Impact of the factors of animal production and welfare on robotic milking frequency

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Abstract – The objective of this work was to evaluate the impact of production factors on milking frequency and the latter's effect on animal welfare in robotic milking. The experiment was performed with Holstein Friesian cows housed in free-stall barns. To assess the impact of production factors, body condition score, milk yield, and concentrate intake were determined. To determine the effects of the milking frequency on welfare, the locomotion and teat-end scores and serum cortisol level were evaluated. Three experimental groups were formed according to the daily average milking frequency: 1.0 to 1.9, 2.1 to 2.9, and above 3.0 milkings per day. The decrease in milking frequency during lactation was related to the reduction in concentrate intake and milk yield. The increase in milking frequency was related to the reduction in the milk flow and the increase in milking duration. Milking frequency was affected by concentrate intake, locomotion problems, milk yield, and lactation stage. The cortisol level and teat-end conditions were not affected by milking frequency. Milking frequency impacts milk flow, duration of milking, and milk composition; however, there is no effect on the indicators of animal welfare.

Index terms: cortisol, free-stall system, lactation, milk yield, voluntary milking system.

Impacto dos fatores de produção e bem-estar animal sobre a frequência de ordenha robotizada

Resumo – O objetivo deste trabalho foi avaliar o impacto dos fatores de produção na frequência de ordenha e a influência desta sobre o bem-estar animal em ordenha robótica. O experimento foi realizado com vacas da raça Holandesa confinadas em "free stall". Para a avaliação do impacto dos fatores de produção, foram determinados escore corporal, produção de leite e consumo de concentrado. Na determinação dos efeitos da frequência de ordenha sobre o bem-estar, foram avaliados os escores de locomoção e de tetos e o cortisol sérico. Formaram-se três grupos experimentais de acordo com a frequência média diária de ordenha: 1,0 a 1,9; 2,1 a 2,9 e acima de 3,0 ordenhas. A diminuição da frequência de ordenha durante a lactação foi relacionada à redução do consumo de concentrado e da produção de leite. Já o aumento da frequência de ordenha foi relacionado à redução do fluxo de leite e ao aumento da duração da ordenha. A frequência de ordenha foi influenciada por consumo de concentrado, problemas de locomoção, produção de leite e estágio de lactação. O nível de cortisol e as condições de tetos não foram afetados pela freqüência de ordenha. A frequência de ordenha tem impacto no fluxo de leite, na duração da ordenha e na composição do leite; porém, não há efeito sobre os indicadores de bem-estar animal.

Termos para indexação: cortisol, sistema free stall, lactação, produção de leite, sistema voluntário de ordenha.

Introduction

The increasing lack of labor resources and the wish of the milk producers to improve aspects of the milk activity, such as handling techniques, quality of life and professionalization of dairy workers, have led to the adoption of the automatic milking system (AMS) (Castro et al., 2012). Among the advantages of the AMS, are the reductions in necessary labor resources and costs related to milking, as well as the increase in milk yield due to the increase in milking frequency (Svennersten-Sjaunja & Pettersson, 2008).

The milking capacity of an AMS is frequently expressed by the daily number of milkings, but other criteria must also be considered, including milk yield, milking interval, teat-cup attachment success rate,

milking duration, size of the herd, and cow traffic (Gygax et al., 2007). Other factors, such as number of passages through the access gate to the robot, concentrate supply, locomotion and body condition scores, can also interfere with the frequency of milking (Jacobs & Siegford, 2012). The AMS milking process is different from conventional milking with regard to the frequency, interval, and daily distribution of milking, cleaning, and teat disinfection procedures. Therefore, the AMS is a milking management system in which feeding, cow traffic, animal behavior and welfare should be taken into account to allow for its more efficient use (Svennersten-Sjaunja & Pettersson, 2008).

The variation in the interval between milking is considered one of the main challenges of robotic milking. Most studies have reported a milking interval variation of 7 to 9 hours (Hogeveen et al., 2001; Gygax et al., 2007). However, longer intervals may lead to a lower milk yield due to the decrease in the mammary blood flow, which decreases the ability of the gland to extract nutrients from the blood (Delamaire & Guinard-Flament, 2006). For an ideal average milking interval (without problems in milk quality and teats), Castro et al. (2012) suggested 9 to 10 hours, which means from 2.4 to 2.6 milkings per day.

The increase in milking frequency in early lactation, as well as other techniques, such as the use of hormones (bovine somatotropin), has been an alternative to increase milk yield in herds with high genetic potential for milk production. According to Wall & Mcfadden (2007), increasing milking frequency increases milk production due to the cellular dynamics of the mammary gland. Svennersten-Sjaunja & Pettersson (2008) observed that the greater milking frequency in the AMS may increase milk production by approximately 13% in multiparous cows and 17% in primiparous cows, when the increase in frequency is kept constant throughout lactation. Another procedure to increase milking frequency is to offer concentrate throughout milking, which has been shown to increase the number of visits to the robot (Migliorati et al., 2009).

Some studies also point out the effect of health and comfort of the cows on the number of visits to the milking station. According to Borderas et al. (2008), cows that visit the AMS less frequently show a greater locomotion score than those that visit the robot more frequently. Regarding animal welfare, the robotic milking process may be stressful to the cows and may modify their blood cortisol levels (Abeni et al., 2005). When comparing the stress level of milked cows between conventional and robotic systems, Lexer et al. (2009) found no difference in blood cortisol levels. However, in their study, the heart rate was more elevated in the cows using the AMS.

As the AMS represents a considerable investment, it is important that the economic return is maximized. According to Pettersson et al. (2011), the annual production of milk per robot is fundamental for a positive economic performance, which, in turn, depends on the number of daily milkings. The milking frequency and the average of the interval between milkings are decisive factors in obtaining a high occupation rate of the AMS and, therefore, a higher milk production.

The objective of this work was to evaluate the impact of production factors on milking frequency and the latter's effect on animal welfare in robotic milking.

Materials and Methods

The experiment was conducted in a commercial farm located in the municipality of Castro, in the state of Paraná, Brazil, during four months, from March 26 to July 1, 2014. Primiparous and multiparous Holstein Friesian cows housed in free-stall barns and milked with two automatic milking systems were used. Milk samples were collected during a week, each month, to obtain data for the official milk records.

Two studies were performed to assess the impact of production factors on milking frequency and the effect of milking frequency on milk yield, milk composition, and animal welfare.

In the first study, 390 cows were used, of which 173 were multiparous and 217 were primiparous, with days in milk (DIM) between 7 and 305 days. The cows were divided into three experimental groups according to the weekly average of daily milking frequency (1.0 to 1.9, 2.1 to 2.9, and above 3.0 milkings per day). For the formation of these groups, milking frequency data during the collection week were obtained from the herd management software DelPro (DeLaval, Tumba, Sweden). Data on lactation order, DIM, daily milking number, duration of milking, milk yield and flow, and concentrate intake were also determined using the

DelPro software. Individual milk composition was obtained monthly from official milk records. When the experimental groups were established, the average milking frequency of the herd was 2.34±0.39 times per day. The number of lactations was 1.72±1.04, DIM was 124.25±77.95 days, and average production was 35.78±7.99 kg milk per day. The factors that affected milking frequency were days in lactation, milk yield, interval between milking, concentrate intake, body condition score (BCS), and locomotion problems.

In the second study, 60 cows were used; this group included five randomly selected cows of each of the three experimental groups. The parameters affected by milking frequency were production level, milk fat and protein contents, teat-end conditions, and level of cortisol in the blood. There was no collection of repeated data for the same cow in the experimental group. That is, all groups consisted of 20 different cows. Cows with fewer than four functional teats, sick cows, and those with high locomotion difficulty were excluded from the study. The average milking frequency in this group was 2.50±0.59 times per day, lactation number was 1.75±0.79, DIM was 131.78±90.67 days, and average milk yield was 37.02±6.94 kg per day.

In both studies, the values for all variables were the average of the seven data-collection days, except for milk fat and protein contents, for which only the result per month was considered.

The cows were feed a partially mixed ration (PMR), composed on a dry matter basis, of: 52.93% corn silage, 16.94% ryegrass silage, 6.35% oat silage, 4.36% alfalfa hay, 8.17% corn proteins, 8.05% soybean meal, 0.91% bypass fat, 1.81% buffer feeding, and 0.48% mineral mix, processed in an electronic mixer wagon and supplied twice a day at 5:30 a.m. and 1:30 p.m. The concentrate supply (18% crude protein and 80% total digestible nutrients) was offered in the milking unit (1.5 kg per animal per milking) and complemented (4 to 12 kg per animal per day) in the FSC400 individual feed station (DeLaval, Tumba, Sweden) using feeding boxes placed in the feeding area. Access to the feeding boxes was through an automatic selection gate located before the Voluntary Milking System (VMS, DeLaval, Tumba, Sweden). The amount of daily concentrate was set in the DelPro software according to the DIM and milk yield: 4 kg per animal per day (DIM < 6 days); 6 kg per animal per day (DIM = 6 to 10 lactation days); 6 to 8 kg per animal per day (DIM = 11 to 15 days); 8 to 10 kg per animal per day (DIM = 16 to 20 days); 10 to 12 kg per animal per day (DIM = 21 to 110 days) and; 7 to 12 kg per animal per day (DIM > 110 days).

Access to the VMS was determined based on the minimum interval between the milkings or on the potential volume of milk stored in the udder, estimated from the average production of the last seven days by the management software. Primiparous cows could access the AMS every 4 hours or 7 kg milk (DIM < 31 lactation days), every 7 hours or 8 kg milk (DIM = 31 to 280 days), and every 9 hours or 7 kg milk (DIM > 280 days). Multiparous cows accessed the AMS every 5 hours or 8 kg milk (DIM < 31 lactation days), every 6 hours or 9 kg milk (DIM > 30 to 31 days before the predicted dry period), and every 8 hours or 8 kg milk (last 30 days). The cows that were late were fetched starting at 13:30 hours after the previous milking. Lactotropin (Elanco, São Paulo, SP, Brazil) was applied only in animals with milk production lower than their productive potential; this was done from 100 to 200 lactation days, every 14 days. From 200 lactation days, Boostin (MSD, São Paulo, SP, Brazil) was applied every 12 days.

For the official milk records, each month, a milk sample (morning milking) was collected from all four mammaries at the same time, using the Shuttle automatic sample collector (DeLaval, Tumba, Sweden). For the analysis of milk composition, samples were collected in standard flasks containing the preservative bronopol (2-bromo-2-nitropropane-1,3-diol) and analyzed by the laboratory of Programa de Análise de Rebanhos Leiteiros do Paraná of Associação Paranaense de Criadores de Bovinos da Raça Holandesa, located in the municipality of Curitiba, in the state of Paraná, Brazil. Milk composition was analyzed using infrared absorption in the Bentley 2000 instrument (Bentley Instruments, Inc., Chaska, MN, USA) in alignment with the Standards (141C) of International Dairy Federation (Whole.., 1996). Milk production was converted into energy-corrected milk (ECM) and protein using the following equation (Tyrrell & Reid, 1965): ECM = [(0.327×MY) + (12.95×%F×MY)/100 + (7.65×%P×MY)/100], where MY is the milk yield in kg per day, F is the fat percentage and, P is the protein percentage.

Evaluations were carried as body condition score (BCS), locomotion score (LCS), teat-end score (TES),

and blood collection for determination of serum cortisol concentration. The evaluations were carried out between 8 and 9 a.m. The flasks with blood samples were stored in a refrigerated box with ice and delivered on the day of collection to the Veterinária Preventiva laboratory (Curitiba, PR, Brazil). Samples were then sent for examination to the Tecsa laboratory (Animal Health Technology, Belo Horizonte, MG, Brazil) after being frozen at -21ºC.

For the BCS assessment, the scale used was adapted from Ferguson et al. (1994), varying from 1 (very thin) to 5 (very fat). The methodology described by Thomsen et al. (2008) was used to evaluate the LCS in a scale from 1 (normal locomotion) to 5 (severe lameness). The TES was analized according to the methodology developed by Neijenhuis et al. (2000). For plasmatic cortisol determination, the chemiluminescence methodology using the Immulite 1000 equipment (Siemens Medical Solutions Diagnostics, NJ, USA) was employed. The present study was approved by the ethics committee on animal use of the Universidade Federal de Santa Catarina, protocol number 1.64.13.

The obtained data were assessed through multivariate analyses (factorial and cluster analyses) using the SAS statistical package, version 9.2 (SAS Institute, Cary, NC, USA). The data were previously standardized by the Standard procedure. The factorial analysis was performed to evaluate the relation between variables, while the cluster analysis aimed to confirm the relations expressed by the averages, which allowed understanding the extent of the interference of each variable.

In the factorial analysis, the variables were selected through their communality and the number of factors was based on the eigenvalues, composing the four following factors: animal behavior, stage of lactation, milking performance parameters, and animal welfare. The analysis was performed using the Factor procedure with Promax rotation.

In the cluster analysis, the Fastclus and Cluster procedures were used to form the groups based on the Euclidian distance. The Discrim procedure using the Stepdisc method was used to select the variables that composed the final model. The comparison between the experimental groups was performed by multivariate analysis of variance using the PROC GLM procedure, and the averages were compared by Turkey-Kramer's test, at 5% probability.

From the data of all the cows (n=390), curve graphs were generated for milk yield and content, concentrate intake, and milking performance parameters. This was done according to the stage of lactation by nonlinear regression techniques using the Nlin procedure and the Wood (1967) function with the equation: $M = At^b e^{-ct}$, where M is the average in the time (instant) t; A is the constant associated with the average at the beginning of lactation; b is the constant related to the average rate of increase or decrease until reaching maximum production; c is the constant referring to the average rate of the increase or decrease after reaching the peak; and e is the base of the Naperian logarithms.

Results and Discussion

All cows were milked an average of 2.64 times per day (Figures 1 and 2) at the beginning of lactation (average DIM = 12 days), and 2.31 times at the end (DIM > 300 days). A more accentuated reduction was observed between the beginning and the peak of lactation (DIM = 105 days), showing an average frequency of 2.46 milkings per day. The average daily milking frequency was 2.34, which was lower than the values reported in other studies: 2.7 milkings per day by Castro et al. (2012) and 2.5 milkings per day by Gygax et al. (2007).

The average milk yield per milking varied from 13.32 kg at the beginning of lactation to 15.48 kg at its peak, reaching 13.36 kg at the end of lactation (Figure 1 A). The ECM and protein production (Figure 1 B) was 35.10 kg per cow per day at the beginning of lactation, 36.68 kg per cow per day at the peak of lactation, and 33.43 kg per cow per day at the end of lactation. Therefore, the curves for milk yield per milking, ECM and protein production, and milking frequency decreased throughout lactation.

The average concentrate intake per cow per day was 7.43 kg at the beginning of lactation, 11.70 kg at 150 days of lactation, and 9.90 kg at the end of lactation (Figure 1 A).

There was a decrease in milking frequency throughout lactation due to the reduction in concentrate intake and the decrease in milk yield, showing that the milking interval is dependent on the milk yield level (Hogeveen et al., 2001). The peak milk yield observed at 105 days of lactation may have been caused by the higher concentrate intake at this lactation stage and

the higher number of daily milkings at the beginning of lactation. This finding justifies the lower milking frequency at the peak of lactation rather than at the beginning. Additionally, the use of bovine somatotropin may explain in part the longer time elapsed between the beginning of lactation and its peak, as well as the low variation in milk yield in this period.

Milk composition varied because of the lactation stage and milking interval. The average fat content varied from 3.60% at the beginning of lactation to 3.90% at the end, and a decrease was observed between the beginning of lactation and the peak of milk yield (3.17%) (Figure 2 A). From this point on, there was a gradual increase until the end of lactation, while milk yield and milking frequency decreased. This variation in fat content was affected by milk yield and concentrate intake, among other factors. Similar results were reported by Bruckmaier et al. (2001), who found that fat content increased gradually with advancing lactation stage and decreased due to the
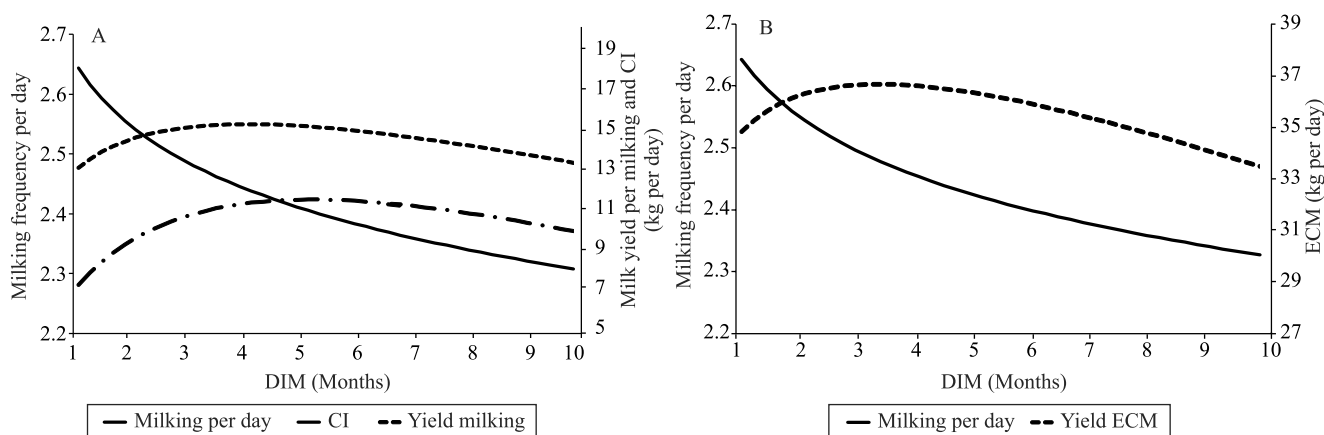

Figure 1. Comparison of milking frequency per day, milk yield per milking, and concentrate intake (CI) per day (A), as well as of milking frequency and energy-corrected milk (ECM) yield (B) according to days in milk (DIM) of 390 yielding Holstein Friesian cows.
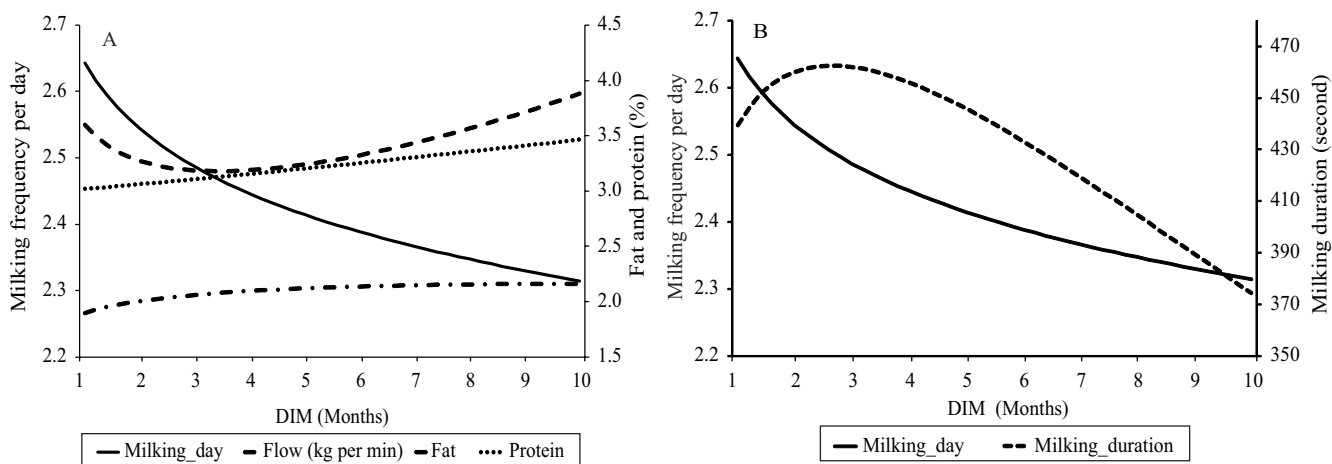

Figure 2. Comparison of milking frequency per day, milk fat and protein contents, and milk flow (A), as well as of milking frequency per day and milking duration (B) in according to the days in milk (DIM) of 390 yielding Holstein Friesian cows.

increase in the milking interval. The average milk protein content increased from 3.0% at the beginning of lactation to 3.5% at the end, which was attributed to the reduction in milking frequency and milk yield in this period. Nielsen et al. (2005), however, did not observe any effect of the milking interval on milk protein content.

The average milk flow increased from 1.89 kg of milk per minute at the beginning of lactation to 2.16 at the end. This value increased mainly starting at the peak of milk yield and decreased with the reduction in milking frequency (Figure 2 A). The increase in milk yield and flow is affected by longer milking intervals, but this effect is not observed in high-yielding cows (Hogeveen et al., 2001). Therefore, milk flow tends to be greater in longer milking intervals, regardless of milk yield. Milking duration was 439, 462, and 374 seconds at the beginning, peak, and end of lactation, respectively (Figure 2 B). Milking duration followed the milk yield and daily milking frequency curves, which means that it decreased throughout lactation. Milk flow increased with the reduction in milking frequency, as also reported by Hogeveen et al. (2001), suggesting that longer milking intervals can decrease milking time and optimize the use of the robot.

In the factorial analysis, the first four factors, including variables of production, concentrate intake, parameters of milking performance, and animal welfare indicators, explained 69.5% of the accumulated variability of the data (Table 1).

Factor 1 (animal behavior) explained 26.7% of the variance of the factorial analysis. A strong relationship was observed between the number of milkings per cow per day and of passages through the selection gate before the VMS and, to a lesser degree, with concentrate intake. It should be noted that the greater the milk yield and the lower the LCS, the higher the milking frequency. Milking frequency depends on the number of passages through the gate before the VMS and on concentrate intake throughout milking. Therefore, the higher the concentrate intake is, the greater is the activity of the cow and the higher is the number of daily milkings. These variables contribute to the increase in ECM and protein production (Table 2). According to Melin et al. (2006), feeding is the main reason why cows visit the robotic milking system.

Factor 2 (lactation state) explained 18.4% of the variance of the factorial analysis and related the increase of the BCS and the reduction of the ECM and protein production with the increase in DIM. In this factor, it was possible to observe that the cows that produced less milk presented greater BCS and that milking duration reduced with the increase in DIM. When comparing the groups of cows milked twice and four times a day, Soberon et al. (2010) did not find differences in the body condition score.

Factor 3 (parameters of milking performance) associated the increase of teat-end lesions with milking duration and explained 12.8% of the variance.

Table 1. Factorial charges, communality, and variance percentage for the parameters of milking performance, concentrate intake, lactation days, milk yield, and indicators of animal welfare of 60 yielding Holstein Friesian cows.

Variable	Factor[1]				Communality
	1	2	3	4	
Passages through the gate before the VMS[2]	0.9041	-0.1443	0.0182	-0.1016	81.8
Number of milkings per day	0.8839	-0.1150	0.0092	0.2838	80.1
Milking duration (s)	-0.2574	-0.3574	0.6677	0.0543	75.2
Concentrate intake (kg fresh matter per day)	0.5976	0.3082	0.2480	-0.0270	51.9
Days in milk	-0.1657	0.8254	-0.0804	0.1971	68.6
ECM[3] and protein	0.5326	-0.6205	-0.1042	0.0063	59.4
Body condition score	0.1871	0.7116	0.0341	-0.0867	60.2
Teat-end score	0.2102	0.1736	0.8779	-0.0292	78.4
Locomotion score	-0.3892	-0.1778	0.1433	0.5252	58.8
Cortisol (nmol L^{-1})	0.1898	0.1677	-0.0504	0.8967	82.0
Variance (%)	26.7	18.4	12.8	11.6	

[1]Factors: 1, animal behavior; 2, lactation stage; 3, parameters of milking performance; and 4, animal welfare, formed by the factorial analysis. [2]VMS, voluntary milking system. [3]ECM, energy-corrected milk.

Therefore, the conditions of the extremity of the teat depend on the duration of the milkings.

Lastly, factor 4 (animal welfare) explained 11.6% of the variance and related the LCS with the level of serum cortisol. Therefore, in the present study, the LCS was the variable that had the greatest impact on animal welfare. According to Nixon et al. (2009), some of the main reasons cows are conducted to the AMS are lameness, lesions on the udder or teats, and mastitis. Gellrich et al. (2015) observed an increase in the cortisol levels in the milk of cows showing lameness, associated with pain and metabolic stress. These authors also pointed out that the assessment of the frequency of visits to the AMS can contribute to the early detection of locomotion problems, while the measurement of cortisol levels in milk can be used as an indicator of painful symptoms such as lameness.

Observing the discriminant analysis, the most important variables in the differentiation of the groups in the cluster analysis were passages through the gate before the VMS, milking duration, concentrate intake per day, ECM, LCS, milk flow, BCS, and cortisol. In the cluster analysis, group 3 differed from the others by presenting a higher number of daily milkings and passages through the gate per day before the VMS and a lower LCS (Table 2). Groups 2 and 3 were similar due to the higher concentrate intake, greater BCS, and shorter milking duration. Groups 1 and 3 had similar ECM and protein production and lower LCS and milk flow. Group 1 differed by presenting cows with

lower concentrate intake and BCS and greater milking duration. Groups 1 and 2 were characterized by higher LCS and fewer milkings per day and passages through the gate before the VMS. Group 2 showed cows with greater DIM and milk flow. However, there was no difference between the groups regarding serum cortisol and TES.

It was verified that the higher the concentrate intake is, the larger are the milking frequency, the number of passages through the gate before the VMS and BCS, and the lower is the LCS (Table 2). Therefore, in the AMS, feeding, correct traffic, and good locomotion conditions are important factors for a higher milking frequency and better body conditions. Cows with lower DIM had higher milk yield and milking duration and lower milk flow and BCS. Milking frequency did not impact BCS, animal welfare, and teat-end conditions. Miguel-Pacheco et al. (2014) reported a reduction in feeding time and number of visits to the AMS for high yielding cows with lameness and managed in AMS. Bach et al. (2007) found a decrease in the visits to the AMS and higher fetching rate for cows with high locomotion scores (score ≥3) in relation to cows with low locomotion scores.

In the present study, the mean serum cortisol levels for the groups of cows averaging 1.91, 2.19, and 2.90 milkings per day were 17.65, 28.96, and 20.69 nmol L^{-1}, respectively, which reveals stress according to baseline values of 14 nmol L^{-1}, as proposed by Forslund et al. (2010). Wiktorsson et al. (2003) observed that,

Table 2. Results of the cluster analysis for parameters of milking performance, concentrate intake, lactation days, milk flow, milk yield, and animal welfare indicators in 60 yielding Holstein Friesian cows[1].

Variable	Unit	Group[2]			p-value
		1	2	3	
Passages through the gate before the VMS[3]	Number	5.40b	7.15b	13.77a	<0,0001
Milkings per day	Number	1.91b	2.19b	2.90a	<0.0001
Milking duration	Seconds	566.81a	394.21b	423.33b	<0.0001
Concentrate intake	kg fresh matter per day	8.53b	10.98a	11.34a	0.0004
Days in milk	days	61.39b	199.58a	112.16b	<0.0001
Energy-corrected milk and protein	kg per day	35.22ab	33.39b	39.83a	0.0003
Milk flow	kg milk per min	2.03b	2.40a	2.05b	0.0310
Body condition score	1 to 5	2.35b	2.77a	2.68a	0.0005
Teat-end score	1 to 4	2.45	2.23	2.43	0.7241
Locomotion score	1 to 5	3.00a	2.15a	1.45b	0.0002
Cortisol	nmol L^{-1}	17.65	28.96	20.69	0.2901
Observations	Number	10	19	31	

[1]Means on the same line followed by distinct letters differ from each other by the Tukey test at 5% significance. [2]Groups formed by the cluster analysis. [3]VMS, voluntary milking system.

during rest, the cows of low and high social hierarchy in the automatic milking systems had cortisol values of 12 and 17 nmol L^{-1}, respectively. The AMS can cause cortisol elevation in cows milked automatically, indicating chronic stress (Abeni et al., 2005).

Both factorial and cluster analysis showed that the cows with lower DIM produced more milk than those with higher DIM and had lower BCS (Tables 1 and 2). Therefore, the increase in DIM increased BCS and milk composition and flow per minute and decreased milk yield and milking duration. In both analyses, it was clear that milking frequency and the greater activity of the cows depended on concentrate intake. Regarding animal welfare, the results of both analyses were similar; that is, cows with lower LCS had more milkings per day and higher concentrate intake and milk yields. Milking frequency was not a stress factor for the cows and did not modify teat-end conditions. However, teat-end conditions were modified by milking duration. According to Mollenhorst et al. (2011), among the various factors that may affect udder health due to the milking interval, the most significant would be milking duration as a result of damage to the teats and risk of bacterial invasion during and after milking.

Conclusions

1. The increase in milking frequency observed in the automatic milking system (AMS) is affected by factors such as concentrate intake, days in milk, milk yield, and locomotion problems.

2. Milking frequency impacts milk yield and protein content and, to a lesser degree, fat content.

3. Milking frequency does not affect mammary gland health or animal welfare.

4. In AMS, locomotion problems had a more significant impact on animal welfare.

5. The AMS allows the management of the evaluated data in real time, facilitating decision making to improve management, milk yield, and animal welfare.

Acknowledgments

References

ABENI, F.; CALAMARI, L.; CALZA, F.; SPERONI, M.; BRETONI, G.; PIRLO, G. Welfare assessment based on metabolic and endocrine aspects in primiparous cows milked in a parlor or with an automatic milking system. **Journal of Dairy Science**, v.88, p.3542-3552, 2005. DOI: 10.3168/jds.S0022-0302(05)73039-3.

BACH, A.; IGLESIAS, C.; CALSAMIGLIA, S.; DEVANT, M. Effect of amount of concentrate offered in automatic milking systems on milking frequency, feeding behavior, and milk production of dairy cattle consuming high amounts of corn silage. **Journal of Dairy Science**, v.90, p.5049-5055, 2007. DOI: 10.3168/jds.2007-0347.

BORDERAS, T.F.; FOURNIER, A.; RUSHEN, J.; PASSILLÉ, A.M.B. Effect of lameness on dairy cows' visits to automatic milking systems. **Canadian Journal Animal Science**, v.88, p.1-8, 2008. DOI: 10.4141/CJAS07014.

BRUCKMAIER, R.M.; MACUHOVA, J.; MEYER, H.H.D. Specific aspects of milk ejection in robotic milking: a review. **Livestock Production Science**, v.72, p.169-176, 2001. DOI: 10.1016/S0301-6226(01)00277-9.

CASTRO, A.; PEREIRA, J.M.; AMIAMA, C.; BUENO, J. Estimating efficiency in automatic milking systems. **Journal of Dairy Science**, v.95, p.929-936, 2012. DOI: 10.3168/jds.2010-3912.

DELAMAIRE, E.; GUINARD-FLAMENT, J. Longer milking intervals alter mammary epithelial permeability and the udder's ability to extract nutrients. **Journal of Dairy Science**, v.89, p.2007-2016, 2006. DOI: 10.3168/jds.S0022-0302(06)72268-8.

FERGUSON, J.D.; GALLIGAN, D.T.; THOMSEN, N. Principal descriptors of body condition score in Holstein cows. **Journal of Dairy Science**, v.77, p.2695-2703, 1994. DOI: 10.3168/jds.S0022-0302(94)77212-X.

FORSLUND, K.B.; LJUNGVALL, O.A.; JONES, B.V. Low cortisol levels in blood from dairy cows with ketosis: a field study. **Acta Veterinaria Scandinavica**, v.52, p.1-6, 2010. DOI: 10.1186/1751-0147-52-31.

GELLRICH, K.; SIGL, T.; MEYER, H.H.D.; WIEDEMANN, S. Cortisol levels in skimmed milk during the first 22 weeks of lactation and response to short-term metabolic stress and lameness in dairy cows. **Journal of Animal Science and Biotechnology**, v.31, p.1-7, 2015. DOI: 10.1186/s40104-015-0035-y.

GYGAX, L.; NEUFFER, I.; KAUFMANN, C.R.; HAUSER, R.; WECHSLER, B. Comparison of functional aspects in two automatic milking systems and auto-tandem milking parlors. **Journal of Dairy Science**, v.90, p.4265-4274, 2007. DOI: 10.3168/jds.2007-0126.

HOGEVEEN, H.; OUWELTJES, W.; KONING, C.J.A.M. de; STELWAGEN, W. Milking interval, milk production and milk flow-rate in an automatic milking system. **Livestock Production Science**, v.72, p.157-167, 2001. DOI: 10.1016/S0301-6226(01)00276-7.

JACOBS, J.A.; SIEGFORD, J.M. Invited review: the impact of automatic milking systems on dairy cow management, behavior,

health, and welfare. **Journal of Dairy Science**, v.95, p.2227-2247, 2012. DOI: 10.3168/jds.2011-4943.

LEXER, D.; HAGEN, K.; PALME, R.; TROXLER, J.; WAIBLINGER, S. Time budgets and adrenocortical activity of cows milked in a robot or a milking parlour: interrelationships and influence of social rank. **Animal Welfare**, v.18, p.73-80, 2009.

MELIN, M.; HERMANS, G.G.N.; PETTERSSON, G.; WIKTORSSON, H. Cow traffic in relation to social rank and motivation of cows in an automatic milking system with control gates and an open waiting area. **Applied Animal Behaviour Science**, v.96, p.201-214, 2006. DOI: 10.1016/j.applanim.2005.06.013.

MIGLIORATI, L.; SPERONI, M.; STELLETTA, C.; PIRLO, G. Influence of feeding flavouring-appetizing substances on activity of cows in an automatic milking system. **Italia Journal Animal Science**, v.8, p.417-419, 2009. DOI: 10.4081/ijas.2009.s2.417.

MIGUEL-PACHECO, G.M.; KALER, J.; REMNANT, J.; CHEYNE, L.; ABBOTT, C.; FRENCH, A.P.; PRIDMORE, T.P.; HUXLEY, J.N. Behavioural changes in dairy cows with lameness in an automatic milking system. **Applied Animal Behaviour Science**, v.150, p.1-8, 2014. DOI: 10.1016/j.applanim.2013.11.003.

MOLLENHORST, H.; HIDAYAT, M.M.; VAN DEN BROEK, J.; NEIJENHUIS, F.; HOGEVEEN, H. The relationship between milking interval and somatic cell count in automatic milking systems. **Journal of Dairy Science**, v.94, p.4531-4537, 2011. DOI: 10.3168/jds.2011-4244.

NEIJENHUIS, F.; BARKEMA, H.W.; HOGEVEEN, H.; NOORDHUIZEN, J.P.T.M. Classification and longitudinal examination of callused teat-ends in dairy cows. **Journal of Dairy Science**, v.83, p.2795-2804, 2000. DOI: 10.3168/jds.S0022-0302(00)75177-0.

NIELSEN, N.I.; LARSEN, T.; BJERRING, M.; INGVARTSEN, K.L. Quarter health, milking interval, and sampling time during milking affect the concentration of milk constituents. **Journal of dairy science**, v.88, p.3186-3200, 2005. DOI: 10.3168/jds.S0022-0302(05)73002-2.

NIXON, M.; BOHMANOVA, J.; JAMROZIK, J.; SCHAEFFER, L.R.; HAND, K.; MIGLIOR, F. Genetic parameters of milking frequency and milk production traits in Canadian Holsteins

milked by an automated milking system. **Journal of Dairy Science**, v.92, p.3422-3430, 2009. DOI: 10.3168/jds.2008-1689.

PETTERSSON, G.; SVENNERSTEN-SJAUNJA, K.; KNIGHT, C.H. Relationships between milking frequency, lactation persistency and milk yield in Swedish Red heifers and cows milked in a voluntary attendance automatic milking system. **Journal of Dairy Research**, v.78, p.379-384, 2011. DOI: 10.1017/S0022029911000471.

SOBERON, F.; LUKAS, J.L.; VAN AMBURGH, M.E.; CAPUCO, A.V.; GALTON, D.M.; OVERTON, T.R. Effects of increased milking frequency on metabolism and mammary cell proliferation in Holstein dairy cows. **Journal of Dairy Science**, v.93, p.565-573, 2010. DOI: 10.3168/jds.2009-2345.

SVENNERSTEN-SJAUNJA, K.M.; PETTERSSON, G. Pros and cons of automatic milking in Europe. **Journal of Animal Science**, v.86, p.37-46, 2008. Supplement 1. DOI: 10.2527/jas.2007-0527.

THOMSEN, P.T.; MUNKSGAARD, L.; TOGERSEN, F.A. Evaluation of a lameness scoring system for dairy cows. **Journal of Dairy Science**, v.91, p.119-126, 2008. DOI: 10.3168/jds.2007-0496.

TYRRELL, H.F.; REID, J.T. Prediction of the energy value of cow's milk. **Journal of Dairy Science**, v.48, p.1215-1223, 1965. DOI: 10.3168/jds.S0022-0302(65)88430-2.

WALL, E.H.; MCFADDEN, T.B. The milk yield response to frequent milking in early lactation of dairy cows is locally regulated. **Journal of Dairy Science**, v.90, p.716-720, 2007. DOI: 10.3168/jds.S0022-0302(07)71555-2.

WIKTORSSON, H.; PETTERSSON, G.; OLOFSSON, J.; SVENNERSTEN-SJAUNJA, K.; MELIN, M. Welfare status of dairy cows in barns with automatic milking. Relations between the environment and cow behaviour, physiologic, metabolic and performance parameters. In: REPORT EU PROJECT IMPLICATIONS OF THE INTRODUCTION OF AUTOMATIC MILKING ON DAIRY FARMS, 2003, Uppsala. p.1-46. Available at: <http://www.automaticmilking.nl>. Accessed on: July 30 2014.

WHOLE milk: determination of milkfat, protein and lactose content: guidance on the operation of mid-infrared instruments. Brussels: International Dairy Federation, 1996. 12p.

WOOD, P.D.P. Algebraic model of the lactation curve in cattle. **Nature**, n.216, p.164-165, 1967. DOI: 10.1038/216164a0.