

Effects of milking frequency in automatic milking systems on salivary cortisol, immunoglobulin A, somatic cell count and melatonin

S. Helmreich, B. Wechsler, R. Hauser, L. Gyga

Food Safety and Veterinary Office FSVO, Centre for Proper Housing of Ruminants and Pigs, Switzerland

Summary

In barns with an automatic milking system (AMS), both the milking frequency and the number of nighttime milkings vary between cows. A low milking frequency might indicate problems in gaining access to the milking unit. Also, nighttime lighting in the waiting area of the AMS and in the milking unit increases exposure to light at night and could suppress nocturnal melatonin synthesis. These effects could result in increased stress, suppressed immune response, and poor udder health. A total of 125 cows (14–16/farm) on 8 farms with AMS were selected based on their average milking frequency. Eight to 10 saliva samples per cow were taken over the course of 4 days, and cortisol, IgA and melatonin concentrations were determined. Somatic cell counts (SCC) were determined in milk samples. Milking frequency had no significant relationship with mean cortisol and IgA levels, but a higher milking frequency tended to be associated with lower SCC levels. Nocturnal melatonin levels tended to be negatively associated with the number of nighttime milkings. In conclusion, no indication of increased stress or reduced immune defense was found in relation to milking frequency on farms with an AMS.

Keywords: dairy cow, automatic milking system, milking frequency, stress, melatonin secretion

Einfluss der Melkfrequenz in Automatischen Melksystemen auf Speichelkortisol, Immunoglobulin A, Zellzahl und Melatoninkonzentration

In Ställen mit Automatischem Melksystem (AMS) variieren die Melkfrequenz und die Anzahl nächtlicher Melkungen zwischen den Kühen. Eine tiefe Melkfrequenz könnte Probleme beim Zugang zur Melkeinheit anzeigen. Zudem setzt die Beleuchtung des Wartebereichs und der Melkeinheit die Kühe nachts vermehrt Licht aus, was die Melatoninsekretion reduzieren könnte. Diese Effekte könnten zu Stress sowie einer Beeinträchtigung der Immunantwort und der Eutergesundheit führen. Für die vorliegende Untersuchung wurden 125 Kühe (14–16/Betrieb) auf 8 Betrieben aufgrund ihrer Melkfrequenz ausgewählt. Acht bis 10 Speichelproben pro Kuh wurden während des Verlaufs von 4 Tagen entnommen und die Konzentrationen von Kortisol, IgA und Melatonin bestimmt. Die Melkfrequenz zeigte keinen signifikanten Zusammenhang mit den mittleren Kortisol oder IgA Konzentrationen, doch ging eine höhere Melkfrequenz tendenziell mit einer tieferen Zellzahl einher. Melatoninkonzentrationen in der Nacht waren tendenziell negativ mit der Anzahl nächtlicher Melkungen assoziiert. Insgesamt ergaben sich keine Hinweise auf erhöhten Stress oder eine reduzierte Immunantwort im Zusammenhang mit der Melkfrequenz auf Betrieben mit AMS.

Schlüsselwörter: Milchkühe, Automatische Melksysteme, Melkfrequenz, Stress, Melatoninsekretion

DOI 10.17236/sat00054

Received: 10.07.2015
Accepted: 09.09.2015

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Introduction

The use of an automatic milking system (AMS) influences cow behavior in several ways. The animals can attend the milking unit at day and night, and milking frequency may vary considerably between individuals (Jacobs and Siegford, 2012). Depending on the cow traffic system, gates control the access to the waiting area in front of the milking unit, the feeding area, or the lying area. As a consequence, the cows' time budget and use of these areas vary in relation to the milking frequency (Helmreich et al., 2014).

Most studies on stress in dairy cows milked in barns with an AMS focused on the milking process (Hopster et al., 2002; Wenzel et al., 2003; Hagen et al., 2004; Gyax et al., 2008). However, restricted access to different barn areas and resources induced by the cow traffic system could result in stressful situations outside the milking unit. For example, dominance interactions are likely to occur at the selection gates and in the waiting area in front of the AMS, and less competitive cows were found to have difficulties in gaining access to the milking unit and to experience longer waiting times than dominant cows (Ketelaar-de Lauwere et al., 1996; Melin et al., 2006; Lexer et al., 2009). Some cows even give up waiting in front of the AMS and return to the resting area (Melin et al., 2006). This may not only result in a lower milking frequency but also induce a stress response.

Increased stress can also affect the immune system of cows (Amadori et al., 2009). Measurements of salivary concentrations of immunoglobulin A (IgA) have already been reported as a non-invasive method to assess the

immune status of cows (Iqbal et al., 2014). Moreover, salivary IgA has been used as a marker of chronic stress in humans (Green et al., 1988), squirrel monkeys (Carver and Hau, 2000) and rats (Guhad and Hau, 1996). To examine the relationship between milking frequency and udder health, somatic cell counts (SCC) were recorded in milk samples of individual cows in the present study. SCC is mainly affected by mammary-gland infections (Dohoo and Meek, 1982), but may also be influenced by physiological and management factors such as milking frequency (Harmon, 1994; Wiktorson and Sørensen, 2004) and stress (Whittlestone et al., 1970; Olde Riekerink et al., 2007).

To facilitate cows' visits to the AMS throughout the night, most dairy farmers provide artificial lighting in the waiting area in front of the AMS and in the AMS unit. Cows with a higher number of nighttime milkings thus might spend longer periods of time in these illuminated areas than cows with fewer nighttime milkings, with possible effects on melatonin secretion. Lawson and Kennedy (2001) and Muthuramalingam et al. (2006) observed that a light intensity of 50 lx during an 8-hour nighttime period reduced nocturnal melatonin levels in dairy heifers by 50 to 70%, whereas light intensities of 10 lx or less had no effect on plasma melatonin concentration. In castrated bulls, a 1-hour light exposure (500 lx) during the night inhibited nocturnal melatonin secretion (Kasuya et al., 2008).

In the present study, we assessed the relationship between milking frequency and potential indicators of stress in cows on farms with AMS. As cow age and days in milk (DIM) contribute to variation in milking frequency (Dzidic et al., 2004), we considered these vari-

Table 1: Number of focal cows, their milking frequency, and number of nighttime milkings in the period of recruitment as well as their milking frequency, number of nighttime milkings (the main design variables), daily milk yield, DIM, and age at the time of data collection per farm.

Farm	Number of focal cows	Milking frequency ^a	Number of nighttime milkings/d ^a	Milking frequency ^b	Number of nighttime milkings/d ^c	Daily milk yield [kg] ^a	DIM ^d	Age [yr] ^d
1	14	2.7 ± 0.6	0.5 ± 0.4	2.5 ± 0.5	0.4 ± 0.4	28.8 ± 8.0	173 ± 78	6.3 ± 1.2
2	16	2.5 ± 0.7	0.7 ± 0.2	2.5 ± 0.8	0.7 ± 0.4	23.7 ± 4.1	81 ± 53	6.3 ± 3.3
3	16	2.7 ± 0.6	0.7 ± 0.3	2.8 ± 0.6	0.8 ± 0.3	27.1 ± 6.5	135 ± 36	4.7 ± 2.3
4	16	2.7 ± 0.3	0.6 ± 0.2	2.7 ± 0.3	0.8 ± 0.3	21.6 ± 5.7	141 ± 85	4.7 ± 1.7
5	16	2.3 ± 0.4	0.7 ± 0.2	2.3 ± 0.4	0.5 ± 0.3	24.8 ± 6.8	129 ± 42	5.3 ± 1.8
6	16	2.3 ± 0.6	0.7 ± 0.2	2.5 ± 0.7	0.7 ± 0.3	28.6 ± 8.6	118 ± 43	5.4 ± 1.8
7	15	2.5 ± 0.7	0.7 ± 0.2	2.5 ± 0.6	0.6 ± 0.3	28.1 ± 8.5	117 ± 66	4.7 ± 2.2
8	16	2.6 ± 0.6	0.7 ± 0.4	2.4 ± 0.4	0.5 ± 0.3	30.8 ± 6.7	116 ± 56	4.4 ± 1.4

^a Mean (±SD) of 14 days' data prior to the start of the experimental phase (used for selection of focal cows).

^b Mean (±SD) of 7 days' data recorded during the experimental phase (used as the main design variable in the analysis of salivary cortisol and IgA concentrations as well as SCC).

^c Mean (±SD) of 4 days' data recorded during the saliva-sampling phase (used as the main design variable in the analysis of salivary melatonin concentrations).

^d Data (means ± SD) based on the first day of the experimental phase.

ables as possible confounders in the statistical analysis. Moreover, we aimed to determine whether and how strongly the frequency of nighttime milkings coincided with a suppression of nocturnal salivary melatonin concentration in dairy cows.

Animals, Material and Methods

Animals

The study was conducted on 8 commercial dairy farms in Switzerland with an AMS that had been in operation for at least 6 mo. Four farms were equipped with the Lely automatic milking system (Model A 2, Lely Industries N.V., Maassluis, The Netherlands), the other 4 farms with the DeLaval voluntary milking system (VMS, DeLaval International AB, Tumba, Sweden). Median herd size was 53 lactating cows (ranging from 30 to 66). A total of 125 focal cows were recruited for data collection based on their average milking frequency during a 14-day period prior to the experiment (as recorded by the AMS, Tab. 1). On each farm, cows covering a wide range of milking frequencies were chosen. Except for the first farm, where 14 focal cows were selected, there were 16 focal cows on all other farms. However, one cow (farm no. 7) developed mastitis during the experimental phase and was thus excluded from the analysis. Each farm was visited for an experimental phase of 2 to 3 weeks between July 2007 and December 2009. Before the start of the experimental phase, all focal cows were examined and declared clinically healthy in that there was no current treatment for any disease, including claw disorders.

Feeding

The feeding times on 7 farms were identical, with one feeding taking place in the morning and one in the evening. One farm had an automatic feeding system with 10 feeding times evenly distributed over the day and a feeding break at night from 22.30 to 04.00 h. Cows were fed grass-maize silage on 6 farms, fresh grass on 1 farm, and a mixture of potatoes, sugar-beet chips, and maize kernels on 1 farm. Hay was provided as a supplement on all farms.

Light intensity

During the night, all barns used dim guiding lights (<10 lx) throughout all areas. Light intensity in the AMS unit and the waiting area ranged from 60 to 280 lx, measured with a luxmeter (ELVOS LM-1010, ELVOS GmbH, Ludwigsburg, Germany) at a height of 1.5 m. As a light intensity of 50 lx was found to affect night plasma melatonin levels in cattle (Muthuramalingam et al., 2006), an influence of the artificial lighting in the AMS unit and the waiting area on the cows' melatonin levels could be expected on all investigated farms. The number of

nighttime milkings as recorded by the AMS was used as an indication of the time a given cow spent in these illuminated barn areas. Nighttime milkings were defined as milking processes taking place between 2200 and 0500 h. This time period is the minimum duration of the natural dark phase in the summer season. In all barns, light intensity during the day was stronger than 200 lx, and natural daylight was supplemented by artificial lighting in the barns in the morning and evening. The sum of the daylight hours and the hours of artificial lighting was viewed as the light period in the barn and ranged from 13 to 19 h.

Saliva sampling

Two sampling sequences were conducted on each farm over 4 days. Ten samples per cow were collected starting on day 1, with samples being taken at 12.00, 16.00, 20.00, 23.00, and 02.00 h, a sampling schedule that was repeated on day 3 (2×5 samples of 16 focal cows per farm). During nighttime sampling, the investigators wore head lamps with a dim red light (<8 lx) and avoided direct illumination of the cows' eyes to preclude an influence of the saliva collection on melatonin secretion. Saliva sampling was performed with a saliva pump specifically developed for this purpose and consisting of an electronic pipette controller (Profiller™ 446, Socorex Isba S.A., Switzerland), a plastic pipette, and tubes approximately 60 cm in length. A sample of clear saliva was collected by aspiration from the cow's cheek pouch. Four mL of saliva were then filled into vials and immediately cooled in water at 4°C to avoid bacterial growth. Once the collection process was finished, the saliva was cleansed of food particles via centrifugation for 5 min at 2,000×g, and the clear supernatant was transferred to a new vial. Samples were then stored at -20°C until assayed. A total of 1'232 saliva samples were collected on the 8 farms.

Analysis of cortisol, IgA and melatonin

A high-sensitivity cortisol enzyme immunoassay (EIA) kit (Salimetrics Europe, Ltd., UK) was used to quantify bovine salivary cortisol concentration.

A bovine IgA enzyme-linked immunosorbent assay (ELISA) kit (Bethyl, Montgomery, TX, USA) was used to determine the concentration of IgA in the saliva.

A direct melatonin radioimmunoassay (Bühlmann Laboratories AG, Switzerland) was used to analyze the saliva samples. The mean melatonin concentration of samples collected during night (see definition) was taken as each cow's average nocturnal melatonin concentration. Daytime melatonin samples were taken during daylight hours, and the melatonin concentration was averaged per cow.

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Milk sampling and SCC analysis

Milk samples were collected with a shuttle milk-sampling unit (Lely) or an automatic milk sampler (DeLaval) on 2 to 3 consecutive days during the period of the saliva sampling. Between 1 and 8 milk samples per cow were taken on each farm, depending on individual milking frequency and the operational reliability (e.g., mixing of some samples on 1 farm) of the sampling unit. One focal cow (farm no. 2) died before the start of the milk-sampling period. A total of 447 milk samples from 124 focal cows were collected on the 8 farms.

Statistical analysis

Data were analyzed using linear mixed-effect models (Pinheiro and Bates, 2000) in R 2.11.0 (R Development Core Team, 2011). The farm effect was considered to be

random, and it controlled for similarities among cows of a given farm. Assumptions of the models were checked graphically. Residuals and random effects were plotted to assess normality and homoscedasticity. All response variables were log-transformed to meet statistical assumptions.

Cortisol and IgA concentrations as well as average SCC were each used as response variables, whereas milking frequency served as the main design variable. The mean cortisol and IgA concentrations of all saliva samples taken for a given cow and the mean SCC of all milk samples taken for a given cow were used in the analysis. The mean milking frequency was calculated based on AMS data from 7 consecutive days during the experimental phase. To minimize experimental interference,

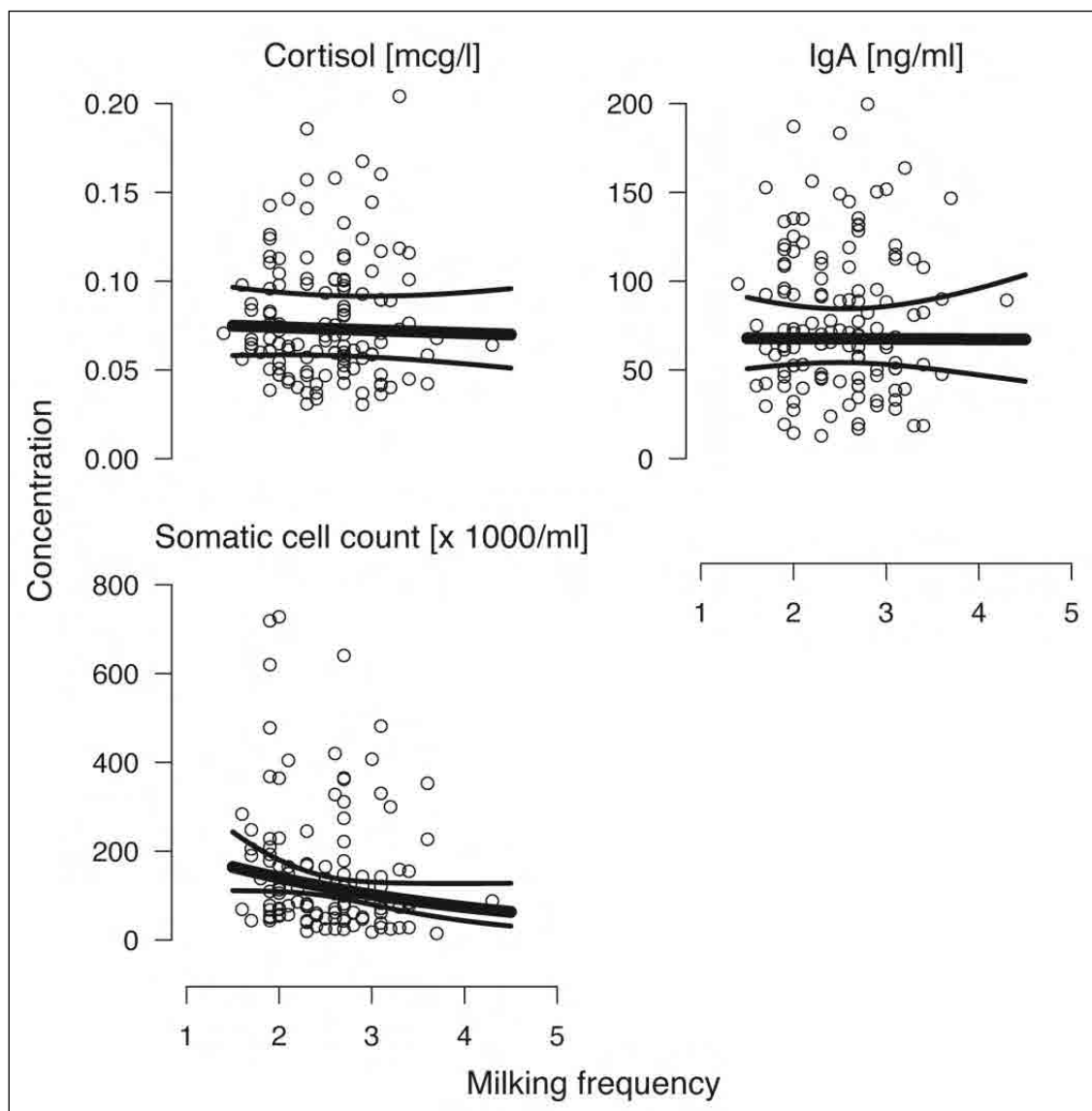


Figure 1: Mean salivary cortisol concentration (mcg/L), salivary IgA concentration (ng/mL), and SCC (x 1,000/mL) as a function of the average milking frequency over 7 days.

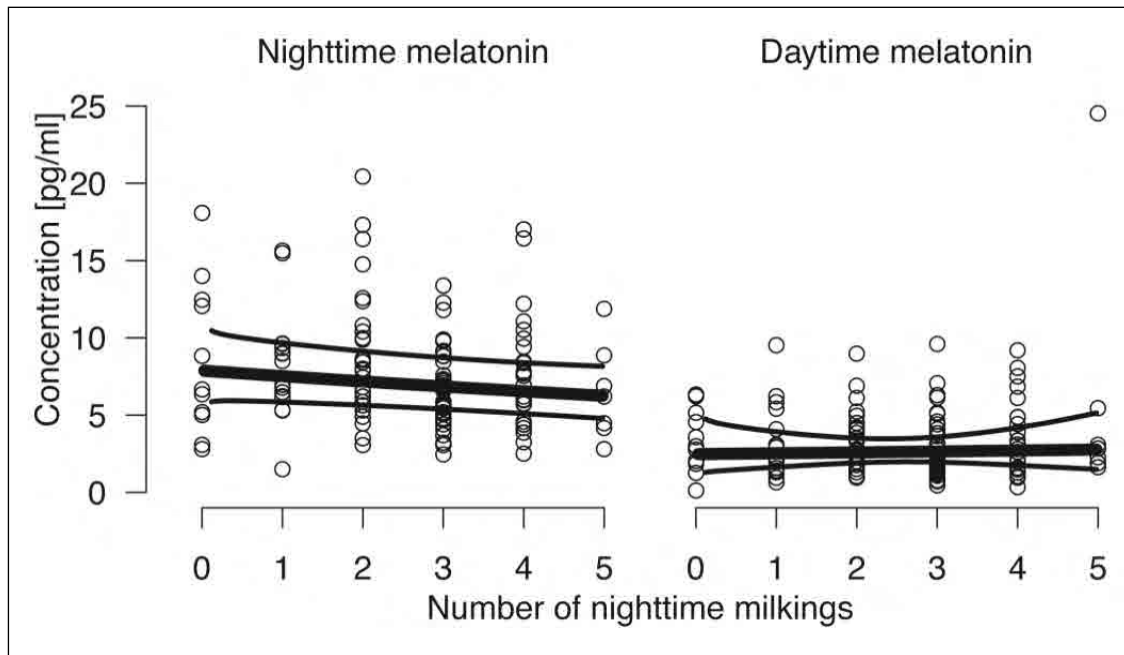


Figure 2: Mean nighttime and daytime melatonin concentration (pg/mL) as a function of the number of nighttime milkings over 4 days (including the saliva-sampling period).

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these 7 days were separated from the saliva and milk sampling by a period of 1 to 6 days. Age and DIM were considered possible confounders and were included in the model as additional continuous main effects.

Nighttime and daytime melatonin concentrations were each used as a response variable, and the number of nighttime milkings during the 4-day saliva-sampling period served as the main design variable. The duration of the light period in the barn per 24 h, varying between seasons and depending on the lighting schedule in a given barn, was considered a possible confounder and included as an additional continuous main effect.

Results

Milking frequency, age or DIM did not influence stress or immune status of cows on farms with AMS. Neither cortisol nor IgA concentrations in the saliva showed a statistically detectable relationship with milking frequency (cortisol: $F_{1,114} = 0.17$, $P = 0.68$; IgA: $F_{1,114} = 0.002$, $P = 0.97$; Fig. 1). Similarly, age and DIM did not significantly influence cortisol (age: $F_{1,114} = 2.63$, $P = 0.11$; DIM: $F_{1,114} = 1.95$, $P = 0.17$) or IgA levels (age: $F_{1,114} = 1.66$, $P = 0.20$; DIM: $F_{1,114} = 0.03$, $P = 0.86$).

SCC levels were only weakly influenced by milking frequency. Cows with a higher milking frequency tended to have lower SCC levels than cows with a lower milking frequency ($F_{1,113} = 3.41$, $P = 0.07$; Fig. 1). Age ($F_{1,113} = 0.23$, $P = 0.63$) and DIM ($F_{1,113} = 0.12$, $P = 0.73$) did not appear to influence the average SCC in the milk samples.

Nighttime milking frequency had a slight influence on salivary melatonin concentrations. Cows with a higher number of nighttime milkings tended to have lower nocturnal melatonin concentrations than cows with a lower number of nighttime milkings ($F_{1,116} = 3.66$, $P = 0.06$; Fig. 2). No such relationship could be detected for daytime melatonin concentrations ($F_{1,116} < 0.05$, $P = 0.82$). The duration of the light period per 24 h in the barn had no detectable impact on melatonin concentrations (night: $F_{1,6} = 0.45$, $P = 0.529$; day: $F_{1,6} = 1.33$, $P = 0.29$).

Discussion

Previous studies on stress in relation to AMS compared cows milked in an AMS and those milked in a conventional milking parlor and did not detect major differences between the 2 milking systems. (Hagen et al., 2005; Gygax et al., 2006; Jacobs and Siegford, 2012). The fact that salivary cortisol concentration was not related to milking frequency in the present study indicates that less competitive cows, although they may have difficulties to access the milking unit (Melin et al., 2006; Lexer et al., 2009), are not markedly stressed on farms with AMS. As an alternative explanation, other effects such as the specific barn layout or the rearing history of the cows could have masked such a relationship. However, in our analysis, we considered two major possible confounders, cow age and DIM (the latter being correlated with milk yield), both of which did not influence the indicators of stress and udder health.

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As IgA concentration was not influenced by milking frequency, the amount of stress may not have varied sufficiently with milking frequency to have an immunosuppressive effect. In a study with goats, Hernandez-Castellano et al. (2011) found that IgG and IgM concentration in the milk decreased with increasing milking frequency, whereas IgA concentration was not clearly associated with milking frequency.

In line with Mollenhorst et al. (2011) we found that cows with a higher milking frequency tended to have lower SCC levels than cows with a lower milking frequency. They also noted that the effect of milking frequency on SCC is likely to be small when corrected for other variables such as age and lactation stage in our study. In contrast, Osterman et al. (2005) reported no differences in SCC in cows with an extended calving interval milked either 2 or 3 times per day. Similarly, Shields et al. (2011) found no difference in SCC between udder halves milked 4 and 2 times daily.

Our results indicate that exposure to light before, during, and after a nighttime milking process in the AMS unit as well as in the waiting area has an influence on melatonin secretion. Although there has been no previ-

ous research on the relationship between melatonin and health in cows, a disturbed melatonin secretion was found to have an impact on the cardiovascular system (Paulis and Simko, 2007) as well as on body weight and energy balance (Barrenetxe et al., 2004) in humans.

In conclusion, we did not find evidence showing that the stress level or the immune status of dairy cows on farms with AMS was influenced by milking frequency. However, exposure to the artificial lighting in the waiting area and AMS unit at night could result in reduced nocturnal melatonin levels.

Acknowledgments

The authors gratefully acknowledge the laboratory assistance provided by Myrtha Arnold and Mariana Graber (Institute of Physiology and Behaviour, ETH Zurich, Switzerland). Our thanks go to Stefan Kury for his help during saliva sampling and laboratory analysis. We are also grateful to the owners of the farms for allowing us to study their herds. This study was funded by the Federal Food Safety and Veterinary Office (project 2.02.06).

Influence de la fréquence de traite dans les systèmes de traite automatique sur le cortisol salivaire, les immunoglobulines A, le nombre de cellules et la concentration de mélatonine

Dans les étables équipées d'un système de traite automatique, la fréquence ainsi que le nombre de traites nocturnes varient d'une vache à l'autre. Une fréquence basse pourrait être le signe de difficultés d'accès à l'unité de traite. D'autre part, l'éclairage dans la zone d'attente et dans l'unité de traite expose les vaches à plus de lumière durant la nuit, ce qui pourrait réduire la sécrétion de mélatonine. Ces éléments pourraient amener un stress et avoir une influence sur la réponse immunitaire et la santé de la mamelle. Pour la présente étude, 125 vaches provenant de 8 exploitations (14–16 animaux par exploitation) ont été choisies en fonction de leur fréquences de traite. On a prélevé 8 à 10 échantillons de salive sur une période de 4 jours et y a mesuré la concentration de cortisol, d'IgA et de mélatonine. On n'observe pas de rapport significatif entre la fréquence de traite et les concentrations moyennes de cortisol et d'IgA mais une fréquence de traite plus élevée a tendance à être corrélée avec un nombre de cellules plus faible. Les concentrations de mélatonine durant la nuit avait ten-

Influsso sulla frequenza della mungitura con i sistemi automatici su cortisolo nella saliva, immunoglobulina A, numero di cellule e concentrazione di melatonina

Nelle stalle con sistema di mungitura automatico (AMS) varia la frequenza della mungitura e il numero di mungiture notturno tra le mucche. Una frequenza bassa di mungitura potrebbe indicare dei problemi di accesso all'unità di mungitura. Inoltre, la notte alle mucche viene imposta un'illuminazione della zona di attesa e dell'unità di mungitura, cosa che può portare ad una riduzione della secrezione di melatonina. Questi effetti possono implicare maggiore stress, e compromettere la risposta immunitaria e la salute delle mammelle. Per il presente studio, sono state esaminate 125 mucche (14–16 per azienda) provenienti da 8 aziende scelte a causa della loro frequenza di mungitura. Sono stati prelevati da 8 a 10 campioni di saliva per mucca durante 4 giorni e sono state determinate le concentrazioni di cortisolo, IgA e melatonina. La frequenza della mungitura non era rilevante per la media del cortisolo e le concentrazioni di IgA, mentre un'alta frequenza tendeva ad un numero inferiore di cellule. Le concentrazioni di melatonina di notte tendevano ad essere associate negativamente al numero di mungiture notturne. Nel complesso, non è

dance à être associées de façon négative avec le nombre de traites nocturnes. De façon générale, on n'a pas d'indice montrant un stress plus élevé ou une réduction de la réponse immunitaire en fonction de la fréquence de traite dans les exploitations équipées d'un système de traite automatique.

stato rilevato nessun aumento dello stress o una ridotta risposta immunitaria in relazione alla frequenza delle mungiture negli allevamenti con AMS.

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Corresponding author

Lorenz Gygax
Centre for Proper Housing of Ruminants and Pigs
Food Safety and Veterinary Office FSVO
Agroscope, Institute for Livestock Sciences ILS
Tänikon
CH-8356 Ettenhausen
Switzerland
Tel.: +41 58 480 33 84
Fax: +41 52 365 11 90
E-Mail: lorenz.gygax@agroscope.admin.ch