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Risk factors of high somatic cell count and differential somatic cells in early lactation associated with selective dry cow therapy

S. Mondini, G. Gislon, M. Zucali, A. Sandrucci, A. Tamburini *, L. Bava

Department of Agricultural and Environmental Sciences, University of Milan, via Celoria 2, 20133 Milan, Italy

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ABSTRACT

The routine use of intramammary antimicrobial products in all dairy cows at the beginning of the dry period is no longer allowed in European Union (**EU**) countries due to the new Regulation (EU) 2019/6 to reduce antimicrobial resistance. This study investigated the application of a selective dry cow therapy scheme and the risk factors of high individual milk somatic cell count (**SCC**) and individual neutrophil count in early lactation, as a response to the application of a selective dry cow therapy (**SDCT**) protocol. The study was carried out on three commercial farms, and a total of 243 lactating cows were monitored at the end of lactation and at the beginning of the next one, 91 of which were dried off without the use of antimicrobials (**NoT**) based on milk SCC, differential somatic cell count (**DSCC**), and the response of Vetscan DC-Q milk analyser, using a secret algorithm. The remaining 152 cows received antimicrobials (**T**). After calving, similar means were observed between the two treatment groups for SCC (4.8 vs 4.9 $\log_{10}$ cells/ml for T and NoT, respectively, $P = 0.5$) and total milk leucocyte count (**TLC**) (5 vs 5.1 $\log_{10}$ cells/ml for T and NoT, respectively, $P = 0.7$) in milk. However, the use of antimicrobials led to a lower DSCC (58 vs 64% for T and NoT, respectively, $P = 0.01$) and lower percentage of neutrophils (59 vs 64% for T and NoT, respectively, $P = 0.05$), although the levels of DSCC and percentage of neutrophils in cows dried off without antimicrobials remained lower than the risk threshold suggested by the international literature. A logistic regression was computed after the application of selective dry cow therapy to identify risk factors of high milk SCC ($\geq$100 000 cells/mL) at the beginning of lactation. Increased milk SCC after calving was related to high SCC at the end of lactation and abandonment of antimicrobial therapy at dry-off. Moreover, the length of the dry period, milk protein content, and flank cleanliness in the last test day before dry-off were other factors in the logistic regression. Neutrophil counts at the beginning of the next lactation were affected by the same factors that influenced SCC, together with milk production, TLC, and macrophages on the last test day. The results obtained in these studied farms showed that selective dry cow therapy may be applied without adversely affecting the next lactation.

Implications

Routine use of intramammary antimicrobial products in all the cows at the beginning of the dry period is no longer allowed in Europe, due to the new Regulation (European Union) 2019/6 for reducing antimicrobial resistance. The study highlighted that a high milk somatic cell count at dry-off and abandonment of antimicrobial therapy were the most important risk factors for increased somatic cell and neutrophil counts after calving. However, farmers can reduce the risks by managing some aspects of the dry period, such as the length and cow cleanliness.

Introduction

Since the 1970s, dairy farmers have used intramammary antimicrobial products on most of their cows at the beginning of the dry period (blanket dry cow therapy) to prevent new intramammary infections and treat any infections that may be present (Rajala-Schultz et al., 2011). However, several studies (e.g., Sykes, 2010; Ferroni et al., 2022) showed that careless use of antimicrobials can cause antimicrobial resistance in microorganisms in farmed animals that, through food products of animal origin, can represent a problem for human health. In light of the new Regulation (European Union (**EU**)) 2019/6 on veterinary medicinal products, which came into force in January 2022, antimicrobial products should not be applied routinely, and prophylactic/metaphylactic use of intramammary antimicrobial products should be

* Corresponding author.
E-mail address: alberto.tamburini@unimi.it (A. Tamburini).

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strongly limited. Therefore, a blanket approach to dry cow treatment is no longer allowed, and a selective dry cow therapy (**SDCT**) protocol needs to be applied. A SDCT protocol states that only cows with an intramammary infection at the end of lactation (Rajala-Schultz et al., 2011) or cows that had udder health problems during lactation due to bacterial infection can be dried off with intramammary antimicrobial products (Halasa et al., 2010). Internal teat sealants are recommended for all the cows for reducing the risk of new intramammary infection at the start of the next lactation (Winder et al., 2019).

There are different methods that can be used with a SDCT protocol to select cows for antimicrobial therapy at dry-off that need to be treated during the dry period, and the most specific one is the milk microbiological culture, which detects the presence of pathogens in a single udder quarter. However, considering the economic cost and time consumption of microbiological analyses that implies an aseptic sample collection, the somatic cell count (**SCC**) of milk is the preferred method for cow selection (Zecconi et al., 2020). The SCC value on the last milk test day before dry-off has been applied for SDCT protocol in the Netherlands since 2013 without deterioration of udder health (Vanhoudt et al., 2018).

Moreover, some algorithms have been developed to help farmers to make more reliable and simple decisions about treatment during dry-off. The SCC value and intramammary infection history (Vasquez et al., 2018) or the bacterial count performed in milk using a rapid test (Cameron et al., 2014; Kabera et al., 2020) were included in these algorithms. Their application did not show differences in SCC or risk of new intramammary infection in early lactation between cows dried off with or without antimicrobial products. Rowe et al. (2020) evaluated lactate dehydrogenase rapid tests and esterase sticks for cow selection; however, these methods showed poor agreement with microbiological culture tests.

Another criterion for selecting cows to be treated with antimicrobials during the SDCT protocol is based on the differential counting of the main types of cells that constitute milk SCC, i.e., neutrophils (**NEU**), macrophages (**MAC**), and lymphocytes (**LYM**). Differential cells are used to calculate the differential somatic cell count (**DSCC**), defined as the sum of NEU and LYM as a percentage of the total SCC. According to Damm et al. (2017), this index (DSCC) increases during intramammary infections. The combination of DSCC and SCC better describes the udder health status (Wall et al., 2018; Schwarz et al., 2019) rather than SCC alone. Therefore, DSCC and single fractions of differential SCC were used in recent studies for new SDCT protocols to select cows suitable for no antibiotic therapy at dry-off (Costa et al., 2021).

The management of dry-off and dry periods affects the udder health status after calving. According to Santman-Berends et al. (2021), it is possible to forecast bulk milk SCC or the percentage of cows with high SCC after calving, starting from the management practices applied during dry-off. Niemi et al. (2021) and Vasquez et al. (2018) estimated individual SCC after calving, starting from general information about herd treatment during the dry period (approximate proportion of treated cows) or by including only cows with low SCC at the end of lactation in the model. Moreover, the management of some dry period aspects, such as barn hygiene, is a crucial point for the application and feasibility of SDCT approaches (Huang et al., 2022). To the best of our knowledge, no studies in the international bibliography describe the forecast of differential SCC after calving due to the dry-off therapy.

This study aimed to evaluate the relationship between SCC and the single fraction of differential somatic cells at the end of lactation and the differences in them in milk at the beginning of lactation between cows dried off with (**T**) or without (**NoT**) intramammary antimicrobial products. The second objective of this study was to identify risk factors of high individual milk SCC and neutrophil counts in early lactation as a response to the application of an SDCT protocol.

Material and methods

Experimental design

A total of 243 cows (experimental units) were enrolled in the trial. A SDCT protocol was applied to three dairy herds and the method of selecting cows to be dried off with intramammary antimicrobial products is described in detail below. Three farms in Lombardy (Italy) are involved in the trial, according to farmer availability to apply an SDCT protocol because, before the experimental trial, all the cows were treated with intramammary antimicrobial products (blanket dry cow therapy) at dry-off, following abrupt cessation of milking. The cows were bred in intensive confined livestock housing, with no pasture, which is the most common farming system in Northern Italy. The lactating cows were milked twice daily in a milking parlour. Dry cows were housed in a barn with cubicles with sawdust (farm A) or straw (farm C) as bedding material, while on farm B, dry cows were housed in a permanent litter with straw without cubicles. Lactating cows were housed in cubicles with straw (farm B and farm C) or sawdust (farm A) as bedding materials.

Sample collection

Members of research team visited farms on a monthly basis for cows' selection. During each visit, individual cow composite milk samples were collected from milking equipment by farm personnel during afternoon milking. During evening milking, individual milk samples from each cow were obtained during the last month of lactation (last 30 days) and during the first month of the next lactation (in the first 60 days). Milk of cows in the first seven days in milk was not collected or collected in the visit of the next month. Hygiene and Teat Apex Score were assessed during monthly visits.

Hygiene and Teat Apex Score

The hygiene score of lactating cows was assessed by the investigators (previously trained for two sessions) through direct observation during milking, following the scheme proposed by Schreiner and Ruegg (2003). Udder, flank, and leg hygiene levels were expressed as scores on a 4-point scale system, in which score 1 indicates very clean skin and 4 indicates skin completely covered with dirt. The Teat Apex Score was assigned at the teat quarter level to lactating cows by direct observation during milking before cluster attaching. The Teat Apex Score is a 4-point scale system used to describe teat conditions, as suggested by the Teat Club International guidelines (Mein et al., 2001). Teat Apex Scores of 1 or 2 were associated with teat apexes in good condition, and Teat Apex Scores of 3 or 4 were associated with hyperkeratosis and lesions.

Milk analyses

All milk samples were transported to the laboratory under refrigeration (4 °C) and processed for analyses no later than 12 h after collection. Milk was analysed for fat and protein content (MilkoScan FT6000; Foss Analytical A/S, Hillerod, Denmark), standard plate count (Bactoscan FC, Foss Analytical A/S, Hillerod, Denmark), milk SCC (SCC, FOSSOMATIC TM 7 Electronic cell counter, FOSS, A/S, Hilleroed, Denmark), and DSCC (Foss DSCC Method Cell Staining), as described by Damm et al. (2017). The DSCC is expressed as follows:

$$\mathrm{DSCC}\ (\%) = \frac{(\mathrm{NEU} + \mathrm{LYM})}{\mathrm{total\ SCC}} \times 100$$

Differential cells were also counted using a Vetscan DC-Q milk analyser (**AAD** Advanced Animal Diagnostics, NC, USA) that provides the concentration (cells/ml) of total milk leucocyte count (**TLC**), defined as SCC without epithelial cells, described as follows:

$$\mathrm{TLC}\ (\mathrm{cells/ml}) = \mathrm{NEU} + \mathrm{LYM} + \mathrm{MAC}$$

Moreover, the Vetscan DC-Q milk analyser provided the concentrations of MAC, NEU, and LYM separately and their percentage of total TLC. The Vetscan DC-Q milk analyser uses fluorescence imaging, as described by Godden et al. (2017), and interprets the results through secret algorithms that identify intramammary infections. The Vetscan DC-Q milk analyser responses may be 'negative', 'borderline', or 'positive'. Individual milk yields at dry-off and on the sampling days were obtained by using farm milking management software: Dairy Comp 305 (Valley Agricultural Software, CA, USA) and AFI milk (Afimilk, Kibbutz Afikim, Israel). Moreover, no milk samples were collected on the day of dry-off, and only data about daily production at dry-off were known.

Selective dry cow therapy protocol

The SDCT protocol was based on SCC and DSCC of individual udder milk samples collected during the last monthly visit before dry-off. Milk with the following characteristics was associated with cows without udder inflammation at the end of lactation, that is, cows that were not dried off with intramammary antimicrobial products (NoT group), but only internal teat sealant was applied:

- SCC for primiparous cows < 100 000 cells/ml, for pluriparous cows < 200 000 cells/ml (Zecconi et al., 2019b)
- DSCC < 69.3% (Zecconi et al., 2019a)
- 'Negative' sample response of Vetscan DC-Q milk analyser

Other cows, with milk not respecting all the three criteria, were treated with intramammary antimicrobial products (betalactamine and miloglycoxides antimicrobials) and internal teat sealant (T group). Dry-off treatments were carried out by farmers.

Statistical analysis of results

Data were analysed using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The results obtained for standard plate count, SCC and TLC, MAC, NEU, and LYM were then transformed to $\log_{10}$ for statistical analysis to obtain a normal distribution of the residuals in linear statistical models. Descriptive analyses of the three farms were performed by using PROC MEANS and PROC FREQ, and a power analysis was performed as reported in Supplementary Material S1.

Relationship between somatic cell count and differential somatic cell count at the end of lactation

Crude univariable regressions between TLC and NEU count and between TLC and NEU expressed as percentage of TLC at the end of lactation were performed with PROC REG. Assumed zero mean, normal distribution and homogeneity, PROC MEANS and PROC FREQ were used for the description of Vetscan DC-Q milk analyser results of milk at the end of lactations.

Milk production of cows dried off with or without intramammary antimicrobial products at the end and at the beginning of lactation

General Linear Model (**GLM**) analysis was performed on milk production (on the test day and at dry-off), standard plate count, SCC, TLC, and differential somatic cells (MAC, LYM, NEU, DSCC) using the following models for observation at the end of lactation (model 1, Supplementary Material S2) and at the beginning of the next one (model 2, Supplementary Material S3). All dependent and independent variables were obtained at the test day (at the end of lactation or at the beginning of lactation, respectively, for Model 1 and Model 2), except milk production at dry-off.

$$Y_{ijklmno} = m + Farm_i + Group_j + Parity_k + HS_l + TA_m + Protein_n + FG_{ij} + e_{ijklmno} \quad (1)$$

$$Y_{ijklmn} = m + Farm_i + Group_j + Parity_k + HS_l + TA_m + FG_{ij} + \beta(\text{dry period length}) + e_{ijklmn} \quad (2)$$

where

$Y_{ijklmno}$ = dependent variable: milk production at dry-off and at test day, standard plate count, SCC, TLC, MAC, LYM, NEU, DSCC.
m = overall average of dependent variables.
$e_{ijklmno}$ = error term of dependent variables.

The fixed effects assumed were:

- $Farm_i$ = farms A, B, and C (i = 1–3).
- $Group_j$ = dry-off group (T or NoT, j = 1–2).
- $Parity_k$ = parity (primiparous or multiparous cows at the end of lactation, k = 1–2).
- HS_l = udder hygiene score (hygiene score 1–2 or hygiene score 3–4, l = 1–2).
- TA_m = mean value of the Teat Apex Score of four-quarters (Teat Apex Score 1–2 or Teat Apex Score 3–4, m = 1–2).
- $Protein_n$ = class of protein content in the last collected milk before dry-off (≤3.6% or > 3.6%, n = 1–2)
- FG_{ij} = interaction factor between farm and group effect
- β*(dry period length)* = coefficient of dry period length (days).

Season effect on quarter basis was not statistically significant (P = 0.8 in Model 1 and P = 0.7 in Model 2), and it was not included in the models. Description of independent and dependent variables of Model 1 and Model 2 are reported in Supplementary Tables S1–S5.

Risk factors for high individual milk somatic cell count and neutrophil counts in early lactation

The relationship between SCC content at the beginning of the new lactation and potential risk factors at the end of the lactation was analysed using logistic regression (PROC LOGISTIC, Supplementary Material S4). The dependent variable was the class of SCC content at the beginning of the new lactation (<5 or ≥5 $\log_{10}$ SCC, equal to <100 000 cells/ml or ≥100 000 cells/ml), and the independent variables at the end of the lactation were reclassified in new categorical variables. Dry-off treatment groups (T and Not) were two levels of class variable. Milk protein content, SCC, hygiene score of the flank and dry period length variables were reclassified into two levels using median values for each variable (Supplementary Table S6). For the logistic regression, classes entered in the model were the variables that fit with P < 0.1. In PROC LOGISTIC, the stepwise option was used to select significant classes to be added in the final model.

To study the relationship between NEU count at the beginning of the new lactation period and independent variables at the end of lactation, a stepwise regression was performed (PROC GLMSELECT with adjusted R^2 criterion, the significance level for entry was P < 0.15, Supplementary Material S5). The entered independent variables in the model were continuous variables referencing the end of lactation (TLC, MAC, LYM, milk protein content, hygiene score of the flank, milk production (on the last test day), and length of dry period). In the same model, the independent categorical variable was dry-off treatment groups (T and NoT).

Validation of the models

Validations of GLM models on dependent parameters at the end of lactation (model 1, Supplementary Material S2) and at the beginning of the next one (model 2, Supplementary Material S3) were performed with Diagnostic Panel of PROC GLM. For TLC and SCC, figures were inserted in Supplementary Material S2 and S3. Validation of the logistic regression was performed with plots in Proc LOGISITC (Supplementary Material Fig. S1). A Bootstrap approach (on two different series of 500 replicates) was performed to control ROC of the logistic model.

Results

Herds and sample description

Table 1 reports the main characteristics of the dry period management applied in the three farms before the starting of the trial. The length of dry period (mean ± SD) was 56 ± 12 days, and cows had a high production at dry-off (mean 19.7 ± SD 6.6 kg/day on average). The average SCC of the herd was close to 100 000 cells/ml, and the percentage of cows with a high average SCC (>200 000 cells/ml) during the year before the start of the trial was between 18% and 30% (Table 1).

Cow composite milk samples (one per cow) were collected from cows at 18 ± 11 days (mean ± SD) before the expected dry-off (318 ± 60 days in milk). Dry-off dates (mean 340 ± SD 63 days in milking) were set by farmers. Moreover, milk samples (one per cow) were collected after calving (mean 24 ± SD 15 days in milk). A total of 243 cows were enrolled in the trial. Not for all cows all variables were available so the lowest counted is paired to 204 at the end of lactation and 224 at the beginning of lactation.

Milk differential somatic cell count

The crude regression (non-independence due to herd not accounted for, after outliers deleted) between TLC and NEU is shown in Fig. 1. Fig. 1A shows a positive relationship between TLC and NEU count (r^2 = 0.95) at the end of lactation. If NEU was expressed as a percentage of TLC, the relationship with TLC was positive but low (r^2 = 0.19; Fig. 1B). Table 2 shows the results of the Vetscan DC-Q milk analyser analyses. One-third of the cows (68 cows) were categorised as 'positive' with a high concentration of TLC (5.6 $\log_{10}$ cells/ml, 398 000 cells/ml) and a predominant fraction of NEU (67.4%). A group of 30 cows (12%) was categorised as 'borderline,' with a TLC of 5.1 $\log_{10}$ cells/ml (126 000 cells/ml) and an NEU percentage of 64.3%. The third group of animals (139 cows, equal to 59%) was classified as 'negative': The TLC was on average 4.7 $\log_{10}$ cells/ml (50 119 cells/ml) while the percentage of NEU was 51.9%, lower than the percentage of 'positive' cows. Furthermore, the MAC in the milk of the 'negative' cows was higher (22.2%) than in that of the 'positive' cows (11.2%).

Application of selective dry cow therapy protocol

A total of 243 cows from three farms were involved in the trial and 91 cows (39, 26, and 26 cows from farms A, B, and C, respectively) were not treated with intramammary antimicrobial products (NoT group) because their milk respected all the three parameters described in the SDCT protocol. The remaining 152 cows (51, 67, and 34 cows from farms A, B, and C, respectively) were treated with antimicrobials at dry-off (T group).

Considering the use of intramammary antimicrobial products at dry-off, an overall reduction in antimicrobial usage of 37.5% was estimated in the three farms (28% for farm B and 43% for farm A and C), compared to blanket dry cow therapy applied in these herds before the experimental trial. The low percentage of reduction observed for farm B was due to the high mean SCC of herd (Table 1). The total number of cows treated with NoT (n = 91) was lower than suggested by the Vetscan DC-Q Milk Analyser (n = 139), because the other cows did not respect the other two criteria about SCC and DSCC previously declared.

As shown in Table 3 and in Supplementary Table S7, at the end of lactation, the cows in the T and NoT groups had similar means in milk yield on the test day or dry-off. However, there were statistically significant differences ($P < 0.05$) in the concentrations of SCC, TLC, and fractions composed of differential somatic cells between the T and NoT groups, as expected. Specifically, cows in the T group showed statistically higher values for SCC (155 132 vs 48 686 cells/ml) and TLC. Moreover, there were statistically significant differences between NEU, MAC, and LYM counts in the milk of cows in the T or NoT groups. Cows in the T group showed statistically significantly higher values for all three fractions than those in the NoT group. In addition, the percentage of NEU and cumulative index of DSCC were statistically significantly higher in the T group cows than in those in the NoT group. In contrast, the percentage of LYM was statistically significantly lower ($P = 0.02$) in the T group (22.2%) than in the NoT group (26.7%). In addition, the standard plate count was statistically significantly higher ($P < 0.01$) in the T group than in the NoT group (4.79 vs 4.58 $\log_{10}$ colony-forming units/ml, for T and NoT, respectively).

As shown in Table 3, at the beginning of lactation, milk production of the cows in the two treatment groups were similar. Moreover, similar means were observed in SCC (69 695 vs 79 378 cells/ml), TLC, MAC (both as count and percentage), LYM count, NEU count and Standard Plate Count. However, NEU percentage and DSCC were statistically significantly higher in cows in the NoT group than in the T group ($P = 0.05$). The percentage of LYM

Table 1
Dry period management in the studied dairy cow farms, on year basis, before starting the trial.

Farm	A		B		C	
	Mean	SD	Mean	SD	Mean	SD
Total lactating cows, n	160		500		138	
Monitored cows, n	90		93		60	
Dry period length, days	59.6	12.9	50.2	10.6	60.3	10.5
Milk production at 304 ± 12 days in milk, kg/day	29.5	10.0	25.0	5.8	26.6	7.5
Milk production at dry-off, kg/day	19.5	6.9	22.7	5.73	15.1	4.3
SCC of milk sample per cow during the year, cells/ml (average on $\log_{10}$ basis)	77 158		116 252		90 572	
Cows with mean value of SCC > 200 000 cells/ml during the year, %	18%		30%		21%	
Month with the highest number of cows with SCC > 200 000 cells/ml	July (summer)		June (summer)		May (spring)	
Month with the lowest number of cows with SCC > 200 000 cells/ml	January (winter)		February (winter)		December (winter)	

Abbreviations: SCC = Somatic Cell Count.

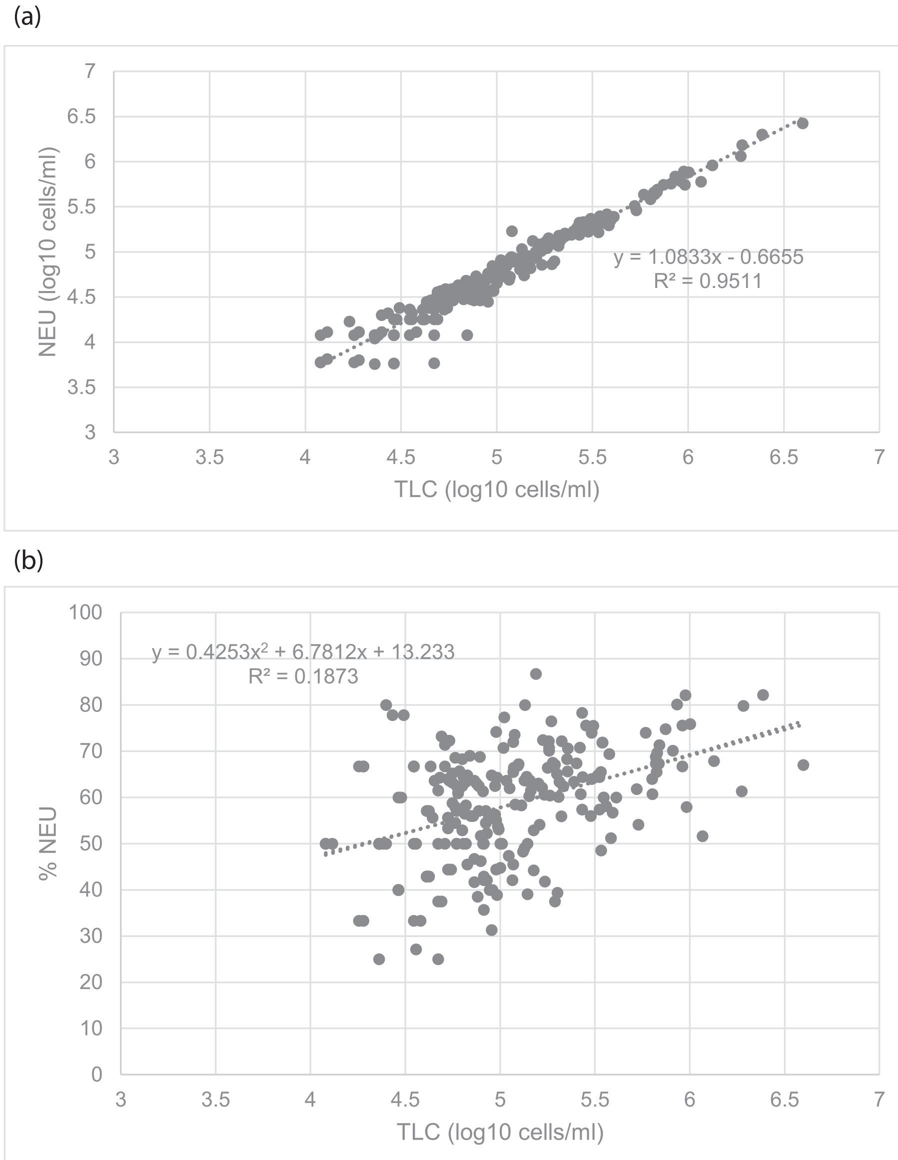

Fig. 1. Relation between Total milk Leucocyte Count (TLC) and neutrophils (NEU) (A = log_{10} cells/ml; B = %) in cow milk at the end of lactation.

Table 2
Cow milk differential cell count and Total milk Leucocyte Count (TLC) for 'negative', 'positive' and 'borderline' samples defined by the algorithm of Vetscan DC – Q Milk Analyser (at the end of lactation).

Item	Mean	SD	Minimum	Maximum
'Negative' (N = 139)				
TLC, log_{10} cells/ml	4.72	0.26	4.08	5.31
Lymphocytes, % TLC	26.1	15.6	1.0	75.0
Macrophages, % TLC	22.2	16.7	1.0	66.7
Neutrophils, % TLC	51.9	14.7	1.0	96.0
'Borderline' (N = 30)				
TLC, log_{10} cells/ml	5.09	0.11	4.84	5.32
Lymphocytes, % TLC	19.2	5.5	7.1	27.9
Macrophages, % TLC	16.6	8.3	1.0	32.5
Neutrophils, % TLC	64.3	5.7	52.9	77.3
'Positive' (N = 68)				
TLC, log_{10} cells/ml	5.62	0.31	5.13	6.60
Lymphocytes, % TLC	21.4	7.7	4.8	38.7
Macrophages, % TLC	11.2	6.9	1.0	35.5
Neutrophils, % TLC	67.4	7.9	48.5	86.7

Abbreviations: TLC = Total milk Leucocyte Count.

Table 3
Milk production, milk somatic cell count and differential cell counts for cows dried-off either with (T) or without (NoT) intramammary antimicrobials products (LS means).

Item	At the end of lactation (n = 204)				At the beginning of lactation (n = 224)			
	T	NoT	SE	*P*	T	NoT	SE	*P*
Milk Production								
At test day, kg/day	25.4	25.8	0.66	0.6	40.7	41.3	0.92	0.6
At dry-off, kg/day	19.5	19.6	0.66	0.9				
Total somatic cell count								
SCC, log_{10} cells/ml	5.19	4.69	0.05	<0.001	4.84	4.90	0.07	0.5
TLC, log_{10} cells/ml	5.21	4.76	0.05	<0.001	5.04	5.08	0.06	0.7
Differential somatic cell count								
Lymphocytes, log_{10} cells/ml	4.47	4.07	0.07	<0.001	4.17	4.16	0.09	0.9
Macrophages, log_{10} cells/ml	4.23	3.82	0.07	<0.001	4.20	4.25	0.08	0.7
Neutrophils, log_{10} cells/ml	5.00	4.47	0.05	<0.001	4.76	4.86	0.08	0.3
Lymphocytes, % TLC	22.2	26.7	1.48	0.02	19.6	15.8	1.50	0.04
Macrophages, % TLC	15.9	18.1	1.60	0.3	21.9	20.8	2.08	0.7
Neutrophils, % TLC	62.1	55.5	1.59	0.002	58.6	63.5	1.94	0.05
DSCC, % SCC	62.7	50.3	1.63	<0.001	58.1	64.1	1.75	0.01
Standard Plate Count, log_{10} CFU/ml	4.79	4.58	0.05	0.002	4.62	4.70	0.05	0.3

Abbreviations: T = cow group dried-off with intramammary antimicrobial products; NoT = cow group dried-off without intramammary antimicrobial products; SCC = Somatic Cell Count; TLC = Total milk Leucocyte Count; DSCC = Differential Somatic Cell Count;

was statistically significantly lower in the NoT group than in the T group (15.8 vs 19.6% for the NoT and T groups, respectively). Considering the variation in SCC before dry-off and after calving, it is plausible to conclude that there was a decrease in milk SCC at the beginning of next lactation in T group (−5.4%) and an increase in NoT group (+3.9%) ($P < 0.0001$), but this increase is not practically relevant, also because the SCC is under the risk threshold. Results are supported also by Effect Size analyses, as reported in Supplementary Table S7. In fact, Cohen's coefficients were high (>0.44) for variables with statistically significant differences between T group and NoT group.

Hygiene Score and Teat Apex Score

The mean values of the Hygiene Score and Teat Apex Score were similar for the cows in the two treatment groups. At the end of lactation, the Hygiene Score of flank, legs and udder of all cows (mean ± SD) were respectively 1.9 ± 0.9, 2.3 ± 0.8 and 2.2 ± 0.9. Teat Apex Score (mean ± SD) of all cows was 2.1 ± 0.6. At the beginning of lactation, the Hygiene Score of flank, legs and udder of all cows (mean ± SD) were paired respectively to 2.2 ± 1.0, 2.8 ± 0.8 and 2.5 ± 0.8 and Teat Apex Score (mean ± SD) of all cows was 1.9 ± 0.6.

Risk factors of high somatic cell count and neutrophil count

Table 4 presents the results of the final model for the odds ratio of SCC ≥ 100 000 cells/ml at the beginning of lactation with reference to variables at the end of the previous lactation period on the last test day. The model had 71% of the concordant observations. The odds ratio of a cow having high SCC (≥100,000 cells/ml) in the next lactation was 0.31 for the T group compared to the NoT group ($P < 0.01$). High SCC in milk at the last test before dry-off (≥100 000 cells/ml), long dry period (>55 days), and dirty flanks (hygiene score = 3–4) enhance the possibility of having high SCC (≥100 000 cells/ml) in next early lactation. Moreover, the odds ratio was 2.1 for low protein milk content (≤3.6%) compared to high protein milk content (>3.6%) at the last test before dry-off, indicating that low milk protein is a risk factor for cows at the beginning of lactation.

The second model considered milk NEU count at the beginning of lactation after the application of SDCT with or without intramammary antimicrobial products (Table 5) and was based on variables referred to the end of the previous lactation. Although low R^2 of the model, the variables at the end of the previous lactation that were positively associated with NEU count increase in the early next lactation were MAC count, dry period length, TLC, and hygiene score of the flanks. The variables, that were statistically significantly negatively associated with NEU count, were milk protein content ($P = 0.04$) and milk production on the last test day before dry-off ($P = 0.04$). There was a statistically significant decrease in NEU count at the beginning of lactation when the cows were treated with antimicrobials (T group) during dry-off ($P = 0.02$).

Discussion

Dry period management

The length of the dry period in the three studied farms was similar to that reported by Zucali et al. (2020), suggesting that 50–60 days interval is the most used by farmers. Moreover, this length of dry period may be the optimal for obtaining high milk production in the next lactation (Kok et al., 2019). The milk production of the three farms on the day of dry-off was higher than the quantity of 15 kg/d, as suggested by Vilar and Rajala-Schultz (2020),

Table 4
Risk factors for somatic cell count ≥ 100 000 cells/ml (5 $\log_{10}$ cells/ml) in cow milk at the beginning of lactation.

Effect	Coefficient	SE	*P*	Odds ratio Point Estimate	Odds ratio 95% Confidence Limits	
Intercept	−0.24	0.19	0.2			
SCC[1] at last test			<0.001			
<100 000 cells/ml (5 $\log_{10}$ cells/ml)	−0.76	0.19		0.22	0.11	0.45
≥100 000 cells/ml (5 $\log_{10}$ cells/ml)	Referent					
Selective dry cow therapy			0.002			
T[2]	−0.58	0.19		0.31	0.15	0.65
NoT[3]	Referent					
Dry period length			0.02			
≤55 days	−0.36	0.16		0.49	0.26	0.9
>55 days	Referent					
Milk protein			0.02			
≤3.6%	0.37	0.16		2.1	1.13	3.9
>3.6%	Referent					
Hygiene score of flank			0.08			
Clean	−0.33	0.19		0.52	0.25	1.08
Dirty	Referent					

Abbreviations: [1]SCC = Somatic Cell Count; [2]T = cow group dried-off with intramammary antimicrobial products; [3]NoT = cow group dried-off without intramammary antimicrobial products.

Table 5
Relationship of neutrophils ($\log_{10}$ cells/ml) in cow milk at the beginning of lactation and independent variables.

Effect	Estimate	SE	*P*	Cumulative R^2
Intercept	3.2	1.02	0.002	0.00
Macrophages, $\log_{10}$ cells/ml	0.003	0.00	0.04	0.08
Dry period length, days	0.009	0.00	0.02	0.10
TLC, $\log_{10}$ cells/ml	0.46	0.15	0.002	0.12
Selective dry cow therapy				0.14
T	−0.24	0.10	0.02	
NoT	0			
Hygiene score flank	0.102	0.05	0.05	0.15
Milk protein, %	−0.28	0.13	0.04	0.16
Milk production at the last test day, kg/day	−0.017	0.01	0.04	0.17
Milk production at dry-off, kg/day	0.009	0.01	0.2	0.17
Lymphocytes, $\log_{10}$ cells/ml	−0.001	0.00	0.3	0.17

Abbreviations: TLC = Total milk Leucocyte Count; T = cow group dried-off with intramammary antimicrobial products; NoT = cow group dried-off without intramammary antimicrobial products.

probably because all farmers involved in the study chose to apply an abrupt dry-off. Farm A and farm B did not apply a gradual reduction in feed intake and/or milking frequency, while farm C used these practices. This explains the different production at dry-off. Abrupt cessation of milk production is a common practice in Italy (Zucali et al., 2020). This practice is easy to implement, especially in large herds; however, gradual cessation through reduction of milking frequency reduces milk yield before dry-off, hastens mammary gland involution, and improves natural protective factors in the udder, playing an important role in mammary gland defence during the dry period (Vilar and Rajala-Schultz, 2020).

Milk differential somatic cell count

In this study, SCC, TLC, DSCC, and differential cell fractions (MAC, NEU, and LYM) were measured in cow composite milk samples following the suggestion of Wall et al. (2018), who found a strong correlation between DSCC and SCC values measured in quarter milk composite and in cow composite samples. In the present study, a high correlation was found between TLC and NEU count ($\log_{10}$), although a moderate correlation was found when NEU was expressed as a percentage. NEU is a component of DSCC, and the trend of this differential fraction is strongly related to DSCC development. However, despite the positive correlation between SCC and DSCC at the end of lactation (Schwarz et al., 2019), the magnitude and duration of this relationship differ under several conditions (for example days in milk and parity) (Kirkeby et al., 2020). The increase in DSCC is related to the causal agent of intramammary infection (Schwarz et al., 2020). For this reason, considering that in this study no information on microbial culture was collected, it was not possible to determine whether the percentage of NEU trend was related to a causal agent. As expected, the TLC increased from the group classified by Vetscan DC-Q milk analyser as 'negative' to the group defined as 'positive' with a very high value for the 'positive' group. The same trend was observed for the percentage of NEU, while, at the same time, the percentage of MAC decreased slightly. This result confirms that the instrument Vetscan DC-Q milk analyser categorised samples at different levels of udder inflammation and underlined the different distribution of differential cells with increasing SCC.

Application of selective dry cow therapy protocol

At the end of lactation, the average level of SCC and TLC in both treatment groups suggested a moderate risk of mastitis (<200 000 cells/ml), while the DSCC gave an interesting indication of udder inflammatory status. The higher NEU percentage, together with the lower LYM percentage in the T group compared to the NoT group, may be explained by the fact that a large proportion of NEU is associated with current infection, while lymphocytes become dominant in the later course of infection (Wagner et al., 2021). The values observed for MAC (%) were similar to the results obtained by Damm et al. (2017). MAC recognise invading pathogens and initiate an immune response; however, they are also active phagocytic cells capable of ingesting bacteria, cellular debris, and accumulated milk components.

The means of SCC and TLC were similar in the two treatment groups after calving and confirmed that the correct criteria were used to select the cows at the end of lactation. Similar results were found by Vasquez et al. (2018), who reported similar means in SCC between cows dried off only with external teat sealant and dried off with antimicrobials and external teat sealant; however, only healthy cows at the end of lactation were included in the study. The results obtained for SCC and TLC were similar to those obtained by Vanhoudt et al. (2018), who reported that the application of SDCT protocol based on the SCC threshold (150 000 cells/ml for primiparous and 250 000 cells/ml for multiparous cows) at the last milking did not affect udder health.

The increase in DSCC at the beginning of the next lactation compared to DSCC at the end of the lactation in the NoT group highlighted that antimicrobial therapy at dry-off is very effective in reducing udder inflammation and that not applying dry cow therapy could worsen the latent inflammatory status in cows after calving. However, the DSCC for NoT cows was lower than the suggested risk threshold proposed by Zecconi et al. (2019a). The statistically significant higher fractions of NEU in the NoT group at the beginning of lactation are consistent with the results obtained by Costa et al. (2021), who reported an increase in NEU in milk samples from NoT cows, but the prevalence of clinical mastitis events during lactation were similar between T or NoT cows.

The low udder hygiene score could be a consequence of the farmers' attention to bedding management, such as the frequent addition of clean materials and manure removal. This was also confirmed by the low level of dirty of legs and flanks. In addition, Zucali et al. (2011) suggested that the cleaner the udder, the lower the standard plate count and SCC in milk. In the present study, the standard plate count was low and well below the EU limit of 100 000 colony-forming units/ml.

Risk factors of high somatic cell count and neutrophil count

High levels of SCC in milk at dry-off (SCC > 100 000 cells/ml) and abandonment of antimicrobial treatment were related to increased SCC after calving. This was consistent with the results obtained by Niemi et al. (2021). The extension of dry period length is another important risk factor, and as reported in the review by Kok et al. (2019), the effect of this management practice has important and diverse consequences not only on udder health but also on energy balance and fertility.

In this study, the increase in SCC negatively affected the milk protein percentage. We suggest that it could be due to the reduction in the synthetic and secretory capacity of the mammary gland caused by damage to the milk-producing epithelial cells and a reduction in milk precursor availability during inflammation. A similar negative association between SCC and milk quality traits was observed by Pegolo et al. (2021). Moreover, the logistic regression analysis underlined the important role of animal cleanliness because dirty flanks could compromise udder health with increase of SCC, as also reported by Schreiner and Ruegg (2003). NEU counts were strongly correlated with TLC. Furthermore, the results showed that the same factors influencing SCC affected NEU count at the beginning of lactation, particularly the use of intramammary antimicrobial products, hygiene score of the flank, milk protein percentage at the end of lactation, and dry period length. Furthermore, high milk production at the end of lactation causes an increase in neutrophils at the beginning of lactation due to the risk of milk leakage and open teat sphincters, so the probability of contracting an intramammary infection is higher (McDougall et al., 2021). Cows with low milk production during lactation (<7700 kg/year) (described by the last milk production on the test day) usually have a high SCC (Jones et al., 1984), and with an increase in DSCC (composed of NEU), milk yield decreased, as reported by Mariani et al. (2022). In these study results, a dilution effect cannot be excluded as suggested by Green et al. (2006).

The application of the SDCT protocol based on the combination of milk SCC and differential cells allowed the avoidance of antimicrobial therapy during dry-off in approximately one-third of the cows. The results highlighted that the cows dried off without antimicrobials had a 3.9% increase in SCC at the start of the next lactation compared to their last control in the previous lactation. This study evaluated the risk factors for an increase in milk SCC or neutrophil count after calving, with the application of two different dry-off treatments (T and NoT). The study showed that high SCC at dry-off and application of SDCT protocol were the two most important risk factors of high SCC and neutrophil count after calving. Management practices, such as length of the dry period and cow cleanliness, play a role and can support the choice to avoid blanket dry cow therapy. More studies are necessary to understand the evolution of udder health in cows that have been dried off without antimicrobials.

Supplementary material

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Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository, available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

Author ORCIDs

Sara Mondini: https://orcid.org/0000-0002-2446-7399.
Giulia Gislon: https://orcid.org/0000-0003-0389-7960.
Maddalena Zucali: https://orcid.org/0000-0002-2380-3274.
Anna Sandrucci: https://orcid.org/0000-0002-1014-0088.
Alberto Tamburini: https://orcid.org/0000-0002-3371-6254.
Luciana Bava: https://orcid.org/0000-0001-6521-3195.

Author contributions

L.B. and **S.M.** designed and directed the work; **S.M, M.Z., G.G.** and **A.T.** processed and interpreted the experimental data; **S.M., G.G., A.S.** and **L.B.** drafted the article; **L.B., A.S.** and **M.Z.** critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of interest

None.

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