Behavioural response of dairy cows with and without calf-contact to hair of own and alien calves presented in the milking parlour

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Abstract

In systems where dairy cows are milked and additionally suckle their calves during the first months of lactation, problems with alveolar milk ejection during machine-milking occur. As olfaction is a key sense for kin recognition and acceptance at the udder, olfactory stimulation might alleviate this challenge. In this pilot study, we investigated whether cows behaviourally respond to calf hair presented in the 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 hours 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 simultaneously presented. 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. 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, we conclude 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.

Keywords: olfaction, calf rearing, maternal discrimination, young recognition, cattle

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., 2015). 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 and Cernoch, 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 Animals, materials and methods

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 hours post partum (p.p.). Seventeen multiparous cows (10 GRP, 7 GH) and six heifers (2 GRP, 4 GH) had free contact with their calves for 12 weeks p.p. ('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 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 days, mean=16, SD=3 days, 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 days of age, mean=42, SD=18 days). 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 minutes and allowed to air dry.

2.3 Experimental trials

Starting between the 12th and 20th day 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)

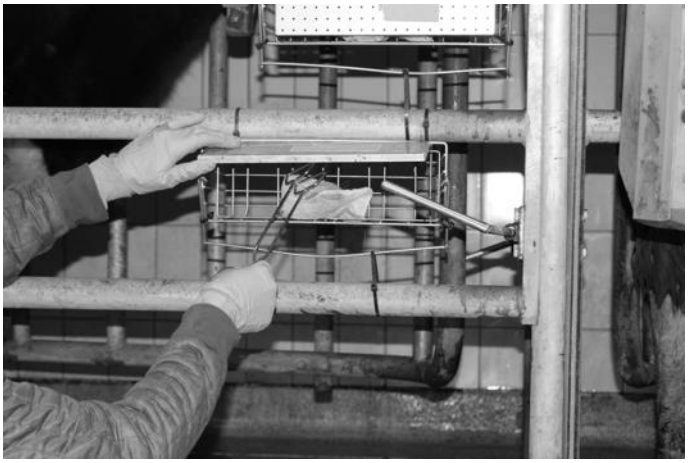

Figure 2: Placing a cloth bag with calf hair in the lower basket with tongs (Source: Kerstin Hofmann)

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, 640 x 480 pixels, Axis Communications AB, Lund, Sweden).

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 minutes, 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 seconds. 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 Table 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=0.000, n=36; licking: r=0.986, p=0.000, n=36). The observer was blind to the treatment.

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' versus 'control') and parity (primi- versus 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 $r=Z/\sqrt{N}$ (Rosenthal 1991, p. 19). Odds ratio was used in combination with Pearson Chi²-tests.

3 Results

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’) (Table 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

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) (Table 3).

The duration of responses among ‘contact’ animals differed between stimuli, being longest towards ‘own’, followed by ‘alien’ and ‘no’ (Table 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 (Table 3).

Table 3: Response duration (sniffing and licking) in % of observation time (median ± interquartile range) towards the different stimuli (‘own’, ‘alien’ or ‘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

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).

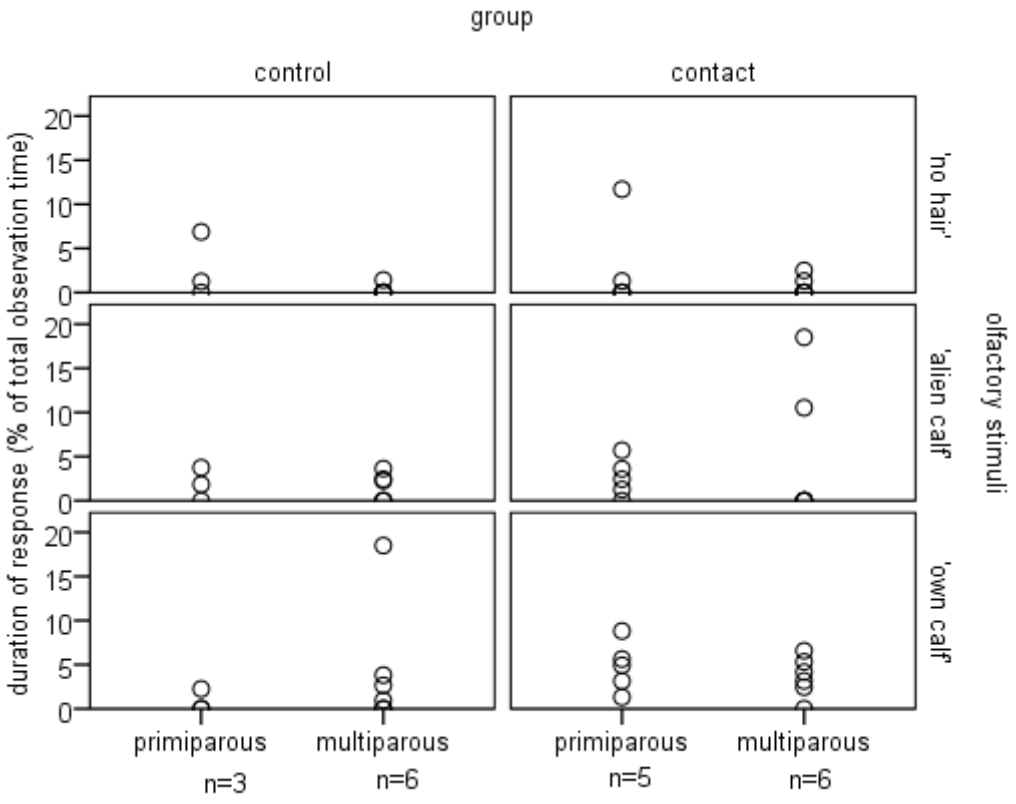

Figure 3: Relative duration of sniffing or licking of responsive cows towards an olfactory stimulus consisting of hair of the own or an alien calf or a control without hair in the parlour for primi- or multiparous cows with or without calf-contact.

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).

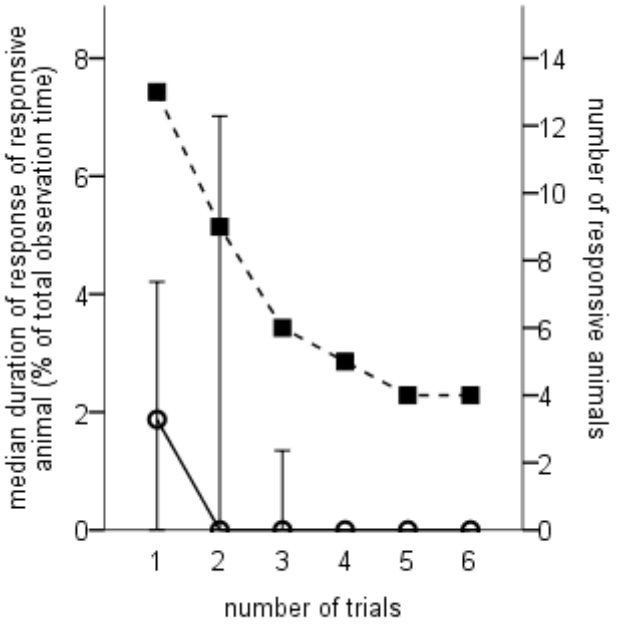

Figure 4: Responses (sniffing or licking) to calf hair (‘own’ or ’alien’) over successive trials (relative duration: ○ solid line, median and 95% confidence interval, and number of responsive animals: ■ broken line, n=20).

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 Figure 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: 1st, 2nd, >2nd 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 & 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 days, mean=42, SD=18 days) than ‘own’ samples (10-21 days, mean=16, SD=3 days) 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.

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.

6 Acknowledgments

We express our thanks to Gabriele Kümmritz, Kerstin Hofmann, Jacqueline Felix and all persons who helped us in the cow dairy and laboratory of the Thünen Institute in Trenthorst, and to Christina von Roedern who helped with the English. We are also grateful to the University of Kassel for providing a dissertation scholarship.

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