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Interaction between dairy cow physiology and milking technology

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Abbreviations:

AC	Automatically milked cows
ACTH	Adrenocorticotropic hormone
AFR	Average flow rate
AMS	Automatic milking system
AUC	Area under curve
C	Control milking
DOA	Dioxoandrostanes
EC	Experienced cows
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organisation
FIL	Feedback inhibitor of lactation
HAB	Heart rate above basal
IMP	Intramammary pressure
LCB	Liner closed before milking
MP	Milking parlour
MPV	Maximum pulsation vacuum during vibration stimulation
OA	Ovarian acyclicity
PC	Parlour cows
PFR	Peak flow rate
UC	Unexperienced cows
VO	Vacuum needed to open the teat canal
VO-C	Vacuum needed to open the teat canal at cessation of milk flow
VO-S	Vacuum needed to open the teat canal at start of milk flow

Summary

One central aspect in milk production is the removal of the milk by the milking machine. In the present study the regulatory background of milk secretion and milk removal were intensively studied. The focus of interest was the interaction between dairy cow physiology and milking technology.

A study on the interaction between the teat anatomy, teat functionality and milk flow profiles showed that the teat anatomy and the vacuum required to open the teat canal could partly explain quarter specific milk flow characteristics. In various experiments the effects of milking routines on oxytocin release, milk ejection and milking performance were investigated. Minimal mechanical impulses on the teat were sufficient to induce oxytocin release and to start the milk ejection. An individually adjusted pre-stimulation according to the actual degree of udder filling improved the milk flow rate and reduced the vacuum load to the teat. At the end of milking a quarter specific removal of teat cups reduced quarter specific machine-on time by approximately 20 %. Automatic milking systems (AMS) differ considerably from conventional systems. The adaptation during the changeover from conventional to automatic milking was studied in cows with and without previous experience in AMS. The heart rate during the first visits to the AMS was remarkably elevated in the latter group and the milk yield was reduced by 15 % due to the changeover. Unexperienced cows entered the AMS voluntarily at day 12 after the changeover. The adaptation capacity between individuals differed and was related to the adrenal cortex sensitivity. Experienced cows immediately visited the AMS voluntarily without human intervention. Furthermore the heart rate was not elevated during their first AMS visits after the changeover. The milk production and the milk fat content were reduced when milking intervals were prolonged while protein and lactose contents as well as somatic cell counts were not affected. These relationships are of importance, because AMS milking results in coincidental milking intervals. The time to regain ovarian cyclicity and days open were not affected by enhanced milking frequencies in AMS as compared to conventional twice daily milking.

An optimal balance between the physiological demands of the dairy cow and the milking machine can reduce negative impacts on the teat anatomy and therefore avoid negative effects on biological teat functions. AMS do not overstrain the adaptation or coping capacity of dairy cows, but during the changeover period an accurate management is essential to avoid stress load on the cow and economical losses of the farmer. The demonstrated interactions between dairy cow physiology and milking technology improve the knowledge about dairy cow husbandry and can help to enhance the resource efficiency of milk production systems.

Zusammenfassung

Ein wichtiger Aspekt der Milchproduktion ist die maschinelle Milchgewinnung. In der vorliegenden Arbeit wurden die physiologischen Hintergründe der Milchsekretion einerseits sowie der Milchabgabe andererseits eingehend untersucht. Dabei stand die Wechselwirkung zwischen der Physiologie der Milchkuh und der Melktechnik im Mittelpunkt des Interesses.

Korrelationsanalysen zwischen der Zitzenanatomie, funktionellen Merkmalen der Zitze und Milchflusskurven auf Viertelebene zeigten, dass die Milchflussverläufe teilweise durch die Zitzenmerkmale erklärt werden können. In mehreren Versuchen wurde die Bedeutung von Melkroutinen auf die Ocytocinfreisetzung, die Milchejektion und die Milchabgabe untersucht. Nur minimale mechanische Reize an der Zitze waren notwendig, um eine Ocytocinfreisetzung und damit die Milchejektion auszulösen. Eine auf den Grad der Euterfüllung abgestimmte, schonende Stimulation vor dem eigentlichen Melkbeginn erhöhte den Milchfluss und reduzierte die Vakuumbelastung der Zitze. Am Melkende konnte die Maschinenhaftzeit durch eine viertelspezifische Abnahme der Zitzenbecher um etwa 20 % vermindert werden. Automatische Melksysteme (AMS) unterscheiden sich erheblich von konventionellen Systemen. Von besonderem Interesse war die Umstellungsphase vom konventionellen zum automatischen Melken bei Kühen mit und ohne vorhergehender AMS-Erfahrung. Unerfahrene Kühe zeigten während der ersten AMS-Besuche erheblich erhöhte Herzfrequenzwerte, zusätzlich reduzierte sich die Milchleistung durch die Umstellung um etwa 15 %. Ab dem 12. Tag besuchten die Tiere weitgehend selbständig das AMS. Die Anpassungsfähigkeit des Einzeltieres gegenüber dem AMS variierte und stand in Bezug zur Sensitivität der Nebennierenrinde. Erfahrene Kühe ließen sich sofort selbständig melken und zeigten auch keine erhöhte Herzfrequenz während der ersten AMS Besuche. In AMS treten systembedingt erheblich schwankende Zwischenmelkzeiten auf. Es konnte gezeigt werden, dass sich die Milchsekretion und der Fettgehalt mit steigender Zwischenmelkzeit verringerten, der Eiweiß- und Laktosegehalt und auch der Gehalt an somatischen Zellen jedoch nicht beeinflusst wurden. In der vorliegenden Untersuchung waren weder die Dauer der azyklischen Phase nach der Kalbung noch die Güstzeit durch eine höhere Melkfrequenz des AMS im Vergleich zum üblichen zweimal täglichen Melken beeinflusst.

Eine optimale Abstimmung zwischen den physiologischen Anforderungen der Milchkuh und der Melktechnik vermeidet negative Einflüsse auf die Zitzenanatomie und verhindert daher eine Beeinträchtigung der biologischen Aufgaben der Zitze. Automatische Melksysteme überfordern nicht das Anpassungsvermögen der Milchkuh, stellen aber insbesondere während der Umstellungsphase erhöhte Anforderungen um eine übermässige Stressbelastung und ökonomische Einbussen für den Landwirt zu vermeiden. Die gezeigten Wechselwirkungen zwischen der Physiologie der Milchkuh and der Technologie des Milchentzuges verbessern die Kenntnisse über die Milchproduktion und können dazu beitragen, Milchproduktionssysteme effizienter zu gestalten.

1 Introduction

Today, there is a population of about 1.4 billion head of domestic cattle worldwide. Bovine milk and meat are the most important source of animal based food. Except for smaller populations of buffalo, gaur, yak and banteng, the modern high yielding dairy cow (bos taurus) and the zebu breed (bos indicus) are the two main types of domesticated bovinae. Both were domesticated independently about 8.000 to 10.000 years ago (Loftkus et al. 1994, Kumar and Hedges 1998).

Bovine products provide only 6 % of nutritional energy supply of men (FAO 2003). However, bovine milk is the most important source of fat (12 % of total fat supply) and an important source of protein (milk 8 %, wheat 21 %, rice 14 %) in human nutrition. In many developing countries cattle is the most important power supply of agriculture (Wilson 2003). The symbiosis of bovinae with ruminal microorganisms to use β-glycosil bonded carbohydrates like cellulose and hemicelluloses enables the use of feed, which is not suitable for human nutrition. This is probably the main factor for their important role in agricultural production. However, the rumination of cattle is one of the main sources of methane emission (Leng 1993) and an important contribution to the man-made greenhouse effect. It is therefore a relevant goal to continue cattle production with the objective to improve the resource efficiency of bovine products.

Dairy cows' physiological regulations determine their production capacity. However, the genetically possible capacity can only be utilized if the environmental conditions meet the demands of the cow. A key to improve the efficiency of milk production is therefore to improve the knowledge about the interaction between dairy cow physiology and milking technology. The present thesis aimed at the investigation of these interactions. This knowledge should help to improve husbandry systems, in particular milking systems, to meet the requirements of the dairy cows and to increase both milk quality and production. The objective was to further improve the resource efficiency of milk production without an excessive demand of the dairy cow's capacity.

1.1 Anatomy and physiology of lactation

1.1.1 Lactation and reproduction

The biological reason for lactation is to provide the offspring with a secretion containing all essential nutrients. The preparation for lactation coincides with gravidity. Therefore the hormonal regulation of lactation is closely connected to regulation of reproduction. The development of the mammary gland (mammogenesis), the induction (lactogenesis) and the maintenance of lactation (galactopoiesis) are controlled by different hormones (Schams 1983b, Akers 2000 and 2002). During mammogenesis oestrogens, progesterone, prolactin, corticoids and growth hormone are essential in various species (Cowie et al. 1980, Schams 1994). Although specific regulations are not understood in detail, prolactin has been demonstrated as essential during mammogenesis (Schams 1994, Tucker 2000). Several other local factors are additionally involved in the development of the mammary gland (Mol and Clegg 1999). Lactogenesis is triggered by the declining progesterone concentration resulting from the luteolysis shortly before parturition (Kuhn 1977). Inhibiting prolactin during galactopoiesis resulted in an inhibition of milk secretion in rats, rabbits and dogs whereas only minor effects were observed in cows, sheep and pigs (Karg et al. 1972, Karg and Schams 1974, Schams et al. 1994). The importance of prolactin is probably reduced in the latter species as a result of domestication and intensive breeding. In order to maintain the lactation a periodical evacuation of the mammary gland is essential (Wilde and Peaker 1990).

Since a basic function of lactation is to improve the prospects of the offspring, the antagonistic interaction between galactopoiesis and follicle development post partum is not astonishing. In pigs the maturation of ovarian follicles is blocked during the sucking period until the removal of the litters (Edwards 1982). In mares, sheep and goats the reproductive cycle can be triggered due to the length of the photoperiod (Legan et al. 1977, Dathe and Scheibe 1994). In cows a retarded onset of the ovulatory cycle is known in suckled cows (Lamb et al. 1999, Yavas and Walton 2000). The specific mechanisms are not understood in detail. Lamb et al. 1999 demonstrated that the maturation of follicles in beef cows remained unchanged during frequent sucking of the calf. Probably a lack of the luteinizing hormone peak impeded the ovulation (Yavas and Walton 2000).

1.1.2 Udder anatomy

The bovine udder consists of 4 separate glands located in the inguinal region of the ventral side of the cow. Each gland consists of secretory and connective tissue connecting teat, cistern and milk ducts with the alveoli (Schmidt 1971, Sandholm et al. 1995). Alveoli are cage-like structures wherein milk is synthesized and secreted. An alveolus is the discrete milk producing unit. A single layer of secretory epithelial cells lines the lumen of each alveolus. Contractile myoepithelial cells surround the epithelial lining, which contract in response to the hormone oxytocin (OT) (Schams 1983a).

The Fürstenberg's venous ringfold, a strong venous circle, is located under the skin between the teat cistern and the udder cistern (Sandholm et al. 1995). Front teats are about 5 to 7 cm long and have a diameter of 2.5 to 3 cm. Rear teats are usually 1 - 2 cm shorter and 0.5 – 0.8 cm thinner than front teats (Michel and Rausch 1988, Le Du et al. 1994). The teat wall consists of numerous longitudinal and circular smooth muscles (Lecourt 1982).

The teat canal is the single orifice in the gland between the internal milk secretory system and the external environment. The teat canal is the main barrier against mastitis infections. The inner surface consists of a skin-like epidermis lined with keratin, a waxy antimicrobial material (Sandholm 1995). The teat canal is surrounded by a large number of longitudinal and circular smooth muscle fibres. Lefcourt (1982) demonstrated their rhythmic contractibility. The influence of teat canal characteristics on milking performance has been demonstrated by Andreae (1958).

1.1.3 Milk secretion and storage

The milk has to be stored within the udder between milkings or suckings, because the secretory cells produce milk continuously. The alveolar lumen is filled, then the milk moves to the small ducts and finally milk is moved to the udder cistern (Knight et al. 1994). The milk stored in the large milk ducts and the cistern can be removed by overriding the teat canal barrier. Contrary, the milk present in the alveolar lumen and the small milk ducts is fixed by capillary forces. Therefore this milk can only be removed by contraction of the alveoli. The glandular milk stored in the udder is therefore classified due to its availability in alveolar and cisternal milk (Cowie et al. 1951). If compared with sheep and goats the amount of cisternal milk in cows is quite low with 10 – 20 % of the totally stored milk (Pfeilsticker et al. 1996). After short milking intervals cisternal milk can even be absent (Knight et al. 1994).

After a regular milking about 10 % of the obtained milk yield can be additionally removed by administration of a supraphysiological dose of OT, which then is defined as residual milk (Brandsma 1978). A chronic injection of exogenous OT can increase the milk yield by an enhanced udder evacuation (Nostrand et al. 1991, Ballou et al. 1993, Bruckmaier 2003).

The amount of milk stored in the udder increases with time after previous milking, which results in a continuous increase of intramammary pressure (Mielke 1969). Shortly after a previous milking, less than 1 kPa IMP was observed (Kuwada 1984). After a milking interval of 11 to 13 h the IMP ranged between 1.4 to 3.8 kPa (Bruckmaier 1988).

With increasing IMP the milk secretion is reduced (Schmidt 1971). A milk protein component FIL (feedback inhibitor of lactation) is discussed as a regulative factor (Wilde and Peaker 1990). Incomplete udder evacuation or extremely prolonged milking intervals enhance apoptosis of secretory tissue (Stefanon et al. 2002). On the other hand, an improved udder evacuation e.g. as a result of more frequent milking causes an increased milk yield. However, specific regulatory mechanisms are not yet understood in detail (Elliot 1961, Hillerton et al. 1990, Erdman and Varner 1995).

1.1.4 Milk ejection and milking

The cow needs to contribute to the removal of the milk stored in the alveolar tissue by contracting the myoepithelial cells (Lefcourt and Akers 1983, Bruckmaier and Blum 1998). The myoepithelial contraction (milk ejection) is activated when the concentration of OT in blood plasma is elevated above a threshold of about 5 ng/l (Schams 1983a).

OT is a peptide hormone secreted from the pituitary gland as a result of tactile stimulation of the teats (neuro-endocrine reflex). Already Ott and Scott (1910) observed a milk ejection in response to the injection of pituitary gland extractions. Although Vigneaud et al. reported the synthesis of OT in 1953, reliable assays to determine physiological concentrations were not available until the first radioimmunoassays for OT were described (Schams et al. 1979, Gorewit et al. 1979, Schams 1983a).

Bruckmaier et al. (1994) demonstrated that continuously elevated OT concentrations are necessary throughout the milking process and essential to complete the udder evacuation. The milking pulsation by the milking machine seems to be as effective as special pre-stimulation routines in terms of OT release (Bruckmaier and Blum 1996). However, additional sucking by the calf can further enhance OT release and milk yields (Bar-Peled et al. 1995).

The milk ejection and therefore a successful milk removal can be disturbed on either central or peripheral level (Wellnitz and Bruckmaier 2001). The milk ejection can be inhibited due to a lack of OT release by the pituitary gland (central blockade), or the milk ejection can be blocked by a contraction of smooth muscles of the milk ducts (peripheral blockade). The peripheral blockade can only be demonstrated by a supraphysiological administration of α-adrenergic substances (Bruckmaier et al. 1997). A central blockade of the milk ejection often occurs when cows are milked in unfamiliar surroundings (Bruckmaier et al. 1996). A disturbance of milk removal under practical conditions is generally due to a central blockade (Tancin et al. 1995, van Reenen et al. 2002).

1.2 Stress physiology and coping strategies

Any influence on the individual, e.g. temperature changes due to weather conditions or metabolic changes due to the start of lactation, can disturb the individual homeostasis. However, since any response to a disturbed homeostasis can be defined as stress response, in the present thesis stress is defined as a condition where expectations and/or objectives do not match the current requirements of the internal or external environment. Likewise factors that cause such mismatches are called stressors. The physiological and behavioural responses that compensate this discrepancy and restore homeostasis are termed stress response (Hopster 1998). In the present thesis, however, long-term factors, for example metabolic changes due to the energy demand of the lactation (Bruckmaier et al. 1998, Aeberhard et al. 2001a und b, Reist et al. 2002), are not considered as stressors.

Studies on how individuals react to stressors can be found throughout the last decades. Although individual reactions are very specific, several researchers tried to classify stress responses. Beginning with basic ideas of Cannons “fight and flight concept” (see overview: Henry and Stephens 1977, Levine and Ursin 1991, Stratakis et al. 1995), this hypothesis was modified in various specifications. The basic idea to classify two basic behavioural coping strategies is still present in today’s concepts e.g. the “ active and passive” approach (Koolhaas et al. 1999).

Both types of coping, the active and the passive one, serve the animals’ ability to cope successfully with the stressor by either reducing the effect of adverse environmental stimuli (active) or by minimising the cost of the response (passive). These different patterns of coping behaviour correspond to the activation of distinct central nervous and neuroendocrine pathways. The active coping is associated with the release of adrenaline and noradrenaline by the adrenal medulla into the bloodstream, which results in an activation of the sympathetic nervous system. This activation enhances the blood supply of both brain and muscles to provide the energy for active reactions (e.g. territorial control, mobility, aggressive behaviour). Passive coping is characterised by behavioural inhibition and a more pronounced activation of the hypothalamus-pituitary-adrenocortical axis.

However, it is generally agreed that both ways of coping may be equally successful in re-establishing homeostasis. Extensive research in human (Henry and Stephens 1977) and animals (Bohus et al. 1987, Benus 1988, Fraser and Broom 1990, Wechsler 1995, Koolhaas et al. 1997, Hopster 1998) indicate that both men and animals respond in rather individual ways when exposed to stressors.

1.3 Milking technology

1.3.1 Machine milking

The first milking machines using a pulsating vacuum were developed at the end of the 19th century (Rabold 1983, Bramley et al. 1992, Mein et al. 2003). Until today many details have been improved. The basic concept, however, the teat cup equipped with a liner has been kept unchanged (Worstorff 1978, Mein et al. 2003). As a result of the pressure difference between milking vacuum and the pulsating chamber vacuum (pulsation vacuum fluctuates between atmospheric pressure and milking vacuum) the liner is periodically collapsing at a frequency of approximately 1 Hz. A milk flow from the teat can be observed during the liner open phase whereas during liner closed phase there is no milk flow present. The collapsing of the liner is important to massage the teat and to prevent negative effects of blood and lymph liquid congestions, which occur during the liner open phase due to the milking vacuum (Mein et al. 1973, Williams et al. 1981).

1.3.2 Automation of machine milking

In order to reduce the workload of milking several technical approaches were developed and integrated in commercial milking installations. Automatic detachers were designed to remove the milking clusters at the end of the milking process (Bramley et al. 1992). To enable a mechanical stimulation before the start of milking various different techniques has been proposed (Osteras and Lund 1980, Whittlestone 1980, Karch et al. 1989). Furthermore, sensor systems were investigated to evaluate the health status of the dairy cow (Maatje et al. 1992, Artmann 1997, Rossing 1999, Ordolff 2001). The last step towards a fully automated milking process without a human assistance was to automate the attachment of the teat cups. The first experimental automatic milking systems (AMS) were developed in the late 1980’s and the first commercial systems were installed in 1992 (Ipema et al. 1992, Kremer 1993).

1.3.3 Public concerns towards innovations

During the last decades several events reduced consumers’ faith in European farming. Events and even scandals like the appearance of the Bovine Spongiforme Enzephalopathie, the extended use of antibiotics in husbandry, the reduction of biodiversity due to increased field size, the appearance of nitrate and pesticides in ground water or the illegal use of hormones in meat production caused a situation in which the consumer became more and more reserved towards innovations in agriculture. Today most consumers do not have any contact to commercial agriculture, which might be a further reason for an increasing gap between commercial farming and consumers’ wishes towards food production systems. Therefore it is essential today to document animals’ welfare status when innovative husbandry systems are introduced in practice. In Switzerland even official tests are required to obtain a marketing license for new systems (Wechsler 2001).

1.4 Interaction between dairy cow physiology and milking technology

1.4.1 Teats and machine milking

The machine milking process differs remarkably from the sucking act of the calf. The peak vacuum forces applied by the calf are even higher as compared to the vacuum applied by the milking machine (Rasmussen and Mayntz 1998). However, resulting impact on the teats seems to be less pronounced during calf sucking than during machine milking (Hamann et al. 1993). This might be due to the reason that machine milking creates a permanent vacuum throughout the milking process, whereas the vacuum applied by the calf oscillates between negative and positive pressures. Furthermore the calf is sucking only one teat per time, whereas machine milking is applied on all four teats. However, even if machine milking and sucking are compared at a teat level, machine milking results in a more pronounced accumulation of blood and lymph fluid in the teat (Hamann et al. 1993). Even well adjusted milking machines cause a remarkable change of teat tissue (Neijenhuis et al. 2001). In case of a failure of milking pulsation or overmilking an increased risk of mastitis infections has been demonstrated (Natzke et al. 1978, Onley and Mitchell 1983, Mein et al. 1986, Hillerton et al. 2002). Milking twice daily causes a direct interaction between the liner and the teat for about 10 to 20 min per day, which means that the milking machine is the machine with probably the most intensive contact to a farm animal at all. An important goal is therefore to reduce negative impacts on dairy cows' teats.

1.4.2 Milk ejection and machine milking

The release of OT by the pituitary gland followed by the ejection of alveolar milk is essential for the milking process, since without the occurrence of milk ejection only the cisternal milk is available to be removed (Knight et al. 1994, Pfeilsticker et al. 1996). Therefore, the challenge for the milking machine is not only to overcome the teat canal barrier by the application of a milking vacuum, but also the stimulation of OT release throughout the milking process (Bruckmaier et al. 1994). To optimise the timing between milk removal and alveolar milk ejection an application of a fix pre-stimulation prior to milking has been recommended (Mayer 1983, Rasmussen et al. 1990, Rasmussen et al. 1992). Only recently however, Bruckmaier and Hilger (2001) demonstrated additionally a close negative relationship between the actual degree of udder filling and the lag time from the start of teat stimulation until the start of milk ejection. Therefore this finding points out possibilities to optimise the milking process by using an udder filling specific pre-stimulation.

1.4.3 Behaviour and automatic milking

The major difference between conventional milking systems and AMS is the cows' motivation to enter the milking stall. In conventional milking systems cows are driven to the milking parlor twice or thrice daily, whereas in AMS they enter the milking stall voluntarily and get milked throughout the day without human intervention.

The cows' motivation to get milked seems to be weak in general and highly variable (Prescott et al. 1998). Therefore, several approaches to attract an AMS visit have been studied intensively (Winter and Hillerton 1995, Ketelaar-de Lauwere et al. 1998, Harms et al. 2002, Hermans et al. 2003). Concentrate feeding in the AMS milking box positively reinforces AMS visits. Additionally, guided cow traffic systems with roughage only available after passing the AMS are common (Harms et al. 2002, Hermans et al. 2003). In adapted cows these restrictions do not negatively influence dairy cows physiology during milking as compared to conventional systems (Hopster et al. 2002).

2 Objectives of the study

The objectives of the present study were to give insights into the complex interactions between the dairy cow and the milking machine. The focus of interest were the interactions between OT releases, milk ejection, teat anatomy and milking performance. In addition, AMS were used to investigate physiological effects of environmental changes and varying milking intervals.

The individual milk flow performance is assumed to be determined by teat characteristics. In a first approach the teat anatomy and the teat functionality as well as the milk flow performance in 148 quarters of 38 dairy cows were analysed at a quarter based level (4.1).

The rate of udder filling and the distribution of quarter milk yields are supposed to affect both the milk ejection and the milking performance. Milking routines, in particular the pre-milking stimulation and the detachment routine, were therefore thoroughly studied to evaluate their effects on OT release, milk ejection and milking performance (4.2).

In AMS cows have to enter the milking stall voluntarily, whereas conventionally milked cows are driven manually to the parlour. Previous studies showed that guided cow traffic systems under proper management conditions did not create adverse effects in adapted cows. In the present study experiments were performed to evaluate the adaptation (coping) process during the changeover from conventional to AMS (4.3).

The voluntary visit of the AMS consequently results in coincidentally variable milking intervals. Effects of unequal milking intervals are known to affect milk constituents in conventional milking systems. Therefore the effects of coincidental milking intervals on milk yield and composition in AMS were studied (4.4).

The stimulation of the milking process itself as well as a more or less severe negative energy balance as a result of higher milk yields is presently discussed to prolong ovarian acyclicity post partum and to reduce fertility. Therefore, the ovarian acyclicity post partum was analysed in both an AMS and a parlour herd to determine possible interaction with milking frequency and milking system (4.5).

3 Materials and Methods

3.1 Animals and husbandry

Experiments were performed at the Research Stations Veitshof and Hirschau of the Technische Universität München and at the research station Grub of the Bavarian Institute for Agriculture. All cows were housed in free stall barns and milked either conventionally in milking parlours or by AMS (research station Hirschau: VMS, DeLaval, 14721 Tumba, Sweden; research station Grub: Merlin, Lemmer-Fullwood GmbH, 53790 Lohmar, Germany). Parlour cows were milked twice daily. Automatically milked cows were milked during their voluntary visits in the AMS stall. Selectively guided cow traffic was applied to reinforce the visits of the milking stall in AMS (Harms et al. 2002). The diet was maize and grass silage, hay and concentrate according to the individual production level. All cows were housed the year round indoors to guarantee constant conditions.

3.2 Experimental procedures

3.2.1 Sampling and hormone assays

For blood sampling a catheter was inserted into the jugular vein the day before the start of the experiments. Blood samples to determine OT were taken before and during milking. Blood samples to determine cortisol were taken before and after administration of ACTH. All blood samples were treated with EDTA to prevent coagulation and centrifuged at 1500 x g for 15 min within 20 min after sampling. The plasma was stored at – 20 °C until further analysis.

OT was determined by radioimmunoassay as described by Schams (1983a). Cortisol was analysed using an enzymeimmunoassay as described by Sauerwein et al. (1991).

To determine the cortisol metabolites 11,17 dioxoandrostanes (DOA) faecal samples were taken twice daily at 07:00 and 18:00 from the rectum and were immediately frozen at – 20 °C until further analysis. DOA concentrations in the faeces were determined by an enzymeimmunoassay as described by Moestl et al. (2002).

Milk was sampled twice weekly to determine progesterone concentration. Progesterone concentrations were analysed in skim milk by an enzymeimmunoassay according to Meyer et al. (1986).

3.2.2 Milk flow measurements

Milk flow recordings of the whole udder were performed by a stationary strain gauge system and analysed as described previously (Worstorff and Fischer 1996). An especially rebuilt set of Lactocorders (Werkzeug- und Maschinenbau Balgach, 9436 Balgach, Switzerland) as described by Wellnitz et al. (1999) was used to record quarter milk flow profiles. The milk flow data obtained were analysed according to Göft (1992a and 1992b) and Rothenanger et al. (1995). Quarter level data were processed by using visual basic programs (Microsoft 2000).

3.2.3 Intramammary pressure recording

The intramammary pressure (IMP) was measured by using a strain gauge system as described previously (Bruckmaier 1988).

3.2.4 Ultrasonography of the teat

The teats were scanned by b-mode ultrasonography as described previously (Bruckmaier and Blum 1992, SonoVet 2000, Probe: 5 MHz linear array scanner Nr. LV4-7AD, Kretztechnik, 4871 Zipf, Austria). Obtained cross sections of the teats were evaluated for teat diameter, teat wall thickness and teat canal length.

3.2.5 Teat canal functionality

The vacuum needed to open the teat canal was determined by the use of a modified transparent teat cup. The teat cup was equipped with a cut mouth piece of a liner to make it tight. The measurement was performed after forestripping and the application of a 1 min manual pre-stimulation in all 4 teats. The vacuum in this teat cup was gradually increased until milk flow was observed and thereafter decreased again towards zero. The vacuum curve was recorded by using a special vacuum measurement device (BoviPress, A&R Trading GmbH, 21379 Echem, Germany). The handle of the teatcup was equipped with a switch to mark the start and the stop of the milk flow in the recorded vacuum curve. Measurements were performed thrice at three different evening milkings in each animal to evaluate the repeatability of the method.

3.2.6 Heart rate measurements

The heart rate was measured by means of a commercial system developed for horses by using electrodes fixed to a special belt around the chest (Polar Horse Tester, Polar Electro GmbH, 64542 Bütelborn, Germany) (Hopster et al. 1994). The signals were saved as 15-s averages for further analyses. A detailed protocol was established during milkings to reassign the actions of the cow and the AMS to the time scale of the heart rate measurement.

3.2.7 Monitoring of the ovarian activity post partum

Ovarian cyclic activity was evaluated based on the progesterone profile of the individual cow. If progesterone concentration in skim milk exceeded 2.5 nmol/l for a period of at least one week followed by a clear drop in progesterone concentration below 0.5 nmol/l, a corpus luteum was assumed to be present, indicating the occurrence of a previous ovulation (Schopper et al. 1989). A subsequent increase of progesterone concentration within the following 10 days indicated the occurrence of the second ovulation. Fertility was controlled by a herd-fertility-program of the Bavarian Institute for Animal Breeding (Bavarian State Research Centre for Agriculture, Freising).

3.2.8 Data handling and statistical analyses

Results are presented as means ± SEM or means ± SD as indicated. All data were processed by the SAS system (version 8.01). For analyses of variance the MIXED procedure was used. The REG procedure was used to calculate Pearson’s coefficient of correlation. To analyse effects of the milking system on reproduction parameters multivariate cox models were used (PHREG procedure, Cox 1972). The repeatability of the newly developed system to determine the vacuum needed to open the teat canal was calculated according to Essl (1987). Differences were indicated as statistically significant in case of $P < 0.05$, unless stated otherwise. A detailed description of the statistical models is provided in the appendix (0).

The DeLaval AMS recorded various data in an internal database and the Lemmer-Fullwood AMS was equipped with a data logger system to register milking data (Harms et al. 2002). In several experiments the degree of udder filling was estimated as the percentage of the maximum storage capacity. The maximum storage capacity of the mammary gland was estimated as half of the daily milk yield in month 2 of the respective lactation. The heart rate results are presented as heart rate above basal (HAB) to avoid a bias due to the close correlation between baseline heart rate and milk yield.

4 Results and discussion

4.1 Relationship between teat anatomy and milk removal

This study was the first one that used a combination of 3 approaches to characterize teats at a quarter level. The measurements of teat anatomy, teat functionality and milk flow characteristics were performed by using an innovative combination of ultrasonography, vacuum measurement und continuous milk flow recording. The combination allowed a correlation analysis covering all obtained data (Weiss et al. 2004b).

The results concerning teat length, teat diameter, teat wall thickness and teat canal length corresponded to previous investigations (Grindal et al. 1991, Rogers and Spencer 1991, Le Du et al. 1994, Neijenhuis et al. 2000). Former investigations reported in general longer and thicker teats (Andreae 1958, Loppnow 1959), indicating changes obviously due to the breeding progress of the last decades. Despite these changes, the variation between cows and quarters in the present study were remarkably high.

VO was determined by the measurement of vacuum at the start of milk flow (VO-S) and vacuum at the cessation of milk flow (VO-C). VO did not differ in front and rear teats (Weiss et al. 2004b). VO-S corresponds to previous results by Le Du et al. (1994) in a comparable approach. VO-C was substantially lower than VO-S. This corresponds to results of Williams and Mein (1986); wherein the initial force to start the milk flow was remarkably higher than the force needed to maintain an already established milk flow. The observed milking characteristics confirm previous investigations at a quarter level (Rothenanger et al. 1995, Wellnitz et al. 1999).

In the present investigation no correlations between teat canal length and externally measurable anatomical characteristics like teat length or teat diameter were observed (Weiss et al. 2004b). These results are in contrast to former investigations by Loppnow (1959) and Hebel (1978) who found a positive correlation between teat length and teat canal length. However, Loppnow (1959) and Hebel (1978) performed their measurements on teats of slaughtered cows, whereas in the present study in vivo measurements by ultrasound were evaluated. Thus their studies disregarded effects of the tone of teat smooth muscles and intrammamary pressure (Lefcourt 1982, Inderwies et al. 2003b).

The milk yield and PFR were higher in rear than in front quarters and rear teats were shorter and thicker than front teats. Therefore, negative correlations between teat length and various milking characteristics at a quarter level (Weiss et al. 2004b), e.g. milk yield, main milking time, plateau phase and AFR are apparently due to differences between front and rear quarter anatomy. Furthermore, the fact that rear teats were thicker than front teats can explain the positive correlations between teat diameter and PFR, since rear teats had a higher PFR. Indeed, when correlations within cow were analysed no significant relationships were observed. The parallel increase of stripping yield and teat length with increasing number of lactations may explain the positive correlation between teat length and stripping yield (Michel and Rausch 1988, Göft et al. 1994). However, disregarding these apparent correlations between teat anatomy and milking characteristics there was a negative correlation between teat canal length and PFR and a negative correlation between teat canal length and AFR. These findings correspond to previous reports (Grindal et al. 1991) where the length of the teat canal was shorter in quarters with a high PFR. In summary, these results indicate that externally measurable teat anatomy, i. e. teat length and teat diameter did not affect important milking characteristics like PFR and AFR.

Surprisingly, teat canal length was not correlated with VO, neither with VO-S nor with VO-C. But VO was indeed negatively correlated with PFR and AFR. These findings correspond to investigations by Le Du et al. (1994) and Mayntz et al. (1999). Similar results were observed at a quarter and an udder level. Since the milking vacuum was definitely higher than VO a correlation between VO and PFR was not to be expected. Furthermore, the resulting teat canal diameter during milk flow, which was not determined in this study, is reported as the most important aspect concerning PFR (Baxter et al. 1950, Andreae 1958, Mein et al. 1973, Williams et al. 1981). The present method was designed to measure VO. Therefore, no information about intensity and velocity of the observed milk flow and about the teat canal

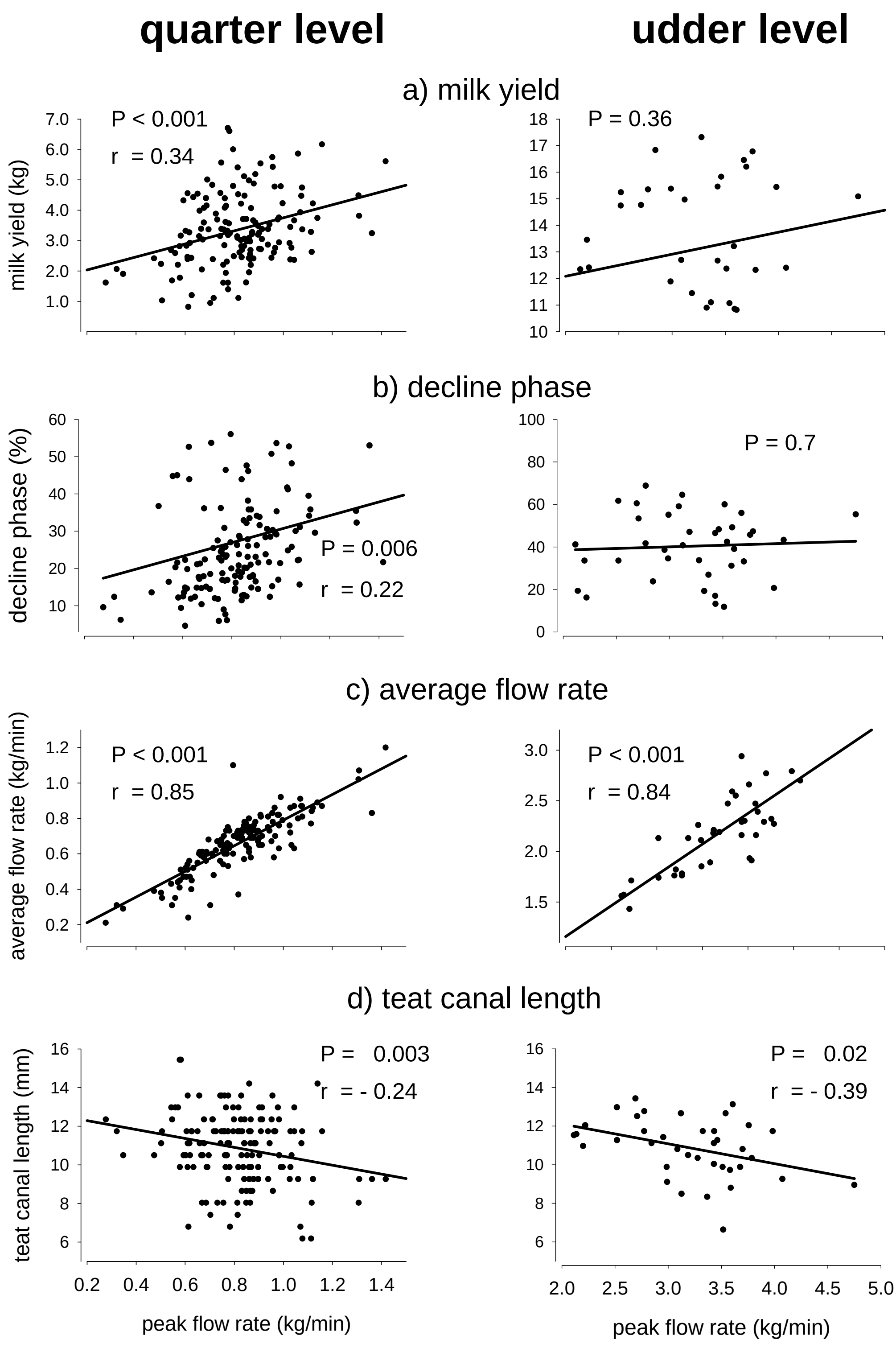

Fig. 1: Relationship between peak flow rate, milk yield (a), relative decline phase (b), average flow rate (c) and teat canal length (d) at a quarter and an udder level, respectively. Levels of significance and correlation coefficients are indicated in the figures.

diameter is available in the present study (Williams and Mein 1986). However, PFR is reported to be correlated to the adrenergic system (Roets et al. 1989, Wellnitz et al. 2001, Inderwies et al. 2003a). Inderwies et al. (2003b) demonstrated in a study that not only the teat canal determines the resulting PFR. Probably, VO reflects the sympathetic tone of the smooth muscles and the presented method provides therefore information about the adrenergic system of the mammary gland.

In cows with extremely high PFR, the supply of alveolar milk during milking can be crucial for the actual milk flow rate (Bruckmaier et al. 1994, Pfeilsticker et al. 1995), because the maximum milk flow rate determined by the milk duct system and the teat might be higher than the supply of alveolar milk by the milk ejection reflex. In this case a short peak with a prolonged decline phase is present in the milk flow curve. The present investigation supports this hypothesis, since a positive correlation between PFR and the decline phase was observed at a quarter level.

A close correlation between PFR and AFR was observed at a quarter and at an udder level (Fig. 1). Interestingly, an increase in AFR was associated with a larger increase of PFR at an udder level than at a quarter level. This difference between quarter and udder level is due to the aspect that at a quarter level the plateau phase (phase of PFR) was more pronounced and resulted therefore in a higher regression coefficient for the relationship between PFR and AFR as compared to an udder level. At an udder level the plateau phase lasted from the start of the plateau phase until the end of the plateau phase in the fastest milking quarter. Contrary, at a quarter level the plateau phase represented the period where cisternal milk is available (Pfeilsticker et al., 1995; Wellnitz et al. 1999). The increase in PFR in case of increased AFR is therefore more pronounced at an udder level than at a quarter level. This aspect is of importance with respect to breeding for milkability. With increasing AFR, the PFR will concomitantly increase to a higher extend. To prevent an excessive increase in PFR and therefore a possible increase in mastitis susceptibility a control of the PFR besides breeding for increased AFR could be a promising option in breeding programs.

In conclusion, the teat canal length and the vacuum to open the teat canal were negatively correlated to peak flow rate and average flow rate at a quarter level. Individual milkability at an udder level is a complex characteristic that is determined by the milkability at a quarter level and the distribution of quarter milk yields. No correlations were present between milkability traits and externally measurable teat characteristics like teat length and teat diameter. To prevent an excessive increase in peak flow rate and therefore an increase in mastitis susceptibility a control of the peak flow rate besides breeding for increased average flow rate could be a promising option in breeding programs.

4.2 Milking routines and milk removal

An optimal machine milking completely evacuates the udder and avoids a damage of teat tissue. Unfortunately, the vacuum forces applied by the milking machine can cause a damage of teat tissue (Hamann et al. 1993, Neijenhuis et al. 2001, Hillerton et al. 2002). Therefore, the vacuum load on the teat should be minimized by optimisation of milking routines and milking machine settings.

4.2.1 Stimulation intensity and oxytocin release

In a first step, the relationship between OT release and teat stimulation intensity was evaluated. The goal of this study was to test the hypothesis that OT release depends on the intensity of the teat stimulus. Although an OT concentration above a low threshold level is sufficient to start the milk ejection (Schams et al. 1983a), the minimum stimulation intensity to release OT and to start the milk ejection was unknown. In addition, it was tested whether a vibration stimulation of one single teat during milking was able induce additional OT release to improve the udder evacuation, without prolongation of the total milking time.

OT and milk flow profiles were established in 4 different treatments. The results showed that the amount of OT released varied with the intensity of teat stimulation. The application of milking vacuum without pulsation (liner-closed position) caused OT release, but at a lower level than that caused by pulsation during normal milking (Fig. 2). The OT release was sufficient to induce milk ejection as pointed out by the absence of bimodal milk flow curves in liner closed position. This indicates the presence of alveolar milk in the cistern already at the start of milking (Bruckmaier and Blum 1996). However, for the liner closed position, OT concentrations similar to these of control milkings were only accessed after the start of liner pulsation. The continuously elevated OT in LCB during the 5 min before the start of milking indicated that not only the attachment procedure of the teat cups but also the applied milking vacuum of the attached teat cup itself was a stimulus for OT release. If attachment alone had been responsible for the OT release, an immediate decrease of OT concentrations would have occurred, owing to the rapid clearance of circulating OT (Wachs et al. 1984, Bruckmaier et al. 1994).

Application of milking vacuum in the liner closed position after the end of normal milking, i.e. the same stimulus as applied before the start of milking, did not cause higher OT concentrations than in control milking (Weiss et al. 2003a) Obviously, OT release ceased after the end of milking. The observed clearance of OT was similar to that seen previously

(Wachs et al. 1984, Bruckmaier et al. 1994). Notably, the same stimulus, which was sufficient to induce a release of OT and to initiate the milk ejection before the start of milking, did not induce any OT release after the end of milking.

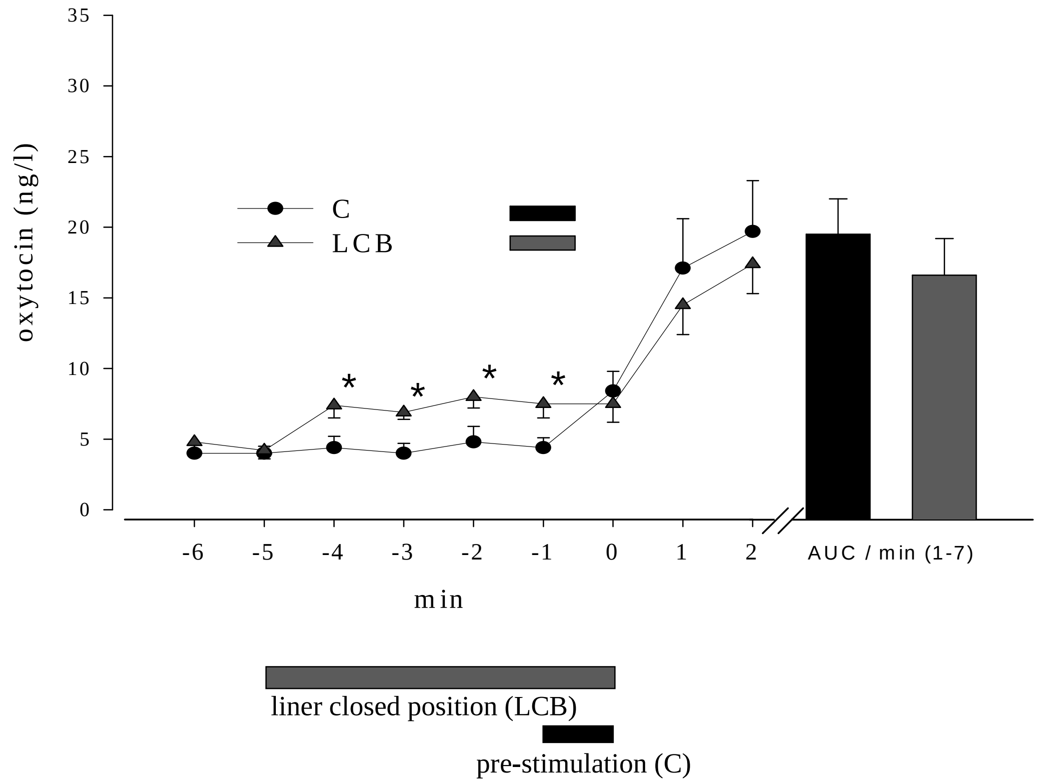

Fig 2. Oxytocin concentrations (means ± SEM for n=12 milkings) in liner closed position before milking (LCB) and control milking (C), 0 min = start of normal pulsation and milking. * Respective means are significantly different between treatments C and LCB ($p<0.05$). Also shown are oxytocin values as areas under the curve (AUC) for the first 7 min of the milkings.

An additional vibration stimulation of one teat during milking had no effect on the OT pattern, i.e. it was not able to induce additional OT release (Weiss et al. 2003a). Obviously, the effects of specific stimuli in the late phases of the milking process were not similar to their effects during the early phase. The continuous pulsation during milking seemed to reduce the responsiveness to teat stimulation. Therefore the applied stimulus (vibration stimulation), which was very effective before the milking, had no effect on the OT pattern during the late phases of milking.

In conclusion, OT release depended on the intensity of the tactile stimulation of the teat. The effect of tactile stimuli on the release of OT was different between early and late phases of milking. However, minimal stimulation intensity before milking was sufficient to start the milk ejection.

4.2.2 *Optimisation of individual pre-milking stimulation*

The lag time between the start of teat stimulation and the start of the milk ejection is related to the actual degree of udder filling (Bruckmaier and Hilger 2001). Therefore, individually adapted pre-stimulation may improve milking performance as compared to a fixed pre-stimulation routine. Since minimal mechanical impulses are sufficient to release OT (Weiss et al. 2003a), a special pre-stimulation pulsation may reduced forces applied to teat tissue. In the present study milk flow and OT profiles were analysed for several modes of tactile pre-stimulation by vibration pulsation.

The basal OT level and the release of OT as result of tactile pre-stimulation were similar to those previously reported (Weiss et al. 2003a, Mayer et al. 1984, Bruckmaier and Blum 1996, Bruckmaier and Hilger 2001). As in previous studies (Bruckmaier and Blum 1996, Bruckmaier and Hilger 2001) milking pulsation caused similar concentrations of OT as compared to a pre-stimulation by vibration pulsation. OT concentrations during the course of milking were not affected by the applied treatments. Therefore the OT concentrations could not be enhanced by the application of pre-stimulation prior to milking as compared to milking without pre-stimulation. The fact that the maximum pulsation vacuum did not influence OT release during vibration stimulation (MPV) confirmed previous results of Weiss et al. (2003a). Therefore a further step towards an optimised pre-stimulation is to analyse the optimal duration of pre-stimulation of the individual cow.

Consequently, and in agreement to several previous investigations (Sagi et al. 1980, Zinn et al. 1982, Karch et al. 1989, Rothenanger et al. 1995, Bruckmaier and Blum 1996), milk yields were not affected by different pre-stimulation routines (Weiss and Bruckmaier, submitted).

According to our and previous results no cisternal milk was available when milking started without pre-stimulation at a low degree of udder filling (Fig. 3 a, Knight et al. 1994, Bruckmaier and Hilger 2001). In this case the milk flow started after the start of the alveolar milk ejection 90 s after the start of milking pulsation. The start of alveolar milk ejection could be shifted when a pre-stimulation before the start of milking pulsation was applied. When 90 s of pre-stimulation was applied the milk flow and therefore the alveolar milk ejection started at

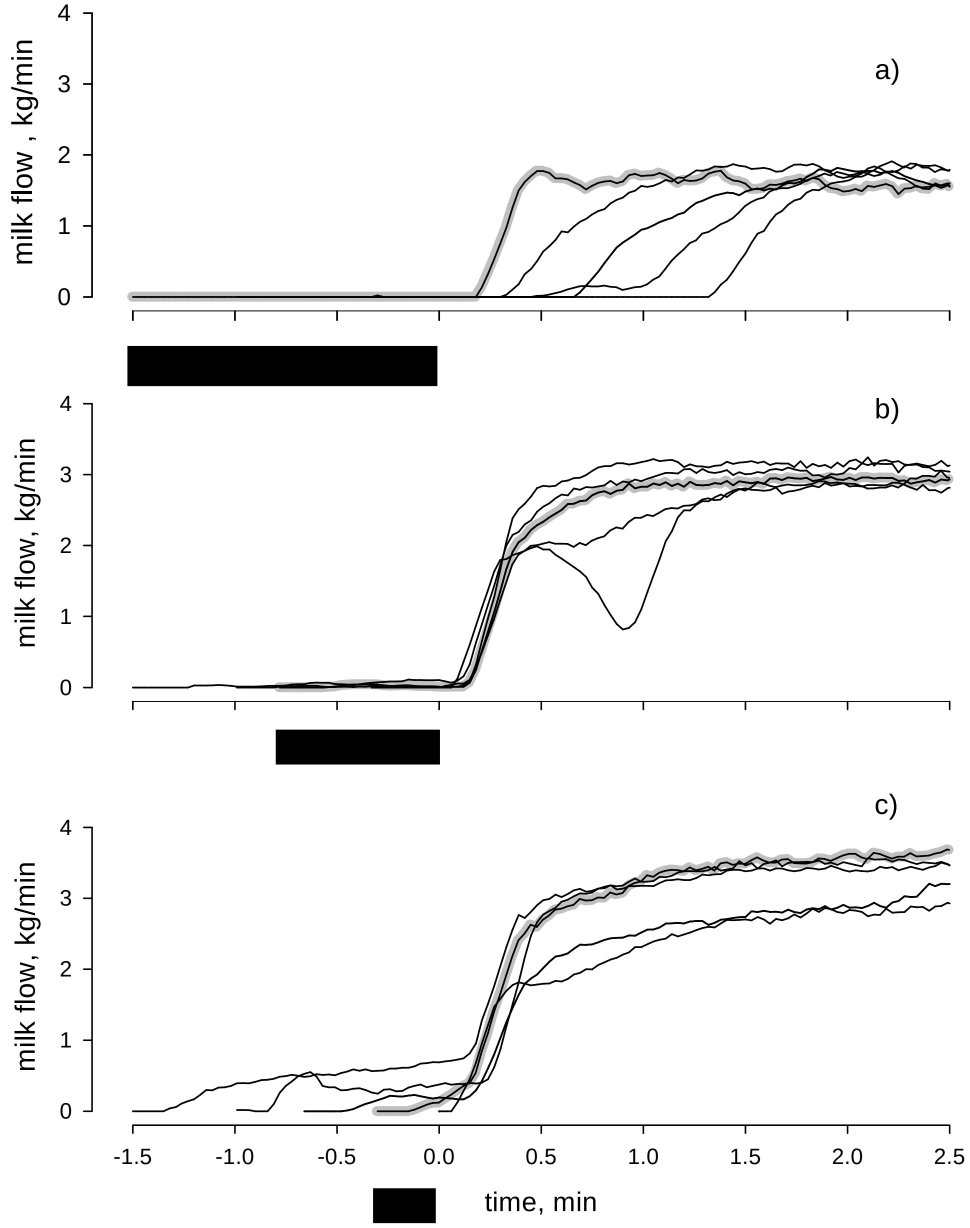

Figure 3: Milking performance after varying pre-stimulation duration (0, 20, 40, 60, 90 s of vibration pulsation) in three different cows, with a) low udder filling, b) moderate udder filling, c) high udder filling. t=0 s represent the start of milking pulsation, each individual line represent the milk flow profile until 2.5 min of milking. The grey, underneath lines represent the optimal milk flow profile. represent the optimal pre-stimulation duration.

the start of milking pulsation. Therefore, the optimal pre-stimulation duration was 90 s in case of an extremely little filled udder (Fig. 3 a). At moderate udder filling cisternal milk was immediately available for milking (Bruckmaier and Blum 1996) and the alveolar milk ejection started about 70 s after the start of pre-stimulation as indicated by the second rise of milk flow (Fig. 3 b, Bruckmaier and Blum 1996, Bruckmaier and Hilger 2001). However the amount of cisternal milk was not sufficient to bridge the gap until the start of the alveolar milk ejection, therefore the milk flow decreased when the cisternal milk was removed. In full udders the amount of cisternal milk was more enhanced (Pfeilsticker et al. 1996) and the lag time until the start of the alveolar milk ejection was further reduced (Fig. 3 c, Bruckmaier and Hilger 2001). Obviously, optimal duration of pre-stimulation, e.g. the minimum duration which is necessary to receive immediate and continuous milk flow after the start of milking varied widely between cows.

However, when discussing optimal duration of pre-stimulation, two effects have to be considered. On the one hand, the lag time from start of teat stimulation until the start of alveolar milk ejection is reduced with increasing udder filling. On the other hand, the amount of cisternal milk is increased with increased udder filling (Knight et al. 1994, Pfeilsticker et al. 1996). However, an estimation of the time until the cisternal milk is removed is unpromising, since the amount of cisternal milk and the individual milk flow rate is very variable between cows (Bruckmaier et al. 1995, Rothenanger et al. 1995 Davis et al. 1998). Therefore, an adjustment of the duration of pre-stimulation according to the actual degree of udder filling provides the best prospects to considerably reduce the time of vacuum application on the teats.

With exception of full udders, pre-stimulation resulted in an increased AFR (Weiss and Bruckmaier, submitted). However, an increased AFR did not result definitely in a reduced milking duration. The optimal duration of pre-stimulation represents the combination of a maximum AFR with a minimum main milking time including the duration of pre-stimulation. These present results basically correspond to previous reports (Zinn et al. 1982, Mayer et al. 1983 and 1984, Karch et al. 1989, Rothenanger et al. 1995, Bruckmaier and Blum 1996), but an udder filling specific effect has not been demonstrated before.

Compared to a fixed pre-stimulation duration of about 30 to 60 s, an individual adjustment of the pre-stimulation may provide two advantages. The milking stall capacity could be improved if milking of full udders was performed after a reduced duration of pre-stimulation. A prolonged pre-stimulation mode at a low vacuum level just sufficient to prevent the fall-off of the milking cluster can reduce the duration of the full milking vacuum in less filled udders. Since negative impacts of the milking vacuum load on teat tissue are well known (Hamann et al. 1993, Neijenhuis et al. 2001, Hillerton et al. 2002) an optimised pre-stimulation, according to the actual degree of udder filling may improve teat condition.

4.2.3 Quarter specific milking routines and their effect on milk removal

In conventional milking systems all 4 teat cups are removed together when milk flow of the last quarter terminated, albeit in single quarters the milk flow terminated earlier (Rothenanger et al. 1995, Wellnitz et al. 1999).

Experiments were designed to determine the efficiency of udder evacuation and the milking performance of three different quarter specific milking routines. Single quarter milk flow profiles were recorded and IMP was measured to monitor the milk ejection before the attachment and after the removal of the first teat cup. Additionally, the residual milk yield was measured to determine the udder evacuation (Weiss et al. 2003b).

A conventional milking routine with removal of all 4 teat cups together was compared with 3 quarter specific treatments: individual teat cup removal was performed at a quarter threshold level of 100 g/min. Quarter specific stripping was immediately performed when milk flow dropped below 200 g/min in the individual quarter. The teat cups were individually removed when the quarter milk flow dropped again below 200 g/min. In a third treatment the pulsation in each quarter stopped at a threshold of 200 g/min while the teat cups remained on the teats in liner-closed position. After the milk flow of the last quarter had dropped below 200 g/min the pulsation of all teat cups was restarted and stripping was performed. All treatments started with the usual pre-milking udder preparation consisting of forestripping, a short udder cleaning (15 s) and vibration pulsation for 1 min. Therefore, the pre-stimulation lasted for 75 s.

Irrespective of the applied modus, quarter specific routines could reduce the duration of vacuum application on the teats by more than 20 %. This observation corresponds to earlier investigations (Wellnitz et al. 1999, Weiss and Worstorff 2001). The residual milk and the peak flow rate did not differ significantly between treatments. Therefore, there were neither negative nor positive effects of the tested milking routines on the udder evacuation.

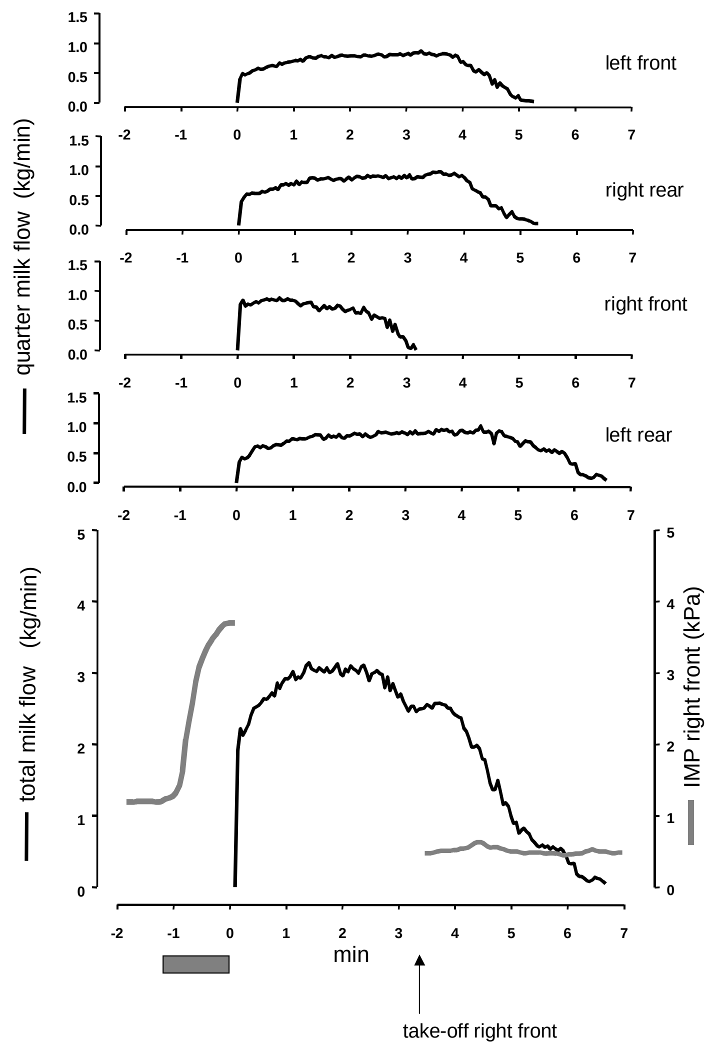

Fig. 4: Quarter milk flow, udder milk flow and IMP in right front quarter of an individual cow with quarter specific teat cup removal (pre-stimulation)

The measurements of baseline IMP and ejection pressure corresponded to earlier investigations (Bruckmaier and Blum 1996). The lack of increase in IMP after the removal of the first teat cup until the removal of the last teat cup indicates that there was no additional milk shifted into the cistern (Fig. 4). Thus the milk ejection in a specific quarter was completed irrespective of elevated oxytocin concentrations and other quarters still being milked. The fact that there was no relationship between quarter stripping yield and order of teat cup removal underlines this conclusion.

4.3 Coping capacity towards a new milking environment

Physiological and behavioural parameters were investigated in dairy cows during the changeover from conventional to AMS. The individual coping capacity towards the changeover was analysed in cows without any experience in AMS. In a second experiment the reactions of cows with and without previous experience in being milked automatically were compared.

4.3.1 Coping capacity during the changeover from conventional to automatic milking

The aim of this study was to quantify the stress reactions of dairy cows during the changeover period from conventional to automatic milking. In addition the hypothesis was tested that the individual coping capacity is related to the individual adrenal cortex sensitivity.

17 dairy cows, which were never milked before in an AMS, were used to study the changeover period from conventional to AMS milking. Heart rate, milk yield and composition, concentration of cortisol metabolites in the faeces and behaviour were evaluated before the changeover, during a 3 d training period and during the first 10 d of automatic milking. Additionally, 12 out of the 17 cows were randomly selected to perform an ACTH stimulation challenge to test the adrenal cortex sensitivity.

During the first visit to the AMS the elevated heart rate above basal (HAB) indicated a high sympathetic activation (Fig. 5). The elevation in heart rate was comparable to results demonstrated by Hopster et al. (1995) after cow-calf separation in dairy cows. Rushen et al. (1999) demonstrated that fear of dairy cows towards an aversive handler resulted in less heart rate elevation compared to the effects observed during the first visit in an AMS. Therefore, the observed effects on heart rate seem to be remarkable. However, it has to be considered that already during the second and third visits the HAB was reduced as compared to the first visit. Within 10 visits to the AMS the HAB normalized, thus indicating a successful coping of the cows to the AMS.

Similarly to the first visits a decrease in HAB could be observed during the first milkings (Weiss et al. 2004a), although the level of HAB during the first milkings was much lower as compared to the first visits. However, the start of milking in the AMS stall seemed to be a new experience since, similar to the first visits, a decrease in HAB could be observed during the first 4 milkings.

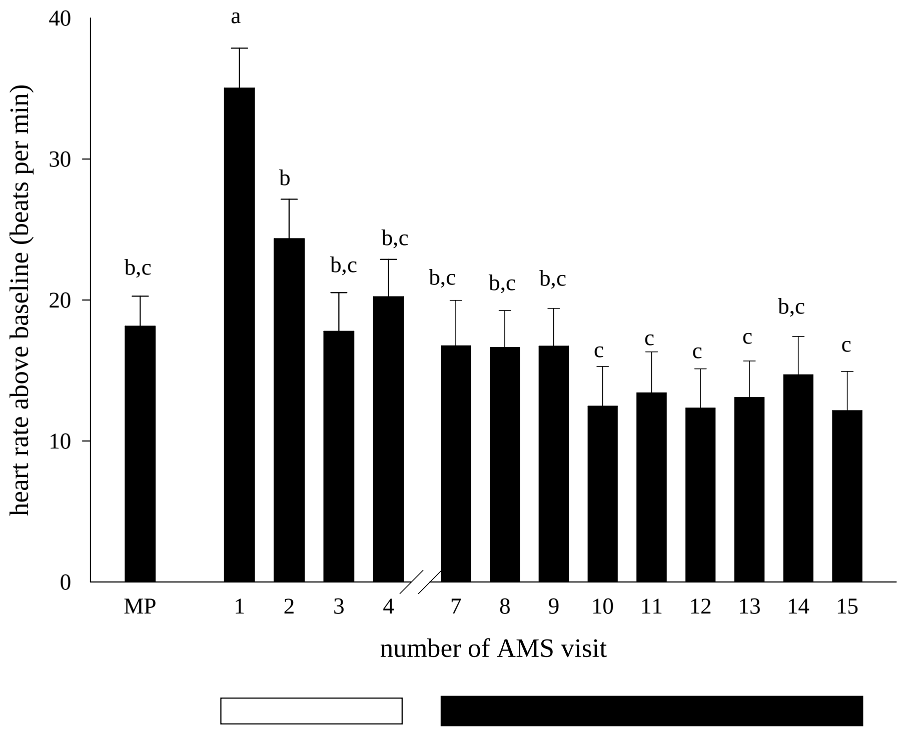

Fig. 5: Heart rate above baseline (mean ± SEM, n = 17 animals) during milking in the parlor (MP) and during visits in the stall of the automatic milking system. a, b, c means without common superscript letter differ significantly ($P < 0.05$). The white bar indicates results during the training period; the black bar indicates results after milking started in the automatic milking system.

However, the milk ejection was adversely affected during the first AMS milkings as milk yields were reduced (Fig. 8, unexperienced cows). The inhibition of milk ejection was reported earlier as a sensitive reaction to environmental changes due to a lack of OT release from the pituitary gland. During repeated milkings in unfamiliar surroundings the release of OT and therefore the occurrence of spontaneous milk ejection gradually normalised (Bruckmaier et al. 1992, 1993 and 1996, van Reenen et al. 2002). In previous studies the cows was administered exogenous OT at the end of the experimental milking in order to empty the udder completely (Bruckmaier et al. 1996). In the present study no exogenous OT was applied to avoid an additional stress load for the animals. Therefore a leftover of about 30 % of the stored milk remained in the udder after the first milking, which was still present at the second milking (Fig. 9, unexperienced cows). The milk stored before the second milking was therefore theoretically 130 % (100 % for the regular milk secretion during the milking interval and 30 % for the leftover from the previous milking), but the obtained milk yield of about only 100 % documents that there was still a partial inhibition of milk ejection. In a similar way the milk yields of the subsequent milkings have to be interpreted. Milk yield was reduced by 15 % after the first 8 to 10 milkings. Negative effects of the selectively forced cow traffic on milk production can be excluded, since milk composition and SCC were not effected by the changeover. The observed decrease of milk yields was most likely caused by the inhibition of milk ejection during the first milkings. This inhibition caused an incomplete emptying of the udder resulting in a reduced milk production during the ongoing lactation (Peaker and Wilde 1996, Bruckmaier and Blum 1998). The reduced milk production likely caused by enhanced apoptosis of the mammary epithelial cells (Murugaiyah et al. 2001, Stefanon et al. 2002).

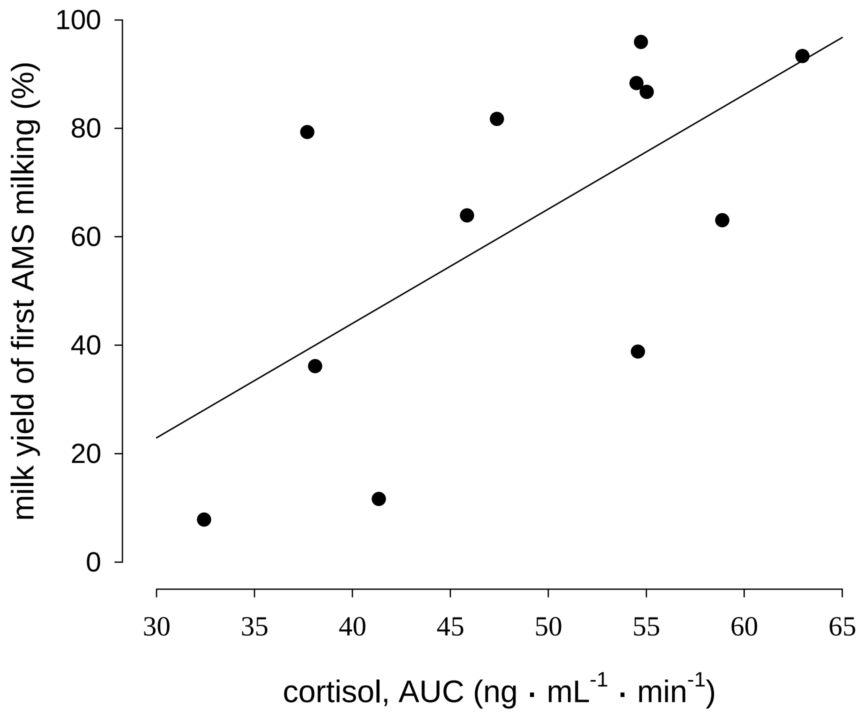

Fig. 6: Relationship between cortisol release during ACTH challenge (area under curve AUC, 0 to 180 min after ACTH application) (x) and the relative milk yield during the first AMS milking (y). Pearson's coefficient of correlations was $y = -40.4 + 2.1\ x$, $r = 0.65$ ($P = 0.02$).

High adrenal cortex sensitivity was signified by a high cortisol release during ACTH challenge. Cows with a high adrenal cortex demonstrated a less distinct disruption of milk ejection during the first milking (Fig. 6). Additionally, in these cows the decrease of milk yield during the first 20 AMS milkings due to the changeover was less pronounced. In the present study the cows visited the AMS milking stall at least 7 times before the first milking was performed. In contrast, in previous experiments milkings took place at the first visit in unfamiliar surroundings (Bruckmaier et al. 1992 and 1996, Rushen et al. 2001, Macuhova et al. 2002). Therefore the milk ejection reflex was probably not completely blocked in the present study, as had been observed before. However, the present results confirm previous investigations by Macuhova et al. (2002), who demonstrated a tendentially negative relationship between the degree of blocked milk ejection in unfamiliar surroundings and the adrenal cortex sensitivity of the individual cow. The individual variation of the disturbance of milk ejection and the HAB during the first milking in the AMS despite a highly standardised treatment for all cows, demonstrates the individual coping capacity towards the changeover to the AMS. For practical application this means that the training period could even be shortened in cows with a high coping capacity. In cows with a low coping capacity a longer training period could possibly prevent a loss in milk yield due to the changeover.

However, the reason for individual differences in adrenal cortex sensitivity in cattle is unclear. Results by Ladewig and Smidt (1989) and Redbo (1998) support the hypothesis that the individual adrenal cortex sensitivity is reduced due to chronic stress overload. Behavioural analyses suggest that a stress overload reduced individual activity and resulted in a decreased exploratory activity (Redbo 1998). If the exploratory activity is reduced, the time needed to adapt to a changing environment is enhanced and the time until successful coping therefore prolonged.

Although all cows adapted within days to the AMS, the individual ability to cope varied widely and was related to the adrenal cortex sensitivity. These results suggest a considerable importance of the hypothalamic-pituitary-axis for the coping process in cattle.

4.3.2 The changeover from conventional to automatic milking in dairy cows with and without previous experience

The reaction towards the changeover from conventional to automatic milking in cows without any experience in automatic milking and in cows that were at least milked for one lactation in the AMS were compared in this study. The hypothesis was tested if the individual cow's reaction differed remarkably due to their previous experience (Weiss et al. a, submitted).

Unexperienced cows (UC) had never been milked by an AMS before while experienced cows (EC) were milked for one lactation in the AMS. Interestingly, EC entered the AMS instantaneous without any human intervention after they were moved to the AMS herd. The rate of voluntary visits on d 1 was reduced; because it was essential to teach the AMS the teat coordinates of the individual cow before the start of the first automatic milking (Fig. 7).

However, this visit because of technical reasons was the sole exception of manually moving EC to the AMS milking stall throughout the experimental period. The fact that EC had not been using the AMS for about 80 d (dry period of 6 wk and 35 d parlour milking), and were thereafter immediately familiar with AMS visits, documented the memory capacity of the dairy cow. These findings correspond to results of Kilgour (1981) and Kovalcik and Kovalcik (1986).

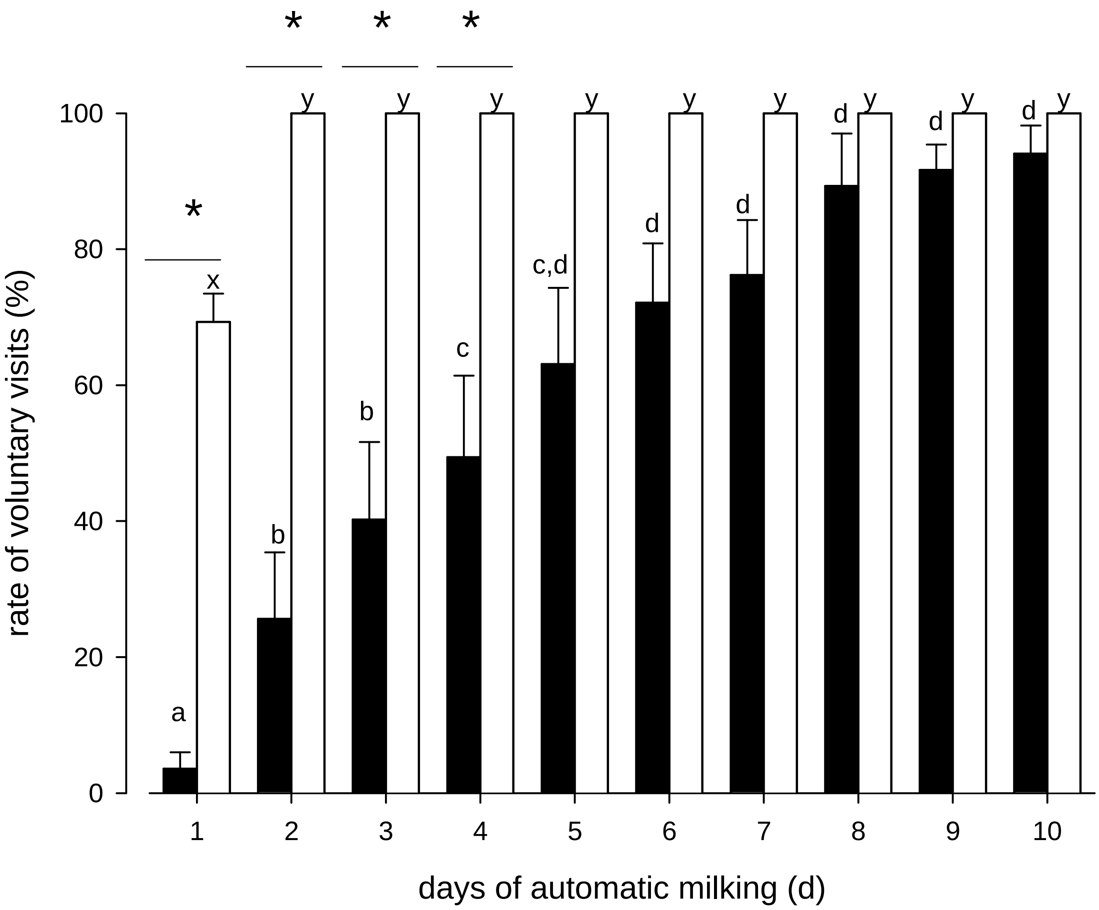

Fig. 7: Rate of voluntary visits (mean ± SEM, n = 17 UC and 9 EC) to the AMS milking stall during the first 10 d of automatic milking. Unexperienced cows (UC) were represented by the black bars, experienced cows (EC) by the white bars. * represent significant differences between EC and UC; a, b, c; x, y means without common superscript letter differ significantly within treatment (EC and UC) ($P < 0.05$).

UC never entered the AMS voluntarily within the training period. The rate of voluntary visits achieved levels of more than 90 % after d 9 of AMS milking. The rate of voluntary visits after successful adaptation to AMS milking corresponded to previous investigations in adapted cows (Ketelaar-de Lauwere et al. 1998, Winter and Hillerton, 1995, Harms et al. 2002, Hermans et al. 2003). However, it has to be pointed out that this level was approached not until d 9 of AMS milking. Therefore, considering the training period of 3 d, a successful automatic milking without an excessive use of labour to move the cows did not take place until d 12 in the AMS.

During the first visit to the AMS the elevated HAB in UC indicated a high sympathetic activation (Fig. 8). In agreement to the previously discussed behavioural results, HAB was not elevated in EC during the first AMS visits.

Milk constituents were not affected due to the changeover process. Due to this fact, negative effects of the selectively guided cow traffic on feed intake can be excluded. Therefore the observed effect of a reduced milk yield in UC and enhanced milk yields in EC were probably

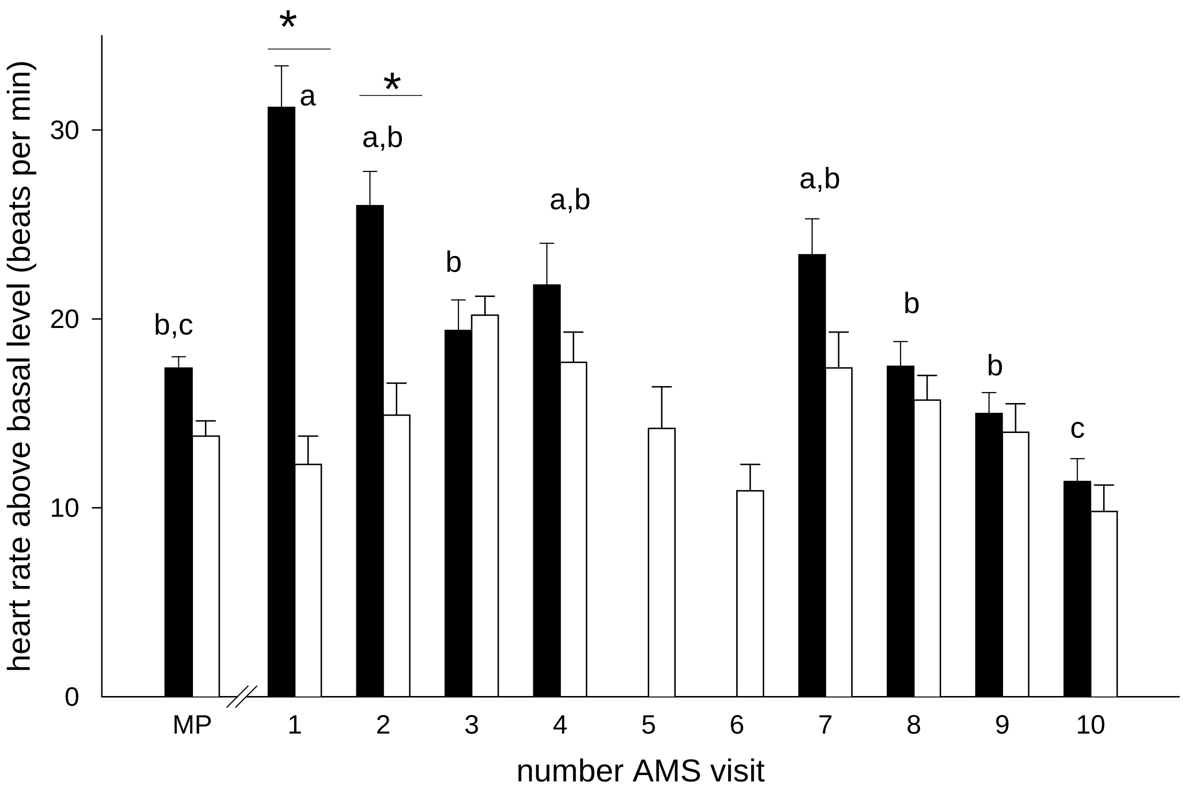

Fig. 8: Heart rate above baseline (mean ± SEM, n = 17 UC, 9 EC) during milking in the parlour (MP) and during visits in the AMS stall. Unexperienced cows (UC) were represented by the black bars, experienced cows (EC) by the white bars. * represent significant differences between EC and UC; a, b means without common superscript letters differ significantly ($P < 0.05$).

due to a local effect in the mammary gland (Fig. 9). In case of an increased milking frequency these local regulations could enhance milk secretion and proliferation of secretory tissue. Probably the enhanced milk yields in EC were due to the same regulatory background like the reduced milk yields in UC.

In conclusion, the change from conventional to automatic milking was remarkably different between dairy cows with and without previous experience. This points at the considerable memory potential of the dairy cow. Even after handling in another environment, experienced cows were immediately able to cope with AMS conditions. However, the change to automatic milking was a challenge for unexperienced cows. Therefore great efforts must be undertaken to minimise negative effects during the first few milkings. Once cows were adapted successfully to automatic milking, the change to AMS seemed to be unproblematic.

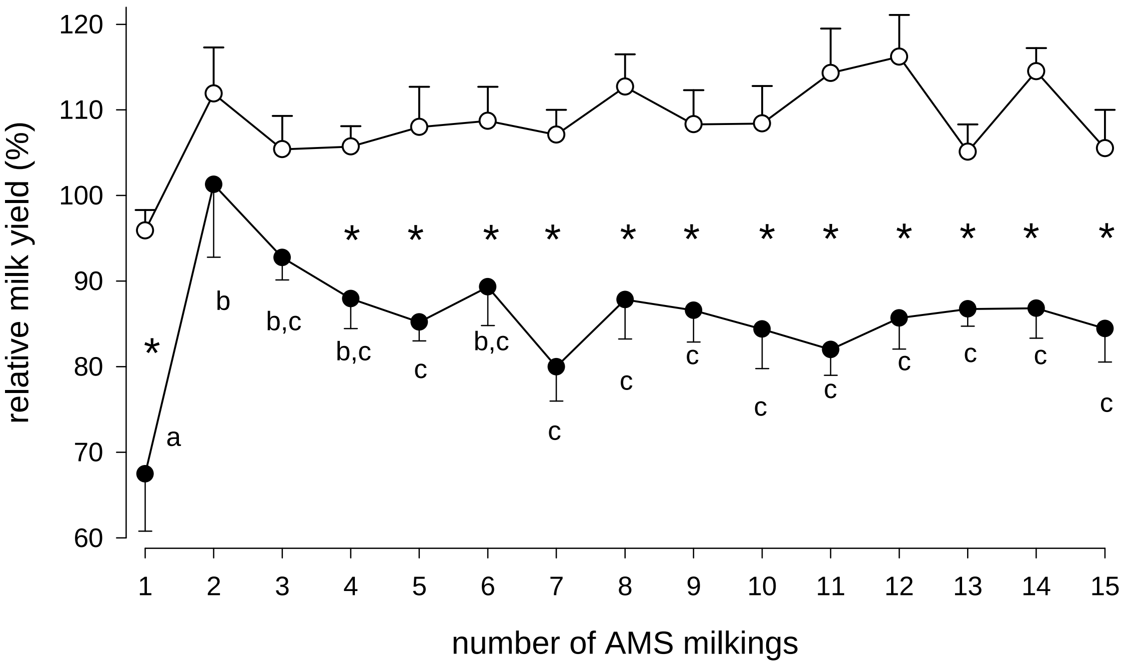

Fig. 9: Milk yield (mean ± SEM, n = 17 UC, 9 EC) expressed as relative values of parlour results (100% = mean parlour results of 10 d prior to the changeover). Black dots represent unexperienced cows (UC), white dots represent experienced cows (EC). * represent significant differences between EC and UC; a, b, c means without common superscript letters differ significantly ($P < 0.05$).

4.4 Variable milking intervals and milk composition

The effects of coincidental milking intervals in AMS on milk production and milk composition were investigated. Our results demonstrate a decrease of fat content with increasing milking interval (Fig. 10). This observation agrees with previous findings of decreasing fat content with increasing milking intervals in cows (Bar-Peled et al. 1992, Elliot et al. 1961, Ontsouka et al. 2003) and ewes (Negrao et al. 2001) in short-term experiments. However, these results are in contrast to observations on a herd based decrease of fat content with continuously increased milking frequency (Erdmann and Varner 1995). Changes in nutritional status due to the increased milk yield in the last mentioned experiments may explain these contrasts to our own findings (Buchberger 1979). There seem to be different effects if either the milking interval is regularly changed (increased milking frequency in conventional milking systems) or milking intervals alternate (short-term experiments or AMS conditions), resulting in a permanent variation of udder filling rates at milking. Probably a certain amount of fat remains in the alveoli and the milk duct system from previous milk removal (Weiss et al. 2002). This fat is not moved to the cistern between two milkings. Solely if a new milk ejection occurs this fat is shifted into the cistern. It is less diluted if a milk ejection takes place after a short milking interval, because in this case only a small amount of milk is shifted into the cistern. In case of a long milking interval the amount of remaining alveolar fat is unchanged, but much more milk is ejected and therefore the fat is more diluted. However, the increased fat production rate

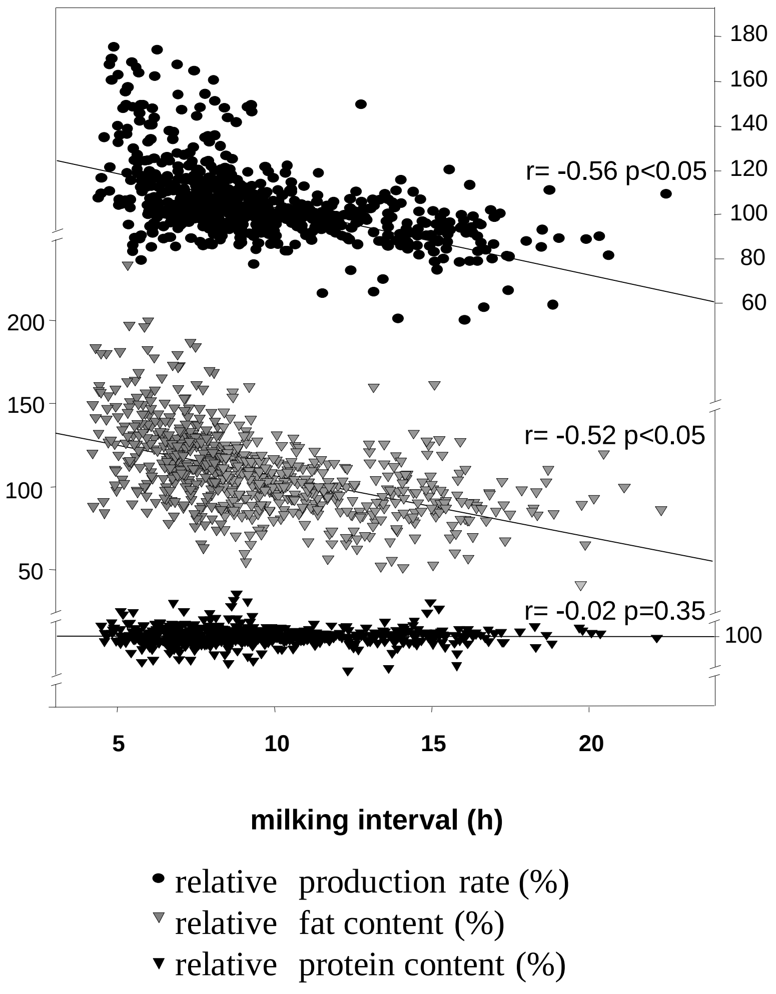

Fig. 10: Effect of milking interval on relative milk production rate, milk fat and milk protein content. ● Relative production rate (%), ▼ relative fat content (%), ▼ relative protein content (%). Values of each experimental milking were expressed as a percentage of the mean value of each individual cow at the milking intervals from 8 to 10 h.

following increasing milking frequency remains unexplained. According to results of Negrao et al. (2001) in ewes there seems to be an additional metabolic change in the mammary tissue. It seems possible that an increased shift of milk fat globules into the alveolar lumen occurred shortly after the milking procedure, therefore the rate of milk fat exocytosis in the alveolar lumen was higher with shorter milking intervals.

The milk protein and lactose content and the SCC remained without significant differences due to the milking interval. This corresponds to previous findings (Bar-Peled et al. 1992, Elliot et al. 1961, Erdmann and Varner 1995, Ontsouka et al. 2003). Lactose is the major osmotically active component of the milk because of its small molecular weight. The lactose secretion mainly determines the influx of water into the milk, i.e. the milk volume. It is therefore not surprising that the lactose content remained similar at different milking intervals.

Our results show a clear correlation between the milk production and the milk fat content with the milking interval. In AMS there is a wide range of possible milking intervals (Ketelaar-de Lauwere et al. 1996, Wendl et al. 1999). Especially when extreme milking intervals occur, the precision of milk sampling once daily is not sufficient to estimate the real milk fat content. Therefore, the shown linear regression can contribute to improve the precision of test day models in AMS.

4.5 The acyclic period post partum in automatic and conventional milking

In this study primiparous and multiparous cows were milked either in an AMS or in a conventional milking parlour under identical housing and management conditions (Weiss et al. b, submitted). The early start of ovarian activity post partum observed in the present study (Table 1) corresponds to previous investigations (Schopper et al. 1989, McLeod and Williams 1991, Reist et al. 2003). The period of post partum ovarian acyclicity (OA) did not differ between AMS cows (AC) and parlour cows (PC). In agreement with result of Darwash et al. (1997) an earlier onset of ovarian cyclicity in primiparous as compared to multiparous cows was observed. However, neither milking frequency nor standard deviation in milking frequency was related to the period of OA and further evaluated reproductive parameters. These results correspond to findings of Kruip et al. (2002) in field studies and to data of Lamb et al. (1999) in beef cows. However, prolonged OA in conventional thrice daily milked dairy cows as compared to twice daily milking were reported by Stevenson et al. (1997) and Amos et al. (1985). Furthermore Smith et al. (2002) figured out a reduction in fertility by increased milking frequency. However, it is remarkable that these studies were examined under field conditions (Smith et al. 2002, Kruip et al. 2002). In addition, some resulted in a significant increase in milk production as a result of increased milking frequency (Amos et al. 1985, Stevenson et al. 1997). Therefore, these studies have to deal with bias by different herd and management conditions or due to a changed milk yield. In the present study milking frequency was elevated in AC as compared to PC, whereas both groups were managed under identical conditions. Additionally, the milking frequency in AC varied continuously between 2.1 and 5.9 milking per day, which enabled an analysis within AC. However, in the present study neither an effect of the milking system nor an effect of the milking frequency within the AC was observed.

Table 1: Onset of ovulatory cycle and reproductive parameters in primiparous and multiparous cows differing for the milking system.

	Milking system	
Item	**AMS**	**Parlour**
Primiparous cows		
Time until first ovulation (d) [1]	22.1 ± 7.1 (12-39) [a]	23.2 ± 6.3 (16-36) [a]
Time until second ovulation (d) [1]	33.8 ± 8.4 (20-49) [a]	36.3 ± 9.2 (24-55) [a]
Time until first service (d) [2]	49.2 ± 14.9 (31-95)	57.1 ± 13.4 (40-87)
Time from first ovulation until first service (d) [2]	26.6 ± 16.1 (0-73) [a]	33.8 ± 15.3 (11-71) [a]
Days open (d) [2]	66.2 ± 25.2 (40-125)	83.9 ± 36.2 (46-160)
Number of services per conception [2]	2.2 ± 1.1 (1-4)	1.6 ± 0.8 (1-3)
Conception at first service (%) [2]	35	56
Multiparous cows		
Time until first ovulation (d) [3]	29.3 ± 9.1 (15-55) [b]	28.5 ± 7.9 (18-49) [b]
Time until second ovulation (d) [3]	42.4 ± 11.1 (22-69) [b]	39.6 ± 9.0 (25-60) [b]
Time until first service (d) [3]	50.9 ± 17.1 (19-100)	46.9 ± 10.0 (30-64)
Time from first ovulation until first service (d) [4]	22.2 ± 15.7 (0-67) [b]	18.0 ± 11.0 (0-37) [b]
Days open (d) [4]	71.2 ± 29.9 (35-157)	70.5 ± 29.6 (41-147)
Number of services per conception [4]	1.8 ± 1.1 (1-5)	1.6 ± 0.6 (1-3)
Conception at first service (%) [4]	58	47

a, b means with different superscript letters in the corresponding rows (primiparous vs. multiparous cows) within the same column are significantly different ($P < 0.05$). Values in brackets represent the full range of observed values. [1] 23 AC and 37 PC, [2] 17 AC and 17 PC, [3] 40 AC and 24 PC, [4] 26 AC and 16 PC.

Recent studies focused on the impact of the energy balance (Beam and Butler 1999, Butler 2000, Koller et al. 2003, Reist et al. 2003), indicating that a more severe negative energy balance reduces reproduction performance post partum. Beam and Butler (1999) and Butler (2000) discussed effects on both OA and conception rate. In contrary Reist et al. (2003) observed only a reduction of conception rate, while OA was not affected.

Because increasing milking frequencies result in increased milk yields (Kruip et al. 2002, Smidt et al. 2002), previously reported interactions between an increased milking frequency and a reduced reproduction performance are likely due to a more pronounced negative energy balance as a result of increased milk yields.

Although in the present study an increased milk yield was observed in AC, the obtained milk yields were on a moderate level, due to the used dual-purpose breed German Fleckvieh. Therefore an excessive negative energy balance at the start of lactation as reported for high yielding dairy cows was not to be expected (Reist et al. 2003).

In conclusion, milking frequencies with up to 5 milkings per day, as observed in the present study, did neither considerably affect the onset of the ovarian cyclicity nor the fertility in dairy cows. A reduced fertility as a result of an increased milking frequency is probably due to a more severe negative energy balance in case of very high milk yields.

5 Conclusions and implications

The focus of the present thesis is the interaction between dairy cow physiology and milking technology. The results point out that there are several prospects to improve the balance between animal and technology.

An understanding of the relationship between teat anatomy and milkability can help to introduce new aspects into current breeding schemes. The present results demonstrated several relationships between teat anatomy and milkability. This knowledge can contribute to improve the genetic potential of dairy herds.

Various experiments demonstrated that there is a noteworthy potential to reduce the vacuum load on the teats by adjusting the milking technology individually to the cows' demand. A proper timing between the start of milking, the oxytocin release and thus the alveolar milk ejection results in an immediate and continuous milk flow after the start of milking and results in a short duration of milking. Although the obtained oxytocin concentrations depended on the intensity of the tactile stimulation of the teat, minimal stimulation intensity before milking was sufficient to start the milk ejection. Therefore a special pre-stimulation routine before milking can avoid a phase of low milk flow already at the start of milking and can reduce the vacuum load of the milking machine. The optimal duration of pre-stimulation was related to the actual degree of udder filling. Hence, short pre-stimulation enhances the milking stall capacity milking full udders and a prolonged pre-stimulation reduces the total vacuum load on the teat milking little filled udders. An individually adjusted duration of pre-stimulation is therefore recommended to optimise the milking process. At the end of milking the milk ejection in single quarters was completed when the milk flow ceased, even if other quarters were continued to be milked. Therefore the individual removal of single teat cups avoided an overmilking of single quarters, thus preventing an additional stress on teat tissue. These results demonstrate potentials to improve the milking technology.

Experiments in automatic milking systems showed that there were no negative impacts of automatic milking systems on cows' welfare, but the individual adaptation capacities of dairy cows varied widely and were related to their adrenal cortex sensitivity. These results suggest a considerable importance of the hypothalamic-pituitary axis for the coping process in cattle. However, the change from conventional to automatic milking was a challenge for unexperienced cows. Therefore a cow individual management during the transition period may reduce negative impacts on the cow and the milk yield. An accurate management is essential to avoid stress load on the cow and economical losses for the farmer. Once cows adapted towards automatic milking the main difference between conventional and automatic milking systems was a higher milking frequency at coincidental milking intervals in the latter one. These variable milking intervals affected the milk fat content. In contrast, fertility and reproduction were unchanged. However, traditional systems to evaluate dairy cows performance have to be rearranged to automatic milking conditions. These insights can help to improve the management skills.

The interaction between dairy cow physiology and milking technology is a complex neuroendocrine cross talk between the dairy cow and the milking machine. The present thesis demonstrates that there is a potential to improve the machine milking process at a genetical, technological and a management level. The knowledge about the physiological background of the biological system dairy cow and their mammary gland provides the basic information for a well-adjusted balance between the dairy cow and the milking machine.

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7 Curriculum vitae

Name	Daniel Weiß
Date of birth	16.2.1974
Place of birth	Schopfheim
Children	Thea and Zacharias

School

1980-1984	Primary School Gresgen
1984-1994	Gymnasium Schönau

Vocational training

1995-2000	study of Agricultural Science at the Technische Universität München
2000	graduation with diploma (Dipl. Ing. agr.)
2000-2004	employee at Physiology Weihenstephan for doctoral thesis

Practical work

1994-1995	civil service hospital Zell i.W.
1995	work experience Statz’s farm Wisconsin, USA (dairy farm) – 2 months
	work experience Advanta seeds, Wisconsin, USA (maize breeding) – 2 months
1998	work experience Advanta GmbH Grünberg, Germany (seed production and distribution) – 2 months
	work experience agriculture cooperation Köchelstorf, Germany (crop and dairy farm) – 8 months
2003/2004	educational journey to Brasil and Chile – 3 months

7.1 List of personal publications

7.1.1 Original publications in peer reviewed scientific journals

Publications printed or "in press"

Weiss, D., Weinfurtner, M., Bruckmaier, R.M. Teat anatomy and its relationship with quarter and udder udder milk flow characteristics in dairy cows. Journal of Dairy Science (in press)

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Bruckmaier, R.M., Weinfurtner, M., Weiss D. 2004 Teat anatomy and teat sphincter closure: relationship with quarter milk flow. National Mastitis Council 43rd Annual Meeting, February 1-4, 2004, Charlotte, North Carolina, USA

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Appendix (original puplications)

Teat anatomy and its relationship with quarter and udder udder milk flow characteristics in dairy cows

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Interpretative Summary

The teat anatomy and functionality and the milk flow characteristics in 148 quarters of 38 dairy cows were analyzed. Teat canal length and the vacuum required to open the teat canal were negatively correlated with peak flow rate. Neither teat length nor teat diameter correlated significantly to teat canal length or to milk flow traits. The anatomical and functional characteristics of single teats could partly explain the milk flow characteristics of individual quarters.

Abstract

Anatomical and functional characteristics of the teat are supposed to have considerable influence on milk flow performance. In the present study various teat and milking characteristics in 148 quarters of 38 cows were analyzed via 3 different approaches. Teat canal length, teat wall thickness and teat diameter were measured by ultrasound. In addition, the vacuum needed to open the teat canal (VO) was determined and milk flow profiles were measured in each quarter separately.

Rear teats were shorther and thicker than front teats, whereas teat canal length and teat wall thickness did not differ for quarter position. Milk yield and peak flow rate (PFR) were higher in rear than in front quarters. Teat canal length and VO were negatively correlated with PFR and average flow rate (AFR), but no correlations were observed between milkability traits and externally measurable teat characteristics like teat length or teat diameter.

In conclusion, individual milkability at an udder level is a complex characteristic that is determined by the milkability at a quarter level and the distribution of quarter milk yields. The anatomical and functional characteristics of single teats can partly explain the milk flow characteristics of individual quarters.

Keywords: teat anatomy, teat canal, milk flow

Abbreviation key: **AFR** = average flow rate, **PFR** = peak flow rate, **VO** = vacuum needed to open the teat canal, **VO-C** = vacuum needed to open the teat canal at cessation of milk flow, **VO-S** = vacuum needed to open the teat canal at start of milk flow

Introduction

During machine milking the teat represents the interface between the mammary gland and the teat cup liner. Therefore, the anatomical and functional characteristics of the teat are supposed to have considerable effects on milking performance of the individual quarter and cow. According to earlier studies teat canal measures are related with the PFR (Baxter et al., 1950; Andreae, 1958; Loppnow, 1959). Besides milkability the anatomy of the teat canal is also related to udder health. Grindal et al. (1991) demonstrated an increased infection risk in quarters with short teat canals. In most studies milk flow was analyzed based on an udder or a half udder level (Rogers and Spencer, 1991; Le Du et al., 1994; Slettbakk et al., 1995), although there is a considerable variability in milk flow profiles between the quarters of one udder (Rothenanger et al., 1995; Wellnitz et al., 1999; Weiss et al., 2003). To the best of our knowledge there is no information available on the relationship between quarter milking characteristics and teat morphology at a quarter level.

The aim of the present study was to demonstrate possible relationships between teat anatomy and teat functionality using three different approaches. Teat anatomy was determined by ultrasound cross sections. Milk flow profiles at a quarter level were recorded and the vacuum needed to open the teat canal (VO) was measured. The hypothesis was then tested if characteristics of the teat considerably influenced the milking characteristics.

Materials and Methods

Animals and Milking

The 38 experimental cows (Brown Swiss x German Braunvieh) were in months one to eight of their first to fifth lactation. The diet consisted of corn and grass silage, hay and concentrate according to the individual production levels. The cows were kept in loose housing and were milked in a 2 x 2 tandem milking parlour. Milking was performed twice daily at 5:00 and 16:00 at a vacuum of 40 kPa, a pulsation rate of 60 cycles/min and a 60:40 pulsation ratio. The milking routine consisted of udder cleaning and stripping of the first milk squirts in addition to manual pre-stimulation. The teat cups were attached 1 min after the first touch of the udder. For each quarter teat cups with an individual ventilation system (Bio-Milker, WestfaliaSurge GmbH, 59299 Oelde, Germany) and with separate long milk tubes were used. At the end of milking stripping was applied when total milk flow decreased below 0.3 kg/min.

Experimental design

All experiments were performed during evening milkings starting at 16:00. Teats were scanned by B-mode ultrasonography as described previously (Bruckmaier and Blum, 1992; SonoVet 2000, Probe: 5 MHz linear array scanner Nr. LV4-7AD, Kretztechnik, 4871 Zipf, Austria). Cross sections of the teats were performed after a 1-min manual pre-stimulation, i.e. at a well filled teat cistern as a result of the initiated milk ejection reflex. Teats were dipped in a plastic cup filled with water and the probe was attached to the cup wall by using an ultrasound gel. The teat canal was used as the longitudinal scan axis. From the ultrasound images the teat canal length, the teat wall thickness and the teat diameter were determined as indicated in Figure 1. A gauge was used to determine the teat length from the teat tip to the teat base.

Milk flow was recorded for individual quarters by using 4 mobile recording units (Lactocorder, Werkzeug- und Maschinenbau Balgach, 9436 Balgach, Switzerland) as previously described (Wellnitz et al., 1999). The milk flow parameters were evaluated at a quarter and an udder level according to Bruckmaier et al. (1995). Plateau and decline phases of the milk flow curves were determined based on the slope of the milk flow profile, as previously described by Göft (1992a and 1992b). The decline phase lasted from the end of the plateau phase until the milk flow dropped below a threshold of 0.3 kg/min and 0.1 kg/min at an udder and at a quarter level, respectively. Main milking time was defined from the start of milking until the end of the decline phase. Average flow rate (AFR) was the quotient of main milk yield and main milking time. Milk flow recording was performed during two evening milkings in each cow and means were used for further correlation analyses.

A special device based on a previously described approach by Le Du et al. (1994) was developed to determine VO. A transparent teat cup was used equipped with the mouthpiece of a liner to avoid air leakage between teat cup and teat (Figure 2). The vacuum in this teat cup was gradually increased until milk flow was visible, and was subsequently decreased again

(Figure 3). A vacuum measurement device was used to record the vacuum within the teatcup throughout the measurement (BoviPress, A&R Trading GmbH, 21379 Echem, Germany). The handle of the teat cup was equipped by a switch to mark the start and the cessation of milk flow within the recorded vacuum curve. The VO was measured after forestripping and the application of a 1-min manual pre-stimulation to ensure the start of milk ejection. Measurements were performed anti-clockwise starting at the front left teat. The VO measurement within one quarter lasted for about 7 s as shown at a representative vacuum profile of one cow (Figure 3). Therefore the total measuring cycle, including the handling time, was about 30 to 60 s for one udder. For calculation of the repeatability the measurements were performed on three days in each animal.

Statistical analysis

The results are presented as means ± SEM. For statistical analysis, the SAS program package release 8.01 (SAS 1999) was used. The Mixed procedure was used to determine significant effects. Differences were localized with student's t-test. The model included the quarter nested within the animal and the date of the measurement. The quarter within the animal was defined as a repeated factor while the date was defined as a random factor. The REG procedure was employed to calculate Pearson's coefficients of correlation between the analyzed parameters. Results were indicated as statistically significant at $P < 0.05$, unless stated otherwise. The data contained information on 148 quarters of 38 cows; four cows were only milked on three teats due to previous mastitis in one quarter. Repeatability within the teat was calculated for VO according to Essl (1987).

Results

Teat anatomy

Front teats were longer than rear teats (Table 1). The diameter of the front teats was smaller

Table 1: Teat anatomy at a quarter level.

	Teat length (mm)	Teat diameter (mm)	Teat wall thickness (mm)	Teat canal length (mm)
Left front	66±1.5 [a]	27±0.4 [a, b]	8±0.3	11±0.3
Right front	67±1.6 [a]	27±0.4 [a]	8±0.3	11±0.3
Left rear	57±1.5 [b]	28±0.4 [b]	7±0.2	11±0.3
Right rear	56±1.6 [b]	28±0.3 [b]	7±0.2	11±0.3

a, b: different superscript letters indicate significant differences between quarters ($P < 0.05$).

than that of the rear teats. Teat wall thickness and teat canal length did not differ significantly between front and rear quarters.

Vacuum needed to open the teat canal (VO)

The repeatability (w) of vacuum needed to start the milk flow (VO-S) and vacuum at cessation of the milk flow (VO-C) was 0.71 and 0.87, respectively. VO-S and VO-C did not

Table 2: Vacuum needed to open the teat canal at the start of milk flow (VO-S) and at cessation of milk flow (VO-C).

	VO-S (kPa)	VO-C (kPa)
Left front	17.8±0.9 [a]	7.1±0.5 [b]
Right front	19.5±0.8 [a]	7.9±0.4 [b]
Left rear	20.3±0.9 [a]	8.6±0.5 [b]
Right rear	21.0±0.9 [a]	8.9±0.5 [b]

a, b: different superscript letters indicate significant differences between vacuum at start of milk flow and vacuum at cessation of milk flow ($P < 0.05$).

significantly differ between teat positions (Table 2). VO-C was lower than VO-S, but VO-S and VO-C were correlated on a highly significant level ($r = 0.82$, $P < 0.0001$).

Milking characteristics

Figure 4 shows an exemplary udder- and quarter milk flow profile. PFR, duration of the plateau phase, duration of the decline phase and machine stripping are indicated at a quarter and an udder level. The start of the plateau phase was similar at a quarter and an udder level. However, the end of the plateau phase at a quarter level was determined by the availability of milk in the specific quarter, whereas the end of the plateau phase at an udder level was determined by the plateau length of the shortest milking quarter. The decline phase lasted until the cessation of milk flow in the slowest milking quarter. In Table 3 the milking characteristics referring to the quarters are shown. Total milk yield was 13.10 ± 0.45 kg, total stripping yield was 0.25 ± 0.04 kg. Milk yield was higher and milking time was longer in rear as compared to front quarters. Stripping yield did not differ between quarters. PFR and AFR were lower in front as compared to rear quarters.

Results of the plateau phase and the decline phase are presented in relation to the respective main milking time. Relative plateau phase was shorter in front than in rear quarters, whereas the decline phase was longer in front quarters. At an udder level the relative plateau phase (30.1 ± 1.8 %, $P < 0.05$) was shorter as compared to a quarter level. Consequently, the relative decline phase (40.4 ± 1.8 %, $P < 0.05$) was prolonged at an udder level as compared to a quarter level (Table 3).

Correlations

Pearson's coefficients of correlation at a quarter level are presented in Table 4. Teat wall thickness was positively correlated with teat diameter and teat canal length. No relationship was observed between teat length, teat diameter and teat canal length.

VO-S and VO-C were closely correlated; furthermore VO-S and VO-C did basically not differ in their relationship to all other investigated parameters. VO was not correlated to any anatomical parameter.

Milk yield was positively correlated with main milking time, plateau phase and AFR, but negatively with decline phase. Stripping yield was not affected by milk yield, PFR, main milking time and AFR. However, stripping yield was negatively correlated with plateau phase and positively correlated with decline phase. PFR was negatively correlated with main milking time and plateau phase, whereas positive correlations were observed with milk yield, decline phase and AFR. However, at an udder level no relationship between PFR and milk

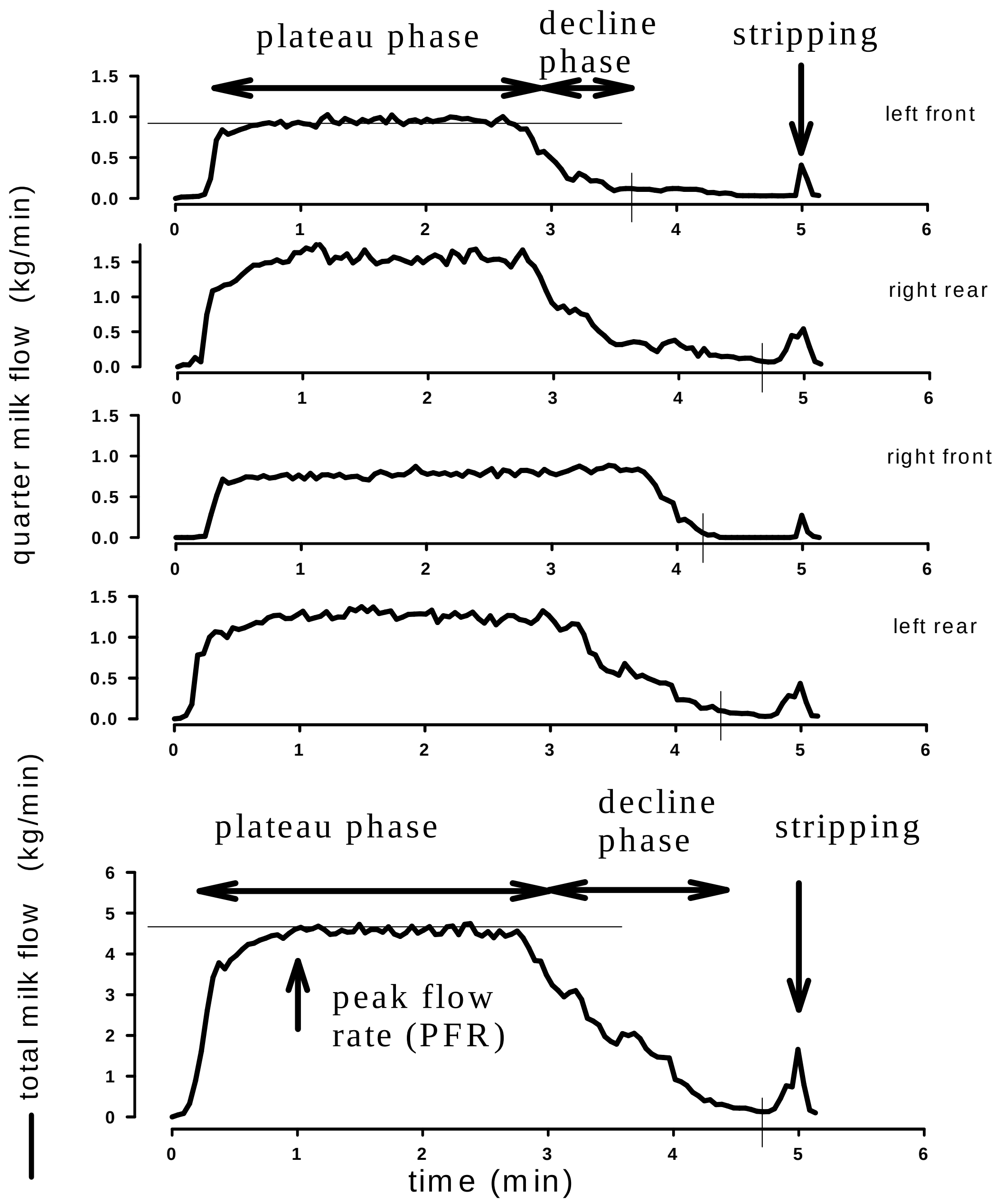

Figure 4: An exemplary quarter and udder milk flow curve. The peak flow rate (PFR), plateau phase, decline phase and stripping phase are indicated in one quarter and the udder milk flow profile. The end of the decline phase (end of main milking) is indicated by the vertical line in each milk flow profile.

yield and between PFR and decline phase was observed (Fig. 5 a, b). Contrary PFR and AFR were similarly correlated at a quarter and at an udder level. (Fig. 5 c).

The regression coefficient between PFR and AFR was b = 0.73 at a quarter level and b = 0.61 at an udder level. This means that at a quarter level an increase of the AFR by one unit was associated with an increase in PFR by 1.37 units. At an udder level a one unit increase in AFR was associated with an increase even by 1.61 units of PFR. Main milking time was positively correlated with plateau phase and negatively with decline phase. Plateau phase and decline phase were negatively correlated. Several correlations between teat anatomy and milkability were observed. Teat length was negatively correlated with milk yield, plateau phase and AFR. Contrary, teat length was positively correlated with stripping yield and decline phase. Teat diameter was positively correlated with PFR, whereas teat canal length was negatively correlated with PFR and AFR. The negative correlation between teat canal length and PFR was observed similarly at a quarter at an udder level (Fig. 5 d). Similarly teat canal length and AFR were negatively correlated at a quarter and an udder level.

Surprisingly, neither at a quarter level, nor at an udder level teat anatomy and VO were correlated, (Fig. 6 a). Contrary, VO was negatively correlated with PFR at an udder and at a quarter level (Fig. 6 b). Furthermore VO was negatively correlated with decline phase and AFR, but VO was positively correlated with main milking time and plateau phase (Table 4).

Table 4: Pearson's coefficients of correlation between teat anatomy, vacuum needed to open the teat canal and milk flow. Values above the diagonal show correlations at an udder level and figures below the diagonal show correlations at a quarter level.

	Teat length	Teat wall thickness	Teat diameter	Teat canal length	VO-S	VO-C	Milk yield	Stripping yield	Peak flow rate	Main milking time	Plateau phase	Decline phase	Average flow rate
Teat length		-0.09	0.26	0.04	0.00	0.02	-0.14	0.27	0.11	-0.29	-0.23	-0.15	0.10
Teat wall thickness	0.03		0.41*	0.57**	0.15	0.15	0.02	0.25	-0.33*	0.17	0.05	0.23	-0.26
Teat diameter	0.13	0.26**		0.17	-0.07	-0.07	0.03	0.11	0.05	0.03	-0.30	-0.17	0.00
Teat canal length	0.13	0.50***	0.05		0.31	0.30	0.27	0.08	-0.39*	0.23	0.08	-0.11	-0.33*
VO-S	-0.05	0.01	0.03	0.08		0.84***	-0.14	0.16	-0.52**	0.18	0.35*	0.14	-0.49**
VO-C	-0.08	-0.02	-0.01	0.10	0.82***		-0.14	0.16	-0.52**	0.18	0.28	0.27	-0.44**
Milk yield	-0.42***	0.00	0.15	-0.02	0.08	0.00		0.07	0.15	0.72**	0.59***	0.35*	0.29
Stripping yield	0.19*	0.14	0.13	0.13	0.11	-0.13	0.04		-0.19	-0.10	0.22	-0.05	0.00
Peak flow rate	-0.08	-0.12	0.18*	-0.24**	-0.35***	-0.34***	0.34***	0.02		-0.46**	-0.39*	0.05	0.84***
Main milking time	-0.37***	0.04	0.02	0.14	0.31***	0.23**	0.69***	0.06	-0.33***		0.73***	0.70***	-0.43**
Plateau phase	-0.30**	0.04	-0.15	0.03	0.37***	0.29**	0.44***	-0.23**	-0.32***	0.58***		0.27	-0.19
Decline phase	0.26**	0.12	0.15	0.02	-0.36***	-0.31***	-0.39***	0.27**	0.22**	-0.45***	-0.94***		-0.19
Average flow rate	-0.18*	-0.08	0.15	-0.19*	-0.24**	-0.24**	0.57***	-0.01	0.85***	-0.16	0.02	-0.12	

*, ** and *** : indicate significant Pearson's coefficients of correlation on the level of $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. VO-S: Start of milk flow during determination of the vacuum needed to open the teat canal. VO-C: Cessation of milk flow during determination of the vacuum needed to open the teat canal.

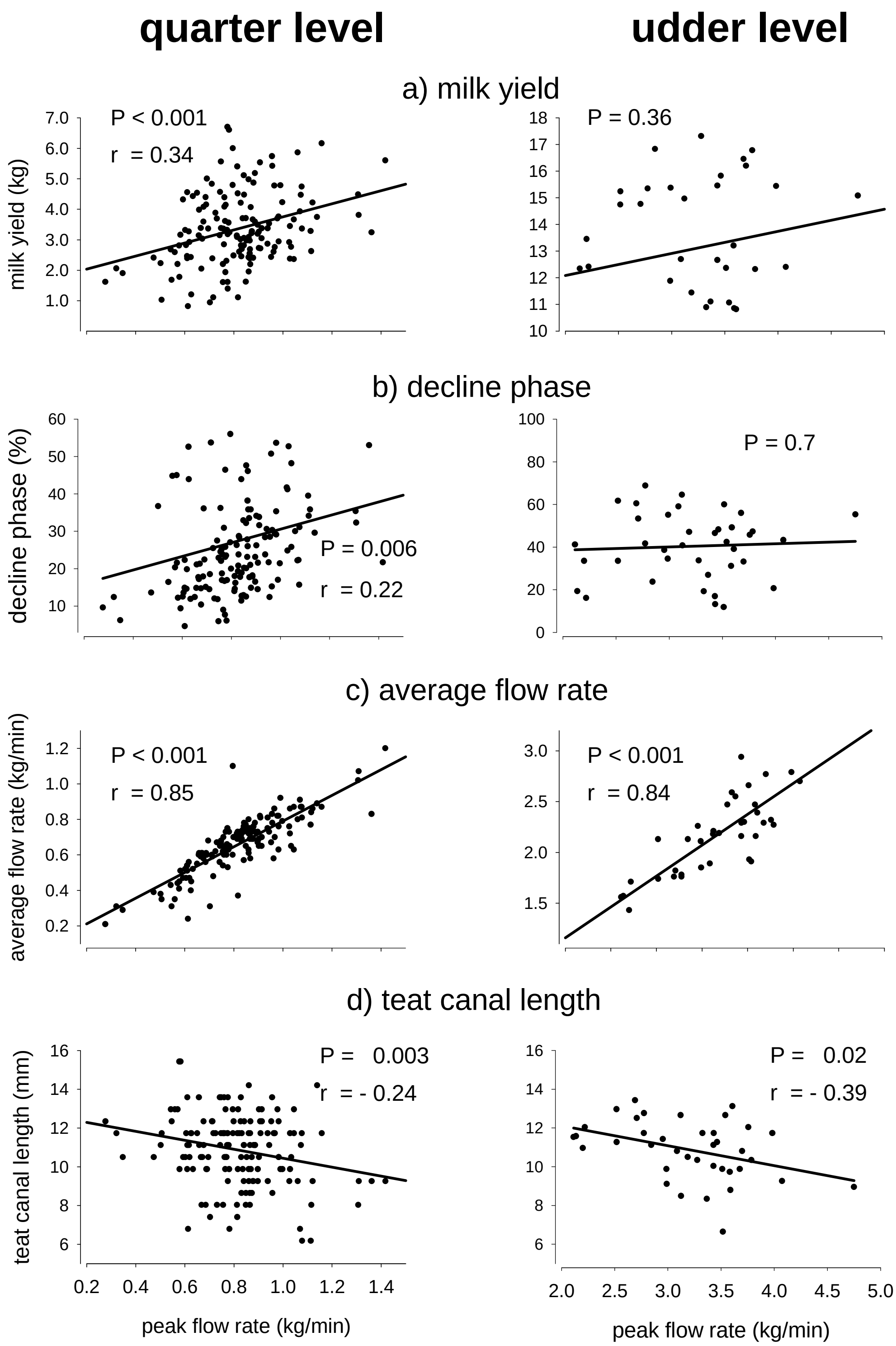

Figure 5: Relationship between peak flow rate, milk yield (a), relative decline phase (b), average flow rate (c) and teat canal length (d) at a quarter and an udder level, respectively. Level of significance and correlation coefficients are indicated in the figures.

Discussion

This study was to our knowledge the first one that used a combination of 3 approaches to characterize teats at a quarter level. The measurements of teat anatomy, teat functionality and milk flow characteristics were performed using an innovative combination of ultrasonography, vacuum measurement und continuously milk flow recording. The combination allowed a correlation analysis covering all obtained data.

Teat length, teat diameter, teat wall thickness and teat canal length were similar as in recent studies (Grindal et al., 1991; Rogers and Spencer 1991; Le Du et al., 1994; Neijenhuis et al., 2001). Earlier investigations reported longer and thicker teats (Andreae, 1958; Loppnow, 1959), indicating changes due to the breeding progress of the last decades. Despite these changes, the variation between cows and quarters were remarkably high in the present study.

VO was determined by the measurement of VO-S and VO-C. VO did not differ between front and rear teats. VO-S corresponds to previous results by Le Du et al. (1994) in a comparable approach. VO-C was substantially lower than VO-S. This corresponds to results of Williams and Mein (1987), who showed that the initial force required for the start of the milk flow was remarkably higher than the force needed to maintain an already established milk flow. Although the present data basically correspond to previous investigations it has to be considered that the available literature dealing with VO is based on different methods. Therefore the comparability of the published data is limited (Williams and Mein, 1987; Le Du et al., 1995; Mayntz et al., 1999). The observed milking characteristics confirm previous investigations at a quarter level. (Rothenanger et al., 1995; Wellnitz et al., 1999; Weiss et al., 2003).

Correlations

In the present investigation no correlations between teat canal length and externally measurable anatomical characteristics like teat length or teat diameter were observed. These results are in contrast to former investigations by Loppnow (1959) and Hebel (1978) who

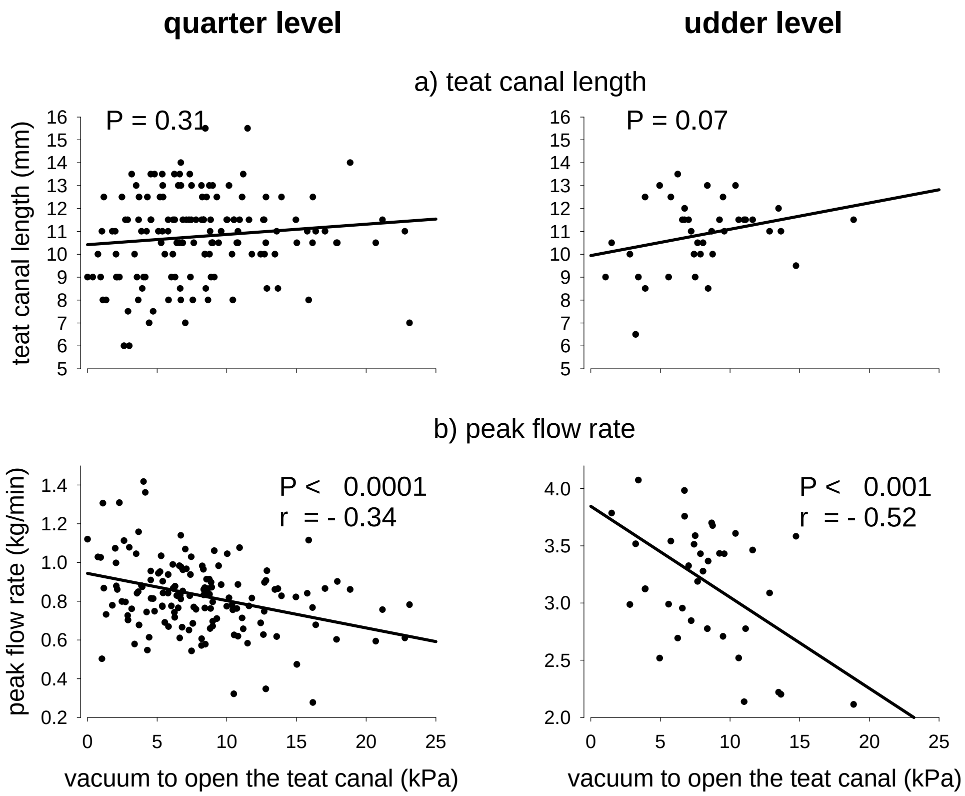

Figure 6: Relationship between the vacuum needed to open the teat canal at cessation of milk flow (VO-C), teat canal length (a) and peak flow rate (b) at a quarter and an udder level respectively. Level of significance and correlation coefficients are indicated in the figures.

found a positive correlation between teat length and teat canal length. However, Loppnow (1959) and Hebel (1978) performed their measurements on teats of slaughtered cows, whereas in the present study in vivo measurements by ultrasound were evaluated. Thus their studies disregarded effects of the tone of teat smooth muscles and intrammamary pressure (Lefcourt, 1982; Inderwies et al., 2003b). The observed positive correlation between teat canal length and teat wall thickness are not unexpected, since the teat canal crosses the teat wall at the teat tip.

Negative correlations between teat length and various milking characteristics, e.g. milk yield, main milking time, plateau phase and AFR are apparently due to differences between front and rear quarters, because milk yield and PFR were higher in rear than in front quarters and rear teats were shorter and thicker than front teats. Furthermore, the fact that rear teats were thicker than front teats can explain the positive correlations between teat diameter and PFR, since rear teats had a higher PFR. Indeed, when correlations within cow were analyzed separately no significant relationships were observed. The parallel increase of stripping yield and teat length with increasing number of lactations may explain the positive correlation between teat length and stripping yield (Michel and Rausch, 1988; Göft et al., 1994). However, disregarding these apparent correlations between teat anatomy and milking characteristics there was a negative correlation between teat canal length and PFR and a negative correlation between teat canal length and AFR. These findings correspond to previous reports (Grindal et al., 1991) where the length of the teat canal was shorter in quarters with a high PFR. In summary, these results indicate that externally measurable teat anatomy, i.e. teat length and teat diameter did not affect important milking characteristics like PFR and AFR.

Surprisingly, teat canal length was not correlated with VO, neither with VO-S nor with VO-C. But VO was indeed negatively correlated with PFR and AFR. These findings correspond to investigations by Le Du et al. (1994) and Mayntz et al. (1999). Similar results were observed at a quarter and an udder level. Since the milking vacuum was definitely higher than VO a correlation between VO and PFR was not to be expected. Furthermore, the resulting teat canal diameter during milk flow, which was not determined in this study, is reported as most important aspect concerning PFR (Baxter et al., 1950; Andreae, 1958; Mein et al., 1973; Williams et al., 1981). The present method was designed to measure VO, although no information about intensity and velocity of the observed milk flow and about the teat canal diameter is available by this method, the obtained results reflects information comparable to an approach proposed by Williams and Mein (1980). However, the opening process of the teat canal is caused by tangential and longitudinal forces along the teat canal (Scott and Reitsma 1978). The present measurement of VO reflects solely the start of the opening of the teat canal. No information about the force that is necessary open the teat canal to a maximum is available. In previous studies a relationship between PFR and the adrenergic system has been demonstrated (Roets et al., 1989; Wellnitz et al., 2001; Inderwies et al. 2003a). Inderwies et al. (2003b) demonstrated in a study that not only the teat canal determines the resulting PFR. Probably, VO reflects the sympathetic tone of the smooth muscles and the presented method provides therefore information about the adrenergic system of the mammary gland.

In cows with extremely high PFR, the supply of alveolar milk during milking can be crucial for the actual milk flow rate (Bruckmaier et al., 1994; Pfeilsticker et al., 1995), when the maximum milk flow rate determined by the milk duct system and the teat might be higher than the supply of alveolar milk by the milk ejection reflex. In this case a short peak with a prolonged decline phase is present in the milk flow curve. The present investigation supports this hypothesis, since a positive correlation between PFR and the decline phase was observed at a quarter level.

A close correlation between PFR and AFR was observed at a quarter and at an udder level. Interestingly, an increase in AFR was associated with a larger increase of PFR at an udder level than at a quarter level. This difference between quarter and udder level is due to the aspect that at a quarter level the plateau phase (phase of PFR) was more pronounced and resulted therefore in a higher regression coefficient for the relationship between PFR and AFR as compared to an udder level. At an udder level the plateau phase lasted from the start of the plateau phase until the end of the plateau phase in the fastest milking quarter. Contrary, at a quarter level the plateau phase represent the period where cisternal milk is available (Pfeilsticker et al., 1995; Wellnitz et al. 1999). The increase in PFR in association with an increased AFR is therefore more pronounced at an udder level than at a quarter level. This aspect is of importance with respect to breeding for milkability. With increasing AFR, the PFR will concomitantly increase to a higher extend. To prevent an excessive increase in PFR and therefore an increase in mastitis susceptibility a control of the PFR besides breeding for increase AFR could be a promising option in breeding programs.

Conclusions

The teat canal length and the vacuum to open the teat canal were negatively related to peak flow rate and average flow rate at a quarter level. Individual milkability at an udder level is a complex characteristic that is determined by the milkability at a quarter level and the distribution of quarter milk yields. No correlations were present between milkability traits and externally measurable teat characteristics like teat length and teat diameter.

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Effect of stimulation intensity on oxytocin release before, during and after machine milking

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Effect of stimulation intensity on oxytocin release before, during and after machine milking

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Release of oxytocin (OT) is essential for milk ejection in dairy cows (Lefcourt & Akers, 1983; Bruckmaier & Blum, 1998). During milk ejection, alveolar milk is shifted into the cistern, which causes an increase of intracisternal pressure (Bruckmaier et al. 1994). To initiate maximum milk ejection at the start of milking, increasing OT concentration beyond a threshold level is sufficient (Schams et al. 1983). Increasing OT concentration beyond this threshold has no additional effect on intracisternal pressure, i.e., milk ejection (Bruckmaier et al. 1994). Stimulatory effects of milking by hand or by machine or by suckling are well documented (Gorewit et al. 1992; Bar-Peled et al. 1995; Tancin et al. 1995; Bruckmaier & Blum, 1996). At the start of milking, stimulatory effects of machine milking without pre-stimulation or with a manual pre-stimulation and subsequent machine milking cause the release of comparable amounts of OT (Gorewit & Gassman, 1985; Mayer et al. 1985; Bruckmaier & Blum, 1996), whereas the timing of the applied pre-stimulation is important for the shape of the milk flow curve. Should the pre-stimulation period be too short, or absent altogether, the start of the main milk flow is delayed resulting in a bimodal milk flow profile (Bruckmaier & Blum, 1996). Furthermore, the stimulation of only one teat causes an OT release similar to that caused by stimulation of all four teats (Bruckmaier et al. 2001). However, milk production is greater for hand milking or suckling than for machine milking, possibly owing to higher OT concentrations (Gorewit et al. 1992; Bar-Peled et al. 1995). In addition, exogenous OT in supraphysiological doses improves udder evacuation and enhances milk production (Nostrand et al. 1991; Ballou et al. 1993; Bruckmaier et al. 1994; Knight, 1994). It has been shown that OT concentrations must be elevated throughout the entire milking process to allow complete milk removal (Bruckmaier et al. 1994). It is possible that the importance of OT concentrations beyond the pre-milking threshold might change towards the end of milk removal. The quarter distribution and the quarter milking time are often very unequal (Wellnitz et al. 1999). Therefore, in most cows individual quarters are over-milked while milk is still removed from others. The goal of this study was to test the hypothesis that OT release depends on the intensity of the stimulus and that a stimulus of low intensity is more effective at the start of milking than at the end of milking. Therefore the stimulatory effect of attached teat cups without pulsation before and after milking was tested. In addition, we tested whether a vibration stimulation of one single teat, without prolongation of the total milking time, towards the end of milking was able to induce additional OT release to improve udder evacuation.

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Materials and Methods

Animals and milking

The six experimental cows (Brown Swiss × German Braunvieh) were in months 2–12 of their first-to-fourth lactation. The cows were kept in loose housing and were milked in a 2 × 2 tandem milking parlour. The diet was maize and grass silage, hay and concentrate given according to individual milk production. Milking was performed with a vacuum of 42 kPa, a pulsation rate of 60 cycles/min and a 60 : 40 pulsation ratio using separate pulsators (Stimopuls CP, Westfalia Landtechnik GmbH, D-59299 Oelde) for each quarter. Machine stripping was performed after total milk flow dropped below 0·3 kg/min. "Bio-Milker" teat cups (Westfalia Landtechnik GmbH, D-59299 Oelde) with separate long milk tubes for each quarter were used.

Experimental design

Experiments were performed at the usual milking times starting at 05.00 and 16.00 for 4 consecutive days in a completely balanced crossover design. Three different treatments were tested during two milkings (a.m. and p.m.) in each cow. The treatments were randomly assigned to the individual cow at each experimental milking. Control milking (C) corresponded to the daily milking routine and included udder preparation by forestripping, short

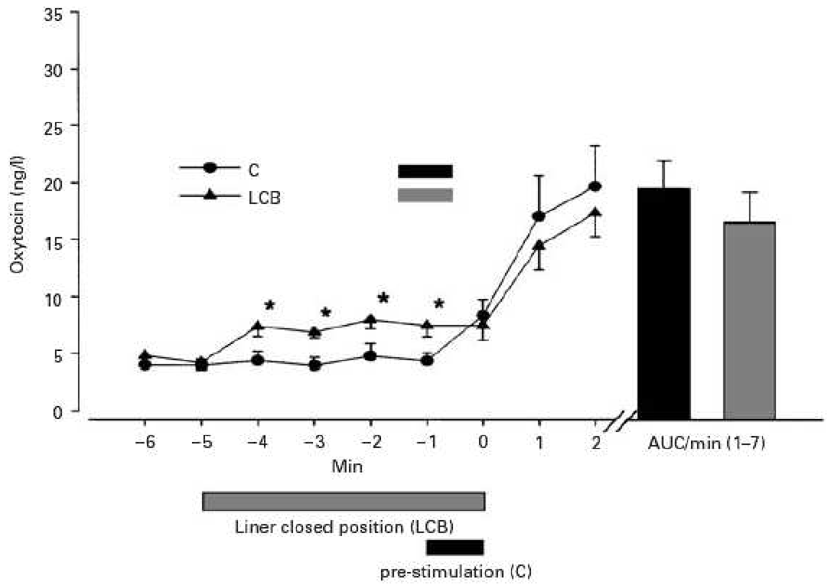

Fig. 1. Oxytocin concentrations (means±SEM for $n=12$ milkings) for liner closed before milking (LCB) and control milking (C), 0 min = start of normal pulsation and milking. * signifies respective means are significantly different between treatments C and LCB ($P<0·05$). Oxytocin values are also shown as areas under the curve (AUC) for the first 7 min of the milkings.

dry-paper cleaning for 15 s and additional vibration pulsation (Stimopuls, 300 min^{-1}, 25 kPa pulsation vacuum) for 60 s. In the treatment in which the liner was closed before milking (LCB) the teat cups were attached without pulsation and without touching the udder before attachment. After 5 min in the liner-closed position, milking pulsation was started without any additional pre-stimulation. In the treatment in which the liner was closed after milking (LCA), the milking process started with the usual routine (C), while pulsation was stopped after stripping, and teat cups remained attached in the liner-closed position for 5 min. In the treatment for which stimulation was applied during milking (STI), milking was started as for control milking. The quarter with the shortest milking time had been determined previously for each cow. One minute before the expected end of milk flow in this quarter, a 1-min vibration stimulation (Stimopuls) was applied to this quarter to provide additional stimulation during milking without prolongation of the total machine on-time.

Milk flow was recorded for individual quarters using four mobile recording units (Lactocorder, Werkzeug- und Maschinenbau Balgach, 9436 Balgach, Switzerland) as previously described (Wellnitz et al. 1999). Various milking characteristics were evaluated according to Bruckmaier et al. (1995). Total milk yield, stripping yield, peak flow rate, average flow rate and total milking time were analysed.

Cows were catheterized for blood sampling from the jugular vein the day before the start of the first experiment. Blood samples for determination of OT concentrations were taken at 1-min intervals. For C and LCB, blood sampling started 6 min before the start of milking. In C and LCA, blood sampling ended 5 min after the end of milking. For STI and LCA, blood sampling started 1 min before the start of teat stimulation. For STI and LCB, blood sampling was stopped after the end of milking. Blood samples were treated with EDTA to prevent coagulation and centrifuged at 1500 ***g*** for 15 min immediately after each milking. Plasma was stored at −20 °C until used for radioimmunological determination of oxytocin concentration according to Schams (1983).

Statistical analysis

Results are presented as means±SEM. For statistical analysis, the SAS program package release 8.01 (SAS, 1999) was used. Treatment effects were tested for significance ($P<0·05$) using the General Linear Model (GLM) procedure. The model included the individual animal and the treatment; the animal was defined as repeated subject. The date and the milking time entered the model as random factors. Differences between treatments were identified by using the Least Significant Difference (LSD) test.

Results

Oxytocin concentrations

Pre-milking OT concentrations (Figs 1, 2 and 4) were low and similar in all treatments. In LCB (Fig. 1) OT values

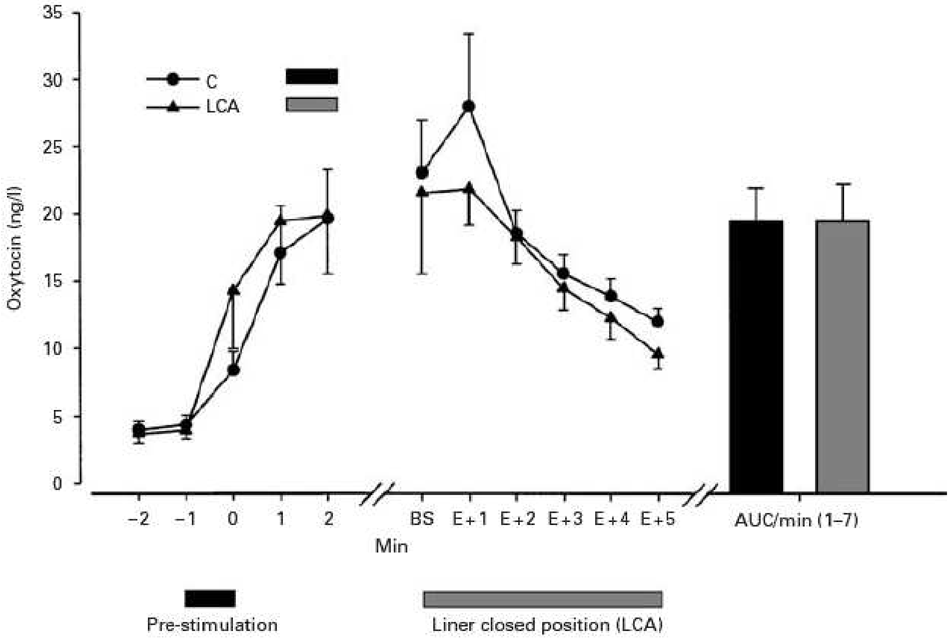

Fig. 2. Oxytocin concentrations (means±SEM for $n=12$ milkings) for liner closed after milking (LCA) and control milking (C), 0 min=start of milking after 1 min vibration stimulation, BS=before stripping, E=end of milking+1–5 min. Oxytocin values are also shown as areas under the curve (AUC) for the first 7 min of the milkings.

after cluster attachment were elevated and significantly higher than the basal levels in C. During subsequent milking, however, OT values increased further and were significantly higher than those during the liner-closed phase. Within 1 min after attachment of the teat cups (i.e., the start of pre-stimulation treatments in C, LCA and STI and start of milking in treatment LCB), OT values increased similarly for all treatments. OT values remained elevated over baseline levels for the entire milking and did not differ significantly from the control. Likewise, the area under the curve (AUC) of OT from 1–7 min of milking did not differ between LCB and C.

For LCA (Fig. 2) the OT pattern during milking was similar to that of the control. After cluster removal in C and the liner-closed phase in LCA, no differences in the steady decline of OT concentrations were observed between the two treatments.

For STI (Figs 3 and 4) OT release during milking was comparable to that of C, with no significant increase in response to the additional stimulation. Before and after the application of the additional 1-min vibration stimulation, OT values did not differ between STI and C.

Milking characteristics

As shown in Table 1, total milk yield, milking time, peak flow rate, average flow rate and stripping yield did not differ significantly between treatments. During the liner-closed phase in LCB and LCA, no milk flow was seen. In treatment STI a decrease in milk flow during the additional stimulation phase in the specific quarter could be observed (Fig. 3), without any significant change for the mentioned parameters. Bimodal milk flow curves, which indicate separate removal of cisternal and alveolar milk fractions due to delayed milk ejection, were not seen for any treatment.

Discussion

Release of OT in response to different tactile teat stimuli in cows has been described before (Lefcourt & Akers, 1983; Bruckmaier & Blum, 1998). Stimulatory effects are due to the milker's hands, movement of the liner or the sucking of the calf. Our results show that the amount of OT released varies with the intensity of teat stimulation. Application of the milking vacuum without pulsation (liner-closed position) caused OT release, but on a lower level than that caused by pulsation during normal milking. This OT release was sufficient to induce milk ejection, as indicated by the absence of bimodal milk flow curves in LCB, i.e., the presence of alveolar milk in the cistern already at the start of milking (Bruckmaier & Blum, 1996). For LCB, however, OT concentrations similar to these of C were only reached after the start of liner pulsation. The continuously elevated OT in LCB during the 5 min before the start of milking indicates that not only the attachment

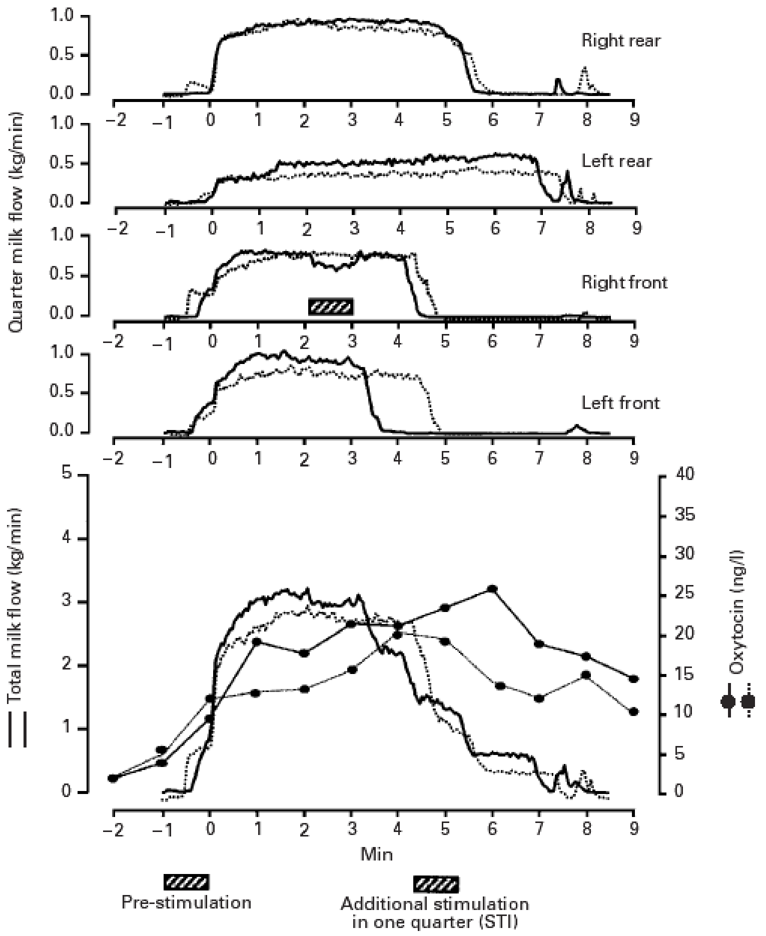

Fig. 3. Oxytocin concentrations (●) and quarter and udder milk flow with (—— STI) and without (········ C) an additional 1-min stimulation of the right front teat of one individual cow during milking.

procedure of the teat cups but also the applied milking vacuum, or the attached teat cup itself, was a stimulus for OT release. If attachment alone had been responsible for the OT release, an immediate decrease of OT concentrations would have occurred, owing to the rapid clearance of circulating OT (Wachs et al. 1984; Bruckmaier et al. 1994). After the start of the normal milking pulsation in LCB, the OT concentrations increased further, indicating that the amount of OT released is modulated by the intensity of the tactile stimulation of the teats.

Application of milking vacuum in the liner-closed position (LCA) after the end of normal milking, i.e., the same stimulus as applied in LCB, did not cause higher OT concentrations than C. Obviously, for both treatments, OT release ceased after the end of milking. The observed clearance of OT was similar to that seen previously (Wachs et al. 1984; Bruckmaier et al. 1994). Notably, the stimulus sufficient to induce a release of OT and to initiate the milk ejection before the start of milking had no effect at all on OT release after the end of milking. It should be remembered, however, that OT concentrations in this phase might have been too high to allow an effect of the stimulus during the liner-closed position in LCA. Nevertheless, OT values in LCA were numerically even lower than those in C during this period.

As for LCA, an additional vibration stimulation of one teat during milking (STI) had no effect on the OT pattern, i.e., it could not induce additional OT release. Obviously, the effects of specific stimuli in the late phases of the milking process were not similar to their effects during the early phase. The continuous pulsation during milking seemed to reduce the responsiveness to teat stimulation. Therefore the applied stimulus (vibration stimulation) in treatment STI, which was very effective before the milking,

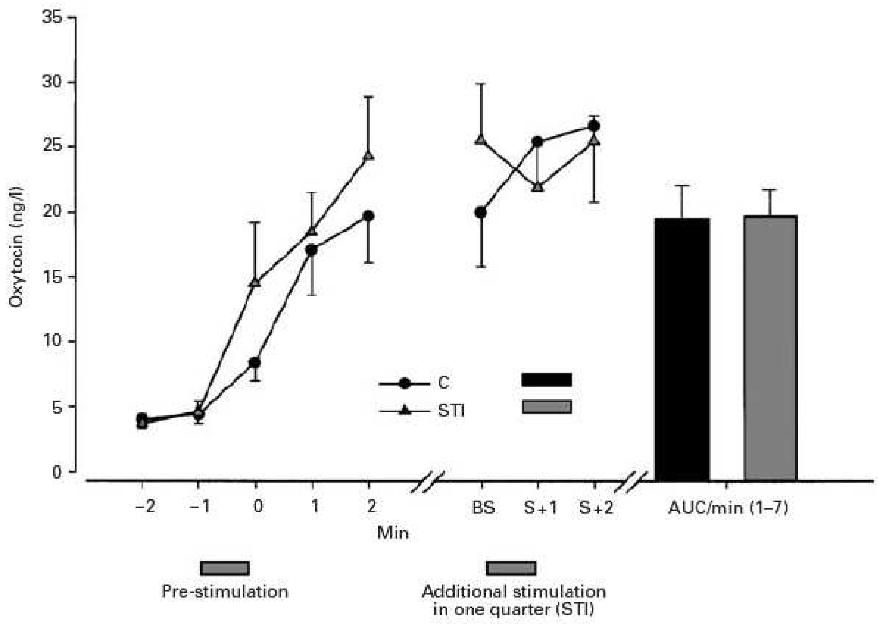

Fig. 4. Oxytocin concentrations (means ± SEM for $n = 12$ milkings) with additional stimulation during milking (STI) and control milking (C), 0 min = start of milking after 1 min vibration stimulation, BS = before additional stimulation, S = stimulation + 1–2 min. Oxytocin values are also shown as areas under the curve (AUC) for the first 7 min of the milkings.

Table 1. Milking characteristics during control milking (C), liner closed before milking (LCB), liner closed after milking (LCA) and additional stimulation during milking (STI)

Values are means ± SEM for $n = 6$

Parameters	C	LCB	LCA	STI
Total milk yield, kg	13·1 ± 1·3	13·0 ± 1·0	13·7 ± 1·3	13·7 ± 1·1
Stripping yield, kg	0·2 ± 0·1	0·6 ± 0·3	0·2 ± 0·1	0·2 ± 0·1
Peak flow rate, kg/min	3·0 ± 2·3	3·0 ± 2·2	3·0 ± 2·1	3·0 ± 2·2
Average flow rate, kg/min	1·6 ± 0·2	1·6 ± 0·1	1·7 ± 0·1	1·6 ± 0·1
Milking time, min	8·4 ± 0·3	8·0 ± 0·3	8·1 ± 0·5	8·2 ± 0·5

had no effect on the OT pattern during the late phases of milking.

The importance of enhanced OT concentrations throughout the whole milking has been shown previously (Bruckmaier et al. 1994). Therefore it is not surprising that milking with non-pulsating teat cups leads to lower lactation yields than milking with pulsating liners (Whittlestone, 1980). At the start of milking, when the udder is full, a relatively small increase in OT concentration is enough to induce milk ejection, as shown for treatment LCB. The degree of udder filling, however, is crucial for the start of milk ejection (Bruckmaier & Hilger, 2001). Therefore it is possible that the threshold at the start and at the end of milking is different. In this study the applied stimuli during and after milking were of too low an intensity to influence the OT pattern. However, the forces that are applied to the teat by a sucking calf are much greater than those applied by the milking machine (Rasmussen & Mayntz, 1998). It is possible that the stronger stimulus during sucking results in enhanced OT concentrations, which may improve udder evacuation.

In conclusion, OT release depends on the intensity of the tactile stimulation of the teat. The effect of tactile stimuli on the release of OT is different between early and late phases of milking, i.e., an OT response to low-intensity stimulation is observed only before the start of milking.

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Optimization of individual pre-milking stimulation in dairy cows

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Interpretative Summary

Oxytocin (OT) release and milking characteristics were investigated in 43 dairy cows after the application of various pre-stimulation routines by vibration stimulation between 0 and 90 s. The optimal pre-stimulation was prolonged in less filled udders and shortened in well filled udders. An optimized pre-stimulation duration can reduce vacuum load in less filled udders and reduce time requirements for milking in well filled udders.

Abstract

The application of a pre-stimulation results in an enhanced milking performance as compared to milking without pre-stimulation. In the present study Oxytocin (OT) release and milking characteristics were investigated in 43 dairy cows after the application of various pre-stimulation routines by vibration stimulation between 0 and 90 s. Additionally, different maximum pulsation vacuum settings during vibration stimulation were investigated. The actual degree of udder filling was calculated as a percentage of the estimated storage capacity. The amplitude of OT release, total milk yield and stripping milk yield did not differ between pre-stimulation routines. An increased maximum pulsation vacuum during vibration stimulation resulted in a milk flow during pre-stimulation, but did not negatively influence milking characteristics. The lag time from the start of teat stimulation until the start of milk ejection was negatively correlated with the degree of udder filling. Obviously this relationship was the reason for the variations in optimal duration of pre-stimulation. The optimal duration of pre-stimulation to receive immediate and continuous milk flow at the start of milking was 90 s in little filled udders whereas in well filled udders the optimal duration of pre-stimulation was only 20 s. By an individual adjustment of pre-stimulation prior to the start of milking the duration of milking vacuum application and hence the vacuum load on the teats could be reduced.

Keywords: pre-stimulation, oxytocin, milk flow, cattle

Abbreviations: **AFR** = Average flow rate; **kPa** = kiloPascal; **MPV** = maximum pulsation vacuum during vibration stimulation; **PFR** = peak flow rate; **OT** = oxytocin

Introduction

The release of oxytocin (OT) from the pituitary is essential for the ejection of alveolar milk (Lefcourt and Akers, 1983; Bruckmaier and Blum, 1998). OT is released in response to tactile teat stimulation. The stimulation by the liner during milking pulsation has been shown to be as effective to induce OT release as manual pre-stimulation (Bruckmaier and Blum, 1996). Even cluster attachment without liner pulsation caused a sufficient OT release to induce the alveolar milk ejection (Weiss et al., 2003). However, OT concentrations need to be elevated throughout milking to ensure a complete udder evacuation (Bruckmaier et al., 1994).

Before the onset of the alveolar milk ejection only the milk stored in the cisternal cavities is available for machine milking (Bruckmaier and Blum, 1996; 1998). The application of a fix pre-stimulation prior to milking between 30 and 60 s has been recommended to receive immediate and continuous milk flow after start of milking (Mayer et al., 1985; Rasmussen et al., 1990; Rasmussen et al., 1992). However, recent investigations demonstrated the importance of the udder filling on the course of milk ejection (Bruckmaier and Hilger, 2001; Dzidic et al., 2004).

In the present study the hypothesis was tested that the optimal duration of pre-stimulation, in order to minimize the machine milking time, is determined by the degree of udder filling. OT concentrations during the course of pre-stimulation and milking were determined to evaluate effects of pre-stimulation on the release of OT. Additionally, the effect of vibration stimulation settings on OT release and milk flow characteristics was evaluated.

Material and Methods

Animals and husbandry

43 experimental cows (Brown Swiss x German Braunvieh) were twice daily milked in a 2x2 tandem milking parlor. Milking started at 0500 and 1600. Therefore, the resulting milking interval was 13 h during the night and 11h during daytime. The cows were in the 1st to 10th month of lactation, whereas one cows was in their 23rd month of lactation due to unsuccessful breeding. Parity ranged from 1 to 6 lactations. The diet consisted of corn and grass silage, hay and concentrate according to the animals’ individual production levels. Milking was

performed at a vacuum of 40 kPa, a pulsation rate of 60 cycles/min and a 60:40 pulsation ratio. Machine stripping was applied after milk flow dropped below a threshold of 0.3 kg/min.

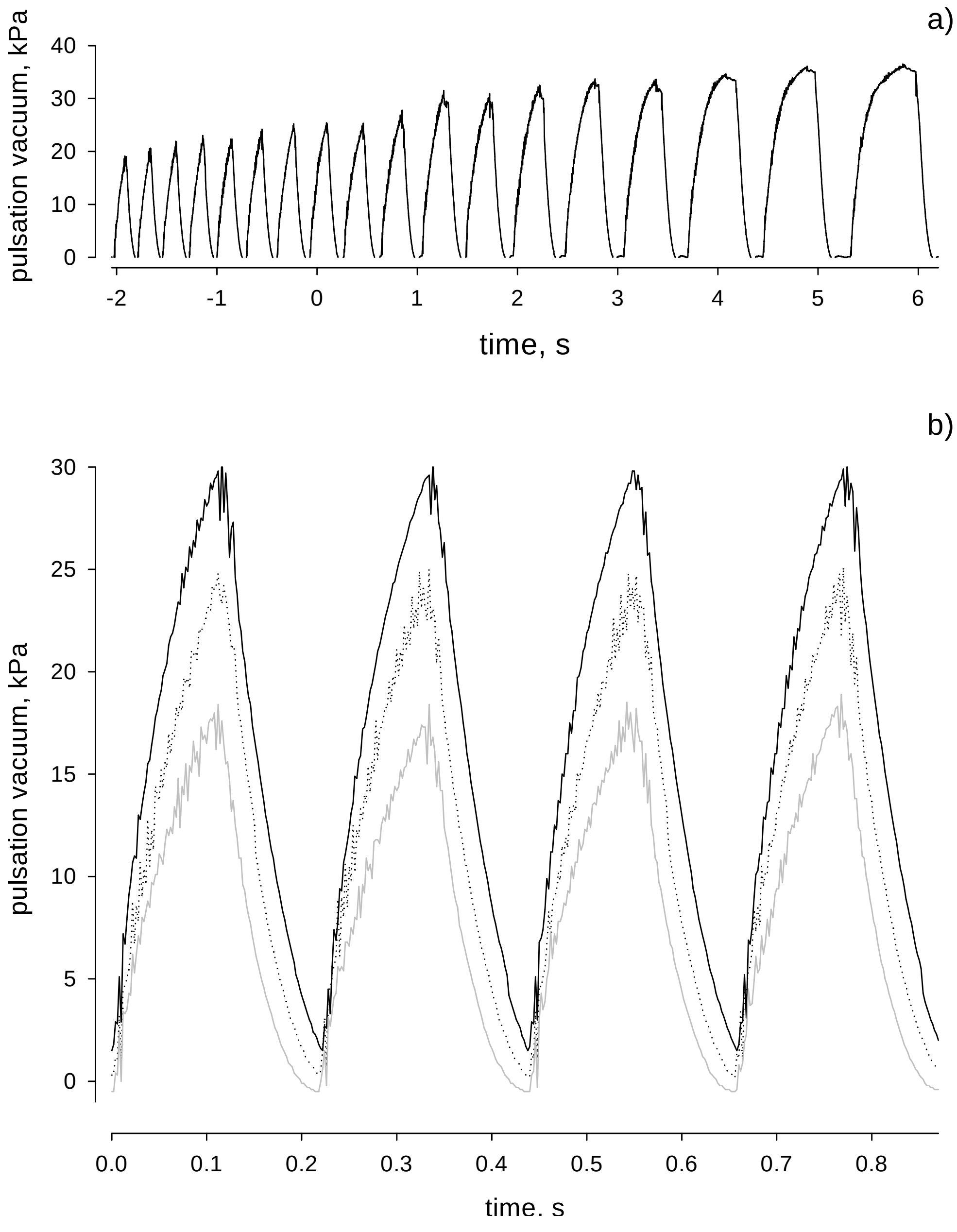

Figure 1: Pulsation chamber vacuum, a) changeover from vibration stimulation to milking pulsation, t = 0 s : changeover to milking pulsation, b). Pulsation vacuum curve during the course of vibration stimulation differing for three different vacuum settings —— 30 kPa of maximum pulsation vacuum (MPV) , - - - - 24 kPa of MPV and —— 17 kPa of MPV.

A conventional milking cluster (no 7021-2620-310, WestfaliaSurge GmbH, 59299 Oelde, Germany) equipped with the liner no 7021-2725-230 was used during the experiments.

Experimental design

Immediately before the start of milking a vibration stimulation at 300 cycles/min was applied in different treatments in a completely balanced crossover design (Karch et al., 1989; Stimopuls P, WestfaliaSurge GmbH). To avoid additional stimulatory effects the teats were not cleaned, not fore-stripped and not touched before teat cups were attached. Thus the duration of the pre-stimulation by vibration stimulation was exactly the time span between the first contact to the teats and the start of milking pulsation. Each treatment block was applied in a sequence of four milkings and was repeated once in a randomized order. Additionally, two milkings with the respective settings were performed before experimental recordings. The duration of vibration stimulation was varied in five steps; 0, 20, 40, 60 and 90 s at a maximum pulsation vacuum (MPV) of 24 kPa (Figure 1). Additionally, in a separate experiment, two treatments were applied with a MPV adjusted to 17 and 30 kPa during a 60 s vibration stimulation. Thus, a total of seven modes of vibration stimulation before the start of milking pulsation were applied. Milk flow profiles were registered by strain gauge recorder jars and processed using a program package as previously described (Worstorff and Fischer, 1996). The obtained milk flow profiles were visually checked for plausibility and were rejected in case of failings, e.g. cluster fall off or in case of an additionally applied cleaning due to extremely dirty teats. Milking characteristics were evaluated according to Bruckmaier et al. (1995).

OT concentrations during milkings were recorded in a separate experiment with two milkings each treatment (a.m. and p.m.) in six cows. Cows were catheterized in one jugular vein for blood sampling on the day before the start of the first experimental milking. The milking routine was identical to that described above. However, due to technical reasons only four treatments could be applied including blood sampling. Treatments performed were milking without pre-stimulation, a pre-stimulation for 60 s at a MPV of either 17 kPa or 30 kPa and a pre-stimulation for 90 s at a MPV of 24 kPa. Blood samples were taken from 1 min prior to the start of stimulation. From the start of stimulation until 1.5 min after the start of stimulation the sampling interval was 15 s. Thereafter blood samples were performed in 1 min intervals until cluster removal at the end of milking. Blood samples were treated with EDTA to prevent coagulation and centrifuged at 1500 x g for 15 min within 20 min after sampling. Plasma was stored at -20°C until used for radioimmunological determination of OT concentration according to Schams (1983).

Statistical analyses

Results are presented as means ± SEM. For statistical analysis, the SAS program package release 8.01 (SAS 1999) was used. Actual milk yield as a percentage of maximum storage capacity was estimated to describe the degree of udder filling in classes of 0 - 20, 20 - 40, 40 - 60, 60 - 80 and 80 – 100 %, respectively, as previously described (Bruckmaier and Hilger, 2001; Weiss et al., 2002). Maximum storage capacity of the mammary gland was estimated as half of the daily milk yield in month two of the respective lactation. The mean udder filling of individual cows throughout the experimental period was calculated separately for morning and evening milkings. Therefore, an average udder filling rate was obtained for morning and evening milkings of the individual animal. The lag time between the start of stimulation and the start of the alveolar milk ejection was visible either due to a prolonged period of milking pulsation in the absence of milk flow or due to a transient reduction of milk flow due to the limited amount of cisternal milk within the first min of milking. The following increase of the milk flow indicated the start of milk ejection (Bruckmaier and Blum, 1996; Bruckmaier and Hilger, 2001). Treatment effects were tested for significance ($P < 0.05$) by using the MIXED procedure. The model included treatment, the udder filling class of the individual animal and the interaction of udder filling class and treatment as fixed effects. Day of milking, time of milking (morning - evening), stage of lactation and lactation number were defined as random variables. The individual animal nested in the treatment block entered the model as repeated factor. The Least Significant Difference test identified differences between treatments. The REG procedure was used for regression analyses.

Results

Oxytocin concentrations

Pre-milking OT concentrations were low and similar in all treatments (Figures 2, 3). A significant increase in OT concentrations was observed within 30 s after the start of stimulation, irrespective of the applied treatment. In all treatments the OT concentrations were enhanced during the course of milking. Neither OT profiles during the course of stimulation and milking nor the area under the curve for OT concentration during milking was influenced by any applied pre-stimulation treatment, including the omission of pre-stimulation (P = 0.31). During milking without pre-stimulation (Figures 2, 3 d) OT concentrations increased only after the start of milking. If pre-stimulation was applied (Figures 2, 3 a, b, c) OT concentrations increased after the start of stimulation and were already high at the start of milking.

Milk flow profiles

Examples of representative milk flow and OT profiles during four different treatments are presented in Figure 3. Already during pre-stimulation milk flow was observed at a MPV of 30 kPa (Figure 3 a), whereas during pre-stimulation with a reduced MPV (Figure 3 b, c) only a

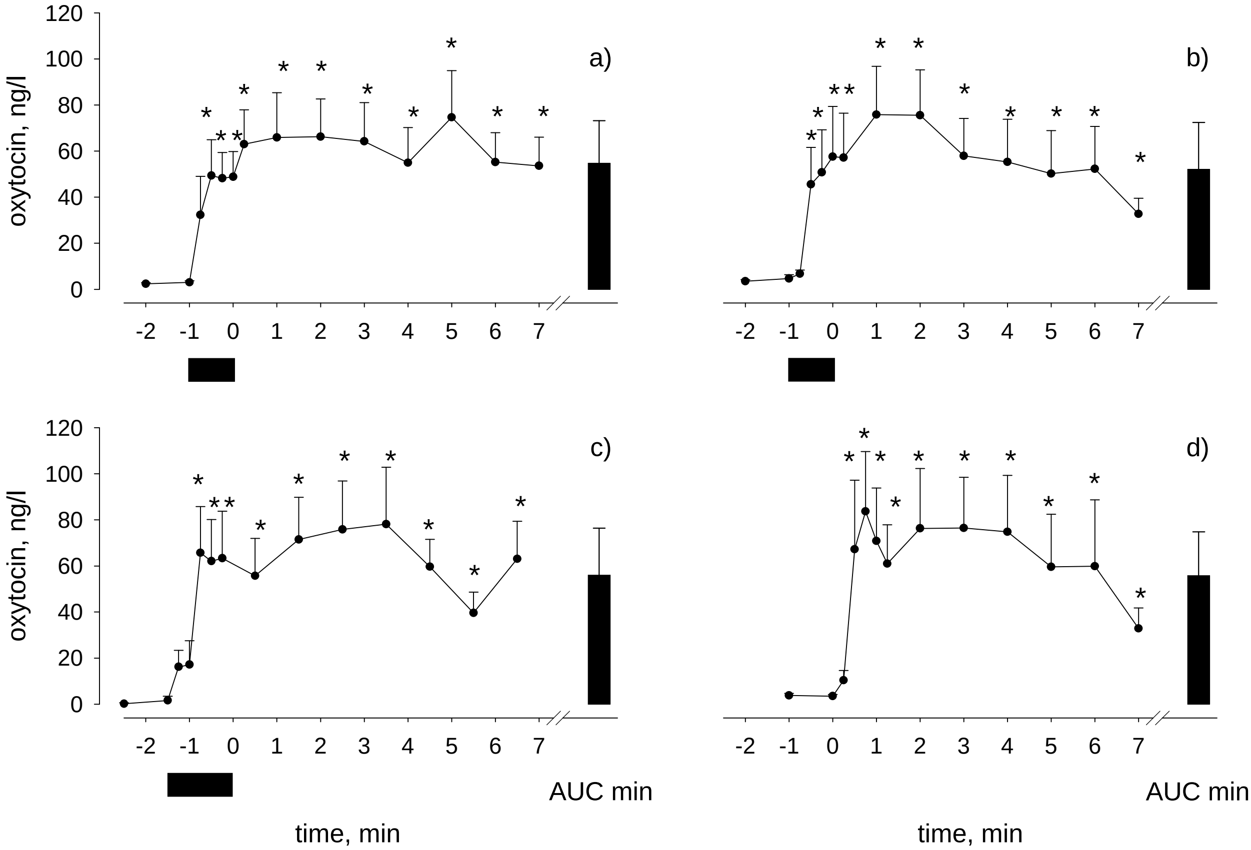

Figure 2: Means ± SEM of oxytocin concentrations during the course of pre-stimulation and milking in four treatments. t = 0 s : start of milking pulsation. indicate pre-stimulation. a) 60 s vibration stimulation at a maximum pulsation vacuum (MPV) of 30 kPa, b) 60 s vibration stimulation with MPV of 17 kPa, c) 90 s of vibration stimulation with MPV of 17 kPa, d) start of milking without previous pre-stimulation. The applied pre-stimulation duration is indicated by the black bar. AUC, area under the curve during milking (0-7 min). * indicate significant differences to basal concentration one min before the start of stimulation.

marginal milk flow was observed. During milking without pre-stimulation (Figure 3 d) the milk flow raised immediately after the start of milking, however a transient reduction of milk flow was observed similar as in Figure 3 a.

In Table 1 various milking characteristics are presented differing for the duration of pre-stimulation. Milk yield and stripping yield did not differ between treatments. Total milking time including pre-stimulation and stripping was shortest without pre-stimulation and longest when a 90 s pre-stimulation was applied. Contrary, main milking time without the duration of pre-stimulation and without machine stripping (MMT -) was shortest with longest duration of pre-stimulation and vice versa. Peak flow rate (PFR) was highest at longest duration of pre-stimulation. Similarly, average flow rate (AFR) was increased with prolonged duration of pre-stimulation.

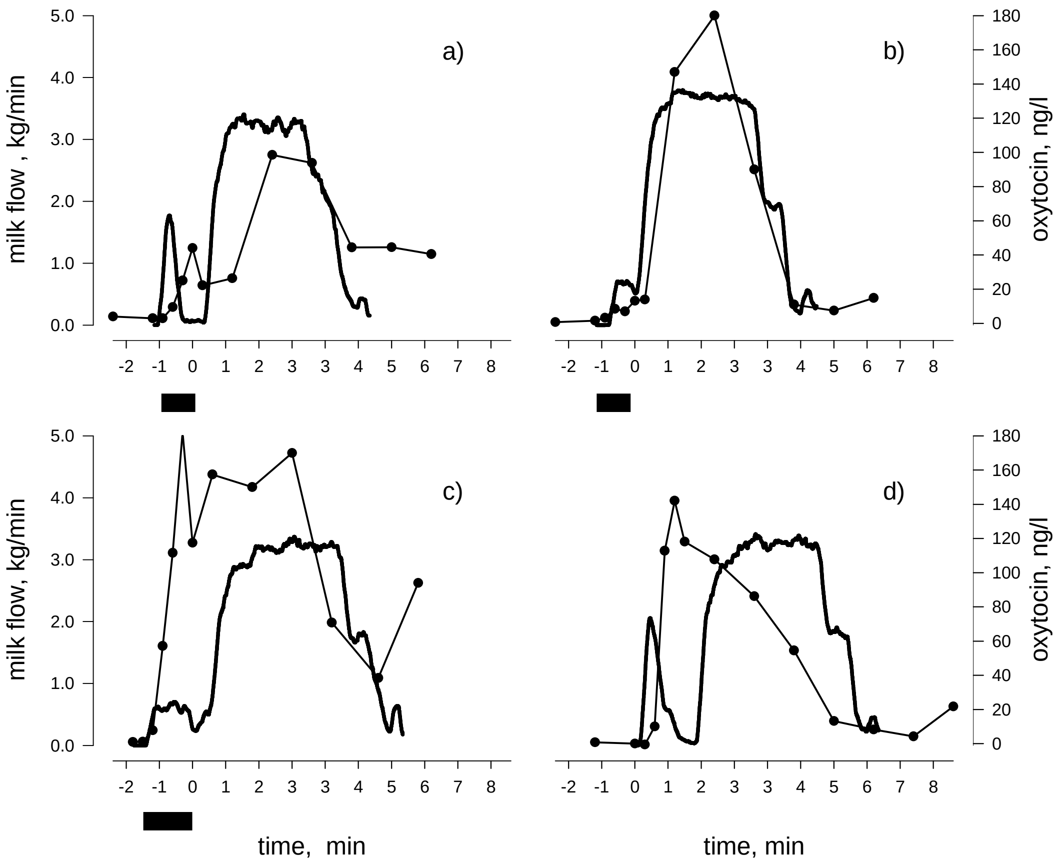

Figure 3: Oxytocin concentration and milk flow of one exemplary animal during the course of pre-stimulation and milking in four different treatments t = 0 s : start of milking pulsation. maximum pulsation vacuum (MPV) of 30 kPa, b) 60 s vibration stimulation with MPV of 17 kPa, c) 90 s of vibration stimulation with MPV of 17 kPa, d) start of milking without previous pre-stimulation.

In Table 2 milking characteristics are presented differing for MPV. However, no significant difference in milking characteristics between a MPV of 17 and 30 kPa was observed.

Figure 4 illustrates effects of the degree of udder filling and the duration of pre stimulation on milk yield, main milking time including the duration of pre-stimulation, PFR and AFR. Mean udder filling of the individual cow was calculated separately for morning and evening milkings. Number of observations were 14, 23, 31, and 18 for udder filling classes of 20-40, 40-60, 60-80 and 80-100%, respectively, e.g. a total number of 86 milkings in 43 cows. However, it has to be considered, that the mean udder filling throughout the experimental period was used to cluster the individual cow. Milk yield was not significantly influenced by pre-stimulation. Main milking time including the duration of pre-stimulation was prolonged with increasing duration of pre-stimulation in full udders. Contrary, a prolonged pre-stimulation did not prolong main milk flow rate when little filled udders were milked. Similar effects were observed for PFR and AFR, while in well filled udders an additional stimulation did not enhance the milk flow rate. In case of little filled udders flow rate was enhanced with a prolonged duration of pre-stimulation.

Figure 5 illustrates the relationship between udder filling and lag time until the start of alveolar milk ejection. This result is based on milkings without pre-stimulation. Additionally, lag time in milk flow profiles in further treatments were considered, when the start of the alveolar milk flow could be clearly identified by a bimodal milk flow pattern or by a prolonged period of milking pulsation in the absence of milk flow. The lag time was about 50 to 60 s in full udders and increased up to 120 s with decreasing udder filling. Udder filling and lag time until start of milk ejection was negatively correlated with a Pearson's coefficient of $r = -0.57$ ($P < 0.001$). [$y = 117.27 – 0.69 x$; x = udder filling (%), y = lag time from start of stimulation until milk ejection (s)].

Representative milk flow profiles of three cows with different udder fillings are shown in Figure 6. Time t = 0 s indicates the start of milking pulsation. Each chart represents the milk flow profiles of five milkings differing for the applied duration of pre-stimulation (0, 20, 40, 60, 90 s, respectively). Milking of a little filled udder (approximately 20 % udder filling) without pre-stimulation resulted in a lack of milk flow until 90 s after the start of milking (Figure 6 a). Milk flow from the start of milking and a transient decrease of milk flow at 0.7

Table 1: Milk yield, stripping yield, total machine on time, main milking time, peak flow rate and main milk flow rate at different duration of vibration stimulation.

Pre-stimulation (s)	0 n = 258	20 n = 267	40 n = 259	60 n = 272	90 n = 254	**Treatment effect (P)**
TMY (kg)	13.56±0.23	13.58±0.25	13.49±0.25	13.58±0.24	13.10±0.25	0.53
SMY (kg)	0.22±0.02	0.26±0.03	0.21±0.02	0.23±0.02	0.26±0.02	0.36
TMT (min)	8.54±0.14 [b]	8.81±0.13 [ab]	8.79±0.14 [ab]	9.05±0.13 [a]	9.08±0.14 [a]	0.03
MMT – (min)	7.24±0.13 [c]	7.05±0.12 [bc]	6.86±0.13 [abc]	6.72±0.12 [ab]	6.38±0.13 [a]	<0.001
PFR (kg/min)	2.77±0.05 [c]	2.81±0.05 [bc]	2.85±0.05 [abc]	2.86±0.05 [ab]	2.91±0.05 [a]	0.001
AFR (kg/min)	1.81±0.03 [e]	1.86±0.03 [d]	1.91±0.03 [c]	2.00±0.03 [b]	2.02±0.03 [a]	<0.001

TMY: total milk yield; **SMY**: stripping milk yield; **TMT**: total milking time, including pre-stimulation and including stripping; **MMT** -: Main machine milking time without pre-stimulation and without stripping; **PFR**: Peak flow rate, **AFR**: Average flow rate, quotient of main milk yield (without stripping yield) and MMT -; **Treatments:** . 0, 20, 40, 60, 90 s of pre-stimulation by vibration stimulation with 24 kPa of maximum pulsation vacuum (MPV).

Table 2: Milk yield, stripping yield, total machine on time, main milking time, peak flow rate and main milk flow rate at maximum pulsation vacuum of 17 and 30 kPa for 60 s of vibration stimulation.

Vacuum (kPa)	17 n = 130	30 n = 117	**Treatment effect (P)**
TMY (kg)	12.68±0.36	12.52±0.35	0.83
SMY (kg)	0.13±0.00	0.21±0.03	0.21
TMT (min)	7.77±0.17	8.01±0.18	0.60
MMT – (min)	6.29±0.16	5.84±0.17	0.32
PFR (kg/min)	2.97±0.07	2.92±0.07	0.75
AFR (kg/min)	2.01±0.05	2.12±0.05	0.38

TMY: total milk yield; **SMY**: stripping milk yield; **TMT**: total milking time, including pre-stimulation and including stripping; **MMT** -: Main machine milking time without pre-stimulation and without stripping; **PFR**: Peak flow rate, **AFR**: Average flow rate, quotient of main milk yield (without stripping yield) and MMT -; **Treatments:** .60 s of pre-stimulation by vibration stimulation with 17 and 30 kPa of maximum pulsation vacuum.

min was observed in a medium filled udder (Figure 6 b). Milking a full udder without pre-stimulation (Figure 6 c) resulted in a prolonged increase phase, but no transient decrease in milk flow was observed. A fast increase and a steady plateau phase was observed after an udder filling specific duration of pre-stimulation. The optimum duration of pre-stimulation was 90, 40 and 20 s for little, medium and well filled udders, respectively.

Discussion

In the present study milk flow and OT profiles were analyzed for several modes of tactile pre-stimulation by vibration stimulation. The basal OT level and the release of OT as result of tactile pre-stimulation were similar to those previously reported (Mayer et al., 1984; Bruckmaier and Blum, 1996; Bruckmaier and Hilger, 2001; Weiss et al., 2003). As in previous studies (Bruckmaier and Blum, 1996; Bruckmaier and Hilger, 2001) milking pulsation resulted in similar concentrations of OT as compared to a pre-stimulation by vibration stimulation. OT concentrations during the course of milking were not affected by

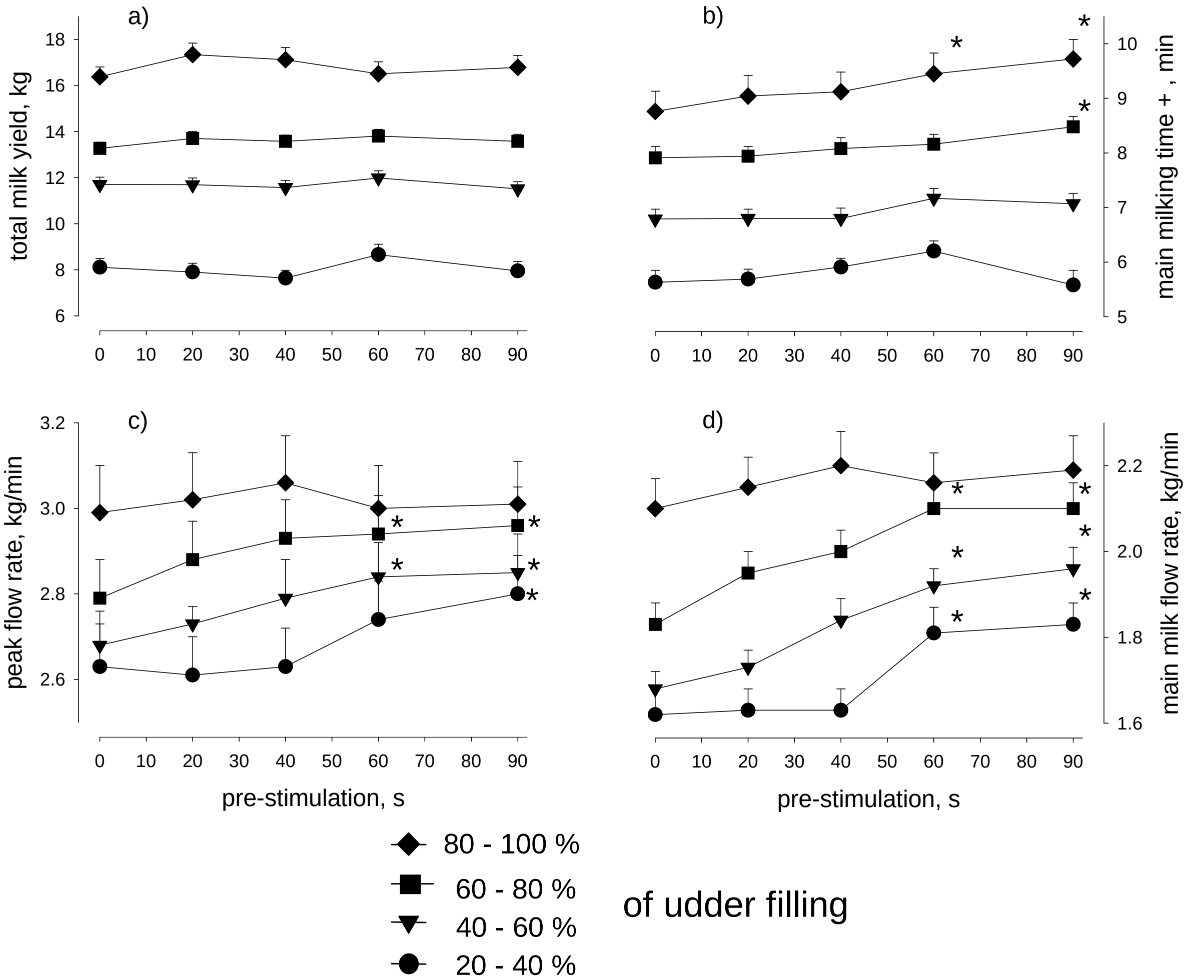

Figure 4: Means ± SEM differing for the degree of udder filling and the duration of pre-stimulation. The symbols indicate clusters of udder filling (20-40, 40-60, 60-80 and 80-100 % of udder filling, respectively). a) total milk yield, b) main milking time including pre-stimulation duration (MMT+), c) peak flow rate (PFR), d) main milk flow rate (AFR). ***** indicate significant differences as compared to milking without pre-stimulation within one udder filling cluster. Significant differences between udder filling clusters were not indicated.

the applied treatments. Therefore, the OT concentrations could not be enhanced by the application of pre-stimulation prior to milking as compared to milking without pre-stimulation. Consequently and in agreement to several previous investigations (Sagi et al., 1980; Zinn et al., 1982; Karch et al., 1989; Rothenanger et al., 1995; Bruckmaier and Blum, 1996), milk yields were not affected by different pre-stimulation routines.

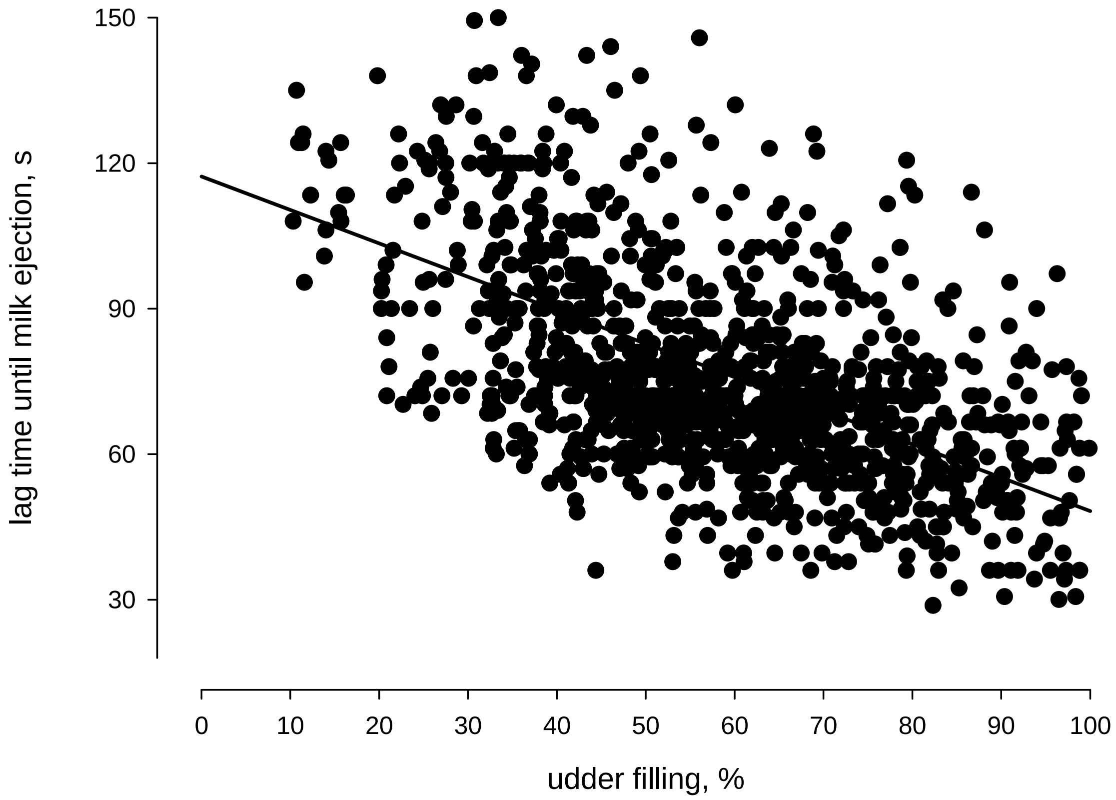

Figure 5: Relationship between udder filling and the lag time from start of teat stimulation until the start of alveolar milk ejection (n = 1191 milkings).

The enhancing effect of pre-stimulation on PFR have been previously demonstrated (Sagi et al., 1980; Zinn et al. 1982; Mayer et al., 1984; Bruckmaier et al., 1996). Interestingly, this effect was absent in well filled udders.

The total machine-on time represents the time from start of pre-stimulation until the end of stripping. The total machine-on time included therefore the machine stripping time and the time span between the end of the main milk flow and the start of machine stripping. The stripping yield was not influenced by the treatment. Therefore, special attention was paid to the main milking phase defined as milk flow from the start of milking pulsation until the milk flow dropped below a threshold of 0.3 kg/min.

An increased MPV during vibration stimulation resulted in a removal of milk already during pre-stimulation. MPV during vibration stimulation did not influence OT release. MPV of 17 and 24 kPa resulted in a vibration of the liner in liner closed position. Only when a MPV of 30 kPa was applied, the liner started to open at peak pulsation vacuum. An MPV of 30 kPa during vibration stimulation enabled a considerable milk flow already during the application of pre-stimulation. However, the fact that milkability was not affected by an enhanced MPV point out that a partial removal of the cisternal milk did not benefit the alveolar milk ejection, even in well filled udders.

The fact that OT release was not influenced by MPV confirms previous results that minimal mechanical impulses are sufficient to release OT and to start the milk ejection (Weiss et al., 2003). Therefore, a further step towards an optimized pre-stimulation is to analyze the optimal duration of pre-stimulation in the individual cow.

According to our and previous results no cisternal milk was available when milking started without pre-stimulation at a low udder filling (Knight et al., 1994; Bruckmaier and Hilger, 2001). Therefore, the milk flow started after the start of the alveolar milk ejection at 90 s. The start of alveolar milk ejection relative to the start of milking pulsation (t = 0 s) could be manipulated by the application of pre-stimulation. The start of milk flow was gradually shifted relative to the start of milking by the application of pre-stimulation in little filled udders. When a 90 s pre-stimulation was applied the milk flow started when milking pulsation started. Without pre-stimulation teats were milked without milk flow until the alveolar milk ejection occurred. Therefore, the optimal pre-stimulation duration was 90 s in case of a little filled udder. At moderate udder filling cisternal milk was immediately available for milking (Bruckmaier and Blum, 1996) and the alveolar milk ejection started about 70 s after the start of pre-stimulation as indicated by the second rise of milk flow (Bruckmaier and Blum, 1996; Bruckmaier and Hilger, 2001). However, the amount of cisternal milk was not sufficient to bridge the gap until the start of alveolar milk ejection.

In this case the milk flow decreased when the cisternal milk was removed. In full udders the amount of cisternal milk was further enhanced (Pfeilsticker et al., 1996) and the lag time until the start of the alveolar milk ejection was further reduced (Bruckmaier and Hilger, 2001). Optimal duration of pre-stimulation, i.e. the minimum duration that is necessary to receive immediate and continuous milk flow after the start of milking varied widely between cows. However, discussing optimal duration of pre-stimulation according to the degree of udder filling there are obviously two effects to consider. An increasing udder filling reduces the lag time from start of teat stimulation until the start of alveolar milk ejection (Bruckmaier and Hilger, 2001). Furthermore, the amount of cisternal milk is higher with increased udder filling

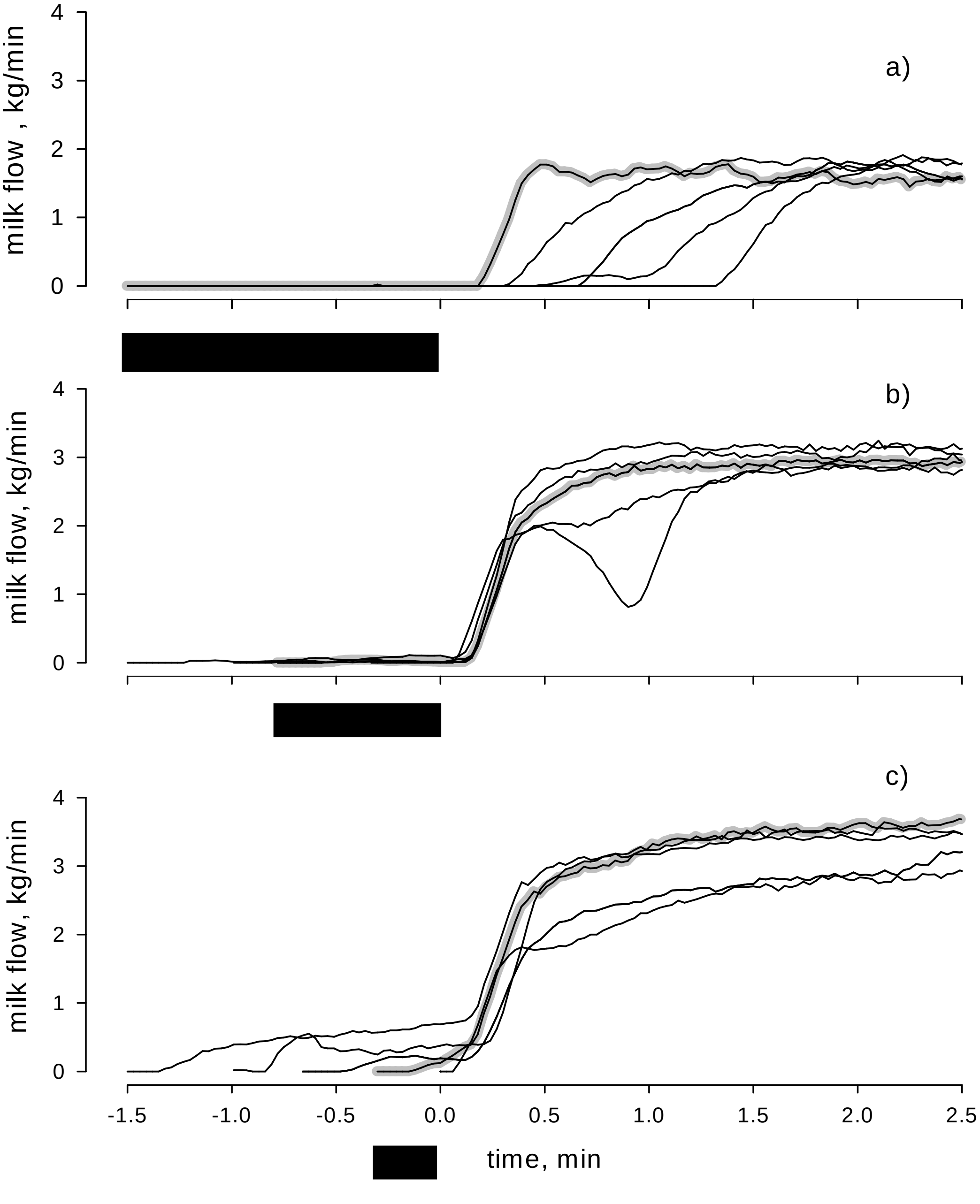

Figure 6: The start of the milk flow profile after varying pre-stimulation duration (0, 20, 40, 60, 90 s of vibration stimulation) in three different cows, with a) low udder filling, b) moderate udder filling, c) high udder filling. t = 0 s represent the start of milking pulsation, each individual line represents the milk flow profile until 2.5 min of milking. The gray, underneath line represent the optimal milk flow profile. ▬ represent the optimal duration of pre-stimulation.

(Knight et al., 1994; Pfeilsticker et al., 1996). However, since the amount of cisternal milk and the individual milk flow rate is very variable between cows (Bruckmaier et al., 1995; Rothenanger et al., 1995; Davis et al., 1998), the estimation of the buffer time of cisternal milk removal is inauspicious. However, there is a close relationship between lag time until the start of milk ejection and rate of udder filling. The actual degree of udder filling provides therefore an important information to optimize the pre-stimulation duration.

Pre-stimulation resulted, except for full udders in an increased AFR. However, an increased AFR does not result definitely in a reduced time requirement for the milking process, since the pre-stimulation duration is not considered in the current definition of AFR. The optimal duration of pre-stimulation represents therefore the combination of a high AFR and a minimized milking time including the duration of pre-stimulation.

Compared to a fixed pre-stimulation duration of about 30 to 60 s, an individual adjustment of the pre-stimulation may provide two advantages. The milking stall capacity could be improved if milking of full udders was performed after a reduced duration of pre-stimulation. A special pre-stimulation modus thus the application of a minimal vacuum level, just sufficient to prevent the fall-off of the milking cluster, can partly substitute the milking vacuum pulsation in less filled udders. Since negative impacts of the milking vacuum load on teat tissue are well known (Hamann et al., 1993; Neijenhuis et al., 2001; Hillerton et al., 2002), an optimized pre-stimulation may improve teat condition.

Conclusions

Immediate and continuous milk flow after the start of milking results in a short duration of milking. A short pre-stimulation enhances milking stall capacity milking full udders and a prolonged pre-stimulation reduces the total vacuum load of the teat milking little filled udders. In dairy practice it should be considered that any mechanical impulses to teat prior to milking, e.g. teat cleaning or fore stripping, cause a pre-stimulation and hence oxytocin release. Additional mechanical pre-stimulation by the milking machine should therefore be adjusted to the farm specific milking routine.

Acknowledgements

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Quarter specific milking routines and their effect on milk removal in cows

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Quarter specific milking routines and their effect on milk removal in cows

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Milking characteristics and udder evacuation in 3 different quarter specific milking routines were evaluated. The findings are based on single quarter milk flow profiles of 10 cows. Each treatment was applied in blocks of 4 subsequent milkings. After the fourth milking of each treatment 10 I.U. oxytocin were *i.v.* injected to obtain the residual milk. The intramammary pressure was recorded during pre-milking stimulation and after the teat cup removal to study the course of the milk ejection. Quarter specific milking routines avoided an overmilking of single teats, and partially decreased the total machine on-time. In comparison to non-stripping routines, the stripping routines had no benefit on udder evacuation or machine on-time. The milk ejection in single quarters was completely terminated when the milk flow ceased and the teat cups were removed. The degree of udder evacuation was therefore not influenced by the tested quarter specific routines.

Viertelspezifische Melkroutinen, Effekte auf die Milchabgabe bei Kühen

Der Einfluss von viertelspezifischen Melkroutinen auf die Milchabgabe und die Euterentleerung wurde in 3 Versuchsvarianten geprüft. Die Auswertung basierte auf Einzelviertel-Milchflusskurven von 10 Kühen. 10 I.U. Ocytocin wurde jeweils nach der letzten Melkung eines Versuchblockes von 4 Melkungen *i.v.* verabreicht, um den Grad der Euterentleerung anhand der Residualmilchmenge zu beurteilen. Der Verlauf des Intramammärdruckes wurde während der Vorstimulation und nach Abnahme der Zitzenbecher gemessen. Blindmelkzeiten können durch die viertelspezifische Abnahme der Zitzenbecher vermieden werden. Auf der Ebene der Einzelviertel kann die Maschinenhaftzeit damit um etwa 20% verkürzt werden. Melkroutinen mit Nachmelken haben gegenüber Routinen ohne Nachmelken keinen positiven Einfluss auf die Euterentleerung oder die Maschinenhaftzeit. Die Milchejektion ist nach Versiegen des Milchflusses einzelner Viertel beendet. Nach- bzw. Teilejektionen einzelner Viertel über das Melkende hinaus wurden nicht beobachtet. Der Grad der Euterentleerung wurde durch viertelspezifische Routinen nicht beeinflusst.

04 Milking routine (Milk removal) **04 Melkroutine** (Milchabgabe)

1. Introduction

The basic mechanisms of milk ejection and milk removal have been intensively investigated, as reviewed by BRUCKMAIER and BLUM (4) and LEFCOURT AND AKERS (8). Teat stimulation induces oxytocin (OT) release, followed by myoepithelial contraction, thus shifting alveolar milk into the cisternal cavities. Manual teat stimulation and machine milking of even one single teat induces a sufficient release of OT to evoke maximum milk ejection in all quarters (3, 5).

Various studies describe an uneven distribution of quarter milk yield in dairy cows. The simultaneous removal of all teat cups caused an overmilking of the less yielding quarters at the end of milking (13, 12, 16). Quarter specific routines with an individual teat cup removal avoid overmilking of individual teats (5, 15). However, OT concentrations during milking are elevated until the last teat cup is removed (5). In the case milk ejection in single quarters would not be completed at the time of teat cup removal, an accumulation of milk in the cistern of the respective quarter would be expected.

This study was designed to determine the efficiency of udder evacuation in 3 different quarter specific milking routines. Single quarter milk flow profiles were recorded and intramammary pressure (IMP) was measured to monitor milk ejection before the attachment and after the removal of the first teat cup.

2. Materials and methods

2.1 Animals

Ten experimental cows (Brown Swiss x German Braunvieh) were in months 2 to 12 of their first to fourth lactation. The cows were kept in a loose housing system and were milked in a 2 x 2 tandem milking parlour. The feeding consisted of maize and grass silage, hay and concentrate according to their individual production levels.

2.2 Experimental design

The experiments were performed during routine milking times starting at 5 a.m. and 4 p.m. On two consecutive days 3 different quarter specific routines were applied during 4 milkings each. After the fourth milking (p.m. milking) 10 I.U. OT were *i.v.* injected in the subcutaneous abdominal vein to obtain the residual milk. Between each treatment block there was at least one day of routine milking without OT application to prevent carry over effects. All treatments started with the usual pre-milking udder preparation consisting of forestripping, a short udder cleaning (15 s) and vibration pulsation (300 cycles $min^{-1,}$ Stimopuls, Westfalia Landtechnik GmbH, D-59299 Oelde) for 1 min. Therefore, the pre-stimulation lasted for 75 s. The control milking (C) was the normal milking routine, starting stripping at a whole udder milk flow threshold of 320 g/min until milk flow dropped below this threshold again. Individual teat cup removal (treatment QR) was performed at a quarter threshold level of 100 g/min. During additional milkings IMP was measured as described by PFEILSTICKER *et al.* (11). The IMP was recorded during pre-stimulation and after teat cup removal in the quarter that terminated the milk flow first, lasted until the last teat cup was removed. In treatment QS quarter specific stripping was immediately performed when milk flow dropped below 200 g/min in each individual quarter. The teat cups were individually removed when the quarter milk flow again dropped below 200 g/min. In treatment SS the pulsation stopped in each quarter at a threshold of 200 g/min while the teat cups remained on the teats in liner-closed position. After the milk flow of the last quarter had dropped below 200 g/min, the pulsation of all teat cups was restarted and stripping was performed.

The milking was performed at a vacuum of 42 kPa, a pulsation rate of 60 cycles min^{-1} and a 60:40 pulsation ratio using separate pulsators (Stimopuls CP, Westfalia Landtechnik, D-59299 Oelde) for each quarter. Quarter sensors based on conductivity measurement monitored the milk flow threshold in each quarter. Bio-Milker teat cups (Westfalia Landtechnik, D-59299 Oelde) with an individual long milk tube for each quarter and a quarter milk flow recording device (Lactocorder, Werkzeug- und Maschinenbau Balgach, CH-9436 Balgach) were used as previously described (16). The measurements were carried out as described by BRUCKMAIER *et al.* (2). Total milk yield, quarter yield, quarter stripping yield, peak flow rate, total machine on-time, average teat cup on-time and average flow rate were evaluated.

2.3 Statistical evaluation

The results are presented as means ± SEM. For statistical evaluation the SAS program package release 8.01(SAS 99) was used. Treatment effects were tested for significance ($p < 0.05$) using the Mixed procedure. The model included the treatment, the individual animal, the milking time, the quarter and the day of milking. The milking time and the day of milking was modelled as a random factor. The animal entered the model as a repeated factor, using the covariance structure Compound Symmetry (CS). Differences between treatments were localised using the Least Significant Difference test (LSD).

3. Results

3.1 Milking characteristics

The total milk yield (Table 1) was lower in QS ($p < 0.05$) than in QR and SS, resp. Peak flow rate did not differ between treatments. The total milking time was shorter in QR and QS than in C and SS, resp. ($p < 0.05$). The milking time per quarter was highest in C and lowest in QS ($p < 0.05$). The overmilking time in C was longer in front than in rear quarters ($p < 0.05$). The stripping yield in C, SS and QS (example Figs. 2 and 3) did not differ significantly. The average milk flow rate was numerically higher in OT and QS than in C and SS, respectively ($p < 0.05$). When quarters

Table 1: Milking characteristics during control milking (C), quarter removal (QR), quarter stop and stripping (SS) and quarter specific stripping (QS)

Parameter		C	QR	QS	SS
Total milk yield, kg		13.2 ± 1.7[ab]	13.4 ± 1.7[b]	12.6 ± 1.7[a]	13.4 ± 1.7[b]
Highest milk flow, kg/min		3.43 ± 0.17	3.40 ± 0.17	3.44 ± 0.17	3.53 ± 0.17
Stripping yield, kg	Front right	0.13 ± 0.07	n.d.	0.08 ± 0.07	0.23 ± 0.08
	Front left	0.23 ± 0.07	n.d.	0.14 ± 0.07	0.30 ± 0.07
	Rear right	0.21 ± 0.06	n.d.	0.30 ± 0.07	0.29 ± 0.07
	Rear left	0.20 ± 0.07	n.d.	0.33 ± 0.09	0.36 ± 0.08
Total machine on-time, min		7.27 ± 0.41[a]	6.73 ± 0.41[b]	6.38 ± 0.41[b]	7.24 ± 0.41[b]
Average teat cup on-time, min		7.27 ± 0.41[a]	5.24 ± 0.32[b]	4.95 ± 0.32[b]	5.38 ± 0.32[b]
Average flowrate , kg/min		1.79 ± 0.15	1.98 ± 0.14	1.93 ± 015	1.89 ± 0.15
Overmilking time, min	Front right	2.20 ± 0.30	n.d.	n.d.	n.d.
	Front left	2.40 ± 0.26	n.d.	n.d.	n.d.
	Rear right	1.86 ± 0.30	n.d.	n.d.	n.d.
	Rear left	1.19 ± 0.18	n.d.	n.d.	n.d.
Residual milk yield, kg		0.90 ± 0.32	1.10 ± 0.28	1.51 ± 0.29	1.00 ± 0.28

[a,b] Means without a common superscript letter within a line are significantly different ($P<0.05$), n.d.: not defined

were ordered by their individual milking time in the sequence of the teat cup removal, quarter stripping yield was not significantly influenced by treatments or by milking time order (Fig. 1).

3.2 Residual milk

The residual milk yield was not significantly different between treatments with numerically lowest values in SS and highest values in QR.

3.3 Intramammary pressure

The IMP increased significantly from baseline to ejection pressure. The IMP after removal of the first teat cup (Table 2) was lower than ejection pressure as well as the baseline pressure. No changes in IMP were found from the time of removal of the first teat cup until the removal of the last teat cup (Fig. 3).

4. Discussion

The numerically higher residual milk yields in QS could have been responsible for the decreasing milk yields in this treatment. However, considering that the treatments lasted over a period of 4 weeks, the lower yields in treatment QS (as the last tested treatment) could be explained by a lactational decline in milk yield. Since the peak flow rate and the average milk flow were not significantly influenced by any treatment an effect on the course of milk ejection can be excluded.

Irrespective of the applied modus, quarter specific routines can reduce the duration of vacuum on the teats by more than 20%. This observation corresponds to earlier investigations (15, 16). As reported by WEISS and WORSTORFF (15), the total machine on-time and the average teat cup on-time was significantly reduced in QR and QS. However, we could not show the described advantage of stripping routines compared to non-stripping routines in this study. The residual milk and the peak flow rate did not differ significantly between treatments. Therefore, there were neither negative nor positive effects of the tested milking routines on udder evacuation.

The measurements of baseline IMP and ejection pressure corresponded to earlier investigations (11). The lack of increase in IMP after removal of the first teat cup until the removal of the last teat cup indicates, that there was no additional milk shifted into the cistern. Following from that, the milk ejection in a specific quarter was com pleted, irrespective of other quarters still milking and oxytocin concentrations still being elevated. The fact that there is no relationship between quarter stripping yield and order of teat cup removal underline this conclusion.

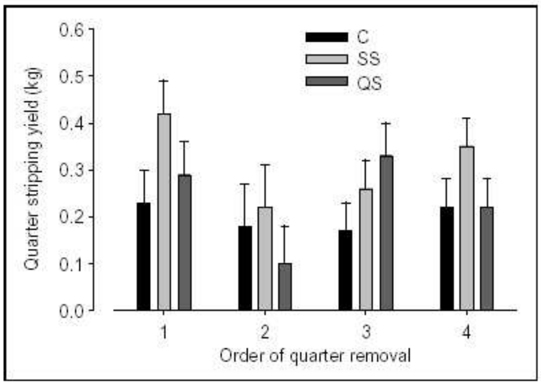

Fig. 1: Quarter stripping yields for control milking (C), stop and common stripping (SS) and quarter specific stripping (QS), ordered in arrange of their milking time

Table 2: Intramammary pressure in the fastest milking quarter

Intramammary pressure	Unit	
Baseline	kPa	1.5 ± 0.2[a]
Ejection	kPa	4.0 ± 0.3[b]
Removal first quarter	kPa	0.7 ± 0.3[c]
Removal last quarter	kPa	0.7 ± 0.3[c]

[a,b,c] Means without a common superscript letter are significantly different ($P<0.05$)

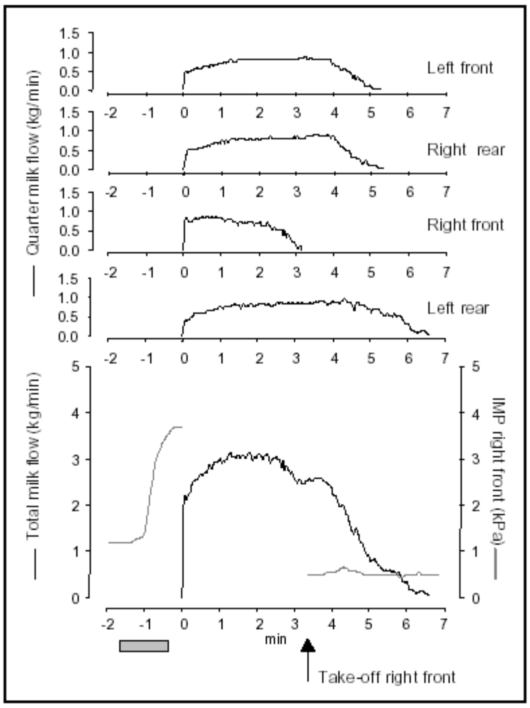

Fig. 2: Quarter milk flow, udder milk flow and IMP in right front quarter of an individual cow with quarter individual quarter removal (QR) (▬ stimulation)

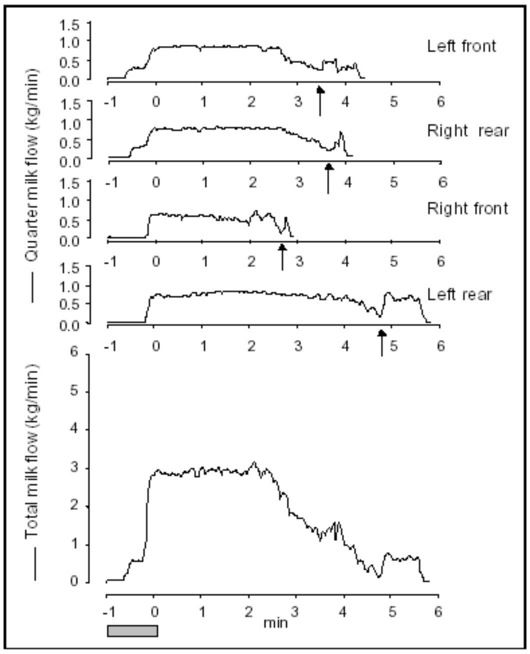

Fig. 3: Quarter milk flow and udder milk flow of an individual cow with quarter specific stripping (QS) (▬ Stimulation; ↑ start of quarter stripping)

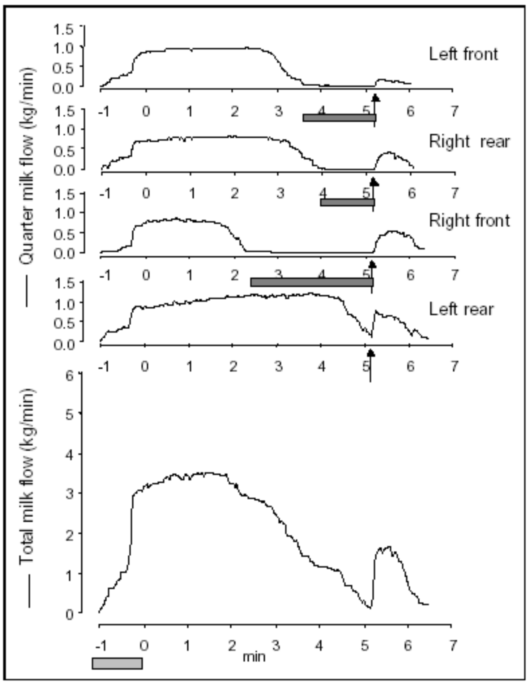

Fig. 4: Quarter milk flow and udder milk flow of an individual cow with stop of pulsation and common stripping (SS) (▬ stimulation; ↑ start of stripping; ▬ quarter individual stop of pulsation)

Milking of empty teats can cause a damage of the teat tissue (7), and can reduce the bactericidal defence function of the teat sphincter. Although no evidence has been shown that the overmilking in conventional systems as result of unequal quarter yields have a direct effect on somatic cell count (16), the infection rate in udders treated by overmilking was elevated (10). This is likely because an overload in vacuum application inhibits the antimicrobial defence of the teat (9). Therefore, quarter specific routines are preferable because they reduce the vacuum load of the teats.

5. Conclusion

Quarter specific milking routines avoid overmilking time of single quarters, thus preventing damage of the teat tissue. The milk ejection in single quarters is completed when milk flow ceases, even if other quarters are continued to be milked.

Acknowledgment

This study was supported by a grant of the Technical University Munich and by Westfalia Landtechnik GmbH, Oelde (Germany). The help of Mr A. Knon and Mr M. Schmölz during the animal experiments are gratefully appreciated.

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Coping capacity of dairy cows during the change from conventional to automatic milking

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Coping capacity of dairy cows during the change from conventional to automatic milking[1]

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ABSTRACT: In conventional milking systems, dairy cows are driven to the milking stall twice or thrice daily, whereas in automatic milking systems (AMS), the cows enter the milking stall voluntarily. In this study, noninvasive methods were used to analyze the physiological reaction of 17 cows toward the changeover from conventional to automatic milking. Milk yield and composition were analyzed. Heart rate was recorded continuously, and feces was sampled twice daily to determine cortisol metabolites (11, 17-dioxoandrostanes) for a period of 2 wk. During the first visit to the AMS (without milking), heart rate was elevated compared with parlor milking by 35 ± 3 beats per minute (bpm) above basal heart rate ($P < 0.05$). Heart rate during the first milking in AMS (eighth visit) was already similar to the heart rate previously measured during milking in the parlor (18.1 ± 2.2 bpm above basal level). Concentration of fecal cortisol metabolites was unchanged during the changeover compared with parlor milking. A decreased ($P < 0.05$) milk yield of 68 ± 7% relative to previous parlor yield during the first AMS milking indicated a disturbance of milk ejection in most cows. Individual yields ranged from 8 to 96% of the previous parlor yield. To examine the relationship between adrenal cortex sensitivity and the coping process, an ACTH challenge experiment was performed after the changeover period. Cows that released more cortisol after ACTH injection, indicating a higher adrenal cortex sensitivity, had a less enhanced heart rate and a near normal milk ejection during the first AMS milkings ($P < 0.05$). In conclusion, the reactions toward the changeover to AMS milking varied widely within cows. Adaption to the AMS was easier in animals with a higher adrenal cortex sensitivity to ACTH.

Key Words: Adaptation, Adrenal Cortex, Dairy Cattle, Milking

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Introduction

The coping of farm animals to environmental changes is a complex neuronal and endocrine process. Although the measurement of endocrine responsiveness contributes to the understanding of the coping process, the interaction between different hormonal systems in this process has only been partially understood (Rushen, 1991; von Borell, 2001). Besides the multiple functions of hormones, the sampling of blood to determine hormones is problematic because the sampling itself can act as a stressor. Therefore, noninvasive methods were used in the present study to record the reactions of dairy cows during the changeover period from a conventional parlor to an automatic milking system (**AMS**).

The cow's motivation to enter the milking stall is the major difference between AMS and conventional milking systems. In conventional milking routines, the cows are driven to the milking parlor two to three times daily. In AMS, the cows enter the milking stall voluntarily and are milked throughout the day without human intervention.

In order to achieve this voluntary visit, several management systems are in use. Usually, concentrate is available in the AMS to attract a visit. In a system called forced cow traffic, roughage is only available after the cow passes the milking stall (Harms et al., 2002; Hopster et al., 2002). In adapted cows, the restricted access to the feeding lane seems to be without importance because AMS milking did not show any negative effects compared with conventional milking (Hopster et al., 2002). However, when cows change from conventional milking to an AMS, they have to overcome several

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environmental changes. The aim of the present study was to quantify the stress reaction of dairy cows during the changeover period from conventional to automatic milking. In addition, the hypothesis was tested that the individual coping capacity is related to the individual adrenal cortex sensitivity. Therefore, an ACTH challenge test was performed to classify the individual adrenal cortex sensitivity after the changeover period and to analyze the relationship between adrenal cortex sensitivity and individual coping capacity.

Material and Methods

Animals and Husbandry

Seventeen healthy cows were used to analyze the transition period from parlor to AMS milking (DeLaval, Tumba, Sweden). Automatically milked cows and those milked in the parlor (total number 100 Red-Holstein/Simmental cows) were kept in one barn, divided in two herds. Except for the milking system, the management, the feeding, and the barn layout were identical for parlor and AMS area. Routine milking times in the parlor started at 0430 and at 1530. Experimental cows were in their first to fifth lactation and between 26 and 316 d in milk. The cows assigned to the experiment had not been previously milked in an AMS. The animals were divided into two groups of eight and nine cows. The groups were balanced for lactational stage, age, and milk yield. All cows were under the same feeding and management regimen and received a PMR consisting of corn silage, chopped grass silage, and concentrates. The training period of the second experimental group started 2 wk after the training period of the first group in order to avoid an overload of the AMS due to the training. Management, feeding, and barn staff were unchanged for both groups. During the changeover period of the first group, the AMS regularly milked an additional 30 cows, and for the second group, an additional 38 cows. The daily yield in the parlor before the changeover ranged from 14.7 to 39.2 kg with a mean of 26.9 ± 2.2 kg/d. In the AMS area, selectively forced cow traffic (Harms et al., 2002) was applied. The feeding area was separated from the resting area by one-way gates, which allowed the cows free access to the cubicles also without being milked. However, they were obliged to pass the AMS before entering the feeding area, with a bypass exception for those cows, which had recently been milked. Cows had to pass the milking stall if milk yields of more than 7 kg were expected. Additionally, AMS visits were positively reinforced by concentrate feeding in the AMS milking box.

Experimental Procedure

The experimental cows were trained to the AMS during daytime for 3 d (Figure 1). These cows were collected after the morning milking from the parlor and moved to the AMS area. They were manually driven to the AMS stall twice daily during the training period between 0600 to 0800 and between 1430 and 1600. Shortly before the evening milking in the parlor, these cows were moved to the parlor area, where they were regularly milked and remained until the next morning milking in the parlor herd. After the morning milking in the parlor on d 4, the experimental cows were moved finally to the AMS and milked there for the first time in the afternoon. During the changeover period, the staff of the stable did not change. For the first and second AMS milking, all cows were manually driven to the AMS, whereas for further milkings, cows were collected when milking intervals exceeded 12 to 14 h.

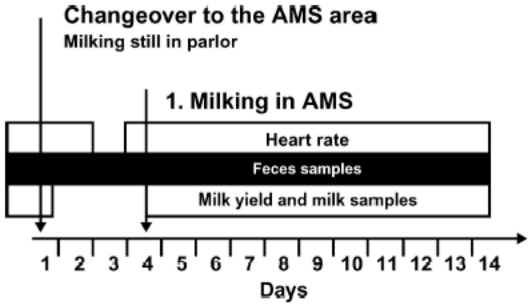

Figure 1. Experimental protocol: The first arrow indicates the start of the training period. The cows were driven twice daily to the stall of the automatic milking system (AMS) but were still milked twice daily in the parlor. The second arrow indicates the start of milking in the automatic milking system; cows remained finally in the automatic milked herd. Milk recordings, heart rate measurement and feces sampling were carried out as indicated by the bars.

Except for a period of 24 h on d 3 of the training period (Figure 1), heart rate was recorded continuously throughout the experiment, during two successive parlor milkings before the changeover, during the training period, and during AMS milking until d 14 in the AMS. The heart rate was measured by means of a commercial system developed for horses using electrodes fixed to a special belt around the chest (Polar Horse Tester, Polar Electro GmbH, Bütelborn, Germany) (Hopster et al., 2002). The signals were saved as 15-s averages for further analyses. To determine the cortisol metabolites, 11,17 dioxoandrostanes (**DOA**), fecal samples were taken twice daily at 0700 and 1800 from the rectum when the animals were fixed at the feeding gate and were immediately frozen at −20°C until further analyses, which were performed according to the method described by Moestl et al. (2002). Milk yield and composition were recorded during the last 10 d of parlor milking before the AMS training and during AMS milking from d 4 (first AMS milking) until d 14. To determine milk yield and for milk sampling, the Lactocorder system (Werkzeug- und Maschinenbau Balgach, Balgach, Switzerland) was used during parlor milkings. In the AMS, the standard milk meter and sampling devices, as provided by the manufacturer, were used. Milk samples were analyzed for fat, protein, lactose and somatic cell count (Milko Scan 6000, Foss GmbH, Hamburg, Germany).

In addition, an ACTH challenge test was performed 4 wk after the changeover of the second group and 6 wk after the changeover of the first group. For technical reasons, 12 animals only were used to test their adrenal cortex responsiveness to exogenous ACTH. The animals were randomly chosen from the total group of 17 experimental cows. The 12 cows used to test the adrenal cortex sensitivity were in their 2.0 ± 0.39 lactation, were 208 ± 38 d in milk, and yielded 26.6 ± 3.2 kg of milk per day before their changeover to automatic milking. Characteristics for the total 17 experimental cows used in the present study were 1.76 ± 0.29 lactations, 203 ± 30 d in milk, and 26.3 ± 2.3 kg of daily milk yield in the parlor, respectively. The day before the test, a catheter was inserted into the jugular vein. The $ACTH_{1-24}$ (80 μg, Synacthan, Novartis, Basel, Switzerland) was intravenously injected to each animal according to Macuhova et al. (2002) to obtain a standardized stimulation of cortisol release of the adrenal cortex, as previously described by Verkerk et al. (1994). Blood samples for cortisol determination were taken at −60, −30, 0, 15, 30, 45, 60, 75, 90, 120, 150, and 180 min relative to the ACTH administration. Feces samples from the rectum were taken at −18 (before catheterization), −2, 5, and 9 h relative to ACTH administration and stored at −20°C until analyses. The blood samples were treated with EDTA to prevent coagulation and centrifuged at 1,500 × g for 15 min within 20 min after each sampling. The plasma was stored at −20°C until cortisol analysis using a competitive enzymeimmunoassay as previously described for bovine plasma by Sauerwein et al. (1991). The cortisol metabolites DOA were determined in the feces according to the method described previously (Moestl et al., 2002).

Data Processing and Statistical Analyses

Data are presented as means ± SEM. Due to the variable milking intervals in AMS, milk yields were handled as production rate per hour, as a quotient of the actual milk yield and the corresponding milking interval (Weiss et al., 2002). To demonstrate any effects of the changeover, milk yields obtained in the AMS were expressed as relative values of mean parlor yields during 10 d before AMS training. Likewise, the milk constituents during AMS milkings were expressed as relative values of parlor results.

The mean of the lower 30th percentile of the dataset of each individual cow (total duration of 262 h) was defined as the basal heart rate. The heart rates in the parlor and in the AMS were defined as average heart rates above baseline (**HAB**). Heart rate during the first 2 min after entering the milking stall was analyzed.

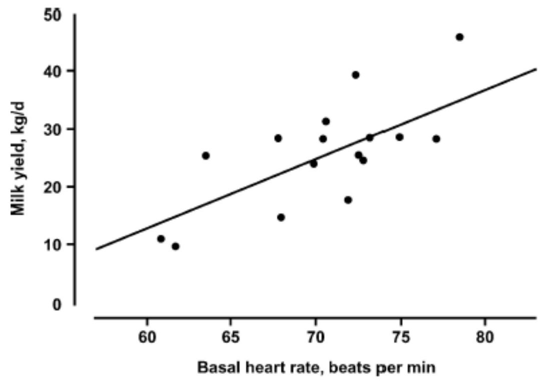

Figure 2. Relationship between the basal heart rate (x) and the daily milk yield (y): $y = -59 + 1.2x$; $r = 0.74$ ($P < 0.001$).

For statistical evaluation, the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC; version 8.01) was used. The model included day, time of the day, and animal group as random variables and the number of the visit, the number of the milking, the lactational stage, and the lactation number as fixed effects. The cow was included in the model as a repeated effect using the covariance structure compound symmetry. Significant differences ($P < 0.05$) were localized using the LSD test. The REG procedure was used to calculate Pearson's coefficient of correlation. Results were indicated as statistically significant at $P < 0.05$, unless stated otherwise.

Results

Behavioral Observations

At their first visit, all cows had to be pushed manually into the AMS stall. The number of cows that needed only a gentle drive to the AMS stall increased during the training period. The rate of voluntary visits was 0, 32, 48, 56, 81, 86, 91, 94, 93, and 97% during the first 10 d of AMS milking (d 4 to 14), respectively.

Heart Rate

Obvious artifacts, such as missing data or errors (heart rates of more than 200 bpm), of heart rate recording were detected and eliminated from records. The basal heart rate varied between 61 and 83 bpm. As shown in Figure 2, the relationship between the basal heart rate and the daily milk yield was linear and positively correlated. Pearson's coefficient of correlation was $r = 0.74$ ($P < 0.001$).

The HAB results are presented separately for AMS visits and AMS milkings because no AMS milkings were performed during the training period (Figure 1), and after the start of milking during half of the visits,

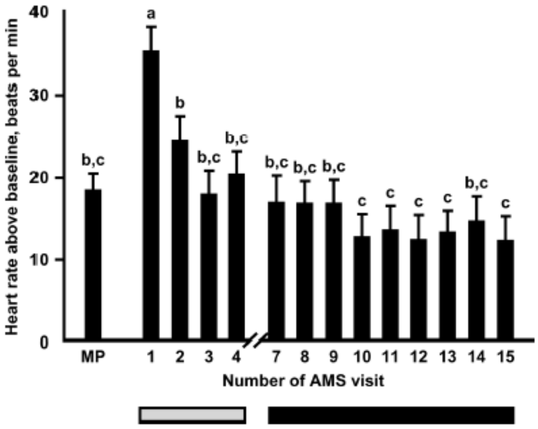

Figure 3. Heart rate above baseline (mean ± SEM, n = 17 animals) during milking in the parlor (MP) and during visits in the stall of the automatic milking system. Means without common letters differ ($P < 0.05$). The gray bar indicates results during the training period; the black bar indicates results after milking started in the automatic milking system (AMS).

cows were not milked. The HAB during the first visit in the AMS was significantly higher compared with the parlor milkings (Figure 3). By the second visit to the AMS stall, mean HAB was similar to results obtained in the parlor. No heart rate measurements were performed on d 3 (Figure 1). Therefore, no data were available during visits five and six.

Overall, the HAB during AMS milking (Figure 4) differed only slightly from results obtained in the parlor. Even during the first milking in the AMS, on d 4, the mean HAB was not significantly elevated compared with parlor milkings.

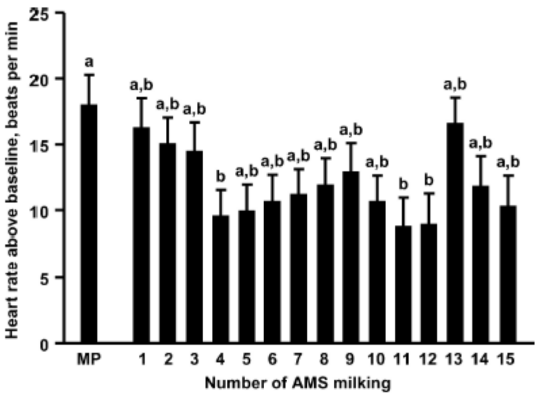

Figure 4. Heart rate above baseline (mean ± SEM, n = 17 animals) during milking in the parlor (MP) and visits when milking was performed in the automatic milking system (AMS). Means without common letters differ ($P < 0.05$).

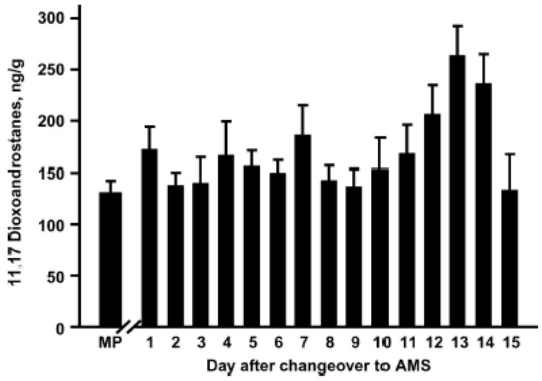

Figure 5. 11,17 Dioxoandrostanes (DOA) concentration (fresh-matter basis) in feces (means ± SEM, n = 17 animals) in the parlor (MP) and the automatic milking system.

11,17 Dioxoandrostane

The DOA concentration (fresh-matter basis) in the feces during the control period was 134 ± 12 ng/g (Figure 5). Although the DOA content was not significantly changed during the changeover period, the SEM increased twofold compared with the data obtained in the milking parlor. The time of day during which sampling took place had no significant influence on DOA concentration in feces.

Milk Yield and Milk Composition

The average milk yield during the first milking in the AMS was 68 ± 7% compared with the yield obtained in the parlor before the changeover procedure (Figure 6). Individual milk yield at the first milking, in the AMS, varied between 8 and 96% of the yield previously obtained in the parlor. During the three following milkings, the milk yield was similar to that obtained in the parlor. However, after the sixth milking in the AMS, milk yield was decreased ($P < 0.05$) compared with previous yield in the parlor. On average, for the first 20 milkings, yields were 85% of the previous yields in the parlor, although milking frequency was increased compared with parlor milking. The milking interval for the first 20 milkings was 10.6 ± 0.2 h (milking frequency was 2.26 ± 0.04 milkings per day).

Milk fat content and somatic cell count were was highly variable between individual cows and between milkings, whereas milk protein had lower variance.

ACTH Challenge

The cortisol response to ACTH peaked at 75 min after the ACTH administration. The area under the curve

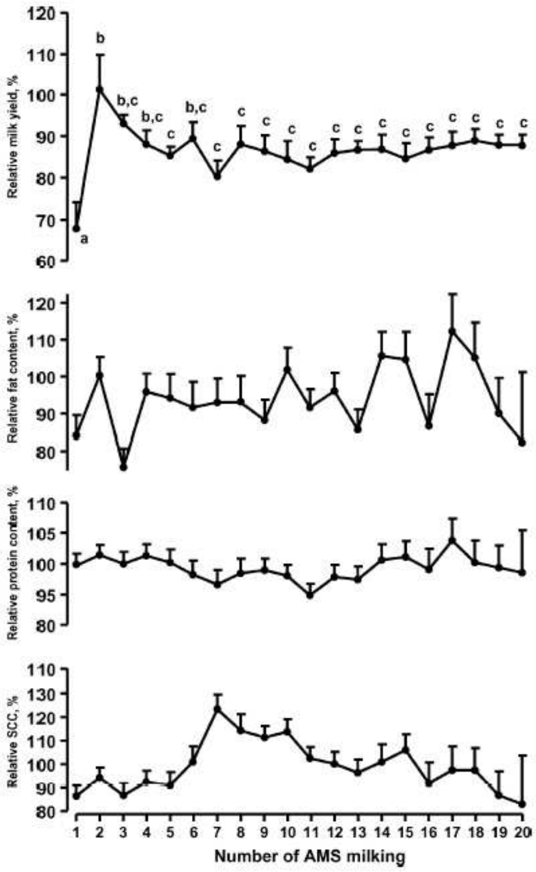

Figure 6. Milk yield and milk composition (mean ± SEM, n = 17 animals) expressed as relative values of parlor results (100% = mean parlor results of 10 d before the changeover). Means without common letters differ ($P < 0.05$).

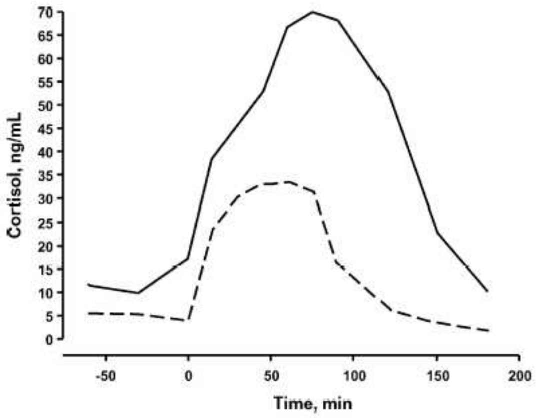

Figure 7. Cortisol response during ACTH challenge in two exemplary cows with high (—) and low (----) adrenal cortex sensitivity. The ACTH was administrated at t = 0 min.

from 0 to 180 min after application of ACTH varied in individual cows from 38 ng/(mL × min) to 63 ng/(mL × min) (Figure 7). The adrenal cortex sensitivity (area under the curve of cortisol response) and the relative milk yield during the first milking had a positive linear relationship (Figure 8). Pearson's coefficient of correlation was r = 0.65 ($P = 0.02$). However, the HAB during the first and second milking was closely, but negatively, correlated with the cortisol responsiveness. Pearson's coefficient of correlation for the first milking was r = −0.60 ($P = 0.05$) and r = −0.75 ($P = 0.008$) for the second milking. The cortisol responsiveness and the relative milk yield during the first 20 AMS milkings were positively correlated. Pearson's coefficient of correlation was r = 0.65 ($P = 0.076$). In contrast, cortisol response and heart rate during the first visits in the AMS were not significantly correlated.

Concentrations of DOA were basal before and at 4 h after ACTH administration, whereas 9 h after ACTH challenge, the concentrations were elevated (Figure 9; $P < 0.05$).

Discussion

In the present study, the stress response of dairy cows toward the changeover from conventional to AMS milking was evaluated. A close positive correlation be-

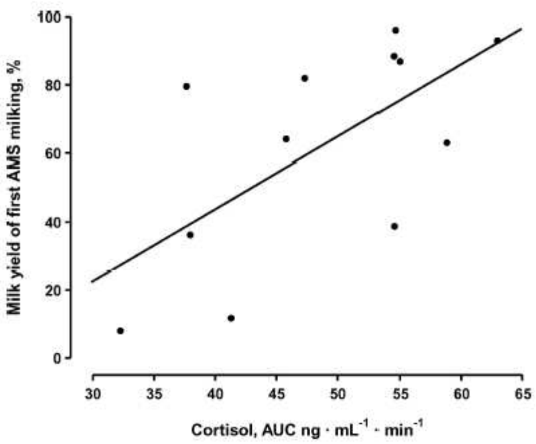

Figure 8. Relationship between cortisol release during ACTH challenge (area under curve [AUC], 0 to 180 min after ACTH application; x) and the relative milk yield during the first AMS milking (y): y = −40.4 + 2.1x; r = 0.65 ($P = 0.02$).

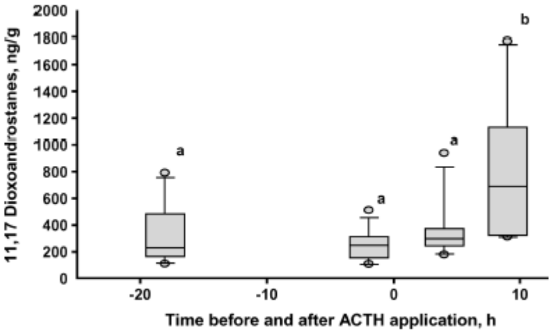

Figure 9. Boxplot of the feces 11,17 dioxoandrostanes concentrations (fresh-matter basis) before and during ACTH administration. Each box shows the median and the upper and lower quartile value; the whiskers show the 10th and the 90th percentiles. The circles represent data points that were outside the centiles. Means without common letters differ ($P < 0.05$).

tween daily milk yield and basal heart rate was demonstrated. The high variability of the daily milk production and the basal heart rate of individual cows pointed toward the need to perform a correction of absolute heart rate values. Therefore, the elevation of heart rates above basal was used for all calculations. During the first visit to the AMS, the elevated HAB indicated a high sympathetic activation. The elevation in heart rate was comparable to results demonstrated by Hopster et al. (1995) after cow–calf separation in dairy cows. Rushen et al. (1999) demonstrated that dairy cows' fear toward an aversive handler resulted in a lower heart rate elevation compared with the effects observed during the first visit. Therefore, the observed effects on heart rate seem to be remarkable. However, it has to be considered that by during the second and third visits, the HAB was reduced. Within 10 visits to the AMS, the HAB normalized, thus indicating a successful coping of the cows to the AMS. The tendency of lower HAB during AMS milkings compared with milking in the parlor corresponds to previous investigations in adapted cows (Hopster et al., 2002).

Similar to the first visits, a decrease in HAB could be observed during the first milkings, although the level of HAB during the first milkings was much lower compared with the first visits. However, the cows seemed to adapt to being milked in the AMS stall since a reduction in HAB was observed within the first four milkings.

The DOA concentrations in the feces were not affected during the changeover period. This result is in contrast with situations like the transport of cattle, where DOA concentrations were elevated 10-fold (Palme et al., 2000). It has to be considered that DOA concentrations are influenced by other factors, such as passage rate or feed type. However, the cortisol concentrations after ACTH application were shown by a significant increase in DAO concentrations 9 h later (Figure 9). Considering that the cortisol concentrations during the first days after tethering of bulls in a previously reported experiment (Ladewig and Smidt, 1989) resulted in levels comparable to those during the ACTH challenge, the feces DOA measurement that was used appears sufficiently sensitive to reflect adrenal cortex activity. Therefore, we conclude that the cows did not respond with increased cortisol secretion during the changeover process.

The introduction of AMS milking was obviously less stressful than tethering. Because the cows entered the AMS milking stall voluntarily after being collecting within two to four visits, and because the HAB normalized within this time, the adaptation to the AMS was unexpectedly fast. However, the milk ejection was adversely affected during the first AMS milkings as milk yields were reduced. The inhibition of milk ejection was reported earlier as a sensitive reaction to environmental changes and is due to a lack of oxytocin release from the pituitary gland (Bruckmaier et al., 1996). During repeated milking in unfamiliar surroundings, the release of oxytocin, and therefore the occurrence of spontaneous milk ejection, gradually normalized (Bruckmaier et al., 1996). In previous studies, the cows were administered exogenous oxytocin during the last part of the experimental milking in order to empty the udder completely. In the present study, no exogenous oxytocin was applied to avoid an additional stress load for the animals. Therefore, a leftover of approximately 30% of the stored milk remained in the udder after the first milking, which was still present at the second milking. The milk stored before the second milking was therefore theoretically 130%. The obtained milk yield of approximately 100% documents that there was still a partial inhibition of milk ejection. The milk yields of the subsequent milkings have to be interpreted in a similar way. Milk yield was reduced by 15% after the first 8 to 10 milkings. Negative effects of the selectively forced cow traffic on milk production can be excluded since milk composition and somatic cell count were not affected by the changeover. The observed decrease of milk yields was most likely caused by the inhibition of milk ejection during the first milkings. This inhibition caused an incomplete emptying of the udder, resulting in reduced milk production during the ongoing lactation (Peaker and Wilde, 1996; Bruckmaier and Blum, 1998). The decreased milk production likely caused by enhanced apoptosis of the mammary epithelial cells (Murugaiyah et al., 2001; Stefanon et al., 2002). However, a recent experiment indicated that the phenomenon of reduced milk yield due to the changeover to AMS milking, appeared only in cows without previous experience in AMS milking. In cows with previous experience in AMS milking even after a transient period of parlor milking, reduced milk yields after the changeover to the AMS were not observed (Weiss and Bruckmaier, 2003).

The cortisol release as a result of ACTH injection was comparable to results reported earlier and varied widely between cows (Figure 8, Ladewig and Smidt, 1989; Hopster et al., 1998; Macuhova et al., 2002). Whether the adrenal cortex sensitivity is determined genetically or by environmental effects has been discussed for the last 30 yr (Ward, 1972; Ladewig and Smidt, 1989; Janssens et al., 1995; de Jong et al., 2000). The rise in adrenal cortex sensitivity and an increase of adrenal cortex weight as a result of chronic stress has been reported in pigs (Janssens et al., 1995; de Jong et al. 2000; von Borell, 2001). In contrast, there is evidence that chronic stress decreases the adrenal cortex sensitivity in cattle (Ladewig and Smidt, 1989; Redbo, 1998). A further explanation for this variation between species may be the different extent of metabolic function of glucocorticoids in monogastric animals and ruminants.

In the present study, cows with a high adrenal cortex sensitivity (high cortisol release during ACTH challenge) demonstrated a less distinct disruption of milk ejection during the first milking, and less increased HAB during the first and the second milking in the AMS. Additionally, in these cows, the decrease of milk yield during the first 20 AMS milkings due to the changeover was less pronounced. In the present study, the cows visited the AMS milking stall at least seven times before the first milking was performed. In contrast, in previous experiments, milking took place at the first visit in unfamiliar surroundings (Bruckmaier et al., 1996; Rushen et al., 2001; Macuhova et al., 2002). Therefore, the milk ejection reflex was probably not completely blocked in the present study, as has been observed before. However, the present results confirm previous investigations by Macuhova et al. (2002) that demonstrated a negative relationship between the degree of blocked milk ejection in unfamiliar surroundings and the adrenal cortex sensitivity of the individual cow. The individual variation of the disturbance of milk ejection and the HAB during the first milking in the AMS despite a highly standardized treatment for all cows demonstrates the individual coping capacity toward the changeover to the AMS. This means that the training period could be further shortened in cows with a high coping capacity. In cows with a low coping capacity, a longer training period could possibly prevent a loss in milk yield due to the changeover.

The reason for individual differences in adrenal cortex sensitivity in cattle is unclear. Results by Ladewig and Smidt (1989) and Redbo (1998) support the hypothesis that the individual adrenal cortex sensitivity is reduced due to chronic stress. Behavioral analyses suggest that an overload reduced individual activity and resulted in a decreased exploratory activity (Redbo, 1998). If the exploratory activity is reduced, the time needed to adapt to a changed environment is enhanced and the time until successful coping will be prolonged.

Implications

This study analyzed the physiological reactions of dairy cows during the transition from conventional to automatic milking systems. Although all cows adapted within days to the automatic milking system, the individual ability to cope varied widely and was related to the adrenal cortex sensitivity. These results suggest a considerable importance of the hypothalamic-pituitary axis for the coping process in cattle.

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The changeover from conventional to automatic milking in dairy cows with and without previous experience

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Abstract

The effects of the changeover from conventional parlour to an automatic milking system (AMS) on behaviour and physiological parameters in dairy cows were investigated. The experimental cows had either no previous experience in AMS milking (n = 17) or were milked in the experimental AMS during the previous lactation (n = 9). Experienced cows (EC) were milked in the parlour for 36 ± 5 d after parturition before they were moved to AMS milking. Milk yield and composition was analysed 10 d prior to the changeover until d 6 of automatic milking. Heart rate was measured continuously and faeces samples were taken twice daily to determine cortisol metabolites before the changeover and during automatic milking until d 6. The rate of voluntarily visits to the AMS stall was evaluated during 10 d. The milk yield in the parlour was 26.3 ± 2.3 kg/d in UC and 37.6 ± 1.7 kg/d in EC. Although the visit in the AMS stall was attracted by concentrate UC had to be pushed into the milking stall of the AMS for the first one or two visits. Heart rate was higher in UC than in EC during the first AMS visit (35 ± 5 and 17 ± 1 beats per min, respectively, $P < 0.05$). EC entered the AMS milking stall voluntarily without any intervention by the staff. In UC the rate of voluntary visits was 4, 26, 40, 49, 63, 72, 76, 89, 91 and 94 % during the first 10 d of AMS milking, respectively. Faecal cortisol metabolites were not affected by the changeover. In UC milk ejection was disturbed during the first visits, mean milk yield at the first milking in the AMS was significantly lower as compared to that in the parlour (67 ± 7 %, $P < 0.05$), whereas milk ejection in EC was not disturbed. The total milk yield of the first 15 milkings differed significantly in UC (87.3 ± 2.4 %) and EC (108.8 ± 3.3 %) as compared to parlour yields (100 %, $P < 0.05$). In conclusion, cows with previous experience to AMS milking did not need a new adaptation period in the AMS after a transient period of parlour milking. In contrast, unexperienced cows do need an intensive adaptation to the AMS in order to minimise production loss.

Keywords: automatic milking; cattle; coping; cortisol; experience; heart rate

Introduction

Cattle respond to a changed environment with physiological and behavioural adaptations. Milking in automatic milking systems (AMS) is associated with environmental changes as compared to conventional parlour milking (Hopster et al., 2002; Weiss et al., 2004). The main difference between AMS and conventional milking is the cow's motivation to enter the milking stall. In conventional milking routines the cows are driven to the milking parlour, whereas in AMS the cows enter the milking stall voluntarily. However, a cow's motivation to get milked seems to be weak and very variable (Prescott et al., 1998). Therefore several approaches to attract an AMS visit have been intensively studied (Winter and Hillerton, 1995; Ketelaar-de Lauwere et al., 1998; Harms et al., 2002; Hermans et al., 2003). Concentrate feeding in the AMS milking box positively reinforces AMS visits. Additionally, forced or selectively forced cow traffic systems with roughage only available after passing the AMS are common (Harms et al., 2002). In adapted cows these specific changes did not negatively effect dairy cows' physiological regulation during milking as compared to conventional systems (Hopster et al., 2002). However, the change from conventional to automatic milking is associated with elevated heart rates and adverse behaviour towards the AMS during the first visits. Furthermore, the milk yield can be reduced due to the changeover (Weiss et al., 2004). These reactions do not represent a long-term negative effect for cow's welfare. Dairy cows are able to cope successfully within days to AMS milking. Comparable effects have been demonstrated due to the change from tie stall milking towards parlour milking (Macha et al., 1981).

There are indications for a remarkable memory potential in cattle (Kovalcik and Kovalcik, 1986). Thus indicates that dairy cows recognise a previously known environment even after a longer period being handled elsewhere. However, the effects of previous experience in being milked automatically on the change from conventional milking to AMS were for the best of our knowledge never studied before. The aim of the present study was to quantify the reactions of dairy cows, with and without previous experience in AMS milking, towards the change from conventional to AMS milking. The hypothesis was tested, that the individual cow's reaction differs according to their previous experience.

Material and Methods

Animals and Husbandry

Nine cows with and 17 cows without experience to be milked in an AMS were tested for their physiological and behavioural reactions to the changeover from conventional parlour milking towards automatic milking. The experienced cows (EC) were previously milked during one whole lactation in the experimental AMS. EC were dried off and housed during the dry period in a separate barn. After parturition these cows were milked for 36 ± 5 d of their second to sixth lactation in the milking parlour. The unexperienced cows (UC) were never before milked in an AMS. UC were in their first to their fifth lactation, primiparous UC were milked for 112 ± 4 d in the parlour prior to the changeover. All cows belonging to a herd of 100 Red-Holstein/Simmental crossbreed cows. The herd was housed in one single barn under identical feeding and management conditions. The diet consisted of maize and grass silage and concentrate according to the individual milk production. A maximum of 12 kg concentrate per day (approximately 50 % of the total ratio) was offered if daily milk yields exceeded more than 40 kg. Concentrate was omitted when daily milk yields declined below 14 kg. One half of the herd was milked in the AMS VMS (Delaval, 14721 Tumba, Sweden), the other half in a conventional herringbone milking parlour (DeLaval). Routine milking times in the parlour started at 4.30 and at 15.30. In the AMS cows were milked during their voluntary visits. A selectively forced cow traffic (Harms et al., 2002; Weiss et al., 2004) was applied. The feeding area was separated from the resting area by one-way gates, which allowed free access to the cubicles also without being milked. However, the cows were obliged to pass the AMS before entering the feeding area. A bypass was available for those cows, which had recently been milked. Cows had to pass the milking stall if milk yields of more than 7 kg were expected. Additionally concentrate feeding in the AMS milking stall positively reinforced AMS visits. When milking intervals exceeded 12 h the respective cows were manually driven to the AMS. UC were introduced in two groups in the AMS herd, balanced for age, lactational stage and milk yield, to prevent an overload of the AMS as previously described (Weiss et al., 2004). The first group of 8 UC was analysed whereas another 30 cows were regularly milked in the AMS. The second group of 9 UC switched to AMS milking in a herd of 38 AMS milked cows. The 9 EC changed from parlour to AMS milking whereas additionally 45 cows were milked in the AMS.

Experimental procedure

UC were trained to the AMS during 3 d, and the first milking was performed 4 d after the start of the training period (Fig. 1). During the training period UC were kept during daytime in the AMS area and were twice daily manually driven into the AMS stall. They were milked in the parlour twice daily and remained in the parlour herd during the night. After the start of milking in the AMS UC were driven to the AMS after milking intervals exceeded 12 h. EC were moved after the morning milking in the parlour to the AMS area. However, since all EC entered the AMS milking stall voluntarily within 5 h after the changeover to the AMS herd, no training period was applied in EC. Starting at 13.00, EC were manually driven to the AMS milking stall on d 1 to determine teat coordinates in the AMS milking stall.

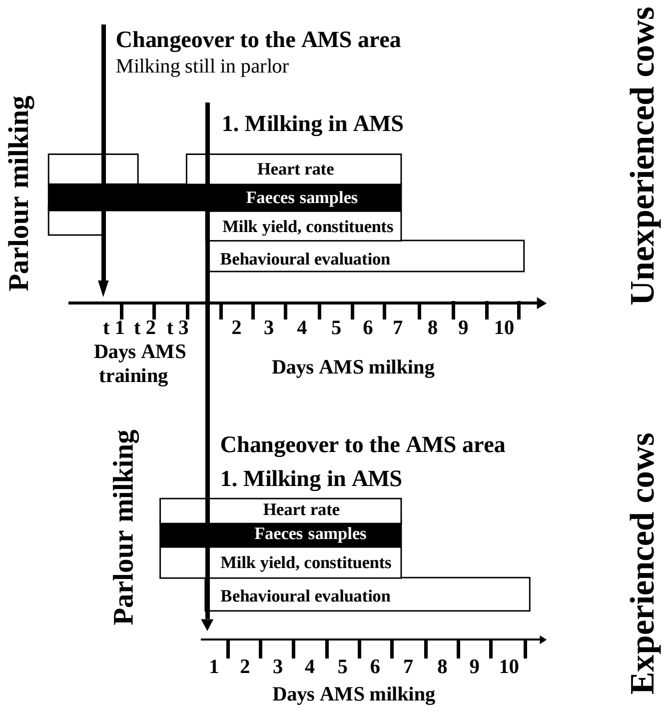

Fig. 1. Experimental protocol: In unexperienced cow (UC) a training period of 3 d was applied, whereas in experienced cows (EC) the training period was omitted. In UC the first arrow indicates the start of the training period. UC were driven twice daily to the automatic milking stall but were still milked twice daily in the parlour. The second arrow indicates the start of milking in the AMS, UC remained finally in the automatic milking area. EC were immediately milked in the automatic milking system. Milk recordings, heart rate measurement and faeces sampling were carried out as indicated by the bars.

Except for a period of 24 h on d 3 of the training period in UC the heart rate was recorded continuously in UC and EC throughout the experiment until d 6 of automatic milking (Fig. 1).

Furthermore heart rate was recorded during two successive parlour milkings in EC and UC before the changeover. The heart rate was measured by means of a commercial system developed for horses using electrodes fixed to a special belt around the chest (Polar Horse Tester, Polar Electro GmbH, 64542 Büttelborn, Germany) (Hopster et al., 2002; Weiss et al., 2004). The heart rate signal was saved as 15-s averages for further analyses. To determine the cortisol metabolites 11,17 Dioxoandrostanes (DOA) faecal samples were taken twice daily at 7.00 and 18.00 from the rectum (about 10 g) and were immediately frozen at –20 °C until further analyses. The DOA concentrations in the faeces was analysed as previously described by Möstl et al. (2002). The milk yield and composition were recorded during parlour milking the last 10 d before the changeover to the AMS and during AMS milking until d 6. To determine milk yield and for milk sampling Lactocorders (Werkzeug- und Maschinenbau Balgach, 9436 Balgach, Switzerland) were used during parlour milkings. In the AMS the installed standard milk meter and sampling device were used. Milk samples were analysed for fat, protein, lactose and somatic cell count (Milko Scan 6000, Foss GmbH, 22769 Hamburg, Germany).

Data processing and statistical analyses

Data are presented as means ± SEM. Statistical significance was set at $P < 0.05$. Due to the variable milking intervals in AMS, milk yields were handled as production rate per hour. Production rate was calculated as the quotient of the actual milk yield and the corresponding milking interval (Weiss et al., 2002; Weiss et al., 2004). To demonstrate effects of the changeover, milk yields obtained in the AMS were expressed as relative values of mean parlour yields during 10 d prior to AMS training. Likewise the milk constituents during AMS milkings were calculated as relative values of parlour results.

The mean of the lower 30^{th} percentile of the dataset of each individual cow was defined as basal heart rate. The heart rates in the parlour and in the AMS were defined as average heart rate above basal level (HAB) as previously described (Weiss et al., 2004). Data during the first 2 min after entering the milking stall were analysed.

For statistical evaluation the MIXED procedure of the SAS 8.01 (SAS, 1999) program package was used. The model included the date, the time of the day and the lactation number as random variables. The number of visits, the number of milkings, the lactational stage and the treatment (UC and EC) entered the model as fixed variables. The cow was included into the model as repeated effect using the covariance structure compound symmetry. Significant differences ($P < 0.05$) were localized by using the least significant difference test.

Results

Behavioural observations

At their first visit during the training period UC had to be pushed manually into the AMS stall. During the second and third visits the number of UC increased which needed only a gentle drive to the AMS stall to make them enter. However, after the third training day all UC

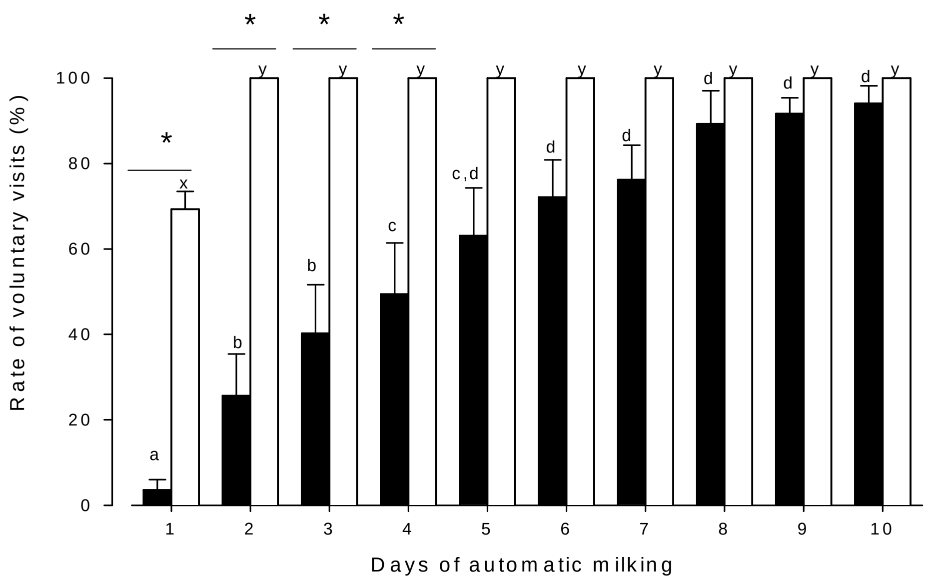

Fig. 2. Rate of voluntary visits (mean ± SEM, n = 17 UC and 9 EC) in the AMS milking stall during the first 10 d of automatic milking. Unexperienced cows (UC) were represented by the black bars, experienced cows (EC) by the white bars. * represent significant differences between EC and UC; a, b, c; x, y means without common superscript letter differ significantly within treatment (EC and UC) ($P < 0.05$).

were able to enter the milking stall without physical forces after they were driven into the waiting area in front of the milking stall. All EC entered the AMS milking stall voluntarily within 5 h when they were moved to the AMS herd after morning milking in the parlour. EC were once manually driven to the AMS stall starting at 13.00 to register the teat coordinates in the AMS stall. Thereafter, EC needed no more to be manually driven to the AMS during the experimental period. The rates of voluntary visits differing for UC and EC are shown in Fig. 2. Except for the voluntary visit of one cow all UC needed to be driven to the AMS on d 1 of milking. Throughout the first 10 d of milking a steadily increasing rate of voluntary visits was observed in UC. The rate of 90 % of voluntary visits in UC was achieved not until d 9 of automatic milking.

Heart rate

The basal heart rate varied between 61 to 85 beats per min (bpm). HAB during the first 10 visits in the AMS milking stall are shown in Fig. 3. HAB during parlour milking was similar

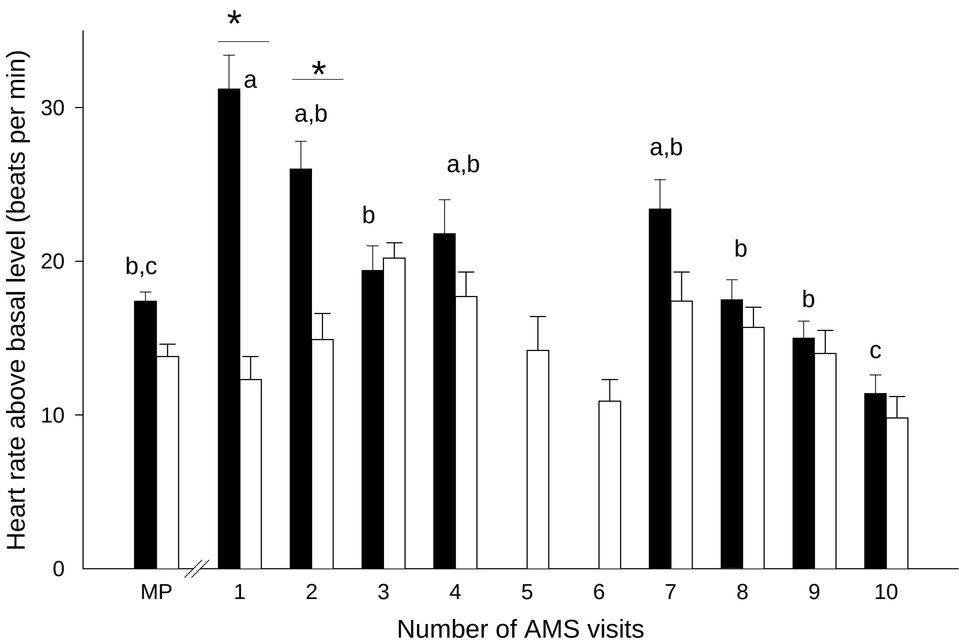

Fig. 3. Heart rate above baseline (mean ± SEM, n = 17 UC, 9 EC) during milking in the parlour (MP) and during visits in the AMS stall. Unexperienced cows (UC) were represented by the black bars, experienced cows (EC) by the white bars. * represent significant differences between EC and UC; a, b means without common superscript letters differ significantly ($P < 0.05$).

in UC and EC. HAB was higher during the first visit of UC in the AMS milking stall than in the parlour and also higher than at the first AMS visit of EC. During the 2nd visit HAB was still elevated in UC as compared to EC, but did not significantly differ from that in the parlour. In UC HAB results of the 5th and 6th visit are missing because heart rate measurements were not performed on d 3 of the training period in UC (Fig. 1). HAB in EC did not differ between parlour and AMS stall and did not significantly change with number of AMS visits.

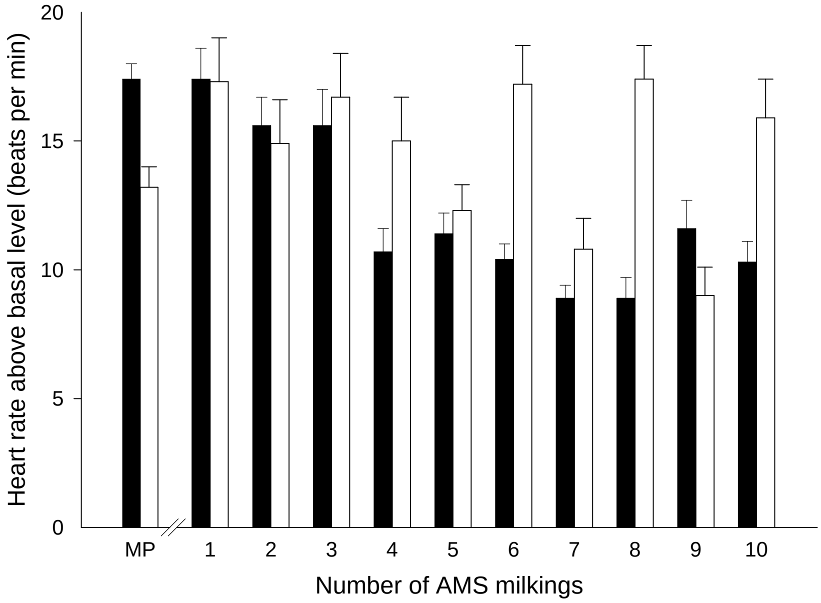

Fig. 4. Heart rate above baseline (mean ± SEM, n = 17 UC, 9 EC) during milking in the parlour (MP) and during visits were milking was performed in the automatic milking system. Unexperienced cows (UC) were represented by the black bars, experienced cows (EC) by the white bars. Neither an effect by the treatment (EC vs. EC) was observed nor effects due to the number of milkings.

HAB results during the first 10 AMS milkings are shown in Fig. 4. Neither an effect of the number of milkings nor an effect of previous experience in AMS milking (UC vs. EC) was observed. Furthermore, HAB during parlour milking was similar to that obtained in the AMS.

11,17 Dioxoandrostanes

The faecal DOA concentrations during the control period in the parlour were significantly higher in EC (243 ± 29 ng/g) than in UC (131 ± 12 ng/g). DOA concentrations during parlour milking and during the first days in AMS are shown in Fig. 5. DOA concentrations did not

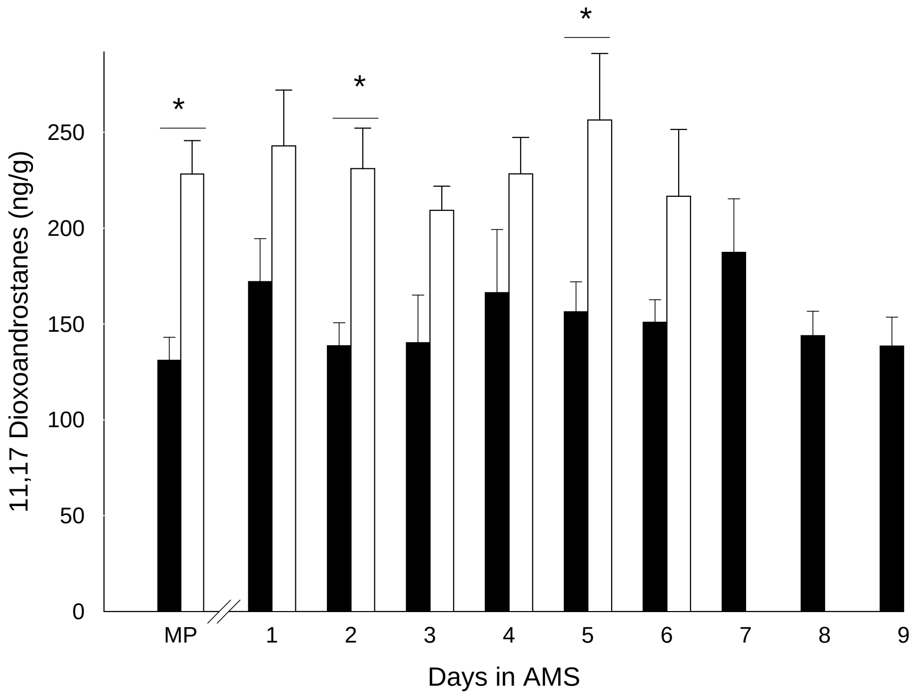

Fig. 5. 11.17 Dioxoandrostanes (DOA) concentrations in faeces (mean ± SEM, n = 17 UC, 9 EC) in the parlour (MP) and during the first d in the automatic milking system (6 d of AMS milking in EC, white bars; 3 d of training and 6 d of AMS milking in UC, black bars). * represent significant differences between EC and UC ($P < 0.05$).

differ between morning and evening sampling (159 ± 8, 184 ± 8 ng/g in UC and 228 ± 13, 222 ± 12 ng/g in EC for morning and evening sampling throughout the experimental period, respectively). DOA concentrations were significantly higher in EC than in UC during parlour milking and at the 2nd and 5th d after the changeover to AMS. However, the changeover itself had no effect on DOA concentrations, since neither in UC nor in EC the DOA results obtained in the parlor did not differ to any day after the changeover to AMS milking (effect of the day: $P = 0.48$). Fig. 6 presents mean DOA concentrations clustered for three levels of milk yield. DOA concentrations were high in high yielding cows irrespective of their previous experience to automatic milking. DOA concentrations tended to be lower ($P < 0.1$) in low yielding cows.

Milk yield and composition

The milk yield at the first milking in the AMS was higher in EC (96 ± 2 % of that obtained in the parlour) than in UC (67 ± 7 % of that obtained in the parlour). Milk yields of the first 15 AMS milkings are presented in Fig. 7. Individual milk yields in UC during the first milking

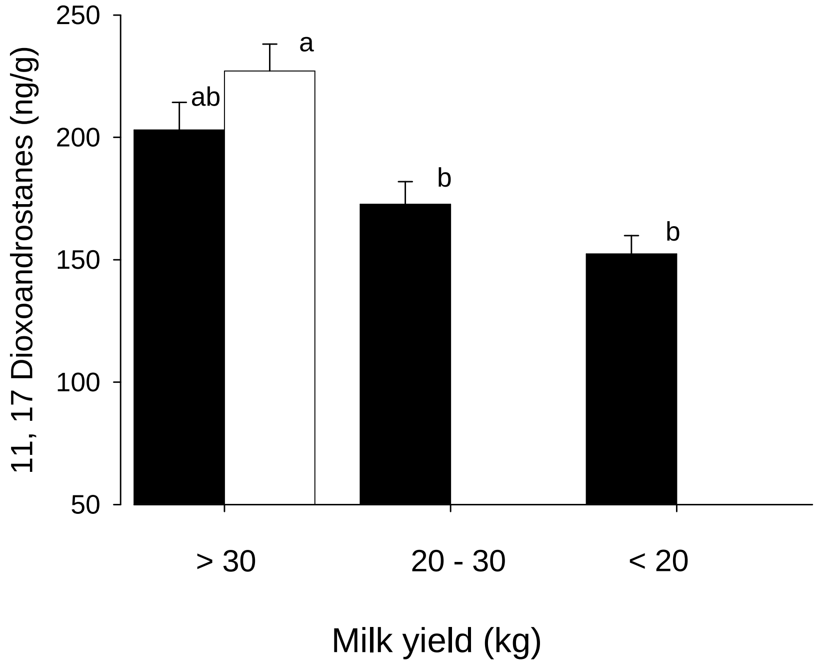

Fig. 6. 11.17 Dioxoandrostanes (DOA) concentrations in faeces (mean ± SEM) during the first 6 d of automatic milking in experienced cows (EC) and during the first 9 days in unexperienced cows (UC) clustered for daily milk yield (> 30 kg/ d: 4 UC and 9 EC, 20 - 30 kg/d 9 UC, < 20 kg/d 4 UC). Black bars represent UC, white represent bars EC. a, b means without common superscript letters tended to be different ($P < 0.1$).

varied between 8 % and 96 % of previous parlour yields. During 2nd and 3rd AMS milking milk yields in UC were similar to parlour yields whereas in further milkings milk yields were significantly reduced. Except for the second milking, relative milk yields were significantly higher in EC than in UC throughout the experimental period. Individual milk yields of the first AMS milking in EC varied between 85 % and 106 %. Milk yield in EC did not significantly differ from parlour yield. However, the milk yield during the first 15 AMS milkings was higher in EC (108 ± 1 % of that obtained in the parlour) than in UC (87 ± 1 % of that obtained in the parlour).

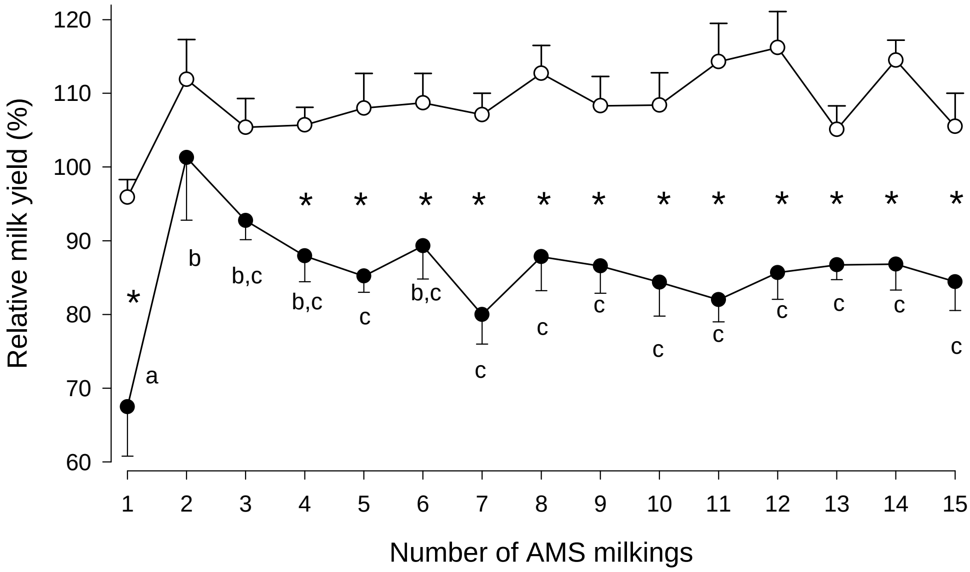

Fig. 7. Milk yield (mean ± SEM, n = 17 UC, 9 EC) expressed as relative values of parlour results (100% = mean parlour results of 10 d prior to the changeover). Black dots represent unexperienced cows (UC), white dots represent experienced cows (EC). * represent significant differences between EC and UC; a, b, c means without common superscript letters differ significantly (*P* < 0.05).

Relative milk yields during the first 15 AMS milkings are shown in Fig. 8. Lactational stages varied in UC between 24 and 316 d in milk, whereas all EC were in early lactation. As indicated relative milk yield during the first 15 AMS milkings did not differ between lactational stages in UC. Relative milk yield was higher in EC than in UC irrespective of the stage of lactation. However it has to be pointed out that no interaction between the stage of lactation and the relative milk yield after the changeover was observed in UC.

The milking interval for the first 15 AMS milkings was shorter in EC (7.8 ± 0.2 h) than in UC (9.8 ± 0.2 h). The milking frequency was therefore 3.07 ± 0.01 milkings per day in EC and 2.45 ± 0.02 milkings per day in UC. Neither milk constitutions nor somatic cell counts were significantly influenced by the changeover to automatic milking.

Discussion

In the present study physiological and behavioural effects of the change from conventional to automatic milking were evaluated. The investigated dairy cows varied with respect to their previous experience to milking in the AMS. Management and feeding was identical in both herds, except for the milking system. Furthermore both herds were housed in two compartments of the same barn. Therefore, the tested animals had to adapt only to the differences in the milking system. Interestingly, EC entered the AMS instantaneous without

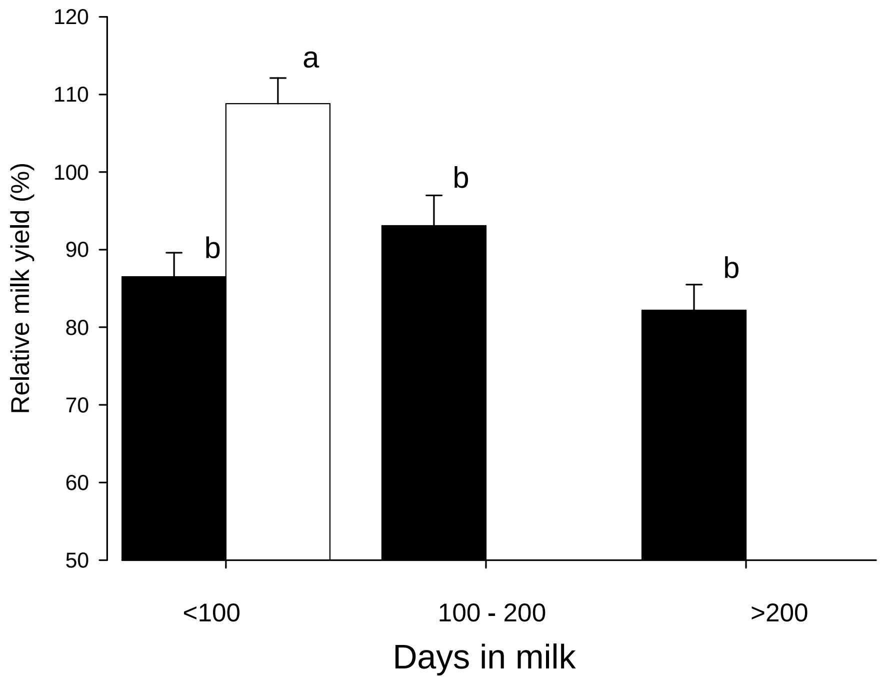

Fig. 8. Milk yield (mean ± SEM) expressed as relative values of parlour results clustered for lactational stage (< 100 d in lactation: 6 unexperienced cows (UC) and 9 experienced cows (EC), 100 - 200 d in lactation: 3 UC, > 200 d in lactation: 8 UC; 100% = mean parlour results of 10 d prior to the changeover). Black bars represent UC, white bars represent EC. a, b means without common superscript letters differ significantly ($P < 0.05$).

any human intervention after they were moved to the AMS herd. The rate of voluntary visits on d 1 was reduced, because it is essential to adjust the AMS according to the teat coordinates of the individual cow before the start of the first automatic milking. However, this visit, because of technical reasons, was the sole exception of manually moving EC to the AMS milking stall throughout the experimental period. The fact that EC did not use the AMS for about 80 d (dry period of six wk and 35 d parlour milking) and their immediate voluntary visit of the AMS, documents the considerable memory capacity of the dairy cow. These findings correspond to results of Kovalcik and Kovalcik (1986).

UC never entered the AMS voluntarily within the training period. Although one cow entered the AMS voluntarily at the first day of milking, the rate of voluntary visits achieved levels of more than 90 % not until d 9 of AMS milking. The rate of voluntary visits after successful adaptation to AMS milking corresponds to previous investigations in adapted cows (Ketelaar-de Lauwere et al., 1998; Winter and Hillerton, 1995; Harms et al., 2002; Hermans et al., 2003). However, it has to be pointed out that this level was approached not until d 9 of AMS milking. Therefore, considering the training period of 3 d, a successful automatic milking, without an excessive use of labour to move the cows, did not take place until d 12 (training and first days of milking) in the AMS.

Heart rate above basal level (HAB) in the individual cow was calculated. This was performed to prevent bias to the close correlation between baseline heart rate and daily milk yield as previously demonstrated (Weiss et al. 2004). During the first visit to the AMS the elevated HAB in UC indicated a high sympathetic activation. The elevation of heart rate was comparable to results demonstrated by Hopster et al. (1995) after cow-calf separation in dairy cows. Rushen et al. (1999) demonstrated that fear of dairy cows of an aversive handler resulted in a less pronounced heart rate elevation compared to the remarkable effects observed during the first visit to the AMS. However, it has to be considered that already during the second and third visits the HAB was similar to those recorded in the parlour. In agreement to the previously discussed behavioural results, HAB was not elevated in EC during the first AMS visits. The present results are in contrast to observations of Hopster et al. (2002), where reduced heart rates during AMS milking as compared to parlour milking were observed. This difference might be due to the fact that Hopster et al. (2002) calculated absolute heat rate values in contrast to the present investigation where heart rate was calculated on HAB basis. Furthermore in the present study HAB were calculated as means of the first two min after closing the gate in AMS or after entering the milking stall in the parlour whereas Hopster et al. (2002), demonstrated the progression during waiting before milking, until the end of milking.

DOA concentrations were not affected by the changeover, whereas Palme et al. (2000) reported a twice to trice fold increase in DOA concentrations as result of transportation in cattle. Obviously, the change to AMS milking did not cause a prolonged activation of the hypothalamic-pituitary-adrenal axis, neither in UC nor in EC. Although an elevation in the sympathetic activation in UC was observed, the change to AMS milking seems to be a minor stressor.

However, the differences in DOA concentrations between UC and EC cannot be explained. The management, the housing, the barn staff and, except for the used charges of feed, the feeding was similar in UC and EC. Furthermore the time of the year was almost similar, since UC were tested in October and November and EC in January. DOA concentrations may be affected by the level of milk production. However, a direct effect of the milk yield on plasma cortisol concentration is unlikely (Schwalm and Tucker, 1978). With increasing milk yields the fraction of concentrate in the total ration is increased, this might change the formation of microorganisms in cows intestine. Possible this could have been affected faecal DOA concentrations (Möstl et al., 1999).

Milk constituents were not affected due to the changeover process. Therefore the observed effect of a reduced milk yield in UC and enhanced milk yields in EC are probably due to a local effect in the mammary gland. As detailed discussed in a previous study (Weiss et al. 2004), the milk ejection in UC was obviously reduced during the first AMS milkings due to a disturbance of milk ejection (Bruckmaier et al., 1992; Bruckmaier et al., 1996; Rushen et al., 2001). This resulted in an additional leftover of milk in the udder (additional to the residual milk). A left-over of milk in the bovine udder can reduce milk secretion and immediately enhances apoptosis of the mammary secretory tissue by local regulation (Peaker and Wilde, 1996; Stefanon et al., 2002). Contrary, in case of an increased milking frequency, these local regulations can enhance milk secretion and can enhance proliferation of secretory tissue. Probably the enhanced milk yields in EC were due to the same regulatory background as the reduced milk yields in UC.

Conclusion

The change from conventional to automatic milking was remarkably different between dairy cows with and without previous experience. This points at the considerable memory potential of the dairy cow. Even after handling in another environment, experienced cows are immediately able to cope with AMS conditions. However, the change to automatic milking is a challenge for unexperienced cows. Therefore great efforts must be undertaken to minimise negative effects during the first few milkings. Once cows are adapted successfully to automatic milking, the change to AMS seems to be unproblematic.

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Variable milking intervals and milk composition

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Variable milking intervals and milk composition

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Milk composition was investigated at different milking intervals, lactational stages and degrees of udder filling in 3 experimental series. In experiment 1 18 dairy cows in early, mid and late lactation were milked at intervals of exactly 4, 8 and 12 h from previous milking. In experiment 2 38 cows were voluntary milked in an automatic milking system (AMS), at milking intervals ranging from 4 to 22 h. Actual yields of the experimental milkings were calculated as a percentage of the estimated storage capacity. Cisternal, main and residual milk samples were collected from 10 cows in experiment 3 at two subsequent milkings.

Milk fat and protein content were higher in late than in early lactation. Milk protein, lactose and somatic cell count were not influenced by the milking interval, whereas fat content decreased as well as milk production rate with increasing milking interval. Milk fat content was lowest at highest degrees of udder filling. The relation between milking interval, production rate and milk fat content followed linear regressions.

Variierende Melkintervalle und die Milchzusammensetzung

Zur Bestimmung des Einflusses variierender Zwischenmelkzeiten auf die Milchinhaltsstoffe und die Milchsynthese wurden 3 Versuche durchgeführt. Im ersten Versuch wurden 18 Kühe, jeweils 6 in früher, mittlerer und später Laktation exakt 4, 8 und 12 h nach der vorhergehenden Melkung gemolken. In einem zweiten Versuch wurden 38 Kühe in einem automatischen Melksystem (AMS) gemolken. Dabei schwankten die Zwischenmelkzeiten zwischen 4 und 22 Stunden. Für alle Melkungen wurde die Euterfüllung als Prozentsatz der maximalen Euterfüllung im zweiten Laktationsmonat angegeben. Lineare Regressionen für die Produktionsrate und die Milchinhaltsstoffe in Abhängigkeit der Zwischenmelkzeit wurden berechnet. In einem dritten Versuch wurde bei 10 Kühen mit Hilfe von exogenem Oxytocin die Residualmilch gewonnen und der Einfluss auf die Milchinhaltstoffe in der Zisternenmilch und im Hauptgemelk in der nächsten Melkung untersucht.

Milchfett- und Eiweißgehalte stiegen gegen Ende der Laktation an. Milcheiweiß- und Laktosegehalte sowie die somatische Zellzahl wurden durch die Zwischenmelkzeit nicht beeinflusst, aber der Milchfettgehalt wie auch die Produktionsrate verringerte sich mit zunehmender Zwischenmelkzeit. Der Milchfettgehalt war am geringsten bei höchster Euterfüllung. Für die Beziehung zwischen Produktionsrate, Milchfettgehalt und Zwischenmelkzeit konnten lineare Regressionen errechnet werden.

04 Milking (milking intervals, milk composition)

04 Milchgewinnung (Melkintervall, Milchzusammensetzung)

1. Introduction

Increased milk production with increasing milking frequency was repeatedly shown (1, 5, 6). Extremely prolonged milking intervals, e.g. milking once daily, result in decreasing production rates. Extremely short intervals with low degrees of udder filling cause a delay of milk ejection (3). Regulative mechanisms on milk production like paracrine inhibition (13) or intramammary pressure (2) are discussed, whereas specific effects on single milk components remain unclear. Effects of variable milking intervals as occurring in automatic milking systems (AMS) are therefore to be expected. In the present study effects of different milking intervals and different degrees of udder filling on milk production and milk composition were investigated. In addition, the effect of removal of the residual milk, containing extremely high amounts of fat, on the milk fat content of the succeeding milking was studied.

2. Materials and methods

2.1 Animals and husbandry

Clinically healthy experimental cows of the Holstein Friesian, German Fleckvieh and German Braunvieh X Brown Swiss breeds were used.

In experiment 1 each lactational stage (day 1 to 100, day 101 to 200 and day 201 to 300) was represented by 6 cows. All experimental milkings were performed during afternoon milking starting at 4 p.m., with fixed milking intervals of exactly 4, 8 and 12 h respectively. The 12 h interval experiment were performed exactly 12 h after morning milking. In order to obtain 8 and 4 h milking intervals, cows were additionally milked at 8 and 12 a.m. respectively. During all experimental milkings proportional milk samples were automatically taken via mobile milk flow recording system (Lactocorder, Foss GmbH D-22769 Hamburg).

In experiment 2 38 cows were voluntarily milked in an AMS (Merlin, Lemmer-Fullwood, D-53790 Lohmar) at coincidental milking intervals as determined by the cow. 16 cows were in early lactation (day 1 to 100), 13 in mid lactation (day 101 to 200) and 9 in late lactation (day 201 to 300). Milk samples of each milking were taken by an automatic sampling device (Shuttle, Lely NL-3155 PD MAASLAND) during a period of 2 weeks. Milk yields were determined by the measuring device of the AMS (Fullflow-Milkmeter, Lemmer-Fullwood, D-53790 Lohmar). The milking threshold for each cow was set at a minimum level of 4 kg of expected milk yield. Cows with prolonged milking intervals of more than 15 h were manually moved to the AMS.

In experiment 3 cisternal milk samples of 10 cows were taken by hand before milking. Additionally proportional samples were taken of the main fraction including stripping and of the residual fraction obtained after i.v. injection of 10 i.u. of oxytocin. During subsequent milking again samples of the cisternal and main milk fractions were collected.

2.2 Measurements of milk composition

Milk samples were analysed by the laboratory of the Milchprüfring Bayern e.V., D-80336 München, for fat, protein, lactose and somatic cell count (SCC) (Milko-Scan 4500, Foss Electric, DK-3400 Hillerod).

2.3 Mathematical calculations and statistical analysis

Data are presented as means ± SEM. Actual milk yield as percentage of maximum storage capacity was estimated to describe the degree of udder filling in classes of 0–20, 20.1–40, 40.1–60, 60.1–80 and 80.1–100%, respectively. Maximum storage capacity of the mammary gland in experiment 1 was estimated as half of the daily milk yield in month 2 of the respective lactation. In experiment 2 maximum storage capacity was estimated as the ratio of daily milk yield in month 2 of the respective lactation and the respective average number of daily milkings in month 2.

To avoid confounding of animal, lactational and nutritional status with the effect of milking interval standardised values of milk production rate and milk composition of each milking were calculated for linear regression analysis in experiment 2. Values of each experimental milking was expressed as a percentage of the individual mean value of each cow at the milking interval from 8 to 10 h.

For statistical evaluation analysis of variance (ANOVA) was calculated based on least square means using the GLM procedure of SAS (SAS 8.01). Influence of udder filling, animal, milking interval and stage of lactation were tested. The Least-Significant-Difference (LSD) test was used to distinguish between treatment means. The REG procedure of SAS was used to calculate linear regression functions.

3. Results

3.1 Experiment 1

Milk composition changed with lactational stage and milking interval. Milk fat content (Table 1) was higher in late than in early lactation and diminished with increasing milking interval. Milk protein content increased in late lactation, but stayed constant at different milking intervals (33.7 ± 21.2, 42.3 ± 1.6 g/l in early and late lactation, respectively). Lactose content was similar at all lactational stages and all milking intervals (48.1 ± 1.1 g/l).

Only in early lactation up to 100 % udder filling was observed (Table 2). As expected with decreasing udder filling degrees the mean lacational stage increased. Milk fat and protein contents were reduced with increasing degree of udder filling. Lactose content was highest with udder filling degrees of 0–20% and 60–80%. SCC was highest at lowest udder filling degree.

The observed classes of udder filling ranged from 20 to 100% (Table 3). In contrast to experiment 1 the degree of udder filling was related to the lactational stage, because cows reduced their milking frequency with progressive lactation. Average milking intervals were 8.58 ± 0.2, 9.15 ± 0.2 and 12.0 ± 0.3 h in early, mid and late lactation, respectively.

Table 1: Milk fat content at different milking intervals in different stages of lactation, Experiment 1

Parameter	Unit	Milking interval (h)	Early lactation	Mid lactation	Late lactation
Fat	g/l	4	47.0 ± 5.2^{Aa}	50.4 ± 6.2^{ab}	56.8 ± 3.8^{b}
		8	37.5 ± 4.8^{Ba}	46.2 ± 8.4^{ab}	54.8 ± 2.4^{b}
		12	34.5 ± 4.8^{Ba}	47.9 ± 9.8^{b}	51.7 ± 4.3^{b}

A,B: Means without common superscript letter within column are significantly different (p<0.05)
a,b: Means without common superscript letter within line are significantly different (p<0.05)

Table 2: Milk composition as a function of udder filling, Experiment 1

Parameter	Unit	0–20%	20.1–40%	40.1–60%	60.1–80%	80.1–100%
Observations	n	6	18	14	13	3
Lactation days	d	207 ± 20^{a}	159 ± 10^{b}	167 ± 20^{ab}	96 ± 20^{c}	50 ± 0^{c}
Yield	kg	2.4 ± 0.3^{a}	6.6 ± 0.4^{b}	10.9 ± 0.4^{c}	14.4 ± 0.8^{d}	19.0 ± 1.2^{e}
Mean udder filling	%	14.1 ± 1.6^{a}	28.5 ± 1.5^{b}	50.4 ± 1.5^{c}	68.0 ± 2.8^{d}	84.6 ± 1.1^{e}
Fat	g/l	68.5 ± 4.5^{ab}	51.9 ± 3.2^{a}	39.7 ± 2.3^{bc}	36.8 ± 3.6^{bc}	33.4 ± 5.3^{c}
Protein	g/l	43.6 ± 1.8^{a}	39.0 ± 1.0^{ab}	37.3 ± 0.7^{ab}	36.3 ± 1.2^{b}	33.4 ± 1.1^{c}
Lactose	g/l	48.2 ± 0.6	47.5 ± 0.3	47.4 ± 0.4	48.5 ± 0.2	48.0 ± 0.3
SCC	log_{10}/ml	5.38 ± 0.1	5.27 ± 0.1	5.20 ± 0.1	5.12 ± 0.1	5.01 ± 0.2

a, b, c, d, e: Means without common superscript letter within line are significantly different ($p<0.05$)

Table 3: Milk composition as a function of udder filling, Experiment 2

Parameter	Unit	20.1–40%	40.1–60%	60.1–80%	80.1–100%
Observations	n	15	219	326	113
Lactation days	d	110 ± 19^{ab}	136 ± 4^{b}	121 ± 4^{a}	110 ± 7^{a}
Yield	kg	6.0 ± 0.3^{a}	7.6 ± 0.1^{b}	9.5 ± 0.1^{c}	12.8 ± 0.3^{d}
Mean udder filling	%	36.6 ± 0.7^{a}	52.3 ± 0.3^{b}	69.0 ± 0.3^{c}	89.6 ± 0.6^{d}
Fat	g/l	48.7 ± 0.2^{a}	44.9 ± 0.5^{a}	41.0 ± 0.4^{b}	37.1 ± 0.6^{c}
Protein	g/l	35.8 ± 0.1	36.1 ± 0.2	35.9 ± 0.2	35.2 ± 0.3
Lactose	g/l	46.3 ± 0.1	47.1 ± 0.1	47.3 ± 0.1	47.6 ± 0.1
SCC	log_{10}/ml	4.92 ± 0.2	4.58 ± 0.0	4.54 ± 0.0	4.56 ± 0.0

a, b, c, d: Means without common superscript letter within line are significantly different ($p<0.05$)

3.2 Experiment 2

Milk fat content decreased with increasing udder filling whereas protein content was similar in all classes. Lactose content was slightly diminished at low udder filling. SCC was highest with lowest degree of udder filling.

As shown in Fig. 1 milk production rate and milk fat content were correlated with the milking interval. The calculated b gradients were –5.08%/h and –3.83%/h for production rate and milk fat content, respectively.

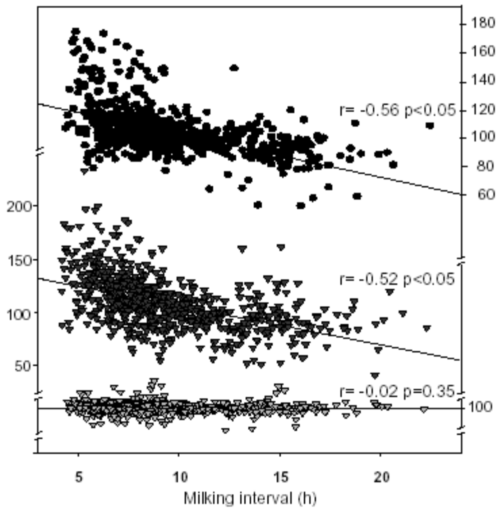

Fig. 1: Effect of milking interval on relative milk production rate, milk fat and milk protein content. ● Relative production rate (%), ▼ relative fat content (%), ▼ relative protein content (%)

3.3 Experiment 3

Fat content in cisternal milk was lowest compared to other milk fractions and did not differ before and after oxytocin treatment (Fig. 2). Collection of residual milk with supraphysiological amounts of oxytocin resulted in a reduced fat content of the main milk fraction of the subsequent milking compared to the milking before. Fat content of the cisternal milk fraction, however, remained unchanged. Fat production rate per hour was not changed when the residual fat yield was added to the following milking ($47.15 \pm 1{,}09$ g/h and $45.72 \pm 1{,}34$ g/h, respectively).

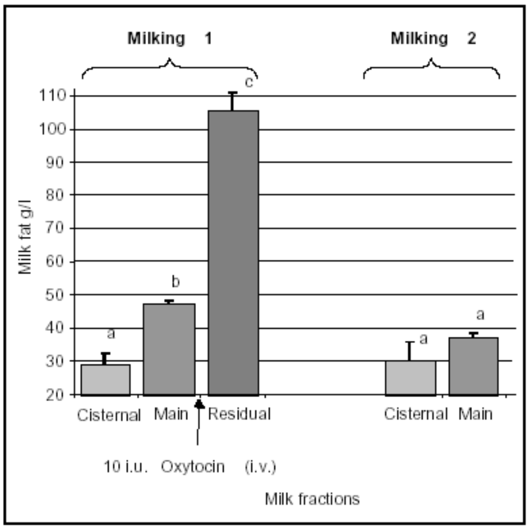

Fig. 2: Milk fat content in different milk fractions before an after application of oxytocin (10 i.u. i.v.)

4. Discussion

The degree of udder filling depends on milking interval and milk production rate, which is correlated with lactational status of the cow. If cows have free access to the milking stall they prolong their milking interval towards the end of lactation. This results in higher udder filling degrees towards the end of lactation in AMS compared to conventional milking. Due to a minimum milking threshold in the AMS (experiment 2) there were no milkings of extremely low udder filling.

Although there are differences in the distribution of the udder filling rates in experiment 1 vs. 2, both basically correspond concerning milk composition. The observed differences, the decrease in milk protein content and the faster decrease in milk fat content with increasing udder filling in experiment 1 compared to experiment 2, can be explained as effects of the lactational status. Lower milk fat and protein contents in early compared to late lactation was reported earlier (4), in experiment 1 the effects of lactational status and udder filling added up.

Our results demonstrate a decrease of fat content with increasing degree of udder filling. This observation agrees with previous findings of decreasing fat content with increasing milking intervals in cows (1, 5, 10) and ewes (9) in short-term experiments. These results are in contrast to observations on a herd based decrease of fat content with continuously increased milking frequency (6). Changes in nutritional status due to the increased milk yield in the last mentioned experiments may explain this contrast to our findings. Likewise it seems to have different effects if the milking interval is chronically changed (increased milking frequency in conventional milking systems) compared to alternating milking intervals (short-term experiments or automatic milking) resulting in a permanent change of udder filling at milking. The close correlation to the degree of udder filling, influenced by either the milking interval or the lactational stage, has previously not been shown. The continuos increase of milk fat throughout milking with highest values in the residual milk fraction was observed before (10). The unchanged milk fat content of the cisternal milk fraction and milk fat production rate at the subsequent milking after removal of residual milk favours the hypothesis that a certain amount of fat remains in the alveoli and the milk duct system from previous milk removal. This fat is not moved to the cistern between milking during transfer of cisternal milk. Only when a new milk ejection occurs this fat is shifted into the cistern. It is less diluted if less milk is ejected in case of a shorter milking interval. However, the increased fat production rate following increasing milking frequency remains unexplained. According to results of NAGRAO *et al.* (9) in ewes there seems to be an additional metabolic change in the mammary tissue. It seems possible that an increased shift of milk fat globules into the alveolar lumen occurs shortly after the milking procedure, therefore the rate of milk fat exocytosis in the alveolar lumen is higher with shorter milking intervals.

The decrease of milk protein content with increasing degree of udder filling observed in experiment 1 is due to changes in lactational stage. Udder filling has no influence on milk protein content which is shown in experiment 2: differences in degrees of udder filling are mainly related to different milking intervals. This leads to the conclusion that there the different lactational stages influence the milk protein content. Protein content is not influenced by different degrees of udder filling or different milking intervals.

The milk lactose content and the SCC remained without significant differences on comparable levels at all degrees of udder filling. This corresponds to previous findings (1, 5, 10, 9, 6). Lactose is the major osmotically active component of the milk because of its small molecular weight. The influx of water into the milk, *i.e.* the milk volume, is mainly determined by the lactose secretion. It is therefore not surprising that the lactose content remained similar at different degrees of udder filling.

Our results show a clear correlation between milk production and milk fat content with the degree of udder filling. In AMS there is a wide range of possible milking intervals (7, 12). Especially when extreme milking intervals occur, the precision of milk sampling once daily is not sufficient to estimate the real milk fat content. The shown linear regression can help to improve the precision of test day models in AMS.

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The acyclic period post partum in automatic and conventional milking

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Summary

The acyclic post partum period in cows is prolonged due to frequent sucking as compared to twice daily milking. In conventional milking systems twice daily milking is common, while in automatic milking systems (AMS) three or more milkings are performed in early lactation. In this study the hypothesis was tested that an increased milking frequency in AMS causes a delay in resumption of ovarian cyclicity post partum and increases days open in German Fleckvieh cows (n=124). Calvings were equally distributed throughout the one-year experimental period. All cows were housed in one barn. Sixty-three cows were milked in an AMS (AC) and 61 cows were twice daily milked in a conventional milking parlour (PC). Forty-two AC and 36 PC remained in the herd until conception. After parturition, milk samples were analysed for progesterone twice weekly to record the ovarian activity until pregnancy was diagnosed. The first and the second ovulation were determined by the progesterone profile of each individual cow.

The mean milking frequency during the first three weeks after parturition was higher in AC (3.17 ± 0.09 milkings/d) than in PC (milked twice daily). The individual mean milking frequency of AC for the first three weeks of lactation ranged from 2.0 to 5.9 daily milkings. The time span from parturition until the first and second ovulation did not differ between AC and PC. However, first ovulation was observed earlier in primiparous (23 ± 1 d) than in multiparous cows (29 ± 1 d, $P < 0.05$). Within AC no relationship between milking frequency and duration of the acyclic period was observed. Days open did not significantly differ between AC and PC and between primiparous and multiparous cows.

In conclusion, the increased milking frequency in AMS did neither cause a delay in resumption of ovarian cyclicity post partum nor did it increase days open.

Introduction

A delayed onset of post partum ovarian cyclicity in suckled beef cows as compared to non-suckled or twice daily milked beef cows was repeatedly reported (Yavas and Walton, 2000; Williams, 1990). However, effects of milking or sucking frequency on ovarian acyclicity (OA) in dairy cows are diversely discussed. A delayed first ovulation (Stevenson et al., 1997) and a decline in reproductive performance (Amos et al., 1985; Smith et al., 2002) occurred as a result of increased milking frequency in dairy cows. Milking frequency may affect OA post partum by two different pathways. The stimulation by the milking process itself may inhibit a final maturation of the follicles (Yavas and Walton, 2000). On the other hand an increased milking frequency may result in increased milk yields, which is associated with a more severe negative energy balance during early lactation (Reist et al., 2003).

Table 1: Number of ovulatory cows, parity, daily milk yield, milking frequency and reasons for withdrawal from the herd in the automatic milking system (AMS) and the parlour.

	Milking system	
Item	**AMS**	**Parlour**
Primiparous cows		
Cows observed	23	37
Parity	1 ^{A}	1 ^{A}
Daily milk yield during the first 21 d post partum	21.9 ± 4.9 (13.2 - 34.1) $^{A, a}$	17.9 ± 3.9 (11.3 - 28.8) $^{A, b}$
Number of daily milkings during the first 21 d post partum	3.3 ± 1.0 (2.1 - 5.9)	2
Excluded from the herd		
Low yield	1 ^{a}	12 $^{A, b}$
Reproduction problems	-	2
Milking problems or mastitis	2	-
Lameness	-	-
Breeding	1	4
Use for embryo transfer	-	-
Others	2	2
Multiparous cows		
Cows observed	40	24
Parity	2.8 ± 1.2 (2 - 6) ^{B}	2.8 ± 1.5 (2 - 8) ^{B}
Daily milk yield during the first 21 d post partum	31.0 ± 5.5 (22.3 - 42) $^{B, a}$	27.1 ± 4.3 (15.0 - 36.0) $^{B, b}$
Number of daily milkings during the first 21 d post partum	3.1 ± 0.5 (2.2 - 4.4)	2
Excluded from the herd		
Low yield	-	1 ^{B}
Reproduction problems	2	1
Milking problems or mastitis	3	3
Lameness	1	-
Breeding	2	2
Use for embryo transfer	3	1
Others	2	1

a, b: means with different superscript letters within a row are significantly different ($P < 0.05$). A, B: means with different superscript letters of corresponding parameters in primiparous and multiparous cows within a column are significantly different ($P < 0.05$). Values in brackets represent the full range of observed values.

In automatic milking systems (AMS), the milking frequency is determined by the individual animal which visits the milking stall voluntarily. The cows' individual milking intervals and milking frequencies vary widely within one herd (Weiss et al., 2002). During early lactation daily milking frequencies of three and more milkings are reported in AMS (Hogeveen et al., 2001). However, milking in AMS results in a high variability in cows' individual milking frequencies within one herd.

In the present study OA and days open were studied in AMS and conventional parlour milking twice daily. The hypothesis was tested that different milking systems and hence milking frequencies may influence the onset of post partum ovarian cyclicity.

Material and Methods

Animals and husbandry

Data from 124 German Fleckvieh dairy cows of parities one to eight were collected from January 2001 to December 2001. Calvings were equally distributed throughout the period of investigation. All cows were kept in a naturally ventilated loose housing barn. Feeding consisted of corn and grass silage and concentrate according to the individual production levels. Except for the milking system all management and housing conditions were identical for all animals.

63 cows (AC) were milked by an AMS (Merlin, Lemmer-Fullwood, 53790 Lohmar, Germany), whereas 61 cows (PC) were milked in a conventional herringbone milking parlour (Westfalia Landtechnik, 59299 Oelde, Germany). PC were milked twice daily at 05.00 and 16.00. AC were milked voluntarily throughout the day. AC which did not visit the milking stall for 14 h were manually driven to the AMS. The onset of ovarian cyclicity was observed in each cow. Because 48 cows were excluded from the herd before a successful conception, only 76 cows remained for evaluation of days open and number of services per conception (Table 1).

Ovarian activity and reproduction parameters

Skim milk samples were quantitatively analysed for progesterone by an enzymeimmunoassay (Meyer et al., 1986) twice weekly from parturition until pregnancy. Cyclic activity was evaluated based on the progesterone profile of the individual cow. If progesterone concentration in skim milk exceeded 2.5 nmol/l for a period of at least one week followed by a clear drop in progesterone concentration below 0.5 nmol/l, a corpus luteum was assumed to be present, indicating the occurrence of a preceding ovulation (Schopper et al., 1989). A subsequent increase of progesterone concentration within the following 10 days indicated the occurrence of the second ovulation. Fertility was controlled by a herd-fertility-program of the Bavarian Institute for Animal Breeding (Bavarian State Research Centre for Agriculture). A careful gynaecological examination was performed in each cow every second week to characterise the status of reproductive tract function and to detect possible health problems, from week two after parturition until a regular ovarian cycle was observed. Therefore, the determination of ovarian cyclicity by individual progesterone profiles was verified by the results of the herd-fertility database. The intervals between calving and first and second ovulation could thus be determined. The intervals from calving until first service, from first ovulation until first service and from calving until conception (days open) as well as the number of services per conception and the conception rate of the first service were calculated.

Milking frequency and milk yields

In both milking systems the manufacturers’ devices determined individual milk yields at each milking. In AC the date, the time and the yield of each milking was recorded. Milking frequency in AMS was calculated as milkings per 24 h over the first 21 days post partum and was used for further analyses. AMS milkings with unsuccessful attachments of single teat cups or incomplete milkings due to a kick off of teat cups were included.

Statistical analyses

Statistical analyses were performed using the SAS program package 8.01. Data are presented as means ± SD. The level of significance was set at $P < 0.05$. Means are presented separately for primiparous and multiparous AC and PC. Distribution of the number of cows in groups (primiparous and multiparous AC and PC, respectively), and the incidence of health disorders, were examined by binomial tests (LOGISTIC procedure). Differences between successfully inseminated cows and those excluded from the herd and between groups (primiparous and multiparous AC and PC, respectively) were analysed by ANOVA, based on least square means (GLM procedure).

Multivariate Cox models (PHREG procedure) were fitted by backward elimination procedures to determine significant interactions between OA and milking traits (Cox et al., 1972; Reist et al., 2003). Using a stepwise elimination the variables parity (dichotomised as 1 and >2), milking system (AMS or parlour), daily milking frequency (during the first 21 d post partum), standard deviation of daily milking frequency (during the first 21 d post partum) and the daily milk yield (during the first 21 d post partum) were analysed for significant effects. All variables were included in the basic models; factors were stepwise excluded until remaining factors were statistically significant ($P < 0.05$).

Results

Number of animals, yields, parity and the reasons for withdrawal of the herd are summarised in Table 1. In 63 AC and 61 PC the onset of the ovarian cycle was determined. AC yielded more than PC ($P < 0.05$), irrespective of parity. The milking frequency during the first 21 days post partum was 3.3±1.0 milkings per day in primiparous AC and 3.1±0.5 in multiparous AC, whereas PC were milked twice daily. The period of OA was studied in 23 primiparous and 40 multiparous AC, as well as in 37 primiparous and 24 multiparous PC. A total of 48 cows were excluded from further evaluation: 23 cows left the herd for breeding or due to low yields and 25 cows left the herd due to health problems. Thus, 76 cows remained in the experiment until conception.

In Table 2 the numbers of cows, milk yield and frequency and period of OA are presented separately for all cows and for those animals which were successfully inseminated. None of the studied parameters differed significantly between all studied cows and cows without a successful insemination.

The first and second ovulations were delayed in multiparous cows as compared to primiparous cows (Table 3). Contrary, the period from first ovulation until first service was prolonged in primiparous as compared to multiparous cows. Milk yield and milking frequency was higher in AC than in PC, but OA and conception parameters did not differ between AC and PC. The period from parturition until first service was similar in AC and PC. No effects of the parity on period from parturition until first service was observed.

Table 2: Number cows, parity, milk yield, milking frequency and ovarian acyclicity in the automatic milking system (AMS) and the parlour differing for the occurrence of conception.

Item	**Milking system**			
	AMS		**Parlour**	
	Interval from calving to first ovulation [1]	Interval from calving to conception [2]	Interval from calving to first ovulation [1]	Interval from calving to conception [2]
Primiparous cows				
Cows observed	23	17	37	17
Parity	1	1	1	1
Daily milk yield during the first 21 d post partum	21.9 ± 4.9	22.7 ± 4.6	17.9 ± 3.9	19.6 ± 3.6
Number of daily milkings during the first 21 d post partum	3.3 ± 1.0	3.4 ± 1.1	2	2
Time until first ovulation (d)	22.1 ± 7.1 ^{A}	22.7 ± 5.7 ^{A}	23.2 ± 6.3 ^{A}	23.4 ± 6.3 ^{A}
Time until second ovulation (d)	33.8 ± 8.4 ^{A}	35.2 ± 7.8 ^{A}	36.3 ± 9.2 ^{A}	36.3 ± 9.6 ^{A}
Multiparous cows				
Cows observed	40	26	24	16
Parity	2.8 ± 1.2	2.6 ± 1.2	2.8 ± 1.5	2.8 ± 1.7
Daily milk yield during the first 21 d post partum	31.0 ± 5.5	32.0 ± 5.2	27.2 ± 4.3	28.7 ± 3.5
Number of daily milkings during the first 21 d post partum	3.1 ± 0.5	3.2 ± 0.5	2	2
Time until first ovulation (d)	29.3 ± 9.1 ^{B}	29.0 ± 8.9 ^{B}	28.5 ± 7.9 ^{B}	28.6 ± 8.3 ^{B}
Time until second ovulation (d)	42.4 ± 11.1 ^{B}	42.2 ± 9.9 ^{B}	39.6 ± 9.0 ^{B}	40.6 ± 9.3 ^{B}

A, B: means with different superscript letters of corresponding parameters in primiparous and multiparous cows within a column are significantly different ($P < 0.05$). [1] Cows in which the first and second ovulation was observed. [2] Cows which were successfully inseminated.

The basic multiple cox model for analyses of the interval from calving until the onset of the ovarian cycle comprised milking system, parity (dichotomised as 1 or >2), milk yield, milking frequency and standard deviation of milking frequency (for the first 21 d after calving). The analyses included all data (primiparous and multiparous AC and PC). Coefficients, Hazard Ratio (HR), 95 % confidence intervals and P values of variable in the final model are presented in Table 4. Similar to results of the ANOVA the period between parturition and the start of the ovulatory activity was delayed in primiparous as compared to multiparous cows. The time from the first ovulation until first service was prolonged in primiparous cows compared with multiparous cows. However, none of the studied parameters was related to the milking system, the milking frequency or the milk yield.

The relative number of anovulatory AC and PC are presented in a lifetime analyses plot (Fig. 1). Cox proportional hazard models revealed no significant difference between AC and PC.

Discussion

In this study primiparous and multiparous cows were milked in an AMS or in a conventional milking parlour under identical housing and management conditions. Daily milking frequency and variation of milking frequency in AC were in the range described previously (Weiss et al., 2002). For technical reasons, mean parity was higher in AC than in PC. Therefore AC and PC data were presented separately as primiparous and multiparous AC and PC. Incidence of health disorders did not differ significantly between the four evaluated groups. An intensive breeding program was performed in the evaluated herd, including the use of an embryo-transfer-program. Therefore all available heifers at the farm were impregnated, in case of poor pedigree the heifers received an embryo. All heifers that obtained an embryo were milked in the parlour after parturition. In case of low milk yield they were removed from the herd. The rate of separation due to health disorders was similar to previous investigations (Reist et al., 2003). Cows that were excluded from the herd did not differ in parity, milking frequency and OA as compared to cows that were successfully inseminated.

Higher milk yields in AC were probably due to higher milking frequency in AC as compared to PC (Hogeveen et al., 2001, Kruip et al., 2002). Another reason might be that AC were selected for udder shape to ensure automatic teat cup attachment in AMS (Wufka and Willeke, 2001).

Table 3: Onset of ovulatory cycle and reproductive parameters in primiparous and multiparous cows in the automatic milking system (AMS) and the parlour.

	Milking system	
Item	**AMS**	**Parlour**
Primiparous cows		
Time until first ovulation (d) [1]	22.1 ± 7.1 (12-39) [a]	23.2 ± 6.3 (16-36) [a]
Time until second ovulation (d) [1]	33.8 ± 8.4 (20-49) [a]	36.3 ± 9.2 (24-55) [a]
Time until first service (d) [2]	49.2 ± 14.9 (31-95)	57.1 ± 13.4 (40-87)
Time from first ovulation until first service (d) [2]	26.6 ± 16.1 (0-73) [a]	33.8 ± 15.3 (11-71) [a]
Days open (d) [2]	66.2 ± 25.2 (40-125)	83.9 ± 36.2 (46-160)
Number of services per conception [2]	2.2 ± 1.1 (1-4)	1.6 ± 0.8 (1-3)
Conception at first service (%) [2]	35	56
Multiparous cows		
Time until first ovulation (d) [3]	29.3 ± 9.1 (15-55) [b]	28.5 ± 7.9 (18-49) [b]
Time until second ovulation (d) [3]	42.4 ± 11.1 (22-69) [b]	39.6 ± 9.0 (25-60) [b]
Time until first service (d) [3]	50.9 ± 17.1 (19-100)	46.9 ± 10.0 (30-64)
Time from first ovulation until first service (d) [4]	22.2 ± 15.7 (0-67) [b]	18.0 ± 11.0 (0-37) [b]
Days open (d) [4]	71.2 ± 29.9 (35-157)	70.5 ± 29.6 (41-147)
Number of services per conception [4]	1.8 ± 1.1 (1-5)	1.6 ± 0.6 (1-3)
Conception at first service (%) [4]	58	47

a, b: means with different superscript letters of corresponding parameters in primiparous and multiparous cows within a column are significantly different ($P < 0.05$). Values in brackets represent the full range of observed values. [1] 23 AC and 37 PC, [2] 17 AC and 17 PC, [3] 40 AC and 24 PC, [4] 26 AC and 16 PC.

The early start of ovarian activity post partum observed in the present study corresponds to previous investigations (Schopper et al., 1989; McLeod and Williams, 1991; Reist et al., 2003). The period of post partum OA did not differ between AC and PC. In agreement with results of Darwash et al. (1997) an earlier onset of ovarian cyclicity in primiparous as compared to multiparous cows was observed. However, neither milking frequency nor standard deviation of milking frequency was related to the period of OA and further evaluated reproductive parameters. These results correspond to findings of Kruip et al. (2002) in field studies and to data of Lamb et al. (1999) in beef cows. However, previously prolonged OA in conventional thrice daily milking as compared to twice daily milking were reported by

Stevenson et al. (1997) and Amos et al. (1985). Furthermore, Smith et al. (2002) figured out a reduction in fertility by increased milking frequency. However, it is remarkable that these

Table 4: Effects of the parity (primiparous vs. multiparous cows) on the interval from calving to first and second ovulation and on the interval from first ovulation to first service.

Observed effect	Trait	Category score	Coef.	S.E.	HR	95 % Confidence interval (HR)	P value
First ovulation	**Parity**	**Primiparous**	0.000	–	1.000	–	–
		Multiparous	-0.764	0.191	0.466	0.320 – 0.678	<0.001
Second ovulation	**Parity**	**Primiparous**	0.000	–	1.000	–	–
		Multiparous	-0.591	0.194	0.554	0.379 – 0.809	0.0023
Time from first ovulation until first service	**Parity**	**Primiparous**	0.000	–	1.000	–	–
		Multiparous	0.537	0.237	1.711	1.075 – 2.724	0.0236

Parity (primiparous vs. multiparous cows) significantly affected the indicated parameters ($P < 0.05$). None of the studied milking parameters were related to the occurrence of the first and second ovulation post partum ($P < 0.05$). Coef.: coefficient of the linear predictor; HR: hazard ratio; S.E.: standard error.

studies were examined either under field conditions (Smith et al., 2002; Kruip et al., 2002) or resulted in a significant increase in milk production as a result of increased milking frequency (Amos et al., 1985; Stevenson et al., 1997). Therefore, these studies have to deal with bias by different herds and management conditions or due to a changed milk yield. In the present study the milking frequency was higher in AC than in PC, whereas both groups were managed under identical conditions. Additionally, the milking frequency in AC varied

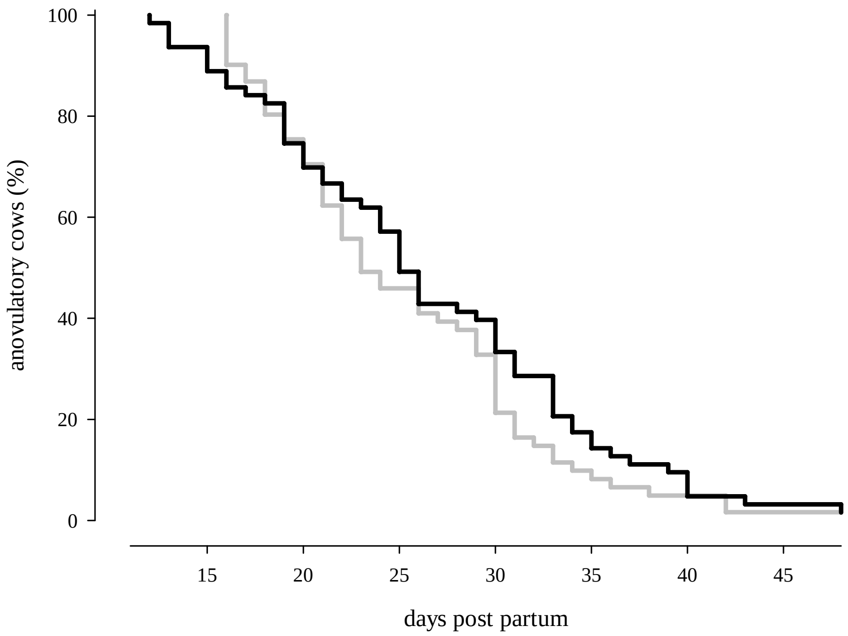

Fig. 1. Lifetime analyses plot, for automatic milked cows (AC) and parlour cows (PC), relative number of anovulatory cows vs. days post partum. Cox proportional hazard models revealed no differences between AC and PC.

continuously between 2.1 and 5.9 milking per day, which enabled an analysis within AC. However, in the present study neither an effect of the milking system nor an effect of the milking frequency within the AC was observed.

Recent studies focused on the impact of the energy balance (Beam and Butler, 1999; Butler, 2000; Koller et al., 2003; Reist et al., 2003) indicating that a more severe negative energy balance reduces reproduction performance. Beam and Butler (1999) and Butler (2000) discussed effects on both, OA and conception rate. Contrary Reist et al. (2003) could observe only a reduction of conception rate, while OA was not affected.

Increased milking frequency results in increased milk yield (Kruip et al., 2002; Smidt et al., 2002). Previously reported interactions between an increased milking frequency and reduced reproduction performance are likely due to a more pronounced negative energy balance as a result of increased milk yields. Although an increase in milk yield was observed in AC, the obtained milk yields in the present study were on a moderate level, due to the used dual-purpose breed German Fleckvieh. Therefore an excessive negative energy balance at the start of lactation, as reported for high yielding dairy cows (Reist et al., 2003), was not to be expected in this study. Probably not the stimulation by the milking process itself, but rather enhanced milk yields are responsible for a reduced fertility of more frequently milked dairy cows.

Conclusions

Milking frequencies with up to 5 milkings per day, as observed in the present study, did neither considerably affect the onset of the ovarian cyclicity nor the fertility in dairy cows. A reduced fertility as a result of an increased milking frequency is probably due to a more severe negative energy balance in case of very high milk yields.

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