

Different Types of Milk Flow Curves and Factors Affecting Milkability in Buffalo Species

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Abstract: Buffaloes are characterized by longer teats and teat canals and stronger muscular resistance of the teat wall than cattle; it is necessary to have a high vacuum level to open the teat canal and begin milk ejection. In buffalo milking management, milk yield, and flow profiles are essential parameters to record and evaluate. The milking machine is a critical point, and the characteristics of the milking vacuum and the pulsation rate are closely related to milk flow observations. In Italy, the most used vacuum levels are 44-46 kPa (range 40-53 kPa). The data on the milkability traits of the Mediterranean Italian breed made it possible to classify eight different types of milk flow curves due to anatomical, physiological, and management differences. This study aims to evaluate the main factors influencing milkability in dairy buffaloes. The results suggest the detachment of the milking cluster to reduce the decreasing and blind phases with the following advantages: reduction of the total milking time and consequently of the worker's time, improvement of the farmer's profitability and milk quality through decreasing the incidence of mastitis. Milk ability is influenced by physiological, sanitary, management, and genetic factors. In Mediterranean Italian buffaloes milked with the Automatic Milking System (AMS), a considerable variation in milk ejection and, consequently, in the milk flow curve was found compared to the conventional one, with better pre-stimulation, independent milk ejection for each teat, optimal milking of all quarters.

In conclusion, continuous milkability monitoring will help optimize milking practices by reducing labor time and increasing farmers' income through better milk quality. In addition, the identification of buffaloes with desirable types of milk flow curves could be helpful for buffalo breeders' associations to address farmer management and to define potential new breeding objectives.

Keywords: Dairy buffalo, milkability, milk flow curves, factors affecting milkability.

1. INTRODUCTION

The buffalo (*Bubalus bubalis*) is a species of worldwide importance, reared for producing milk, meat, hides, and other by-products and often used as a working animal in marginal rural areas [1, 2]. Although the milk produced by buffaloes is an irreplaceable source of nutrients and energy, especially in some countries or confined environments, its extraction from the mammary gland is not always easy. Milk removal is essential both for production purposes and to ensure the health of the organ; it can be done in two ways:

- a) natural extraction by suckling the calf,
- b) artificial extraction by manual or mechanical milking

In dairy species, milking is the foremost essential operation affecting production efficiency. A general definition of "milkability" is 'the ability of an animal to provide regular, complete, and rapid milk secretion from the mammary gland in response to correct milking

technique.' The primary method of assessing "milkability" is based on the analysis of the milk flow curve; in this way, the electronic milk meter (Lacto Corder ® device) records milk yield in the whole milking, electrical conductivity, and the main parameters of the milk flow curve, including the Total Milking Time (TMT). Since 1999, the Milk Quality laboratory of the "Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri" (Rome, Italy) has been engaged in the study of milk emission kinetics by recording milk flow curves in the main dairy species (buffalo, cattle, goat, sheep, and donkey).

This study aimed to provide a brief overview of the "state of the art" of milkability in dairy buffaloes and to evaluate the main factors influencing it, namely physiological, health, management, and genetic factors.

2. MILK EJECTION AND CLASSIFICATION OF MILK FLOW CURVES

2.1. Phases in Milk Flow Curves

To the best of our knowledge, the first studies on the milking ability of dairy buffaloes using electronic milk meters to evidence milk flow curves were carried

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out in the early 2000s, respectively, on the Italian Mediterranean buffalo [3-6] and the Murrah buffalo [7, 8].

A graphical representation of milk ejection can be displayed through milk flow curves, which differ according to the dairy species [4, 5]. The milk flow curves are subdivided into three main phases and a fourth one (Figure 1). The first phase is the “Lag Time” (LT) or ascending time, represented by the time elapsed between the attachment of the milking clusters and the time until there is a constant milk flow. The second is the “Plateau Phase” (PPT), where the milk flow is constant. The third phase is the “Decreasing Phase” (DPT), which represents the time from the PPT until milk flow drops below 0.20 kg/min. The fourth phase may be the “Blind Phase” (BT). The BT (milk flow below 0.2 kg/min) occurs between the end of the DPT and the detachment of the milking cluster [3-6]. Usually, the detachment is not performed promptly to collect the small amount of milk, the residual fraction, by stripping (often obtained by manual traction of the milking cluster by a milker) followed by further over-milking before detachment of the milking cluster. This milk fraction is called Stripping Milk (SM). Milk letdown is influenced by several factors: anatomical, physiological, sanitary, and managerial [8-12]. Many studies performed in different countries have shown that buffalo have difficulty being milked because there is a delay in milk ejection. Because of the udder anatomy, buffaloes are characterized by a longer teat with a longer teat canal, stronger muscular resistance of the teat wall, and a different cisternal fraction of milk

than dairy cattle [13-16]. Although each animal has its own anatomical and physiological characteristics, the milking machine can affect milkability and milking time.

2.2. Teat Anatomy

There is little information in the literature on teat anatomy in the dairy buffalo, especially for the Indian Murrah and the Italian Mediterranean, with significant differences in teat anatomy, particularly in the teat canal length. Recently, an important field study has been carried out to determine the main parameters of teat anatomy in different dairy buffalo breeds. The preliminary result of the study conducted on five breeds (Nili Ravi, Mediterranean Italian, Egyptian, Bulgarian Murrah, and Azeri) respectively raised in Pakistan, Italy, Egypt, Bulgaria, and Iran showed the following average values: Teat canal length (TCL) 24.13 mm, Teat diameter 30.46 mm, Cisternal diameter 17.80 mm, Teat wall 7.12 mm, Teat length 67.61 mm. We want to focus on TCL and its variability. The Authors found significant differences in the TCL among the breeds: Azeri (29.19 mm), Bulgarian Murrah (28.32 mm), and Egyptian (26.71 mm) showed significantly higher estimates in comparison to Mediterranean Italian (21.79 mm) and Nili Ravi (14.70 mm) [17].

Therefore, a higher vacuum level is needed to open the teat canal and start milk ejection in this species. In the Mediterranean Italian buffalo, the vacuum required to open the teat canal was measured using a method established for dairy cows [10, 13, 16]. The continuously increasing vacuum (no pulsation) caused

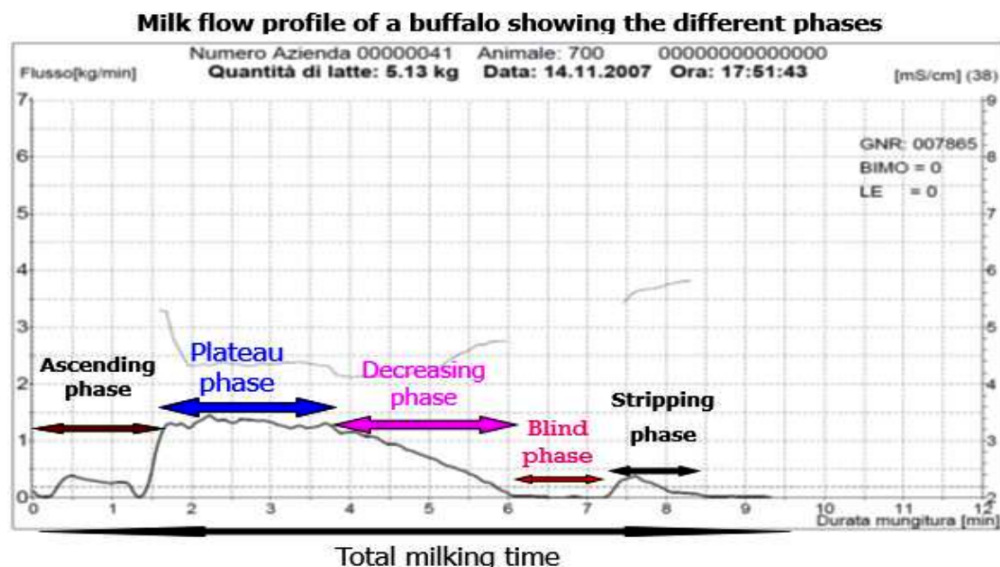


Figure 1: A representative milk flow curve showing the different phases (x-axis: Time in minutes; y-axis left: milk flow in kg/min; y-axis right: electrical conductivity in mS/cm).

milk flow in only 12 of 30 quarters up to a vacuum of 45 kPa, while 18 quarters showed no milk flow during this experiment [13]. Milk flow started at 29.0 ± 0.8 kPa, ranging between 16 and 40 kPa [13]. These results suggest that milk flow is also related to the vacuum required to open the teat canal [13]. In Italy, the most used vacuum levels are 44–46 kPa (range 40–53 kPa).

2.3. Different Types of Milk Flow Curves in Mediterranean Italian (MI) Breed

In a recent field study of 2,419 MI buffaloes reared on 187 farms in central Italy, Boselli *et al.* [18] classified eight types of milk flow curves based on anatomical, physiological, and management differences. The classification of curves was based on visual inspection of curve shape, milk yield, and milk flow parameters. Only the 2,288 milk flow curves have been classified into eight different types. The most represented curve was type 3 (Figure 2A, similar time between PPT and DPT, 27.32%), followed by type 6 (Figure 2B: 17.79%), characterized by a very long plateau phase. The least represented curve was type 1 (Figure 2C: 4.41%), characterized by a long lag time and low peak flow rate.

According to the analysis of variance, the milk yield ranged from 2.21 to 5.22 kg per milking for types 1 and 6, respectively, while the peak flow rate was minimum (0.50 kg/min) and maximum (1.73 kg/min) for types 1 and 4 respectively [18, 25]. Concerning the main milkability parameters, the results show that the TMT averaged 11.29 min; the lag time and the milk emission time averaged 2.19 min and 4.30 min, respectively. The 12.5% of the total recorded curves were classified as bimodal (two different peaks of milk flow are evident; the first peak is due to the removal of the cisternal milk fraction, while the second peak is due to the action of oxytocin, which allows the removal of the alveolar milk fraction).

Based on the literature, type 4 curves are representative of very short teat canals and very high milk flow. The average somatic cell score was 3.63 units, with a maximum value found for type 1 and a minimum for type 6. The highest milk yield at milking, the lowest somatic cell score, and the shortest milking time characterized buffaloes showing curves of type 5 and 6. The results of this study showed that such traits could be used as indicators to improve udder health and milk ability in dairy buffaloes. The classification proposed in our field study shows significant differences among the milk flow curves, which could

impact milk production and udder health. The results showed a high prevalence of over-milking, which may be responsible for adopting a higher TMT in buffaloes than in cows. Appropriate pre-milking udder stimulation should be used to reduce LT, increase Average Flow Rate (AFR), and limit TMT. In addition, proper milking practices would result in reduced labor time and improved farmer income due to better milk quality and fewer udder diseases. The results of this study allowed the identification of optimal milk flow curve types for the MI breed in terms of milk production and udder health; these results could be helpful to buffalo breeders' associations in herd management and in defining potential new breeding goals.

3. FACTORS AFFECTING MILK ABILITY

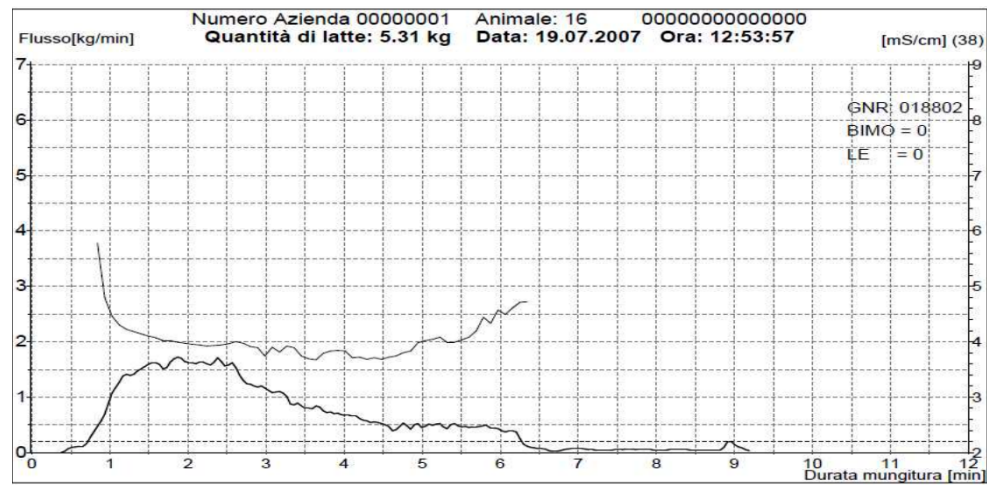
3.1. Physiological Factors

Regarding the effects of pre-stimulation and oxytocin release, studies show that mean measurements of teat anatomy vary with the reduction of the teat canal and teat wall, while cisternal teat and teat diameter increase. Similar results were obtained for exogenous oxytocin administration before milking [13, 14, 18]. Ozenc *et al.* [19], in a field study conducted on Anatolian buffaloes, observed that the post-stimulation mean TCL values were significantly higher in nervous buffaloes than those of teats in docile buffaloes. It seems that animal temperament affects milking efficiency. In nervous buffaloes, the stimulation alone may not be sufficient to open the teat canal and achieve complete milking.

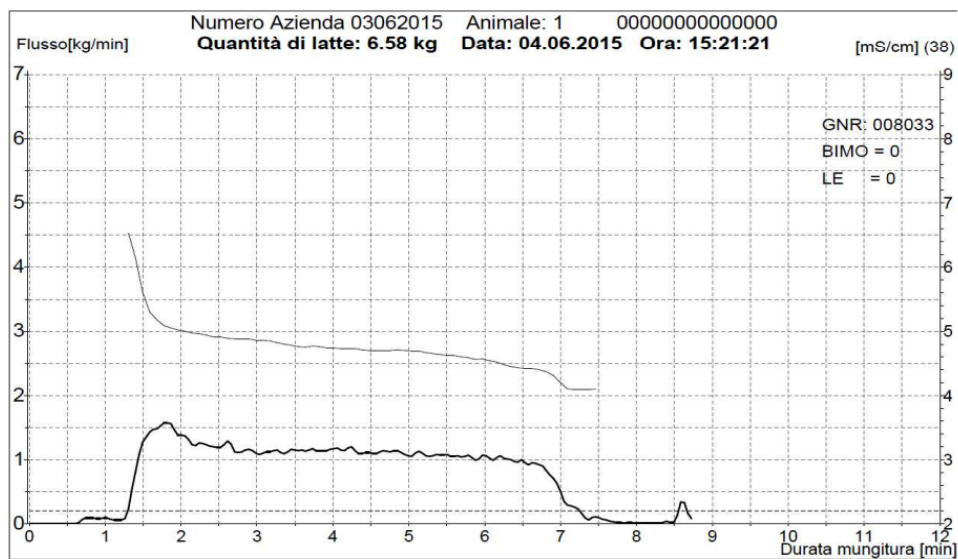
The delay in alveolar milk ejection correlates with the concentration of oxytocin in the blood, as shown by Thomas [8], which can already be observed at concentrations $>3-5$ ng/l for all treatments (no stimulation, stimulation, and stimulation combined with the administration of concentrate).

3.2. Sanitary Factors

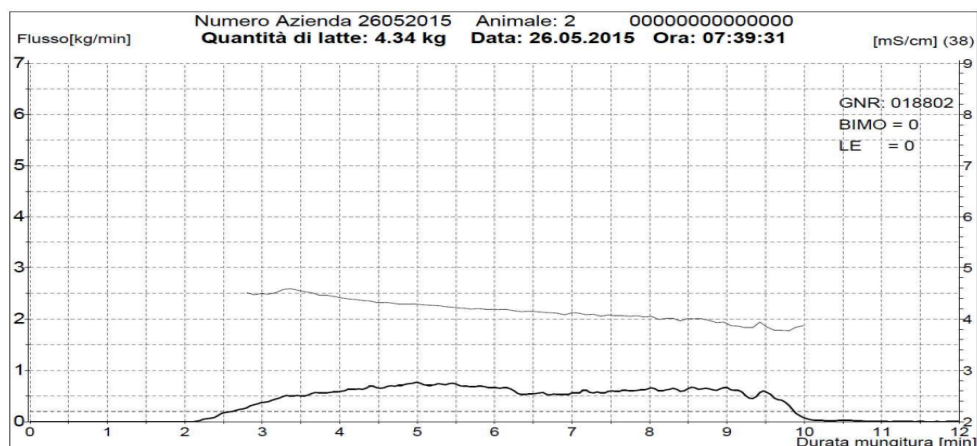
It is well established that mastitis is often subclinical in buffalo, as latent inflammation with no signs, and frequently may become chronic [20, 21]. It is well-established that udder health influences milk composition and milk flow parameters in dairy species, including buffalo. As in other dairy species, the milk somatic cell count (SCC) is the most adopted indicator for udder health and mastitis in buffalo. Overall, in healthy conditions, buffaloes showed lower milk SCC than bovines. The level of SCC is influenced mostly by environmental factors, in particular, milking practices



A



B



C

Figure 2: A: Typical “type 3” mik flow curve, with similar time between PPT (from 2 to 3 min of milking) and DPT (from 4 to 5 min of milking) phases; (x-axis: Time in minutes; y-axis left: milk flow in kg/min; y-axis right: electrical conductivity in mS/cm).

B: Typical “type 6” milk flow curve, characterized by a very long PPT phase (from 2 to 7 min of milking); (x-axis: Time in minutes; y-axis left: milk flow in kg/min; y-axis right: electrical conductivity in mS/cm).

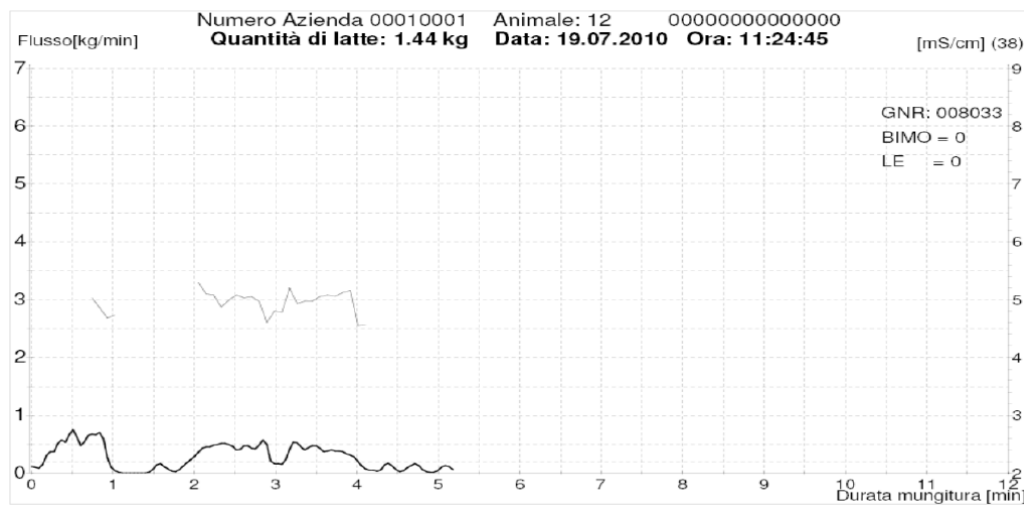
C: Typical “type 1” milk flow curve, characterized by a long lag time (from the start of milking to 4 min of milking) and low peak flow rate (x-axis: Time in minutes; y-axis left: milk flow in kg/min; y-axis right: electrical conductivity in mS/cm).

and farm hygiene. Several Authors report correlations between SCC and milk yield in Italian buffaloes. Negative correlation coefficients have always been determined and linked to SCC levels [22].

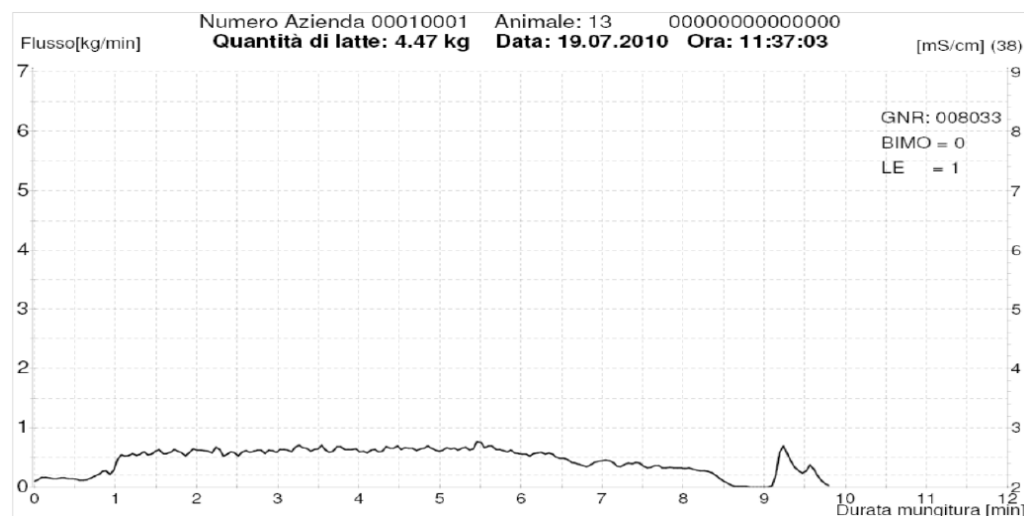
3.3. Management Factors

In buffalo milking management, similarly to what has been observed in bovine species [23], the milking routine and machine set-up are critical, and the milking vacuum levels and pulsation rate are strictly connected with milk flow observations. A working vacuum level of up to 45 kPa for MI breeds is generally ineffective unless alveolar milk ejection has occurred [13].

Generally, different studies conducted in different parts of the world in which the vacuum level varies in the range of 45-68 kPa for buffalo, while In Italy, the most used vacuum levels are 44-46 kPa (range 40-53 kPa) [11, 12]. Regarding this point, in a field study, Caria *et al.* [11, 12] tested different vacuum levels (37-40-43-46-49-52 kPa). There was an increase in the decline phase (DPT) at lower vacuum levels ($P < 0.001$), although these differences were not significant up to 46 kPa and between 49 and 52 kPa. A long DPT may indicate low or absent milk flow. This is associated with a higher risk of mastitis infection due to the possible passage of pathogens through the open teat canal.



A



B

Figure 3: A: Typical milk flow curves recorded from AMS by a field study (Boselli *et al.* 2011, on Mediterranean Italian buffalo milked with Lely Astronaut, incomplete milking); (x-axis: Time in minutes; y-axis left: milk flow in kg/min; y-axis right: electrical conductivity in mS/cm).

B: Typical milk flow curves recorded from AMS by a field study (Boselli *et al.* 2011, on Mediterranean Italian buffalo milked with Lely Astronaut, complete milking); (x-axis: Time in minutes; y-axis left: milk flow in kg/min; y-axis right: electrical conductivity in mS/cm).

The study showed that the buffalo MI breed could be milked at different levels of operating vacuum (37, 40, 43, 46, 49, and 52 kPa) without affecting the milk yield and consequently completely emptying the udder; average and peak milk flow rate (AFR and PFR) increased significantly along with an increase in working vacuum level. Vacuum levels of 37 and 40 kPa provided good milk ability conditions in which the plateau phase (PPT) was longer than the decline phase (DPT), while lag time (LT) was not affected by vacuum level.

We must consider the Automatic Milking System (AMS), which has been available since 1992 and used as a method of voluntary milking in cattle and, more recently, in dairy buffaloes. Only a few Authors [25-28] report data on the adaptability of buffaloes to AMS. Boselli *et al.* [25] measured data on buffalo with a portable milkmeter LactoCorder installed after the tubes connecting the milking cups (Figures **3A**, **3B**). The TMT measured between the beginning of the cleaning of the first teat and the detachment of the last teat ranged from 8.80 min [25] to 8.3 min [26]. Regarding the number of milkings per day, Faugno *et al.* [27] reported 2.3 milkings per buffalo per day, Sannino *et al.* [28] and Boselli *et al.* [25] reported about 2.5 milkings per buffalo per day. Sannino *et al.* [28] analyzed the factors causing missed milking visits for one year, representing about 10% of the AMS working time. Approximately 27% of missed visits were due to cow behavior, while 72% were due to robot malfunctions (e.g., unexpected size and/or orientation of one or more teats; obstructed teat view). This study aimed to shed light on the operational and functional aspects of AMS in buffaloes and their relationships with milk yield and quality. The research was conducted over 3 years on a dairy buffalo farm equipped with both a traditional tandem (TT) parlor and four AMSs, each serving approximately 50 cows. The efficiency of the AMS was evaluated considering three aspects: (1) yield and quality of milk drawn; (2) system capacity, i.e., number of buffaloes milked by the AMS per year; (3) number of visits due to missed milking, i.e., failures of the robot in attaching the milking cluster. Cows milked by AMS had a higher number of milkings/days and higher milk production ($p < .05$) compared to those milked with the TT system, while no differences were found in milk composition.

The differences are as follows (Figures **3A**, **3B**): better pre-stimulation in AMS, allowing a positive endogenous release of oxytocin, with a reduced milk letdown phase; independent milk ejection for each teat, with optimal milking of all quarters, with a reduction in

overmilking; better milking hygiene during the milking routine, limiting the incidence of mastitis and with a low somatic cell score; adverse effects in AMS due to failed or incomplete milkings (17% of the total milkings, higher than the 10% found by Sannino *et al.* over a 1 year of daily observations), which limit the potential performance and efficiency of AMS; frequent air leakage, which could cause alterations in milk composition. AMS is suitable for buffaloes and opens a new strategy for recording and managing many milkability traits in dairy buffalo milking.

3.4. Genetic Factors

Regarding MI in a field study [24] carried out in the Lazio Region, the Authors found these values: the average milk yield was 3.77 ± 0.07 kg per milking session per animal, as similarly reported by different authors in MI buffalo [3-6], while TMT was 10.24 ± 0.22 min. Considering that the farms with a TMT higher than 10.00 min represent 49.04% (51/104) of the total farms, it could be easy to reduce the milking time. The Plateau milking time was 4.24 ± 0.09 min (41.41% of the TMT). As, at this time, 88.86% of the total milk yield was produced, the goal would be to reduce the DPT and the **BT**. The main principal phases related to milk ejection were PPT (1.77 ± 0.05 min) and DPT (2.12 ± 0.06 min); also, LT (1.98 ± 0.10 min) could be considered a phase of partial milk emission because in this first phase is extracted the cisternal fraction, which in buffalo is very low.

The stripping milk (SM 0.22 ± 0.03 min) could be considered another phase, not always present, during which a small quantity of milk will be extracted.

The blind milking time is very long (3.80 ± 0.17 min). During this time, the teat canal is opened at intermittent phases, with poor or without milk ejection, allowing the possibility of bacterial infections and mastitis risk.

The absence of pre-stimulation, associated with inadequate pre-milking routines (teat cleaning), is the main cause of the highest milk time. In fact, the average time of about two minutes corresponds to the time between the attachment of the milking clusters and the time until there is a constant milk flow; it represents the time necessary to reach the threshold of oxytocin in the blood.

There is little data available in the literature on the Murrah breed, which can be traced back to two different studies by Thomas *et al.* [7, 8]. In the first

study [7], the researchers found a lag time for milk ejection in the Murrah breed, which varied on average from 2.50 ± 0.11 to 6.33 ± 0.13 minutes with combined treatment (pre-stimulation and concentrate administration) and without pre-stimulation. As for the mean flow rate, it varied on average from 0.49 ± 0.10 to 0.10 ± 0.10 kg/min with the combined treatment (pre-stimulation and concentrate administration) and without pre-stimulation; for the same treatments, the total machine-on time varied on average from 9.83 ± 2.55 to 13.66 ± 2.95 minutes without significant differences.

In a second study [8], for the same breed, they found a Lag Time that varied on average from 61 to 161 s (1.02 to 2.68 min), depending on the different milking times and treatments.

The maximum and average flow rates varied on average from 0.93 to 1.18 kg/min and from 0.24 to 0.42 kg/min, respectively, while the time of milking (machine time) varied on average from 587 to 775 s (9.78 to 12.92 min), depending on the different time of milking and treatments, although without significant differences.

The comparison of milk ability traits between the two buffalo breeds showed a lower Lag Time and higher PFR and AFR for MI than for Murrah, while mechanical milking times were similar between the two breeds.

CONCLUSIONS

In conclusion, it is possible to know the milk ability of the herd by monitoring milk flow curves. Continuous milk ability monitoring will help to optimize milking practices by reducing labor time and increasing farmers' income through better milk quality and fewer udder diseases. In addition, identifying buffaloes with desirable types of milk flow curves could be helpful for buffalo breeders' associations to address farmer management and define potential new breeding objectives. However, further research is needed to investigate the variability of these phenotypes at the population level and to understand whether they can be used as trait indicators for reproductive purposes.

In the future, we will invite breeders, researchers, universities, research Institutes, etc., to implement milk ability data in other breeds besides Mediterranean Italian and Murrah.

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