

**PRODUCTION AND MANAGEMENT:** *Original Research*

# Comparison of cow milk components between daily actual and AM-PM composite samples from 2 consecutive milkings by Dairy Herd Improvement

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## ABSTRACT

**Objective:** This study was undertaken to evaluate the level of agreement of milk fat, protein, lactose, MUN, and SCC concentrations between daily actual and AM-PM composite milk samples taken at 2 consecutive DHI milkings and to assess factors affecting their level of agreement.

**Materials and Methods:** Milk samples from 2 consecutive milkings were collected using in-line milk meters on 4,340 Holstein cows in 100 Canadian commercial dairy herds. Three milk samples per cow were analyzed for major components: (1) evening samples; (2) morning samples; and (3) an AM-PM composite sample obtained by visually mixing equal volumes of milk from 2 consecutive milkings. Daily actual milk component concentrations were computed proportionally to milk yields. Equal 50:50 composite milk component concentrations were the average of evening and morning samples.

**Results and Discussion:** Concordance correlation coefficients (CCC) between actual and equal 50:50 samples varied from 0.997 to 1.000. For the comparison between daily actual and AM-PM composite milk component concentrations, CCC ranged from 0.911 to 0.964. This suggested that AM-PM composite samples were not always composed of an equal 50:50 volume of milk from evening and morning milkings. A great variation of CCC was observed between herds, indicating differences in their assessment of volumes of milk to pour into vials at each milkings.

**Implications and Applications:** Assuming that milk samples were homogeneous, AM-PM composite samples predicted daily actual milk components with great precision and accuracy. However, this was not the case in all

herds. Visually assessing milk volumes to pour into vials when creating AM-PM composite milk samples was the major cause of a decrease in level of agreement when predicting daily actual milk component concentrations, which varied between herds. One recommendation might be to add indicators on DHI vials to guide in mixing an equal milk volume from 2 consecutive milkings. Because DHI records are used in decision making, it is important that predicted daily milk component concentrations are as close as possible to daily actual milk component concentrations. Producers can make an informed decision on which sampling scheme to choose according to their management objectives.

**Key words:** precision, accuracy, dairy herd improvement, milking scheme, prediction

## INTRODUCTION

For many years, records collected through the DHI program have been used for cattle genetic selection (Cole et al., 2021), nutrition and management decisions (Svennersten-Sjaunja et al., 1997), and health and welfare status evaluation (Warner et al., 2020). Hence, it is important to ensure that both the collection of milk samples and data recording are reliable. The DHI agency in Canada follows the International Committee for Animal Recording guidelines. According to the guidelines (ICAR, 2017), the reference milk recording scheme is performed by an official representative of the DHI agency every 4 wk, with milk samples and weights separately taken at 2 consecutive milkings for cows milked twice daily. Then, a daily proportional sample, weighted according to milk yield and obtained by milk pipetting, is analyzed for milk composition. However, the reference milk recording scheme is the most expensive scheme. Therefore, several trials have aimed to evaluate different strategies to reduce milk recording costs for dairy producers (Schaeffer and Rennie, 1976; Hargrove and Gilbert, 1984; Cassandro et al., 1995). The most

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studied strategy is the alternated one-milking recording scheme, in which milk weights and samples are collected at one milking, alternating between morning and evening (AM-PM) milkings, and adjustment factors are used to compute daily milk yield and fat concentrations (Lee and Wardrop, 1984; Liu et al., 2000; Jenko et al., 2010). Čandek-Potokar et al. (2006) reported that the alternated one-milking testing scheme results in a loss of precision. Another recognized milk sampling scheme is called equal measure sampling (ICAR, 2017), which consists of taking an equal volume of milk at evening and morning milkings, mixed on farm without pipetting. About one-third of Canadian herds uses this scheme. This strategy could lead to errors in predicting daily milk component concentrations (Thompson et al., 1960), because milk yield is greater in the morning than in the evening, even for cows milked at 12-h intervals (Gilbert et al., 1973). In addition, it has been shown that milk fat and urea concentrations are greater, and milk protein concentrations are slightly greater in the milk from evening milkings, irrespective of milking intervals, with stable milk lactose concentrations and no clear pattern for SCC concentrations (Gilbert et al., 1973; Godden et al., 2001; Quist et al., 2008).

To our knowledge, the level of agreement between daily major component concentrations of actual and AM-PM composite milk samples has never been reported. Hence, this study aimed at assessing the level of agreement of milk fat, protein, lactose, MUN, and SCC concentrations between daily actual and AM-PM composite samples taken at 2 consecutive milkings during a DHI test. Factors affecting the level of agreement, such as the variability among herds, were also evaluated. In addition, current results were compared with those reported in a companion study evaluating the alternated one-milking recording scheme (Duplessis et al., 2019a).

## MATERIALS AND METHODS

All procedures followed in this experiment were approved by the Animal Care Committee from Université Laval, Québec, QC, Canada, in accordance with the guidelines of the Canadian Council on Animal Care (2009).

### Participating Herds

The inclusion criteria for herds were based on a previous trial using the same data set (Duplessis et al., 2019b): DHI milk recording service is used; cows are milked twice a day; the herd has Holstein cows; and evening and morning milk samples can be collected. Dairy herds that met the inclusion criteria were contacted over the phone and recruited on a voluntary basis. Participating herds were previously described by Duplessis et al. (2019a). Briefly, 100 Holstein dairy herds (98 tiestall and 2 freestall barns; with 16 to 113 cows in lactation) located in the province of Québec, Canada, were enrolled. Herds were visited once during 2 consecutive DHI milkings (evening and morning milkings) between October 2014 and June 2015.

### Data Collection and Analyses

A total of 4,340 Holstein cows (1,484 first, 1,093 second, and 1,763 third or more lactations) had their milk sampled. Three separate 35-mL milk samples per animal were collected on the farm and analyzed; they consisted of (1) an evening milking sample; (2) a morning milking sample; and (3) an AM-PM composite milk sample. The latter was composed of an approximately equal volume of milk from evening (17.5 mL) and morning (17.5 mL) milkings mixed on farm. Specifically, vials were filled halfway on the basis of a visual assessment during the evening milking and stored overnight at room temperature, and then, the other half was visually filled during the morning milking. The AM-PM composite samples were taken by either the producer or an employee of the DHI agency. All milk samples were collected using in-line milk meters certified [e.g., Waikato milk meters (Coburn) and Tru-Test milk meters (Datamars)] and calibrated according to ICAR guidelines (ICAR, 2017) and then poured into a bowl and transferred into another at least twice before being poured into DHI vials. This reduced the risk for a possible deviation of components due to on-farm milk handling. Hence, it had been assumed that milk samples put into vials were homogeneous and representative of whole milkings. Milk samples were preserved with bronopol and then immediately sent to the laboratory (Lactanet, Canadian Network for Dairy Excellence, Sainte-Anne-de-Bellevue, QC, Canada) for milk fat, CP, lactose, MUN, and SCC concentration analyses (MilkoScan FT 6000 combined with a Fossomatic FC for SCC determination, Foss). Milk yields and the time when each of the 2 milkings started during the DHI tests were recorded.

### Calculations and Statistical Analyses

Daily actual milk component concentrations were calculated as follows: {[evening milk component concentration (% , mg/dL, or cells/mL) × evening milk yield (kg)] + [morning milk component concentration (% , mg/dL, or cells/mL) × morning milk yield (kg)]} / daily milk yield (kg). For MUN and SCC concentrations, a milk density of 1.03 kg/L was considered in the calculation. Equal 50:50 composite milk component concentrations were calculated as follows: [evening milk component concentration (% , mg/dL, or cells/mL) × 0.5] + [morning milk component concentration (% , mg/dL, or cells/mL) × 0.5]. Actual and equal 50:50 composite milk component concentrations were used to evaluate the precision and accuracy of the AM-PM composite sampling method in the current study.

The milking interval between the 2 milking tests was computed as the beginning time of the morning milking minus the beginning time of the evening milking. For AM-PM composite samples, an adjustment factor was applied to daily milk fat concentrations if the milking interval was less than 10 h or more than 14 h (ICAR, 2017), as follows: corrected daily milk fat concentration (%) = milk fat concentration (%) + 0.69 - 1.3 × [morning milk yield

(kg)/daily milk yield (kg)]. Two herds ( $n = 98$  cows) had a milking interval of greater than 14 h and required a correction of AM-PM composite milk fat concentrations.

All 3 milk samples, i.e., evening, morning, and AM-PM composite, were required from each cow for the analysis. Records for milk fat, protein, and lactose concentrations outside 2 to 7%, 2 to 5.5%, and 3.5 to 5.5%, respectively, were deemed to be not biologically possible and were excluded. One herd ( $n = 40$  cows) had missing values for lactose and MUN data. One herd ( $n = 25$  cows) was excluded from the analysis because its data were considered unreliable. In contrast with that of other herds, the concordance correlation coefficient (CCC) between equal 50:50 and AM-PM composites was close to zero for all milk components.

Descriptive statistics (average, SD, minimum, and maximum) were obtained using Proc UNIVARIATE of SAS (SAS Institute Inc.). The relationships between milk component concentrations were fitted by using Proc MIXED of SAS with herd as the random effect to obtain regression equations and to check for normality and homoscedasticity assumptions. Linearity between dependent and independent variables was evaluated with PROC GPLOT of SAS. Milk SCC data were log transformed as they were not distributed normally (Shook, 1993). Prediction errors related to the AM-PM composite samples were evaluated using the relative prediction error (RPE, which represents root mean squared prediction error as a percentage of the mean of reference values), mean (i.e., error in central tendency), slope (i.e., error due to regression), and dispersion (i.e., random disturbance) biases as a percentage of the mean squared prediction error (Theil, 1966; Tedeschi, 2006) and CCC (Lin, 1989) and using the epiR package (Stevenson and Sergeant, 2021) of R software (version 1.1.419). Concordance correlation coefficient was used as a measure of agreement that assesses both precision and accuracy (Petrie and Watson, 2013). Concordance correlation coefficients and RPE were also evaluated for each herd. The level of agreement was considered very accurate (RPE <5%), accurate (RPE <10%), or acceptable (RPE <15%; Pacheco et al., 2012). Box plots were created using an Excel spreadsheet (2016, Microsoft Corp.) to evaluate the variation of CCC and RPE among herds. Outliers were considered beyond the limits of the following calculation: lower or upper quartile  $- 1.5 \times$  interquartile range (Kaps and Lamberson, 2017).

## RESULTS AND DISCUSSION

### Descriptive Statistics

As previously reported in the companion study (Duplessis et al., 2019a), evening and morning milk yields averaged  $15.1 \pm 4.6$  and  $16.9 \pm 5.1$  kg, respectively. The interval between evening and morning milkings averaged  $12.6 \pm 0.6$  h. Slight average differences of <0.01 percentage point were noted between actual, AM-PM composite,

and equal 50:50 composite milk fat, protein, and lactose concentrations (Table 1). Compared with the present study, Hargrove (1994) documented a greater difference in milk fat concentration between actual and equal 50:50 composite samples for a herd with an evening-morning milking interval of 14 h, although milk protein and SCC differences were similar between the 2 trials. The difference for milk fat concentrations between these 2 studies may be explained by the fact that there was an average evening-morning milking interval of 12.6 h in the current assessment. Averaged SCC and MUN concentration differences from the 3 different types of milk samples were <2,000 cells/mL and <0.06 mg/dL, respectively (Table 1). The difference between daily actual and AM-PM composite milk fat concentration could be as high as about 1 percentage point (Table 2).

### Prediction Errors Related to AM-PM Composite Milk Component Concentrations

Given the lowest RPE and the highest CCC results in Table 3, we observed that predictions based on AM-PM composite samples were more accurate for daily actual milk protein and lactose concentrations than for daily actual milk fat, MUN, and SCC concentrations. There was also less variation between daily actual and AM-PM composite samples for these 2 milk components as suggested by scatter plots (Figure 1). This is not surprising, as it has been shown that milk protein concentrations have slight differences between evening and morning milkings (Quist et al., 2008) and that milk lactose is the least variable milk component (Svennersten-Sjaunja et al., 1997). It has been well documented that milk fat concentration varies by about 0.3 to 0.5 percentage point between evening and morning milkings (Gilbert et al., 1973; Quist et al., 2008), explaining why the prediction of daily actual milk fat concentration is more affected when not considering milk weights. Regarding milk SCC concentrations, Quist et al. (2008) did not observe a clear pattern between evening and morning milkings, and Deng et al. (2021) noticed several 30-d milk SCC fluctuation patterns among cows, highlighting its pattern complexity. The assessment of RPE indicated that predictions based on AM-PM composites were very accurate (RPE <5%) for daily milk protein and lactose concentrations, accurate (RPE <10%) for daily milk fat and SCC concentrations, and acceptable (RPE <15%) for MUN concentrations. Mean biases of mean squared prediction error breakdown were minimal for all milk components (i.e., <0.5%). However, slope biases between 4.5 and 8.4% indicated a regression deviation (Table 3). The slope bias was greatest for MUN (Table 3 and Figure 1d). Moreover, the lowest CCC and the highest RPE were obtained for MUN concentrations (Table 3). One possible explanation might be that Godden et al. (2001) and Wattiaux et al. (2005) have found a substantial MUN concentration difference of about 0.72 to 1.00 mg/dL between evening and morning milking samples. Thus,

**Table 1.** Descriptive statistics of data used to evaluate the relationships between daily actual and composite milk component concentrations<sup>1</sup>

Records	Cows (no.)	Average	SD	Minimum	Maximum
Actual milk components <sup>2</sup>					
Fat, %	4,252	4.13	0.61	2.21	6.95
Protein, %	4,265	3.34	0.36	2.40	4.98
Lactose, %	4,224	4.57	0.19	3.56	5.15
MUN, mg/dL	4,226	11.52	2.94	0.52	23.61
SCC, × 10 <sup>3</sup> cells/mL	4,266	206	563	3	12,909
AM-PM composite milk components <sup>3</sup>					
Fat, %	4,252	4.14	0.62	2.11	6.97
Fat corrected, <sup>4</sup> %	4,252	4.14	0.62	2.11	6.97
Protein, %	4,265	3.35	0.37	2.43	4.97
Lactose, %	4,224	4.57	0.20	3.53	5.11
MUN, mg/dL	4,226	11.58	3.02	1.00	24.30
SCC, × 10 <sup>3</sup> cells/mL	4,266	208	585	1	10,405
Equal 50:50 composite milk components <sup>5</sup>					
Fat, %	4,252	4.14	0.61	2.21	6.95
Protein, %	4,265	3.35	0.36	2.41	4.99
Lactose, %	4,224	4.57	0.19	3.54	5.15
MUN, mg/dL	4,226	11.55	2.94	0.55	23.45
SCC, × 10 <sup>3</sup> cells/mL	4,266	208	570	3	13,317

<sup>1</sup>One herd (n = 25 cows) out of 100 was excluded from the whole analysis. One herd (n = 40 cows) had missing values for milk lactose and MUN concentrations. Records for milk fat, protein, and lactose concentrations were deemed not biologically possible and were excluded from the analysis if outside the range of 2 to 7%, 2 to 5.5%, and 3.5 to 5.5%, respectively. Cows with at least one missing milk sample were excluded.

<sup>2</sup>Daily actual milk component concentrations = {[evening milk component concentration (% , mg/dL, or cells/mL) × evening milk yield (kg)] + [morning milk component concentration (% , mg/dL, or cells/mL) × morning milk yield (kg)]} / daily milk yield (kg). For MUN and SCC concentrations, a milk density of 1.03 kg/L was considered in the calculation.

<sup>3</sup>AM-PM composite milk samples were mixed on the farm using an approximately equal volume of milk from evening and morning milkings.

<sup>4</sup>A correction was applied to the AM-PM composite milk fat concentration if the milking interval was less than 10 h or greater than 14 h. Two herds (n = 98 cows) had a milking interval of greater than 14 h. Corrected milk fat concentration = analyzed milk fat concentration + 0.69 – 1.3 × (morning milk yield/daily milk yield) (ICAR, 2017).

<sup>5</sup>Equal 50:50 composite milk component concentrations = [evening milk component concentration (% , mg/dL, or cells/mL) × 0.5] + [morning milk component concentration (% , mg/dL, or cells/mL) × 0.5].

type of milk samples, considering or not milk yield proportion at each milking, can have a great effect on MUN concentration prediction.

Levels of agreement were high in the current study (CCC between 0.911 and 0.964), suggesting that, overall, the equal measure sampling scheme is reliable for predicting daily milk component concentrations. Given the fact that milk volume mixing of AM-PM composite samples relied on a visual assessment, one question was whether highest levels of agreement and lowest slope deviations would have been achieved if the AM-PM composite samples had been composed of an exactly equal 50:50 volume of milk from evening and morning milkings. We started from the assumption that if AM-PM composite milk samples were taken according to ICAR guidelines, half their volume should be from the evening milking and half from the morning milking. Given that separate evening and morning milking samples were collected in the current study, it

was possible to compute the equal 50:50 composite milk component concentrations and compare these data with AM-PM composite milk component concentrations. Although perfect agreement between equal 50:50 composite and AM-PM composite data was expected, we noted that RPE and CCC values from this analysis were similar to values from the actual and AM-PM composite data comparison (the RPE was 5.0% for fat, 2.92% for protein, 1.20% for lactose, 10.92% for MUN, and 6.83% for SCC, and the CCC was 0.943 for fat, 0.964 for protein, 0.959 for lactose, 0.912 for MUN, and 0.950 for SCC). This suggests that AM-PM composite samples did not correspond exactly to equal 50:50 composite samples, indicating that the on-farm sample mixing method was probably the first issue affecting prediction precision and accuracy. We analyzed the association between actual and equal 50:50 composite milk component concentrations (Table 4) to evaluate what the level of agreement would be if the sampling

**Table 2.** Absolute differences of milk components between daily actual or equal 50:50 composite and AM-PM composite samples<sup>1</sup>

Item	Median	Percentile 1	Percentile 99
Absolute differences between actual and AM-PM composite milk components			
Fat, %	0.03	0.00	0.99
Protein, %	0.01	0.00	0.53
Lactose, %	0.01	0.00	0.28
MUN, mg/dL	0.76	0.01	4.08
SCC, × 10 <sup>3</sup> cells/mL	5	3	732
Absolute differences between equal 50:50 and AM-PM composite milk components			
Fat, %	0.03	0.00	0.97
Protein, %	0.01	0.00	0.52
Lactose, %	0.01	0.00	0.29
MUN, mg/dL	0.75	0.00	3.95
SCC, × 10 <sup>3</sup> cells/mL	6	0	720

<sup>1</sup>Daily actual milk component concentrations = {[evening milk component concentration (% , mg/dL, or cells/mL) × evening milk yield (kg)] + [morning milk component concentration (% , mg/dL, or cells/mL) × morning milk yield (kg)]}/daily milk yield (kg). For MUN and SCC concentrations, a milk density of 1.03 kg/L was considered in the calculation. Equal 50:50 composite milk component concentrations = [evening milk component concentration (% , mg/dL, or cells/mL) × 0.5] + [morning milk component concentration (% , mg/dL, or cells/mL) × 0.5]. The AM-PM composite milk samples were mixed on the farm using an approximately equal volume of milk from evening and morning milkings.

**Table 3.** Summary of AM-PM composites for the prediction of daily actual milk component concentrations<sup>1</sup>

Item	RPE <sup>2</sup> (%)	CCC <sup>3</sup>	MSPE <sup>4</sup> bias breakdown (%)		
			Mean	Slope	Dispersion
Fat corrected, <sup>5</sup> %	5.09	0.941	0.5	5.1	94.4
Protein, %	2.93	0.964	0.5	4.5	94.9
Lactose, %	1.19	0.960	0.5	5.5	94.0
MUN, mg/dL	11.01	0.911	0.2	8.4	91.5
Log SCC, <sup>6</sup> × 10 <sup>3</sup> cells/mL	6.88	0.949	0.4	4.5	95.1

<sup>1</sup>Actual daily milk component concentrations = {[evening milk component concentration (% , mg/dL, or cells/mL) × evening milk yield (kg)] + [morning milk component concentration (% , mg/dL, or cells/mL) × morning milk yield (kg)]}/daily milk yield (kg). For MUN and SCC concentrations, a milk density of 1.03 kg/L was considered in the calculation. AM-PM composite milk samples were mixed on the farm using an approximately equal volume of milk from evening and morning milkings.

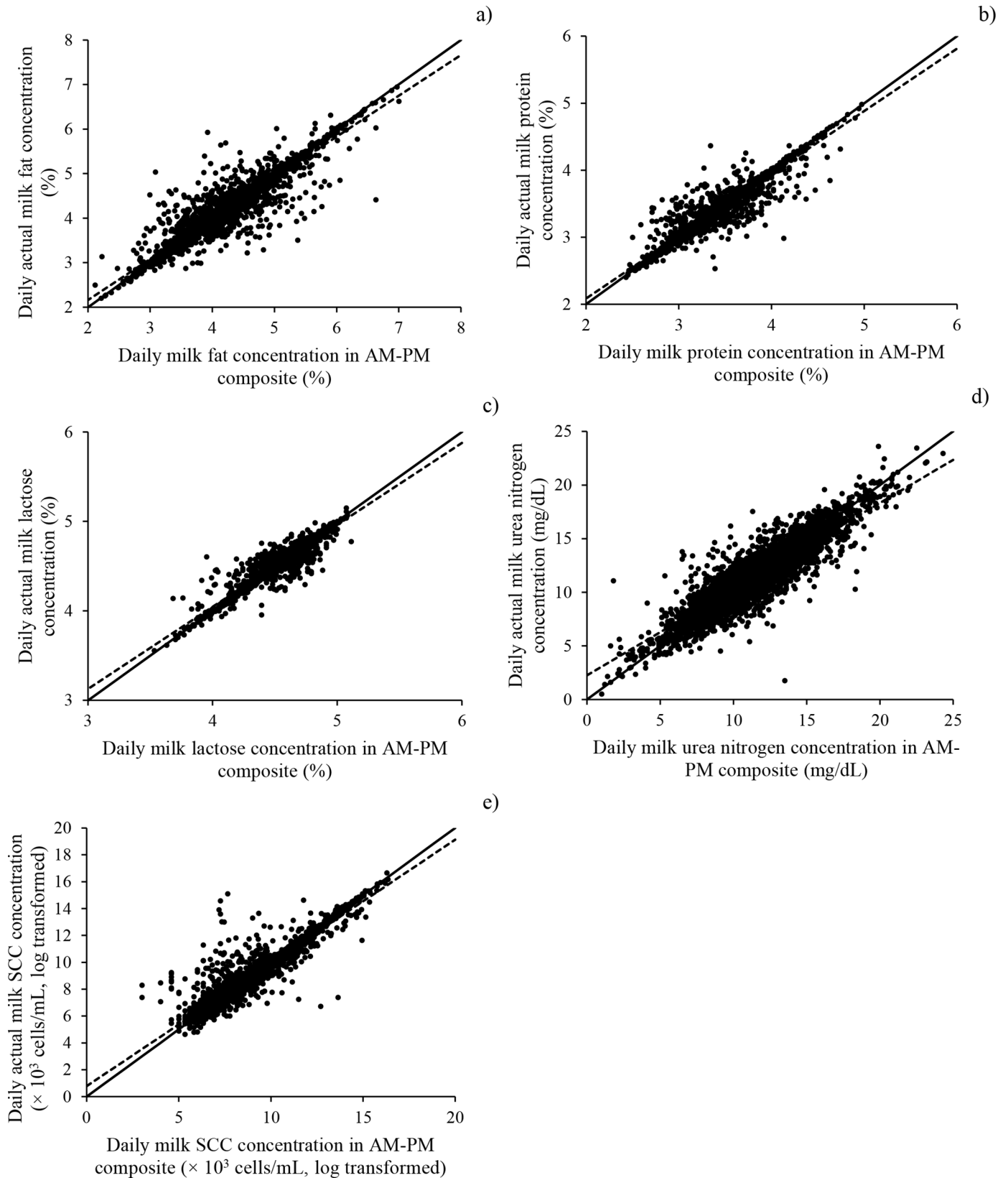
<sup>2</sup>RPE = relative prediction error or root mean squared prediction error expressed as a percentage of the average of daily actual milk component concentrations.

<sup>3</sup>CCC = concordance correlation coefficient as calculated by Lin (1989).

<sup>4</sup>MSPE = mean squared prediction error decomposed in percentages due to mean (overall bias of prediction), regression (deviation of slope from unity), and random disturbance biases.

<sup>5</sup>If the milking interval was less than 10 h or greater than 14 h, the daily milk fat concentration was corrected as follows: milk fat concentration + 0.69 – 1.3 × (morning milk yield/daily milk yield) (ICAR, 2017).

<sup>6</sup>Raw SCC data were log transformed for normality.



**Figure 1.** Relationship between daily actual and AM-PM composite milk component concentrations: (a) fat, (b) protein, (c) lactose, (d) MUN, and (e) SCC. Actual daily milk component concentrations =  $\{[\text{evening milk component concentration (\%, mg/dL, or cells/mL)} \times \text{evening milk yield (kg)}] + [\text{morning milk component concentration (\%, mg/dL, or cells/mL)} \times \text{morning milk yield (kg)}]\} / \text{daily milk yield (kg)}$ . For MUN and SCC concentrations, a milk density of 1.03 kg/L was considered in the calculation. AM-PM composite milk samples were mixed on the farm using an approximately equal volume of milk from evening and morning milkings. Solid lines are the line of unity, and dashed lines are the regression line.

was done as stated in the ICAR guidelines. We obtained CCC values at or very close to perfect agreement (CCC between 0.997 and 1.000), and all RPE were smaller than previously obtained (<1.18%). Moreover, biases due to slope were minimal in all predictions (<0.3%). However, the mean and slope biases should be interpreted cautiously because they are reported as a percentage of mean squared prediction error, which was small. This means that if AM-PM composite samples were composed of an exactly equal 50:50 volume of milk from evening and morning milkings, predicted milk component concentrations would be very close to daily actual milk component concentrations. One way to improve the sampling method might be to add 2 indicators on DHI vials to show the sampler what volume of milk is required from each milking to obtain an equal 50:50 composite sample from 2 consecutive milkings.

Using the same data set as in the current study, Duplessis et al. (2019a) assessed the Canadian model for predicting actual daily milk fat concentration from single-milking DHI samples. Using either evening or morning milking sample to predict daily milk fat concentrations, they obtained CCC of 0.897 and 0.917, respectively. In the present study, where we obtained a CCC of 0.941 between actual and AM-PM composite data, it can be concluded that, for milk fat concentration, the equal measure sampling scheme is more precise and accurate than the alternated one-milking recording scheme. However, the equal measure sampling scheme can be less convenient as it requires more labor because milk tests are done during 2 consecutive milkings compared with one for the alternated one-milking recording scheme. In Canada, about two-thirds

of herds are using the alternated one-milking recording scheme, thus suggesting that this is a highly valuable alternative.

### Variability Among Herds

To answer the question of whether the prediction error was the same or variable among herds, each dairy herd was evaluated for the level of agreement between equal 50:50 composite and AM-PM composite milk component concentrations. Figure 2 shows that RPE and CCC statistics varied among herds, with some herds having RPE and CCC outside the whiskers of the box plots. In more than 75% of herds, CCC were above 0.900 for daily milk fat, protein, lactose, and SCC concentration predictions, whereas in one herd, the CCC was as low as 0.494 for milk fat concentrations. Relative prediction errors were within 5% in about 75% of herds for milk fat, protein, and lactose concentrations, indicating a very accurate prediction of equal 50:50 composite concentrations from AM-PM composite samples for those herds. For MUN, the CCC was above 0.900 and the RPE was within 10% in about 25% of herds. These results highlight that, in some herds, AM-PM composite samples were very similar to the equal 50:50 composite samples, whereas in others, this was not the case. For the former, it can be suggested that no effect on decision making would occur, whereas for the latter, this can be associated with major differences between actual and predicted values and lead to erroneous management decisions. For instance, a difference of 3.95 mg/dL was observed between equal 50:50 and AM-PM composite

**Table 4.** Summary of equal 50:50 composites for the prediction of actual daily milk component concentrations<sup>1</sup>

Item	RPE <sup>2</sup> (%)	CCC <sup>3</sup>	MSPE <sup>4</sup> bias breakdown (%)		
			Mean	Slope	Dispersion
Fat, %	1.07	0.997	7.6	0.3	92.1
Protein, %	0.32	1.000	5.5	0.3	94.3
Lactose, %	0.14	0.999	2.9	0.0	97.0
MUN, mg/dL	1.18	0.999	5.2	0.1	94.7
Log SCC, <sup>5</sup> × 10 <sup>3</sup> cells/mL	0.52	1.000	3.4	0.0	96.6

<sup>1</sup>Actual daily milk component concentrations = {[evening milk component concentration (% , mg/dL, or cells/mL) × evening milk yield (kg)] + [morning milk component concentration (% , mg/dL, or cells/mL) × morning milk yield (kg)]}/daily milk yield (kg). For MUN and SCC concentrations, a milk density of 1.03 kg/L was considered in the calculation. Equal 50:50 composite milk component concentrations = [evening milk component concentration (% , mg/dL, or cells/mL) × 0.5] + [morning milk component concentration (% , mg/dL, or cells/mL) × 0.5].

<sup>2</sup>RPE = relative prediction error or root mean squared prediction error expressed as a percentage of the average of daily actual milk component concentrations.

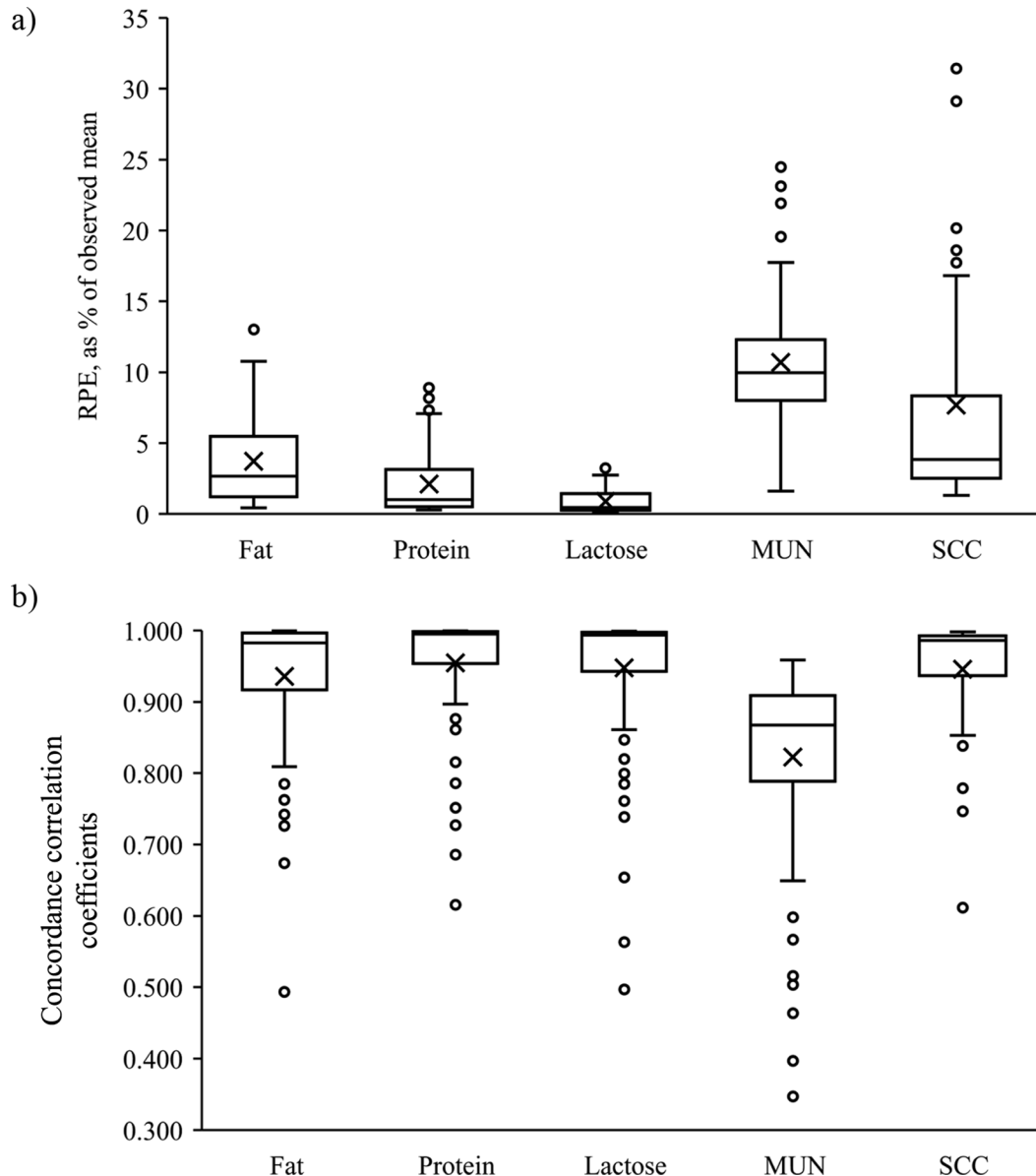
<sup>3</sup>CCC = concordance correlation coefficient as calculated by Lin (1989).

<sup>4</sup>MSPE = mean squared prediction error decomposed in percentages due to mean (overall bias of prediction), regression (deviation of slope from unity), and random disturbance biases.

<sup>5</sup>Raw SCC data were log transformed for normality.

MUN concentration (Table 2). If this is the case for several cows within a feeding group, this can cause a nonnecessary ration adjustment for protein. Some components are more variable between evening and morning milkings, and their concentrations are therefore more affected by the use of composite samples composed of an unequal volume of milk. It seems that some workers are more skilled than others at visually assessing the volume of milk to pour into vials during evening and morning milkings to obtain

an exactly equal 50:50 composite sample mixed on farm. Unfortunately, there is no record of who performed the AM-PM composite sampling. It is therefore not possible to determine whether there was a sampler effect (producer vs. DHI employee). Animal misidentification might also have occurred in some cases. Moreover, sample preparation before pouring milk into DHI vials can also explain some of the variability between herds. Indeed, proper milk sample handling is necessary to get reliable results.



**Figure 2.** Box plots on a herd basis, showing the distribution of the relative prediction errors (RPE; a) and the concordance correlation coefficients (CCC; b) between equal 50:50 and AM-PM composite milk concentrations of fat, protein, and SCC (99 herds) and lactose and MUN (98 herds). For each component, the “x” represents average RPE and CCC, the box represents the 25th (lower quartile) and 75th (upper quartile) percentiles, the midline is the median, the length of the whiskers is 1.5 times the interquartile range, and open circles are outlier data determined as being outside the upper and lower limits. Equal 50:50 composite milk component concentrations = [evening milk component concentration (% , mg/dL, or cells/mL) × 0.5] + [morning milk component concentration (% , mg/dL, or cells/mL) × 0.5]. AM-PM composite milk samples were mixed on the farm using an approximately equal volume of milk from evening and morning milkings.



## APPLICATIONS

This study was conducted to evaluate the precision and the accuracy of using AM-PM composite milk samples taken at 2 consecutive milkings to predict actual daily milk fat, protein, lactose, MUN, and SCC concentrations. We obtained CCC and RPE ranging from 0.911 to 0.964 and 1.19% to 11.01%, respectively, between daily actual and AM-PM composite milk component concentrations. Milk protein and lactose had the lowest RPE and the highest CCC, whereas it was the opposite for MUN. For each component, there was a slope bias ranging from 4.5 to 8.4% of the mean of actual data. Overall, this means that the equal measure sampling scheme can be used to predict actual daily milk component concentrations with great precision and accuracy. However, further analyses on a herd basis showed that the on-farm sampling was variable because the AM-PM composite samples were not always composed of an equal 50:50 volume of milk from 2 milkings. If we assume that milk samples are homogeneous, a suggestion might be to add clear indicators of 50:50 volume on DHI vials or to use a pouring container with a predefined volume to ensure an equal volume of milk at each DHI milking ends up in the DHI vial. Based on milk fat concentrations from a companion paper, the equal measure sampling scheme appeared more precise and accurate than the alternated one-milking recording scheme. To our knowledge, this is the first study to assess the agreement of milk components between daily actual and AM-PM composite milk samples. It was the missing piece of information allowing producers to make an informed decision about which sampling scheme to choose according to their management objectives. Variation in CCC and RPE between herds also stressed that AM-PM composite sample mixing volume at the farm is very important to get reliable results, and it also showed that visually assessing the correct volume to pour into the vial is a difficult task. Because DHI records are used for management decision making, it is of great importance that dairy producers, advisors, and veterinarians be aware of the overall performance of the DHI milk sampling scheme used. However, other factors might affect the choice of a sampling scheme, such as its convenience.

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