Viscosity Measurements of Milk to Investigate Risk Factors for Milk Leakage in Dairy Cows Before and After Dry-Off

J. Bachmann[1], S. Bertulat[1], S. Seiffert[2], W. Heuwieser[1]

[1] Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Königsweg 65, 14163 Berlin, Germany
[2] Institute of Physical Chemistry, Johannes Gutenberg-Universität Mainz, Duesbergweg 10-14, 55128 Mainz, Germany

Date submitted:31/05/2017 *Date accepted: 11/10/2017* *Volume/Page(s): 70/20-24*

Abstract

Milk leakage is a prevalent phenomenon in dairy cows and it is known to negatively affect udder health, as it increases the risk for clinical mastitis and new intramammary (IMM) infections. The determination of risk factors for milk leakage might be the first step towards the development of potential prevention measures. Therefore, the objective of this in vitro study was to evaluate the effect of the teat canal diameter, IMM pressure, milk temperature, milk composition, dry-off and antibiotic dry cow therapy on the occurrence of milk leakage in dairy cows. Two Ubbelohde viscometers (type no. 50100 and 50110 according to DIN 51 562 Part 1; SI Analytics GmbH, Mainz, Germany) were used to mimic the teat canal diameter, IMM pressure and milk temperature by suitable choice of the capillary diameter, hydrostatic pressure and milk sample temperature. Nineteen quarter foremilk samples each were collected on the day of dry-off and 2d after dry-off in cows dried off with and without antibiotic dry-cow therapy, respectively. All milk samples were analysed for milk composition (protein, fat, lactose, somatic cell count (SCC)) by the local Dairy Herd Improvement Association. The viscometers were used to measure the efflux time of all milk samples and afterwards the dynamic viscosity was calculated on basis of the Hagen–Poiseuille equation. Parameters that lead to shorter efflux times and smaller dynamic viscosities were considered as potential risk factors for milk leakage in vivo, because the milk could flow faster and more easily. In our study, the efflux time was shorter at wider capillary diameter, higher hydrostatic pressure, higher milk sample temperature and lower concentrations of fat and protein ($P < 0.001$). The dynamic viscosity was determined to be smaller at higher milk sample temperature and lower concentrations of fat and protein ($P < 0.001$). These results indicate that wider teat canal diameter, higher IMM pressure, higher milk temperature and lower concentrations of fat and protein might be risk factors for milk leakage in dairy cows.

Key words: milk leakage, dairy cow, dynamic viscosity, dry-off

Introduction

Milk leakage is defined as milk dripping or flowing from the teat in the absence of active milking. In dairy cows it frequently occurs shortly before milking [1], when cows were recumbent between milkings [2] and during the first days after dry-off [3]. While there are several studies describing milk leakage in lactating cows, milk leakage before or after dry-off has rarely been investigated. Klaas et al. [1] reported that overall 5.3% of lactating cows showed milk leakage while entering the milking parlor. After dry-off, however, approximately 30% of cows had milk leakage [3, 4]. Milk leakage should be considered a serious condition, because it is associated with a 4.0 and 6.0 times greater risk for clinical mastitis and new intramammary (IMM) infections, respectively [4-7]. Especially, an association with clinical mastitis caused by Escherichia coli has been reported [8].

There are few studies that have assessed the risk factors for milk leakage on cow level. Reported external risk factors are short teat canals, teat canal protrusion, high peak milk flow and high IMM pressure [1, 9] for lactating cows and high milk yield and high extramammary udder pressure for dry cows [3, 10]. Rovai et al. [9] detected that the reason for the high IMM pressure in lactating cows is a large amount of cisternal milk. Persson Waller et al. [2] concluded that the high IMM pressure can be caused by an activated milk ejection and that milk leakage occurs when the IMM pressure overcomes the closing mechanism of the teat canal. The teat canal is known to be a barrier for preventing milk leakage and defending the udder against new IMM infections [11]. Several studies investigated the influence of teat canal length and diameter on udder health and associated shorter and wider teat canals with an increased risk for mastitis [12-14], but an association with milk leakage was not evaluated.

Rovai et al. [9] discovered decreased concentrations of lactose in milk leaking from teats compared to foremilk collected after udder preparation. Based on that finding, we hypothesized that parameters of the milk composition might be potential internal risk factors for milk leakage. Changes in milk composition are caused by several factors, such as nutrition [15, 16], season [17-20], milking omission [15, 21] or mammary involution after dry-off [22]. The relationship between milk composition changes and milk leakage and the influence of antibiotic dry cow therapy on milk leakage after dry-off, however, have not yet been investigated.

In order to investigate risk factors for milk leakage in a field study, a

ISSN 2567-9538

high number of cows and measurements would be necessary due to variance between cows, quarters and teats. For this purpose, a measuring method evaluating risk factors for milk leakage under controlled conditions would be advantageous in order to reduce the use of study animals [23]. Therefore, a laboratory model composed of two capillary viscometers was developed to determine risk factors for milk leakage under various conditions. Capillary viscometers are used to measure the efflux time of fluids (time interval that the fluid needs to descend from the upper timing mark to the lower timing mark of the viscometers' measuring sphere) and calculate their dynamic viscosity (internal resistance of a fluid to the application of pressure) [24]. Fluids with short efflux time and low dynamic viscosity flow fast and easy. In food engineering, milk composition and viscosity of milk are determined as important factors for manufacturing processes of dairy products [20, 24]. Alcântara et al. [24] reported increased dynamic viscosity values for milk as contents of fat, protein and lactose increased and decreased values as temperature increased. Physical properties such as the viscosity of milk might be useful factors to optimize milk processing [20] and they might also be plausible factors to investigate risk factors for milk leakage. We hypothesized that milk with decreased efflux time and decreased dynamic viscosity has a higher likelihood to leak from the teat.

Overall, the objective of this in vitro study was to evaluate plausible risk factors for milk leakage in dairy cows before and after dry-off. Specifically, we set out to determine the effects of 1) teat canal diameter, IMM pressure and milk temperature, 2) milk composition, 3) dry-off, and 4) IMM dry cow therapy on efflux time and dynamic viscosity of quarter foremilk samples.

Materials and Methods

Laboratory model:

Two calibrated Ubbelohde viscometers (type no. 50100 and 50110 according to DIN 51 562 Part 1; SI Analytics GmbH, Mainz, Germany) were used in this in vitro study. Capillary diameters (0.36 and 0.63 mm, referred to as capillary No. 0 and I) of the viscometers were chosen based on teat canal diameters reported in the literature [12, 14, 25, 26]. The viscometers were placed in a tempered water bath, for heating the milk sample to a certain temperature (26, 32 and 38°C) and for keeping this temperature constant during the measurements. Temperatures were chosen based on previously reported teat [27] and milk temperatures [28]. In order to simulate varying udder pressures values, different hydrostatic pressures (60, 80 and 100 mbar) were applied on the capillary tubes of the viscometers. Pressure values were chosen based on previously reported IMM pressure values [29, 30] and were adapted to actual milk yields at dry-off (15 to 20 kg). The two viscometers were used to measure the efflux time and calculate the dynamic viscosity of quarter foremilk samples before and 2 d after dry-off, with and without antibiotic dry-cow therapy.

Sample Collection:

Six multiparous Holstein-Friesian crossbreed dairy cows from the Clinic of Animal Reproduction (Freie Universität Berlin, Berlin, Germany) were enrolled in this study. The six cows were in the first to sixth lactation and their 305d milk yield of the previous lactation averaged 8,019 ± 1,449 kg. All cows were in good health, had 4 functional quarters and were milked twice daily. All cows were fed grass silage, haylage and hay ad libitum supplemented with standard dairy concentrate according to their milk yield (MLF 18/3 Standard; BKF Belziger Kraftfutter GmbH, Bad Belzig, Germany). Fresh water was available ad libitum.

Quarter foremilk samples were collected in two trials conducted between September and November 2015. During the first trial, three hundred and fifty mL foremilk samples were collected from each quarter on the day of dry-off (d0) and 2 d after dry-off (d2), because this is the day with the highest likelihood of the occurrence of milk leakage after dry-off [3]. All samples were collected immediately after examining foremilk from each quarter for signs of clinical mastitis (clots, flakes) utilizing a pre-milking cup. Quarters with signs of clinical mastitis were withdrawn from the study. Thirty mL of the 350 mL quarter foremilk samples were send to the local DHIA (Landeskontrollverband Berlin-Brandenburg e. V., Waldsieversdorf, Germany) for milk composition analysis (fat, protein, lactose, SCC). All samples were analysed using the CombiFoss (Foss, Hilleroed, Denmark). After sample collection (samples d0) and milking on d0, each quarter was treated with an IMM dry cow antibiotic (150 mg of cefquinome; Virbactan, Virbac Ltd, Carros, France). Antibiotic dry cow therapy (AB) was administered according to the summary of product characteristics provided by the manufacturer. After antibiotic treatment on d0 cows were not milked for 48h for simulating dry-off until sample collection on d2 (samples d2 AB).

During the second trial the procedures were repeated as described above using the same cows, however, cows were not treated with an IMM dry cow therapy on d0. Foremilk samples were again collected from each quarter 2d after dry-off (samples d2). The efflux time of all samples (samples d0, samples d2 AB and samples d2) was measured the same day as samples were collected using the two viscometers.

Viscosity Measurements:

Seventy mL of each milk sample were transferred into the viscometers and tempered to the starting temperature (26°C). The time interval (efflux time) that the milk sample needed to descend from the upper timing mark to the lower timing mark of the measuring sphere was measured manually with a stopwatch. Each combination of the 2 capillary diameters, 3 pressure values and 3 temperature values was measured with 3 repetitions for every milk sample. The dynamic viscosity η (mPa s) was calculated on the basis of the Hagen–Poiseuille equation:

$$\eta = K \times \Delta p \times t.$$

The viscometer constant K was determined individually for both viscometers by measurements with distilled water at 38°C. Delta p (mPa) is the sum of the pressure difference between the capillary ends and the additional hydrostatic pressure applied on the capillary tubes, and t (s) is the mean efflux time for each capillary-temperature-pressure combination. The mean and SD of the efflux time and the dynamic viscosity for each capillary-pressure-temperature combination were calculated.

Statistical Analysis:

Data were entered into Excel spreadsheets (version 2010; Microsoft Corp., Redmond, WA) and statistical analyses were performed with IBM SPSS Statistics for Windows software (version 22.0; IBM Deutschland GmbH, Ehningen, Germany). The association and differences between efflux times and dynamic viscosities of capillary 0 and I, were investigated using Pearson`s correlation and paired t-test. Further analyses of efflux time and dynamic viscosity were carried out with two mixed model ANOVAs. Models were built according to the model building strategies developed by Dohoo et al. [31]. The effect of capillary diameter, hydrostatic pressure, milk sample temperature, fat, protein and lactose content, SCC, dry-off and their potential interactions on efflux time were tested in the first mixed model ANOVA. The effect of capillary diameter, hydrostatic pressure, milk sample temperature, fat, protein and lactose content, SCC, dry-off, antibiotic dry cow therapy and their potential interactions on dynamic viscosity were tested in the second mixed model ANOVA. The random effect of cows was included in both models and days were considered as the repeated measure. All values reported are LSM ± SEM. The significance level was set at $P < 0.05$.

Results

Twenty-four quarters of six cows were enrolled in the study. Five quarters had to be excluded from final analysis due to deviations from the study protocol (e.g., clinical mastitis) and these quarters were treated with an IMM antibiotic for lactating cows. A total of 57 foremilk samples from 19 quarters (19 samples d0, 19 samples d2 and 19 samples d2 AB) were collected and analysed for milk composition. Three thousand and seventy-eight efflux time measurements were carried out, and 1026 dynamic viscosity values were calculated. Both decreased efflux time and decreased dynamic viscosity of the quarter foremilk samples were considered as a measure for a higher likelihood of occurrence of milk leakage, because the milk could flow and potentially leak faster and more easily. Therefore, parameters which lead to decreased efflux time and decreased dynamic viscosity were considered as potential risk factors for milk leakage in the field.

Capillary Diameter, Hydrostatic Pressure and Milk Sample Temperature:

Efflux time and dynamic viscosity differed ($P < 0.001$) between capillary 0 and capillary I, respectively, but showed a high correlation ($r = 0.996$; $r = 0.989$, $P < 0.001$). The efflux time decreased whereas the dynamic viscosity increased with wider capillary diameter ($P < 0.001$; Table 1).

An overall effect of the hydrostatic pressure and milk sample temperature ($P < 0.001$) on the efflux time and dynamic viscosity could be evaluated. The efflux time decreased as the hydrostatic pressure and milk sample temperature increased ($P < 0.001$; Table 1). The dynamic viscosity, however, just slightly increased as the hydrostatic pressure increased (Fig. 1a), but decreased as the milk sample temperature increased ($P < 0.001$; Fig. 1b). In addition to the effect of the capillary diameter, the hydrostatic pressure and milk sample temperature on the efflux time and dynamic viscosity, an interaction between the capillary diameter and hydrostatic pressure and furthermore between the capillary diameter and milk sample temperature could be demonstrated ($P < 0.001$). A slightly greater decrease of efflux time by increasing hydrostatic pressure was detected in capillary 0 and a greater and consistent increase of dynamic viscosity was seen in capillary I. A greater decrease in efflux time and dynamic viscosity by increasing milk sample temperature was seen in capillary 0.

Table 1: Differences in efflux time and dynamic viscosity in quarter foremilk samples on the day of dry-off (d0) and 2 d after dry-off (d2) with and without antibiotic dry cow therapy

	Efflux time (s)			Dynamic viscosity (mPa s)		
Variable	**LSM**	**SEM**	**P-value**	**LSM**	**SEM**	**P-value**
Dry-off						
d0	102.95	4.80	< 0.001	1.93	0.02	< 0.001
d2	108.61	3.40		2.03	0.01	
Antibiotic						
with	-	-	0.485	2.04	0.17	< 0.001
without	-	-		1.98	0.12	
Temperature						
26°C	122.58	5.40		2.27	0.01	
32°C	105.75	4.69	< 0.001	1.98	0.01	< 0.001
38°C	91.84	4.11		1.74	0.01	
Pressure						
60 mbar	132.17	5.70		1.97	0.02	
80 mbar	103.25	4.47	< 0.001	2.00	0.02	< 0.001
100 mbar	84.76	3.70		2.02	0.02	
Capillary						
0 (0.36 mm)	189.67	1.92	< 0.001	1.93	0.01	< 0.001
1 (0.63 mm)	23.78	0.55		2.03	0.01	
Antibiotic × Lactose			-			< 0.001
Antibiotic × SCC			-			0.025
Capillary × Temperature			< 0.001			< 0.001
Capillary × Pressure			< 0.001			< 0.001

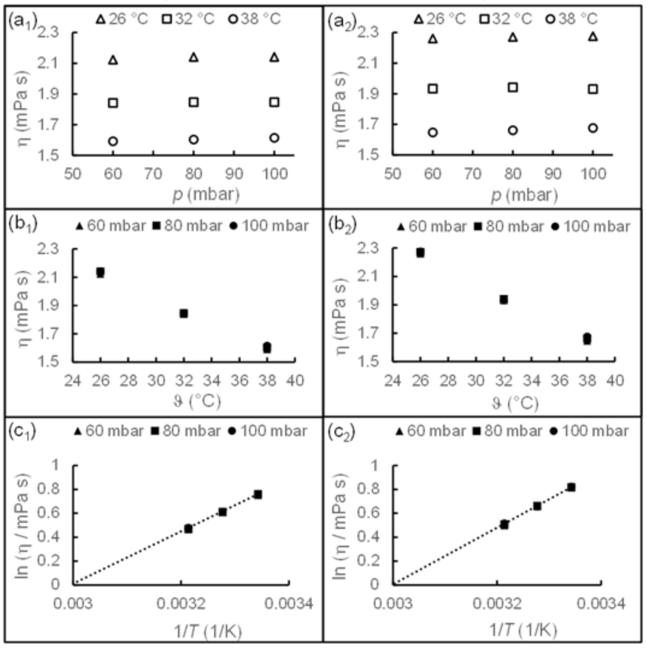

Figure 1: Average dynamic viscosities, η, of samples d0 (left column, panel label index "1") and samples d2 (right column, panel label index "2") as a function of (a) hydrostatic pressure, *p*, and (b) sample Celsius temperature, ϑ. (c) Replotting the data in a form of ln η vs. the inverse Kelvin temperature, 1/*T*, yields a linear Arrhenius plot that allows the energy of activation on flow to be determined from the slope of the linear fit (dotted line). All error bars are smaller than the data symbols.

Milk Composition, Dry-off and Antibiotic Dry Cow Therapy:

There were considerable changes in the milk composition between samples d0 and samples d2. The protein content increased from 3.80 ± 0.39 in samples d0 to 4.44 ± 0.39% in samples d2 ($P < 0.001$), SCC increased from 84,153 ± 22,671 to 151,153 ± 22,671 SCC/mL ($P < 0.001$) and the lactose content decreased from 4.57 ± 0.14 to 4.13 ± 0.14%, respectively ($P < 0.001$). No difference in the fat content could be evaluated between samples d0 and samples d2 (1.83 ± 0.29 and 1.87 ± 0.29%, $P = 0.340$). Changes in the milk composition between samples d2 AB and samples d2 could be detected as well. In these samples, the fat content decreased from 2.50 ± 0.38 in samples d2 AB to 1.88 ± 0.38% in samples d2 ($P < 0.001$), SCC decreased from 467,819 ± 52,240 to 132,345 ± 52,240 SCC/mL ($P < 0.001$), and the lactose content increased from 3.63 ± 0.15 to 4.13 ± 0.15%, respectively ($P < 0.001$). No difference in the protein content could be observed between samples d2 AB and samples d2 (4.44 ± 0.42 and 4.45 ± 0.42%, $P = 0.296$).

Changes in the milk composition affected the efflux time and dynamic viscosity. The efflux time and dynamic viscosity increased as the fat and protein content increased ($P < 0.001$). The lactose and SCC content, however, had no effect on the efflux time ($P = 0.495$ and $P = 0.423$, respectively). An effect of the interaction between the lactose content and antibiotic dry cow therapy ($P < 0.001$) and between SCC and antibiotic dry cow therapy ($P = 0.025$) on the dynamic viscosity could be evaluated. In samples d2 AB, the dynamic viscosity increased with increasing lactose content and in samples d2, the dynamic viscosity decreased with increasing lactose content. Furthermore, in samples d2 AB, the dynamic viscosity decreased with increasing SCC, however, in samples d2 the dynamic viscosity was not affected by SCC.

An overall effect of dry-off on the efflux time and dynamic viscosity could be evaluated. In samples collected after dry-off, the efflux times and dynamic viscosities were higher than in samples before dry-off ($P < 0.001$), respectively. Antibiotic dry cow therapy had no effect on the efflux time ($P = 0.485$). The interaction between antibiotic dry cow therapy and lactose content and between antibiotic dry cow therapy and SCC on the dynamic viscosity is described above.

Discussion

Increasing milk yield resulting in higher udder pressure before and after dry-off might aggravate the occurrence of milk leakage in dairy cows. Factors such as missed or incomplete milking in automatic milking systems [2] and an abrupt dry-off [32] without reduction of feed allowance during the dry-off period [10] can increase the prevalence of milk leakage. Considering milk leakage as a risk factor for clinical mastitis, the investigation of its aetiology is important for the development of potential prevention methods. To our knowledge, this is the first study evaluating risk factors for milk leakage in lactating cows and 2 d after dry-off with and without antibiotic dry-cow therapy in an in vitro study utilizing a laboratory model. Our results demonstrate that a wider capillary diameter, higher hydrostatic pressure, higher milk sample temperature and lower concentration of fat and protein are associated with a decreased efflux time of quarter foremilk samples. Furthermore, we detected an association between higher milk sample temperature, lower concentration of fat and protein and a decreased dynamic viscosity of quarter foremilk samples. As we considered a decreased efflux time and decreased dynamic viscosity as a measure for higher likelihood of the occurrence of milk leakage in the field, we identified a wider teat canal diameter, higher IMM pressure, higher milk temperature and lower concentrations of fat and protein as potential risk factors for milk leakage.

Capillary Diameter:

The efflux time decreased with a wider capillary diameter, which is well expectable based on the Hagen–Poiseuille equation:

$$\frac{dV}{dt} = \frac{\pi \times r^4 \times \Delta p}{8 \times \eta \times l}.$$

The equation states that the volumetric flow rate (dV/dt) of a fluid through a capillary depends on the fourth power of the capillary radius (r), which is half the capillary diameter. Consequently, the efflux time (t) decreases with wider radius and diameter, respectively. Transferred to milk leakage, these findings indicate that cows with wider teat canal diameter would be more likely to show milk leakage than cows with narrow teat canal diameter. Nevertheless, the influence of the teat canal diameter on the occurrence of milk leakage has not been investigated in a field study, yet. Shorter and wider teat canals were associated by several authors with an increased risk for mastitis [12-14], as was milk leakage [4-7]. A higher association between milk leakage and mastitis caused by Escherichia coli than by Staphylococcus aureus has been detected [8]. As milk leakage is a sign for an open teat canal, the udder might be more susceptible for IMM infections caused by environmental pathogens. An association between teat canal configuration, milk leakage and mastitis has not yet been investigated.

In our study, dynamic viscosities calculated from the measured efflux times using the Hagen–Poiseuille equation differed between the two capillaries but were highly correlated. We speculate that uncertainties in the manual measurements or in the determination of the viscometer constant, K might be the potential reason for discrepancy between the two capillaries.

Hydrostatic Pressure:

The efflux time of the quarter foremilk samples was affected by hydrostatic pressure and decreased as the hydrostatic pressure increased. Transferred to the teat, this finding indicates that higher IMM pressure might facilitate milk leakage in the field. This conclusion confirms earlier results of Bertulat et al. [3], who demonstrated that the occurrence of milk leakage after dry-off was significantly associated with udder pressure and that cows with a high udder pressure were more likely to show milk leakage than cows with low pressure values. A similar relationship between greater IMM pressure and milk leakage was demonstrated by Rovai et al. [9] in lactating cows. These authors hypothesized that the elevated IMM pressure caused by a large amount of cisternal milk is the trigger for milk leakage. Along with the efflux time, the dynamic viscosity of the quarter foremilk samples was also affected by hydrostatic pressure. The dynamic viscosity increased slightly as the hydrostatic pressure increased (Fig. 1a). Transferred to the teat, those findings indicate that high IMM pressure leads to faster but not easier flowing or leaking of milk.

Milk Sample Temperature:

Viscosity measurements were carried out at three different temperatures, aiming to represent milk temperatures in the teat cistern at different ambient temperatures. Direct data on milk temperature in the teat cistern are not available, but indirect estimates have been reported. We speculate that temperatures of milk in the teat cistern were lower than in the udder cistern and more affected by ambient temperatures, as it is physiological for acra. Therefore, we have chosen 26, 32 and 38°C as experimental temperatures, thereby representing cisternal milk temperatures during a cool, moderate and hot period.

In our study an overall effect of temperature on the efflux time and dynamic viscosity of the quarter foremilk samples could be evaluated. The efflux time and dynamic viscosity decreased as the temperature increased (Fig. 1b), similar to results from previous studies analysing dynamic viscosities of whole or raw milk samples [24, 33, 34]. In our study, the plot of the milk viscosity against inverse temperature is linear (Fig. 1c), substantiating Arrhenius-type thermal activation of the milk flow. For all our samples, an energy of activation of 19 ± 1 kJ/mol could be determined from this type of data evaluation, in quantitative agreement to earlier findings from Alcântara et al. [24]. Those results indicate that the likelihood of occurrence of milk leakage might be higher when the ambient temperature, and with that, the teat and cisternal milk temperatures increase. This observation warrants further research on teat and cisternal milk temperatures depending on the ambient temperatures, and with that, seasonality of milk leakage in the field.

Milk Composition, Dry-off and Antibiotic Dry Cow Therapy:

In response to simulated dry-off, the milk protein content and SCC increased, whereas the lactose content decreased. These changes in the milk composition can be attributed to changes in the mammary epithelial tissue permeability in response to milking omission. Alveolar distension due to milk accumulation leads to impaired tight junctions and an efflux of milk lactose and influx of blood proteins [21]. In response to antibiotic dry cow therapy, the milk fat content and SCC increased, whereas the lactose content decreased. The physiological background of these antibiotic dry cow therapy related changes in the milk composition remains unclear. The increased fat content might be related to the paraffin content of the dry cow antibiotic.

An overall effect of the fat and protein content on the efflux time and dynamic viscosity could be demonstrated. The efflux time and dynamic viscosity increased as the fat and protein content increased. A similar relationship between the dynamic viscosity and fat and protein content was reported by several authors [24, 33, 34]. McCarthy [34] reported that lactose and whey proteins have a relatively small influence on the dynamic viscosity of whole milk and the fat and casein content had a major and the greatest influence, respectively. Transferred to the teat, those findings indicate that a decrease of the fat and protein content might facilitate milk leakage due to faster and more easily flowing of the milk. Furthermore, it might be hypothesized that breeds with a naturally higher fat and protein content (e.g., Jersey cows) are less likely to show milk leakage. Again, further studies are warranted to elucidate this association.

In the current study, the lactose content and SCC had no effect on the efflux time. An interaction between the lactose content and antibiotic dry cow therapy and between SCC and antibiotic dry cow therapy on the dynamic viscosity, however, could be detected. Similar to the results of Alcântara et al. [24] the dynamic viscosity decreased with decreasing lactose content in samples with antibiotic dry cow therapy. In samples without antibiotic dry cow therapy, however, dynamic viscosity increased with decreasing lactose content. Rovai et al. [9] detected lower concentrations of lactose in milk leaking from teats of lactating cows, which let us hypothesize that low lactose content might facilitate milk leakage. Our results contradict this hypothesis, because the lactose content was not found to have any effect on the efflux time and decreasing lactose content of samples without antibiotic dry cow therapy let to an increased dynamic viscosity. To our knowledge, there are no studies that report an effect of SCC on dynamic viscosity of raw milk. In the current study, increased SCC resulted in a decreased dynamic viscosity in samples with antibiotic dry cow therapy. But in samples without antibiotic dry cow therapy the dynamic viscosity was not affected by SCC.

The efflux time and dynamic viscosity increased after dry-off, which is in agreement with the effects of dry-off related changes in milk composition. Those findings, however, are in contrast to the prevalence of milk leakage, which is with approximately 30% [3, 4] 6 times higher in cows the first days after dry-off than in lactating cows with 5.3% [1]. Transferred to the teat, dry-off and dry-off related changes in milk composition might not be a risk factor for milk leakage and not the reason for the higher prevalence of milk leakage after dry-off.

Antibiotic dry cow therapy does not affect the efflux time but does affect the dynamic viscosity in interaction with lactose content and SCC, respectively. Transferred to the teat, antibiotic dry cow therapy in interaction with decreased lactose content and increased SCC might be a risk factor for milk leakage on the milk level. To our knowledge, there are no other studies investigating the effect of antibiotic dry cow therapy on the likelihood of the occurrence of milk leakage.

Conclusions

To our knowledge our laboratory model is the first in vitro approach for a bovine teat. We are aware that the anatomical and physiological traits of a living teat and teat canal which are influenced by physiological changes such as blood flow, tissue tonus or contractions of smooth muscles could not be perfectly simulated by the constant diameter of a cylindrical shaped capillary in a viscometer. The laboratory model allowed us to reduce our study animals and to perform less invasive procedures on them. It might be useful in pilot studies, to provide indications for parameters which should be studied more extensively or it might be a good basis for further, improved models. The results of this in vitro study indicate that various factors influence the efflux time and dynamic viscosity of milk, which might influence the occurrence of milk leakage in the field. Considering decreased efflux time and decreased dynamic viscosity as a measure for higher likelihood of the occurrence of milk leakage, we confirmed wider teat canal diameter, higher IMM pressure, higher milk temperature and lower concentrations of fat and protein as plausible risk factors for milk leakage in dairy cows. Future research studies should investigate the influence of a teat sealant on the occurrence of milk leakage, the potential seasonality of milk leakage regarding the milk temperature and the potential lower prevalence for milk leakage in breeds with a naturally higher milk fat and protein content, which could be a useful information for future breeding programs.

Acknowledgments

We thank the staff of the DHIA (Landekontrollverband Berlin-Brandenburg e. V., Waldsieversdorf, Germany) for their superb cooperation. Joana Bachmann was funded in part by a scholarship from Tiergyn Berlin e. V. (Berlin, Germany).

References

1. Klaas IC, Enevoldsen C, Ersbøll AK, Tölle U. Cow-related risk factors for milk leakage. J Dairy Sci. 2005; 88:128-136.
2. Persson Waller K, Westermark T, Ekman T, Svennersten-Sjaunja K. Milk leakage—an increased risk in automatic milking systems. J Dairy Sci. 2003; 86:3488-3497.
3. Bertulat S, Fischer-Tenhagen C, Suthar V, Möstl E, Isaka N, Heuwieser W. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yields. J Dairy Sci. 2013; 96:3774-3787.
4. Schukken YH, Vanvliet J, Vandegeer D, Grommers FJ. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. J Dairy Sci. 1993; 76:2925-2930.
5. Waage S, Ødegaard SA, Lund A, Brattgjerd S, Røthe T. Case-control study of risk factors for clinical mastitis in postpartum dairy heifers. J Dairy Sci. 2001; 84:392-399.
6. Waage S, Sviland S, Ødegaard SA. Identification of risk factors for clinical mastitis in dairy heifers. J Dairy Sci. 1998; 81:1275-1284.
7. Peeler EJ, Green MJ, Fitzpatrick JL, Morgan KL, Green LE. Risk factors associated with clinical mastitis in low somatic cell count British dairy herds. J Dairy Sci. 2000; 83:2464-2472.
8. Schukken YH, Grommers FJ, van de Geer D, Erb HN, Brand A. Risk factors for clinical mastitis in herds with a low bulk milk somatic cell count. 2. Risk factors for Escherichia coli and Staphylococcus aureus. J Dairy Sci. 1991; 74:826-832.
9. Rovai M, Kollmann MT, Bruckmaier RM. Incontinentia lactis: Physiology and anatomy conducive to milk leakage in dairy cows. J Dairy Sci. 2007; 90:682-690.
10. Tucker CB, Lacy-Hulbert SJ, Webster JR. Effect of milking frequency and feeding level before and after dry off on dairy cattle behavior and udder characteristics. J Dairy Sci. 2009; 92:3194-3203.

11. Paulrud CO. Infrared thermography and ultrasonography to indirectly monitor the influence of linear type and overmilking on teat tissue recovery. Vet Res Commun. 2005; 29:215-245.
12. McDonald JS. Radiographic method for anatomic study of the teat canal: changes between milking periods. Am J Vet Res. 1975; 36:1241-1242.
13. Hamana K, Motomura Y, Yasuda N, Kamimura S. Bovine teat morphology and ultrasonic tomography related to milk quality and bacteria. Pages 377-380 in Proc. of the XVIIIth World Buiatrics Congress. 1994; Bologna, Italy.
14. Klein D, Flöck M, Khol JL, Franz S, Stüger HP, Baumgartner W. Ultrasonographic measurement of the bovine teat: breed differences, and the significance of the measurements for udder health. J Dairy Res. 2005; 72:296-302.
15. Lacy-Hulbert SJ, Woolford MW, Nicholas GD, Prosser CG, Stelwagen K. Effect of milking frequency and pasture intake on milk yield and composition of late lactation cows. J Dairy Sci. 1999; 82:1232-1239.
16. Bauman DE, Griinari JM. Nutritional regulation of milk fat synthesis. Annu Rev Nutr. 2003; 23:203-227.
17. Quist MA, LeBlanc SJ, Hand KJ, Lazenby D, Miglior F, Kelton DF. Milking-to-milking variability for milk yield, fat and protein percentage, and somatic cell count. J Dairy Sci. 2008; 91:3412-3423.
18. Heck JML, van Valenberg HJF, Dijkstra J, van Hooijdonk ACM. Seasonal variation in the Dutch bovine raw milk composition. J Dairy Sci. 2009; 92:4745-4755.
19. Lambertz C, Sanker C, Gauly M. Climatic effects on milk production traits and somatic cell score in lactating Holstein-Friesian cows in different housing systems. J Dairy Sci. 2014; 97:319-329.
20. Chen B, Lewis MJ, Grandison AS. Effect of seasonal variation on the composition and properties of raw milk destined for processing in the UK. Food Chem. 2014; 158:216-223.
21. Charton C, Larroque H, Robert-Granié C, Pomiès D, Leclerc H, Friggens NC, Guinard-Flament J. Individual responses of dairy cows to a 24-hour milking interval. J Dairy Sci. 2016; 99:3103-3112.
22. Hurley WL. Mammary function during the nonlactating period: enzyme, lactose, protein concentrations, and pH of mammary secretions. J Dairy Sci. 1987; 70:20-28.
23. Russell WMS, Burch RL. The principles of humane experimental technique. 1959; Methuen, London, UK.
24. Alcântara LAP, Fontan RCI, Bonomo RCF, Souza EC, Sampaio VS, Pereira RG. Density and dynamic viscosity of bovine milk affect by temperature and composition. Int J Food Eng. 2012; 8:1556-3758.
25. McDonald JS. Radiographic method for anatomic study of the teat canal: changes with lactation age. Am J Vet Res. 1968; 29:1207-1210.
26. Capuco AV, Mein GA, Nickerson SC, Jack LJW, Wood DL, Bright SA, Aschenbrenner RA, Miller RH, Bitman J. Influence of pulsationless milking on teat canal keratin and mastitis. J Dairy Sci. 1994; 77:64-74.
27. Paulrud CO, Clausen S, Andersen PE, Rasmussen MD. Infrared thermography and ultrasonography to indirectly monitor the influence of linear type and overmilking on teat tissue recovery. Acta Vet Scand. 2005; 46:137-147.
28. West JW, Mullinix BG, Bernard JK. Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. J Dairy Sci. 2003; 86:232-242.
29. Mayer H, Bruckmaier R, Schams D. Lactational changes in oxytocin release, intramammary pressure and milking characteristics in dairy cows. J Dairy Res. 1991; 58:159-169.
30. Bruckmaier RM, Hilger M. Milk ejection in dairy cows at different degrees of udder filling. J Dairy Res. 2001; 68:369-376.
31. Dohoo IR, Martin SW, Stryhn H. Veterinary Epidemiologic Research. 2nd ed. University of Prince Edward Island. 2009; Charlottetown, PEI, Canada.
32. Zobel G, Leslie K, Weary DM, von Keyserlingk MAG. Gradual cessation of milking reduces milk leakage and motivation to be milked in dairy cows at dry-off. J Dairy Sci. 2013; 96:5064-5071.
33. Rohm H, Müller A, Hend-Milnera I. Effects of composition on raw milk viscosity. Milchwissenschaft. 1996; 51:259-261.
34. McCarthy OJ. Rheology of milk and dairy products | Liquid products and semi-solid products. Pages 2445-2456 in Encyclopedia of Dairy Sciences. 2002; Elsevier, Oxford, UK.