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Milking of Brown Swiss and Austrian Simmental cows in a herringbone parlour or an automatic milking unit

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Abstract

Automatic milking systems (AMS) are becoming increasingly common on dairy farms and should be evaluated for their effect on cow welfare. Dairy cows' stress responses during normal successful milking in a 2 × 6 herringbone milking parlour (HMP) were compared with their responses during normal successful voluntary milking in an automatic unit (AMU). We observed 42 cows: 12 Simmental and 11 Brown Swiss in the AMU, and 10 Simmental and 9 Brown Swiss in the HMP. Both prior to and during the observations each cow was milked in one system only. Behaviour during milking and durations of different phases of the milking procedure were observed directly two to six times per cow. Heart rate was recorded telemetrically. Samples of composite milk were analysed for cortisol using an enzyme-immunoassay. Milking lasted longer in the HMP than in the AMU (general mixed model: $F_{1,39} = 12.06$, $P = 0.0013$), after significant effects of milk yield, day of lactation and time of day had been taken into account. Location of the teats by the robot took longer in Simmental than in Brown Swiss cows (Mann–Whitney-U-test: $U_{11,12} = 32$, $P = 0.037$). Kicking and stepping with the hind legs was less frequent in the AMU than in the HMP ($U_{23,19} = 76.5$ for kicks; $U_{23,19} = 85$ for steps; $P < 0.001$ in both cases). Brown Swiss cows stepped less than Simmental cows in the AMU ($U_{11,12} = 32$, $P = 0.036$). In the HMP, kicks occurred more frequently during the time period from the beginning of udder cleaning until cluster attachment was completed, than at other times (Friedman test: $F_{19,4} = 32.41$, $P < 0.001$; Bonferroni-corrected post-hoc-comparisons: $P < 0.01$). There was no difference between groups in heart rate during milking. The AMS group had higher milk cortisol values than the HMP group ($U_{23,19} = 130.5$, $P = 0.026$). This was probably not related to milking itself, but to other aspects

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of the system, and absolute values were not very high. In conclusion, Simmental cows may be more vulnerable during milking than Brown Swiss. Limiting the study to successful voluntary milking events without AMU attachment failure, there were no indications of more stress in the AMU than in the HMP.

Keywords: Dairy cattle; Automatic milking; Robotic milking; Voluntary milking; Welfare; Stress; Heart rate; Milk cortisol; Behaviour

1. Introduction

Automatic milking systems (AMS), consisting of one or more automatic milking units (AMU) in a loose-housing system, have become increasingly common in recent years. The milking process as well as the management in AMS differ from conventional systems and may influence the health and welfare of cows in various ways. For example, the daily rhythm and herd synchronisation is influenced as cows in the AMS get milked singly at any time of day. Depending on the type of cow traffic, the cows' access to resources may be limited in the AMS. The herdsman's traditional opportunity for screening each cow's state during milking is not given in the AMS, and it has to be replaced by active health monitoring of the cows in the barn. High yielding cows are milked more often in the AMS and may thus experience less udder pressure. Whereas cows in milking parlours are driven to the parlour and interact with other cows and humans before and during milking, cows in AMS may interact with others before, but not during milking, and humans are not involved in the milking process. The details of udder cleaning, teat cup attachment, milk removal and teat cup detachment are different, both in terms of the mechanics and in terms of the timing. In this paper, we address the details of the milking process and its effects on cows' stress responses.

Previous studies of dairy cows' stress responses during milking in AMU have yielded ambiguous results. Hopster et al. (2002) compared physiological and behavioural indicators of stress in Holstein cows milked in an AMU or in a tandem milking parlour. They observed behaviour and heart rate, as well as plasma levels of adrenaline, noradrenaline, oxytocin and cortisol during milking, and concluded that the cows were not adversely affected by being milked in the AMU. However, Wenzel et al. (1999) measured behaviour, heart rate and milk cortisol in Holstein and German Simmental cows, and concluded that cows were more stressed during milking in an AMU than in a tandem milking parlour.

Some of these discrepancies probably reflect methodological and interpretational differences, while others may be caused by differences in management, milking system and cows. We aimed to further elucidate stress responses during milking in an AMU compared to a herringbone milking parlour (HMP). One important factor in the response to milking systems may be breed. As AMS are now being introduced in Austria and Switzerland where Brown Swiss and Austrian Simmental are common, we chose to investigate the responses of these breeds specifically. We consider it important to distinguish between the process of normal successful milking and problems that may be caused by failed teat cup attachment and delayed milking. Here, we focus on the comparison of successful, undisturbed milking.

Several non-invasive stress parameters were investigated simultaneously to ensure a broad basis for interpretation: behaviour, heart rate and milk cortisol.

2. Animals, materials and methods

2.1. Animals, milking systems and housing

The study was conducted in November and December 2001 as part of a larger research project on robotic milking at the *Landwirtschaftliche Bundesversuchswirtschaften GmbH* in Wieselburg, Austria. Two herds that consisted of 30 cows (15 Austrian Simmental and 15 Brown Swiss) were matched for lactation, milk yield and pregnancy, and kept in separate loose-housing pens in the same uninsulated building. The AMS group was milked in a single-box AMU Lely Astronaut Series 30 (Lely Industries NV, Maasland, The Netherlands) with partially-forced cow traffic. The other group was milked twice daily in a 2 × 6 herringbone milking parlour with a Happel milking system (System Happel GmbH, Friesenried, Germany). Both systems had been in operation for approximately 1 year at the time of the study. Each individual cow had experience of one system only and was kept in this system throughout.

The cows in the AMS group had access to the AMU around the clock, apart from when it was in cleaning cycles. Twice a day, around 8:00 and 18:00 h, cows that had at that time not been milked for more than 14 h were herded into the robot for milking. The AMU was equipped with silicon liners, the pulsation rate was 60 cycles/min with a 65:35 pulsation ratio, and the milking vacuum was set at 42 kPa. Teat cups were automatically detached separately depending on the milk flow of each quarter. Although the system was equipped with an electrical movement inductor to make cows leave the AMU after milking, this inductor was not in use during the study. The cows in the HMP group were milked in the mornings between 5:00 and 6:30 h and in the evenings between 15:30 and 17:00 h. The Happel milking system was equipped with acrylonitrile butadiene rubber liners, and it applied the special pulsation technique S90 including the Robotex® automatic stripping system as specified by the manufacturer (for details, see http://www.happel-system.de). Detachment was automatic for the entire cluster depending on total milk flow.

The herds were kept socially stable, and dry cows were left in the group. Cows were primiparous or in second lactation, and they were at evenly distributed stages of lactation and pregnancy. A total of 42 lactating cows were included in the present study: 12 Simmental and 11 Brown Swiss in the AMS group, 10 Simmental and 9 Brown Swiss in the HMP group.

The loose-housing pens (Fig. 1) had slatted floors, and each pen had 30 cubicle boxes with rubber mats and small amounts of straw. Each of the pens was equipped with two water troughs, one scratching/rubbing brush, two concentrate feeding stations (the AMU containing one of them in the AMS group), and a separate roughage feeding place per animal (The Calan Broadbent Feeding System, American Calan Inc., Northwood, USA). A mixed ratio was fed ad libitum once daily, and 1 kg daily of additional concentrate per cow was given to attract the cows in the AMS group to the AMU. These concentrates were given during milking in the AMS group (the concentrate feeding station in the barn was not

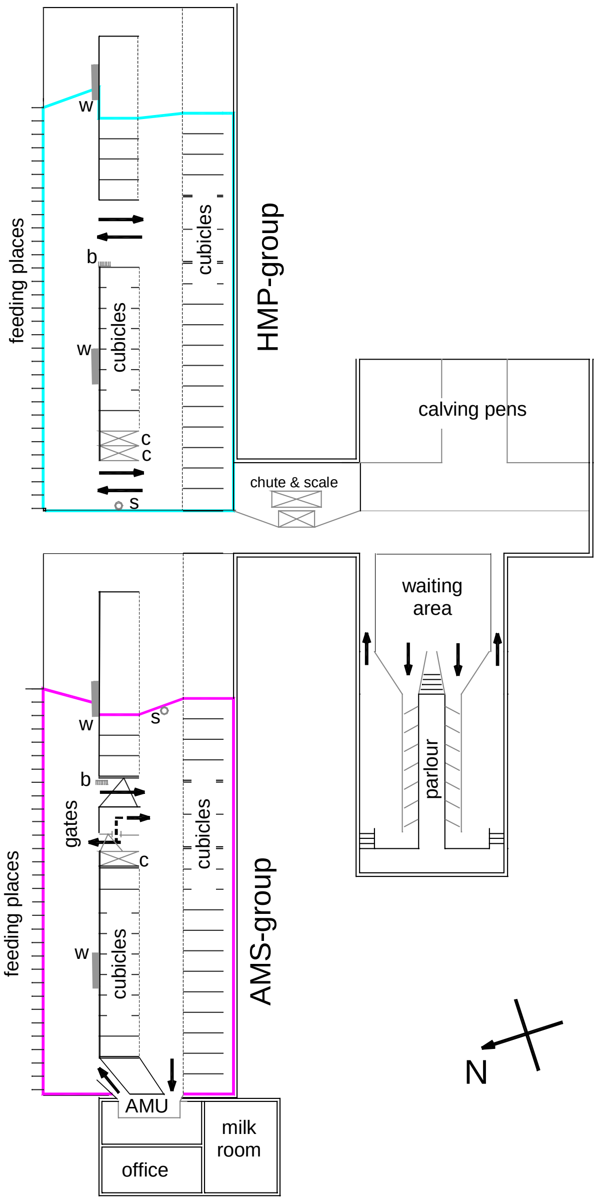


Fig. 1. Ground map of the parts of the barn used in the study. Arrows denote possible cow traffic. At the selection gate in the AMS group, cows that had recently been milked according to the AMU computer programme could pass from the lying area to the feeding area, whereas cows that had to be milked were re-directed back into the lying area if they attempted to pass through (partially-forced cow traffic). Abbreviations: b, brush; c, concentrate feeding station; s, salt lick; w, water trough.

used during this part of the experiment), and in the concentrate feeders in the barn in the HMP group.

From 4:00 to 20:00 h the barn was fully lit by artificial light and/or natural daylight, whereas at night the light was dimmed to about 5 lx. The mean air temperature in the barn during the study was 3.9 °C (minimum −3.7 °C, maximum 15.9 °C), the mean air humidity was 84.7% (minimum 48.7%, maximum 95.3%), both measured throughout in 30 min intervals using a permanently installed data logger (HotDog, Elpro-BuchsAG, Switzerland).

2.2. Observations and data

All feeding, milking and handling of the animals that was not directly related to the experiments was carried out by the regular farm workers. The cows were in addition handled and observed by one team of three experimenters to whom the cows were accustomed. In both groups, data were recorded during normal milking. This means that we did not herd cows into the AMU or influence their order of entry into it. When such procedures were carried out, the data were excluded from analysis. Equally, milkings with teat cup attachment failure, i.e. when milking failed because the robotic arm did not succeed in attaching all teat cups, were excluded.

We simultaneously recorded the timing of the milking process and the behaviour, heart rate (HR) and milk cortisol for individual cows during one to six milkings per cow in both groups. In addition, production data such as milk yield were automatically measured by both systems. In the AMS group, we fitted HR monitors on cows and waited for them to be milked in the AMU, where they were observed and a milk sample was taken automatically. In the HMP group, we fitted cows with HR equipment prior to the morning and afternoon milking sessions. Up to five of these cows could be observed per milking session, and milk samples were obtained from them.

The milking process is not similar in the two systems. For comparison, the functional phases of milking were defined (Table 1) and observed directly using the Observer software (Noldus Information Technologies, Wageningen, The Netherlands) on handheld computers (Husky Computers Ltd., Coventry, England). In the AMU, observers took turns to observe cows from the adjacent office, thereby being largely out of view of the animals. In the HMP, direct observations were carried out by two observers standing in the parlour. Each observer concentrated on one focal animal at a time.

During the direct observations, observers also recorded any special events, problems with the milking machine, interactions between the farm workers and the cows, and the cows' behaviour: steps and kicks, as well as incidents of urination, defaecation and vocalisation. A step was defined as lifting of a leg without the tip of the claw passing the other leg's dew claw, and a kick was defined as a higher lift with a potential change of direction. The cows were videotaped at most times by CCTV cameras mounted to the roof of the barn and milking parlour, to validate and illustrate the directly observed data, and for synchronisation of the internal clocks of the handheld computers and the HR monitors.

Heart rate was recorded with a telemetric system (horse trainer transmitters and S810 monitors from Polar Electro, Helsinki, Finland) that recorded interbeat intervals. The transmitters were attached to a horse girth and fitted to cows as described by Hopster and Blokhuis (1994). The cows were not shorn or shaved where the electrodes were placed, but ample

Table 1
Definition of the functional phases during milking in the two systems

Phase		Description
In AMU	In HMP	
Before	Before	In both groups: idle time after entrance, i.e. from entry into the AMU or HMP, until first contact with the udder
	Wet	In the HMP: from spraying of the udder with water until first manual contact with the udder for cleaning or manual milk ejection
Clean		In the AMU: during cleaning of the teats, system-defined at 40 s
Locate		In the AMU: after cleaning, until a cup is attached to first teat
Attach		In the AMU: from attachment of cup to the first teat until all cups have been attached
	Clean-attach	In the HMP: from the first touch to the udder for cleaning or manual milk ejection, including a break, until all teat cups have been attached (corresponds to the three phases clean, locate and attach in the AMU)
Main	Main	In both groups: while all teat cups are attached and four teats are being milked simultaneously without Robotex®
	Last	In the HMP: while all teat cups are attached but milk flow has got below 800 g/min and the machine applies the automatic stripping device Robotex®
Part		In the AMU: from the first teat cup is detached because of low milk flow, until all teat cups have been taken off
After	After	In both groups: idle time before exit, i.e. from when the entire cluster has been detached, until the brisket-bar (HMP) or front gate (AMU) opens
Out	Out	In both groups: from the opening of the brisket-bar or front gate until the cow has gone out

AMU, automatic milking unit; HMP, herringbone milking parlour.

electrode gel was used to ensure good contact, and an extra elastic girth was used to keep the transmitters in place. Prior to the study, cows were accustomed to this procedure, and the belts were applied at least 10 min before milking to ensure that measurements were not influenced. Heart rate was averaged over the milking duration.

Cortisol was measured in milk in order to avoid invasive sampling. In the AMU, the “shuttle” (Lely Industries NV, Maasland, The Netherlands) was used for automatically taking samples of composite milk from most milkings, including those that were not observed, between 5:00 and 17:00 h on observation days. The extra samples made it possible to take into account that the circadian cortisol secretion rhythm in cattle (Thun, 1987) could lead to differences between samples taken at different times of day. In the AMU, a total of 21 samples were taken in the mornings between 5:00 and 8:00 h, 145 samples were taken later during the day before 15:00 h, and 58 samples were taken between 15:00 and 18:00 h. In the milking parlour, measuring cups attached to the milking machine were used to obtain samples of composite milk from those cows whose behaviour was being observed, a total of 40 samples from morning milking and 41 samples from afternoon milking.

From each milk sample, 5 ml were frozen at −22 °C within one hour. Samples were later thawed and centrifuged, 500 μl skimmed milk per sample were extracted with 5 ml diethyl-ether and dissolved in 0.5 assay puffer. Thereafter, cortisol concentration was determined using an enzyme-immunoassay (Palme and Möstl, 1997). The intra- and interassay coefficients of variation were 8.9 and 11.1%, respectively.

Apart from the extra milk samples to test for effects of time of day on milk cortisol, data sets that were not complete, i.e. when heart rate, behaviour or milk cortisol data were missing, were excluded from analysis. When the distribution of the data was normal or could be normalised with common transformations, the data were analysed with general mixed linear models (GLMM, Pinheiro and Bates, 2000). Pseudo-replication was avoided by including the identity of cows as a random factor in the model, thereby achieving the appropriate degrees of freedom. Different terms were included in the analyses of the various dependent variables, but all models were derived from the following general structure:

$$\begin{aligned} y = C + (G_i + R_j + M_{\mathrm{cov}} + L_k + Lday_{\mathrm{cov}} + T_{\mathrm{m}} + Tday_{\mathrm{cov}} \\ + Z_{\mathrm{cov}} + A_{\mathrm{cov}} + P_{\mathrm{cov}})^2 + cow_n + error \end{aligned}$$

where y is the dependent variable, e.g. the duration of a milking phase or heart rate. In some cases the variable was square-root or ln-transformed; C is a constant; $(\cdot)^2$ represents that the factors and covariates in brackets were also analysed for possible effects of first degree interactions; G is the treatment group (AMS group ($i = 1$), HMP group ($i = 2$)); R the breed (Simmental ($j = 1$), Brown Swiss ($j = 2$)); M the milk yield at a particular milking (covariate, minimum = 4.5 kg, mean = 10.4 kg, maximum = 19.9 kg): alternatively daily milk yield, i.e. average milk yield of a cow during the experimental period (covariate, minimum = 7.0 kg, mean = 21.6 kg, maximum = 38.0 kg); L the lactation (first ($k = 1$), second or later ($k = 2$)); $Lday$ the day of lactation (covariate, minimum = 8, mean = 209, maximum = 456); T the pregnancy (pregnant ($m = 1$), not pregnant ($m = 2$)); $Tday$ the day of pregnancy (covariate, minimum = 4, mean = 103, maximum = 250); Z the time of day (categorised to entire hours, covariate, minimum = 5:00 h, maximum = 18:00 h); A the average number of milkings per day of a cow during the experimental period (covariate, minimum = 1.0, mean = 2.3, maximum = 3.8; only in the AMS group, constant = 2 in the HMP group); P the position of the cow in the milking parlour (categorical, minimum = 1, maximum = 6); cow the cow (random factor within the treatment groups; $1, 2, \ldots, n = 23$ in the AMS group, $1, 2, \ldots, n = 19$ in the HMP group; 1–6 observations per cow) and $error$ the unknown random error.

The residuals of the models were inspected graphically and tested for deviation from the normal distribution with the Shapiro–Wilk test. All calculations of models were carried out in the statistics environment R (Ihaka and Gentleman, 1996) using restricted maximum likelihood methods. Presentation of results is based on the ANOVA output of the reduced models. When individual differences contributed significantly to the variation in the GLMM, this effect was confirmed by Pearson's correlation of first and second measurements.

Non-parametric tests based on Siegel and Castellan (1988) were used where appropriate, averaging the repeated measures for each individual cow so that the sample size equalled the number of cows that were observed. These averages were also used for graphical presentation of results in boxplots.

3. Results

The treatment groups did not differ with regard to mean daily milk yield during the observation period (individual total milk yield divided by number of days; group mean ± S.D. $= 22.09 \pm 7.24$ kg, $N = 23$ in AMS group; 21.50 ± 6.07 kg, $N = 19$ in HMP group). The daily milk yield was largely explained by the day of lactation in both groups ($r = -0.75$, $N = 23$, $P < 0.001$ in AMS group; $r = -0.88$, $N = 19$, $P < 0.001$ in HMP group). In the AMS, cows were milked more than twice, but less than three times a day ($N = 23$, mean ± S.D. $= 2.26 \pm 0.55$, minimum 1.00, maximum 3.80; one-sided Wilcoxon signed-ranks test: $V_{23} = 99.5$, $P = 0.013$ for difference from 2; $V_{23} = 12$, $P < 0.001$ for difference from 3). Brown Swiss cows were milked more often than Simmental (Mann–Whitney-*U*-test: $U_{11,12} = 24.5$, $P = 0.0089$). Three cows (all Simmental in early lactation) contributed strongly to this effect as they had frequent milking failures, which they compensated with increased yield per milking. Exclusion of these special cases revealed positive relationships in the rest of the group between daily milk yield and number of milkings ($r = 0.90$, $N = 20$, $P < 0.001$), as well as daily milk yield and yield per milking ($r = 0.56$, $N = 20$, $P = 0.011$).

Cows spent on average 510 s in the AMU (minimum 297, maximum 1037), and 990 s in the HMP (minimum 586, maximum 1471). Phases that allow direct comparison took significantly longer in the HMP group (Table 2). For the phases that included milk removal, the effect of milk yield at the particular milkings was accounted for in the analysis. When combined durations of the phases *attach*, *main*, *part* and *last* were compared instead of the main milking phase *main* only, the durations were always longer in the HMP. The phase *locate* in the AMS group took longer for Simmental than for Brown Swiss cows, and in the HMP group, the automatic stripping phase *last* took longer in Simmental than in Brown Swiss cows (Table 2). The random factor of individual identity of the cows contributed significantly to the variation in durations that were related to milk removal. Correspondingly, the duration of *main* was correlated between first and second measures taken of individuals in both groups ($r = 0.85$, $N = 23$, $P < 0.001$ in AMS group; $r = 0.68$, $N = 19$, $P < 0.001$ in HMP group). The duration of *part* was correlated between first and second measures in the AMU ($r = 0.71$, $N = 23$, $P < 0.001$). The durations of *last* was correlated between first and second measures in the HMP ($r = 0.85$, $N = 19$, $P < 0.001$).

Overall, steps and kicks were more frequent in the HMP than in the AMU (Table 3). Steps with the hind legs were more frequent in Simmental than in Brown Swiss cows (Fig. 2). The relationship between the milking process and frequencies of steps and kicks is illustrated in Figs. 3 and 4. Only hind leg frequencies are shown; inclusion of front leg frequencies led to very similar results. Frequencies of stepping and kicking with the hind legs were not correlated with each other in either group. Cows in the HMP group kicked more in the phase clean-attach than at other times. Individual differences were reflected in correlations between first and second measurements of frequencies of stepping ($r = 0.78$, $N = 42$, $P < 0.001$) and kicking ($r = 0.56$, $N = 42$, $P < 0.001$).

We did not observe vocalisations during milking in either of the groups. Defaecation and urination were observed rarely: in the AMS group, in 82 cases of observed milking no urination was seen, and one cow defaecated once. In the HMP group, in 81 observed milkings, urination was seen six times and defaecation once.

Table 2
Analysis of the durations of the functional phases of the milking procedure

Phase	Duration (s) (mean ± S.D.)		Analysis (distribution; GLMM; Mann–Whitney-*U*-test)
	AMU ($N = 82$)	HMP ($N = 81$)	
Before	17 ± 15	77 ± 54	Poisson distribution; AMU: not normalised; HMP: normalised by ln-transformation, no effects in GLMM; treatment groups differed: $U_{23,19} = 3$, $P < 0.001$
Wet		64 ± 34	Distribution not normalised, no effects
Clean	36 ± 7		Normal distribution, no effects
Locate	41 ± 23		Bimodal distribution, not normalised; longer in Simmental than in Brown Swiss cows: $U_{11,12} = 32$, $P = 0.037$
Attach	31 ± 31		Normal distribution after ln-transformation and exclusion of one extreme value; longer the more milkings per day ($F_{1,21} = 9.52$, $P = 0.0056$) and the earlier in the day ($F_{1,57} = 4.95$, $P = 0.030$)
Clean-attach		77 ± 51	Normal distribution after ln-transformation; trend for longer duration the further in the front of the milking parlour cows stood ($F_{1,60} = 6.30$, $P = 0.056$)
Main	246 ± 106	380 ± 167	Normal distribution after exclusion of four extreme values; longer in HMP ($F_{1,39} = 12.06$, $P = 0.0013$) and longer the more milkings per day ($F_{1,39} = 4.91$, $P = 0.032$), longer the higher yield at milking ($F_{1,115} = 124.28$, $P < 0.0001$) and longer the earlier in the day ($F_{1,115} = 12.74$, $P < 0.001$)
Last		114 ± 145	Normal distribution after ln-transformation and exclusion of extreme values in one cow; longer the lower yield at milking ($F_{1,58} = 4.16$, $P = 0.046$), and longer in Simmental cows ($F_{1,16} = 5.91$, $P = 0.027$)
Part	78 ± 72		Normal distribution after square-root-transformation; longer the higher yield at milking ($F_{1,58} = 9.45$, $P = 0.0032$), and a trend for longer in Simmental cows ($F_{1,21} = 3.88$, $P = 0.062$)
After	10 ± 2	266 ± 203	Bimodal; normal distribution in the groups separately, no effects in GLMM; treatment groups differed: $U_{23,19} = 0$, $P < 0.001$
Out	43 ± 65	15 ± 10	Bimodal distribution; longer in the AMU; normalised after ln-transformation in separate groups; AMU: longer, the later in the day ($F_{1,57} = 5.97$, $P = 0.018$) and trend for longer the earlier in the lactation ($F_{1,57} = 2.98$, $P = 0.090$); HMP: no effects
Total	510 ± 147	919 ± 143	

AMU, automatic milking unit; HMP, herringbone milking parlour; GLMM, general linear mixed model, with *F*-statistic when normal distribution was obtained directly or after appropriate transformation.

No effects of treatment group or breed on mean heart rate during milking were identified (Fig. 5). Heart rate was not related to behaviour during milking, but it was higher earlier in the day than later (random covariate *time of day* in the GLMM: $F_{1,116} = 16.07$, $P < 0.001$). Individuals' heart rates during first and second measurements were correlated ($r = 0.45$, $N = 42$, $P < 0.01$).

The AMS group had significantly higher milk cortisol values than the HMP group (Mann–Whitney-*U*-test: $U_{23,19} = 130.5$, $P = 0.026$). There were no breed differences. Milk cortisol was not correlated with heart rate, and not related to day of lactation or to day of pregnancy, nor to time of day: concentrations in the morning or afternoon did not

Table 3
Steps and kicks per minute in the two treatment groups

Behaviour	Mean number/minimum ± S.D.		Mann–Whitney test statistic, *U*	*P*-value
	AMS (*N* = 23)	MP (*N* = 19)		
Kicks				
With front legs	0.013 ± 0.024	0.10 ± 0.25	125.5	0.0092
With hind legs	0.071 ± 0.10	0.29 ± 0.25	76.5	0.00017
Steps				
With front legs	0.67 ± 0.54	1.13 ± 1.13	146.5	0.069
With hind legs	1.01 ± 1.14	2.22 ± 1.60	85	0.00047

differ either in the AMS group ($N = 15$, mean ± S.D. $= 0.74 \pm 0.57$ nmol/l in the morning; 0.67 ± 0.34 nmol/l in the afternoon, Wilcoxon signed-ranks test: $V = 69$, $P = 0.64$) or in the HMP group ($N = 19$, mean ± S.D. $= 0.47 \pm 0.26$ nmol/l in the morning; 0.43 ± 0.28 nmol/l in the afternoon, Wilcoxon signed-ranks test: $V = 123.5$, $P = 0.26$). Individuals' milk cortisol levels in the first measurement were not correlated with subsequent measures. However, subsequent measures were correlated among each other (r varied from 0.52 to 0.77 in six correlations, N varied from 34 to 14, $P < 0.05$ in all cases).

Heart rate, milk cortisol and movements during milking were not related to each other in either group.

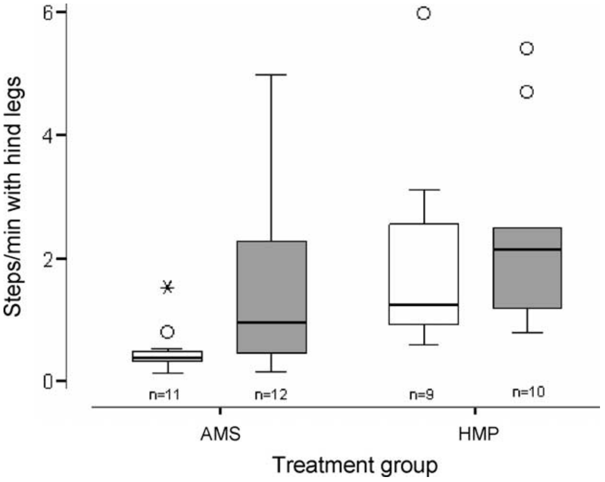
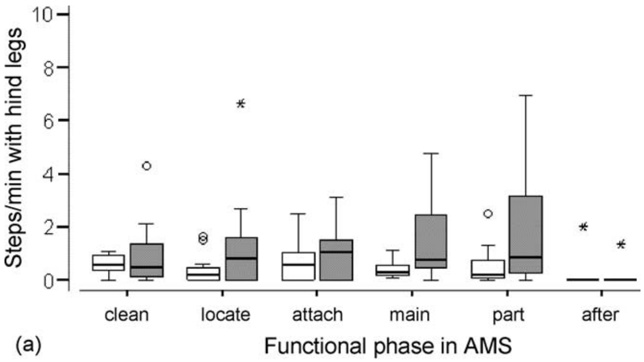


Fig. 2. Frequency of steps with the hind legs in the two groups in relation to breed. In the AMS group the breeds (open boxes, Brown Swiss; grey boxes, Simmental) differ (Mann–Whitney-*U*-test: $U_{11,12} = 32$, $P = 0.036$), in the HMP group they do not ($U_{9,10} = 36$, $P = 0.50$). The difference between treatment groups is statistically significant overall (Table 2) and within Brown Swiss cows ($U_{11,9} = 7$, $P < 0.001$), but not within Simmental cows ($U_{12,10} = 35$, $P = 0.11$). Data are given as box-whisker plots with medians (lines in the boxes), 25 and 75% quartiles (boxes), 10 and 90% ranges (whiskers), outliers (dots) and extremes (stars).



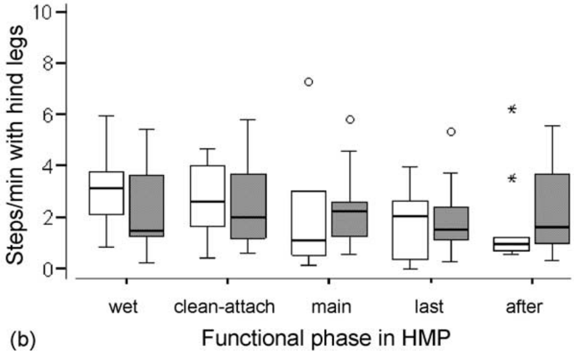


Fig. 3. Frequency of steps with the hind legs in the two groups in the different functional phases of milking, for Brown Swiss (open boxes, $N = 11$ in AMS, $N = 9$ in HMP) and Simmental cows (grey boxes, $N = 12$ in AMS, $N = 10$ in HMP). Data are given as box-whisker plots (for a description, see legend of Fig. 2). (a) In the AMS group, phases differed significantly within each of the breeds (Friedman test: Brown Swiss: $F_{11,5} = 12.04$, $P = 0.034$; Simmental: $F_{12,5} = 15.11$, $P = 0.0099$). Post-hoc comparisons showed that there was less stepping in the phase *after* than in each of the others ($P < 0.01$). (b) In the HMP group, stepping did not differ within phases in the Brown Swiss cows. In the Simmental cows, phases differed overall ($F_{10,4} = 13.05$, $P = 0.011$), but not in post-hoc comparisons.

4. Discussion

None of the potential stress indicators that were measured simultaneously were correlated. This is a common finding (Ramos and Mormède, 1998) and underlines the importance of measuring multiple parameters. Van Reenen et al. (2002) equally did not find correlations between measures of cortisol release, heart rate response and behavioural responses during milking. They found individual differences in these measures in primiparous heifers at the beginning of lactation, and our results indicate individual differences for cows at different stages of lactation.

The average daily number of milkings per animal (2.26) in the AMS was low compared to an average of three milkings per day advertised by the producers. However, this is a common finding: Neuhaus et al. (1998) observed milking frequencies similar to ours, Thune (2000) reported an average of 2.56 daily milkings with forced cow traffic and 2.39

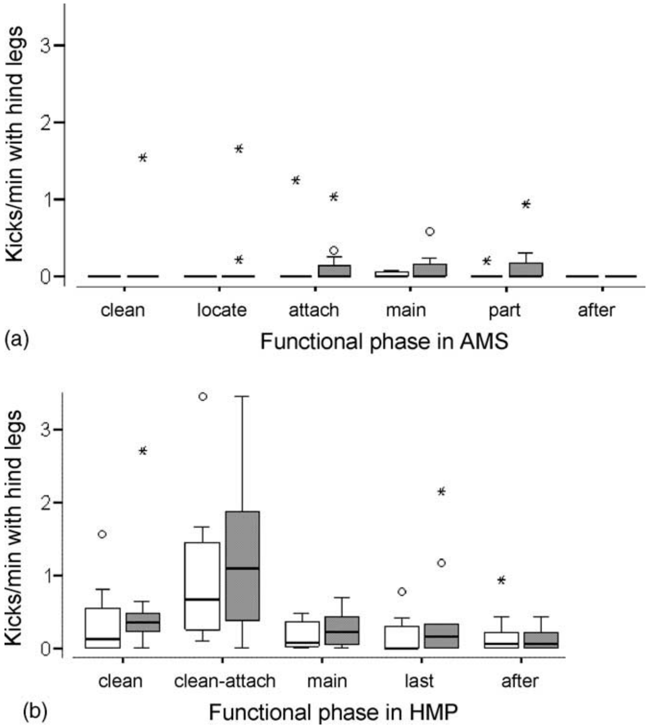


Fig. 4. Frequency of kicks with the hind legs in the two groups in the different functional phases of milking, for Brown Swiss (open boxes, $N = 11$ in AMS, $N = 9$ in HMP) and Simmental cows (grey boxes, $N = 12$ in AMS, $N = 10$ in HMP). Data are given as box-whisker plots (for a description, see legend of Fig. 2). (a) In the AMS group, phases did not differ within Simmental cows, and although they differed overall in Brown Swiss (Friedman test: $F_{11,5} = 16.14$, $P = 0.0064$), post-hoc tests did not reveal significant pairwise differences. (b) In the HMP group, phases differed within both breeds (Brown Swiss: $F_{9,4} = 16.58$, $P = 0.0028$; Simmental: $F_{10,4} = 18.33$, $P = 0.0011$): the phase *clean-attach* differed clearly from the subsequent phases ($P < 0.01$) and tended to differ from the phase *wet* ($P = 0.092$).

with partially-forced cow traffic, and in a study by Harms et al. (2002), cows were milked 2.63 times daily in forced cow traffic and 2.56 times daily in partially-forced cow traffic. In a study by Hopster et al. (2002), AMS cows were milked three times a day, but they were all in the beginning of lactation.

Like Neuhaus et al. (1998), we found a positive correlation of higher daily milk yield with higher milk yield per milking, as well as with higher number of milkings. In other words, cows with more milk were milked more often and also gave more milk per milking. The interactions between milk yield, number of milkings, feeding regime, AMS system and cow traffic are complex (Wiktorsson et al., 2003).

The teat location and attachment times that we found were comparable to those found by Huschke and Klimetschek (2000). In a study by Wenzel (1999, p. 39), also with a Lely Astronaut, the time from recognition of a cow until all teat cups were attached was only 40 s. The AMU must in that case have been programmed for a short udder cleaning period,

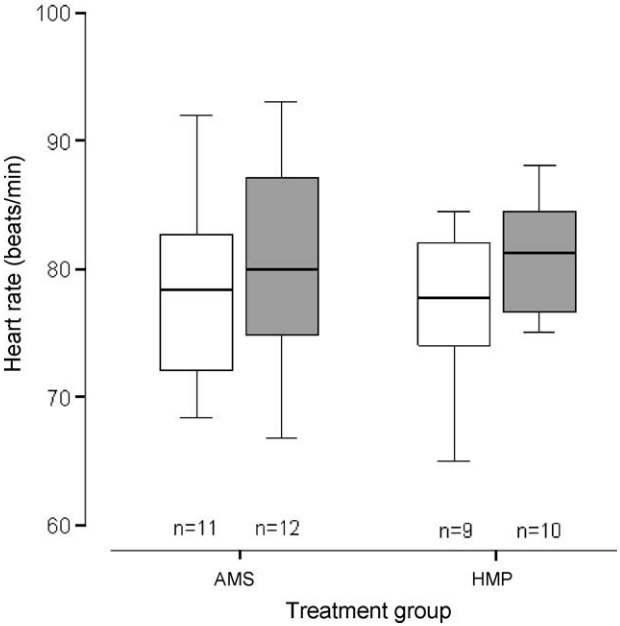


Fig. 5. Heart rate (beats/min) in the two treatment groups by breed (open boxes, Brown Swiss; grey boxes, Simmental), based on individual cows' means. Data are given as box-whisker plots (for a description, see legend of Fig. 2).

which probably resulted in insufficient udder and teat preparation. Given these differences in cleaning, the average time spent in the AMU in Wenzel's study (7 min 16 s) is comparable with our findings (8 min 30 s). Hopster et al. (2002) measured durations of milking phases in Holstein cows in a Lely Astronaut AMU similar to ours, and the average time spent in the AMU was 9 min 26 s. The teat location/attachment phases and the main milking phase in the AMU had similar durations to those reported here. However, the detachment of teat cups (phase *part* in our study) took more than double as long in Hopster et al.'s study.

The substantially longer waiting times that we found for the cows in the HMP before and after milking is a trivial effect explained by the fact that the AMU handles only one cow at a time, whereas in the HMP each side of the parlour has room for six cows that have to enter and leave together. While the time from touching the udder until the cluster was completely attached was comparable in the two systems, milk removal itself took longer in the HMP than in the AMU. This result was not explained by differences in yield, as the cows in the two groups were matched in that respect, and the influence of the yield per milking was taken into account in the analysis. Post-hoc inspection of maximal and average milk flow data stored in the systems' computers confirmed our suspicion, that milk flow was less in the HMP than in the AMU (K. Luger, personal communication). In the original allocation to groups, cows had been matched for milkability. One reason for less milk flow in the HMP could be that cows in the AMS, but not in the HMP, were fed concentrates during milking, which can increase oxytocin secretion (Svennersten et al., 1995). We also suspect that there may have been some degree of stress in the HMP resulting from the design of the herringbone system. It was equipped with a prototype brisketbar for exiting, which appeared to have the disadvantage of not helping to place the cows appropriately in relation to each other. It also meant that cows that had left the stall sometimes remained in close vicinity of the cows that were milked after them. It may also generally be stressful to cows to stand uncomfortably close to each other during milking in herringbone parlours.

The reduced milk flow could be seen in relation to the finding that cows in the HMP kicked and stepped more than those in the AMU, which may indicate more discomfort or aversion. However, interpretation of the behavioural responses of cows to potentially aversive stimuli is not straightforward. Rushen et al. (1999) found that cows that distinguished between an aversive and a gentle handler moved more when the aversive handler was present than when the gentle handler was present. On the other hand, Munksgaard et al. (2001) reported that tied dairy cows moved their legs less often in the presence of an aversive handler, from whom they kept a greater distance. Bremner (1997) suggested that low activity levels are seen in cows with a very high as well as a very low level of fear, and this suggestion has recently been supported with regard to behavioural responses during milking (Van Reenen et al., 2002). The level of aversiveness, individual differences between cows and the specific situation seem to interact in determining the stepping and kicking responses of cows.

In our study, kicking and stepping were not directly related, although both occurred more frequently in the HMP. It may thus be useful to separate the two. The term “flinch, step and kick response” (FSK) was first coined by Willis (1983) to describe a typical series of responses that occurs in apparently nervous cows during milking. Hemsworth et al. (1987) reported that cows that were more habituated to human presence showed less FSK during milking. Hemsworth et al. (1989) subsequently distinguished between FSK and “flinch and step responses” (FS), as they found evidence that higher frequencies of FSK were positively related to parameters indicating fear of humans, but no such evidence was found for FS.

The absence of humans in the AMU may explain why cows in our study kicked less in the AMU than in the HMP. This interpretation is supported by the fact that kicking in the HMP was mainly observed during cleaning and attachment, when contact with humans is most intense and might cause discomfort. This also corresponds to the observation that cows in herringbone parlours showed about 50% of the stepping and kicking related to human interaction during udder preparation before attachment (Waiblinger, unpublished data from 30 farms). The frequencies of kicking in our study did not correspond to those reported by Wenzel et al. (1999), who observed comparable or lower frequencies in the parlour, but much higher frequencies in the AMU.

Stepping is probably not so much a sign of fear, but rather a sign of discomfort or restlessness. It has also been suggested that cows step or shuffle more in relation to being fed or expecting food during milking (Prescott et al., 1998). This cannot explain our results, as the cows in the AMU were fed but stepped less. Like us, Hopster et al. (2002) also found less stepping during attachment in the AMU than in the parlour. However, they found more frequent stepping in the AMU during detachment. In the study by Wenzel et al. (1999), the frequencies of stepping in the AMU were much higher than in our study. The frequencies in the milking parlour were much lower than in our study, which could be related to the smoother cow traffic in a tandem compared to a herringbone parlour. On the other hand, there may have been different human–cow interactions in the parlour, and it is difficult to generalise about a system where the human and management factors play such an important role.

Simmental cows stepped more than Brown Swiss cows, especially in the AMU. This could be an indication that they experienced more discomfort, and may be related to the observation that they had longer durations of teat location and detachment. This is probably related to breed-specific udder characteristics, as the Simmental is a less specialised dairy breed.

In the study by Wenzel et al. (1999), cows in the AMU, but not in the parlour, had elevated heart rates during the first 5 min of milking compared to before and after milking, but mean heart rates did not differ. Hopster et al. (2002) observed lower heart rates in the AMU than in the parlour. The average heart rate observed here was about 80 beats/min, which is comparable to that reported in some of the literature (Hemsworth et al., 1989; Rushen et al., 2001) and lower than that reported in previous studies of AMS (Hopster et al., 2002; Umstätter and Kaufmann, 2002; Wenzel et al., 1999). We found no factors other than time of day that influenced heart rate during milking. We conclude that further studies and more detailed analysis are required, although it is possible that heart rate will not prove to be a suitable measure of stress in a situation where differences in feeding regimes may have a strong confounding effect.

We observed much lower levels of milk cortisol (<1 nmol/l) than those reported by others: between 1.15 and 2.66 by Wenzel (1999), and 2.5 by Hemsworth et al. (1989). The low levels were probably caused by the type of biochemical analysis, as previously discussed by Sixt et al. (1997). Cows in the AMS group had higher levels of cortisol in their milk than cows in the HMP group. This result corresponds to the findings of Wenzel et al. (1999). However, it is unlikely that the group difference reflects differences in response during milking, because cortisol concentration in milk reflects the average plasma cortisol concentration during the period of milk synthesis (Bremel and Gangwer, 1978). Although Termeulen et al. (1980) reported that milk cortisol levels followed plasma cortisol levels closely and without delay after ACTH-injection, cows in their experiment had been milked prior to the ACTH-injection and subsequent milk sampling. Therefore, the milk samples in their study reflected short periods of milk synthesis. In our study, milk cortisol levels may reflect much longer times of milk synthesis, and the contribution of milking itself to the cortisol concentration was therefore probably minor.

In summary, cows showed more behavioural signs of discomfort and aversion in the HMP than in the AMU, in particular during the time from beginning of cleaning until the cluster had been attached, and they may have had less average milk flow. We interpret this as an effect of the design or management of the herringbone parlour and discomfort of udder preparation by milkers. However, cows had higher milk cortisol levels in the AMU, suggesting some degree of stress in the AMS system that was probably not directly related to milking. There is some indication that Simmental cows have more difficulties in the AMS than Brown Swiss. Generally, heart rate was not particularly high, and there were no indications of more stress than usual in either system. However, it should be kept in mind that our data relate only to successful voluntary milking events without AMU attachment failure. Also, we looked at two very specific systems within one particular farm environment, and the stocking density in the AMS was only about half the commercial stocking density. Generalisations to other breeds, to other types of AMS and to other types of milking parlour systems should be made with caution.

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