Milk Cortisol Concentration in Automatic Milking Systems Compared with Auto-Tandem Milking Parlors

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ABSTRACT

Milk cortisol concentration was determined under routine management conditions on 4 farms with an auto-tandem milking parlor and 8 farms with 1 of 2 automatic milking systems (AMS). One of the AMS was a partially forced (AMSp) system, and the other was a free cow traffic (AMSf) system. Milk samples were collected for all the cows on a given farm (20 to 54 cows) for at least 1 d. Behavioral observations were made during the milking process for a subset of 16 to 20 cows per farm. Milk cortisol concentration was evaluated by milking system, time of day, behavior during milking, daily milk yield, and somatic cell count using linear mixed-effects models. Milk cortisol did not differ between systems (AMSp: 1.15 ± 0.07; AMSf: 1.02 ± 0.12; auto-tandem parlor: 1.01 ± 0.16 nmol/L). Cortisol concentrations were lower in evening than in morning milkings (1.01 ± 0.12 vs. 1.24 ± 0.13 nmol/L). The daily periodicity of cortisol concentration was characterized by an early morning peak and a late afternoon elevation in AMSp. A bimodal pattern was not evident in AMSf. Finally, milk cortisol decreased by a factor of 0.915 in milking parlors, by 0.998 in AMSp, and increased by a factor of 1.161 in AMSf for each unit of ln(somatic cell count/1,000). We conclude that milking cows in milking parlors or AMS does not result in relevant stress differences as measured by milk cortisol concentrations. The biological relevance of the difference regarding the daily periodicity of milk cortisol concentrations observed between the AMSp and AMSf needs further investigation. Key words: dairy cow, milk cortisol, automatic milking system, auto-tandem parlor

INTRODUCTION

Only a few studies have addressed welfare issues of automatic milking systems (AMS) based on physiological measurements of stress, and their results are contradictory. Hopster et al. (2002) found lower epinephrine and norepinephrine reactions and a tendency for higher blood cortisol concentrations during the first phase of milking in an AMS with forced cow traffic compared with an auto-tandem parlor (ATM). They did not find a difference in the concentration of fecal cortisol metabolites, which might have indicated more general differences in the type or number of stressful situations that cows faced in AMS compared with ATM. In contrast, Wenzel et al. (2003), Hagen et al. (2004), and Abeni et al. (2005) reported higher cortisol concentrations in milk and blood for AMS with partially forced cow traffic in comparison with an ATM and a herring-bone parlor, respectively, although all studies reported low absolute cortisol values.

Hopster et al. (2002) concluded that their measured physiological stress response to the milking procedure in an AMS was low and in the typical range for milking, whereas Wenzel et al. (2003) and Hagen et al. (2004) suspected some degree of stress on a farm with an AMS. However, they suggested that stressful social interactions in the waiting area in front of the milking parlor or evasion to less desirable times of day for milking by low-ranking cows were more likely related to the elevated levels of milk cortisol than the milking procedure in the AMS. All studies were limited in that only one type of AMS was investigated (Lely Astronaut), cow traffic did not follow the company's recommendation (promoting free cow traffic), only 1 herd was observed per milking system (1 group of dairy cows in the AMS and another group in the milking parlor), and all observations took place on only 1 experimental farm. Thus, it is difficult to assess how much the results were influenced by the specific farm and whether they can be generalized to other farms or even other types of AMS.

In the study presented, we compared 2 types of AMS (Lely Astronaut and DeLaval VMS) with ATM on 4 farms, each under practical conditions. We hypothesized that milk cortisol might be higher in the AMS independent of the specific cow traffic in comparison with the ATM and that this difference would be more pronounced for high-performance dairy cows, those with an impaired state of health, or those showing restless behavior during milking. Within the AMS, we ex-
Table 1. Description of the farms and animals, sample sizes, and descriptive statistics on the measured variables for automatic milking systems with partially forced (AMSp) and free (AMSf) cow traffic and for auto-tandem milking parlors (ATM)

<table>
<thead>
<tr>
<th>Item</th>
<th>AMSp</th>
<th>AMSf</th>
<th>ATM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farms, n</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Animal to feeding place ratio</td>
<td>≤1:1</td>
<td>≤1:1</td>
<td>≤1:1</td>
</tr>
<tr>
<td>Animal to lying cubicle ratio</td>
<td>≤1:1</td>
<td>≤1:1</td>
<td>≤1:1</td>
</tr>
<tr>
<td>Cubicles with bedding material</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cubicles with soft lying mat</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cubicles with both</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Floor in the activity area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid (concrete or mastic asphalt)</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Partially or fully slatted concrete</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rubber coated (slatted or solid)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Type of feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass and concentrate in feeder</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hay and concentrate in feeder</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Silage and concentrate in feeder</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Partial or TMR</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Light at night</td>
<td>Off</td>
<td>Off</td>
<td>Off</td>
</tr>
<tr>
<td>No. of animals with samples</td>
<td>180</td>
<td>182</td>
<td>106</td>
</tr>
<tr>
<td>No. of animals sampled</td>
<td>30 to 54</td>
<td>37 to 49</td>
<td>20 to 40</td>
</tr>
<tr>
<td>Average no. of lactations</td>
<td>2.2 to 3.4</td>
<td>2.6 to 3.9</td>
<td>2.4 to 3.2</td>
</tr>
<tr>
<td>DIM</td>
<td>142 to 195</td>
<td>140 to 169</td>
<td>97 to 168</td>
</tr>
<tr>
<td>Breed (no. of farms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mainly Brown Swiss</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mainly Holstein-Friesian</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mainly Red Holstein</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Brown Swiss/Red Holstein</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mainly Swiss Simmental</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Data collection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average milking frequency</td>
<td>2.3 to 2.5</td>
<td>2.2 to 2.7</td>
<td>2</td>
</tr>
<tr>
<td>Milk cortisol (no. of sampling days)</td>
<td>2 to 4</td>
<td>2 to 4</td>
<td>1 to 2</td>
</tr>
<tr>
<td>Milk cortisol (no. of samples)</td>
<td>2 to 4 (1 to 7)</td>
<td>3 to 4 (1 to 6)</td>
<td>2 to 4 (1 to 4)</td>
</tr>
<tr>
<td>Behavior (no. of observed milkings)</td>
<td>3 to 8 (2 to 11)</td>
<td>5 to 9 (3 to 14)</td>
<td>5 to 6 (3 to 6)</td>
</tr>
<tr>
<td>Variable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk cortisol, nmol/L</td>
<td>1.15 ± 0.07</td>
<td>1.02 ± 0.12</td>
<td>1.01 ± 0.16</td>
</tr>
<tr>
<td>Mean daily milk yield, L</td>
<td>24 ± 2</td>
<td>22 ± 2</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>SCC (×1,000)</td>
<td>219 ± 18</td>
<td>280 ± 13</td>
<td>113 ± 28</td>
</tr>
<tr>
<td>Stepping, no./min</td>
<td>1.64 ± 0.08</td>
<td>1.10 ± 0.08</td>
<td>1.23 ± 0.12</td>
</tr>
<tr>
<td>Foot-lifting, no./min</td>
<td>0.18 ± 0.05</td>
<td>0.13 ± 0.03</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Kicking, no./min</td>
<td>0.05 ± 0.03</td>
<td>0.00 ± 0.00</td>
<td>0.02 ± 0.01</td>
</tr>
</tbody>
</table>

1Range over farms.
2Range of median over farms (range over cows).
3A median (mean for daily milk yield) was calculated for each cow. The mean for each of the farms was then calculated. The values report the mean ± SE of these farm values.

Expected the system with partially forced cow traffic to disrupt daily periodicity in milk cortisol compared with the system with free cow traffic because cow behavior is more restricted with forced traffic (Ketelaar-de Lauwere et al., 1998), possibly resulting in stressful situations for the cows.

MATERIALS AND METHODS

Experimental Design and Farms

The investigation included 12 farms (Table 1), 4 with conventional ATM [2 with products of DeLaval (Tumba, Sweden), and 2 with products of Westfalia (Westfalia-Surge GmbH, Oelde, Germany), and 8 farms with 1 of 2 AMS. The 2 AMS differed in that they were managed with free cow traffic (AMSf) or partially forced cow traffic (AMSp). Farms had used their respective milking system for at least 6 mo, and farms with an AMS had been recommended by the manufacturers. All dairy cows were habituated to the system at least for the current lactation. Data collection took place during 4 periods (November 2001, May to June 2002, October to November 2002, and January to February 2003). In each period, data on 1 farm per system were collected except for the fourth AMSf farm, which was investigated in the penultimate period.

As recommended by the manufacturers, cow traffic was partially forced in 1 type of AMS (AMSp: DeLaval VMS, DeLaval International AB) and free in the other (AMSf: Lely Astronaut, Lely Industries N.V., Maas-
sluis, the Netherlands). All 4 farms with AMSp had a waiting area in front of the milking unit, which could be accessed through a 1-way gate. On 2 of these farms, cows wanting to move from the lying area to the feeding area could pass through a selection gate, which was independent of the milking unit. On the other 2 farms, cows could choose to go through a selection gate in the waiting area instead of the milking unit if they had recently been milked. On the 4 farms with AMSf, cows could freely move between the lying and feeding areas. However, 3 of these farms had a waiting area in front of the milking unit, which was accessed through a 1-way gate and was the only place where water was provided (contrary to Swiss animal welfare regulations). At 1 farm, the restriction only applied during the summer, and on another, cows could access water through the waiting area fence. Cows in the AMSf that had not been milked for a long time were herded to the milking unit. This was done infrequently and unsystematically on the farms studied.

The 2 AMS differed in several respects apart from cow traffic, such as the specific technique used in the milking unit to clean, find, and attach cups to the teats. Nevertheless, they are referred to as AMSp and AMSf herein because milk cortisol is thought to reflect a wider time window than just the milking process itself.

On the farms with ATM, cows were milked twice daily, whereas slightly higher average milking frequencies were found on farms with AMSp and AMSf (Table 1). Milkings in the ATM started between 0500 and 0600 h and between 1630 and 1800 h and lasted about 1 h.

On the farms with AMS, milk samples were collected with automatic milk sampling units on at least 1 d with the intention of collecting at least 2 samples per cow (Table 1). Where conditions allowed, milk samples were taken on 3 consecutive days. In the ATM, milk samples were generally collected on 1 d, and a second person was needed for milk sampling. A total of 1,457 milk samples of uninterrupted milkings from 468 cows were collected for analyses (Table 1).

Variables Measured

Milk Cortisol Concentrations. Circadian patterns of cortisol concentration were measured based on milk cortisol data, which were evenly distributed over 24-h periods. We refer to the estimated circadian pattern as daily periodicity because only a couple of milk cortisol measurements were available for each individual cow. To measure total milk cortisol (free and bound), the cortisol in 0.5 mL of skimmed milk was extracted with 2 mL of dichloromethane. A 20-μL aliquot of this extraction was used in the luminiscence immunoassay (LIA) kit for the cortisol measurement (LIA kit no. RE 620 11, IBL-Hamburg, Germany), which was read by a luminometer (MPL-1, Zylux Corporation, Oak Ridge, TN). The interassay variation was 10.1%, and the intraassay variation was 5.3%. For the statistical analysis, measurements below the detection limit (0.44 nmol/L) were set arbitrarily at 0.4 nmol/L (77/1,457 milk samples, 5.3%). The response variable was the measured cortisol concentrations multiplied by the measurement of 1 sample of known concentration, which was run in each assay and divided by this known concentration.

It has been shown that measurements of cortisol concentrations in milk and blood correlate closely (Shutt and Fell, 1985; Verkerk et al., 1998). If cows are milked for short intervals, milk cortisol instantaneously reflects changes in blood cortisol (Termeulen et al., 1981). Whereas Shutt and Fell (1985) found a 1:1 relation for absolute levels of free cortisol in blood and milk, they and others (Bremel and Gangwer, 1978; Termeulen et al., 1981; Verkerk et al., 1998) found total milk cortisol to be one-sixth of blood values.

For each cow, the following variables were calculated (1 constant value per cow) as explanatory variables in the analysis of milk cortisol as a general characterization of the cows:

Mean Milk Yield Per Day. Milk yield data were collected for approximately 3 d on each farm. Mean milk yield was calculated for each cow as the summed milk yield of the single milkings of a cow divided by the time interval in which milk yield data were collected (Table 1).

SCC. The median SCC of all milk samples taken for a given cow was calculated (Table 1; SCC analysis was conducted by the Swiss Brown Cattle Breeders’ Federation, Zug, Switzerland).

Behavioral Variables. Behavior during milking was observed by video in 20 focal cows per farm on 3 d (Table 1). Cameras were installed such that the hind legs and udder of the cow being milked were visible. Median frequencies of steps (shifting weight from 1 hind foot to the other, lifting the foot <10 cm), foot lifts (foot lifted >10 cm), and kicks (a fast movement often directed at items around the cow) during milking were calculated for each cow. Focal cows were selected according to the criteria of good general health, absence of lameness, and calm behavior during milking, based on the advice of the farmers. On 2 smaller farms, 18 (AMSp) and 16 (ATM) cows fulfilled these criteria. In the ATM system, behavioral and milk yield data were collected during the days directly before or after the milk samples for the milk cortisol analysis were taken because collecting the milk samples was considered a potential source of disturbance.
**Statistical Analysis**

Milk cortisol was compared between AMSp, AMSf, and ATM using linear mixed-effects models (Pinheiro and Bates, 2000). Farm and individual were included as nested random effects to account for the repeated milkings and the potential dependency among cows on each farm.

Milk cortisol and the median SCC were log transformed before inclusion in the model to satisfy statistical assumptions. Model assumptions (i.e., the distribution of the residuals and random effects) were checked graphically for normality, homogeneity, and independence of the explanatory variables in each of the models. In some of the models, heterogeneity was observed among the different milking systems. Thus, a term that accounted for different variability per milking system was included in all the models for consistency reasons, although it did not lead to a statistically significant improvement in each model.

Three main models were calculated that differed in their sets of explanatory variables. The structure of all the models can be written as subsets of

\[ y_{ijkl} = \mu + b_i + b_j + \alpha_k + \left( \beta_1 X_1 \right) + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \ldots + \varepsilon_{ijkl} \]

where \( \mu \), the intercept, the fixed effects \( \alpha_k \), type of milking system (factor with \( k = 3 \) levels and treatment contrasts: ATM, AMSp, AMSf), time of day (either \( \beta_i \), factor with \( l = 2 \) levels and treatment contrast: morning and afternoon, or \( X_l \), continuous: seconds since midnight), \( X_2 \), mean daily milk yield (continuous), \( X_3 \), median SCC (continuous), \( X_4 \), median frequency of stepping (continuous), \( X_5 \), median frequency of foot-lifting (continuous), \( X_6 \), median frequency of kicking (continuous), and \( \ldots \), all possible 2- and 3-way interactions, and the nested random effects \( b_i \), farm and \( b_j \), the individual cow. Statistical assumptions are that \( \varepsilon_{ijkl} \sim N(0, \sigma^2) \) iid, \( b_i \sim N(0, \sigma^2_i) \) iid, and \( b_j \sim N(0, \sigma^2_j) \) iid (iid = independently identically distributed).

**Simple Approach.** A simple model with only the type of milking system as explanatory variable was calculated because no clear daily pattern in milk cortisol was obvious, and thus the question of a general difference between the systems could be addressed. All 1,457 milkings were included in this analysis.

**Complex Approach.** A model including milking system, time of day (morning vs. afternoon), daily milk yield, median SCC, and their interactions was calculated including all milkings in the ATM and those milkings in AMSp and AMSf that were observed at the same time of day (between 0400 and 0800 h and 1600 and 2000 h). There were 559 milkings (1 to 4 per cow) of 321 cows (14 to 40 cows per farm) on all 12 farms included in this analysis. In another model, which included the median frequencies of steps, foot lifts, and kicks as explanatory variables, 297 milkings (1 to 4 per cow) of 159 cows (8 to 20 of the focal cows per farm) fulfilled the temporal criteria.

**Daily Periodicity.** A model including milking system, daily milk yield, SCC, time of day (seconds since midnight), and their interactions was evaluated using the data for the AMS farms to investigate the daily periodicity. The 1,008 milkings (1 to 7 per cow) of 345 cows (26 to 54 cows per farm) on 8 AMS farms were included. A similar model that included the behavioral variables was restricted to 449 milkings (1 to 7 per cow) of 153 of the focal animals (17 to 20 cows per farm).

To allow an a priori unrestricted smooth shape of the daily periodicity, natural splines were used for the effect of time (Venables and Ripley, 2002). The number of knots in the splines necessary to represent the daily pattern was found by continuously increasing this number as long as the increase resulted in a statistically significant improvement of the model. In the model including all the data from the AMS farms, 9 knots were necessary. In the model including the behavioral variables, a greatly increased number of knots led to a better model fit. The fitted curve was very rugged and was taken as an indication of overfitting. Thus, the same number of knots was used as in the model with all the data.

Model selection began with the models described above. Interactions were included up to 3-way interactions. Random terms for fixed effects were tested in addition to the random term of the intercept. Then, the fixed effects were reduced until only statistically significant effects and main effects that were still included in interactions remained in the model.

In the reduced models, random terms for fixed effects were checked to see whether they were necessary. Such terms were not necessary in any of the models, and thus only a random term for the intercept was included in each of the models presented here. In the models including the behavioral variables, it was not possible to include all 3-way interactions because of the available number of degrees of freedom. Thus, the same interactions as in the model without the behavioral variables were allowed plus the main effects of the behavioral variables. After the selection steps described above, behavioral variables were checked to see if there were any 2-way or 3-way interactions. All evaluations were carried out using R 1.8.1 (R Development Core Team, 2003) and the method “lme” (Pinheiro and Bates, 2000).
RESULTS

Influence of the Milking System—Simple Approach

The general milk cortisol concentration was low. Most measurements ranged from 0.25 to 4 nmol/L (Figure 1, Table 1). There was no detectable difference in milk cortisol concentrations of cows milked in AMSp, AMSf, and ATM ($F_{2,9} = 0.11, P = 0.90$).

Influence of the Milking System—Complex Approach

A decrease in milk cortisol concentration by a factor of 0.84 was found between the morning (0400 and 0800 h) and the evening milkings (1600 and 2000 h; $F_{1,237} = 9.54, P = 0.002$, Figure 1). In the ATM there was a slight tendency for cows with higher SCC to have lower milk cortisol concentrations, whereas the relation was negligible in cows milked in AMSp but positive in cows milked in AMSf (interaction: $F_{2,306} = 6.22, P = 0.002$).

In the model that included the behavioral variables, the reduction of milk cortisol in the evening compared with the morning milking was by a factor of 0.73, 0.46, and 0.95 for the ATM, AMSp, and AMSf, respectively (interaction: $F_{2,135} = 4.36, P = 0.015$). A similar interaction of milking system and SCC was found as in the model without behavioral variables; that is, in ATM, cows with an elevated SCC had reduced milk cortisol, whereas these were increased in cows from AMSp and were even higher in cows from AMSf ($F_{2,141} = 6.38, P = 0.002$).

Figure 1. Milk cortisol concentration vs. time of day (m = morning; a = afternoon) in auto-tandem milking parlors (ATM) and automatic milking systems with partially forced (AMSp) or free (AMSF) cow traffic obtained on 4 different farms each (rows designate a total of 12 farms). Data points represent the individual milkings. AMSp and AMSf: black bars on time axis reflect time of morning and evening milkings with inlaid box-plots based on the data in that period; thick black lines = predictions of the model on daily periodicity without the behavioral variables; dashed black lines = loess smoother (local polynomial regression) based on all individual milkings.
animals. In the respective model, the decrease in milk cortisol was increased in cows that stepped or lifted their feet more often but did not increase additively if both values were high in the same cow.

**Daily Periodicity**

Daily periodicity of milk cortisol differed between AMSp and AMSf (interaction: F_{9,655} = 3.25, P = 0.001). In AMSp, there was a clear early morning peak in milk cortisol and a broader late afternoon elevation, whereas milk cortisol had several small peaks in AMSf with the highest level of milk cortisol around noon (Figure 1).

An interaction of time × milking system was found in the model that included the behavioral variables (F_{9,278} = 2.67, P = 0.005). The behavioral variables themselves had no significant influence on milk cortisol concentrations. The estimated effect of time of day had a qualitatively similar pattern (not shown) as in the model without behavioral variables.

**DISCUSSION**

**General Differences Between AMS and ATM**

In this study, no difference in milk cortisol was found when comparing 4 farms with ATM and 8 farms each with AMSp or AMSf and the milk cortisol concentration was low (0.25 to 4 nmol/L in milk = 1.5 to 24 nmol/L in blood = 0.5 to 8.5 ng/mL; conversion factor of 6 from milk to blood; conversion factor of 0.36 from nmol/L to ng/mL; Amador, 1999). This finding agrees with Weiss et al. (2004), who did not find changes in fecal cortisol metabolites during a change from a milking parlor to an AMS, but contrasts Hopster et al. (2002), who found systematically (but nonsignificantly) elevated acute blood cortisol during milking in an AMS with forced cow traffic (average values of up to about 12 ng/mL in blood), as well as Wenzel et al. (2003), Hagen et al. (2004), and Abeni et al. (2005), who demonstrated elevated milk cortisol in an AMS.

We did not find differences among the milking systems in how much milk cortisol values decreased from morning to afternoon milkings, which implies that there are no differences in the milk cortisol change throughout the day. Although Hagen et al. (2004) did not find differences between morning and afternoon milkings, Wenzel et al. (2003) indicated that differences between milk cortisol in morning and evening milkings were smaller in the ATM than in the AMS. Our results support these changes, if we include behavioral variables of restlessness and restrict the data to the focal animals. In the respective model, the decrease in milk cortisol was greater in AMSp than in the ATM, whereas AMSf had similar values to the ATM. Thus, AMSp appears to have a more pronounced change in the milk cortisol throughout the day than was observed in the ATM and in AMSf.

Milking seems to be a stress in itself, in that milking increases cortisol, whereas being suckled by a calf does not (Lupoli et al., 2001). In agreement with this, cows are willing to forgo a reward if they can avoid being milked. Visits to a feed bunk decline to one-third when cows are milked there while being fed (Grimm et al., 1980). A possible aversive effect of milking is reflected by the increase of blood cortisol during milking to about 10 to 25 ng/mL (Rushen et al., 2001; Hopster et al., 2002), although machine milking results in lower values than hand milking (Gorwit et al., 1992).

We measured milk cortisol, which does not reflect the milking process proper because it is thought to be an integrated measure of the stress axis in the period 2 to 4 h prior to milking (Fox et al., 1981; Verkerk et al., 1998). Thus, differences in milking and housing systems might be difficult to detect if the milking process or a situation that usually precedes milking more than these 4 h was extreme and caused stress to dairy cows.

Although absolute values of cortisol concentrations may be influenced by the method of biochemical analysis, our milk cortisol measurements (corresponding to values of 0.5 to 8.5 ng/mL in blood) are well below the blood cortisol levels reported for acute stress responses: 10 ng/mL after transportation (Bremel and Gangwer, 1978), 20 to 25 ng/mL during milking in a novel environment (Rushen et al., 2001), and 20 to 35 ng/mL during sham, freeze, and hot-iron branding (Lay et al., 1992). The cortisol concentrations measured reflect a level that can be considered easily tolerable.

Based on our cortisol concentrations we would concur with Hopster et al. (2002), who concluded that AMS and ATM are equally acceptable regarding welfare, and we do not share the caution expressed by Wenzel et al. (2003), who claimed that AMS could be problematic for the cows due to the lack of the soothing effect of human–animal interactions and increased social interference in the waiting area of an AMS.

**Influences Other Than Milking System**

We found that cows with higher milk cortisol concentrations tended to show more behavioral patterns that are thought to reflect unrest (stepping, foot-lifting, kicking). Thus, behavioral unrest during milking coincided with elevated basal milk cortisol levels. The simplest explanation is that these cows are easily excited, though the stress reactivity would be more meaningful than the basal cortisol levels.
Cows with higher SCC had lower milk cortisol in the ATM, whereas the milk cortisol of such cows was elevated in AMSp and AMSf. Thus, it seems that milk cortisol is increased with an added strain such as SCC, or that the animals that had elevated milk cortisol in the AMS were more likely to develop high SCC compared with cows in ATM. This is in parallel to the findings of Rushen et al. (2001), who stressed cows during milking and observed increased residual milk yield, which may in turn lead to increases in SCC (Harmon, 1994). As average daily milk yield dropped with all the statistical models, there was no indication that high-performing dairy cows reacted more strongly to the different milking systems.

**Differences Between AMSp and AMSf**

The daily periodicity comparing the 2 AMS in the fitted model showed a clear early morning peak in milk cortisol and a broader late afternoon elevation in AMSp but did not show a clear pattern in AMSf. This difference became visible even though most of the AMSf were similar to the AMSp because they were equipped with a waiting area separated by 1-way gates or water was only provided in the waiting area. The elevations in milk cortisol in the daily periodicity of the cows milked in AMSp coincide well with the elevations found during a night-morning and midday period in cycling heifers by Thun (1987), though Lefcourt et al. (1993) only found one peak at about 0600 h in lactating cows. Light seems to be a time giver sufficient to induce circadian patterns in cows (Thun, 1987). Thus, the absence of a clear daily periodicity in AMSf may be a deviation from an expected circadian pattern and could be an indication for stressful conditions that disrupt the “normal” circadian pattern (Zähner, 2001).

It is hard to judge how far the circadian patterns of cortisol concentrations found in earlier studies with cattle (Thun, 1987) could have been induced by external time givers in addition to daylight, such as fixed milking or feeding times (Saito et al., 1989), or even the regularly repeated process of blood sampling itself. Thus, we suggest that the absence of a strong daily periodicity seen in cows in AMSf could reflect a “natural” state given minimal restrictions of behavior over time. The common assumption is that there exists a circadian pattern in undisturbed cattle (Thun, 1987). Thus, we would conclude that the dairy cows in AMSf were subjected to a situation that was more stressful on the basis of the absence of a clear daily periodicity. These findings in milk cortisol are supported by the observation that heart rate at rest was elevated in AMSf (our unpublished data). It is unclear whether the restricted access to water in some of the AMSf farms might have caused this pattern of stress. In the comparison between morning and afternoon milkings, which are often taken to reflect daily periodicity, the focal cows kept in ATM lacked a strong decrease in cortisol concentrations similar to the cows in AMSf. This suggests that the situation in the waiting area was more similar and more stressful for the cows kept in the ATM and AMSf in contrast to those kept in AMSp.

Although Hopster et al. (2002) argued that a comparison of an AMS to an ATM is sufficient for a judgment regarding animal welfare because the latter is “widely accepted as meeting current animal welfare standards”, the question of which is the natural circadian pattern of cortisol remains because hormonal changes in cows in an ATM may be caused by time givers and may not reflect an original state. This question could be addressed by measuring the circadian pattern in suckler cows, cows under (semi)feral conditions, or by experimentally disrupting a clear circadian pattern with increased stress.

**CONCLUSIONS**

There was no consistent difference in milk cortisol concentrations between auto-tandem milking parlors and automatic milking systems with partially forced or free cow traffic. We conclude that milking cows in ATM or AMS did not result in biologically relevant stress. It seemed that the daily periodicity was less pronounced in the AMS with free cow traffic than in the AMS with partially forced traffic, which may indicate strain. The relationship between such a change in the circadian pattern of cortisol concentrations and parameters of animal welfare needs further investigation.

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