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Effects of chronic mastitis and its treatment with ketoprofen on the milk ejection curve

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In this Research Communication we report the results of a controlled study conducted under field conditions in which we analysed milk ejection curves in cows with chronic mastitis, and assessed the influence of antinflammatory treatment with ketoprofen. Total milking time was reduced in chronic mastitis cows, irrespective of ketoprofen treatment, and the proportion of bimodal flow curves was increased. This latter effect was partially reversed by ketoprofen. To our knowledge, this is the first study showing that chronic mastitis has a significant effect on the milk ejection curve. Antinflammatory treatment with ketoprofen was shown to be efficacious in reducing these negative effects, re-establishing a pattern close to the one observed in healthy cows.

Keywords: milk ejection curve, chronic mastitis, inflammation, NSAIDs.

Introduction

Milking machine characteristics and milking procedures can affect udder health status, milk somatic cell count (SCC) and teat conditions (Rasmussen & Madsen, 2000; Weiss & Bruckmaier, 2005; Zecconi & Hamann, 2006). Another association between mastitis and milking is repre- sented by the influence on the milk ejection curve. Indeed, an inflammatory status could affect milk ejection by inhibition of oxytocin activity (Wellnitz et al. 1997; Wellnitz & Bruckmaier, 2001).

Among the several forms of mastitis, chronic mastitis is a pathological condition with several peculiar features, includ- ing a decrease in milk yield, consistently high SCC and very poor cure rate for antimicrobial treatment. Thus, farmers have only two options: to cull chronic cows as soon as pos- sible or to keep them until they are dried off. This latter option implies that cows are regularly milked until dry-off, despite the presence of an inflammatory status and, very likely, of udder pain (Leslie & Petersson-Wolfe, 2012).

Relatively few studies have addressed the effects of mas- titis on the milk ejection curve, and these have mainly been in subclinical mastitis (Tancin et al. 2007; Zucali et al. 2009; Tamburini et al. 2010), but not in cows with chronic mastitis.

Based on previous considerations, this paper reports the results of a controlled study conducted under field conditions aiming to analyse milk ejection curves and to assess the influence of anti-inflammatory treatment with ketoprofen on milk ejection curves in cows with chronic mastitis.

Materials and methods

Study design

Three different herds were considered for this study. In all three, cows were milked twice a day with a milking vacuum of 42 kPA, and a pulsation ratio of 42. One herd

(A) had a tandem parlour, the lowest number of lactating cows (22) and an average milk yield of 24·6 kg/d. The other two herds, B and C, had herringbone parlours, respect- ively 117 and 85 lactating cows and an average milk yield of 21·5 kg/d for herd B and 30·8 kg/d for herd C (see also online Supplementary Table S1).

Cow selection

Chronic cows were identified by the Farmer Association monthly individual cows SCC evaluation, applying the fol- lowing inclusion criteria: SCC >400·000/ml in the last two monthly records; no blind quarters. Exclusion criteria were the following: clinical mastitis in the last two months, other clinical diseases in the last two months, antimicrobial or anti-inflammatory treatment in the last two months for any reason, time to

drying off <90 d. Enrolled chronic cows were confirmed by performing SCC on individual milk sample 3–4 d before entering into the trial. Randomly chosen healthy cows milked contemporarily to the diseased ones were enrolled in the study representing a negative-healthy control group. The inclusion criteria for these cows were their contemporary presence with diseased ones and the absence of chronic or clinical mastitis. Within this group, only cows with at least four milking session recorded at the end of the follow-up period were included in the final database.

Treatments

The herd veterinarian assigned chronic cows randomly to the treated or untreated group once they were enrolled in the study. The treatment protocol was: 15 ml ketoprofen (Dinalgen™ 15%, Ecuphar, Bruges, B) administered intra- muscularly every Monday and Thursday after milking for 4 weeks. Healthy cows did not receive any treatment during the entire follow-up period.

Milk ejection curve assessment

Milk flow curves of each enrolled cow were recorded with a continuous electronic milk flow meter (Lactocorder™, WMB, Balgach, CH) twice a week in combination with treatments and once more 4 d after the end of treatments.

Data collected by Lactocorder™ were transferred, and then elaborated by software LactoPro™ (WMB, Balgach, CH). The raw data were exported to a database and com- bined with cow data for the statistical analysis.

Statistical analysis

Data were analysed by a mixed model for repeated mea- surements (Littel et al. 1996) using PROC MIXED of SAS (SAS Institute, Cary NC USA, rel. 9·4).

The model included as fixed effects: herd (HERD), the number of parturition (AGE), days in milk (DIM), time of sampling from enrolment (TIME), health status and treatment (GROUP), and the interactions of GROUP with the other fixed effects.

The frequency of bimodal curves (due to the temporary collapse in the milk flow registered after removal of milk cis- ternal fraction) in the three groups of cow considered was analysed by $\chi 2$ test using PROC FREQ of SAS (SAS Institute, Cary NC USA, rel. 9·4).

Results

The study included 102 cows, 34 of them were classified as chronic and among these cows, 19 were treated with keto- profen, while 15 were left untreated. Milking data from 68 cows classified as healthy and milked in the same sessions as the chronic ones were also included in the study (see also online Supplementary Table S1). Overall, 583 milking curves were included, chronic untreated cows (Chronic) represent 23·2% of all data, chronic treated cows (Treated) 28·7% and healthy ones (Healthy) 48·1%. Characteristics of the enrolled cows are the following: 43% of them were in their first lactation, 30% in the second, 27% in the third and higher; 34% of samples were collected when cows were between 60 and 120 DIM, 36% of cows between 121 and 240 DIM and 30% of cows with >240 DIM.

Data on overall milking and mean values (± SD) of the three parameters (MGG, tMGG, AMF) for the three groups of cows (Chronic, Treated and Healthy) are reported in Table 1 and the results of statistical analysis are reported in online Supplementary Table S2. The statistical analysis shows as total milk yield (MGG) was significantly influenced by HERD, DIM, TIME and by the interaction of GROUP with HERD. GROUP and interaction of GROUP with TIME and AGE influenced the length of total milking period (tMGG), while only HERD and DIM significantly affected average milk flow (AMF).

Results of statistical analysis of parameters related to main milking process are reported in online Supplementary Table S3. GROUP and its interaction with HERD and TIME significantly influenced the time of main milking process (tMHG). The interaction of GROUP with TIME significantly influenced average main milking process (DMHG), (see also Supplementary Fig. SF1), and maximum peak flow per min (HMG), while TIME, among the fixed factors, was the only one affecting the variability of all the three parameters considered.

The statistical analysis of incline and plateau phases (online Supplementary Table S4) showed as HERD and TIME influenced all the parameters considered, out of the time of plateau phase (tPL). Three parameters related to respectively to the first, second and third minutes of milking, MG1 (see also Supplementary Fig. SF2), MG2, MG3, were significantly influenced by the interaction of GROUP with TIME.

Decline, overmilking and stripping phases were poorly influenced by the factors considered (online Supplementary Table S5).

Statistical analysis of electrical conductivity (EC) para- meters showed as GROUP, but none of its interactions with the other fixed factors, had a significant influence on any of the parameters measured (online Supplementary Tables S6 and S7).

The frequency of bimodal curves was 24·3% in Healthy group, 32·3% in Treated group and 45·2% Chronic group ($P = 0{\cdot}0001$; $\chi 2$ test) with a significant difference between the frequencies observed in Chronic group

115 ($P < 0{\cdot}05$) and the frequencies observed in Treated and Healthy group. The frequencies between these last
116 two groups were not statistically different.

117 Discussion

118 Cows with chronic mastitis represent a peculiar group of animals within a dairy herd, which poses a number
119 of problems to dairy farmers and to veterinarians. If keeping a chronic cow in production can be justified
120 for economic reasons, from a welfare point of view, it cannot be ignored that a distress related to udder
121 inflammation during milking can be present (Langford & Stott, 2012).

122 Scientific data on milk ejection curves in chronic cows are not available. To our knowledge, this is the first
123 study under field conditions designed to analyse milk ejection curves in chronic cows and to assess the
124 influence of antinflammatory treatment of milk ejection curves in cows with chronic mastitis.

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Table 1. Mean values (± SD) observed for parameters related to total milking in the three group of cows considered

	Healthy	Chronic	Treated
Total milk yield (kg, MGG)	13·29 ± 3·76	9·95 ± 3·67	12·37 ± 4·49
Total milking period (min, tMGG)	6·55 ± 1·91	5·76 ± 2·66	5·88 ± 1·86
Average milk flow (kg/min AMF)	2·10 ± 0·61	1·87 ± 0·76	2·14 ± 0·73
Duration of rise phase (min, tAN)	0·76 ± 0·47	0·95 ± 0·58	0·79 ± 0·45
Time of plateau phase (min, tPL)	2·53 ± 1·32	1·38 ± 1·22	1·74 ± 1·32
Time of decline phase (min, tAB)	2·63 ± 1·28	2·74 ± 1·88	2·59 ± 1·19

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127 When initial and plateau phases were considered, the main factor influencing the variability of the
128 parameters describing these phases was HERD. However, milk yield in the initial three minutes of milking was
129 significantly influenced by the interaction between GROUP and TIME. Indeed, chronic untreated cows
130 showed always a lower milk yield after the first three sampling when compared both to chronic treated and
131 healthy cows. These results, all together, suggest that the presence of a chronic mastitis affects mainly the
132 initial and plateau phases of the milk ejec- tion curve. This observation is supported by the analysis of the
133 frequency of bimodal curves that was significantly higher in chronic untreated cows when compared with
134 the other two groups.

135 The presence of a significant effect on the initial and plateau phases of milking indirectly suggest an
136 impairment of oxytocin activity during chronic mastitis due to the presence of inflammation and, probably,
137 pain. Therefore, alteration of the milk ejection curve could be reduced, decreasing the inflammatory
138 response into the udder by the application of a treatment with an NSAID (i.e. ketoprofen).

139 The results of this study support this hypothesis. Indeed, when the treated chronic cows group is considered,
140 the EC mean values were higher than in Healthy, but lower than in Chronic group suggesting that the
141 treatment was able to reduce the inflammation.

The presence of a positive effect of ketoprofen is sup- ported by the significant influence observed on DMHG in the main milking process, with Treated group mean values significantly higher than in Chronic and close to Healthy. Likewise, the pattern of DHMG in Treated group was diver- gent from the one observed in Chronic group, and very similar to Healthy group, after three treatments. The positive results are also supported by the significant reduction in the frequency of bimodal curves in Treated group, when compared to Chronic group, probably thank to a more efficient oxytocin activity at the beginning of milking (Wellnitz & Bruckmaier, 2001).

Conclusions

The results of this field trial show that chronic mastitis has a significant effect on the milk ejection curve at initial and plateau phase. Antinflammatory treatment with ketoprofen was shown to be efficacious in reducing these negative effects, re-establishing a pattern close to the one observed in healthy cows.

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