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QFL QUALITE FILIERE LAIT
QMK QUALITÄT DER MILCHKETTE

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TEAT DIMENSIONS OF DAIRY COWS AND THEIR RELATION WITH UDDER HEALTH AS ASSESSED USING A NOVEL 2D-VISION-BASED DEVICE

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LIST OF ABBREVIATIONS

CCC	concordance correlation coefficient
CS	compound symmetry
CV	coefficient of variation
DHIA	Dairy Herd Improvement Association
DIM	days in milk
ICAR	International Committee for Animal Recording
IDF	International Dairy Federation
IMI	intramammary infection
ISO	International Organization for Standardization
LnqSCC	natural logarithm of quarter milk somatic cell count
LSM	least squares means
MPCV	mouthpiece chamber vacuum
NMC	National Mastitis Council
qSCC	quarter milk somatic cell count
SCC	somatic cell count
WHFF	World Holstein-Friesian Federation

PREFACE

Sustainable development has become increasingly important and is no longer a “hollow” term. It covers all sectors, including agriculture and thus dairy farming. Sustainability, meaning “producing for the needs of the present generation without harming third parties (including people and animals), future generations and the environment”, implies that a sustainable animal farming system should be economically viable, environmentally sound and socially acceptable (Blaha and Köfer, 2009). One pathway to achieve these goals is by optimizing resource efficiency, through feeding, breeding and animal health (Gerber and Steinfeld, 2010). Improved animal health, in particular udder health, will result in more milk production, less discarded milk, lower cull rate and longer shelf life of food products, factors which will also reflect in the financial outcome of higher milk price and lower costs. Furthermore, the environmental impact will be reduced as improved milk production per cow and lower replacement rate mitigate greenhouse gas emissions from dairy farms (Vellinga et al., 2011).

One of the most important diseases that threatens the sustainability of dairy farms is mastitis. Because of the multifactorial character of the disease, a holistic approach is needed to prevent and control mastitis. Much progress has been made in this respect but there is still room for improvement. Due to its close interaction with the teatcup liner, the teat, which acts as the primary defence mechanism against infectious agents, is currently receiving more attention.

CHAPTER 1

GENERAL INTRODUCTION

MILKING MACHINES

HISTORY AND DEVELOPMENT OF MILKING MACHINES

Milk is regarded as one of the most valuable food sources in nature since it is balanced for most nutrients and has a high caloric value. All mammals depend on this secretion as first sustenance in their early stages of development (Park and Haenlein, 2006). When in ancient Mesopotamia humans started animal domestication for meat and hides, milk and its derived yoghurt- and cheeselike products appeared to be unlooked-for benefits (Mendelson, 2008). Not surprisingly, milk meant survival, replenishment and fecundity in many ancient cultures (Vatsyayana, trans. 1999; Valenze, 2011). For example, in India all cattle were given full protection by religious law because a fertile cow’s milk could feed more people than its carcass (De Vylder, 2003). In other cultures, however, its perishability and the natural lactose intolerance of humans that generally occurrs with aging, aroused suspicion, fear and repulsion for the liquid. Nevertheless, milk was valued and even considered as building block of some civilizations (Valenze, 2011).

Although milk had proven to be an important product, not only in the development of the individual but also in the history of mankind, it was generally scarce. Growing populations increased the need for more supplies. As a result, herd sizes increased, and time and labour for milking, which happened generally by hand for centuries, intensified. To replace the heavy work of hand milking, ideas for machine milking became apparent approximately 175 years ago. Milking machines were constructed according to one of three principles (Erf, 1906; Hall, 1959; Hall, 1977).

The first principle, i.e. cannulae milking, consisted of inserting tubes into the cow’s teat. By means of gravity and intramammary pressure, milk could flow from the udder (Hall, 1977). This method threatens the udder and the teat through contamination and injury. Therefore, it was not generally accepted, albeit in certain conditions, as for milking mastitic udders, it may be practical (Erf, 1906).

The second principle imitates hand milking by applying pressure to the teat. At the end of the 19th century, a wide variety of pressure devices were developed and patented. Many used plates, bars, rollers or belts, controlled either mechanically, hydraulically or pneumatically (Erf, 1906). An example of a milking machine that used rollers to squeeze the milk from the teats is depicted in Figure 1A. Difficulties in operating and cleaning, the common belief of possible teat damage and low cost-effectiveness however, resulted in the disappearance of milking machines based on this principle before 1920 (Hall, 1977).

The third and eventually most successful principle uses suction to extract milk from the udder. Although mainly intended as a way to extract gunshot from wounds and for other medical procedures, the idea of using vacuum to milk cows probably came from two British inventors, Hodges and Brockenden, who patented it in 1851 (Hall, 1977). In 1860, milking machines with single-chambered teatcups and hand-operated pumps were developed. An example is presented in Figure 1B. These machines subjected the cow’s teat to constant vacuum, causing congestion and oedema. Furthermore, the milking machines suffered from fluctuating vacuum associated with the use of the hand pump. A new design used a reciprocating vacuum pump that produced cyclic pressure changes with each stroke and prevented the continuous milk flow. Udder problems, however, were reported with these vacuum changes. The idea of relieving the teats of constant vacuum was adopted and the next significant inventions concerned a “pulsator” and double-chambered teatcups (Hall, 1959; Hall, 1977). The principle of the double-chambered teatcups is used until today, and will be discussed in more detail.

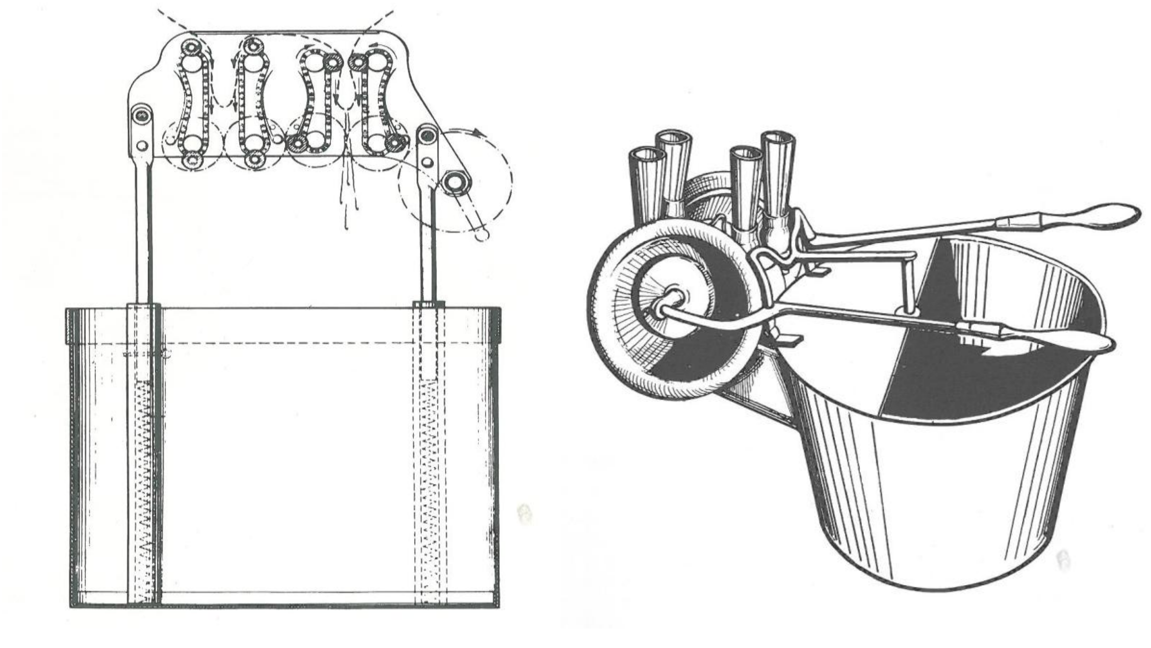


Figure 1. The Crees lactator (1881) using rollers on travelling chains to squeeze the milk from a pair of teats alternately (A). The Colvin hand-operated vacuum milker (1860) (B) (Source: Hall, 1977)

ACTION OF MILKING MACHINES WITH DOUBLE-CHAMBERED TEATCUPS

Most milking machines throughout the world use double-chambered teatcups to milk cows or other dairy animals. The double-chambered teatcup consists of an outer casing and an inner lining, thus forming an annular airspace in between, i.e. the pulsation chamber. A teatcup liner is defined as “a flexible sleeve, having a mouthpiece and a barrel, which may have an integral short milk tube” (ISO 3918:2007 (E/F)). The components of the double-chambered teatcup are presented in Figure 2.

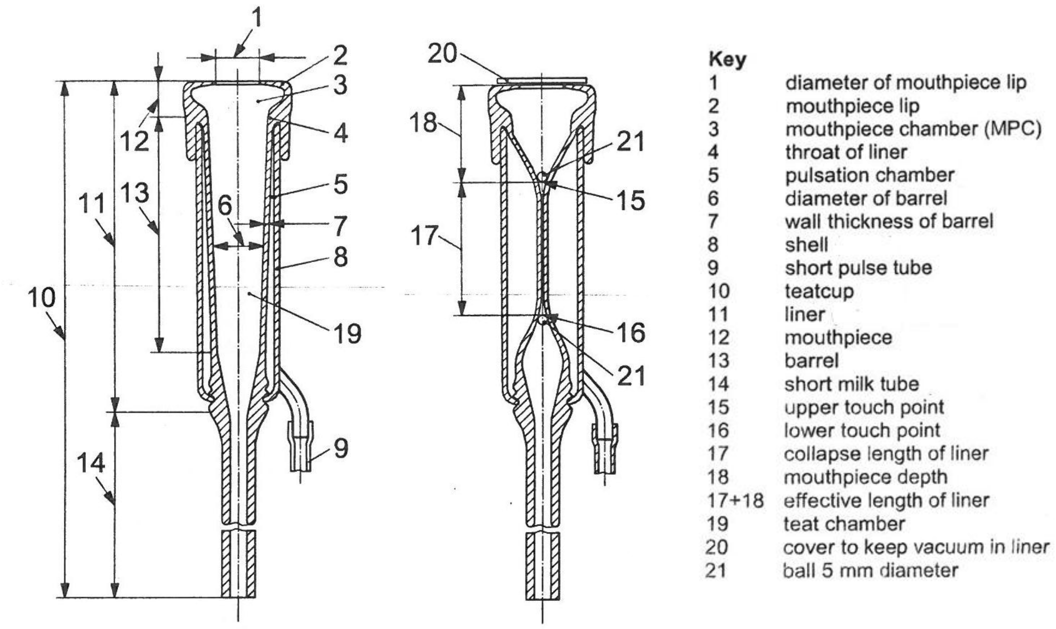


Figure 2. Components of the double-chambered teatcup (Source: ISO 3918:2007 (E/F))

Via the short milk tube the liner is connected to a milk claw and continuous vacuum. When the teat enters the liner, it is subjected to vacuum, which forces the teat canal to open by reducing the external air pressure, and thus milk flows from the udder. The vacuum has the additional function of transporting the milk to a collecting unit. Resistance occurs during transport of the milk through the milking pipelines. This resistance depends on the length of the pipelines and the difference in height. Recommendations for plant vacuums vary between the different types of installations. Plant vacuum levels of 48-50 kPa, 44-47 kPa, 40-44 kPa, and 40-45 kPa are recommended for highline milking parlours, milking parlours with recorders, lowline milking parlours, and automatic milking systems respectively (Remmelink et al., 2009). Both low vacuum levels (possibly resulting in cluster fall offs and liner slips) and high vacuum levels (possibly harmful to the teats by increasing the mechanical impairment of the teat tissue) can result in improper milking conditions (Hamann et al., 1994).

The ISO standard recommends an average vacuum applied to the teat end of 32-42 kPa during the peak flow period to ensure most cows will be milked quickly, gently and completely (ISO 5707:2007(E)). Continuous application of vacuum to the teat results in congestion and oedema, therefore pulsation is used to massage the teat. Pulsation is created by producing an alternating vacuum inside the pulsation chamber. Due to pressure differences caused by constant vacuum inside the liner and intermittent atmospheric pressure in the pulsation chamber, the liner collapses around the teat (Fig. 3). This action does not relieve the teat from constant vacuum but blood circulation is maintained (Dodd and Clough, 1959).

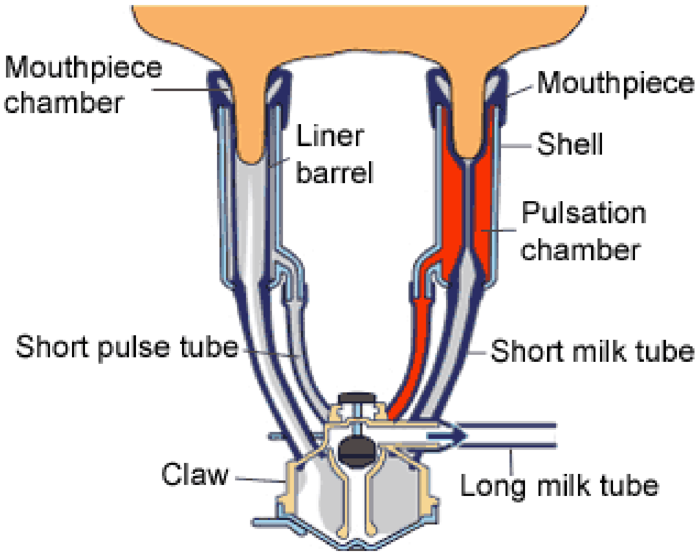


Figure 3. Action of the double-chambered teatcup cluster (Source: http://en.delaval.cn)

The mechanisms for the success of this principle were not completely understood at the time of first development. The main objectives described in the patent of the teatcup by Gillies in 1902, were to simulate hand milking by applying external pressure to the teat, but, unlike for hand milking or suckling of the calf, there is no squeezing of the milk from the teat in machine milking (Dodd and Clough, 1959). The need for the teat end to be completely surrounded by the liner to obtain adequate massage, was not recognized at that time.

Today, the main function of the teatcup liner to cyclically massage the teat to avoid congestion and oedema is widely acknowledged. Moreover, the function of all components and settings of the milking machine (vacuum level, pulsation rate, pulsation ratio, duration of the different pulsation phases, etc.), and milking procedures (premilking udder preparation, cluster attachment and detachment, overmilking, etc.), and their correlations with regard to the treatment of the teat during milking and related new intramammary infection risk are acknowledged as well, although even currently not completely understood.

TEAT DIMENSIONS

The udder consists of four quarters or mammary glands. Each quarter is a separate milk-secreting unit and possesses its own teat through which milk is drained from the udder (Cowie, 1959).

The teat consists of three parts (Fig. 4). The teat base forms the connection with the udder. The teat apex is the most distal part of the teat and comprises the teat canal. The intermediate part between teat base and teat apex is the largest and forms the teat barrel.

The length of the teat is generally determined as the distance from the teat apex to the point of connection to the udder.

Most studies determine the diameter at the middle of the teat (Binde and Bakke, 1984; Seykora and McDaniël, 1986; Lin et al., 1987; Coban et al., 2009), but diameters at other heights of the teat have been measured as well. Higgins et al. (1980) and Johansson (1957) have reported proximal and distal diameters. Using ultrasonographic scanning, the diameter just above the teat canal (Neijenhuis et al., 2001b; Gleeson et al., 2002; Gleeson et al., 2004), and 1.5 cm and 1 cm above the Fürstenberg rosette (Klein et al., 2005; Seker et al., 2009) have been measured. Other studies determined the teat diameter at a defined distance from the teat end or teat base (Chrystal et al., 1999; Borkhus and Rønningen, 2003; Weiss et al., 2004; Ambord and Bruckmaier, 2010).

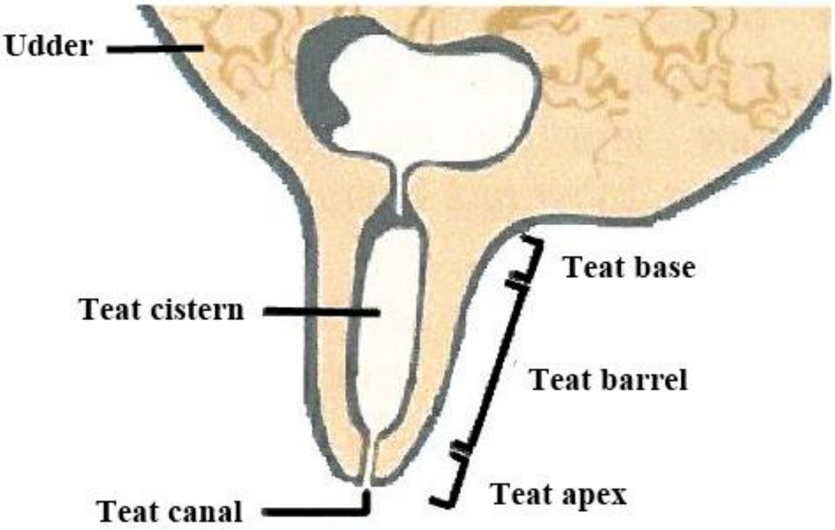
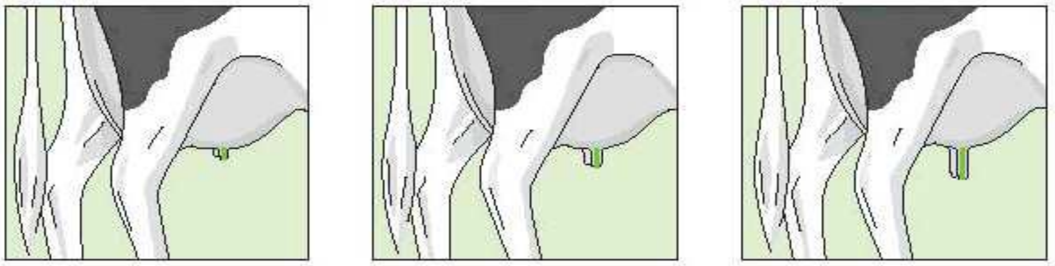


Figure 4. Cross-section of the udder and the teat with indication of the different parts of the teat (Adapted from https://www.teatseal.com.au/)

MEASURING METHODS

Teat length

Visual scoring. Visual scoring is a very fast, inexpensive and easy-to-use technique that allows data collection on a large scale. Not surprisingly, it is the general method to assess type traits in dairy cattle, including teat length. Traditionally, scoring was performed in relation to a desired optimum. Thompson et al. (1981), however, demonstrated that linear scoring, which uses a scale bounded by biological extremes that describes the degree of the trait and not its desirability, allows measurement of more genetic variation, and the results are easier to interpret. Therefore, type evaluation with linear scale was recommended. Nowadays, the use of linear assessment to determine teat length is customary. However, the subjectivity of the technique is recognized as a major source of variation (White, 1974). Different countries use different scoring scales (Marie-Etancelin et al., 2002). Some countries use a 50-point-scale. Sapp et al. (2003) suggest that a large number of score classes could lead to more inconsistency. In their study, they confirmed that some misclassification may be present with the 50-point-scale. The 10 class method is proposed to decrease the inconsistency by enabling better distinction of classifiers between classes and to potentially reduce the costs of data collection. To provide uniform and standardised information, the World Holstein-Friesian Federation (WHFF) has introduced the type harmonisation program (World Holstein-Friesian Federation, 2005). This program contains a list of approved standard traits which need to be included in the classification reports of different countries. The principles of the type harmonisation program have been adopted by the International Committee for Animal Recording (ICAR), which has formulated guidelines intended to standardise the methods of assessment. A linear scale from 1 to 9 is recommended (1 = short, 5 = intermediate, 9 = long) (Fig. 5). For application, the scale should cover the expected biological extremes of the population in the country of assessment. Either front or hind teats can be scored, but the choice of quarter position should be consistent in the entire system (ICAR, 2010). Although ICAR has formulated some recommendations to improve the accuracy and transparency of the visual scoring method (ICAR, 2010), the method remains limited in accuracy and precision due to its subjective nature.



Score 1 = short teat

Score 5 = intermediate teat

Score 9 = long teat

Figure 5. Nine-point scale for the linear visual scoring of teat length with score 1-3 for short teats, score 4-6 for intermediate teats, and score 7-9 for long teats (Source: ICAR, 2010)

Measuring tape, calliper and ruler. A variety of simple, quantitative methods have been used to objectively measure teat length for research purposes such as measuring tapes, callipers, and rulers (Johansson, 1957; Hickman, 1964; Rathore, 1976; McKusick et al., 1999; Tilki et al., 2005). During measurements the tool is placed next to the teat and the teat length is read from the scale. These methods are fast, inexpensive and easy-to-use. To our knowledge, however, no studies have determined their accuracy and precision.

Transparent open-ended tubes. Several studies have used transparent, open-ended tubes marked with a graduated scale to determine teat length in research studies. The internal diameter of the tubes varied between 30 and 45 mm. The teat is placed into the tube until the upper end of the tube touches the teat base. The internal diameter of the tube thus defines the teat base. The teat length is read either direct from the scale (Fig. 6A) (Neijenhuis et al., 2001a; Gleeson et al., 2002) or a piston in the lower end of the tube is raised until it contacts the teat apex to help determine the teat length (Fig. 6B) (Bakken, 1981; Hamann et al., 1993). The fixed opening of the tube reduces the variation in teat length measurements.

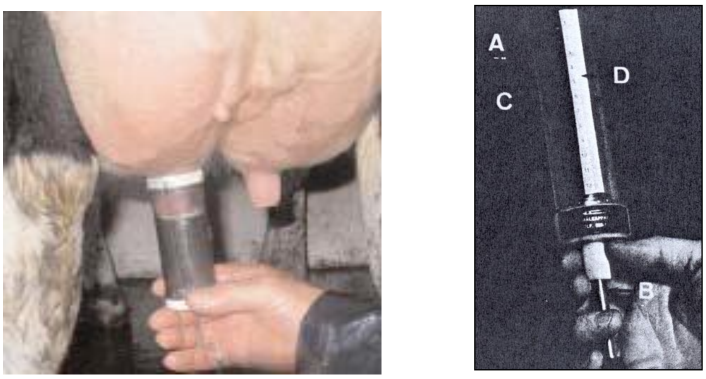


Figure 6. Transparent open-ended tube with graduated scale (A) without piston (Source: Gleeson et al., 2002) and (B) with piston (Source: Bakken, 1981)

Teat diameters

Visual scoring. Visual scoring of teat diameter is not as generally applied as teat length scoring. However, in some countries, such as Denmark, Sweden, and Finland, teat diameter is recorded in the national classification reports (Interbull, 2012). Consequently, it has been included in the type harmonization program of the WHFF as optional trait, but, unlike the "approved standard traits", it is not obligatory (World Holstein-Friesian Federation, 2005). Since teat diameter is commonly used by breeding organisations world-wide, ICAR has also adopted teat diameter in their guidelines for conformation recording in dairy cattle (ICAR, 2010). In general, the diameter at the middle of the teat is scored on a linear 9-point-scale (1 = thin, 5 = intermediate, 9 = thick).

Callipers and rulers. As for teat length, various studies have used simple, fast, inexpensive and quantitative methods to measure teat diameters such as callipers and rulers (Hickman, 1964; Higgins et al., 1980; Bakken, 1981; McKusick et al., 1999; Tilki et al., 2005). The diameter at a certain point of the teat is determined by placing the teat between the jaws of the calliper or by placing the ruler against the teat at that point, and consequently reading the diameter from the scale (Fig. 7).

Figure 7. Teat diameter measurement using a ruler

(Source: http://www.milkingmanagement.co.uk/contents/teat_health_studies.htm)

Cutimeter. The cutimeter is a device widely used to measure changes in skin thickness of cattle in reaction to tuberculosis tests. A modified version of this device, further referred to as modified cutimeter, has been described to measure teat diameter, teat barrel thickness, and generally teat end thickness (Hamann et al., 1996). The instrument consists of one fixed and one movable spring-loaded jaw plate (20 x 20 mm) connected to a calibrated calliper scale.

By positioning the teat end carefully between the open jaws and applying the spring pressure to the teat for a predefined time sufficient to allow the jaws to reach a steady state position on the teat (generally 1 or 2 seconds), the teat end thickness could be determined from the calibrated scale as the distance between the spring-loaded jaws. Differences in this distance before and after milking show the direction and the extent of changes in the mass of fluid and other teat tissues compressed between the jaws and have been defined as changes in teat end thickness (Hamann and Mein, 1988). Teat barrel thickness can be measured in the same way. Teat diameter can also be determined using the cutimeter, either by removing the calliper spring or by holding the jaws open manually to neutralize the spring pressure (Fig. 8).

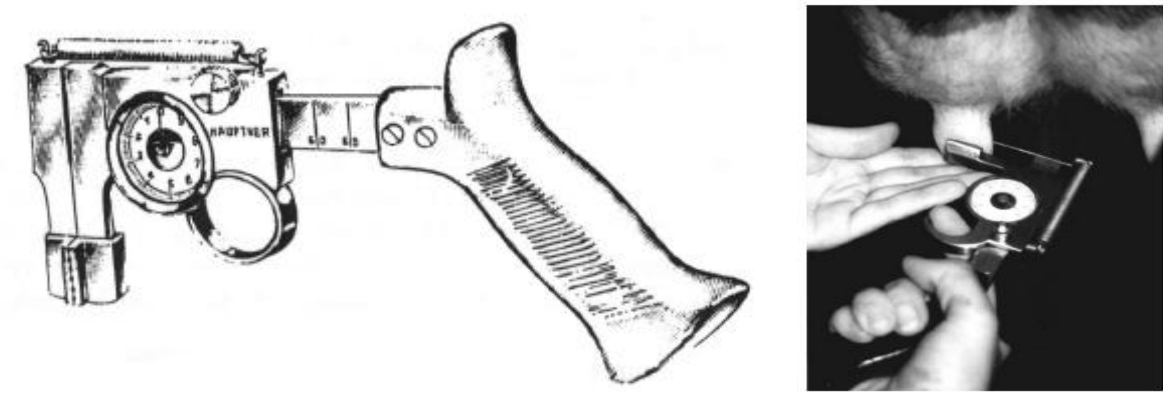


Figure 8. Teat diameter measurement using the cutimeter (Source: Hamann and Mein, 1988)

The accuracy and precision of the modified cutimeter have not been studied in regard to teat diameter, but they have been studied in regard to changes in teat end thickness. A high degree of accuracy (± 2%) was reported by Hamann and Mein (1988), although it is not clearly stated how this result was obtained. Isaksson and Lind (1992) reported an accuracy of 0.1 mm, i.e. pointer reading. A high degree of repeatability (r = 0.99) can be obtained provided reasonable care is taken by the operator (Hamann et al., 1996). The jaws of the cutimeter should be positioned on the same region of the teat, and with the same or similar orientation of the instrument per measurement. In addition, since teats become significantly thicker during teat contractions, measurements should be avoided when contractions are occuring. These recomendations also apply to teat diameter measurements. A similar degree of repeatability (r = 0.99) was found by Isaksson and Lind (1992) for changes in teat end thickness. In a study by Hamann et al. (1988), a coefficient of variation of 8-10% was reported for teat end thickness measurements performed with the modified cutimeter by the same operator. However, the repeatability of the device, although acceptable, remains questionable. The modified cutimeter measures teat thickness in respons to an applied pressure. This pressure depends on the applied force and the contact region between the cutimeter and the teat, and may therefore be influenced by the teat diameter and the teat shape.

Electronic calliper device. To overcome some disadvantages of the cutimeter, an electronic calliper device was developed to measure teat end thickness (Hamann et al., 1988). The device contains a pressure sensor placed at the fixed jaw plate, and thus enables to perform measurements at identical pressures, independent of the teat diameter or shape. Analogue to the cutimeter, the electronic calliper device consists of one fixed and one movable jaw plate, each with a surface area of 400 mm² (20 x 20 mm). The movable jaw is connected to a motor which moves with incremental steps of 0.5 mm. The pressure applied to the teat and the thickness of the teat are recorded at these closure intervals of the jaws until the predefined maximum pressure (generally 20 to 25 kPa) is reached. A schematic overview of the device is given in Figure 9. Because the recordings start at very low pressure (0.125 kPa), the first reading is a measurement of the diameter of the teat apex. A coefficient of variation as low as 2% between repeated measurements was reported for this device. However, as for the modified cutimeter, the obtainable precision depends on the care taken by the operator, i.e. the device should be placed at the same position on the teat and measurements during teat contractions should be avoided. When these precautions are taken, operators with no special training or skills can obtain precise results.

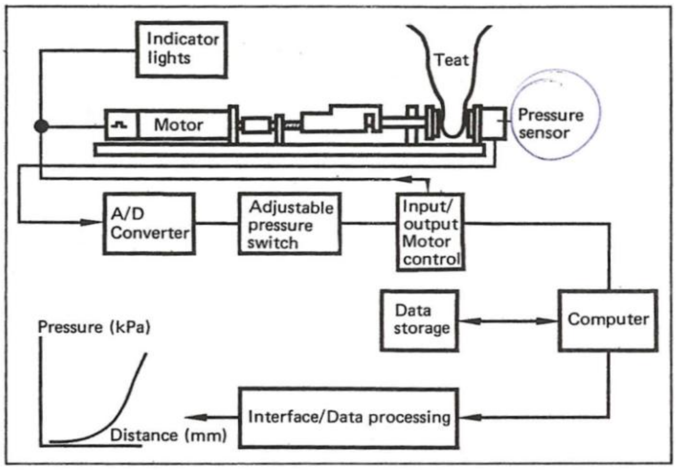


Figure 9. Schematic overview of the electronic calliper device (Source: Hamann et al., 1988)

Ultrasonography. Ultrasonography visualizes body structures by recording the echoes of ultrasonic waves directed into the tissue (Paulrud et al., 2005). This noninvasive technique has been extensively used for various animal measurements, such as gynaecological examination, as well as examination of the heart, lungs, liver, urinary tract, etc. (Nyland and Mattoon, 2002). Ultrasonographic scanning of the udder and teat is generally performed to diagnose milk flow disorders but is increasingly used as alternative method to measure teat morphology and teat tissue changes (Franz et al., 2009). To prevent deformation of the image of the teat, direct contact between the probe and the teat is avoided by placing the teat in a latex bag filled with warm water (35°C) (Fig. 10A). A lubricating gel is applied on the probe to improve the contact with the bag. When a clear image is obtained, the image is frozen on screen and stored for further analysis. Various teat characteristics can be determined from the image, such as teat canal length, cistern diameter, teat wall thickness, and teat diameter (Fig. 10B), either direct or by using a software program. Teat diameter has been measured just above the distal end of the teat canal (Neijenhuis et al., 2001b; Gleeson et al., 2002; Gleeson et al., 2004; Seker et al., 2009), 25 mm above the teat apex (Weiss et al., 2004; Ambord and Bruckmaier, 2010), 1 cm (Seker et al., 2009) and 1.5 cm above the Fürstenberg rosette (Klein et al., 2005). High repeatability (coefficient of variation of 3.6% between duplicate measurements and 4.4% between days) was reported for teat end diameter measurements (Neijenhuis et al., 2001b). However, a well-trained observer and substantial measuring time is needed to perform measurements, therefore the method is limited to experimental conditions (de Koning et al., 2003).

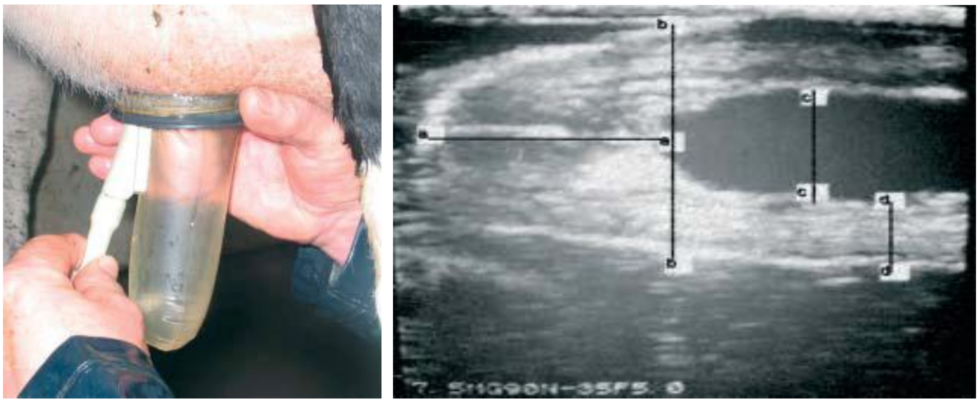


Figure 10. Teat placed in a latex bag filled with water for ultrasonographic scanning (A) and ultrasonographic scan with measurement of teat canal length (a), teat diameter (b), cistern diameter (c), and teat wall thickness (d) (B) (Source: Gleeson et al., 2002)

Teat length and diameters

Teat load monitor. Rønningen (2000) presented the "teat load monitor", a device initially constructed to measure the forces acting on the teat during milking, and in addition determines teat end position and teat end diameters. It concerns a modified version of the 3D dynamic photogrammetry method described by Maalen-Johansen (1992). The method uses mirrors and a camera in a housing to make photographic images of the teatcup liner which is placed in a teatcup shell with a transparent window. A grid of circular retroreflective targets marked on the liner enables the construction of a 3D profile of the liner (Fig. 11). The collapsed liner applies pressure on the teat, and thus reflects the shape of the compressed teat. The accuracy of the system is within 0.2 mm in the x, y, and z-direction. The teat end position is estimated from the profile and is a key factor in determining the other teat characteristics. The length of the teat during milking is the position of the teat end relative to the mouthpiece of the liner. The teat end diameters are determined from the cross section of the profile at fixed distances (5, 10 and 15 mm) from the estimated teat end position. The method was reported to have good repeatability. Diameters other than those at the teat end could be measured in the same way (Rønningen, personal communication). However, the method is based upon the fact that the liner touches the teat in the area of interest. It is questionable whether the liner is in contact with the teat at higher regions, such as the teat base, therefore accuracy might be poor at those positions. Since the curvature of the liner around the teat during milking is needed to perform teat measurements, and vacuum and the collapsing of the liner may alter the teat diameters, determination of the actual teat end diameters is not possible. Hence, the measurements differ from those performed by the other methods.

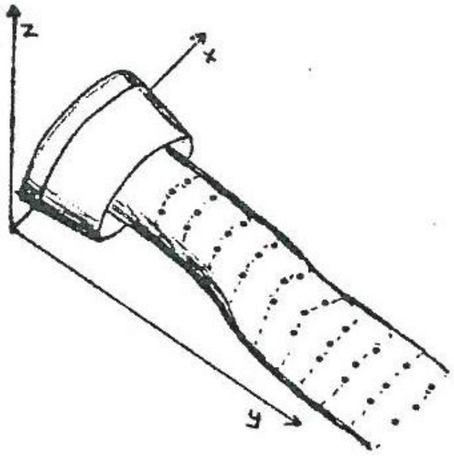


Figure 11. Collapsed liner with a grid of circular retroreflective targets, and axis orientation in the 3D-system (Source: Rønningen, 2000)

Image analysis. Imaging systems have the advantage of enabling determination of multiple teat characteristics without manipulating the teat, with decreased risk for the operator and with reduced effort. Due to the high accuracy of imaging systems in combination with the positive price evolution and robustness of cameras, the use of these techniques in different agricultural sectors has increased considerable during the last decades (Qian et al., 2008; Hijazi et al., 2010). Some studies have used photographs or video camera recordings to measure teat length and teat diameters manually (Amin et al., 2002; Borkhus and Rønningen, 2003). The progress of digital technology and image analysis software offers new perspectives for measuring teat dimensions, and image analysis techniques have been proposed to measure udder and teat morphology in dairy ewes (Marie-Etancelin et al., 2002). Although the mean teat length and diameter at the teat base measured with image analysis were not substantially different from those measured *in vivo* using a calliper, the correlations between the two methods were low (0.57). Repeatability was lower for the teat length measurements extracted by image analysis compared to direct measurements (0.65 vs 0.76) whereas for diameter at the teat base repeatability was higher (0.65 vs 0.61). Reproducibility was low for diameter at the middle of the teat (0.64), but moderate to high for diameter at the teat base and teat length (correlation of 0.80 and 0.96 respectively). It is stated that better results could be obtained by improving the quality of the image and the lightening of the lowest part of the udder. The technique is more objective compared to scoring and appears to be very promising. The application of the technique, however, is elaborate and for the moment only possible under experimental conditions.

Baert et al. (2008a; 2008b) have presented a 3D teat measuring device based on image analysis (Fig. 12).

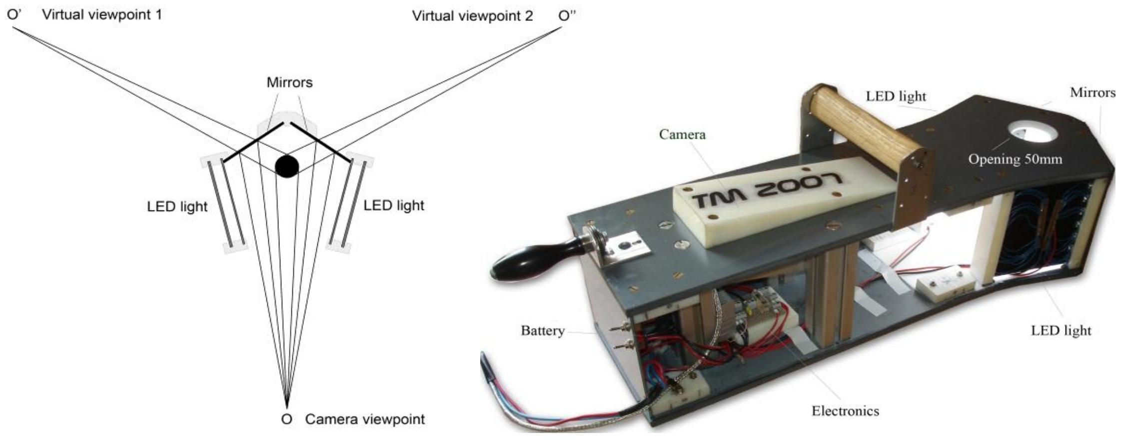


Figure 12. Vision-based measuring device for teat morphology (Source: Baert et al., 2008b)

The device uses two mirrors to obtain a full surrounding view of the teat. Using image processing, teat length and diameters are calculated and a 3D model of the teat is constructed (Fig. 13). Besides measuring teat dimensions of the whole teat in a single measurement, the measuring method has the advantage that additional information may be extracted from the image, such as teat (end) shape, teat colour and the orientation of the teat in the measuring device. The measuring device is fast and easy-to-use, however, due to its weight (9 kg) and size, it is ergonomically not feasible to do large scale measurements in a short time period. Furthermore, the device has experienced some problems with reaching certain teats due to their position on the udder (Baert, personal communication). As for the method presented by Marie-Etancelin (2002), the technique is very promising, although at least for the moment restricted to experimental conditions.

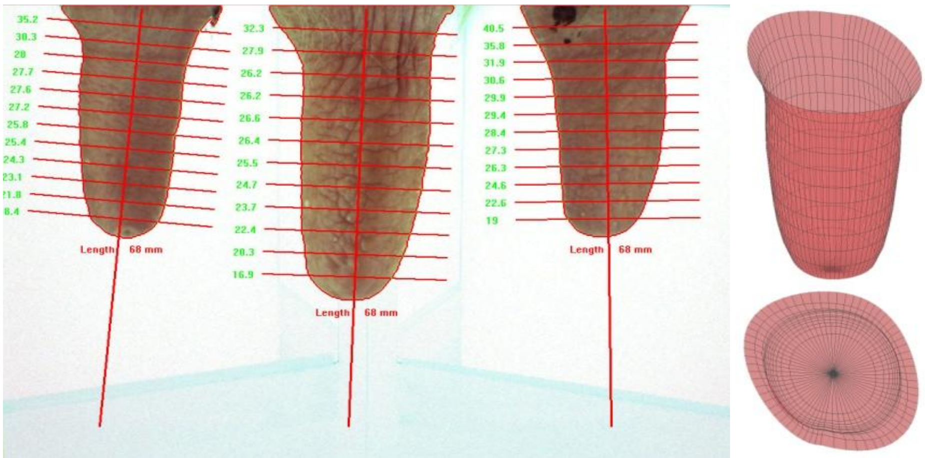


Figure 13. Automatic determination of teat dimensions and construction of a 3D model (Source: Baert et al., 2008b)

Conclusions

Many of the described teat dimension measuring methods are widely used (scoring, tapes, callipers, rulers, transparent open-ended tubes, cutimeters). However, their accuracy and precision are often unknown, low or questionable. Furthermore, apart from scoring and imaging systems (including ultrasonography), the measuring methods require measurements at different points of the teat to evaluate the whole teat, which is inevitable time-consuming. As a result, gathering information on a large scale is difficult. In this respect, the imaging systems have many advantages over the other methods, however, for the moment their applications are restricted to experimental conditions.

It can be concluded that the existing methods all have their advantages and disadvantages, but until now no objective, accurate, precise and fast method capable of measuring teat dimensions on a larger scale and with reduced effort for the operator is available. A method that meets these requirements, however, would be a large step forward in collecting data on teat morphology. The use of digital image processing appears very promising.

VARIATION IN TEAT DIMENSIONS

Teat length and diameters

Tables 1 and 2 give an overview of average teat lengths and teat diameters for front and hind quarters reported in literature with indication of number of cows or quarters in the study, measuring method, breed and parity.

Table 1. Overview of average teat length measurements (in cm)

	N_{cows}	$N_{quarters}$	Method	Breed	Parity	Front	Hind
Johansson (1957)	434		Calliper	SRB[2]	-	5.6	4.7
	308			SLB[3]	-	7.7	6.7
Andreae (1963)	60		Measuring tape	SBT[4]	1	6.5	5.4
					2	7.0	5.8
					3	7.6	6.0
Higgins et al. (1980)	402		-	HF[5]	-	5.6	4.5
Bakken (1981)	504		Transparent tube	NR[6]	1	5.0	4.3
Batra & McAllister[1] (1984)	713		-	HF	1	5.1	4.3
	446			A[7]	1	4.5	3.9
Binde & Bakke (1984)	138		Transparent tube	NR	-	4.9	4.4
Seykora & McDaniël (1985)	898		-	HF	-	5.0	
Seykora & McDaniël (1986)	-	5934	Special device	HF			
					1	5.0	4.2
					2	5.3	4.4
					3	5.5	4.6
					4	5.6	4.6
					5	5.6	4.8
Michel & Rausch (1988)	29		-	SMR[8]	1	5.7	
	27				2	6.1	
	29				3	6.9	
	21				4	7.1	
	15				5	7.1	
Rogers & Spencer (1991)	97		Special device	HF	-	5.9	4.9
Weiss et al. (2004)	38		Gauge	BS x GB[9]	-	6.7	5.7
Graff (2005)	2.109		Ruler	SBT	-	5.5	4.5
Tilki et al. (2005)	18		Measuring tape	BS[10]	1	5.7	4.8
	21				2	5.3	4.4
	15				3	5.9	4.9
	13				4	6.8	5.5
	10				5	5.9	5.0
	17				5+	6.1	5.2

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[1] Measurements within 24 h after calving

[2] Swedish Red Breed

[3] Swedish Holstein

[4] Schwarzbunt

[5] Holstein Friesian

[6] Norwegian Red

[7] Ayrshire line

[8] Schwartzbuntes Milchrind

[9] Brown Swiss x German Braunvieh

[10] Brown Swiss

Table 2. Overview of average teat diameter measurements (in mm)

	N_{cows}	$N_{quarters}$	Method	Breed	Parity	Position	Front	Hind
Johansson (1957)	434		Calliper	SRB[2]	-	B[12]	26.7	25.9
	308			SLB[3]	-	B	31.8	31.6
Higgins et al. (1980)	402		Calliper	HF[4]	-	B	36.4	33.8
						M[13]	24.4	24.8
						A[14]	17.5	17.7
Bakken (1981)	504		Calliper	NR[5]	1	M	21.7	21.4
Batra & McAllister[1] (1984)	713		Calliper	HF	1	M	25.0	25.0
	446			A[6]	1	M	25.0	25.0
Binde & Bakke (1984)	138		Calliper	NR	-	M	22.0	22.1
Seykora & McDaniël (1985)	898		-	HF	-	M	21.0	
Seykora & McDaniël (1986)	-	5934	Calliper	HF				
					1	M	23.0	22.4
					2	M	24.8	24.0
					3	M	25.6	24.9
					4	M	26.0	25.3
					5	M	26.1	25.5
Rogers & Spencer (1991)	97		Calliper	HF	-	M	25.0	25.8
Weiss et al. (2004)	38		Ultrasonography	BS x GB[7]	-	A + 2.5 cm	27	28
Graff (2005)	2.109		Template	SBT[8]	-	A + 2 cm	23.8	23.5
Klein et al. (2005)	68	195	Ultrasonography	HF	-	F[15]	22.4	
	87	221		BS[9]	-	F	23.3	
	83	283		S[10]	-	F	25.2	
	31	102		S x RP[11]	-	F	25.0	
	68	119		HF	-	F + 1.5 cm	25.9	
	87	192		BS	-	F + 1.5 cm	27.8	
	83	196		S	-	F + 1.5 cm	28.3	
	31	67		S x RP	-	F + 1.5 cm	29.1	
Tilki et al. (2005)	18		Calliper	BS	1	M	20.6	20.5
	21				2	M	20.3	20.3
	15				3	M	21.6	20.8
	13				4	M	24.6	23.8
	10				5	M	22.8	21.7
	17				5+	M	23.0	22.0
Seker et al. (2009)	17	33	Ultrasonography	HF	-	F	21.1	
	29	56		BS	-	F	21.2	
	14	27		S	-	F	22.6	
	17	33		HF	-	F + 1 cm	26.8	
	29	56		BS	-	F + 1 cm	28.2	
	14	27		S	-	F + 1 cm	28.7	

[1] Measurements within 24 h after calving
[2] Swedish Red Breed
[3] Swedish Holstein
[4] Holstein Friesian
[5] Norwegian Red
[6] Ayrshire line
[7] Brown Swiss x German Braunvieh
[8] Schwarzbunt
[9] Brown Swiss
[10] Simmental
[11] Simmental x Red Pied crossbreed
[12] Diameter at the teat base
[13] Diameter at the teat middle
[14] Diameter at the teat apex
[15] Diameter at the Fürstenberg rosette

Factors associated with teat length and diameters

Breed. Teat length differs between breeds of cows (Wufka and Willeke, 2001). Teat diameter at the Fürstenberg rosette significantly differed between pure breeds (Holstein Friesian, Brown Swiss, and Simmental), but not between pure and crossbred Simmental in a study by Klein et al. (2005), whereas Seker et al. (2009) reported only a significant difference between Simmental and crossbreeds. Furthermore, teat diameter 1 cm above the Fürstenberg rosette did not differ significantly between Holstein cows, Brown-Swiss, Simmental and crossbreeds in the study by Seker et al. (2009), contrary to the significant difference in diameter 1.5 cm proximal of the Fürstenberg rosette between all breeds found by Klein et al. (2005). The differences between breeds at other heights of the teat have not yet been studied.

Quarter position. Front teats are generally longer and smaller than hind teats (Weiss et al., 2004; Tilki et al., 2005).

Parity. Teat length and teat diameter increase with parity, although not always significant (Seykora and McDaniël, 1986; Tilki et al., 2005; Seker et al., 2009).

Lactation stage. Little agreement exists on the relation between stage of lactation and teat dimensions. An increase in teat length during early lactation was noted by some authors (Seykora and McDaniël, 1986; Wufka and Willeke, 2001; Graff, 2005) and Tilki et al. (2005) found a significant increase with advancing lactation only in hind teats in a study on Brown Swiss cows. In contrast, Michel and Rausch (1988) reported no significant association between lactation stage and teat length. According to some studies, teat diameters do not change significantly as the lactation progresses (Tilki et al., 2005; Seker et al., 2009), whereas one study detected a significant decrease between first and subsequent stages of lactation (Graff, 2005).

Conclusions

General agreement exists on the association between teat dimensions and quarter position and parity, but consensus on the effect of lactation stage and breed is lacking. Contradictory findings between studies may be due to different breeds, classifications, measuring methods and low precision of some methods. To gain better insight in the existing variation in teat dimensions, studies performed using an accurate and precise measuring method are required. In addition, no studies have quantified the variation in teat dimensions present at the different levels of the hierarchy (herd, cow, quarter). Knowledge on the distribution of the variation is, however, a prerequisite to improve the herd management with regard to teat dimensions most effectively.

TEAT DIMENSIONS AND UDDER HEALTH

Udder health

Mastitis, or inflammation of the mammary gland, is a response to injury caused by infectious agents and their toxins, physical trauma or chemical irritants. Although an intrammamary infection (IMI) is not required for mastitis to exist, nearly all mastitis cases are due to IMI caused by microorganisms. In respons to the infection, the cow's immune system is activated and neutrophil migration from the blood to the milk is promoted, causing an increase in milk somatic cell count (SCC). Two forms of mastitis can occur. *Clinical mastitis* is characterized by visible changes in the udder and the milk, such as swelling or redness of the udder, or milk with abnormal appearance, and may lead to lethargy, anorexia, and in severe cases even death. *Subclinical mastitis* occurs without obvious clinical signs but SCC is elevated and milk production is generally decreased, although not as pronounced as for clinical mastitis (Harmon, 1994).

Mastitis is considered to be one of the most common and costly diseases in dairy cattle. Economic losses are due to reduced milk yield, veterinary and treatment costs, higher probability of culling, etc. (Halasa et al., 2007). Good quality milk is also of major importance for the dairy processing industry since high SCC is associated with lower cheese yield, slower rate of curd formation, decreased shelf life and altered organoleptic properties of pasteurized milk (Le Roux et al., 2003). In addition, the potential presence of antibiotic residues in the milk from treated cows can impair the processing because bacteria are needed for the manufacturing of most dairy products. The use of antibiotics is also of great concern for public health since antimicrobial drug use may contribute to the development of antimicrobial resistance of human pathogens. For public health, the absence of pathogenic bacteria is essential as well (LeBlanc et al., 2006). Most dairy products are produced on the basis of heat treated milk, thus inactivating the vegetative bacteria present in the raw milk. Mastitis also threatens animal welfare and is therefore important from an ethical point of view. Furthermore, increasing consumer awareness directs the demand for high quality milk of healthy cows (Heringstad et al., 2000).

As the vast majority of IMI in dairy cows results from bacteria gaining entry to the mammary gland through the teat canal, it is not surprising that the teat has several anatomic features to prevent penetration of bacteria into the udder. Prevention occurs either i) by minimizing the number of bacteria that may potentially enter the teat canal, ii) as physical obstruction, or iii) by removing or eliminating bacteria that have entered the teat canal. First, the teat skin, and in particular the *stratum corneum*, has a broad set of protective properties which limit pathogen colonization, such as low water content, acidic pH, resident microflora, and antimicrobial lipids and proteins deposited at the surface (Elias, 2007). Second, the *stratum corneum* acts as physical barrier through the structural organization of the corneocytes which resemble a “brick wall”. In addition, the teat end contains sphincter muscles that maintain tight closure between milkings and thus prevent entrance of environmental agents (Rainard and Riollet, 2006). Another physical obstruction to bacteria is the keratin plug which seals the teat canal during the nonlactating period. Third, a layer of keratin generally covers the teat canal. Keratin is a waxy material that can physically entrap certain bacteria. During milking, the superficial layers of keratin with the entrapped microorganisms are removed. Continuous replacement of the keratinized cells occurs by outward differentiating epithelial cells (Capuco et al., 1994; Paulrud, 2005; Rainard and Riollet, 2006). Furthermore, bacteriostatic and bactericidal properties are reported for keratin. Another anatomic feature is the Fürstenberg rosette, situated at the base of the teat canal where it forms the boundary with the teat cistern. The epithelial cells associated with the Fürstenberg rosette are believed to secrete bacteriostatic agents and thus eliminate bacteria that have passed through the teat canal (Reece, 2009).

Mastitis is a complex, multifactorial disease in which the pathogens, the cow as well as the management determine whether a quarter will become infected. Cow factors that are associated with udder health include the stage of lactation (Bunch et al., 1984; Houben et al., 1993), the level of milk production (Schukken et al., 1991; Houben et al., 1993), the udder health history of the cow (Rupp and Boichard, 2000; Piepers et al., 2009), and genetics (Schukken et al., 1999). Management factors associated with udder health include housing, milking and cleaning procedures, and the milking machine (O'Shea, 1987; Schukken et al., 1991; Capuco et al., 1994). Because of the multifactorial character of the disease, a holistic approach is enivitable to prevent and control mastitis. The National Mastitis Council (NMC) has provided a program that focuses on management. This well-known 10-point plan consists of establishment of goals for udder health; maintenance of a clean, dry, comfortable environment; proper milking procedures; proper maintenance and use of milking equipment; good record keeping; appropriate management of clinical mastitis during lactation; effective dry cow management; maintenance of biosecurity for contagious pathogens and marketing of chronically infected cows; regular monitoring of udder health status; and periodic review of mastitis control program (NMC, 2012). The succesful implementation of holistic mastitis control programs has resulted in decreased incidence of the disease (Bradley, 2002).

Teat condition

Since the teat acts as primary defense mechanism against infections, a healthy teat condition is of paramount importance. Changes in teat tissue, especially from the teat skin, teat canal, and the teat end, may predispose the quarter to IMI due to increased colonization with bacteria or due to inferior resistance against bacteria. Teat tissue changes are either machine milking-induced or originate from environmental or infectious factors (Hillerton et al., 2001). Changes can occur after short, medium or long term (Mein et al., 2001). As indicated earlier, the teat skin acts as inhibitor to bacterial growth. Bruises, lesions, chemical irritation, warts, etc. may impair this function, thus forming a reservoir of mastitis infections. Particularly *Staphylococcus aureus* and *Streptococcus dysgalactiae*, contagious pathogens with fibronectin binding surface components, can colonize the epithelium and adhere to tissue lesions (McGavin et al., 1993; Fox and Norell, 1994; Blowey and Edmondson, 2010). Furthermore, alterations in the barrier function of the *stratum corneum* may decrease the resistance to ingress of pathogens (Elias, 2007). Severe teat end callosity or hyperkeratosis may also provide a reservoir of mastitis infections because the cracks can act as refuge for bacteria to multiply. Teat end callosity does not result from erosion, eversion or prolapse of the teat tissue or the orifice, but consists of localized hyperplasia of the *stratum corneum*, as indicated by histological studies (Neijenhuis et al., 2001c). Although teat end callosity is often considered as risk factor for IMI for the abovementioned reasons, consensus is lacking and evidence is thin (Sieber and Farnsworth, 1981; Bakken, 1981; Neijenhuis et al., 2001a). Teat end callosity may also affect the rate and completeness of the teat canal closure (Neijenhuis et al., 2001c), as can fatigue of the sphincter (Neijenhuis and Hillerton, 2002), congestion, and oedema (Paulrud, 2005). Congestion and oedema may develop when the existing removal of blood and interstitial fluids due to massage is insufficient. These conditions may impair the blood supply to the tissue which is necessary for optimal functioning of the defence against pathogens, such as neutrophil movement and activity (Hamann and Osteras, 1994), and may thus result in inferior resistance. Changes in teat tissue thickness are generally used as indicator for fluid accumulation in the teat tissue. Increases in teat end thickness of 5% or more were found to be significantly associated with increased teat duct colonization with environmental pathogens (Zecconi et al., 1992) and with new IMI (Zecconi et al., 1996). Hamann & Mein (1996) formulated a lower threshold of -5% change in teat end thickness post milking compared with pre milking because too high cyclic pressures applied to the teat by the liner might remove fluid from the blood vessels and thus restrict the blood supply to the tissues. Circulatory impairment will influence the keratinization of the teat canal lining, the muscle capacity to close the teat canal, the movement of neutrophils from blood to milk, and oxygen concentrations needed for elimination of pathogens by phagocytosis (Hamann and Osteras, 1994).

Teat dimensions and udder health

The physical characteristics of the teat may influence the predisposition to poor teat condition and udder health.

The length of the teat has been found not to be significantly associated with mastitis incidence (Hickman, 1964), mastitis prevalence (Binde and Bakke, 1984), and SCC (Seykora and McDaniël, 1986; Coban et al., 2009). However, Rogers et al. (1991) suggested that longer teats might be more prone to injury. Intermediate scores were associated with the lowest risk of culling in Czech Flekvieh cows (Zavadilova et al., 2009). Furthermore, positive genetic correlations were identified for teat length and clinical mastitis (Van Dorp et al., 1998), for teat length and SCC (Rogers et al., 1991), and a negative genetic correlation was found between teat length and functional longevity (Larroque et al., 1999), indicating that cows with longer teats are genetically more likely to develop mastitis or to be culled.

The diameter of the teat is reported to have a significant effect on SCC, with increasing SCC for wider teats (Higgins et al., 1980; Seykora and McDaniël, 1986; Chrystal et al., 1999). A tendency towards higher incidence of mastitis with increased teat diameter was found by Hickman (1964), and larger than herd-average teat diameter was identified as risk factor for mastitis by Slettbakk et al. (1995). Similar, cows with extremely thick teats were more likely to be culled than those with thin ones, but an intermediate optimum exhibited the lowest risk of culling (Zavadilova et al., 2009). Larger diameter teats tend to have larger teat orifices and wider teat canals (Rathore and Sheldrake, 1977; Chrystal et al., 1999), what may explain the observations between teat diameter, SCC and mastitis. Some authors, however, found no relationship between teat diameter and IMI (Bakken, 1981), mastitis prevalence (Binde and Bakke, 1984), and SCC (Porcionato et al., 2010). A low positive genetic correlation was reported between teat diameter and SCC (Seykora and McDaniël, 1985).

Teat dimensions and teatcup liner design, and teat condition and udder health

Because of the close interaction between teat and teatcup liner, teat morphology is an important factor in choosing the most optimal liner for a herd. An ideal match between teat morphology and liner design should provide an efficient and complete milk out and should result in good teat condition and udder health (McGrath and O'Shea, 1972).

Mismatching between the liner and the teat can be very diverse. For example, teats with a large diameter may have difficulties in penetrating the liner through a (too) small mouthpiece opening (Rasmussen et al., 2004), whereas when the bore of the liner is too wide for a given teat, the mouthpiece chamber vacuum (MPCV) increases. High level MPCV is suggested to cause congestion and discomfort (Newman et al., 1991; Rasmussen, 1997). When the combination of liner and teat fails to maintain a seal between the teat and the mouthpiece lip, air leakage through the mouthpiece opening may occur, resulting in abrupt loss of vacuum (low MPCV) and liner slips (Borkhus and Rønningen, 2003). These conditions may transfer bacteria or bacteria-contaminated milk droplets from outside the teat into the teat canal or teat cistern and thus increase the incidence of IMI (Baxter et al., 1992). Since both a very low and very high MPCV are associated with poor udder health, a medium high MPCV (range 10-30 kPa) should be obtained (Ronningen and Rasmussen, 2008). Teats that are too short for a given liner will not reach the collapsing point of the liner, while teats that are too long, penetrate the liner below this point. In both cases, the liner cannot massage the teat end, resulting in poor teat end condition (Fig. 14) (Mein et al., 2004; Rasmussen et al., 2004). The effective length of the liner depends on the liner bore. Since teats penetrate deeper in wide bore liners, a larger effective length is needed.

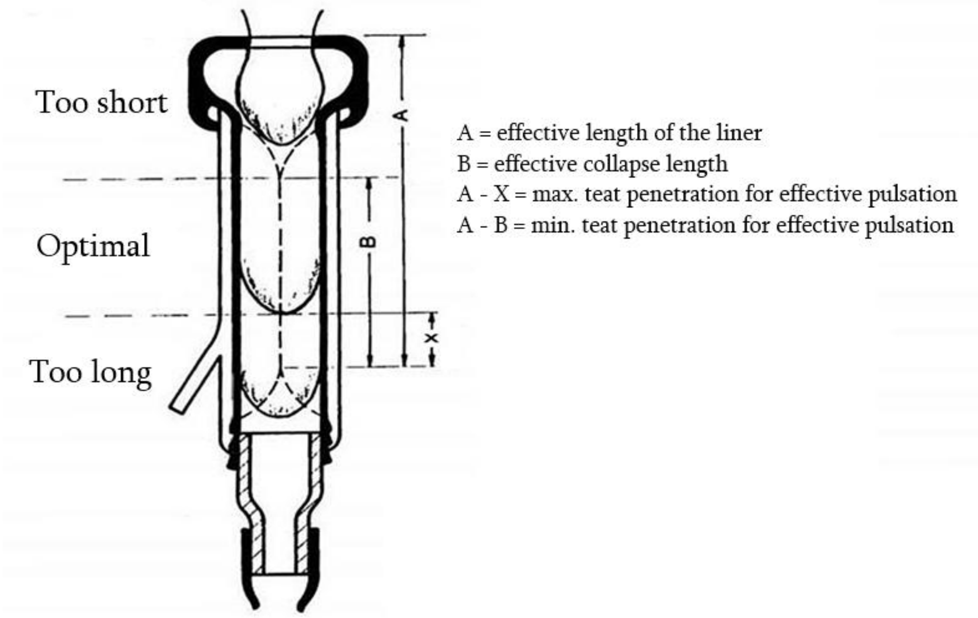

Figure 14. Teat penetration in relation to effective pulsation of the liner (Adapted from Mein et al., 2004)

Conclusions

Despite some agreement on the relation between teat dimensions and udder health, there is no general consensus. Different definitions of mastitis, study designs, and breeds may account for contradictory results. Furthermore, short-term changes in the teat tissue (induced by a single milking) are known to potentially impair the defence mechanism of the teat against IMI. Nevertheless, no studies have reported on the effect of changes in teat length or diameters due to milking on udder health. Knowledge on the association between teat dimensions and their milking-induced changes, and udder health, however, might help in safeguarding or improving the udder health status of the herd.

CONCLUSIONS

Mastitis is one of the most important diseases threathening the sustainability of dairy farms. Because of the multifactorial character of the disease in which the cow and the management play a major role, a holistic approach is needed to prevent and control mastitis. As teat dimensions, and possibly the milking-induced changes in teat dimensions, affect udder health, insight in the existing variation in teat dimensions is required to improve the udder health. Currently, teat dimension measurements are generally performed using methods which are subjective, lack accuracy, and have low or unspecified precision. Knowledge on the factors associated with teat dimensions is therefore often contradictory and the distribution of the variation over the different levels (herd, cow, quarter) is unknown. Using an accurate and precise measuring method the variation in teat dimensions, the associated factors, as well as the associations between teat dimensions, their milking-induced changes and udder health can be clarified. A schematic overview of some factors associated with udder health with indication of the area of interest of this thesis is depicted in Figure 15. Figure 16 presents the area of interest in more detail with indication of the gaps leading to the aims of this study.

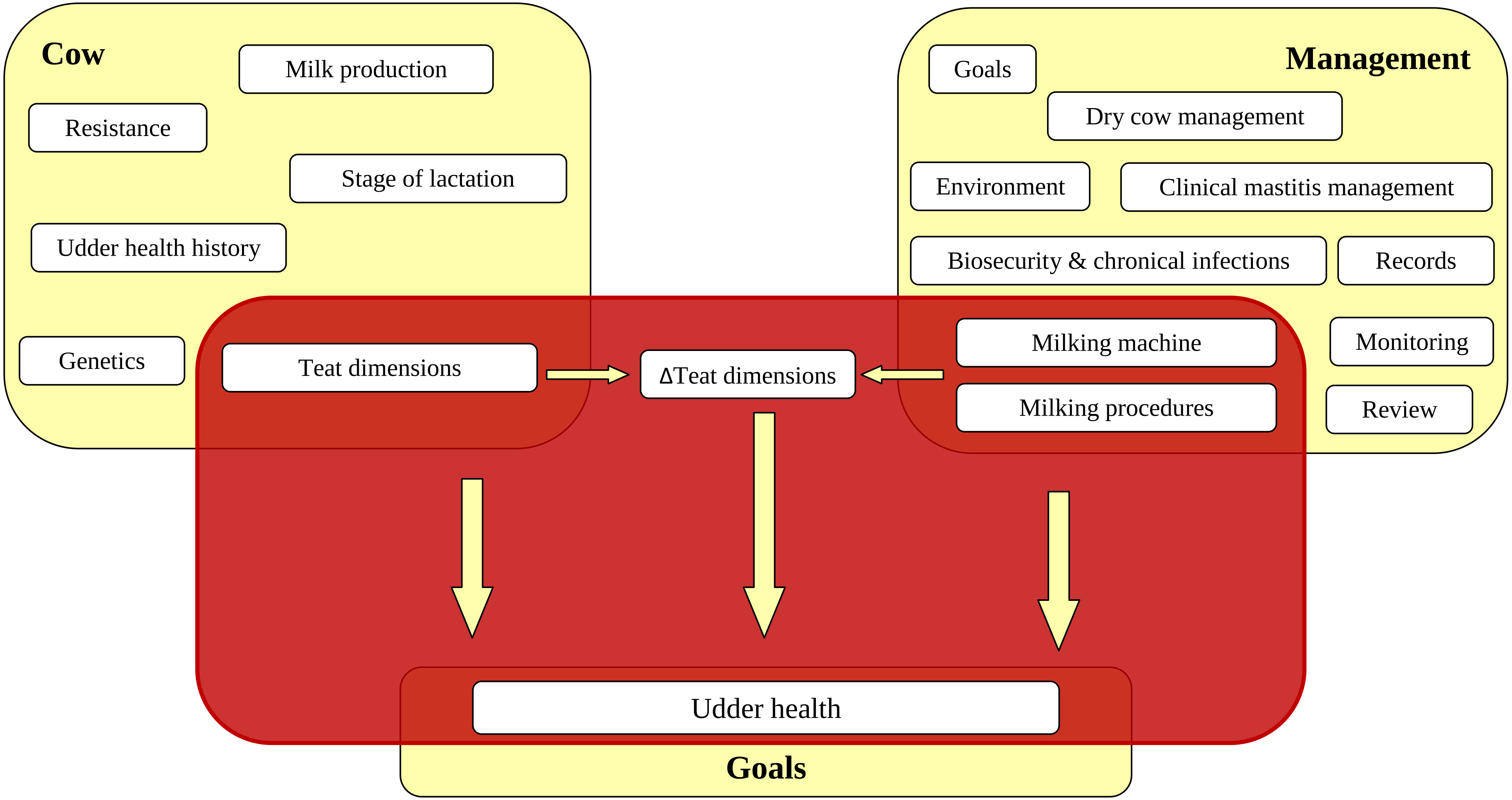

Figure 15. Schematic overview of some cow factors associated with udder health and the 10 points of the NMC mastitis control program, with indication of the area of interest of this thesis.

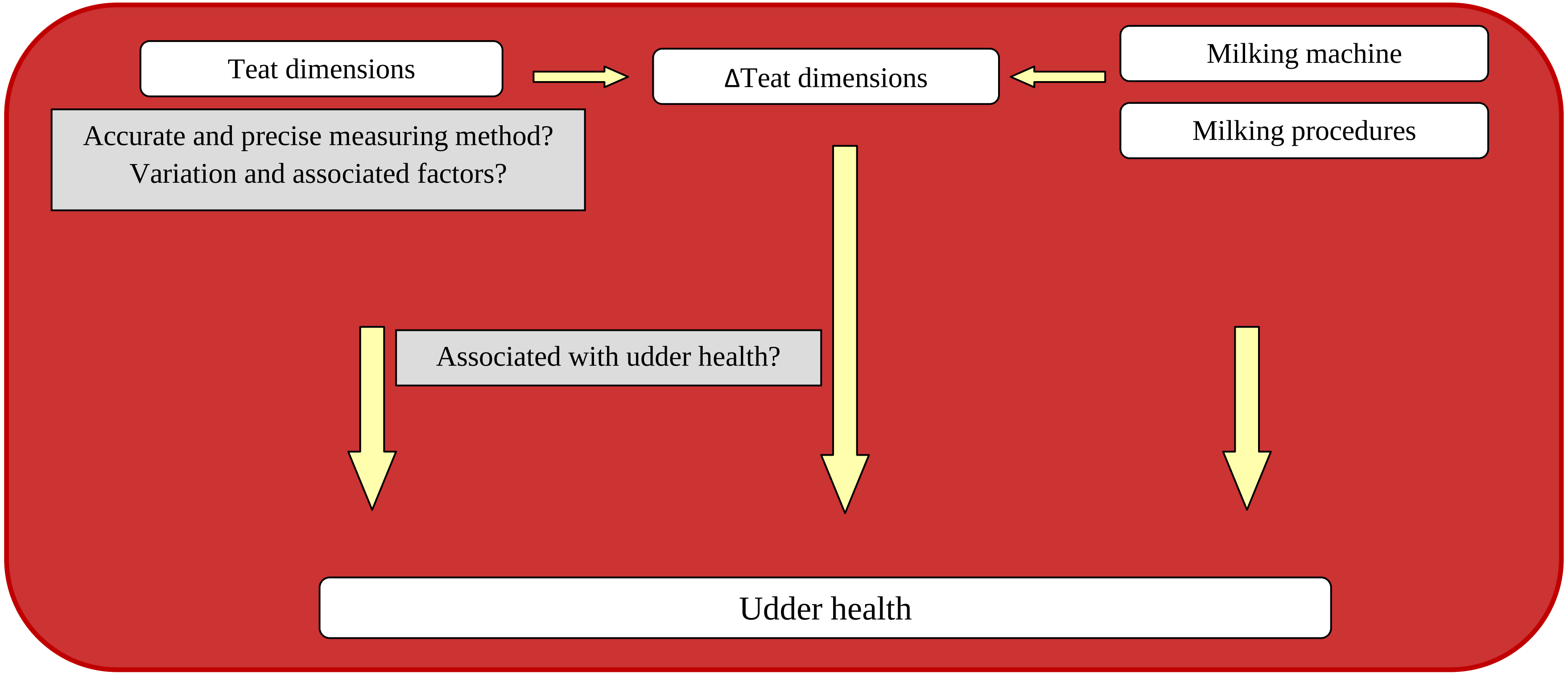

Figure 16. Schematic overview of the area of interest of this thesis with indication of the gaps leading to the aims of this thesis in grey.

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CHAPTER 2

AIMS OF THE THESIS

The general aim of this thesis is to gain insights in the variation in teat dimensions in dairy cows and their relation with udder health as assessed with an accurate and precise measuring device.

The specific aims of this thesis are:

- To develop and validate an objective and practical 2D-vision-based device to measure cows’ teat dimensions (teat length and diameters) accurately and with high precision using image analysis (Chapter 3 & 4).
- To determine the distribution of the variation in teat dimensions of dairy cows at the different levels of the data hierarchy and to identify factors associated with teat length and diameters, using data collected in both a cross-sectional and longitudinal study with the novel 2D-vision-based device (Chapter 5).
- To determine the association between teat length, teat diameters, and the short-term, machine milking-induced changes in these teat dimensions of dairy cows, and udder health (Chapter 6).

CHAPTER 3

OBJECTIVE MEASURING TECHNIQUE FOR TEAT DIMENSIONS OF DAIRY COWS

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ABSTRACT

The interaction between the teat and the teatcup liner can strongly affect the milking characteristics and udder health of dairy cows. Therefore, teat morphology is an important parameter in choosing the most appropriate liner. Nevertheless, information on teat morphology is scarce and rarely sufficient for liner selection. Current techniques for measuring teat morphology are subjective, not always accurate and time consuming, and gathering such information on large scale is difficult. This study presents a new vision-based measuring technique that uses a camera to obtain a 2D image of the teat, and image processing analysis to determine teat length and diameters. The technique was shown to be accurate. Errors were generally limited to 5% for both teat length and diameters and were less than 2% when the angle of the teat in the longitudinal direction was small (-5° to +15°).

NOMENCLATURE

α	angle of the teat in the transverse direction (°)
β	angle of the teat in the longitudinal direction (°)
c1	centre at 33% of the teat length
c2	centre at 80% of the teat length
D	distance from the lens centre to the centre of the teat insertion opening (mm)
d^{p}	measured teat diameter of the final 2D teat shape (T) (pixel)
$d^{p,c}$	measured teat diameter of the final 2D teat shape (T) corrected for the angle in the transverse direction (pixel)
$d^{w,c}$	measured teat diameter of the final 2D teat shape (T) corrected for the angle in the transverse direction (mm)
$f(m,n)$	intensity of the pixel located in the mth column, nth row of the geometrical teat shape (G_t)
G_t	2D teat shape after thresholding
l	teat length (mm)
l^{p}	measured teat length of the final 2D teat shape (T) (pixel)
$l^{p,c}$	measured teat length of the final 2D teat shape (T) corrected for the angle in the transverse direction (pixel)
$l^{w,c}$	measured teat length of the final 2D teat shape (T) corrected for the angle in the transverse direction (mm)
t	threshold
T	final two-dimensional teat shape after cleaning operations

INTRODUCTION

During machine milking, the forces applied on the teats result in physiological and pathological changes, which may counteract the normal teat defence mechanism. Consequently, the teats may become more sensitive for the entry of pathogens with intramammary infections as a result. Therefore, the condition of the teat plays a considerable role in the incidence of mastitis infections (Gleeson et al., 2004; Bhutto et al., 2010). Besides the greater risk on penetration of bacteria into the udder, these changes in teat condition are generally accompanied by discomfort and pain, which negatively influence the milking process. The design of the teatcup liner has a larger effect on teat tissue changes than other machine settings (Gleeson et al., 2004). In addition, the liner design has proven to have a great influence on milking characteristics (Mein et al., 2004). Consequently, the selection of teatcup liners is a crucial part of good milking management. Because of the interaction between teat and teatcup liner, teat morphology is an important parameter in choosing the most suitable liner for a herd. Yet, to date, liners are usually chosen on a ‘trial and error’ basis (de Koning et al., 2003). Teat morphology is seldom considered in choosing the most suitable liner, mostly because of the lack of knowledge.

Various methods to measure teat morphology have been described. Until today, the most common method to determine teat dimensions, and udder morphology in general, is on sight scoring (World Holstein-Friesian Federation, 2005; Zavadilova et al., 2009). The results of this technique are, however, biased by the operator and the standard used (Sapp et al., 2003). More objective methods have been used to determine teat length and teat diameter, such as measuring tapes, callipers and transparent open-ended tubes with graduated scales (Bakken, 1981; McKusick et al., 1999; Tilki et al., 2005; Rovai et al., 2007). Teat diameters can also be measured using a cutimeter, by removing the calliper spring or by holding the jaws open manually to neutralise the spring pressure (Hamann et al., 1996). An electronic calliper device, an improved version of the cutimeter, has been developed to measure changes in teat end thickness in response to changes in the applied pressure. Because the recordings start at a very low pressure (0.125 kPa), the first readings are a measurement of the diameter of the teat apex (Hamann et al., 1988). Another method used to measure teat morphology and teat tissue changes is ultrasonography (Neijenhuis et al., 2001; Paulrud et al., 2005; Seker et al., 2009; Ambord and Bruckmaier, 2010). This method requires an experienced observer and substantial measuring time and has therefore been restricted to experimental conditions (de Koning et al., 2003). Apart from scoring and ultrasonography, all methods described so far also have the disadvantage of providing per single measurement only one value at one point. Consequently, multiple measurements are needed to evaluate the whole teat; as a result the gathering of information on a large scale is not possible. Furthermore, the accuracy and the repeatability of the methods can be questioned since the results of the measurements depend on the position of the measuring tool on the teat and the moment of measurement; measurements during teat contractions should be avoided (Hamann et al., 1988).

An objective measuring technique capable of measuring teat dimensions accurately, with precision and speed, and with reduced effort for the operator, would be a large step forward in collecting data on teat morphology. Such a technique could be applied by control and consulting organisations either as a handheld system or implemented in automatic milking systems, helping the farmer select the most suitable liner for the herd or, if possible, for a specific cow or perhaps even individual teats. The acquired data could also be used to select more uniform teat dimensions, which might eventually simplify liner choice. Furthermore, a fast and accurate measuring device may be used to investigate the relationship between teat dimensions and udder health. The technique could serve as a useful tool to evaluate milking machines in combination with other monitoring systems, such as milk flowmeters. The progress of digital technology and image analysis software offers new perspectives in this area. An image analysis technique has already been proposed to measure udder morphology in dairy ewes (Marie-Etancelin et al., 2002). The technique is more objective compared to scoring and appears to be very promising. However, the application of the technique is elaborate and, for the moment, only possible to use under experimental conditions.

The aim of this study was to develop an objective and easy-to-use vision based measuring technique that was capable of providing numerous measurements of the whole teat.

MATERIALS AND METHODS

Measuring device and technique

A Super Video Graphics Array camera IEEE1394 (Guppy F-046B, Allied Vision Technologies, Stadtrod, Germany) with a fixed 25 mm C-mount lens was mounted at the end of a rectangular extruded aluminium profile (120 mm x 60 mm x 750 mm, 3 mm thickness) (Fig. 1). Opposite to the camera, a circular opening of 50 mm i.d. was provided in the housing for insertion of the teat. In a previous study (Baert et al., 2007), an opening of this size proved to be large enough to place the teat sufficiently deep in the device to acquire a good image for adequate teat length measurements. Images of the teats were made while each teat was inserted separately in the measuring device so that the upper side of the device was pressed gently against the udder. The 50 mm opening thus defines the teat base. The distance from the centre of the opening to the lens centre was fixed (550 mm). An LED illuminated background at the back of the profile generated high contrasting contours of the teats. This high contrast provided a sharp edge of the contour, necessary for accurate and automated image analysis. The camera was triggered by a push button on the housing that simultaneously switches on the LED-background illumination. The camera and the LEDs were powered by a 4-cell lithium polymer battery (14.4 V, 2000 mA). Electronics stabilised the voltage for the LEDs and the trigger signal. The camera and lens settings were kept constant (shutter, gain, focus and diaphragm) to maintain standard image quality, necessary for comparison between the images. A rugged, waterproof tablet PC (E100, Getac, Düsseldorf, Germany), connected to the camera through FireWire, was used for controlling the camera, automatic storing of the images and visual control of the images of the teats. The latter was needed to make sure the images meet the requirements necessary for further image processing.

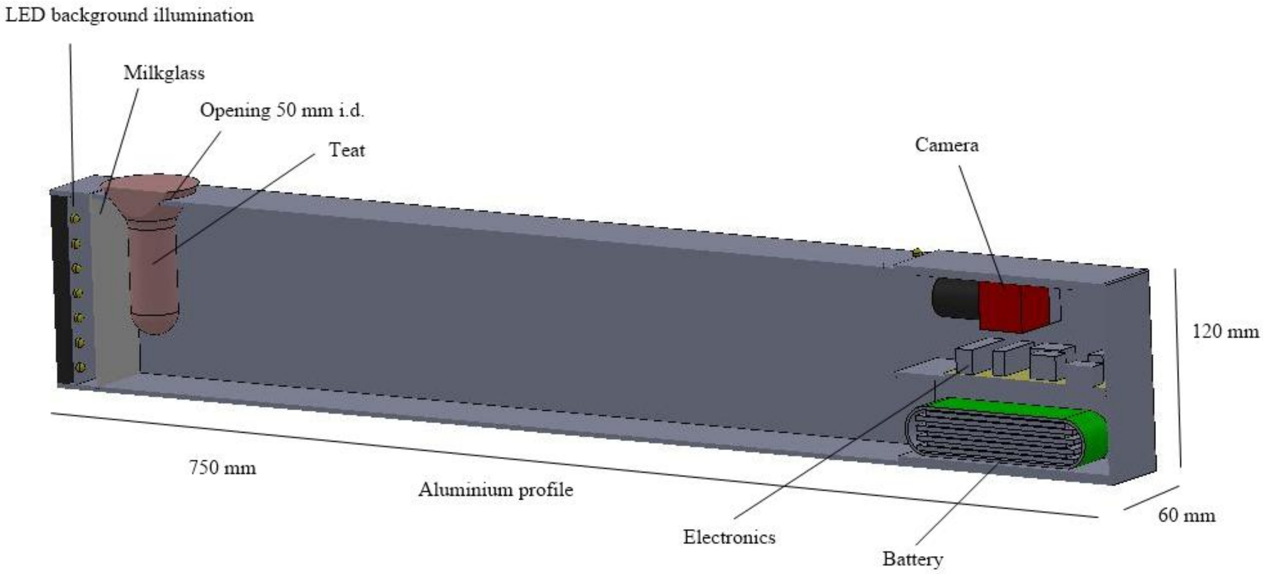

Figure 1. Cross-section of the vision based measuring device for teat dimensions

Vision based analysis

The image analysing software used was developed using Halcon 8.0 (MVTec Software GmbH, München, Germany). A fixed rectangular region of interest was defined in the raw image to eliminate the redundant image fraction. With constant light conditions and high contrast between subject and background, the 8-bit greyscale image can be accurately binarised with a fixed threshold (t).

$$G_t = \{f(m,n): f(m,n) < t\} \tag{1}$$

With $f\,(m,n)$ the intensity of the pixel located in the m^{th} column, n^{th} row. G_t represents the geometrical teat shape but still contains unwanted structures such as hair and dirt spots on the background. An opening operator (erosion followed by dilation) with circular structural element removed most of these unwanted structures. Larger dirt spots were removed based on area size of the discontented regions. The resulting 2D binary teat shape (T) is used for analysing length and diameter of the teat.

The length l^p in pixels is calculated from the highest to the lowest position of T:

$$l^p = Max(n) \in T - Min(n) \in T \tag{2}$$

The diameter d^p in pixels is calculated at a relative length from the base of the teat:

$$d_i^p = \sum_m T(l_i^p, m) \tag{3}$$

With $l_i^p = Max(n) \in T - \left\lfloor i \frac{l^p}{100} \right\rceil \; (i \in Z | 0 \leq i \leq 100)$ (4)

To maintain high accuracy, the diameters and length were corrected for the position of the teat in the measuring device and for the distance of the teat to the camera. Since the angle of the teat in the transverse direction (α) can cause inaccuracies, this angle was determined so a correction factor can be added to the length and the diameters. The angle α was calculated by determining the centres (*c1* and *c2*) of the sections at 33% and 80% of the length of the teat, which were empirically chosen not too low and not too high on the teat (Fig. 2):

$$\alpha = \arctan\left(\frac{x_{c1} - x_{c2}}{y_{c1} - y_{c2}}\right) \tag{5}$$

With

$$x_{ci} = \sum_m T(l_i^p, m).m/d_i^p \tag{6}$$

$$y_{ci} = i\frac{l^p}{100} \qquad i \in \{33, 80\} \tag{7}$$

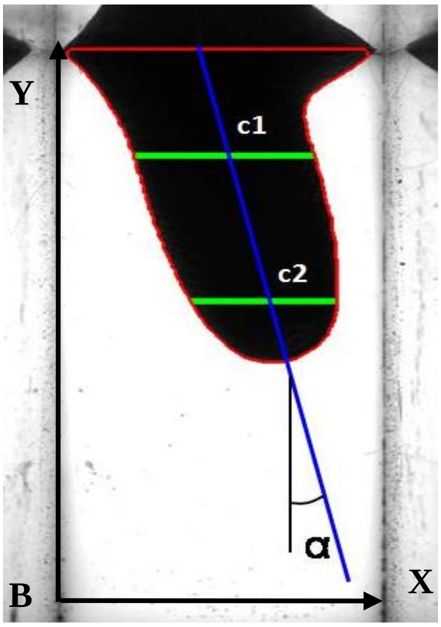

Figure 2. Calculation of the angle of the teat in the transverse direction (α) by determining the centres of the sections at 33% (c1) and 80% (c2) of the teat

The corrected diameters $d^{p,c}$ and lengths $l^{p,c}$ were:

$$l^{p,c} = \frac{l^p}{\cos(\alpha)} \tag{8}$$

$$d_i^{p,c} = d_i^p \cos(\alpha) \tag{9}$$

Real world dimensions ($l^{w,c}, d^{w,c}$) were obtained by conversing the pixel dimensions. The resolution of objects at the distance of 550 mm is 0.189 mm pixel^{-1}.

Accuracy

The image analysing software automatically corrected for deviations of the teat measurements due to the position in the transverse direction. However, the angle of the teat in the longitudinal direction (β) (towards or away from the camera) can also cause errors in the measured teat length and teat diameter. The error on the teat length made by β (Fig. 3) can be calculated by using the following equation:

$$error = \left(\frac{l^{w,c} - \mathrm{l}}{\mathrm{l}}\right).100 \tag{10}$$

with $l^{w,c}$ = measured teat length = $\left(\frac{\mathrm{l}\cos\beta}{\mathrm{D}-\mathrm{l}\sin\beta}\right)\mathrm{D}$

l = actual teat length

D = constant = distance from the lens centre to the centre of the teat insertion opening.

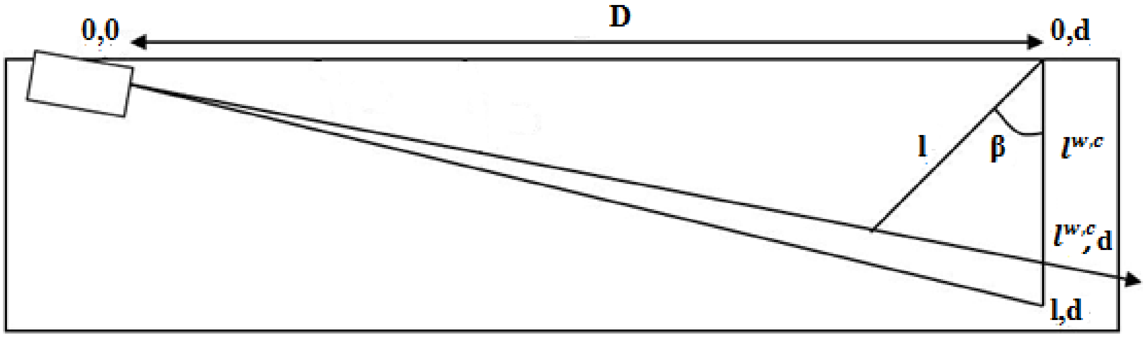

Figure 3. Calculation of the error made on the teat length by the measuring device due to the angle in the longitudinal direction (β)

Deviations of teat length due to movements in longitudinal direction from -20° to 0° (away from the camera) and 0° to 30° (towards the camera) were calculated for different teat lengths varying from 30 mm until 100 mm, which is the range of teat length found in literature, using Eq. 10.

The error on teat diameters made by the measuring device due to the angle the teat makes in the longitudinal direction could not be calculated since this angle was unknown. The accuracy of the measuring technique for teat diameters in the longitudinal direction was therefore obtained by analysing the images of four artificial teats of known dimensions, positioned at different angles relative to the camera. The artificial teats consisted of 200 mm long steel cylinders, respectively 20, 28, 36 and 44 mm in diameters at the barrel and a radius of the sphere of 12.5 mm at the apex. The dimensions were chosen to represent the range of teat dimensions in Holstein cows. The artificial teats were attached to a protractor. The design of protractor and artificial teats allowed the teats to rotate around pivoting points. To examine the influence of teat length a total of four pivoting points were present in the cylinders, located at respectively 30, 50, 70 and 90 mm from the apex. The artificial teats were placed straight in the device (i.e. angle of 0°). Images were taken at incremental steps of 5°, ranging from 0° to 30° towards the camera and to 20° away from the camera. Errors on teat diameters were determined at 75%, 50%, 25% and 10% of the teat length, starting from the teat end (D75, D50, D25 and D10), by comparing the measured diameters relative to the actual teat diameters.

Statistical analyses were performed using IBM SPSS Statistics 19 (SPSS Inc. 2010, IBM corporation, New York, USA). Linear regression was used to investigate whether the diameter measured by the measuring device and the angle of the teat in the longitudinal direction (β) (independent variables) could be used to predict the actual teat diameter at 75%, 50%, 25% and 10% of the teat length (dependent variables). A three-way ANOVA with actual length of the teat, actual diameter of the teat, β and their interactions as fixed factors was performed on the percentage deviation on teat diameters at 75%, 50%, 25% and 10% of the teat (dependent variables). If significant, Scheffé post hoc tests were performed. All data were reported as means. Statistical significance was considered when $P < 0.05$.

Practical use and evaluation on real teats

Preliminary tests were performed in different types of milking parlours to assess the practical use, robustness, and ergonomic aspects of the measuring device and to evaluate the image processing analyses on real teats. The first test was done in a tie-stall; 70 teats from 18 cows were measured. A second test consisted of measuring the teats of 90 cows (352 teats in total) in a side-by-side milking parlour with automatic detachment of the units. A third series of tests was performed at six different herds (1 tandem, 5 herringbone parlours) where in each herd 10 randomly chosen cows were measured.

RESULTS

Teat length

Errors were less than 2% when the teat was put straight in the device or when the angle of the teat in the longitudinal direction was small (-5° to +15°). Errors were limited to 5% in most cases (Table 1). However, larger errors (up to 11%) were possible with angles greater than 25° and 15°, towards and away from the camera, respectively. Under most conditions teat length was underestimated. Only when the teat was directed at the camera at small angles (5° to 15°), and for teats of certain length, was the length overestimated. At the same angle, the calculated error for teats of equal length was greater for teats tilted away from the camera compared to those pointed towards the camera. For teats positioned away from the camera the error was smaller for shorter teats than for larger teats. For teats of the same length, the error rapidly increased with increasing angle. The association was less straightforward for teats that pointed in the direction of the camera. At large angles, the deviation of the calculated length compared to the actual length decreased with teat length and increased with increasing angle.

Table 1. Percentage deviation on teat length due to the angle in the longitudinal direction (-20° to 30° towards the camera) for teats of different length (30 to 100 mm), with deviations > 5% in bold and > 10% in bold and italic, determined using Eq. 10

Teat length (mm)	Angle in the longitudinal direction of the camera (°)										
	-20	-15	-10	-5	0	5	10	15	20	25	30
30	**-7.8**	-4.8	-2.4	-0.9	0.0	0.1	-0.6	-2.0	-4.2	**-7.2**	***-11.0***
35	**-8.0**	-5.0	-2.6	-0.9	0.0	0.2	-0.4	-1.8	-3.9	**-6.9**	***-10.6***
40	**-8.3**	**-5.2**	-2.7	-1.0	0.0	0.3	-0.3	-1.6	-3.6	**-6.5**	***-10.1***
45	**-8.6**	**-5.4**	-2.9	-1.1	0.0	0.3	-0.1	-1.3	-3.3	**-6.1**	**-9.7**
50	**-8.9**	**-5.6**	-3.0	-1.2	0.0	0.4	0.1	-1.1	-3.0	**-5.7**	**-9.3**
55	**-9.1**	**-5.8**	-3.2	-1.2	0.0	0.5	0.2	-0.8	-2.7	**-5.4**	**-8.8**
60	**-9.4**	**-6.1**	-3.4	-1.3	0.0	0.6	0.4	-0.6	-2.4	-5.0	**-8.4**
65	**-9.7**	**-6.3**	-3.5	-1.4	0.0	0.7	0.5	-0.4	-2.1	-4.6	**-8.0**
70	***-10.0***	**-6.5**	-3.6	-1.5	0.0	0.7	0.7	-0.1	-1.8	-4.2	**-7.5**
75	***-10.2***	**-6.7**	-3.8	-1.6	0.0	0.8	0.9	0.1	-1.4	-3.8	**-7.1**
80	***-10.5***	**-6.9**	-3.9	-1.6	0.0	0.9	1.0	0.4	-1.1	-3.4	**-6.6**
85	***-10.7***	**-7.1**	-4.1	-1.7	0.0	1.0	1.2	0.6	-0.8	-3.0	**-6.1**
90	***-11.0***	**-7.3**	-4.2	-1.8	0.0	1.1	1.4	0.9	-0.5	-2.6	**-5.7**
95	***-11.3***	**-7.5**	-4.4	-1.9	0.0	1.1	1.5	1.1	-0.1	-2.2	**-5.2**
100	***-11.5***	**-7.7**	-4.5	-1.9	0.0	1.2	1.7	1.4	0.2	-1.8	-4.7

Teat diameter

Linear regression showed that the diameter of the different artificial teats can be predicted from the diameter measured by the measuring device (adjusted coefficient of determination R^2= 0.999 ± 0.274, 0.991 ± 0.811, 0.997 ± 0.445 and 0.970 ± 1.507 for D75, D50, D25 and D10, respectively). These already high values of R^2 were slightly increased ($P < 0.001$) by adding the angle of the artificial teat in the longitudinal direction (β) to the model 1.00 ± 0.185, 0.994 ± 0.673, 0.999 ± 0.269 for D75, D50 and D25, respectively. No significant contribution of β was found for D10 ($P = 0.842$).

The percentage deviation of the teat diameters at 75%, 50% and 25% of the teat was significantly affected by the teat diameter of the artificial teat ($P < 0.001$), whereas no significant influence of the teat diameter on the percentage deviation was found for D10 ($P = 0.272$). In addition, the percentage deviations at all heights significantly depended on the interaction between teat length and β ($P < 0.001$) (Table 2). Teats positioned straight through the opening of the profile gave almost no inaccuracies for all teat lengths. Errors increase with increasing angles in both directions. Yet, these errors are restricted to 5% for D75, D50 and D25. At very small angles, the error was even lower than 2%. Moreover, the errors made by the device at different angles for different teat lengths were not significantly different from the errors when the teat was placed straight in the device, except at large angles. In contrast, at the teat end (D10), large underestimations of diameters were observed for most angles and the errors were larger for shorter teats than for longer teats. The data showed an increase in deviation of measured diameters compared to the actual diameters from teat base to barrel, both for teats pointing towards (overestimation) and away (underestimation) from the camera.

Table 2. Average percentage deviation on teat diameters at 75%, 50%, 25% and 10% of the teat, starting from the teat end, for artificial teats of different length (30 mm, 50 mm, 70 mm and 90 mm) under various angles in the longitudinal direction (-20° to 30° towards the camera), with deviations > 5% in bold

Angle in the longitudinal direction of the camera (°)	Teat length															
	30 mm				50 mm				70 mm				90 mm			
	D75	D50	D25	D10	D75	D50	D25	D10	D75	D50	D25	D10	D75	D50	D25	D10
-20	-0.6	-1.3	**-5.1***	**-11.7**	-1.1*	-1.4	-4.4	**-7.5**								
-15	-0.4	-1.1	-3.9	**-9.2**	0.1	-1.0	-3.3	**-5.9**	-1.1	-2.2*	-4.1	**-5.8**				
-10	-0.4	-0.9	-2.4	**-6.9**	0.2	-0.2	-2.4	**-5.5**	-0.6	-0.8	-3.1	-4.6	-1.1	-1.4	-3.0	-4.9
-5	-0.3	-0.7	-1.3	-3.1	0.3	-0.3	-1.8	-2.1	0.2	-0.3	-1.9	-2.7	-0.1	-0.6	-1.1	-2.6
0	-0.1	-0.6	-0.1	0.6	0.6	0.3	-1.2	-0.7	0.5	0.1	-1.3	-1.1	0.5	0.2	-0.6	-1.0
5	0.2	-0.2	-0.5	-1.3	0.8	0.5	-0.9	0.5	0.4	0.4	-0.6	0.8	0.3	0.6	0.5	0.4
10	0.2	-0.2	-0.7	-4.8	0.9	1.0	-0.1	-1.0	0.7	1.1	0.4	0.4	1.0	1.3	1.6	1.7
15	0.2	0.0	-2.1	**-12.9**	1.2	1.4	0.4	-3.3	1.0	1.5	1.4	-0.5	1.4	2.1*	2.5	1.8
20	0.3	0.5	-2.9	**-11.4**	1.3	1.7	0.9	**-5.6**	1.4	2.3*	2.1	-1.0	1.8	2.6*	3.2	1.0
25	0.7	0.6	-3.6	**-12.7**	1.4	2.3*	0.7	**-7.1**	1.5	2.9*	2.7	-2.7	2.2*	3.4*	4.3*	1.1
30	0.6	0.7	-4.0	**-11.6**	1.8	2.5*	1.3	**-6.5**	2.0*	3.1*	3.5*	-2.3	2.4*	4.0*	**5.1***	1.0

* Significantly different from value at 0° within column (*P* < 0.05)

Practical use and evaluation on real teats

Due to the small shape and the light weight (3.6 kg) the measuring device was easy to operate and allowed ergonomic measuring of the teats. Two operators were needed to perform measurements of the teats: one person to operate the device and a second person to visually assess the images (Fig. 4). The device was practical in the various types of conventional milking parlours. Nearly all teats could be measured without difficulties. The position of the teat on the udder and objects in the milking parlour such as bars and milking clusters, occasionally hindered the measurements. Measurements in a side-by-side milking parlour were performed fastest but teats could be measured without noteworthy prolonging the milking process in other types of milking parlours as well.

Figure 4. Teat measurements using the 2D vision-based measuring device in a tie stall.

DISCUSSION

The images of the teats taken with the measuring device were all of good quality for image processing analysis. Due to the regularly dispersed background light, the contour of the teat could be easily identified. For teats that made an angle in the transverse direction, a standard correction was integrated in the software program for both teat length and teat diameter. In this study, the accuracy of the teat measuring device was under investigation, i.e. the ability to give a true measure of the object of interest. The calculated error on the length of teats that make an angle in the longitudinal direction was less than 2% for teats at small angles and was in general less than 5%, although larger angles caused larger errors. Since this angle cannot be measured under field conditions, no correction factor can be incorporated in the analysis. It was therefore concluded that care should be taken by the operator to put the teat in the device as straight as possible.

For diameters, regression analysis indicates that the measured teat diameter was a good predictor for the teat diameter at all heights and for different teat lengths. Adding β to the regression model only slightly increased the precision of the diameter prediction. Errors were mostly limited to 5%. These errors significantly depended on the interaction between teat length and β. Errors at different angles for different teat lengths did not significantly differ from the errors when the teat was placed straight in the device, except at large angles but even then they were mostly less than 5%. Errors generally increased with increasing deviation in the longitudinal direction due to the increasing shift away from the centre towards or away from the camera. As errors in the diameter were calculated at relative heights, the height at which the diameter was measured varied with the teat length. For this reason, the error on the teat diameter also depended on the error of the teat length at a certain angle. This emphasised the importance of placing the teat in the device as straight as possible. Errors at the teat base (D75) were usually smaller than those at the barrel (D50) since, under the same angle, lower diameters are moved more towards or away from the camera. For the same reason, errors at the teat base and the barrel increase with increasing length. Large errors, that exceed 10%, occured at the teat end (D10). In contrast, the largest errors at the teat end were found for short teats and the errors decreased with increasing length. It should be kept in mind that diameters at different heights of the teat were being compared. For a short teat of 30 mm the diameter at 10% of the teat was measured at 3 mm from the top, whilst for a long teat of 90 mm this corresponded to a point 9 mm from the top. Some researchers determine teat diameter at a defined distance from the teat end (Chrystal et al., 1999; Borkhus and

Rønningen, 2003). These absolute measures are more clear-cut. However, both relative as well as absolute measures contain valuable information. From the images made with the 2D vision based measuring device, both relative as absolute teat dimensions can be deduced, providing a large amount of information that can be used for a wide range of purposes. Depending on the topic under investigation, relative or absolute dimensions will be used. Research on the selection of teatcup liners based on teat dimensions will use absolute measures. To determine whether a liner is too small or too broad at a specific point for a certain teat, the diameter of the liner has to be compared to the diameter of the teat at that point. Consequently, absolute measures are required in liner selection, whilst relative measures are mainly used for descriptive herd studies since it is important to compare the same area (teat end, barrel or base), when comparing teats.

As for teat length, this is the first study, to our knowledge, that calculates the percentage difference of the measured values compared to the actual diameters of artificial teats. A high degree of accuracy (± 2%) was reported for the modified cutimeter (Hamann and Mein, 1988). However, it was not clearly stated how this result was obtained. A similar accuracy of 2% was found with the 2D vision based measuring technique presented in this study, both for teat length and diameters, except for diameters at the teat end and as long as the instructions for the measuring device are followed, i.e. when the teat is put straight in the device. When this is done, the accuracy of the technique is sufficient to measure the variation in teat length between front and rear teats (difference on average 8 to 19%) and between parity; Wufka & Willeke (2001) reported a difference of 5.3% between first and second lactation, and of 2.5% between second and third lactation. Since the errors at the teat end can be greater than 10%, further research is needed to examine whether the measuring technique could be applied in determining the diameters at the teat end. Artificial teats of various teat shapes as well as real teats should be subject of this study.

Besides accuracy, a coefficient of variation of 8-10%, a measure for repeatability, was observed for the modified cutimeter, whilst better results (2%) were found for the electronic calliper device (Hamann et al., 1988). Although the cutimeter can measure teat diameters, it is usually used to measure changes in teat tissue due to milking. As a result, the high degree of accuracy and repeatability could be applicable to changes in teat tissue instead of teat diameter measurements. Good repeatability (3.6% difference between duplicate measurements and 4.4% difference between days) was also attained by use of ultrasonography for teat end diameter (Neijenhuis et al., 2001). These results reflect the consistency of measurements repeated with the same technique, regardless of whether or not the values are correct, as a measure for precision, which differs from the accuracy investigated in this study. To assess if the developed measuring device can be used to determine teat dimensions in field conditions precise and accurate, determination of the repeatability and reproducibility of the device, as well as comparison with a standard, will be subjects of subsequent studies.

CONCLUSION AND FUTURE PERSPECTIVES

A new 2D vision based measuring method has been presented in this study. The use of image analysis permits a fast and objective measurement of teat shape parameters. The method has been proven to be accurate for teat dimensions: errors are generally less than 5% and less than 2% when the angle of the teat in the longitudinal direction is small (-5° to +15°). Special attention must therefore be paid on putting the teats straight into the measuring device. Further research is needed to evaluate the repeatability and the reproducibility of the measuring technique under field conditions and to examine whether the measuring technique can be applied in determining the diameters of the teat end and consequently be used for teat (end) shape classification. Artificial teats of various shapes as well as real teats should be subject of this study. Because the measuring technique makes it possible to gather information on a large scale, both in experimental and field conditions, it allows identification and investigation of the factors influencing teat dimensions. Additionally, since information of the whole teat is available, liner performance can be evaluated in relation to teat dimensions by assessing udder health and milking characteristics. In the short term the information generated using the measuring device may enable better selection of teatcup liners adapted to the herd. In the longer term, the information may lead to more uniform teat dimensions within a herd through selective breeding, which may simplify liner choice. Better liner selection may in turn, result in better teat condition, decreasing the incidence of intramammary infections and thereby improving the quality and the quantity of the milk yield.

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CHAPTER 4

INTRAOPERATOR REPEATABILITY AND INTEROPERATOR REPRODUCIBILITY OF DEVICES MEASURING TEAT DIMENSIONS IN DAIRY COWS

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Submitted

ABSTRACT

Various methods have been applied to measure teat dimensions. However, the accuracy and precision needed to obtain reliable results are poor or have not yet been investigated. To determine the precision of the ruler, the calliper and a recently developed 2D-vision-based measuring device under field conditions, for respectively teat length, teat diameter and both teat length and diameter, two experiments were conducted in which the consistency of measurements within operators (repeatability) and between operators (reproducibility) was tested. In addition, the agreement of the 2D device with the ruler and the calliper was studied. Although the ruler and the 2D device poorly agreed, both methods were precise in measuring teat length when the operators had experience in working with cows. The calliper was repeatable in measuring the teat diameter, but was not reproducible. The 2D device was also repeatable in measuring the teat diameter, and reproducible when the operators had experience with the device. The methods had poor agreement, most likely due to the operator-dependent pressure applied by the calliper. Because the 2D device has the advantage of measuring both teat length and teat diameters in a single measurement, is accurate and practical, this method allows efficient and fast collection of data on a large scale for various applications.

INTRODUCTION

In dairy cows, the teat represents the interface between the mammary gland and the environment through which milk is drained, and which serves as primary defense mechanism against infections. Consequently, the teat dimensions and the (mis)match of the teat and the teatcup liner may affect milking characteristics and may influence teat condition and udder health.

Until now, a wide variety of methods to measure teat dimensions have been applied [measuring tapes (Tilki et al., 2005), callipers (Tilki et al., 2005), transparent open-ended tubes with graduated scale (Gleeson et al., 2004), modified cutimeters (Hamann et al., 1996), electronic calliper devices (Hamann et al., 1988) and ultrasonography (Neijenhuis et al., 2001; Gleeson et al., 2004)], although none is accurate (strong agreement between the measured value and the actual value of a variable), precise, i.e. highly repeatable and reproducible (strong agreement between measurements performed by the same operator and performed between operators, respectively), and practical to use, i.e. limited measuring time and labor, and no intensive training needed prior to use.

Recently, we developed a new 2D-vision-based device for measuring teat dimensions (further referred to as the 2D device) (Zwertvaegher et al., 2011). The method uses image analysis, permits fast and objective measurements, and has been shown to be highly accurate (error < 5%) during evaluation on artificial teats. Nevertheless, this method has not yet been assessed under field conditions, neither has it been compared with other available methods.

The aim of this study was to determine the precision of the ruler, the calliper, and the recently developed 2D device, respectively for measuring teat length, teat diameter, and for both teat length and diameter, when used under field conditions. This was done by evaluating the consistency of measurements (repeatability) and operators (reproducibility) per method. In addition, the agreement of the results obtained by the 2D device with those recorded by the ruler and the calliper was assessed.

MATERIALS AND METHODS

Experiment 1: Precision and Agreement of Ruler, Calliper and 2D Device

Study design and data collection. The teat length and the diameter at the middle of the teat of 8 Holstein-Friesian cows were measured with the 2D device. In addition, the teat length and the teat diameter were determined using a ruler and a calliper, respectively. Measurements were performed in duplicate and by 2 different operators (2 x 2), which had both experience in working with cows, in a tie-stall, 2 hours after morning milking. A total of 64 measurements were performed per method per operator. The measurements were performed randomly within each cow (method, operator, outcome variable teat length and diameter at the middle of the teat), but the teats within each cow were measured according to a defined order. The results were noted, and the images made by the 2D device were checked to ascertain that the requirements needed for further image processing were met, i.e. the teats are placed straight into the 2D device and are not leaking milk (Zwertvaegher et al., 2011). When these requirements were not fulfilled, a new image was taken. Measurements during teat contractions were avoided.

Repeatability was studied within each method for teat length (ruler and 2D device) and for teat diameter (calliper and 2D device) by comparing duplicate measurements, whereas reproducibility of each method was studied for teat length (ruler and 2D device) and for teat diameter (calliper and 2D device) by comparing measurements performed by operator A with the measurements performed by operator B. Agreement between the 2D device and the ruler (for teat length) and the calliper (for teat diameter) was calculated by comparing the collected data. Reproducibility and agreement were assessed by comparing only one set of data of the measurements, whereas for repeatability the duplicate measurements were used for obvious reasons.

Statistical analysis. Prior to statistical analysis, data were checked for normality using Kologmorov-Smirnoff test and by visual assessment of the Q-Q plots using SPSS Statistics 19 (SPSS Inc. 2010, IBM corporation, New York, USA).

First, to determine differences in measurement (repeatability), operator (reproducibility) and measuring method (agreement), paired *t*-tests and Wilcoxon signed-rank tests were performed for teat length and teat diameter, respectively. Statistical significance was considered when $P < 0.05$. Second, the CV were calculated per pair of test results and reported as mean CV over all pairs as a measure for precision (repeatability and reproducibility). Furthermore, the overall levels of consistency were reflected by calculating the concordance correlation coefficients (**CCC**). Bland-Altman plots were produced to visualize the difference between the pairs of test results relative to their mean value with 95% limits of agreement. Additionally, scatterplots were generated, and regression lines were fit to the data to define the difference relative to the line of perfect agreement (slope 45° and intercept zero) (Arunvipas et al., 2003).

All analyses were done using Excel 2007 (Microsoft Corporation, Redmond, USA), except Wilcoxon signed-rank tests, which were performed using SPSS Statistics 19 (SPSS Inc. 2010, IBM corporation, New York, USA).

Experiment 2: Precision of the 2D Device

An additional experiment was conducted to investigate the precision (repeatability and reproducibility) of the 2D device in practice more extensive.

Study design and data collection. The teat length and the diameter at the middle of the teat were measured with the 2D device. For studying repeatability, the teats of 19 Holstein-Friesian cows were measured 5 times by 1 operator, with measurements being performed in a tie-stall, 3 hours before evening milking. Reproducibility was assessed by measuring the teats of 8 Holstein-Friesian cows once by 8 different operators, with measurements being done 3 hours after morning milking in a tandem milking parlour. Five of the operators were familiar with working with cows (= cow experience) and 3 of those 5 were also experienced in working with the 2D device (= tool experience). All measurements were performed as reported before.

Statistical analysis. Prior to statistical analysis, data were checked for normality using Kologmorov-Smirnoff test and by visual assessment of the Q-Q plots (SPSS Statistics 19, SPSS Inc. 2010, IBM corporation, New York, USA).

First, to determine whether teat length was associated with measurement (repeatability), operator, cow experience or tool experience of the operators (reproducibility), respectively, repeated measures ANOVA’s were performed, whereas for teat diameter, non-parametric Friedman tests were used (SPSS Statistics 19, SPSS Inc. 2010, IBM corporation, New York, USA). Statistical significance was considered when $P < 0.05$. Second, CV were calculated for all measurements within operator (repeatability), for all operators in general, and for all operators with cow or tool experience (reproducibility), respectively. In addition, CCC, scatterplots and Bland-Altman plots were generated for teat length and teat diameter. For repeatability, the first measurement was compared with the last measurement. For reproducibility, the effect of cow and tool experience on teat length and diameter at the middle of the teat was examined and visualized for 2 randomly selected persons with cow experience (teat length) and tool experience (teat diameter).

Analyses were done using Excel 2007 (Microsoft Corporation, Redmond, USA).

RESULTS

Experiment 1

Teats were on average 55.79 mm long and ranged between 35.00 and 90.00 mm. The mean diameter at the middle of the teat was 25.88 mm and varied between 14.78 and 39.13 mm.

Precision of the ruler. Non-significant differences, low CV, and strong correlations were present between duplicates and between operators for teat length determined with the ruler (Table 1). The mean differences were close to zero with the 95% limits of agreement varying between 7.25 and -7.31 mm, and 6.09 and -7.40 mm, for repeated measurements and for operators, respectively (Table 1). The points of the scatterplots clustered around the lines of perfect agreement (Fig. 1A).

Precision of the calliper. Duplicates of the diameter at the middle of the teat measured with the calliper showed non-significant differences, low CV, and strong correlations (Table 1). The mean difference was close to zero and the 95% limits of agreement of the Bland-Altman plot varied from 2.71 to -3.17 mm (Table 1). The points of the scatterplot tended to cluster near the line of perfect agreement (Fig. 2A). Measurements significantly differed between operators, CV was high and correlation was poor (Table 1). The mean difference in measurements performed by the 2 operators was 2.51 mm and the 95% limits of agreement were broad (Table 1). The linear regression line fitted to the scatterplot clearly differed from the line of perfect agreement (Fig. 2A).

Precision of the 2D device. For teat length measured with the 2D device, non-significant differences, low CV, and strong correlations were found between duplicates and between operators (Table 1). The mean differences were close to zero and the 95% limits of agreement were situated at 7.26 and -8.97 mm, and 8.07 and -7.36 mm, for duplicates and for operators, respectively (Table 1). The regression lines differed only slightly from the lines of perfect agreement (Fig. 1B).

Non-significant differences, low CV, and strong correlations were found between repeated measurements and between operators of the diameter at the middle of the teat measured with the 2D device (Table 1). The mean differences were close to zero and the 95% limits of agreement were narrow (Table 1). The linear regression lines fitted to the data approached the line of perfect agreement (Fig. 2B).

Table 1. Level of statistical significance, coefficients of variation (CV), concordance correlation coefficients (CCC), mean difference and 95% upper and lower limits of agreement of the Bland-Altman plots for teat length measured with the ruler and the 2D device, and for diameter at the middle of the teat measured with the calliper and the 2D device.

		Teat length		Diameter at the middle of the teat	
		Ruler	2D device	Calliper	2D device
Repeatability	Sign. Level[1]	NS[11]	NS	NS	NS
	CV[2]	2.94	4.43	3.31	1.56
	CCC[3]	0.95	0.92	0.93	0.97
	Mean difference (limits of agreement)[4]	-0.03(-7.31 – 7.25)	-0.85 (-8.97 – 7.26)	-0.23 (-3.17 – 2.71)	-0.13 (-1.75 – 1.48)
Reproducibility	Sign. Level[5]	NS	NS	< 0.001	NS
	CV[6]	2.86	4.19	8.37	1.89
	CCC[7]	0.96	0.94	0.65	0.95
	Mean difference(limits of agreement)[8]	-0.66 (-7.40 – 6.09)	0.36 (-7.36 – 8.07)	-2.51 (-7.16 – 2.14)	0.26 (-1.77 – 2.29)
Agreement	Sign. Level[9]	< 0.001		< 0.001	
	CCC[10]	0.86		0.50	

[1] Level of significance of the paired *t*-test (teat length) and the Wilcoxon-signed rank test (teat diameter) between duplicates within each method

[2] Coefficient of variation between duplicates within each method (in %)

[3] Concordance correlation coefficient between duplicates within each method

[4] Mean difference (and 95% lower and upper limit of agreement) of the Bland-Altman plots between duplicates within each method (in mm)

[5] Level of significance of the paired *t*-test (teat length) and the Wilcoxon-signed rank test (teat diameter) between operators within each method

[6] Coefficient of variation between operators within each method (in %)

[7] Concordance correlation coefficient between operators within each method

[8] Mean difference (and 95% lower and upper limit of agreement) of the Bland-Altman plots between operators within each method (in mm)

[9] Level of significance of the paired *t*-test (teat length) and the Wilcoxon-signed rank test (teat diameter) between methods

[10] Concordance correlation coefficient between methods

[11] Not significant ($P < 0.05$)

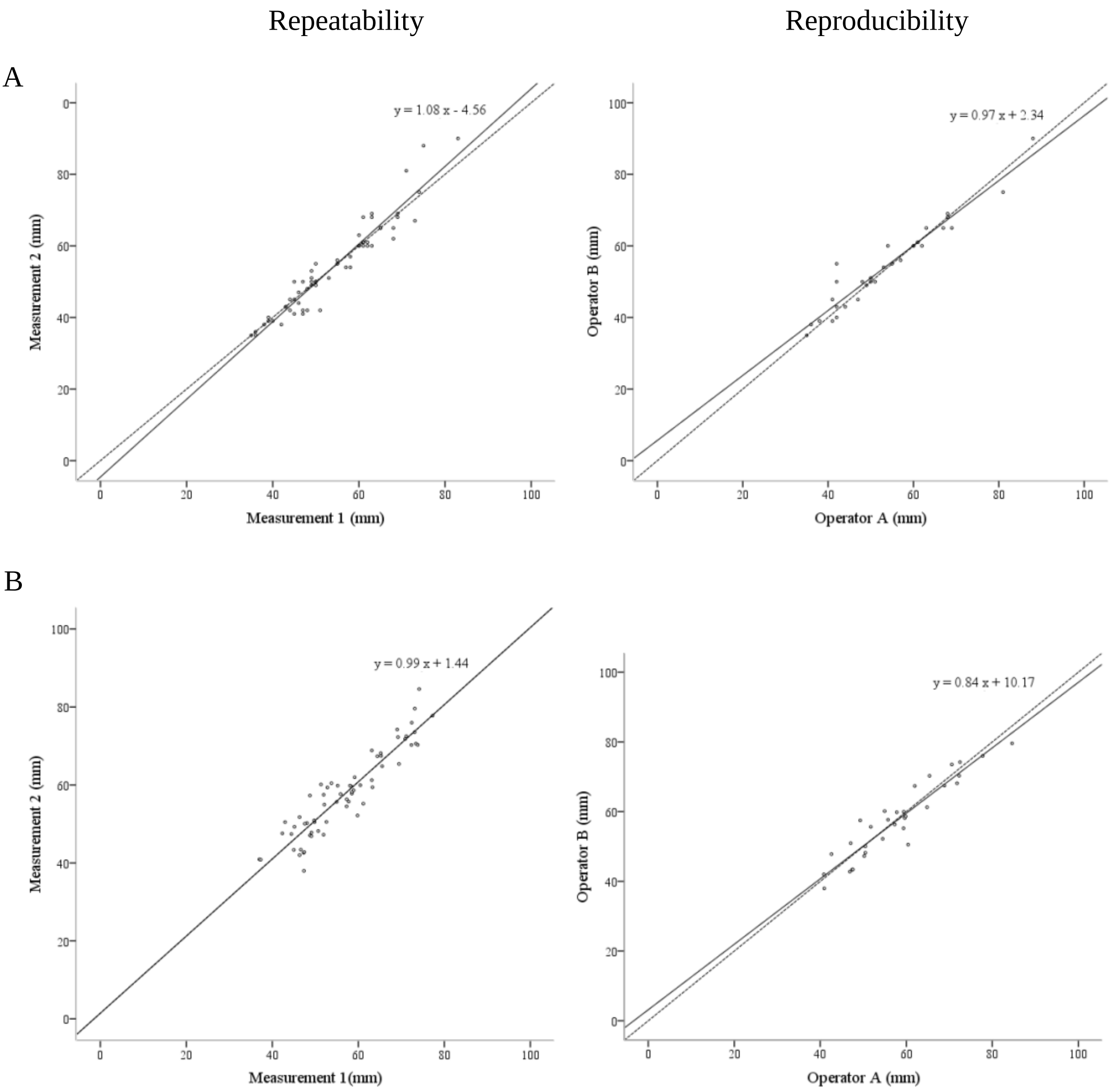

Figure 1. Scatterplots (-------- line of perfect agreement; —— linear regression line fit to the data) for repeatability and reproducibility of teat length measured with the ruler (A) and the 2D device (B).

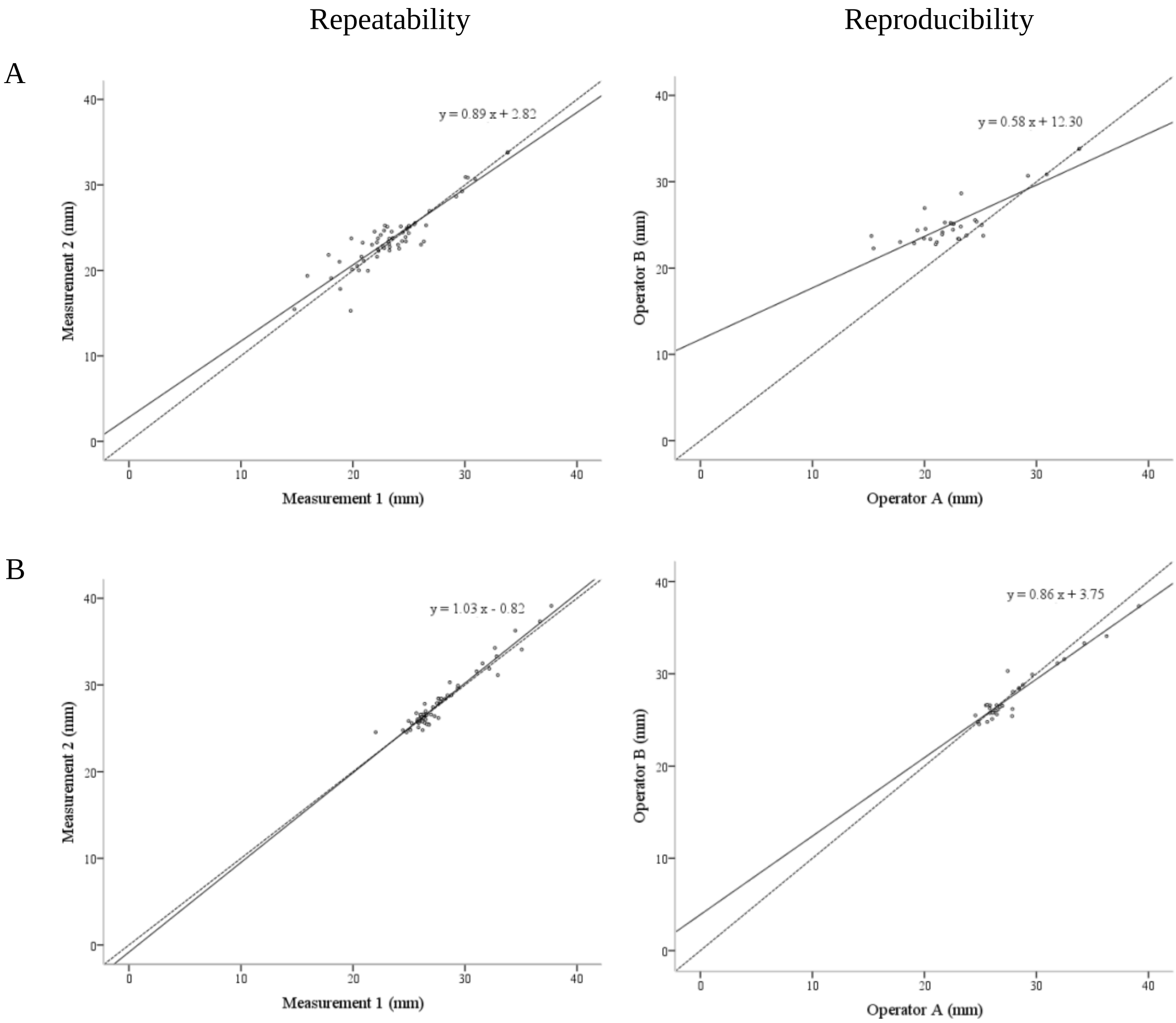

Figure 2. Scatterplots (-------- line of perfect agreement; —— linear regression line fit to the data) for repeatability and reproducibility of diameter at the middle of the teat measured with the calliper (A) and the 2D device (B).

Agreement of the ruler and 2D device. The mean teat length measured with the 2D device was significantly longer from that obtained by the ruler (57.79 ± 10.89 mm vs. 54.23 ± 12.37 mm) (Table 1). Although CCC was 0.86, the regression line differed from the line of perfect agreement when the values of the 2D device were plotted against those of the ruler (Fig. 3A). The mean difference between the 2 methods was 3.56 mm. The upper and lower limit of agreement were respectively 13.61 mm and -6.49 mm (Fig. 3A).

Agreement of the calliper and 2D device. The average diameter at the middle of the teat measured with the 2D device was significantly broader than that measured with the calliper (27.82 ± 3.24 mm vs. 24.11 ± 3.94 mm). Correlation was poor (Table 1) and the scatterplot shows a clear difference of the points with the line of perfect agreement (Fig. 3B). The mean difference was 3.71 mm and 95% limits of agreement were 8.50 mm and -1.07 mm (Fig. 3B).

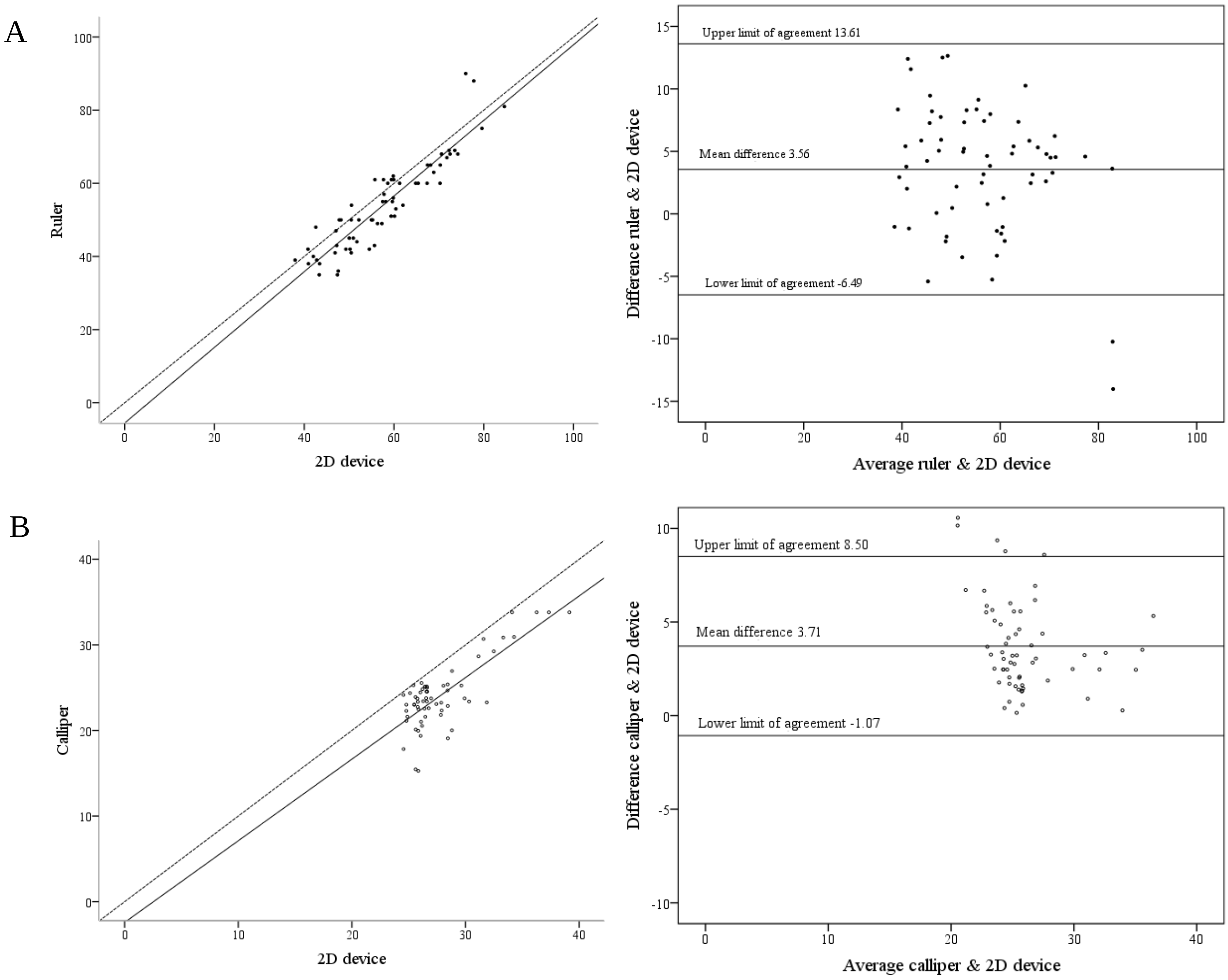

Figure 3. Scatterplots (-------- line of perfect agreement; —— linear regression line fit to the data) and Bland-Altman plots with indication of mean difference, 95% upper and lower limits of agreement for agreement of teat length measured with the ruler and the 2D device (A) and of diameter at 50% of the teat measured with the calliper and the 2D device (B).

Experiment 2

Repeatability. Teat length was on average 55.13 mm and ranged between 34.29 and 87.23 mm. No significant difference was found with the repeated measures ANOVA between teat length measurements performed by the same operator ($P > 0.05$). Coefficient of variation over all measurements was low (3.26%). The scatterplot shows a strong correlation between measurements 1 and 5 (CCC = 0.92) and the mean difference was 0.07 mm with upper and lower limits of agreement ranging between 6.94 and -6.81 mm.

The mean diameter at the middle of the teat was 26.88 mm and teats varied between 21.18 and 36.79 mm. The Friedman test indicates that measurement had no significant effect on the diameter at the teat middle ($P > 0.05$). The CV of all diameter measurements was low (1.74%). Measurements 1 and 5 were strongly correlated (CCC = 0.94) and the mean difference between the two measurements was -0.09 mm. The upper and lower limits of agreement ranged between 1.83 and -2.01 mm.

Reproducibility. Teats were on average 54.77 mm long and ranged between 32.16 and 94.67 mm. The repeated measures ANOVA's show that operator significantly affects teat length measurements ($P < 0.001$), whereas no significant differences were present within the group of operators with cow experience ($P > 0.05$), nor within the group of operators with tool experience ($P > 0.05$). The CV were 7% for operators in general, 6.02% for operators familiar in working with cows, and 5.27% for tool-experienced operators. A strong correlation (CCC = 0.93) was found between the teat length measurements of the 2 randomly selected operators with cow experience. Measurements differed on average 1.43 mm. The 95% upper and lower limits of agreement were 10.85 and -7.98 mm, respectively.

The mean diameter at the middle of the teat was 25.07 mm and ranged between 19.82 and 34.48 mm. The Friedman test indicates that diameter measurements at the middle of the teat significantly differ between operators ($P < 0.001$) and between operators with cow experience ($P = 0.006$), but operators with tool experience did not significantly differ from each other ($P > 0.05$). The CV were 2.3%, 2.16% and 1.88% for operators in general and for operators with cow and tool experience, respectively. The diameter measurements performed by the 2 randomly selected operators with tool experience were strongly correlated (CCC = 0.97). The mean difference between the 2 operators was 0.25 mm and the 95% limits of agreement of the Bland-Altman plots were situated at 1.91 mm and -1.42 mm.

DISCUSSION

Based on a number of analyses, we conclude that the ruler was both repeatable and reproducible in measuring teat length when the operators had experience in working with cows. As operators in field conditions are assumed to have cow experience, the effect of cow experience was not examined. Similar, the calliper was highly repeatable in measuring diameter at the middle of the teat but was shown not to be reproducible. We hypothesize that the pressure applied by the calliper on the teat during measurements will influence the results. A low CV was found between duplicates performed by the same operator (approximately 3%), whereas a much higher CV was present between operators (approximately 8%). This high CV between operators suggests that the applied pressure is operator-dependent. To obtain precise results with the calliper, measurements should therefore be performed by one operator or operators should measure at equal pressure. In a previous study by Hamann et al. (1988), a CV of 8 to 10% was reported for teat end thickness measurements performed with the modified cutimeter by the same operator. A higher degree of precision is obtained with the electronic calliper device that uses a pressure sensor which ensures that identical pressure is applied to the teat (Hamann et al., 1988).

The 2D device was repeatable in measuring teat length, although experience in working with cows is needed to obtain reproducible results. Furthermore, the method was shown to be repeatable and, when the operators have experience with the tool, to be reproducible in measuring diameters of the teat. The effect of cow and tool experience on repeatability was not examined, nevertheless, some training in working in close interaction with cows and with the measuring device is advised.

The 2D device measured on average significantly longer teats than the ruler (mean difference 3.6 mm). This finding can be explained by the fact that the point at which the udder ends and the teat begins is not anatomically discernible at the exterior of the teat, potentially hindering teat measurements. A part of the udder might be inserted through the opening of the 2D device during measurements and consequently be included in the teat length values. Equally, the ruler, but also other measuring methods, might experience problems with distinguishing the teat from the udder. Nonetheless, the two methods were found to be repeatable and reproducible when operators were familiar with cows, and they had similar precision in measuring teat length.

Poor agreement was found between the 2D device and the calliper. The 2D device measured consistently and significantly broader teats than the calliper. Smaller diameters measured with the calliper were probably due to the pressure applied by operators, which is inherent to the instrument.

Although the studied teat measuring devices poorly agreed in teat length and teat diameter measurements, this is trivial when comparing measurements performed with the same device, and for the calliper by the same operator. Therefore, all three methods allow to be used for various purposes. First, the devices can be used in practice as advisory tools for consulting organizations to help farmers select the most suitable liner for their herd or to select towards more uniform teat dimensions. Second, the methods can be applied in research to investigate the relationship between teat dimensions and teat condition or teat dimensions and udder health.

CONCLUSIONS

In conclusion, the ruler might be slightly more precise in measuring teat lengths than the 2D device, and modifications to the calliper, such as adding a pressure sensor, might result in a more acceptable precision. Nevertheless, the 2D device could be more advantageous for applications in practice, because it is a fast and semi-automated method that gives more information of the teat per measurement, and thus allows to gather information on a large scale with reduced effort, albeit some training in working with the measuring device and, not surprisingly, with cows is needed.

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CHAPTER 5

VARIANCE COMPONENTS OF TEAT DIMENSIONS IN DAIRY COWS AND ASSOCIATED FACTORS

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ABSTRACT

Traditionally, all cows within a herd are milked with the same teatcup liner, although it is hypothesized that considerable variation in teat dimensions exists between cows and quarters within cows. To study at which level (herd, cow, quarter) most variation in teat dimensions resides, and to identify factors explaining (part of) this variation, both a cross-sectional ($n_{\text{obserservations}}$ = 2,715) and a longitudinal study ($n_{\text{observations}}$ = 8,678) were conducted. Using an objective and easy-to-use measuring device, teat length and teat diameters were determined. In both studies, most variation in teat dimensions was present at the cow or within-cow level, and not at the herd level, indicating that choosing a teatcup liner that is identical for all cows in a herd is far from optimal. Quarter position (front versus hind), parity and lactation stage were identified as factors associated with teat length and teat diameters. Generally, front teats were longer and broader than hind teats. Teat length and diameters increased with parity, although the increase in teat length was not significant from second parity onwards in front teats based on observations from the longitudinal study. After the first 30 days in milk, teat length substantially and significantly increased, whereas teat diameters decreased. We suggest that better results in teat condition, and eventually in udder health, might be yielded when different teatcup liners are chosen for front versus hind teats or for cows of different parity or lactation stage, with special attention to the first 30 DIM. However, before practical application, the biological relevance of these differences should be examined first.

INTRODUCTION

During the last 20 years, genetic selection in dairy cows has resulted in on average smaller teats (Theron and Mostert, 2004; Graff, 2005; Dube et al., 2009). Nevertheless, it looks as if there is still considerable variation in teat dimensions and morphology between cows in a herd (Rasmussen et al., 2004). Cows within a herd, however, are typically milked with the same type of teatcup liner. If the liner does not fit the teat properly, its main function to cyclically massage the teat to avoid congestion and oedema will be strongly impaired (Mein et al., 2004), negatively influencing milking characteristics and potentially also udder health. Consequently, the choice of a liner whose dimensions match to those of the teat is critical.

Mismatching between the liner and the teat can be very diverse. For example, teats that are too short for a given liner do not reach the collapsing point of the liner, whereas teats that are too long, penetrate the liner below the collapsing point. In both cases, the liner cannot massage the teat end, resulting in poor teat end condition (Mein et al., 2004; Rasmussen et al., 2004). During milking, forces between the teat and the teatcup liner are needed for the liner to maintain a stable position on the teat. The main sources are the frictional force between the teat and the barrel of the liner or the mouthpiece lip. A good seal is therefore required and depends on the combination of teat dimensions and liner design. A higher mouthpiece chamber vacuum (MPCV) increases the friction between liner and teat (Mein et al., 1973). A medium high MPCV (range 10-30 kPa) should be obtained since both a very low and very high MPCV are associated with poor udder health (Ronningen and Rasmussen, 2008). When the combination of liner and teat fails to maintain a seal between the teat and the mouthpiece lip, air leakage through the mouthpiece opening may occur, resulting in abrupt loss of vacuum (low MPCV) and liner slips (Borkhus and Ronningen, 2003). These conditions may transfer bacteria or bacteria-contaminated milk droplets from outside the teat into the teat canal or teat cistern and thus increase the incidence of intramammary infections (IMI) (Baxter et al., 1992). High MPCV is suggested to cause congestion and discomfort (Newman et al., 1991; Rasmussen, 1997). These higher MPCV levels results from a good seal at the mouthpiece opening but an inadequate seal between the teat and the liner barrel (Borkhus and Ronningen, 2003), and thus occur when the bore of the liner is too broad. Certain problems associated with machine milking could be avoided if teat size could be standardized more within a herd, e.g. by genetic selection.

Knowledge of the level (herd, cow, quarter) at which most variation in teat dimensions resides and identification of factors explaining (part of) this variation is needed to select towards more uniform teat sizes in a herd or to formulate advices on how to deal with the existing variation. Multilevel or mixed models analysis that adjust for clustering of teats within cows and cows within herds can estimate the contribution of those levels to the total variance in teat dimensions (Dohoo et al., 2001). A limited number of studies have performed multilevel analysis studying teat dimensions but, to our knowledge, none have quantified the variance components.

Teat length mainly depends on breed of cows (Wufka and Willeke, 2001), whereas teat diameter 1 cm above the Furstenberg rosette does not differ significant between Holstein cows, Brown-Swiss, Simmental and crossbreeds (Seker et al., 2009). The differences between breeds for diameters at other heights of the teat have, however, not yet been studied. An increase in teat length and teat diameter with parity, although not always significant, has been reported (Seykora and McDaniël, 1986; Tilki et al., 2005; Seker et al., 2009). Less agreement exists on the relation between stage of lactation and teat dimensions. An increase in teat length during early lactation was noted by some authors (Seykora and McDaniël, 1986; Wufka and Willeke, 2001; Graff, 2005) and Tilki et al. (2005) found a significant increase with advancing lactation only in hind teats in a study on Brown Swiss cows. In contrast, Michel and Rausch (1988) reported no significant association between lactation stage and teat length. According to some studies, teat diameters do not change significantly as the lactation progresses (Tilki et al., 2005; Seker et al., 2009), whereas one study detected a significant decrease between first and subsequent stages (Graff, 2005).

Different methods are used to measure teat morphology. Most are subjective, lack accuracy, and have low or unspecified reproducibility and repeatability. Contradictions between studies might therefore be caused by different measuring methods or low reproducibility of some methods. For that reason, we recently developed and validated a measuring device able of recording teat dimensions in an objective, accurate and precise way (Zwertvaegher et al., 2011).

The aims of this study were (1) to determine at what level of the hierarchy (herd, cow, quarter, and observation within quarters) most of the variation in teat dimensions (length and diameter) was present using a recently developed and validated measuring device, and (2) to identify cow and quarter level factors associated with the teat dimensions. This was done using data collected in both a cross-sectional and a longitudinal field study.

MATERIALS AND METHODS

Cross-sectional Study

Herds and Animals. A cross-sectional study was conducted on 15 randomly selected Flemish dairy herds. All herds participated in the Dairy Herd Improvement Association (DHIA) program in Flanders (CRV, Oosterzele, Belgium). Teat measurements were performed within 7 days relative to the DHIA recordings. Between October 2008 and February 2009, 2,715 teats from 683 Holstein Friesian cows were measured. Table 1 shows the structure of the data and gives an overview of some cow characteristics.

Data collection. Teat length and diameters were determined using an 2D-vision-based measuring technique developed at the Institute for Agricultural and Fisheries Research (ILVO) in Flanders, Belgium (Zwertvaegher et al., 2011). All measurements were performed prior to evening milkings and between teat cleaning and fore-stripping to avoid interference of dirt particles or milk droplets present on the teats. The teat was positioned straight into the device and a picture was taken. All measurements were performed by the same person. After a picture was taken, a second person performed visual control of the image to ascertain the requirements needed for further processing were met and when this was not the case, a new picture was made. All pictures were analyzed using a software program developed at ILVO determining teat length as well as teat diameters at 3 different heights of the teat, i.e. at 75% (teat base), 50% (teat barrel) and 25% (teat tip) relative to the teat end. Different cow and quarter level factors potentially associated with teat length and diameter were recorded (Table 2).

Statistical analysis. Prior to statistical analysis, all data were checked for unlikely values. No data were excluded for this reason. To approximate normality, a reciprocal transformation of teat diameter (1/D75, 1/D50, 1/D25) multiplied by 1,000 was used.

To evaluate the proportion of variance present at the different levels of the data, three-level (herd, cow, and quarter) null-models (intercept only) were fit for teat length and 1/D75, 1/D50, and 1/D25 as dependent variables, respectively, using SAS 9.3 (PROC MIXED, SAS Institute Inc, NC, USA). Herd and cow were included as random effects to correct for clustering of cows within herds and quarters within cows, respectively. The reduction in variance estimates by the factors significantly associated with teat length and diameters in the final linear mixed regression model was evaluated numerically.

Table 1. Structure of the data and descriptive statistics (mean and range) of number of cows measured per herd, age, parity, days in milk (DIM), milk yield and somatic cell count (SCC) of cows at test-day of the cross-sectional and longitudinal study, respectively.

	Cross-sectional study			Longitudinal study		
	n	Mean	Range	n	Mean	Range
Herds	15	…	…	8	…	…
Cows	683			250		
Number per herd	…	45	21 – 72	…	31	10 – 96
Age (days)	…	1,638	695 – 5,105	…	1,745	660 – 4,781
Parity	…	2.6	1 – 11	…	3.0	1 – 11
DIM	…	191	5 – 819	…	177	0 – 631
Milk yield at test-day	…	27.7	5.6 – 56.8	…	26.7	3.1 – 56.8
SCC at test-day	…	201,400	4,000 – 5,926,000	…	243,680	4,000 – 8,047,000
Quarters	2,715	…	…	988	…	…
Observations	2,715	…	…	8,678	…	…

The regression-model building process to identify these factors and thus to build the final model, involved several steps as described previously (De Vliegher et al., 2004). First, univariable associations were tested between all independent (Table 2) and the four dependent variables. Statistical significance in this step was assessed at $P < 0.15$. Second, Pearson correlation and Spearman correlation coefficients were calculated among the significant independent variables to detect multicollinearity. If two variables had a correlation coefficient ≥ 0.6, only one was selected for further analysis. Strong correlations (≥ 0.6) were found between age and parity, and between milk production and lactation stage. Cow level variables parity and lactation stage, respectively, were selected for further analyses. In a third step, multivariable models were fit. The significance level for this step was set at $P < 0.05$. Finally, all two- and three-way interactions were tested and removed when non-significant ($P > 0.05$). The fit of the final models was evaluated by examination of the normal probability plots of residuals and by inspection of the residuals plotted against the predicted values. Least squares means (LSM) were calculated.

Table 2. Overview of recorded herd, cow, quarter and observation level factors potentially associated with teat length and teat diameters in the cross-sectional (herd, cow, quarter) and longitudinal (cow, quarter, observation) study, respectively.

Herd level
  Month of measurement (October – November; December; February)
Cow level
  Age (days) – Parity (1, 2, 2+) – Lactation stage (0-30, 31-60, 61-120, 121-180, 181-240, 240+
Quarter level
  Quarter position1 (Front, Hind) – Quarter position2 (Left, Right)
Observation level
  Measurement number (1 – 13)

Longitudinal Study

Herds and Animals. A longitudinal study was conducted on 8 Flemish dairy herds from May 2008 to May 2009. All herds participated in the Dairy Herd Improvement Association (DHIA) program in Flanders (CRV, Oosterzele, Belgium). On 2 of the 8 herds, all cows in lactation at the moment of the measurement participated in the study. On the 6 remaining herds, a cohort of 10 cows per herd was randomly selected within parity blocks (4 heifers, 3 cows of second parity, and 3 cows of third or higher parity) at the beginning of the study. Before the end of that study, 20 out of 60 cohort cows were culled, as described before (Verbist et al., 2011). All cohort cows culled before the 12th month of the study (n = 14) were replaced by randomly selected herd mates of the same parity. In total, 8,678 measurements were conducted on 988 teats from 250 Holstein Friesian cows. The structure of the data and an overview of some cow characteristics is given in Table 1.

Data collection. Monthly (n = 13), teat length and diameters were measured. All measurements were performed as reported above. All measurements within a milking were done by the same person. Table 2 gives an overview of different cow, quarter and observation level factors potentially associated with teat length and diameter that were recorded.

Statistical analysis. Statistical analyses were performed as described above, although an extra level was included because of the repeated measurements within quarters: four-level null-models (herd, cow, quarter, and observation within quarter) were fit for teat length and 1/D75, 1/D50 and 1/D25 as dependent variables, respectively, using SAS 9.3 (PROC MIXED, SAS Institute Inc, NC, USA). Herd, cow, and quarter were included as random effects to correct for clustering of cows within herds, quarters within cows, and repeated measurements within quarters, respectively. Measurement number was forced into all models as fixed effect to model the repeated measurements. The covariance between repeated measurements was modeled using the Compound Symmetry (CS) structure.

As in the cross-sectional study, strong correlations were found between age and parity, and between milk production and lactation stage, and only parity and lactation stage were selected for further analyses. Two- and three-way interactions were tested for all significant factors, but not with measurement number.

RESULTS

Cross-sectional Study

Variance components. The variance components are presented in Table 3, both for the null-model and the final model. The largest proportion of variation in teat length (58.5%) was present at the quarter level, i.e. between quarters, whereas for the different teat diameters the largest proportion of variation resided at the cow level (> 50%). Only a small part of the total variation in the four teat dimension variables was present at the herd level.

Including the significant fixed effects in the model (resulting in the final models), reduced the unexplained variance estimates for teat length at quarter level with 46%. A decrease at cow level of 23, 24, and 21% was found for 1/D75, 1/D50, and 1/D25, respectively.

Factors associated with the teat dimensions. All independent variables were significantly associated with the four dependent variables in the univariable models, except for quarter position1, quarter position2, and month of measurement: quarter position1 was not associated with 1/D75, quarter position2 not with teat length and 1/D75, and month of measurement was not associated with any of the dependent variables. Quarter position1, parity and lactation stage were significantly associated with teat length in the final model (Table 4).

Table 3. Variance components at each level of the null-models (intercept only) and the final models of the cross-sectional study for teat length and the reciprocal transformations of teat diameters at 75% (1/D75), 50% (1/D50) and 25% (1/D25) of teat length relative to the teat end, respectively. In bold, the proportion of variation present at the level where the largest variation resides.

Dataset	Data hierarchy	Variance components			
		Null-model		Final model	
		Var.est.[1]	%[2]	Var.est.	%
Teat length	Herd	1.4	2.0	1.4	2.6
	Cow	28.5	39.5	30.4	55.9
	Quarter	42.2	**58.5**	22.6	41.5
	Total variance	72.1	100	54.4	100
1/D75	Herd	2.2	12.9	2.2	14.5
	Cow	9.2	**53.3**	7.1	46.9
	Quarter	5.8	33.8	5.8	38.6
	Total variance	17.2	100	15.1	100
1/D50	Herd	2.3	13.1	2.2	15.0
	Cow	10.8	**62.6**	8.2	56.3
	Quarter	4.2	24.3	4.2	28.7
	Total variance	17.3	100	14.6	100
1/D25	Herd	1.4	6.6	1.4	7.7
	Cow	13.2	**62.1**	10.5	57.9
	Quarter	6.7	31.3	6.2	34.4
	Total variance	21.3	100	18.1	100

[1] Variance estimate.

[2] Variance proportion present at the different levels.

Hind teats (LSM: 47.2 mm) were significantly shorter than front teats (LSM: 54.9 mm) and teat length significantly increased with increasing parity. The longest teats were observed between 121 to 180 DIM (LSM: 52.0 mm) and the shortest at the start of the lactation (0 to 30 DIM) (LSM: 49.1 mm). Hind teats were slightly but significantly smaller at the tip (D25) than front teats. Teat diameters at all heights significantly increased with parity and decreased in early lactation. Furthermore, the interaction between quarter position1 and parity was significant at the barrel (D50) of the teat: the difference between front and hind teats was only significant in cows of parity three or higher (Fig. 1A). The interaction term between quarter position1 and lactation stage was also significant for diameter at 50% of the teat (Fig. 1B).

Table 4. Final multilevel linear models of the cross-sectional study describing the factors associated with teat length and the reciprocal transformations of teat diameters at 75% (1/D75), 50% (1/D50) and 25% (1/D25) of teat length relative to the teat end, respectively.

Independent variable		$N_{quarters}$	Teat length				1/D75				1/D50				1/D25			
			β[1]	SE[2]	*P*-value	LSM[3]	β	SE	*P*-value	LSM[4]	β	SE	*P*-value	LSM[4]	β	SE	*P*-value	LSM[4]
Constant			49.9	0.6	…		35.9	0.4	…		38.0	0.5	…		44.4	0.4	…	
Quarter position1					< 0.001				NSU[6]				0.02				< 0.001	
	Front	1,358	ref.[5]	…		54.9					ref.	…		25.9	ref.	…		22.3
	Hind	1,357	-7.7	0.2		47.2					0.2	0.1		25.8	1.2	0.1		21.8
Parity					< 0.001				< 0.001				<0.001				< 0.001	
	1	959	ref.	…		48.8	ref.	…		26.5	ref.	…		24.7	ref.	…		21.1
	2	583	2.3	0.6		51.1	-1.4	0.3		27.6	-1.8	0.3		25.9	-2.1	0.4		22.1
	2+	1,173	4.3	0.5		53.1	-2.8	0.3		28.7	-3.4	0.3		27.0	-3.8	0.3		23.0
Lactation stage					0.03				< 0.001				<0.001				0.01	
(DIM)	0-30	236	ref.	…		49.1	ref.	…		29.2	ref.	…		27.1	ref.	…		22.8
	31-60	232	1.9	1.1		51.0	1.7	0.5		27.8	1.7	0.6		26.0	1.8	0.6		21.9
	61-120	631	1.4	0.9		50.5	2.3	0.5		27.4	2.2	0.5		25.6	1.9	0.5		21.9
	121-180	346	2.9	1.0		52.0	2.2	0.5		27.5	2.0	0.5		25.7	1.9	0.6		21.9
	181-240	321	2.6	1.0		51.7	2.8	0.5		26.9	2.4	0.5		25.5	1.9	0.6		21.9
	240+	949	2.6	0.9		51.7	3.1	0.4		26.8	2.7	0.5		25.3	1.8	0.5		21.9
Quarter position1 x parity[8]		2,715			NSM[7]				NSM				0.04				NSM	
Quarter position1 x lactation stage[8]		2,715			NSM				NSM				0.02				NSM	

[1] Linear regression coefficient.

[2] Standard error.

[3] Least Squares Means (in mm).

[4] Untransformed Least Squares Means (in mm).

[5] Reference.

[6] Not significant in univariable model.

[7] Not significant in multivariable model.

[8] Estimates not shown.

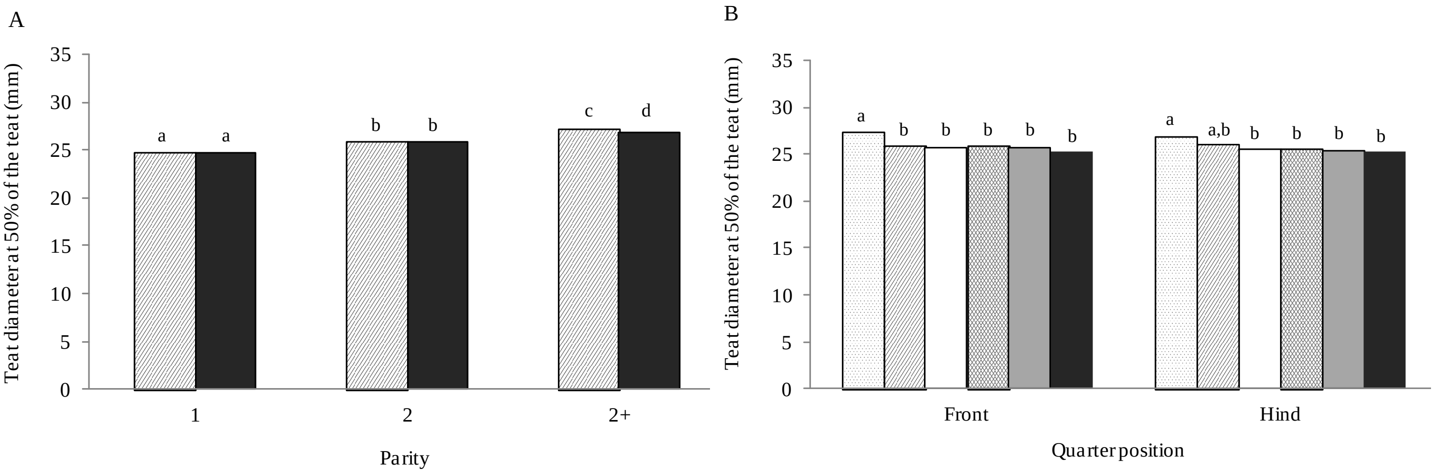

Figure 1. Untransformed least squares means [(1/LSM)*1,000] (in mm) of the cross-sectional study for diameter at 50% of the total teat length (A) for front (▨) and hind (■) quarters in first, second and older parity cows, and (B) for front and hind quarters in 0-30 (░), 31-60 (▨), 61-120 (□), 121-180 (▩), 181-240 (▒) and 240+ (■) DIM. Different superscripts within the same parity and quarter position denote statistical significance at $P < 0.05$.

Longitudinal Study

Variance components. The largest proportion of variation in teat length (41.5%) and diameters (> 50%) resided at the cow level (Table 5), whereas only a minority of the variation in the four different teat dimension variables was present at the herd level.

In the final models, the unexplained variance estimates for teat length were reduced with only 5% at cow level, whereas a large reduction (70%) was found at quarter level, the level where the second largest proportion of variation was situated (31.2%). For teat diameters, the estimates at cow level were reduced with 23%, 32% and 32% for 1/D75, 1/D50 and 1/D25, respectively. Furthermore, substantial decreases, varying from 16 to 22%, were found at observation level for teat diameters.

Factors associated with the teat dimensions. In the univariable models, quarter position2 was not associated with any of the dependent variables and milk production was not associated with teat length. Factors significantly associated with the different teat dimensions in the final model were measurement number, quarter position1, parity and lactation stage (Table 6). Hind teats (LSM: 50.9 mm) were significantly shorter than front teats (LSM: 58.6 mm). Hind teats lengthened with parity, whereas front teats only significantly increased in length from first to second parity (Fig. 2A). Within parities, the increase in teat length from the first stage of lactation (0-30 DIM) to the second (31-60 DIM) was significant and substantial with little change thereafter, except within the first parity where the increase in teat length at the start of the lactation was more gradual (Fig. 2B).

At all heights of the teat, front teats were significantly broader than hind teats. Teat diameters increased with parity. Generally, diameters significantly decreased at the beginning of the lactation but, depending on the relative height on the teat (75%, 50% or 25%) and the parity, continued to decrease or stabilized. Figure 3 illustrates the interaction between parity and lactation stage for diameter at 50% of the teat. In addition, the interactions between quarter position1 and parity, and between quarter position1 and lactation stage were significant at the teat end (D25) (Fig. 4A and 4B, respectively).

Table 5. Variance components at each level of the null-models (intercept only) and the final models of the longitudinal study for teat length and the reciprocal transformations of teat diameters at 75% (1/D75), 50% (1/D50) and 25% (1/D25) of teat length relative to the teat end, respectively. In bold, the proportion of variation present at the level where the largest variation resides.

Dataset	Data hierarchy	Variance components			
		Null-model		Final model	
		Var.est.[1]	%[2]	Var.est.	%
Teat length	Herd	4.2	4.8	6.6	9.6
	Cow	36.5	**41.5**	34.8	50.7
	Quarter	27.4	31.2	8.3	12.1
	Observation	19.8	22.5	19.0	27.7
	Total variance	87.9	100	68.7	100
1/D75	Herd	1.1	6.6	1.5	10.9
	Cow	8.2	**51.1**	6.3	45.7
	Quarter	1.8	11.3	1.8	13.0
	Observation	5.0	31.0	4.2	30.4
	Total variance	16.0	100	13.8	100
1/D50	Herd	1.5	8.6	1.8	13.2
	Cow	9.2	**53.1**	6.3	46.3
	Quarter	1.5	8.8	1.5	11.0
	Observation	5.1	29.5	4.0	29.4
	Total variance	17.3	100	13.6	100
1/D25	Herd	2.1	10.1	2.2	13.5
	Cow	10.9	**51.3**	7.4	45.4
	Quarter	3.1	14.7	2.4	14.7
	Observation	5.1	23.9	4.3	26.4
	Total variance	21.2	100	16.3	100

[1] Variance estimate.

[2] Variance proportion present at the different levels.

Table 6. Final multilevel linear models of the longitudinal study describing the factors associated with teat length and the reciprocal transformations of teat diameters at 75% (1/D75), 50% (1/D50) and 25% (1/D25) of teat length relative to the teat end, respectively.

Independent variable		$N_{quarters}$	Teat length				1/D75				1/D50				1/D25			
			β[1]	SE[2]	*P*-value	LSM[3]	β	SE	*P*-value	LSM[4]	β	SE	*P*-value	LSM[4]	β	SE	*P*-value	LSM[4]
Constant			53.3	1.1	…		34.0	0.5	…		36.7	0.5	…		43.0	0.6	…	
Measurement number					< 0.001				< 0.001				< 0.001				< 0.001	
	1	682	ref.[5]	…		55.1	ref.	…		29.7	ref.	…		27.5	ref.	…		23.3
	2	593	0.8	0.2		55.9	-0.6	0.1		30.2	-0.3	0.1		27.9	-0.3	0.1		23.5
	3	610	0.8	0.3		55.9	-0.7	0.1		30.3	-0.7	0.1		28.2	-0.7	0.1		23.8
	4	622	0.01	0.3		55.1	-0.2	0.1		29.9	-0.1	0.1		27.7	-0.2	0.1		23.5
	5	594	-0.2	0.3		54.9	-0.3	0.1		29.9	-0.3	0.1		27.9	-0.4	0.1		23.5
	6	599	-0.2	0.3		54.8	0.2	0.1		29.5	0.5	0.1		27.2	0.4	0.1		23.1
	7	676	0.02	0.3		55.1	0.2	0.1		29.4	0.3	0.1		27.3	0.2	0.1		23.2
	8	703	-1.3	0.3		53.8	0.7	0.1		29.1	0.8	0.1		27.0	0.7	0.1		22.9
	9	705	-0.6	0.3		54.5	0.6	0.1		29.2	0.7	0.1		27.1	0.4	0.1		23.1
	10	730	-1.1	0.3		54.0	0.6	0.1		29.2	0.8	0.1		27.0	0.6	0.1		23.0
	11	719	-1.3	0.3		53.8	0.6	0.1		29.2	0.7	0.1		27.1	0.6	0.1		23.0
	12	732	-0.3	0.3		54.8	0.8	0.1		29.0	0.6	0.1		27.1	0.5	0.1		23.1
	13	713	-1.3	0.3		53.8	0.5	0.1		29.2	0.6	0.1		27.2	0.4	0.1		23.1
Quarter position1					< 0.001				< 0.001				< 0.001				< 0.001	
	Front	4,337	ref.	…		58.6	ref.	…		29.8	ref.	…		27.6	ref.	…		23.7
	Hind	4,341	-7.7	0.2		50.9	0.5	0.1		29.3	0.6	0.1		27.1	1.6	0.1		22.8
Parity					< 0.001				< 0.001				< 0.001				< 0.001	
	1	2,241	ref.	…		51.9	ref.	…		27.9	ref.	…		25.8	ref.	…		22.2
	2	2,527	3.4	0.3		55.4	-2.3	0.2		29.9	-2.8	0.2		27.7	-2.5	0.2		23.5
	2+	3,937	4.9	0.5		56.9	-3.4	0.2		30.9	-4.2	0.2		28.8	-3.8	0.3		24.2
Lactation stage					< 0.001				< 0.001				< 0.001				< 0.001	
(DIM)	0-30	828	ref.	…		52.4	ref.	…		30.8	ref.	…		28.5	ref.	…		23.9
	31-60	750	2.2	0.2		54.5	1.0	0.1		29.9	1.1	0.1		27.6	1.0	0.1		23.4
	61-120	1,610	2.6	0.2		55.0	1.6	0.1		29.3	1.5	0.1		27.3	1.3	0.1		23.2
	121-180	1,518	3.1	0.2		55.4	1.8	0.1		29.2	1.8	0.1		27.1	1.5	0.1		23.1
	181-240	1,451	3.2	0.2		55.5	1.9	0.1		29.1	2.0	0.1		27.0	1.6	0.1		23.0
	240+	2,521	3.2	0.2		55.5	2.0	0.1		29.0	2.3	0.1		26.7	1.9	0.1		22.9
Quarter position1 x parity[7]					0.002				NSM				NSM				0.002	
Quarter position1 x lactation stage[7]					NSM[6]				NSM				NSM				0.003	
Parity x lactation stage[7]					< 0.001				< 0.001				< 0.001				< 0.001	

[1] Linear regression coefficient.

[2] Standard error.

[3] Least Squares Means (in mm).

[4] Untransformed Least Squares Means (in mm).

[5] Reference.

[6] Not significant in multivariable model.

[7] Estimates not shown.

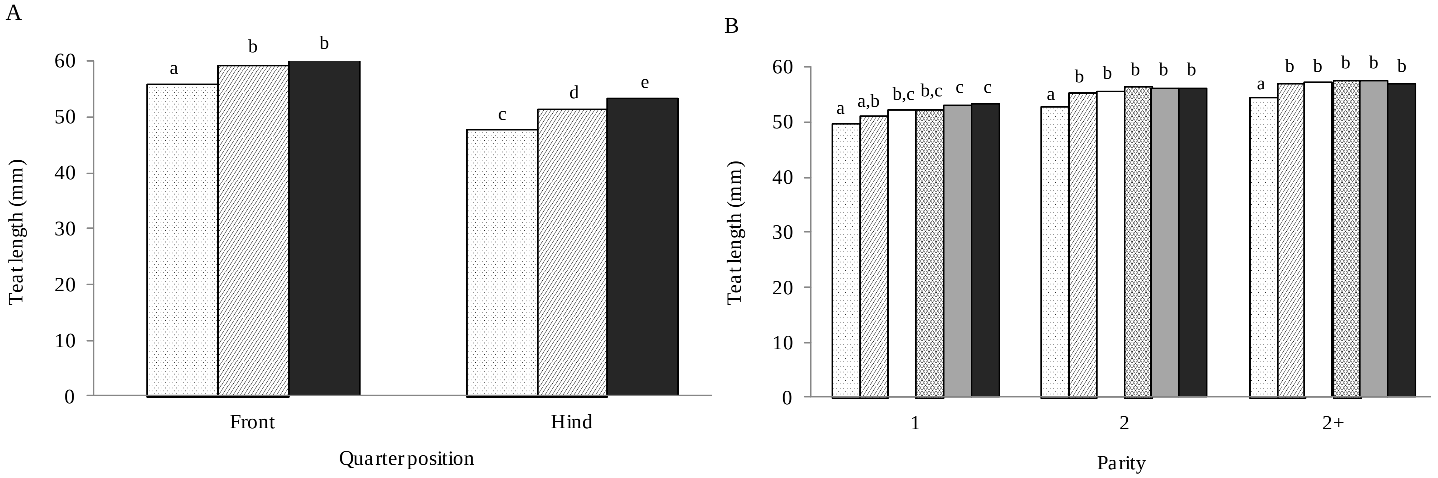

Figure 2. Least squares means (in mm) of the longitudinal study for teat length (A) for front and hind quarters in first (⬚), second (▨) and older parity (■) cows, and (B) for first, second and older parity cows in 0-30 (⬚), 31-60 (▨), 61-120 (□), 121-180 (▩), 181-240 (▒) and 240+ (■) DIM. Different superscripts within the same quarter position and parity denote statistical significance at $P < 0.05$.

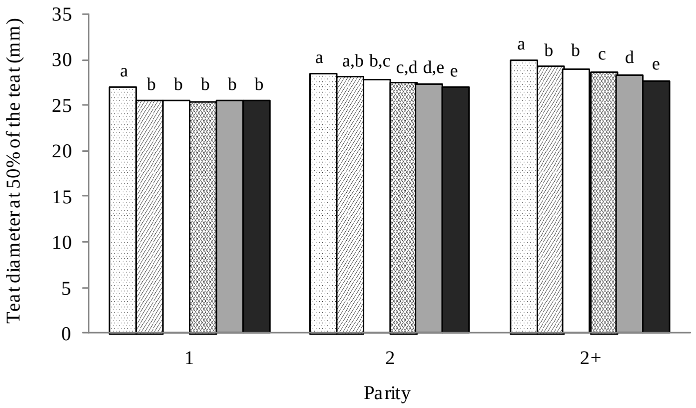

Figure 3. Untransformed least squares means [(1/LSM)*1,000] (in mm) of the longitudinal study for diameter at 50% of the total teat length for first, second and older parity cows in 0-30 (⬚), 31-60 (▨), 61-120 (□), 121-180 (▩), 181-240 (▒) and 240+ (■) DIM. Different superscripts within the same parity denote statistical significance at $P < 0.05$.

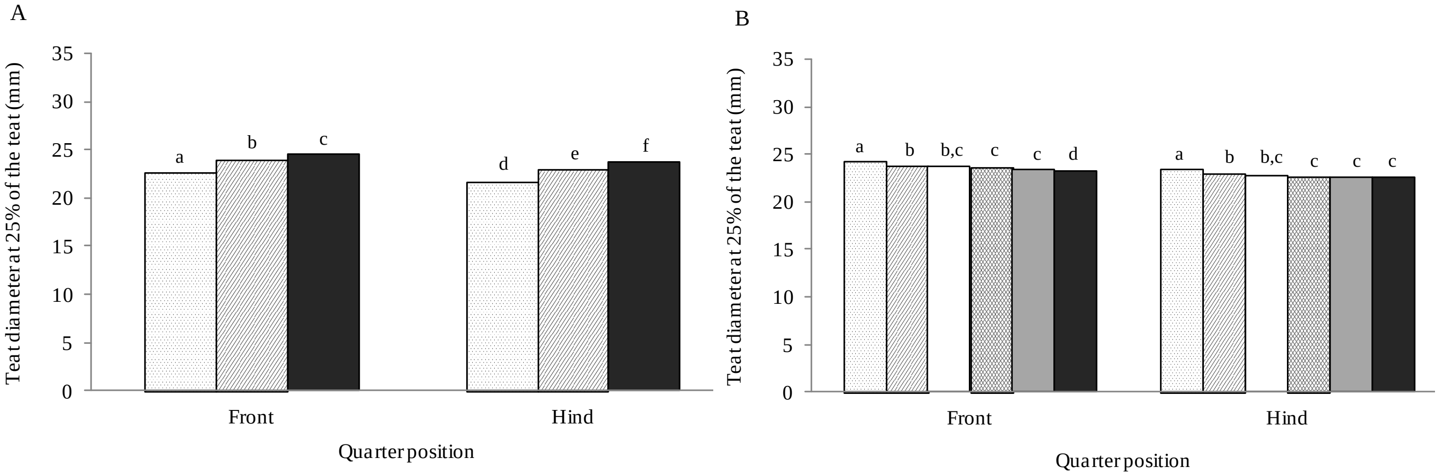

Figure 4. Untransformed least squares means [(1/LSM)*1,000] (in mm) of the longitudinal study for diameter at 25% of the total teat length (A) for front and hind quarters in first (░), second (▨) and older parity (■) cows, and (B) for front and hind quarters in 0-30 (░), 31-60 (▨), 61-120 (□), 121-180 (▩), 181-240 (▒) and 240+ (■) DIM. Different superscripts within the same quarter position and parity denote statistical significance at $P < 0.05$.

DISCUSSION

A cross-sectional and a longitudinal study were performed resulting in a large dataset of teat dimensions of almost 1,000 Holstein Friesian cows collected with a recently developed, easy-to-use and objective measuring device. Both studies revealed that most of the variation in teat length and teat diameters resided at the cow or the within-cow level, and not at herd level. As well, in both studies, a number of similar factors associated with teat dimensions were identified.

The herd level was identified as the level at which the least variation in teat dimensions is present. Because a mismatch between teats and teatcup liners will result in inefficient milking, potentially resulting in udder health problems, the choice of identical teatcup liners to milk all cows in a herd is far from ideal. Rather, as the majority of variation in teat dimensions is present at the cow, quarter and even within quarter level, differences between and within cows should be handled in a more appropriate way. Still, as herds within this study had comparable herd characteristics (i.e. similar breed and genetic background of the animals, but also herd size, milk production, general management and milking system) it could well be that the proportion of variation in teat dimensions at the herd level would be somewhat higher if herds which for example milked cows of different breeds, were included. The somewhat higher proportion of variation at the cow and quarter level in the cross-sectional study compared to that in the longitudinal study can be explained by the portions of variation at those levels that actually reside at the additional level (observation) in the latter.

In general, front teats were longer and slightly broader than hind teats. Previous studies also found longer front teats, but reported no or limited differences in diameter, both in Holstein Friesian cows and other breeds (Seykora and McDaniël, 1986; Tilki et al., 2005; Rovai et al., 2007). Those studies measured diameters using callipers, and as callipers slightly press the teat during measurements, the method may conceal the natural variation present. Weiss et al. (2004) noted that hind teats are thicker than front teats at 25 mm above the teat apex in Brown Swiss cows after prestimulation. Since this is an absolute measurement that is measured at a well-defined point on the teat (25 mm from the teat apex), and front teats are found to be longer than hind teats, the results can not unambiguously be compared with those found in studies that use relative measures such as ours.

In both our studies, teat diameters increased with parity, corresponding with previous findings (Seykora and McDaniël, 1986; Tilki et al., 2005). However, to our knowledge, this is the first study that examined the effect of parity on diameters other than at the barrel of the teat. Teat length also increased with parity but in the longitudinal study the increase in front teats was not significant from second parity onwards. The increase in teat dimensions is generally interpreted as the age-dependent evolvement of the udder and the teat (Graff, 2005).

A slight increase in teat length was noted in the first 30 DIM with little change thereafter. Although there is no clear consensus on the significance of lactation stage for teat length, the majority of studies reported an increase during the first months of lactation (Seykora and McDaniël, 1986; Graff, 2005; Tilki et al., 2005). Diameters at all heights of the teat generally decrease at the beginning of the lactation, as found in some studies (Seykora and McDaniël, 1986; Graff, 2005), but in our study, depending on the diameter (75%, 50% or 25%) and the parity, they continue to decrease or stabilize. Some studies found an overall decrease of teat diameters with lactation stage (Seykora and McDaniël, 1986; Graff, 2005), but others did not report any trend or significant effect (Tilki et al., 2005; Seker et al., 2009). The contradictory findings from those studies may be due to the choice made in classifying the lactation stage differently. The variation in teat dimensions might be determined by a combination of factors throughout the lactation. Teat length and diameter may be associated with milk yield, as suggested in dairy ewes (Fernandez et al., 1995). Furthermore, the action of the milking machine may cause long-term changes in teat dimensions. For example, after machine milking for several days Hamann and Stanitzke (1990) noticed a development of teat end thickness prior to milking and suggested the tissue might have adapted to machine milking. The age dependent or pregnancy related evolvement of the udder and the teat may also affect teat dimensions. As well, udder oedema can influence the dimensions of the teat (Waage et al., 2001). Therefore, pathologic udder oedema or the resolving of physiologic udder oedema 2 to 4 weeks after calving (Divers and Peek, 2007) may indirectly influence the teat dimension measurements, either by altering the actual dimensions of the teat or by hindering the measuring methods from measuring them accurately. This is consistent with the large decrease in teat diameter and increase in teat length from the first stage of lactation (0-30 DIM) to the second (31-60 DIM) found in these studies.

Because of the significant difference between front and hind teats, adapting the type of liner to the quarter position will take into account quarter level variance and might therefore contribute to better milking performances and reduce the incidence of IMI. One could imagine the use of milking clusters that hold liners that differ between front and hind quarters. As robots already allow for quarter individual milking, manufacturers could be challenged to provide various teatcups holding different liners, from which the automated milking system can choose when quarters with different teat dimensions are milked. Furthermore, since a considerable proportion of variation resides at the cow level, milking cows in groups of comparable teat sizes, such as the same parity or lactation stage, with adapted milking equipment could be helpful in safeguarding teat condition and udder health better. Although practically difficult, automated milking systems could be adapted to accomplish this.

Albeit significant differences in teat dimensions were found (e.g. between different parities), they were small and therefore their biological relevance still needs to be investigated. A study that examines the relationship between milking performance, teat condition and udder health on the one hand and teat dimensions on the other hand would be helpful to determine whether the findings of this study are relevant, and should help to focus on the most important steps in milking different cows or quarters with adapted milking equipment, accounting for the differences reported here. As part of the variation remained unexplained by the factors included, research regarding more factors potentially associated with teat dimensions might help in understanding the variability in teat dimensions and should focus more on differences between cows and differences between quarters within cows than on differences between herds because of the distribution of the variance over the different levels.

CONCLUSIONS

Teat length and diameter measurements performed with a newly developed, objective measuring device conducted in a cross-sectional and a longitudinal study on 15 and 8 dairy farms in Flanders, respectively, show that the largest proportion of variation resides at the cow or within-cow level and not at the herd level, indicating that choosing a teatcup liner that is identical for all cows in a herd is far from optimal. The results confirm that quarter position (front versus hind), parity and lactation stage are important factors associated with teat dimensions. Further, future studies should include more factors potentially associated with teat dimensions to unravel the remaining unexplained variation and should focus on the biological relevance of the differences in teat dimensions such as the relation with udder health.

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CHAPTER 6

ASSOCIATIONS BETWEEN TEAT DIMENSIONS AND MILKING-INDUCED CHANGES IN TEAT DIMENSIONS, AND QUARTER MILK SOMATIC CELL COUNTS IN DAIRY COWS

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Submitted

ABSTRACT

Although many studies have examined the relation between a wide range of factors and quarter milk SCC (qSCC), including physical characteristics of the teat and changes in teat tissue due to milking, the effect of short term, milking-induced changes in teat dimensions on SCC has not yet been investigated. To identify teat dimensions and milking-induced changes in teat dimensions associated with qSCC, a longitudinal study ($n_{observations}$ = 1,939) was conducted. Parity, stage of lactation, teat barrel diameter and changes in teat barrel diameter during milking were identified as factors associated with qSCC. Teats with wider barrels had higher qSCC. Negative changes in the diameter of the teat barrel during milking (i.e. thinner teats post milking compared with pre milking) were associated with lower qSCC, whereas positive changes (i.e. thicker teats post milking compared with pre milking) were associated with higher qSCC. Selection towards more optimal teat characteristics may therefore result in improved milk quality and udder health. However, it is suggested that a threshold exists for the maximum reduction in teat barrel diameter below which udder health is negatively influenced. If so, changes in teat barrel diameter might serve as indicator for suboptimal milking and incorrect teatcup liner choice of milking machine settings, and thus help improve the management of the herd.

INTRODUCTION

Mastitis is one of the most common and costly diseases in dairy cattle (Halasa et al., 2007). During intramammary infection (IMI), the milk somatic cell count (SCC) increases as part of the inflammatory response. In the absence of clinical symptoms, measuring of the SCC is the most frequently used indirect measure to detect subclinical mastitis (Beaudeau et al., 2002). Many studies have examined the relation between managerial, environmental, cow and quarter factors, including the physical characteristics of the teat, and SCC. Teat length was not significantly associated with SCC (Seykora and McDaniël, 1986; Coban et al., 2009), whereas SCC generally increased with increasing teat diameter (Seykora and McDaniël, 1986; Chrystal et al., 1999). One study, however, reported the absence of an association between SCC and teat diameter in Gir cows (Porcionato et al., 2010). It is well known that machine milking induces changes in both teat tissue (such as congestion and hyperkeratosis) and teat dimensions. Although changes in teat thickness have been associated with udder health (Zecconi et al., 1996) and guidelines for acceptable changes in teat end thickness have been formulated (Hamann and Mein, 1996), no studies have actually examined the effect of changes in teat dimensions on udder health. The objectives of this study were to examine potential associations between teat dimensions and short term, milking-induced changes in teat dimensions and quarter milk SCC.

MATERIALS AND METHODS

A longitudinal study including 72 Holstein-Friesian cows from 6 Flemish dairy herds, was conducted between June 2008 and May 2009. During one year, teat dimension and quarter milk somatic cell count (qSCC) measurements were performed monthly. Per herd, a cohort of 10 clinically healthy cows was randomly selected within parity blocks (4 heifers, 3 cows of second parity, and 3 cows of third or higher parity) at the beginning of the study. Before the end of the study, 18 out of 60 cohort cows were culled. All cohort cows culled before the 11th month of the study (n = 12) were replaced by randomly selected herd mates of the same parity. Quarter milk samples were collected and SCC was determined by means of a Fossomatic 5000 FC (Foss, Minnesota, USA). A natural logarithmic transformation of qSCC (LnqSCC) was performed to obtain a normal distribution.

Within 15 days relative to the qSCC recordings, teat dimensions (length and diameters) were determined using an 2D-vision-based measuring technique (Zwertvaegher et al., 2011) both immediately before and after evening milking. All pictures were analyzed using a software program determining teat length as well as teat diameters at three different heights of the teat, i.e. at 75% (further referred to as the teat base), 50% (teat barrel) and 25% (teat apex) relative to the teat end. Absolute and relative changes in teat length and teat diameters due to milking were calculated. This resulted in 16 different teat dimensions available for further study: teat length, teat diameter at teat base, teat barrel, and teat apex, respectively, as measured pre and post milking, as the absolute changes [post milking value – pre milking value], and as % changes relative to the pre milking value [(post milking value – pre milking value)/pre milking value x 100].

A four-level (herd, cow, quarter, and observation) model was fit with LnqSCC as dependent variable using SAS 9.3 (SAS Institute Inc, NC, USA). Herd, cow, and quarter were included as random effects to correct for clustering of cows within herds, quarters within cows, and repeated measurements within quarters, respectively. Measurement number was forced into all models as fixed effect to model the repeated measurements. The covariance between repeated measurements was modeled using the AR(1) structure.

The regression-model building process involved several steps as described previously (De Vliegher et al., 2004). In addition to the 16 different teat dimensions, the effects of parity (1, 2, 2+), stage of lactation (0-30, 31-60, 61-120, 121-180, 181-240, 240+ DIM), and quarter position (front, hind) on LnqSCC were assessed. First, univariable associations were tested between all independent variables (16 teat dimensions, parity, stage of lactation, and quarter position) and LnqSCC. Statistical significance in this step was assessed at $P < 0.15$. Second, Pearson correlation and Spearman correlation coefficients were calculated among the significant independent variables to avoid multicollinearity. If two variables had a correlation coefficient ≥ 0.6, only one was selected for further analysis. In a third step, multivariable models were fit using a backwards stepwise procedure at $P < 0.05$. Finally, the relevant two-, three-, and four-way interactions were tested between the fixed effects included in the final model, and removed in a backward stepwise manner when non-significant ($P > 0.05$). Least squares means (LSM) were calculated for the independent variables in the final model. The fit of the final model was evaluated by examination of the normal probability plots of residuals and by inspection of the residuals plotted against the predicted values.

RESULTS AND DISCUSSION

Descriptive statistics (mean, standard deviation and range) for the 16 different teat dimensions are presented in Table 1. The largest proportion of variation in LnqSCC as studied using a four-level null-model (no fixed effects included) resided at the observation level (62.2%), followed by the quarter (20.3%), cow (13.5%) and herd level (4.1%), respectively. Including the significant fixed effects, the model explained 20% of the total variance of LnqSCC.

Table 1. Descriptive statistics (mean, standard deviation and range) of teat length, diameter of the teat base, teat barrel, and teat apex as measured pre and post milking, as the absolute changes, and as the relative changes.

Teat dimensions	Mean ± SD	Range
Pre milking (in mm)		
Teat length	54.3 ± 9.2	28.5 – 83.1
Diameter teat base[1]	29.7 ± 3.8	21.2 – 52.0
Diameter teat barrel[2]	27.8 ± 3.3	19.1 – 48.9
Diameter teat apex[3]	23.7 ± 2.5	16.4 – 37.5
Post milking (in mm)		
Teat length	59.1 ± 10.7	27.8 – 93.5
Diameter teat base	29.8 ± 3.2	21.7 – 48.6
Diameter teat barrel	27.0 ± 2.6	20.7 – 45.7
Diameter teat apex	23.7 ± 2.1	16.9 – 37.1
Absolute change[4] (in mm)		
Teat length	4.8 ± 6.2	-15.2 – 31.6
Diameter teat base	0.0 ± 2.8	-10.3 – 12.2
Diameter teat barrel	-0.8 ± 1.9	-9.2 – 5.5
Diameter teat apex	0.0 ± 1.5	-6.3 – 4.7
Relative change[5] (in %)		
Teat length	9.2 ± 11.7	-25.6 – 64.4
Diameter teat base	0.7 ± 9.2	-24.8 – 45.4
Diameter teat barrel	-2.4 ± 6.5	-22.9 – 25.0
Diameter teat apex	0.3 ± 6.1	-18.6 – 23.1

[1] Diameter measured at 75% of the teat relative to the teat end.

[2] Diameter measured at 50% of the teat relative to the teat end.

[3] Diameter measured at 25% of the teat relative to the teat end.

[4] Post milking value – pre milking value

[5] [(post milking value – pre milking value)/pre milking value x 100]

Table 2. Final multilevel (herd, cow, quarter, observation) linear model describing teat dimensions associated with the natural logarithmic transformation of the quarter somatic cell count (LnqSCC).

Independent variable		$N_{measurements}$	LnqSCC			
			β[1]	SE[2]	*P*-value	LSM[3]
Constant			3.7	0.5	…	
Measurement number					0.06	
	1	159	ref.[4]	…		3.5
	2	138	0.0	0.1		3.5
	3	165	0.0	0.1		3.5
	4	150	0.0	0.1		3.6
	5	145	0.3	0.1		3.8
	6	144	0.2	0.1		3.7
	7	164	0.1	0.1		3.7
	8	182	0.0	0.1		3.5
	9	192	0.0	0.1		3.6
	10	159	-0.2	0.1		3.3
	11	198	0.0	0.1		3.5
	12	143	0.0	0.1		3.5
Parity					0.002	
	1	310	ref.	…		3.2
	2	712	0.4	0.1		3.6
	2+	917	0.6	0.2		3.9
Lactation stage					< 0.001	
(DIM)	0-30	147	ref.	…		3.2
	31-60	159	-0.3	0.1		2.9
	61-120	369	0.0	0.1		3.2
	121-180	368	0.5	0.1		3.7
	181-240	318	0.9	0.1		4.1
	240+	578	1.3	0.1		4.5
Pre milking diameter teat barrel[5]		1,939	0.05	0.0	0.007	
% change diameter teat barrel[6]		1,939	0.02	0.0	< 0.001	

[1] Linear regression coefficient.
[2] Standard error.
[3] Least Squares Means (in mm).
[4] Reference.
[5] Diameter measured at 50% of the teat relative to the teat end (in mm).
[6] Relative change in diameter measured at 50% of the teat post milking compared to pre milking (in %).

Similar to other studies (Reneau, 1986; Bartlett et al., 1990) LnqSCC significantly increased with parity and followed a non-linear curve over lactation stage (Table 2). LnqSCC significantly increased with increasing diameter of the teat barrel (Table 2), corresponding with previous findings (Higgins et al., 1980; Seykora and McDaniël, 1986; Chrystal et al., 1999). Quarters with larger teat diameters tended to have more clinical and subclinical mastitis (Hickman, 1964), and larger than herd-average teat diameter was identified as risk factor for mastitis by Slettbak et al. (1995). Larger diameter teats tend to have larger teat orifices and wider teat canals (Rathore and Sheldrake, 1977; Chrystal et al., 1999), what may explain the association between teat diameter, SCC and mastitis. Some authors, however, found no relationship between teat diameter and SCC (Porcionato et al., 2010), IMI (Bakken, 1981), or mastitis prevalence (Binde and Bakke, 1984). Different teat dimension measuring methods, different definitions of mastitis and IMI, differences between statistical analyses and breeds may explain contradictory results.

Negative changes in teat barrel diameter (i.e. thinner teats post milking compared with pre milking) were associated with lower LnqSCC, whereas positive changes (i.e. thicker teats post milking compared with pre milking) were significantly associated with higher LnqSCC (Fig. 1).

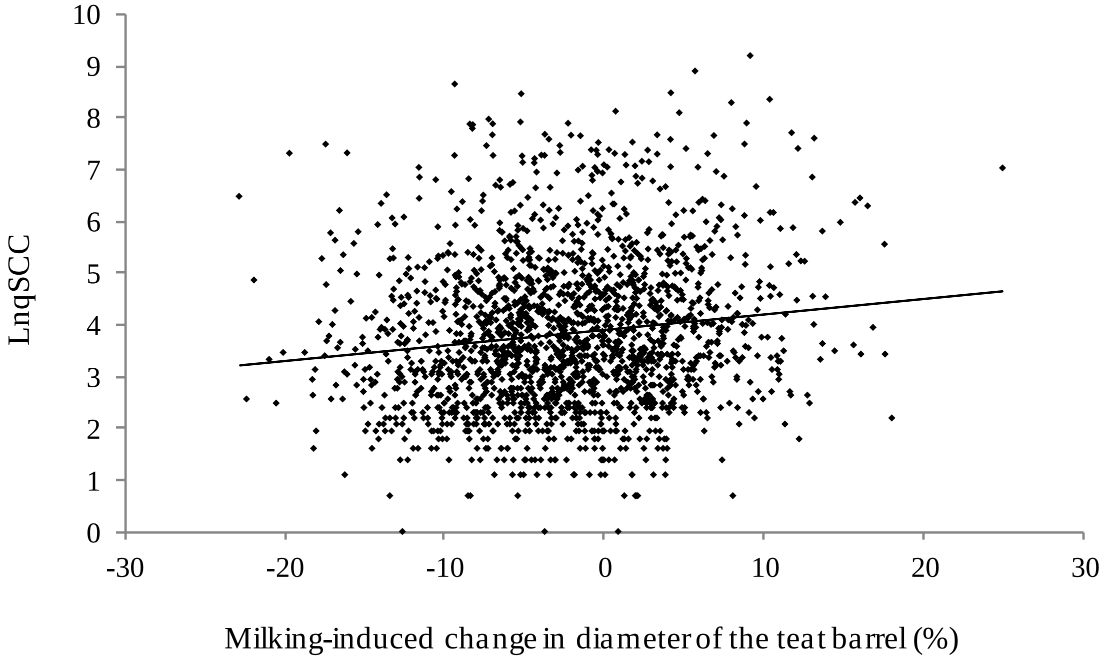

Figure 1. Scatterplot for LnqSCC plotted against the % change in the teat barrel diameter post milking compared to pre milking (not corrected for clustering or other factors).

The described changes are milking-induced changes of either cistern diameter, teat wall thickness or a combination of both. Reduced intramammary pressure and muscle tone (Hamann et al., 1993), and elongation of the teat (Isaksson and Lind, 1992) may account for a decrease in teat diameter after milking, whereas accumulation of fluid in the teat wall results in positive teat wall thickness changes (Hamann and Mein, 1990; Hamann et al., 1993). Congestion or oedema may impair the blood circulation and thus reduce the efficiency of the teat defence mechanisms such as neutrophil activity (Hamann and Osteras, 1994), what may explain the increased SCC with increasing positive diameter changes at the teat barrel found in this study. On the other hand, large decreases in teat diameter due to milking might also affect udder health since too high cyclic pressures applied to the teat by the liner might remove fluid from the blood vessels and thus restrict the blood supply to the tissues. Therefore, despite the significant decrease in LnqSCC with decreasing diameter change at the barrel reported in this study, it is suggested that a threshold exists for the maximum reduction in diameter after milking at this region of the teat, below which udder health is negatively influenced. Once this range is determined, the changes in teat dimensions can serve as indicators for suboptimal milking and improper teatcup liner choice or milking machine settings. Such a range has already been described for teat end thickness. Hamann & Mein (1996) suggested a threshold of 5% increase and 5% decrease in teat end thickness post milking compared to pre milking to evaluate the effectiveness of pulsation (in relation to vacuum level and liner type). In our study, however, no relation of relative changes in diameter of the teat apex and qSCC was found. This may be because diameters were not determined low enough on the teat, or no classification (comparable to the threshold of ± 5%) was made in teat end diameter changes.

Although no significant relationships were found between LnqSCC and teat length, which is in agreement with other studies (Seykora and McDaniël, 1986; Coban et al., 2009), nor for pre milking diameters of the apex and the base of the teat, it is known that the ratio of teat dimensions (length and diameters) with liner characteristics is important for good teat condition and milking characteristics (Mein et al., 1983; Rasmussen et al., 2004). In this respect, knowledgde on how the teatcup liner should relate to the teat under certain milking machine settings (i.e. pulsation and vacuum) is required for better liner selection.

As the diameter of the teat barrel and the relative changes in this diameter are associated with SCC, selection towards specific teat characteristics might result in improved milk quality and udder health. Knowledgde of the level (herd, cow, quarter, observation) at which most variation in these teat dimension factors resides and identification of factors explaining (part of) this variation is a prerequisite for more directed selection. A risk factor analyses including herd, cow, and quarter factors, as well as milking machine settings and milking characteristics should be conducted, and could also include culture results rather than SCC values. However, albeit significant, the biological relevance of these teat dimensions on udder health might be limited compared to other factors. Future research should therefore focus on the relative importance of these associations.

CONCLUSIONS

In conclusion, both the pre milking teat barrel diameter and the relative change of the teat barrel diameter due to milking are significantly associated with qSCC, indicating that these teat dimensions could be useful to improve milk quality and udder health, either i) by directed (genetic) selection towards more optimal teat characteristics, or ii) as indicators for improper milking and incorrect machine settings, including evaluation of the teatcup liner. Still, the relative importance of these associations should be determined first.

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CHAPTER 7

GENERAL DISCUSSION

INTRODUCTION

From an economical and animal welfare perspective, the dairy industry is becoming more interested in teat dimensions and the effect they have in combination with teatcup liners on milking characteristics and udder health. This information is of interest for any dairy farmer as well.

The scope of this thesis was to gain insights in the variation in teat dimensions and their possible association with udder health, in order to advise on potential improvements of the management of the dairy herd.

An objective 2D-vision-based method to measure teat dimensions (teat length and diameters) accurately and with precision was developed (Chapter 3) and validated (Chapter 4). Such a method was lacking and was a prerequisite for further study, to understand the factors associated with teat dimensions, and the relation between teat dimensions and udder health, but also as potential management tool. Factors at the cow and quarter level associated with teat length and diameters and the distribution of the variation in teat dimensions over the different levels (herd, cow, quarter) were identified in a cross-sectional and longitudinal study (Chapter 5). Finally, the relation of teat dimensions and their milking-induced changes with udder health was studied in more detail (Chapter 6).

TEAT DIMENSION MEASURING TOOL

The lack of a method that was capable of measuring teat dimensions accurately and precisely under field conditions has among others resulted in only limited research on teat dimensions and the research that was actually performed often reported contradictory findings. In addition, it has hindered the use of teat morphology in herd management, such as founded teatcup liner choice and directed (genetic) selection towards more uniform teat sizes potentially resulting in fewer problems associated with machine milking. This triggered us to develop a new vision-based measuring tool that uses a black-and-white camera to obtain a 2D image of the teat, and image processing analysis to determine teat length and diameters at different heights of the teat. The technique is **highly accurate**, as was shown when measuring artificial teats (Chapter 3). Because of the high resolution of the objects in the device at a distance of 550 mm from the lens centre (0.189 mm pixel^{-1}), this is not surprising. However, care should be taken to put the teats as straight as possible into the device. The technique was furthermore **repeatable and reproducible** in determining teat length and diameters (Chapter 4), although some training in working with the device itself, and not surprisingly, with cows as well, is advised. Besides being accurate and precise, the method is also **practical and fast**. Over the different field studies (Chapter 4 – 6), nearly all teats could be measured without difficulties in the various types of conventional milking parlours. Occasionally certain teats were harder to reach due to their position on the udder or because an object in the milking parlour obstructed measurements. Nevertheless, all teats could be measured prior to milking without noteworthy prolonging the milking process and little delay occured when teats were measured post milking.

Nevertheless, **optimization** of the tool is possible. In a comparative study between the 2D device and a ruler, the first measured on average significantly longer teats (Chapter 4). We hypothesize this is due to a part of the udder being inserted through the opening of the 2D device and consequently being included in the teat length values. Although the point where the udder ends and the teat begins is not anatomically discernible at the exterior of the teat, a **threshold value** based on the curving of the udder, which could be a good indicator for this point, could be decided on and incorporated into the software algorithms.

To improve the efficiency of the device it should be adjusted to make it **operable by one person** as currently it still needs two operators; one person to operate the device and measure the teats, and a second person to perform visual assessment of the images. A device operable by one person would require a screen on which the operator could easily see the image of the teat. A component and matching software program that allow to code the image with the necessary information (e.g. cow number and quarter position) in real time and thus transfers it to the hard disk of the PC for storage, would further increase the efficiency, as manual coding afterwards would no longer be required.

Besides improved efficiency, the 2D device could also be optimized by ascertaining the error due to the **angle of the teat in the longitudinal direction** is small since the errors in teat dimensions increase with increasing deviation in this direction. Although the accuracy of the method was tested on artificial teats, similar results are expected for *in vivo* measurements and we emphasize the importance of placing the teat in the device as straight as possible. However, the angle the teat makes in the longitudinal direction can currently not be determined under field conditions. A camera located at the bottom of the measuring device that makes an image of the teat simultaneously with the camera that records the frontal side of the teat, and subsequent image analysis could be used to calculate this angle. A correction factor could then be determined and incorporated in the image analysing software. Notwithstanding, regression analysis indicates that adding the angle in the longitudinal direction in the regression model only slightly increases the precision of the teat diameter estimation based on the measured diameter (Chapter 3). Moreover, the measuring method was repeatable and reproducible in determining the teat dimensions (Chapter 4), indicating that the angle under which the teat is measured is generally comparable between measurements. Including a second camera into the device would furthermore require a larger aluminium profile, which might reduce its ergonomics. Therefore, the gained accuracy might not justify the modifications to the device. Another possibility is to position a mirror relative to the frontal camera so the teat orifice can be visualized. Such a construction might aid in putting the teat in the device as straight as possible, and consequently increase the accuracy.

Furthermore, both solutions, the extra camera and the mirror, could be used to determine the presence and the degree of **teat end callosity** when the quality of the image is satisfactory, and could thus result in a potential **additional application** of the 2D device. Teat end callosity is often considered as a risk factor for IMI but consensus is lacking (Sieber and Farnsworth, 1981; Bakken, 1981; Neijenhuis et al., 2001). An accurate and precise method that allows the gathering of information on a large scale, and potential objective classification, may help in clarifying the association with udder health.

In addition, the 2D device could be further developed for the determination of **teat shape and teat end shape**. Both are important factors with respect to udder health. Generally, funnel-shaped teats have lower SCC and lower frequency of mastitis compared to cylindrical-shaped teats (Hickman, 1964; Rathore, 1976). It is suggested this is due to a lower incidence of teatcup crawl during milking. Short-term changes in teat shape, such as swelling near the teat base, often result from teatcup crawl, high mouthpiece chamber vacuum, or overmilking (Mein et al., 2001). A method that objectively determines teat shape and the changes due to milking might help the farmer to improve udder health by serving as indicator for improper milking and by choosing a liner adapted to the shape of the teats to prevent harmful milking conditions. Less agreement exists on the relationship between teat end shape and the risk of developing mastitis (Johansson, 1957; Bakken, 1981; Seykora and McDaniël, 1985; Chrystal et al., 1999; Chrystal et al., 2001). The lack of consensus may be due to the subjective scoring of teat end shape in those studies. An objective method could help to elucidate this relationship and may eventually improve the management of the herd in that area.

The 2D device could also be adjusted to measure teat **colour changes** due to milking. After cluster removal, teats can be noticeable red at the apex or over the entire length of the teat, or redden after some time (30 to 60 seconds). In extreme cases, teats appear blue or become bluish, indicating cyanosis (Hillerton et al., 1998). Acute colour changes of the teat indicate an impairment of the blood circulation and can occur after a single milking. During milking, teats are constantly under vacuum. High vacuum levels may lead to inadequate blood flow to the tissue. In addition, overmilking (Hillerton et al., 2002) and heavy cluster weight (Hillerton et al., 1998) may cause discolouration of the teats. Massage is provided by the pulsating liner to avoid accumulation of blood and interstitial fluids in the teat tissue. If pulsation fails, blood circulation is impaired. Pulsation failure occurs when the liner cannot fully collapse around the teat. This may be due to teats penetrating to deep into the liner as a consequence of milking with too short teatcup liners (Mein et al., 1983), or because the teat does not reach the collapsing point of the liner if teats are too short for a given liner (Mein et al., 2004; Rasmussen et al., 2004) or if the mouthpiece chamber of the liner is too high (Rasmussen et al., 1998). Furthermore, massage may be insufficient when the duration of liner closure per pulsation cylce (d-phase) is too short. To date, colour changes are used to evaluate the milking management or to detect shortcomings in the milking machine. However, the only available method to assess colour changes of the teat skin is by visual scoring, either direct or from a photograph, and many veterinarians and advisers apply their own classification system. Simpler and more standardised methods that reduce time and effort for the evaluator, and minimize interference with the milking routine are requested (Mein et al., 2001). Adjustments to our 2D device to measure these changes include the replacement of the black-and-white camera by a colour camera. In addition, illumination that enlightens the front of the teat and constant light conditions are required. By incorporating a calculation of the average Red, Green and Blue components of different sections of the teat and determination of the average Red/Green value in the image analysing software, the relative changes of the colour of the teats post versus prior to milking could be defined.

The 2D device was validated for measuring teat dimensions of Holstein-Friesian cows, but teats of **other breeds or even species** (goats, sheep, buffaloes, etc.) could be measured as well, provided some adjustments are made, such as increasing or decreasing the size of the opening through which the teat is inserted, and validation is performed.

The 2D tool is a **relatively expensive** investment due to the costly camera, lens and software licence. However, the tool is robust and low in maintenance costs. Additional expenses are therefore limited under standard applications. The hardware without the tablet PC costs approximately € 1500. The costly tablet PC (€ 2500) could be replaced by a less expensive processor and screen for an estimated cost of about € 300. Production on a larger scale and positive price evolutions in cameras and lenses might further reduce the costs.

TEAT DIMENSIONS: VARIATION, ASSOCIATED FACTORS, AND THE RELATION WITH UDDER HEALTH

Good udder health management is of major importance on dairy farms. Since mastitis is a multifactorial disease, a holistic approach is required. Such an approach is provided by the well-known and succesfully implemented 10-point mastitis prevention and control program (NMC, 2012). In this program, the milking procedures and the milking equipment are adressed as two key factors. A good match between teatcup liner and teat, besides other aspects such as pre milking teat preparation, is crucial for a good milking process, and consequently teat morphology is an important factor in choosing the most suitable liner. Besides the management, cow-factors also determine whether a quarter will become infected. The dimensions of the teat itself may predispose the quarter to poor udder health. As a result, more insights in teat dimensions and the associated factors could help in safeguarding or improving the udder health status of the herd.

Our study confirms that **wider teat diameters at the barrel** are associated with higher quarter milk SCC (Chapter 6). From an udder health management perspective, selection for smaller teats might therefore be desired. Considering the positive genetic correlation between teat diameter and SCC (Seykora and McDaniël, 1985), which implies that selection for one trait results in changes in the other trait because they are influenced by common genes, selection towards smaller teats might also be beneficial. Heritability of the teat diameter is high [$h^2 = 0.44$ in a study by Seykora and McDaniël (1986)] and thus genetic selection would be effective. Nevertheless, a considerable part (29.5%) of the variation in teat barrel diameter resides at the observation level, as was shown in Chapter 5. This indicates that, although the largest proportion in variation is present at the cow level, teat diameters alter over time. Little is known about the long term changes in teat diameter. With increasing age of the cow the epithelial layers of the teat increase in thickness (Hamann, 1987). Parity and lactation stage only explain little of the variation in teat diameter (Chapter 5). Research regarding additional factors potentially associated with the teat diameter is therefore required. We suggest that udder oedema may influence the teat diameter, as well as the temperature in the milking parlour. These factors should therefore be monitored in further studies. Furthermore, the teat tissue might adapt to the machine milking (Hamann and Stanitzke, 1990). However, to our knowledge, few studies have been performed on the long-term effects of teat tissue changes, and thus the influence of the milking machine on teat dimensions. Milked teats show a greater thickness in circular muscle layer than unmilked teats. Machine milking is involved in causing metaplasia of the teat wall epithelium and fibrosis of the blood vessel walls, although the mechanisms are unclear (Hamann et al., 1994). A longitudinal study conducted on heifers at first calving in which 1 front and 1 hind quarter are machine milked and the other 2 quarters are milked with a reference system in a split-udder design, could give more insight in the long-term changes in teat dimensions due to machine milking compared to the physiological changes over time. The reference system used to evaluate changes in teat dimensions could either be calf suckling, hand milking or cannula milking, as suggested by Hamann and Burvenich (1994). Milk withdrawal by cannula consists of inserting tubes into the cow’s teat and by means of gravity and intramammary pressure milk flows from the udder, whereas calf suckling and hand milking apply pressure to the teat and cause larger changes in teat tissue (Hamann and Stanitzke, 1990; Hamann and Mein, 1990). Therefore, cannula milking is recommended as reference system. In addition, the short-term and long-term changes in teat dimensions induced by different liner types, vacuum levels and pulsation settings compared to physiological changes may be tested using this study design.

As SCC increases with increased positive **machine milking-induced changes in diameter at the barrel of the teat** (i.e. thicker teats after milking compared with prior to milking) and decreases with increased negative changes (i.e. thinner teats after milking compared with prior to milking) (Chapter 6), it may be rewarding to aim for the latter situation. However, thresholds of physiological acceptable changes are expected but were not looked for in this study. We used the 2D device to measure changes in teat dimensions, however, the device was not evaluated for this application. Nevertheless, the device was precise in measuring teat dimensions prior to milking and is hypothesized to measure teat dimensions precisely post milking as well, although guidelines should be formulated on within what time relative to pre milking udder preparation and to cluster removal teats should be measured.

As some variation in changes at the barrel of the teat is present at the cow level (19.6% of the total variation, unpublished results), desirable changes could be achieved by selecting for cows with a natural predisposition for a milking-induced reduction in teat diameters. However, the vast majority of variation resides at the observation level (72.5%). Lactation stage could only explain little of this variation, indicating that other factors were associated with the changes in teat diameter. These changes are either milking-induced changes in cistern diameter or teat wall thickness or a combination of both. Reduced intramammary pressure and muscle tone (Hamann et al., 1993), and elongation of the teat (Isaksson and Lind, 1992) may account for a decrease in teat diameter after milking, whereas accumulation of fluid in the teat wall contributes to positive teat wall thickness changes (Hamann and Mein, 1990; Hamann et al., 1993). Future research regarding factors potentially associated with changes in teat dimensions should therefore focus on the milking process and differences within quarters over time, such as vacuum, pulsation settings, duration of milking, milk yield, compressive load and teatcup liner design and characteristics. Some of these factors have been found to affect teat end thickness (Hamann and Mein, 1988; Le Du and Taverna, 1989; Hamann et al., 1993; Hamann and Mein, 1996; Gleeson et al., 2004). The information might eventually serve as indication for improper milking procedures, milking machine settings or liner choice.

Although **other teat dimensions** were not significantly associated with SCC in the multivariable model in our study (Chapter 6), the diameter at the teat base (D75) was unconditionally associated with quarter milk SCC but omitted from the multivariable model because of high correlation with the diameter at the teat barrel. Ditto for the relative changes at this point.

In addition, negative relationships with udder health may occur if the teatcup liner does not fit the teat properly (Mein et al., 1983). Other teat dimensions might therefore be associated with poor udder health when the ratio between teat and teatcup liner is suboptimal. An optimal ratio could not yet be determined but reducing the variation in teat dimensions as a management practice to simplify the liner choice and to avoid certain problems associated with machine milking is nevertheless advised.

In our study, we measured **quarter milk SCC as an indicator for udder health** to determine the relation of teat dimensions and their milking-induced changes with udder health. In our study, we measured quarter milk SCC within 15 days relative to the teat dimension measurements. Considering the variation in teat dimensions over the lactation stage, measuring quarter milk SCC within a predefined number of days might be more ideal. Also, using bacteriological culture of milk to define an **IMI status** of a quarter, rather than measuring quarter milk SCC, might be a better approach. Recently an international definition for IMI has been formulated (Andersen et al., 2010; Dohoo et al., 2011), which could contribute to the consensus in udder health research. However, **time and expenses** limit the use of IMI status for research purposes. Despite the **lack of consensus on the use of SCC as indirect trait** for reducing the incidence of mastitis (Detilleux, 2009), SCC is generally regarded as a good indirect measure for udder health. Our study on the association between teat dimension factors and SCC is therefore a good indication for the relation between teat dimensions and udder health.

Although 20% of the total variance in quarter milk SCC was explained by including parity, lactation stage, diameter of the teat barrel and relative changes in this diameter in the model, a **large portion of the variation in quarter milk SCC remained unexplained**. Considering the multifactorial character of mastitis, this is not surprising. Research regarding more factors associated with quarter milk SCC might help in understanding the variation in SCC and result in improved management. These studies should focus on differences in time within quarters of cows because of the large variance situated at the observation level (62.2%), and should include teat dimensions as independent variables.

We wish to emphasize that the final study of this thesis (Chapter 6) focused on the relation between teat dimensions and udder health. However, teat dimensions and their combination with teatcup liners, also influence milking characteristics and teat condition. Therefore, management practices that favour udder health may not be beneficial for other factors such as milk yield, milk flow rate, and milking time. For example, milking teats with narrower bore liners might result in less congestion and oedema as compared to wider bore liners due to the lower vacuum applied to the teat, but consequently milking time can be longer (Gleeson et al., 2004). Liners with wider bore might however reduce the incidence of liner slips but on the other hand stripping yield might be increased (Mein et al., 2004). As a result, compromises will have to be made when choosing a liner. Nevertheless, the choice of the teatcup liner design should focus on safeguarding teat condition and udder health as this will indirectly affect milking characteristics. Furthermore, although consensus is lacking on the relationship between teat dimensions (length and diameters) and udder health, some agreement exists on the higher risk for mastitis and increased SCC with wider teats, as was found in this study (Chapter 6). As indicated earlier, selection towards smaller teats might be desired from an udder health management perspective. In addition, smaller teats were found to milk faster (Batra and Mcallister, 1984). Also, as a positive genetic correlation was observed between the diameter at the barrel of the teat and the teat length (Batra and Mcallister, 1984), selection for smaller teats would indirectly result in shorter teats. Since, negative correlations were reported between teat length and longevity (Larroque et al., 1999), milk production (Hickman, 1964; DeGroot et al., 2002), milk flow rate, milking time, and labor (Blake and McDaniël, 1979), breeding for cows with smaller and shorter teats might be beneficial for economically important traits. However, these correlations might differ between breeds. Fore example, Harris et al. (1992) reported a positive correlation between teat length and milk production in Guernsey cows. Nevertheless, in our opinion extreme teat dimensions might not be desired and it is suggested that an optimal range exists, comparable to the intermediate optimums described for both teat diameter and teat length in Czech Flekvieh cows in relation to the risk of culling (Zavadilova et al., 2009). Therefore, before implementing new traits in the breeding program, thorough knowledge about genetic correlations of the different breeds is needed to avoid undesirable side effects for other traits and animal welfare (Rauw et al., 1998; Buch et al., 2011) and the optimal range of teat dimensions should be determined.

FUTURE RESEARCH AND POTENTIAL APPLICATIONS

The work performed in this PhD thesis has provided an accurate and precise method for measuring teat dimensions and has contributed to the knowledge on variation in teat dimensions and their relation with udder health (Fig. 1). Additional adjustments, as suggested before, could turn our device in an even more practical and useful tool. In its current and future form it will be helpful in filling the gaps in our knowledge with regard to teat dimensions, teat end callosity, teat shape, teat end shape, teat colour, and their physiological and milking-induced changes.

An overview of potential future research is given in Figure 2. First, **guidelines** need to be formulated on whithin what time relative to pre milking udder preparation and to cluster removal teats should be measured before further application of the measuring tool to determine teat dimension changes. Second, for teat dimensions and their milking-induced changes to be used as preventive measures for udder health, elaborate research to **explain more of the existing variation and the factors potentially associated** with these teat dimensions is needed, as discussed earlier. Third, the **range of acceptable changes in teat dimensions** should be determined in a study that assesses the effect of teat dimension changes on udder health. The changes could then be used as indicator for improper liner choice, milking machine settings or milking procedures. Furthermore, it is likely that a similar **optimal range** exists for teat dimensions with regard to udder health. Therefore, future studies should focus on describing this range. Given the importance of a good match between the teat and the teatcup liner during milking to obtain good milking conditions, such as medium high mouthpiece chamber vacuum and sufficient massage, the **ratio between the teat and the teatcup liner** is likely to be highly associated with udder health. Future research should focus on defining the range of acceptable ratio’s based on its effect on udder health. The determination of this range is a prerequisite to select the most appropriate liner for a given teat that preserves the cow from poor udder health. Finally, as the teat dimensions, the ratio between teat and teacup liner, and possibly changes in teat dimensions affect **teat condition and milking characteristics**, similar studies should be performed for these traits.

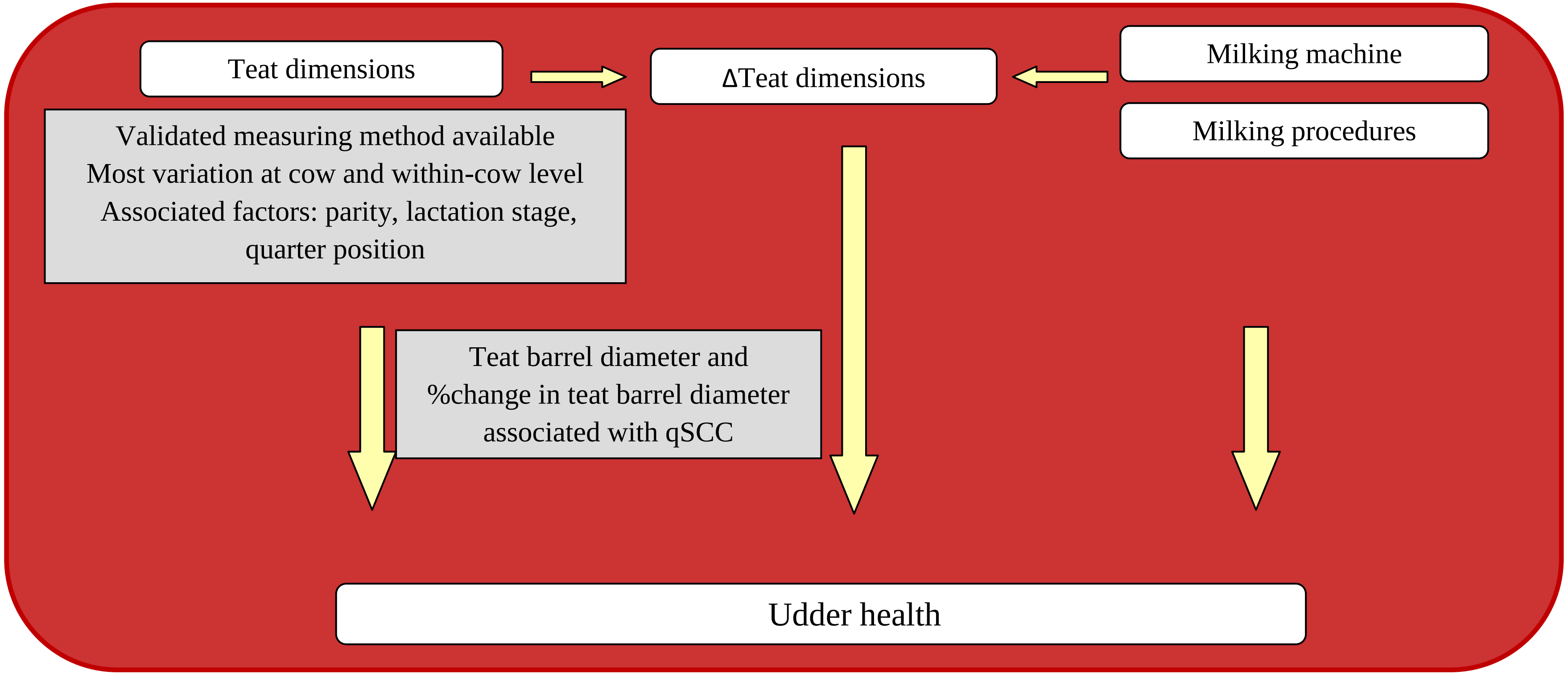

Figure 1. Schematic overview of the area of interest of this thesis with indication of the acquired knowledge on teat dimensions and changes in teat dimensions in grey.

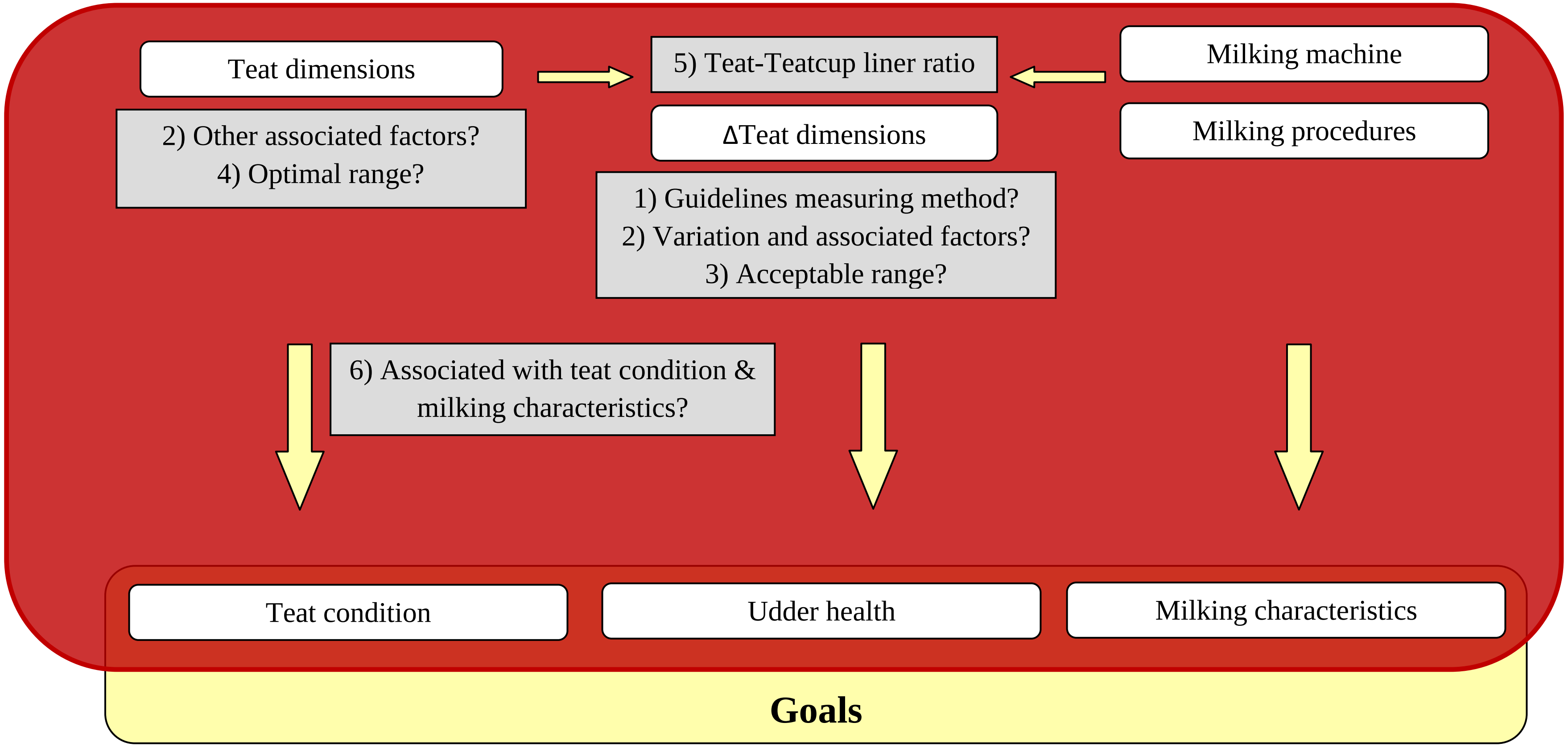

Figure 2. Schematic overview of the area of interest of this thesis with indication of possible future research in grey.

Determining the ratio between teat and teatcup liner remains a challenge. During the peak milk flow-rate, the contact area between the liner barrel and the teat is the major source of friction that keeps the liner on a stable position on the teat (Mein et al., 1973b). After the decline of milk flow, friction mainly stems from the force between the teat and the mouthpiece lip. When problems occur during milking, such as when the seal between the liner and the teat is lost, resulting in too high or low mouthpiece chamber vacuum, the dimensions of the teat relative to those of the teatcup liner indicate poor fit and thus improper ratio. These dimensions should therefore be determined. A model that provides information on how the teat will act during milking based on the teat dimensions prior to milking and associated cow and quarter factors (e.g. lactation stage, parity, quarter position) under certain milking machine settings (e.g. vacuum, pulsator ratio, pulsation rate) could be used to select a teatcup liner.

A schematic overview of a possible teatcup liner selection process is depicted in Figure 3. The goal is to construct a model in which knowledge on A) teatcup liner characteristics, B) the range of acceptable ratio’s between teat and teatcup liner, and C) the action of teat dimensions during milking, based on input on D) the dimensions of the teat, E) information of the cow (e.g. parity, lactation stage), F) information of the milking system (e.g. vacuum level, cluster type), and G) the preferences defined by the farmer, will result in a suggestion for an optimal teatcup liner (= output). The preferences of the farmer can be related to teat condition, udder health, milking characteristics or a combination, but probably compromises are required since it is expected that no liner design can grant all demands. For example, farmers that prefer to reduce milking time may use liners designed to milk fast, although these liners tend to leave more strippings behind in the udder (Dodd and Clough, 1959). Similar, a liner designed primarily to reduce cup slips may be less comfortable for the cows (Mein et al., 2004).

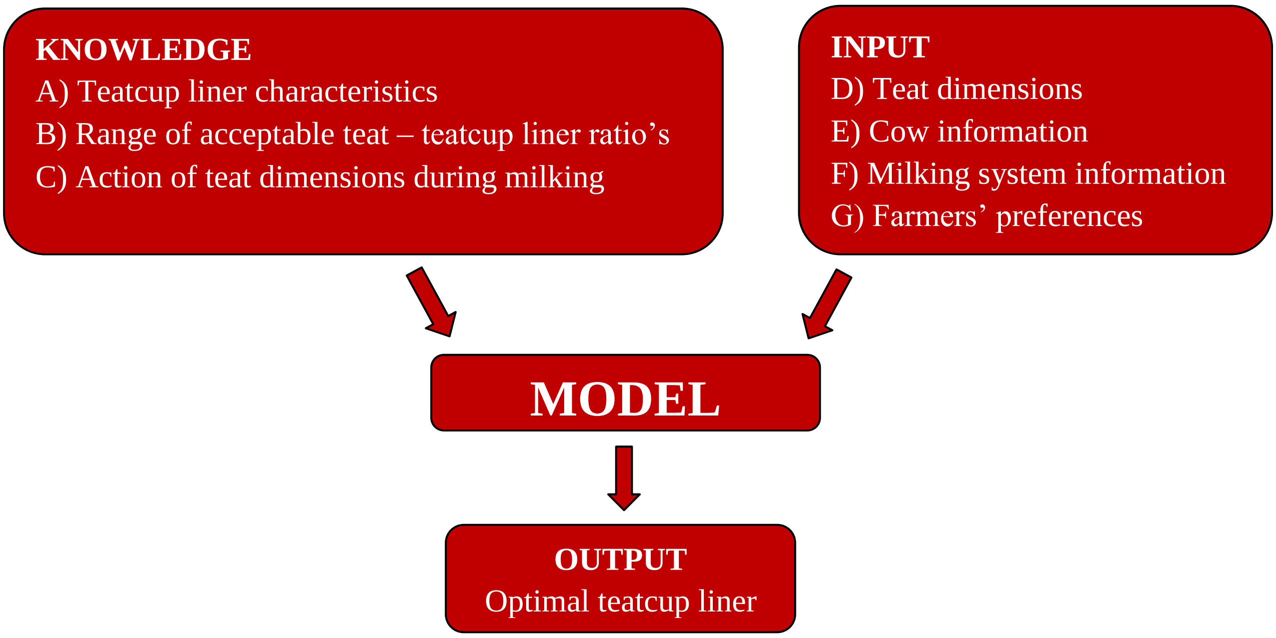

Figure 3. Schematic overview of teatcup liner selection based on a model, with indication of required knowledge and input.

Nevertheless, no practical method to dermine teat dimensions during milking is available. The most succesful technique was radiography (Mein et al., 1973a), but this method is laborious and can only be applied under experimental conditions. The teat load monitor presented by Rønningen (2000) is capable of measuring teat end position and teat end diameters but not diameters at other heights of the teat. Other methods determine liner wall movement but do not allow for teat dimension measurements, such as the method described by Spencer and Jones (2000) using a laser sensor, and by Schuiling (2003) using a series of light beams that were projected on the liner and were converted to a light sensitive cell by a lens.

In the absence of a method to determine teat dimensions during milking, to date the only viable approach to evaluate the fit between teat and teatcup liner is a more empirical one. Quarter level recordings of milk flow and vacuum in the liner and evaluation of cow discomfort during milking, and assessment of teat condition post milking indicate when the combination between liner and teat is not optimal. By comparing the dimensions of the teat with those of the teatcup liner prior to milking an estimated ratio can be defined. A large dataset comprising of a wide range of teatcup liner – teat combinations is therefore required.

The future applications of the 2D tool and the acquired knowledge are multiple. Even more so when the additional applications of the tool to determine teat end callosity, teat shape, teat end shape and colour changes can be realized, and when the use of the tool is extended to other breeds and species. Because of the rather large investment, the tool is more likely to be used by **consulting and breeding organisations, research institutes, and manufacturers**. They would benefit from a fast, accurate and precise method to measure teat dimensions, by i) allowing better teatcup liner selection and evaluation of the milking machine settings and milking procedures, ii) efficient gathering of data for breeding purposes, or iii) enabling the research on the relationship between teat dimensions and teat condition, udder health and milking characteristics. The tool could serve as reference for other teat dimension measuring techniques, or even be implemented in an automatic milking system. One could imagine the robot measuring the teat pre milking, determining the teat dimensions, teat shape and teat end shape, and selecting the teatcup liner most appropriate for milking the specific teat from a provided number of teatcup liners with different designs. Futher, the robot could determine teat end callosity and teat colour pre milking and changes in teat dimensions, teat shape, teat end shape, and teat colour post milking. Based on the thus acquired information the robot could decide to send an alarm to the farmer to adjust or replace the milking equipment or to attend a specific cow. The generated information may thereby result in more efficient milking performances, better cow teat condition and decreased incidence of mastitis.

In conclusion, we advise the farmer to **select towards more uniform teats**, since this would simplify liner choice. Selection towards **smaller teats** is suggested, although extremely small teats should be avoided as this might adversely affect longevity. We recommend to **focus on choosing the most optimal liners and milking machine settings** for the average teat or even for a specific teat. In this respect, adapting the milking equipment and, in particular, the type of liner to the **quarter level** is desired. In addition, we encourage frequent evaluation of the milking equipment to prevent and control mastitis. Besides proper milking machine settings and components, we emphasize the need for a **holistic approach** for an udder health management program to be successful.

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CHAPTER 8

GENERAL CONCLUSIONS

This study aimed to get insight in the variation in teat dimensions (length and diameters) in dairy cows and their relation with udder health using an accurate and precise measuring device.

A new 2D-vision-based measuring device was presented. The device is accurate in measuring teat dimensions. Errors are generally less than 5% and are less than 2% when the angle of the teat in the longitudinal direction is small (-5° to +15°). Special attention must therefore be paid on putting the teats straight into the measuring device. The method is furthermore precise, i.e. highly repeatable and reproducible, practical, and allows to gather information efficiently on large scale, which is an advantage over other methods, such as rulers and callipers. Some training in working with the device itself and with cows as well is advised. Suggestions to optimize and extend the applications of the device include making the 2D device operable by one person, determining teat end callosity, teat shape, teat end shape, and teat colour changes, and measuring teat dimensions of other breeds and species.

From a cross-sectional and a longitudinal study on 15 and 8 dairy farms in Flanders, respectively, it became apparent that the largest proportion in variation in teat dimensions resides at the cow or within-cow level and not at the herd level. This indicates that choosing a teatcup liner which is identical for all cow in a herd is not optimal. Quarter position (front versus hind), parity, and lactation stage were identified as factors associated with teat dimensions. Adapting the liner type to the quarter position or to groups of cows of the same parity or stage of lactation might therefore contribute to better milking performances and udder health. It is suggested that automatic milking systems could be adapted to milk quarters with different liners. As part of the variation remained unexplained, research regarding more factors potentially associated with teat dimensions might help in better understanding the variability in teat dimensions.

The pre milking teat barrel diameter and the relative change in the teat barrel diameter were significantly associated with qSCC. These teat dimensions could be used to improve milk quality and udder health by directed (genetic) selection towards more optimal teat characteristics, or as indicator for improper milking procedures and incorrect milking machine settings. Nevertheless, the relative importance of these associations compared to others should be determined first.

SUMMARY

For an animal farming system to be sustainable it should be economically viable, environmentally sound and socially acceptable. Mastitis is one of the most important diseases threatening the sustainability of dairy farms. Because of the multifactorial character of the disease, a holistic approach is needed to prevent and control mastitis. In this respect, the teat, which acts as the primary defence against infectious agents, is currently receiving more attention.

The review of the literature on teat dimensions and their relationship with udder health, described in **Chapter 1**, indicates that teat dimensions, and especially the combination with teatcup liner design and possibly the milking-induced changes in teat dimensions, affect udder health. To improve the herd management with regard to teat dimensions, insight in the existing variation in teat dimensions is required. However, the lack of a method that is capable of measuring teat dimensions accurately and precisely under field conditions has resulted in only limited research on teat dimensions, often reporting contradictory findings.

The aims of this thesis are outlined in **Chapter 2.** The main aim was to gain insights in the variation in teat dimensions in dairy cows and their relation with udder health as assessed with an accurate and precise measuring device. The specific aims were to develop an accurate, precise and practical 2D-vision-based device to measure teat length and teat diameters of dairy cows, and to validate it, to determine the variation in teat dimensions and to identify associated factors, and to evaluate the potential association between teat dimenions and their milking-induced changes, and udder health.

Various methods have been described to measure teat dimensions, such as visual scoring, rulers, callipers, modified cutimeters, ultrasonography and methods based on image analysis. However, most of these methods are subjective, lack accuracy, have low or unspecified precision, and are time consuming, thus hindering the gathering of information on a larger scale. In **Chapter 3**, a new 2D vision-based measuring tool, further referred to as the 2D device, was presented. The tool uses a camera to obtain a 2D image of the teat and image processing analysis to determine teat length and diameters at different heights of the teat. The

accuracy of the tool was assessed during evaluation on artificial teats. The tool was shown to be highly accurate; errors were generally limited to 5% for both teat length and diameters and were less than 2% when the angle of the teat in the longitudinal direction was small. It is nevertheless advised that care should be taken by the operator to put the teat in the device as straight as possible.

The precision of the developed 2D device was determined for teat length and teat diameters under field conditions in **Chapter 4**. In addition, the precision of the ruler and the calliper, 2 widely used measuring methods, to measure teat length and teat diameters, respectively, was assessed. This was done in 2 experiments in which the consistency of measurements within operators (repeatability) and between operators (reproducibility) was tested. Furthermore, the agreement of the 2D device with the ruler and the calliper was studied. Although the ruler and the 2D device poorly agreed, both methods were precise in measuring teat length when the operators had experience in working with cows. The calliper was repeatable in measuring the teat diameter, but lacked reproducibility. The 2D device was also repeatable in measuring the teat diameter, and reproducible when the operators had experience in working with the device. The methods poorly agreed, most likely due to the operator-dependent pressure applied by the calliper. It was concluded that all three methods can be used for various applications. The 2D device, however, has the advantage of measuring both teat length and teat diameters in a single measurement, is accurate and practical, and therefore allows efficient and fast collection of data on a larger scale, albeit some training in working with the measuring device, and not surprisingly also with cows, is advised.

Traditionally, all cows within a herd are milked with the same teatcup liner. However, if the liner does not fit the teat properly, its main function to cyclically massage the teat to avoid congestion and oedema will be strongly impaired, negatively influencing milking characteristics and udder health. Consequently, the choice of a liner whose dimensions match to those of the teat is critical. It is hypothesized that considerable variation in teat dimensions exists between cows and quarters within cows, but no studies have actually determined the variation present at the different levels of the hierarchy (herd, cow, quarter, observation). In **Chapter 5**, the level at which most variation in teat dimensions resided and some factors explaining (part of) this variation were identified. Teat length and teat diameters were determined using the 2D device in both a cross-sectional ($n_{\text{obserservations}}$ = 2,715) and a longitudinal study ($n_{\text{observations}}$ = 8,678). In both studies, most variation in teat dimensions was present at the cow or within-cow level, and not at the herd level, indicating that choosing a teatcup liner that is identical for all cows in a herd is far from optimal. Quarter position (front versus hind), parity and lactation stage were identified as factors associated with teat length and teat diameters. Generally, front teats were longer and broader than hind teats. Teat length and diameters increased with parity, although the increase in teat length was not significant from second parity onwards in front teats based on observations from the longitudinal study. After the first 30 DIM, teat length substantially and significantly increased, whereas teat diameters decreased. It was suggested that better results in teat condition, and eventually in udder health, might be yielded when different teatcup liners are chosen for front versus hind teats or for cows of different parity or lactation stage, with special attention to the first 30 DIM.

However, although the differences in teat dimensions reported in Chapter 5 were significant, they were small and their biological relevance needed to be examined before more concrete advices can be formulated. In **Chapter 6**, the association between teat dimensions and their short term, milking-induced changes, and quarter milk somatic cell count (qSCC) was investigated in a longitudinal study ($n_{\text{observations}} = 1{,}939$) using data collected with the 2D device. Parity, lactation stage, teat barrel diameter and changes in teat barrel diameter during milking were identified as factors associated with qSCC. Teats with wider barrels had higher qSCC. Negative changes in the diameter of the teat barrel during milking were associated with lower qSCC, whereas positive changes were associated with higher qSCC. Selection towards more optimal teat characteristics may therefore result in improved milk quality and udder health. However, as too high cyclic pressures, which may result in decreased teat diameters after milking, might restrict blood supply to the tissues, it is suggested that a threshold exists for the maximum reduction in teat barrel diameter due to milking below which udder health is negatively influenced. If so, changes in teat barrel diameter might serve as indicator for suboptimal milking and incorrect teatcup liner choice of milking machine settings, and thus help improve the management of the herd.

In **Chapter 7**, the overall results are discussed, as well as some suggestions are made to optimize the 2D-vision-based measuring device that was used in our studies, and for future research. As the combination between teat and teatcup liners is crucial for good milking management, future studies should focus on this subject.

SAMENVATTING

Om duurzaam te zijn dient een dierproductiesysteem economisch levensvatbaar, ecologisch verantwoord en sociaal aanvaard te zijn. Mastitis of uierontsteking is één van de belangrijkste ziektes die de duurzaamheid van melkveebedrijven bedreigt. Vanwege de multifactoriële aard van de ziekte is een holistische aanpak nodig om mastitis te voorkomen en te bestrijden. Het is in dit opzicht dat de speen, die fungeert als de eerstelijnsbescherming tegen infectieuze agentia, momenteel meer aandacht krijgt.

De literatuurstudie over speendimensies en hun relatie met uiergezondheid, beschreven in **Hoofdstuk 1**, geeft aan dat speendimensies, en in het bijzonder in combinatie met tepelvoeringen en mogelijks de veranderingen in speendimensies door toedoen van melken, een effect hebben op uiergezondheid. Om het bedrijfsmanagement met betrekking tot speendimensies te verbeteren is inzicht in de bestaande variatie in speendimensies vereist. Het gebrek aan een methode die in staat is speendimensies accuraat en precies op te meten onder praktijkomstandigheden heeft echter geresulteerd in slechts een beperkt aantal studies naar speendimensies, vaak met tegenstrijdige resultaten.

De doelstellingen van deze thesis werden beschreven in **Hoofdstuk 2**. De voornaamste doelstelling was het verwerven van inzicht in de variatie in speendimensies en hun relatie met uiergezondheid met behulp van een accurate en precieze meetmethode. De specifieke doelstellingen waren het ontwikkelen van een accurate, precieze en praktische methode om speenlengte en speendiameters op te meten en ze te valideren, het bepalen van de variatie in speendimensies en de ermee geassocieerd factoren, en het evalueren van het verband tussen speendimensies, hun veranderingen veroorzaakt door melken, en uiergezondheid.

Verscheidene methodes om speendimensies op te meten zijn beschreven zoals visuele scoring, meetlinten, schuifpassers, aangepaste cutimeters, ultrasonografie en methodes op basis van beeldanalyse. De meeste methodes zijn echter subjectief, gebrekkig accuraat, hebben een lage of onbepaalde precisie, en zijn tijdrovend wat het verzamelen van informatie op grotere schaal bemoeilijkt. In **Hoofdstuk 3** werd een nieuwe meetmethode op basis van 2D beeldverwerking voorgesteld, hierna het 2D toestel genoemd. De methode maakt gebruik van een camera om een 2D beeld van de speen te bekomen én van beeldverwerking om speenlengte en speendiameters op verschillende hoogtes van de speen te bepalen. De accuraatheid van de methode werd nagegaan door kunstspenen te evalueren. De methode is uiterst accuraat; fouten zijn in het algemeen beperkt tot 5% voor zowel speenlengte als speendiameters en zijn kleiner dan 2% wanneer de hoek die de speen maakt in de longitudinale richting klein is. Het wordt evenwel aan de bediener aangeraden om voldoende aandacht te schenken om de speen zo recht mogelijk in het toestel te plaatsen.

De precisie van het ontwikkelde 2D toestel werd bepaald voor speenlengte en speendiameters onder praktijkomstandigheden in **Hoofdstuk 4**. Daarnaast werd ook de precisie van de meetlat en de schuifpasser, 2 veelgebruikte meetmethodes, om respectievelijk speenlengte en speendiameters op te meten, nagegaan. Hiertoe werden 2 experimenten uitgevoerd waarin de overeenkomst van metingen binnen bedieners (herhaalbaarheid) en tussen bedieners (reproduceerbaarheid) onderzocht werd. Verder werd ook de overeenstemming van het 2D toestel met de meetlat en de schuifpasser bestudeerd. Hoewel de overeenstemming tussen meetlat en het 2D toestel laag was, zijn beide methodes precies in het meten van speenlengte wanneer de bedieners ervaring hebben in het werken met koeien. De schuifpasser is herhaalbaar maar niet reproduceerbaar in het meten van speendiameters. Het 2D toestel is eveneens herhaalbaar in het opmeten van speendiameters en daarenboven reproduceerbaar wanneer de bediener ervaring heeft in werken met het toestel. Deze methodes vertonen beperkte overeenkomst, vermoedelijk doordat de druk uitgeoefend door de schuifpasser afhankelijk is van de bediener. Er kon worden besloten dat alle drie de methodes kunnen aangewend worden voor verschillende toepassingen. Het 2D toestel heeft echter het voordeel dat zowel speenlengte als speendiameters kunnen opgemeten worden in één enkele meting, dat het daarenboven accuraat en praktisch is, en aldus toelaat om snel en efficiënt data te verzamelen op grotere schaal, hoewel enige training in het werken met het toestel en, niet verrassend ook met koeien, gewenst is.

Traditioneel worden alle koeien op een bedrijf gemolken met hetzelfde type tepelvoering. Nochtans zal, wanneer de tepelvoering niet geschikt is voor de speen, de hoofdfunctie van de tepelvoering om de speen cyclisch te masseren om congestie en oedeem te vermijden, nadelig beïnvloed worden, met een potentieel negatief effect op de melkkarakteristieken en uiergezondheid. Bijgevolg is de keuze van een tepelvoering waarvan de afmetingen passen bij deze van de speen kritisch. Er wordt verondersteld dat er aanzienlijke variatie bestaat in speendimensies tussen koeien en tussen kwartieren binnen koeien, maar geen enkele studie heeft werkelijk de variatie aanwezig op de verschillende niveaus van de hierarchie (bedrijf, koe, kwartier, observatie) bepaald. In **Hoofdstuk 5** werd het niveau waar de meeste variatie zich bevindt en enkele factoren die (een deel van) deze variatie verklaren onderzocht. Speenlengte en speendiameters werden bepaald met behulp van het 2D toestel in zowel een cross-sectionele ($n_{observaties}$ = 2715) als een longitudinale studie ($n_{observaties}$ = 8678). In beide studies was de meeste variatie in speendimensies aanwezig op het koe-niveau of binnen het koe-niveau, wat erop wijst dat de keuze van een tepelvoering welke gelijk is voor alle koeien binnen een kudde verre van optimaal is. Kwartierpositie (voor versus achter), pariteit en lactatiestadium werden aangetoond als factoren die verband houden met speenlengte en speendiameters. Voorspenen zijn langer en breder dan achterspenen. Speenlengte en speendiameters nemen toe met pariteit, hoewel op basis van de metingen in de longitudinale studie deze toename in speenlengte bij voorspenen enkel significant was van de eerste naar de volgende pariteiten. Na de eerste 30 dagen in lactatie nam speenlengte aanzienlijk en significant toe terwijl speendiameters afnamen. Er wordt geopperd dat betere resultaten in speenconditie en uiteindelijk in uiergezondheid zouden kunnen bekomen worden wanneer verschillende tepelvoeringen gekozen worden voor voor- versus achterspenen of voor koeien van verschillende pariteit of lactatiestadium, met bijzondere aandacht voor de eerste 30 dagen in lactatie.

Hoewel de verschillen in speendimensies gevonden in Hoofdstuk 5 significant waren, waren ze eveneens klein en hun biologische relevantie diende dan ook onderzocht te worden alvorens meer concrete adviezen konden geformuleerd worden. In **Hoofdstuk 6** werd het verband tussen speendimensies en hun korte termijn veranderingen door toedoen van melken, en het somatisch celgetal op kwartierniveau (kCG) onderzocht in een longitudinale studie ($n_{observaties}$ = 1939). Pariteit, lactatiestadium, de diameter van de speenschacht en veranderingen in deze diameter tijdens melken werden aangetoond als factoren die geassocieerd zijn met kCG. Kwartieren waarvan de spenen wijder zijn aan de schacht hadden gemiddeld een hoger kCG. Negatieve veranderingen in de diameter van de speenschacht tijdens melken waren geassocieerd met een lager kCG, terwijl positieve veranderingen geassocieerd waren met een hoger kCG. Selectie voor optimalere speenkarakteristieken zou daarom kunnen leiden tot verbeterde melkkwaliteit en uiergezondheid. Aangezien te hoge cyclische druk, welke kan resulteren in verminderde speendiameters na melken, de bloedtoevoer naar de weefsels kan beperken, wordt echter verondersteld dat een drempelwaarde bestaat in de afname van de diameter van de speenschacht waaronder de uiergezondheid negatief beïnvloed wordt. In dat geval kunnen veranderingen in de diameter van de speenschacht dienen als indicator voor suboptimaal melken en onjuiste tepelvoeringskeuze of melkmachine-instellingen, en aldus helpen het bedrijfsmanagement te verbeteren.

In **Hoofdstuk 7** worden de algemene resultaten besproken, alsook enkele voorstellen gedaan ter verbetering van de meetmethode die werd aangewend in onze studie, en voor toekomstig onderzoek. Aangezien de combinatie tussen speen en tepelvoering cruciaal is voor goed management, zou toekomstig onderzoek zich moeten richten op dit onderwerp.

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Ingrid Zwertvaegher werd geboren op 5 november 1985 te Gent. Na haar middelbare studies Moderne Talen – Wetenschappen aan het Sint-Pietersinstituut te Gent begon ze in 2003 de studie Biologie aan de Universiteit Gent, waar ze in 2007 het diploma Master in de Biologie – optie Dierkunde behaalde met onderscheiding. In 2008 studeerde zij met onderscheiding af als Master in de Milieusanering en het Milieubeheer, eveneens aan de Universiteit Gent.

Vanaf november 2008 was zij tewerkgesteld aan het Instituut voor Landbouw- en Visserijonderzoek, eenheid Technologie & Voeding (ILVO T&V) te Merelbeke als wetenschappelijk attaché op het IWT-project (nr. 50670) “Ontwikkelen van een meetmethode ter bepaling van de gemiddelde speengrootte als objectieve parameter bij de tepelvoeringskeuze voor een melkveestapel”. In november 2009 startte zij daar in samenwerking met de Faculteit Diergeneeskunde, Vakgroep Voortplanting, Verloskunde en Bedrijfsdiergeneeskunde, een doctoraatsonderzoek dat zich toespitste op speendimensies, geassocieerde factoren en de relatie met uiergezondheid. Gedurende deze periode begeleidde zij 2 thesisstudenten bij het uitvoeren van hun Bachelor- of Masterproef.

Ingrid Zwertvaegher is auteur en co-auteur van verscheidene wetenschappelijke publicaties en gaf presentaties op verschillende nationale en internationale studiedagen en congressen.

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