

Short-Term Pre-Milking Stimulation and Milk Ejection Reflex in Dairy Cows

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Enhancing cow productivity and maintaining udder health are among the key factors for the timely activation of the milk ejection reflex, which is directly associated with the effectiveness of pre-milking stimulation. However, despite existing studies addressing these issues, the physiological response of cows to these procedures remains insufficiently substantiated. It has been established that the milking technology for Brown Swiss cows in a milking parlor equipped with a "Parallel" (2 × 20) system, accompanied by light classical music, ensures, first, conditioned-reflex stimulation of the milk ejection reflex prior to the start of preparatory procedures (98.6 s). Second, unconditioned-reflex stimulation occurs through short-term (4.5 s) wet cleaning and massage of the teat surface using the brushes of a manual massager-cleaner. The second conditioned-reflex stimulation is ensured by the delay time before attaching the milking unit to the cows' udders (62.8 s). It has been established that in cows with a single milking yield of 20.5 kg, the average and maximum milk flow rates are 3.39 and 4.74 kg·min⁻¹, respectively, whereas in cows with a productivity level of 14.5 kg, these indicators do not fall below 3.49 and 5.23 kg·min⁻¹, respectively. It has been determined that cows' readiness for milking defines the level of milk ejection activity, whereas the magnitude of a single milking yield is not of significant importance. Stereotypical conditioned and unconditioned reflex stimulations ensure a high level of animals' readiness for milk ejection.

Keywords: cows, milking, preparation, milk ejection reflex

1 Introduction

Significant changes and fundamental transformations of established practices and methods of dairy cattle management are currently taking place both in industrial complexes and on small farms.

The process of re-equipping livestock production systems is gaining priority importance. Under these conditions, there is a clear trend towards the development of technology not only to support existing processes but also to create effective technological solutions based on fundamentally new machines and equipment. The introduction of innovative technical means into production should be maximally based on the consideration of the biological needs of the animal organism within the "human – machine – animal – environment" system.

Under intensive production technologies, animals develop a stable pattern of behavioral responses that ensures the preservation of health and the maintenance of a consistent level of milk productivity (Pishchan et al., 2024). At the same time, under the influence of new environmental factors, the physiological status (homeostasis) of animals may change, and adaptation disorders may arise, since the organism cannot always effectively adjust to altered environmental conditions, which are sometimes arbitrarily modified without taking into account the biological characteristics of the animal's body.

The breeding of dairy cattle in large industrial complexes has led to the emergence of animals well adapted to the requirements of dairy farming, including machine milking in milking parlors. Nevertheless, despite significant progress in selection, the basic

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physiological regulation of lactation in cows remains unchanged. Machine extraction of milk accumulated in the udder requires its complete and rapid removal under appropriate stereotypical and sanitary-hygienic conditions to save animal health and ensure further high productivity.

In this regard, the quality and duration of pre-milking preparation of cows become critically important, depending on the degree of udder filling with milk and the time delay between the start of stimulation and the attachment of the milking machine, leading to the realization of the milk ejection reflex (Wieland et al., 2025). Complete emptying of the udder during each milking is essential for maintaining both high productivity and udder health (Müller et al., 2024), which has key economic significance. In general, the milking of lactating animals is a rather labor-intensive process that requires significant labor and energy inputs. It is therefore not surprising that the issue of introducing innovative solutions to reduce manual operations in machine milking systems and to increase the throughput of milking installations is highly relevant today. Large industrial complexes, as noted by Jafri et al. (2024), successfully exploit the effect of scale, thereby reducing production costs per unit of output and increasing the overall productivity level of cows. On cattle farms, the quantity of milk obtained is still often considered more important than its quality, although the quality of cow's milk directly affects the profitability of the dairy sector.

It is well known that no machine or technological equipment involved in milk production comes into such close contact with the animal as the milking installation, particularly its main working mechanism – the milking machine. The design of the milking installation has a significant impact on the milking process, particularly on the realization of the milk ejection reflex in cows, which determines the amount of milk obtained, as well as its quality and technological properties (Wieland et al., 2021). According to Sederevičius et al. (2023), indicators of udder health in cows include not only external appearance but also the components of the milk secretion obtained after machine milking, such as somatic cell count and organoleptic characteristics.

Under traditional cow milking technology, operators perform a range of preparatory operations to induce a complete milk ejection reflex: washing the udder with clean warm water, drying with a towel (or disposable cloth) combined with udder massage (Lutsenko et al., 2021). The reproduction of classical music during milking has been shown to positively affect both milk production and quality, as well as cow welfare, by reducing stress and excitability under intensive production systems

(Lemcke et al., 2021; de Castro Lippi et al., 2023). Existing recommendations for pre-milking udder preparation include teat sanitation – namely disinfection prior to milking, mechanical cleaning, and drying – which ensures stimulation of the milk ejection reflex before the onset of milking (Singh et al., 2024).

The principal objective of pre-milking udder preparation is to ensure the gentlest, most complete, and rapid extraction of milk from cows. However, in large industrial complexes there is a trend toward reducing the duration of stimulation to increase the throughput of milking parlors. Insufficient pre-milking stimulation, however, can lead to reduced milk productivity and impaired milk quality (Ivanyos et al., 2020). At present, there is a lack of up-to-date information regarding the most effective methods for stimulating the receptor system of lactating animals during the pre-milking period. Moreover, most of the available studies date back several decades.

The aim of the study was to substantiate specific elements of milk production technology based on a comprehensive assessment of the characteristics of the milk ejection reflex and the risks of bimodality under stereotypical pre-milking conditioned and short-term unconditioned stimulation of this process, performed without manual contact with the udder and without stripping the first streams of milk.

2 Material and Methods

2.1 Ethical Statement

The ethical approval for conducting research and performing manipulations with experimental animals was obtained from the Bioethics Committee of Dnipro State University (Protocol № 55, 2025). The studies conducted on Brown Swiss lactating cows fully complied with the Law of Ukraine “On the Protection of Animals from Cruelty” (№ 5456-VI, October 16, 2012), the “General Ethical Principles of Animal Experiments” adopted by the First National Congress on Bioethics (Kyiv, 2001), and the international bioethical standards of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, March 18, 1986, ETS № 123). The researchers have extensive experience in conducting experimental work, possess profound knowledge in animal biology and welfare assurance methods, and take full responsibility for the reliability and transparency of the results obtained.

2.2 Housing, Milking, and Feeding Conditions of Experimental Animals

The study was carried out at the largest breeding farm of the Brown Swiss breed (“Big Brown Swiss”) within

the “Yekaterynoslavsky” dairy production complex (Dnipropetrovsk region, Ukraine), with a capacity of 4,500 head of cattle, including 1,450 lactating cows. The productive herd was housed in lightweight barns divided into sections, each containing 150 animals. Cows rested in individual cubicles (120 × 120 × 170 cm) equipped with rubber mats.

Milking of lactating cows was performed three times daily at 8-hour intervals in a milking parlor equipped with a “Parallel” (2 × 20) system (DeLaval) and the herd monitoring system “Dairy Comp-350.” The milking units were attached from the rear, so that the milk-vacuum tube passed caudally between the hind limbs. Two operators worked in the milking parlor, each servicing 10 cows. During milking, light classical music was played. For pre-milking preparation, operators used a handheld device designed to clean and wash each teat sequentially. The device, weighing approximately 2 kg, was equipped with a detergent spray system. By pressing a button on the handle, the operator activated a light for visibility of the udder and initiated the inward movement of rotating brushes, which simultaneously sprayed the cleaning solution “Kenopur” ($t = 29\text{ }^{\circ}\text{C}$). The rotating brushes enclosed the teat and, together with the detergent, cleaned and massaged the teat tissue. The duration of sanitary-preparatory operations before milking was recorded for each cow, and the mean values (s) were calculated.

After milking and automatic detachment of the milking unit from the udder, operators disinfected each teat by immersing it into a special cup containing the “Ketomix” solution, prior to the next milking. Throughout both preparatory and concluding operations, direct manual contact of operators with the teats or the udder was completely excluded.

The animals were fed a total mixed ration (TMR), which was distributed onto the feed table of the barns twice daily using a multifunctional SPM-27 feeder. Feed push-up to the feed barrier was performed autonomously by the “Lely Robot” feed pusher. Each barn section was equipped with self-feeders supplying mineral supplements: sodium chloride (NaCl), calcium carbonate (CaCO_3), and sodium carbonate (Na_2CO_3).

2.3 Study of the Milk Ejection Reflex in Cows

The research was conducted in 2025 on lactating Brown Swiss cows in the 2nd–4th month of lactation. The time of entry and duration of stay of the animals in the milking stall, environmental conditions, operators’ voices, light classical music, and the general background noise of the milking parlor were considered as conditioned stimuli of the milk ejection reflex (min, s). In contrast,

all manipulations involving the teats of the udder were regarded as unconditioned stimuli of the receptor apparatus (s).

Chronometric observations of the milking process were carried out from May to August and included: the time required to fill one side of the installation with animals (20 head, min) and the time needed to empty it after milking (s). During milking, the identification number of each cow and the minute-by-minute milk flow (kg) were recorded using a stopwatch until the detachment of the milking unit. Based on these data, the total milking time per cow (min, s) and the amount of milk obtained (kg) during the second (midday) milking were determined.

The average milk flow rate (Q , $\text{kg}\cdot\text{min}^{-1}$) was calculated using the following formula:

$$Q = \frac{q}{t}$$

where: q – the amount of milk yield per milking (kg);
 t – the duration of machine milking (min, s)

The maximum milk flow rate ($\text{kg}\cdot\text{min}^{-1}$) was determined by analyzing data from minute-by-minute recording of milk yield. The minute with the highest recorded milk output was taken as the result for evaluating maximum milk ejection intensity. The completeness of udder emptying was assessed as a percentage of the total milk yield obtained during the first, second, and third minutes of milking machine operation. Based on graphs of minute-by-minute milk flow dynamics, the activity of the milk ejection reflex in cows was evaluated.

Considering the single milk yield of Brown Swiss cows ($\Sigma = 83$ head) and the timing of maximum milk ejection as indicated by the milk flow curve dynamics, six groups were formed. Group I ($n = 17$) had an average yield of 11.3 kg at the second (midday) milking. Group II ($n = 15$) had a yield of 14.5 kg, Group III ($n = 27$) – 16.2 kg, Group IV ($n = 9$) – 20.5 kg, Group V ($n = 4$) – 23.0 kg, and Group VI ($n = 11$) – 12.4 kg. The groups of cows were formed according to the principle of analogs based on breed, line affiliation, live weight, single milking yield, and the time since the last calving.

2.4 Statistical Analysis

The obtained data were processed using methods of variation statistics, including the calculation of arithmetic means (M), standard errors of the mean ($\pm m$), and significance levels (P). Differences between the mean values of the compared samples were considered statistically significant at $P < 0.05$. Calculations were performed using licensed software: Microsoft Office

Excel 2010, Statistica for Windows (Version 5.5), and SPSS (Version 20).

3 Results and Discussion

Innovative milk production technologies are characterized by large-group housing of cows in lightweight modern facilities and milking on high-performance systems such as “Parallel” installations. Milking remains the key technological operation both in large industrial complexes and on small dairy farms, as it determines not only the quantity and quality of milk but also the health of the animals. As noted by Kumar et al. (2024), rational milking practices increase cow productivity and ensure the healthy condition of teats and the udder. According to Dahl & Wieland (2024), udder health ultimately defines the welfare status of dairy cows in milk production enterprises.

One of the most important steps in the milking process is pre-milking preparation (Krupelnyskyi, 2023), which consists of several consecutive operations necessary for the most complete udder emptying, improvement of milk quality, maintenance of udder health, and effective machine milking. At most dairy enterprises, pre-milking udder preparation includes teat sanitation (disinfection), washing, wiping with a clean towel or cloth, stripping of the first milk streams, and the interval from the start of preparation to the attachment of the milking unit (lag time). Particularly important is the fact that pre-milking preparation of lactating cows is usually performed manually; therefore, its execution may vary not only among different operators but also from day to day, which in turn affects the efficiency of the milking process.

Milking of Brown Swiss cows in the milking parlor with a “Parallel” system involves placing a group of cows in the holding area and alternately filling the two sides of the parlor, 20 animals each. Depending on the activity of the cows, the complete filling of one side of

the installation required on average 98.6 seconds (Table 1). In general, the familiar environment of the holding area, the movement of animals into the milking parlor where light classical music was played (Lechuga, K. K. dos S. L. et al., 2023), and the positioning of cows in the stalls had a conditioned-reflex effect on the excitation of the milk ejection reflex. This was fully confirmed by the fact that in some cows milk already began to drip from individual teats during movement, while in others complete milk streams from all four teats were observed.

After the animals were fixed in the stalls by the front restraining barrier, operators immediately proceeded with sanitary preparation of the udder before connecting the milking unit. All preparatory manipulations consisted of activating a handheld cleaning-massager device, which was used to wash and clean the surface of each teat individually. Through the inward rotation of the brushes combined with spraying of the cleaning solution “Kenopur” the teat surface was cleaned of dirt and gently massaged. Cleaning and massaging of teats with soft brushes had the effect of unconditioned stimulation of the milk ejection reflex in cows. Notably, no direct manual contact of the operator with the udder or teat tissues occurred. The cleaning solution temperature was approximately 29 °C, which also exerted a stimulating influence on the excitation of the milk ejection reflex.

Overall, the sanitary preparation of the cow’s udder was to some extent comparable to the stimulation of teat tissues by the warm, moist mouth of a calf. However, while a calf stimulates the milk ejection reflex continuously throughout the entire sucking process (8.3–9.3 min; Kour et al., 2021), unconditioned stimulation by the handheld cleaning-massager was relatively short, averaging only 4.5 seconds. Nevertheless, this duration of sanitary preparation was sufficient to ensure that each teat was cleaned and fully prepared for the attachment of the milking unit. The sanitary cleaning of one teat using the cleaning-massager required approximately

Table 1 Pre-milking and post-milking operations of cows in a “Parallel” milking parlor ($M \pm m$, $n = 83$)

Indicator	Parametric statistics, s		
	$M \pm m$	Cv (%)	Limit
Filling one side of the installation (20 cows)	98.6 $\pm$ 1.773	14.04	65–120
Mechanical cleaning and washing of teats with cleaning solution	4.5 $\pm$ 0.08	13.45	3.3–5.5
Consumption of cleaning solution per teat (mL)	$\approx$ 15	–	–
Delay time before attachment of the milking unit (latent period of milk ejection)	62.8 $\pm$ 0.40	4.99	55–68
Duration of milking unit attachment to the udder	5.8 $\pm$ 0.09	11.81	6.3–4.0
Teat disinfection with “Ketomix” solution (post-milking)	3.6 $\pm$ 0.02	4.07	3.4–3.8
Group release of milked cows (20 head)	9.4 $\pm$ 0.18	15.04	8–12
Total milking time for 20 cows (min)	9.4 $\pm$ 1.43	–	–

15 ml of the cleaning solution. The primary focus of preparatory operations was the achievement of a visibly clean teat surface, as assessed by the operator, rather than the duration of teat massage aimed at stimulating the milk ejection reflex.

It should be noted that such preparatory operations performed before attaching the milking unit to the cow's udder are practically independent of the operator's skills or proficiency and are therefore consistently repeated in the same mode from one milking to the next. Although tactile stimulation of the teat tissues using the handheld cleaning-massager is short-term, it plays a significant role in activating milk ejection, during which the alveolar fraction of milk is released into the gland cisterns.

Regarding the duration of milk ejection reflex stimulation through mechanical action on the teat tissues of cows, a considerable number of studies have been conducted. According to Kaskous & Bruckmaier (2011), pre-milking stimulation should last for about 60 seconds to achieve a full milk ejection reflex. It is considered that the duration of pre-milking stimulation should correspond to the degree of udder filling: with a well-filled udder, 20 seconds is sufficient, whereas with a less filled udder, stimulation should be extended to 30 seconds, with a latent period of not less than 60 seconds (Kaskous & Bruckmaier, 2011).

Most researchers agree that mechanical pre-stimulation of the udder tissue lasting around 90 seconds is optimal to achieve an immediate and continuous milk flow from the udder that contains only a small residual fraction. Watters et al. (2012) found that udder preparation combined with forestripping improves outcomes in terms of milk flow rate and milking time in cows milked three times per day. In contrast, Vetter et al. (2014) suggested that a 15-second pre-stimulation period is already sufficient to induce oxytocin release, but a certain latent period is still required before milk ejection begins. Short pre-milking stimulation lasting 15 seconds, followed by a latent period of up to 45 seconds, was found to be an effective alternative to continuous stimulation for initiating the milk ejection reflex. Moreover, milking efficiency improved when tactile stimulation for 15 seconds was followed by a 30-second latent period, compared to 45 seconds of continuous stimulation.

Manual tactile stimulation of udder tissues by operators for 24.0 seconds before attaching the milking unit ensured complete milking without reducing total milk yield (Pishchan, 2015). Moore-Foster et al. (2019) reported that as herd size increases, less time – on average 14.5 seconds (ranging from 2.0 to 40.8 s) – is spent on teat stimulation, thereby reducing the overall pre-milking preparation time. The effort to increase the throughput

of milking installations also leads to a reduction in both the stimulation period and the delay time before milking unit attachment.

After short-term sanitary preparation for milking, the operators immediately attached the milking units to the udders – importantly, without stripping the first streams of milk, which would normally serve as a signal stimulus for initiating the milk ejection reflex. On average, one operator spent 62.8 seconds on the preparation and stimulation (exteroceptive massage) of the teat tissues of ten cows. This indicates that the time interval between the completion of sanitary preparation and the attachment of the milking unit to each cow's udder was approximately one minute, specifically 62.8 seconds.

It should be noted that during this delay period (waiting phase), the animals received a second conditioned stimulation of the milk ejection reflex. Thus, in the milking parlor, lactating cows were exposed to a total of approximately 161.4 seconds of conditioned stimulation of the milk ejection reflex before the onset of milking.

As is well known, an important condition for effective machine milking of cows is, first, the optimal duration of teat tissue stimulation (unconditioned stimulation of the milk ejection reflex), and second, the delay between the completion of teat sanitation and the attachment of the milking unit – that is, the interval between the end of hygienic preparation and the start of milking. After the completion of pre-milking preparation, operators connected the milking units within a very short period, requiring only 5.8 seconds. Active milk flow from the udders of lactating cows began immediately, indicating that there was no "dry milking" phase at the start of machine operation.

This finding suggests that during the 62.8-second delay, cows experienced a latent period of the milk ejection reflex, which ensured active milk removal once the milking unit was attached. Our results are consistent with the data reported by Wieland et al. (2021), who indicated that the delay time during pre-milking preparation in large industrial dairy complexes averages about one minute (57 seconds). It is noteworthy that such stimulation is restricted to the teat tissues rather than affecting the entire udder.

Regarding the delay between udder preparation and the attachment of the milking unit, researchers hold considerably different opinions. Bruckmaier (2013) reported that a latent period of two minutes or longer is not recommended, since in the absence of tactile stimulation of the udder tissues the blood concentration of oxytocin declines rapidly. In contrast, the studies of Watters et al. (2015) demonstrated a positive effect

of preparation delay time on the activation of the milk ejection reflex and on the completeness of milking.

In general, in recent years the milking routine with a relatively long delay between udder preparation and milking onset has tended to change with increasing herd size. Moore-Foster et al. (2019) emphasized that the optimal delay time between adequate pre-stimulation and attachment of the milking unit, to prevent a reduction in milk yield and an extension of milking duration, should not exceed 90 seconds. According to Singh et al. (2024), consideration of the latent period corresponding to a 90-second delay during pre-milking stimulation ensures adequate activation of the milk ejection reflex in cows. Moreover, such a prolonged latent period may mitigate the negative effects of vacuum-induced forces on teat tissue, improve udder health, and promote animal welfare (Upton et al., 2023). Nevertheless, from a practical herd-management perspective, a shorter latent period between udder preparation and the start of milking may be more rational.

Thus, short-term sanitary preparation of cows for milking lasting 4.5 seconds, performed according to the “massager-teat surface” principle, has an unconditioned-reflex significance

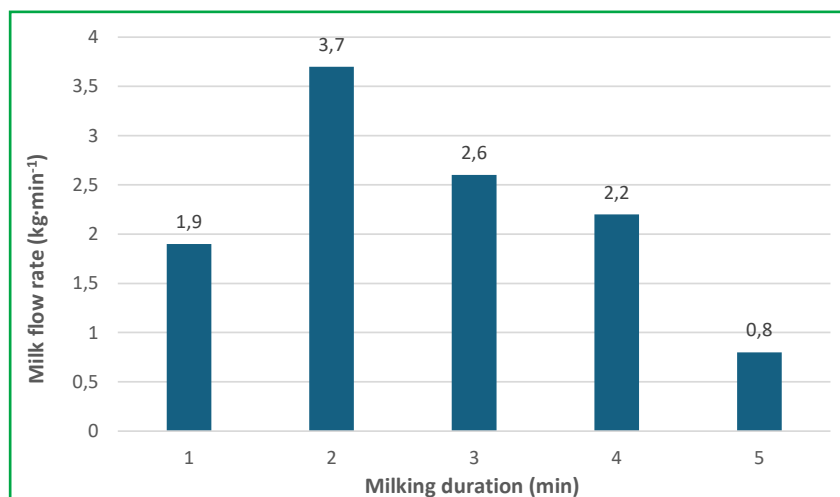


Figure 1 Dynamics of milk flow rate in Group I cows with a single milk yield of 11.3 kg

for stimulating the milk ejection reflex. Under general conditioned stimulation of the lactation reflex lasting 161.4 seconds, the delay time averaged 62.8 seconds, during which the latent period of milk ejection occurred. Upon completion of milk removal, the milking cluster was automatically detached from the udder, and the operators immersed each teat into a cup containing the disinfectant solution “Ketomix”. This teat disinfection (post-milking) procedure lasted 3.6 seconds. The total time required to milk 20 cows on one side of the “Parallel” installation was less than 10 minutes (9.4 min). Group release of milked cows from the milking installation took an average of 9.4 seconds.

Cows of Group I during the second (midday) milking were characterized

by the relatively lowest milk yield, averaging no more than 11.3 kg. The removal of this amount of milk accumulated in the udder required an average of 4.4 minutes. The short duration of machine milking in these cows was ensured by a high activity of the milk ejection reflex. The average milk flow rate was 2.54 kg·min⁻¹, while the maximum flow rate averaged 3.72 kg·min⁻¹.

The single milk yield of Brown Swiss cows from Groups I to VI and their milk ejection parameters are presented in Table 2.

It is well known that milk flow curves provide a basis for characterizing both the activity of the milk ejection reflex in cows and its possible inhibition. Analysis of milk flow dynamics offers valuable information for improving

Table 2 Activity of the milk ejection reflex in Brown Swiss cows under stereotypical milking conditions in a “Parallel” milking parlor (M ± m)

Group of animals	Single milk yield (kg)	Udder emptying (%)		Milking duration (min)	Milk flow rate (kg·min ⁻¹)	
		first minute	first two minutes		average	maximum
I, n = 17	11.3 ± 0.09	16.8	49.9	4.43 ± 0.018	2.54 ± 0.019	3.72 ± 0.034
II, n = 15	14.5 ± 0.08	31.2	67.4	4.14 ± 0.026	3.49 ± 0.025	5.23 ± 0.033
III, n = 27	16.2 ± 0.16	21.1	45.6	5.24 ± 0.030	3.08 ± 0.024	3.98 ± 0.038
IV, n = 9	20.5 ± 0.41	17.3	40.4	6.06 ± 0.166	3.39 ± 0.042	4.74 ± 0.094
V, n = 4	23.0 ± 0.33	24.1	46.5	6.55 ± 0.014	3.51 ± 0.054	5.55 ± 0.104
VI, n = 11	12.4 ± 0.06	10.9	21.8	5.13 ± 0.014	2.41 ± 0.015	4.36 ± 0.045

the efficiency of machine milking and maintaining animal health.

In cows of Group I, a classical pattern of milk ejection reflex realization was observed. Immediately after the attachment of the milking unit, milk flow increased during the first minute and reached its peak in the second minute, after which it gradually declined until the end of milking (Figure 1). Specifically, during the first minute, 1.9 kg of milk was withdrawn from the udder, while in the second minute this amount increased to 3.7 kg – 1.9 times higher than during the first minute.

As noted by Moore-Foster et al. (2019), with adequate tactile stimulation of the receptor apparatus in the udder tissues and an optimal latent period (i.e., the delay time before attaching the milking unit), a steep increase in milk flow is observed, followed by the highest flow phase. Conversely, insufficient stimulation, an inadequate delay time, or a combination of both factors may result in inhibition of the milk ejection reflex.

Several researchers (Kaskous & Bruckmaier, 2011) have noted that the shape of the milk flow curve during the first two minutes of milking is directly dependent on

the effectiveness of pre-milking udder preparation, which stimulates the milk ejection reflex. In the present study, it was established that by the third minute of machine milking, the amount of milk extracted had decreased to 2.6 kg, and by the fourth and fifth minutes – to 2.2 kg and 0.8 kg, respectively.

During the first minute of milking, only 16.8% of the total milk yield was obtained, whereas by the end of the second minute this value increased to 49.9%. Thus, nearly half of the total milk yield was obtained within the first two minutes of milking. Milk flow decreased significantly during the third and fourth minutes, averaging 2.6 kg and 2.2 kg, and dropped to only 0.8 kg in the fifth minute. Within the first three minutes of milking, the udders of the cows were emptied by 73.3%. Overall, the average milk flow rate in these cows was $2.54 \text{ kg} \cdot \text{min}^{-1}$, and the maximum rate reached $3.72 \text{ kg} \cdot \text{min}^{-1}$.

Cows of Group II, with a single milk yield of 14.5 kg (Figure 2), exhibited a milk flow pattern similar to that of Group I animals, but their yield was 22.1% higher ($P < 0.001$). Due to the high readiness of Group II cows for milk ejection, 4.5 kg of milk was withdrawn from the udder during

the first minute of milking and 5.2 kg during the second minute, which was 13.5% greater ($P < 0.001$).

During the first minute of milking, the udders of these cows were emptied by only 31.2%, while within the first two minutes this value increased to 67.4%. In the third minute, 3.8 kg of milk was extracted, after which milk flow declined sharply, reaching minimal values of 0.6 kg and 0.3 kg during the fourth and fifth minutes, respectively. Within the first three minutes of machine milking, the cows were practically completely milked, as the udders were emptied by 93.3% of the accumulated secretion.

The similarity in the milk flow dynamics of Groups I and II cows was that, after the attachment of the milking unit, the milk ejection reflex was activated during the first minute and reached its maximum in the second minute. The high milk ejection rates, combined with the considerably higher milk yield of Group II cows compared to Group I, resulted in a short machine milking duration, averaging 4.14 minutes. The average milk flow rate in Group II cows was $3.49 \text{ kg} \cdot \text{min}^{-1}$, while the maximum flow rate reached $5.23 \text{ kg} \cdot \text{min}^{-1}$.

Thus, the combination of soft classical music in the milking parlor, short-term tactile stimulation of the teat tissues lasting 4.5 seconds during sanitary preparation with a handheld cleaning massager, and a 62.4-second delay before milking unit attachment was sufficient to induce a high level of milk ejection reflex activity in cows with single milk yields of 11.3 and 14.5 kg.

In cows of Group III, with a single milk yield of 16.2 kg – 10.5% higher than that of Group II animals – a high level of milk ejection reflex activity was maintained during the first three minutes of milking,

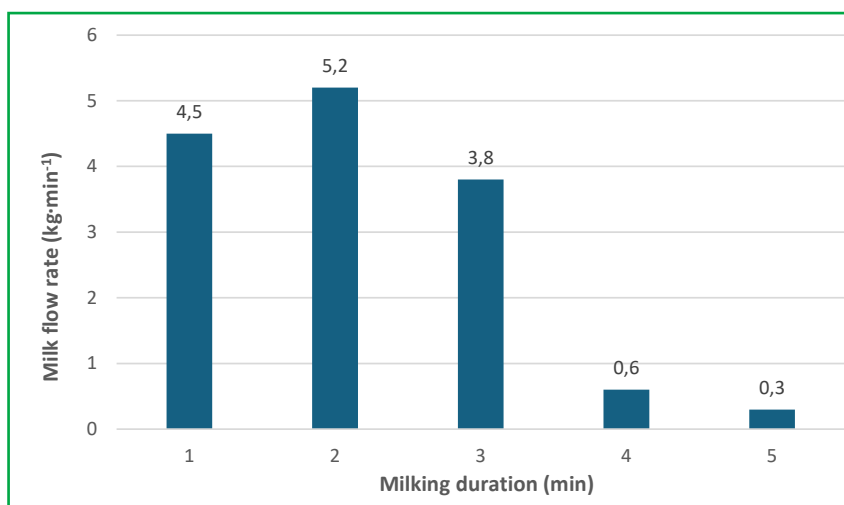


Figure 2 Dynamics of milk flow rate in Group II cows with a single milk yield of 14.5 kg

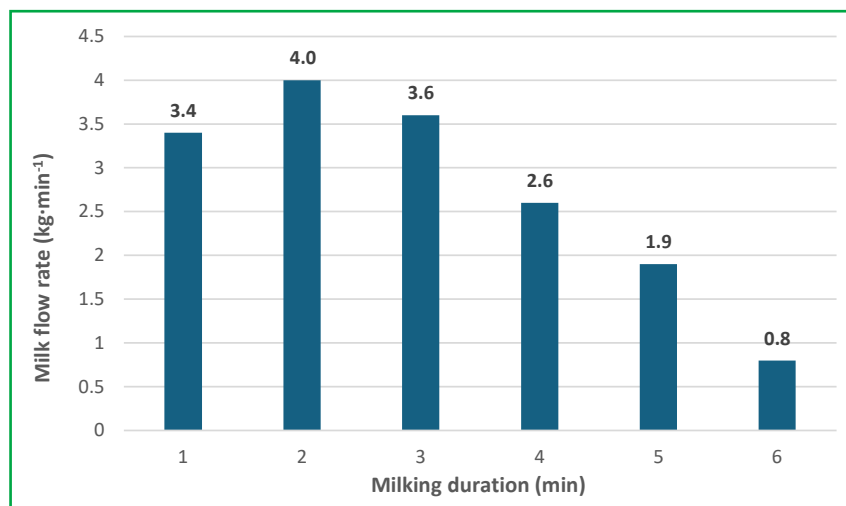


Figure 3 Dynamics of milk flow rate in Group III Brown Swiss cows with a single milk yield of 16.2 kg

after which the milk flow became relatively sluggish (Figure 3), extending the overall milking time. During the first minute, 3.4 kg of milk was extracted from the udder, and 4.0 kg during the second minute, an increase of only 12.5%. In the third minute, 3.6 kg of milk was obtained, which was 10.0% less than in the second minute but only 5.6% higher than in the first. In the fourth and fifth minutes, milk flow declined substantially to 2.6 kg and 1.9 kg, respectively, and reached only 0.8 kg in the final (fifth) minute.

Within the first two minutes of milking, the udders of these cows were emptied by 45.6%, and within three minutes – by 67.6%. The decline in milk ejection reflex activity after the third minute resulted in a relatively prolonged machine milking period, averaging 5.2 minutes. The slightly extended milking time corresponded to moderate milk flow indicators: the average milk flow rate was 3.08 kg·min⁻¹, and the maximum rate reached 3.98 kg·min⁻¹. Although the single milk yield of Group III cows exceeded that of Group II animals by 10.5%, their average milk flow rate was 13.3% lower and the maximum rate 31.4% lower.

Thus, in Group III cows with a single milk yield of 16.2 kg, milked in a “Parallel” milking parlor under short-term preparatory stimulation lasting 4.5 seconds and with a 62.4-second delay before milking unit attachment, a high level of milk ejection reflex activity was maintained during the first three minutes of machine milking – corresponding to 3.4, 4.0, and 3.6 kg·min⁻¹, respectively.

A distinct feature of milk ejection was observed in cows of Group IV, with a single milk yield of 20.5 kg, and Group V animals, with a milk yield of 23.0 kg during the second milking (Figure. 4). Cows of Group IV exhibited a classical pattern of milk ejection reflex realization, in which the peak milk flow was recorded during the second minute of milking, followed by a steady decline until completion. During the first minute, an average of 3.5 kg of milk was extracted from the udder, increasing by 25.5% ($P < 0.001$) to 4.7 kg in the second minute. By the third minute, milk flow had decreased by 6.8% compared to the second minute, amounting to 4.4 kg.

In Group IV cows, with a single milk yield of 20.5 kg, the average and maximum milk flow rates were 3.39 and 4.74 kg·min⁻¹, respectively.

Milk flow during the first, second, and third minutes of milking was not characterized by high intensity and amounted to 1.93, 2.87, and 2.31 kg, respectively. This indicates that continuous udder stimulation lasting 43.5 seconds followed by a 65-second delay before milking unit attachment provides a similar realization of the milk ejection reflex as short-term stimulation lasting 4.5 seconds with a 62.8-second delay.

Moreover, while milk flow during the first minute of milking was nearly identical under both preparation methods, the second- and third-minute flow rates were even higher when short-term stimulation was applied. These findings are consistent with those of Lutsenko and Popkov (2025), who reported that during relatively prolonged preparatory operations (43.5 s) on a “Parallel” milking parlor (2 × 16), which included udder washing with warm water (40–45 °C), wiping with a towel, forestripping, and a 65-second delay before milking unit attachment, the maximum milk flow rate occurred in the second minute of machine milking.

Overall, it should be noted that the present research was conducted on Brown Swiss cows using a “Parallel”-type milking installation, which also played an important role in the complete realization of the milk ejection reflex. Studies of the milk ejection reflex in Brown Swiss cows on an industrial dairy complex, where an extended set of preparatory operations was performed (24.0 s) – including teat treatment with a cleaning solution for 3.6 seconds, drying of udder and teat tissues with a clean towel for 17.9 seconds, and forestripping for 2.5 seconds – ensured high milk flow rates, with an average intensity of 3.3 kg·min⁻¹ and a maximum of 5.4 kg·min⁻¹ (Pishchan, 2015).

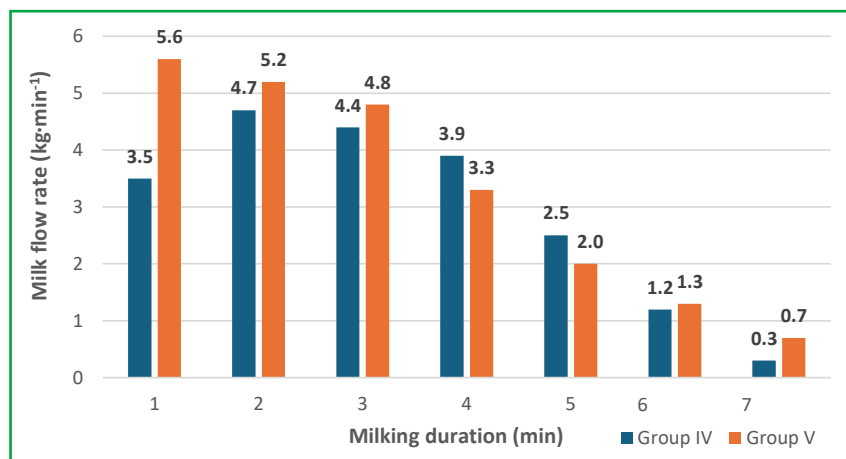


Figure 4 Dynamics of milk flow rate in Brown Swiss cows with single milk yields of 20.5 and 23.0 kg

Furthermore, Lutsenko et al. (2021) found that pre-milking preparation technology implemented in a “Parallel”-type milking parlor provides a 30% higher expression of the milk ejection reflex compared to a carousel-type milking machine, as confirmed by the milk flow rate during the first minute of milking – 2.97 kg·min⁻¹ versus 1.85 kg·min⁻¹.

A different milk flow dynamic was observed in cows of Group V, with a single milk yield of 23.0 kg. In these animals, the maximum milk output was recorded during the first minute of machine milking and averaged 5.6 kg. During the second minute, milk flow decreased by only 7.7%, amounting to 5.2 kg. In the third minute of milking, 4.8 kg of milk was extracted from the udder, which was 16.7% lower than in the first minute and only 8.3% lower than in the second.

Thus, in the high-producing cows of Group V, the first three minutes of machine milking were characterized by the highest milk flow rates. Within the first two minutes, the udders of these cows were emptied by 46.5%, and within three minutes – by 67.4%. During the fourth, fifth, and sixth minutes, milk flow gradually declined to 3.3, 2.2, and 1.3 kg, respectively. The seventh minute of machine milking completed the process, with

an additional 0.7 kg of milk extracted from the udder.

Halai and Lutsenko (2018) reported that significant effects on milking time parameters – such as increased milk flow rate and reduced operating time of the milking unit – are observed in high-producing cows milked three times per day in a “Parallel”-type milking parlor, particularly when the latency period before milking is at least 22.5 seconds.

It was found that, at high levels of single milk yield, cows from Groups IV and V exhibited similar durations of machine milking. The udders of cows in Group IV were completely emptied within 6.1 minutes,

while those in Group V required 6.6 minutes. Consequently, these animals were characterized by high milk flow intensity. The average milk flow rate in Group IV cows was 3.39 kg·min⁻¹, while in Group V it was only slightly higher (+3.4%), averaging 3.51 kg·min⁻¹. In contrast, the maximum milk flow rate in Group IV cows reached 4.74 kg·min⁻¹, whereas in Group V cows it was 7.9% higher ($P < 0.01$), averaging 5.55 kg·min⁻¹.

Thus, in high-producing cows with single milk yields exceeding 20 kg, under stereotypical short-term preparatory operations, the realization of the milk ejection reflex largely depends on the individual response to machine milking. In cows of Group IV, with a single yield of 20.5 kg, the maximum activity of the milk ejection reflex occurred during the second minute of milking, corresponding to the standard pattern of reflex realization, whereas in more productive cows (23.0 kg), it was observed during the first minute.

The study demonstrated that under uniform short-term preparatory procedures and overall standardized machine milking conditions, the peak activity of the milk ejection reflex may also occur during the third

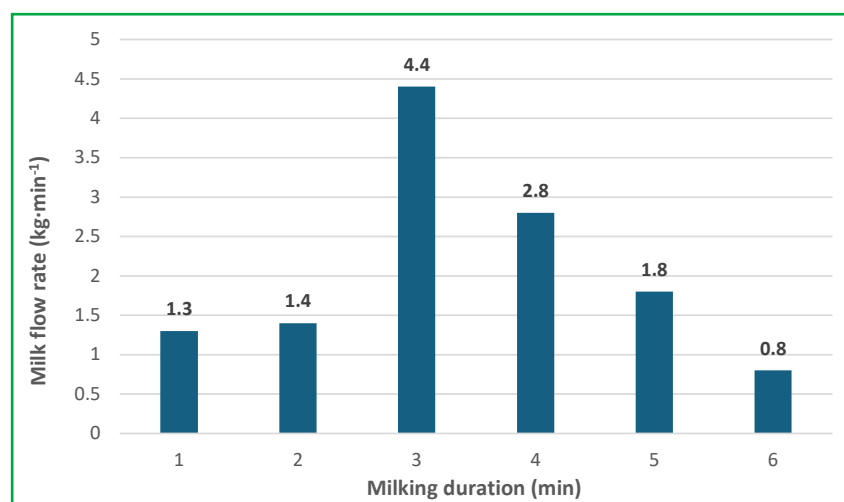


Figure 5 Dynamics of milk flow intensity in Brown Swiss cows with a single milk yield of 12.4 kg

minute of milk extraction. As shown by the dynamics of milk flow in cows of Group VI (Figure. 5), both the first and second minutes of milking were characterized by relatively low milk flow parameters, which did not exceed 1.3 and 1.4 kg, respectively. However, in the third minute, a sharp activation of the milk ejection reflex was observed, resulting in 4.4 kg of milk being extracted from the udder – nearly three times more than in the first and second minutes. Subsequently, during the fourth and fifth minutes, milk flow rates decreased to 2.8 and 1.8 kg, respectively.

Under this pattern of the milk ejection reflex in cows of Group VI, the udder was emptied by only 21.8% during the first two minutes of milking, whereas within three minutes it reached 57.2%. The low activity of the milk ejection reflex immediately after the milking unit was attached resulted in a total milking duration of 5.13 minutes at a single yield of 12.4 kg. Consequently, the average milk flow rate did not exceed 2.41 kg·min⁻¹. The activation of the reflex during the third minute of milking ensured the maximum milk flow rate of 4.36 kg·min⁻¹.

It is likely that the three-time milking schedule per day and the considerable physical load on teat tissues caused by the milking unit, combined with a stereotyped conditioned pre-milking stimulation lasting 98.6 seconds and an unconditioned stimulation of 4.5 seconds with a delay of 62.4 seconds, cannot always ensure the classical pattern of the milk ejection reflex that supports complete udder emptying. In Brown Swiss cows, this type of reflex manifestation was characterized by the absence of a distinct double-peak maximum throughout the milking process. According to Fernandes et al. (2023), delayed milk flow, which most often appears as bimodality, is caused by a disruption in oxytocin delivery to the myoepithelial cells, interrupting the milk flow between the cisternal and alveolar fractions. Bimodality may indicate an insufficient pre-milking stimulation protocol, particularly when the duration of teat stimulation is too short or the delay before cluster attachment is inadequate (Singh et al., 2024).

4 Conclusions

Soft classical music in the milking parlor equipped with a Parallel-type installation, combined with short-term stimulation of teat tissues during hygienic treatment with a handheld cleaning-massager lasting 4.5 seconds and a delay of 62.4 seconds before attaching the milking unit v without forestripping – proved sufficient to induce the milk ejection reflex. With a single milk yield of 11.3–20.5 kg, the highest milk flow activity was observed

during the second minute of machine milking (3.72–5.23 kg·min⁻¹). The average milk flow rate ranged from 2.54 to 3.49 kg·min⁻¹, while udder emptying during the first two minutes of milking reached 40.4–67.4%.

Under stereotyped pre-milking procedures in the milking parlor, highly productive cows with a single yield of 23.0 kg demonstrated the highest milk flow already during the first minute of milking (5.55 kg·min⁻¹). In such animals, the conditioned stimulating factors during movement to the milking stall (98.6 s) and the 62.8-second delay before cluster attachment ($\Sigma = 161.4$ s) occurred within the latent phase of the milk ejection reflex.

The activity of the milk ejection reflex depended less on the total milk yield than on the physiological readiness of the udder for milking, which determined the flow rate achieved by the milking unit. In moderately productive Brown Swiss cows (12.4 kg), inhibition of the milk ejection reflex may occur even under stereotyped pre-milking operations. As a result, only minimal milk volumes (on average 1.3 and 1.4 kg) were obtained during the first and second minutes, while maximum milk flow (4.4 kg·min⁻¹) occurred in the third minute, corresponding to an average flow intensity of 2.41 kg·min⁻¹.

Author Contributions

Conceptualization, I.P. and S.P.; Methodology, S.P., I.K. and L.M.; Validation, L.L. and A.H.; Formal analysis, S.P. and I.K.; Data curation, I.P. and S.P.; Writing – original draft preparation, S.P. and I.P.; Writing – review and editing, L.L., L.M., A.H. and I.K.; Visualization, L.M.; Project administration, S.P.

Statement

During the preparation of the manuscript, no generative AI tools or AI-assisted technologies were used.

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