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Changes of inner teat morphology caused by the milking process and by incomplete milking during dry-off as assessed by innovative technologies

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Für meine Eltern

Changes of inner teat morphology caused by the milking process and by incomplete milking during dry-off as assessed by innovative technologies

In dairy farming, the timing of the lactation cycle and the way of milk removal differ considerably from the natural conditions. Ensuring good health and wellbeing of farm animals is of great interest from both an economic and ethical point of view. Through the use of innovative technologies in this thesis, new insights into the effects of milking on the inner teat morphology as well as the effects of incomplete milking for gently preparing the mammary gland to dry-off were acquired.

By machine milking, the teat morphology is subject to modulating influences that may impede the local defense mechanisms of the teat canal. These internal effects were repeatably visualized and analyzed using high-resolution ultrasound technology (18 MHz). Focusing on the distal teat canal, three new traits of inner teat morphology were established, and associations with udder health-related and animal specific parameters were revealed.

In addition, a protocol for successive incomplete milking was established based on the development of an innovative milking software, by which the milking cluster is taken off, when a specified amount of milk is removed instead of the common practice that is based on reduced milk flow indicating the emptying of the udder. The new protocol allows for reducing the milk yield of dairy cows in order to prepare the animals for the dry period. The targeted performance depression was likewise as successful as the maintenance of good udder health conditions in cows that were identified as healthy before beginning of the experiment (somatic cell counts < 100.000 cells/mL without bacteriological findings in each quarter milk). The expected early induction of involution by the long-term retention of residual milk in the udder was confirmed by increased concentrations of the acute phase protein haptoglobin in skim milk. Internal teat morphology was also assessed by high-resolution ultrasound in this trial. As expected, significantly less changes in the morphological traits could be verified when milking with the early induced cluster take-off.

The present thesis contributes to a better assessment of the effects of the milking process on teat tissue and, in addition, presents a novel way to prepare healthy dairy cows in an animal friendly and gradual manner for the critical management phase at the end of lactation end, by application of innovative technologies.

Veränderungen der inneren Zitzenmorphologie durch den Melkprozess und Effekte von unvollständigem Melken vor dem Trockenstellen: neue Erkenntnisse durch Anwendung innovativer Technologien

In der modernen Milchviehhaltung unterscheiden sich der Verlauf des Laktationszyklus und die Art des Milchentzugs deutlich von den Bedingungen unter natürlichen Gegebenheiten. Die Gewährleistung von guter Gesundheit und Wohlbefinden unserer Nutztiere ist sowohl aus ökonomischen, als auch aus ethischen Gesichtspunkten von größtem Interesse.

Durch Anwendung innovativer Technologien konnten im Rahmen der vorliegenden Arbeit neue Einblicke in die Einflüsse des Melkens auf die innere Zitzenmorphologie, sowie die Einflüsse von unvollständigem Melken vor dem Trockenstellen gewonnen werden. Durch maschinelles Melken unterliegt die Milchkuhzitze modulierenden Einflüssen, welche die lokalen Abwehrmechanismen des Zitzenkanals einschränken können. Diese inneren Auswirkungen konnten mit Hilfe von hochauflösender Ultraschalltechnik (18 MHz) wiederholbar visualisiert und analysiert werden. Mit besonderem Fokus auf die Morphologie des distalen Zitzenkanals wurden drei neue Messparameter etabliert und zudem Zusammenhänge zu eutergesundheitsrelevanten und einzeltierspezifischen Parametern aufgedeckt. Mittels einer innovativen Melksoftware wurde ein Verfahren für sukzessiv unvollständiges Melkens erarbeitet, welches zur Milchmengenreduzierung bei hochleistender Milchkühe in Vorbereitung auf die Trockenstehphase dient. Die gezielte Leistungsdepression war hierbei ebenso erfolgreich wie die Beibehaltung einer guten Eutergesundheit bei entsprechender Eignung des Tieres zu Beginn des Versuchs (somatische Zellzahl < 100.000 Zellen/mL ohne bakteriologischem Befund in allen Vierteln). Die vermutete frühzeitige Einleitung der Involution durch das langfristige Belassen von Restmilch im Euter wurde anhand von der Konzentration des Akut-Phase-Proteins Haptoglobin in der Milch bestätigt. Auch in diesem Versuch wurde die zuvor etablierte Evaluierung der Einflüsse der Melktechnik auf die interne Zitzenmophologie mittels Ultraschalltechnik angewendet. Wie zu erwarten, konnte mit der vorzeitigen Melkzeugabnahme deutlich geringere Veränderungen in den Messmerkmalen verifiziert werden als bei konventionellen Melkbedingungen. Die erarbeiteten Methoden dieser Arbeit tragen zu einer verbesserten Bewertung der Auswirkungen des Melkprozesses bei, und eröffnen zudem eine neuartige Möglichkeit gesunde Milchkühe schonend und schrittweise auf die kritische Managementphase des Abmelkens am Laktationsende vorzubereiten.

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List of abbreviations

AUTODRY	software module, early removal of milking clusters (incomplete milking)
BHB	Beta-hydroxybutyrate
BSA	Bovine serum albumin
cfu	colony forming units
CON	control cows, conventional milking (cluster removal at milk flow < 0.3 kg/min)
CV	Coefficient of variation
DIM	Days in milk
DTCp	Distal teat canal`s perimeter
DTCs	Distal teat canal`s surface
FT	Front teat
Hp	Haptoglobin
IgG	Immunoglobulins
IMI	Intermammary infection
NEFA	Non-esterified fatty acids
PRH	Prolactin releasing hormone
RT	Rear teat
SCC	Somatic cell count
SCS	Somatic cell score
SD	Standard deviation
SE	Standard error
SED	Standard error of the difference between means
T0	Ultrasonographic scans conducted before milking
T1	Ultrasonographic scans conducted after milking
TCD	Teat canal diameter
TCL	Teat canal length
TCW	Teat cistern width
TEW	Teat end width
TMR	Total mixed ration
TOR	Distal teat canal`s orifice
TRH	Thyrotropin releasing hormone
TW	Teat width
TWT1	Teat wall thickness of the lower teat wall
TWT2	Teat wall thickness of the upper teat wall

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Chapter 1
General overview

1.1 Introduction

Selective breeding during the last decades has successfully led to high producing dairy cows (Oltenacu and Broom, 2010). Due to this genetically determined potential and optimized housing and feeding conditions, the cows` metabolism underlies increasing performance demands. To ensure constantly good productivity and sufficient health conditions of dairy cows, prudent and scientifically substantiated management techniques are required.

1.2 The teat and the teat canal of lactating dairy cows

Since mastitis is one of the major culling reasons in current dairy farming systems (Halasa et al., 2007), it is important to pay special attention to the delicate gland tissue. Previous studies have already shown that shape of udder and teats (Seykora and McDaniel, 1985; Nickerson, 2011; Guarín et al., 2017; Miles et al., 2019), microbiota of the teat skin and inner mucosa (Derakhshani et al., 2018; Svennesen et al., 2019) and the mechanical stimulus of machine milking (Neijenhuis, 2011; Zwertvaegher et al., 2013) are influencing the teats` defense mechanisms and therefore can affect the mammary gland`s susceptibility to mastitis.

1.2.1 Anatomical structure

The bovine mammary gland comprises four separated gland quarters. Each quarter is structured into two functional sections: a complex of gland parenchyma for synthesis, secretion and storage of milk and a teat for milk removal (Krömker, 2014). Size and shape of teats, as well as inner morphology differs among breeds (Klein et al., 2005; Bobić et al., 2014), between cows and quarters within cows (Rasmussen et al., 2004; Zwertvaegher et al., 2012) and is furthermore influenced by the milking procedure

(Gleeson et al., 2004; Guarín and Ruegg, 2016). Genetic selection of high yielding dairy cows during the last decades has resulted in (on average) shorter teats (Graff, 2005). Front teats are usually longer and broader than hind teats (Weiss et al., 2004; Zwertvaegher et al., 2013). Rear quarters tend to produce more milk and therefore need longer cluster on time to be milked completely (Weiss et al., 2004). Teat length and diameter seem to increase with parity, although the observed trend was not always significant (Tilki et al., 2005; Seker et al., 2009; Zwertvaegher et al., 2013).

A schematical visualization of the adult bovine teat is shown in figure 1.1. Stratified squamous epithelium covers the teat externally. The transition from the gland sinus into the teat sinus is marked by the venous ring of Fürstenberg. The teat consists of the surrounding teat wall, the apex with the teat canal and the teat sinus. The teat sinus is lined by a very flexible two-layered cuboidal epithelium. The teat wall is compound by different layers: the epithelium of the teat sinus or teat canal, a connective tissue layer and smooth muscle layer. The connective tissue layer contains large blood vessels that become engorged with blood at vacuum application during the milking or the suckling process, respectively (Steiner, 2016). The rosette of Fürstenberg forms the inner orifice from the teat canal into the teat sinus. Beneath, the sphincter muscle is located, consisting of circularly oriented bundles of smooth muscle fibers.

The teat canal is located at the apex of the teat and is connecting the teat sinus to the outside; it is responsible for preventing both leakage of milk and entry of potential harmful pathogens (Paulrud, 2005). It is lined with stratified squamous epithelia and is characterized by a layer of proliferating keratinocytes. The keratin in the teat canal reinforces the epithelial cells to a better resistance and plasticity for physical stress (Paulrud, 2005). The teat canal is surrounded with a net-like integrated musculoelastic system facilitating opening and closure during milk extraction. Descriptions about the

dimensions of the teat canal in the literature are varying. Length specifications from 5.0 to 18.3 mm (Krömker, 2014; Steiner, 2016) can be found and definition of the diameter range from 1.7 to 2.95 mm (Steiner, 2016).

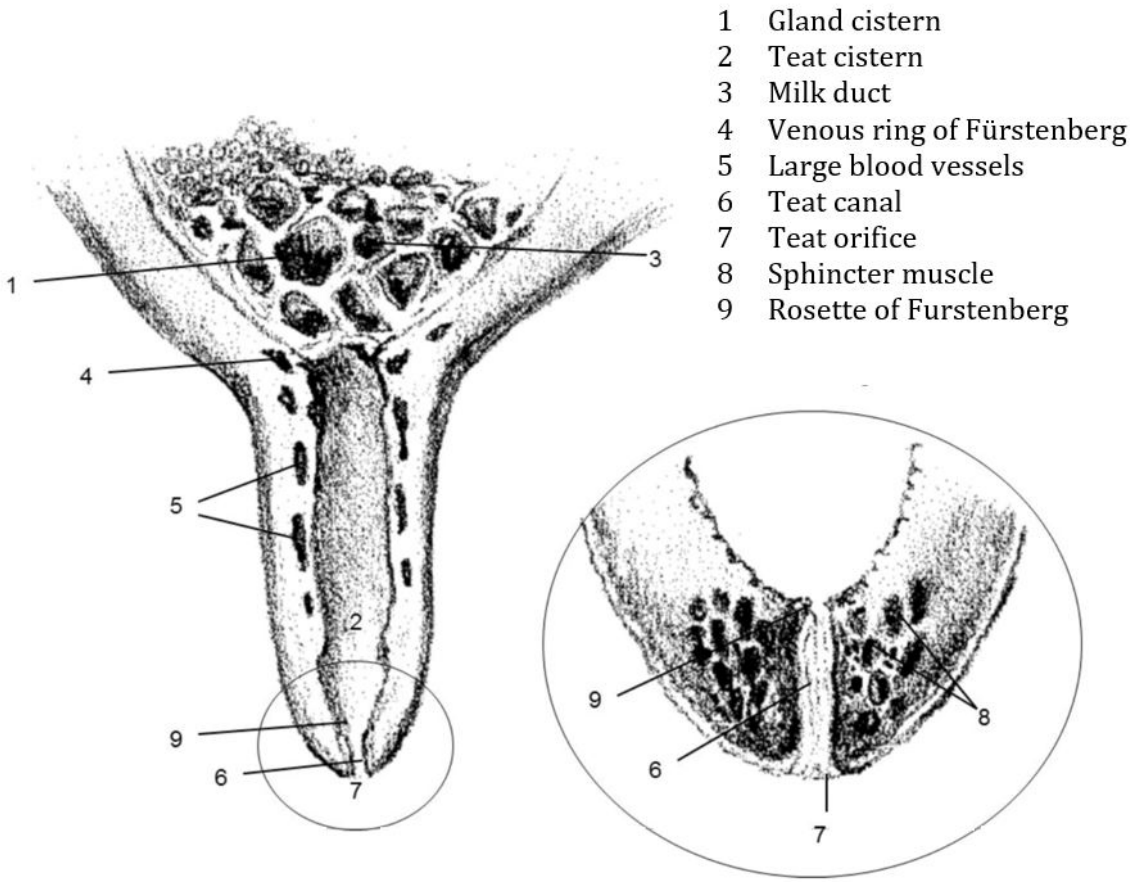

Figure 1.1: Schematic illustration of the bovine teat and teat canal

(modified from Krömker, 2014)

1.2.2 The importance of the teat canal for udder health

Since the distal orifice of the teat canal forms the entrance gate for potentially harmful pathogens into the lumen of the teat, the importance of the teat canal for udder health is very high (Krömker, 2014; Paulrud, 2005). In addition to this physical barrier functions a variety of further defense mechanisms are known.

The keratin in the teat canal fulfills functions that are beneficial for udder health. Microorganisms that enter the lumen can be absorbed by the keratin filaments and

during milking, the cellular detritus together with the keratin filaments and attached potential pathogens are sloughed from the teat canal`s surface.

In the dry period a keratin plug builds up, that closes the teat canal`s entrance gate against external influences. Dingwell et al. (2004) state that formation of this plug early in the non-lactating period can significantly reduce the potential of new intramammary infections (**IMI**) in multiparous cows.

The keratinocytes are furthermore involved in the emergence of local inflammatory processes, due to production of cytokines and activation of leukocytes, which mostly occurs in the region of the rosette of Fürstenberg (Nickerson and Pankey, 1984). The distal orifice of the teat canal can be characterized by hyperkeratosis, that in mild expression has the main function to protect the teat skin and support the closure of the teat canal and the defense against invading bacteria. Strong forms of hyperkeratosis hinder the closure of the distal orifice after milking, which leaves the teat in an unprotected state and facilitates the entrance of potential pathogens (Neijenhuis et al., 2001b). Due to the rough surface, hyperkeratotic teat ends are often difficult to clean (Haverkamp and Krömker, 2010), and are more densely populated with microorganisms than teat ends with a smooth surface (Timms et al., 1998; Paduch et al., 2012). Many authors found a connection between strong degrees of hyperkeratosis and compromised udder health (Neijenhuis et al., 2001b; Gleeson et al., 2004; Potrafki, 2005; Zucali et al., 2009). Extreme forms of hyperkeratosis are often induced by inaccurate milking machine settings, which put too much stain on the sensitive teat tissue.

Some researchers found connections between the shape of the teat canal and the health status of the respective quarter or udder. Mielke and Michel (1994) reported wider teat canal diameters in not udder healthy cows (1.0 - 1.25 mm) in comparison to udder healthy cows (0.4 - 0.55 mm). The length of the teat canal is also discussed to have an

influence on the defense against bacteria. Grindal et al. (1991) as well as Lacy-Hulbert and Hillerton (1995) state, that there is a trend for shorter teat canals increasing the risk of new infections.

The teat canal also has chemical defense mechanisms. Long-chain fatty acids (C16, C18, C18:1; Hogan et al, 1986) and antimicrobial proteins (Hibbitt et al., 1969; Senft et al., 1990) can impede the bacterial growth of mastitis-causing bacteria such as *Streptococcus agalactiae, Staphylococcus aureus* and *Corynebacterium bovis.* It is assumed, that the microbial population of the teat canal influences the defense mechanisms (Derakhshani et al., 2018). Some bacteria of the microflora (*Corynebacterium xerosis, Bacillus sp*. and *Aerococcus viridans*) were found to inhibit bacterial growth of potential pathogens *in vitro* (Woodward et al., 1987 and 1988). Typical skin-associated bacteria of the healthy bovine teat canal microbiota are *Corynebacteria* and *coagulase negative Staphylococci* (Braem et al., 2013; Adkins et al., 2018; Mahmmod et al., 2018; Derakhshani et al., 2018) which display a large diversity among the present species (Braem et al., 2012). Overviews of bacteria cultured in quarter milk samples during our experiments are given in table 2.5 (chapter 2) and in table 3.2 (chapter 3).

1.2.3 Effects of machine milking on teat morphology

During machine milking mechanical forces exerted by the induced vacuum and the collapsing liner cause changes in the overall teat dimensions (Hamann and Stanitzke, 1990). The pressure of the collapsed liner is unevenly distributed over the teat (van der Tol et al., 2010). The highest mechanical load is exerted in the middle region of the teat canal (Mein et al., 2003). Vacuum is applied to the teat to open the teat canal. It makes the milk available but also leads to congestion of blood and lymph in the teat`s tissue (Hamann et al., 1993). Poor milk machine settings can impede the enclosure of the teat

canal`s distal orifice. Application of too intense vacuum settings, overmilking, inadequate pulsation or inappropriate rubber liners can therefore hinder the different defense mechanisms of the teat canal and harm the cow's udder health.

To enable a better understanding of morphological changes of the external and internal teat characteristics due to milk removal, in the following a brief description of pressure rations during the natural process of suckling of a calf is provided: In contrast to mechanical milk extraction, the suckling of a calf applies compressive pressure near the teat base. Milk flow is induced by a combination of suckling and overpressure in the teat cistern caused by dynamic movement of the tongue (van der Tol et al., 2010). While the milk is swallowed, teat pressure decreases due to a reduction in vacuum (Fischer, 2007). Therefore calf suckling causes less teat tissue swelling (Hamann and Stanitzke, 1990) and does not stress the sensitive area of the teat end, as observed during machine milking (evaluated with the aid of a pressure-sensitive sensor by van der Tol et al., 2010).

1.2.3.1 Measurement of the external morphology

Changes in the external morphology of the teat can be assessed, for example, by measuring the alterations of dimensions like teat length or teat diameter with a translucent measuring ruler (Guarín and Ruegg, 2016) or a 2-dimensional vision-based measuring technique (Zwertvaegher et al., 2013). Other external characteristics that can be observed are teat color (creation of reddening or blueness), firmness of the tissue or visual assessment whether the teat duct orifice is open after milking or not (Hillerton et al., 2000). It has to be taken into account that these methods are mostly subjective, lack accuracy, and have low or unspecified reproducibility and repeatability (Zwertvaegher et al., 2012). Guarín and Ruegg (2016) compared pre- and postmilking external anatomical characteristics of teats by utilizing a translucent measuring ruler. The

authors found the teats to be prolonged and narrowed at both the barrel and the apex, due to the machine milking process. The mean length of the teats increased from 44.3 ± 0.20 to 45.6 ± 0.20 mm, whereas the mean diameter of the teats decreased from 23.9 ± 0.09 to 21.7 ± 0.07 mm measured at the barrel and from 19.6 ± 0.06 to 19.0 ± 0.05 mm measured at the apex. Data in this study is provided as means ± standard error (**SE**). Zwertvaegher et al. (2013) utilized a 2-dimensional vision-based measuring technique to observe changes in teats due to milking. The authors also found teats to be prolonged (relative change of 9.2 ± 11.7% (mean ± standard deviation (**SD**)) in teat length) and narrowed (relative change of - 2.4 ± 6.5%) at the barrel. On the contrary, the diameter of the teat in the region of the base and the apex due to the mechanical milk extraction increased about 0.7 ± 9.2% and 0.3 ± 6.1%, respectively. Hillerton et al. (2000) suggested that the principal risk factors due to machine milking may be high cluster weight, overmilking, vacuum applied during the overmilking phase and the design of the liner mouthpiece. Studying the effects of milking settings on teat load with a pressure-indicating film, Demba et al. (2018) found the teat load to increase with higher machine vacuum and pulsation rate.

1.2.3.2 Measurement of the internal morphology

For assessing changes in the internal morphology of the teat, ultrasonography can be utilized. Most commonly, teats are examined with the help of the water bath technique (Stocker et al., 1989), during which the teat is immersed into a plastic container filled with lukewarm water and the scanning probe is moved along the container to avoid deformation of the tissue. To ensure a sufficient quality of ultrasonographic images a frequency of no less than 7.5 MHz is recommended (Fasulkov, 2012). There are several studies available that investigate milking induced changes in inner teat morphology by the use of ultrasonographic probes up to a frequency of 13 MHz. An overview of

utilized equipment, enrolled animals and measured traits of teat morphology that are applied in these studies is given in table 1.1.

Table 1.1: Overview of scientific studies utilizing ultrasonographic examinations to evaluate changes in inner teat morphology caused by milking

Reference	Utilized frequency	Experimental cows	Measured traits of teat morphology
Neijenhuis et al., 2001a	7.5 MHz	18 primi- and multiparous Holstein cows; free of clinical mastitis; free of leg problems; average DIM[1] 170	Teat end width Teat canal length Teat wall thickness (lower teat wall) Teat cistern width Calculated trait: ratio between teat wall thickness and teat cistern width
Szencziová et al., 2013	7.5 - 13 MHz	26 Holstein cows in first and second lactation	Teat canal length Teat canal diameter Teat wall thickness
Bobić et al., 2014	4.5 - 8 MHz	30 Holstein and 30 Simmental cows (primi- and multiparous), up to DIM 250	Teat canal length Teat end width Teat wall thickness Teat cistern width
Fasulkov et al., 2014	5 - 12 MHz	12 primiparous Black and White cows; clinically healthy	Teat canal length Teat canal diameter at the rosette of Fürstenberg Teat wall thickness Teat cistern diameter (middle and base position)
Strapák et al., 2017	7.5 MHz	70 Holstein cows	Teat canal length Teat canal diameter
Melvin et al., 2019	5- to 10-MHz linear array transducer	80 Holstein cows, left front and right hind teats, milked three times per day	Teat canal length Teat canal diameter (proximal) Teat canal diameter (distal) Test canal diameter (at the midpoint between proximal and distal)

[1] DIM = Days in milk

Figure 1.2 provides an example of how inner teat morphology can be visualized by the use of high-resolution ultrasonography of 18 MHz. In ultrasonographic images of high quality the teat canal can be identified as a hyperechoic line, bordered by two parallel hypoechoic bands, which are located centrally in the teat tip (Franz et al., 2001). The rosette of Fürstenberg is visualized as a homogenous hyperechoic structure above the teat canal (Franz et al., 2009). The three layers of the teat walls of bovine teats can be differentiated by ultrasonography: the teat skin, which represents the outer layer, appears as a lucent hyperechoic line, the middle layer (connective tissue and musculature) shows as a homogenous, thick structure if moderate echogenicity (hypoechoic layer), while the innermost layer (mucosa) is clearly differentiated because it appears hyperechoic (Fasulkov, 2012). In some cases, hypoechoic blood vessels in the connective tissue layer can be seen. The teat cistern can be visualized most appropriately when filled with milk (Franz et al., 2001), therefore in most studies teats are scanned after the foremilking procedure to ensure properly filled cisterns. In ultrasonographic images, the milk filled cisteral area appears aneachoic (Santos et al., 2004).

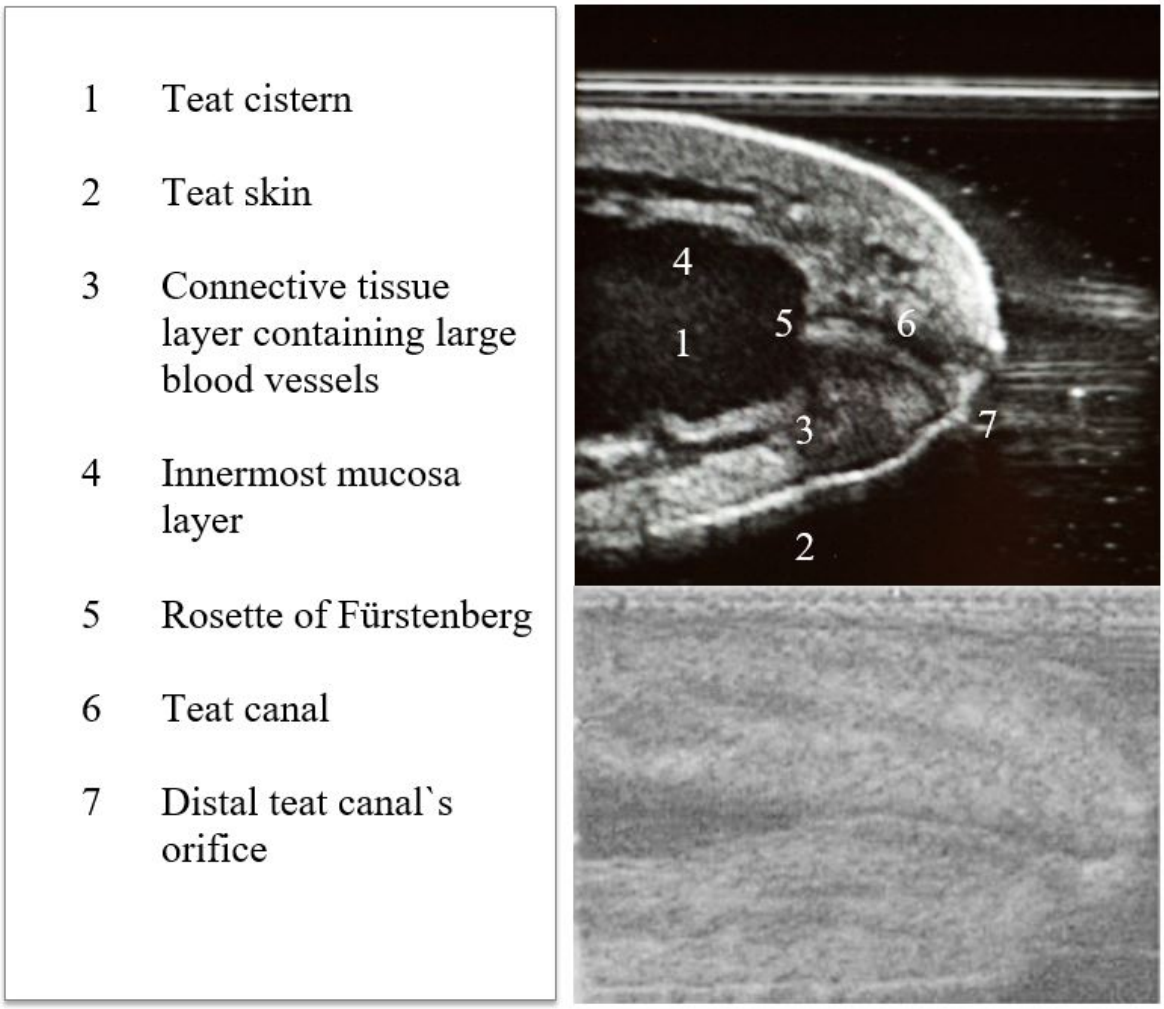

Figure 1.2: Inner morphological structure of the bovine teat visualized with an 18 MHz ultrasonographic probe (upper image) and with a 7.5 MHz ultrasonographic probe (lower image; Neijenhuis et al., 2001)

Common traits of inner teat morphology that are measured in ultrasonographiy studies are: diameter of the teat at different positions, width of the teat cistern and the teat walls as well as length and diameter of the teat canal (Neijenhuis et al., 2001a; Weiss et al., 2004; Szencziová et al., 2013; Bobić et al., 2014; Fasulkov et al., 2014; Strapák et al., 2017; Wieland et al., 2018). Specifications of changes in the respective traits of inner teat morphology due to the milking procedure and the absolute dimensions before and after milking assed by ultrasonography that can be found in the literature are summarized in table 1.2.

Table 1.2: Influence of the milking process on traits of inner teat morphology as assessed by ultrasonography; T0: Before cluster attachment; T1: After milking; FT: front teats, RT: rear teats

Trait	T0 (mean ± SD), in mm	T1 (mean ± SD), in mm	Average change	*P* -value	Reference
Teat canal length (mm)	10.0	11.2	+ 1.2 mm	< 0.05	Neijenhuis et al. 2001
	10.7 ± 1.4	13.1 ± 1.5	+ 2.4 mm	< 0.01	Szencziova et al. 2013
	FT: 12.37 ± 2.11; RT: 12.27 ± 2.1	FT: 14.16 ± 2.36; RT: 13.76 ± 2.2			Bobić et al., 2014
	8.48 ± 1.41	6.68 ± 1.01	+ 20.5 %	< 0.001	Fasulkov et al., 2014
	FT: 10.67 ± 1.53; RT: 9.36 ± 1.79	FT: 12.86 ± 1.52; RT: 12.44 ± 1.77			Strapák et al., 2017
Lower teat wall diameter (mm)	6.6	9.8		< 0.01	Kuchler 2011
	6.0 ± 0.9	8.7 ± 0.8		< 0.01	Szencziova et al. 2013
	FT: 6.34 ± 1.42; RT: 5.90 ± 1.38	FT: 7.5 ± 1.34; RT: 7.81 ± 1.57			Bobić et al., 2014
	5.3 ± 0.7	7.3 ± 0.8		< 0.01	Fasulkov et al. 2014
Teat cistern diameter (mm)	11.8	5.4	- 6.4 mm	< 0.05	Neijenhuis et al. 2001
	FT: 12.44 ± 3.34; RT: 14.72 ± 3.21	FT: 8.26 ± 2.67; RT: 8.61 ± 2.77			Bobić et al., 2014
	13.5 ± 2.2	7.9 ± 2.5	- 5.6 mm	< 0.01	Fasulkov et al. 2014
Teat end width (mm)	FT: 21.42 ± 1.74; RT: 21.79 ± 1.95	FT: 21.21 ± 1.72; RT: 21.59 ± 1.23			Bobić et al., 2014
	16.52 ± 1.42	16.58 ± 2.18		n.s.	Fasulkov et al., 2014
Teat width (mm)	21.2	21.7	+ 0.5 mm	< 0.05	Neijenhuis et al. 2001
	FT: 1.11 ± 0.10; RT: 1.09 ± 0.09	FT: 1.21 ± 0.12; RT: 1.19 ± 0.10			Strapák et al., 2017
Teat canal diameter (mm)	1.92 ± 0.38	1.73 ± 0.37		< 0.01	Fasulkov et al., 2014
	1.94 ± 0.41 2.11 ± 0.49 1.76 ± 0.46	1.87 ± 0.40 1.99 ± 0.49 1.69 ± 0.50		0.68 0.23 0.95	Melvin et al., 2019 (midpoint between proximal and distal, proximal, distal)

This thesis provides a more detailed visualization of inner teat morphology than available in the literature, by utilizing a higher frequency probe of 18 MHz. The measurement traits known from the literature were combined with three newly established measurement traits of the distal teat canal. The extent of modifications of the inner teat traits due to the milking process depends on the machine settings, such as machine-on time, applied vacuum level or type of teatcup liners (Hamann et al., 1993; Hillerton et al., 2000; Paulrud et al., 2005; Penry et al., 2017b). Changes of inner teat morphology caused by milking under a standard milking pattern as observed via high-resolution ultrasound were described in the second chapter. The third chapter deals with the changes in inner teat morphology after software-induced incomplete milking process of automated early teatcup removal in comparison to morphological changes after a standard milking pattern.

1.3 Drying off high yielding cows: a management challenge

Under natural conditions cows nurse their calves for 8 to 12 months (Reinhardt et al., 1986), whereby milk production increases rapidly until a peak is reached, approximately around the 7th week of lactation. Frequent suckling of the calf stimulates a higher milk production rate by increasing the frequency of udder emptying (Bar-Pelled et al., 1995). After peak lactation, milk production slowly declines simultaneously with the increasing nutritional independence of the suckling calf. Decrease of milk synthesis therefore naturally occurs successively over a long time period and reduction of milk production is caused by the diminishing need of the offspring. In contrast to this, calves are separated from their mother directly after birth in most commercial dairy systems. With the motivation of milk collection for human consumption, cows are required to yield persistently high milk amounts over the complete lactation phase, following

natural milk yield curves. Milk yield decreases after peak lactation, but milk production mostly remains high even in late gestation, induced by the application of high levels of udder emptying (by automated cluster removal based on low milk flow rates) until milking is ceased for a short period of approximately 60 days (Bachman and Schairer, 2003), before the birth of the new offspring.

The lactation cycle of modern dairy cows is structured by the economically driven target setting of obtaining one calf per cow and year (Strandberg and Oltenacu, 1989). The dry period is recommended to last about 60 days (Bachman and Schairer, 2003), leaving 305 days of lactation, which can be split into three phases: early, mid and late lactation. Current studies indicate, that shortening the dry period to 30 to 40 days may improve postpartum energy balance by lowering milk production in the subsequent early lactation, which has potential positive effects on metabolic health (Grummer and Rastani, 2004; O'Hara et al., 2019). However, reported effects of this restructured management technique on udder health are ambiguous (van Hoeij et al., 2016) and an average milk loss of 4.5% in the next lactation have to be taken into account (van Knegsel et al., 2013).

The transition from a lactating to a non-lactating state for dry-off is conventionally induced by an abrupt cessation of milking. This induces the mammary gland to go through acute involution. Zobel et al. (2015) explain that this process would rarely occur under natural conditions and could only be caused by the loss of the offspring. Acute involution is not recommended in humans, mainly due to concomitant tissue engorgement, milk leakage and pain but also health concerns like an increased risk of breast cancer (Betzold, 2007; Lawrence and Lawrence, 2011; Silanikove, 2014).

1.3.1 Relevance and risks of the dry period

The benefit of allowing milk-producing animals a rest of lactation has been acknowledged for a long time and many studies have been conducted, aiming to understand the processes that occur during the dry period (Hurley, 1989; Capuco and Akers, 1999; Andersen et al., 2005). If dairy cows are not allowed to experience a resting phase from milk synthesis, production amounts during the subsequent lactation are clearly limited as shown in previous studies. Andersen et al. (2005) observed milk yield in early lactation to be decreased about 22% in high yielding dairy cows that were managed without a dry period in comparison to cows with an equally high production potential that were dried off for seven weeks. The omission of the dry period resulted in 5.6 kg less milk per day compared to herdmates that underwent a 60 d dry period, when studied by Remond et al. (1997). This depression of milk synthesis, when not allowing for a non-lactating period can be explained by the important remodeling processes of regression, proliferation and differentiation of the mammary epithelial component that occur during the resting phase and which allow for a high production potential of the tissue (Capuco et al., 1997).

1.3.1.1 Involution

Mammary involution is a remodeling process through which the gland returns into a non-lactating state and renews secretory tissue. It starts approximately two days after cessation of milking (Holst et al., 1987). Under the common practice of abrupt cessation of milking the cisternal ducts and alveoli of the cow`s udder become engorged with milk and intramammary pressure rises (Oliver and Sordillo, 1989), because although milked is not extracted anymore the gland continues to synthesize milk for a few days. This physical event triggers the abrupt involution of the mammary tissue (Wilde et al.,

1997). Mammary involution is characterized by changes in mammary signaling. The expression of genes involved in apoptotic pathways increases and the rate of de-differentiation and apoptosis of mammary epithelial cells rises (Singh et al., 2005; Piantoni et al., 2010; Singh et al., 2016; Jena et al., 2019). This process is controlled by changes in systemic galactopoietic hormone levels. When mechanical milk removal or suckling ceases, the secretion of prolactin stops, which in turn triggers apoptosis (Wilde et al., 1997). Accumulation and stasis of milk after cessation of milk removal initiates recruitment of immune cells (leukocytes) and changes the abundance of various host-defense associated proteins. For example lactoferrin (Sordillo et al., 1987; Sordillo et al., 1997) and haptoglobin (Boggs et al., 2015) concentrations increase. Thus, inhibiting bacterial growth due to the ability of binding iron that consequently is not available for the demand for growth of certain pathogens. Increased levels of immunoglobulins (**IgG**) act against major gram-negative bacteria (Bushe and Oliver, 1987). Furthermore, the enzyme activities, such as those of plasmin, plasminogen activator and matrix metalloproteinases increase during involution. When involution proceeds, the permeability of the tight junctions of mammary epithelian cells, which are strongly closed during lactation increases, causing changes in mammary gland fluid composition because of enabling para-cellular transport between the interstitial space and milk (Nguyen and Neville, 1998).

For evaluating the progress of occurring involution, concentrations of different markers can be measured in samples of milk and mammary secretions: bovine serum albumin (**BSA**), Na+-to-K+ ratio, lactoferrin, citrate (Ponchon et al., 2014) as well as lactate dehydrogenase and proteolytic activity (matrix metalloproteinase 2 and 9) (Lanctôt et al., 2017) or plasmin and plasminogen (Politis et al., 1989).

1.3.1.2 Common risks of the dry period

Although a resting phase from milking is essential for proper cell renewal of the mammary gland, research activities have already shown, that cows are at their highest risk for IMI during modulation of the secretory tissue (Dingwell et al., 2003). Two time points are defined as the most risky phases when new IMIs are most likely to occur: on the one hand during resumption of milk synthesis during early lactation and on the other hand during modification of the udder from a lactating into a non-lactating state directly after cessation of milking (e.g. early involution). In the resting state of achieved involution, the risk of new infections is relatively low. Therefore, approaches that promote the involution of the mammary gland after cessation of milking might reduce the incidence of mastitis after dry-off (Lanctôt et al., 2017).

Bradley and Green (2004) partition intramammary infections that are present during the dry period into two categories: those that were carried into the dry period from the previous lactation (existing infections), and those entering between the time of dry-off and calving (new infections). The risk of a new infection during the non-lactating period is influenced both by management factors such as the rate of exposure to potential harmful pathogens from the environment (hygiene) or the effectiveness of preventive medical protection (such as antibiotic dry cow therapy or teat sealants; Robert et al., 2006) and the individual cow`s susceptibility to the respective infection (Dingwell et al., 2004). Whereby the animal`s vulnerability surely not only depends on genetic determination, but also on management affected constitution parameters such as the metabolic health status. A well-managed dry period should provide the prerequisite for an existing infection to heal.

Due to selective breeding over the last decades, milk yields in modern dairy cows have reached high values and often milk production is still at a high level at the end of

lactation. Rajala-Schultz et al. (2005) found that cows entering the dry period with high milk yields are more likely to develop IMI, probably due to increased occurrence of milk leakage and delayed teat plug formation, leaving the teat canal open for potential pathogenic invading (Rovai et al., 2007; Bertulat et al., 2013). Another explanation for the vulnerability of high yielding mammary glands in late lactation may be their weaker immune defense status. Lower amounts of natural protective factors such as phagocytic cells, lactoferrin, and IgG were found in udders producing high amounts of milk before cessation of milking, giving the mammary glands a less effective antibacterial response in this crucial phase (Bushe and Oliver, 1987). For example, Silanikove et al. (2013) showed that milk production levels affect leukocyte populations. Cows entering the dry period with less than 14 kg/d produced higher numbers of lymphocytes and macrophages, when compared with cows producing more than 25 kg/d and therefore experiencing a forced involution.

Since public interests and research activities in the field of animal welfare grow (von Keyserling et al., 2009), concerns have been raised whether abrupt cessation of milking (especially in high yielding cows) is still appropriate in modern dairy systems (Bertulat et al., 2013; Silanikove et al., 2013; Chapinal et al., 2014; Zobel et al., 2015). It is not clearly understood how the animals experience this practice, but it has been assumed that they may feel discomfort and pain if milk production is not reduced before dry-off, as they undergo a phase of high internal udder pressure after cessation of milking that can lead to painful tissue damage (O`Driscoll et al., 2011; Bertulat et al., 2013; Silanikove et al., 2013). Challenges in measuring pain and discomfort directly have mainly led to studies observing changes in the behavior of the cows. A decrease in lying time was found in connection with a high milk yield at dry-off (Zobel et al., 2013) and these behavioral changes seem to be particularly notable in combination with an abrupt

cessation of milking in those high yielding animals (Chapinal et al., 2014; Rajala-Schultz et al., 2018). The authors concluded, that the engorged udder tissue causes discomfort in a laying position, wherefore affected cows reduced their lying time. Silanikove et al. (2013) recorded increased vocalization in cows yielding more than 25 kg/day when dried off abruptly. The authors suggest that this distinctive behavioral change is an indicator for udder engorgement related discomfort. As a consequence of the described problems of high milk yield at dry-off, a production depression before cessation of milking seems to be beneficial for both health and wellbeing of the cow.

1.3.1.3 Use of antibiotics for dry cow therapy

A well thought udder health management is important for the maintenance of a healthy and profitable dairy herd. Especially as the associations between the genetically higher production potential of modern dairy cows and increased risk of production diseases, as well as reduced fertility and threats for animal welfare aspects are well known (Ingvartsen et al., 2003, Bertoni et al., 2009; Oltenacu and Broom, 2010). This susceptibility is even more exacerbated in times of reduced immunocompetence like during the transition from a high milk yield to the non-lactating dry period (Trevisi and Minuti, 2018). Therefore, dry cow therapy with intramammary antimicrobials after cessation of milking has long been recommended as an essential element of udder health management on dairy farms (Dodd et al., 1969). Antimicrobial dry cow therapy is an important component of mastitis control programs with the potential to eliminate existing intramammary infections during the non-lactating period. During blanket dry cow treatment, antimicrobials are also applied preventively to inhibit the development of new infections during the dry period in udder healthy cows. Thus, the utilization of

antibiotic substances during dry-off can be differentiated between therapeutical and preventive purpose. Therapeutic application after cessation of milking is indispensable if a clinical or subclinical infection exists. In contrast to this, the usefulness of general antibiotic treatment at cessation of milking is questioned increasingly (Scherpenzeel et al., 2014; Scherpenzeel et al., 2018). More and more selective dry cow therapy is recommended, during which the decision whether or not to dry-off with antimicrobials is made for each cow individually, based on respective udder health variables such as Somatic Cell Count (**SCC**), bacteriological findings and clinical mastitis history (Torres et al., 2008; Cameron et al., 2015; Kiesner et al., 2016). In the Netherlands, dry cow therapy already changed from mainly blanket to selective antimicrobial utilization. Supported by a national guideline, which recommends the individual SCC at the last milk recording before dry-off as the main selection criterion. A study by Vanhoudt et al. (2018) demonstrated that the selection of dairy cows for dry-off without antimicrobials, based on recommended SCC thresholds of < 150,000 cells/mL for primiparous cows, and < 50,000 cells/mL for multiparous cows, was possible without negative side effects on udder health. According to Vilar et al. (2018) in Finnish dairy herds selective dry cow therapy is already practiced on 78% of dairy farms across the country. Currently, also the feasibility of quarter-individual antimicrobial dry-off is discussed (Skarbye et al., 2018).

The main driving force for reducing antimicrobials in dairy farming is the public threat for human health by generating resistant organisms, which is caused by overusing antimicrobials (for example for preventive purposes), and particularly not pathogen-specific application of the active substances. The increasing occurrence of multidrug resistant bacteria in Europe was already highlighted as a threat for public health in 2009 in a report of the European Centre for Disease Prevention and Control

and the European Medicines Agency (ECDC/EMEA, 2009). The generation of antimicrobial resistances is among others caused by the overuse of antibiotics in modern livestock farming systems (Oliver et al., 2011). Due to the transmission of resistant bacteria along the food chain, the societal and political debate on preventive antibiotic use in livestock farming has intensified and strategies to reduce the use of antibiotics in dairy farming are prospected (Trevisi et al., 2014; Garcia et al., 2019). A good udder health status before cessation of milking allows for dry-off without antimicrobials devoid of unwanted side effects. A reduction of milk yield at the end of lactation could promote the prerequisites for an antibiotic free dry-off (Rajala-Schultz et al., 2005) and thus reduce the overall amount of antimicrobials used in modern dairy farming.

1.4 Common practices of milk yield reduction and the new approach of incomplete milking

For lowering milk production in late lactation, mainly two management techniques are utilized in modern dairy systems: low energy diets and reduced milking frequencies (Tucker et al., 2009; Gott et al., 2016).

1.4.1 Limitation of energy intake and skipping of individual milking times

The first approach reduces milk production by limiting energy and nutrient intake of the animal. The reduced nutrient supply decreases blood flow and prolongs transit time of blood through the mammary gland. A down-regulation of the mammary glucose transport system leads to a depression of milk synthesis (Shennan and Peaker, 2000). This strategy for lowering milk yield before dry-off is well established in the feed regiment of late lactating cows (Bushe and Oliver, 1987). Limitation of energy supply can be achieved by composing a total mixed ration (**TMR**) with lower energy density,

restricting the fed amount of the conventional TMR (which cows had received during the whole lactation) or by changing to give access only to low-energy roughage like hay (Zobel et al., 2015).

In a study by Tucker et al. (2009) feed restriction about 50% over a time period of six days reduced milk yield from 9.1 to 6.9 ± 0.95 kg/d, whereby limitation of feed intake was realized by reduced pasture access and pasture silage provision.

The second approach aims to reduce milk production by skipping individual milking times and is therefore also known as intermitted dry-off. Milk synthesis is inhibited due to milk stasis in the udder during the extended milking interval, which causes regression of mammary secretory epithelial cells (Stefano et al., 2002). Reviewing the effects of reduced milking frequencies on milk production, Stelwagen et al. (2013) stated that once daily milking reduces milk yield by approximately 22%, whereby the extent of production depression varies substantially and ranges from 7 to 40% in different lactation stages. Milking cows once instead of twice daily in the week before dry-off was found to effectively reduce milk yield in a study by Tucker et al. (2009) (7.0 vs. 8.9 ± 0.95 kg/day). Gott et al. (2016) found a depression of 33.4% on milk production caused by skipping from twice to once daily milking seven days before cessation of milking. The last daily milk yield before dry-off was effectively reduced in comparison to abruptly dried off cows (13.2 vs. 19.8 kg, respectively). Further benefits of the intermitted cessation in comparison to abrupt cessation are defined by Zobel et al. (2013): Skipping milking times over five days before dry-off reduced milk leakage (27% vs. 75%; overall leakage incidence e.g. either leaking or not leaking, tested with a 2-tailed Fisher's exact test over the course of the four observation days following complete cessation of milking) and motivation to be milked, interpreted from the time the cows spent standing at the parlor`s entrance gate anticipating routine

milking. This opens the conclusion that increased standing after abrupt cessation is caused by both frustration due to routine changes and by discomfort from udder engorgement. Similar to other management fields in dairy farming, gradual routine changes seem to cause less negative behavioral responses than when these changes are performed abruptly, providing the animals time to adapt to this new situation (Zobel et al., 2015).

In a study by Valizaheh et al. (2008) both management techniques of feed restriction and skipping milking times before dry-off to reduce milk yield were combined. Forty-two late lactation cows were fed a late lactation TMR for six days and then had ad libitum access to either grass or oat hay for another six days and were subjected to the same milking frequency schedule. On the day before dry-off cows were not milked at all and during d 2 to 4 before dry-off there were only milked once. By d 12 milk production had effectively declined from 16.4 ± 6.1 kg/day to 4.7 ± 0.37 kg on oat hay and 7.8 ± 0.42 kg on grass hay. Dancy et al. (2019) prepared Holstein dairy cows with a reduced milking frequency over five days before dry-off and compared behavioral and physiological variables of two groups, differing in the nutrient density of the mixed ration they received. As a mean for both treatment groups, milk yield at dry-off was reduced by approximately 10 kg after the experimental period. Cows that were fed the lower nutrient density diet produced 0.9 kg less milk at the time of dry-off in comparison with the group of cows that received the higher nutrient density diet.

Interestingly, recent evidence suggests that these common practices of reducing milk production itself may impede the welfare status of the cow (Zobel et al., 2015).

Odensten et al. (2007a,b) define that restricting nutritional feed supply in late lactation may result in compromising the cow metabolically during this challenging phase of high pregnancy. Greater blood non-esterified fatty acid (**NEFA**) and cortisol levels were

found in cows that were fed with limited energy intake before dry-off. Furthermore, it seems obvious that under such circumstances cows may not be able to reach satiety and may experience hunger. Tucker et al. (2009) noted increased vocalization in cows that were fed only half of their former ration (8 vs. 16 kg of dry matter per day) when being compared to those that received the full amount until dry-off (0.8 vs. 0.2 ± 0.15 calls/min for 8 and 16 kg of dry matter per day, respectively). In the study by Valizaheh et al. (2008) close to dry-off cows were divided into two groups and six days before dry-off were given ad libitum access to either oat hay or grass hay. The cows that received oat hay vocalized more often and ingested only about half of the daily amount of the grass hay group. Therefore, the authors concluded that the ad libitum access to grass hay enabled better satiety, due to the conditions that it is more palatable (e.g. the cows were motivated to eat more) and it contains higher amounts of protein than the oat hay.

The practice of gradual cessation of milking by skipping individual milking times was found to increase the risk of IMI at calving for multiparous cows, whereas abrupt cessation increased the risk of IMI in primiparous cows (Gott et al., 2016). In the cited study the odds of IMI of primiparous cows at calving were 3.5 times higher for abruptly dried off quarters than for quarters milked once daily for the final week of lactation. Furthermore, gradual cessation is discussed to frustrate the cow due to changes in the milking routine. Increased waiting at the pen`s exit gate was assumed to be a nonfunctional behavior and an indicator for frustration. Stefanowska et al. (2000) showed that an increase in this standing behavior was connected to missed milkings in lactating cows. In a study by Tucker et al. (2009) skipping from twice to only once daily milking was not associated with increased standing behavior. An experiment by Zobel et al. (2013) showed that after dry-off cows that were dried off abruptly were more

likely to spend time anticipating milking in comparison to cows that were dried off via intermitted milking over five days.

1.4.2 Prolactin inhibitors and other approaches

Prolactin is a single chain polypeptide, synthesized and released principally by anterior pituitary lactotroph cells. The main function of prolactin is the regulation of lactation by stimulating the growth and development of mammary gland tissue, milk synthesis and maintenance of milk secretion (Saleem et al., 2018). Prolactin is released in response to several signals acting mainly indirectly by modulation of tuberoinfundibular dopaminergic system (inhibitory control by hypothalamic dopamine) or directly on lactotropes in the pituitary. Prolactin releasing hormone (**PRH**), Thyrotropin releasing hormone (**TRH**) and neural stimuli during milking or suckling stimulate the release of prolactin. Also dopamine antagonists promote the release of prolactin, whereas dopamine and dopamine agonists inhibit prolactin secretion (Saleem et al., 2018).

Consequently, milk secretion can be reduced by reducing galactopoietic hormones, such as prolactin, in the blood and by that inhibiting the lactogenic signal, which is driven by prolactin (Ollier et al., 2012; Bach et al., 2015; Lacasse et al., 2016; Bertulat et al., 2017). One prolactin-release inhibitor at the level of the pituitary gland is cabergoline, a selective dopamine D2 receptor agonist that reduces blood prolactin concentrations for up to eight days when injected after abrupt dry-off (Boutinaud et al., 2016). Consequently udder pressure, milk leakage and signs of udder pain were found to be reduced after applying cabergoline at dry-off (Bach et al., 2015; Bertulat et al., 2017).

Another specific inhibitor of prolactin release (dopamine D2 receptor agonist) that was tested in several studies is quinagolide. Ollier et al. (2014) compared the effects of prolactin-release inhibition via quinagolide to the common approach of feed restriction aiming to lower milk production. Cows in the feed restriction group were only fed dry

hay during the last five days before dry-off. The treatment group received the same lactation diet as the control cows but received injections of quinagolide twice daily from d 5 before dry-off to d 13 after cessation of milking. Concentrations of prolactin (in blood serum and in skim milk) and the citrate to lactoferrin ratio (in skimmed milk) were found to be reduced and both BSA and Na^+-to-K^+ ratio increased faster in both groups (compared to the control group). Milk production was depressed to an average milk yield of 17.9 kg/day and 10.1 kg/day at dry-off for the quinagolide and hay group, respectively. In comparison, control cows had still high milk yields at cessation of milking with an average of 24.8 kg/day. Production depression caused by feed restriction led to decreased blood glucose and amino acid concentrations and increased the concentrations of Beta-hydroxybutyrate (**BHB**) and NEFA in blood. In the quinagolide group there was only an increase in blood glucose measured. These findings suggest, that inhibiting prolactin release by injecting quinagolide might serve as an alternative to lower milk production before dry-off without perturbation of the cows` metabolism as it could be observed during the common practice of feed restriction. In a similar study design by Ollier et al. (2015), milk production in cows before cessation of milking was limited either by feed restriction or quinagolide injection and quarters were challenged by dipping teats in a solution containing *Streptococcus agalactiae* to investigate the effect of the respective milk yield reduction method on susceptibility to new intramammary infections. The number of *Streptococcus agalactie* colonies found in mammary secretion samples collected after dry-off and the number of *Streptococcus agalactiae* infected quarters were lower for the cows treated with quinagolide. On d 14 after dry-off in 57.5% of the control quarters were infected with *Streptococcus agalactiae,* in contrast to this only 17.2% of the quarters that were treated with quinagolide were infected. The authors therefore suspect

prolactin release inhibition to have a positive effect on intrammamry infection rate at dry-off.

Boutinaud et al. (2016) studied the ability of inhibiting prolactin secretion to accelerate the involution process after dry-off. A treatment with the prolactin release inhibitor cabergoline at the time of dry-off decreased lactose concentrations and the citrate:lactoferrin molar ratio and increased lactoferrin concentrations, SCC and fat content in milk samples; leaving the authors to conclude that the cabergolin injection effectively accelerated mammary involution. Administration of cabergoline at dry-off was also found to effectively reduce udder engorgement and milk leakage after cessation of milking and improve lying time at the day following dry-off (Bach et al., 2015).

Another experiment was carried out testing the ability of prolactin release inhibitors to lower milk production and limiting energy deficit in cows subjected to acute nutritional stress in early/mid lactation (Ollier et al., 2016). Twenty-three cows were provided only 55.9% of their previous ration over 5 days and received injections of either quinagolide, dexamethasone or water (control group). Feed restriction reduced milk production but the change was greater in the quinagolide and dexamethasone cows than in the control cows. The limited energy supply led to a decrease in plasma glucose concentration and to an increase in NEFA and BHB concentrations. Cows that were injected with quinagolide showed higher glucose and lower NEFA and BHB concentrations. Limiting milk production by inhibiting prolactin release under the circumstances of acute nutritional stress limited the reduction of the energy balance. Analyzing the concentration and the activity of polymorphonuclear leukocytes, the authors also state that injections of quinagolide did not impede the cows` immune function.

Vanacker et al. (2017) tested how inhibiting the lactogenic signal through injections of quinagolide affects milk production and immune status in cows after calving. Eight quinagolide treatments every twelve hours after calving led to a lower milk production, higher blood glucose and calcium concentrations and lower blood BHB concentrations during the first week of lactation. There was no residual effect on longer term cow productivity and limiting the prolactin peak at the beginning of lactation led to an enhancement of the proportion of immune cells that entered oxidative burst.

There are also other scientific approaches that indirectly influence the milk yield before dry-off by aiming to speed up the involution process of the mammary gland after cessation of milking by different intramammary infusions at dry-off. In a study by Lanctôt et al. (2017) low- and high viscosity hydrogels of chitosan, a natural polysaccharide derived from chitin, which is able to trigger host immunity, were infused into cow teats at dry-off. In comparison to the control group (which was infused with water) the treatment group showed signs of immune response and involution faster. Physiological reactions were apparent in the increase in SCC, BSA, lactoferrin and lactate dehydrogenase concentrations in milk samples, which were collected after dry-off. The authors concluded, that chitosan hydrogel infusions could promote and hasten the involution process after cessation of milking and therefore shorten the hazardous time span of involution during which the risk of acquiring new intramammary infections is increased. Ponchon et al. (2014) had similar findings when infusing casein hydrolysate (70 mg) at dry-off and comparing the effects to reactions after ethylene glycol-bis(β -aminoethyl ether)-N,N,N,N-tetraacetic acid (5.7 g) and lactose (5.1 g) infusions. SCC and BSA concentrations in milk samples of quarters that were infused with casein hydrolysate after cessation of milking were higher and lactoferrin concentrations increased faster. The citrate to lactoferrin ratio was lower in treated

quarters and the Na^+-to-K^+ ratio increased faster. The casein infusion was also found to lead to an increase in proteolytic activity, obvious in greater matrix metalloproteinase 9 activities after dry-off. In contrast to this, infusions of ethylene glycol-bis(β -aminoethyl ether)-N,N,N,N-tetraacetic acid only increased SCC and infusions of lactose had no effect on any of the involution parameters. Accordingly Ponchon et al. (2014) suggested that casein hydrolysate represents a local inhibitor for milk secretion during milk stasis that accelerates mammary involution.

Leitner et al. (2007) also treated high producing cows (25 kg/day at dry-off) with intramammary casein hydrolysate at dry-off. The authors found the treated cows to spend more time lying down than nontreated cows after cessation of milking, due to significant difference in udder engorgement. Udder firmness in untreated cows increased for the first four days after dry-off, whereas it immediately decreased in the treated cows.

In a study by Maynou et al. (2018) acidogenic boluses were ultilized to lower milk production in dairy cows before dry-off. The authors aimed for inducing a mild and temporary metabolic acidosis at dry-off to facilitate the transition from lactation to the dry period. Administration of oral agidogenic mineral boluses containing anionic salts decreased feed intake and milk production before dry-off (- 2.56 kg/day, - 1.15 kg/day measured two days after treatment with two or one bolus, respectively). Bolus cows also showed lower udder pressure and increased lying time after dry-off but incidence of milk leakage did not differ between treated animals and control group.

1.4.3 The new approach of incomplete milking

For scientific purposes there have also been approaches aiming to reduce milk production of dairy cows in different lactation phases by means of incomplete milking.

Periods of milk stasis were proven to reduce milk yield not only caused by extended milking intervals but also by incomplete milking. After removing the milking clusters during a single milking interval in mid lactation (55 ± 9 days in milk (**DIM**)) when 40% or 70% of the expected milk amount was extracted, Albaaj et al. (2018) found milk production to be decreased about 5.3 kg for the 40% group and about 1.3 kg for the 70% group. Investigating effects of incomplete milking half of each contralateral udder of 12 cows over six weeks, Penry et al. (2017a) detached the teat cups early aiming to leave approximately 30% of the total milk yield behind. The effect of this treatment on milk production rate was found to be significant, with the average treatment half-udder producing 0.24 kg/h less in comparison to the average control half-udder (mean over the 6 week treatment period). Cows in this study were enrolled during early lactation (from 5 to 47 DIM).

Not emptying udders completely has traditionally been assumed to pose a threat for the cows` health situation (Woodward et al., 1936). Residual milk may serve as a growth medium for potentially harmful pathogens and therefore incomplete milking was traditionally suspected to impede udder health. Even though most studies on incomplete milking found slight increases of SCC in the quarters that received a reduced level of emptying there is no current experiment that found udder health to be impeded by this practice. After a single interval of incomplete milking Albaaj et al. (2018) found Somatic Cell Score (**SCS**) to be increased, but the effect was not longer apparent after milking the experimental cows under conventional settings for five further times. Penry et al. (2017a) reported SCC to rise only slightly after increasing the amount of milk remaining in the cisternal compartment after cluster removal over six weeks. Average SCC was 26,300 cells/mL for control half-udders and 48,300 cells/mL for incompletely milked half-udders. No information regarding the bacterial status of the enrolled udders

was provided in this study, which would have been essential for interpreting the causal interrelations between incomplete milking and changes in SCC.

So far, there are no studies investigating the effect of incomplete milking on milk production or udder health in late lactation, but there are several recent publications about the implications and benefits of incomplete milking in early lactation. The incipient milk production after calving induces an abrupt increase in the demands for energy and nutrients in dairy cows. This periparturient period presents a challenging time span for both metabolism and the immune system. Recent studies indicate that slowing down the production increase during the first days of lactation by only partial udder emptying can decrease the risk of potential metabolic or immunological disturbances during early lactation.

The approach of limiting milk harvest while maintaining milk stimulus in early lactation was initially studied by Carbonneau et al. (2012). Forty-seven Holstein cows were allocated to three groups: the first group was milked completely twice daily from calving (control), the second group was also milked twice a day but only about a third of the expected milk amount was extracted during the first 5 DIM (incomplete) and the third group was left to nurse their calves until 5 DIM and milked once a day from DIM 3 to 5 (nursing). From DIM 6 to 61 all cows were conventionally milked twice a day to study long term effects on cow productivity and metabolic status. During the treatment period incompletely milked cows received the following schedule: 6 L on DIM 1, 8 L on DIM 2, 10 L on DIM3, 12 L on DIM 4 and 14 L on DIM 5. The authors did not observe a residual effect of this milking protocol on long-term milk production (average milk yield between week in milk 2 and 9: 47.8 kg, 45.7 and 46.4 kg/day for the control, incomplete and nursing group, respectively). Interestingly, metabolic stress in cows of the incomplete and nursing group was lower compared to the conventionally milked

cows, concluded from higher blood concentrations of glucose as well as lower blood concentrations of NEFA and BHB. This effect was significant until DIM 28. The assumption, that incomplete milking in early lactation might lead to elevated intramammary pressure and changes in lying behavior was disproved by Krug et al. (2017). While milking cows incompletely (10-14 L/day) during the first five days of lactation, no significant effect of treatment on lying time was found.

Morin et al. (2018) state, that such a practice of incomplete milking in the early postpartum period could help to limit the negative energy balance in dairy cattle. Utilizing a comparable treatment protocol as seen in Carbonneau et al. (2012), the authors found incomplete milking to effectively reduce ketonemia and the prevalence of hyperketonemia in early lactation.

Randomly allocating multiparous cows (n = 846 cow lactations) into a control group and a treatment group, Krug et al. (2018a,b) studied the effects of incomplete milking during the first 5 DIM on culling hazard, milk production, milk composition and on udder and reproductive tract health. Experimental cows in the treatment group were milked incompletely with a maximum of 10, 10, 10, 12 and 14 L/day collected on DIM 1, 2, 3, 4 and 5, respectively. The study period lasted from 2 to 44 weeks in milk. During this observed period the authors did not find an effect of treatment group on culling hazard, milk weight, milk fat or milk protein concentrations. A difference in energy corrected milk was noted during the 38th week in milk: cows that had been milked incompletely before produced less amounts than cows that started in lactation under conventional settings. However, this discrepancy in production performance was only observed during this single week. Therefore the authors conclude, that milking incompletely had negligible effects on cow productivity (Krug et al., 2018b). Moreover, there were also no effects of incomplete milking on the incidence of clinical mastitis

during the first 90 DIM or on the health status of the reproductive tract investigated at 35 DIM (Krug et al., 2018a). Krug et al. (2018a) emphasized that milking incompletely during the first five days in milk even increased the odds for a decrease in SCS from 8 to 11 DIM.

The commonly used practices of modern dairy farming to reduce milk yield before dry-off (limitation of energy intake and skipping of individual milking times) can be accompanied by a variety of unwanted side effects for both welfare and health situation of the cow, as described before. For scientific purpose, there are further approaches of induced production depression but so far, none of them seems to serve as an appropriate on-farm management technique. The need of exploring new practicable alternatives to prepare high yielding cows for dry-off has already been emphasized in the literature (Zobel et al, 2015). Especially against the background of the growing importance of selective dry cow therapy (Scherpenzeel et al., 2014; Scherpenzeel et al., 2018) due to increasing public concerns regarding the blanket preventive application of antibiotics (Barkema et al., 2015), preparation for dry-off should enable a gentle and uncomplicated cessation of milking that does not require antibiotic treatment to maintain good udder health. Zobel et al. (2015) state that, due to consumer demands and health issues around the dry period that are at least partially attributed to the management of this phase, current dairy industry may soon be motivated to work out animal friendly methods for a more natural structure of the lactation cycle. Aiming for a more gradual transition from a non-lactating into a high lactating state and concomitant reduced metabolic or immunological disturbances in early lactation, practices of milk yield reduction seem to be beneficial not only before dry-off but also during the crucial phase of early lactation, by limiting milk production and slowing the overall production increase.

1.5 Objectives

The distal teat canal`s orifice is considered to be the main entrance gate for pathogens into the teat`s lumen. Therefore, its unimpeded functionality of opening for milk release and closure after milking is essential for a sufficient udder health status. Even though there are some ultrasonographic studies existing, describing the changes of inner teat morphology due to mechanical milk removal, hardly any study has focused on alterations of the teats` tip area. This motivated us to particularly investigate the morphological structure of the distal teat canal and its orifice via high-resolution ultrasonographic scanning. This thesis aimed:

1. to visualize and analyze the inner teat morphology in a detailed way by utilizing a probe of higher frequency than ever applied in the literature before (18 MHz),
2. to establish new measurement traits of the distal teat canal, and
3. to describe the changes in the teats` overall morphology caused by milking and their associations with other variables of the individual cow such as parity and particular aspects of milkability and udder health.

Since milk yield has severely increased during the last decades (more than doubled during the previous 50 years; Oltenacu and Broom, 2010), the cessation of milking in high yielding cows forms a demanding challenge for modern dairy farming. Currently there is no universal management technique available that enables milk yield reduction before dry-off as a gentle preparation before cessation of milking without potentially impeding the cows` wellbeing or health. Therefore, this thesis also aimed for:

4. programming of a software module that enables automated cluster removal at a targeted milk amount (see patent specification in the annex), and by applying this software in late lactation
5. testing a procedure of successive incomplete milking that will cause an adaptation of the cows` milk yield, without negative impact on udder health when the cow entered the program in a state of good health condition. With this successive milk yield reduction gradual involution of the mammary gland already prior to cessation of milking, the reduction of the milking machine induced modifications of the teats` tissue and no impairments of milk production or udder health in the subsequent lactation were targeted. The software induced preparation for cessation of milking may lay the foundation for an antibiotic free dry-off.

1.6 References

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Chapter 2

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Evaluation of inner teat morphology by using high-resolution ultrasound: Changes due to milking and establishment of measurement traits of the distal teat canal

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2.1 Abstract

The teat canal is important in the defense against invading pathogens, but its functional features can be impeded by the milking process. The objective of our study was to compare teat morphology before and after a standard milking procedure using high-resolution ultrasonography. Tissue changes were determined by measuring inner traits of teat morphology: teat width, teat end width, teat cistern width, diameter of the lower and upper teat wall, teat canal length and teat canal diameter. Additionally, three traits describing the distal teat canal and its external orifice were established: diameter of the distal teat canal`s orifice, distal teat canal`s perimeter and distal teat canal`s surface. In a first trial we verified the repeatability of scanning over time with a mixed model. During the second trial significant changes after milking were observed for all measured traits of teat morphology, except in teat end width. The traits from the distal teat canal and its orifice were remarkably changed by milking: distal teat canal`s orifice: + 28.9%, distal teat canal`s perimeter: + 25.0% and distal teat canal`s surface: + 41.5%. Comparing multiparous versus primiparous cows, higher values of teat width, teat end width and teat canal length were observed in the older animals. Testing the effect of milk yield on teat dimensions, cows with milk yields > 11.0 kg/afternoon milking were found to have larger teat widths, teat end widths and cistern widths before attachment of the cluster. Furthermore we observed associations of inner teat morphology towards bacterial counts in the appropriate milk. Regarding this udder health related parameter especially the newly established traits indicated to have a connection. Teats whose appropriate milk showed bacterial growth had larger distal teat canals` perimeters and distal teat canals` surfaces. High-resolution ultrasonographic scanning of dairy teats allowed a detailed visualization of the inner morphology. The applied procedure can therefore serve as a useful tool for comparison and evaluation of different milking

techniques by analyzing the resulting changes of the morphological traits. The thorough description of teat tissue can also be applied for drawing conclusions on the status of the teat canal`s physical and mechanical defense function.

2.2 Introduction

Maintaining a healthy mammary gland in dairy cows is indispensable for good production performance and the animals` wellbeing. Inner morphology of the lactating bovine teat and its modifications during the milking procedure play an important role in the defense against mastitis. The teat canal is highly specialized in its function of preventing leakage of milk and entry of microorganisms. Complex anatomical and immunological defense mechanisms are known (Krömker, 2014). A smooth muscle sphincter allows opening and closure of the distal teat canal`s orifice during the milking process; this sphincter is considered to form the first physical and mechanical barrier against invasion of bacteria by closing the most important portal of entry for pathogens into the mammary gland (O`Shea, 1987). Consequently, teat canal penetrability is an important aspect for udder health and by that also affects health management strategies on dairy farms. Teat morphology differs among breeds (Klein et al., 2005; Bobić et al., 2014), between cows and quarters within cows (Zwertvaegher et al., 2012) and is furthermore influenced by the milking procedure (Gleeson et al., 2004; Guarín and Ruegg, 2016). Machine milking may induce changes in teat dimension as well as in the teats tissue, such as congestion and hyperkeratosis (Neijenhuis et al., 2001b). Vacuum applied to the teat to open the teat canal makes the milk available but also leads to accumulation of blood and lymph in the teats tissue (Hamann et al., 1993). The magnitude of change in the teat canal is influenced by the milking machine settings such as machine-on time, applied vacuum level or type of teatcup liners (Hamann et al.,

1993; Hillerton et al., 2000; Paulrud et al., 2005; Penry et al., 2017). Various studies have been conducted to analyze the morphology of the bovine teat and its changes due to milk removal. External studies utilized a 2-dimensional vision-based measuring technique (Zwertvaegher et al., 2013) or a translucent measuring ruler (Guarín and Ruegg, 2016). For investigations of the teats` inner morphology ultrasound has been used and a variety of traits are known to be useful for measurements in ultrasonographic studies of the bovine teat (Neijenhuis et al., 2001a; Weiss et al., 2004; Szencziová et al., 2013; Bobić et al., 2014; Fasulkov et al., 2014; Strapák et al., 2017; Wieland et al., 2018). However, hardly any study has focused on the teats` tip area. As the teat canal is considered to be the main infectious path for pathogens, its unimpeded functionality is essential for a sufficient udder health. This motivated us to particularly investigate the morphological structure of the distal teat canal and its orifice via ultrasonographic scanning and thereby establishing additional measurement traits describing this delicate area. Ultrasonographic investigations are easy to apply, noninvasive, and thus allow for visualizing the teats' internal structure and their morphological changes during milking. However, the morphological dimensions of the teat canal are very small and scans of excellent image quality together with appropriate software are required for ensuring high accuracy and reliability which in turn allow for identification of influencing factors such as the milking process or peculiarities of individual cow rather than interpreting measurement errors. Therefore the objective of our study was to visualize and analyze the inner teat morphology in a detailed way by applying a high-resolution probe of 18 MHz and establishing new measurement traits. Furthermore our aim was to describe the changes in the teats` overall morphology caused by milking and their associations with other variables of the individual cow such as parity, and particular aspects of milkability and udder health.

2.3 Materials and Methods

2.3.1 Animals and Sampling

The experiments were carried out at the Frankenforst research station of the University of Bonn (Königswinter, Germany). The experimental procedures (June 2016 to October 2017) performed in this study were approved by the relevant authority (Landesamt für Natur-, Umwelt- und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen (84-02.04.2016.A047) and were in strict accordance with the German animal protection law. The Holstein dairy herd (n = 72) was loose-housed in a two-row open free-stall barn with cubicles and concrete floor. All animals were fed with a mixed ration and additional concentrate depending on the individual performance. The cows were milked twice per day at 05:30 a.m. and 16:30 p.m. Milking was performed in a double-four in-line milking parlor (GEA Farm Technologies GmbH, Bönen, Germany) at a pulsation rate of 62 pulses/min, a pulsation ratio of 64:36 and a vacuum level of 40 kPa; the milk pipelines were placed below cow standing level thus the milk was transported to the tank without lifting. The clusters were removed automatically when total milk flow decreased below 0.3 kg/min. Premilking treatment consisted of udder cleaning with a wet paper towel and stripping of the first milk squirts in addition to mechanical prestimulation (300 pulses/min during the first 30 s). Milk yield, milking duration and peak flow rate were recorded at the level of the udder by Metatron C21 (GEA Farm Technologies GmbH, Bönen, Germany).

For monitoring bacterial contents and somatic cell counts, we collected aseptic milk samples at the quarter level between the routine premilking treatment and the ultrasonographic scanning. Before sampling, the first strippings of milk from each quarter were discarded, teat ends were then disinfected with 70% alcohol and allowed to

dry. After that milk samples were taken into sterile vials and transported refrigerated to a commercial laboratory.

2.3.2 Bacteriological Culture and Somatic Cell Count

Bacteriological analyses were carried out according to the recommendations of the German Veterinary Society regarding the isolation and identification of mastitis pathogens (DVG, 2009). Milk samples were plated (10 µL) on blood agar (Oxoid, Wesel, Germany), incubated (37 °C) and optically evaluated after 24 h and 48 h for overall extent of bacterial growth. Accordingly, the quarters were classified either as group 0 (no growth of pathogens detectable) or group 1 (growth of pathogens detectable). Pathogens were then cultivated in pure culture on plate count (VWR, Darmstadt, Germany) or blood agar and identified via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) by the Chemical and Veterinary Investigation Office (CVUA, Stuttgart, Germany). Somatic cell counts in the quarter milk samples were quantified by using an automated cell counter (DCC Cell Counter; DeLaval GmbH, Glinde, Germany). For the procedure 60 µL of milk sample were aspirated into a small cassette. A DNA-specific fluorescent reagent bound to the SCC nuclei, which were then counted by an integrated digital camera. SCC values are given as base ten logarithms.

2.3.3 Experimental Design and Ultrasonographic Scanning

We used a MyLab TM Five Vet scanner with a multi-frequency (6-18 MHz) linear array probe (Esaote Biomedica GmbH, Cologne, Germany) to record ultrasound images of 50 (Trial 1) and 138 teats (Trial 2), respectively. The trials are described in detail below. The scans were performed on the longitudinal cross-section of the left and right front teats at the afternoon milking with B-mode 18 MHz. To avoid deformation of the

teat image by direct contact of the probe we used a plastic cup (7x5x12 cm) filled with lukewarm tap water to immerse the teat. Lateral to the teat the probe was held against the cup. To ensure an adequate contact between probe and cup we used ultrasonic gel (Aquasonic 100, Parker Laboratories Inc., Fairfield, USA). Scans before milking (**T0**) were carried out after routine teat preparation and collection of milk samples, to ensure a sufficiently filled teat cistern. Scans after milking (**T1**) were conducted immediately after milking, not later than three minutes after automatic cluster removal. The real-time scanning was observed on the screen of the scanning device. Taking the longitudinally folded cylinder-shaped structure of the teat canal (Paulrud, 2005) into account, we aimed to conduct each scan at the respective point of widest diameter. Therefore the probe was slowly moved on the plastic cup along the horizontal line of the teat, visualizing the teat canal in its overall morphology. The scanning procedure was performed until the resulting picture showed a sufficient quality (visibility of the whole teat at least 10 mm proximal the Fürstenberg`s rosette, adequate sharpness, good visibility of the ideally widest point of the teat canal`s diameter). As soon as the teats morphology appeared in adequate sharpness, the "freeze button" was pushed. The scanning device makes the previous 400 images available, and thus we were able to select the respective picture with the highest quality regardless whether or not the "freeze button" was pressed in the very second. The resulting pictures were stored in the internal memory of the scanning device. After the milking time the pictures were transferred to a computer via USB device. Measurements were conducted at a computer using MyLabDesk software (Version 10.0, Esaote Biomedica GmbH, Cologne, Germany) with an accuracy of 0.1 mm and were all conducted by the same person. We assessed ten different morphological characteristics of the teat as shown in figure 2.1.

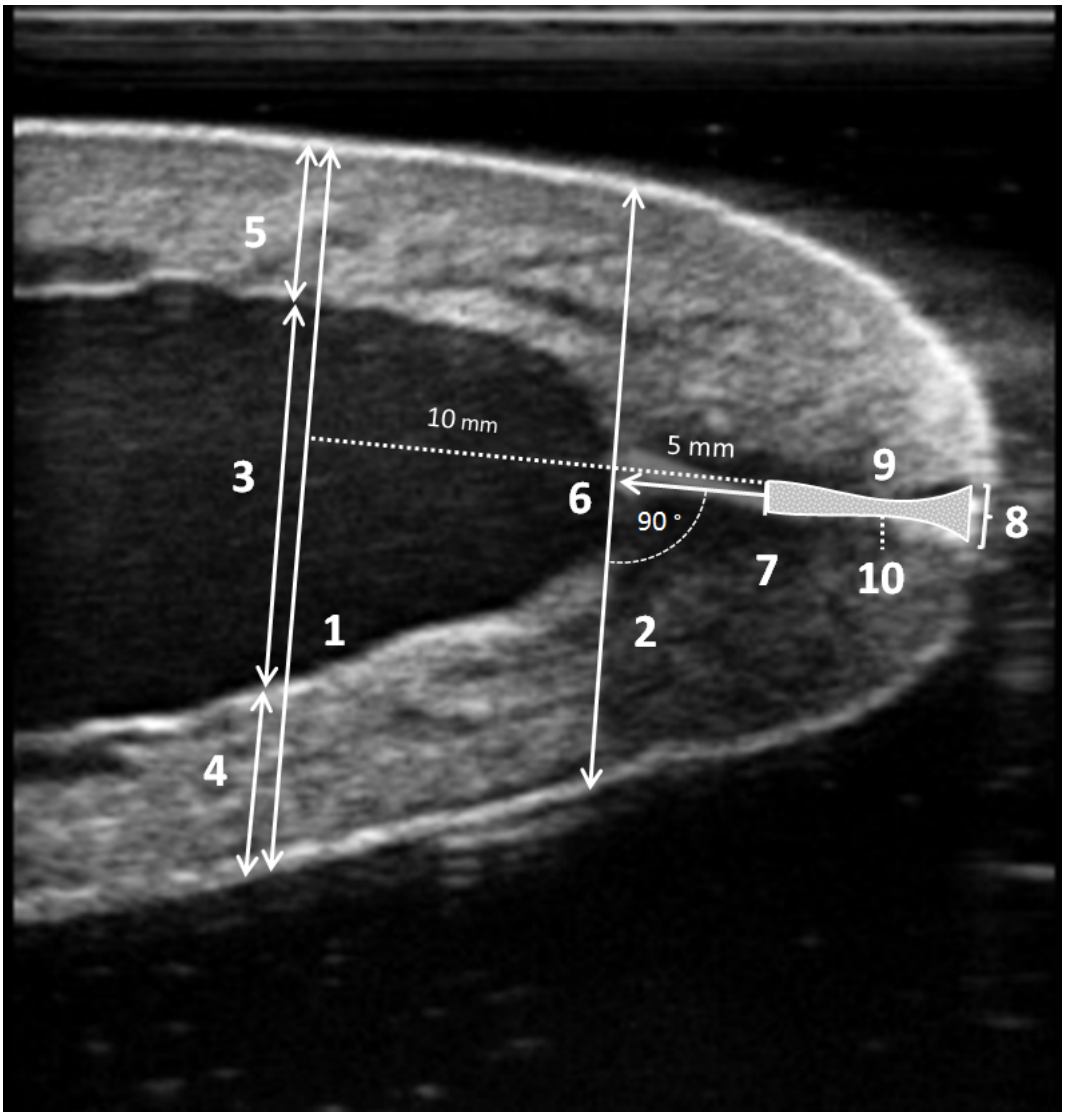

Figure 2.1. Measurement positions of the traits of inner teat morphology. 1 – TW (mm) width of the teat 10 mm proximal the rosette of Fürstenberg; 2 – TEW (mm) width of the teat end at the rosette of Fürstenberg; 3 – TCW (mm) width of the teat cistern 10 mm proximal the rosette of Fürstenberg; 4 – TWT1 (mm) diameter of the lower teat wall 10 mm proximal the rosette of Fürstenberg; 5 – TWT2 (mm) diameter of the upper teat wall 10 mm proximal the rosette of Fürstenberg; 6 – TCL (mm) length of the teat canal; 7 – TCD (mm) diameter of the teat canal 5 mm distal the rosette of Fürstenberg; 8 – TOR (mm) width of the teat canals distal orifice; 9 – DTCp (mm) perimeter of the distal teat canal beginning 5 mm distal the rosette of Fürstenberg until the distal orifice; 10 – DTCs (mm^2) surface of the distal teat canal referring to DTCp

Teat width (**TW** in mm) was measured orthogonally to the teat canal, 10 mm proximal the rosette of Fürstenberg. The diameter of the teat on the same orthogonal axis at the point of the rosette of Fürstenberg was measured as teat end width (**TEW** in mm). Diameters of teat cistern (**TCW** in mm), lower (**TWT1** in mm) and upper teat wall (**TWT2** in mm) were measured at the vertical line of TW. The length of the teat canal (**TCL** in mm) was measured from the rosette of Fürstenberg up to the distal end of the teat canal. The resulting axis of the teat canal served as a direction for measurements of teat dimension as mentioned before. The diameter of the teat canal (**TCD**) was measured 5 mm distal of the rosette of Fürstenberg. In addition to these seven traits of teat morphology that have already been applied similarly in previous studies (cf. Neijenhuis et al., 2001a; Strapák et al., 2017) three traits focusing on the area of the distal teat canal were assessed. At the distal end of the teat canal we measured the width of the teat canal`s orifice (**TOR** in mm). The perimeter (**DTCp** in mm) of the distal teat canal was measured starting at 5 mm distal the rosette of Fürstenberg at the measurement point of TCD up to the distal orifice (TOR). The perimeter was defined by manually setting measuring points hemming the hyperechoic area of the distal teat canal utilizing the feature for determining perimeters of the MyLabDesk software. The measurement software displayed the distal teat canal`s marked perimeter in mm (DTCp) and relating thereto, calculated the surrounded surface (**DTCs** in mm^2).

Trial 1 was performed to characterize the repeatability of the scans. Twentyfive randomly selected cows (average lactation number 3.0 ± 1.7) from the herd were assigned to three sequentially repeated ultrasonographic scans at weekly intervals. At each scanning date ultrasonographic scans of every front teat were done: One scan before (T0) and one scan after (T1) milking in order to evaluate the effects of the milking process. In total, 300 ultrasonographic scans from 50 teats were recorded.

Trial 2 was conducted to compare teat morphology before and after milking, aiming for evaluation of the effects of the milking process. Scans were done in 69 cows (average lactation number 2.4 ± 1.4) of the herd and included two ultrasonographic scans of every front teat, i.e. before (T0) and after (T1) milking. In total, 276 ultrasonographic scans from 138 teats were recorded.

All cows included in both trials had to be free of clinical mastitis and udder abnormalities. Number of lactations, milk yields and milking traits of the experimental cows are shown in table 2.1.

Table 2.1: Descriptive statistics of lactation number, milk yield, and milkability traits from the experimental cows

Trait	Unit	Trial1 (n = 25)				Trial 2 (n = 69)			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Lactation number		3.0	1.7	1	8	2.4	1.4	1	7
Daily milk yield	kg/day	33.8	10.0	18.0	49.2	27.1	10.0	8.2	49.2
Milk yield[1]	kg	14.7	4.9	8.2	22.6	12.3	4.8	3.4	21.8
Milking duration[1]	min	6.27	1.6	4.9	9.2	5.8	2.0	2.6	12.9
Highest milk flow[1]	kg/min	4.28	1.09	2.23	6.09	4.23	1.22	1.58	7.11

[1] as assessed during the afternoon milking (1630 h)

2.3.4 Statistical Analyses

The obtained data were analyzed using SPSS Statistics (Version 24.0, SPSS Inc., Chicago, IL). Data were tested for normal distribution using the Shapiro-Wilk test. Variance homogeneity was checked with the Levene`s test ($P > 0.10$). During Trial 1, teat scans of the same 25 cows before and after the afternoon milking were done in weekly intervals across three weeks, to characterize the repeatability of teat scanning of

the various traits from measurements over days. *Cow* was considered as the experimental unit and linear mixed models were fitted to analyze the effect of time on the measured traits. Dates of measurements were included as fixed effects and cow as a random effect. In addition, the standard error of the difference between means (**SED**) of the three measuring days was calculated to verify the effect of time. To provide the relation of the SED to the total mean for each trait percent values were calculated with reference to Neijenhuis et al. (2001a) as follows:

$$\% = \frac{SED\ trait}{total\ mean} * 100$$

In Trial 2, scans of the front teats (n = 138) during the exact milking time (T0, T1) were used to assess the milking-related changes in teat morphology and their potential associations with parity as well as particular aspects of milking characteristics and udder health. The experimental unit during these analyses was the individual teat. Taking variance homogeneity into account, we performed a paired Student`s t-test between the recorded values of each of the measured traits at T0 and T1 milking to determine their changes during milking. We used Student`s t-tests to compare the subgroups of the experimental animals that were formed according to threshold values for different traits (multiparous vs. primiparous, milk yield > or < 11.0 kg, peak flow rate > or < 4.0 kg/min; both on udder level). At the teat level subgroups were formed according to the microbiology results of 138 quarter milk samples, i.e. with or without bacterial growth in the appropriate milk. For estimation of variance MANOVA models were built. Measurements of teat traits at T0, T1 and respective values of differences in the measured values in comparison of the time points were put into the model as dependent variables. Cow and lactation number (multiparous vs. primiparous) were included as fixed effects, milk yield (> or < 11.0 kg) and peak flow rate (> or < 4.0 kg/min) at the respective afternoon milking as well as bacteriological

findings in the corresponding milk (with or without bacterial growth) served as covariates. We calculated Pearson`s coefficients of correlation for determining potential associations between the different traits of teat morphology. Results were considered as significant at $P < 0.05$. Values are given as means ± SD.

2.4 Results and Discussion

During Trial 1, aiming on characterizing the repeatability, we conducted 300 scans (25 cows, 50 teats, two scans per teat immediately before and after milking). Of the theoretically possible 3,000 measurements (10 traits per scan) 2,898 measurements were actually done. To analyze the changes of teat morphology caused by the milking process (Trial 2) we included 276 ultrasonographic scans in the data set (69 cows, 138 teats, two scans per teat immediately before and after milking). We were able to conduct 2,704 measurements of the theoretically possible 2,760. The total of 158 missing values was due to a few scans of inadequate quality, in which not all traits could be measured. The portion of 2.7% unavailable data represents the high quality of the scans and a good applicability of the method.

2.4.1 Repeatability of the ultrosonographic measurements

During Trial 1, the date of scanning was not associated with any of the variables assessed (average effect of time: $P = 0.54$). Thus all ten variables of teat morphology were repeatable at least over three weeks. Neijenhuis et al. (2001a) stated that differences between days are comparable with differences between duplicate measurements, therefore and because of the intense nature of the scanning procedure we renounced on duplicating scans. To analyze the mean differences in response to day of measurement we provided the SED for each measured trait (Table 2.2). The mean difference of ultrasound measurements of the various teat parameters between days varied from 1.6% for TW after milking to 10.9% for DTCs before milking.

Table 2.2: Differences in measurements of internal teat traits of the same 25 cows on three different days before (T0) and after (T1) milking (Trial 1)

Trait	Unit		N[1]	Total Mean	Measurement days			Effect of time		
					Mean day 1	Mean day 2	Mean day 3	SED[2]	%[3]	*P*-value
Teat width (TW)	mm	T0	150	27.4	27.6	27.2	27.5	0.52	1.9	0.61
		T1	143	25.9	26.0	25.9	25.9	0.42	1.6	0.38
Teat end width (TEW)	mm	T0	150	23.0	23.1	22.8	23.0	0.41	1.8	0.33
		T1	148	23.1	23.3	22.9	23.1	0.49	2.1	0.52
Teat cistern width (TCW)	mm	T0	150	13.7	14.2	13.4	13.7	0.82	6.0	0.91
		T1	144	7.05	6.77	7.38	6.99	0.68	9.7	0.68
Teat wall thickness lower teat wall (TWT1)	mm	T0	150	6.83	6.66	6.94	6.89	0.32	4.8	0.60
		T1	143	9.41	9.41	9.48	9.32	0.35	3.7	0.34
Teat wall thickness upper teat wall (TWT2)	mm	T0	150	6.77	6.71	6.81	6.79	0.38	5.6	0.49
		T1	145	9.49	9.73	9.36	9.38	0.68	3.6	0.82
Teat canal length (TCL)	mm	T0	150	10.7	10.6	10.8	10.7	0.46	4.3	0.63
		T1	148	12.7	12.7	12.7	12.7	0.39	3.1	0.65
Teat canal diameter (TCD)	mm	T0	140	1.01	0.90	1.05	1.07	0.07	6.5	0.29
		T1	141	1.16	1.12	1.16	1.19	0.06	5.2	0.54
Distal teat canal`s orifice (TOR)	mm	T0	143	2.05	1.97	2.00	2.17	0.15	7.1	0.10
		T1	145	2.66	2.69	2.61	2.69	0.19	7.3	0.77
Distal teat canal`s perimeter (DTCp)	mm	T0	138	16.3	16.2	16.5	16.1	0.89	5.5	0.45
		T1	140	20.9	21.0	21.1	20.7	0.89	4.2	0.44
Distal teat canal`s surface (DTCs)	mm^2	T0	138	7.12	6.80	7.55	7.04	0.78	10.9	0.51
		T1	140	10.5	10.3	10.8	10.3	0.91	8.7	0.79

[1]N = number of measurements included
[2]SED = Standard error of the difference
[3]% = (SED trait/total mean) * 100

Differences between days measured by Neijenhuis et al. (2001a) ranged from 4.4% for TEW up to 19.9% for TCW. Based on our relatively small variation of means between days and the independence of time, it can be stated that the applied method was characterized by a good repeatability. Aiming to analyze teat morphology in greater detail, we established three additional measurement traits with focus on the distal teat canal. Repeatability of these traits was equally well as of the measurement traits already applied in ultrasound studies of lactating teats in the literature (Neijenhuis et al., 2001a). The utilization of a high-resolution scanning device in our trials showed that it could be applied easily and without stress for the animals due to the noninvasive nature of the technique. Furthermore, it was fairly easy to operate for the user. Precise measurements were not only enabled by a high image quality, but were also based on implementation of measurement software of high accuracy. The conduction of all image analyses through the same observer is emphasized by Wieland et al. (2018), mentioning the risk of decreased precision in estimates and power to detect coherences of interest when performing measurements by various observers.

2.4.2 Changes of teat morphology during milking

Except for TEW all traits were associated with the milking process ($P < 0.001$), i.e. different values were obtained before and after milking as shown in Table 2.3.

Table 2.3: Changes in internal teat traits before (T0) and after (T1) milking (Trial 2)

			T0			T1			
Trait	Unit	N[1]	Mean	SD	SE	Mean	SD	SE	%[2]
Teat width (TW)	mm	132	27.2[a]	2.14	0.19	25.5[b]	1.93	0.17	- 6,07
Teat end width (TEW)	mm	134	22.6[a]	1.68	0.15	22.7[a]	1.77	0.15	+ 0,15
Teat cistern width (TCW)	mm	133	14.0[a]	3.21	0.28	7.21[b]	2,87	0.25	- 48,5
Teat wall thickness lower teat wall (TWT1)	mm	133	6.61[a]	1.45	0.13	9.21[b]	1.43	0.12	+ 39,4
Teat wall thickness upper teat wall (TWT2)	mm	133	6.40[a]	1.51	0.13	8.99[b]	1.51	0.13	+ 40,6
Teat canal length (TCL)	mm	135	10.6[a]	1.95	0.17	12.7[b]	1.90	0.16	+ 19,0
Teat canal diameter (TCD)	mm	132	0.99[a]	0.26	0.02	1.22[b]	0.25	0.02	+ 22,6
Distal teat canal`s orifice (TOR)	mm	133	2.02[a]	0.69	0.06	2.61[b]	0.75	0.07	+ 28,9
Distal teat canal`s perimeter (DTCp)	mm	131	16.7[a]	4.18	0.36	20.9[b]	4.29	0.38	+ 25,0
Distal teat canal`s surface (DTCs)	mm^2	131	7.04[a]	2.77	0.24	10.0[b]	3.28	0.29	+ 41,5

[a-b] Means within a row with different superscripts differ ($P < 0.001$)
[1]N = number of measurements included
[2]Percentage change of trait from T0 to T1

In our study TCW showed the highest relative changes after milking. Neijenhuis et al. (2001a) had similar findings with an average decrease of 45.8% in cistern width. During the milking process the milk is withdrawn from the teat cistern and as the cistern`s volume decreases, the dimension of the teat walls increases. During the vacuum phase strain is generated in the teats` tissue which leads to a dilatation of blood vessels. Swelling of teat walls can be explained by the machine induced accumulation of fluid (blood and lymph) (Hamann et al., 1993). However, appropriate milking machine settings should provide pulsation characteristics that enable effective teat massage and venous flow, so the interstitial fluid can drain off when the liner compresses during the massage phase (IDF, 1987). Teat shape in its overall width was only slightly affected, which was in accordance to previous studies (Paulrud et al., 2005; Kuchler, 2011; Bobić et al., 2014). In our experiment average change in TEW was about 0.15%. Therefore we can conclude that the machine settings did not lead to an intense accruement of edema. Stretching of the teats` tissue in its length dues to the vacuum and the weight of the cluster attached to the teat. Strapák et al. (2017) had similar findings, i.e. in front teats a 20.5 % average elongation of the teat canal. Even higher changes (27%) were reported by Szencziová et al. (2013). Neijenhuis et al. (2001a) found less stretching of the teat canal of 12%. These divergent outcomes might be explained by application of varying milking techniques and morphological variation of the experimental animals` teat tissue. Consequently, in our study general teat shape was much more affected in its length than in its width as a consequence of the milking procedure. Dimensions of teat width scaled down about an average of 6.07 % and a maximum of 8.96% (for TW) while extension of TCL reached 19% in average with a maximum of 49%. The same effect of overall shape adjustment was observed by Neijenhuis et al. (2001a). Using a translucent measuring ruler, Guarín and Ruegg (2016) also found post milking teats to be longer

and narrower than pre milking teats. Regarding the diameter of the teat canal, Strapák et al. (2017) found changes of 9% from 1.11 mm to 1.21 mm, also based on measurements in Holstein cows` front teats. The higher extension of the average diameter recorded in our study may not only be explained by different milking machine settings or the varying initial condition of the studies animals morphological characteristics before milking. Application of software tools for measuring the scans, varying in their accuracy probably also has an impact on the resulting values.

In addition to the classical teat canal traits, three innovative characteristics were established herein, describing the area of the distal teat canal and its external orifice in greater detail. Milk flow through the teat canal did not only extend the diameter of the teat canal at 5 mm proximal the rosette of Fürstenberg (TCD) but also led to a widening ($P < 0.001$) of the orifice (TOR) as well as the perimeter (DTCp) and surface (DTCs) of the distal teat canal. For evaluating a standard operating procedure for ultrasound-based measurements of teat canal dimensions, Wieland et al. (2018) measured a related trait defined as the teat canal diameter at the distal end of the teat canal using a 5-10 MHz probe. Obtained data was of comparable scale (2.42 ± 0.43 mm).

Associations between the measured traits of teat morphology were identified by correlation analysis (Pearson). The DTCp and DTCs were correlated to TCL, due to the fact that measurement of both traits describing the distal teat canal started 5 mm distal the rosette of Fürstenberg. Therefore with increasing length of the teat canal higher values of DTCp and DTCs were observed (DTCp: T0 r = 0.91, T1 r = 0.88; DTCs: T0 r = 0.63, T1 r = 0.57; at $P < 0.01$). Regarding the distal teat canal we found correlations between all three newly established characteristics. A great distal orifice (TOR) was relate to larger perimeter (DTCp) and surface (DTCs) of the distal teat canal (DTCp: T0 r = 0.31, T1 r = 0.40; DTCs: T0 r = 0.59, T1 r = 0.69; at $P < 0.01$). Positive correlations

were also detected between TCL and the diameters of the teat walls (TWT1: T0 r = 0.0.49, T1 r = 0.0.33; TWT 2: T0 r = 0.40, T1 r = 0.33; at $P < 0.01$). These findings are in accordance with Weiss et al. (2004) who found a correlation of r = 0.50 ($P < 0.001$).

In this study we also analyzed the connection between animal specific data, such as parity or traits related to udder health and milkability, and teat morphology. Milk yield was found to be connected to the inner morphology of the teat. For comparison of the experimental animals we formed two groups among a threshold value of 11.0 kg amount of afternoon milk yield (> 11.0 kg, n = 36; < 11.0 kg, n = 33). This value corresponds to the median afternoon milk yield of the cows (11.0 kg) and was used since milk yield data were not normally distributed. Cows with an afternoon milk yield > 11.0 kg not only had larger ($P = 0.01$) cistern (14.5 ± 3.07 mm in contrast to 13.0 ± 3.53 mm in lower yield cows) and teat width ($P = 0.001$) (TW 27.0 ± 1.75 mm and TEW 23.0 ± 1.56 mm in contrast to TW 26.3 ± 2.20 mm and TEW 22.1 ± 1.67 mm in lower yield cows) before attachment of the clusters due to the teats bulging with milk, but also showed a higher difference in the measured values in comparison of the time points. Consistent with the bigger milk volume removed in cows with > 11.0 kg milk yield, the differences in TW and TCW were higher ($P = 0.005$ and $P = 0.002$, respectively) than in cows with milk yields < 11.0 kg. TCW was reduced by 7.39 ± 3.12 mm in cows with higher yields and by 5.69 ± 2.92 mm in cows with lower yields in the course of removal of the milk. Bobić et al. (2014) also emphasized a slight connection (r = 0.27, $P < 0.05$) between dimensions of the teat cistern and milk yield in front teats. Connections between the measured values of TW and milk yield could not be verified by Weiss et al. (2004). This might be explained by a higher average milk yield during our experiment (27.1 ± 9.98 kg) compared to the milk production of cows enrolled by

Weiss et al. (2004) (13.1 ± 0.45 kg) and consequently a higher variation in the amount of teat swelling due to heavily filled cisterns. Furthermore, Weiss et al. (2004) stated that an association with milkability traits was not observed when examining externally measurable teat characteristics, such as the teat diameter. Therefore ultrasonographic examination to analyze inner characteristics seems more appropriate for studying such relationships. In our experimental cows with a peak flow rate recorded at afternoon milking > 4.0 kg/min (n = 36) showed a trend for wider teat canal diameters before (P = 0.055) and after (P = 0.067) milking than cows with peak flow rates < 4.0 kg/min (n = 33) (> 4.0 kg/min: T0 1.03 ± 0.03 mm, T1 1.25 ± 0.27; < 4.0 kg/min: T0 0.94 ± 0.02 mm, T1 1.17 ± 0.23 mm). The threshold value reflects the average peak flow rate observed in the experimental cows during Trial 2 of 4.23 ± 1.22 kg/min (data were normally distributed). The observed trends might be interpreted as wider TCD enable a greater volume of milk to pass through the teat canal per unit time. Milk yield and milk flow were both recorded at the udder level during our study. Expanding such data to the quarter level in future ultrasonographic studies will deepen our insight into the relationships between milking traits and inner teat morphology.

Parity was identified as a factor associated with changes of teat morphology during milking. Comparing multiparous (n = 50) to primiparous (n = 19) cows, we observed differences in various traits of inner teat morphology (Table 2.4).

Table 2.4: Internal teat traits of multiparous and primiparous cows before (T0) and after (T1) milking (Trial 2)

			Multiparous cows (n = 50)			Primiparous cows (n = 19)			
Trait	Unit		Mean	SD	SE	Mean	SD	SE	*P* - Value
Teat width (TW)	mm	T0	27.6	2.20	0.22	26.1	1.52	0.25	< 0.01
		T1	25.8	1.91	0.19	24.8	1.79	0.29	< 0.01
Teat end width (TEW)	mm	T0	23.1	1.55	0.16	21.6	1.58	0.26	< 0.01
		T1	23.0	1.37	0.14	21.8	2.34	0.38	< 0.01
Teat cistern width (TCW)	mm	T0	14.3	3.41	0.34	13.0	2.88	0.47	0.044
		T1	7.45	3.16	0.32	6.62	1.79	0.29	0.059
Teat canal length (TCL)	mm	T0	10.8	2.06	0.21	10.1	0.16	0.27	0.042
		T1	12.8	2.03	0.21	12.2	0.14	0.23	0.034
Distal teat canal`s perimeter (DTCp)	mm	T1	21.3	4.5	0.46	19.8	3.44	0.56	0.063

Teats of multiparous cows were wider before and after milking ($P < 0.01$) and showed larger cistern dimensions before milking ($P = 0.044$ at T0) than teats of primiparous cows. These differences in teat shape might be explained by the lower average milk yield of the heifers as compared to the multiparous cows (heifers: 9.47 ± 2.17 kg/day; multiparous: 13.50 ± 5.05 kg/day) and thus a less filled cistern and a less swollen teat. Zwertvaegher et al. (2012) reported similar findings of narrower teats in heifers using a 2-dimensional vision based measuring technique to estimate the teat barrels diameter in an external study. Parity did also affect length of the teat canal. We found longer TCL in teats of multiparous cows compared to those of heifers. Comparing Holstein cows in the first and second lactation by using a 7.5 MHz probe, Szencziová et al. (2013) also detected differences ($P < 0.05$) in length of the teat canal. Referring to the newly established traits of distal teat morphology, the perimeter of the distal teat canal (DTCp) after milking tended to be larger in multiparous than in primiparous cows, however the level of significance was not reached in this case ($P = 0.063$). Taking the effects of parity together, we suggest that exposure of teat tissue to mechanical milk removal increases the dimensions of inner morphological traits with advancing lactations. We thus suggest that special attention should be paid to the influence of milking machines on teat tissue with increasing age.

With regard to udder health, no major pathogens were detected in the quarter milk samples during Trial 2 (n = 138). An overview of the occurrence of cultured udder pathogens is shown in table 2.5.

Table 2.5: Overview of bacteria cultured in quarter milk samples (n = 138, Trial 2)

Classification	Bacteria	Number of affected quarter milk samples (%)[1]	Number of cfu (Min-Max)[2]
Coagulase-negative staphylococci	*Staphylococcus chromogenes*	6 (4.3)	1-34
	Staphylococcus haemolyticus	2 (1.4)	1-7
	Staphylococcus equorum	4 (2.9)	1-8
	Staphylococcus cohnii	4 (2.9)	1-6
	Staphylococcus auricularis	1 (0.7)	2
	Staphylococcus vitulinus	1 (0.7)	1
Corynebacteria	*Corynebacterium bovis*	15 (10.9)	1-9
	Corynebacterium confusum	2 (1.4)	2-5
	Corynebacterium frankenforstense	4 (2.9)	1-7
	Corynebacterium resistens	1 (0.7)	1
Aerococci	*Aerococcus viridans*	3 (2.2)	2
	Aerococcus suis	1 (0.7)	1
Micrococci	*Micrococcus luteus*	1 (0.7)	1
	Micrococcus kocuria carniphila	2 (1.4)	1
Negative culture		100 (72.5)	
Contaminated samples[3]		1 (0.7)	

[1]A quarter milk sample was defined as affected by the presence of 1 or more colonies of the same type
[2]Number of colony forming unit (cfu) identified in the individual cultured quarter milk samples per 10 µL
[3]Samples were defined as contaminated when 2 or more colonies of 2 or more different bacteria (without distinct excess of one type of colony) were identified

The quarters examined during trial 2 had a relatively low average somatic cell count (log_{10}SCC = 4.16 ± 0.57 cells/mL, or SCC = 49,036 ± 178,603 cells/mL, respectively), indicating a good udder health status in the herd. In 11 out of the 138 enrolled quarters we detected SCC > 100,000 cells/mL, but without bacteriological findings and clinical symptoms. These quarters were examined for udder pathogens two further times with an interval of one week between samplings confirming the negative findings. Therefore these quarters were classified as showing unspecific mastitis (DVG, 2002). Bacterial growth in the appropriate milk was the trait connected to udder health and vulnerability to mastitis, enrolled in our study. We were able to expose that especially the newly established traits of teat morphology were associated to this udder health related characteristic. We classified the scanned teats by the bacterial growth in the corresponding milk samples (n = 138; group 0: no growth of pathogens detectable, n = 100; group 1: growth of pathogens detectable, n = 37; contaminated samples: n = 1). Whereby, in general only minor pathogens were found during our bacteriological analyses. Samples were considered as contaminated when two or more colonies of two or more different bacteria (without distinct excess of one type of colony) were identified. Therefore one sample was excluded. The overall low bacterial growth may indicate that the determined bacteria originated rather from the teat canals mucosa than from a colonialization of the whole quarter. Measured values of the newly established internal teat traits of the distal teat canal at T0 and T1 in the respective groups are shown in table 2.6.

Table 2.6: Distal teat canals traits of teats with no bacterial growth (group 0) and bacterial growth (group 1) in the corresponding milk before (T0) and after (T1) milking (Trial 2)

		T0							T1						
		Group 0			Group 1			*P* - Value	Group 0			Group 1			*P* - Value
Trait	Unit	Mean	SD	SE	Mean	SD	SE		Mean	SD	SE	Mean	SD	SE	
Distal teat canal`s orifice (TOR)	mm	1.90	0.53	0.07	2.14	0.69	0.11	0.052	2.41	0.62	0.08	2.71	0.79	0.13	0.058
Distal teat canal`s perimeter (DTCp)	mm	15.8	4.13	0.52	18.1	4.04	0.67	0.019	20.3	4.07	0.52	22.2	3.94	0.66	0.003
Distal teat canal`s surface (DTCs)	mm^2	6.48	2.42	0.30	7.78	2.89	0.48	0.008	9.12	2.34	0.30	11.06	3.35	0.56	0.024

The sphincter has the function to close the teat canal`s external orifice between milking times to prevent infiltration of potential mastitis causing pathogens. Thus a widely opened orifice after milking represents a critical risk factor for udder health. In our study teats with bacterial growth in the appropriate milk tended show higher values describing the teat canals external orifice (TOR), even though statistical significance was narrowly missed in this case. Consequently not only texture of the teats tissue is affected by mechanical milk removal, also defense mechanisms of the teat canal can be compromised by factors of the milking technique. Greater values of perimeter and surface of the teat canal connote a high amount of mucosal tissue at the distal teat duct and by that represent a larger area for invading microorganisms to colonize. This may explain why we determined larger DTCp (T0: $P = 0.019$; T1: $P = 0.003$) and DTCs (T0: $P = 0.008$; T1: $P = 0.024$) in teats whose appropriate milk showed bacterial growth. The bacterial growth related differences detected in the newly established traits of teat morphology, even when not including cows with udder health issues point to the importance of the distal teat canal and its orifice for the animals` health. Integration of the three newly established traits in future ultrasound studies is even more intriguing since none of the common morphological traits known from the literature was demonstrated to be related to udder health in our experiments. Whereas Klein et al. (2005) found TCL and TCD in infected quarters (SCC > 100.000 cells/mL and detection of udder pathogens) to be higher ($P < 0.001$) compared to healthy quarters. Utilizing a translucent measuring ruler Guarín and Ruegg (2016) indicated an increased risk of clinical mastitis with enlarged premilking diameter of the teat apex. These mentioned traits did not show connections to udder health in the present study, but it should be noted that no inflamed or infected quarters (SCC > 100,000 cells/mL and detection of udder pathogens) were enrolled. In connection with the finding of parity

effect, results of an enlarged DTCp in multiparous cows after milking may be connected to an increased risk of mastitis with advancing lactation number (Breen et al., 2009). Investigating dairy cows` teat canals by swab technique and highlighting a connection of teat end callosity and microbial load of the appropriate teat canal, Paduch et al. (2012) emphasized the need of identifying further factors affecting the extent of microbial incidence in teat canals. During our study we could verify the dimensions of the distal teat canal (DTCp, DTCs) as being connected to the amount of bacterial growth in the respective milk sample. Therefore these traits can be classified as a useful measurement option and ultrasonographic investigation may be considered for complementing common examination methods in further studies, aiming on the evaluation the teat canal`s defense mechanisms and microbial load.

Despite all detected influencing factors, variance estimation showed that cow was the factor accounting for the largest proportion of variance in teat traits measured before (73% TCL; 71% TCW; 69% DTCp; 66% TWT1; 58% DTCs; 52% TEW, TW and TWT2; 51% TOR; not significant for TCD) and after milking (75% TCL; 74% DTCp; 66% TCW; 62% DTCs; 59% TOR; 55% TW; 54% TWT1; 49% TWT2; 45% TEW; not significant for TCD). These findings were in accordance with previous studies by Neijenhuis et al. (2001) and Zwertvaegher at al. (2012). Animal specific data (multiparous vs. primiparous, milk yield > or < 11.0 kg, peak flow rate > or < 4.0 kg/min and bacteriological findings in the corresponding milk: with or without bacterial growth) explained only marginal parts of variance during the multivariate statistics. Estimation of variance for the differences in the measured values in comparison of the time points (relative changes in teat traits from T0 to T1) showed that for only three out of ten traits variance was partly explainable by the individual cow. This may be engendered by the fact that measured values at T0 and T1 refer to the teats` individual

morphology, whereas relative changes in teat traits were caused by the external effect of the milking technique, which formed a relatively equal influencing variable for all experimental animals. Inner teat morphology differs among individual cow and quarter within cow. Whereby during our study teat position (left front versus right front) did account for negligible variance and was therefore left out of the model. However, it should be taken into account that the relative dimensions of teat tissue changes due to the milking procedure for rear teats might be divergent, and thus both front and rear teats should be included in future studies. Rear quarters commonly contain more milk than front quarters. Removing the milking cluster in our experiments was based on and milk flow threshold value at the udder level, but the mechanical stain in front quarters might have differed from the hind quarters. Furthermore, observation of external teat traits in future studies may provide easy determinable additional information and thus enable investigation of associations between internal and external teat traits as performed by Weiss et al. (2004) and Ambord and Bruckmaier (2010). The present study refers to a data base of high producing, healthy Holstein cows. Investigation and comparison of the distal teat canal`s morphology in other breeds might form a further interesting research approach.

Allowing for analyses of morphological changes, teat scanning has already been used for the assessment of different milking machine settings or liner types (Gleeson et al., 2005; Paulrud et al., 2005; Vetter at al., 2014; Besier and Bruckmaier, 2016). Application of the newly established measurement traits could also improve investigations in this case.

2.5 Conclusions

The vulnerability of the bovine teat canal to invading microorganisms cannot be measured directly by ultrasonography. However, changes in teat morphology after milking are assumed to reflect the teat canal penetrability. Therefore the measurement of traits estimating the relative changes in teat tissue via ultrasound represents an expedient method to assess the teat condition. High-resolution ultrasonographic teat scanning served as a practicable, noninvasive tool to study the morphological changes during milking in a repeatable way. It can therefore be applied for the evaluation of different milking techniques, liners and milking machine settings by analyzing the reaction of the teat. Examination of the newly established traits, referring to the delicate area of the teat canals distal orifice may result in an improved estimation of the individual teats ability of physical defense against invading microorganisms.

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Chapter 3

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Automated gradual reduction of milk yield before dry-off: Effects on udder health, involution and inner teat morphology

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3.1 Abstract

Lowering milk production of high yielding dairy cows before dry-off may be beneficial for both physical health and welfare of the cow. Extending the milking intervals as well as restricting energy intake are the commonly used approaches to decrease milk secretion, but are mostly accompanied by adverse side effects. Our objective was to elaborate a new technique for reducing milk production taking the natural process of weaning into account, i.e. the abatement of the calf`s milk removal when shifting its diet from milk towards solid feed. We tested a software module by which the degree of udder emptying can be gradually decreased through automated early removal of the milking clusters over 10.4 ± 1.8 days on 26 Holstein cows that were close to dry-off and free of mastitis (SCC < 100,000 cells/mL and negative bacteriology in quarter milk samples). Milk yield was compared to 30 conventionally milked control cows, which had the same initial milk yields and fulfilled the same udder health criteria. After the period of incomplete milking, milk production immediately before dry-off was 35.3 ± 12.9% less in the experimental cows compared to the control cows. As expected, the duration of milking decreased with time of incomplete milking. Neither SCC nor bacteriology findings were altered by leaving residual milk in the udder. Ultrasonographic investigation of pre- and post-milking teat characteristics showed that all traits of inner teat morphology except teat end width were less changed through the incomplete milking process when compared to the changes occurring in the conventionally milked control cows (differences in changes ranged from - 2.0% for teat width to - 27.9% for the distal teat canal`s surface). These findings suggest that the mechanical strain on the teat was considerably decreased with termination of the milking process before udders were completely emptied. After several days of incomplete milking, the perimeter and the surface of the distal teat canal of the

experimental teats were even smaller before attachment of the clusters when compared to the pre-milking values of the control teats, which might reflect a strengthening of the teat canals` functional defense mechanisms against the invasion of potential pathogens. Assessments in the following lactation showed that neither milk yield nor udder health were impeded by the targeted automated reduction of milk production before dry-off. The software module tested herein thus provides an effective tool for facilitating the dry-off process.

3.2 Introduction

Milk yield of dairy cows is significantly increased by genetic selection and improved management practices. One of the consequences is that cows maintain high yields until the end of lactation and are thus to be dried off when daily milk yields may still amount > 20 L (Stefanon et al., 2002). After applying high levels of udder emptying and constant milking frequencies during lactation, dry-off is commonly performed by abrupt cessation of milking. This approach is critical with regard to health and welfare. High yielding cows may experience discomfort and pain when being dried off abruptly due to increased intramammary pressure caused by udder tissue engorged with milk (O`Driscoll et al., 2011; Bertulat et al., 2013; Silanikove et al., 2013). This pressure can lead to leakage of milk after cessation of milking, opening the teat canal as a pathway for the entrance of potential pathogens and therefore impeding the health status of the udder (Rovai et al., 2007). Indeed, increased risks of developing IMI during the dry period (Dingwell et al., 2002) or at calving (Rajala-Schultz et al., 2005; Gott et al., 2016), and higher SCC (Gott et al., 2017) in the subsequent lactation were reported as potential consequences. Decreases in lying time were observed in high yielding cows as behavioral concomitants of abrupt dry-off (Chapinal et al., 2014; Rajala-Schultz et al.,

2018). From other studies lying time after dry-off was reported to be generally decreased with increasing milk yields, regardless of the dry-off technique (Zobel et al., 2013). Consequently, decreasing the milk production towards dry-off is desirable and various management tools for achieving this are applied in dairy farming. Restricting energy intake is known to effectively decrease milk production (Bushe and Oliver, 1987), but is also related to distress, likely due to hunger and abrupt ration changes (Valizaheh et al., 2008; Tucker et al., 2009). Limiting the supply of nutrients in late lactation and during dry-off is also considered as substantial metabolic challenge for cows in the third trimester resulting in greater NEFA and cortisol concentrations in the circulation (Odensten et al., 2007a,b). Milk secretion also decreases when milk removal is reduced or entirely stopped (Albaaj et al., 2018). Increasing the intervals between milkings, e.g. by skipping individual milking times to create milk stasis represents another method to reduce milk production (Stelwagen et al., 2008; Gott et al., 2016). This gradual cessation of milking was found to increase the risk of intramammary infections at calving for multiparous cows (Gott et al., 2016) and is furthermore discussed as being frustrating for the cows due to changes in the milking routine based on the observation of increased standing times at the exit gate of the pen (Stefanowska et al., 2000; Zobel et al., 2013). Until now there is no universal management technique available that is appropriate for lowering milk production without considerable side effects for animal health or welfare.

The aim of our study was to elaborate an alternative practice to prepare cows for the cessation of milking in a gradual and gentle way, without changing milking frequency or feed supply and to initially test this new method on cows with healthy udders. A step-down program for reducing the degree of udder emptying by triggering cluster removal at a pre-defined amount of milk removed via implementing a new module into

the milking software was tested. In consideration of the naturally diminishing milk production at weaning when the offspring`s need for milk declines, we hypothesized that (1) reducing milk removal and thus increasing residual milk in the udder will cause an adaptation of the cows` milk production, and that (2) this procedure will not impair udder health provided cows are healthy when entering the program. This procedure might (3) induce accelerated involution of the mammary gland already prior to cessation of milking. We also expected (4) the milking machine induced alterations of teat tissue to be less when compared to standard milking. The latter hypothesis was investigated by high-resolution ultrasound (as described in Martin et al., 2018). Our last hypothesis was, that (5) application of this software module prior to dry-off will not impede milk production or udder health in the subsequent lactation. The present study should serve as an initial attempt, testing the potential of software-induced incomplete milking for preparation for dry-off only on udders of healthy cows. Future research applying less strict enrollment criteria is appropriate before on farm applicability of the presented method.

3.3 Materials and Methods

3.3.1 Animals

All experimental procedures performed in the present study were approved by the relevant authority (Landesamt für Natur-, Umwelt- und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen (84-02.04.2016.A047) and were in strict accordance with the German Animal Protection Law. Fifty-nine Holstein dairy cows of the Frankenforst Research Station of the University of Bonn (Königswinter, Germany) were included in our study.

The herd comprised approximately 70 Holstein dairy cows that were housed in a two-row open free-stall barn with cubicles and concrete floor. A mixed ration was provided ad libitum with fresh feed delivered twice daily after each milking time. No alterations were made to the diet during the study period so all cows remained on the lactation diet until dry-off. Concentrate was additionally provided through separate feeding stations via transponder access according to the individual milk yields. In late lactation cows received only 0.2 kg concentrate per day. All cows had constant ad libitum access to clean water from automated water troughs.

Cows were milked twice daily at 05:30 a.m. and 16:30 p.m. in a double-four in-line milking parlor (GEA Farm Technologies GmbH, Bönen, Germany) with a system vacuum level set at 40 kPa, pulsation rate of 62 pulses/min and a pulsation ratio of 64:36. Udder cleaning with a wet paper towel and forestripping was followed by mechanical prestimulation (300 pulses/min during the first 30 s). Milk yield, milking duration and milk flow in the measuring unit at the moment of cluster removal were recorded automatically at the level of the udder for each cow during each milking by the Metatron C21 (GEA Farm Technologies GmbH) throughout the experiment.

During May 2015 and October 2017, those cows of the herd that were close to dry-off were examined for udder health by collecting twice aseptic quarter foremilk samples (d 205 and 212 of gestation). To be enrolled in the trial, each cow had to be free of clinical symptoms, have SCC < 100,000 cells/mL and a negative bacteriological result (based on the definitions provided by the German Veterinary Society (DVG, 2009)) in each quarter sample from both sampling times. Following these criteria, 56 cows with healthy udders were selected and randomly allocated to either the experimental group (**AUTODRY**, n = 26; average lactation number 1.9 ± 1.1) or the control group (**CON**, n = 30; average lactation number 2.4 ± 1.7).

3.3.2 Software Module and Experimental Design

Before cessation of milking, the AUTODRY cows were milked using the software module and underwent a step-down program of gradually reducing the level of udder emptying for 10.4 ± 1.8 days (average treatment period from d 219 – 228 of gestation). The software module AUTODRY was programmed by Schmidt et al. (2017; German patent DE 10 2017 120 656; International patent WO 2019/048521 A1) and integrated into the milking computer (Metatron C21; GEA Farm Technologies GmbH) on the experimental farm. The settings for selecting individual cows and the specification of targeted yield were made by means of the herd management software removal of the milking clusters at a defined targeted milk amount. The respective amount was calculated based on the cow`s individual milk yield before the trial (mean from d 212 - 218 of gestation) which was then reduced by 5% on a daily basis to achieve a successive lowering of the individual's milk yield. The amount of milk obtained on the last day of this period was defined as targeted end milk amount (kg/day). The last three milkings before cessation of milking in the AUTODRY cows (morning and evening milking on d 229 of gestation, morning milking on d 230 of gestation) were performed using the conventional cluster take-off level according to milk flow rate (0.3 kg/min) to remove the residual milk accumulated in the udder during the study period before dry-off, and to estimate the degree of adaptation to the AUTODRY milking. The achieved end milk amount (kg/day) was calculated as the sum of the last two milkings before dry-off, after the residual milk was removed during the first conventional milking. For the CON cows which were conventionally milked using the cluster take-off level according to milk flow rate (0.3 kg/min) until cessation of milking, the last daily milk amount before dry-off was considered as the achieved end milk amount. We calculated the individuals‘ achieved milk yield reduction as a total amount (kg) over the complete

period of the software application (Total achieved milk yield reduction (kg) = Difference between start milk amount (kg/day) and achieved end milk amount (kg/day)) and as a relative value of daily achieved milk yield reduction:

Daily achieved milk yield reduction (kg/day): $\frac{total\ achieved\ milk\ yield\ reduction\ (kg)}{duration\ of\ milk\ yield\ reduction\ (days)}$

All cows enrolled were examined for clinical symptoms at quarter level during each milking time. Timing of dry-off was based on breeding dates and a targeted dry period length of 50 days. On d 230 of gestation, milking was ceased and all cows received intramammary antimicrobial treatment (Orbenin Extra, Zoetis Deutschland GmbH, Berlin, Germany) and internal teat sealants (Orbeseal, Zoetis Deutschland GmbH) in each quarter after the final milking. Milk yield and udder health were further monitored for 90 days after calving.

3.3.3 Milk Sampling and Analyses

Foremilk samples from both groups were collected aseptically on the quarter level on d 205 and 212 of gestation, when cows from both CON and AUTODRY group were milked conventionally and on d 220, 224 and 228 of gestation when AUTODRY cows were milked with the software module while CON cows were still milked conventionally. Milk samples from both groups were also collected three times after calving on d 10, 18 and 26 of the following lactation. The sampling procedure was performed after the routine premilking treatment and included discarding of the first strippings, disinfection of the teat ends with 70% alcohol and sampling into sterile vials after teats were dried. The samples (n = 1,780) were transported at approximately 4 °C to the microbiological laboratory of the Institute of Animal Science (Bonn, Germany), for assessing bacteriology according to the guidelines of the German Veterinary Society about isolation and identification of mastitis pathogens (DVG, 2009). Using sterile

disposable calibrated loops, 10 µL of each sample were plated onto blood agar (Oxoid, Wesel, Germany). Plates were incubated at 37 °C and bacterial growth was evaluated at 24 and 48 h. Identification of isolates was conducted via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) by the Chemical and Veterinary Investigation Office (CVUA, Stuttgart, Germany) after pure culture cultivation of the pathogens. Somatic cells counts were assessed with the DCC Cell Counter (DeLaval GmbH, Glinde, Germany).

For measuring haptoglobin, additional quarter foremilk samples (n = 406; AUTODRY: n = 238; CON: n = 168) were collected on d 205, 212, 220, 224 and 228 of gestation (i.e., two times before and three times during the treatment period of differential milking). The concentration of this acute phase protein was assessed in skim milk via the in-house developed ELISA described by Hiss et al. (2004). The limit of detection was 0.2 µg/mL, mean intra assay CV was 9% and mean inter assay CV was 13%.

3.3.4 Ultrasonographic Scanning

On d 212 and 228 of gestation, the left and right front teats of all 26 AUTODRY cows and of 22 CON cows were examined by ultrasonography using a high-resolution scanner (MyLab Five Vet, 6-18 MHz; Esaote Biomedica GmbH, Cologne, Germany) as described by Martin et al. (2018). Each of the 96 teats was scanned directly before (**T0**) and after (**T1**) milking. The teat was immersed in a plastic cup filled with lukewarm tap water to avoid deformation of the sensitive tissue and the ultrasonographic probe was moved along the horizontal line of the teat on the plastic cup, which was covered with ultrasonic gel (Aquasonic 100, Parker Laboratories Inc., Fairfield, NJ). We evaluated the 384 resulting scans on a computer utilizing the MyLabDesk software (version 10.0, Esaote Biomedica GmbH). All measurements were done by the same person. Ten

different morphological characteristics of the lactating teat were measured in each image: Teat width (**TW**; in mm), teat end width (**TEW**; in mm), teat cistern width (**TCW**; in mm), diameters of the lower (**TWT1**; in mm) and upper (**TWT2**; in mm) teat wall, length of the teat canal (**TCL**; in mm), diameter of the teat canal (**TCD**; in mm), width of the teat canal's orifice (**TOR**; in mm), perimeter of the distal teat canal (**DTCp**; in mm), and surface of the distal teat canal (**DTCs**; in mm^2). For each trait the relative changes from T0 to T1 were calculated. More detailed information about the applied scanning procedure and the respective positions and boundaries of the measured traits are provided by Martin et al. (2018).

3.3.5 Statistical Analyses

All statistical analyses were done with the SPSS Statistics program (Version 25.0, SPSS Inc., Chicago, IL). The Shapiro-Wilk test was applied for testing normal distribution. Variance homogeneity was checked with the Levene's test ($P > 0.10$). Cow served as the experimental unit regarding milk yield before dry-off or after calving. For comparing the variables related to udder health (SCC, Hp milk concentration and data obtained by ultrasonographv), quarter was considered as the experimental unit. Taking variance homogeneity into account, we performed Student's t-tests for pairwise comparisons between the experimental and the control group. Since the respective data were not normal distributed, the Spearman's Rho coefficient of correlation was calculated to test for dependencies between variables. Linear mixed models were fitted for examining repeated measurements per cow or quarter. Cow or nesting of quarter within cow served as the subject. Sampling date (for analyses of milking characteristics: d 200-229 of gestation before dry-off or day in milk (DIM) 1-90 after calving; for analyses of udder health characteristics day of gestation 205, 212, 220, 224 and 228 before dry-off or DIM 10, 18 and 26 after calving), group (AUTODRY or CON),

interactions between sampling date and group as well as lactation number (1-7) were included in the models as fixed effects. Cow was set as random effect. At $P \leq 0.05$ results were considered as significant and the threshold for a trend was set at $P \leq 0.10$. Values are provided as means ± SD.

3.4 Results and Discussion

The software module worked well throughout the whole study period without any defaults or data losses and was easy to operate.

3.4.1 Milk yield reduction

Table 3.1 shows the milk yield data of the AUTODRY cows in comparison to the CON cows.

Table 3.1 Descriptive statistics of milk yield development in the experimental (n=26) and the control cows (n=30) during the observed period from d 212 of gestation until dry-off on d 230 of gestation

	Unit	AUTODRY Cows				CON Cows			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Lactation number		1.9	1.1	1	4	2.4	1.7	1	7
Duration of milk yield reduction	days	10.4	1.8	7	14	-	-	-	-
Start milk amount[1]	kg/day	20.6	3.8	13.8	27.9	20.6	3.2	14.9	25.8
Targeted end milk amount[2]	kg/day	10.9	3.0	3.9	17.2	-	-	-	-
Achieved end milk amount[3]	kg/day	13.8	4.5	7.4	21.7	19.9	3.7	13.8	27.1
Targeted milk yield reduction[4]	kg	10.4	2.3	5.3	15.6	-	-	-	-
Total achieved milk yield reduction[5]	kg	7.3	2.6	2.0	12.8	0.7	1.2	- 2.3	2.8
Daily achieved milk yield reduction[6]	kg/day	0.7	0.2	0.3	1.1	0.07	0.1	- 0.2	0.3

[1] seven days` mean from d 212 to 218 of gestation
[2] milk yield on the last day of milk yield reduction
[3] AUTODRY cows: physiological milk production after period of milk yield reduction, calculated on last milkings before drying off following conventional cluster take-off level (0.3 kg/min); CON cows: last milk yield before drying off
[4] difference between start milk amount and targeted end milk amount
[5] difference between start milk amount and achieved end milk amount
[6] AUTODRY cows: $\frac{total\ achieved\ milk\ yield\ reduction\ (kg)}{duration\ of\ milk\ yield\ reduction\ (days)}$, CON cows: natural milk yield reduction was observed over the last 10.4 ± 1.8 d before dry-off

Milk yield at the start of the trial was not different between the groups ($P = 0.88$) and thus the starting condition could be considered as equal. Expectedly, the amount of milk withdrawn during the differential milking was lower in the AUTODRY than in the CON cows ($P < 0.001$). The achieved end milk amounts were less; accordingly the milk yield reduction in the observed period of incomplete milking was remarkably higher in the AUTODRY cows ($P < 0.001$). Applying the software module thus enabled the experimental cows to achieve about 10-fold greater reductions than observed in the control cows not receiving a preparation for dry-off. The development of the daily milk amount comparing the experimental and the control cows is illustrated in Figure 3.1.

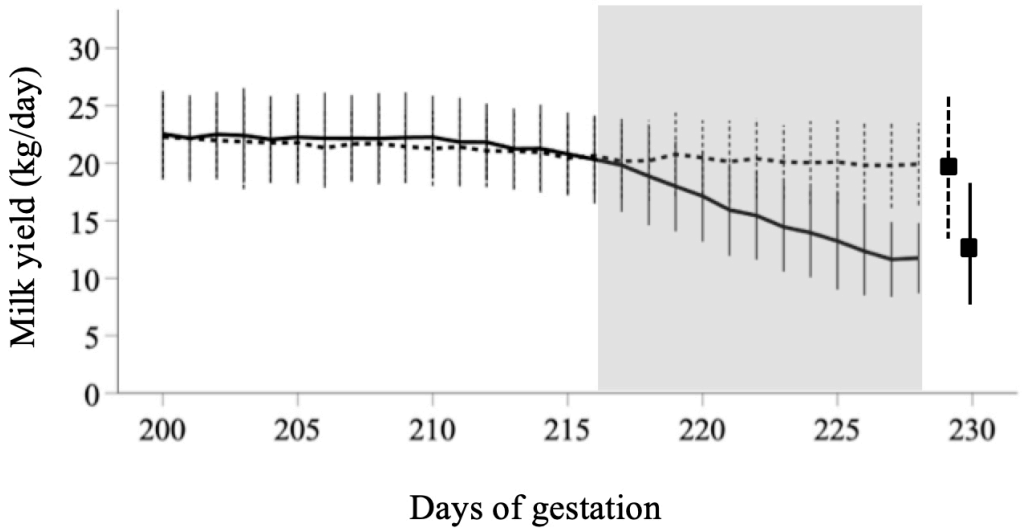

Figure 3.1 Time course of the daily milk yield (means ± SD) in the AUTODRY (solid line, n = 26) and the CON cows (dotted line, n = 30) from d 200 to 230 of gestation. The CON cows were milked following the conventional milking routine with automated removal of the cluster removal at a milk flow rate of 0.3 kg/min. The AUTODRY cows were milked to defined target amounts to reduce udder emptying using the software module for 10.4 ± 1.8 d. This time period is highlighted in grey. During the last three milkings before drying off the AUTODRY cows were returned to the conventional milking routine. The last daily milk yields before drying off were calculated as means from the third and the second conventional milking and are presented separately on d 230. All cows were dried off on d 230.

After the AUTODRY cows had undergone the incomplete milking achieved by the software module (over 10.4 ± 1.8 d), they were milked according to the conventional routine with automated cluster removal at 0.3 kg/min for three additional milking times before complete cessation of milking for dry-off. At the first milking of these additional milkings, more milk than during the preceding milking times was obtained (12.3 ± 3.6 kg; ranging from 4.8 to 18.7 kg). This corresponds to 56.3 ± 10.6% of the respective start milk amount before incomplete milking (range: 34.7% to 74.1%) and was also positively correlated to the start milk amount ($r = 0.88$ at $P < 0.01$). The increased yield obtained by conventional milking after the AUTODRY period likely reflects an accumulation of residual milk retained in the udder during incomplete milking. Albaaj et al. (2018) compared different levels of incomplete milking, achieved by limiting the amount withdrawn to 70, 40 or 0% of the expected yield, to unrestricted milking, i.e. 100% of the expected yield. Incomplete milking was applied only at one milking time in the quoted study; the yields of the following milking using unrestricted milking were the more increased the lesser the udder was emptied in the preceding milking (+ 4.5, + 8, and + 10.2 kg in 70, 40, and 0%, respectively, as compared to 100% removal of the expected yield). Besides the effects on residual milk, periods of milk stasis, be they induced by incomplete milking after manual cluster detachment (Penry et al., 2017; Albaaj et al., 2018) or by extended milking intervals (Davis et al., 1999; Stelwagen et al., 2013), were shown to have carry-over effects reducing milk synthesis. In the aforementioned study with 3 levels of reduced milking, Albaaj et al. (2018) reported reduced milk production in the 2nd and 3rd milking the one-time reduced milking, amounting to 1.3 kg to 12.8 kg less milk after milking 70 or 0% of expected yield, respectively, in early lactation (55 ± 9 DIM). Similarly, Penry et al. (2017) showed decreased milk production rates (- 0.24 kg/h per half udder) after incomplete

milking over several weeks. Stelwagen et al. (2013) expounded that once daily milking reduces milk yield by approximately 22%, whereby the extent of production losses varies substantially and ranges from 7% to 40% in different stages of lactation. The magnitude of performance depression depends on the amount of milk stored in the udder, influenced by the individuals` genetically determined production rate and the time interval as well as the repetition of periods of milk accumulation.

In relation to milked amount, milking frequency and milk stasis, a variety of factors influence the autocrine-paracrine mechanisms of regulating milk secretion. For example, serotonin produced in mammary epithelial cells acts as a homeostatic regulator of lactation (Collier et al., 2012) and accumulation of local signals such as Na+ and K+ concentrations during milk stasis change the integrity of tight junctions (Nguyen and Neville, 1998). Incomplete milking during AUTODRY treatment therefore might have triggered different local stimuli. In the following, we will speculate about the changes in prolactin concentrations as one example of altered factors. The milking process or suckling of the offspring induces the release of prolactin, which in turn stimulates the synthesis of milk (Lacasse et al., 2016). With the AUTODRY protocol used herein, the frequency of the milking stimulus is maintained but the duration of the milking process is decreased. Inhibiting prolactin secretion to decrease milk production before dry-off was demonstrated to hasten the involution process, consequently reducing udder health problems (Lacasse et al., 2016). The prolactin status in the current experiment is not known, but based on reports about the milking-induced prolactin surge in late lactation being decreased as compared to earlier stages of lactation (Bernier-Dodier et al., 2011), we speculate that the AUTODRY treatment might have provoked an accelerated decrease of prolactin secretion which in turn supports involution as it is known from inhibiting prolactin release (Ollier et al.,

2014). However, the milk yield reduction achieved by the AUTODRY software module in our study was effective in general (mean decrease of 35.3 ± 12.9%), but there was considerable inter-individual variation (see Table 3.1). The inhibitory effect on milk synthesis (calculated as reduced amount at the end of the reduced milking/starting milk amount) ranged from 8.5% to 56.7%.

3.4.2 Further milking characteristics

As expected, duration of milking decreased with the application of the software module (Figure 3.2).

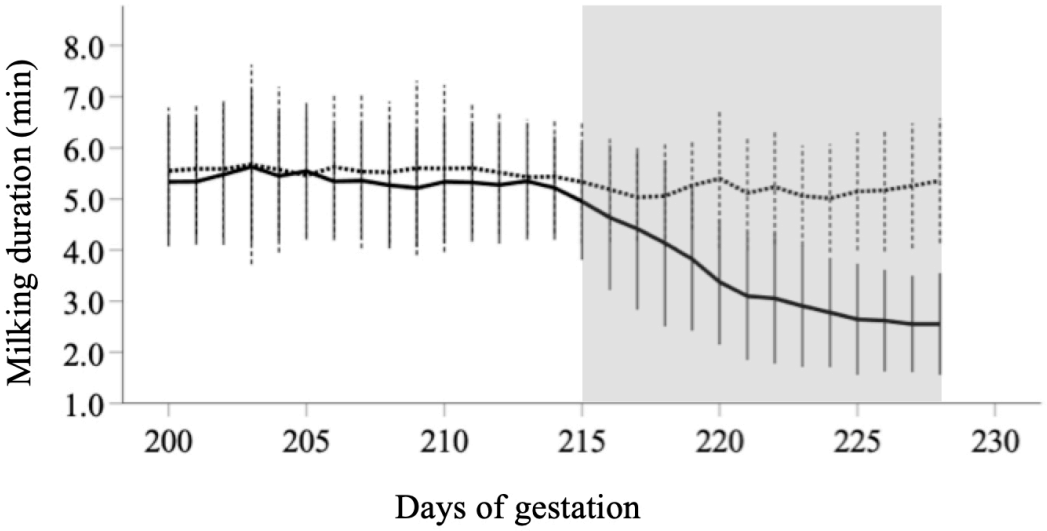

Figure 3.2 Time course of average milking duration (means ± SD) in the AUTODRY (solid line, n = 26) and the control (CON) cows (dotted line, n = 30) from d 200 to 228 of gestation. The CON cows were milked following the conventional milking routine with automated removal of the cluster removal at a milk flow rate of 0.3 kg/min. The AUTODRY cows were milked to defined target amounts to reduce udder emptying using the software module for 10.4 ± 1.8 d before dry-off. This time period is highlighted in grey.

Over the complete time period of incomplete milking, cluster on time was lower ($P < 0.01$) in the experimental cows than in the control cows. The average milking duration of the AUTODRY cows was 3.1 ± 1.3 min (ranging from 1.5 to 8.7 min) at morning milkings and 2.5 ± 1.0 min (ranging from 1.5 to 6.9 min) at afternoon milkings. In the CON cows the corresponding values were 5.2 ± 1.4 min (ranging from 1.9 to 9.2 min) at morning milkings and 4.8 ± 1.4 min (ranging from 1.5 to 12.6 min) at afternoon milkings, respectively. This effect of reduced milking duration might be

beneficial for optimizing time management for herds with individual cows whose required milking time exceeds the herd`s mean. The software module might thus be implemented in the organization of the overall milking routines for improving work efficiency and saving labor costs.

For determining milk flow rates in the measuring unit at the moment of cluster removal during the application of the software module, we analyzed the time course of milk flow during each milking and the respective last value (or the penultimate value if the last one was 0 kg/min) was included in the data set. Average milk flow over the complete time period of incomplete milking was 3.7 ± 1.2 kg/min (ranging from 0.4 to 6.5 kg/min) when the software module triggered cluster removal in the AUTODRY cows. In contrast, clusters were removed at a threshold of 0.3 kg/min in the CON cows. The high milk flow rate recorded at the time of the software-induced early cluster takeoff demonstrates incomplete milk removal in the experimental cows. The course of milk flow at cluster detachment during the experimental period in the AUTODRY group is visualized in figure 3.3.

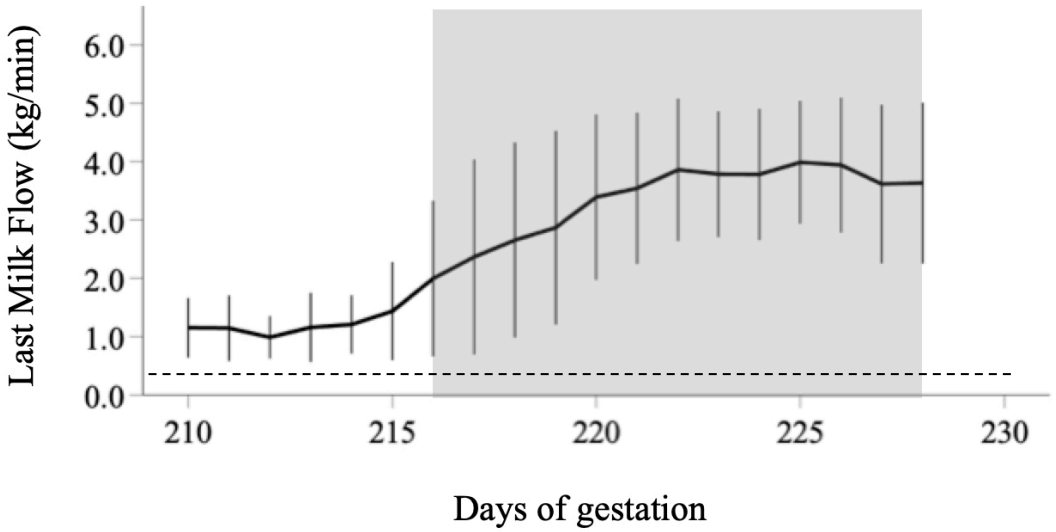

Figure 3.3 Time course of the last milk flow in the measuring unit recorded at the moment of cluster detachment (mean ± SD, in kg/min) in the AUTODRY cows (solid line, n = 26) from d 200 to 228 of gestation. The AUTODRY cows were milked to defined target amounts to reduce udder emptying using the software module for 10.4 ± 1.8 d before dry-off. This time period is highlighted in grey. CON cows were milked using the conventional cluster take-off level according to milk flow rate (0.3 kg/min), which is illustrated by the dotted line.

The software module allowed for an effective reduction of the level of udder emptying and values of last milk flow were considerably higher than cluster remover thresholds applied for practical or scientific purpose in the literature (Magliaro et al., 2005; Edwards et al., 2013; Krawczel et al., 2017). The reduction achieved with the AUTODRY procedure did not impede udder health, as presented in the following section.

3.4.3 Udder health

All experimental cows underwent the study period of reduced udder emptying without showing signs of impaired udder health. In none of the 104 AUTODRY quarters

involved IMI was detected and no cases of increased SCC or positive bacteriological findings were observed. Udder health in the CON group was comparable. No case of IMI was detected in the 120 quarters tested and elevated SCC were detected in 6 out of 360 milk samples (1.7%) during the trial. Figure 3.4 shows the time course of SCC in the quarter milk samples from the AUTODRY and from the CON group. During the time period of incomplete milking SCC was slightly higher ($P < 0.001$) in the CON quarters in comparison to the AUTODRY quarters, whereby both means were far below the threshold for mastitis (SCC < 100,000 cells/mL; according to DVG, 2009).

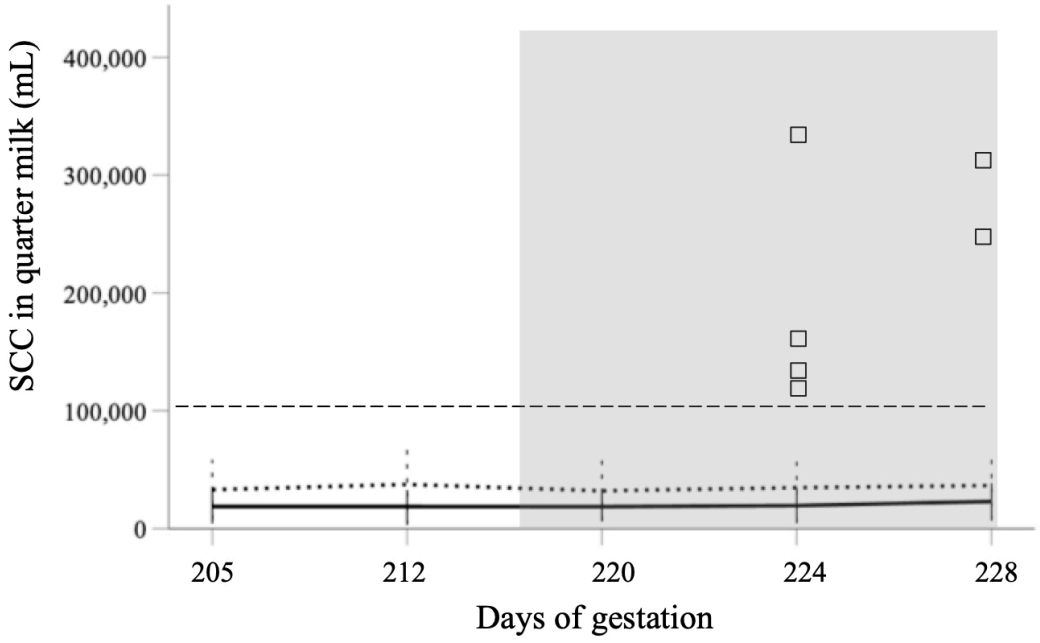

Figure 3.4 Time course of SCC (mean ± SD) in foremilk samples of AUTODRY quarters (solid line, n = 104), CON quarters (dotted line, n = 120) from d 205 to 228 of gestation. The CON cows were milked following the conventional milking routine with automated removal of the cluster removal at a milk flow rate of 0.3 kg/min. The AUTODRY cows were milked to defined target amounts to reduce udder emptying using the software module for 10.4 ± 1.8 d. This time period is highlighted in grey. CON quarters with elevated SCC (increase by < 50,000 cells/mL to < 100,000 cells/mL) are presented by squares (n = 6). The horizontal dashed line designates the SCC threshold for healthy quarters, i.e. < 100,000 cells/mL (DVG,2009)

The results from bacteriology are summarized in table 3.2. In 405 out of the 1,120 quarter milk samples tested, bacterial growth was observed. Positive culture results were limited to minor pathogens, mostly coagulase negative *Staphylococci* (252/405) and *Corynebacteria* (222/405). In general, the number of colony forming units (**cfu**) was low in both groups. In milk from AUTODRY cows less cfu were observed than in CON cows (2.3 versus 4.2 cfu/mL; $P < 0.001$). We speculate that these minor pathogens may originate from the teat canal mucosa rather than from the teat or the gland itself (Derakhshani et al., 2018). Collectively the results reflect the good udder health status of the enrolled cows. The results also support the notion that leaving residual milk in the udders did neither increase in SCC nor bacterial growth and thus did not impair udder health.

Residual milk in the udder was considered as a risk for udder health for long times since it may serve as substrate for pathogens. However, the results of our study are not in support of this concept. Similarly, other studies also did not find udder health to be impaired in consequence of incomplete udder emptying. Albaaj et al. (2018) found SCC to be increased after milking incompletely only once, but after five further conventional milkings, SCC had returned to basal levels. After milking cows incompletely over several weeks, Penry et al. (2017) reported SCC to increase only slightly (average SCC: control quarters: 26,300 cells/mL; experimental quarters: 48,300 cells/mL). Krug et al. (2018a) emphasized that milking incompletely by manual early cluster removal during the first five days of lactation had no effect on the incidence of clinical mastitis and even increased the odds for decreasing SCC from 8 to 11 DIM.

Table 3.2 Overview of bacteria cultured in milk samples of healthy quarters before dry-off

	Bacteria	Day of gestation	Quarter milk samples from experimental cows (n = 520)		Quarter milk samples from control cows (n = 600)		Total quarter milk samples (n = 1,120)	
			Affected samples (%)[1]	Cfu (Min-Max)[2]	Affected samples (%)[1]	Cfu (Min-Max)[2]	Affected samples (%)[1]	Cfu (Min-Max)[2]
Coagulase-negative Staphylococci	*S. chromogenes*	205	4	3-51	6	1-47	10	1-47
		212	7	1-31	3	10-40	10	1-40
		220	5	1-30	7	2-62	12	1-62
		224	3	15-21	7	1-16	10	1-21
		228	3	1-23	9	2-28	12	1-28
		Total	22	1-51	32	1-62	54	1-62
	S. haemolyticus	205	1	3	-	-	1	3
		212	5	1-3	1	4	6	1-4
		220	5	1-35	4	1-4	9	1-35
		224	4	1-8	3	6-15	7	1-15
		228	4	1-14	4	1-20	8	1-20
		Total	19	1-35	12	1-20	31	1-35
	S. equorum	205	3	1-15	4	1-2	7	1-15
		212	7	1-11	2	1	9	1-11
		220	3	1-4	1	4	4	1-4
		224	3	1-2	2	3-7	5	1-7
		228	4	1-3	1	2	5	1-3
		Total	20	1-15	10	1-7	30	1-15
	S. cohnii	205	6	1-24	6	1-7	12	1-24
		212	8	1-15	4	1-18	12	1-18
		220	10	1-20	8	1-11	18	1-20
		224	8	1-5	9	1-12	17	1-12
		228	14	1-10	9	1-10	23	1-10
		Total	46	1-24	36	1-18	82	1-24

		205	8	1-4	2	1-11	10	1-11
		212	8	1-6	2	1-4	10	1-6
	Other *coagulase negative staphylococci*[3]	220	4	1-12	6	1-5	10	1-12
		224	11	1-7	6	2-8	17	1-8
		228	6	1-3	4	1-20	10	1-20
		Total	37	1-12	18	1-20	55	1-20
	Total		144		108		252	
		205	3	1-5	16	1-18	19	1-18
		212	4	1-4	25	2-20	29	1-20
		220	5	1-9	31	1-20	36	1-20
	C. bovis	224	3	1-3	23	1-15	26	1-15
		228	4	1-2	21	1-21	25	1-21
		Total	19	1-9	116	1-21	135	1-21
		205	3	1-6	3	1-6	6	1-6
		212	3	1-3	3	1-7	6	1-7
		220	5	1-4	-	-	5	1-4
	C. confusum	224	1	1	1	10	2	1-10
		228	12	1-6	3	1-2	15	1-6
Corynebacteria		Total	12	1-6	10	1-10	22	1-10
		205	4	1-7	2	3-7	6	1-7
		212	1	2	4	1-5	5	1-5
		220	12	1-6	-	-	12	1-6
	C. frankenforstense	224	2	2-4	-	-	2	2-4
		228	15	1-8	-	-	16	1-8
		Total	34	1-8	6	1-7	40	1-8
		205	2	6	2	3-6	4	3-6
		212	2	1-2	5	1-5	7	1-5
	Other *Corynebacteria*[4]	220	4	1	7	1-3	11	1-3
		224	2	1	2	1-3	4	1-3
		228	5	1-9	1	3	6	1-9

		Total	11	1-9	14	1-6	25	1-9
	Total		76		146		222	
Aerococci	*Aerococcus viridans and Aerococcus suis*	205	10	1-5	1	1	11	1-5
		212	7	1-2	2	1	9	1-2
		220	9	1-10	4	1-8	13	1-10
		224	5	1-8	1	1	6	1-8
		228	8	1-6	2	1	10	1-6
		Total	39	1-10	10	1-8	49	1-10
Other[5]		205	3	2-6	-	-	3	2-6
		212	10	1-7	4	1-6	14	1-7
		220	2	1-2	3	2-3	5	1-3
		224	1	1	1	2	2	1-2
		228	9	1-7	1	2	10	1-7
		Total	28	1-7	9	1-6	37	1-7
Total affected samples[1]			170 (32.7)		235 (39.2)		405 (36.2)	
Negative culture			348 (66.9)		360 (60.0)		708 (63.2)	
Contaminated samples[6]			2 (0.4)		5 (0.8)		7 (0.6)	

[1]A quarter milk sample was defined as affected by the presence of 1 or more colonies of the same type
[2]Number of cfu identified in the individual cultured quarter milk samples per 10 µL
[3]Other *coagulase negative Staphylococci - Staphylococcus arlettae, Styphylococcus epidermidis, Staphylococcus squiuri, Staphylococcus warneri, Staphylococcus hominis, Staphylococcus succinus, Staphylococcus saprophyticus, Staphylococcus pasteuri, Staphylococcus auricularis, Staphylococcus gordonii, Styphylococcus xylosus*
[4]Other *Corynebacteria - Corynebacterium stationis, Corynebacterium camporealensis, Corynebacterium lactis, Corynebacterium aurimucosum, Corynebacterium phoceense*
[5]Other - *Acinetobacter (Acinetobacter iwoffii, Acinetobacter guillouiae), Micrococcus kocuria carniphila, Microbacteria (Microbacterium liquefaciens, Microbacterium foliorum, Microbacterium hydrocarbonoxydans, Microbacterium marytipicum), Tetragenococcus solitarius, Streptococcus lutetiensis, Stenotrophomonas rhizophila, Pseudomonas libanensis, Bacillus megaterium, Bacillus licheniformis, Pantotea agglomerans*
[6]Samples were defined as contaminated when 2 or more colonies of 2 or more different bacteria (without distinct excess of one type of colony) were identified

3.4.4 Haptoglobin

Haptoglobin (**Hp**) is one of the major acute phase proteins in cattle (Eckersall and Conner, 1988; Ceciliani et al., 2012). The liver is the main source of Hp but extrahepatic expression was also demonstrated, amongst other tissues also in mammary epithelia (Hiss et al., 2004; Thielen et al., 2007). Increased concentrations in blood and milk were demonstrated during clinical but also subclinical mastitis (Eckersall et al., 2001; Grönlund et al., 2005, Eckersall et al., 2006, Hiss et al., 2007). As expected, the Hp concentrations in milk from the enrolled cows were relatively low and remained well below the threshold of 2.2 µg/mL suggested by Hiss et al. (2007) for differentiating healthy from (subclinically) mastitic quarters (AUTODRY 0.40 ± 0.47 µg/mL, CON 0.43 ± 0.47 µg/mL; at P= 0.58; Figure 3.5).

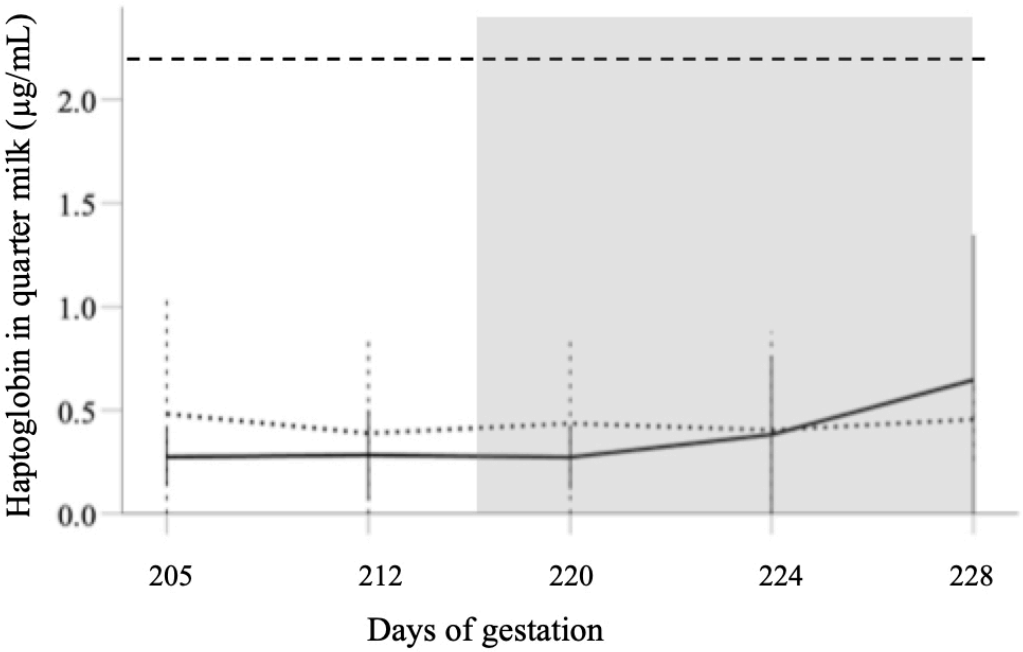

Figure 3.5 Time course of the haptoglobin concentrations (means ± SD) in foremilk samples of AUTODRY quarters (solid line, n = 104), CON (dotted line, n = 120) from d 205 to 228 of gestation. Quarters were defined as not healthy if SCC > 100,000 cells/mL or positive bacteriological findings, or both. CON cows were milked following the conventional milking routine with automated cluster removal at 0.3 kg/min. In the AUTODRY cows, milking was limited to targeted amounts for reducing udder emptying for 10.4 ± 1.8 d before dry-off. This time period is highlighted in grey. The horizontal dashed line at 2.2 µg/mL designates the threshold value for differentiating healthy from infected quarters as defined previously (Hiss et al., 2007).

The coefficient of correlation (Spearman Rho) between SCC and Hp concentrations over all samples collected during conventional milkings was r = 0.66 ($P < 0.01$). Similar relationships were reported in the literature (Simões et al., 2018: r = 0.68 at $P < 0.001$; Hiss et al., 2007: r = 0.8 at $P < 0.01$). When assessing the correlation at the individual sampling times in both groups, the values were quite constant (r = 0.51 - 0.72; $P < 0.01$) over time. The only exception was the last sampling in the AUTODRY cows when SCC and Hp were no longer correlated (r = 0.09, P= 0.53), due to an increase of Hp while

SCC remained constant. Involution of the mammary gland has been associated with increased mRNA expression of acute phase proteins in mice (Stein et al., 2004: Serum Amyloid A3 (SAA3); Nazemi et al., 2014: SAA3, alpha-1-acid glycoprotein (AGP) and Hp), but also in cows (Molenaar et al., 2009: SAA3). For Hp mRNA, Piantoni et al. (2010) did not observe an increase with involution. At the level of the protein, lactoferrin is long known to increase in milk during involution (Welty et al., 1976; Lanctôt et al., 2017), and for Hp increasing concentrations were observed in milk using proteomics (Boggs et al., 2015). Eight days after dry-off, 4-fold greater Hp concentrations than before were reported in the latter study. In view of these earlier observations about increased expression of acute phase proteins during involution, we interpret the increase of Hp in the AUTODRY cows as indicative for initiated involution rather than an impairment of udder health. In support of this notion, Silanikove at al. (2013) reported that the inflammatory response after cessation of milking was greater in cows approaching natural involution (milk yield before dry-off < 14 L/day) than in high yielding cows (milk yield before dry-off: 25 - 35 L/day) approaching forced involution. Considering that the accumulation of milk resulting from incomplete milking implies a dilution of Hp, but also SCC and bacterial counts, the observed effects might be underestimated. Taken together, limiting milk synthesis by incomplete milking at the end of lactation is likely to stimulate involution of the mammary gland that typically occurs at the end of the lactation cycle.

3.4.5 Ultrasonographic scanning of the teats

Teat morphology was assessed by ultrasonography at two scanning dates (d 212 and 228 of gestation, i.e., before and at the end of differential milking), each with scans done before (**T0**) and after milking (**T1**). The 10 traits measured are described in detail

by Martin et al. (2018). From the targeted 3,840 measurements (4 assessments per teat, 2 teats per cow, 48 cows (26 AUTODRY and 22 CON) and 10 variables), 129 could not be used due to compromised image quality. On the first scanning date (d 212; when both groups were milked following the conventional cluster removal settings) the teat traits at T0 and T1 were not different between the AUTODRY and the CON teats ($P > 0.1$). For the CON teats also no significant ($P > 0.1$) deviations were observed when comparing the two scanning dates. Overall changes of teat morphology due to the standard milking pattern (including all measurements on the first scanning date, of both AUTODRY and CON teats, and the measurements of the CON teats on the second scanning date, n = 2,800) were similar to those described previously in the literature (Neijenhuis et al., 2001; Martin et al., 2018) and are presented in comparison to the values obtained during the reduced level of udder emptying (AUTODRY teats on the second scanning date) in table 3.3.

Table 3.3 Influence of conventional and incomplete milking on traits of inner teat morphology

		Conventional milking pattern[1]			Reduced level of udder emptying[2]			
Trait		Mean	SD	SE	Mean	SD	SE	P – value[3]
Teat width (TW, in mm)	T0	26.1	1.51	0.16	26.5	1.76	0.25	0.99
	T1	25.0	1.73	0.18	25.8	1.85	0.26	**0.018**
	%[4]		- 4.48, P<0.001			- 2.46, P<0.001		**0.001**
Teat end width (TEW, in mm)	T0	21.9	1.82	0.19	21.8	1.55	0.22	0.21
	T1	22.2	1.91	0.20	22.2	1.52	0.21	0.97
	%[4]		+ 1.12, P=0.12			+ 1.57, P=0.07		0.35
Teat cistern width (TCW, in mm)	T0	12.9	2.92	0.30	13.4	3.09	0.43	0.40
	T1	7.33	2.98	0.31	11.2	3.47	0.49	**< 0.001**
	%[4]		- 43.4; P<0.001			- 16.3, P<0.001		**< 0.001**
Teat wall thickness lower teat wall (TWT1, in mm)	T0	6.68	1.62	0.17	6.58	1.43	0.20	0.43
	T1	8.86	1.46	0.15	7.24	1.43	0.20	**< 0.001**
	%[4]		+ 32.6, P<0.001			+ 9.97, P=0.001		**< 0.001**
Teat wall thickness upper teat wall (TWT2, in mm)	T0	6.54	1.55	0.16	6.22	1.42	0.20	0.09
	T1	8.75	1.58	0.16	7.02	1.81	0.25	**< 0.001**
	%[4]		+ 33.8, P<0.001			+ 12.9, P<0.001		**< 0.001**
Teat canal length (TCL, in mm)	T0	10.7	2.10	0.22	10.1	1.83	0.26	**0.04**
	T1	12.8	1.69	0.17	10.9	1.65	0.23	**< 0.001**
	%[4]		+ 19.2, P<0.001			+ 7.88, P<0.001		**< 0.001**
Teat canal diameter (TCD, in mm)	T0	0.99	0.27	0.03	0.94	0.30	0.04	0.24
	T1	1.25	0.26	0.03	1.01	0.30	0.04	**< 0.001**
	%[4]		+ 26.4, P<0.001			+ 7.91, P=0.004		**< 0.001**
Distal teat canal`s orifice (TOR, in mm)	T0	1.99	0.71	0.08	1.78	0.67	0.09	0.09
	T1	2.57	0.70	0.07	1.95	0.65	0.09	**< 0.001**
	%[4]		+ 29.3, P<0.001			+ 9.44, P=0.019		**< 0.001**
Distal teat canal`s perimeter (DTCp, in mm)	T0	16.8	4.51	0.48	14.73	3.73	0.53	**0.005**
	T1	20.9	3.84	0.41	16.25	3.26	0.46	**< 0.001**
	%[4]		+ 24.2, P<0.001			+ 10.3, P=0.03		**< 0.001**
Distal teat canal`s surface (DTCs, in mm^2)	T0	6.81	2.72	0.29	5.62	2.01	0.29	**0.003**
	T1	9.60	2.91	0.31	6.34	2.29	0.32	**< 0.001**
	%[4]		+ 40.7, P<0.001			+ 12.8, P=0.049		**< 0.001**

[1] Including all scans of the control teats and the scans of the experimental teats during the first scanning date (n = 280 scans and 2,800 measurements)
[2] Including the scans of the experimental teats during the second scanning date (n = 104 scans and 1,040 measurements)
[3] P-Value is given for the comparison of the respective value obtained during the conventional or the incomplete milking procedure
[4] Percentage change and significance of trait from T0 to T1

Percentage values are given as the mean difference in the relative changes from T0 to T1 when comparing the morphological changes due to the standard milking pattern with the AUTODRY milkings. Except for teat end width (**TEW**), all measured teat traits showed remarkably lower relative changes in their morphology due to the milking procedure (T0 vs. T1) during use of the software module, thus indicating that AUTODRY milking was more gentle.

Milk removal reduces the volume of the milk filled teat cistern. The amount of milk extracted from the teat was related to the extent of cistern shrinking ($r = 0.33$; $P < 0.05$). At the end of the step down program (milk yield AUTODRY cows: day of gestation 212: 22.2 ± 3.4 kg/day; day of gestation 228: 10.9 ± 3.0 kg/day), the observed decrease of the cisterns` diameter was 27.1% less compared to the values obtained under conventional milking. Accordingly, the teat cistern variables at T1 during incomplete milking were higher ($P < 0.001$) than obtained under the standard milking pattern, which indicates that during AUTODRY milking, the milk left in the udder was at least partially located in the teat cistern. Albaaj et al. (2018) measured the cisternal area of incompletely milked teats via ultrasonography and also found higher values after the partial udder emptying in comparison to the completely emptied teats.

Swelling of teat walls can be explained by a vacuum-induced accumulation of blood and lymph in the tissue (Hamann et al., 1993). Applying the AUTODRY software module halved the time of milking from 4.6 ± 1.9 min to 2.2 ± 1.1 min (evening milking d 212 and d 228 of gestation, respectively) and thus likely lessened the intensity of strain on the tissue. This might explain for the lesser swelling of the teat walls (- 22.6% for teat wall thickness of the lower teat wall (**TWT1**) and - 20.9% for teat wall thickness of the upper teat wall (**TWT2**)), the reduced extension of the teat canal length (**TCL**: - 11.3%) and diameter (**TCD**: - 18.5%) and the lower effects on the traits of the

distal teat canal (orifice of the distal teat canal (**TOR**): - 19.7%, perimeter of the distal teat canal (**DTCp**): - 13.9%, surface of the distal teat canal (**DTCs**): - 27.9%) when comparing the AUTODRY to all conventional milkings. At the end of the software-induced decrease of milk yield, the decrement of the distal teat canal`s perimeter and surface were even found to be smaller before attachment of the clusters (T0). In previous experiments we observed that increased DTCp and DTCs were associated with bacterial growth in the corresponding milk sample (Martin et al., 2018). These traits of distal teat morphology are thus likely related to the health status of the teat. The reduction of the perimeter and surface of the distal teat canal in incompletely milked cows might be explained by a smaller area of mucosal tissue in this particular area, making it more difficult for potential pathogens to colonize. Furthermore, the reduced diameter of the distal teat canals orifice (TOR) after milking under the circumstances of incomplete milking also points to a narrowed entrance for pathogens. As stated before, the application of the software module did not affect the bacterial growth in the milk samples of the healthy teats. However, based on the results from ultrasonography, we conclude that the software application may decrease the impairment of the teats` defense mechanisms caused by mechanical milk removal. Preparing cows for the dry period with this technique might not only reduce their milk amount effectively but also have a positive effect on the extent of mechanical strain on the delicate teat tissue and thus on teat health status.

3.4.6 Milk yield and udder health in the subsequent lactation

Milk yield and udder health of all cows enrolled were investigated during the first 90 d of the subsequent lactation. One cow of the AUTODRY group did not enter the next lactation for reasons not related to the trial. Milking characteristics were recorded during each milking and cows and foremilk were visually checked for clinical signs of

mastitis twice daily. Sterile quarter milk samples were collected on DIM 10, 18 and 26. All 660 quarter milk samples were investigated for SCC and bacteriological analyses were conducted. We observed elevated SCC in three quarters of one CON cow and in two quarters of one AUTODRY cow, whereas udder pathogens were not detected in any of these samples. The respective AUTODRY cow was injured at both front teats what was likely driving the increase of SCC rather than the experimental set-up. The average SCC (excluding the two aforementioned cows) on the days of sampling were 20,147 ± 16,533, 14,982 ± 15,827 and 13,474 ± 16,365 cells/mL in the AUTODRY cows and 39,456 ± 37,116, 20,932 ± 36,808 and 11,787 ± 12,226 cells/mL in the CON cows, respectively. The time course of daily milk yield during the first 90 DIM is shown for both groups in figure 3.6.

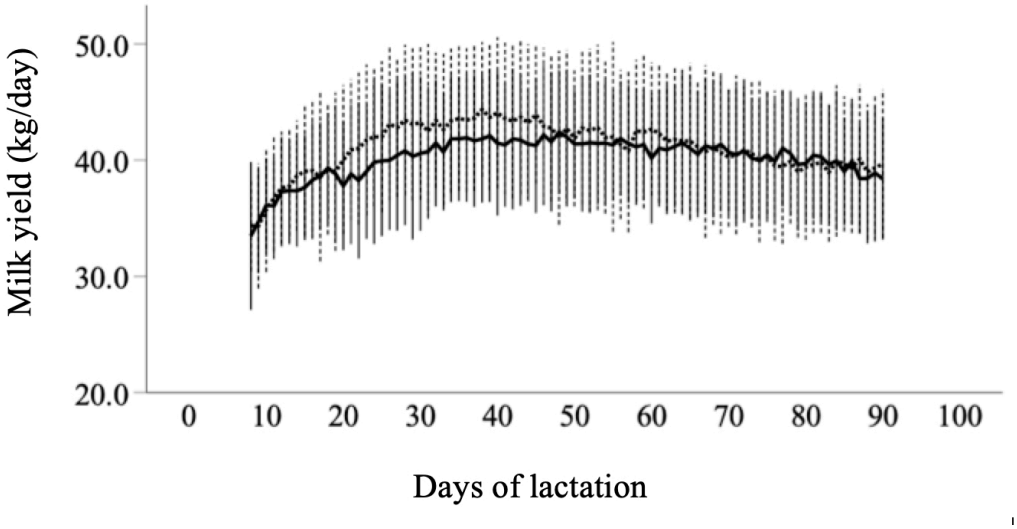

Figure 3.6 Time course of daily milk yields (mean ± SD) from cows dried off by conventional (n = 30, dotted line) or AUTODRY milking (solid line, n = 26) during the first 90 d of their subsequent lactation. All cows included had no signs of mastitis before dry-off

Milk yield did not differ between the experimental and the control cows ($P = 0.33$). As expected, lactation number had an effect ($P = 0.05$) on the amount of milk produced in the early lactation phase. Studies in which cows were prepared for the dry period by a software-induced successive decrease of udder emptying are not available, but gradual cessation of milking (milking once daily for the final week of lactation) was also found to have no sustained effects on milk yield and SCC in the subsequent lactation (Ferris et al., 2008; Gott et al., 2017). Stelwagen et al. (2013) stated that negative carry over effects of once daily milking only occur during the respective lactation. The dry period allows for recovery of mammary tissue and thus likely prevents impairments of productivity to be taken along into the next lactation. We thus conclude that the period of milk stasis, induced by incomplete udder emptying by means of the software module tested herein or by extended milking intervals, neither affected milk yield in the subsequent lactation nor did it compromise udder health after calving.

3.4.7 Further applications of the software

Milking technologies available on the market only allow for automated cluster removal based on flow rate or cluster-on time. The new software module AUTODRY made it possible to stop milking when a targeted amount of milk was reached, independent on the remaining residual milk in the udder. This invention simplifies studies on incomplete milking or residual milk in dairy udders and may also be applicable for other utilizations beside research. For example, the newly invented software module might serve as a tool to better manage the cow`s metabolism during early lactation by limiting milk yield during the first days in milk, as investigated by Carbonneau at al. (2012), and thus allows for reducing the potential health risk of metabolic stress (Krug et al., 2018a,b; Morin et al., 2018).

Despite the effective and non-hazardous applicability of the software-induced milk yield reduction for cows with healthy udders demonstrated herein, no recommendations can be provided at present for cows with compromised udder health. The cows tested herein met strict criteria of selection before being allocated to the AUTODRY milking (SCC < 10,000 cells/mL and no udder pathogens in all quarters of two consecutive samplings). Further research is needed to investigate the effects of incomplete milking on udder quarters that are not entirely healthy. For on-farm use of the software before cessation of milking, the decision about the suitability of individual cows for incomplete milking should be combined with a selective dry cow treatment. The period of successively reduced level of udder emptying before dry- off forms a basis for an uncomplicated and animal friendly antibiotic-free dry-off.

3.5 Conclusions

Lowering milk yield of high producing dairy cows before dry-off reduces the cows' vulnerability to potential health and welfare hazards during and after dry-off. The common approaches for reducing milk production (restricted feed intake and increased milking intervals) before final cessation of milking are often accompanied by adverse side effects. An alternative approach for an automated gradual reduction of the amount of milk removed via a software module was presented herein. Application of this software on cows with healthy udders yielded an effective reduction of milk production without negative side effects for udder health. However, implications on cows that do not meet the strict criteria of udder health might differ. Increasing the portion of residual milk in the udder seemed to initiate the acute phase reaction that naturally occurs during involution of the mammary tissue already before cessation of milking. Ultrasonographic investigations showed that inner teat morphology was less affected after the incomplete

milking process as compared to conventional milking which stresses the natural defense mechanisms of the teat canal. Furthermore, milk yield and udder health in the subsequent lactation were not affected by the automated reduction of milk production before dry-off. This gentle preparation for dry-off may contribute to improving welfare and health of dairy animals.

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Chapter 4

General discussion and conclusion

4.1 Evaluation of inner teat morphology using high-resolution ultrasound – limitations and perspectives of the applied method

For visualizing the small morphological dimensions of the bovine teat canal, ultrasonography has been proven to be a useful tool (Franz et al., 2009). Due to the noninvasive nature of this technique it can be applied easily during the milking routine and therefore exerts no stress for the animals. Using an ultrasonographic probe of higher resolution than ever used before (18 MHz), we were able to visualize the structures of the teat in more detail and to establish additional traits of the distal test canal. The morphological traits in the resulting images were assessed with software of high accuracy and yielded to a good repeatability of the method.

The ultrasound technique was used to asses the morphological changes of the teat morphology caused by different milking machine settings. The application of ultrasonography is an effective method for evaluating different milking machine settings or components concerning their influence on the teat tissue: Gleeson et al. (2005) evaluated the effects of two milking systems, differing in cluster weight (3.2 kg vs. 1.65 kg), claw volume (150 mL vs. 275 mL), diameter of the liner (wide vs. narrow) and pulsation pattern (simultaneous vs. alternate), on milking characteristics, teat tissue changes and new infection rate. The authors found that the external teat diameter tended to be higher ($P = 0.08$) when milking took place with the heavier cluster weight milking system. Paulrud et al. (2005) used 7.5 MHz ultrasonography and thermography to indirectly monitor the influence of liner type and overmilking on teat tissue recovery after milking. Overmilking tended to increase the diameter of the teat walls and milking with a soft liner accelerated recovery of teat canal elongation after milking when compared to recovery time after milking with a standard liner under extra high tension. In a study by Vetter et al. (2014) the effects of a periodic reduction of the vacuum

during the massage phase from 43 kPa to 12-15 kPa on inner teat tissue conditions were monitored using a 5 MHz ultrasonographic probe. Teat wall thickness five min after milking with the reduced vacuum was found to be smaller ($P = 0.05$) when compared to the postmilking dimensions of the conventionally milked teats. Another study that utilized ultrasound to investigate teat tissue changes due to milking was conducted by Besier and Bruckmaier (2016). Using a 5 to 10 MHz probe, the authors found teat wall thickness to be increased and teat cistern diameter to be decreased after milking with a system vacuum of 50 kPa and a minimum claw vacuum of 34 kPa in comparison to milking with a system vacuum of 42 kPa and either a minimum claw vacuum during milk flow of 33 kPa or claw vacuum drop during milk flow down to 24 kPa. The assessment of different milking machine settings, liner or cluster types by the use of ultrasonography was complemented by utilization of high-resolution ultrasonography as conducted in chapter 2. This opens the possibility to measure additional traits of the distal teat canal and to evaluate how the respective milking treatment affects the anatomical defense mechanisms of the bovine teat canal.

As already mentioned in the study by Paulrud et al. (2005), there is endeavor to scientifically understand how the dimensions of the bovine teat recover after the mechanical milk removal. Through application of vacuum, the teat canal opens to led milk flow out. After the milk removal, an expeditious closure of the teat canal is essential for the defense against the entry of potentially harmful pathogens. Changes in the pliability of the tissue as caused by edema or congestion can impede this resistance function of the teat canal (O`Shea, 1987). Neijenhuis et al. (2001) observed the recovery of cow teats by ultrasonographic scanning with a 7.5 MHz probe up to 8 h after milking. Scans of all teats, immersed in a water filled latex bag, of 18 cows were conducted

every hour. Milking took place using conventional milk machine settings and clusters were removed automatically when milk flow decreased below 0.2 kg/min. Generally, the authors state that recovery of cow teats took longer than expected, particularly teat end width and teat canal length did not reach their initial pre milking values within the observed time period. However, it should be taken into account that the pre milking value does not represent the "resting state" of the teat between milking. Scanning immediately before milking took place after teat preparation to ensure a sufficiently filled teat cistern and good preconditions for ultrasonographic imaging. Paulrud et al. (2005) defined that approxiamtely 80% of variability in teat canal length from before teat preparation to after milking can be explained by changes during teat preparation. Therefore, interpretations of teat tissue recovery after milking should not assume the premilking morphology to represent an uninfluenced situation of proper defense possibilities. Since the teat canal contains muscular elements, it is expected to undergo constant dynamic modulations, which are induced by both internal (e.g. hormone release) and external factors (e.g. temperature changes, tactile stimuli). Thus defining a "resting state" with unaffected defense mechanisms might be hardly possible.

Studying cows that were milked three times daily, Melvin et al. (2019) also suggested, that the teat canal and its surrounding tissue are in a state of near-constant remodeling. Further research of teat scanning over a minimum time interval from one milking to the following is needed to understand the changes and deformations of the teat tissue between milking times. The integration of the newly established measurement traits could support this research approach, especially regarding analyses of the distal teat canals` defense mechanisms. Changes in TOR, DTCp and DTCs over an enlarged time period and the definition of a "resting state" of the teat canal in which it is neither recovering from the pervious milking nor influenced by preparations for the subsequent

milking might be interesting to define optimal milking interval lengths that enable defense mechansisms of the teat canal to function properly.

Consistent with the differing external dimensions of front and hind teats, Neijenhuis et al. (2001) found the teat cistern width to vary dependent on teat position. Interestingly the authors also found that recovery of the cisternal dimension took considerably more time in front teats than in hind teats (> 8 h vs. 3 h). These findings suggest that reactions of teat tissue to the milking process and their modulation after milk extraction might vary considerably between different teat positions. The front quarters usually contain less milk than the hind quarters (Weiss et al., 2004), thus if no quarter individual detachment is installed, vacuum might still be applied even though front quarters are already emptied. We found no effects on teat position (left front vs. right front), but rear teats were not included. This lead to the suggestion that during future ultrasonographic studies utilizing the high-resolution probe the consideration of hind teats could be interesting. Measurement of the newly established traits of inner teat morphology in hind teats and comparison of the respective findings to those generated in front teats might give new insights into the morphology and defense mechanisms of cow teats and lead to conclusions concerning quarter individual cluster detachment. Possibly, relative changes of teat morphology that were observed in front teats are transferable to hind teats, even though absolute dimensions differ depending on teat position. In this context it should be noted, that the practical implementation of teat scanning was experienced to be easier conductible on front teats compared to hind teats, due to the partial location of the udder behind the cow`s hind leg, when approached from the side as carried out during our experiments. Depending on the construction of the milking parlor, the feasibility of investigating different teat positions might differ.

During our studies, only udder healthy cows were included. Evaluation of inner teat morphology of clinically or subclinically infected cows by means of the established method surely would enable interesting inferences regarding the importance of the defense mechanisms of the teat canal. This approach is supported by the finding of enlarged DTCp and DTCs in teats whose appropriate milk samples showed bacterial growth. It might also be conceivable to visualize inner teat morphology via high-resolution ultrasound after challenging the teat with infectious pathogens to study the morphological reactions and for drawing conclusions on the defense mechanisms.

In conclusion, the first three objectives of the thesis were successfully reached in chapter 2. The inner teat morphology could be visualized and analyzed in a more detailed way by application of high-resolution ultrasonography. This enabled the establishment of new measurement traits of the distal teat canal, which were proven to be related to parameters of udder health. Changes in the overall inner morphology could be evaluated and coherences to other variables of the individual cow were pointed out. After our experiments were performed, Wieland et al. (2018) came up with a similar research approach of measuring the diameter of the teat canal at different positions, which emphasizes the relevance and currency of our objectives. The authors aimed to develop a standard operating procedure for ultrasound-based measurements of teat canal dimensions in dairy cows by conducting 64 scans of the right hind and left front teats from 16 cows before and after milking. In the obtained images the following teat dimensions were measured: teat canal length, teat canal diameters (at the proximal end of the teat canal, at the midpoint between the proximal and distal ends of the teat canal, and at the distal end of the teat canal), teat end diameter at the midpoint between the proximal and distal ends of the teat canal, and the teat canal cross-sectional area. Implementation of the developed standard operating procedure when evaluating the

scans by three operators increased both the interoperator reproducibility and the intraoperator repeatability. The authors concluded, that measurements of teat canal dimensions can be conducted with satisfactory precision when strict and consistent guidelines are followed during the measurements of the teat dimensions. These findings support the methodical approach that was applied during our experiments: all images were visually evaluated by the same person, measurements were conducted following a standardized procedure, and only images of good quality were included. The high precision of the ultrasound-based measurements performed in this study is clearly reflected by the good repeatability that is presented in chapter 2.

4.2 Software induced milk yield reduction – limitations and improvement suggestions of the conducted experiment

Surprisingly, the mean adaptation of milk production in our experiment was even higher than average numbers given for production depression during once daily milking (Stelwagen et al., 2013). Despite the long exiting common believe that residual milk is harmful for udder health, incomplete milking of healthy quarters induced by the software module was possible without impeding the health situation. These findings are in accordance to previous investigations on the effects of incomplete milking on udder health (Penry et al., 2017; Albaaj et al., 2018; Krug et al., 2018a). As already mentioned, the limitation of enrolling only quarters that meet the strict definition criteria for udder health (SCC < 100,000 cells/mL and negative bacteriological finding) is emphasized. Incomplete milking and thus leaving residual milk in the udder was possible without impeding the health status of quarters that were defined as healthy before the start of the program of successive milk yield reduction. Nevertheless, it has to be acknowledged that beside all the positive outcomes of the study, no conclusions

can be drawn on the applicability of the software module on cows whose quarters do not meet the health prerequisites. Effects of incomplete milking of not udder healthy cows (for example with subclinically infected quarters or elevated SCC) have not yet been studied adequately in the literature (Cording et al., 2013). A study by Napper and Williamson (1983) indicated that residual milk might increase SCC in quarters with subclinical mastitis. In contrast, Clarke et al. (2008) found no detectable effect of incomplete milking on SCC in either infected or uninfected quarters. Further research is needed before the suitability of the software module for cows that are not entirely udder healthy can be evaluated.

Even though the average adaptation of milk production was very effective throughout our study, it was not possible to differentiate the production depression between the individual quarters. Since milking data were collected at the level of the udder, the quarters might have reacted not equally to the applied procedure of incomplete milking. Data collection at the level of the quarter would allow for assessing the reaction of individual quarters and might give rise to new findings regarding the adaptation of milk production. Especially measuring the last milk flow before cluster detachment for each quarter individually might lead to surprising outcomes since this parameter is likely not equal for all quarters and commonly hind quarters need more time to be milked out, since they contain more milk than front quarters (Weiss et al., 2004).

During our study early induced involution of the bovine mammary gland was assessed by measuring the content of the acute phase protein haptoglobin in skimmed milk samples. As described previously the involution process of the udder is characterized by various changes in mammary signaling and alterations in the concentration of proteins. There is a variety of parameters that has been measured in the literature for evaluating

the involution process. Especially the measurement of lactoferrin and BSA in milk are used more commonly in studies dealing with the involution of the bovine mammary gland (Ponchon et al., 2014; Lanctôt et al., 2017). Integration of these markers in further studies using the software induced milk yield reduction before dry-off might enable a more extensive evaluation of involution during incomplete milking. Changes in endocrine signaling for lowering milk production could also be assessed by measuring prolactin.

4.3 Assessment of animal welfare

As described before, the transition from a (highly) lactating into a non-lactating state can lead to impairment of the animals` welfare and therefore should be managed cautiously (Zobel et al., 2015). Different approaches can be found in the literature that aim to evaluate the cows` well-being during dry-off under various circumstances for example by conducting behavioral observations or evaluations of external and internal parameters:

Cessation of milking leads to accumulation of milk in the udder, which can cause situations of swollen and firm udders due to painfully high internal udder pressure. Estimation of extra-mammary udder pressure via utilization of a hand-held dynamometer in a study by Bertulat et al. (2013) showed that results in udder firmness dependent on the last milk yield before dry-off. As expected udder pressure after cessation of milking was highest in high yielding cows that were dried off abruptly. The implementation of such measurements of udder pressure in further experiments studying the production adaptation caused by software application would be easy to conduct and give insight into the individual reaction of the udder. Measuring udder pressure might also be interesting in connection with observing milk leakage in cows both during the procedure of successive incomplete milking and after dry-off. High

internal pressure can lead to dripping of milk, leaving the teat canal open and enabling potential pathogens to penetrate the teat lumen (Rovai et al., 2007). Findings of monitoring milk leakage could be integrated in the evaluation of udder health-related findings and ultrasonographic investigations of the inner teat morphology. Observations of milk leakage during milking times could be tied in into the experimental schedule easily. Potential impairments of well-being of cows due to high udder pressure are often assessed by means of changes in the lying behavior (Zobel at al, 2013; Chapinal et al., 2014; Rajala-Schultz et al., 2018). The swollen and firm udder tissue can impede the resting behavior of the animal because lying down in a comfortable position is no longer possible, or the cows experience lying down on the swollen udder to be bearable only for a limited time. Other behavioral investigations refer to increased vocalization of cows as possible signs for discomfort or frustration (Valizaheh et al., 2008; Tucker et al., 2009; Silanikove et al., 2013). Observing these different behavioral aspects during preparation for dry-off with the innovative software module and also after dry-off comparing experimental and control cows could provide insights into how cows experience the procedure of incomplete milking. However, it has to be admitted that conducting these ethological observations will lead to considerable extra work during the experiment. Both changes in cow behavior and udder pressure would be especially interesting to monitor in consideration of the high amount of accumulated residual milk we collected during the first conventional milking after the time period of incomplete milking.

Potential distress cannot only be assessed by visible or externally measurable parameters; there are also internal metabolic markers that indirectly refer to the welfare situation of the animal. In the study by Bertulat et al. (2013) concentrations of fecal glucocorticoid metabolites were measured after dry-off and the authors found these

markers to be highest in cows that entered the dry period with high milk yields. Putman et al. (2018) emphasized that many of the biomarkers associated with early-lactation metabolic stress also changed during the transition from late lactation to the early dry period. Blood samples were collected to quantify the changes in these biomarkers related to nutrient metabolism, oxidative stress, and inflammation (Ca, NEFA, BHB, BSA, haptoglobin, cortisol, reactive oxygen and nitrogen species, and others) during the transition of cows into the early dry period. The authors state that further research is needed to investigate the coherences between the magnitude and duration of deflection of these indicators and impairments of health and well-being of cows after cessation of milking. An integration of an extended biomarker analysis when studying the effects of incomplete milking could enable a more objective view on how the cows` experience this procedure of successive milk yield reduction.

4.4 Further applicability of the software module

As already pointed out in chapter 3, the software module (see patent specification in the annex) could be used to speed up the milking process when applied on individual cows that hold up the workflow due to disproportionate milking duration.

The software module can not only facilitate the transition from a lactating into a non-lactating state but also opens the opportunity to gently manage the onset of milk production after calving. The effectiveness of incomplete milking during early lactation to slow down the production increase for decreasing metabolic stress has already been proven in the literature (Carbonneau et al., 2012; Krug et al., 2018a,b; Morin et al., 2018).

Another advantage of the software module becomes apparent with regard to the statement of Besier and Bruckmaier (2016), who suggested that an early cluster

detachment skirting the lowest milk flow at the end of milking should be considered to avoid the increased mechanical strain on the delicate teat tissue. In our study, we could clearly verify that the internal teat morphology was less changed during the software-induced milking procedure of incomplete milking. The application of high-resolution ultrasound confirmed the considerable lower mechanical strain on teats when clusters were detached early. In conclusion, our findings support the statement of Besier and Bruckmaier (2016), and the software tool might thus allow for gentle milking procedures that preserve the sensitive teat tissue, regardless of the lactation stage for example after teat injuries or edematous changes in the teat tissue.

4.5 Summary

The primary aim of the present thesis was to obtain new insights into an optimal health and welfare oriented milking management of high producing dairy cows through the use of high-resolution ultrasound technology on the one hand and the development of an innovative milking software module for facilitating the dry-off process on the other hand.

Against the background of the great importance of the teat canal for udder health, the internal morphology of the teat and its changes during the milking process were first analyzed by visualization with an 18 MHz ultrasonographic probe. The applied methodology allowed for the establishing three additional measuring traits of the distal teat canal (TOR: distal orifice of the teat canal, DTCp: perimeter of the distal teat canal, DTCs: surface of the distal teat canal). These new traits as well as the traits known from the literature could be assessed in a repeatable way. Changes in the characteristics of the internal morphology were monitored in a second experiment and, in addition, associations with animal-specific variables were revealed. The dimensions of teat width,

teat end width and length of the teat canal were greater in multiparous cows compared to primiparous cows. Particularly interesting was the exposure of a relationship between the dimensions of the newly established traits of the distal teat canal and an udder health related parameter: teats whose milk showed bacteriological growth displayed larger values of perimeter and surface of the distal teat canal. The more detailed visualization of the internal teat structure, made possible by the high-resolution technique, allowed for a more extensive evaluation of the effects of the milking process. In future studies, utilizing the high-resolution technique and the new measurement traits can thus be applied for the evaluation of different milking machine settings regarding their mechanical strain on the teat. By visualization of the distal teat canal, conclusions can be also be drawn on the important defense functions of the teat canal.

The development of an innovative technique to prepare high yielding dairy cows for the dry period was the objective of a trial. Reducing milk yield at the end of lactation is recommended both for health reasons and for the well-being of the animal during this critical management step. Through the development and patenting of a software module, which enables automated cluster removal at a targeted milk amount, a program for successive milk yield reduction was established. Twenty-six udder healthy cows were milked increasingly incomplete over 10.4 ± 1.8 days prior to the scheduled termination of milking. Without compromising the health status, this procedure reduced milk production by about 35.3 ± 12.9%. A trend for increasing the concentrations of the acute phase protein haptoglobin suggested an induction of early involution in the experimental animals. The methodology of high-resolution ultrasound examination was also implemented and showed, that the early removal of the milking cluster lead to significantly smaller changes in the traits of inner teat morphology when compared to the farm`s conventional milking settings. After several days of incomplete milking,

surface and area of the distal teat canal showed even lower values before the start of the milking process. Milk yield and udder health in the subsequent lactation were not negatively impacted by the software-induced program. The software module can prepare udder healthy cows for cessation of milking in an animal-friendly and successive way, and support antibiotic free dry-off. In addition, the findings also open up further potential applications of innovative technology. For example, it could also be utilized to limit milk yield in early lactation for metabolic stabilization.

The experiments conducted in this thesis give an insight into the possibilities of using innovative technologies to better understand and manage the health maintenance of high yielding dairy cows. The presented results serve as a basis for subsequent studies and implementation in practice.

4.6 Zusammenfassung

Das primäre Ziel der vorliegenden Arbeit war es, durch die Anwendung hochauflösender Ultraschalltechnik einerseits und die Entwicklung eines neuartigen Melksoftwaremoduls (Patentschrift siehe Anhang) andererseits, neue Erkenntnisse hinsichtlich eines für Tiergesundheit und Wohlbefinden optimalen Melkmanagements von hochleistenden Milchkühen zu erlangen.

Im Rahmen des ersten Manuskripts wurde vor dem Hintergrund der großen Bedeutung des Zitzenkanals für die Eutergesundheit die innere Morphologie der Zitze und deren Veränderungen durch den konventionellen Melkprozess mittels Visualisierung mit einer 18 MHz Sonde analysiert. In einem ersten Versuch konnte gezeigt werden, dass die angewendete Methodik die Etablierung dreier ergänzender Messmerkmale des distalen Zitzenkanals erlaubt (TOR: distale Öffnung des Zitzenkanals; DTCp: Umfang des distalen Zitzenkanals; DTCs: Fläche des distalen Zitzenkanals) und die Bewertung dieser neuen, sowie der altbekannten Messparameter wiederholbar möglich ist. Veränderungen in den Merkmalen der inneren Zitzenmophologie konnten in einem zweiten Versuch anschaulich präsentiert werden und zudem wurden Zusammenhänge zu tierindividuellen Einflussgrößen aufgedeckt. So zeigten die Maße der Breite der Zitze, der Breite des Zitzenendes und der Länge des Zitzenkanals höhere Werte bei mehrkalbigen Tieren im Vergleich zu Färsen. Interessant war im Rahmen dieses Versuches vor allem die Darstellung des Zusammenhangs der Ausprägung der neu etablierten Merkmale des distalen Zitzenkanals und einer eutergesundheitsrelevanten Messgröße. So wiesen Zitzen, in deren Milch bakteriologisches Wachstum verifiziert werden konnte, größere Ausprägungen von Umfang und Fläche des distalen Zitzenkanals auf. Die durch die hochauflösende Technik realisierte, detailliertere

Visualisierung der inneren Morphologie ermöglichte so eine ausführlichere Evaluierung der Auswirkungen des Melkprozesses. Die Verwendete Technik kann unter Anwendung der neu etablierten Parameter somit zukünftig auch zur Bewertung der mechanischen Belastungen verschiedener Melkverfahren genutzt werden. Zudem können Rückschlüsse auf die wichtige Abwehrbarriere des distalen Zitzenkanals gezogen werden.

Die Erarbeitung einer innovativen Möglichkeit hochleistende Milchkühe auf die nötige Trockenstehphase vorzubereiten war Inhalt des zweiten Manuskripts. Nach Stand des Wissens ist eine Reduzierung der Milchleistung vor Beendigung des Melkens sowohl aus gesundheitlichen Aspekten zu empfehlen, als auch förderlich für das Wohlbefinden des Tieres während dieses kritischen Managementschrittes. Durch Entwicklung und Patentierung eines Softwaremoduls, welches die automatisierte Abnahme des Melkzeuges nach absolut gemolkener Menge ermöglicht, wurde ein Programm zur sukzessiven Milchmengenreduzierung erarbeitet. Sechsundzwanzig eutergesunde Kühe wurden über 10.4 ± 1.8 Tage vor geplanter Beendigung des Melkens zunehmend unvollständig gemolken. Ohne Beeinträchtigung des guten Gesundheitszustandes führte diese Prozedur zu einer Verringerung der Milchleistung um 35.3 ± 12.9%. Tendenzen in der Konzentrationsentwicklung des Akute Phase Proteins Haptoglobin ließen die Indizierung frühzeitiger Involution bei den Versuchstieren vermuten. Auch im Rahmen dieser Fragenstellung fand die Methodik der Ultraschalluntersuchung Anwendung. So konnte gezeigt werden, dass die frühzeitige Abnahme des Melkzeuges zu deutlich geringeren Veränderungen in den Merkmalen der inneren Zitzenmorphologie führt, verglichen mit den konventionellen Melkeinstellungen des Betriebs. Umfang und Fläche des distalen Zitzenkanals zeigten nach mehreren Tagen des unvollständigen Melkens sogar geringere Ausprägungen vor Beginn des Melkprozesses. Milchmenge

und Eutergesundheit in der darauffolgenden Laktation wurden durch das softwareinduzierte Programm nicht negativ Beeinträchtigt. Das Softwaremodul kann somit als sinnvolle Möglichkeit dienen, eutergesunde Kühe tierschonend und sukzessive auf die Beendigung des Melkens vorzubereiten. Zudem eröffnen die Erkenntnisse auch weitergehende Anwendungsmöglichkeiten der innovativen Technik. Zum Beispiel könnte sie ebenfalls zwecks Stoffwechselstabilisierung zur Limitierung der Milchmenge in der Frühlaktation genutzt werden.

Die Versuche im Rahmen dieser Arbeit geben einen Einblick in die Möglichkeiten der Anwendung innovativer Technologien zum besseren Verständnis und Management der Gesunderhaltung von hochleistenden Milchkühen. Die hier gezeigten Ergebnisse dienen als Basis für nachfolgende Studien sowie zur Umsetzung in der Praxis.

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6. Research publications

Peer reviewed published articles

Martin, L. M., C. Stöcker, H. Sauerwein, W. Büscher, and U. Müller. 2018. Evaluation of inner teat morphology by using high-resolution ultrasound: Changes due to milking and establishment of measurement traits of the distal teat canal. J. Dairy Sci. 101:8417–8428.

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Martin, L. M., W. Büscher, and U. Müller. 2018. Automatisierte Milchmengenreduktion vor dem Trockenstellen. Tagungsband der Veranstaltung MSD Expertise 2018, 23.-24.10.2018, Unterschleißheim, Deutschland. (Abstract, oral presentation)

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Annex – Patent specification

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(81) Bestimmungsstaaten *(soweit nicht anders angegeben, für jede verfügbare nationale Schutzrechtsart)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN,

(54) **Title:** METHOD FOR REDUCING THE INDIVIDUAL-SPECIFIC MILK PRODUCTION OF MILK-PRODUCING ANIMALS

(54) **Bezeichnung:** VERFAHREN ZUR REDUZIERUNG DER TIERINDIVIDUELLEN MILCHPRODUKTION VON MILCHGEBENDEN TIEREN

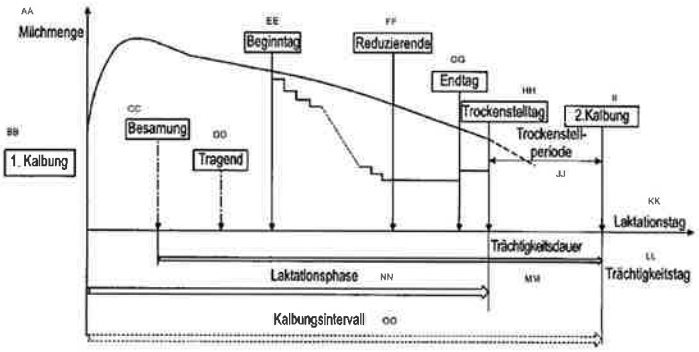

Fig. 2

AA Milk quantity
BB 1st calving
CC Insemination
DD Pregnant
EE First day
FF Reducing
GG Final day
HH Drying off day
II 2nd calving
JJ Drying off period
KK Lactation day
LL Pregnancy day
MM Pregnancy duration
NN Lactation phase
OO Calving interval

(57) **Abstract:** The invention relates to a method for milking a milk-producing animal, in particular a cow. According to this method, a target milk quantity which is lower than a possible expected milk quantity is determined. The milking process is carried out. During milking, the milked quantity is determined. The milked quantity is compared with the target milk quantity. Milking is terminated when the milked quantity corresponds substantially to the target milk quantity.

(57) **Zusammenfassung:** Zum Melken eines milchabgebenden Tieres, insbesondere einer Kuh, wird ein Verfahren vorgeschlagen, bei dem eine Zielmilchmenge, welche geringer ist als eine mögliche zu erwartende Milchmenge bestimmt. Der Melkvorgang wird durchgeführt. Während des Melkvorgangs wird die ermolkene Milchmenge ermittelt. Es findet ein Vergleich zwischen der ermolkenen

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[Fortsetzung auf der nächsten Seite]

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— *mit internationalem Recherchenbericht (Artikel 21 Absatz 3)*

Milchmenge mit der Zielmilchmenge stat. Der Melkvorgang wird beendet, wenn die ermolkenen Milchmenge im Wesentlichen der Zielmilchmenge entspricht.

Verfahren zur Reduzierung der tierindividuellen Milchproduktion von milchgebenden Tieren

Der Gegenstand der Erfindung bezieht sich auf Verfahren zum Melken eines Milch abgebenden Tiers, insbesondere einer Kuh, sowie auf eine zur Durchführung des Verfahrens geeignete und bestimmte Vorrichtung.

Obwohl im Folgenden die Erfindung in Verbindung mit einer Melkanlage zum Melken von Kühen beschrieben wird, wird darauf hingewiesen, dass sich der Gegenstand der Erfindung, insbesondere auch auf Verfahren und Vorrichtungen zum Melken von Schafen, Ziegen, Lamas, Kamelen, Dromedaren, Büffeln, Stuten, Eseln, Yaks sowie anderen Milch abgebenden Tieren eignet. Die Erfindung kann sowohl bei robotergestützten Melkanlagen als auch bei vollautomatischen, halbautomatischen oder konventionellen Melkanlagen verwendet werden.

Sowohl beim traditionellen Handmelken, welches ein Druckmelken ist, als auch bei maschinellen Saugmelkverfahren wird die gesamte im Euter befindliche Milch ermolken, um maximalen Ertrag zu erhalten und um das Euter zur weiteren Milchproduktion anzuregen.

Bei maschinellem Melken besteht die Gefahr, dass Zitzen zu lange gemolken werden, obwohl kein Milchfluss mehr vorhanden ist. Oder die Zitze ist verdreht oder abgeknickt und gibt gar keine Milch frei. Oder die milchführenden Kanäle der Zitzen werden durch ungünstige Umstände zu früh verschlossen. Es ist bekannt, dass bei einem zu starken Ausmelken es zu einer Beeinträchtigung der Zitzenkonditionen kommen kann. Insbesondere besteht die Gefahr der Hyperkeratosen (Strichkanalausspülungen).

Es sind unterschiedliche Verfahren bekannt, um solche negativen Auswirkungen beim vollständigen Ausmelken zu verhindern. So schlägt zum Beispiel die DE 36 09 275 A1 vor, dass die zeitliche Änderung der Milchmenge und/oder des Milchflusses innerhalb einzelner Pulszyklen charakteristische Besonderheiten aufweist, die bewertet werden können. Damit können Melkparameter über die Steuerung angepasst oder das Melkende bestimmt werden, so dass die Melksteuerung die Melkzeugabnahme ausführen kann. Durch die EP 0 534 565 B1 ist ein Verfahren zum automatischen Melken bekannt, das mittels Strömungssensoren den Milchfluss aus den einzelnen Zitzen überwacht und bei Versiegen des Milchflusses das Melken der Zitze beendet. In der DE 28 44 562 B1 ist ein weiteres Verfahren zum vollständigen Ausmelken bei maschinellem Milchentzug beschrieben. Wenn der Milchfluss unter eine bestimmte Schwelle sinkt, wird die Abnahme des Melkzeugs bewirkt.

Im Hinblick darauf, dass sich der Melkertrag und die Milchzusammensetzung im Laufe einer Laktation verändert, wird eine Kuh zur Vorbereitung auf die folgende Laktation trockengestellt. Unter einem Trockenstellen wird der Übergang aus der laktierenden Phase in eine nicht laktierende Phase verstanden. Während der Trockenstehperiode erfolgt im Wesentlichen kein Milchentzug, da dem Tier die Möglichkeit gegeben werden soll, eine Regeneration der Reserven für den nächsten Laktationsstart zu erreichen.

Es ist festgestellt worden, dass insbesondere bei Tieren mit hoher Milchleistung und hoher Tagesmelkleistung zum Zeitpunkt des Trockenstellens eine Neuinfektionsrate mit Mastitis sehr hoch sein kann (Volker Krömker; Kurzes Lehrbuch Milchkunde und Milchhygiene; Parey in MVS Medizinverlage Stuttgart; 2007). Um das Risiko für die Eutergesundheit zu verringern, kommen antibiotische Langzeitpräparate, die auch als sogenannte Trockensteller bekannt sind, zum Einsatz. Durch die Verwen-

dung von insbesondere antibiotischer Langzeitpräparate, die auch präventiv Tieren gegeben werden, können Eutergesundheitsprobleme zumindest verringert werden.

Andererseits ist Rohmilch ein bedeutsames Lebensmittel und ein wichtiger Rohstoff für die Nahrungsmittelindustrie. Zum Schutz des Verbrauchers, zur technischen Verarbeitungsfähigkeit sowie zur Marktlenkung muss Rohmilch sowohl nationalen als auch internationalen Qualitätsanforderungen genügen. Wird ein Tier medikamentös behandelt, so darf die Milch des Tiers während der medikamentösen Behandlung und innerhalb einer vorgegebenen Zeitspanne nach dem Ende der medikamentösen Behandlung nicht als verkehrsfähige Milch klassifiziert werden. Außerdem fördert der verbreitete Einsatz von Antibiotika Resistenzen bei Bakterienstämmen in der gesamten Umwelt.

Hiervon ausgehend liegt der vorliegenden Erfindung die Zielsetzung zugrunde, ein verbessertes Verfahren zum Melken eines Milch gebenden Tiers, insbesondere einer Kuh, anzugeben.

Diese Aufgabe wird durch ein Verfahren zum Melken eines Milch abgebenden Tiers, insbesondere einer Kuh, mit den Verfahrensschritten gemäß Patentanspruch 1 gelöst. Weitere vorteilhafte Ausgestaltungen und Ausführungen des Verfahrens sind in den abhängigen Patentansprüchen angegeben. Die in den Patentansprüchen einzeln aufgeführten Merkmale sind in beliebiger, technologisch sinnvoller Weise miteinander kombinierbar und können durch erläuternde Sachverhalte aus der Beschreibung ergänzt werden, wobei weitere Varianten der Erfindung aufgezeigt werden.

Nach dem erfindungsgemäßen Verfahren zum Melken eines Milch abgebenden Tiers, insbesondere einer Kuh, wird vorgeschlagen, dass eine

Zielmilchmenge bestimmt wird. Die Zielmilchmenge ist dabei geringer als eine mögliche zu erwartende Milchmenge. Während des Melkvorgangs wird die ermolkene Milchmenge ermittelt. Es findet ein Vergleich zwischen der ermolkenen Milchmenge und der Zielmilchmenge statt. Der Melkvorgang wird beendet, wenn die ermolkene Milchmenge im Wesentlichen der Zielmilchmenge entspricht. Dies kann durch eine automatische Abnahmesteuerung erfolgen oder manuell durch Melkpersonal, das den maschinellen Melkvorgang beaufsichtigt oder Handmelken ausführt.

Durch dieses erfindungsgemäße Verfahren wird insbesondere eine schonende Behandlung der Zitzen eines Tiers, insbesondere einer Kuh, erreicht.

Das Verfahren ist insbesondere vorteilhaft im Zusammenhang mit der Einleitung einer Trockenstehperiode einer Kuh. So wird vorgeschlagen, dass ein Beginntag, der vor einem Trockenstelltag liegt, bestimmt wird und von dem an der Melkvorgang beendet wird, wenn eine Zielmilchmenge ermolken wurde. Durch diese bevorzugte Ausgestaltung des Verfahrens wird eine Involution der Milchdrüse aktiv eingeleitet. Durch diesen aktiven Vorgang vereinfacht sich die Trockenstellung des Milch gebenden Tiers, insbesondere kann im Wesentlichen auf die Verwendung antibiotischer Trockenstellpräparate verzichtet werden.

Vorzugsweise wird die Zielmilchmenge zumindest für den Beginntag aus historischen Daten ermittelt. Es ist bekannt, dass moderne Milchviehbetriebe über ein Herdenmanagementsystem verfügen. In dem Managementsystem sind zahlreiche Daten hinterlegt, die einzelnen Tieren oder Tiergruppen zugeordnet werden. Verfügt der Milchviehbetrieb über ein solches Herdenmanagementsystem, so stehen insbesondere tierindividuelle Daten zur Verfügung. Hierbei kann es sich um Daten handeln, aus denen erkennbar ist, welchen Milchertrag ein Tier an einem bestimmten

Laktationstag abgegeben hat. Auf der Basis eines solchen Datensatzes mit den historischen Daten kann die Zielmilchmenge zumindest für den Beginntag ermittelt werden. Die historischen Daten enthalten vorzugsweise sämtliche für das Melken relevanten Daten.

Es besteht auch die Möglichkeit, dass die Zielmilchmenge zumindest für den Beginntag aus historischen Daten ermittelt wird, wobei hier die historischen Daten nicht zwingend tierindividuell sein müssen. Die Milchmenge, welche ein Tier einer bestimmten Rasse, physischer Kondition etc. abgibt, ist an und für sich geläufig und kann als Basis für die Bestimmung oder Ermittlung der Zielmilchmenge für den Beginntag postuliert werden.

Wird ein Tier nach dem erfindungsgemäßen Verfahren während einer Laktation gemolken, so werden diese Daten, insbesondere die Milcherträge pro Melkvorgang bzw. pro Melkertrag, gespeichert. Diese gespeicherten Daten stellen auch historische Daten dar. Wird das Tier nach dem erfindungsgemäßen Verfahren in einer weiteren Laktation gemolken, so kann auf die historischen Daten aus zumindest einer vorhergehenden Laktation zurückgegriffen werden. Ggf. wird eine Korrektur der historischen Daten durchgeführt in Abhängigkeit davon, in welcher Laktation sich das Tier befindet. Ist bspw. bekannt, dass der Milchertrag während der Laktation nach der zweiten, dritten oder weiteren Kalbung stets abnimmt, so kann eine Korrelation zwischen den historischen Daten und den weiteren Kalbungen bzw. weiteren Laktationen gestellt werden, um die als erwartete im Euter befindliche Milchmenge genauer zu schätzen bzw. die reduzierte Zielmilchmenge genauer zu berechnen.

Wird das Verfahren bei einem Tier durchgeführt, welches nach der ersten Kalbung laktierend ist, so wird auf historische Daten vergleichbarer Tiere zurückgegriffen.

Bei den historischen Daten kann es sich auch Daten handeln, die sich auf vorangegangene Melkvorgänge der aktuellen Laktation beziehen.

Die Zielmilchmenge wird vorzugsweise für jeden einzelnen Melkvorgang bestimmt, von denen jedes Tier in der Regel mehrere pro Tag hat. Diese Vorgehensweise ist insbesondere dann vorteilhaft, wenn das Tier zu exakt vorgegebenen Tageszeiten gemolken wird.

Wird das Tier in einem Milchviehbetrieb gehalten, in dem ein freier Tierverkehr praktiziert wird, so ist es vorteilhaft, wenn die Zielmilchmenge für jeden Tag festgelegt wird. Beim freien Tierverkehr ist nicht sichergestellt, dass ein Tier an jedem Tag zum gleichen Zeitpunkt gemolken wird. Dies ist insbesondere dann der Fall, wenn robotergestützte Melkeinrichtungen freiwillig von den Tieren aufgesucht werden können. Ist dies der Fall, so ändert sich je nach Zeitabstand zwischen zwei aufeinanderfolgenden Melkvorgängen die zur Verfügung stehende Milchmenge im Euter.

Auch bei einem gelenkten Tierverkehr mit geplanten Melkzeiten, die jeweils nur ein Zeitfenster vorher bestimmen, lässt sich der Zeitpunkt für einen Melkvorgang eines bestimmten Tieres nicht genau vorhersagen. Dann ist es ebenfalls vorteilhaft, wenn die Zielmilchmenge für jeden Tag festgelegt wird.

Vorteilhafterweise wird die Zielmilchmenge für einen ganzen Tag nach verschiedenen Anwendungsfällen und Verfahren ermittelt und daraus eine Zielmilchmenge für einen einzelnen Melkvorgang abgeleitet. Dies ist, insbesondere beim freien Tierverkehr oder bei Angabe einer Melkzeit als Zeitfenster, zu bevorzugen. Wird die Zielmilchmenge (ZMM) für ein einzelnes Melken auf der Basis einer Tages-Zielmilchmenge (TZM) bestimmt, so ist es vorteilhaft, wenn der zeitliche Abstand (S) zwischen zwei aufeinanderfolgenden Melkvorgängen berücksichtigt wird. So kann bspw. die

Zielmilchmenge aus dem Produkt der Tages-Zielmilchmenge und dem Quotienten aus der Zeit zwischen zwei aufeinanderfolgenden Melkvorgängen pro 24 Stunden berechnet werden. Für die Zielmilchmenge soll gelten:

$$ZMM = TZM * (S\,[h] / 24[h])$$

Im Hinblick darauf, dass die Milchproduktion im Euter seit einem letzten Melken nicht unendlich steigen kann, wird vorgeschlagen, dass die Zeitdauer seit dem letzten Melkvorgang für die Berechnung der Zielmilchmenge für ein einzelnes Melken auf einen Maximalwert begrenzt wird. Eine solche Situation kann bspw. eintreten, wenn eine unvollständige Datenerfassung zu einem Tier erfolgt ist und der letzte aufgezeichnete Melkvorgang nicht der letzte tatsächliche Melkvorgang ist. Wird bspw. festgestellt, dass in Abhängigkeit von der geplanten Melkfrequenz, das heißt der Anzahl der Melkvorgänge pro 24 Stunden, ein vorgegebener Zeitabstand zu einem vorhergehenden bekannten Melkvorgang den Maximalwert weit überschreitet, so kann ein angenommener Zeitabstand zwischen zwei aufeinanderfolgenden Melkvorgängen zur Bestimmung der Zielmilchmenge pro Tag basierend auf der Tages-Zielmilchmenge herangezogen werden, wobei hier die geplante Melkfrequenz, das heißt die Anzahl der geplanten Melkungen pro Tag, berücksichtigt wird.

Bevorzugt wird hierbei von dem nachfolgend in der Tabelle wiedergegebenen Werten ausgegangen:

Geplante Melkfrequenz	*Zeitabstand zu einem letzten bekannten Melkvorgang*	*Angenommener Zeitabstand*
1	*36h*	*24h*
2	*24h*	*12h*
3	*16h*	*8h*
4	*12h*	*6h*

Soll bspw. entsprechend der Tabelle ein Tier zweimal am Tag gemolken werden (geplante Melkfrequenz), was einem angenommenen Zeitabstand von 12 Stunden pro Tag entspricht, und ist festgestellt worden, dass zwischen zwei aufeinanderfolgenden Melkvorgängen mehr als 24 Stunden vergangen sind, so wird für die Bestimmung der Zielmilchmenge für das anstehende Melken ein Zeitabstand zwischen zwei aufeinanderfolgenden Melkvorgängen von 12 Stunden angenommen, obwohl der tatsächliche Zeitabstand kleiner oder größer sein kann.

Diese Verfahrensführung ist insbesondere dann vorteilhaft, wenn vor einem Melkvorgang eine Identifikation des zu melkenden Tiers erfolgt.

Gemäß einer noch weiteren vorteilhaften Gestaltung des erfindungsgemäßen Verfahrens wird vorgeschlagen, dass der Beginntag für das Melken mit reduzierter Zielmilchmenge in Abhängigkeit vom geplanten Trockenstelltag eines Tieres festgelegt wird. Der geplante Trockenstelltag bestimmt sich tier-, betriebs- oder rassespezifisch aus der Anzahl der Tage seit dem Tag der Besamung bzw. des Deckvorgangs, für die in einer späteren Trächtigkeitsuntersuchung die Trächtigkeit festgestellt wurde. Der Beginntag für das Melken mit reduzierter Zielmilchmenge liegt eine bestimmte, tier-, betriebs- oder rassespezifische Anzahl Tage vor dem geplanten Trockenstelltag. Die Größe der Änderung der Zielmilchmenge kann an die Zeitspanne zwischen Beginntag und geplantem Trockenstelltag angepasst werden.

Insbesondere wird vorgeschlagen, dass die Zielmilchmenge eines Tages aus der Zielmilchmenge des vorigen Tages verringert um einen Änderungswert M bestimmt wird. Der zu verringernde Vortageswert am Beginntag selbst, im Folgenden als Referenzmenge bezeichnet, kann die durchschnittliche Tagesmilchleistung des Tieres sein, berechnet aus den Milchmengen historisch aufgezeichneter Melkvorgänge oder gemäß der

bereits vorgestellten Verfahren zur Schätzung gemäß Laktationstag und -kurve oder Schätzverfahren, die frühere Laktationsleistungen oder vergleichbare Tiere benutzen. Alle Werte können auch zitzenindividuell bestimmt werden.

Der Änderungswert M kann ein konstanter Wert sein, der für jeden Tag subtrahiert wird. Vorzugsweise wird er durch einen Faktor K aus dem Vortageswert ermittelt bzw. am Beginntag aus der ermittelten Referenzmenge. Der Faktor K für eine Reduktion der Menge muss kleiner als 1 sein. Für später vorgestellte Anwendungen des Melkens mit begrenzter Zielmilchmenge, wo eine schrittweise Erhöhung gewünscht wird, kann er andere Werte haben. Änderungswert M oder Faktor K sind vorzugsweise konstant und einstellbar. Insbesondere wird vorgeschlagen, dass sie von einer Zeitspanne zwischen Beginntag und Trockenstelltag und/oder Laktationsstand abhängige Größen sind. Dadurch wird eine noch bessere Anpassung der Zielmilchmenge und der damit verbundenen angestrebten Involution der Milchdrüse erreicht. Die Reduktion kann auch andere Einflussfaktoren berücksichtigen, insbesondere auch die Rasse, Physiognomie und den tierindividuellen Gesundheitszustand. Die Anpassung kann linear oder asymptotisch auf einen bestimmten Zielwert hin erfolgen.

Gemäß einer noch weiteren vorteilhaften Weiterentwicklung des Verfahrens wird vorgeschlagen, dass zum Ende der Laktationsperiode hin das Tier wenigstens einem vollständigen Melkvorgang unterzogen wird. Hierdurch soll erreicht werden, dass am Trockenstelltag das Euter des Tiers auch tatsächlich vollständig ausgemolken wird.

Das erfindungsgemäße Verfahren ist nicht nur im Zusammenhang mit dem angestrebten Trockenstellen des Tiers von Vorteil, sondern es berücksichtigt auch, dass eine verbesserte Tiergesundheit und insbesondere eine verbesserte Energiebilanz erreicht werden kann. Es ist bekannt, dass

das Tier für die Milchproduktion eine bestimmte Energie aufbringen muss. Um diese Energie bereitzustellen, werden dem Tier passende Nährstoffe in optimaler Menge zugeführt. Das Tier soll nicht zu fett werden, da es zu Schwierigkeiten bei der folgenden Kalbung kommen kann. Ein zu hoher Eiweißeintrag führt zu Stoffwechsel- und Gesundheitsproblemen des Tiers. Insbesondere bei hoher Milchleistung ist es bei einem raschen Anstieg der Milchproduktion, insbesondere nach der Kalbung in einem Frühstadium der Laktation, oft schwierig, ein Gleichgewicht zwischen einer adäquaten Fütterung und Milchproduktion herzustellen. Eine rasche Änderung der Futtermenge führt nicht zu dem gewünschten Ergebnis, da sich das Verdauungssystem stets an die geänderte Menge anpassen muss. Es besteht die Gefahr, dass es zu Verdauungserkrankungen kommt, wenn die Futtermenge zu hoch wird. Bei einem schnellen Anstieg der Milchproduktion entsteht daher ein Defizit an der zugeführten Energiemenge, und es besteht die Gefahr einer Ketose-Erkrankung.

Des Weiteren kann die Menge des konzentrierten Kraftfutters nicht beliebig erhöht werden, da das Verdauungssystem einen bestimmten Mindestanteil an Rohfasern im Verhältnis zur aufgenommenen Energiemenge benötigt, so dass es Grenzen durch das Volumen des Gesamtfutters gibt. Bei Kühen besteht daher die Gefahr, dass es zu einer negativen Energiebilanz kommt, was das Immunsystem und die Fruchtbarkeit beeinträchtigt.

Nach dem erfindungsgemäßen Verfahren besteht daher auch die Möglichkeit, den Anstieg der Milchproduktion insbesondere zum Anfang der Laktationsperiode zu beeinflussen. Liegt die Zielmilchmenge unterhalb der möglichen zu ermelkenden Milchmenge, so wird die Produktion reduziert. Der Anreiz für die Kuh, die Milchproduktion zu steigern, wird verringert, was sich positiv auf die Gesamtenergiebilanz und somit auch für die Tiergesundheit des Tiers auswirken kann.

Das erfindungsgemäße Verfahren kann auch für eine bestimmte Zeitspanne während der Laktationsperiode zum Einsatz kommen. Dadurch wird bspw. eine Drosselung der Milchproduktion während der Laktation erreicht. Die gezielte Drosselung der ermolkenen Milchmenge und damit einhergehenden Reduktion der Milchproduktion bei dem Tier ist insbesondere dann von Vorteil, wenn das Tier eine gesundheitliche Beeinträchtigung aufweist. Eine Drosselung der Milchproduktion entlastet den Stoffwechsel des Tiers, was zur Stärkung des Immunsystems und somit zu einer Verbesserung des Gesundheitszustands des Tiers führen kann.

Gemäß einem weiteren erfinderischen Gedanken wird eine Melkvorrichtung zur Durchführung eines Verfahrens nach einem der Ansprüche 1-16 vorgeschlagen. Die Melkvorrichtung weist eine Melkeinrichtung auf. Insbesondere handelt es sich bei der Melkeinrichtung um eine robotergestützte Melkeinrichtung. Die Melkvorrichtung weist des Weiteren Mittel zur Tieridentifikation auf. Hierbei kann es sich um herkömmliche, bekannte Mittel zur Tieridentifikation handeln. Die Melkvorrichtung weist des Weiteren Mittel zur Erfassung einer während eines Melkvorgangs ermolkenen Milchmenge auf. Des Weiteren ist eine Steuereinrichtung vorgesehen, welche zur Steuerung der erfindungsgemäßen Melkeinrichtung geeignet und bestimmt ist.

Insbesondere weist die Steuerung einen Computer umfassend einen Speicher mit einem darin hinterlegten Computerprogramm und einen digitalen Prozessor auf.

Gemäß einem noch weiteren erfinderischen Gedanken wird ein Computerprogrammprodukt vorgeschlagen, welches zur Durchführung des Verfahrens nach einem der Ansprüche 1-16 bestimmt und eingerichtet ist.

Des Weiteren wird ein maschinenlesbares Speichermedium, auf dem ein Computerprogrammprodukt gemäß Anspruch 19 gespeichert ist, vorgeschlagen.

Weitere Vorteile und Einzelheiten der Erfindung werden anhand des in der Zeichnung dargestellten Ausführungsbeispiels erläutert, ohne dass der Gegenstand der Erfindung auf dieses Ausführungsbeispiel beschränkt ist. Es zeigen:

Fig. 1: schematisch eine Melkvorrichtung zur Durchführung des erfindungsgemäßen Verfahrens und

Fig. 2: schematisch einen Verlauf einer Milchleistung über die Zeit.

Fig. 1 zeigt schematisch einen Melkbereich 1 mit einem Melkplatz 2. Der Melkplatz 2 wird vom Tier 4 über den Eingangsbereich 3 betreten und verlassen.

Im Bereich des Melkplatzes 2, in dem dargestellten Ausführungsbeispiel im Kopfbereich des Melkplatzes, sind Mittel zur Tieridentifikation 8 vorgesehen. Damit werden die einzelnen Tiere einer Identifikation unterzogen. Die Mittel zur Tieridentifikation 8 arbeiten vorzugsweise berührungslos. Sie weisen insbesondere eine Sende- und/oder Empfangseinheit auf. Mittels der Identifikationsmittel 8 werden die von den Tieren getragenen Tieridentifikationsmittel, in denen tierindividuelle Kenngrößen bzw. Daten hinterlegt sind, ausgelesen und an bspw. ein Herdenmanagementsystem übergeben. Das Herdenmanagementsystem ist signaltechnisch auch mit einer Steuereinrichtung 6 zur Steuerung der Melkeinrichtung 5 am Melkplatz 2 verbunden. Das Herdenmanagementsystem kann auch ein Teil der Steuereinrichtung 6 sein. Es ist jedoch nicht zwingend, dass ein Herdenmanagementsystem vorhanden sein muss, es ist jedoch

vorteilhaft, wenn dieses zur Verfügung steht. Die Anordnung der Mittel zur Tieridentifikation im Kopfbereich des Melkplatzes stellt eine Ausführungsform dar. Diese können auch beispielsweise im Gangbereich zum Melkplatz angeordnet sein. Während eines Melkvorgangs mittels einer Melkeinrichtung 5 wird die ermolkene Milchmenge erfasst, wozu Mittel 7 vorgesehen sind.

Betritt ein Tier 4 den Melkplatzbereich 2, so wird dieses durch die Mittel zur Tieridentifikation 8 identifiziert. Die Identifikationsinformation wird vorzugsweise an ein Herdenmanagementsystem gegeben. Ist im Herdenmanagementsystem hinterlegt und das entsprechende Signal an die Steuerungseinrichtung 6 gesendet worden, dass zu dem konkreten Tier 4 ein Melkvorgang durchgeführt werden soll, so werden die nicht dargestellten Melkbecher an die Zitzen des Tiers angesetzt und der Melkvorgang durchgeführt.

In dem Herdenmanagementsystem ist auch hinterlegt, ob das Tier nach einem Melkvorgang entsprechend dem erfindungsgemäßen Verfahren gemolken werden soll. Ist dies der Fall, so wird dies vor Beginn des Melkvorganges der Steuereinrichtung 6 mitgeteilt. Während des Melkvorgangs wird die ermolkene Milchmenge ermittelt. Bei den Tieren, die mit dem erfindungsgemäßen Verfahren zu melken sind, findet vorzugsweise ein kontinuierlicher Abgleich der ermolkenen Milchmenge mit einer vorzugsweise tierindividuell bestimmten Zielmilchmenge statt. Entspricht die ermolkene Milchmenge im Wesentlichen der Zielmilchmenge, so wird der Melkvorgang beendet. Um einen Tierschutz zu gewährleisten und ein für das Tier gesundheitsschädigendes Melken zu verhindern, können zusätzlich Abbruchkriterien definiert werden, die ein Melkende vor einem Erreichen der Zielmilchmenge einleiten.

Die erfindungsgemäße Verfahrensführung kann auch für Melkvorgänge während der Laktationsperiode angewendet werden, wobei die Zielmilchmenge abhängig vom Laktationsstand des Tiers ist. Insbesondere wird das erfindungsgemäße Verfahren im Zusammenhang mit dem angestrebten Trockenstellen des Tiers verwendet.

Fig. 2 zeigt einen schematischen Verlauf der Milchleistung während einer Laktation in Abhängigkeit von der Zeit. Eine Laktation ist der Zeitraum nach einer Kalbung. Der nullte Laktationstag ist der Tag der Kalbung. Von da an werden die Laktationstage gerechnet. Kurz nach einer Kalbung hat ein Tier die höchste Milchleistung. Im Laufe einer Laktation gibt ein Tier immer weniger Milch ab, was aus dem fallenden Verlauf der Kurve ersichtlich wird. Der Milchfluss (Kilogramm pro Minute) wird hierbei immer geringer.

Während der der Laktation erfolgt wenigstens eine Besamung des Tiers. In einer Trächtigkeitsuntersuchung wird festgestellt, ob das Tier tragend ist und welche Besamung erfolgreich war. Der nullte Trächtigkeitstag ist der Tag der Besamung. Von diesem Tag an gerechnet werden die Tage auch Trächtigkeitstage für das Tier genannt. Vor der nächsten Kalbung erfolgt in der Regel ein Trockenstellen des Tiers. Ab dem Trockenstelltag wird das Tier für einige Tage vor der nächsten Kalbung nicht mehr gemolken. Diese Trockenstellperiode führt zu einer Verbesserung der Energiebilanz des Tiers, so dass es gesünder in die Kalbung und den Anfang der nächsten Laktation geht. Die Laktationszahl bezeichnet die Zahl, wie oft ein Tier bereits gekalbt hat.

Der in der Fig. 2 dargestellte Verlauf der Milchproduktion kann für unterschiedliche Tiere verschieden sein. Der Verlauf kann auch in Abhängigkeit von der Laktationszahl eines Tiers veränderlich sein.

Aus dem in der Fig. 2 dargestellten Verlauf ist erkennbar, dass am Trockenstelltag die Milchproduktion sprunghaft auf Null reduziert wird, was dadurch bedingt ist, dass ab dem Trockenstelltag keine Melkvorgänge mehr durchgeführt werden. Nach den bisherigen Verfahren wird das Euter eines Tiers während der Laktation leer gemolken. Durch das Leermelken wird das Euter maximal angereizt, weitere Milch zu produzieren, so dass ein theoretischer weiterer Verlauf der Laktation möglich wäre, wie dies vorstehend in der Fig. 2 in der Trockenstellperiode gestrichelt dargestellt ist.

Nach dem erfindungsgemäßen Verfahren wird ein Tier gemolken, wobei der Melkvorgang beendet wird, sobald eine vorgegebene Zielmilchmenge erreicht worden ist oder die klassischen Abnahmeverfahren aus Tierschutzgründen eher greifen.

Ist der Trockenstelltag bekannt, so wird ab einem Beginntag, der vor einem Trockenstelltag liegt, mit einer Zielmilchmenge gemolken. Die Zielmilchmenge ist eine Milchmenge, die geringer ist als eine mögliche zu erzielende Milchmenge. Die Zielmilchmenge wird zumindest für den Beginntag aus historischen Daten ermittelt. Hierzu kann auf geeignete Verfahren, insbesondere Schätzverfahren zurückgegriffen werden. Wird das Tier nach dem erfindungsgemäßen Verfahren gemolken und die Zielmilchmenge im Laufe der Tag reduziert, so sind die Milchproduktion und der Euterinnendruck am Trockenstelltag so gering, dass die Anwendung von Antibiotika unnötig oder zumindest in erheblich geringerem Umfang verwendet wird. Das Verfahren fördert die Involution der Milchdrüsen. Die langsame Abnahme der Milchproduktion führt auch zu einer schonenderen Veränderung der Fütterungsnotwendigkeit, die das Tier nicht mehr so viel Energie aus dem Futter für die Milchproduktion benötigt. Das Tier gerät somit nicht so leicht ins energetische Ungleichgewicht.

In der Fig. 2 ist schematisch zwischen Beginntag und Reduzierende der Verlauf der Zielmilchmenge dargestellt. Ab einem Beginntag, der vorzugsweise in Abhängigkeit vom Trockenstelltag definiert wird, wird das Tier mit der Zielmilchmenge gemolken. Bis zum Beginntag wird das Tier nach dem klassischen Melkverfahren gemolken. Mit dem Beginntag wird vorzugsweise die durchschnittliche Tagesmilchmenge des Tiers aus den historischen aufgezeichneten Gemelksmengen der vorangegangenen Tage berechnet. Vorzugsweise wird die Gemelksmenge der vorangegangenen 7 (sieben) Tage berücksichtig. In Kenntnis der historischen Daten wird ein erster Reduzierschritt vorgenommen, durch den die zu erzielende Zielmilchmenge am Beginntag definiert wird.

Die Zielmilchmenge für einen einzelnen Melkvorgang wird aus der Tages-Zielmilchmenge und der Zeit berechnet, die seit dem vorigen Melken vergangen ist, wobei ein Schätzwert angenommen werden kann, wenn der Abstand zur letzten aufgezeichneten Melkung nicht plausibel ist. Es besteht auch die Möglichkeit, dass Zielmilchmengen für jeweilige Melkvorgänge eines Tages direkt definiert werden.

Aus der Fig. 2 ist ersichtlich, dass die Zielmilchmenge, die der tatsächlichen ermolkenen Milchmenge entspricht, stufenweise ab dem Beginntag bis zu einem Reduzierende abnimmt. Hierbei handelt es sich um eine bevorzugte Ausführungsform des Verfahrens. Es ist nicht zwingend, dass eine Reduktion der Zielmilchmenge von Tag zu Tag erfolgt. Tritt bspw. der Fall ein, dass die erfasste Milchmenge nicht oder unvollständig gespeichert wurde, so wird der darauf folgende Melkvorgang mit der letzten Zielmilchmenge fortgesetzt. Der Änderungswert M kann auch variabel sein, so dass bspw. im Verlauf der Laktation der Änderungswert M kleiner wird. Dies bedeutet, dass die Differenz zwischen den Zielmilchmengen aufeinanderfolgender Tage geringer wird.

Während des Melkvorgangs wird die ermolkene Milchmenge erfasst. Wird die erfasste Milchmenge zitzenindividuell ermittelt, so kann auch die Zielmilchmenge zitzenindividuell eingestellt werden.

Eine Reduktion der Zielmilchmenge erfolgt bis zu einem vorgegebenen Reduzierende. Zwischen dem Reduzierende und einem Endtag, der vor dem Trockenstelltag liegt, wird vorzugsweise mit einer konstanten Zielmilchmenge gemolken. Das Reduzierende und der Endtag werden vorzugsweise in Abhängigkeit von der Trächtigkeitsdauer des Tiers eingestellt. Der Endtag sollte kurz vor dem geplanten Trockenstelltag liegen. Zwischen dem Endtag und dem Trockenstelltag wird das Tier vollständig ausgemolken. Hierdurch soll sichergestellt werden, dass das Euter vor dem Trockenstellen leergemolken wurde. Dies ist insbesondere dann zweckmäßig, wenn eine „versiegelnde" Substanz in das Euter eingeführt wird. Vorzugsweise wird zwischen dem Endtag und dem Trockenstelltag das Tier zwei- bis dreimal vollständig ausgemolken, damit sichergestellt ist, dass die Milchproduktion tatsächlich reduziert wurde. Die bei einem vollständigen Melkvorgang erfasste Milchmenge kann auch als Überprüfung der Wirksamkeit der Verfahrensführung mit der Zielmilchmenge genutzt werden. In Abhängigkeit davon, welche tatsächliche Milchmenge beim vollständigen Melkvorgang vorliegt, kann beim betreffenden Tier, der Tiergruppe oder der Herde eine Anpassung des Änderungswerts M ermöglicht werden.

Diese Verfahrensführung hat insbesondere den Vorteil, dass die Eutergesundheit gesteigert wird. Die Verwendung antibiotischer Trockenstellepräparate kann im Wesentlichen vermieden werden.

Aus der Darstellung nach Fig. 2 ist ersichtlich, dass zu Beginn der Laktation eine große Steigung der Milchproduktion des Tiers verzeichnet werden kann. In dieser frühen Laktationsphase, in der auch das Kolostrum

vorliegt, ist der Energiebedarf des Tiers sehr hoch. Das Kolostrum oder auch Biestmilch genannt, wird in zahlreichen Ländern als nicht verkehrsfähige Milch betrachtet. Es ist daher nicht zwingend, dass ab dem Tag der Kalbung eine hohe Milchproduktion einsetzt. Wünschenswert wäre es, wenn die hohe Milchproduktion frühestens nach Ende der Kolostrumperiode eintritt, was meistens bei Kühen ab dem fünften Laktationstag der Fall wäre.

In der frühen Laktation ist der Energiebedarf des Tieres für den starken Anstieg der Milchleistung sehr hoch und kann unter Umständen nicht durch das aufgenommene Futter gedeckt werden. Um das Ketoserisiko zu verringern, wird die Milchproduktion begrenzt.

Vorstehend wurde dargelegt, dass ab einem Beginntag die Zielmilchmenge reduziert wird. Das Verfahren ist auch vorteilhaft in der Beginnphase der Laktation. Mit der Kalbung, das heißt ab dem nullten Laktationstag oder einem späteren Tag, wird das Tier mit einer Zielmilchmenge gemolken, wobei an jedem weiteren Tag eine Erhöhung der Zielmilchmenge vorgenommen wird. Die Erhöhung der Zielmilchmenge kann bspw. ein bestimmter, einstellbarer Prozentsatz des Vortageswerts sein. Es besteht auch die Möglichkeit, dass die Erhöhung um einen bestimmten Betrag erfolgt. Die Erhöhung in Abhängigkeit vom Laktationsstand kann konstant oder auch variabel sein.

Das Verfahren zum Melken eines Tiers mit reduzierter Zielmilchmenge kann auch für eine Drosselung der Milchproduktion während der Laktation erfolgen. Es ist bspw. festgestellt worden, dass die Nahrungsaufnahme des Tiers gestört ist oder das Tier aus anderen Gründen mit einer reduzierten Zielmilchmenge gemolken werden soll, so kann ab einem Beginntag bis zu einem vorgegebenen Reduzierende während der Laktation das Tier mit einer reduzierten Zielmilchmenge gemolken werden.

Durch das erfindungsgemäße Verfahren wird ein schonendes Melken eines Tiers erreicht. Des Weiteren kann auf antibiotische Trockenstellpräparate im Wesentlichen verzichtet werden. Der Gesundheitszustand des Tiers wird durch das erfindungsgemäße Verfahren gesteigert.

Bezugszeichenliste

1	Melkbereich
2	Melkplatz
3	Eingangsbereich des Melkplatzes
4	Tier
5	Melkeinrichtung
6	Steuereinrichtung
7	Mittel zur Erfassung einer ermolkene Milchmenge
8	Mittel zur Tieridentifikation

Patentansprüche

1. Verfahren zum Melken eines milchabgebenden Tieres, insbesondere einer Kuh, umfassend die folgenden Schritte:

Bestimmung einer Zielmilchmenge, welche geringer ist als eine mögliche zu erwartende Milchmenge,
Durchführen eines Melkvorgangs;
Ermittlung einer ermolkenen Milchmenge während eines Melkvorgangs;
Vergleich der ermolkenen Milchmenge mit der Zielmilchmenge;
Beendigung des Melkvorgangs, wenn die ermolkenen Milchmenge im Wesentlichen der Zielmilchmenge entspricht.

2. Verfahren nach Anspruch 1, bei dem ein Beginntag, der vor einem Trockenstelltag liegt, bestimmt und von dem an mit einer Zielmilchmenge gemolken wird.

3. Verfahren nach Anspruch 1 oder 2, bei dem die Zielmilchmenge zumindest für den Beginntag aus historischen Daten ermittelt wird.

4. Verfahren nach Anspruch 3, bei dem die Zielmilchmenge zumindest für den Beginntag tierindividuell ermittelt wird.

5. Verfahren nach einem der Ansprüche 1 bis 4, bei dem die Zielmilchmenge für jeden einzelnen Melkvorgang bestimmt wird.

6. Verfahren nach einem der Ansprüche 1 bis 4, bei dem die Zielmilchmenge für jeden Tag bestimmt wird.

7. Verfahren nach einem der Ansprüche 1 bis 6, bei dem vor einem Melkvorgang eine Identifikation des zu melkenden Tieres erfolgt.

8. Verfahren nach einem der Ansprüche 1 bis 7, bei dem die Zielmilchmenge für einen auf einen Melkvorgang folgenden Melkvorgang der Zielmilchmenge des vorhergehenden Melkvorgangs entspricht, wenn keine oder unvollständige Daten über die ermolkene Milchmenge des vorherigen Melkvorgangs vorliegen.

9 Verfahren nach einem der Ansprüche 1 bis 8, bei dem die Berechnung der Zielmilchmenge eines einzelnen Melkvorgangs aus der Tages-Zielmilchmenge angenommene Werte für die Berechnung verwendet, wenn der aufgezeichnete vorangegangene Melkvorgang keinen plausiblen Zeitabstand hat.

10. Verfahren nach einem der Ansprüche 2 bis 9, bei dem der Beginntag in Abhängigkeit von einem geplanten Trockenstelltag des Tieres, festgelegt wird.

11. Verfahren nach einem der Ansprüche 2 bis 10, bei dem zumindest ab dem auf den Beginntag folgenden Tag die Zielmilchmenge aus einer Referenzmenge um einen Änderungswert M verändert wird.

12. Verfahren nach Anspruch 11, bei dem der Änderungswert M konstant ist.

13. Verfahren nach Anspruch 11, bei dem der Änderungswert M eine insbesondere von einer Zeitspanne zwischen Beginntag und Trockenstelltag und/oder Laktationsstand abhängige Größe ist.

14. Verfahren nach Anspruch 11, 12 oder 13, bei dem der Änderungswert M tierindividuell, insbesondere zitzenindividuell ist.

15. Verfahren nach einem der Ansprüche 10 bis 14, bei dem zumindest ab dem auf den Beginntag folgenden Tag die Zielmilchmenge wenigstens in Abhängigkeit von einer Zeitspanne zwischen dem Beginntag und dem Trockenstelltag bestimmt wird.

16. Verfahren nach einem der Ansprüche 10 bis 15, bei dem die Melkvorgänge mit einer Zielmilchmenge bis zu einem Endtag, der vor dem Trockenstelltag liegt, durchgeführt werden, wobei in der Zeitspanne zwischen dem Endtag und dem Trockenstelltag wenigstens einmal vollständig ausgemolken wird.

17. Melkvorrichtung zur Durchführung eines Verfahrens nach einem der Ansprüche 1 bis 16 umfassend:
eine Melkeinrichtung (5);
Mittel zur Tieridentifikaton (8);
Mittel (7) zu Erfassung einer während eines Melkvorgangs ermolkenen Milchmenge;
eine Steuereinrichtung (6) zur Steuerung der Melkeinrichtung (5).

18. Melkvorrichtung nach Anspruch 17, wobei die Steuerung einen Computer, umfassend:
einen Speicher mit einem darin hinterlegten Computerprogramm, und
einen digitalen Prozessor
aufweist.

19. Computerprogrammprodukt, welches zur Durchführung eines Verfahrens nach einem der Ansprüche 1 bis 16 bestimmt und eingerichtet ist.

20. Maschinenlesbares Speichermedium, auf dem ein Computerprogrammprodukt nach Anspruch 19 gespeichert ist.

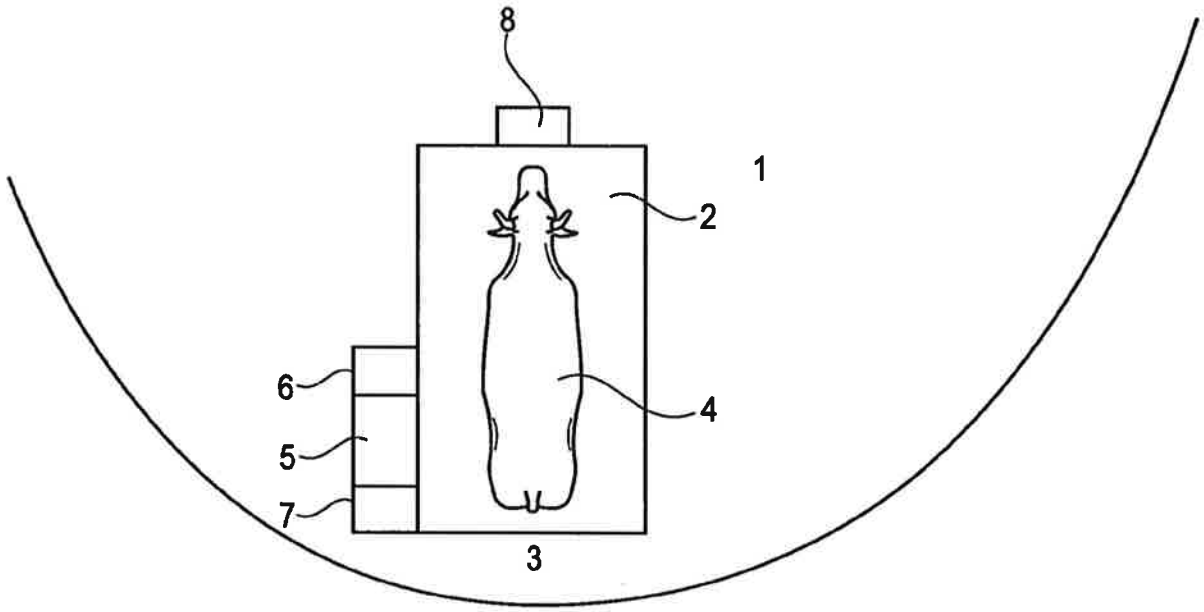

Fig. 1

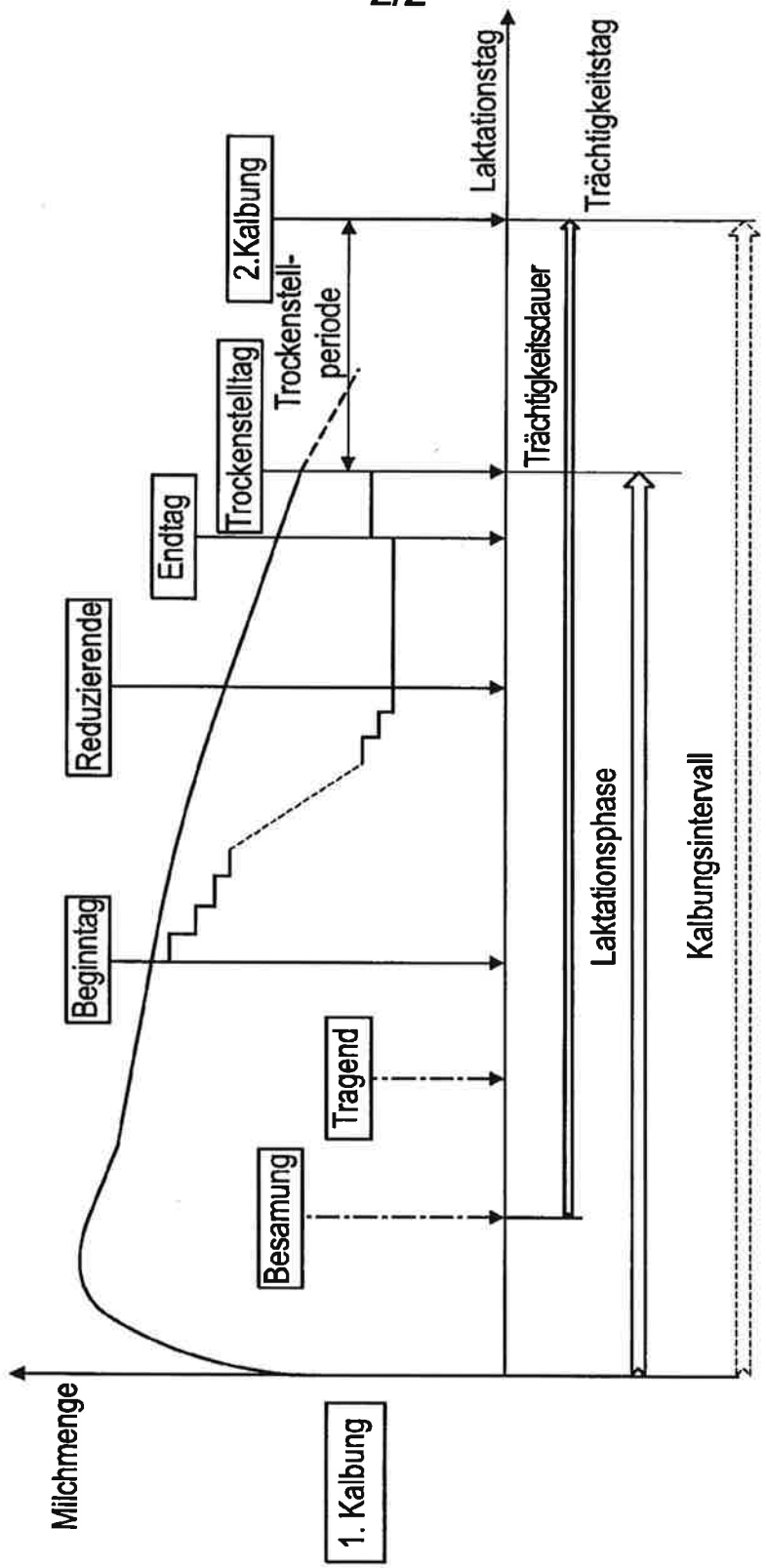
Laktationstag
Trächtigkeitstag
2.Kalbung
Trockenstell-
periode
Trockenstelltag
Trächtigkeitsdauer
Endtag
Reduzierende
Beginntag
Tragend
Besamung
Laktationsphase
Kalbungsintervall
Milchmenge
1. Kalbung

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/073948

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01J5/007
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	EP 1 125 492 A2 (MAASLAND NV [NL]) 22 August 2001 (2001-08-22) paragraph [0016] ----	1,5-9, 17-20 2-4, 10-16
X	WO 2006/068568 A1 (DELAVAL HOLDING AB [SE]; PETTERSON TORBJOERN [SE]; LUNDIN SOEREN [SE]) 29 June 2006 (2006-06-29) page 5 ----	1,5-9, 17-20

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 2018	22/11/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Moeremans, Benoit

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/073948

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1125492 A2	22-08-2001	DE 69429804 D1	21-03-2002
		DE 69429804 T2	27-03-2003
		DE 69434922 T2	08-11-2007
		DK 0657097 T3	27-05-2002
		DK 1125492 T3	04-06-2007
		EP 0657097 A2	14-06-1995
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		NL 9400630 A	16-06-1995
WO 2006068568 A1	29-06-2006	US 2008184936 A1	07-08-2008
		WO 2006068568 A1	29-06-2006

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
PCT/EP2018/073948

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
INV. A01J5/007
ADD.

Nach der Internationalen Patentklassifikation (IPC) oder nach der nationalen Klassifikation und der IPC

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
A01J

Recherchierte, aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)
EPO-Internal, WPI Data

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X A	EP 1 125 492 A2 (MAASLAND NV [NL]) 22. August 2001 (2001-08-22) Absatz [0016] ----	1,5-9, 17-20 2-4, 10-16
X	WO 2006/068568 A1 (DELAVAL HOLDING AB [SE]; PETTERSON TORBJOERN [SE]; LUNDIN SOEREN [SE]) 29. Juni 2006 (2006-06-29) Seite 5 ----	1,5-9, 17-20

☐ Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen ☒ Siehe Anhang Patentfamilie

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Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
15. November 2018	22/11/2018

Name und Postanschrift der Internationalen Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Moeremans, Benoit

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INTERNATIONALER RECHERCHENBERICHT

Angaben zu Veröffentlichungen, die zur selben Patentfamilie gehören

Internationales Aktenzeichen
PCT/EP2018/073948

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WO 2006068568 A1	29-06-2006	US 2008184936 A1	07-08-2008
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