli et al. (2020). In the 17 farms involved a standard pre-milking routine, as widely described by Pazzona et al. (2009), was applied and included: teats cleaning, brief hand massage, drying, and attachment of milking cluster. On average, 2 min were needed for the pre-milking phase, with small differences across farms due to management, personnel, milking parlour structure, and number of heads milked at the same time. In all the farms enrolled, the manual detachment of milking cluster was performed. The Lactocorder® started to record and store information as soon as the first teat cup was attached and identification of milk flow phases was performed by the LactoPro software (WMB, Balgach, Switzerland); a comprehensive list and description of the storable information is available in Steidle et al. (2000). The recorded traits comprised:

- MY (kg): total milk yield during the whole mechanical milking;
- MY_1 (kg): milk yield recorded in the first 60 s of mechanical milking;
- PFR (kg/min): peak flow rate in the main milking process (where the majority of milk is released) within a time interval of 8 measuring points recorded within a time interval of 22.4 s.
- AFR (kg/min): average milk flow rate during the total milk emission time;
- LT (min): lag (or latency) time from beginning of measurement until a milk flow of 0.50 kg/min;
- MET (min): milk emission time, calculated as the time between a milk flow rate over 0.50 kg/min and a milk flow rate of 0.20 kg/min;
- PPT (min): plateau phase time as the duration of plateau, i.e. where a high constant milk flow is observed, from the vertex of the incline phase to the vertex of the decline phase;
- DPT (min): time of decline phase, i.e. from end of PPT to a milk flow rate below 0.20 kg/min.
- BT (min): mechanical overmilking, if present, recorded from end of DPT to a milk flow below 0.10 kg/min.
- TMT (min): total milking time, including BT if present; i.e. the time elapsed beginning the attachment of milking cluster at mammary gland and the manual detachment at end of milking.

2.3. Statistical analysis

The MFC_{TYPE} classification was carried out considering presence of bimodality, length of phases, and appearance. In the present study, curves (1 per each ewe) were classified using criteria defined by Mačuhová et al. (2020):

- Single peak (P_1): 1 milk emission peak (Fig. 1A);
- Double peak (P_2): presence of bimodality, i.e. 2 milk emission peaks (Fig. 1B);
- Long plateau (P_L): 1 milk emission peak with a PPT greater than 20 s (Fig. 1C).

Pearson correlations were calculated in SAS software (version 9.4, SAS Institute, Cary, NC, US) through the PROC procedure; in the same software, the analysis of variance was carried out on MY, MY_1, and milkability traits by imputing the following model in the MIXED procedure:

$$y_{ijklmn} = \mu + LS_i + PO_j + CT_k + (LS \times PO)_{ij} + (LS \times CT)_{ik} + (PO \times CT)_{jk} + YC_l + CF_m + e_{ijklmn}$$

where *y* is the vector of phenotypic observation of the analysed trait; *μ* is the intercept of the model; *LS* is the effect of ith lactation stage (6 levels: 1st, 2nd, 3rd, 4th, 5th, and ≥6th months after parturition); *PO* is the fixed effect of the jth parity order (primiparous and multiparous); *CT* is the effect of the kth MFC_{TYPE} (P_1, P_2, and P_L); (*LS x PO*) is the interaction between the ith *LS* and the jth *PO*; (*LS x CT*) is the interaction between the ith *LS* and the kth *CT*; (*PO x CT*) is the interaction between the jth *PO* and the kth *CT*; *YC* represents the fixed effect of the mth year class (5 classes: 2007–2009, 2010–2012, 2013–2015, 2016–2018, and 2019–2021); *CF* represents the random effect of the mth commercial farm; *e* is the random residual term. Frequency of records available in each lactation stage, parity, and MFC_{TYPE} is reported in Table 1; as regards the interactions (*LS* × *PO*), (*LS* × *CT*), (*PO* × *CT*), the minimum frequency of records was 15, 5, and 23, respectively. The year class with the greatest and the lowest number of records were the second (2010–2012) and the fifth (2019–2021), respectively. Differences

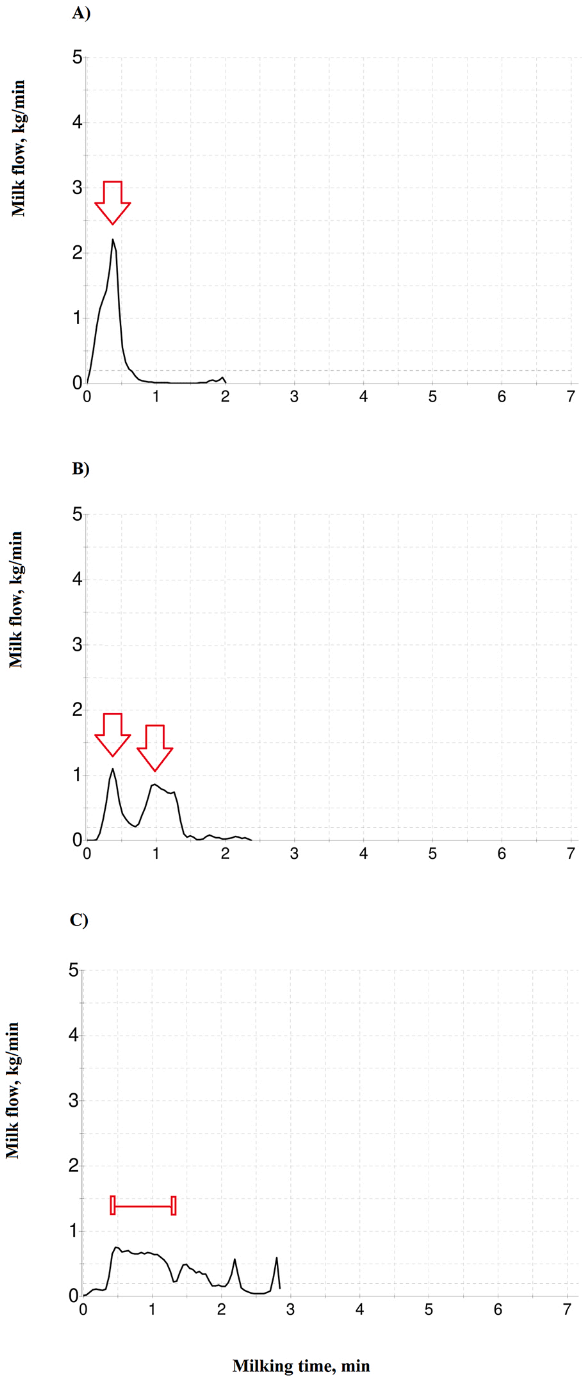

Fig. 1. Milk flow curves characterized by A) single emission peak, B) double emission peak, or C) plateau phase length greater than 20 s.

between least squares means (LSM) were tested with the Bonferroni *post hoc* test with significance given at $P < 0.05$.

3. Results

Table 1 contains the frequency of records for all the levels of fixed effects. The 6 lactation stages were represented in the dataset and primiparous ewes accounted for the 23 % of total animals. The frequency of P_1, P_2, and P_L curves was 57.54, 20.77, and 21.69 %, respectively. An overview of descriptive statistics is provided in Table 2. The average MY was equal to 0.59 kg and was extracted in 1.46 min (Table 2). Most of the MY was excreted in the first 60 s of milking, as the averages of MY and MY_1 were not too far from each other's (Table 2). The flow rates recorded, namely AFR and PFR, differed by 0.20 kg/min (Table 2) and MET represented the 51 % of the TMT, with a PPT of 0.27 min and a DPT of 0.33 min. The LT length was 18 % of the TMT duration, while BT accounted for about a quarter of TMT (Table 2).

Table 1
Number of ewe (= records) in each lactation stage, parity order, and milk flow curve type.

Fixed effect		n
Lactation stage		
1		88
2		82
3		83
4		97
5		99
6		95
Parity		
Primiparous	124	
Multiparous	420	
Milk flow curve type		
Single peak (P_1)		313
Double peak (P_2)		113
Long plateau (P_L)		118

The analyses of variance revealed that lactation stage and MFC_{TYPE} significantly affected all the traits investigated, with few exceptions (Table 2); on the other hand, parity had a significant effect exclusively on MY, MY_1, and PFR, with a tendency for PPT.

Considering the LSM of lactation stage, MY ranged from 0.28 ± 0.04 to 0.82 ± 0.03 kg and the maximum was recorded in the lactation stage 2, i.e. at second month of lactation. Estimated MY at second month of lactation was significantly different from MY estimated in subsequent stages but statistically similar to MY at first month of lactation (Table 3). Similarly, the peak of MY_1 was observed at the second lactation stage, followed by a linear decrease afterwards (Table 3). In addition, MY_1 was affected by parity ($P<0.05$), with primiparous ewes being less productive than multiparous (0.46 ± 0.02 kg *vs* 0.50 ± 0.02 kg; data not shown).

The LSM estimated for the fixed effect of MFC_{TYPE} are given in Table 4. In the case of MY, the greatest estimate was found for P_L curves and was significantly different than P_1 and statistically similar to P_2 (Table 4). The same can be extended to MY_1, as the estimate was numerically the greatest and the lowest in P_L and P_1, with an intermediate value in P_2 (Table 4). However, the differences among the LSM were not significant in this case.

Both PFR and AFR were the greatest in the first 3 months of lactation and LSM were significantly different than those of subsequent stages (Table 3); on the other hand, LT was the highest in lactation stage 6. Some comparisons between the 6 estimates of LT were not significant likely due to large standard errors.

The PFR estimated for primiparous was significantly ($P < 0.001$) lower than the PFR of multiparous, being 0.79±0.05 and 0.88±0.04 kg/min, respectively; AFR was significantly affected by lactation stage and by MFC_{TYPE} (Fig. 2); in particular, the estimate of P_L curves in the last lactation stage was significantly different ($P < 0.05$) from that of P_1 and P_2 curves and the estimate of the first 3 lactation stages were significantly different from those of subsequent stages ($P < 0.05$). Overall, PFR and AFR were the highest in P_1 (Table 4), with estimates equal to 0.94 ± 0.04 and 0.76 ± 0.04 kg/min.

In general, LSM of MET revealed that milk emission tends to be faster in early than late lactation (Table 3), with duration of MET phase being on average equal to 0.66 min for lactation stage 4, 5, and 6 and equal to 0.90 min for lactation stage 1, 2, and 3. Numerically, TMT was maximum in the second month of lactation, but the large standard errors did not allow detection of significant differences (Table 3). The effect of MFC_{TYPE} affected both milking lengths, i.e. MET and TMT, with the shortest, the intermediate and the longest estimates obtained for P_1, P_2,

Table 2
Mean, standard error of mean (SEM), and significance[1] of fixed effects for the traits[2] recorded in Sarda ewes.

Trait	Mean	SEM	Lactation stage	Parity	Milk flow curve type	Year	Lactation stage × Parity	Parity × Milk flow curve type	Lactation stage × Milk flow curve type
MY, kg	0.59	0.01	***	*	*	ns	ns	ns	ns
MY_1, kg	0.50	0.01	***	*	ns	ns	†	ns	ns
PFR, kg/min	0.91	0.02	***	**	***	ns	ns	*	ns
AFR, kg/min	0.71	0.01	***	ns	***	ns	ns	ns	*
LT, min	0.27	0.01	**	ns	***	ns	ns	ns	ns
MET, min	0.74	0.02	***	ns	***	ns	ns	ns	ns
PPT, min	0.27	0.01	*	†	***	***	ns	ns	**
DPT, min	0.33	0.01	***	ns	ns	ns	ns	†	*
BT, min	0.39	0.02	ns	ns	*	ns	ns	ns	ns
TMT, min	1.46	0.03	*	ns	**	ns	ns	ns	ns

[1] *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; † $P < 0.10$; ns = not significant.
[2] MY = milk yield per milking; MY_1 = milk yield in the first 60 s of milking; PFR = peak milk flow rate; AFR = average milk flow rate; LT = lag time; MET = milk emission time; PPT = time of plateau phase; DPT = time of decline phase; BT = time of blind phase; TMT = total milking time.

Table 3
Least squares means (± standard error) of the milkability traits[1] estimated for the fixed effect of lactation stage. Superscript letters within row indicate significant difference at $P < 0.05$ according to the Bonferroni post-hoc comparison test.

Trait	Lactation stage					
	1	2	3	4	5	6
MY, kg	0.73 ± 0.03 ^{ab}	0.82 ± 0.03 ^{a}	0.70 ± 0.03 ^{b}	0.51 ± 0.04 ^{c}	0.43 ± 0.04 ^{cd}	0.28 ± 0.04 ^{d}
MY_1, kg	0.61 ± 0.03 ^{ab}	0.67 ± 0.02 ^{a}	0.59 ± 0.02 ^{b}	0.43 ± 0.03 ^{c}	0.36 ± 0.03 ^{c}	0.23 ± 0.04 ^{d}
PFR, kg/min	1.02 ± 0.06 ^{a}	1.10 ± 0.05 ^{a}	1.00 ± 0.05 ^{a}	0.80 ± 0.06 ^{b}	0.65 ± 0.07 ^{bc}	0.44 ± 0.07 ^{c}
AFR, kg/min	0.77 ± 0.05 ^{a}	0.77 ± 0.04 ^{a}	0.77 ± 0.04 ^{a}	0.62 ± 0.05 ^{b}	0.51 ± 0.06 ^{b}	0.44 ± 0.06 ^{b}
LT, min	0.25 ± 0.03 ^{b}	0.25 ± 0.03 ^{b}	0.26 ± 0.03 ^{b}	0.29 ± 0.04 ^{ab}	0.24 ± 0.04 ^{b}	0.40 ± 0.04 ^{a}
MET, min	0.89 ± 0.07 ^{ab}	0.99 ± 0.06 ^{a}	0.81 ± 0.06 ^{abc}	0.75 ± 0.07 ^{bc}	0.68 ± 0.08 ^{bc}	0.56 ± 0.07 ^{c}
PPT, min	0.37 ± 0.03 ^{a}	0.37 ± 0.03 ^{a}	0.34 ± 0.03 ^{a}	0.26 ± 0.03 ^{a}	0.26 ± 0.04 ^{a}	0.34 ± 0.04 ^{a}
DPT, min	0.36 ± 0.05 ^{ab}	0.47 ± 0.05 ^{a}	0.30 ± 0.05 ^{b}	0.33 ± 0.06 ^{ab}	0.25 ± 0.06 ^{a}	0.20 ± 0.06 ^{b}
BT, min	0.33 ± 0.07 ^{a}	0.35 ± 0.07 ^{a}	0.31 ± 0.06 ^{a}	0.29 ± 0.08 ^{a}	0.39 ± 0.09 ^{a}	0.33 ± 0.09 ^{a}
TMT, min	1.52 ± 0.11 ^{a}	1.68 ± 0.10 ^{a}	1.46 ± 0.10 ^{a}	1.39 ± 0.11 ^{a}	1.35 ± 0.13 ^{a}	1.29 ± 0.14 ^{a}

[1] MY = milk yield per milking; MY_1 = milk yield in the first 60 s of milking; PFR = peak milk flow rate; AFR = average milk flow rate; LT = lag time; MET = milk emission time; PPT = time of plateau phase; DPT = time of decline phase; BT = time of blind phase; TMT = total milking time.

and P_L curves (Table 4).

Length of PPT and DPT was variable across lactation stages and no linear trends were observed (Table 3). The MFC_{TYPE} affected PPT but no DPT (Table 4); in fact, PPT was the maximum in P_L, intermediate in P_2, and minimum in P_1. Furthermore, similarly to AFR, PPT was significantly affected by the interaction between lactation stage and MFC_{TYPE} (Fig. 2). Fig. 3 shows the LSM of PFR for the interaction between fixed effect of parity and MFC_{TYPE} ($P < 0.05$). Significant differences were observed within primiparous, with P_1 being characterized by a greater PFR compared to other curves (Fig. 3).

Moreover, parity tended ($P < 0.10$) to affect PPT, in fact the length of this phase was shorter in primiparous than multiparous (0.30 ± 0.03 *vs* 0.35 ± 0.02 min). Finally, BT was significantly affected only by MFC_{TYPE} (Table 2), with P_1 and P_L curves showing the longest (0.41 ± 0.05 min) and the shortest LSM (0.22 ± 0.07 min), respectively.

Table 4
Least squares means (± standard error) of the milkability traits[1] estimated for the fixed effect of milk flow curve type. Superscript letters within row indicate significant difference at $P < 0.05$ according to the Bonferroni post-hoc comparison test.

Trait	Milk flow curve type		
	Single peak (P_1)	Double peak (P_2)	Long plateau (P_L)
MY, kg	0.53 ± 0.02 ^{b}	0.58 ± 0.03 ^{ab}	0.61 ± 0.03 ^{a}
MY_1, kg	0.47 ± 0.02 ^{a}	0.48 ± 0.02 ^{a}	0.50 ± 0.03 ^{a}
PFR, kg/min	0.94 ± 0.04 ^{a}	0.80 ± 0.05^{b}	0.76 ± 0.05 ^{b}
AFR, kg/min	0.76 ± 0.04 ^{a}	0.64 ± 0.04 ^{b}	0.54 ± 0.05 ^{b}
LT, min	0.24 ± 0.02 ^{b}	0.24 ± 0.03 ^{b}	0.36 ± 0.03 ^{a}
MET, min	0.59 ± 0.05 ^{c}	0.79 ± 0.06 ^{b}	0.97 ± 0.07 ^{a}
PPT, min	0.17 ± 0.02 ^{c}	0.28 ± 0.03 ^{b}	0.52 ± 0.03 ^{a}
DPT, min	0.32 ± 0.04 ^{a}	0.27 ± 0.05 ^{a}	0.36 ± 0.05 ^{a}
BT, min	0.41 ± 0.05 ^{a}	0.37 ± 0.06 ^{ab}	0.22 ± 0.07 ^{b}
TMT, min	1.29 ± 0.08 ^{b}	1.48 ± 0.09 ^{ab}	1.57 ± 0.11 ^{a}

[1] MY = milk yield per milking; MY_1 = milk yield in the first 60 s of milking; PFR = peak milk flow rate; AFR = average milk flow rate; LT = lag time; MET = milk emission time; PPT = time of plateau phase; DPT = time of decline phase; BT = time of blind phase; TMT = total milking time.

4. Discussion

In the present study, milk flow curves of 544 ewes were classified according to Mačuhová et al. (2020). Briefly, curves showing single peak (P_1), double peak (P_2), and single peak with a long plateau phase (P_L) were compared in terms of milkability and milk yield. For this purpose, fixed effects included in the model accounted for variability related to lactation stage, parity, and farm, other than for MFC_{TYPE}. The criteria used to identify 'long' PPT differ across studies conducted in sheep; for instance, Marie-Etancelin et al. (2006); Tančin et al. (2011); Boselli et al. (2012), and Mačuhová et al. (2020) considered as P_L those curves with a PPT ≥ 17, 10, 30, and 20 s, respectively, i.e. PPT ≥ 0.28, 0.17, 0.5, and 0.33 min.

In agreement with Dzidic et al. (2004) and Mačuhová et al. (2012), in the present study the milk flow curve with the greatest frequency was P_1 followed by P_2 and P_L (Table 1). Overall, findings indicated that almost all the traits were affected by lactation stage and MFC_{TYPE}, while only few variables were affected by parity, namely MY, PFR and PPT (Table 2).

Evolution of MY across lactation in sheep has been extensively described in the literature (Pulina et al., 2007; Casu et al., 2008). The typical lactation curve shows a peak in early stages and a progressive reduction afterwards up to the end of lactation. In the present study, the trend of MY resembled that of MY_1. This was expected, since on average the amount of milk excreted in the first 60 s, i.e. MY_1, accounted for 85

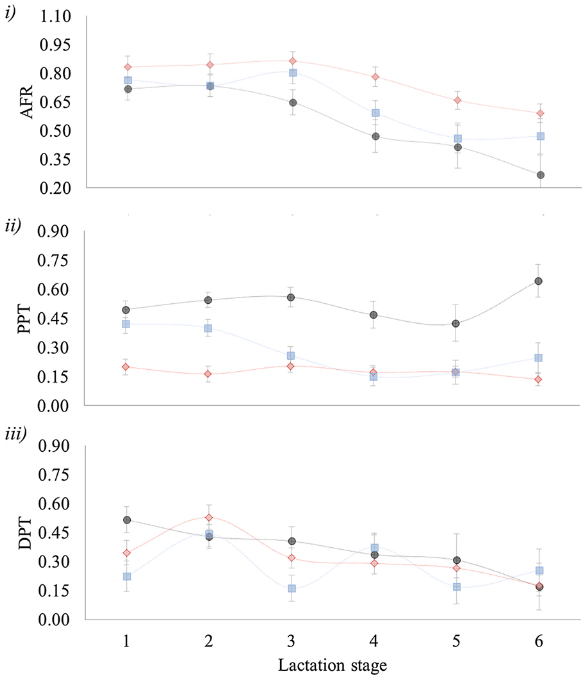

Fig. 2. Least squares means of *i)* average milk flow rate (AFR, kg/min), *ii)* plateau phase (PPT, min), and *iii)* decline phase (DPT, min) estimated for the interaction ($P < 0.05$) between fixed effect of milk flow type[1] and lactation stage. Standard errors ranged from 0.05 to 0.11, from 0.03 to 0.09, and from 0.05 to 0.14 for AFR, PPT, and DPT, respectively, and are graphically represented by bars.

[1] ◆ = single peak; ■ = double peak; ● = long plateau.

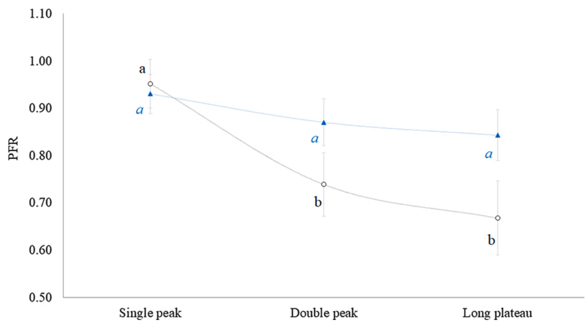

Fig. 3. Least squares means of peak milk flow rate (PFR, kg/min) estimated for the interaction ($P<0.05$) between fixed effect of milk flow type and parity. According to the Bonferroni *post hoc* comparison test: letters indicate significant ($P < 0.05$) differences among milk flow curves within primiparous (○, roman font) and within pluriparous (▲, italic font). Standard errors ranged from 0.04 to 0.08 and are graphically represented by bars.

% of MY (Table 2).

The estimated LSM of MY were comparable to those reported by Boselli et al. (2006) in Sarda and those observed in other breeds, namely Tsigai, Improved Valachian and Lacaune (Oravcová et al., 2006). The official Italian statistics of Sarda sheep report MY per lactation in 2020 equal to 291 ± 89 and 224 ± 80 l in Latium and Sardinia region, respectively (AIA, 2021) and an average production of 203 l for a 162-d lactation was reported for Italian Sarda ewes by Carta et al. (2009). Considering Assaf, Sarda, Lacaune, Comisana, and Sopravissana breed, Boselli et al. (2012) found an average MY of 0.44 kg/milking with differences between breeds. In particular, the minimum (0.22 ± 0.01 kg) and the maximum (0.64 ± 0.02 kg) MY were observed in Sopravissana and Assaf ewes, respectively. Literature shows that persistency of MY during lactation can be affected by a variety of factors, including rearing conditions, breed, health status, and milking practices (Oravcová et al., 2006; Cuccuru et al., 2011). Findings of this study highlighted that MY was greater in P_2 compared to P_1, corroborating what Mačuhová et al. (2008) found using data from Tsigai, Improved Valachian and their crosses with Lacaune ewes. Mačuhová et al. (2008, 2012) defined as “plateau I” those curves with peak milk flow rate greater than 0.4 l/min and with no clear distinction between the 2 peaks; animals with such a curve were found to be more productive in terms of MY (0.481 ± 0.045 L) than the others (Mačuhová et al., 2008). In addition, the same authors reported that MY_1 was significantly lower in single-peaked curves (0.169 ± 0.035 L) compared to double-peaked (0.302 ± 0.028 L) and “plateau I” curves (0.388 ± 0.043 L). According to the literature, the optimal oxytocin release seems to occur in P_L ewes, i.e. those where there is no clear separation between cisternal and alveolar milk fraction.

Both mean PFR and AFR (Table 2) were in accordance with previous studies on sheep, e.g. Caria et al. (2008) and Boselli et al., (2006, 2012). In fact, in multiparous Sarda ewes PFR and AFR were equal to 1.07 ± 0.45 kg/min and 0.55 ± 0.20 kg/min with working vacuum of 28 kPa. As indicated by Marie-Etancelin et al. (2006), PFR and AFR significantly decreased through the lactation, particularly after the third month, as a result of the decrease in intra-mammary cisternal pressure due to the progressive reduction of milk productivity. Such results are in agreement with Casu et al. (2008) who reported similar trends for 967 Sarda × Lacaune back-cross ewes. On the contrary, the milk flow rate in dairy cows is reported to be more stable across the lactation (Tančin et al., 2006) likely due to the different udder anatomy and milk storage and secretion. In fact, most of the milk excreted during milking is cisternal rather than alveolar in cows. Alveolar fraction derives from both the alveolar lumen and small ducts, while cisternal fraction is stored in large ducts, cistern, and teat cisterns. The cisternal milk fraction is excreted at the very beginning of milking, while the alveolar milk fraction is removed from the udder in response to an increase in plasma oxytocin concentration after stimuli (Bruckmaier and Blum, 1992). The cisternal milk fraction in dairy ewes typically varies between 59 and 77 % (Marnet and McKusick, 2001; Rovai et al., 2008) with variation across breeds due to different storage capacity (McKusick et al., 2002).

Both PFR and AFR were greater in P_1 curves than in other types (Table 4), suggesting that milk is released rapidly, in a short time window, with predominance of the cisternal rather than of the alveolar milk fraction. This can happen in high-producing animals in early lactation (Dzidic, 2019). Regarding this, a distribution of MFC_{TYPE} across lactation stages is reported in Table 5. Frequencies show that P_L are less frequent in late lactation animals, i.e. those with an overall lower MY (Table 4). The same animals present instead very high percentage of P_1 curves, e.g. 82 % in lactation stage 6. The significant Pearson correlation calculated between MY and AFR (0.44, Supplementary Table 1), between MY and PFR (0.63, Supplementary Table 1), and between AFR and PFR (0.75, Supplementary Table 1) demonstrated and supported the idea that there is a positive and strong association among the three traits.

Considering LT, the average (0.27 min) was lower than the one (0.48 min) reported by Marie-Etancelin et al. (2006) in different lines of Lacaune breed. Although not significantly affected by parity, LT seemed to increase from lactation stage 1 onwards likely due to changes in udder compartments filling degree along the lactation curve. Nevertheless, additional data are needed to confirm such trend.

The P_L curves were characterized by longer LT compared to P_1 and P_2

Table 5
Number of curve for each milk flow curve types across lactation stages.

Milk flow curve type	Lactation stage					
	1	2	3	4	5	6
Single peak (P_1)	36	36	54	49	66	71
Double peak (P_2)	20	27	22	23	11	10
Long plateau (P_L)	39	36	21	11	5	6

ones (Table 4). This difference can be attributed to either peculiar anatomical characteristics of teat canal (Caja et al., 2000) or physiological reasons (Bruckmaier et al., 1997).

According to Table 2, MET represented more than half of TMT. This is in agreement with a study carried out on Sarda breed (Caria et al., 2008), where authors reported similar values under different working vacuum level, i.e. MET of 0.89 and 076 min, respectively. Average TMT (Table 2) was similar to that reported by Casu et al. (1.44 min; 2008) and Boselli et al. (1.42 min; 2012). The same was greater compared to TMT obtained in Caria et al. (2008) under different vacuum levels, i.e. 1.09 and 0.93 min. Based on the LSM reported in Table 3, the ratio between MET and TMT numerically reduced during the lactation, being 0.56, 0.54, 0.51, 0.51, 0.45, and 0.39 in lactation stage 1, 2, 3, 4, 5, and 6, respectively (data not shown). This means that, regardless of total milking length, milk was removed in a shorter time in late compared to early lactation. Regarding MFC_{TYPE}, milk was ejected in a shorter time in the P_1 class, but no differences were observed among curves in terms of TMT (Table 4).

Finally, MFC_{TYPE} significantly affected BT, i.e. the indicator of overmilking recorded from end of declining phase to the moment where milk flow reached 0.10 kg/min. The BT was significantly lower in P_L compared to P_1 but at the same time was similar to P_2 (Table 4). Differences between P_L and P_2 and between between P_1 and P_2 resulted not significant likely due to large standard errors of the LSM. Confirming this finding, it is quite obvious that ewes with high PFR and at the same time low MET (P_1 curves) are at greater risk of overmilking than the others.

It derives that monitoring milk emission would be an optimal practice in order to adjust the TMT and other milking practices based on the MFC_{TYPE} and to avoid overmilking, particularly in ewes with the P_1 curve. In large herds, milking ewes according to their lactation stage is a common practice, however this does not allow to implement customized milking practices based on the individual specific milkability. Ideally, a potential efficient strategy would be to separate lactating animals based on both lactation stage or productivity level and MFC_{TYPE} allowing tailored milking procedures to be adopted within P_1, P_2, and P_L groups. Although this is demanding in practical terms and feasibly implementable only in large and well managed farms,it is worth to mention that the average herd size is sizeable in sheep farms, with 167.8 ewes/farm officially recorded in 2020 in Sardinia, i.e. the Italian region that delivers the vast majority of the milk used for production of the Pecorino Romano PDO cheese that in 2020 hosted the 76 % of the national Sarda ewes under official recording (AIA, 2021). Additionally, when focusing on the Sarda breed exclusively, the average herd size increases up to 170.3 ewes/farm (AIA, 2021).

5. Conclusion

The aim of the present study was to estimate the effect of MFC_{TYPE} on milkability traits recorded in Sarda ewes farmed in the production area of Pecorino Romano PDO cheese (Italy). The lowest MY was found in ewes with a P_1 curve, which were characterized by the highest PFR and AFR and the shortest MET compared to those with either a P_2 or a P_L curve. Moreover, overmilking was longer and thus more unfavourable in presence of a P_1 curve. Farmers are recommended to pay more attention to milk emission curves to limit the overmilking within the herd, particularly in animals that show P_1 curves. The farms present in the PDO area are characterized by sizeable herd size, thus separating animals based on MFC_{TYPE} and lactation stage would be feasible and would result in optimal milking. In fact, in this way specific customized milking practices could be adopted within groups. In the long term, monitoring milk emission curves and adopting tailored practices will i) improve milkability, ii) limit mammary gland stress and incidence of related issues, and iii) reduce antimicrobial use due to poor udder health in ewes farmed in the area of the Pecorino Romano PDO cheese.

Author contribution

Angela Costa: Data curation; Formal analysis; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing **Carlo Boselli:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Validation; Visualization; Roles/Writing - original draft **Massimo De Marchi:** Writing - review & editing **Giuseppe Todde**: Conceptualization; Writing - review & editing **Maria Caria:** Visualization; Writing - review & editing

Funding

This work was supported by the “Fondo di Ateneo per la Ricerca 2020” of University of Sassari.

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.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.smallrumres.2021.106584.

References

Associazione Italiana Allevatori (AIA), 2021. Statistiche Ufficiali. http://bollettino.aia.it/Contenuti.aspx?CD_GruppoStampe=RS&CD_Specie=C4.

Boselli, C., Rosati, R., Giangolini, G., Filippetti, F., Amatiste, S., Fagiolo, A., 2006. Reliefs on the electrical conductivity in sarda milk sheep in the lazio region – Italy. In: Proceedings of the 27th IDF World Dairy Summit and Congress. 16-23 October 2006, Shanghai, China.

Boselli, C., Giangolini, G., Giacinti, G., Filippetti, F., Bicocchi, R., Campagna, M.C., Amatiste, S., 2012. Curve Di Emissione Del Latte Registrate in Allevamenti Ovine, in: Proceedings of the Italian Society of Sheep and Goat Pathology and Production (SIPAOC), 26-29 September 2012, Siracusa, Italy.

Boselli, C., De Marchi, M., Costa, A., Borghese, A., 2020. Study of milkability and its relation with milk yield and somatic cell in Mediterranean Italian water buffalo. Front. Vet. Sci. 7, 432. https://doi.org/10.3389/fvets.2020.00432.

Bruckmaier, R.M., Blum, J.W., 1992. B-mode ultrasonography of mammary glands of cows, goats and sheep during α- and β- adrenergic agonist and oxytocin administration. J. Dairy Res. 59, 151–159. https://doi.org/10.1017/S0022029900030399.

Bruckmaier, R.M., Paul, G., Mayer, H., Schams, D., 1997. Machine milking of Ostfriesian and Lacaune dairy sheep: udder anatomy, milk ejection and milking characteristics. J. Dairy Res. 64, 163–172. https://doi.org/10.1017/s0022029997002173.

Caja, G., Such, X., Rovai, M., 2000. Udder morphology and machine milking ability in dairy sheep. In: Proceedings of the 6th Great Lakes Dairy Sheep Symposium, 2-4 November 2000. Guelph, Ontario, Canada.

Caria, M., Murgia, L., Pazzona, A., 2008. Effect of low vacuum on sheep milking. In: Proceedings of the International Conference on Innovation Technology to Empower Safety, Health and Welfare in Agriculture and Agro-Food Systems. 15-17 September 2008, Ragusa, Italy.

Carta, A., Casu, S., Salaris, S., 2009. Invited review: current state of genetic improvement in dairy sheep. J. Dairy Sci. 92, 5814–5833. https://doi.org/10.3168/jds.2009-2479.

Casu, S., Marie-Etancelin, C., Robert-Granié, C., Barillet, F., Carta, A., 2008. Evolution during the productive life and individual variability of milk emission at machine milking in Sardinian×Lacaune back-cross ewes. Small Rumin. Res. 75, 7–16. https://doi.org/10.1016/j.smallrumres.2007.07.003.

CLAL, 2021. Italy: import/export Summary. https://www.clal.it/en/?section=riepilogo_istat.

Cuccuru, C., Meloni, M., Sala, E., Scaccabarozzi, L., Locatelli, C., Moroni, P., Bronzo, V., 2011. Effects of intramammary infections on somatic cell score and milk yield in Sarda sheep. N. Z. Vet. J. 59, 128–131. https://doi.org/10.1080/00480169.2011.562862.

Dzidic, A., Rovai, M., Poulet, J.L., Leclerc, M., Marnet, P.G., 2019. Milking routines and cluster detachment levels in small ruminants. Animal 13, 86–93. https://doi.org/10.1017/S1751731118003488.

Herve, L., Lollivier, V., Quesnel, H., Boutinaud, M., 2018. Oxytocin induces mammary epithelium disruption and could stimulate epithelial cell exfoliation. J. Mammary Gland Biol. Neoplasia 23, 139–147. https://doi.org/10.1007/s10911-018-9400-8.

Kulinová, K., Mačuhová, L., Uhrinčať, M., Tančin, V., 2012. The effect of stressful treatment before and during milking on milkability of dairy ewes. Vet. ir Zootech. 57, 39–43. https://vetzoo.lsmuni.lt/data/vols/2012/57/pdf/kulinova.pdf.

Mačuhová, L., Uhrinčať, M., Mačuhová, J., Margetín, M., Tančin, V., 2008. The first observation of milkability of the sheep breeds Tsigai, Improved Valachian and their crosses with Lacaune. Czech J. Anim. Sci. 53, 528–536. https://doi.org/10.17221/369-CJAS.

Mačuhová, L., Tančin, V., Uhrinčať, M., Mačuhová, J., 2012. The level of udder emptying and milk flow stability in Tsigai, Improved Valachian, and Lacaune ewes during machine milking. Czech J. Anim. Sci. 57, 240–247. https://doi.org/10.17221/5922-cjas.

Mačuhová, L., Tančin, V., Mačuhová, J., Uhrinčať, M., Oravcová, M., Vršková, M., Tvarožková, K., 2020. Effect of somatic cell count on milkability and milk composition of ewes. Potr. S. J. F. Sci. 14, 1035–1041. https://doi.org/10.5219/1411.

Marie-Etancelin, C., Manfredi, E., Aurel, M.R., Pailler, F., Arhainx, J., Ricard, E., Lagriffoul, G., Guillouet, P., Bibe, B., Barillet, F., 2006. Genetic analysis of milking ability in Lacaune dairy ewes. Genet. Sel. Evol. 38, 183–200. https://doi.org/10.1186/1297-9686-38-2-183.

Marnet, P.G., McKusick, B.C., 2001. Regulation of milk ejection and milkability in small ruminants. Livest. Prod. Sci. 70, 125–133. https://doi.org/10.1016/S0301-6226(01)00205-6.

McKusick, B.C., Thomas, D.L., Berger, Y.M., Marnet, P.G., 2002. Effect of milking interval on alveolar versus cisternal milk accumulation and milk production and composition in dairy ewes. J. Dairy Sci. 85, 2197–2206. https://doi.org/10.3168/jds.S0022-0302(02)74299-9.

Olechnowicz, J., 2012. Chapter 10: the course of machine milking in small ruminants. In: Chaiyabutr, N. (Ed.), Milk Production – an up-to-Date Overview of Animal Nutrition, Management and Health. Headquarters IntechOpen Limited, London, UK, pp. 185–216.

Oravcová, M., Margetín, M., Peškovičová, D., Daňo, J., Milerski, M., Hetényi, L., Polák, P., 2006. Factors affecting milk yield and ewe's lactation curves estimated with test-day models. Czech J. Anim. Sci. 51, 483–490. https://doi.org/10.17221/3968-CJAS.

Pazzona, A., Caria, M., Murgia, L., 2009. Effects of a low vacuum level on vacuum stability and milking parlor performance for sheep. Trans ASABE. 52, 247–252.

Pulina, G., Nudda, A., Macciotta, N.P.P., Battacone, G., Rassu, S.P.G., Cannas, A., 2007. Non-nutritional factors affecting lactation persistency in dairy ewes: a review. Ital. J. Anim. Sci. 6, 115–141. https://doi.org/10.4081/ijas.2007.115.

Pulina, G., Atzori, A.S., Dimauro, C., Ibba, I., Gaias, G.F., Correddu, F., 2021. The milk fingerprint of Sardinian dairy sheep: quality and yield of milk used for Pecorino Romano P.D.O. Cheese production on population-based 5-year survey. Ital. J. Anim. Sci. 20, 171–180. https://doi.org/10.1080/1828051X.2021.1875896.

Rovai, M., Caja, G., Such, X., 2008. Evaluation of udder cisterns and effects on milk yield of dairy ewes. J. Dairy Sci. 91, 4622–4629. https://doi.org/10.3168/jds.2008-1298.

Steidle, E., Goeft, H., Immler, S., Rosenberger, E., Korndoerfer, R., Duda, J., Trogere, F., Bruckmaier, R., Worstorff, H., Model, I., Harch, M., Deneke, J., 2000. . Lactation Consulting With Milk Flow Curves. Bayrische Landesanstalt Fuer Tierzucht Grub. http://www.lactocorder.ch/pdf/MEEN.zip.

Tančin, V., Ipema, B., Hogewerf, P., Mačuhová, J., 2006. Sources of variation in milk flow characteristics at udder and quarter levels. J. Dairy Sci. 89, 978–988. https://doi.org/10.3168/jds.S0022-0302(06)72163-4.

Tančin, V., Mačuhová, L., Oravcová, M., Uhrinčať, M., Kulinová, K., Roychoudhury, S., Marnet, P.G., 2011. Milkability assessment of Tsigai, improved valachian, Lacaune and F1 crossbred ewes (tsigai × lacaune, improved valachian × lacaune) throughout lactation. Small Rumin. Res. 97, 28–34. https://doi.org/10.1016/j.smallrumres.2011.01.007.