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

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

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

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Introduction

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

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

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

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

Material and Methods

Animals and Husbandry

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

Experimental Procedure

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

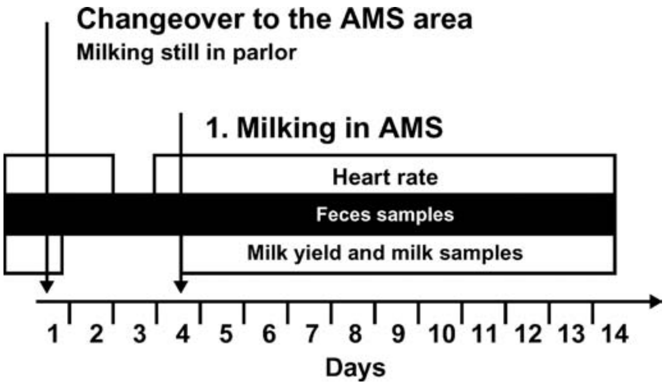

Figure 1. Experimental protocol: The first arrow indicates the start of the training period. The cows were driven twice daily to the stall of the automatic milking system (AMS) but were still milked twice daily in the parlor. The second arrow indicates the start of milking in the automatic milking system;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

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

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

Data Processing and Statistical Analyses

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

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

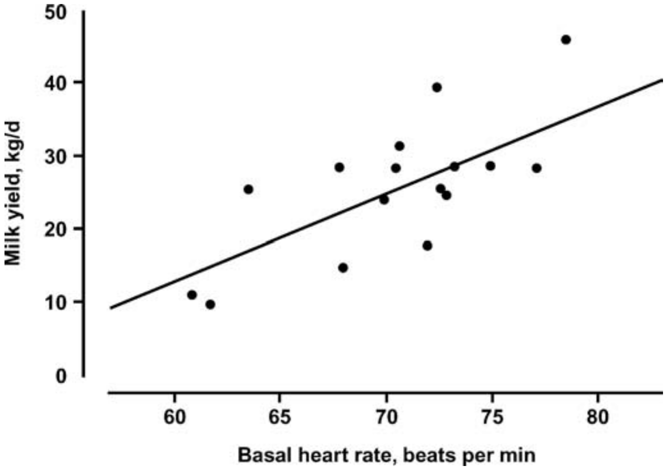

Figure 2. Relationship between the basal heart rate (x) and the daily milk yield (y): $y = -59 + 1.2x$; $r = 0.74$ ($P < 0.001$).

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

Results

Behavioral Observations

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

Heart Rate

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

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

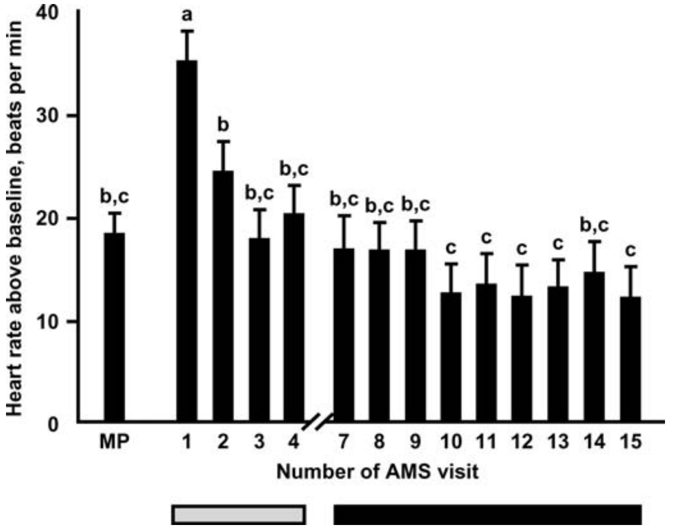

Figure 3. Heart rate above baseline (mean ± SEM, n = 17 animals) during milking in the parlor (MP) and during visits in the stall of the automatic milking system. Means without common letters differ ($P < 0.05$). The gray bar indicates results during the training period; the black bar indicates results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

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

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

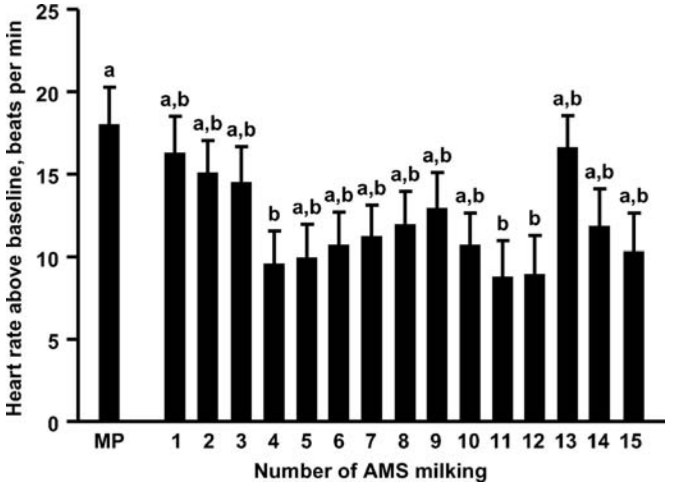

Figure 4. Heart rate above baseline (mean ± SEM, n = 17 animals) during milking in the parlor (MP) and visits when milking was performed in the automatic milking system (AMS). Means without common letters differ ($P < 0.05$).

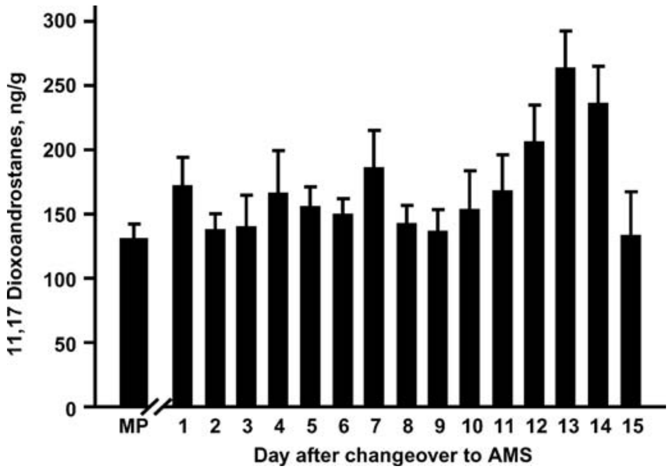

Figure 5. 11,17 Dioxoandrostanes (DOA) concentration (fresh-matter basis) in feces (means ± SEM, n = 17 animals) in the parlor (MP) and the automatic milking system.

11,17 Dioxoandrostane

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

Milk Yield and Milk Composition

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

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

ACTH Challenge

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

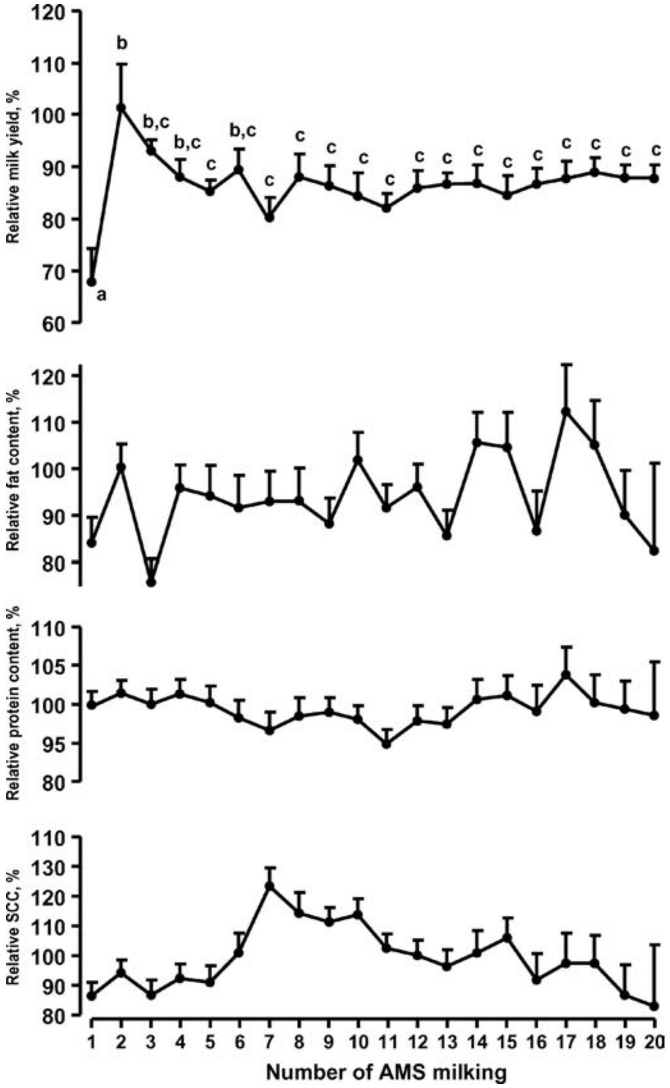

Figure 6. Milk yield and milk composition (mean ± SEM, n = 17 animals) expressed as relative values of parlor results (100% = mean parlor results of 10 d before the changeover). Means without common letters differ ($P < 0.05$).

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

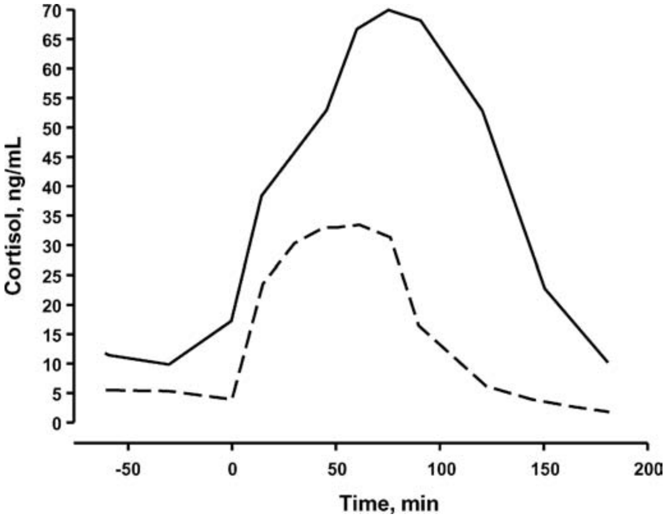

Figure 7. Cortisol response during ACTH challenge in two exemplary cows with high (—) and low (----) adrenal cortex sensitivity. The ACTH was administrated at t = 0 min.

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

Discussion

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

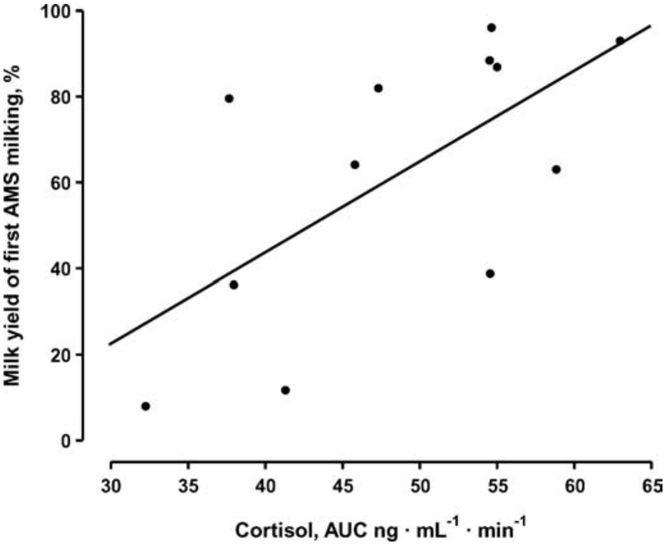

Figure 8. Relationship between cortisol release during ACTH challenge (area under curve [AUC], 0 to 180 min after ACTH application; x) and the relative milk yield during the first AMS milking (y): y = −40.4 + 2.1x; r = 0.65 (*P* = 0.02).

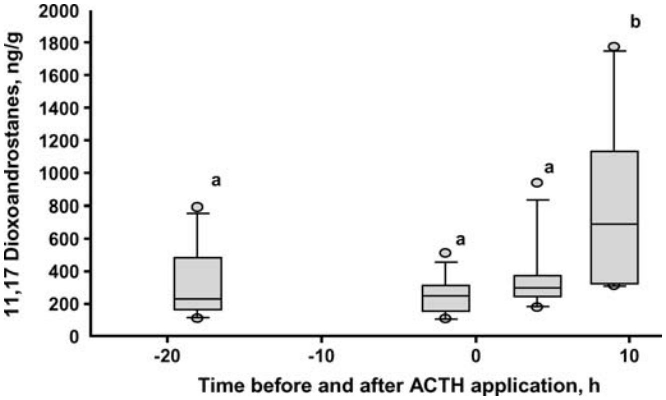

Figure 9. Boxplot of the feces 11,17 dioxoandrostanes concentrations (fresh-matter basis) before and during ACTH administration. Each box shows the median and the upper and lower quartile value; the whiskers show the 10th and the 90th percentiles. The circles represent data points that were outside the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

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

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

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

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

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

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

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

Implications

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

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