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RESEARCH ARTICLE

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Characterisation of milk ejection dynamics in dairy buffaloes based on *Lactocorder®* measurements

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ABSTRACT

Current milking protocols for dairy buffaloes are often adapted from cattle, despite physiological and behavioural differences between the species. This study aimed to characterise milk ejection dynamics in Italian Mediterranean buffaloes and provide indications for species-specific milking protocols. Data was collected from 798 lactating buffaloes across four commercial farms between March and September 2024, for a total of 2768 individual milking records. Milk ejection traits were measured using a *Lactocorder®* device, recording peak milk flow rate (HMF), maximum milk yield per minute (HMG), total milking time (tMGG), milk yield during the first three minutes, and overmilking, defined as the time from milk flow <0.20 kg/min to cluster detachment. Traits were analysed using a generalised mixed model including farm, days in milk (DIM), parity, milk yield class, and month as fixed effects. On average, 21% of milk was released in the first minute, 46% by the second, and 66% by the third. Farms differed significantly in tMGG and overmilking ($p < 0.05$), which in turn were positively correlated ($r = +0.43$). HMF and HMG peaked in early lactation and declined over time ($p < 0.05$), whereas DIM did not affect tMGG or overmilking. Parity influenced most traits: second-parity buffaloes had the highest HMF and HMG (1.82 and 1.66 kg/min), while tMGG was longest in ≥5-parity buffaloes (7.83 min) and shortest in primiparous animals (7.00 min). These results indicate that extended milking may be unnecessary, increasing overmilking risk, and that multiparous buffaloes require closer monitoring and farm-specific detachment settings to improve efficiency, udder health, and welfare.

HIGHLIGHTS

- Current cattle-derived milking protocols do not reflect buffalo physiology.
- Tailored protocols can improve efficiency, udder health, and welfare in buffaloes.

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Flowrate; lactation; milk flow; overmilking; welfare

1. Introduction

Buffalo breeding represents a key economic sector in Italy, particularly in the southern regions, where most of buffalo farms are located (CLAL 2023). Italian Mediterranean buffalo population exceeds 430,000 animals (BDN 2025), with approximately 121,000 individuals officially monitored by the Italian National Breeders Association (ANASB 2025). In recent years, this sector has shown remarkable growth, as evidenced by the increase in the national number of reared buffaloes from 402,796 in 2019 to 434,773 in

2025, driven by constant management development and increasing consumer demand for high-value buffalo-derived products, especially 'mozzarella di Bufala Campana' (Zicarelli 2021; VETINFO 2025). Mozzarella cheese obtained protected designation of origin status (PDO) in 1996 (EC Regulation No. 1107/96) and is currently the third most economically valuable and the fourth most produced PDO product in Italy.

To sustain the growing market and improve farm profitability, it is essential to enhance both the efficiency and quality of milk production while

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safeguarding animal welfare (Matera et al. 2023), by preventing pain and minimise stress during milking. Achieving this goal requires an integrated strategy that combines optimised management practices, the adoption of innovative technologies, and close consideration of the physiological and behavioural specificities of buffalo species. In this context, milking protocols play a crucial role, as they can directly affect milk yield, composition, and udder health (Edwards et al. 2013; Moore-Foster et al. 2019). These protocols not only involve the routine management of animals during milking but also include the technical configuration of the milking system, such as vacuum level, pulsation ratio, stimulation time, and cluster removal settings. When inadequately managed, for instance, prolonged machine-on time can lead to overmilking, which negatively impacts udder health by causing teat tissue congestion and delaying teat canal closure, thereby increasing the risk of intramammary infections (Odorčić et al. 2019).

Despite anatomical and physiological differences between dairy buffaloes and cows, milking protocols applied to buffaloes are largely adapted from those originally developed for dairy cattle (Caria et al. 2011; Costa et al. 2020a). Buffaloes exhibit distinct udder morphology, characterised by less developed suspensory ligament (Borghese et al. 2013) and a predominance of the rear quarters, which contain 25%–50% more secretory tissue than the forequarters and contribute to 55%–60% of the total milk secretion (Napolitano et al. 2022; FAO 2024; Mota-Rojas et al. 2024). Furthermore, while cows store a considerable volume of milk in the gland cistern, buffaloes retain approximately 90%–95% of their milk in the alveolar compartments (Ambord et al. 2010; Mota-Rojas et al. 2024), necessitating active ejection stimulated by oxytocin. As a result, buffaloes generally require longer pre-stimulation before milking to ensure effective milk letdown, and exogenous oxytocin is required to facilitate milk release (Boselli et al. 2004). This condition, in which alveolar milk is expelled only after the cisternal fraction has been emptied, may produce a characteristic bimodal milk flow curve, characterised by an initial drop in flow rate followed by a second peak as alveolar milk is finally released into the teat cistern (Sandrucci et al. 2007; Tančin et al. 2007). The presence of bimodality, together with elevated somatic cell count ($>500 \times 10^3$ cells/mL), has been associated with reduced peak flow rates and prolonged overmilking phases, thereby exacerbating stress on udder tissues and reducing milking efficiency (Tančin et al. 2007).

Several factors may influence the onset of delayed milk ejection, and in turn bimodality, including parity, lactation stage, milk yield, and variations in milking protocols or equipment settings (Wieland et al. 2022). While such aspects have been widely studied in dairy cattle, leading to the extensive optimisation of milking protocols (Dzidic et al. 2004; Bobić et al. 2020; Wieland et al. 2021), similar efforts in dairy buffaloes remain limited, highlighting a significant knowledge gap (Matera et al. 2023). In addition, although buffaloes have traditionally been considered less susceptible to mastitis than dairy cows, a recent study indicated a rising prevalence of subclinical intramammary infections in this species (Costa et al. 2020b). This emerging trend underscores the need to develop and implement milking protocols specifically tailored to the anatomical and physiological characteristics of buffaloes. The *Lactocorder*[®], a portable ICAR-certified electronic milk flow meter, offers a non-invasive and reliable method to monitor key milking ejection traits, including milk flow rate, total milking and overmilking times, while providing a detailed graphical representation of milk flow curves (Wieland et al. 2024). The present study aimed to investigate milk ejection dynamics in Italian Mediterranean buffaloes using the *Lactocorder*[®] device, with the focus on identifying the main factors contributing to its variability. These insights are intended to support the development of milking protocols specifically adapted to the physiological characteristics and needs of dairy buffaloes.

Materials and methods

Milking data was collected during routine operations on commercial farms, therefore, ethical approval for the use of experimental animals was not required.

Farm description and data collection

The study was conducted in collaboration with the Associazione Italiana Allevatori (AIA) and the Associazione Regionale Allevatori Campanie e Molise (ARACM). Data was collected between March and September 2024 from four commercial buffalo farms (designated as Farm 1 to 4) located in the province of Salerno (Campania, Italy). Individual milking parameters were collected during the official test-day sessions. All farms operated under comparable management conditions, were equipped with 3+3 tandem milking parlours, and did not use automatic milking cluster removal systems. Average vacuum

pump levels were 43.0 ± 2.35 kPa on Farm 1, 44.0 ± 2.14 kPa on Farm 2, 38.4 ± 0.71 kPa on Farm 3, and 40.0 ± 2.63 kPa on Farm 4. Farms 1 and 2 used a pulsation ratio of 60:40, whereas Farms 3 and 4 operated with a 65:35 ratio. All farms employed single-mode pulsators operating at 60 cycles per minute and did not use oxytocin during the studied period. Somatic cell counts, measured in bulk milk throughout the study period, averaged $169,500 \pm 39,000$ cells/mL on Farm 1, $85,500 \pm 34,000$ cells/mL on Farm 2, and $83,200 \pm 31,900$ cells/mL on both Farms 3 and 4.

Milking ejection traits were recorded during each individual milking session through the *Lactocorder*[®] device (Lactocorder S, WMB AG, Switzerland). The device was installed at the milking point, positioned between the milk claw and the milk line before each milking session. Animal identification was managed using a radio frequency identification system (Global Super Maxi Female, Allflex, Madison, USA), which automatically registered individual data including date of birth, days in milk (DIM) and parity. To ensure measurement accuracy, the *Lactocorder*[®] was calibrated prior to the beginning of the trial and re-checked at regular intervals following the manufacturer's (https://www.quality-certification.com/meter_manuals/lactocordermanual.pdf) and ICAR guidelines (<https://www.icar.org/certifications/milk-meters/>). The same device was used across all farms, and calibration was verified before each recording session by testing with standardised reference weights and volumes. All data were processed with LactoPro 6.0.95 software (WMB AG, Switzerland). Moreover, to guarantee uniformity and minimise operator-related variability, a single trained technician was responsible for device handling, data acquisition, and subsequent processing throughout the entire study. The following milking traits were extracted: peak milk flow (HMF, kg/min), defined as the highest instantaneous milk flow rate recorded during milking session; maximum milk flow over one continuous minute (HMG, kg/min), representing the highest average milk flow sustained over one-minute interval; milk yield during the first, second, and third minutes of milking (1MG, 2MG and 3MG, kg), determined based on flow measurements recorded from milking cluster attachment; total milking time (tMGG, min), calculated as the time elapsed from teat cup attachment to detachment; overmilking (min) was calculated as the sum of two parameters recorded by the *Lactocorder*[®]: (i) post-stripping time, corresponding to the interval between the decline of milk flow below 0.20 kg/min and its complete cessation at 0 kg/min

and (ii) blank milking time, defined as the period during which the milking cluster remains attached to the teats in the absence of milk flow. A graphical representation of the variables is presented in Figure 1.

Data manipulation and editing

Records with implausible values were excluded from the analysis. Specifically, only data with HMF greater than 0.20 kg/min, considered a standard threshold for milk flow detection (Upton et al. 2025), and tMGG between 1 and 30 min were retained. Moreover, buffaloes over 300 DIM were excluded from the analysis, as the standard lactation length for buffaloes is conventionally set at 270 days. Subsequently, DIM were classified into ten categories of 30 days each (from day 5 to 300). Parity was categorised into five classes, with the fifth class including all animals in their fifth or greater lactations (≥ 5). To assess the influence of milk yield on milk ejection traits, animals were stratified into five production classes according to the percentile distribution of their total milk yield: class 1 (1.00–6.80 kg), class 2 (6.81–8.50 kg), class 3 (8.51–10.1 kg), class 4 (10.2–12.4 kg), and class 5 (>12.4 kg). Following data cleaning, the final dataset included 2,768 milking records from 798 buffaloes, of which 25.1% were primiparous and 74.9% multiparous. The proportion of primiparous and multiparous animals was comparable among farms (Supplementary Figure 1), ensuring homogeneous herd structures and minimising potential bias arising from farm-specific parity composition. The distribution of milking records across farms was balanced, with 834, 477, 855 and 602 observations respectively from Farm 1, Farm 2, Farm 3, and Farm 4. Furthermore, approximately 80% of buffaloes contributed with at least two milking records (Supplementary Figure 2). On average, each animal was represented by 2.64 ± 1.84 records (range: 1–11), providing within-animal replication that improved the robustness of the analyses and the overall representativeness of the sample.

Statistical analysis

All statistical analyses were conducted using R software (version 2023.03.0; RStudio PBC, Boston, MA). Data visualisation and editing were performed with the *tidyverse* and *dplyr* packages (Wickham et al. 2016). To evaluate the sources of variation in milk ejection traits, the following generalised mixed model with repeated measurements was fitted:

$$Y_{ijklmn} = \mu + Farm_i + DIM\ class_j + Parity_k + Milk\ class_l \\ + Month_m + Animal\ ID_n + e_{ijklmn},$$

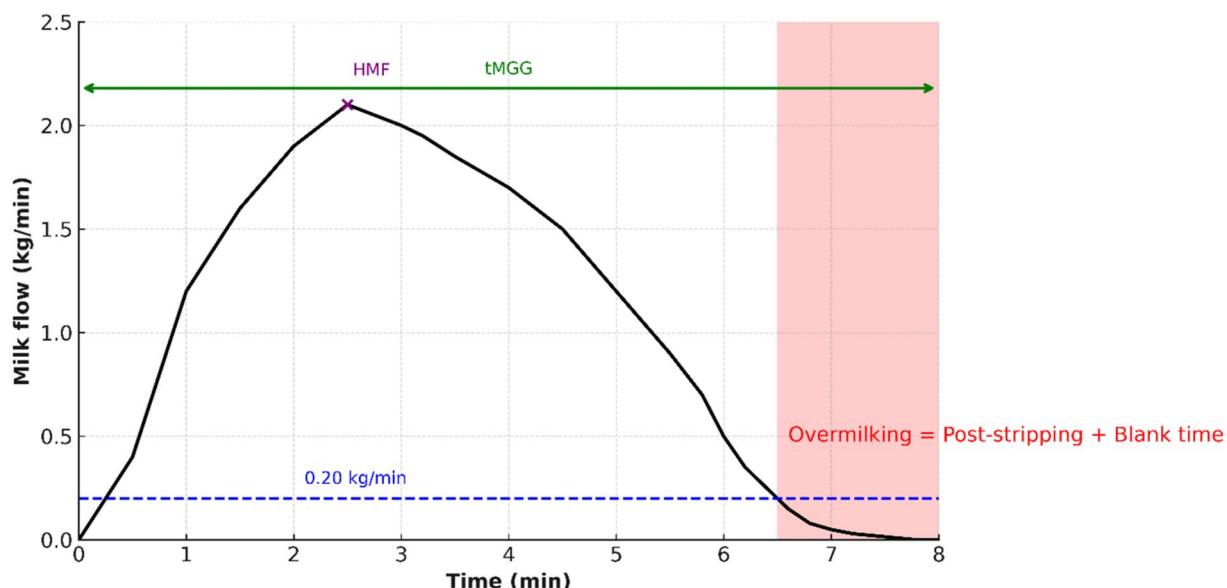


Figure 1. Illustrative milk flow curve recorded with *Lactocorder*® device, highlighting the main milking traits: total milking time (tMGG), post-stripping phase (from milk flow <0.20 kg/min to 0 kg/min), blank time (interval when the milking unit remains attached in the absence of milk flow to detachment), and peak milk flow rate (HMF).

where y_{ijklm} is the dependent variable (HMF, HMG, tMGG, overmilking); μ is the population mean for the evaluated traits; $Farm_i$ is the fixed effect of the i^{th} farm of sampling ($i = \text{from 1 to 4}$); $DIM\ class_j$ is the fixed effect of the j^{th} class of DIM ($j = \text{from class 1 to 10}$); $Parity_k$ is the fixed effect of the k^{th} parity order of the animals ($k = \text{from 1 to 5}$); $Milk\ class_l$ is the fixed effect of the l^{th} class of milk yield ($l = \text{from class 1 to 5}$); $Month_m$ is the fixed effect of the m^{th} month of sampling (March, April, May, June, July, September); $Animal\ ID_n$ is the uncorrelated random effect of the n^{th} $Animal \sim N(0, \sigma^2_{Animal\ ID})$, where $\sigma^2_{Animal\ ID}$ is the animal variance; and e_{ijklmn} is the random residual $\sim N(0, \sigma^2_e)$, where σ^2_e is the residual variance. Each dependent variable was analysed in a separate model. Multicollinearity among fixed effects was assessed prior to model fitting, with all predictors showing variance inflation factor values below 2, indicating the substantial absence of collinearity. Because the distribution of overmilking was right-skewed, a \log_2 transformation was applied to normalise the data, and results were then back-transformed for interpretation. Pairwise comparisons among factor levels were performed using the least significant difference (LSD) *post-hoc* test, with statistical significance declared at $p < 0.05$.

Results

Descriptive statistics of milk ejection traits

Table 1 presents the descriptive statistics of the milk ejection traits recorded using the *Lactocorder*® device.

All evaluated parameters displayed moderate variability, as evidenced by coefficients of variation (CV) consistently exceeding 35.0%. Among the analysed traits, tMGG displayed the lowest variability (CV = 37.4%), whereas overmilking duration was the most variable, with a CV of 49.0%. The average tMGG was 7.35 min, with values ranging from 0.37 to 24.4 min, while the mean duration of overmilking was 2.09 min. As illustrated in Figure 2, milk production during the first three minutes of milking (1MG, 2MG, and 3MG) progressively decreased as DIM increased. On average, 21% of the milk yield was ejected within the first minute, 46% by the second, and 66% by the end of the third minute. Buffaloes within the first 30 days recorded the highest 1MG, averaging 1.15 kg, significantly exceeding all other DIM classes ($p < 0.05$). Similarly, animals between 30 and 60 DIM demonstrated the highest 2MG and 3MG (2.56 and 3.86 kg, respectively) compared to other DIM classes. Parity order did not have a significant impact on milk production during the first three minutes of milking ($p > 0.05$; Table 2).

Sources of variation of milking ejection traits

Supplementary Table 1 reports the significance levels of minimum selected models. Farm effect significantly affects HMG, tMGG and overmilking ($p < 0.05$). Both DIM class and parity significantly affected HMF and HMG ($p < 0.05$), whereas only parity had a significant effect on tMGG ($p < 0.05$). Milk yield class and month

of sampling were both significant in explaining the variability of all analysed traits ($p < 0.05$), except for overmilking which was not affected by milk yield-classes ($p > 0.05$).

The least squares means of HMF, HMG, tMGG and overmilking according to farm effect are presented in Table 3. The highest HMG values were observed in Farms 2 and 4 (1.65 and 1.59 kg/min, respectively), while Farm 3 recorded the longest tMGG and

overnilking duration (7.97 and 2.16 min, respectively). In contrast, Farm 1 exhibited the lowest level of overmilking (1.43 min). Figure 3 depicts the effect of DIM on HMF, HMG, tMGG and overmilking duration. The greatest values of HMF and HMG were recorded in the earliest stage of lactation, specifically between DIM 0 and 30 ($p < 0.05$). Whereas both HMF and HMG significantly declined as DIM increased ($p < 0.05$). On the contrary, no significant differences were observed across DIM classes for tMGG and overmilking duration ($p > 0.05$). The least squares means of HMF, HMG, tMGG and overmilking duration according to parity order effect are presented in Table 4. The highest values of both HMF and HMG were recorded in second-parity buffaloes in respect to other parity classes

Table 1. Descriptive statistics of the milk ejection traits collected with *Lactocorder*^{®1} device.

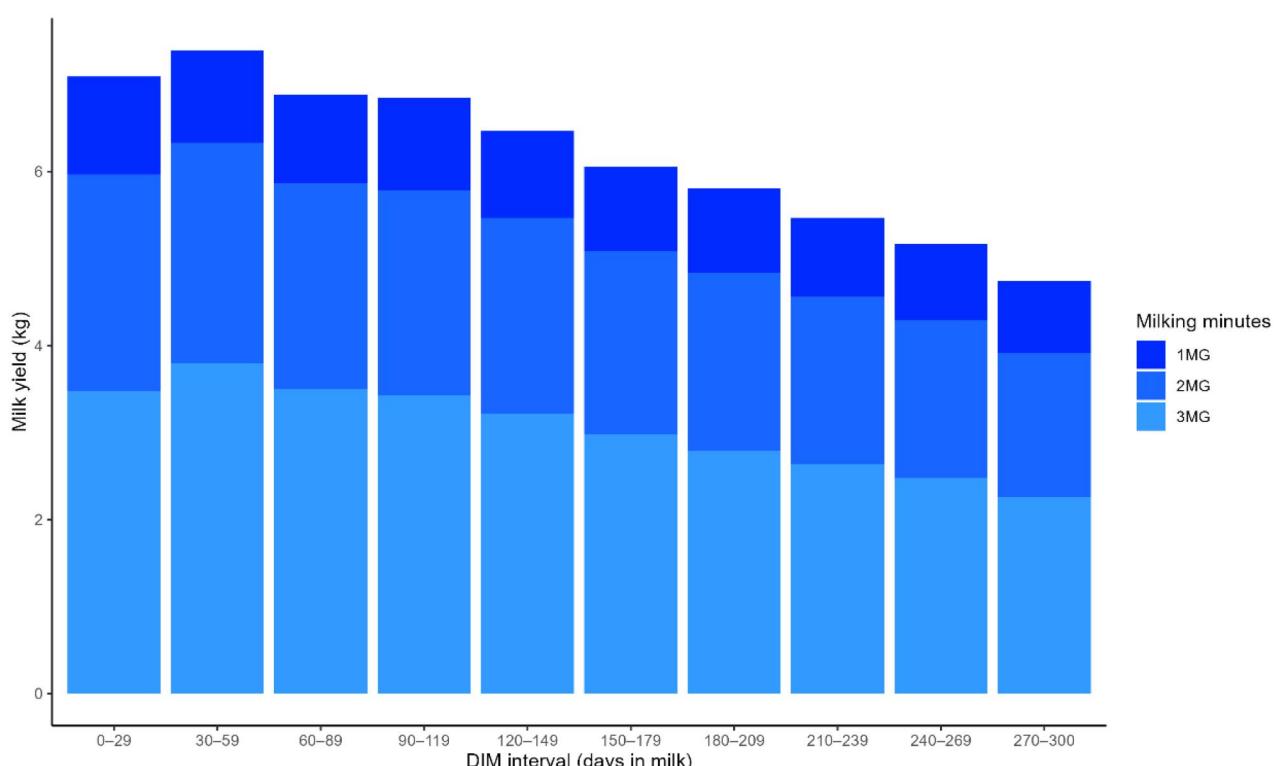
Traits ¹	Mean	Minimum	Maximum	SD
HMF (kg/min)	1.77	0.22	6.30	0.80
HMG (kg/min)	1.59	0.21	5.73	0.68
tMGG (min)	7.35	0.37	24.4	2.75
1MG (kg)	1.01	0.05	3.55	0.57
2MG (kg)	2.25	0.08	6.80	1.09
3MG (kg)	3.22	0.08	9.52	1.39
Overmilking (min)	2.09	0.05	5.00	1.03

¹*Lactocorder*[®] milk ejection traits: HMF, highest instantaneous milk flow rate recorded during the milking session; HMG, highest average milk flow sustained over one-minute interval; 1MG, 2MG, and 3MG, daily milk yield during the first, second, and third minutes of milking; tMGG, total milking time calculated as the time elapsed from teat cup attachment to detachment; overmilking: calculated as the sum of two parameters recorded by the *Lactocorder*[®](i) post-stripping time, corresponding to the interval between the decline of milk flow below 0.20 kg/min and its complete cessation at 0 kg/min and (ii) blank milking time, defined as the period during which the milking cluster remains attached to the teats in the absence of milk flow.

Table 2. Proportion of milk yield ejected during the first three minutes of milking¹ according to the effect of parity order.

Traits ¹	Parity				
	1	2	3	4	≥ 5
1MG (%)	22.0	21.6	20.9	20.0	19.4
2MG (%)	48.6	48.8	46.9	44.1	42.8
3MG (%)	69.3	68.8	66.9	63.6	62.7

¹1MG, 2MG, and 3MG, represent the proportion of the milk yield released during the first, second, and third minutes of milking, respectively.



¹1MG, 2MG, and 3MG, represent the proportion of the milk yield released during the first, second, and third minutes of milking, respectively.

Figure 2. Milk yield (kg) during the first, second, and third minutes of milking¹ according to the effect of days in milk (DIM) classes.

Table 3. Least square means and standard error of the mean (*SEM*) of *Lactocorder®* milk ejection traits¹ according to farm effect.

Traits ¹	Farm				<i>SEM</i>
	1	2	3	4	
HMF (kg/min)	1.72	1.80	1.67	1.76	0.03
HMG (kg/min)	1.50 ^b	1.65 ^a	1.52 ^b	1.59 ^a	0.02
tMGG (min)	7.23 ^b	7.09 ^b	7.97 ^a	7.12 ^b	0.22
Overmilking (min)	1.43 ^c	1.83 ^b	2.16 ^a	2.09 ^{ab}	0.17

¹*Lactocorder®* milk ejection traits: HMF, highest instantaneous milk flow rate recorded during the milking session; HMG, highest average milk flow sustained over one-minute interval; tMGG, total milking time calculated as the time elapsed from teat cup attachment to detachment; overmilking: calculated as the sum of two parameters recorded by the Lactocorder®(i) post-stripping time, corresponding to the interval between the decline of milk flow below 0.20 kg/min and its complete cessation at 0 kg/min and (ii) blank milking time, defined as the period during which the milking cluster remains attached to the teats in the absence of milk flow.

^{a-c}Least-square means with different superscript letters within a row differ significantly according to LSD *post-hoc* multiple comparison adjustment ($p < 0.05$).

($p < 0.05$; 1.82 and 1.66 kg/min, respectively), while tMGG increased as parity order increased, with the longest tMGG observed in animals beyond the fifth parity ($p < 0.05$; 7.83 min). In contrast, overmilking duration did not significantly differ across parity classes ($p < 0.05$). Table 5 reports the least squares means of milk ejection traits according to milk yield classes. A significant increase in HMF, HMG, and tMGG was observed with increasing milk yield ($p < 0.05$). In contrast, overmilking duration did not differ significantly among milk yield classes ($p > 0.05$), representing the only trait not following the general trend.

Discussion

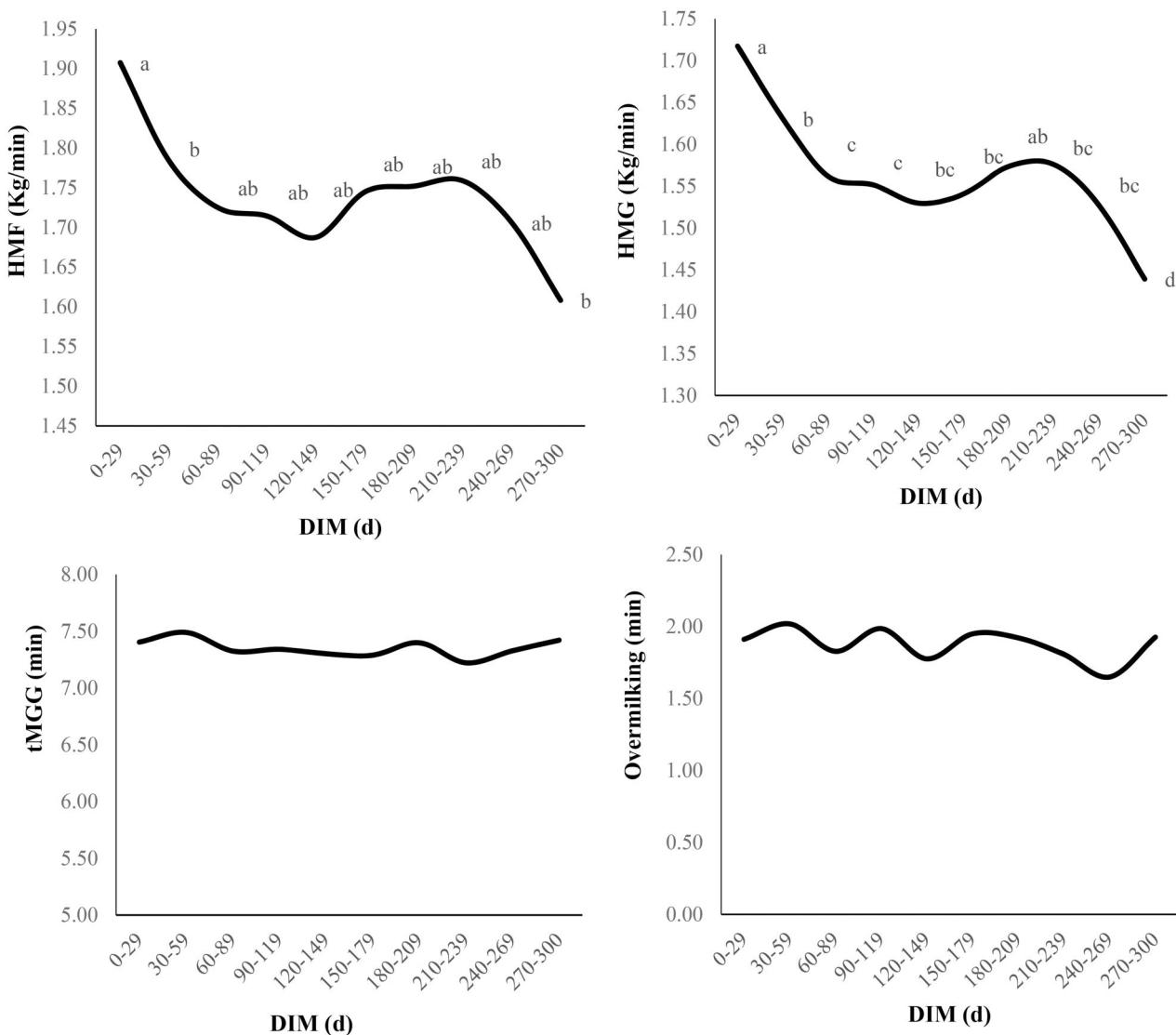
This study aimed to deepen the understanding of milk ejection dynamics in Italian Mediterranean buffaloes, offering a scientific foundation for enhancing efficiency and designing species-specific milking protocols. Indeed, as previously introduced, buffaloes display unique behavioural, anatomical, and physiological traits that necessitate tailored management strategies (Bertoni et al. 2020).

Descriptive statistics of milk ejection traits

Descriptive statistics indicated mean values of HMF and HMG of 1.77 and 1.59 kg/min, respectively. These values align with previous studies in buffaloes, although the average HMF was slightly higher than the 1.43 kg/min reported by Bava et al. (2007). This difference may be attributed to differences in milking procedures and equipment settings (Vermaak et al. 2022), as well as to genetic improvement in recent

years. Indeed, selection programs aimed at increasing milk yield (ANASB, 2025) may have indirectly contributed to the higher HMF and HMG observed in the present study, as animals with greater milk production also tend to exhibit faster and more sustained milk flow rate. To support this hypothesis, dairy cattle, characterised by higher milk yields, generally display quicker and more efficient milk ejection dynamics. Strapák et al. (2011) documented HMF values of 2.49–2.56 kg/min and HMG values of 3.74–4.17 kg/min in Holstein cows, with an average tMGG of around 5 min. In the present study, the mean tMGG was 7.35 min, showing moderate variability (CV = 37.4%), which is consistent with the values reported in a large-scale study conducted across 104 buffalo herds (Borghese et al. 2013). On average, buffaloes require approximately 8.00 min to complete milking, though individual variability exists, particularly in animals with higher HMF. However, longer tMGG, averaging 11–12 min, have been reported by Caria et al. (2011) in a single-farm study involving 450 buffaloes.

Overmilking exhibited the highest variability among all traits (49.0%), with a mean duration of 2.09 min. This is markedly lower than the 4.81 min recorded by Di Palo et al. (2007) in the same region nearly two decades ago, suggesting progress in minimising unnecessary machine attachment. Similarly, the current overmilking average was lower than the 3.64 min reported by Atigui et al. (2024), highlighting the persistence of management differences among Italian buffalo farms. The observed reduction in overmilking compared to previous studies is advantageous for udder health, as prolonged overmilking has been associated with teat-end lesions, elevated somatic cell count, particularly in late-lactating multiparous cows, and an increased risk of mastitis (Edwards et al. 2013). These variations can be attributed to multiple management factors, such as milking routines, the timing and effectiveness of pre- and post-dipping procedures (Watters et al. 2012), which may affect milking speed and efficiency, thereby playing a key role in the variability observed. On average, overmilking accounted for 28.4% of the tMGG, in line with previous estimates of 33% (2.78 min) by Bava et al. (2007). By contrast, a study that used automatic milking cluster removal systems in dairy cattle reported significantly lower overmilking, representing only 16% of tMGG (Sandrucci et al. 2005). The absence of automatic milking cluster removal systems may contribute to this variability (Magliaro and Kensinger 2005; Vermaak et al. 2022), suggesting that the implementation of *Lactocorder®* in milking could play a role in



¹Lactocorder® milk ejection traits: HMF, highest instantaneous milk flow rate recorded during the milking session; HMG, highest average milk flow sustained over one-minute interval; tMGG, total milking time calculated as the time elapsed from teat cup attachment to detachment; overmilking, calculated as the sum of two parameters recorded by the Lactocorder®:(i) post-stripping time, corresponding to the interval between the decline of milk flow below 0.20 kg/min and its complete cessation at 0 kg/min and (ii) blank milking time, defined as the period during which the milking cluster remains attached to the teats in the absence of milk flow. ^{a-d}Least squares means with different superscript letters differ significantly according to LSD post-hoc multiple comparison adjustment ($p < 0.05$).

Figure 3. Least square means of Lactocorder® milk ejection traits¹ according to days in milk (DIM) effect.

reducing overmilking duration in buffalo herds. Manual detachment often results in delayed cup removal, prolonging machine-on time, increasing the risk of teat-end stress and udder tissue damage (Tančin et al. 2006). Prolonged mechanical stimulation not only deteriorates teat tissue but also increases the incidence of hyperkeratosis, thereby compromising udder defences and predisposing animals to mastitis (Edwards et al. 2013; Moore-Foster et al. 2019). While these negative outcomes are well documented in dairy cattle (Hillaert et al. 2002; Edwards et al. 2013), further research is needed in buffaloes,

particularly to optimise milking equipment settings. Therefore, targeted research on buffaloes is needed to calibrate optimal detachment thresholds and teat-end vacuum levels that prevent long-term udder damage.

Analysis of milk flow dynamics revealed that buffaloes tend to release most of their milk in the early milking phase. On average, 21% of the total daily milk yield was released within the first minute, 46% by the second, and 66% by the end of the third minute. These values are in line with previous studies conducted on buffaloes reared under comparable conditions (Bava et al. 2007; Di Palo et al. 2007), and closely

Table 4. Least square means and standard error of the mean (SEM) of Lactocorder® milk ejection traits¹ according to parity order effect.

Traits	Parity					SEM
	1	2	3	4	≥5	
HMF (kg/min)	1.71 ^{bc}	1.82 ^a	1.81 ^{ab}	1.67 ^c	1.69 ^{bc}	0.05
HMG (kg/min)	1.54 ^{bc}	1.66 ^a	1.63 ^{ab}	1.49 ^c	1.51 ^c	0.04
tMGG (min)	7.00 ^c	7.04 ^c	7.27 ^{bc}	7.63 ^{ab}	7.83 ^a	0.56
Overmilking (min)	1.79	1.84	1.85	1.99	1.92	0.10

¹Lactocorder® milk ejection traits: HMF, highest instantaneous milk flow rate recorded during the milking session; HMG, highest average milk flow sustained over one-minute interval; tMGG, total milking time calculated as the time elapsed from teat cup attachment to detachment; overmilking: calculated as the sum of two parameters recorded by the Lactocorder®(i) post-stripping time, corresponding to the interval between the decline of milk flow below 0.20 kg/min and its complete cessation at 0 kg/min and (ii) blank milking time, defined as the period during which the milking cluster remains attached to the teats in the absence of milk flow.

^{a–c}Least squares means with different superscript letters within a row differ significantly according to LSD *post-hoc* multiple comparison adjustment ($p < 0.05$).

resemble patterns observed in dairy cattle. Sandrucci et al. (2007) reported that in dairy cows, 67% of the total milk yield was extracted within the first three minutes of milking. Similarly, Lee and Choudhary (2006) observed that Holstein cows released 42% of their milk within the first two minutes and 62% within the first three minutes. The high proportion of milk ejected in the initial minutes is largely attributed to effective *pre-milking* stimulation, which facilitates endogenous oxytocin release and accelerates alveolar milk letdown (Lee and Choudhary 2006; Singh et al. 2024). Conversely, inadequate stimulation can lead to delayed milk ejection and bimodal flow curves, with reduced 1MG due to insufficient oxytocin-mediated milk mobilisation (Strapák et al. 2011). The critical role of *pre-milking* management has been emphasised by Costa et al. (2020a) and Napolitano et al. (2022), who suggested that timely and adequate stimulation shortens the lag time, enhances milk flow efficiency, and ultimately reduces tMGG. These findings are in line with the present study, where efficient early milk ejection is associated with shorter tMGG values.

When evaluating the effect of lactation stage, the proportion of milk yield ejected within the first three minutes declined as DIM increased, confirming the expected physiological reduction in milk yield as lactation progresses. Notably, buffaloes in early lactation (0–30 DIM) displayed the highest 1MG (1.15 kg), while animals between 30 and 60 DIM achieved the highest 2MG and 3MG values (2.56 and 3.86 kg, respectively). In contrast, parity had no significant effect on milk yield during the initial three minutes. These results are not in line with findings in Holstein cows, where Strapák et al. (2011) reported higher 1MG percentages

Table 5. Least square means and standard error of the mean (SEM) of Lactocorder® milk ejection traits¹ according to milk yield class effect.

Traits	Milk class (kg)					SEM
	1.00–6.80	6.81–8.50	8.51–10.1	10.2–12.4	>12.4	
HMF (kg/min)	1.38 ^e	1.56 ^d	1.73 ^c	1.92 ^b	2.10 ^a	0.04
HMG (kg/min)	1.18 ^e	1.41 ^d	1.57 ^c	1.74 ^b	1.93 ^a	0.29
tMGG (min)	6.42 ^d	6.72 ^d	7.39 ^c	7.84 ^b	8.39 ^a	0.13
Overmilking (min)	1.97	1.82	1.97	1.83	1.79	0.10

¹Lactocorder® milk ejection traits: HMF, highest instantaneous milk flow rate recorded during the milking session; HMG, highest average milk flow sustained over one-minute interval; tMGG, total milking time calculated as the time elapsed from teat cup attachment to detachment; overmilking: calculated as the sum of two parameters recorded by the Lactocorder®(i) post-stripping time, corresponding to the interval between the decline of milk flow below 0.20 kg/min and its complete cessation at 0 kg/min and (ii) blank milking time, defined as the period during which the milking cluster remains attached to the teats in the absence of milk flow.

^{a–e}Least squares means with different superscript letters within a row differ significantly according to LSD *post-hoc* multiple comparison adjustment ($p < 0.05$).

in multiparous cows beyond their third lactation (21.0%), compared to second parity (19.3%) and primiparous cows (18.3%). However, since they did not evaluate 2MG or 3MG, direct comparison with the current results remains limited. This discrepancy suggests that parity may not exert a significant influence in dairy buffaloes, possibly due to species-specific lactation physiology or the relatively uniform mammary gland development observed across parities in the present population.

Sources of variation of milking ejection traits

To better elucidate the sources of variability in milking performance, this study compared key milking traits across four farms operating under comparable structural and management conditions. As expected, no significant differences in HMF were observed among farms, supporting the notion that this parameter exhibits high intrinsic stability. Such consistency is likely attributable to its strong dependence on physiological mechanisms and udder morphology, which are inherent animal characteristics and therefore less influenced by farm-specific operational practices. Conversely, HMG exhibited a modest but statistically significant variation (range: 1.50–1.65 kg/min), although the limited numerical spread suggests that these differences are more likely due to individual animal variability rather than systematic farm-level effects. In contrast, both tMGG and overmilking showed significant variation across farms. Notably, Farm 3, which contributed the largest number of animals in the study ($n=247$), recorded the longest average values for both parameters, consistent with the

positive association observed between these traits (Spearman's correlation = + 0.43). This outcome may reflect not only the farm's technical settings, characterised by the lowest vacuum level (38.4 ± 0.71 kPa) and a 65:35 pulsation ratio, but also the larger herd size, which may have resulted in less consistent attention to pre-milking stimulation and ultimately contributed to prolonged tMGG. By contrast, Farms 1 ($n=240$) and 2 ($n=137$), which operated at slightly higher vacuum levels (43.0 and 44.0 kPa, respectively) with a 60:40 pulsation ratio, maintained shorter overmilking times, indicating more efficient control of tMGG. Importantly, both farms also remained below the 2-min overmilking threshold proposed by Edwards et al. (2013) as a benchmark for minimising teat-end stress in dairy cows. Farms 2 and 4 ($n=174$), which showed the highest HMG values (1.65 and 1.59 kg/min, respectively), maintained comparatively low bulk milk SCC levels ($85,500 \pm 34,000$ and $83,200 \pm 31,900$ cells/mL), suggesting a favourable balance between equipment settings and herd performance. Overall, these findings suggest that vacuum intensity, pulsation dynamics, and operator management may interact with herd size to shape milking efficiency and udder health outcomes, as previously observed in other studies (Borghese 2007; Sandrucci et al. 2007; Caria et al. 2011). Nevertheless, given that only four farms were included, the scope for speculation remains limited and the generalisability of the results should be interpreted with caution.

Moreover, both HMF and HMG were significantly influenced by the stage of lactation. Specifically, the early lactation phase (0–30 DIM) was associated with the highest milk flow rates, with mean HMF and HMG values of 1.91 and 1.72 kg/min, respectively, this finding suggests that it is a period characterised by high alveolar pressure and marked neuroendocrine responses. These findings are consistent with previous research by Bava et al. (2007), who reported elevated milk flow in buffaloes during the first 90 DIM (average HMF: 1.80 kg/min), followed by a progressive decline as DIM increased. Similar trends have been observed in dairy cattle, where early lactation coincides with peak mammary gland activity and enhanced responsiveness to endogenous oxytocin stimulation (Strapák et al. 2011). According to Bruckmaier and Hilger (2001), this increased flow is primarily driven by peak milk production and optimal neuroendocrine regulation during the initial post-partum period. In Holstein cattle, HMF and HMG have been shown to peak within the first 100 DIM, reaching values of 2.80 ± 0.95 kg/min and 4.21 ± 1.55 kg/min, respectively (Strapák et al. 2011).

Although absolute flow rates in Holsteins are higher, the temporal trend across DIM classes appears conserved, suggesting common physiological mechanisms despite species-specific differences in anatomy and production potential.

In contrast, tMGG and overmilking did not significantly vary across DIM classes. This result diverges from the findings of Bava et al. (2007), who observed a significant reduction in tMGG with advancing lactation in buffaloes, and from those of Sandrucci et al. (2007), who reported a comparable trend in dairy cows. One plausible explanation is the lack of automatic milking cluster removal systems on all farms evaluated in this study, which may have led to uniform machine-on times regardless of actual milk yield or lactation stage. As a result, any potential reductions in tMGG during later lactation stages could have been masked by manual detachment procedures. Nevertheless, the absence of significant variation in overmilking across DIM classes aligns with previous observations by Bava et al. (2007), reinforcing the hypothesis that milking management practices, rather than physiological factors alone, exert a dominant influence on this trait. These findings further underscore the importance of optimising milking routines and equipment settings to improve efficiency and animal well-being across all stages of lactation.

Buffaloes in their second and third lactations exhibited the highest HMF and HMG values, indicating greater milk flow efficiency during these intermediate parities. Conversely, animals in their fourth lactation or beyond showed reduced flow rates, which did not differ significantly from those of primiparous buffaloes. This decline may be linked to the cumulative effects of successive lactations, during which the mammary gland undergoes repeated functional stress and progressive physiological wear. Such processes can impair milk ejection capacity and reduce milk flow rate. Moreover, advancing age is frequently associated with structural alterations in mammary tissue and an increased incidence of microtraumas, both of which may further contribute to the overall reduction in milk flow rate. This pattern supports the hypothesis that full physiological maturation of the mammary gland occurs during the second and third lactations in buffaloes, resulting in increased milk ejection (Borghese et al. 2007). Similarly, Catillo et al. (2002) reported peak of milk flow rates during the third or fourth lactation in Italian Mediterranean buffaloes, reinforcing the view that, optimal milkability is achieved during mid-parity stages. Similar patterns have been observed

in dairy cattle, as noted by Berry et al. (2013), who reported that peak milk flow rates increased from the second parity onward in Irish dairy cows, while primiparous cows exhibited significantly lower values. Similarly, Tančin et al. (2006) reported lower HMF and HMG in first-lactation cows (3.67 kg/min) compared to multiparous counterparts (ranging from 3.96 to 4.22 kg/min). Although absolute milk flow rates in buffaloes are generally lower than those in dairy cows, the relative trend across parities observed in this study mirrors that reported in bovine species (Sandrucci et al. 2005), further highlighting the influence of parity on milk ejection dynamics across ruminant dairy systems. Our study also revealed that buffaloes in greater parities experienced a gradual increase in tMGG, with the longest duration recorded from the fifth parity onwards (average 7.83 min). The increase in tMGG from the fifth parity onward suggests a progressive decline in udder efficiency, potentially linked to tissue elasticity loss or subclinical conditions that merit further investigation. These findings align with Bobić et al. (2020), who reported that older Holstein and Jersey cows showed longer tMGG and lower milk flow rates. Similarly, Tamburini et al. (2007) observed extended tMGG values in multiparous Brown cows, averaging 8.58 min in second-parity cows and 9.28 min in those beyond the third parity, suggesting a reduction in milking efficiency with age and repeated calvings. Together, these results may inform decision-making regarding optimal retention strategies and highlight the importance of tailored milking protocols in older animals to compensate for declining flow efficiency.

Analysis by milk yield class revealed a progressive increase in HMF, HMG, and tMGG with higher yield levels, indicating that animals producing greater milk volumes also tend to exhibit faster flow rates and longer milking durations. This association can be explained by physiological factors, as higher-yielding animals generally possess greater cisternal and alveolar capacities, resulting in larger milk volumes that require more time for complete evacuation. From a technical perspective, the larger volume prolongs machine-on time, thereby increasing both the maximum flow and the duration of milk emission. These findings are consistent with those of Milanesi et al. (2024), who reported similar trends in dairy cattle, reinforcing similar physiological basis of this association. In contrast, overmilking remained relatively stable across milk yield classes, without significant differences or a

discernible trend. This lack of correlation may be attributed to uniform milking protocols and fixed cluster detachment timings, regardless of individual yield, particularly in the absence of automated milking control systems. Such practices can result in overmilking being equally distributed across milk yield classes, underscoring the importance of individualised milking management. Overall, these results highlight the dual need to balance efficient milk extraction in high-producing animals while preventing unnecessary mechanical exposure in all classes. In buffaloes, where the adoption of automated cluster removal is still limited, refining the switch point settings and improving operator sensitivity could reduce teat-end stress and promote udder health.

Conclusions

This study demonstrated that milk ejection traits in Italian Mediterranean buffaloes differ substantially from those observed in dairy cattle and are significantly influenced by lactation stage, parity, milk yield level, and farm-specific milking management. These findings underscore the importance of developing species-specific and context-adapted milking protocols for buffaloes. Such protocols should incorporate adjustable switch-point thresholds, the adoption of automatic cluster removal systems where feasible, and routine adaptations according to DIM and parity. The implementation of these strategies is expected to optimise milk flow dynamics, reduce overmilking duration, and ultimately promote udder health and animal welfare.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Ethic statement

Milking data was collected during routine operations on commercial farms, therefore, ethical approval for the use of experimental animals was not required.

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Data availability statement

None of the data were deposited in an official repository. Data will be made available upon reasonable request.

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