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# Investigating the genetic background of novel behavioral indicators of robotic milking efficiency in North American Holstein cattle

Hugo Bérat, <sup>1,2</sup> • Nicolas Gengler, <sup>2</sup> • Jacob M. Maskal, <sup>1</sup> • Jacquelyn P. Boerman, <sup>1</sup> • and Luiz F. Brito <sup>1</sup>Department of Animal Sciences, Purdue University, West Lafayette, IN 47907 <sup>2</sup>TERRA Teaching and Research Center, Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium

# **ABSTRACT**

The adoption of automated milking systems (AMS) across worldwide dairy farms has grown considerably over the last few decades. Automated milking systems contribute to reducing labor costs, increasing milk performance, improving cow welfare, and generating large-scale data on a routine basis that can be used for deriving novel traits for breeding purposes. Therefore, the primary objectives of this study were to (1) derive behavioral traits from AMS data and assess their phenotypic variability during lactation in US Holstein cattle, and (2) estimate variance components and genetic parameters for these traits. Daily AMS records from 5,645 US Holstein cows, collected at 36 robotic milking stations between 2018 and 2021, were analyzed. Evaluated traits included average milking time (AMT, min), total milking time (TMT, min), time interval between milkings (INT, h), number of attempted visits to the AMS (NoV), number of successful entries within the AMS (NSE), percentage of successful milkings (PSM, %), and cow preference consistency score (PCS, score unit). Variance components and genetic parameters were estimated using repeatability models with the restricted maximum likelihood method. The heritability estimates were similar between the 2 models for most traits: 0.46 versus 0.46, 0.27 versus 0.28, 0.08 versus 0.10, 0.10 versus 0.10, 0.10 versus 0.11, and 0.05 versus 0.06, for AMT, TMT, INT, NoV, NSE, and PSM, respectively. However, a notable difference was observed for PCS, with heritability estimates of 0.09 and 0.24 depending on the model fitted. The SE for the heritability ranged from 0.001 to 0.03. Repeatability estimates were 0.74 to 0.71 (AMT), 0.52 to 0.49 (TMT), 0.34 to 0.27 (INT), 0.29 to 0.25 (NoV), 0.29 to 0.30 (NSE), 0.20 to 0.18 (PSM), and 0.55 to 0.53 (PCS). Positive genetic correlations were observed for trait pairs AMT-PSM (0.38–0.35),

Received August 15, 2024. Accepted March 24, 2025. INT-PSM (0.71–0.64), INT-PCS (0.50–0.40), and PSM-PCS (0.37), whereas other correlations were unfavorable or near zero. All cow behavioral traits related to AMS efficiency evaluated in this study were found to be heritable, suggesting that their inclusion in selection schemes could contribute to improving dairy cow milking efficiency and welfare in dairy farms using AMS. Future studies will model these traits using random regression models and estimate their genetic correlation with other relevant traits in dairy breeding programs. **Key words:** automated milking systems, behavioral genetics, dairy cattle, genetic parameters, milking robots

#### INTRODUCTION

The adoption of automated milking systems (AMS; commonly termed milking robots) has become increasingly prevalent on dairy farms worldwide in recent years (Bhoj et al., 2022). In the previous decade, these on-farm systems revolutionized the milking sector and were used to milk over 1.2 million dairy cows in more than 10,000 herds worldwide (Moyes et al., 2014; Dechow et al., 2020). By 2018, more than 25,000 AMS-equipped farms had been counted worldwide, with ~38,000 individual devices installed (Penry et al., 2018). Since 2020, this figure has exceeded 50,000 AMS counted on several continents, with a concentration of over 90% in Europe, ~9% in North America, and 1% in Asia, a number that is constantly increasing according to reports from various manufacturers (Simões Filho et al., 2020; Cogato et al., 2021). The main goals of using AMS are to increase overall farm efficiency by reducing labor intensity, labor expenditure, promoting animal welfare (e.g., giving cows the choice of the time to be milked, and thus alleviating udder pressure and discomfort in high-producing cows), and collecting a large volume of daily information related to milk production, behavior, health, and milk quality (King et al., 2021; Pedrosa et al., 2023). Several traits such as the volume of milk produced, milking speed, SCC (Castro et al., 2018), electrical conductivity (EC; Wethal and Heringstad, 2019), and milking box time can be auto-

<sup>\*</sup>Corresponding author: britol@purdue.edu

matically recorded and stored by AMS devices (Brito et al., 2020; Pedrosa et al., 2023). In addition, behavioral and personality traits of dairy cattle can be derived from AMS data. These traits have been shown to influence both the milking process and milk volume harvested by AMS units and most of them tend to be under genetic control (Siewert et al., 2018; Wethal and Heringstad, 2019; Brasier et al., 2023). Furthermore, beyond cow behavior, AMS data allows for the derivation of additional traits related to cow efficiency, such as milk production per quarter and udder conformation. For instance, udder conformation can be assessed using cartesian coordinates recorded by AMS units (Medeiros et al., 2024), providing detailed insights into the anatomical features affecting the milking process.

Personality traits in animals can be defined as individual behavioral variations that tend to remain stable over time and under different environmental conditions (Hedlund and Løvlie, 2015; Finkemeier et al., 2018; Foris et al., 2024). The study of these traits is of paramount importance for both productive efficiency and animal welfare, as they directly influence how animals react and interact with their environments (Finkemeier et al., 2018). Personality plays a crucial role in the voluntary participation of cows in AMS milking activities (Brasier et al., 2023). Furthermore, the identification of behavioral traits in cows, such as milking failure (MFAIL) or milking refusals (MREF), could play an important role in the amount of milk harvested over time, which has been reported to range from 2,500 to 3,000 L per AMS unit per day (Dechow et al., 2020). Milking failures could be due to the cow behavior, but other factors may include poor cow posture, udder and teat conformation, or equipment failures. Milking refusals represents the number of times that a cow approached the milking robot unit but was not allowed to be milked. This is mainly due to very frequent visits or repeated visits with short intervals in between them. Although there are studies in the literature reporting heritability estimates for general milking traits, few studies have focused on cow behavior uniquely derived from AMS, such as MREF, MFAIL, and cow preference for certain AMS units.

Cows make multiple decisions on a daily basis in farms with AMS, especially when faced with the choice of multiple AMS units for milking (Løvendahl and Buitenhuis, 2022). In general, cows milked by AMS can choose to be milked at different times of the day, establish a specific passage order through the milking robots, develop milking routines, show preference for certain AMS stations, or show greater flexibility on the AMS units used. Behavioral responses are influenced by the animal's environmental conditions and their genetics. Therefore, when routines are maintained over long periods, they reveal a certain consistency, suggesting the existence of a behavioral basis determined by genetic factors (Løvendahl et al., 2016; Brasier et al., 2023). Selecting individuals according to

their behavior and milking efficiency could therefore enhance productivity and efficiency in farms using AMS (Dechow et al., 2020). The heritability estimates ( $\pm$ SE) reported in the literature for MFAIL and MREF are 0.02  $\pm$  0.01 and 0.09  $\pm$  0.01 with a genetic correlation between them of 0.25  $\pm$  0.02 (Pedrosa et al., 2023). As behavioral traits tend to be heritable in cattle populations (Chang et al., 2020; Alvarenga et al., 2021; Pinto et al., 2025), they can be altered through selective breeding.

Combining genomic data with AMS records can enable the derivation and implementation of genomic selection for various novel traits. In this study, we focus specifically on behavioral indicators related to milking efficiency in AMS, including average milking time (AMT), total milking time (TMT), milking interval (INT), number of robot visits (NoV), number of successful entries (NSE), percentage of successful milkings (PSM) and preference consistency score (PCS). These traits are closely linked with the way cows interact with and adapt to the AMS environment. In this context, the primary objectives of this study were to derive cow behavioral indicators of AMS efficiency, assess their phenotypic variability throughout lactation, and estimate variance components and genetic parameters for the derived traits in North American Holstein cattle. These genetic parameters are important for the design or refinement of selection indexes to improve dairy cattle production efficiency, health, and welfare in precision farms, and thus, the long-term sustainability of the dairy industry.

# **MATERIALS AND METHODS**

### **Ethics Statement**

Approval from an Institutional Animal Care and Use Committee was not required for the purpose of this study, as all necessary information was obtained from preexisting databases.

# **Data Collection**

A total of 7,136,116 phenotypic records, from 5,645 Holstein cows, collected between 2018 and 2021 on a large commercial dairy farm located in Indiana, was available for this study (before quality control). The cows were milked by 36 Lely Astronaut robotic milking units (Maassluis, the Netherlands). Information per visit to the AMS units was stored in the Lely database and retrieved through the Purdue Animal Sciences Research Data Ecosystem platform (Boerman et al., 2025). The variables used in this study included cow identifiers; birth and calving dates; milking start and end times; the number of successful, rejected, and failed milkings for each cow, as well as the AMS identifiers collected for

each milking. All the cows were assigned to a pen at the beginning of their lactation but could be moved to a different pen with access to new AMS units throughout the observation period. Cows had access to 3 different AMS units per pen, and all pens had the same number of AMS units. Daily records from AMS were used to derive novel indicators of dairy cow behavior, such as the AMT (min) and TMT (min), INT (h), NoV, PSM (%), NSE, and PCS (as suggested by Løvendahl and Buitenhuis, 2022). In this study, "milking time" (average and total) refers specifically to the length of time during which milk is actively flowing, from the moment milk starts flowing until it stops. This definition is distinct from that of "milking box time," commonly used in the literature, which refers to the total time spent by a cow in the AMS unit, including preparation and postmilking phases. By focusing on milking time rather than time in the AMS box, we aimed to capture the efficiency of the milking process itself.

#### **Trait Definitions**

The NoV trait reflects the total number of visits to the AMS per day. A visit is defined as the voluntary approach of a cow to an AMS unit regardless of the milking outcome. We used 2 traits (MFAIL and MREF) previously reported in the literature by Pedrosa et al. (2023) to define the NoV trait for quantifying the level of engagement of cows with the AMS units, along with the number of successful milkings (RMILK), the animal identifiers, and the time stamp of each event. Both MFAIL and RMILK indicate that the cow entered the AMS unit, whereas MREF indicates that the cow had an AMS entry refused. Therefore, NoV was defined as

$$NoV = RMILK + MREF + MFAIL.$$

To assess cow behavioral response to AMS milking efficiency, NSE was calculated as

$$NSE = NoV - MREF$$
,

where NSE represents the number of successful AMS entries for each cow per day, and it indicates the milking sessions authorized within the AMS for each cow over a 24-h period. A successful entry can be considered as a milking that initiated and completed, that initiated but stopped before completion, or a milking event that was never initiated. In this case, MREF are deliberately not included in the calculation, as these events are distinct and could be influenced by other specific behavioral factors, such as social interactions and stress or individual variations in cow daily habits. Therefore, NSE quantifies the cow's adaptation to visiting the AMS units.

Accurate assessment of milking session duration (i.e., milking time and not box time as previously explained)

may be essential for understanding and interpreting cow welfare in AMS. We quantified 2 aspects of milking duration: AMT and TMT in minutes per cow per day. To do this, the start and end times of each milking session were used. A simplified approach was employed by attributing the entire milking session to the milking start date. This was done to analyze records with milking sessions extending beyond midnight and overlapping 2 d. The AMT was calculated by dividing the sum of the durations of all milking sessions for each day by the number of milking sessions, as shown in the following formula:

$$AMT = \frac{\sum_{i=1}^{n} Duration_{i}}{n},$$

where *n* represents the number of milking sessions.

The trait TMT was calculated as the sum of the durations of all milking sessions per day, as in the following:

$$TMT = \sum_{i=1}^{n} Duration_{i}$$
.

The trait AMT provides information on the milking efficiency of each cow, and TMT reflects the overall workload and time required for milking the cow. These 2 indicators are complementary and could be useful in assessing animal comfort in relation to the AMS, and thus, define whether certain animals require special attention in relation to their efficiency using these milking systems.

The trait INT was found to be associated with milk production and welfare, as research suggests that it is closely linked to the cow social dominance in the herd (Hogeveen et al., 2001; Jacobs and Siegford, 2012; Santos et al., 2018). The INT was calculated as

$$INT_n = T_{start, n} - T_{end, n-1}$$
.

The time interval between the end time of milking  $(T_{end, \, n-1})$  and the start time of milking  $(T_{start, \, n})$  was calculated for each pair of milking sessions. Events with overlapping midnight milkings had each part of the time assigned to the respective date. A logarithmic transformation of INT was performed to meet the assumption of normality of the residuals from the models used.

Percentage of successful milking is a variable used to evaluate cow success in AMS, calculated as the percentage of the total number of RMILK in relation to daily NoV:

$$PSM = \frac{RMILK}{NoV}.$$

A high PSM value indicates a high proportion of successful milkings compared with the number of failed or rejected milking attempts.

**Table 1.** Overall descriptive statistics of the derived cow behavioral traits in North American Holstein cattle used for the estimation of genetic parameters<sup>1</sup>

Trait	No. of cows	No. of records	Mean	SD	Minimum	Maximum
Average time of milking (min)	4,075	1,732,378	7.23	1.93	2.08	12.08
Total time of milking (min)	4,075	1,732,378	20.22	6.56	3.32	36.4
Milking time interval (h) <sup>2</sup>	4,075	1,732,378	0.93	0.15	0.56	1.21
Number of visits	4,077	1,729,928	3.65	1.97	1	9
Number of successful entries	4,077	1,746,982	2.84	0.88	1	4
Percentage of successful milking	4,077	1,750,708	83.3	22.87	17.14	100
Preference consistency score	4,249	142,491	0.42	0.20	0.087	0.95

<sup>&</sup>lt;sup>1</sup>Only records from genotyped animals that passed the data editing and quality control, as described in Supplemental Tables S1 and S2, are summarized in this table.

Lastly, we calculated PCS of the cows for the different AMS units present in each pen as proposed by Løvendahl and Buitenhuis (2022). The PCS measures the cow's preference to be milked by a certain AMS unit over time. We define a period of 15-d segments to capture this behavioral pattern (as suggested by Løvendahl and Buitenhuis, 2022), in which each segment represents a distinct interval for the calculation of PCS. For each specific lactation segment, the number of successful visits per cow to each AMS unit was calculated, and the most frequented AMS unit for each cow was identified and assumed to be the preferred AMS unit over that specific time period. The value for PCS was then calculated based on the actual frequency of visits to this preferred robot relative to the total number of successful visits, adjusted to account for the total number of AMS units available:

$$PCS = 1.00 \times \frac{\left[actual\ frequency - \left(\frac{1}{number\ of\ choices}\right)\right]}{\left[1 - \left(\frac{1}{number\ of\ choices}\right)\right]}.$$

The PCS is a standardized measure that reveals the cow's preference for a particular milking robot when the score is close to 1, whereas a score close to 0 indicates no preference for any AMS unit.

# **Data Editing and Quality Control**

All phenotypic data were processed using the R software (R Core Team, v 4.2.2). The raw data obtained was filtered, edited, and used to create 2 data subsets for further analyses. Dataset 1 contained daily measurements from the AMS (i.e., records for various parameters taken daily from 2018 to 2021). Dataset 2 contained records collected at individual milking events. All the animals initially included in the dataset 2 were also present in the dataset 1. The quality control steps that were carried out

for the 2 datasets, along with the number of phenotypic records and animals remaining after the quality control are shown in Supplemental Tables S1 and S2 (see Notes). Subsequently, 7 traits were derived. The traits derived from dataset 1 were NoV, NSE, and PSM, whereas the traits derived from dataset 2 included AMT, TMT, INT, and PCS. All traits were measured up to 350 d. The records for all traits, except PCS, were presented per day and per animal. Preference consistency score differed from the other traits because it was based on a score assigned to the different AMS units. When plotting the distribution of phenotypic values, records were summarized by calculating a daily average per DIM (or lactation segment in the case of PCS) within parity to facilitate the visualization of general trends. Phenotypic records deviating 3.5 SD from the mean were eliminated from further analyses. After this filtering step, the 2 datasets were merged for subsequent genetic analyses. Only animals with more than 10 records per parity were included in the analyses. The descriptive statistics for each trait, including the final number of phenotypic records and animals retained after quality control, are presented in Table 1.

# **Genomic Information**

All analyses were based on genomic information as pedigree information was not available. A total of 5,645 genotyped animals with genomic information for 62,029 SNPs were available for this study. Genotyped animals with no phenotypic records, after the phenotypic data editing and quality control, were removed from subsequent analyses. Table 1 presents the number of individuals included in the genetic analyses for each analyzed trait. Animals were genotyped using different SNP chip panels and imputed to a common SNP panel density as described in Chen et al. (2023). All the genomic analyses were performed using the BLUPF90 family of programs (Misztal et al., 2024). During the quality control stage, SNPs with a call rate below 90% and a minor allele frequency

<sup>&</sup>lt;sup>2</sup>Variable was log10 transformed to meet the model assumption of normality of the residuals.

(MAF) below 0.05 were eliminated. A total of 4,747 SNPs were eliminated due to low MAF, but none were removed due to the low call rate criteria. Furthermore, no animals were excluded due to low call rate (<90%). No parent-progeny conflicts were identified due to the lack of pedigree information. After these genomic data quality checks, 57,282 SNPs were used for the analyses. The remaining SNPs had an average allele frequency of 0.713. The initial rate of missing genotypes was 7.65%, but it was reduced to 0.00% after the quality control.

#### Statistical Models and Genetic Parameter Estimation

The variance and covariance components  $\left(\sigma_a^2, \sigma_{pe}^2, \text{ and } \sigma_e^2\right)$  and the genetic parameters (i.e., heritability  $\left[h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}\right] \text{ and repeatability} \left[t = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}\right]$ 

for AMT, TMT, INT, NoV, NSE, PSM, and PCS were estimated using the BLUPF90 software suite (Misztal et al., 2024). We used the expectation-maximization algorithm for the first iterations and then the average-information restricted maximum likelihood (EM-REML and AI-REML, respectively) method. The single-trait repeatability animal models fitted can be described as:

$$y = Xb + Za + Wpe + e$$

where y represents the vector containing the repeated phenotypic records for each trait analyzed, b is the vector of fixed effects containing DIM, parity number (1, 2, 3, or 4+), the enclosures (i.e., pens) containing the AMS units (from 1 to 12), and 19 contemporary groups represented by the calving year-season (with seasons defined as September to November, March to May, June to August, December to February); a is the vector of random additive genetic effects with  $\mathbf{a} \sim \mathrm{N} \left( 0, \; \mathbf{G} \sigma_a^2 \right)$ , where  $\mathbf{G}$ represents the genomic relationship matrix based on the first method proposed by VanRaden (2008) and  $\sigma_a^2$  is the additive genetic variance for each trait; pe is the vector of random permanent environmental effects in y with  $\mathbf{pe} \sim \mathrm{N}\left(0, \ \mathbf{I}\sigma_{pe}^2\right)$ , where  $\mathbf{I}$  represents an identity matrix and  $\sigma_{ne}^2$  the variance associated with the permanent environmental effects; e is the vector containing the random residual effects with  $\mathbf{e} \sim \mathrm{N}\left(0, \; \mathbf{I}\sigma_e^2\right)$ , where I represents an identity matrix and  $\sigma_{\rho}^2$  the residual variance. **X**, **Z**, and **W** are the incidence matrices linking phenotypic observations of each trait to the fixed effects, random effects due to additive genetic effects, and permanent environmental effects, respectively.

Bivariate linear models were also fitted to estimate the genetic correlations of all pairs of traits based on the following equations:

$$\mathbf{y}_1 = \mathbf{X}_1 \mathbf{b}_1 + \mathbf{Z}_1 \mathbf{a}_1 + \mathbf{W}_1 \mathbf{p} \mathbf{e}_1 + \mathbf{e}_1 \text{ and } \mathbf{y}_2$$
  
=  $\mathbf{X}_2 \mathbf{b}_2 + \mathbf{Z}_2 \mathbf{a}_2 + \mathbf{W}_2 \mathbf{p} \mathbf{e}_2 + \mathbf{e}_2$ .

These models included the same fixed and random effects as those previously described for each trait. Written in a different way into a single system of equations, the genetic parameters were obtained by the following multitrait repeatability model:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & 0 \\ 0 & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{W}_1 & 0 \\ 0 & \mathbf{W}_2 \end{bmatrix} \begin{bmatrix} \mathbf{p} \mathbf{e}_1 \\ \mathbf{p} \mathbf{e}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix},$$

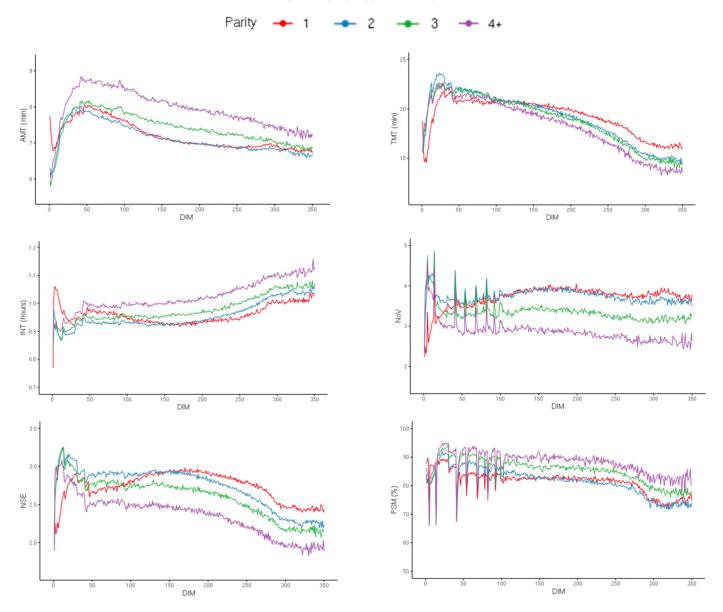
where y<sub>i</sub> represents the vector of repeated phenotypic observations for the traits considered 2 by 2; b<sub>i</sub> is the vector for fixed effects that are identical to those described for the single-trait analyses; a<sub>i</sub> and pe<sub>i</sub> are the vectors containing additive genetic and environmental permanent random effects, respectively, of phenotypic observations of animals included in v<sub>i</sub>; e<sub>i</sub> is a vector containing random residual effects. Incidence matrices linking observations to fixed and random effects are denoted respectively by  $X_i$ ,  $Z_i$ , and  $W_i$ . In this multitrait model (MT), it was assumed that E[y] = Xb;  $var(a_{MT}) = G_0 \otimes A$ ;  $var(pe_{MT}) =$  $P_0 \otimes I$ ; and  $var(e_{MT}) = R_0 \otimes I$ , where E[y] indicates the expected values of the variable y,  $G_0$  is the genomic relationship matrix, A is the covariance matrix for additive genetic effects,  $P_0$  the covariance matrix for the effects of the permanent environment,  $\mathbf{R}_0$  the covariance matrix for residual effects, I is an identity matrix and ⊗ represents the operator for the Kronecker product of the matrices. The vectors of random effects a and pe and the vector of residual effects e were assumed to be uncorrelated.

For these analyses, 2 sets of distinct repeatability models were fitted: the first accounting for the effect of the permanent environmental effect between parities, and the second fitting both the effects of the permanent environment within and across parities. In the second case, animal identifiers and parities were concatenated to create a new variable that uniquely accounts for each animal at each parity. In this way, the effect of the permanent environment specific to each parity was considered for each animal.

# **RESULTS**

# Phenotypic Variability Across DIM and Within Parities

Figure 1 presents the phenotypic averages of the behavioral traits AMT, TMT, INT, NoV, NSE, and PSM de-

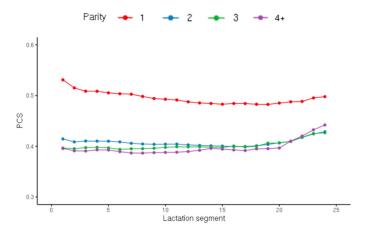


**Figure 1.** Phenotypic average for average milking time (AMT), total milking time (TMT), number of visits (NoV), number of success entries (NSE), time milking interval (INT) and percentage of milking success (PSM) and preference consistency score (PCS) in estimated in North American Holstein cattle in first, second, third, and fourth parity.

rived from the daily data records across DIM and within parity. Figure 2 shows the phenotypic averages for PCS by lactation segment within each parity. For each trait, with exception of INT, NoV, NSE, and PCS, which stood out slightly for first-parity cows, the appearance of the curves remained consistent between the different parities throughout lactation.

For all parities combined, AMT showed an initial upward trend, peaking at around 50 DIM, followed by a gradual, steady decline. The AMT for cows of the first 3 parities were similar, with averages ranging from 7.13 to

7.41 min (SD = 0.38 to 0.43). In contrast, higher values were observed for animals from the fourth parity onward, with an average of 7.94 min (SD = 0.52). For TMT, the pattern of progression was similar to that of AMT, with the difference being the evolution over the DIM between parities. As with AMT, TMT increased gradually during the first week of lactation. The AMT peak was reached in the fifth week of lactation before decreasing steadily. However, for TMT, the order of the curves observed between parities differed over time. Indeed, during the first 100 DIM, TMT ( $\pm$ SD) decreased in the following parity



**Figure 2.** Phenotypic average for preference consistency score (PCS) by lactation segment estimated for first-, second-, third-, and fourth-parity North American Holsteins.

order: parity 2 (19.1  $\pm$  2.53), parity 3 (18.9  $\pm$  2.51), parity 4+ (18.3  $\pm$  2.70), and parity 1 (19.2  $\pm$  1.78); beyond this, the order became parity 1, parity 2, parity 3, and parity 4+. Finally, INT had higher values during the first week of lactation, before gradually decreasing until the second week and increasing again, this time slowly, from this stage onward. For parities 2, 3, and 4+, the curves are relatively constant, with a marked position from the start of the lactation. Parity 1 differed, however, as there was more pronounced variation from the beginning of the lactation and a gradual change between 100 and 150 DIM, resulting in a shorter time interval than the other parities. The average INT was 0.96 ( $\pm 0.03$ ) for parity 1, 0.95 ( $\pm 0.04$ ) for parity 2, 0.97 ( $\pm 0.04$ ) for parity 3, and 1.02 (±0.05) for parity 4+. The majority of milking events, for all parities combined, took place between 1600 h and 1000 h. The longest time intervals between milkings were between 1000 h and 1600 h.

Concerning NoV, the average number of attempts per parity was 3.72 ( $\pm 0.26$ ) for parity 1, 3.74 ( $\pm 0.18$ ) for parity 2, 3.34 ( $\pm 0.22$ ) for parity 3, and 2.87 ( $\pm 0.284$ ) for parities 4+. First-parity cows showed different aspects from the other parities, with less pronounced fluctuation in AMS visits in the first week and during the first 10 wk. Regarding NSE, values increased in early lactation for all parities. For multiparous cows (parity 2:  $2.74 \pm$ 0.26; parity 3:  $2.60 \pm 0.26$ ; parity 4+:  $2.36 \pm 0.27$ ) the peak was reached after the first 2 wk. For primiparous cows (2.73  $\pm$  0.18), the peak shifted 2 wk later to 171 DIM. Shortly after these peaks, all parities combined, a more pronounced decrease was observed up to 50 DIM, before showing a gradual decline over time for multiparous cows. As observed for NoV, first-parity cows showed a different behavior within the AMS, compared with other parities. The behavior of primiparous cows

slightly increased after the drop reached around 50 DIM, whereas other parities showed a decrease in the phenotypic trait. Overall, second and third parity cows entered the AMS units more times than first-parity cows during the first 100 DIM. At ~150 DIM, however, first and second parity cows had similar numbers of entries into the AMS units. This trend changed after 150 DIM, when first-parity cows had more entries than later-parity cows. In contrast, parity 4+ cows had the fewest AMS entries over almost the whole lactation. Regarding PSM (parity 1:  $81.3 \pm 4.04$ ; parity 2: 81.4 $\pm$  4.94; parity 3: 85.0  $\pm$  4.58; parity 4+: 87.8  $\pm$  4.26), rates fluctuated for all parities between 65% and 95% during the first 7 wk up to 50 DIM, and between 75% and 95% between wk 6 and 15 as cows approached the first 100 DIM. After these fluctuations for the remainder of the evaluation period, PSM rates remained stable before decreasing during the last 50 DIM. In addition, greater variability was observed for the 4+ parity than for the others. Finally, the PCS revealed variability in the use of available milking robots (Figure 2). Once again, the first-parity cows showed a more pronounced preference for available robots during milking, whereas cows in later parities appeared less selective. The average preference score for parity 1 (0.49  $\pm$  0.01) was significantly higher than for the other parities over the whole lactation period. The PCS decreased during the first 30 DIM, then throughout lactation, PCS remained consistently lower than it was during the first 30 d. Compared with cows in other parities, initial scores were similar and constant throughout lactation (parity 2:  $0.41 \pm 0.01$ ; parity 3:  $0.40 \pm 0.01$ ; parity 4+:  $0.39 \pm$ 0.01). However, parity 4+ individuals showed a greater increase in PCS at the end of lactation, which slightly distinguished them from parities 2 and 3.

# Repeatability, Heritability, and Variance Components of Cow Behavioral Traits

The various genetic and statistical parameters estimated, such as heritability, repeatability, variance components, and Akaike information criterion (AIC) for the different cow behavioral traits evaluated are shown in Table 2. The results presented considered a model fitting 2 permanent environmental effects (within and across lactations) due to the lower AIC values, showing a better fit of data variability than the model with a single environmental effect (Supplemental Table S3, see Notes). To facilitate subsequent interpretation, heritability estimates below 0.15 were considered as low, those between 0.15 and 0.30 as moderate, and those above 0.30 were considered as low, those between 0.30 and 0.60 as moderate, and those above 0.60 as high.

**Table 2.** Additive genetic variance  $\left(\sigma_a^2\right)$ , permanent environmental variance between parities  $\left(\sigma_{pe}^2\right)$ , permanent environmental variance within parities  $\left(\sigma_{pe2}^2\right)$ , residual variance  $\left(\sigma_e^2\right)$ , heritability (h<sup>2</sup> ± SE), repeatability (t ± SE), and Akaike information criterion (AIC) of cow behavioral traits derived from data obtained from automated milking system in North American Holstein cattle

Trait <sup>1</sup>	Parity	$\sigma_a^2$	$\sigma_{pe}^2$	$\sigma^2_{\it pe2}$	$\sigma_e^2$	$h^2$	t	AIC
AMT	1–6	1.75	0.72	0.34	1.02	$0.46 \pm 0.02$	$0.74 \pm 0.01$	43,285,107.54
TMT	1–6	11.25	5.67	5.17	20.24	$0.27 \pm 0.01$	$0.52 \pm 0.01$	88,645,192.63
INT	1–6	0.0021	0.0017	0.0049	0.017	$0.08 \pm 0.01$	$0.34 \pm 0.01$	-18,811,306.51
NoV	1–6	0.33	0.31	0.37	2.42	$0.10 \pm 0.01$	$0.29 \pm 0.01$	6,510,470.94
NSE	1–6	0.070	0.074	0.12	0.47	$0.10 \pm 0.01$	$0.29 \pm 0.01$	31,673,689.80
PSM	1–6	26.83	33.21	41.38	402.47	$0.05 \pm 0.01$	$0.20 \pm 0.004$	134,026,410.47
PCS	1–6	0.0044	0.0076	0.015	0.022	$0.09 \pm 0.01$	$0.55 \pm 0.01$	-14,954,900.58
PCS	1	0.00067	0.0084	0.0059	0.023	$0.018 \pm 0.01$	$0.39 \pm 0.01$	-5,541,312.64
PCS	2	0.0011	0.013	0.0088	0.020	$0.026 \pm 0.001$	$0.53 \pm 0.01$	-5,281,107.93
PCS	3	0.0019	0.0165	0.0115	0.0213	$0.037 \pm 0.02$	$0.58 \pm 0.01$	-2,699,536.76
PCS	4+	0.0003	0.0178	0.0181	0.022	$0.005\pm0.03$	$0.62\pm0.01$	-1,460,013.49

<sup>1</sup>AMT = average time of milking; TMT = total time of milking; INT = time interval between milkings; NoV = number of visits; NSE = number of successful entries into the AMS units; PSM = percentage of milking success; PCS = preference consistency score.

The results show a diverse disparity between the traits studied in terms of heritability and repeatability estimates, suggesting varying levels of genetic and environmental influence on the studied traits. Average milking time and TMT are highly (0.46) and moderately (0.27) heritable, respectively, suggesting a substantial genetic component (Table 2). In contrast, the other 5 traits had lower heritability estimates (ranging between 0.05 and 0.10), highlighting a more significant environmental influence on their phenotypic variability. With regards to the repeatability estimates, AMT (0.74) for cows of parity 1 to 6 and PCS (0.55) for cows of parity 4+ showed relatively high values. Conversely, other traits, such as TMT (0.52) and INT (0.34) for cows in parities 1 to 6, and PCS for cows in parities 2 and 3, showed moderate values, suggesting a certain stability and consistency of these traits over time. Conversely, NoV (0.29), NSE (0.29), and PSM (0.20), had lower repeatability values, this time suggesting greater variability in trait performance over time. Estimates of repeatability and heritability for all traits were compared between the 2 models used. The results were similar in both situations, except for PCS for all parities combined from 1 to 6, for which the model with a single permanent environmental effect yielded a significantly higher heritability estimate (0.24). With regards to the variance components, high levels of additive genetic variance were observed for all traits, indicating that substantial genetic progress can be achieved through direct genetic selection. These findings are reflected in the 2 models evaluated.

# **Genetic Correlations**

Estimates of genetic correlations for all traits are presented in Table 3. The results take into account the model

involving 2 permanent environmental effects, due to lower and significantly better AIC values, showing a better model fit of the data. The results for the model with a single permanent environmental effect are presented in Supplemental Table S4 (see Notes). The genetic correlations between traits derived from AMS data revealed significant and complex relationships. Correlations above 0.70 (or below -0.70) were considered as high, those between 0.30 and 0.70 (or between -0.30 and -0.70) as moderate, and those between -0.30 and 0.30 as low.

Three pairs of traits showed high and positive genetic correlations. The high and positive genetic correlation observed between NoV and NSE indicates that cows that frequently visit the AMS are more likely to be able to enter the AMS units. Second, the high and positive genetic correlation observed between AMT and TMT suggests that cows with longer average milking times tend to accumulate a higher total milking time. Third, a highly positive, though less pronounced, genetic correlation between PSM and INT shows that cows with longer milking intervals tend to have increased milking success when they do visit the AMS units. High and negative genetic correlations were also observed. For example, a high genetic correlation was observed between INT and NoV, as well as between INT and NSE, revealing that cows that visit the AMS less often have longer milking intervals due to fewer entries into the AMS units. Furthermore, the strong negative genetic correlations between PSM and NoV and NSE suggest that cows that frequently visit and enter the AMS do not necessarily achieve a proportionally high milking success rate.

As shown in Table 3, 7 pairs of moderate correlations were also observed. Individuals with a longer AMT tend to have a higher PSM, but fewer visits and therefore fewer entries into the AMS. Individuals expressing a preference for a certain AMS unit tend to show longer

**Table 3.** Genetic correlations (±SE) table for derived traits from data obtained by the automated milking system for North American Holstein cattle with permanent environment effect within and between parities

Trait <sup>1</sup>	TMT	INT	NoV	NSE	PSM	PCS
AMT	$0.84 \pm 0.02$	$0.24 \pm 0.05$	$-0.36 \pm 0.01$	$-0.34 \pm 0.05$	$0.38 \pm 0.05$	$-0.045 \pm 0.06$
TMT	_	$-0.26 \pm 0.05$	$0.12 \pm 0.05$	$0.22 \pm 0.05$	$0.012 \pm 0.06$	$-0.20 \pm 0.07$
INT	_	_	$-0.90 \pm 0.02$	$-0.98 \pm 0.05$	$0.71 \pm 0.04$	$0.50 \pm 0.06$
NoV	_	_	_	$0.92 \pm 0.01$	$-0.94 \pm 0.01$	$-0.44 \pm 0.06$
NSE	_	_	_	_	$-0.72 \pm 0.04$	$-0.35 \pm 0.07$
PSM	_	_	_	_	_	$0.37\pm0.07$

<sup>1</sup>AMT = average time of milking; TMT = total time of milking; INT = time interval between milkings; NoV = number of visits; NSE = number of successful entries; PSM = percentage of milking success; PCS = preference consistency score.

intervals between milking events and a higher PSM. Furthermore, at the genetic level, cows with a stronger preference for a specific AMS tended to visit the robots less frequently in general and therefore entered them fewer times. Moreover, 7 pairs of traits showed weak or nonsignificant correlations (Table 3). Of these associations, TMT was the trait least correlated with the others at the genetic level. A longer INT tends to be associated with reduced TMT. For AMT, the genetic correlation with INT was positively weak, illustrating a different underlying dynamic to that of TMT. Furthermore, a longer TMT seems to be weakly genetically correlated with an increase in the number of visits, and with the number of successfully initiated entries. Finally, genetic correlations between TMT and PSM, as well as between AMT and PCS, although positive and negative, respectively, remained low, indicating a weak genetic relationship between these traits. In both sets of models (fitting 1 or 2 permanent environmental effects), genetic correlations showed similar general trends.

#### **DISCUSSION**

Automated milking systems allow automation of the milking process and play a crucial day-to-day role in improving breeding practices and cattle herd management. These AMS units are integral in the collection of large datasets, as they are capable of precisely recording numerous variables about performance, behavior, and health indicators (e.g., EC) on an individual animal. The effectiveness of AMS is greatly influenced by the cows' adaptation to its use and their learning behavior, which are partially determined by the genetic background of the animals (Schafberg et al., 2022). Therefore, AMS provide an opportunity in the field of phenomics to derive novel traits for advancing genetic selection for dairy cattle (Dechow et al., 2020; Pedrosa et al., 2023). Several behavioral and milking performance indicators such as milking speed, milking box time, EC, and milk efficiency, have already been evaluated using AMS data (e.g., Santos et al., 2018; Pedrosa et al., 2023). In addition, other traits more related to the temperament of dairy cows, such as the number of refused, missed, or incomplete milkings, have also been the subject of multiple studies (e.g., Vosman et al., 2014; Santos et al., 2018; Brito et al., 2020).

In this study, 7 traits associated with cow behavior in US Holstein cattle were derived based on data routinely recorded by AMS. Previous studies have investigated the genetic background of temperament in dairy cattle from a quantitative genetic perspective or in the context of selection objectives (Gutiérrez-Gil et al., 2008; Jensen et al., 2008; Haskell et al., 2014; Brito et al., 2020). However, many other cow behavior traits can still be derived based on routinely collected data and could be used for refining dairy cattle breeding schemes. To do this, it is essential to determine whether traits are heritable, repeatable, and if there are genetic relationships with each other or with other relevant traits already under selection. In this study, we aimed to derive and evaluate the genetic background of traits that may contribute to the selection of cows optimal for milking with AMS (Wethal and Heringstad, 2019; Chen et al., 2020; Dechow et al., 2020; Piwczyński et al., 2021).

# Phenotypic Behavior of Selected Traits Along the DIM for Dairy Cattle

Milking time of dairy cows was studied in 2 aspects, namely TMT and AMT. These are essential traits to consider when evaluating cow behavior and performance in AMS (Carlström et al., 2013). Milking time represents the time required for milk to flow when the AMS cups are attached to the cow's teats. Milking sessions that are too long can increase stress, discomfort, health problems (Wethal and Heringstad, 2019) such as teat irritation, and the risk of mastitis (Hogeveen et al., 2001; Jiang et al., 2017). Conversely, high milking speed can also affect mammary health (Dechow et al., 2020). Finding the optimum milking duration can help prevent teat lesions. In the present study, the average TMT was 20.22 min, which is comparable to the average of 20.43 min reported

by Dechow et al. (2020). In this study, TMT decreased faster throughout lactation for multiparous cows than for primiparous cows (Figure 1). Others have reported that frequency of AMS visits decreases with age as a function of time (Løvendahl and Buitenhuis, 2022). Furthermore, Siewert et al. (2019) observed that, in free circulation systems, primiparous cows visit the AMS less at the beginning of lactation, as much as multiparous cows in mid lactation, then more at the end of lactation. Our results show similar but staggered trends: the TMT of primiparous cows was initially lower due to reduced milking frequency, then equivalent to that of multiparous cows in mid lactation, before increasing at the end of lactation with higher milking frequency. The trait AMT, which is different in its interpretation but closely related to TMT, has been less studied in the literature. Average milking time could enable us to better capture potentially significant variations at the individual level, including the detection of inefficiencies in the milking process that may be associated with discomfort for cows, which might cause anxiety, pain, or other pathologies in dairy cows. Hogeveen et al. (2001) reported an AMT of 5 min, whereas we observed 7.23 min in this study. Milking box time is another related trait reported in the literature (Carlström et al., 2013; Santos et al., 2018; Wethal and Heringstad, 2019), which is defined as the time the cows spends in the AMS. Milking box time considers not only the time that it takes for milking, but also the time spent on subsidiary operations that may occur before and after the milking, such as the time needed to identify the animal, clean the udder, attach the teat cups, and disinfect the teats. Although defined differently than AMT, Santos et al. (2018), Carlström et al. (2013), and Wethal and Heringstad (2019) observed values of 6.27, 7.40, and 7.46 min, respectively, for milking box time, which is similar to the value of AMT observed in this study (7.23 min). The differences across studies may be due to farm management practices (e.g., animal traffic patterns, type of AMS used), number of recordings, and data collection period.

One of the main benefits of AMS is that it enables cows to milk voluntarily. So, INT offers a nuanced perspective on cow milking practices, including the number of visits to the AMS and consequently, the time interval between milkings. In this study, the majority of milkings, across all parities, took place between 1600 h and 1000 h the next day. The longest intervals between milkings were observed between 1000 and 1600 h, which was expected as cows generally eat or rest during this period (Krohn and Munksgaard, 1993; DeVries and von Keyserlingk, 2005). Santos et al. (2018) observed this pattern for both milking time and longer intervals between milkings. Irregular milking intervals can reduce daily milk production by limiting milk synthesis (Bach and Busto, 2005;

Jacobs and Siegford, 2012). In addition, short intervals increase milking failures and the risk of mammary infections (Hogeveen et al., 2001). An intermediate INT therefore seems optimal. Average INT values reported in the literature range from 9.2 to 9.9 h/d, with an average milking frequency of 2.4 to 2.6 daily visits (Hogeveen et al., 2001; Gäde et al., 2006; Carlström et al., 2013; Santos et al., 2018; Wethal and Heringstad, 2019). In the present study, the average INT was 8.5 h/d, which is equivalent to a milking frequency of 2.8 daily visits. This difference in the results may be attributed to the specific conditions of each farm, including milk yield levels, nutritional management practices, and access to outdoors space. The analytical methods may also differ across studies. For example, Carlström et al. (2013) removed records with intervals of less than 5 h and more than 30 h from the analyses, as these values were considered to be outliers. This was not the case in all other studies. We found a constant increase in INT during lactation, which may be a result of the changes in milk production across lactation (Dzidic et al., 2004; Jacobs and Siegford, 2012). Moreover, we observed that INT for primiparous cows before 25 DIM was higher than for multiparous cows (Figure 1). Santos et al. (2018) also reported a more consistent time interval for first-parity cows. This observation is particularly consistent with the explanations of Siewert et al. (2019), illustrating that in free-flow systems primiparous cows in early lactation produce less milk and visit the AMS less frequently than multiparous cows. This trend reverses over the course of the lactation days. Thus, longer INT may be associated with lower milk production in primiparous cows, as they tend to have fewer milkings compared with multiparous cows, which are milked more frequently and tend to produce more milk. Two other major factors may explain a longer INT at the start of DIM for primiparous cows. First, primiparous cows are not well adapted to the AMS, and second, the hierarchical ranking of the herd, established by older cows, may limit access to these devices for individuals of lower social ranking (Santos et al., 2018). It is worth emphasizing that future studies should evaluate indirect genetic effects (social interactions). However, this was not done in this study due to the current data structure. As our database grows, this will be evaluated in subsequent studies.

Selection for greater milking frequency may contribute to improving both udder health and milk yield (Hogeveen et al., 2001; Jiang et al., 2017; Siewert et al., 2019; Simões Filho et al., 2020). Examples of average milking frequencies reported in the literature are 2.4 (Prescott et al., 1998), 2.7 (Hermans et al., 2003), 2.5 (Nixon et al., 2009), 3.2 (Munksgaard et al., 2011), 2.4 (Carlström et al., 2013), 2.6 (Wethal and Heringstad, 2019), and 2.9 (Løvendahl and Buitenhuis, 2022)

times/d. In our study, the frequency of milking in AMS was evaluated by NSE, which considers the number of entries in the AMS unit, including successful and unsuccessful milking events. The average NSE observed in this study was 2.84 passages through the AMS units, which is similar to the estimates reported in the literature. We also observed that primiparous cows had fewer milking entry successes at the start of lactation (during the first 30 DIM), but the NSE increased with DIM. This is in concordance with findings previously reported by Siewert al. (2019) in Holsteins. The opposite situation was observed for multiparous cows, where later-parity cows tended to have fewer successful milking entries. Finally, toward the end of the lactation, the NSE rate dropped for all parities. This could be explained by the fact that we studied lactations up to 350 DIM instead of the 305 DIM, which is more common. Number of visits reflects the adaptation and efficiency of cows milked in AMS. Optimizing the NoV contributes to saving farm labor by avoiding the need to push cows through AMS that may have not been milked over a long period. The number of voluntary visits of cows to the AMS units can be related to the cows' temperament. Prescott et al. (1998) and Munksgaard et al. (2011) reported a daily average NoV of 4.1 and 5.30, respectively, which is higher than the average NoV observed in this study (3.7). These differences may be due to the fact that in the 2011 study, Red Danish cows were considered in addition to Holsteins and to an equal time period for both studies of 42 and 24 d, respectively, which is lower than the time period evaluated in this current study. Short-term studies may not take into account seasonal variations or changes in behavior over time. Hermans et al. (2003), also studying NoV in AMS with traffic systems that were semiclosed or closed, reported an average of 3.95 and 6.00 NoV, respectively. The result for the semiclosed system is similar to our average NoV value for a free-flow traffic situation. The results of our study reflect the same trend in parity as explained above for the NSE, with 2 differences. First, the lack of a marked decrease toward the end of lactation shows that dairy cows are more likely to be refused entry into AMS although they may still visit the AMS units. This can be explained by the restrictions on the maximum number of visits imposed by the farmers on certain animals. Second, a more intense variation in NoV was observed between 0 and 100 DIM, corresponding to the period when milk yield increased before peaking and gradually decreasing. During this lactation period, cows return more frequently to the AMS to relieve udder pressure and avoid engorgement. They also seek to increase their intake of concentrate to compensate for the negative energy balance that occurs during the first 15 wk of lactation (Siewert et al., 2019; Hanzen, 2021). Finally, the last trait related to milking frequencies is PSM, which pro-

vides a relative measure of the milking success rate by taking into account the total number of visits, unlike the NSE. This indicator can be particularly useful for identifying animals likely to encounter difficulties in milking with AMS due to health problems or adaptation to AMS. This measure may reflect greater adaptation of cows to AMS and also be useful for estimating efficiency of AMS usage, enabling herd managers to optimize procedures and parameters. To our best knowledge, this trait has not yet been studied before. We observed an inversion in the order of the parity curves during DIM in comparison to NSE, which is explained by the inclusion of NoV in the calculation of PSM as multiparous cows had a lower NoV than primiparous cows.

Preference consistency score is a measure that provides a quantifiable assessment of a cow's preference behavior, reflecting both their comfort and their compatibility with the AMS units. A score of 0 indicates an apparent lack of preference for a particular AMS unit in the pen, whereas a higher score reflects a stronger preference for a specific milking robot. However, it is important to note that, in some cases, a low score may not reflect an intrinsic lack of preference, but rather an external influence, such as the possibility of dominant animals driving out less dominant individuals from certain AMS units. This potential bias could affect the measurement of PCS, particularly in cows with lower social ranks, and this is therefore a limitation to be considered. Interestingly, Løvendahl and Buitenhuis (2022) reported an average PCS score of 0.43 in Danish Holstein cows, which is similar to the value of 0.42 observed in this study, indicating that PCS may be consistent across Holstein populations (another evidence of its genetic control). Throughout lactation, first-parity cows had the highest PCS and older cows had lower PCS (Figure 2). One reason for this relatively high score in primiparous cows may be their lack of experience with AMS. In addition to a natural curiosity that may exist at the outset, learning by repetition in young animals is often effective, as there have never been any pre-established competing behaviors. This could justify the slight decrease in PCS over time for first-parity cows. Although their average PCS score had a high value, cows could identify a certain similarity between stations and thus vary over time. However, another element that could justify a higher score in primiparous cows would be a feeling of reluctance or stress in some individuals in their new environment, restricting them to visiting unfamiliar AMS units. Moreover, with regards to multiparous cows, MacKay et al. (2014) and Brasier et al. (2023) reported that older cows had bolder personalities, which could explain why multiparous cows had lower PCS. These individuals, even with pre-existing individual preferences, have already had several experiences with AMS, which may lead them to make less consistent choices during lactation.

# Variance Components, Heritabilities, and Repeatability Between and Across Lactations

Several new derived phenotypes associated with behavior and milking efficiency in North American Holstein cows have shown to be under genetic control. Although there are many reports of heritability estimates for milking-related traits in the literature, a limited number of studies have reported genetic parameters for behavioral and milking efficiency traits in AMS. The estimated heritabilities for TMT for the 2 models fitted in the present study were 0.27 and 0.28. Dechow et al. (2020) estimated a heritability of 0.26 for TMT and Pedrosa et al. (2023) reported heritability estimates in the same population ranging from 0.22 to 0.28. However, previous studies reported a higher estimate of 0.38 (Gäde et al., 2006; Carlström et al., 2014). These results indicate that TMT is heritable and could be incorporated into genetic selection programs to increase the efficiency of dairy farms utilizing AMS. The heritability estimates for AMT (0.46) reported in this study is, to our knowledge, the first in the literature. Previously, AMT was compared with the milking box time trait, which has heritability estimates ranging from 0.21 to 0.44 (Løvendahl and Chagunda, 2011; Carlström et al., 2013; Wethal and Heringstad, 2019). Both TMT and milking box time are similarly heritable traits.

The heritability estimates for INT (0.08–0.10) observed in this study are within the range of estimates previously reported by Nixon et al. (2009) in Canadian and Dutch Holstein cattle, which ranged from 0.02 to 0.14. Santos et al. (2018) also reported a heritability of 0.07 for German Holstein cattle, which is similar to the one observed in our population. Additionally, Wethal and Heringstad (2019) observed a slightly lower estimate of heritability of 0.02 for Norwegian Red cows. Other estimates of heritability ranging from 0.09 to 0.26 have been reported in the literature (Løvendahl and Chagunda, 2011; Carlström et al., 2013). Genetic selection for optimal milking interval could be considered in a selection subindex aiming to improve performance and welfare in robotic milking farms. Milking frequency should be adapted to different stages of lactation to have a positive impact on both cow productivity and health (Jacobs and Siegford, 2012). However, from the cow's perspective, different interval lengths can affect milk production (André et al., 2010), with shorter intervals generally leading to increased daily milk yield due to higher milking frequency.

The low heritability estimates observed for NSE (0.10–0.11) in the 2 models of this study are close to the estimates (0.14–0.15) of the trait milking success reported by Schafberg et al. (2022) in dairy cattle. The heritability for milking frequency in dairy cattle has been reported to range from 0.03 to 0.15 (Nixon et al., 2009; Carlström et al., 2013; Wethal and Heringstad, 2019), which is comparable to the NSE trait evaluated in this study. As for NoV, the heritability estimates found in our study according to the 2 models were both 0.10. Again, Schafberg et al. (2022) reported estimates in the same range as for NSE, with values between 0.14 and 0.15. Santos et al. (2018) reported heritability estimates of 0.08 and 0.05 for NoV to AMS greater than 3 and 4, respectively. These 2 traits depend on the context and the objectives to be set, so although they can be genetically modified, it is difficult to give clear recommendations on the selection direction, as there is no clear consensus on the optimum values for these traits. Compared with PSM (0.05-0.06), the heritabilities found are lower and no additional heritabilities have been found in the literature for comparison purposes. All 3 derived traits are related to AMS efficiency. An intermediate milking frequency is desirable as too few milking events could have a negative impact on both cows and farmers by requiring extra work, whereas too many visits to AMS units could reduce the ability of other cows to use the AMS during the day, which would have a direct impact on production levels (Wethal and Heringstad, 2019). Selection programs need to be optimized and the traits, NSE, NoV, and PSM, could become good indicators to assess activity rate and cow adaptation to AMS, while maximizing milking efficiency. However, an important subsequent study needs to be the estimation of genetic correlations between these traits and other relevant traits in dairy cattle breeding programs. Intermediate values for the number of daily visits are also desirable. An excessive number of AMS entry attempts will result in more refusals, which could cause stress to the cows. On the other side, too few visits could lead to lower milk yield and require extra labor to fetch the cows that have not been milked yet. In addition, there is a greater risk of udder infection if the cows are not milked on a regular basis. Optimum NSE and PSM values ensure that cows are milked regularly and efficiently. NSE, NoV, and PSM have low heritability but substantial additive genetic variance. These 3 cow behavioral indicators of AMS in dairy cows' require further investigation in future studies to determine whether or not they should be included in selection schemes.

We observed a greater difference between the heritability estimates obtained for PCS (0.09–0.24) based on the 2 models fitted considered within and both within

and across lactation permanent environmental effects than the other traits. Løvendahl and Buitenhuis (2022) reported heritability estimates for PCS ranging from 0.05 to 0.13 using a similar model. The best model in our study based on AIC values was the one considering 2 permanent environmental effects. Thus, introducing additional complexity into the simple repeatability model containing a single permanent environmental effect enabled us to obtain a better model fit. By adding a second permanent environmental effect to capture variations within parities, this model takes into account a greater proportion of permanent environmental variability by reflecting a more complex covariance structure. Moreover, we analyzed PCS per parity as different traits. For both models, the heritabilities found for each parity were similar. Environmental variations specific to each parity were well captured by both models. When data from all parities were considered together, the model with 2 permanent environmental effects better captured the total environmental variance.

#### **Correlations Between Cow Behavioral Traits**

In addition to evaluating the genetic background of individual traits, knowledge of the genetic relationship among novel and routinely evaluated traits is essential when designing or refining selection indexes. The high and positive genetic correlation between AMT and TMT (0.84–0.82) was expected. If a cow has several milking sessions per day and each session takes longer on average, the total accumulated time will be higher. Similar genetic correlation was also reported by Carlström et al. (2013) between milking box time and total milking time. The low to moderate and favorable genetic relationship between AMT and PSM (0.38-0.35) indicates that selection for greater milking time could have a positive impact on the rate of successful milking in AMS. Too short milking time can have a negative impact on udder health (Dechow et al., 2020). In fact, milking times that are too short can lead to incomplete udder emptying, creating an environment conducive to bacterial development. Moreover, too high milking speed can lead to a deterioration in udder health and an increase in SCC, which is an indicator of mastitis (Gäde et al., 2007; Vosman et al., 2014). It is conceivable that prolonged milking periods could have a negative impact on animal health and welfare, due to the increased time spent in the AMS. Average milking time had a moderate and negative genetic correlation with NoV (-0.36). A lower NoV when the AMT tends to increase could be explained by the temperament of the cows and a reduced motivation to go to the AMS due to too much time spent inside the AMS unit. On the other hand, we observed a moderate and negative genetic correlation of the same order of magnitude between AMT and NSE (-0.34), reflecting a drop in the number of milking entries when milking time spent in the AMS is extended. If each milking session takes longer on average, each cow spends more time in the AMS and in return fewer daily visits to the AMS are needed. If a cow manages to enter the AMS several times over a period, not all milkings might prove effective and thus be shortened. A similar finding was reported by Wethal and Heringstad (2019) in Norwegian Red cattle between milking box time and milking frequency. Another relevant element observed is the favorable and high genetic relationship between INT and PSM (0.71 and 0.64), indicating more successful milkings would be expected when the interval between milkings increases. This highlights that optimal milking intervals could reduce milking failures and refusals. The strong and negative genetic correlation between PSM and NoV (-0.94 and -0.93) indicates that selection for cows that visit the AMS units too frequently could result in less successful milkings. This is expected as farmers set interval limits between milkings, which will result in lower PSM. On the other hand, there was a strong negative correlation between PSM and NSE (-0.72 and -0.64). As previously indicated, a large number of entries into the AMS could lead to inefficient milking. This relationship highlights that high NSE may be associated with poorer relative performance in terms of milking success percentage as the proportion of failed milkings increases. In other words, a high NSE could indicate a situation where the cow often enters AMS but with a relatively low success rate, thus lowering PSM. On the other hand, if the number of entries to AMS within the pens increases and becomes saturated, the number of refusals could increase due to cows still attempting to milk, which may reduce the PSM. Other logically deductive results in support of what has been said are worth noting. We observed a strong and positive genetic correlation between NoV and NSE (0.92 and 0.89), NSE being a direct component of NoV, it is obvious that if it increases, so does NoV. We also observed high negative genetic correlations between INT and NoV and NSE (-0.90, -0.87, and -0.98). These results indicate a logical increase in the number of visits, and therefore AMS entries, as the time between milkings decreases, which could indicate a tendency for cows to be milked more often but inefficiently and could be unfavorable. A higher number of entries in a shorter period could indicate insufficient preparation of cows for milking. Wethal and Heringstad (2019) reported a genetic correlation of -0.99 between milking frequency and time interval. Finally, weak positive correlations were observed between TMT and the NoV and NSE traits. TMT does not seem to be a major factor influencing the number of visits, so other factors may be at the origin of visits to AMS, such as motivation and personal

curiosity on the part of the cows. Furthermore, regarding NSE, it seems logical that cows triggering the milking system in the AMS would contribute to an increase in TMT. However, TMT has previously been observed to be strongly correlated with AMT, so genetic selection for AMT will result in correlated responses in TMT. For PCS, we observed a moderate genetic relationship with PSM (0.37). This indicates that selection in favor of individuals with a preference for an AMS unit could result in favorable genetic response on milking success. We also observed a favorable genetic correlation with INT (0.50). Cattle traffic at AMS could also be smoother, leaving cows free to choose an AMS. There was also a moderate negative correlation with NoV (-0.44 and -0.41). This means that cows would be less consistent in their choice of AMS with a high number of visits, which could indicate that comfort is no longer a priority when a high number of passages is imposed. Conversely, if their preference for an AMS increase, the NoV decreases, which could be an indicator that milking is going well, and that the number of refusals is decreasing. We also observed a negative genetic correlation with NSE (-0.35)and -0.25), which is logically explained by the number of visits. A cow showing more consistency of preference for an AMS unit will be less tempted to want to visit other robots, and so will count fewer entries. In addition, Løvendahl and Buitenhuis (2022) found a correlation of -0.38 between milking frequency and PCS, suggesting that milking frequency was lower when AMS usage increased. Finally, we found a weak genetic correlation between PCS and TMT (-0.20 and -0.13), indicating that selection for higher AMS use could help reduce total milking time, as cows were efficiently milked at a station they determined.

Existing relationships between the various traits were examined and evaluated. These results depend on the context but provide directions for potential future choices of temperament-related traits in dairy cattle breeding programs. Some traits stood out with favorable relationships. In particular, milking interval has repeatedly demonstrated its potential in several associations. This trait could become a key lever for improving both milking efficiency and the well-being of dairy cows, through more successful milking and greater comfort for cows using AMS. In fact, INT could positively influence milking time, the PSM, as well as allowing cows' preference for an AMS. In turn, milking time and PCS could contribute positively to improved milking success rates. In addition, we observed that the number of visits and successful entries can vary according to the traits considered. Different aspects studied from different angles are therefore important for optimizing dairy cow behavior within the herd and for implementation of optimal management practices.

#### **Limits and Future Directions**

New estimates of genetic parameters for AMS-derived traits have been carried out for dairy cows. The analyses carried out presented elements for North American Holstein cattle that could prove essential in terms of future genetic progress. In this study, we used repeatability models as they are commonly used in dairy cattle national genetic evaluations around the world due to its simplicity and ability to capture interindividual variability across lactation. A next step should be the evaluation of random regression models (Oliveira et al., 2019) for modeling variability of the behavioral traits across DIM. One advantage of this model is that it can incorporate stage of lactation for random genetic and environmental effects over a given period. Thus, combining such a model with genomic information, which is currently increasingly used in genetic evaluations, could provide more accurate estimates throughout lactation. Based on the circumstances that AMS collects various types of information, there is an opportunity to evaluate the genetic relationship of behavioral traits with traits directly linked to milk production, udder health, resilience, and health. Although the derived traits are more directly related to milking robotic farms, the genetic responses obtained could still be of values in farms without AMS. For example, selection for certain temperament traits could benefit dairy cows milked in conventional milking systems (e.g., milking parlors). In this study, we used data from one AMS provider (Lely), although there are other AMS providers in the market. Future studies should evaluate the genetic correlations between traits collected by both AMS types to assess their consistency on the recorded variables.

Given the genetic background of the traits evaluated in this study, a key next step will be GWAS to identify genomic regions and candidate genes underlying their phenotypic variability.

### **CONCLUSIONS**

All the AMS-derived cow behavioral traits evaluated in this study (AMT, TMT within the AMS unit, INT, NoV, NSE, PSM, and PCS for each AMS unit) are heritable with substantial additive genetic variance. A wide range of genetic correlations were observed among all trait pairs. Some trait pairs, such as AMT-PSM, INT-PSM, INT-PCS, and PSM-PCS, showed positive and favorable genetic correlations, which could be explored in dairy cattle breeding programs.

#### **NOTES**

This research was funded by the Agriculture and Food Research Initiative Competitive Grant number 2022-

67021-37022 from the USDA National Institute of Food and Agriculture (Washington, DC). The first author was financially supported by the International Relations Department of the University of Gembloux Agro-Bio Tech in Belgium and would particularly like to thank the various authors who participated in the review of this paper. We are very grateful for the support received from the Purdue College of Agriculture and Purdue Ag Data Services for supporting the development of the Purdue Animal Sciences Data Ecosystem (PASDE). The authors thank Homestead Dairy LLC and especially Brian and Jill Houin for inviting us on their farm and for allowing our research group to use their farm records. We also thank Lely for giving us access to their system for extracting the datasets. Supplemental material for this article is available at https://doi.org/10.6084/m9.figshare .28825784. Approval from an Institutional Animal Care and Use Committee was not required for the purpose of this study, as all necessary information was obtained from pre-existing databases. The authors have not stated any other conflicts of interest.

Nonstandard abbreviations used: AIC = Akaike information criterion; AMS = automated milking systems; AMT = average milking time; EC = electrical conductivity; INT = milking interval; MAF = minor allele frequency; MFAIL = milking failure; MREF = milking refusals; NoV = number of robot visits; NSE = number of successful entries; PCS = preference consistency score; PSM = percentage of successful milkings; RMILK = number of successful milkings; TMT = total milking time.

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# **ORCIDS**

Hugo Bérat, © https://orcid.org/0009-0003-2546-4610
Nicolas Gengler, © https://orcid.org/0000-0002-5981-5509
Jacob M. Maskal, © https://orcid.org/0009-0006-9561-2625
Jacquelyn P. Boerman, © https://orcid.org/0000-0002-0336-8295
Luiz F. Brito © https://orcid.org/0000-0002-5819-0922