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ABSTRACT

The aim of the study was to investigate the relationships between milk flow traits, milk Somatic Cell Count (SCC) and teat apex score in primiparous cows during the first 120 days of lactation.
A total of 82 primiparous Holstein cows were randomly selected in 6 herds characterized by different milking machines and management. The cows were monitored monthly during the first 120 days of lactation. Each month, milk flow curves of the whole udder of each primiparous cow were registered with continuous electronic milk flow meters (Lactocorder), quarter milk samples were aseptically collected, somatic cells were counted, and teat apex scores were assessed. Quarter milk samples were divided in two groups according to SCC: <200,000 and >200,000 cells/ml, respectively. At udder level each cow was classified as low SCC (LSCC) when all quarters had <200,000 cells/ml and high SCC (HSCC) in all other situations, separately for each control. Animals with at least one HSCC control during the first 120 days of lactation (n=33), compared to subjects always classified as LSCC cows (n=49), showed significantly higher average milk flow (2.32 *vs* 2.13 kg/min; P<0.05), peak milk flow (3.71 *vs* 3.14 kg/min; P<0.05), lower duration of plateau phase (2.33 *vs* 3.27 min; P<0.05), longer duration of increase phase (0.81 *vs* 0.66 min; P<0.05), and higher average SCC (4.07 *vs* 3.45 Log_{10} SCC; P<0.05). Moreover, primiparous cows with at least one HSCC control had a significant increase in peak milk flow during the first four months of lactation, while the other cows showed only a slight increase. Teat apex scores significantly increased in both groups of primiparous cows as lactation progressed but values in subjects always classified as LSCC animals were constantly lower with respect to cows with at least one HSCC control. The results obtained in this study support the relationship between SCC, teat apex score and milk flow traits.

Key words: Primiparous cows, Milking, Milk flow, SCC, Teat apex score.

RIASSUNTO

CURVE DI EMISSIONE DEL LATTE, CELLULE SOMATICHE E CONDIZIONE DEI CAPEZZOLI IN BOVINE PRIMIPARE ALL'INIZIO DELLA LATTAZIONE

Scopo del lavoro è stato quello di valutare le interazioni tra curve di emissione del latte, contenuto di cellule somatiche e condizioni dell'apice dei capezzoli di bovine primipare nel corso dei primi 120 giorni di lattazione. Il lavoro ha coinvolto 82 bovine primipare di razza Frisona selezionate in 6 aziende caratterizzate da differenti impianti e diverse modalità di mungitura. I rilievi sono stati effettuati a cadenza mensile per i primi 120 giorni di lattazione e hanno riguardato: le curve di emissione del latte, misurate tramite flussometri elettronici (Lactocorder), la valutazione delle condizioni dell'apice dei capezzoli e le analisi citologiche del latte di ogni quarto mammario. I campioni di latte di quarto sono stati suddivisi in due classi a seconda che il contenuto di cellule somatiche fosse, rispettivamente, inferiore o superiore al limite di 200.000 cellule/ml. A livello di mammella quindi sono state definite due classi: basso contenuto di cellule somatiche (LSCC), quando tutti i quarti avevano conte cellulari inferiori a 200.000 cellule/ml, e alto contenuto di cellule somatiche (HSCC) in tutte le altre situazioni, separatamente per ciascun controllo. Dai risultati ottenuti si è evidenziato che le bovine con almeno un controllo HSCC nei primi 120 giorni di lattazione (n=33), rispetto agli animali classificati in tutti i controlli LSCC (n=49), sono stati caratterizzati da valori significativamente più elevati di flusso medio (2,32 vs *2,13 kg/min; P<0,05), di flusso massimo (3,71* vs *3,14 kg/min; P<0,05), da una minor durata della fase di plateau (2,33* vs *3,27 min; P<0,05), da una maggior durata della fase ascendente (0,81* vs *0,66 min; P<0,05) e da un più alto valore medio di cellule somatiche (4,07* vs *3,45* Log_{10} *SCC; P<0,05). Inoltre gli animali con almeno un controllo HSCC hanno mostrato un significativo incremento del flusso massimo nei primi 120 giorni di lattazione rispetto alle bovine classificate in tutti i controlli come LSCC, che hanno invece fatto registrare solo un lieve incremento. Entrambi i gruppi hanno mostrato un peggioramento della condizione dell'apice del capezzolo con il progredire della lattazione, più marcato negli animali HSCC. I risultati ottenuti confermano la relazione tra le caratteristiche delle curve di emissione, le cellule somatiche del latte e le condizioni dell'apice dei capezzoli. Lo studio della curva di emissione lattea può quindi essere uno strumento utile per la valutazione non solo della modalità di mungitura ma anche dello stato della mammella.*

Parole chiave: *Bovine primipare, Mungitura, Flusso di eiezione lattea, Cellule somatiche, Condizioni del capezzolo.*

Introduction

Modern machine milking requires complete and fast removal of milk from the udder under optimal hygienic conditions to maintain high milk yield, product quality and animal health at a low cost (Bruckmaier and Hilger, 2001).

Milking is a well-known management factor that can affect udder health status. Many authors demonstrated relationships between milking routine (stimulation time, forestripping, pre-dipping, post-dipping), milking machine and udder health, in terms of somatic cell count (SCC) and teat tissue integrity (Rasmussen *et al.*, 1992; Zecconi and Hamann, 2006; Tancin *et al.*, 2007).

Milking routine and milking machine characteristics also affect the shape of milk ejection curve (Sandrucci *et al.*, 2007). In particular for fresh primiparous cows, the milking process may be stressful, due to the novelty and close interaction with the human handler. The mammary gland of primiparous cows is characterized by a smaller cisternal portion compared to pluriparous ones, partly as a consequence of the smaller total milk yield in primiparous cows (Pfeilsticker *et al.*, 1996). So it is useful to perform an efficient mammary gland stimulation before teat cup attachment in order to induce a proper release of oxytocin and good milk let down of alveolar fraction.

Primiparous cows showed lower peak

milk flow, lower duration of plateau phase and higher percentage of bimodal curves characterized by non-continuous flow, in comparison with pluriparous cows (Dodenhoff *et al*., 1999; Sandrucci *et al*., 2007).

The detailed analysis of milk flow curves using electronic milk flow meters provides useful information for assessing milking process and its relationship with udder health (Weiss *et al*., 2004; Sandrucci *et al*., 2007).

Somatic cell count gives an indication of the inflammatory response to an intramammary infection or another trigger of the immune system. Milk analysis at the quarter level shows the most accurate relationship between intramammary infection and SCC (Schukken *et al*., 2003). Milking technique is one of the management factors that have an influence on the level of bulk SCC (Barkema *et al*., 1998) and on teat conditions. In fact during machine milking, the teat represents the interface between the mammary gland and teat cup liner (Weiss *et al*., 2004). Machine milking can have effects that occur either in the short or in the long term. Short-term effects are, for example, teat swelling and colour changes; long-term modifications are open lesions of teat skin and development of hyperkeratosis as a stress response (Mein *et al*., 2001). Teat apex characterized by rough surface is more difficult to clean during pre-milking preparation, and it is often associated with teat-end lesions which can be frequently colonized by bacteria. Neijenhuis *et al*. (2001) found a correlation between clinical mastitis and teat-end callosity.

This paper reports the results of a field study aimed to investigate the relationships between milk flow traits, milk SCC and teat apex conditions in primiparous cows, during the first 120 days of lactation.

Material and methods

Herds and milking characteristics

A total of 82 primiparous Holstein cows (28.5±3.79 months calving age) were randomly selected in 6 herds, characterized by different milking machines and management (Table 1). Only cows in their first lactation were selected in order to minimize the risk of previous infections. In one herd cows were housed in a tie-stall barn (herd A, experimental farm), while all the other ones were free-stall barns (B, C, D, E, F, commercial farms). Within these latter herds, three had herringbone parlours (B, C, D), one had parallel parlour (F) and one had tandem parlour (E). Milking operations were performed by a single milker in herds A and B and by two milkers in the other herds. Dairy cows were milked twice

Table 1. General description of farms and milking parlours.

Farm	Cows no.	Type of parlour	Milking units no.	Milkers no.	Pulsation ratio	System vacuum (kpa)	Milking frequency (/d)	Milk production (kg/d)
A	10	pipeline tie stall	2	1	60/40	42	2	28.1 ± 4.73
B	69	herringbone	4+4	1	60/40	43	2	35.3 ± 7.28
C	70	herringbone	6+6	2	60/40	42	2	36.0 ± 5.07
D	188	herringbone	12+12	2	60/40	42	2	30.1 ± 7.87
E	104	tandem	5+5	2	60/40	42	2	35.2 ± 4.84
F	87	parallel	10+10	2	60/40	42	3	34.3 ± 7.56

a day in 5 herds and three times a day in one herd. In all the herds milking procedures included: pre-milking cleaning of the teats with a detergent and single-use paper towel, fore-milking, and teat dipping at the end of milking.

Sampling and analysis

Primiparous cows enrolled in the study calved between September and December 2005 and they were monitored monthly; due to slight different intervals between farm visits, it was not possible to have the same number of observations in each month of lactation. Quarter milk samples were collected before milking by an aseptic procedure and immediately delivered to the lab in a refrigerated container. Somatic cells were counted using Bentley Somacount 150 (Bentley, USA).

Udder health conditions

Quarter milk samples were classified in two groups as follow: samples with SCC <200,000 cells/ml and samples with SCC ≥200,000 cells/ml, respectively. At udder level each cow was classified as LSCC when all quarters had <200,000 cells/ml and HSCC in all other situations, separately for each control. Each cow, during the first 120 days of lactation, was classified as 'always LSCC' when all controls were LSCC and 'at least one HSCC control' when at least one control was HSCC at udder level. SCC was expressed as logarithmic transformation.

Teat conditions were assessed by scoring digital pictures of teat apex, transferred on a PC, following the procedure described by Zecconi *et al*. (2006).

Milk flow measurements

Milk flow curves of the whole udder of each primiparous cow were registered once per month, with a continuous electronic milk flow meter (Lactocorder, WMB, Switzerland). The instrument measured milk flow, milk yield, and electrical conductivity throughout the evening milking. Milk flow characteristics were detected every 0.7 s and saved at intervals of 2.8 s. The flow profile was divided into 3 main phases: 1) increase phase (from milk flow rate >0.5 kg/min until the start of the plateau phase); 2) plateau phase (phase of steady flow determined by the slopes of the milk flow profile); 3) decrease phase (from the end of the plateau phase until milk flow dropped below 0.2 kg/min). Overmilking time was calculated as period from milk flow <0.2 kg/min until cluster removal. Peak milk flow was defined as the maximum milk flow during any 22.4 s period and the average milk flow was calculated from duration of main milking (the sum of increase, plateau and decrease phases) and milk yield Sandrucci *et al*. (2007). Bimodality of milk flow was detected when a curve had a flow pattern with 2 increments separated by a clear drop in milk flow for more than 200 g/min within 1 min after the start of milking (Dzidic *et al*., 2004). Stimulation time was measured using Lactocoder from the first touch of the teat to the beginning of milk flow emission (this phase includes teat cleaning operations). Total milking time was calculated as indicated by Sandrucci *et al*. (2007). Time of milk ejection was defined as total milking time minus stimulation time.

Statistical analysis

Data collected during the first 120 days of lactation were analysed by ANOVA by using a generalized linear model (GLM procedure; SAS, 2001). The model was:

$$Y_{ijklm}=\mu+H_i+A_j(H_i)+M_k+S_l+C_m+SC_{lm}+MC_{km}+e_{ijklm}$$

where: Y=dependent variables; μ=general mean; H_i=effect of herd (i=1-6); $A_j(H_i)$=effect of animal nested in herd (j=1-14); M_k=effect of milk production level (k=<30; 30-36; >36 kg/d); S_l=effect of stage of lactation (l=1-4 months); C_m=effect of SCC level (m=always LSCC - at least one HSCC control); SC_{lm}=effect of SCC level x stage of lactation interaction; MC_{km}=effect of milk production level x SCC level interaction; e_{ijklm}=residual error.

Principal Component Analysis (PCA) was used in order to examine relationships among several quantitative variables (milk yield, stimulation time, time of milk ejection, average milk flow, peak milk flow, time of increase phase, time of plateau phase, time of decrease phase), and it was performed by proc Princomp (SAS, 2001). PCA identifies orthogonal directions of maximum variance in the original data and projects them into a lower-dimensionality space formed of a subset of the highest variance components; in particular, the first significant component explains the largest percentage of the total variance, the second one, the second largest percentage.

Results and discussion

Table 1 reports a general description of farms and milking parlours involved in the study. Pulsation ratio and vacuum level were almost the same, but type and size of parlour were different and they likely influenced milking routine and measured data. On a total of 294 observations, milk yield per milking was on average 13.5±3.8 kg and peak milk flow was 3.3±1.1 kg/min. Milking time was, on average, 7.1±2.4 min, longer than milking time found by Tancin *et al*. (2006) for primiparous cows during the whole lactation. Stimulation time was quite high (2.28±1.45 min) with large variations between farms. Increase phase was only 0.77±0.43 min and showed wide variability. Plateau phase had an average duration of 2.8±1.7 min and decrease phase showed an excessive duration (2.5±1.3 min). Frequency of bimodality was 24.8% with a standard deviation of 43.3%, as already reported by Dodenhoff *et al*. (1999). Overmilking phase was 0.76 min on average, characterized by wide variability (±0.82), which is a sign of a irregular milking routine. Overmilking unnecessarily increases machine on time and can promote poor teat apex condition (Rasmussen, 1999). Logarithmic transformation of quarter SCC (Log_{10}/ml) was 4.74±0.51. Teat apex conditions showed similar scores for each quarter and average score was low (1.3±0.46), probably because all the cows were at the beginning of their first lactation.

Milk flow analysis and teat condition assessment

After calving, primiparous cows should adapt to a new social (i.e. interaction with older cows) and physical environment (i.e. milking), while they are still modifying their anatomical and physiological characteristics. A nice example of these complex interactions is represented by the relationship between the milking machine and the teat. It is well-known that the teat increases in length and diameter as the number of lactations proceeds (Hamann and Burvenich, 1994). Some of these changes could significantly affect the risk of new infection through impairment of teat tissue immune defences and by inducing transient or persistent teat lesions (Zecconi *et al*., 1996; Neijenhuis *et al*., 2000; Mein *et al*., 2001). Table 2 reports the comparison of the data obtained during the whole period. Primiparous cows with at least one HSSC control showed significantly higher average milk flow (2.32 *vs* 2.13 kg/min, P<0.05) and peak milk flow (3.71 *vs* 3.14 kg/min, P<0.05) than the other primiparous cows. The higher milk flow suggests that the animals are probably characterized by a wider teat orifice than the other cows, which could allow easier penetration of microorganisms. High SCC and high peak milk flow seem to be related, as found by Sandrucci *et al*. (2007). Milk flow curves

Table 2. Least squares mean values for DIM, milk flow parameters, SCC and teat apex score measured during the first 120 days of lactation of 82 primiparous cows.

		Always LSCC	At least one HSCC control	SEM
	no.	176	118	
DIM		63.5	60.8	2.14
Milk yield	kg/milking	14.2	13.7	0.33
Stimulation time	min	2.30	2.23	0.12
Time of milk ejection	"	7.87[a]	6.74[b]	0.28
Average milk flow	kg/min	2.13[b]	2.32[a]	0.07
Peak milk flow	"	3.14[b]	3.71[a]	0.12
Time of increase phase	min	0.66[b]	0.81[a]	0.05
Time of plateau phase	"	3.27[a]	2.33[b]	0.19
Time of decrease phase	"	2.54	2.65	0.15
Increase: plateau ratio		0.29[b]	0.48[a]	0.05
Decrease: plateau ratio		0.83[b]	1.21[a]	0.09
Bimodality	%	23.5	22.7	4.97
Maximum electrical conductivity*	mS/cm	6.16	6.15	0.05
SCC mean	Log_{10}/ml	3.45[b]	4.07[a]	0.07
Average teat apex score		1.20[b]	1.49[a]	0.05

Means within a row with different superscripts differ (P<0.05).
**Electrical conductivity measured during plateau phase.*

of primiparous cows with at least one HSCC control were characterized by lower duration of the plateau phase (2.33 *vs* 3.27 min, P<0.05) and longer duration of the increase phase (0.81 *vs* 0.66 min, P<0.05) in comparison with the other cows. These results show that the shape of the milk ejection curve can be related to SCC levels, so the milk flow curve could give some information about udder health. The ratio between incline phase and plateau phase and the ratio between decrease phase and plateau phase were significantly higher in cows with at least one HSCC control in comparison to the others. The longer duration of secondary phases (increase and decrease) with respect to the main emission phase (plateau) can suggest a situation of disturbed milk ejection. In fact, milk flow curves characterized by a long duration of increase phase are more susceptible to show bimodality (Sandrucci *et al.*, 2007); moreover, long duration of the decrease phase is reported in association with high SCC (Tancin *et al.,* 2007). Average teat apex score was significantly higher in animals with at least one HSCC control, when compared with cows always classified as LSCC (1.49 *vs* 1.20, P<0.05). Rough teat apices are more difficult to clean and they can more frequently be a ‘source’ of bacte-

Table 3. Least squares mean values for DIM, milk flow parameters, SCC and teat apex score measured during the first 120 days of lactation in primiparous cows 'always LSCC' and 'at least one HSCC control'.

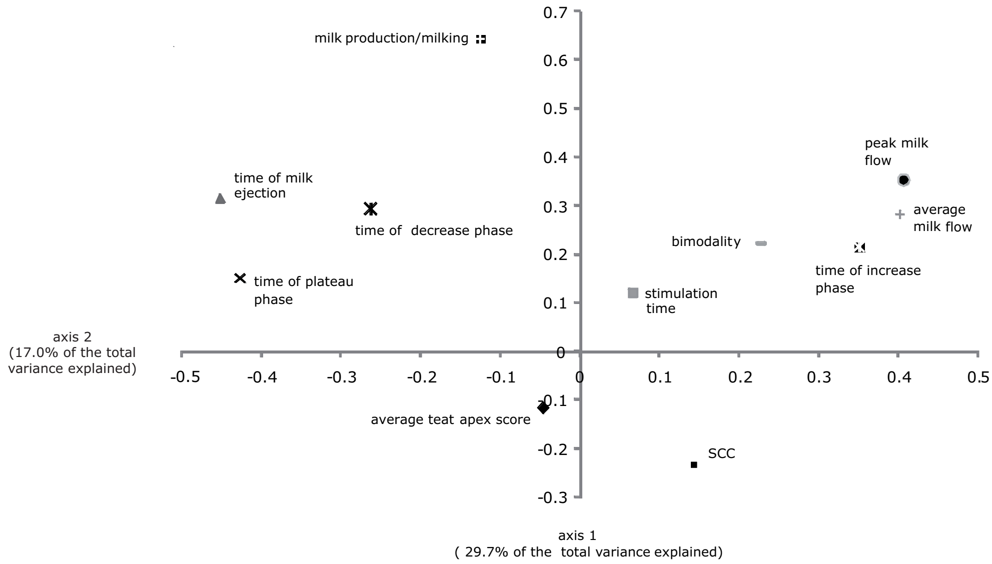
		Always LSCC				At least one HSCC control				
		Month of lactation				Month of lactation				
		1	2	3	4	1	2	3	4	SEM
	no.	33	45	51	47	25	32	33	28	
DIM		16.4^{d}	45.0^{c}	77.1^{b}	106.8^{a}	17.4^{d}	45.1^{c}	76.5^{b}	105.4^{a}	2.53
Milk yield	kg/milking	13.1^{b}	14.7^{a}	14.0^{a}	14.4^{a}	11.7^{b}	14.1^{a}	14.4^{a}	13.9^{a}	0.76
Stimulation time	min	2.72^{a}	2.25^{ab}	2.28^{ab}	2.05^{b}	2.51	2.26	2.10	2.22	0.28
Time of milk ejection	"	7.50	8.40	7.21	7.80	6.40	6.91	7.05	6.02	0.66
Average milk flow	kg/min	1.94^{b}	2.09^{ab}	2.26^{a}	2.26^{a}	2.09^{b}	2.27^{b}	2.38^{ab}	2.62^{a}	0.17
Peak milk flow	"	2.79	3.18	3.29	3.21	3.20^{b}	3.49^{b}	3.95^{a}	4.06^{a}	0.29
Time of increase phase	min	0.54	0.65	0.71	0.72	0.69	0.80	0.85	0.91	0.12
Time of plateau phase	"	3.52	3.48	2.93	3.14	2.07	2.65	2.48	2.05	0.46
Time of decrease phase	"	2.28	2.77	2.83	2.15	2.79	2.63	2.64	2.61	0.36
Bimodality	%	12.4	27.2	26.4	22.9	10.1	15.2	29.1	27.3	11.6
SCC mean	Log_{10}/ml	3.50	3.45	3.44	3.44	4.73	3.87	3.90	3.89	0.14
Average teat apex score		1.05^{b}	1.12^{b}	1.23^{ab}	1.33^{a}	1.24^{c}	1.51^{ab}	1.44^{bc}	1.68^{a}	0.12

Means within a row with different superscripts differ (P<0.05), separately for LSCC and HSCC group.

ria. These data seem to confirm the relationship between poor teat condition and high SCC, according to Neijenhuis *et al*. (2000), although primiparous cows show few teat apex problems. The results confirmed the importance of milking machine characteristics and milking procedures as factors affecting teat conditions, and hence udder health status (Querengasser *et al*., 2002; Sandrucci *et al*., 2007; Tancin *et al*., 2007).

When the results were analysed by months of lactation (Table 3), data showed that primiparous cows with at least one HSCC control had a significant increase in peak milk flow during the first four months of lactation while the other cows had only a slight, not significant, increase. Moreover, peak milk flow values in primiparous cows with at least one HSCC control were always higher than those in the other primiparous cows. Also average milk flow significantly increased in both groups with stage of lactation; cows with at least one HSCC control showed a more consistent increase and higher average milk flow values than animals with always LSCC. Bimodality increased in both groups during lactation, although not significantly, probably because of a reduction of cisternal area during lactation (Caja *et al.*, 2004). Teat apex scores significantly increased in both groups of cows as lactation progressed, but values in those always classified as LSCC animals were constantly lower than scores observed in the other group. Such differences in terms of apex conditions were already observed in

Figure 1. Principal component analysis (PCA).



the first month of lactation, suggesting the importance of a careful evaluation of milking machine characteristics and milking procedures in order to prevent outcome of persistent teat disorders.

PCA showed some interesting correlations between variables (Figure 1). Axis 1 explains 29.7% of the total variance and axis 2 explains 17.0% of the total variance. Peak milk flow, average milk flow, bimodality, time of increase phase and stimulation time are located in the same part of the graph, suggesting that these parameters are related. Bimodality, SCC and stimulation time are close each other on axis 1, which explains most of the variance. Somatic Cell Count is located in the opposite part of the graph in relation to milk production level probably because these two parameters have a negative correlation. Time of milk ejection is positively correlated to time of plateau and decrease phases; on the other hand, it is not associated with duration of increase phase.

Conclusions

The results obtained in this study support the presence of a relationship between SCC, teat apex scores and some milk flow traits. In particular there is a close relation among high peak milk flow, short duration of plateau phase and the high milk SCC in primiparous cows at the beginning of lactation. Moreover, in primiparous cows worse teat apex scores are associated with high SCC already during the early months of lactation. Milk flow trait measurement was confirmed to be a practical and useful means of assessing the adequacy of milking procedures and milking machine settings.

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