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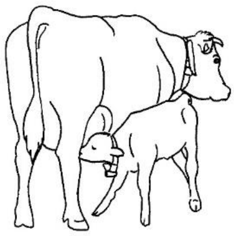

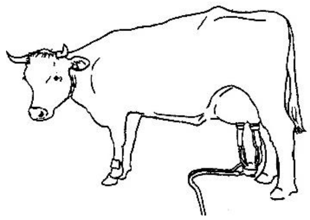

How to tackle alveolar milk ejection problems during milking in dam rearing?

influence of different stimuli in the parlour and effects of half-day compared to free contact

Dissertation zur Erlangung des akademischen Grades

eines Doktors der Agrarwissenschaften (Dr. agr.)

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„Die Mutter spielt im Leben des Kalbes also nicht nur die Rolle einer Nahrungsquelle, sondern auch die eines Sozialpartners höchster Attraktivität.“

(Viktor Reinhardt, 1980, p. 21f.)

„Man darf nie an die ganze Straße“, die man fegen will, „auf einmal denken, verstehst du? Man muss nur an den nächsten Schritt denken, an den nächsten Atemzug, an den nächsten Besenstrich. Und immer wieder nur an den nächsten.“ Wieder hielt er inne und überlegte, ehe er hinzufügte: „Dann macht es Freude; das ist wichtig, dann macht man seine Sache gut. Und so soll es sein.“

(Beppo Straßenkehrer in Michael Ende’s „Momo“)

SUMMARY

In dam rearing, where dairy cows nurse their calves for several weeks while being milked, the farmer may loose considerable amounts of harvested milk, partly due to disturbed alveolar milk ejection. Therefore the objective of this thesis was to improve the machine gained milk yield in dam rearing. The influence of acoustic, olfactory and manual stimulation in the parlour on cows with free or no calf-contact were investigated. Beside parameters of milk let-down, agitation behaviour, heart rate (HR) and heart rate variability (HRV) were assessed to analyse if dams are stressed during milking. This could be a reason for impaired alveolar milk ejection. The second approach was to reduce mother-calf-contact: free, half-day and no cow-calf-contact were compared.

In a preliminary study (Chapter 2), it was investigated whether cows behaviourally respond to calf hair presented in a tandem milking parlour, and whether this is affected by suckling the own calf or not. Discrimination between hair of the own calf in a thin cloth bag ('own'), hair of an alien calf ('alien') and a control cloth bag without calf hair ('no') was tested among 17 multiparous and 6 primiparous cows with free calf-contact ('contact') and 13 multiparous and 4 primiparous cows separated within 12 h after parturition from their calves ('control'). Both groups were milked twice daily in a tandem milking parlour, where they were individually tested in six consecutive milkings (trials) starting between the 12th and 20th day of lactation. Two of three olfactory stimuli were presented simultaneously. Sniffing or licking of the stimuli during the first minutes of milking (response duration in % of total observation time) and number of trials with any response (frequency of responses) were recorded. Data were analysed using non-parametric tests. Calf hair ('own' or 'alien') elicited responses in 60% of the animals at least once, but altogether there were only overt responses in 23% of trials. Significant differences in responsiveness towards the different stimuli were found in terms of frequency of responses for all cows (n = 28 without missing data, p = 0.003). Response duration differed significantly for all responsive multiparous cows (n = 12, p = 0.049) and in tendency for all responsive heifers (n = 8, p = 0.061) and for responsive 'contact' cows and heifers (n = 11, p = 0.034). In all these cases, responses were highest for 'own', intermediate for 'alien' and lowest for 'no'. In the post hoc tests, no significant differences between 'own' and 'alien' could be detected. Despite low response rates to the presented olfactory stimuli in general, it can be concluded that the responsive multiparous cows and 'contact' heifers were able to perceive the presented calf odour and preferred to sniff/lick those stimuli compared to a stimulus with 'no' odour.

Out of the cows of the pilot study 15 dams and 22 'control' animals took part in the next experiment (Chapter 3). Both were conducted at the research farm of the Thünen Institute, Trenthorst, where cows were loose housed in two similar cubicle pens with one calf creep available each. In this study, dams and 'control' cows were compared during milking concerning machine collected milk yield, machine-on time, milk flow characteristics, milk fat content, somatic cell score (SCS), agitation behaviour, HR and HRV. During three consecutive weeks (26th to 50th d in milk) in each week one of three treatments were

conducted during milking in the parlour and results were compared with those from routine milking with vibration stimulation in the same week: played-back calls of calves before milk feeding (acoustic), hair of the own calf in a thin cloth bag (olfactory), teat massage following pre-milking and udder cleaning (in total 60 sec, manual). Mixed models were applied. Over all treatments machine collected milk yield (-9.9 kg per milking), fat content (-0.66%) and milk flow characteristics of dams were lower than in 'control' animals (all tests: $p < 0.0001$, effect size $r > 0.70$). SCS as indicator of udder health did not differ between groups ($p = 0.4111$, $r = 0.13$). There was no impact of 'contact' on rumination, stepping, kicking, HR and some parameters of HRV (RMSSD, SDNN, HF%) in the parlour. Dams showed a tense head position ($p = 0.0007$, $r = 0.56$) and defecated ($p = 0.0125$, $r = 0.50$) at more milkings than cows without calf contact. On the other hand, some characteristics of HRV differed between 'contact' animals and the 'control' (LF%, LF/HF; $p < 0.05$, $r > 0.30$), indicating a higher vagal activity in dams. Reason for this may be a generally higher vagal activity due to suckling, which could also result in higher gut motility and therefore a higher defecation frequency. None of the treatments had great impact on the animals. Manual stimulation increased the mean milk flow during the main milking phase. However, this is possibly due to technical differences compared to vibration stimulation without effects on harvested milk. Acoustic stimulation led to lower SCS compared to routine milking, but only in dams (interaction: $p = 0.0023$). This result is hard to interpret. In conclusion, it was not possible to enhance milk let-down in dams with free calf-contact through acoustic, olfactory and manual stimulation.

As the applied stimuli did not enhance milk let-down in dams, in a third experiment at the research farm of the University of Kassel the influence of different durations of cow-calf-contact during the day was assessed (Chapter 4). After full-day cow-calf-contact for three days post partum in a calving pen, 11 cows had half-day calf-contact between morning and afternoon milking (ca. 10 h 45 min, 'half-day') and 13 cows had free calf-contact (24 h, 'free'). Both groups were housed each in a deep litter pen with a calf creep. Cows of the 'no contact' group were separated from their calf half a day post partum and were moved into a third deep litter area one day after birth. Control calves were moved to individual calf igloos and were group-housed after the first week of life in a deep litter system. They were fed a maximum of 2x3 L heated whole milk d^{-1} from teat buckets. All cows were milked twice daily. After nine weeks of 'nursing', calves with dam-contact were moved to a pen, were they could see, but not suckle or touch the mother, and were trained to drink whole milk from a teat bucket (2x3 L d^{-1}, 'in sight+milk feeding'). In the 11th - 12th week of life these calves were housed with the 'no contact' calves, where they no longer could see their mothers, and gradual weaning started (4 - 2 L d^{-1}, 'out of sight+weaning'). Data assessment ended two weeks after weaning (13th - 14th week of life, 'post weaning'). The influence of contact-treatment and experimental phase on machine milk yield, milk content, SCS and percentage of milkings with SCC >100,000 cells ml^{-1} were analysed with mixed models. ANOVA was used to analyse the effect of treatment on the average daily milk yield of the lactation (220 - 305 days in milk), calving-interval and daily weight gain of calves during 'nursing' and 'in sight+milk feeding'. After the latter phase all male calves were sold for fattening, resulting in a decreased sample size. Incidences of mastitis and calf data during ‘out of sight+weaning’ and ‘post weaning’ were analysed for potential differences using non-parametric tests.

Daily milk yield during ‘nursing’ was on average 9.9 kg lower in ‘half-day’ compared to ‘no contact’ cows ($p = 0.0054$, $r = 0.48$) and 3.6 kg higher compared to ‘free’ cows ($p = 0.0576$, $r = 0.32$). Nearly 80% of the machine milk yield of ‘half-day’ cows could be harvested after the separation from the calf overnight, during morning milking. Over the whole lactation, ‘half-day’ milk yields were in tendency higher than ‘free’ ($p = 0.0889$, $r = 0.31$) and not significantly different from ‘no contact’ ($p = 0.2193$, $r = 0.23$). In both dam rearing groups milk fat content was about 1 percent point lower during the ‘nursing’ phase. The lower machine milk yield and fat content indicates a disturbed alveolar milk ejection in both groups with calf-contact. This, however, did not negatively affect parameters of udder health. Somatic cell counts did not differ between treatments ($p > 0.1$), however mastitis incidences were high in all treatments. Protein content was 0.15 - 0.30 percent points higher and lactose content 0.17 - 0.33 percent points lower in dam rearing groups than in ‘no contact’ animals during ‘nursing’. After ‘nursing’ ended, machine milk yield and fat and lactose contents of dams increased. Protein content in ‘half-day’ and ‘no contact’ differed between phases, but remained stable in ‘free’ cows. Calving interval was not affected by calf-contact ($p = 0.714$). Weight development of dam-reared calves was similar. ‘No contact’ calves’ gained significantly less weight during ‘nursing’ ($p < 0.0001$) and more during ’in sight+milk feeding’ than ‘half-day’ calves ($p < 0.05$). During the following weeks, weight gain of dam reared calves increased, but numerically remained under the level of ‘no contact’ calves ($p > 0.1$). Two weeks after weaning, however, dam reared calves’ body weight was still higher than the body weight of restrictedly fed calves ($p < 0.05$). In conclusion ‘half-day’ contact helped to reduce milk losses during dam rearing, while calf development did not differ from ‘free’ contact.

ZUSAMMENFASSUNG

Bei der muttergebundenen Kälberaufzucht säugen Milchkühe ihr eigenes Kalb über mehrere Wochen hinweg und werden zusätzlich gemolken. Dabei kann der Verlust an ermolkener Milchmenge für den Betrieb sehr groß sein. Zum Teil liegt dies an einer gestörten Alveolarmilchejektion beim Melken. Ziel der vorliegenden Dissertation war es deshalb, die ermelkbare Milchmenge bei der muttergebundenen Kälberaufzucht zu erhöhen. Untersucht wurden mögliche Effekte akustischer, olfaktorischer und manueller Stimulation während des Melkens im Melkstand bei Kühen mit freiem und ohne Kalb-Kontakt. Da Stress beim Melken eine Ursache für eine gehemmte Alveolarmilchejektion im Melkstand sein kann, wurden neben Parametern der Milchabgabe auch Unruheverhalten, Herzfrequenz (HR) und Herzfrequenzvariabilität (HRV) erfasst. In einem weiteren Ansatz wurde untersucht, wie sich eine Reduktion der Kontaktstunden pro Tag auf die Leistung von Kühen und Kälbern auswirkt. Dabei wurde halbtägiger mit freiem Kontakt und einer Kontrolle ohne Kalb-Kontakt verglichen.

In einer Pilotuntersuchung (Kapitel 2) wurde zunächst überprüft, ob sich Kälberhaare als olfaktorischer Stimulus im Tandem-Melkstand eignen. Dazu wurden die Verhaltensreaktionen von 17 multiparen und 6 primiparen Kühen mit freiem Kalb-Kontakt und 13 multiparen und 4 primiparen Kühen ohne Kalb-Kontakt gegenüber drei verschiedenen Geruchsvarianten ausgewertet: (1) Haare vom eigenen Kalb in einem dünnen Stoffsäcken, mit dem das Kalb zuvor abgerieben wurde („Eigen“), (2) Haare von einem fremden Kalb in einem dünnen Stoffsäcken, mit dem das Kalb zuvor abgerieben wurde („Fremd“) oder (3) ein Stoffsäckchen ohne Kälberhaare („Kontrolle“). Zwischen dem 12. und 20. Laktationstag wurden an sechs aufeinanderfolgenden Melkzeiten zu jeder Melkzeit zwei der drei Geruchsvarianten in verschiedenen Kombinationen in Edelstahlkörbchen in den Melkboxen des Tandem-Melkstands präsentiert. Es wurden die Anzahl reagierender Tiere, die Anzahl der Melkzeiten mit Reaktion und die Dauer der Reaktion innerhalb der Zeit vom Einlegen des ersten Säckchens bis zum Melkbeginn (Riechen oder Lecken, in % der Gesamtbeobachtungszeit) erfasst. Die Daten wurden mittels nicht-parametrischer Tests ausgewertet. Es reagierten 60% aller Kühe mindestens einmal auf ein Säckchen mit Kälberhaaren. Allerdings lag der Anteil der Melkzeiten mit Reaktion bei nur 23%. Numerisch zeigten mehr Kühe mit als ohne Kalbkontakt eine Reaktion auf „Eigen“ oder „Fremd“ (70% vs. 47%). Bei Betrachtung aller wenigstens einmal reagierenden Tiere mit vollständigen Datensätzen unterschied sich die Anzahl der Melkzeiten mit Reaktion pro Tier signifikant zwischen den verschiedenen Stimuli (N = 28, P = 0,003). Die Reaktionsdauer der reagierenden multiparen Kühe beider Gruppen und die Reaktionsdauer der regierenden Kontakttiere beider Paritäten unterschieden sich signifikant zwischen den Stimuli (N = 12, P = 0,049; N = 11, P = 0,034). Primipare Tiere beider Gruppen reagierten häufiger, waren jedoch weniger selektiv in ihrem Verhalten. So unterschied sich die Reaktionsdauer bezüglich der verschiedenen Proben nur tendenziell (N = 8, P = 0,061). Haare des eigenen Kalbes lösten stets die stärksten und die „Kontrolle“ die schwächsten Reaktionen aus; die Reaktionen gegenüber „Fremd“ lagen dazwischen.

Post-hoc Tests konnten jedoch keine signifikanten Unterschiede zwischen „Eigen“ und „Fremd“ aufzeigen. Generell sank mit der Anzahl der Testmelkungen die Reaktionsdauer und die Anzahl der reagierenden Tiere. Gründe hierfür könnten einerseits ein Gewöhnungseffekt sein, andererseits könnte auch die Intensität des Geruchs mit zunehmender Lagerzeit abgenommen haben. Obwohl die eingesetzten olfaktorischen Stimuli nur in geringem Maße Verhaltenssreaktionen auslösten, kann geschlussfolgert werden, dass die reagierenden multiparen Kühe beider Gruppen und die primiparen Kontakttiere den Kalbgeruch wahrnehmen konnten und bevorzugt an Proben mit Kälberhaaren rochen oder leckten.

Fünfzehn kalbführende Kühe und 22 Kontrolltiere der Pilotuntersuchung nahmen auch Teil im nächsten Projekt (Kapitel 3). Beide Untersuchungen wurden auf dem Versuchsbetrieb des Thünen Instituts in Trenthorst durchgeführt, wo die Kühe in zwei identisch gestalteten Laufstallabteilen mit Liegeboxen und einem Kälberschlupf pro Abteil gehalten wurden. In diesem Versuch wurden ermelkbare Milchmenge, Maschinenhaftzeit, Milchflusscharakteristika, Milchfettgehalt, somatische Zellzahl (als SCS), Unruheverhalten, HR und HRV erfasst. Während drei aufeinanderfolgender Wochen (26. bis 50. Laktationstag) wurde in jeder Woche an vier Melkzeiten eine von drei Stimulationen im Melkstand angewandt und mit Routinemelkungen während derselben Woche verglichen. Die Stimulationen bestanden aus Abspielen aufgezeichneter Rufe von Kälbern vor der Milchtränke (akustisch), Haaren des eigenen Kalbes in einem dünnen Stoffsäckchen, mit dem das Kalb zuvor abgerieben worden war (olfaktorisch) und Zitzenmassage, die auf das normale Vormelken und die Euterreinigung folgte (insgesamt 60 Sekunden, manuell). Beim Routinemelken wurde nach der beschriebenen Eutervorbereitung von ca. 20 Sekunden, eine 40-sekündige Vibrationsstimulation angewandt. Die Daten wurden mittels gemischter Modelle ausgewertet. Insgesamt waren die ermelkbare Milchmenge (-9,9 kg pro Melkzeit), der Fettgehalt der Milch (-0,66%) und der Milchfluss der kalbführenden Kühe im Vergleich zur Kontrolle reduziert (alle Vergleiche: $P < 0,0001$, Effektgröße: $r > 0,70$). Die somatische Zellzahl (SCS), als Indikator der Eutergesundheit, unterschied sich nicht zwischen den Gruppen mit und ohne Kalb-Kontakt ($P = 0,4111$, $r = 0,13$). Das Verhalten während der Eutervorbereitung, Wiederkäuen, Trippeln und Treten, die HR und einige Parameter der HRV (RMSSD, SDNN, HF%) im Melkstand unterschieden sich nicht zwischen Kühen mit und ohne Kalb-Kontakt. Kalbführende Kühe zeigten während mehr Melkzeiten eine angespannte Kopfhaltung (13,1% vs. 1,2%, $P = 0,0007$, $r = 0,56$) und Abkoten (8.7% vs. 4.6% der Melkzeiten, $P = 0,0125$, $r = 0,50$). Andererseits wiesen einige Parameter der HRV (LF%, LF/HF) auf eine erhöhte parasympathische Aktivität der kalbführenden Kühe hin ($P < 0,05$, $r > 0,30$). Grund hierfür könnte eine generell erhöhte parasympathische Aktivität durch das Säugen und die damit einhergehenden hormonellen Veränderungen sein. Im Zusammenhang damit könnten auch eine erhöhte Darm-Peristaltik und vermehrtes Abkoten stehen. Keine der eingesetzten Stimulationen hatte einen bedeutenden Effekt auf die Tiere. Das durchschnittliche Minutenhauptgemelk war während der manuellen Stimulation verringert ($P = 0.0494$, $r = 0,10$), was jedoch wahrscheinlich durch technische Unterschiede beim Melken mit und ohne Vibrationsstimulation verursacht war. Die robusteren Parameter der Alveolarmilchejektion, wie ermelkbare Milchmenge und Fettgehalt, blieben von der Zitzenmassage unbeeinflusst. Während der akustischen Stimulation war der SCS der Mütter niedriger als beim Routinemelken, während er bei der Kontrollgruppe konstant blieb (Interaktion: P = 0,0023). Hierfür gibt es keine offensichtliche Erklärung. Zusammenfassend hatten die angewandten Stimulationen im Melkstand keinen Effekt auf die gestörte Milchabgabe bei freiem Kuh-Kalb-Kontakt. Das Säugen beeinflusste den physiologischen Zustand der Mütter in positiver Weise, jedoch führte der Melkvorgang zu mehr Anspannung als bei Kühen ohne Kalb-Kontakt.

Da die eingesetzten Stimuli nicht zu einer Verbesserung der Milchabgabe der säugenden Kühe beim Melken führten, wurde in einem dritten Experiment auf dem Versuchsbetrieb der Universität Kassel der Einfluss von unterschiedlich langem Kuh-Kalb-Kontakt pro Tag untersucht (Kapitel 4). Für drei Tage nach der Abkalbung bestand freier Kuh-Kalb-Kontakt in den Abkalbeboxen. Danach hatten 11 Kühe halbtägigen Kalb-Kontakt zwischen dem Morgen- und Abendmelken (ca. 10 h 45 min, „Halbtags“) und 13 Kühe freien Kalb-Kontakt (24 h, „Frei“). Beide Gruppen waren in separaten Tiefstreu-Abteilen mit Kälberschlupf untergebracht. Kühe der Gruppe „ohne Kontakt“ wurden innerhalb des ersten halben Tages post partum vom Kalb getrennt und einen Tag nach der Geburt in einem dritten Tiefstreu-Abteil aufgehalten. Die Kälber „ohne Kontakt“ wurden innerhalb der ersten Woche in Einzeliglus und danach in eine mit Stroh eingestreute Box mit Kälbern ähnlichen Alters aufgestallt. Sie erhielten täglich max. 2x3 L erwärmte Vollmilch mittels Nuckeleimern. Alle Kühe wurden zweimal täglich gemolken. Nach neunwöchigem „Säugen“, wurden die „Halbtags“ und „Frei“ Kälber in ein Abteil umgruppiert, von wo aus sie ihre Mutter sehen konnten; Berührungen oder Säugen war jedoch nicht mehr möglich. Gleichzeitig wurden die Kälber an das Trinken aus dem Nuckeleimer gewöhnt (2x3 L d^{-1}, „in Sicht+Milchtränke“). In der 11. und 12. Lebenswoche wurden diese Kälber mit den Kälbern „ohne Kontakt“ gemeinsam gehalten und es fand schrittweises Absetzen von der Milch statt (4 - 2 L pro Tag). Dort konnten sie ihre Mütter nicht mehr sehen (4 - 2 L d^{-1}, „außer Sicht+Absetzen“). Die Datenaufnahme endete zwei Wochen nach dem Absetzen (13. und 14. Lebenswoche, „nach dem Absetzen“). Mittels gemischter Modelle wurden der Einfluss der Kuh-Kalb-Kontaktdauer und der experimentellen Phase auf die ermelkbare Milchmenge, die Milchinhaltsstoffe, SCS und den Anteil der Melkungen mit einer somatischen Zellzahl >100.000 Zellen ml^{-1} analysiert. Varianzanalysen wurden angewandt, um den Effekt der Kontaktdauer auf die durchschnittliche tägliche Milchmenge der Laktation (220 - 305 Melktage), die Zwischenkalbezeit und die durchschnittliche tägliche Zunahme der Kälber während „Säugen“ und „in Sicht+Milchtränke“ zu berechnen. Nach der zuletzt genannten Phase wurden die männlichen Kälber zur Mast verkauft, wodurch sich die Stichprobengröße verringerte. Die Kälberdaten der nachfolgenden Phasen und die Inzidenzen von Mastitiden und wurden mittels nicht-parametrischer Tests ausgewertet.

Die ermelkbare Milchmenge während des „Säugens“ lag bei den „Halbtags“-Tieren durchschnittlich 9,9 kg unter der Leistung der Tiere „ohne Kontakt“ (P = 0,0054, r = 0,48) und 3,6 kg über der Leistung der „Frei“-Tiere (P = 0,0576, r = 0,32). Fast 80% der täglichen Milchleistung wurde bei den „Halbtags“-Tieren, nach der Trennung vom Kalb über Nacht, am Morgen ermolken. Über die gesamte Laktation hinweg lag die Milchleistung der „Halbtags“-Tiere tendenziell über der Leistung der „Frei“-Tiere (P = 0,0889, r = 0,31) und unterschied sich nicht signifikant von der Milchleistung der Kühe „ohne Kontakt“ (P = 0,2193, r = 0,23). Die Milchmenge, die die Kälber „ohne Kontakt“ per Nuckeleimer bekamen, ist bei diesem Vergleich noch nicht berücksichtigt und würde die Differenz zwischen „Halb“ und „ohne Kontakt“ noch weiter reduzieren. Während des „Säugens“ lag der Milchfettgehalt der Kühe mit Kalb-Kontakt etwa einen Prozentpunkt unter dem der Tiere „ohne Kontakt“. Die Mindererträge bei ermelkbarer Milchmenge und beim Fettgehalt sprechen für eine gestörte Alveolarmilchejektion bei beiden Kontakt-Gruppen. Dies wirkte sich jedoch nicht negativ auf die Eutergesundheit aus. Die somatischen Zellzahlen unterschieden sich nicht zwischen den Gruppen (P > 0,1), die Mastitis-Inzidenzen waren jedoch generell hoch. Während des „Säugens“ lagen der Eiweißgehalt der Milch bei den Müttern 0,15 - 0,30 Prozentpunkte über, der Laktosegehalt 0,17 - 0,33 Prozentpunkte unter dem der Gruppe „ohne Kontakt“. Nach der „Säuge“-Phase stiegen ermelkbare Milchmenge, Fettgehalt und Laktosegehalt der Mütter wieder an. Der Eiweißgehalt von „Halbtags“ und „ohne Kontakt“ variierte im Verlauf der Phasen, wohingegen er bei „Frei“ stabil blieb. Die Zwischenkalbezeit wurde nicht durch die Kalb-Kontaktdauer beeinflusst (P = 0,714). Die Gewichtsentwicklung war über alle Phasen hinweg bei den Kälbern mit Mutter-Kontakt ähnlich. Die täglichen Zunahmen der Kälber „ohne Kontakt“ (N = 12) lagen während des „Säugens“ signifikant unter (Median: 0,64 kg vs. „Halb“ N = 7, „Frei“ N = 10, beide 0,96 kg d^{-1}, P < 0,0001, r = 0,78), während „in Sicht+Milchtränke“ über denen von „Halb“ und „Frei“ ($N_{ohne\ Kontakt}$ = 11, 0,88 kg vs. N_{Halb} = 9, 0,36 kg und N_{Frei} = 10, 0,38 kg pro Tag, „Halb“ vs. „ohne Kontakt“: P = 0,0114, r = 0,46). Während der folgenden Wochen steigerten sich die Gewichtszunahmen der Kontakt-Kälber, blieben jedoch numerisch noch unter dem Level der Kälber „ohne Kontakt“ (P > 0,1, r < 0,20). Zwei Wochen nach dem Absetzen lag das Körpergewicht der „Halb“ (N = 5) und „Frei“ (N = 8) jedoch noch über dem der restriktiv gefütterten Kälber „ohne Kontakt“ (N = 8) (Median: 128,0 kg, 120,5 kg, 109,3 kg, „Halb“ vs. „ohne Kontakt“: P = 0,0294, r = 0,48). Zusammenfassend konnte in vorliegendem Versuch bei ähnlicher Kälberentwicklung, die ermelkbare Milchmenge durch halbtägigen Mutter-Kalb-Kontakt im Vergleich zu freiem Kontakt tendenziell gesteigert werden.

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°	degrees
%	percent
a	corrects for inbalance in sample sizes: $a = \frac{(n_1+n_2)^2}{n_1 n_2}$
AIC	Akaike Information Criterion
a.m.	ante meridiem
ANOVA	analysis of variance
approx.	approximately
BC	before Christ
BMEL	Bundesministerium für Ernährung und Landwirtschaft in Deutschland
bpm	beats per minute
C	Celsius
ca.	circa
cm	centimeter
d	day(s) or
	in case of statistics: standardized mean difference
d^{-1}	per day
df	degrees of freedom
DVG	Deutsche Veterinärmedizinische Gesellschaft
EC	European Commission
Ed.	Editors
EFSA	European Food Safety Authority
e.g.	exempli gratia, English: for example
Est	model estimate
et al.	et alii, English: and others
f	Fig. 10: female
f.	following page

ff.	and the following pages
FiBL	Forschungsinstitut für biologischen Landbau
Fig.	figure
g	gram
GH	German Holstein
GRP	German Red Pied
h	hour
h^{-1}	per hour
HF%	percentage of frequency in the high frequency band of HRV, 0.20-0.58 Hz
HFnorm	normalized power of the high frequency band, 0.20-0.58 Hz
HR	heart rate
HRV	heart rate variability
Hz	hertz
i.e.	id est, English: in other words
incl.	including
IS+MF	in sight+milk feeding
K	Cohen’s Kappa Coeffient
kg	kilogram
kPa	kilopascal
KTBL	Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V.
L	litre
LF%	percentage of frequency in the low frequency band of HRV, 0.05-0.20 Hz
LFnorm	normalized power of the low frequency band, 0.05-0.20 Hz
LF/HF	ratio of low frequency and high frequency of HRV
ln	natural logarithm
log	logarithm
m	meter or in Fig. 10: male
m^2	square meter

mg	milligram
min	minute
min^{-1}	per minute
ml	millilitre
ml^{-1}	per millilitre
ms	millisecond
n	sample size
OR	odds ratio
OS+W	out of sight+weaning
p	p-value: probability value
p.	page
pp.	pages
p.m.	post meridiem
r	in case of observer reliability: Spearman Correlation Coefficient
	in combination with p: Pearson's Correlation Coefficient as measure of effect size
RMSSD	root mean square of successive RR interval differences
SCC	Somatic Cell Count
SCS	Somatic Cell Score
SD	standard deviation
SDNN	standard deviation of normal-to-normal RR intervals
sec	seconds
t	t-value: size of the difference between samples relative to the variation, expressed in standard error
Tab.	table
vs.	versus
wk	week
wks	weeks
z	z-value: 'value of an observation expressed in standard deviation units' (Field et al., 2012, p. 928)

1 GENERAL INTRODUCTION

In common agricultural practice, dairy cows (*Bos taurus*) are usually separated from their calf within a few hours after parturition (reviewed by Keyserlingk and Weary, 2007). If calving took place in a calving pen, cows are re-introduced into the milking herd after separation from the calf. Calves are initially housed singly in most cases, or, especially at later times, in groups of calves approximately their age. They are fed with milk or milk replacer, mostly by teat buckets or automatic milk feeders at amounts of often 10 - 15% of their body weight (4 - 6 kg) (Rushen et al., 2008, pp. 214 ff.). At 4 - 12 wks of age calves are weaned from milk feeding (reviewed by Flower and Weary, 2003). In organic farming, feeding of natural milk for 3 months is required (European Commission, 2008). The European regulation on organic production further says: 'All young mammals shall be fed on maternal milk in preference to natural milk…' (European Commission, 2008, Article 20, 1.). However, this is not mandatory and mainly ignored. Early separation of cow and calf is criticized by different parties: Some scientists describe negative effects on animal welfare (e.g. reviewed by Johnsen et al., 2016; Keyserlingk and Weary, 2017; Rushen et al., 2008, p. 183). The Scientific Panel on Animal Health and Welfare of the European Food Safety Authority (EFSA) states: 'The needs of young calves are met most effectively by the presence and actions of their mothers.' (EFSA, 2006, p. 28). Also consumers (Boogaard et al., 2008, 2010; Busch et al., 2017; Hötzel et al., 2017; Ventura et al., 2013) and animal protection advocates are concerned about early separation (Ventura et al., 2013). Early separation is often disapproved as a routine lacking naturalness (Boogaard et al., 2008, 2010; Ventura et al., 2013). In the following some aspects of maternal behaviour of semi-wild cattle under near natural conditions are explained to clarify what naturalness means in case of mother-calf bond and nursing behaviour. Further the establishment of mother-young recognition is outlined as the perception of different calf-associated cues by the mother. This is a topic in the current dissertation.

1.1 Maternal behaviour and mother-young recognition in cattle

There are a number of studies describing the behaviour of extensively managed domestic cattle (*Bos taurus* and *Bos indicus*) under free-range conditions. As the wild ancestor of cattle is extinct and no reliable information on their behaviour is available, comparisons between wild and domestic cows are not possible. The animals observed were either beef suckler cattle with bulls being in the herd for the mating period only (Kiley-Worthington and de la Plain, 1983; Lidfors and Jensen, 1988; Schleyer, 1998), or no information about bulls were given (Kiley, 1972; Koch and Zeeb, 1969; Kour et al., 2018; Padilla de la Torre et al., 2015; Price et al., 1984/85; Schäffer et al., 1999; Walker, 1962), or the animals were not commercially used at the time of the study with permanent presence of bulls in the herd (Reinhardt, 1980; Reinhardt and Reinhardt, 1981: *Bos indicus*; Schloeth et al., 1958; Vitale et al. 1986). Herds consisted of 11 (Schleyer, 1998) to 380 cows and their calves (Lidfors and Jensen, 1988) with, depending on herd size, one bull (Kiley-Worthington and de la Plain, 1983; Reinhardt, 1980; Reinhardt and Reinhardt, 1981; Schleyer, 1998) four bulls (Vitale et al., 1986) or 10 bulls (Lidfors and Jensen, 1988).

Upon parturition, the cow often leaves the herd. After parturition the calf stays hidden while the mother forages near-by or joins the herd again (Reinhardt, 1980, p. 9; Vitale et al., 1986). Other authors discuss whether calves are hiders or followers, as both has been observed (discussed by Lidfors and Jensen, 1988). At least under commercial conditions, separation of the dam from the herd and the activity of the calf may depend on space and shelter available for the animals (reviewed by Keyserlingk and Weary, 2007; Lidfors and Jensen, 1988). After the calf is born, the mother produces a lot of low pitched sounds (Kiley-Worthington and de la Plain, 1983, p. 76; Padilla de la Torre et al., 2015; Reinhardt, 1980, p. 9), defined as 'mm' and 'purr' calls by Kiley-Worthington and de la Plain (1983, p. 76) who speculate that the reason for this vocalisation might be an ''auditory imprinting' of the cow on the calf' (Kiley-Worthington and de la Plain, 1983, p. 76). However, calves during their first days of life are typically 'lying out' (Hall, 2002, p. 139). Thus, it can also be speculated that imprinting on the calves' side plays a minor important role. It may be more likely that on the side of the dam, that leaves the calf and returns to it, imprinting processes are essential (Reinhardt 1980, p.11). The cow sniffs and licks the calf for a large proportion of time after calving (e.g. Kiley-Worthington and de la Plain, 1983, pp. 72f., 83; Lidfors and Jensen, 1988). This process seems to be crucial for the establishment of mother-calf-attachment (reviewed by Keyserlingk and Weary, 2007).

In case of separation from the herd, the dam re-joins the herd with the calf one to three weeks after parturition. Cows spend most of their time grazing with other cows, and the calf is resting or playing with the youngest, non-grazing calves of the herd, in the so called kindergarten, supervised by a cow or the bull (Reinhardt, 1980, pp. 9f.). Contact searching between mother and calf is often initiated by high pitched calls (Padilla de la Torre et al., 2015; Price et al., 1984/85; Reinhardt, 1980, p.11; Schloeth, 1958). Then either the cow approaches the calf (Reinhardt, 1980, p. 13) or the calf walks towards the cow for suckling (Koch and Zeeb, 1969; Price et al., 1984/85; Reinhardt, 1980, p.11). In the first days after birth, the calf approaches the dam frontally with lowered, but stretched head (Reinhardt, 1980, p. 11; Schloeth, 1958;). This posture can also be observed as submissive behaviour in adult cattle (Schloeth, 1958). At this point the mother produces a low pitched sound with closed mouth, defined as the 'mm' call by Kiley (1972). Reinhardt (1980, pp. 11ff.) suggested that the sound should reassure the calf that the cow is the mother. This is followed by nursing (Reinhardt, 1980, p. 11; Schloeth, 1958) or social licking (Reinhardt, 1980, p. 11). Reinhardt (1980, p. 11) observed in semi-wild Zebu cattle that suckling is often combined with sniffing or licking of the calf's anogenital region. In contrast Schäffer et al. (1999) found an olfactory control of calves by cows in only 11.1% of suckling bouts in European beef sucklers. However, in both studies, the age of the calves is not mentioned. It is reported that the olfactory control of the calf declines soon after parturition (Jensen, 2011; Schloeth, 1958). Soon after birth cow and calf sometimes mistake other cows or calves with their mother or offspring, if only visual cues are available (Reinhardt, 1980, p. 13; Schloeth, 1958). Olfactory cues ensure mother-calf-recognition more reliably. However, for olfactory control a close individual distance is necessary which is sometimes not tolerated by alien animals (Reinhardt, 1980, p. 13). After a solid mother-calf-relationship is established, visual recognition becomes more reliable and the frontal approach of the calf before suckling is omitted (Schloeth, 1958).

Vitale et al. (1986) found a relatively low number of average suckling bouts, 2.5 bouts d^{-1} during the first 10 d of life to 0.6 suckling bouts between the 50^{th} to 60^{th} d of life, compared with 0.18 suckling bouts per hour found by Lidfors and Jensen (1988). Reinhardt and Reinhardt (1981) observed 4-8 bouts d^{-1}, while 3 - 9 month old calves have been found to suckle 3-5 times d^{-1} (Koch and Zeeb, 1969; Walker, 1962). Walker (1962) found differences in suckling frequency according to breed. Mostly it is reported that suckling bouts decrease with calf-age (Kiley-Worthington and de la Plain, 1983, p. 86; Reinhardt and Reinhardt, 1981; Vitale et al., 1986), which was not found when animals were only observed during 4 h in the morning (Lidfors and Jensen, 1988). Observed suckling durations per bout range from 8 - 13 min independent of age (Koch and Zeeb, 1969; Kour et al., 2018; Lidfors and Jensen, 1988; Reinhardt and Reinhardt, 1981), over 1 - 3 min in the third month of life compared to 6 - 7 min in the first month (Schleyer, 1998), to 10 - 17 min, increasing with decreasing bout number (Vitale et al., 1986).

It is not possible to assess how much milk calves ingest under semi-natural conditions with non-invasive methods. Weighing of dam reared calves before and after restricted suckling under farm conditions showed that calves drank 6 - 14 L d^{-1}, depending on calf age (e.g. Boden and Leaver, 1994; Ivemeyer et al., 2016; de Passillé and Rushen, 2006; de Passillé et al., 2008). Suckling is normally conducted in an antiparallel position. Sometimes, if the calf cannot stop the mother from grazing, the calf is suckling from the rear (Koch and Zeeb, 1969; Schloeth, 1958). Schloeth (1958) estimated that less than 8% of suckling bouts are from the rear. Beside affiliative behaviour and suckling (Reinhardt, 1980, p. 11), cow and calf lie and graze together (Koch and Zeeb, 1969).

Under semi-natural conditions, Zebu calves were weaned from milk by the dam at an age of 8.8 - 11.3 months (Reinhardt and Reinhardt, 1981). In extensively kept beef suckler herds (*Bos taurus*) weaning has been observed to occur when calves were about 9.7 month old or after 82.5% of the time between parturitions (Albertsen and Held, 2017). Even after weaning and birth of a next calf, the mother-calf bond persists, evidenced by social licking and grazing next to each other (Reinhardt, 1980, p. 20; Veissier et al., 1990).

1.2 Dam rearing systems

Alternative to early separation, on some dairy farms the contact duration between cow and calf is prolonged to often 6 to 12 weeks and cows are milked additionally to the nursing their calf. This is called dam rearing. Different systems are possible, which are distinguished according to contact duration during the day (reviewed by Johnsen et al., 2016):

- In restricted suckling systems mother and calf meet at specific times, often twice daily, for 15 min to 1 h. This is most common in Germany (own data, unpublished) and Switzerland (Zumbrunnen, 2012). Restricted suckling is often combined with fostering as described by Ivemeyer et al. (2016). On the commercial farm, where the latter study was conducted, mother and calf have free contact (excluding twice daily milking and main feeding) for three weeks post partum, living in a group with other cow-calf-pairs during this phase. The following eight weeks mother and calf meet twice daily for 45-60 min for suckling before milking takes place. During this period, the cow is also foster mother for calves older than 11 wks. After an interim phase from the 11th week post partum onwards the cow is milked only, while the calf may suckle foster mothers until it is gradually weaned at 15 wks of age.

- During half-day contact, where calves have access to their mothers either during day-time or during night-time and are separated from the dam the rest of the day.
- Free or permanent contact systems are characterized by free access between cow and calf 24 h d^{-1}, mostly except for the time the cow spends in the waiting area and milking facility.

In restricted suckling systems, cows and calves often meet at a specific place e.g. in the waiting area. Thus, there is no need for calf-safety precautions in the cow barn as it is in the other two systems. If half-day or free contact is given, calves often may enter the cow barn or at least a part of it. This was tested in deep litter as well as in cubicle systems. Sometimes there is a calf creep where cows do not have access and where calves find a lying area, feed and water (Kälber and Barth, 2014).

Advantages of dam rearing are that affiliative behaviour between cow and calf can be performed. Furthermore, calves have higher weight gains during the suckling period, and cross-sucking, an abnormal behaviour which is a general problem in calf rearing, is considerably decreased or does not occur in dam rearing. Even some positive effects on heifer behaviour and performance have been shown (reviewed by Johnsen et al., 2016).

Although in dam rearing animals may behave more naturally, which is appreciated by consumers, and calves and heifers develop well, it is only seldomly established on commercial farms and farmers hesitate to start with dam rearing. One reason relates to economics. Presently no specific bonuses or labels for milk from dams are available, but dam rearing systems may involve increased costs or less profit. Whether dam rearing requires more or less labour input has not systematically been assessed so far. However, further potential problems are explained in the following paragraphs.

1.3 Reasons for early separation

In contrast to consumers' opinions (Boogaard et al., 2008: n = 39; 2010: n = 127; Ventura et al., 2013: n = 11), in a small North American online survey, 63.6% of farmers (n = 11) and 52.9% of people being familiar with dairy practice (n = 17, including the 11 farmers) preferred early separation (Ventura et al., 2013). Major reasons for early separation given were: (1) reduced separation stress because no bond is established, (2) improved calf health through separation and (3) better milk let-down and cow health (especially udder health) without calf-contact. These statements characterize major concerns of farmers regarding dam rearing and show why dam rearing is not very common in dairy practice (reviewed by Flower and Weary, 2003).

1.3.1 Separation stress

Behavioural responses to separation indicating stress in cow and calf are increasing with prolonged contact (reviewed by Flower and Weary, 2003; Keyserlingk and Weary, 2007). Not only animal welfare is impaired, but also humans living near the stable can be distressed by this (Johnsen et al., 2016), as animals react with high pitched vocalisation during the first days after separation (reviewed by Flower and Weary, 2003; Keyserlingk and Weary, 2007). Some management practices have been shown to minimize separation stress at least in calves. They include fence line separation (dairy cattle: Johnsen et al., 2015b;Verwer and Kok, 2012; beef cattle: Price et al., 2003) and the use of nose flaps (dairy cattle: Barth et al., 2015a; Verwer and Kok, 2012; beef cattle: Burke et al., 2009; Haley et al., 2005; Lambertz et al., 2015; Loberg et al., 2008). It is further possible to use udder nets. Scientific studies on this topic, however, are rare (Loberg et al., 2008: combined with nose flaps). All three methods allow physical contact of dam and calf without suckling. Beside these, giving the calf a different source (bottle, bucket, automatic milk feeder) to ingest milk after separation from the dam (Johnsen et al., 2014, 2015b; Lidfors et al., 2017; Rushen et al., 2016), and gradual reduction of the amounts of milk or milk replacer had beneficial effects (Johnsen et al., 2014). Less information on the impact of two-step weaning on cow welfare is available. Fence-line weaning did not influence cows' vocalisation or lying behaviour in a positive way (Lidfors et al., 2017), while by using nose-flaps such effects could be achieved in beef cattle (Haley, 2006; Ungerfeld et al., 2016).

1.3.2 Calf health

Feeding management has high impacts on incidences of disease and mortality (Rushen et al., 2008, pp. 33, 212). Major feeding factors affecting calf health are colostrum quality and supply, amount of milk fed, temperature of the milk and the way of ingestion. Colostrum intake soon after birth is essential for the development of the calf's immune system (e.g. Spiekers and Potthast, 2004, pp. 275f.). Udder seeking attempts of the calf may not be successful because teats of dairy cows are often at a lower position than they would be naturally. Therefore, calves may need assistance (Edwards, 1982; Kiley-Worthington and de la Plain, 1983, pp. 84f; Kovalčik et al., 1980; Lidfors, 1996; Selman et al., 1970b). Thus, regardless of dam rearing or early separation, the farmer is responsible for safeguarding successful suckling or colostrum supply. Dam rearing allows the calf sucking the milk at an optimal temperature from a teat with a slow milk flow, drinking more often in smaller portions, and often the ingestion of higher amounts of milk than usually provided during commercial rearing of calves. While cross-sucking is minimized (reviewed by Johnsen et al., 2016), there is no clear evidence regarding the impact of dam rearing on incidences of disease and mortality. Grøndahl et al. (2007) observed in 39 calves suckling their mother for 6-8 weeks and later in the young stock no mortality or illness. However, there was no control group. Fröberg et al. (2011) ascertained only low incidences of diarrhoea, and respiratory disease, and there were no differences between calves with free dam contact and calves fed by an automatic milk feeder (not statistically tested). In the review of Krohn (2001) it is only mentioned that nursed calves are 'usually healthy', but no details are given. In dam reared calves occurrence of non-infectious diarrhoea, which did not lead to increased medical treatment, was reported from experimental studies. The reason was possibly high milk consumption (Bar-Peled et al., 1997; Roth et al., 2009a) and farmer interviews (Ehrlich, 2003; FibL et al., 2011; Zumbrunnen, 2012). However, if Johne's disease (*Mycobacterium avium* ssp. paratuberculosis) occurs on a farm, strict separation of calves and adult cattle is recommended, because it can be transferred by milk and faeces of diseased cows (e.g. BMEL, 2014; Groenendaal et al., 2003; Ridge et al., 2010).

1.3.3 Milk let-down, stress and udder health

Nursing cows frequently show impaired alveolar milk ejection during machine milking (reviewed by Johnsen et al., 2016). Causes of disturbed milk let-down are unclear, but stress due to separation from the calf during milking may play a role. Furthermore, the milking process might not be the optimal stimulus to induce milk ejection in nursing cows being used to their calf's stimulation (Johnsen et al., 2016). The milk which cannot be collected by machine milking plus the milk suckled by the calf lead to a considerably decreased amount of sellable milk during the suckling period (reviewed by Johnsen et al., 2016).

Concerning udder health two hypotheses exist: that the remaining milk in the udder has a negative impact, or that the frequent suckling improves udder health. However, several studies could not find differences in udder health between dams and non-nursing cows (reviewed by Johnsen et al., 2016). Nevertheless, possibilities to alleviate the problem of impaired milk let-down or at least to decrease the loss of sellable milk would be an important precondition for a wider commercial application of dam rearing.

1.4 Outline of the dissertation

The challenges described above were tackled in this dissertation in two ways: first, the influence of calf associated stimuli on the dam in the parlour was tested (Chapter 2 and 3), and second, effects of half-day cow-calf contact were evaluated (Chapter 4).

Acoustic, olfactory and manual calf associated stimulation in the milking parlour were investigated in cows with and without calf-contact regarding their effects on milking performance and milk quality, but also on behaviour, heart rate (HR) and heart rate variability (HRV) as indicators of stress (Chapter 3).

However, beforehand in a pilot-study, the suitability of calf-hair as olfactory stimulus in the parlour was tested. In an earlier study (Barth et al., 2010), olfactory stimulation had no effect on milk let-down and it remained unclear whether the cows had perceived the calf-odour. Therefore, an attempt was made to improve the olfactory stimulation and it was assessed, whether cows with and without calf-contact showed different behaviour towards samples with hair of the own or an alien calf or towards a control without calf-hair (Chapter 2).

In restricted suckling the amount of sellable milk is reduced by 7 - 12 kg d^{-1}, while during free contact daily milk losses can reach 20 kg (reviewed by Johnsen et al., 2016). On the other hand, restricted contact also restricts mother-calf-interactions and interactions with the rest of the dairy herd. As a possible compromise between the animals' and the farmers' needs - half-day cow-calf-contact is compared with free and no contact in Chapter 4. Machine gained milk yield, milk composition, udder health, calving interval and weight gain of calves were investigated during the suckling period of nine weeks, as well as during the time after separation and after weaning of calves, which were fed whole milk until 12 weeks of age.

Finally, in Chapter 5 the results of the three studies are jointly discussed and general conclusions are drawn (Chapter 6).

2 BEHAVIOURAL RESPONSE OF DAIRY COWS WITH AND WITHOUT CALF-CONTACT TO HAIR OF OWN AND ALIEN CALVES PRESENTED IN THE MILKING PARLOUR

2.1 Introduction

Dairy calf rearing systems, in which the calves are allowed to suckle their mothers and the cows are additionally milked, have both advantages and disadvantages (Johnsen et al., 2016). One of the challenges to overcome is a decreased milk yield during machine milking due to disturbed alveolar milk ejection, which, in turn, is caused by suppressed oxytocin release (Bar-Peled et al., 1995; de Passillé et al., 2008; Sandoval-Castro et al., 1999). The underlying mechanisms are not completely understood, but a lack of calf-associated stimuli during milking may play a role. The odour of the cow's own young might be an especially strong stimulus. At least in ewes, olfaction is particularly important for the acceptance of the lamb at the udder (Alexander and Stevens, 1981; Alexander et al., 1983). Most scientific research on olfactory young recognition in farm ungulates has been done in ewes, but some studies have been conducted on goats and cattle as well. Olfactory selectivity is established through the prepartum rise of oestrogen, vaginocervical stimulation caused by fetus expulsion and the licking of the neonate, which, in turn, is elicited by an olfactory attraction towards amniotic fluid, and triggers further hormonal and neurophysiological processes in the mother (reviewed by Lévy and Keller, 2009 and Poindron et al., 2007). The influence of olfaction during the establishment of maternal selectivity was demonstrated by comparing intact goats or sheep with animals prenatally rendered anosmic. The latter were not attracted to amniotic fluid (Lévy et al., 1983) and did not form an exclusive bond with their own offspring, but also suckled alien young (ewes: Ferreira et al., 2000; Lévy et al., 1995; Poindron and Le Neindre, 1980; goats: Hernandez et al., 2002; Romeyer et al., 1994). Among anosmic goats, the oxytocin release was the same when suckling their own or an alien young, while intact goats showed a higher oxytocin release when suckling their own kid (Hernandez et al., 2002). Likewise, in beef cattle, anosmic mothers showed lower suckling-mediated inhibition of LH secretion than intact animals while suckling their own calf. This may lead to an earlier oestrus after calving among anosmic animals (Griffith and Williams, 1996).

If calf odour shall be used as a potential stimulus in the milking parlour, the first question that arises is how to present it in a way that is best perceived by the cow. In sheep, scent rather than pheromones is responsible for the development of a selective recognition of the lamb (reviewed by Kendrick et al., 1997). Odours of faeces or urine play a minor role in maternal recognition in ewes (Alexander and Stevens, 1981: 1982/83). The theory that lambs are 'labelled' by their mother's milk or saliva could not be substantiated (Alexander and Stevens, 1982/83; Lévy et al., 1991). Textile materials, with which animals were rubbed or which were worn by an animal or human, have been successfully used in fostering (beef cattle: Dunn et al., 1987; ewes: Martin et al., 1987) and discrimination tests (ewes: Alexander and Stevens, 1982/83; humans: Porter et al., 1983; Porter and Moore, 1981; Lundström et al., 2009; European storm petrel sea birds: Bonadonna and Sanz-

Aguilar, 2012). Therefore, Barth et al. (2010) rubbed calves with cotton cloths, which were used to reproduce an olfactory stimulation in dairy cows in the milking parlour. However, neither behavioural responses were detected nor an increase in milk ejection achieved. It remained unclear whether the cows did not perceive the stimulus or merely did not react to it. Therefore, in this study we attempted to intensify the calf-odour of samples presented to cows in the milking parlour. A source of odour, which worked well in choice tests with ewes, was wool of different body regions (Alexander, 1978; Alexander and Stevens, 1982/83). For acceptance at the udder, the odour from the anogenital region of the lambs was most important (Alexander et al., 1983). Therefore, calf hair from the anogenital region and hind limbs was used as the source of odour in this study to elicit a behavioural response in cows during milking. As proffering each cow the hair of her own calf is too labour intensive for normal farm practices, the response to alien calf hair is of significant interest. This pilot study addressed the questions whether (i) cows behaviourally respond to small amounts of calf hair presented in the parlour (compared to a control without calf hair), (ii) responses are different to hair of the own calf or an alien calf, and whether (iii) cows with and without calf contact behave differently to the olfactory stimuli. The possible influence of olfactory stimulation on milk let-down was tested in another experiment, not presented here.

2. 2 Animals, materials and methods

2.2.1 Animals, housing and treatment groups

The experiment was carried out at the Thünen Institute of Organic Farming in Trenthorst, Germany, during the winter housing period. In total, 40 dairy cows of two different breeds, German Red Pied (GRP; n = 20), a dual-purpose breed, and German Holstein black-and-white (GH; n = 20), were included in the study. The breeds were kept in two separate herds with respectively 45 and 48 cows in two identical sections of a loose housing stable. Maintaining a balance between breed and parity was an important factor for the selection of animals to participate in the study. Thirteen multiparous (6 GRP, 7 GH) cows and four heifers (2 GRP, 2 GH) were separated from their calves within 12 h post partum. Seventeen multiparous cows (10 GRP, 7 GH) and six heifers (2 GRP, 4 GH) had free contact with their calves for 12 weeks post partum ('contact’, n = 23). All animals were milked twice daily in a 2x4 autotandem milking parlour (GEA, Boenen, Germany). Calves of the same breed from the 'contact' and 'control' group were housed together. Via a chip-controlled selection gate, 'contact' calves were able to enter the cows’ lying area unrestrictedly. Thus, 'control' cows potentially had contact to alien calves, but not to their own calves. Suckling of 'contact' calves at 'control' cows was not observed. Calves had neither access to the feeding area of the cows nor the waiting area in front of the parlour. For further details on the stable and general conditions see Wagner et al. (2012).

2.2.2 Preparation of stimuli

With the exception of three, all calves born in the two herds during the duration of the experiment, were part of the study and served as donors for ‘own’ calf hair in the second to third week of life (11 - 19 d, mean = 16, SD = 3 d, hair of one body side). One day before the mother was tested for the first time, hair of the own calf (‘own’) and an alien calf (‘alien’) was shorn with a trimmer around the anogenital region, including tail and hind legs lateral to the tail on one body side. In order to intensify the odour of the samples, after trimming the hair, calves were rubbed with five cloth bags (cloth: Fliselina®, Freudenberg Vliesstoffe KG, Weinheim, Germany, bag size: 12 x 8 cm). The calves were rubbed with each side of the bag on one body side in the form of a lying eight from the blade-bone to the tail. For the experiment on milk let-down (not reported here), hair from the other side of the calves' bodies was shorn between the fifth and seventh week of life. Calves' hair also served as 'alien' samples for alien cows. As nearly all calves born during the experimental time were included in the studies and each hair sample was used only for one cow in the parlour, the hair of calves had to regrow before 'alien' hair samples could be taken. Thus calves were older at this time (19 - 88 d of age, mean = 42, SD = 18 d). Six samples from the three extra calves, whose mothers were not part of the study, were additionally used for 'alien' samples. The latter were evenly allocated to 'control' and 'contact' cows. It was the goal to use 'alien' samples from calves of the same breed as the cow and to assure that the donor calf had no contact to the cow in the barn. Further important considerations were that calves should be the same sex as the cow's own calf and not suffering from diarrhoea. However, in several cases this was not possible to achieve due to the limited number of donor calves: 5x different breed, 10x contact with cow, 16x different sex, 3x diarrhoea.

The shorn hair of each calf was divided into five equal portions of about 0.8 g per visual judgement and filled into the cloth bags. As control stimuli ('no'), clean cloth bags without calf hair were used. The cloth bags with and without calf hair were stored at about 16 - 18°C, each in a separate screw cap glass jar, which was cleaned in a laboratory washer after use. The time of storage until use in the experiment ranged between half a day and three days. From each stimulus category ('own', 'alien', and 'no'), four bags (samples) were used over all trials, the fifth served as a back-up. No sample was re-used, however, the cloth bags were used several times after being washed under running water, sterilized in boiling water for 5 min and allowed to air dry.

2.2.3 Experimental trials

Starting between the 12th and 20th d of lactation, during six consecutive milkings, two of the three olfactory stimuli ('own', 'alien', and 'no') were presented concurrently in the milking parlour. They were placed in two small stainless steel baskets, one above the other, that were installed at the height of the cows' heads at one side of the milking box.

Figure 1: Upper and lower basket with empty cloth bags tied at the baskets when no stimulus was presented in the head region of the milking box (Source: Kerstin Hofmann)

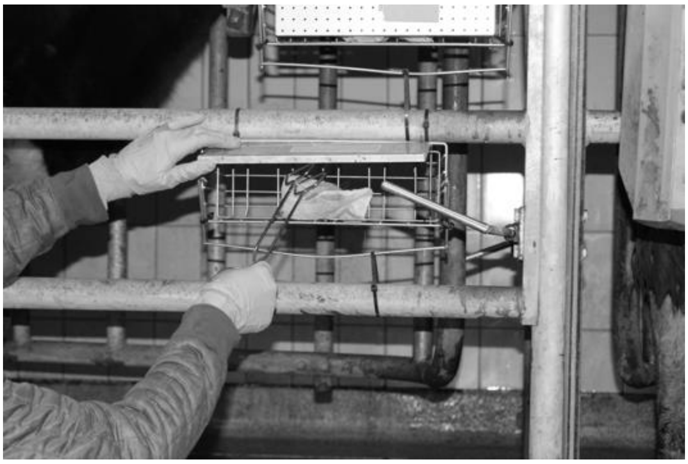

Figure 2: Placing a cloth bag with calf hair in the lower basket with tongs (Source: Kerstin Hofmann)

The order in which the stimuli were presented was randomised and all possible combinations (‘own’ and ‘alien’, ‘own’ and ‘no’, ‘alien’ and ‘no’) and positions of each stimulus (upper or lower basket) were implemented. To habituate the cows to the baskets and bags, the baskets were installed two weeks before the experiment started and clean bags without calf hair were tied in them and renewed every week (Fig. 1). During the trials, those empty bags were removed from the baskets after the animal had entered the milking box. One after the other, the stimulus bags were taken out of the jar with tongs, sprayed with distilled water and placed inside the upper basket first and then into the lower basket (Fig. 2). The ‘no’ hair stimuli always consisted of a fresh cloth bag without hair and were also sprayed with distilled water. During the minute following the placement of the bag in the lower basket, milking of that cow began. The handling of the samples and milking of the animals were always conducted by the same familiar person during the experiment. Behaviour of the animals was videotaped during all milkings (Axis 221 day and night network camera, 640x480 pixels, Axis Communications AB, Lund, Sweden).

2.2.4 Behavioural observations

Videos were analysed by one observer using the programme The Observer® XT 10.5 (Noldus Information Technology, Wageningen, Netherlands). The duration of sniffing and/or licking of the baskets was recorded with continuous focal animal sampling during a period of 3 min, which started when the bag was placed in the upper basket. As the bag in the lower basket was placed later, the observed time regarding this stimulus was reduced by a maximum of 48 sec. To account for the time discrepancy, the total duration of licking and sniffing at one stimulus during a trial in seconds was divided through the time the stimulus was presented (= response [%]). Moreover, for each milking it was noted whether or not a cow showed any licking or sniffing towards the stimulus (frequency of responses).

The behaviours were defined as depicted in Tab. 1.

Table 1: Ethogram of behaviours observed towards olfactory stimuli in the parlour.

behaviour	definition
sniffing	muzzle directed towards the basket or touching it, increased breathing visible through exhalation, slightly moving the head while nosing the basket
licking	tongue visible and touching the basket, possibly followed by licking the muzzle

Inter-observer reliability between the observer and the experimenter was checked and results were good to very good (Pearson correlation for sniffing: $r = 0.878$, $p < 0.001$, $n = 36$; licking: $r = 0.986$, $p < 0.001$, $n = 36$). The observer was blind to the treatment.

2.2.5 Statistical analysis

Non-parametric tests (SPSS 20.0, IBM® SPSS® Statistics) were applied because distribution of data or residuals was not normal (checked graphically). An influence of breed and parity on response behaviour was graphically checked. Parity (primi- versus multiparous) had an impact and was therefore taken into account in the analysis.

The effect of group (‘contact’ vs. ‘control’) and parity (primi- vs. multiparous) on the responsiveness to the stimuli was analysed using Pearson Chi²-Test. In all of the following tests, only data from responding animals without missing values (due to non-analysable videos) were used: 11 from ‘contact’ and nine from ‘control’ cows. The sum of the proportional response duration towards each stimulus during all four trials was calculated per animal. Mann-Whitney-U-Tests were used to test for the possible differences between groups per stimulus. Friedman’s ANOVA was used to test for potential differences in the frequency and response duration of responses towards the three different stimuli, for response duration separately for ‘contact’ and ‘control’ animals as well as for primi- and multiparous cows. Wilcoxon signed-rank test was applied as post hoc test. Friedman’s ANOVA was, furthermore, used to test for a possible time effect (six trials) on the general response duration. A significance level of 5% was used for all tests. Exact two-tailed significance is presented. The effect size for results from the Wilcoxon signed-rank tests and Mann Whitney-tests was calculated with $r = \frac{z}{\sqrt{n}}$ (Rosenthal 1991, p. 19). Odds ratio was used in combination with Pearson Chi²-tests.

2.3 Results

2.3.1 Responsiveness in general

In 26% of trials (61 of 236), a reaction towards a stimulus could be observed and in 23% of trials (54 of 236), there was a reaction towards ‘own’ and/or ’alien’. Of the 40 cows participating in the experiment, 26 (65%) responded at least once to any stimulus. Two cows only reacted to empty bags (‘no’). Thus, 60% of the cows responded to any stimulus with calf hair (‘own’ and/or ’alien’) (Tab. 2). Frequencies of responses towards the three stimuli differed in responding animals without missing values (n = 20, $\chi^2(2) = 11.2$, p = 0.003). Responses towards ‘own’ were observed in altogether 30 of 80 trials, which was significantly more frequent than responses towards ‘no’ with 10 trials (T = 12.0, p = 0.001, r = -0.71). Responses towards ‘alien’ were, with 20 trials, intermediate, tending to differ from ‘no’ (T = 29.5, p = 0.093, r = -0.40), but not from ‘own’ (T = 23.5, p = 0.142, r = -0.35).

Table 2: Number of cows responding to the different stimuli or never responding ('never'), depending on parity and calf-contact.

stimulation	%	n	contact (n = 23)		control (n = 17)	
			primiparous (n = 6)	multiparous (n = 17)	primiparous (n = 4)	multiparous (n = 13)
all stimuli	15.0	6	1	2	1	2
own calf+alien calf	12.5	5	3	1	1	0
own calf+no hair	2.5	1	1	0	0	0
alien calf+no hair	0.0	0	0	0	0	0
only own calf	20.0	8	0	5	0	3
only alien calf	10.0	4	1	2	0	1
only no hair	5.0	2	0	1	1	0
never	35.0	14	0	6	1	7
total	100.0	40	6	17	4	13

2.3.2 Influence of calf-contact

Numerically, but not significantly more animals of the ‘contact’ group (n = 23) responded to ‘own and/or alien’ (70%) or only to ‘own’ in any trial (57%) compared to 'control' cows (n = 17, 47%, 41%, $\chi^2(1) = 2.06$, p = 0.199, odds ratio = 2.57; $\chi^2(1) = 0.92$, p = 0.523, odds ratio = 1.86). Relative response duration towards ‘own’, ‘alien’ or ‘no’ were not significantly different between responsive animals of the two groups (n = 20, ‘own’: U = 29.00, p = 0.130, ‘alien’: U = 47.50, p = 0.891, ‘no’: U = 45.50, p = 0.771) (Tab. 3).

The duration of responses among ‘contact’ animals differed between stimuli, being longest towards ‘own’, followed by ‘alien’ and ‘no’ (Tab. 3). However, in the post-hoc tests only the difference between ‘own’ and ‘no’ tended towards significance (T = 11.00, p = 0.054, r = -0.42). The 'control' group numerically showed the same trend of responses, but weaker and with no significant differences (Tab. 3).

Table 3: Response duration (sniffing or licking) in % of observation time (median ± interquartile range) towards the different stimuli ('own', 'alien', 'no' calf hair) for control and contact animals, results from Friedman's ANOVA.

group	stimuli			X^2-value, p-value
	own calf	alien calf	no hair	
control n = 9	2.61 ± 12.63	2.27 ± 6.28	0.00 ± 4.15	4.563, p = 0.114
contact n = 11	8.42 ± 8.35[a]	1.27 ± 7.87[ab]	0.00 ± 1.32[b]	6.650, p = 0.034

data with different superscripts: tendency (p < 0.1), Wilcoxon signed rank test

2.3.3 Influence of parity

Regardless of calf-contact, numerically, but not significantly more primiparous (90%, n = 10) than multiparous cows (57%, n = 30) reacted at least once in all trials to any stimulus ($\chi^2(1) = 3.66$, $p = 0.123$, odds ratio = 6.88), towards calf hair ('own' and/or 'alien', responsive primiparous: 80%, responsive multiparous: 53%, $\chi^2(1) = 2.22$, $p = 0.263$, odds ratio = 3.50) or only to 'own' (responsive primiparous: 70%, responsive multiparous: 43%, $\chi^2(1) = 2.13$, $p = 0.273$, odds ratio = 3.05). Multiparous cows significantly differed in their response duration towards the different stimuli ($\chi^2(2) = 6.049$, $p = 0.049$, n = 12), while in primiparous cows, this was only a tendency ($\chi^2(2) = 5.871$, $p = 0.061$, n = 8) (Fig. 3). Post-hoc tests confirmed that multiparous cows explored 'own' longer than 'no' ($median_{'own'}$ = 3.49%, $median_{'no'}$ = 0.00%, T = 3.00, p = 0.005, r = -0.54) and 'alien' longer than 'no' ($median_{'alien'}$ = 1.14%, T = 2.00, p = 0.047, r = -0.41). There was no significant difference between 'own' and 'alien' (T = 23, p = 0.413, r = -0.18), likely due to the large variance in responses towards 'alien' in 'contact' cows (Fig. 3).

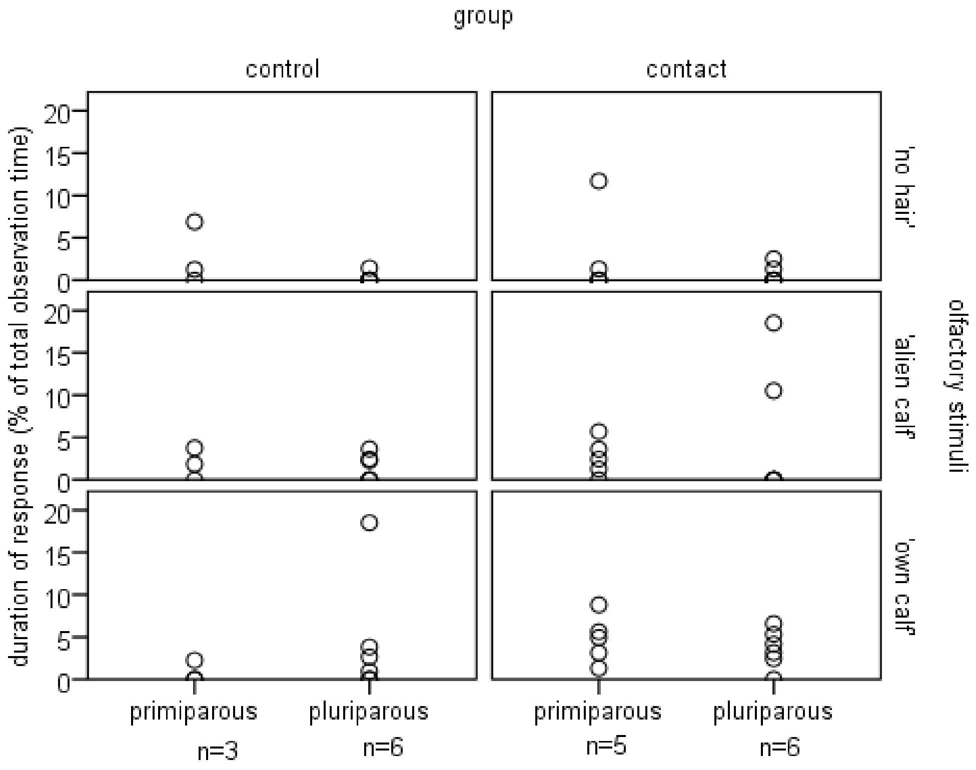

Figure 3: Relative duration of sniffing or licking of responsive cows towards an olfactory stimulus consisting of hair of the own or an alien calf or a control without hair in the parlour for primi- or multiparous cows with or without calf-contact.

2.3.4 Influence of trial number

The response duration towards 'own' or 'alien' significantly decreased over successive trials ($\chi^2(5) = 17.59$, $n = 20$, $p = 0.004$) (Fig. 4).

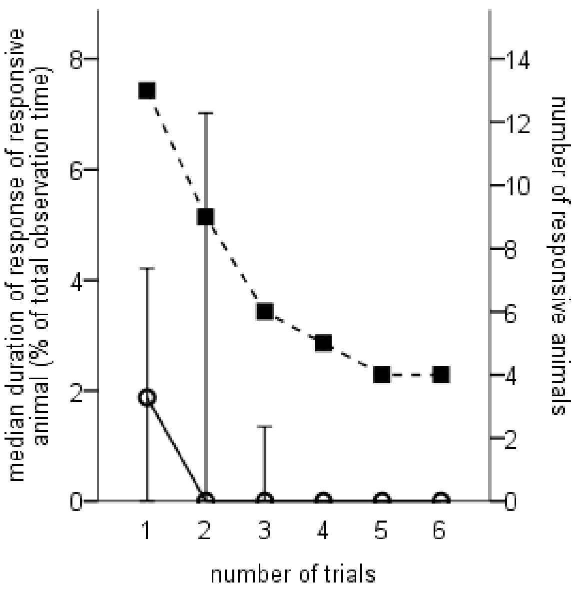

Figure 4: Responses (sniffing or licking) to calf hair ('own' or 'alien') over successive trials (relative duration: ○ solid line, median and 95% confidence interval, and number of responsive animal: ■ broken line, n = 20).

2.4 Discussion

More than half of the cows (60%) reacted at least once to any of the olfactory calf-associated stimuli presented in the milking parlour during milking. However, with overt responses towards such stimuli during only approximately 23% of milkings, the response rate was, in general, rather low. Motivation for exploration may have been hampered in the normal milking situation, either due to fear elicited by handling at the baskets or by distraction through milking. In a specially equipped testing arena, responsiveness might have been higher and distraction lower, but the particular aim of this experiment was to test the cows' perception of olfactory stimulation during milking. Another potential factor in low response rates may have been the distance between the cow's muzzle and the stimuli. The milking boxes were 0.70 m wide with the possible consequence that samples were too far away for the cows to perceive the odours. To our knowledge, the distance at which dairy cows can identify their offspring by smell has not been studied; studies in ewes indicate a span of 0 - 0.25 m (Alexander, 1978; Alexander and Shillito, 1977). However, as the odour of a living lamb is presumed to be more intense than the odour of the stimuli used in this experiment, the maximum distance is supposed to be lower than 0.25 m. The odour might be intensified by increasing the amount of hair (e.g. Alexander and Stevens, 1982/83 used nearly double the weight of hair) and by using dry hair bags. Different from licking a living calf, moistening of the samples with distilled water might have cooled them and, in doing so, diminished the intensity of the odour.

Compared to the experiment of Barth et al. (2010), in which only cloth with calf odour was used, the proportion of responsive cows to calf hair samples was higher, although still too low to render the use of calf hair a promising measure of olfactory stimulation during routine milking. This is compounded by the fact that a decrease in responsiveness of cows over successive trials was observed. However, this might not only reflect habituation (Alexander and Stevens, 1982/83, Ellis and Wells, 2010), but also a change in odour of the calves' hair used for samples over time. From trimming to the test situation, a maximum of three days passed and samples were stored at roughly 16 - 18°C, whereas, in other studies samples were cooled (Alexander and Stevens, 1982/83: 4°C, Lundström et al., 2009: -80°C). Bonadonna and Sanz-Aguilar (2012) stored their samples at a maximum of 15°C and had a comparable amount of non-responding birds (37%), but they did not repeat the measurements.

Despite the generally low response rates, the significantly different response of multiparous cows towards 'own' or 'alien' compared to 'no' (i.e. the longer exploration of 'own' or 'alien'), strongly suggests that the multiparous cows were able to perceive and preferred the samples with calf hair. It is not clear if the cows reacted to 'own' or 'alien' because they recognized it as calf odour or because these samples simply smelled different from 'no'. In 'contact' cows, the data show a trend of longer exploration of 'own' than 'no'. However, the variation in responses was so large that statistically no differences between responses to 'own' and 'alien' could be found.

Data presented in Fig. 3 suggest more unselective responses in 'control' than in 'contact' heifers, which could not be statistically analysed due to the low numbers of heifers and the data distribution. In ewes, maternal olfactory selectivity, in general, is not influenced by parity (Keller et al., 2003: 1^{st}, 2^{nd}, $>2^{nd}$ lambing). However, if there is a separation between mother and young soon after birth, primiparous animals are reported to be less selective and/or less maternal than multiparous mothers (beef cattle: Le Neindre and D'Hour, 1989; Prince et al., 1986, goats: Lickliter, 1982, ewes: Otal et al., 2009). Multiparous cows apparently discriminated calf-associated stimuli regardless of calf-contact. This might be due to higher neuroendocrine responses linked to motherliness during and after parturition in multiparous compared to primiparous animals (ewes: reviewed by Dwyer, 2008). However, regarding the general responsiveness in the milking parlour during this experiment, we did not find a significant effect of parity on behavioural response. Many factors may have affected the cows' responses to the olfactory samples, which could not be sufficiently standardised with the available experimental resources. For instance, 'alien' samples were on average from older calves (19 - 88 d, mean = 42, SD = 18 d) than 'own' samples (10 - 21 d, mean = 16, SD = 3 d) and were comprised of shorter, regrown hair. Responsive 'contact' cows were presented samples with odour from an alien calf of a different sex than their own calf in four cases and with 'alien' samples of a different breed ('contact': once, 'control': twice). It is also possible that there was contact to alien calves in the barn on three ('contact') and two ('control') occasions. However, according to our graphical check, these factors did not cause any obvious bias.

2.5 Conclusions

Despite low response rates to the presented olfactory stimuli in general, we conclude that, at least, the responsive multiparous cows and 'contact' heifers were able to perceive the presented calf odour and preferred to sniff/lick those stimuli compared to a stimulus with 'no' odour. For conclusive results regarding possible effects of calf-contact, especially in heifers, and the cows' ability to discriminate between olfactory samples from their own and alien calves, further investigations with larger samples would be needed.

3 RESPONSES OF DAMS VERSUS NON-NURSING COWS TO MACHINE MILKING IN TERMS OF MILK PERFORMANCE, BEHAVIOUR AND HEART RATE WITH AND WITHOUT ADDITIONAL ACOUSTIC, OLFACTORY OR MANUAL STIMULATION

3.1 Introduction

On most dairy farms with *Bos taurus* cow and calf are separated soon after birth which can be criticized from an animal welfare perspective (e.g. Keyserlingk and Weary, 2007). Consumers in many countries are concerned about early separation (Boogaard et al., 2008, 2010; Ventura et al., 2013). There is growing interest in allowing suckling and additionally milking the cows (Busch et al., 2017; Hötzel et al., 2017). In so-called dam rearing, different nursing strategies are possible (reviewed by Johnsen et al., 2016). A major challenge of dam rearing is incomplete alveolar milk ejection during machine-milking (e.g. Boden and Leaver, 1994; de Passillé et al., 2008; Mendoza et al., 2010) and thus the reduced amount of saleable milk. In addition, milk fat content and milk flow are reduced (e.g. Barth et al., 2010; Mendoza et al., 2010).

Milk is produced continuously in the alveoli of the udder (reviewed by Bruckmaier, 2001). In cows milked twice daily without suckling, the cisternal milk fraction stored in the udder is nearly 20%. This milk is obtainable without milk ejection. However, tactile stimulation of the udder is necessary in order to induce the neuro-endocrine milk ejection reflex targeting the fraction stored in the alveoli. Oxytocin is released and if a certain threshold is reached, the shift of alveolar milk into the cistern is provoked, where it can be collected (Bruckmaier, 2001). The tactile stimulus of pre-milking and udder cleaning is mostly sufficient to induce alveolar milk ejection in non-nursing cows (Bruckmaier and Blum, 1998). Cause for alveolar milk ejection problems in dams are not completely understood, but some mechanisms have been detected. Dams release less oxytocin during machine milking than non-nursing cows (Akers and Lefcourt, 1984; de Passillé et al., 2008). On the other hand, they have higher blood oxytocin levels when nursing the own calf compared to non-nursing cows at machine milking (Akers and Lefcourt, 1982; Bar-Peled et al., 1995; de Passillé et al., 2008; Lupoli et al., 2001; Tancin et al., 2001). However, for dams nursing an alien young, no (goats: Hernandez et al., 2002; beef sucklers: Silveira et al., 1993) or a smaller oxytocin increase (dairy cows: Bar-Peled et al., 1995) is reported. The exclusive response towards the own offspring is likely due to oxytocin being an important hormone in maternal bonding and maternal behaviour (reviewed by Kendrick, 2000; Uvnäs-Moberg et al., 2001). A further possible aspect affecting milk ejection is the occurrence of stress. For instance, Schneider et al. (2007) found indications for higher agitation (combination of eye-wideness, rumination and head position) during milking in dams compared to non-nursing cows. However, the knowledge about the well-being of dams during milking is insufficient.

Peeters et al. (1973) observed milk ejection during calf-contact, when the udder was not touched, in primiparous and multiparous cows, but mainly if the animals showed maternal behaviour towards their offspring. The authors conclude that calf-related stimuli such as sight, sound and odour are suitable to induce milk ejection, even without the experience of suckling. Therefore, calf-associated stimuli in the parlour might alleviate problems with milkability. Barth et al. (2010) tested the influence of olfactory stimulation in the parlour on milk let-down of dams with a cloth their calves were firmly rubbed with before. No effect was found, but it remained unclear whether animals did not perceive the odour, or calf-odour did not influence alveolar milk ejection. In a prior study we examined the behavioural response of cows with and without calf-contact on hair of the own calf, hair of an alien calf and a control without calf-hair (Zipp et al., 2016). Increased responses towards samples with calf-hair suggested that this kind of olfactory stimulation was perceived by the animals. Pollock and Hurnik (1978) stimulated non-nursing cows acoustically with played-back calf calls during udder preparation and thereby gained more milk. Also manual teat stimulation, for example hand milking, can lead to higher oxytocin release (Gorewit et al., 1992), higher milk yield and milk fat content in non-nursing animals (Svennersten et al., 1990). So far it is not known if these kinds of stimuli are able to enhance milk let-down in dams during the milking process.

The aim of this study was therefore to compare milk yield, milk flow characteristics and milk composition between dams and non-nursing dairy cows under conditions of routine milking versus milkings with enhanced olfactory stimulation with calf hair, acoustic stimulation with played-back calf calls or manual teat massage before machine milking. In addition, we took into account agitation behaviour, HR and HRV during the different treatments in the parlour as potential stress indicators.

3.2 Animals, material and methods

3.2.1 Animals, housing, management and experimental groups

The experiment was conducted from November 2010 to May 2011 at the research farm of the Thünen-Institute of Organic Farming in Trenthorst, Germany in two separate horned herds of 45 - 48 German Holstein black-and-white and 45 - 48 German Red Pied cows. They were housed in two identical sections of a loose housing stable with cubicles and were managed in the same way. All animals were fed a total mixed ration and additionally received concentrate provided by automated concentrate feeders according to their automatically measured milk yield (max. 8.5 kg d^{-1}, for details see: Wagner et al., 2012).

The ‘control’ group consisted of 22 animals (three primiparous and ten multiparous German Holstein, two primiparous and seven multiparous German Red Pied). They calved in a calving pen, one for each cow-calf-pair, were separated from their calf within the first 12 h after birth, and were integrated in the milking herd one day after parturition. Milking twice daily started after calving. ‘Control’ calves were group-housed. Colostrum was supplied by bottle and afterwards calves were trained to drink at an automatic milk feeder.

The ‘contact’ group consisted of 18 animals (four primiparous and seven multiparous German Holstein, two primiparous and five multiparous German Red Pied). However, due to cases of clinical mastitis and death of one calf, one primiparous and one multiparous German Red Pied and one German Holstein heifer were excluded from analysis, so that in the end data from 15 dams were analysed. 'Contact' cows also calved in the calving pen, but stayed there for the first five days together with their calf, except for milking and the main feeding time after milking twice daily. Calves were bottle-fed 2 L colostrum immediately after birth and allowed to suckle the dam. After five days they were integrated into the dairy herd. Calves were housed with 'control' calves in the calves' area where hay, silage, concentrate as well as water were provided. 'Contact' calves had no access to the automatic milk feeder, but could enter the dairy cows' lying area unrestrictedly through a transponder controlled selection gate. Thus, 'control' cows had also contact with the 'contact' calves, but no nursing event was observed in 'control' animals.

3.2.2 Preparation and application of treatments

Starting between the fourth to fifth wk of lactation, stimulation tests were carried out for three consecutive weeks (26 - 50 d in milk). The experiment started for each animal on a Monday, therefore the day of lactation of the animals varied (contact: 30 ± 2.3 d; control: 29 ± 2.3 d; mean and SD). Each week one of three treatments was applied during four consecutive milkings in the 2x4 autotandem milking parlour (GEA, Boenen, Germany): acoustic, olfactory and manual stimulation. On four other consecutive milkings of this week routine handling was conducted. Each animal underwent the three treatments, except for six 'control' cows, where no olfactory stimulation was possible, because their calves had died. In all eight milkings milk yield, machine-on time, milk flow characteristics, milk composition, HR and agitation behaviour were recorded. To avoid carry over effects, there were two to four routine milkings without recording between the four recorded routine milkings and the four treatment-milkings. Milking routine consisted of manual pre-milking and cleaning of the udder (approx. 20 sec in total) with a machine washed and tumble dried fabric towel, attaching and positioning of milking cluster, 40 sec vibration stimulation (300 pulses min^{-1}), 38 kPa milking vacuum, automatic stripping starting at a milk flow of 800 g min^{-1} and automatic cluster removal at a milk flow lower than 300 g min^{-1}. Emptiness of udders was checked manually after automatic cluster removal and cluster was attached again when necessary. To prevent calves from intake of disinfectants, only the teats of 'control' cows were dipped after milking.

Acoustic stimulation consisted of played back calf calls recorded approx. 10 h after the last milk feeding from ten 2 - 12 wk old alien calves belonging to another farm. Calf calls were played back at least from the moment cows entered the milking stall until milking clusters were removed. For olfactory stimulation, hair of the own calf was cut half a day before the first stimulation. Hair samples came from the anogenital region, hind legs and tail. For each milking, approx. 0.8 g hair was filled in one thin cloth bag with which the calves had been rubbed before (cloth: Fliselina®, Freudenberg Vliesstoffe KG, Weinheim, Germany). The bags were stored each in a glass jar at approx. 16 - 18°C. When the cow entered the milking stall, the hair bag was taken out of the glass using tongs, sprayed with distilled water and placed in a stainless steel basket, installed in the head region of each milking stall. Damping the hair should simulate the licked hair of calves. Six control cows with dead calves were excluded from this treatment, as already mentioned. During acoustic and olfactory stimulation milking process followed routine milking. For manual stimulation, after pre-milking and udder cleaning, fore- and rear teats of one body side were simultaneously massaged in a rotating downwards movement, alternating between sides for a total stimulation time of 60 sec. Then the milking cluster was attached and machine milking started immediately without vibration stimulation.

Figure 5: Video of the udder preparation during manual stimulation (Source: Kerstin Hofmann) can be downloaded or watched under:
https://www.sciencedirect.com/science/article/pii/S0168159118302302

The same familiar person was milking during all treatments with data collection. The order of treatments during the three weeks of lactation and the order of routine milking and treatment milking during one week was as far as possible randomized for each animal. However, the acoustic stimulation via speakers in the milking parlour could not be applied individually. Therefore every third calendar week all cows were acoustically stimulated. It was randomized if either the acoustic treatment or routine milking was conducted first during this calendar week.

3.2.3 Milk recording

Milk yield (kg), machine-on time (min) and milk flow parameters were measured with LactoCorders® (LC, WMB AG, Balgach, Switzerland). Parameters of milk flow were peak milk flow (kg min^{-1}) and average milk flow during main milking phase (kg min^{-1}). Main milking phase started when milk flow reached 0.50 kg min^{-1} and ended when it fell under 0.20 kg min^{-1}. For further explanations of the LactoCorders® and parameters see Sandrucci et al. (2007). Composite milk samples were taken with Metatron Premium 21 (GEA Boenen, Germany). Milk samples were stored at -18°C and content of fat and somatic cell count were analysed in the laboratory of the milk recording organisation (Landeskontrollverband Schleswig-Holstein e. V.) according to standardised methods (CombiFoss™, FOSS, Hilleroed, Denmark). Somatic cell count of clinically healthy animals was transformed to somatic cell score ($SCS = log_2\left(\frac{SCC}{100{,}000}\right) + 3$; Wiggans and Shook, 1987). Data collection was not possible in one 'contact' and two 'control' cows that had blood in the milk during seven, five and seven milkings respectively, and four 'contact' cows with clinical mastitis during 4 - 9 milkings that were treated with antibiotics. Clots or blood would have clogged the LactoCorder® strainer and the sensors for the analysis of milk composition.

3.2.4 Agitation behaviour sampling

Milkings were videotaped (Axis 221 network-cameras, 640x480 pi zoom lens, Axis Communcations, Lund, Sweden) and later analysed by two observers using The Observer® XT (Version 10.5, Noldus Information Technology, Wageningen, The Netherlands). Inter- and intra-observer reliability were tested on the basis of videos of 12 single milkings. Agreement between outcomes was analysed using Cohen's Kappa, or in case of steps min^{-1} using Spearman correlation. For the latter it was also ascertained that the slope of the regression (forced through the origin) was close to one. Inter- and intra-observer reliability were acceptable before, during and after behavioural recording (Tab. 4). Behavioural measures are listed and defined in Tab. 4 including the phases in which they were recorded.

Table 4: Definitions of agitation behaviour observed in the parlour and observer reliability (before, during and after observation period, each time one inter- and two intra-observer reliability tests; Cohen's Kappa Coefficient for binomial data, Spearman Correlation for metric data).

parameter	definition	observed time	observer reliability
behaviour during udder preparation binomial: calm/agitated	calm: max. 3 steps agitated: > 3 steps or at least 1 kick	udder preparation	K = 0.77 - 1.0
ruminating binomial: no/yes	ructus, chewing and swallowing of bolus ruminating < 50% of time: no ruminating >50% of time: yes	machine-on-time	K = 0.66 - 1.0
posture of the head binomial: calm/tense	Calm: head in a nearly horizontal position, no or few up and down movements of the head Tense: head position higher than withers, or frequent up and down movements of head, or cow is turning her head caudally	machine-on-time	K = 0.76 - 1.0
stepping (number of steps/cow and min)	lifting one hind leg less than at udder height	time in milking box (udder preparation excluded)	r = 0.84 - 0.98
kicking binomial: yes/no	lifting one hind leg at udder height or higher, or clearly directing movement at milking cluster or milker	time in milking box (udder preparation excluded)	K = 0.82 - 1.0
defecating binomial: yes/no	drop of faeces	time in milking box	K = 0.99 - 1.0

3.2.5 Heart rate measurement

HR was measured non-invasively during morning milkings only. Polar S810i and RS800CX (Polar Electro Oy, Kempele, Finland) recorded HR in beat-to-beat intervals. Animals were habituated to wearing the elastic girth during one week before stimulation started. During the experimental phase HR equipment (without monitor-watch) was attached to the animals while they were locked at the feeding rack after afternoon milkings. The coat was not trimmed but soaked with water in the regions where the electrodes were placed and electrode gel (Sonogel®, Vertriebs GmbH, Bad Camberg, Germany) was applied. Electrodes were placed about 10 cm under the withers (+) and in the heart region near the elbow of the left body side (-). To fix the HR equipment safely, a 20 cm wide elastic girth was strapped over it. One German Holstein 'control' cow reacted aggressively to this handling and was therefore excluded from HR measurements. During morning milking, after the animal had entered the milking stall, the monitor watch was activated and fastened at the girth. In some cases electrode gel had to be applied again. If the fit of the equipment was further incorrect, no HR measurement was done at this animal during that milking. Milking started approximately 5 min after this manipulation.

3.2.6 HRV analysis

HR sequences of 5 min starting with the udder preparation were selected and corrected with Polar Trainer 5.0 with medium filter and cut of peaks (Polar Electro Oy, Kempele, Finland). Milkings where > 5% of data (checked in 1 min intervals) needed correction were excluded from analysis. In addition, three 'contact' cows' milkings were frequently shorter than 5 min and therefore excluded from analysis. Data of 21 'control' and 12 'contact' cows were considered. HR (bpm), root mean square of successive beat-to-beat differences (RMSSD, ms), standard deviation of inter beat intervals of the 5 min period (SDNN, ms), relative power of low and high frequency bands (LF%, HF%) and the ratio between LF and HF band powers (LF/HF) were calculated with Kubios HRV 2.2 (Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). The limits of frequency bands were set to LF: 0.005 - 0.20 Hz and HF: 0.2 - 0.58 Hz following the recommendation of von Borell et al. (2007).

3.2.7 Statistical analysis

Due to missing values (in cases of mastitis, blood in the milk, technical problems, short milkings or dead calves regarding olfactory stimulation) sample sizes for different measures differed from a minimum of 8 'contact' and 14 'control' animals to a maximum of 15 'contact' and 22 'control' animals. Mixed effects models (Bates et al., 2014) were used for analyses of the dependent variables milk yield, fat content, SCS, milk flow characteristics, HR and HRV-parameters and steps per min. The other parameters of agitation behaviour were analysed with generalized linear mixed models (Bates et al., 2014) for binomial data. The time animals spent in the milking stall was included as offset variable in case of kicking and defecation, as this period was not only dependent on the milking duration but also on the treatment and whether HR was assessed or not. The maximum model included group (contact, control), treatment (routine, acoustic, olfactory, manual) and their interaction as fixed factors. Through backwards selection, we excluded the interaction, if the Akaike's Information Criterion (AIC) was decreased and therefore the model was improved by it. The minimum model included only group and treatment as fixed factors. Observation week (1 - 3) nested in animal, and separately daytime (morning, afternoon) were random factors in the models for milking performance and behaviour to account for the repeated measurements and the comparison of data within the observation week. Data of HR and HRV during stimulation were not compared with the routine milking of the same week, but with all routine milkings during the whole experiment. This was necessary due to missing values (technical problems). As HR was, furthermore, only measured during morning milkings, random factor in the models for HR and HRV was only animal. Normality and homogeneity of residuals and independence of fixed factors were checked graphically and with values of skew and kurtosis. Normality of random effect 'animal' and absence of bias in the mean errors was checked graphically for generalized mixed models. To fit model assumptions some variables had to be transformed: HR, SDNN, RMSSD, HF% and LF/HF ($ln(x)$), steps per min ($log_{10}(x+1)$) and fat content ($log(\frac{x}{1-x})$) Results are presented as means and standard deviations of observed data, model estimates or odds ratio and if appropriate confidence intervals. If treatment had an effect, Dunnett's post hoc tests were conducted to compare routine milking with the three stimuli. P-values of the post-hoc tests are adjusted. Effect sizes for metric data were calculated with $r = \sqrt{\frac{t^2}{t^2+df}}$ (Rosenthal, 1991, p. 19). Effect sizes for binomial data were calculated with the mean and SD of the percentage of occurrence of the behaviour from observed data with $= \frac{d}{\sqrt{a+d^2}}$ (*d*=standardized mean difference, *a*=((n_1+n_2)²)/(n_1*n_2). All analyses were done with R (version 3.1.2, R Development Core Team 2014).

3.3 Results

3.3.1 Machine collected milk yield, fat content, milk flow and SCS

Effects of acoustic, olfactory and manual stimulation in comparison to routine milking on machine collected milk yield, fat content, milk flow characteristics and SCS were analysed for 19, 16, 22 and 22 animals of the 'control' group and 13, 14, 13 and 15 'contact' animals.

Milk yield ($p < 0.0001$, r = 0.85) and fat content ($p < 0.0001$, r = 0.80) were lower in dams, while stimulation had no effect (machine milk yield: p = 0.9511; fat content: p = 0.2792). 'Contact' cows had on average 9.9 kg less milk per milking and a 0.66% lower fat content than 'controls' during the second month of lactation (Fig. 6, A and B).

Peak milk flow ($p < 0.0001$, r = 0.67) and mean milk flow during the main milking phase ($p < 0.0001$, r = 0.76) were lower in 'contact' animals. The stimuli did not have an effect on peak milk flow (p = 0.2303, Fig. 6, C). However, the mean milk flow of the main milking phase was higher during manual stimulation than during routine milking (p = 0.0494, r = 0.10, Fig. 6, D). Although numerically this difference was only apparent in dams, no interaction with group was detected.

SCS as indicator of udder health did not differ between groups (p = 0.4111, r = 0.13, Fig. 7). However, SCS was lower in dams during acoustic stimulation compared to routine milking, while SCS of 'controls' stayed at a stable level (interaction: p = 0.0023, Fig. 7).

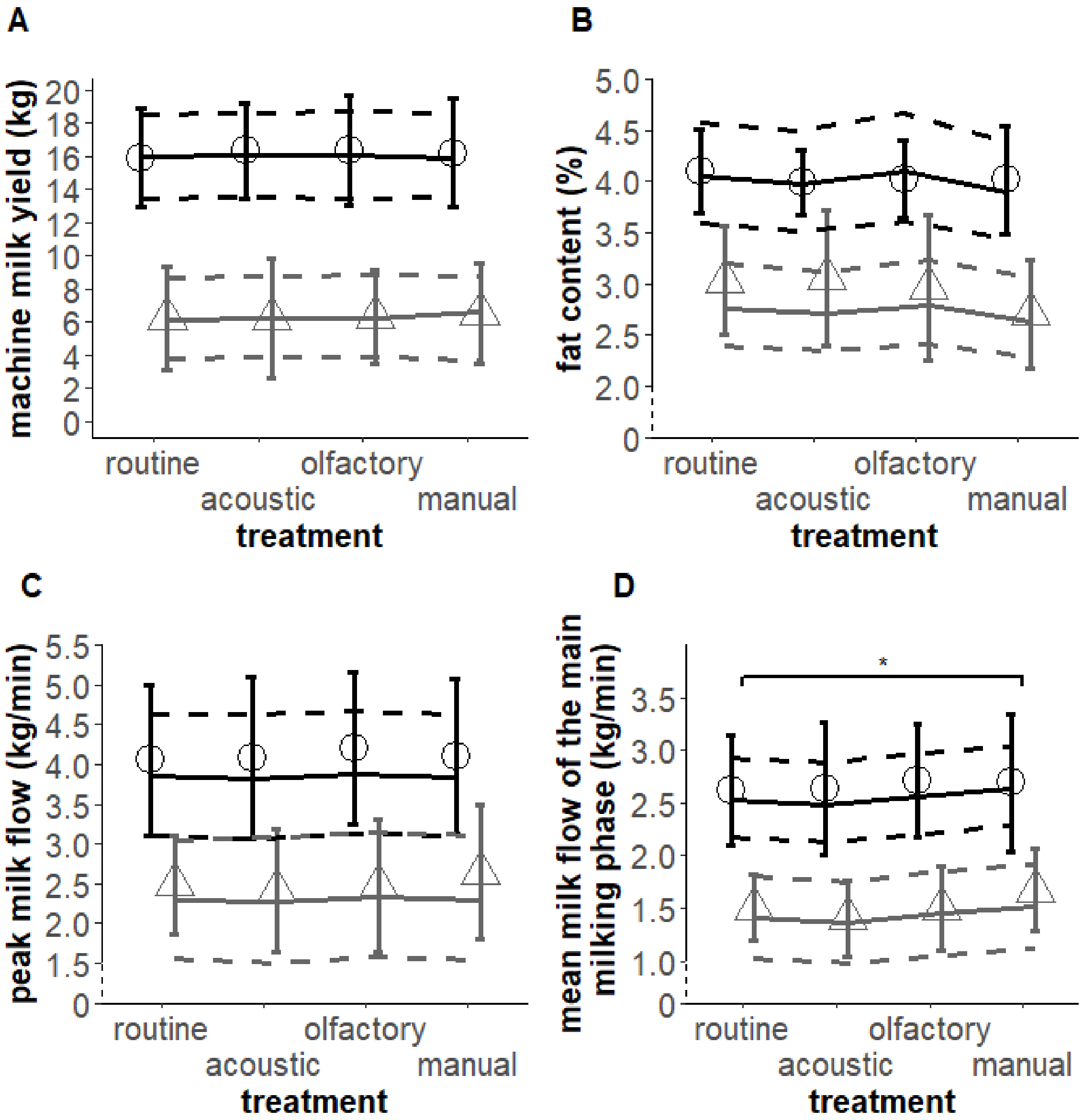

Figure 6: Influence of no cow-calf contact (○, $n_{routine}$ = 22, $n_{acoustic}$ = 19, $n_{olfactory}$ = 16, n_{manual} = 22) or free cow-calf contact (Δ, $n_{routine}$ = 15, $n_{acoustic}$ = 13, $n_{olfactory}$ = 14, n_{manual} = 13) and stimulation during milking on machine collected milk yield (A), fat content (B), peak milk flow (C) and the mean milk flow of the main milking phase during the fourth to seventh week of lactation (shape and whisker: mean ± SD of observed data, solid line: model estimate, dashed lines: 95% confidence interval, star: $p < 0.05$).

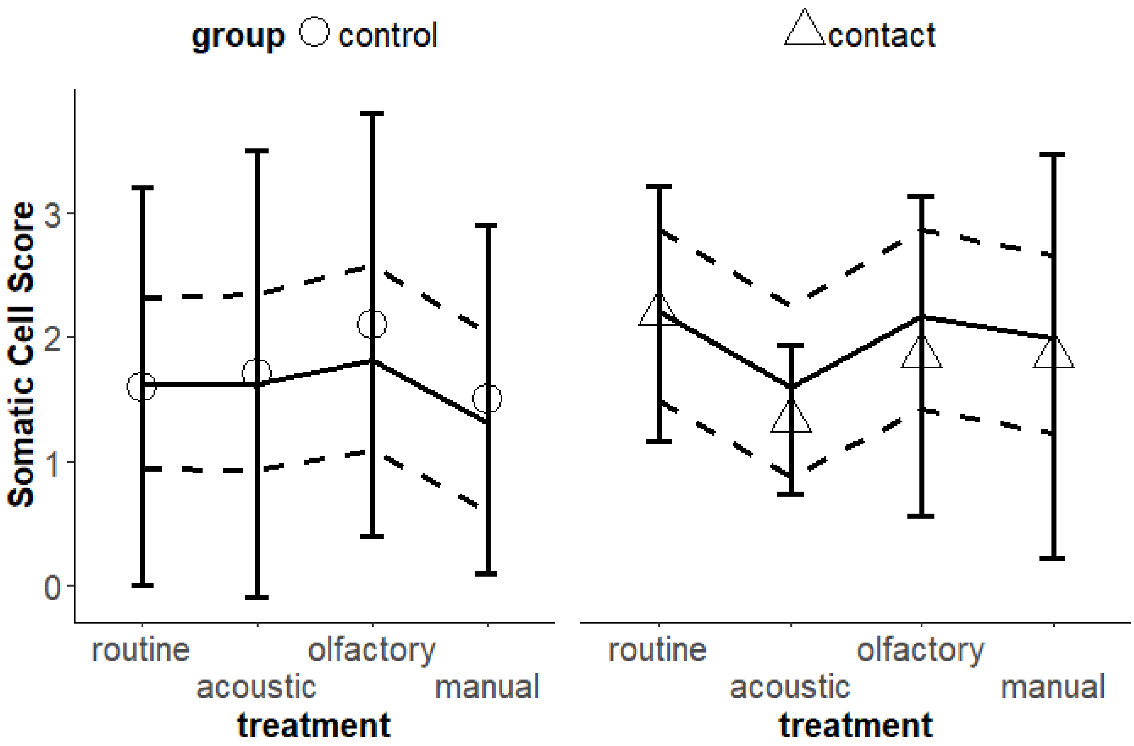

Figure 7: Interaction of no cow-calf contact (○, $n_{routine} = 22$, $n_{acoustic} = 19$, $n_{olfactory} = 16$, $n_{manual} = 22$) or free cow-calf contact (Δ, $n_{routine} = 15$, $n_{acoustic} = 13$, $n_{olfactory} = 14$, $n_{manual} = 13$) and stimulation during milking on Somatic Cell Score (shape and whisker: mean ± SD of observed data, solid line: model estimate, dashed lines: 95% confidence interval).

3.3.2 HR and HRV

Effects of the stimulation in comparison to routine milking on heart rate and HRV were analysed in 16, 15, 18 and 21 'control' animals and 9, 10, 9 and 12 dams, respectively.

'Controls' had higher LF% and LF/HF than 'contact' cows (Tab. 5). Otherwise, calf-contact or stimulation did not influence heart rate and HRV-parameters (Tab. 5).

3.3.3 Agitation behaviour in the parlour

Effects of the stimulation in comparison to routine milking on agitation behaviour were analysed in 19, 16, 22 and 22 'control' as well as 13, 15, 13 and 15 'contact' cows, respectively.

'Contact' animals showed a tense head position and defecation during more milkings than 'control' cows. Other parameters of agitation behaviour were not influenced by calf-contact (Tab. 5). Behaviour was not affected by any stimulus ($p > 0.1$).

Table 5: HR, HRV ($n_{control}$ = 21, $n_{contact}$ = 12) and agitation behaviour ($n_{control}$ = 22, $n_{contact}$ = 15) during routine milking of dairy cows (4th to 7th week of lactation) with free calf-contact or no calf-contact (control): mean ± SD of observed data, in case of binomial variables percentage of occurrence calculated from observed data), significant group-effects in bold ($p < 0.05$; r: effect size; Est = Estimate and OR = Odds ratio: control as reference).

dependent variable	control	contact	p-value r-value	Est or OR
HR (b/min)	74.2 ± 8.5	71.4 ± 6.4	p = 0.3510 r = -0.16	Est = -0.7
RMSSD (ms)	11.1 ± 7.9	9.1 ± 4.8	p = 0.5857 r = -0.09	Est = -0.1
SDNN (ms)	32.6 ± 19.2	27.8 ± 17.8	p = 0.4050 r = -0.14	Est = -0.2
HF%	4.5 ± 4.8	5.5 ± 5.3	p = 0.3795 r = 0.15	Est = 0.3
LF%	22.6 ± 11.3	15.1 ± 8.8	**p = 0.0160 r = -0.37**	**Est = -8.2**
LF/HF	11.2 ± 11.4	7.3 ± 7.1	**p = 0.0251 r = -0.37**	**Est = -0.7**
agitated during udder preparation (% of milkings)	31.0 ± 28.9	46.5 ± 33.5	p = 0.1728 r = 0.24	OR = 2.6
tense head position (% of milkings)	1.2 ± 3.1	13.1 ± 13.3	**p = 0.0007 r = 0.56**	**OR = 19.4**
no rumination (% of milkings)	61.6 ± 18.9	63.4 ± 23.7	p = 0.8500 r = 0.04	OR = 1.1
steps min^{-1}	1.2 ± 0.5	0.9 ± 0.4	p = 0.142 r = -0.24	Est = -0.2
kicks (% of milkings)	18.9. ± 24.3	23.5 ± 26.3	p = 0.6884 r = 0.09	OR = 1.3
defecation (% of milkings)	4.6 ± 15.7	8.7 ± 10.4	**p = 0.0125 r = 0.50**	**OR = 3.3**

3.4 Discussion

3.4.1 Calf-contact

Conforming to other studies (reviewed by Johnsen et al., 2016) machine collected milk yield, fat content and milk flow of dams was significantly lower than in non-nursing cows. Decreased fat content indicates incomplete alveolar milk ejection (Ontsouka et al., 2003). A lower milk flow was also reported from cows with low compared to high udder filling during late lactation (Edwards et al., 2014; Sandrucci et al., 2007; Tancin et al., 2006), so the low milk flow could also be due to the low udder filling in dams because of the additional suckling between the regular milking times. Bruckmaier and Hilger (2001) have shown a linear relationship between degree of udder filling and delay from first touch of the udder until the onset of milk ejection. Consequently, cows with low udder pressure need longer pre-stimulation (Bruckmaier and Hilger, 2001; Weiss and Bruckmaier, 2005). In conclusion, the applied manual and vibration stimulation time of approximately 60 sec may have been insufficient to induce an adequate milk ejection.

Similar to other studies (e.g. Barth et al.; 2007; Krohn 2001), SCS did not differ between groups and was always at a low level. Concerning udder health it must be recognized, however, that six dams and no 'controls' had signs of clinical mastitis during the fourth to seventh week of lactation. Two of the cows were excluded from analysis because clots in the milk made SCC measurement impossible. Nevertheless, a retrospective analysis over the whole lactation did not reveal any difference in mastitis incidence of dams and 'control' cows (data not shown). This confirms the general finding that dam rearing has no effect on the incidence of clinical mastitis (Barth et al., 2007; reviewed by Krohn, 2001).

Behaviour during udder preparation, rumination during machine-on-time as well as stepping and kicking during the time cows spent in the milking box did not differ between groups. Nevertheless, despite low effect sizes ($r < 0.3$), according to odds ratios dams were during 2.6 more milkings agitated during udder preparation than controls. For the milker this might be quite a difference. Furthermore, milkings with a tense head position were increased in dams 19.4 times and with defecation 3.3 times compared to controls. This does not only mean decreased working comfort, but may indicate impaired animal welfare in dams during milking. Defecation can also be a problem for hygienic food production. Also Schneider et al. (2007) found indications for mild stress during milking in nursing cows, though no increased defecation. On the other hand, LF% and LF/HF were significantly lower in 'contact' than in 'control' cows. This indicates an activation of the vagal axis. The other parameters of HRV were not influenced by nursing. However, other studies analysing HRV data of non-nursing cows during milking also found that their treatment only affected some parameters of HRV while others did not differ (Hagen et al., 2005; Gutmann et al., 2013). Compared to the results from Gutmann et al. (2013), we found higher RMSSD and lower LF% and LF/HF values. This consistently points at higher vagal activity of the cows in our study. HRV data of Hagen et al. (2005) are similar to our results. Therefore we conclude that even though not all parameters of HRV pointed in the same direction, there were no contradictions.

The higher vagal activity of dams in our study may be due to the following mechanism: cows with free calf-contact expectedly have several high oxytocin releases over the day during suckling (Akers and Lefcour, 1982; Bar-Peled et al., 1995; Lupoli et al., 2001), and consequently higher basal oxytocin levels (Bar-Peled et al., 1995). Suckling (reviewed by Goldstein et al., 1995) and oxytocin cause increased vagal activity (reviewed by Uvnäs-Moberg and Petersson, 2005). Possibly, in combination the results indicate that the stress experienced by dams during milking was not severe enough to offset the general vagal activation. However, no firm conclusion can be drawn without further investigation of HRV in dams at other times than milking. Furthermore, regarding the interpretation of defecation frequency there are two alternatives: commonly it is used as indicator of stress or fear, e.g. during behavioural tests (reviewed by Forkman et al., 2007; de Passillé et al., 1995; MacKay et al., 2014) or during milking (Das and Das, 2004; Jacobs and Siegford, 2012; Rushen et al., 1999). The concurrent occurrence of tense head position and defecation point into this direction, too. However, increased defecation could also be due to a general activation of gut motility and therefore generally higher defecation frequency over the day through suckling combined with high oxytocin release. Studies about the effect of oxytocin on elimination behaviour are rare: after exogenous oxytocin was applied, guinea pigs urinated more often (Bisset and Lewis, 1962) and higher frequencies of defecation were observed in healthy women (Ohlsson and Sjölund, 2001, cited from Ohlsson et al., 2004) and horses (Maraki et al., 2014). Maraki et al. (2014) found a 2 - 4 h faster intestinal transit time in horses, and the horses defecated on average 12% (0 - 2x) more often than before oxytocin application.

3.4.2 Stimulation

No specific effect on milk let-down of dams with free calf-contact could be obtained by additional acoustic, olfactory or manual stimulation during milking in the fourth to seventh week of lactation.

Manual stimulation induced a higher average milk flow during the main milking phase. While no significant group*stimulation interaction could be detected, numerically the effect was only apparent in dams. A higher milk flow in non-nursing cows after manual stimulation compared with vibration stimulation (300 cycles min^{-1}) has been found by Karch et al. (1988) and Watters et al. (2015). Similar to our study, this was not accompanied by higher milk yield (Karch et al., 1988; Watters et al., 2015). It has been suggested that the change in milk flow is due to technical differences and not a result of a better alveolar milk ejection: In contrast to teat massage, during vibration stimulation a low amount of milk is already collected during the process (Karch et al., 1988: 0.05 - 0.23 kg; Watters et al. 2015: 0.32 kg). This may result in a decreased main milk flow rate (Karch et al. 1988), as intra-mammary pressure decreases. This hypothesis is supported by the fact that other indicators of milk let-down such as machine milk yield, fat content and peak milk flow were not affected by manual stimulation (Fig. 6).

Despite promising reports from the literature on increases in machine gained milk through played back calf calls in non-nursing cows (McCowan et al., 2002; Pollock and Hurnik, 1978) we found no such effects due to acoustic stimulation. Pollock and Hurnik (1978) played-back calf-calls only during udder preparation. This was not possible in our study as milking procedures in the tandem parlour were not synchronous for different animals. Therefore, our acoustic stimulation was not very realistic as our imaginary calves did not stop calling, when ‘they were suckling’ or in reality, milking clusters were attached. However, SCS was lower in dams during acoustic stimulation compared to routine milking, while it stayed at a similar level in ‘controls’. In general the variance in SCS was high, but comparable with other studies (Zhao et al., 2015, mean SD = 1.29). SCC and correspondingly SCS can vary even in an individual cow by 4 - 70% during one day (Harmon, 1994) and by 30 - 35% (Dohoo and Meek, 1982) or at a standard deviation of 0.5 between days (Forsbäck et al., 2010). However, during acoustic stimulation the standard deviation of SCS in dams was with about 1 half as high as during the other treatments. This indicates that response of dams towards acoustic stimulation was rather similar between individuals. SCS is an indicator of udder health, but can also be influenced by stress (Dohoo and Meek, 1982). Heat stress (Lambertz et al., 2014) as well as the animal-human-relationship has been shown to influence SCS (Ivemeyer et al., 2011). These however, are parameters, which appeal to the animals for a longer time. Until now, it is unclear if a sudden stress or positive event during milking can directly influence SCS. Further, we do not know why the calling of hungry calves should have a relaxing effect on dams.

Even though results of our preliminary study on behavioural responses of cows with and without calf contact suggest that both groups perceived the olfactory stimulus of calf hair (Zipp et al., 2016), no effect on the parameters assessed could be found.

3.5 Conclusions

It was not possible to significantly increase the amount of saleable milk or the fat content of dams with free calf-contact through stimulation with played-back calf calls, hair of the own calf or teat massage compared to routine milking. Differences in productivity of dams compared with non-nursing cows were in accordance with literature: lower machine collected milk yield with less fat and lower milk flow, but no differences in SCS. While there were some indications of increased agitation during milking in dams, at the same time they had a generally increased vagal activity (lower LF/HF and LF%), possibly suckling induced, which warrants further investigation.

4 DAM REARING OF DAIRY CALVES: EFFECTS OF HALF-DAY VERSUS FREE COW-CALF CONTACT ON THE PERFORMANCE OF MOTHER AND OFFSPRING

4.1 Introduction

When European dairy cows (*Bos taurus*) are allowed to nurse their calves and are additionally milked, the amount of sellable milk is reduced by 7 - 20 kg d^{-1}compared to non-suckling systems (reviewed by Johnsen et al., 2016). One reason is that nursed calves can drink up to 14 kg of milk d^{-1} (de Passillé et al., 2008). Bucket fed calves usually receive less milk or milk replacer, for example 10% of live weight (e.g. Jasper and Weary, 2002). Under such conditions, more harvested milk can be sold. Another reason for reduced collected milk yield is disturbed milk ejection during milking in dams (i.e. cows rearing a calf) caused by lower oxytocin release (Akers and Lefcourt, 1984; de Passillé et al., 2008). This is reflected by slower milk flow rates (Barth et al., 2010; Mendoza et al., 2010), a higher amount of residual milk after milking (de Passillé et al., 2008) and a reduced fat content in collected milk by 1.0 - 1.5 percent points (reviewed by Johnsen et al., 2016). Beside problems during weaning, the loss of harvested milk is the major challenge of dam rearing systems of dairy calves in terms of feasibility. Otherwise dam rearing carries a number of advantages for the calves' and dams' welfare (Johnsen et al., 2016).

To date no effective and sustainable way to heighten oxytocin release during milking in nursing cows has been found (Johnsen et al., 2016). Another approach to increase milkability may be to decrease the time of cow-calf contact. On some commercial farms restricted suckling is practised where cow and calf meet twice per day for 15 - 60 min. Verbal reports from these farms suggest only minor problems with milk let-down. From scientific studies, losses of harvested milk of 6.9 - 12.4 kg d^{-1} are reported for restricted suckling in Holstein cows (Barth et al., 2007; Boden and Leaver, 1994; Krohn et al., 1990, cited by Krohn, 2001; Mendoza et al., 2010; de Passillé et al., 2008). This is less than found in free contact systems (13.8-20.0 kg d^{-1}; Barth et al., 2009b; Barth et al., 2010; Zipp et al., 2013). However, in restricted suckling systems calves can only drink twice daily, which is not in line with their natural suckling behaviour. Furthermore, depending on contact duration and place, affiliative behaviour and social learning from the mother or other cows are considerably limited. Therefore, half-day contact may be an approach where calves and cows can perform more natural behaviour than in restricted suckling. At the same time, the milkability may be less impaired than in a free contact cow-calf system. Other studies on half-day dam rearing focused on animal behaviour (Veissier et al., 2013) or did not compare with free contact (Johnsen et al., 2015a; 2015c). The aim of this study was to investigate the effect of half-day contact between cow and calf in comparison to free contact and to a control treatment without cow-calf contact in terms of machine collected milk yield, milk composition and calf weight gain. Dam rearing most often had no impact on udder health and calving interval, but there are conflicting results (reviewed by Johnsen et al., 2016 and Kälber and Barth, 2014). Therefore, we further included these variables in the study.

4.2 Animals, material and methods

4.2.1 Animals, housing and management

The experiment was conducted from October 2011 to June 2012 at the organic research farm of the University of Kassel, Germany, and carried out in compliance with the German Animal Welfare Act. At the time of the experiment, the farm kept about 95 dairy cows plus replacement stock of the dual-purpose breed German Black Pied Cattle. They were predominantly horned. Mean milk yield in 2011 was 5,434 kg per year with 4.21% fat and 3.33% protein. A total mixed ration consisting of grass-clover silage, alfalfa silage, grass silage, maize silage, washed raw potatoes and straw were fed without adjustment to individual milk yields. Cows received no concentrate.

The originally 45 experimental cows (lactation numbers 1 - 9) were evenly allocated to three treatments: free whole day calf-contact (‘free’), half-day calf-contact (‘half-day’) and no calf-contact (‘no contact’). However, earlier calving and disease incidences directly after parturition led to reduced and uneven numbers of animals in the treatments (n = 38). From the planned three primiparous cows per group, one changed from ‘half-day’ to the ‘no contact’ group because she had a stillborn calf. Multiparous cows were allocated to the different groups according to expected calving date, as well as milk yield and milk flow of the last one or two lactations. Cows with very high or low milk flow (LactoCorder® data) and high or low milk yield were as far as possible equally distributed over the treatments (± 1 SD from the mean of all experimental cows).

Animals of the three experimental treatments were housed in separate pens in a deep litter barn. Each treatment had a lying area of 6.3 - 7.1 m² per cow and a concrete outdoor run (3.9 - 4.5 m² per cow) with automatic scraper. Animal : feeding place ratio was 0.6 to 1.0 in ‘no contact’, 0.6 - 0.9 in the ‘half-day’ and 0.7 - 0.9 in the ‘free’ treatment. All experimental cows were grouped two weeks before the expected first calving. Thus, dry and lactating cows were mixed in the same pen during the experiment in order to avoid changes of group composition. However, some regrouping became necessary: Although cows did not receive concentrate, some multiparous dry cows were gaining too much weight, as they fed the same ration as the lactating cows. Therefore two ‘no contact’, two ‘half-day’ and three ‘free’ multiparous dry cows were moved to another deep litter area 21 - 67 d before calving. They received lower energy fodder (grass silage and straw) and joined their experimental group after calving. Furthermore, one multiparous cow of the ‘free’ group had a stillborn calf two weeks after the start of the experiment and therefore was moved to the ‘no contact’ pen post partum.

For calving, all cows were kept individually in a deep litter calving pen (3.50 x 5.25 m). ‘No contact’ cows stayed in the pen for one day, dams for three days post partum (see 4.2.2). If independent suckling was not observed half an hour after birth, suckling was assisted to ensure colostrum supply. If cows calved on their own during the night, suckling was assisted in the morning if calves appeared to be hungry or weak. During the time the calves stayed in the calving pen, suckling was assisted three times a day until the calf was observed suckling regularly on its own. Milking twice daily in the milking parlour started after calving.

The 2x6-herringbone parlour (System Happel GmbH, Friesenried, Germany) operated with 40 kPa vacuum, common mode, pulsation rate 60 double pulses min^{-1}, pulsation ratio 60:40. Automatic milk-out started at 1,000 g min^{-1} milk flow and automatic cluster take-off was at 250 g min^{-1}. In one 'no contact' and two 'half-day' cows, milk-out and cluster removal were carried out manually, as otherwise they would not have been milked out properly. This was already routine on the farm before the study started. Udder preparation consisted of foremilking and cleaning of the teats with one to three wet udder wipes per cow. Quarter fill was not systematically checked. About one minute after the start of udder preparation milking clusters were attached. If during the milking one quarter was empty, while others were still giving milk, the milking cluster of this teat was detached and a dummy plug inserted. After removal of all milking clusters, emptiness of quarters was checked manually. If appropriate, milking clusters were attached again. After milking, 'no contact' cows in general and 'half-day' cows in the afternoon were dipped with an iodine-containing agent (Kenostart®, Cid Lines, Ieper, Belgium, 3 mg iodine per g). Cows with free calf-contact were not dipped during the nursing period.

All lactating cows were driven to the waiting area with no access to the calves at about 6:00 h and 17:30 h daily. After milking cows went back into the stable and could re-unite with their calves, if applicable.

4.2.2 Treatments

4.2.2.1 No contact

Four primiparous and ten multiparous cows of this treatment (mean lactation number 3.6 ± 2.6) were separated from their calf half a day after birth. One primiparous and four multiparous cows had stillborn calves. Three of them were originally allocated to the 'half-day' or 'free' groups. One of the latter was moved to the 'no contact' pen after calving, as already described. The other two cows stayed in the pen of the other treatment to prevent regrouping stress. So there was one 'no contact' animal in the 'half-day' and 'free' treatment pen each. It was never observed that they nursed alien calves.

The 'no contact' calves consisted of five female and five male calves (including two twin male calves). To offset the data-losses due to dead calves, additionally weight gains of all calves born during the study period by non-experimental cows were recorded (one male, three females, including two twin female calves). In total, data of six male and eight female calves were analysed. Half a day after birth, calves were moved into individual calf igloos (1.7 m² straw bedded lying area, 1.5 m² outdoor area). During the first week after birth, calves were fed three meals of 1.5 L colostrum d^{-1}. They had access to water, and feed ad libitum. In the second week, they were housed in an outdoor climate barn in groups of up to seven calves in straw-bedded pens (3.1 x 6.0 m²) with a bowl drinker. Thenceforward calves obtained 2 x 3 L whole milk d^{-1}. Milk and colostrum were heated to 39°C and fed by teat bucket. Calves had ad libitum access to the cows' total mixed ration, concentrate and sometimes hay.

4.2.2.2 Free contact

Three primiparous and 10 multiparous animals (mean lactation number 2.7 ± 1.4) had 'free' calf-contact with nursing during the first nine weeks of lactation. During the first three days post partum, they stayed in the calving pen with their calf, except for milking twice daily. On the fourth day post partum, mother and calf were moved into the pen of the 'free' treatment in the cows' barn. Seven female, five male calves and one freemartin (in analysis handled as female) were in the 'free' treatment. The male calf of the mixed sex twins was handled as 'no contact' calf and sold for fattening at two weeks of age. 'Free' calves could freely suckle their dam day and night and move between the cows' area and a calf creep. The calf creep consisted of a group igloo (14.9 m²) with straw bedding and trough with cows' total mixed ration, concentrate and sometimes hay. In an outdoor area (29.0 m²), water was provided in a 10 L bucket. Free cows were separated from their calves during each milking for about 15 - 45 min.

4.2.2.3 Half-day contact

The 'half-day' treatment consisted of two primiparous and ten multiparous animals (mean lactation number 3.1 ± 1.9) with five female, five male calves and two freemartins. One multiparous cow and her freemartin-calf were excluded from analysis because the cow had a serious udder oedema and milking was impaired (leading to n = 11). 'Half day' cows and calves had three days of full contact in the calving pen after birth. After cow and calf were moved to their treatment pen, calves were locked into the calf creep over the night. Cow-calf-contact started after morning milking and ended with the start of afternoon milking (6:45 h - 17:30 h). Beside these experimental conditions, everything was comparable for 'half-day' and 'free' contact cows and calves. Due to farm-related problems, 'half-day' calves accidentally did not have access to the calf creep during the day for up to three days per week from February to end of April. During this time, they had only access to feed and water during the night. During the day, they could ingest straw and some calves were able to feed at the cows' feeding rack. Four calves were affected for 2 - 6 wks and three calves during the whole nursing period.

Male calves of all treatments were sold after the 10th week of life.

4.2.3 Experimental phases

The experimental phases described in Tab. 6 were applied to both contact treatments. Data of 'no contact' animals during the comparable weeks post partum were compared with data of dams. However, conditions for the 'no contact' animals did not change during the phases. One exception was that calves of all treatments were weaned in the same way.

Table 6: Experimental phases, housing and management of 'free' and 'half-day' animals.

phase	weeks of lactation	cow-calf-contact	housing of calves	milk source and amount of milk
nursing[1]	1st - 9th	free or half-day physical contact	cow barn + calf creep[1]	dam, free or half-day access[1]
in sight+ milk feeding	10th	visual and acoustic contact	separation pen	teat bucket, 2x3 L d^{-1}
out of sight+ weaning[2]	11th - 12th	out of sight, acoustic contact	calf barn	teat bucket, 11th wk: 2x2 L d^{-1} 12th wk: 2x1 L d^{-1}
post weaning	13th - 14th	out of sight, acoustic contact	calf barn	no milk access

[1] first three days post partum in the calving pen with free contact for 'half-day' and 'free'

[2] analysis of milk composition until 11th week, milk yield recording until 12th week

The phases 'in sight+milk feeding' and 'out of sight+weaning' started always on a Wednesday. There was one straw-bedded separation pen (5.0 x 5.0 m) per treatment located about two meters apart from the outdoor run of the cows. One to three 'free' or 'half-day' calves were moved to the separation pen at a time and grouped together with a 'no contact' calf of approximately the same age. Therefore, no single calf stayed alone. After this phase, male calves were sold and female calves were grouped with the 'no contact' calves in the calf barn. As during 'in sight+milk feeding' and 'out of sight+weaning' no nursing occurred, for some statistical comparisons these two phases were combined to 'no nursing'.

4.2.4 Data collection

Recording of milk yield and composition started at the earliest five days after calving. Milk yield was assessed with the calibrated automatic milk yield recording of the milking machine (Memolac 2, MM8, System Happel, NEDAP Agri BV, Groenlo, The Netherlands) on four days per week (Thursday to Sunday). Milk yields lower than 1 kg, which could not be recorded by the milking machine, occurred during afternoon milkings in dams and in one primiparous cow of the 'no contact' treatment. In these cases 0.5 kg were taken as yield for this milking. In 'half-day' dams this was necessary during 'nursing' in 43.9% of afternoon milkings, 0% during 'in sight+milk feeding', 5.4% during 'out of sight+weaning' in 'free'-dams in 17.9%, 11.8% and 2.0% of milkings, respectively, and in the 'no contact' treatment in 1.1% during 'nursing'. If the milk yield of a milking was missing due to technical reasons (2.8%), the mean milk yield of the animal of that week was used instead.

Composite milk samples were taken on four milkings per week (Monday afternoon to Wednesday morning) with Meltec milk meters (NEDAP Agri BV, Groenlo, The Netherlands). During 'out of sight+weaning', milk samples were analysed only during the first week. Fat, protein and lactose content were analysed with infrared spectroscopy and somatic cell count with flow cytometry according to milk recording standards.

For each experimental phase, means per animal were calculated from daily milk yield recordings and milk composition analyses per milking. Means of somatic cell count (SCC) were transformed to somatic cell score ($SCS = log_2(\frac{SCC}{100\,000}) + 3$) (Wiggans and Shook, 1987). SCS in cows with clinical signs of mastitis was not assessed. Applying a maximum threshold of 100,000 cells ml^{-1} to be expected in healthy udders (DVG, 2012), we analysed an additional variable: the proportion of milkings with SCC >100,000 cells ml^{-1} including the milkings with clinical mastitis. Furthermore, the incidences of new mastitis cases during the first 12 weeks post partum were recorded. Clinical mastitis was defined as visible or palpable abnormalities of the udder (being red, hard, swollen or hot) or visible abnormalities of the milk (flakes, clots or watery appearance). One ‘half-day’ and one ‘free’ cow had blood in the milk during two milkings. No data were collected in these cases.

For cows with lactation information for at least 220 d, also the mean daily milk yield of lactation was calculated. If the lactation was longer than 305 d, only the milk yield up to this day was integrated in the average daily lactation yield.

Calving intervals of eleven ‘free’, ten ‘half-day’, four ‘no-contact’ as well as for 32 cows of the herd, which were separated from the calf half a day after calving, were calculated based on the milk reports. Cows with assisted calvings were excluded from analysis (two ‘no-contact’ cows). As one cow in each treatment had twins, this factor was not further considered in the analysis of calving interval.

Calves were weighed on the day of birth, after ‘nursing’ (9 wks after birth), after ’in sight+milk feeding’ (10 wks after birth), ‘out of sight+weaning’ (12 wks after birth) and two weeks after weaning with an electronic scale with a precision of ± 1 kg (EziWeigh2, Tru-Test Group Limited, Auckland, New Zealand). Daily weight gains were calculated per animal for the experimental phases. Missing values due to missing or incorrect weighing occurred so that the number of animals per treatment and phase partly differ. As male calves were sold after ’in sight+milk feeding’ in the following phases only data of female calves were available.

4.2.5 Statistical analysis

Linear mixed effects models (Pinheiro et al., 2014) were used for the analysis of the dependent variables milk yield, the content of milk fat, protein and lactose, SCS and percentage of SCC >100,000 cells ml^{-1}. Random factor was always the phase nested in animal (repeated measurements). SCS nested in phase nested in animal was selected as additional random factor for lactose content as dependent variable a priori. Fixed factors in all models were treatment (‘no contact’, ‘half-day’, ‘free’) and phase (‘nursing’, ‘in sight+milk feeding’, ‘out of sight+weaning’). If the relative quality of the model was improved (lower Akaike Information Criterion), the interaction of treatment and phase were included as additional fixed factor in the model. For the dependent variables machine milk yield, SCS and percentage of milkings with an SCC >100,000 cells ml^{-1}, parity (primiparous vs. multiparous) was chosen a priori as additional fixed factor. This was done to account for the unbalanced group structure. Results for parity are not presented. Contrasts were used to compare 'half-day' with 'free' and 'half-day' with 'no contact' treatments. As these contrasts are non-orthogonal, p-values for treatment were adjusted according to Holm (Field et al., 2012, pp. 429 f.). The first contrast for the phases compared 'nursing' with phases with 'no nursing' ('in sight+milk feeding' combined with 'out of sight+weaning'). In a second contrast 'in sight+milk feeding' was compared with 'out of sight+weaning''. Normality and homogeneity of residuals and independence of fixed factors were confirmed graphically and, additionally, values of kurtosis and skew were evaluated.

The incidence of clinical mastitis per treatment was analysed using Fisher's exact test (Warnes et al., 2013). Only data of multiparous cows were taken into account, as only multiparous cows had mastitis and the number of primiparous animals differed between treatments.

ANOVA was used to analyse the effect of treatment on calving-interval, average daily milk yield during the lactation (incl. lactation number as independent variable) and daily weight gain of calves during 'nursing' and 'in sight+milk feeding' (incl. sex as independent variable). Contrasts and p-value adjustment for treatment were the same as for the linear mixed models. Homogeneity of residuals was ascertained with Levene-test (Fox and Weisberg, 2011). Normality of residuals was confirmed using kurtosis, skew and qq-plots. Possible treatment effects on daily weight gain of female calves during 'out of sight+weaning' and 'post weaning' and the live weight of calves two weeks after weaning were analysed by Kruskal-Wallis tests (low animal numbers). If treatment had a significant effect, Wilcoxon-rank-sum test was used as post-hoc test.

For linear mixed models and ANOVA, means and standard deviations of fitted values of the models are presented. All other results are presented as means and standard deviations of raw data, if nothing else is noted. Effect sizes were calculated for results from contrasts of linear mixed models and ANOVA with $r = \sqrt{\frac{t^2}{t^2+df}}$

and from Wilcoxon-rank-sum tests with $r = \frac{z}{\sqrt{N}}$ (Rosenthal, 1991, p. 19). All analyses were done with R (version 3.1.2, R Development Core Team 2014).

4.3 Results

4.3.1 Milk yield

4.3.1.1 Daily milk yield until 12th week of lactation

There was a significant influence of treatment on collected milk yield but also the interaction with phase was significant. Over all phases 'half-day' animals yielded less milk than 'no contact' cows (p = 0.0054, r = 0.48) and gave in tendency more milk than 'free' cows (p = 0.0576, r = 0.32). This was more pronounced during the nursing period. After this phase ended, the milk yield of dams increased (treatment*phase interaction, p < 0.0001, Fig. 8, A). During 'nursing' the distribution of the collected milk yield differed between morning and afternoon milkings in 'half-day' cows compared to the other treatments (Fig. 9).

4.3.1.2 Mean daily milk yield of lactation

Two 'no contact' and one 'half-day' primiparous animals and two multiparous 'free' cows were excluded from analysis of the lactation yield because their lactations lasted shorter than 220 days (leading to $n_{\text{no contact}} = 12$, $n_{\text{half-day}} = 10$, $n_{\text{free}} = 11$).

Mean daily milk yield of lactation was 18.5 ± 2.1 kg, 16.7 ± 3.7 kg and 14.1 ± 2.6 kg for 'no contact', 'half-day' and 'free' respectively. The difference between 'half-day' and 'no contact' was statistically not significant (p = 0.2193, r = 0.23). However, there was a tendency of a difference between 'half-day' and 'free' animals (p = 0.0889, r = 0.31). In general, mean daily milk yield of lactation increased significantly with lactation number (p = 0.0263, r = 0.40).

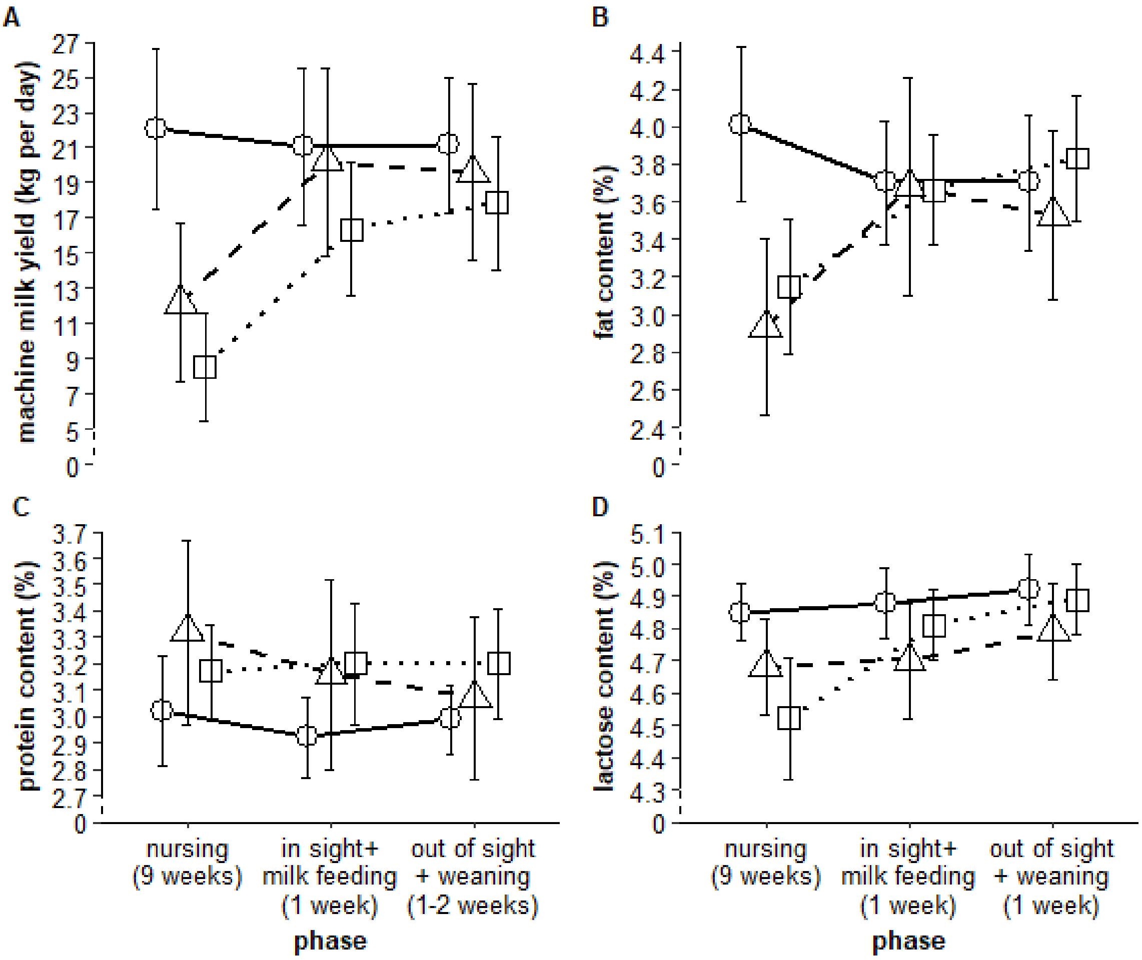

Figure 8: Influence of no cow-calf-contact (○, solid line, n = 14), half-day cow-calf-contact (Δ, dashed line, n = 11) and free cow-calf-contact (□, dotted line, n = 13) on (A) machine milk yield (kg d^{-1}), (B) fat content (%), (C) protein content (%) and (D) lactose content (%) during different phases. Machine milk yield was assessed for two weeks during 'out of sight + weaning', while milk composition was analysed during only one week (mean ± SD of fitted values from linear mixed effects models).

4.3.2 Milk composition

During 'nursing' fat content of treatments with calf-contact were lower than those of 'no contact' animals, while during the later phases fat contents of all treatments were similar (treatment effect over all phases: ‘half-day’ vs. ‘no contact’: p = 0.0054, r = 0.48; ‘half-day’ vs. ‘free’: p = 0.2253, r = 0.20). Therefore there was a significant treatment*phase interaction (p < 0.0001, Fig. 8, B). During ‘nursing’ fat content of dams was low during both, morning and afternoon milking (Fig. 9).

Similarly, a significant treatment*phase interaction could be found for protein contents (p = 0.0004) as protein content of the treatments changed in different directions over the experimental phases (Fig. 8, C). Further, ‘half-day’ animals had in tendency higher protein contents than ‘no contact’ (p = 0.0694, r = 0.35), but there was no significant difference between ‘half-day’ and ‘free’ (p = 0.9194, r = 0.02).

Lactose content was lower in ‘half-day’ than in ‘no contact’ cows (p = 0.004, r = 0.49). There were no differences between dam treatments (p = 0.7470, r = 0.06). However, there was a significant treatment*phase interaction: In ‘free’ cows the increase in lactose content from ‘nursing’ to ‘in sight+milk feeding’ was more pronounced than in the other treatments (p < 0.0001, Fig. 8, D).

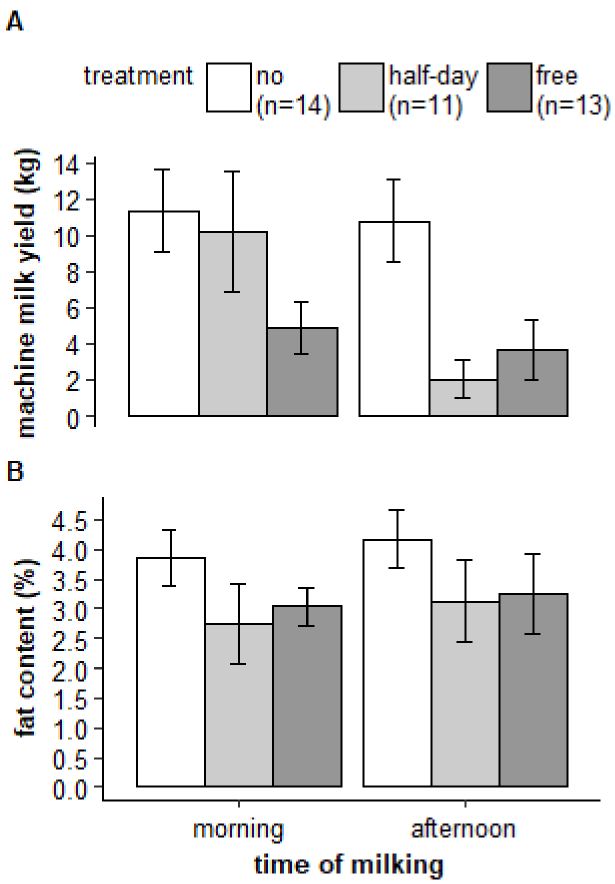

Figure 9: Machine milk yield (kg) and fat content (%) of dairy cows at morning and after-noon milkings with ‘no contact’ (n = 14), ‘half-day’ (n = 11) or ‘free’ calf-contact (n = 13) during nine weeks post partum (‘nursing’ phase for cows with calf-contact) (mean ± SD).

4.3.3 Udder health

There were no significant differences between treatments (Tab. 7) and no significant treatment*phase-interactions (amount of milkings with SCC >100,000 cells ml^{-1}: p = 0.372, SCS: p = 0.9778). However, SCS was higher during 'nursing' in all treatments than during 'no nursing' (Tab. 7). Udder-infections occurred only during the first nine weeks post partum. Two 'free' and one 'no contact' cow had recurrent udder infections, which were treated as one incidence. The incidences of clinical mastitis in multiparous animals during 12 weeks (i.e. all phases) was 30% in 'no contact' (n = 10), 33% in 'half-day' (n = 9) and 50% in 'free' (n = 10) animals. However, these differences were statistically not significant (p = 0.7094).

Table 7: Percentage of milkings with somatic cell count >100,000 cells ml^{-1} and somatic cell score (SCS) of cows with 'no contact' (n = 14), 'half-day' (n = 11) or 'free' calf-contact (n = 13) during nine weeks post partum ('nursing'), one week 'in sight+milk feeding' of calves (IS+MF) and two weeks of 'out of sight+weaning' (OS+W) (see explanation of phases in the text), (mean ± SD of fitted values from linear mixed effects models); effects with p < 0.05 in bold.

	phase	no contact	half-day	free	effect of treatment	effect of phase
SCC >100,000 cells ml^{-1} (% of milkings)	nursing	51.9 ± 40.4	49.9 ± 28.1	41.3 ± 32.3	p = 0.3364	p = 0.3431
	IS+MF	46.3 ± 42.5	55.7 ± 41.2	29.6 ± 28.5		
	OS+W	44.8 ± 43.3	53.1 ± 45.0	29.3 ± 30.2		
SCS	nursing	3.4 ± 1.4	3.4 ± 1.5	2.8 ± 1.4	p = 0. 2521	nursing vs. IS+MF and OS+W: **p = 0.0068, r = 0.31**
	IS+MF	3.0 ± 1.4	3.3 ± 1.6	2.3 ± 1.1		IS+MF vs. OS+W: p = 0.9822, r < 0.01
	OS+W	3.0 ± 1.4	3.3 ± 1.9	2.3 ± 1.3		

4.3.4 Calving-interval

Eight, one and two cows of the 'no contact', 'half-day' or 'free' treatment, respectively, were slaughtered before the next conception. In addition, two 'no contact' cows had an abnormally long period between calvings due to health problems and were therefore excluded from analysis. Data of 32 cows from the rest of the herd, which calved in 2011 and 2012, were added to the remaining four animals of the 'no contact' treatment for analysis (n_{free} = 11, $n_{half\text{-}day}$ = 10, $n_{no\ contact}$ = 36). There were no differences in calving-intervals between treatments (p = 0.714). 'No contact' cows and the rest of the herd had a mean calving interval of 369 ± 41 days compared to 378 ± 42 days in 'half-day' and 363 ± 42 day in 'free' contact.

4.3.5 Daily weight gain and live weight of calves

During all phases, there were no differences in weight gain between 'half-day' and 'free' calves ($p > 0.1$, $r \leq 0.15$, Fig. 10). Sex of calves did not have an influence on daily weight gain during 'nursing' ($p = 0.183$, $r = 0.26$) and 'in sight+milk feeding' ($p = 0.9609$, $r < 0.01$). 'Half-day' calves gained more weight during 'nursing' than 'no contact' calves (Fig. 10, $p < 0.0001$, $r = 0.78$). After the 'nursing' phase ended, there was a growth check in both contact treatments. Weight gain of 'half-day' calves was significantly lower during 'in sight+milk feeding' than those of 'no contact' calves ($p = 0.0114$, $r = 0.46$). During 'out of sight+weaning' and 'post weaning' weight gain of dam-reared calves increased. They numerically still remained below the level of 'no contact' calves during these phases, but statistically there were no differences and effect sizes were low ('out of sight+weaning': $p = 0.2109$, $r = 0.19$, 'post weaning': $p = 0.2466$, $r = 0.16$). Median live weights (± median absolute deviation) of calves two weeks after weaning were 109.3 ± 6.8 kg, 128.0 ± 9.5 kg and 120.5 ± 11.8 kg for 'no contact', 'half-day' and 'free' calves respectively (all calves were female: $n_{\text{no contact}} = 8$, $n_{\text{half-day}} = 5$, $n_{\text{free}} = 8$). 'Half-day' animals weighed significantly more than 'no contact' calves ($p = 0.02939$, $r = 0.48$), but there was no difference compared to 'free' ($p = 0.6358$, $r = 0.10$).

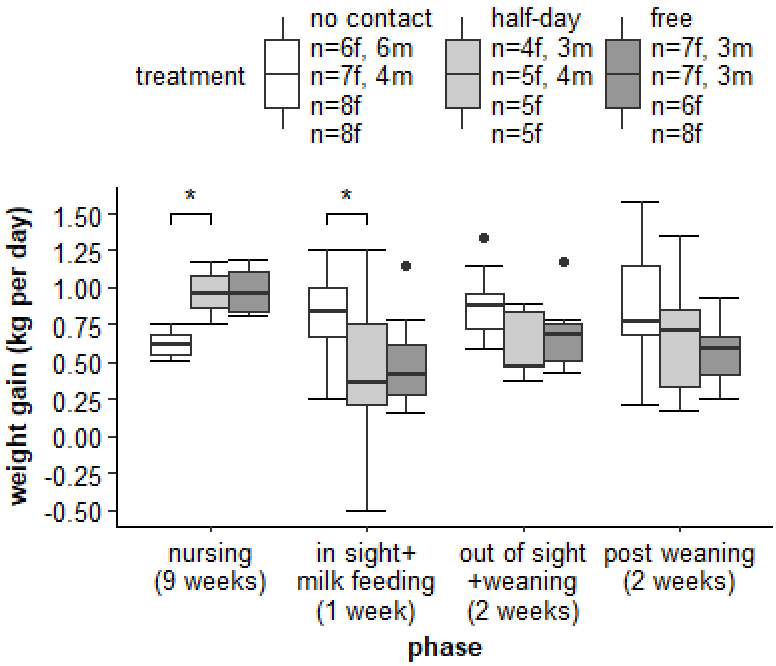

Figure 10: Daily weight gain of female (f) and male (m) calves with no, half-day or free cow-calf contact. 'No contact' calves were teat bucket fed during 'nursing' (6 L d^{-1}). All calves were teat bucket fed during 'in sight+milk feeding' and 'out of sight+weaning' (6 - 2 L d^{-1}). Sample size varied due to missing or incorrect weighing and because male calves were sold after 'in sight+milk feeding' (n top down relate to boxplots from left to right). Boxplot: lower whisker: minimum, lower end of the box: 25% quartile, line in the box: median, upper end of the box: 75% quartile, upper whisker: maximum, dots: outliers, stars: $p < 0.05$.

4.4 Discussion

4.4.1 Daily milk yield

Machine gained milk yield in ‘half-day’ dams was lower than in ‘no contact’ animals during the nursing period. During this time the milk loss was on average 9.9 kg d^{-1} in ‘half-day’ cows compared to ‘no contact’ (with yields of 22.1 kg d^{-1}). This difference in harvested milk was lower than the milk loss in ‘free’ contact in the present study (-13.5 kg d^{-1}), and in other studies with free contact (-13.8 up to -20.0 kg d^{-1}, controls: 25 - 32.5 kg d^{-1}, Barth et al., 2009b; Barth et al., 2010; Zipp et al., 2013). It was also lower than the milk losses through half-day contact during the night (Barth et al., 2015b: 13.8 kg; Johnsen et al., 2015a: -15.2 kg, controls: 29.8 kg d^{-1}). In most studies investigating restricted suckling systems, milk losses were similar or even higher compared to half-day contact in the present study (-9.4 - 12.4 kg; Barth et al., 2007; Boden and Leaver, 1994; Krohn et al., 1990, cited by Krohn, 2001; de Passillé et al. 2008). Only Mendoza et al. (2010) recorded lower milk losses (-6.9 kg). Comparability is, however, limited due to differences in breed, performance level and weeks of contact. In the current study, the in tendency higher milk yield in ‘half-day’ vs. ‘free’ contact resulted from higher milk yields during the morning milking. However, there was a high variation. Afternoon milk yields in ‘half-day’ cows averaged at 2.7 kg. Therefore, for low yielding breeds in half-day rearing systems, milking only once per day might be considered as an option. Implications, e.g. on lactational milk yield, should be further investigated.

After ‘nursing’ ended, machine milk yields of dams increased, although the milk yield remained below the level of ‘no contact’ cows until the end of the recordings (12^{th} wk post partum). There were no significant differences in daily machine milk yields of the lactation between cows with no or half-day contact, which is in accordance with the study of Johnsen et al. (2015a, half-day contact overnight). Over the lactation, ‘half-day’ cows yielded 1.8 kg less d^{-1} than ‘no contact’, which equals 549 kg in 305 d. Therefore, ‘half-day’ cows gained on milk yield, as the milk loss after ‘nursing’ was higher (9.9 kg d^{-1} during 63 d equals 624 kg) than at the end of lactation. Even though this difference was not significant, for a farmer, 549 kg less sellable milk per lactation may be too substantial a loss. However, it should be considered that in organic farming, calves have to be fed whole milk for three months. About 367.5 L was bucket-fed to ‘no contact’ calves. When subtracting this amount from the milk yields of their mothers, there remains only a difference of about -181.5 kg between ‘half-day’ and ‘no contact’. Furthermore, the amount of 6 kg milk per calf and day, although still a rather common management practice, is increasingly criticized as too low (e.g. Rushen et al., 2008, p. 215). The difference of 549 kg milk between ‘no-contact’ and ‘half day’ cows would be equivalent to updated feeding recommendations (e.g. Jensen et al., 2015) and similar to the amount calves drink when they are fed ad libitum (Jasper and Weary, 2002).

The main daily milk yield of the lactation was in tendency higher in ‘half-day’ animals compared to ‘free’. The milk loss in ‘free’ dams was 13.5 kg d^{-1} during 63 d of ‘nursing’, which equals 850.5 kg. The difference between ‘free’ and ‘no-contact’ at the end of lactation equals to about -1,342 kg (-4.4 kg d^{-1} during the 305 d). Thus, the milk yield of ‘free’ cows was lower throughout the lactation. After subtracting the bucket fed milk from ‘no contact’s’ lactational yield there is still a loss of -974.5 kg. As calf growth was comparable in dam-contact treatments, milk intake of calves was probably similar. On the other hand, udder emptying in ‘half-day’ cows might have been better than in ‘free’ during morning milkings. Increased milking frequency during early lactation stimulates milk synthesis and has carryover effects on the remaining lactation (Connor et al., 2008; Eslamizad et al., 2010; Hale et al., 2003). Thus, the frequent nursing combined with milking might cause an overall enhanced milk production. However, in ‘free’ cows, the constantly incomplete udder emptying due to milk ejection problems during milking might decrease milk production even after the ‘nursing’ period. This warrants further investigation as Metz (1987) and Flower and Weary (2001) did not find differences in lactational milk yield between ‘no contact’ and ‘free’ cows, but with calf-contact lasting only 10 - 14 days. Krohn (personal communication, 1999, cited by Krohn, 2001) reported that milk yield after 12 wks of free nursing was ‘normal’ 24 wks after separation, but no information about the total lactation is given.

4.4.2 Milk composition

Independent from the extent of calf-contact, milk fat content of the dams during ‘nursing’ was about 1%point below the values of non-nursing animals. This is in line with a number of other studies (Barth et al., 2009b; Boden and Leaver, 1994; Mendoza et al., 2010; Zipp et al., 2013). The alveolar milk fraction has the highest fat content and remains in the udder when milk ejection problems occur (Ontsouka et al., 2003). The lower fat content in the ‘half-day’ treatment during morning and afternoon milkings (Fig. 2) suggest that there were still milk ejection problems even in the morning. At the same time, high variation in morning milk yields and fat contents reflect high individual differences. This might provide a starting point for breeding selection towards improved milkability of dams. In accordance with Mendoza et al. (2010), fat content after separation did not differ between treatments. Separation of calves and cows happened on Wednesdays. As the next milk samples were taken on Monday, we cannot exclude a possible influence of separation on milk composition, e.g. a drop in milk fat, during the first four days.

Nursing cows had higher protein contents than the ‘no contact’ treatment. The effect size comparing ‘half-day’ and ‘no contact’ was at a lower medium level ($r = 0.35$). This confirms the findings of Boden and Leaver (1994) and Schneider et al. (2007). However, in other studies no effect (Mendoza et al., 2010) or even a lower protein content in dams were found (Cozma et al., 2013). It is unclear why the protein content of ‘half-day’ animals dropped after the ‘nursing’ phase. Currently no information on involved proteins or possible physiological mechanisms are available. Protein contents were in general low, which might be due to low energy rations (e.g. Gross et al., 2011).

Lactose contents were mainly in the normal range of 4.6 - 5.0% but lower in dams. For restricted suckling, also Barth et al. (2007) found an influence of nursing on lactose content, but only during morning milkings. Other studies on restricted suckling did not find any effect (Fulkerson, 1978; Lupoli et al., 2001). In half-day or free contact, milk is removed more frequently through the combination of nursing and suckling. Moreover, incomplete milk removal is more frequent due to disturbed milk ejection during milking and the drinking pattern of the calf. Both, more frequent milk removal (e.g. Delamaire and Guinard-Flament, 2006; Soberon et al., 2010) and incomplete milk removal (Penry et al., 2017) can lead to decreased lactose contents. A possible reason is an increased permeability of tight junctions (e.g. Delamaire and Guinard-Flament, 2006; Stelwagen and Singh, 2014; Penry et al., 2017). Another mechanism might be a higher lactose content in alveolar milk (Ontsouka et al., 2003), which cannot completely be gained when milk ejection is impaired. However, the significant lactose increase in the 'no contact' treatment over the phases remains unexplained. An influence of udder health on the lactose content can be excluded as SCS was used as a random factor in the analysis.

4.4.3 Udder health and calving-interval

Udder health in general was suboptimal, as in 31-56% of milkings SCC was above the threshold for subclinical mastitis (100,000 cells ml^{-1}). Three cows in the 'half-day' and the 'no contact' treatment each, and five cows in the 'free' treatment had clinical mastitis. Statistically, there were no significant differences between treatments regarding any indicator of udder health. This may be due to the low sample size and consequently low power of testing. However, numerous studies found that suckling combined with milking did not negatively affect udder health (Fröberg et al., 2008; Fulkerson et al.,1978; Krohn et al., 1990, cited by Krohn, 2001; Margerison et al., 2002; Wagenaar and Langhout, 2007; Zipp et al., 2013). The higher SCS during nursing than during the other phases might be due to high SCS after calving, which is not automatically a sign of an infection and lasts up to two weeks (reviewed by Dohoo and Meek, 1982).

The calving-interval was not influenced by calf-contact, although again low sample sizes must be considered. While anoestrus can be prolonged in dams (reviewed by Kälber and Barth, 2014), it has been found that calving-conception interval is not impaired or even shorter in cow-calf systems (Krohn et al., 1990, cited by Krohn, 2001; Margerison et al., 2002). This results in similar calving-intervals between groups as found in the current study.

4.4.4 Daily weight gain and live weight of calves

The extent of weight gain advantage in dam-reared compared to 'no contact' calves depends on the amount of milk provided to the bucket fed calves. With 6 L milk d^{-1} (15.7 - 6.2% of mean body weight), the feeding regime in this study was relatively restrictive. Weight gains of 'no contact' calves during the first nine wks post partum did not reach German recommendations (0.75 kg d^{-1}; Kirchgessner, 2008, p. 434). During the time of the experiment, 6 L milk d^{-1} was common practice on the research farm and still largely reflects feeding management on commercial farms in Germany. Therefore, it is no surprise that dam-reared calves gained more weight than 'no contact' calves. This is in line with numerous studies that compared dam-reared calves with calves fed relatively low quantities (Flower and Weary, 2001, milk equivalent to 5% of body weight; Metz, 1987, 8 L milk replacer; Roth et al., 2009a, 8 L milk d^{-1}; Wagenaar and Langhout, 2007, 6 L milk). However, calves without cow-contact receiving 12 L or having ad libitum milk access, had comparable or even higher weight gains than ‘free’ (Veissier et al., 2013) and ‘half-day’ calves (Johnsen et al., 2015a; Veissier et al., 2013).

A main point of interest in the current study is the lacking significant difference in weight gain between ‘free’ and ‘half-day’ calves, which confirms the findings of Veissier et al. (2013). However, after ‘nursing’ stopped, there was a growth check in dam-reared calves, which is in line with the findings of Fröberg et al. (2011). It is well established that feeding lower milk amounts to calves stimulates higher concentrate intake (bucket-fed versus nursed: Fröberg et al., 2008; Fröberg and Lidfors, 2009; Roth et al., 2009a; different bucket feeding levels: Borderas et al., 2009; Guindon et al., 2015; Jensen et al 2015; Kiezebrink et al., 2015; Yunta et al., 2015). In the current study, reasons for the decreased weight gain of dam-reared calves after the ‘nursing’ phase might be the separation from the dam, the challenge to drink from the teat bucket, regrouping and weaning stress, but also reduced intake of solid feed. We expected an increased intake of solid food and consequently a lower weight loss in ‘half-day’ compared to ‘free’ calves, as found by Veissier et al. (2013). However, the ‘half-day’ calf creep, where calves could feed, was accidentally blocked for some time during the day. This probably thwarted our expectations. No significant differences between dam-reared treatments could be found.

During the next phase ‘out of sight+weaning’ differences in weight gain between all treatments were only numerical. Especially between ‘no contact’ and dam-reared calves the lacking significance might be caused by a low number of animals and a high variation within treatments. Nevertheless, live weight two weeks after weaning was on average still 11-19 kg higher in dam-reared calves than in controls. This result must be interpreted with caution due to low sample size (‘no contact’: n = 8, ‘free’: n = 8 and ‘half-day’: n = 5) and group composition. The ‘no contact’ group comprised two twin calves (‘free’: 1, ‘half-day’: 0) that may weigh on average 15% less (Kertz et al., 1997). Furthermore, in each group there was one calf from a primiparous cow (that may weigh on average 7-8% less, Kertz et al., 1997). Due to different sample sizes the proportion of these animals was higher in the ‘half-day’ group. As weight gain is not influenced by birth weight (Martin et al., 1962), these differences will presumably not have affected weight gain.

Behavioural observation of cows and calves was outside the scope of this study. However, it was noted in chance observations that ‘half-day’ calves vocalized when separated from the dam overnight and when morning milking started. When those calves entered the cows’ area after morning milking they directly headed to the mothers’ or another cows’ udder. Cows sometimes kicked even their own calf at these attempts to suckle, maybe due to low udder filling. Thus, future investigations should include behavioural observations and the assessment of stress in the different cow-calf systems including separation and weaning periods.

4.5 Animal welfare implications and conclusion

‘Half-day’ cow-calf contact over the first nine weeks post partum is a promising dam rearing system. It allows increased cow-calf interaction compared to restricted dam rearing systems. At the same time, losses of machine gained milk yield are lower than in ‘free’ contact, while weight gains of calves are at a similarly high level. There was no statistical difference between lactation milk yield of ‘half-day’ and ‘no contact’ cows. The decreased milk fat content during the nursing period indicates that milk ejection problems are still present. No effects on udder health and calving interval were detected, but number of animals were partly low and udder health was suboptimal in general. Possible impairments of welfare due to separation overnight as well as to separation after several weeks of cow-calf-contact need further attention and should be part of future research.

5 GENERAL DISCUSSION

The aim of this thesis was to investigate different attempts to improve the machine collected milk yield in dairy cows nursing their own calf. The first attempt was to use calf-associated stimuli in the parlour, e.g. calf odour. Beforehand it had been evaluated in a pilot study, whether a small amount of calf hair in a thin cloth bag, the calf was rubbed with before, is suitable as olfactory stimulus. The behavioural response towards hair of the own calf, hair of an alien calf and a control without hair was compared in dams with free calf-contact and cows without calf contact. As the animals overtly reacted more frequently to samples with calf hair it could be concluded that cows perceived the odour. In the second experiment, played back calf calls and teat massage were used in addition to hair of the own calf as stimuli in the parlour. The influence on parameters of milk let-down were investigated as well as agitation behaviour, heart rate (HR) and heart rate variability (HRV) to analyse if dams were stressed during milking. This could be a reason for impaired alveolar milk ejection. In the third study the impact of reduced mother-calf-contact was assessed: The performance of cows and calves during free and half-day contact during the day were compared.

The amount of sellable milk from dams could not be increased by the tested stimulation during milking in the parlour, but reducing the contact between dam and calf to half a day led to in tendency higher yields of machine collected milk compared to free contact. During the contact time of nine weeks, milk yield was considerably lower than in control animals that were not nursing their calf and conventionally milked. However, taking into account the whole lactation there were no statistically significant differences between these treatments. In both experiments dams' milk fat content during the contact period was lower than in non-nursing cows, indicating disturbed milk ejection. However, somatic cell count was not affected. Differences in some parameters of HRV during milking indicated an activation of the vagal axis of the autonomous nervous system in dams. Reason for this might be a higher frequency of oxytocin ejections during the day in dams (e.g. Akers and Lefcourt, 1982; Bar-Peled et al., 1995). This might also have caused the higher frequency of milkings with defecation in the parlour in nursing cows. However, dams also had a tense head position during more milkings which cannot be explained by higher oxytocin levels. Therefore it can be suggested that dams' physiological regulation in general was positively influenced by nursing but they were slightly stressed in the parlour. Calves with free and half-day contact gained more weight during nursing than control calves, but had a growth check after separation from the dam. However, after one week, differences were not significant any longer and five weeks after separation dam reared calves still had higher body weights than 'no contact' calves.

In the following chapter these results will be discussed in a wider context. The cues which are necessary for the recognition of the calf by its mother and the use of such isolated stimuli to improve milk let-down, as well as the impact of the different dam rearing systems on various aspects of cow and calf welfare and performance are part of this section.

Concurrently, potential future research directions, are addressed. Finally, the method of HRV analysis in cattle is discussed.

5.1 Mother-calf-recognition and stimulation

In the following section it is discussed, whether cows of the control group would have been able to distinguish between stimuli of their own (olfactory: Chapters 2 and 3) or alien calves (olfactory: Chapters 2, acoustic: Chapter 3). For this purpose, results of experimental studies and the course of natural establishment of mother-young-recognition are incorporated. Further the importance of different cues in mother-calf-recognition and the results of the current and other studies using calf associated or tactile stimuli are discussed.

5.1.1 Vocal recognition and acoustic stimulation

Calves can distinguish between the calls of their mother and the voice of another cow (Barfield et al., 1994; Marchant-Forde et al., 2002; Padilla de la Torre et al., 2016). On the one hand, it is reported that cows do not mistake calls of alien calves for vocalisation of their offspring (Reinhardt, 1980, p. 13; Schloeth, 1958). On the other hand, it was postulated that cows cannot recognize the voice of their calf, because the newborn calf, being a hider, rarely vocalizes (Kiley, 1972; Watts and Stookey, 2000). Marchant-Forde et al. (2002) found that cows, that were separated from their calf one day after birth and tested with played-back calf calls two days post partum, did not distinguish between calf calls of different origin. In contrast, Padilla de la Torre et al. (2016) documented vocal discrimination in cows kept on pasture with their calves: Cows were more likely to move their ears and turn their head or body towards the sound or walk to the speakers, when calls of their own calves were played-back in contrast to alien ones, although the number of calling by the cow during the test and latency to respond did not differ. Cows were more likely to respond when their offspring were younger (range according to Fig. 2 in Padilla de la Torre et al., 2016: about 5 - 110 days). The difference in vocal discrimination between the studies (Marchant-Forde et al., 2012; Padilla de la Torre et al., 2016) may arise by the different calf-contact durations post partum. In the current study (Chapter 3), dams may have recognized that the played-back calf calls in the parlour were not from their own calves, whereas the control cows, that had been separated half a day after birth, may not have had this ability. This could be an explanation for the different impact of acoustic stimulation on SCS in dams compared to control cows. However, it remains an open question why SCS in dams was lowered. While acute stress can increase SCS (Lambertz et al., 2014), SCS on herd-level was not related to fecal cortisol level, which is a parameter for chronic stress (Ivemeyer et al., 2018). However, it is unclear why vocalisations of hungry calves should lower the stress level in dams. Another explanation might be different cell counts in different milk fractions: higher counts in cisternal milk which could have been proportionally decreased in dams due to suckling before milking, and higher counts in later fractions of alveolar milk (Sarikaya et al., 2005), leading to higher SCC in cows with a better milk let-down. However, these explanations are not supported by significant differences in machine milk yield, fat content or milk flow characteristics.

5.1.2 Olfactory recognition and stimulation

After parturition the cow licks her calf frequently, which is essential for olfactory recognition (see 1.1). At this point fostering of alien calves is easiest, especially if the odour of the alien calf is covered with the amniotic fluid of the dam (Hudson, 1977; Waltl et al., 1995). If goats, which are hiders similarly to cattle, are rendered anosmic before parturition, no exclusive bond with the offspring is established (Hernandez et al., 2002; Romeyer et al., 1994). This proofs the special role of olfaction in the process of maternal selectivity. Furthermore, there are indications of a sensitive period after birth, when maternal selectivity can be established: If mother and offspring are separated directly post partum, maternal behaviour is still shown after 1 h of separation in cattle (Le Neindre and D'Hour, 1989), but decreased already after 2 h of separation in goats (Lickliter et al., 1982). Five minutes of contact after birth are sufficient to prolong the period, when maternal recognition is apparent (cattle: Hudson and Mullord, 1977; goats: Klopfer et al., 1964; Ramírez et al., 1996). However, mothers were not selective any longer at a reunion after 24 h (cattle: Hudson and Mullord, 1977; goats: Ramírez et al., 1996). Therefore, regarding the current study (Chapter 2 and 3) it can be supposed that the control animals, that had about 12 h calf-contact post partum, will not have recognized their calf or it's odour as they had already been separated for about 16 (Chapter 2) or about 30 d (Chapter 3). Even so, multiparous control animals numerically preferred to sniff or lick at samples of their own calf (Chapter 2). In dams, more selective or maternal behaviour has been observed in multiparous compared with primiparous animals (Edwards and Broom, 1982; Le Neindre and D'Hour, 1989; Lickliter et al., 1982). It can be speculated that in multiparous control cows other mechanisms than maternal recognition may cause the higher response: it has been shown in choice tests with ewes (Romeyer et al., 1993) and pigs (Maletínská et al., 2002) that mothers responded most to familiar offspring, followed by unfamiliar offspring compared to non-related animals. The pairwise comparisons between the responses towards unfamiliar offspring and unrelated animals, however, were not significant in the study of Maletínská et al. (2002).

Calf-hair was chosen as olfactory stimulus as ewes were able to distinguish their lamb from others by samples of wool (Alexander and Stevens, 1982/83), while other sources like urine and faeces in ewes (Alexander and Stevens 1981; 1982/83), or fabric the offspring was rubbed with in dairy cows were not suitable to elicit the aimed response (Barth et al., 2010). Despite a relatively low response rate of 60% in general, the results of the current pilot study (Chapter 2), suggest that cows were able to perceive the olfactory stimulus, as responsive cows sniffed and licked longer at bags with calf hair than at empty bags. However, olfactory stimulation with hair of the own calf did not influence parameters of milkability, behaviour, HR or HRV in dams or cows without calf-contact (Chapter 3). It cannot be excluded that the way of stimulus storing and presentation may have diminished an effect. Intensity of the odour and therefore the effect of the stimulus might have been increased, if more hair had been used, had been used fresh or stored at cooler temperature, but presented at warmer temperatures (e.g. not sprayed with water). Another option might be using other mechanisms instead, for example conditioning calf-contact with a certain odour. Neatsfoot oil and wool wax for example have been found to improve fostering in lambs (Alexander and Stevens, 1985; Price et al., 1998). However, here the odour of the own lamb was more effective (Price et al., 1998). Prenatal flavour exposure to anise affected growth, health and the behaviour of piglets in a positive way during weaning when they were re-exposed to the odour. However, postnatal exposure to the same flavour had no effect (Oostindjer et al., 2010). It is unclear, whether an operant conditioning of calf-contact with a flavour would lower the stress level during milking (tense head posture, more defecation) and thus might enhance milkability of dams.

5.1.3 Calf-presence and visual stimulation

Positive conditioning of nursing with a blue disk as visual stimulus increased machine milk yield (Willis and Mein, 1981). However, the study was conducted with only three animals. The presence of the calf during milking might be the strongest stimulus. According to a limestone panel dated from the New Kingdom in Egypt (1550 - 1070 BC), tethering of the calf to the forelegs of the cow, was already used to stimulate milk let-down (exhibition venue: Egyptian Museum, Cairo). In Zebu cattle (*Bos indicus*), buffalos (reviewed by Costa and Reinemann, 2003) and in Salers (*Bos taurus*), a French double purpose breed, which has lately been used as beef sucklers, even non-nursing animals show disturbed milk ejection during milking (Cozma et al., 2013; 2016). A short suckling period before milking and sometimes calf-contact during milking is necessary to improve milk let-down in these animals (reviewed by Costa and Reinemann, 2003; Cozma et al., 2013; 2016). This however, is labour intensive (reviewed by Costa and Reinemann, 2003). Therefore Kamboj et al. (2017) tested the effect of a synthetic dummy calf compared to suckling before milking and a control without calf-contact in Zebu cows and buffalos. The visual stimulus of the dummy calf led to comparable time until milk ejection occurred in cows and buffalos compared with animals suckling their calf before milking. Agitation behaviour during milking was similar in Zebu cows in the dummy and suckling treatment. However, buffalos with the dummy calf were more agitated during milking than suckled buffalos. Mean milk yield of the lactation and mean lactation length was improved by the dummy calf compared to no calf-contact, but remained below the results of suckled animals in both species (Kamboj et al., 2017). Compared to Zebu cows, European dairy cows can be milked without impaired alveolar milk ejection, if they are separated from their calf soon after birth (e.g. Prim Holstein: Cozma et al., 2013). However, if *Bos taurus* cows are nursing their calf, suckling before milking (Prim Holstein: Cozma et al., 2013) or udder contact during milking can be used to prevent problems in milk let-down (Prim Holstein: Cozma et al., 2013; Original Brown Cattle: Lucht, 2009; Salers: Tournadre et al., 2008). The presence of the calf during milking without udder-contact has not been found to improve milk let-down (Tancin et al., 2001; Tournadre et al., 2008). Contrary, Peeters et al. (1973) observed milk ejection in cannulated teats of cows in a tied stall which were allowed to interact with their calf, but without udder-contact. As personally witnessed on a farm in Sweden, an option is to build the parlour in a way that cows can have physical contact with their calves during milking without hygiene problems or increased labour costs. Some dam rearing farmers in Germany reported that the presence of the calf in the parlour is used especially in heifers to enhance milk let-down during the first milkings. In dam rearing with Original Brown Swiss (*Bos taurus*) milk yield and milk flow was improved when the calf was suckling one teat during milking (Lucht, 2009). In conclusion, physical calf contact during milking might enhance milk let-down in nursing cows during milking. However, apart from individual solutions, this practice is labour intensive. In contrast to milking in tied housing, milking parlours present problems with regard to available space and hygiene, e.g. if milking clusters are dropped due to calf-contact.

For the practical reasons mentioned above, and because visual recognition is established last in the process of mother-young-bonding (Reinhardt, 1980, p. 13) and further least important in kin discrimination (reviewed by Tang-Martinez, 2001), visual stimuli had not been included in the current study (Chapter 3). However, there is evidence that a visual stimulus during milking without calf-contact may improve milk let-down in nursing cows (Willis and Mein, 1981; Kamboj et al., 2017). A possible future direction of research would be to investigate effects of videos of suckling calves, e.g. in tandem parlours where they would be easy to apply.

5.1.4 Tactile stimulation

According to Lidfors et al. (1994), a suckling bout of a beef calf consists of three phases: Pre-stimulation, milk intake and post-stimulation. During pre-stimulation isolated sucking movements and frequent release of the teat can be observed. Butting occurs during the whole suckling event, however increases before milk let-down. About 90 sec after pre-stimulation started, milk is ingested by rhythmic sucking movements for about 6 min. Thereafter, increased butting occurs, followed by isolated sucking movements and release of the teat (Lidfors et al., 1994). The behavioural pattern of the calf is related to the milk ejection pattern of the cow. If milk ejection is induced by the injection of exogenous oxytocin, there is no pre-stimulation behaviour and milk ingestion starts directly (Mayntz and Costa, 1998).

The release of oxytocin from the pituitary gland starts about 20-30 sec after teat stimulation started. For a few seconds, oxytocin is transported by the blood to the udder tissue (reviewed by Bruckmaier, 2009). However, the delay from start of udder stimulation until milk ejection is highly variable, as it increases with decreasing filling of the udder (Bruckmaier and Hilger, 2001; Weiss and Bruckmaier, 2005). This is likely due to the time needed for myoepithelial contraction of the alveoli until milk is pressed out. More contraction is needed until milk is ejected, if alveoli are filled incompletely (Bruckmaier, 2001). If the filling of the udder was 40 - 100%, a delay of 50 – 70 sec has been found (Bruckmaier and Hilger, 2001). Therefore, a duration of 15 sec stimulation and a latency of 45 - 60 sec until attachment of milking clusters is recommended for non-nursing cows (reviewed by Bruckmaier, 2009). In the current experiments this advice has been considered. The duration of pre-stimulation (udder-preparation plus vibration stimulation or manual stimulation, Chapter 3) was 60 sec and in Chapter 4 udder-preparation lasted 20 sec plus approximately 40 sec of latency time. However, due to the nursing in dams before milking, their udder filling and consequently their udder pressure might have been reduced. The influence of low filling of the udder due to short milking intervals or late in lactation were investigated by Bruckmaier and Hilger (2001). They recorded a delay from start of stimulation until milk ejection of 78 s, if the udder was filled 20.1 - 40.0% and of

107 sec, if udder filling was between 0 and 20%. Future research should therefore include possible effects of stimulating dams until the maximum intramammary pressure is reached, or of the combination of different stimulation and latency durations. Some commercial dam rearing farms, conducting intensive udder cleaning because of raw milk production, report minor problems with milk ejection. However, it can be questioned whether problems are not existing or not detected.

Also the kind of stimulation plays a role for milk ejection in dams. Bruckmaier (2005) reviewed that in non-nursing cows minimum tactile stimulation of the teats is sufficient to induce the neuro-endocrine milk ejection reflex which results in full milk let-down. Other stimuli like hand-milking and vaginal stimulation in non-nursing cows and the suckling of a calf in dams evoke a higher release of oxytocin. However, as milk ejection follows a threshold principle, the amount of ejected milk is not increased by this. Stress can lead to central inhibition of oxytocin release, for example during milking in unfamiliar surroundings, at the onset of milking in primiparous cows and if animals switch between nursing and milking (reviewed by Bruckmaier, 2005). As hand-milking induced higher oxytocin release in non-nursing cows (Gorewit et al., 1992), the effect of manual teat stimulation on dams compared to vibration stimulation has been tested. However, this did not result in a better milk let-down (Chapter 3). While in primiparous animals with disturbed milk ejection during milking, oxytocin release could be induced by vaginal stimulation (Bruckmaier et al., 1992), this was not possible in a preliminary test of three dams (personal communication, Barth K., 12.04.2018). The use of this method can be questioned for ethical reasons. However, the results point out that the central inhibition of dams might be more severe than the inhibition in primiparous animals during their first milkings. Therefore, the neuronal signal for the stimulation of oxytocin release apparently needs to be stronger in dams than in non-nursing cows either with or without disturbed milk ejection. The stimulation applied in the present study (Chapter 3) was not sufficient for dairy cows with free calf-contact. It is possible to overcome inhibited milk let-down by injecting exogenous oxytocin. This, however, leads to desensitization of the udder tissue for oxytocin (Belo and Bruckmaier, 2010; Mačuhová et al., 2004) and therefore is no sustainable solution. Furthermore, a regular use of exogenous oxytocin would be critical, especially in organic farming. Therefore, either stronger stimuli need to be applied or the low machine milk yield under free contact conditions needs to be accepted.

Alternatively, a part time- separation of calf and dam may be an option. During half-day and restricted contact compared to free contact, higher milk yields could be collected (reviewed by Johnsen et al., 2016). However, the decrease in contact duration also influences other parameters. This is tackled in the next chapter of the discussion where different dam rearing systems are compared.

5.2 Comparison of different dam rearing systems

In dam rearing, free, half-day and restricted contact are possible options. Scientific research mainly focused on free and restricted contact (reviewed by Johnsen et al., 2016), whereas less is known about half-day contact (Barth et al. 2015b; Johnsen et al. 2015a; 2015c; Veissier et al., 2013). In commercial dam rearing often restricted contact combined with foster mothers is conducted at least in Germany (own data, unpublished) and Switzerland (Zumbrunnen, 2012). Information about each of the three systems in comparison with milk feeding systems, as well as about foster mothers can be found in Kälber and Barth (2014) and Johnsen et al. (2016). The following appraisal of the three contact types is organized according to the different criteria of comparison.

5.2.1 Machine collected milk yield

In all dam rearing systems, impaired alveolar milk ejection can be found, as indicated by a reduction of milk fat content of about 1%point. At the same time, the yield differs between contact durations (Johnsen et al., 2016). The loss of machine collected milk during calf-contact is often highest in free systems (-13.8 - 20.4 kg d^{-1}; Barth et al., 2010; Krohn 1999, cited by Krohn 2001; Schneider et al., 2007; Chapters 3 and 4). The difference in collected milk in half-day cows can be higher (-13.8 kg - 15.2 kg d^{-1}; Barth et al., 2015b; Johnsen et al., 2015a), similar or even less (-9.9 kg d^{-1}; Chapter 4) than during restricted contact (-6.9 - 12.4 kg d^{-1}; Barth et al., 2007; Boden and Leaver, 1994; Krohn et al., 1990, cited by Krohn, 2001; Mendoza et al., 2010; de Passillé et al., 2008). However, there is no study comparing the latter two systems directly. It can be concluded that milk loss in free contact is highest and that different stimuli to enhance milk let-down were not successful (Chapter 3). Shorter contact durations enhance collected milk yield (Chapter 4). However, there seems to be no linear relationship between the difference in milk loss and contact duration during the day.

In the present study (Chapter 4), fat content of ‘half-day’ dams was on average 1 percent point lower after the separation from the calf overnight, during the morning milking. This is a sign for impaired milk ejection. At the same time the mean milk yield was only 1 kg less than in control cows. Before afternoon milking nursing was possible. Therefore, machine milk yield of half-day animals was only 2 kg, while fat content was again decreased by 1 percent point. A by 1 percent point reduced fat content has also been found in other studies on dam rearing (Chapter 3; reviewed by Johnsen et al., 2016). The question arises if level of fat content reduction gives information about the milk yield, which cannot be removed by milking from the udder. Maybe dams independent of udder filling, deliberately or not, withhold a certain, but every time similar amount of milk for the calf. In this case, machine collected milk yield would be higher, if the time span between nursing and milking is prolonged and thereby udder-filling during milking is increased. In future studies, residual milk of dams with different intervals between suckling and milking should be investigated. To my knowledge residual milk was only measured once in dams. De Passillé et al. (2008) recorded 8.7 ± 0.8 kg residual milk in restricted suckling which was 5.5 kg more than in non-nursing animals.

The milk loss due to dam rearing increases in the course of time of the contact-period (Barth et al., 2009a; Schneider et al., 2007), because older calves drink more milk (e.g. Ivemeyer et al., 2016; de Passillé et al., 2008). Milk losses reported for free contact in Chapter 3 were higher compared to other studies with free contact. Reason for this might be that the study period in Chapter 3 related only to the second month of lactation. One to two month old calves presumably drink more milk than the average drunk by calves during the first 9 (Chapter 4) to 12 wks of life (personal communication Krohn C.C. 1999, cited by Krohn 2001). This theory is supported by the data of Schneider et al. (2007), who estimated the milk loss during the 50th d of lactation in dams with free contact only -2 kg than the milk losses in our study. This also underlines the absence of any specific positive effect of the different stimulations on the dams.

Taking into account the whole lactation (220 to 305 d in milk in Chapter 4), yields tended to be lower in free contact dams compared to the half-day contact group, and the difference to the control cows was relatively large (not statistically analyzed). The lactational yield of dams with half-day (Johnsen et al., 2015c; Chapter 4) or restricted contact (Kišac et al., 2011; Krohn et al., 1990, cited by Krohn, 2001) did consistently not differ compared to non-nursing cows, except in the investigation of Reuter (2014). He estimated a milk loss of 1.6 kg d^{-1} in nursing cows (restricted and free together) during the time without calf-contact (101st - 305th day of lactation). Cows with restricted contact during the first 12 wks post partum, however, gave 0.5 kg d^{-1} more milk than cows with free contact, but statistically no pairwise comparisons between restricted and no and free and no contact had been conducted. In foster cows suckling three calves similar effect of free and restricted suckling has been found. From the end of the nursing period 8 wks post partum until the end of lactation, cows, who nursed twice daily instead of freely, gave 230 kg more milk during milking (Everitt and Phillipps, 1971). Probably in foster cows or dams with half-day or restricted contact, the udder is more often completely emptied compared to free calf contact, because in the latter calves distribute their suckling more evenly and do not ingest high amounts of milk at once. Milk production is regulated, among other factors, by an inhibitory protein in the milk, which acts on the secretory cells. If the udder is not properly emptied, the inhibitory protein downregulates the activity of secretory cells. Even the amount of secretory cells may decrease (reviewed by Svennersten-Sjaunja and Olsson, 2005) which may lead to reduced milk production even after separation from the calf. A further aspect for consideration was the numerically higher incidence of mastitis in the 'free' contact group in Chapter 4, which could have affected milk production of the lactation negatively (e.g. Walsh, 1974).

5.2.2 Fertility of dams

Griffith and Williams (1996) found in intact beef cows that the onset of the reproduction cycle after parturition was inhibited by nursing the own calf. However, if the beef cows nursed their own offspring, but were not able to see and smell them, or nursed an alien calf, secretion of luteinising hormone increased. In contrast, Wettemann et al. (1978) could not find differences in the interval to first oestrus of dams nursing their own or an alien calf. Comparing different suckling systems, the interval until first ovulation or oestrus (beef cows: Álvarez-Rodríguez et al., 2010) and the number of inseminations per conception did not differ between cows with free or restricted contact to their own calf (dairy cows: Reuter, 2014). Also the calving-interval of cows with free or half-day contact was similar (Chapter 4). However, beef cows nursing two calves, had a prolonged anoestrus compared to cows nursing just one own or alien calf, possibly due to higher amounts of milk drunk by the two calves (Wettemann et al., 1978). Similarly, foster mothers nursing three to four calves twice daily without being milked had longer post partum anoestrus intervals (Thomas et al., 1981). In addition, the duration of the nursing period may play a role. Suckling for 10 d after birth led to an earlier conception compared to control cows (Metz, 1987), while in another study 26 weeks of nursing prolonged calving-intervals (Margerison et al., 2002). In conclusion the the dam rearing system has no effect on dam fertility, but the number of suckled calves in foster rearing and the nursing duration have an influence.

5.2.3 Calf growth

Pre-weaning growth

Calves of the different dam rearing systems gained about 1 kg d^{-1} during the nursing period in different investigations (e.g. de Passillé et al., 2008; Grøndahl et al., 2007; Johnsen et al., 2015a). However, in a study of Fröberg et al. (2008) where dams were milked three times daily and had restricted calf-contact for 30 min twice daily for eight weeks, calves gained less than 0.5 kg d^{-1}. This was similar to the weight gain of control calves, but with a higher variation in nursed calves. However, results from free contact studies are also widely varying from 0.85 kg (Veissier et al., 2013) to 1.5 kg d^{-1} (Fröberg et al., 2011). Obviously, weight gain of calves is not only influenced by the duration of dam contact, but also by other factors such as milk yield of the dam and attractiveness of supplied feed. Therefore, caution must be applied when comparing studies on different rearing systems, but also with different breeds and husbandry conditions. Projects where two contact durations were compared could not find great differences between treatments: restricted vs. free contact (Roth et al., 2009), half-day vs. free contact (Veissier et al., 2013; Chapter 4), restricted or free contact of three calves per foster mother (Everitt and Phillipps, 1971). According to these few findings there is no clear linear relationship between contact duration and calf growth during the nursing period.

Post-weaning growth

It might be assumed that systems with temporal separation from the dam motivate the calves to ingest more solid food. As a result of this, the growth check after separation from the dam and/or after weaning might be less severe than in free contact. However, no differences in feeding behaviour (free vs. half-day: Veissier et al., 2013) or feed intake (free vs. restricted: Roth et al., 2009) could be detected. Nevertheless, Veissier et al. (2013) registered higher weight gains post weaning in half-day compared to free contact calves which could not be confirmed in the present study (Chapter 4). However, the accidental temporary blocking of access to the calf creep with fodder in the ‘half-day’ group might have diminished potential differences. However, also Roth et al. (2009) found no differences in weight gain between free and restricted contact calves in the month after abrupt separation and weaning either.

Some weeks after weaning, body weight of ‘free’ and ‘half-day’ calves (Chapter 4), and similarly of free and restricted contact calves (Roth et al., 2009), was still higher compared to calves fed with nipple-buckets or at an automatic feeder, but did not differ between contact-treatments.

Therefore no clear relationship between contact duration and growth development after separation and/or after weaning can be detected. It must be considered that important factors influencing calf growth differed between studies: e.g. the feeding management during and after the contact-period as well as the way of separation (no contact, visual contact, tactile contact) and weaning (abrupt, gradual). So far, there are not enough studies to conclude a systematic effect of these influencing factors on calf growth.

5.2.4 Health

In interviews with 26 Scottish organic dairy farmers without nursing-system (Maxwell et al., 2006) and 14 dam rearing farmers in Switzerland and Germany (Zumbrunnen, 2012), they expressed their high interest in possible improvements of cow and calf health through nursing (Maxwell et al., 2006; Zumbrunnen, 2012). On the other hand, potential animal health problems are one impediment for dam rearing, regularly mentioned by farmers (Maxwell et al., 2006; Ventura et al., 2013). This underlines that farmers are highly interested in the impacts of dam rearing on animal health.

Calf health

There is only one study comparing the health of calves with different dam rearing systems. Roth et al. (2009a) could not find differences in the health status or the number of veterinary treatments between free and restricted contact. According to Bar-Peled et al. (1997) and Roth et al. (2009a) as well as farmers' reports, diarrhoea can occur due to high amounts and/or a high fat content of ingested milk, but farmers report that they have no pathogenic effects (Ehrlich, 2003; FibL et al., 2011; Zumbrunnen, 2012). In future studies it should be assessed systematically, if and if so what kind of pathogens are involved.

Cow health

It has been reported that nursing can impair the cows' body weight and body condition score after calving (Cozma et al., 2016; reviewed by Kälber and Barth, 2014; Margerison et al., 2002). There are no studies comparing this in different dam rearing, however investigations on beef sucklers and foster mothers and an experiment in dairy cows might give some indications: Beef cows nursing their calf once per day gained more weight than cows nursing twice per day or freely (Álvarez-Rodríguez et al., 2010). The authors suggested that this might be due to the higher milk production in animals nursing more than once per day. In another study, Thomas et al. (1981) could not find differences in body condition or live weight development between foster mothers nursing three to four calves and control cows who were milked twice daily. In an experimental study with dairy cows, five days of free calf-contact after calving combined with one milking a day led to reduced metabolic stress compared to two milkings a day, without affecting the milk yield during the following eight weeks (Carbonneau et al., 2012). In conclusion, nursing, even if combined with milking, does not in any case affect body weight and condition negatively. However, more investigations are necessary to compare different dam rearing systems.

It has been suggested that suckling may positively influence expulsion of the after-birth and involution of the uterus (reviewed by Kälber and Barth, 2014). Both is associated with contractions of the uterus in response to oxytocin release. This hormone is mainly ejected during suckling or milking. However, Lidfors (1996) and Krohn et al. (1999) could not find a difference between nursing or separated cows concerning placental retention. As most dam rearing farmers keep mother and calf in the calving pen during the colostrum period, no differences between dam rearing systems are to be expected.

Johnsen et al. (2016) concluded in their review that dam rearing at large has no effect on udder health compared with cows that are milked only, but that in some studies a positive influence of restricted suckling had been found. The present study is the first comparing udder health of different dam rearing systems (Chapter 4). SCS, the percentage of milkings with SCC >100,000 cells ml^{-1} or signs of udder inflammation and the incidence of mastitis did not differ between half-day and free contact dams. A possible advantage of restricted dam rearing is that teats can be dipped with a disinfectant like iodine or chlorine after milking or suckling. This preventive udder health measure cannot be applied in free contact or only once per day in half-day contact cows, as otherwise calves would ingest the dipping agent by suckling. In theory, the high frequency of milk removals by calf and machine in half-day and free contact may lead to an incomplete recovery of teat tissue between milk removals. Further, the in total longer duration of being milked/suckled may lead to teat damage. Milking intervals of less than 6 h did not increase SCC compared to milking intervals of more than 8 h in automated milking (Hamann and Halm, 2004; Mollenhorst et al., 2011). Even though the vacuum which is produced by the calf during suckling is higher than the one produced by the machine, nursing is less invasive for the teat, as the udder quarter is evacuated faster due to a higher suckling cycle (McDonalds and Witzel, 1966). In addition, if only one calf is suckling, nursing occurs at only one teat at one time. Suckling bouts also include suckling breaks, when no pressure is applied to the teats, lasting about 25% of the total duration of the suckling event and teat changes (approx. 8% of time; Hamann and Heermann, 1989). Less pressure is applied to the teat end (Hamann and Stanitzke, 1990; van der Tol et al., 2010) and the diameter of teats are not increased after suckling compared to milking. Oedema due to milking lasted more than 30 min in the study of Hamann and Stanitzke (1990). Therefore and because of the lower amount of udder emptying, systems with both milking and nursing cannot easily be compared with high frequencies of machine milking.

Restricted suckling in combination with several fostered calves per cow, led to worse teat skin condition compared to only milking as a control in experimental studies (Thomas et al., 1981; Rasmussen and Larsen, 1998) and has been reported by farmers (Ehrlich, 2003; Zumbrunnen, 2012). On the other hand, nursing also decreased the number of esculin positive bacteria on the teat skin (Rasmussen and Larsen, 1998), which may lower the risk of mastitis.

As udder health is affected by many factors, a comparison of different dam rearing studies concerning this is not appropriate. A direct comparison of half-day and free contact with restricted contact should be conducted to evaluate the effects of dipping, number of suckling events, number of udder evacuations and time between nursing/milking.

In conclusion, there is a lack of knowledge concerning the effect of different dam rearing systems on calf and cow health. Beside the influence of the different dam rearing systems on the incidence of diarrhoea and mastitis, the general immune response and health problems such as infestation by endoparasites and pneumonia in calves as well as involution of the uterus and metabolic disorders in cows should be part of future studies.

5.2.5 Mother-calf- and social behaviour

When mother and calf are separated soon after birth, they cannot or only briefly perform their natural mother-child behaviour. Besides suckling, mother-calf interactions consist of social licking and rubbing, playing (e.g. horning and mounting) and spending time near each other (Koch and Zeeb, 1969; Schloeth, 1958). It has been suggested that cows (Mandel and Nicol, 2017) and calves (Yayou et al., 2014) separated immediately after birth extensively use a mechanical brush to satisfy their need for physical contact. Cows used the brush in the period after parturition longer than during the rest of the lactation (Mandel and Nicol, 2017: 6 min d^{-1}). Also calves used the brush to a high extent, but data were not compared with another period of time (Yayou et al., 2014: 25 min d^{-1}). Newby et al. (2013) observed cows, that had calf-contact for 100 min post partum and in the time after separation. In their study, cows used the brush only after the calf was separated. However, that hypothesis is very speculative. No information is available about brush use of cow-calf-pairs with and without contact for longer periods, e.g. to exclude possible other reasons for intensive brushing after parturition e.g. dorsal pain.

In dam rearing, the time animals can perform mother-calf-behaviour differs according to the system. In free contact, calf and cow can meet during both day and night (milking excluded) and therefore they can perform mother-child behaviour nearly unrestrictedly. In half-day contact, calves can suckle and interact with their dam during the day, but not during the night (Veissier et al., 2013; Chapter 4) or the other way around (Barth et al., 2015b; Johnsen et al., 2015a; 2015c). Veissier et al. (2013) showed that calves with half-day contact suckled at their dams for longer than free-contact calves during the same period, possibly because the latter were also nursed during the night, when no data were recorded. In restricted dam rearing systems dams and calves meet twice daily for 15 - 60 min. Suckling only twice per day is not according to natural behaviour (e.g. Lidfors and Jensen, 1988; Reinhardt and Reinhardt, 1981). In common calf rearing one solution for this problem are automatic milk feeders and ad libitum feeding of acidified milk. Thus, in terms of suckling frequency as part of mother-calf interactions, restricted dam rearing might be retrograde. On the other hand, it has been suggested by Roth et al. (2009) that 15 min of cow-calf contact twice daily are not comparable to milk-feeding-systems, neither restricted nor ad libitum. Justification for this hypothesis was that total suckling duration per day, health, weight gain and reduction of cross-sucking behaviour of animals with restricted contact were similar than in free contact. Furthermore, it has recently been shown that calves can drink more than 5 kg per meal without milk entering the rumen or behavioural signs of discomfort (Ellingsen et al., 2016). Obviously, free contact meets animals' needs best, as they can choose between all options. Half-day or restricted contact does provide this opportunity only during specific times. However, this might impair the animals' welfare less than restricted feeding in milk-feeding-systems, but some aspects should be considered. These, are part of the next paragraphs.

For half-day systems it is an open question whether calves should have mother-contact during the day or the night. For instance, different circadian distributions of suckling are reported from beef sucklers (Ewbank, 1969; Koch and Zeeb, 1969; Somerville and Lowman, 1979; Walker, 1962). Apparently the distribution of nursing over the day is affected by external factors like housing or grazing, time of daybreak and grazing and resting periods of the dams (Koch and Zeeb, 1969; Schloeth, 1958; Somerville and Lowman, 1979; Vitale et al., 1986; Walker, 1962). Thus it cannot be generalized that nursing only during the night is less natural than only during the day. In dam rearing, milking might trigger suckling during the day: It has been observed unsystematically in free and half-day contact that calves often suckle directly before milking and sometimes are even called by their dams to suckle before milking (personal communication Barth K. 19.04.2018; own observations). As the mother is not only a source of milk for the calves, but a social partner of highest attractiveness (Reinhardt, 1980, pp. 21f), the question arises, if quality and quantity of social interactions with the mother and other social partners are comparable during day- and night-contact. This might influence the effect of dam rearing on the calves' social abilities and cognition because of the differing possibility for learning through observations and experiences. Roth (2008, p. 124) reported that calves with free contact stayed in the calves' creep during most of the night-time. Johnsen et al. (2015c) investigated half-day contacts during the night time only, and found that during 10% of the first 2 contact hours affiliative behaviour was performed, during another 20% calves stayed near the dam and additional suckling took place. Thus, besides suckling, social behaviour occurs during night contact, but the observation period was too short to draw conclusions about the extent. Unpublished data of half-day contact with small sample sizes suggest less affiliative and play behaviour between dam and calf during night-contact compared to free contact of previous studies on the same research farm (personal communication, Barth K., 19.04.2018). Advantages of half-day contact during the night would include an independent management of cows and calves during the day, e.g. grazing and veterinary treatments. Further it is easier to increase the number and quality of human-calf-interactions, if calves are housed only in the calf creep during the day compared to the cow barn. This may improve animal-human-relationship. It would be worthwhile to further investigate possible short- and longer term effects of the timing of cow-calf contacts e.g. on social abilities, cognition and animal-human-relationship of the offspring.

Some studies indicate that dam rearing may influence the social behaviour of calves towards other calves and cows in a positive way (Buchli et al., 2017; Flower and Weary, 2001; Waiblinger et al., 2013), but no studies comparing different contact-systems are available. For example, Buchli et al. (2017) recorded more frequent submissive responses to threats in dam reared compared to no contact calves.

5.2.6 Abnormal and stress associated behaviour

Cross-sucking

Cross-sucking, where calves suck at eachothers ears, navels, udders, scrotums and other body parts of pen mates, is interpreted as redirected sucking behaviour. It is supposed that cross-sucking can lead to bezoars by the ingestion of hair which in rare cases may cause digestive disorders. In the cross-sucked calf inflammation of ears or navel as well as udder damage can occur (reviewed by Broom and Fraser, 2007, pp. 244f). However, in some recent studies cross-sucking has not been found to cause umbilical infections (Gößbacher et al., 2018) or udder health problems (Vaughan et al., 2016). To my knowledge there is no original paper about the prevalence of hairballs due to cross-sucking in dairy calves. Roth et al. (2009b) assessed rumen development of 15 wk old dairy calves, but did not report that bezoars have been found. Beiranvand et al. (2014) could find patches of a sticky mass including hair in rumens of 10 wk old dairy calves fed with 4 L milk d^{-1} and concentrate. As the calves were housed individually the hair must have derived from their own coat. No hair has been found in the same study, when calves had access to hay. Hairballs have also been found in veal calves due to licking or 'grazing' of hair, which is caused by a lack of solid feed or roughage in the diet (e.g. Herd and Cook, 1989; Morisse et al., 1999; Webb et al., 2013). Whether cross-sucking is connected to urine drinking (Lidfors, 1993) and intersucking, which is udder sucking and milk stealing in older animals, is not clear (reviewed by de Passillé, 2001). In non-nursing systems cross-sucking can be suppressed by individual housing (e.g. Dong et al., 2017) or fixation of calves during and 10 - 60 min after milk ingestion (e.g. Gößbacher et al., 2018; Keil et al., 2001). Reasons for cross-sucking are mainly hunger and probably an unsatisfied need for suckling (e.g. Roth et al., 2009a).

In dam rearing, independently of the system, cross-sucking occurs rarely during the time when calves have access to their mothers (e.g. Fröberg et al., 2008; Margerison et al., 2003; Roth et al., 2009a; Veissier et al., 2013). When separation from the dam and weaning are conducted at the same time, cross-sucking has been observed more often in free contact calves than in half-day contact and even less than in calves reared without mother-contact (Veissier et al., 2013). In contrast, Roth et al. (2009a) observed cross-sucking less often in free than in restricted contact calves (Roth et al., 2009a). However, in both studies the method of observation can be questioned. Roth et al. (2009a) observed only 4 h d^{-1} and it is unclear how observations were conducted. Veissier et al. (2013) observed the animals during the whole day, but not during the night, when half-day animals had been separated from the dam. Further, instantaneous scan sampling was used, observing the behaviour in an interval of 5 - 10 min. As cross-sucking occurs largely unpredictably, and it may last shorter than 10 min, continuous behaviour sampling would have been more precise.

In a small student project, connected to the present comparison of free, half-day and no contact, four calves of the half-day contact group and seven of the free group were observed during the nursing period between 6:00 a.m. and 7:30 p.m., allocated over four days with 3.5 h observation time per group each day (Brüne, 2013). Three of four half-day calves and none of the free contact calves were observed cross-sucking. Two of the half-day calves performed cross-sucking only once for 1 - 2 min when they were locked in the calf creep. The other calf was observed cross-sucking several times also during the contact time for up to 9 min. This should be a reason for concern. Re-directed behaviour often occurs in situations when animals experience increased stress (Kerr and Wood-Gush, 1987). Veissier et al. (2013) had observed half-day calves only during the time they had contact with their dams. Even then, cross-sucking occurred in free and half-day contact animals, but to a very low degree and significantly less than in animals without dam-contact (0.4 vs. 1.2% of scans). Thus there is a need for research about cross-sucking during the separation time in half-day or restricted contact.

Licking and nibbling at objects

Nibbling and licking at objects are part of the normal exploration behaviour, but can also develop into abnormal behaviour. It is interpreted as such if context, intensity or frequency of nibbling or licking are overtly different from the norm (Broom and Fraser, 2007, p. 226). There are only a few studies about this behaviour in dam reared calves, therefore results should be interpreted with caution. Brüne (2013) observed all calves of the half-day group nibbling at objects, mainly at the wooden wall of the cow barn. The frequency was four times higher than in the 'free' group, where six of seven calves nibbled objects. However the longest duration of nibbling was observed in a free calf lasting 39 min. Veissier et al. (2013) combined licking objects and licking other calves in their analysis and found free and half-day calves (analysed as one treatment) showing this behaviours more often than calves without dam-contact (8.1 vs. 6.0% of scans). In restricted suckling, licking objects occurred, but to a significantly lower extent than in non-nursed calves (Fröberg et al., 2008). In contrast, Roth et al. (2009a) never observed suckling of equipment in free and restricted contact calves. It can be questioned if licking and sucking objects should be treated as one. Veissier et al. (2013) for example categorized licking objects together with licking other animals and sucking objects together with cross-sucking.

In veal calves, beside breed, different factors have been found to decrease the occurrence of licking and nibbling at objects: type of solid feed (maize silage compared to pellets, muesli or cereal grain), space allowance (>1.8 m² per calf), number of calves in a group (>10) and season (winter; Leruste et al., 2014). De Passillé et al. (1992) observed that milk ingestion increased sucking and butting behaviour, but not licking objects. Further, drinking either from an open bucket or a teat-bucket had no influence on licking fixtures (observed during 30 min after milk feeding; Dong et al., 2017). It is supposed that licking or nibbling at objects may be triggered by other factors than cross-sucking, but derive from a need to show grazing (Veissier et al., 1998) and exploration behaviour (Sato and Wood-Gush, 1988, cited from Leruste et al., 2014 and from de Passillé et al., 1992). In calves with and without dam-contact, studies have shown that with increasing rumination (e.g. Kerr and Wood-Gush, 1987; Margerison et al., 2003; Veissier et al. 1998) and ingestion of solid feed, especially roughage, nibbling or licking at objects decreases (e.g. Margerison et al., 2003; Veissier et al. 1998; Webb et al., 2013). At the same time, an enriched environment stimulates investigative behaviour, including licking and nibbling at objects (Pempek et al., 2017). Kerr and Wood-Gush (1987) observed a higher proportion of investigation behaviour during the day in beef suckler Angus-crossbreed calves on pasture compared to individually housed and bucket-fed Holstein Friesian calves. However, confined housed calves investigated more often, but only for short durations, which was interpreted as a result of the barren environment (Kerr and Wood-Gush, 1987). If the environment is comparable, social interactions with the dam may decrease nibbling and licking objects. Krohn et al. (1999) compared calves in pens either with the dam or without during the first four days of life. Singly housed calves licked more at objects than calves with dam-contact, either nursed or not. Nursed calves showed less licking objects even after separation from the dam. Results of Kerr and Wood-Gush (1987) indicate a negative correlation between the proportion of time spent investigating and the time interacting with the dam.

In conclusion, it is hard to distinguish between normal and abnormal investigative behaviour. There is no natural threshold between a normal and non-normal duration or frequency of licking and nibbling at objects. Similarly, it may be difficult to draw the line between stereotypic and normal behaviour, i.e. to assess the quality of the performed behaviour. Therefore, for the evaluation of different systems of dam rearing, the assessment of oral behaviours directed at objects should be performed in a comparative manner. This requires at least comparable housing conditions between the different treatments, but also definitions of the observed behaviour should be refined. Dam reared calves normally have a more diverse social and housing environment than calves without mother-contact fed by bucket or automat. Roughage is available in both systems. It can be discussed if nibbling at a wooden wall for 39 min may be normal investigative behaviour or if other factors were involved.

Stimulating dam reared calves to ingest considerable amounts of solid feed as soon as possible, does not only decrease licking and nibbling at objects, but facilitates rumen development and rumination and may reduce the growth check after separation from the mother and/or weaning off milk. It is therefore a recommendable measure in any case. Grazing with the mother might be an option. In heifers it has been observed that grazing behaviour is improved when they are grouped with pasture-experienced cows during their first pasture access (Costa et al., 2016).

Self-grooming

Self-grooming as behavioural trait has rarely been studied in dairy calves, however in veal calves it is associated with low fibre intake (e.g. Herd and Cook, 1989; Morisse et al., 1999; Webb et al., 2013). Dam reared calves with half-day contact groomed themselves twice as often as calves in the ‘free’ group (19.5 vs. 8.8; n = 4 vs. n = 7; Brüne, 2013). Kerr and Wood-Gush (1987) found that calves in confined environment licked themselves twice as often as beef suckler calves. Self-grooming, like nibbling objects, is a normal behaviour, and the ability to groom is an important criterion for good housing (Broom and Fraser, 2007, p. 264). At the same time, self-grooming can serve self-stimulation or be a conflict behaviour e.g. due to an environment with little stimuli or as a sign of frustration (Kerr and Wood-Gush, 1987). Again, drawing the line is difficult.

Vocalisation and other stress associated behaviour

Vocalisation ‘may signal the physiological and emotional state, motivations and intentions of the calling animal‘ (Watts and Stookey, 2000, p. 16). Although not systematically investigated, in the present study (Chapter 4) it was apparent that ‘half-day’ calves vocalized when morning milking started, before they were allowed access to the cows’ barn. Calves were standing at the fence directed towards the waiting room, and emitted high-pitched calls (31 vs. 1 calls h^{-1}; Brüne, 2013). Presumably the calls were directed towards the cows or the staff in the waiting room or parlour, and the calves may have been stressed. If calves are weaned or separated from their mother, frequent vocalisations are the result (Watts and Stookey, 2000). This may reflect their negative feelings (e.g. hunger, missing the mother, fear; Watts and Stookey, 2000). Kiley (1972) distinguished between different types of calls in cattle and observed that such calls occur beside others when animals are hungry and they are not fed, even though the stockperson is nearby. In the present study, hunger may be the possible reason for calling as calves headed directly to the mothers' or another cows' udder when they could enter the barn. Cows sometimes threatened or kicked even their own calf at these attempts to suckle, maybe due to low udder filling. In free contact such aversive behaviour also happened, but was observed much less (0.3 - 0.6 vs. 0.0 - 0.2 frequency h^{-1}, during 3.5 h of observation during the day, Brüne 2013). It can be supposed that in restricted systems this problem might occur if nursing is after the milking process instead of before. However, if restricted nursing is conducted before milking, cows in early lactation were tenser during milking compared to free contact cows. They also vocalised more often and spent more time near the gate to the calves' area compared to free contact cows. This can also be interpreted as stress due to separation (Schneider et al., 2007). No data on such behaviour in half-day contact are available so far.

The vocalisation of cows and calves after separation or during weaning is also important concerning not only animal but also human welfare (reviewed by Johnsen et al., 2016). Little is known about the influence of different nursing systems on welfare issues during this period. It can be hypothesized that temporal separation trains the animals to be apart from the dam. In horses progressive habituation to separation has been found to reduce stress in mothers and foals (Lansade et al., 2018). To the contrary, studies on temporal separation in other species and beef cattle showed negative effects on mother and offspring: signs of depression in rodents and primates (reviewed by Newberry and Swanson, 2008), higher parasite levels in lambs (Orgeur et al., 1998) and greater growth check in male but not female beef calves (Holroyd and Petherwick, 1997). Veissier et al. (2013) found higher cortisol levels in free and half-day contact calves after weaning compared to non-nursed calves, but without a significant difference between dam-treatments.

Quantity and quality of vocalisation of calves and cows should be investigated in more detail when calves are separated during the nursing period in half-day and restricted dam rearing. In contrast to HR and Cortisol, where outcomes vary only from low to high and rise both in pleasant and unpleasant situations, analysis of the vocalisation may give more distinct information about the affective state of an animal (Watts and Stookey, 2000). For example, the vocalisation of a cow, when she is hungry or separated from her calf, could be distinguished from other vocalisation by the spectral structure of sound (Ikeda and Ishii, 2008). Thomas et al. (2001) found that frequency as well as number of calls were strongly related to the amount of milk provided to a calf. However, this was not the case when different amounts of milk were supplied by an automated feeder (Vieira et al., 2008). The authors assumed that animals vocalize if they have someone (dam or human) to address. In Precision Livestock Farming vocalisation is already used as indicator of welfare in other species. The development in cattle is in progress (e.g. reviewed by Green et al., 2017; Meen et al., 2015). Vocalisation and other stress-indicating measurements should be used to further evaluate cow and calf welfare in the different cow-calf systems, also including separation and weaning periods.

5.2.7 Animal-human relationship

Some farmers are concerned that dam reared animals are harder to handle due to a weaker animal-human relationship, because calves do not associate humans with milk feeding (Johnsen et al., 2016). Some dam rearing farmers therefore spend additional time positively interacting with the calves to establish a good animal-human relationship (Dieng, 2018; Ehrlich, 2003). The different amounts of necessary daily human-contact in the different rearing systems, decreasing from restricted, to half-day, to free contact, may make a difference in this regard. On the other hand, calves can observe interactions of humans with their mother or other cows to a different extent in the different rearing systems. Munksgaard et al. (2001) reported that cattle partly adapt their response towards different handlers according to the behaviour shown by other animals towards these people.

A general effect of dam rearing on animal-human-relationship has been observed in different studies (Kälber and Barth, 2017; Krohn et al., 1999; Wagner et al., 2011a; 2011b). However, different systems of dam rearing were not compared. Even calves which were nursed only the first four days post partum made less physical contact to a human in an approach test until the 25th week of life, compared to separated calves or non-nursed calves with dam contact. The same animals showed a higher avoidance distance during the 15 - 18th month of life, if they had been in the nursed group, compared to the non-nursed animals, either with or without dam contact (Krohn et al., 1999). However, in each dam rearing system a good animal-human relationship will likely be possible if stockpersons take their time to positively interact with the calves. The existence of sensitive periods, during which minimal human-animal interaction can have long-lasting positive effects, has been suggested: for example in cows during or directly after calving (Hemsworth et al., 1989; Probst et al., 2012). After separation from the dam, there might also be a chance to improve the calf-human relationship. In the present study on half-day and free contact, calves were fed with teat buckets after nine weeks of nursing. The subjective impression was that the calves' fear of people decreased during this period, although dam reared calves still appeared more flighty than control calves until their first calving. Afterwards no differences in avoidance behaviour or agitation behaviour during milking were reported by the milkers. This is in line with reports from Wagner et al. (2011a; 2011b) that the avoidance distance of dam reared animals was significantly higher at 28 days and 15 months of age, but not at 26 months of age (Wagner et al., 2011b) or four months after their first calving (Wagner et al. 2011a). On an experimental farm and on commercial farms, Kälber and Barth (2017) did not find differences in the avoidance distance, estimated at the feeding place, of young stock either reared with or without dam-contact.

Thus, the impact of the different dam rearing systems on the animal-human-relationship should be further investigated, including effects of different quantities and qualities of human contact in the long term.

5.2.8 Long-term effects

Productivity

In a few studies, a positive influence of feeding increased amounts of milk or milk replacer has been found on calves' body weight and size until the 70th day of life, when the study ended (Omidi-Mirzaei et al., 2015) as well as on heifer performance during the first lactation (Soberon et al., 2012). However, in most other studies this was not confirmed (post-weaning growth: e.g. Guindon et al., 2015; Jasper and Weary, 2002; Yunta et al., 2015; first lactation performance: Kiezebrink et al., 2015; Shamay et al., 2005; Yunta et al., 2015). Similarly, results on effects of dam rearing on the productivity of heifers are heterogeneous (Bar-Peled et al., 1997; Broucek et al., 2006; Kälber and Barth, 2017; Ufer, 2014; Wagenaar et al., 2011).

In tendency higher milk yields during the first lactation of dam reared cows have been found by Bar-Peled et al. (1997) and Broucek et al. (2006). Other studies could not find differences in first lactation milk yields according to the rearing system (Kälber and Barth, 2017; Ufer, 2014; Wagenaar et al., 2011; Zipp and Knierim, 2015), although in most studies control animals were fed restrictedly with milk or milk replacer (6 - 8 L d^{-1}; Ufer, 2014; Wagenaar et al., 2011; Zipp and Knierim, 2015).

Nursing did not influence milk content (Kälber and Barth, 2017; Ufer, 2014), udder health (Kälber and Barth, 2017; Ufer, 2014; Wagenaar et al. 2011), number of veterinary treatments (Kälber and Barth, 2017; Ufer, 2014), occurrence of lameness (Kälber and Barth, 2017), body weight or body dimensions (Bar-Peled et al., 1997; Ufer, 2014; Wagenaar et al., 2011; Zipp and Knierim, 2015) during the first lactation. A positive effect of dam rearing on several parameters of fertility have been reported by some authors (Bar-Peled et al., 1997; Kälber and Barth, 2017; Ufer, 2014) while in other studies no difference has been found compared to animals reared without dam or foster mother contact (Wagenaar et al., 2011; Zipp and Knierim, 2015).

In an epidemiological study Kälber and Barth (2017) could not find differences in culling rates of dam or foster mother reared calves or young stock compared to non-nursed animals. During the first lactation, however, in tendency less contact-animals were culled (Kälber and Barth, 2017). In experimental studies differences between culling rates of first lactation cows with restricted and free (Ufer, 2014) or half-day and free contact (Zipp and Knierim, 2015) compared with no mother-contact were not significant.

There is a lack of studies comparing different dam rearing strategies. Ufer (2014) included data of first lactation cows reared either with restricted or with free dam contact in her analysis, but did not compare both contact-systems directly. Only an own small follow-up study on the first lactating cows that had had either half-day or free contact is available, in which no difference regarding age, body weight, height at withers and trunk girth at calving was detected. Also milk yield during the first 100 d of lactation, mean daily milk yield of the total lactation, lactation duration and culling rates did not differ between treatments (Zipp and Knierim, 2015).

Behaviour, cortisol and heart rate

Free and half-day contact calves have access to most parts of the cows barn and have contact with at least a part of the dairy herd, although in half-day contact only between two milkings. The question arises how these factors affect the calves' social development. Studies comparing the long-term effect of different dam rearing systems are rare. In the own follow-up study, free contact, half-day or no contact heifers were observed after their integration into the dairy herd (Zipp and Knierim, 2015). While all heifers showed similarly little lying behaviour (recorded by acceleration sensors) during the first 24 h after integration, free or half-day contact heifers lay significantly or in tendency longer during the next 24 h than no contact heifers, with no significant differences between the dam reared groups.

In another experiment, short- and long-term effects of free, restricted contact (here: 2x15 min d^{-1} during 12 wks) and no contact were investigated. After integration into the milking herd, free contact heifers showed more self-grooming than restricted and no contact heifers. Submissive behaviour tended to differ between treatments, with decreasing frequencies from free to restricted to no contact heifers. However, this did not decrease the number of received agonistic behaviours (Wagner et al., 2012). This study was repeated by Kälber and Barth (2017). They found that automated-raised heifers vocalized nearly twice as often as dam reared animals during the first 12 h after integration into the milking herd, which can be interpreted as a sign of discomfort. However, cortisol level as indicator of stress, did not differ between the two groups. During the integration period submissive behaviour was not shown significantly more frequently by dam reared animals. At day 8 and 9 after the integration only heifers reared with free dam contact showed and received submissive behaviour. However, the latter had in tendency longer lying periods (Kälber and Barth, 2017). In the same study during the 100th d of the first lactation non-nursed cows initiated and received more agonistic interactions than animals which had been nursed as calves. From the 100th - 200th and 300th d of lactation, agonistic interactions both, received and initiated, decreased, which can be interpreted as an integration process. Lesions due to horn butts, however, did not differ between groups during the whole lactation. Submissive behaviour shown by other herd members towards dam reared first lactating cows significantly increased, whereas it decreased in automat-reared conspecifics (100th, 200th and 300th d of lactation, Kälber et al., 2016). This difference in submissive behaviour might have developed due to better learning opportunities in contact with the mother (e.g. Brüne, 2013; Edwards and Broom 1982; Selman et al., 1970a; Wolters, 2006) or alien cows (Waiblinger et al., 2013). Calves with free contact initiated more agonistic interactions compared to control calves, but received to a higher extent agonistic interactions initiated by other cows (Waiblinger et al., 2013). Calves may learn from such experiences, which also occur in restricted contact, especially if combined with foster-mothers (Loberg and Lidfors, 2001), and half-day contact (Brüne, 2013). However, it can be supposed that the number of interactions and therefore the need of the calf to adapt its behaviour may decrease with decreased contact duration to adult conspecifics.

Free contact first lactation cows were more active during an isolation test, but simultaneously had the lowest heart rate (HR). Moreover, they had the lowest HR after re-joining the dairy herd and the lowest basal cortisol level. Wagner et al. (2015) interpreted this as free contact animals having increased sociality and a more active coping style. There were no differences in a novel object test between first lactation cows which were raised with free or restricted contact (Wagner et al., 2015).

In conclusion, there are a few first indications of possible long-term effects that are more pronounced in free compared to half-day or restricted contact.

5.2.9 Labour input

A topic not associated with the performance or welfare of animals is the work the farmer needs to invest. Little is known about necessary labour input for the different dam rearing systems. Junqueira et al. (2005) estimated the time needed for bucket-feeding and cleaning of buckets to be 3.63 min. In the same study the Holstein x Zebu crossbreed cows were milked in the presence of the calf. It took 1.97 min to bring the calf to the parlour and 1.52 min to move them to the pen for suckling. Thus, there was no difference in the amount of work under these conditions (Junqueira et al., 2005). Supposedly more labour input is needed in systems that require moving of the calf (restricted and half-day contact) (reviewed by Johnsen et al., 2016), which was also mentioned by dam rearing farmers in interviews (Ehrlich, 2003). However, the effort for the control of calf health and to sustain a good animal-human-relationship is not considered in this reflection. In farmer interviews in Germany, most farmer said, that the duration of time they spend is less or similar compared to the milk feeding system they conducted before (Ehrlich, 2003, n = 8; Dieng, 2018, n = 4). All noted that they enjoy the work in the dam rearing system more, but some added that at the same time the work is more challenging (Dieng, 2018).

5.3 Analysis of heart rate variability in cattle

Heart rate variability (HRV) has been used as an indicator of stress in cattle in several studies (e.g. Anton and Solcan, 2012; Gutmann et al., 2013; Kovács et al., 2013, 2014, 2015; Lürzel et al., 2015). The software Kubios HRV (Biosignal Analysis and Medical Imaging Group, Kuopio, Finland) is a commonly used tool, and was also applied in the present analyses (version 2.2, Chapter 3). In the following, some challenges regarding method and interpretation shall be discussed.

As recommended by von Borell et al. (2007) for frequency based HRV-parameters, in the current study HR segments lasting 5 min have been used. At the same time, a minimum of 512 data points is set as standard for analysis of frequency based HRV-parameters (von Borell et al., 2007). Cattle have a relatively low HR compared to other farm animals (Chapter 3: about 70 bpm), leading to only about 350 data points in a 5 minute-segment (70 bpm x 5 min). Interpolation of the missing values is needed (von Borell et al., 2007), which is done by the software Kubios HRV automatically. It is unclear how this is done (written communication, Mohr E., 18.12.2015). To ensure comparability with other studies, it is recommended to employ the 5 min intervals and not use for example 8 min intervals for cattle where no interpolation is needed (written communication, Mohr E., 21.12.2015). For future research, in my opinion, it would be advantageous to change standards: the length of HR segments for different species should be chosen according to their normal average HR. Thus no interpolation with an unknown method would be necessary.

Detailed instructions for frequency based HRV analyses for cattle are missing, because the software was originally developed for analysis of human HRV and detection of disease. Authors of animal welfare studies using this software often do not give detailed information which settings for interpolation rate and Fast Fourier transformation were used and why (no information: e.g. Anton and Solcan, 2012; Gutmann et al., 2013; Kovács et al., 2013, 2014, 2015; Lürzel et al., 2015; Wilk and Janczarek, 2015). It is likely that settings were often left to default (written communication, Mohr E., 18.12.2015). Therefore the current study also used default settings. Kovács et al. (2016) published information on the settings of frequency based HRV analysis. They also used the default settings. Whether this is appropriate, is unclear. It would be interesting to compare the effect of different settings on the HRV indicator values. This has been done for the different correction factors which are available in the Kubios HRV software for the analysis of HRV in horses (van Vollenhoven et al., 2016). Results indicate that there were no significant differences in the level of HR and HRV parameters, except for LFnorm, when no, low and medium correction were compared. Mean and median of HR and HRV parameters corrected with the very strong correction factor differed from all other results. In the next step of their study concerning the repeatability of HRV measurements authors chose the strong correction factor as results differed only from no and very strong correction. Further, the strong correction was able to erase 'more artefacts and background noise without dampening the variability in the RR-interval signal' (van Vollenhoven et al., 2016, p. 78).

The advantage of frequency based HRV parameters over time-based ones is that the sympathic and parasympathic nervous system regulate the HR in a frequency-dependent way. The Fast Fourier Transformation separates the HRV during frequency based analysis in different bands, which can be assigned to different physiological functions. Therefore the activity of the two branches of the autonomous nervous system is assessed directly (reviewed by Kovács et al., 2014). Time-based parameters are simple descriptive-statistical measures, which estimate but do not measure the activity of the sympathic and parasympathic nervous system directly (Kovács et al., 2014). However, some parameters of the time domain correlate with frequency or nonlinear domain HRV parameters and results can be interpreted in the same way (e.g. RMSSD with HFnorm; Hagen et al., 2005).

HRV results of the present study regarding RMSSD are similar (Gutmann et al., 2013) or higher (Hagen et al., 2005) while LF% (Gutmann et al., 2013) and LF/HF are lower (Hagen et al., 2005; Gutmann et al., 2013). These findings indicate higher parasympathic or lower sympathic activity in the cows of the current study. Due to limited labour resources, no HR and HRV measures were taken during resting of the study animals. Then it would have been possible to compare data of individuals during milking and during resting as in the study of Gygax et al. (2008). A baseline level of HR and HRV parameter could have been assessed for the individuals to answer the question if there is a greater difference between resting and milking in dams compared to non-nursing cows indicating more stress during milking or if vagal activity in dams is higher independent of the situation due to suckling. The latter would be an advantage of dam rearing.

6 GENERAL CONCLUSIONS

Dairy cows which nurse their calves, release less oxytocin during milking and hence show impaired alveolar milk ejection. However, oxytocin release and milk let-down during nursing are not disturbed (de Passillé et al., 2007). Therefore, in the current dissertation the influence of calf-associated stimuli on milking traits were tested. However, although it can be supposed that olfactory stimulation (hair of the own calf) was perceived by most of the cows, it did not enhance machine milk yield, milk flow and fat content. The same was true for acoustic (played-back calf-calls) and manual stimulation (teat massage). A tense head position and defecation during more milkings indicated, that dams were mildly stressed during milking, which may be one of the reasons for central inhibition of oxytocin release. The applied stimuli did not influence stress-associated behaviour. At the same time, some parameters of HRV led to the assumption that the vagal branch of the autonomous nervous system was more activated in dams compared to non-nursing cows. As HRV was not assessed during resting, no information on the general physiological status of dams is available. This and the impact of nursing on gut motility warrants further investigation. Providing stronger or other stimuli could also be part of future studies, e.g. using a larger amount of calf-hair, prolonging the duration of tactile teat stimulation or latency time, showing videos of suckling calves (acoustic and visual stimuli) or conditioning of nursing with other stimuli. Even, if one of the mentioned stimuli would be successfully alleviating milk ejection problems, free cow-calf-contact would be either connected with considerable milk losses or with expanded effort during milking.

Alternatively, half-day cow-calf-contact during the day have tended to increase machine milk yield during the nursing period and over the whole lactation in the third experiment of the current dissertation. A decreased fat content still pointed at impaired alveolar milk ejection. After the separation from the calf over night, machine milk yield was nearly comparable to non-nursing cows, while in the afternoon the milk yield was less than in free contact. Therefore, in extensive dairy farming, milking dams only once per day could decrease labour input in half-day systems. Among other things, the impact of reduced milking frequency on udder health and lactational milk yield needs to be further investigated. In the current studies no differences in parameters of udder health were found between cows with no, half-day or free calf-contact. Independent from contact duration, an increased protein content during the nursing period was found in dams' milk. In further studies a differenciated analysis of milk proteins may provide information about the physiological mechanisms involved.

Even though harvested milk yields in the half-day contact group tended to be higher, calves with free and half-day contact gained similar weight during the nursing and post-nursing period, suggesting comparable amounts of ingested milk. Non-nursed calves gained less weight than dam reared calves during the nursing period, but after the separation from the mother dam reared calves had a growth check. As all calves were fed with teat buckets in the three weeks between separation and weaning (10^{th} - 12^{th} wk of life), beside lower milk intake, stress due to physical separation from the mother, re-grouping, more human contact and learning to drink from the teat bucket may have been involved. Beside the mitigation of these stressors, an important issue of future research would be to improve the ingestion of solid feed in dam reared calves during the nursing period. At the end of the study, two weeks after weaning, weight gain of dam reared calves had increased, but was still below the level of control calves, body weight however was still higher in dam reared calves. A longer study period would have been necessary to evaluate whether weight gains of contact calves increased until the buffer in body weight had declined.

Compared to restricted cow-calf-contact, further advantages of half-day contact are that calves have prolonged contact to their mothers, other cows of the herd and the cow barn, which is more in compliance with the natural behaviour of cattle (e.g. Koch and Zeeb, 1969; Schloeth, 1958). Further it may positively affect herd structure and calves' cognitive and social abilities. These effects, however, need to be veryfied in future studies as well as possible impairments of welfare due to the sepration overnight in the half-day contact system.

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RELATED PUBLICATIONS AND REPORTS

In the following, publications and reports related to the research project this PhD thesis is based on are listed sorted by document type and date of publication. Publications written in italic letters were used as the basis for the PhD thesis. The reference to the related chapter is given in brackets. These publications have been composed by myself, taking into account inspiration and suggestions from all co-authors. Data collection has been done by Gabriele Kümmritz, Kerstin Hofmann, Jacqueline Felix, Martina Wetzel, Tina von Roedern, Lisa Born, Florian Thaller, and by myself. Videos were analysed by Gabriele Kümmritz, Irina Dettenrieder, Doreen Schilke and by myself. Data were analysed by myself.

Scientific papers

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