Analysis of the accuracy of protocols in robotic milking herds for estimation of 24-hour fat and protein (yields and percentage)

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<u>Abstract</u>

The evolution of the number of farms equipped with Automatic Milking System (AMS), the difficulties in milk recording lead to examine ways to improve and simplify protocols. The aim of this study is to compare six protocols to determine minimal number samples, minimal sampling period to estimate accurate 24-hour fat and protein (yields and percentage). Protocols were tested from unadjusted and adjusted samples. Analysis of results with unadjusted samples shows that the limitation of number samples (only one sample) impact fat percentage and yields (correlations 0.846 and 0.923 respectively). With two samples, correlations are higher than 0.960 for fat yields, protein yields and percent, only fat percent is affected. The limitation of sampling period from 12 hours to 8 hours decreases the accuracy for all traits (mainly for fat percentage) and all cows are not sampled when the sampling period is lower than 12 hours.

Another protocol was tested to improve estimated 24-hour fat and protein with one single sample. Separate regressions for 240 combinations of parity, milking interval, lactation stage, time of sampling milking were fitted in adjusted sample. The accuracy of 24-hour fat percent and yields is better with one single sample adjusted from one sample unadjusted (compared with reference 24-hour). There is little improvement of accuracy in protein yields and percentage. Correlations between true and estimated daily fat percent derived from am (pm) milking increase from 0.804 to 0.840 for first lactations (0.795 to 0.832) and from 0.785 to 0.822 (from 0.772 to 0.810) for later lactations. The analysis of the results between milking intervals confirms better accuracy with one single sample adjusted for fat percentage and yields. However, the level of results obtained with single sample adjusted (mainly fat percentage) does not allow to use data for genetic evaluation. A way of improving estimate 24-hour fat consists to apply a regression model with seven other factors. The first results obtained with this model are interesting.

Keywords : automatic milking system, milk recording, sampling period, 24-hour fat percentage

Introduction

In France, the number of dairy farms equipped with Automatic Milking System (AMS) increased significantly between 2000 and 2011 (from 49 to 1 866 AMS during this period). This evolution creates difficulties for the Milk Recording Organizations: cost of milk recording, use of sampling equipment... Currently, the French guidelines of milk recording suggests to estimate 24-hour milk yields from weights of the last 2 days (48 hours) and to collect and analyze all milk samples with a 12 to 24 hours period. Most breeders with AMS would prefer to limit the duration of a robotic milk sampling to a manageable period of time and the cost in terms of labor and sample analysis.

On the published literature, several studies have been made to answer some of these issues and challenges for automated systems (Bouloc, 2001; Peeters and Galesloot, 2002; Hand *et al.*, 2006). The aim of the current study was threefold. First, to evaluate the impact of a sample number limitation on the estimated fat and protein yields and percentage (P1 and P2). Second, to evaluate the effect of the sampling period reduction from 12 to 24 hours to less than 8, 10 and 12 hours (P3, P4 and P5). Third, to study the possibilities and perspectives of adjusting sampling fat and protein (kg and %) for specific covariates to improve results obtained with a simplified AMS sampling schemes (P6).

Material and Methods

The protocols investigated for estimation of 24-hour fat and protein percent are summarized below.

- Description of 6 alternate AMS sampling protocols

- P1 : use one sample (the first) unadjusted for covariates
- P2 : use two samples (the first two samples) unadjusted for covariates
- P3 : use all samples unadjusted for covariates for which milk intervals are ≤ 8 hours
- P4 : use all samples unadjusted for covariates for which milk intervals are ≤ 10 hours
- P5 : use all samples unadjusted for covariates for which milk intervals are ≤ 12 hours
- P6 : use single sample adjusted for covariates (milking intervals, days in milk, parity, am/pm).

Reminds : current French recommendation for AMS milk recording: 1/ Milk intervals must be greater than or equal to 4 hours for all samples used to estimate 24-hour fat and protein percentage. 2/ The reference 24-hour rate (calculated for fat and protein percentage), use all milk and samples records collected during a 12 - 24 hours period.

Data were collected in France between 2009 and 2011 on four different AMS models. Two data files were used one for testing protocols P1 to P5, another for testing protocol P6 (Table 1). In fact, P6 required an adjustment for days in milk and parity, which are only available from national genetic database. National and AMS database must be crossed. However, AMS animal identification being not formatted this lead to the loss of a large amount of data.

For all tested protocols, at least 2 sampled milking were required per test-day to make sure that reference fat and protein yields and percent were properly estimated. In P6, a single sample was taken into account. To enlarge P6 dataset and make it more representative of diversity (milking interval, time of sampling milking...), we considered all sampled milking of a test-day as independent. In this way, the analysis is based on 58 062 samples from 24 628 test-day records (Table 1). Averages and standard deviations of the 24-hour milk, fat, protein yields and percentage are shown in Table 2 for the 2 datasets.

	Dataset for P1 to P5	Dataset for P6
# Test-day records selected for analysis	52 314	24 628
# Cows	19 783	7 624
# Herds	268	109
# Sample per cow	1.88	2.38
Average length of sampling period	19:03	21:18

Table 1: Description of datasets

Troita	Datase	t for P1 to P5	Dataset for P6			
Traits	Mean	Standard Deviation	Mean	Standard Deviation		
Milk - kg	26.9	9.12	30.2	8.50		
Fat - kg	1.084	0.350	1.182	0.320		
Protein - kg	0.873	0.260	0.961	0.238		
Fat - %	4.125	0.791	3.991	0.692		
Protein - %	3.305	0.390	3.229	0.332		

Table 2: Description of 24-hour reference performance of the selected data

Fat and protein (yields and percentage) adjustment from unique sample is based of multiple linear regression model. Fat yield (protein yield) was estimated by multiplying the estimated 24-hour fat (protein respectively) by the 24-hour milk yield. The model A1 considers separate regressions for every combination of parity (i), milking interval (j), lactation stage (k) and moment of sampling period (l):

$$y_{24-h \text{ estimated}} {}^{[ijkl]} = b_0 {}^{[ijkl]} + b_1 {}^{[ijkl]} y_{1sample} {}^{[ijkl]} + b_2 {}^{[ijkl]} milk_{24-h} {}^{[ijkl]}$$
 mentioned as A1

A second regression model A2 (adapted from M6 model of Peeters and Galesloot, 2002) was fitted to estimate 24-hour fat percentage (yields) from the fat percentage (yields) of the single sampled milking on test-day. The model A2 considers similar 4 regressions factors (even they are not mentioned to simplify equation). For fat percentage, the regression is function of fat percentage, protein percentage, milk yields and milking interval of the sampled milking (Fat‰_n, Pro‰_n, Milk_n, MI_n), milk yield and milking interval of the milking before the sampled milking (Milk_{n-1}, MI_{n-1}) and Milk yields estimated on 24 hours (Milk_{24-h}). Similar equation was applied to fat yields.

 $Fat\%_{24-h \text{ estimated}} = b_0 + b_1 \operatorname{Fat}\%_n + b_2 \operatorname{Pro}\%_n + b_3 \operatorname{MI}_n + b_4 \operatorname{MI}_{n-1} + b_5 \operatorname{milk}_n + b_6 \operatorname{milk}_{n-1} + b_7 \operatorname{milk}_{24-h}$ mentioned as A2

Traits	Number of classes	Class definition
Parity	2	1^{st} lactation, 2^{nd} and later
Milking interval	5	< 6 h ; 6 h - 8 h ; 8 h - 10 h ; 10 h - 12h ; > 12 h
Lactation stage	12	30 days per class (till 360)
Am / Pm (time of sampled milking)	2	Am : 3 h - 15 h / Pm : 15 h - 3 h

Table 3: Definition of effect classes considered in P6

Results

Two kinds of results are presented for P1 to P6 protocols : the bias estimated as the difference between the proposed protocol and the reference method (mean, absolute mean, standard deviation) and correlations between both.

- Effect of sample number : protocols P1 and P2

The aim is to show the loss of accuracy when the number of samples is restricted to one in comparison with two and more for P1, two in comparison with three and more for P2. For P1 (table 4), fat percentage and yields are deeply impacted by the limitation of sample number as shown by correlations (0.846 and 0.923 respectively). For protein yields and percentage, the impact of the number of samples is more limited, correlations are greater than 0.950.

With at least two samples, correlations are higher : over 0.960 for fat yields and protein percentage, and even better for protein yields (0.992). Only fat percentage is affected (0.933).

The aim of P2 is to show the loss of accuracy when the number of samples represents two samples (from three samples and more). Table 4 shows that correlations are quite acceptable for all traits (greater than 0.960), except for fat percentage (0.933).

The results obtained on P1 and P2 are not comparable because they are not on the same dataset.

	P1 (N=36 426)				P2 (N=8 744)			
Traits	Mean	Mean (*abs)	Std	Correlations	Mean	Mean (*abs)	Std	Correlations
F - kg	0.000	0.096	0.141	0.923	0.012	0.059	0.089	0.967
P - kg	0.000	0.020	0.034	0.990	0.004	0.017	0.023	0.992
F - %	0.000	0.329	0.458	0.846	0.034	0.175	0.253	0.933
P - %	0.009	0.071	0.115	0.954	0.014	0.052	0.084	0.970

Table 4: Bias and correlations (R²) from P1 and P2 (effect of number samples)

(*abs) absolute value

- Protocols P3 to P5: sampling period effect

Table 5 shows that the loss of accuracy increases for all traits when the sampling period decreases from 12 to 8 hours. For fat percentage, correlations evolve from 0.924 to 0.883 when the sampling period decreases. For fat yields, correlations are higher but follow the same trend (0.965 to 0.943). For protein percentage, the results of P3 to P5 are greater than 0.960 and at least 0.993 for protein yields. Means and standard deviation are greater for P3 compare to P5.

The limitation of sampling period can create some trouble in dairy traits for genetic evaluation: with a sampling period limited at 8 hours, 22 % of cows are not sampled. This percentage decreases to 13 % with a period between 8 and 10 hours and 8 % with a restriction of 12 hours.

Table 5: Bias and correlations (R²) from P3, P4 and P5 (effect of sampling period)

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	P3 (N=40 794)				P4 (N=45 467)			
Traits	Mean	Mean (*abs)	Std	Correlations	Mean	Mean (*abs)	Std	Correlations
F - kg	0.005	0.075	0.123	0.943	0.006	0.062	0.110	0.954
P - kg	0.002	0.016	0.030	0.993	0.002	0.013	0.027	0.994
F - %	0.014	0.259	0.404	0.883	0.019	0.219	0.367	0.902
P - %	0.008	0.056	0.101	0.965	0.009	0.048	0.092	0.971

	P5 (N=48 205)							
Traits	Mean	Mean (*abs)	Std	Correlations				
F - kg	0.007	0.048	0.094	0.965				
P - kg	0.002	0.010	0.023	0.995				
F - %	0.026	0.171	0.321	0.924				
P - %	0.009	0.037	0.081	0.978				

(*abs) absolute value

- Protocol P6 : single sample effect adjusted for covariates (parity, milking interval, lactation stage, am/pm).

Table 6 to 8 presents elements about accuracy and precision for the prediction of fat and protein percentage and yields by the components of one sampled milking after adjustments. Effects of parity and time of sampled milking are limited on fat and protein yields as shown in table 6. The impact of the time of sampled milking seems to be larger on percentage (around + 0.01 in favor of am milking). Fat percentage is not very well predicted on 24-hour from a single sample. Correlations are around 0.77 to 0.80 (table 5) but can go down till 0.70 with milking interval lower than 6 hours (Table 6).

The proposed adjustment A1 enables to improve correlations for the four traits. Its impact is very limited for protein yields, for which adjustment is not necessary. For protein percentage, adjustment for covariates is more interesting, especially when milking interval is lower than 6 hours (increase of 0.025 – Table 7). For fat traits, adjustments are compulsory with protocols based on a single sampled milking considering the correlations obtained without. For fat percentage, it enables to increase correlations from 0.780 to 0.852 (without distinction of parity, milking interval...). Even if A2 adjustment is more complex than A1, correlations are largely improved for fat percentage (between 0.02 and 0.04).

Table 6: Effect of parity and time of sampled milking on correlations (R²) between single sample and 24-hours performance (P6)

]	L1	L2	2+
Traits	Adjustment	am	pm	am	pm
Number of	samples records	10 119	11 587	17 058	19 298
	No	0.874	0.871	0.875	0.878
F - kg	Yes – Al	0.913	0.907	0.920	0.914
	Yes – A2	0.918	0.911	0.928	0.922
D lea	No	0.981	0.979	0.981	0.979
P - Kg	Yes – Al	0.985	0.983	0.984	0.983
	No	0.804	0.795	0,785	0.772
F - %	Yes – Al	0.840	0.832	0.822	0.810
	Yes – A2	0.865	0.857	0.851	0.842
D 0/	No	0.909	0.896	0.913	0.903
P - %	Yes – A1	0.920	0.909	0.922	0.915

Table 7: Effect of milking intervals on correlations (R²) between single sample and 24-hours (P6)

	-			Milking interval	ls	
Traits	Adjustment	< 6 h	6 - 8 h	8 - 10 h	10 - 12 h	> 12 h
Number of	samples records	4 965	16 016	15 910	10 320	10 851
	No	0.821	0.872	0.875	0.880	0.900
F - kg	Yes – A1	0.879	0.910	0.918	0.923	0.930
	Yes – A2	0.892	0.919	0.926	0.929	0.932
Dha	No	0.966	0.974	0.978	0.981	0.986
P - Kg	Yes – A1	0.977	0.980	0.983	0.985	0.989
	No	0.704	0.786	0.805	0.813	0.837
F - %	Yes - A1	0.750	0.807	0.825	0.829	0.847
	Yes – A2	0.792	0.839	0.857	0.856	0.867
D 0/	No	0.855	0.889	0.899	0.904	0.927
r - %	Yes	0.880	0.902	0.912	0.915	0.934

As it should be, mean bias after adjustment is null. To be analysing, bias is shown in absolute value (Table 8). For fat percentage, mean value decrease from 0.373 without adjustment to 0.259 after adjustment and standard deviation keep high level. For protein traits, the situation is better.

Table 8: Mean and standard deviation of bias (P6)

		Mean			Mean (*abs)		Std	
Adjustment	No	Yes - A1	Yes – A2	No	Yes – A1	Yes – A2	No	Yes – A1	Yes – A2
F – kg	0.022	0.000	0.000	0.117	0.088	0.084	0.173	0.123	0.118
P-kg	0.007	0.000		0.027	0.026		0.048	0.041	
F - %	0.071	0.000	0.000	0.373	0.286	0.259	0.527	0.388	0.358
P - %	0.022	0.000		0.090	0.084		0.149	0.131	
2 .									

(*abs) absolute value

Discussion

Milk Recording Organizations and AMS breeders wish to reduce costs of milk recording and limits effort involve in milk sample collection. Different alternatives were studied to simplify AMS milk recording. However, it is imperative before adopting any protocols, to ensure these protocols lead to estimates that are unbiased and reliable. The above tables show various situations according to the studied traits and large differences between proposed protocols. For protein yields, results are better whatever the tested protocols. On the opposite, fat percentage need to be carefully analysed.

In the first part of the study, we chose to study separately the effects : number of sample and sampling period. The restriction to a single sample has a large impact on the accuracy of the proposed protocol. With P1 the limiting criterion is fat percentage and yields. With P2 the limiting criterion is fat percentage. P2 with at least two sampled milking on a period of 12 to 24 hours can be considered to give a sufficient accuracy for milk recording and genetic evaluation. Reduction of sampling period also has an impact on the accuracy, especially for fat percentage. P3 to P5 give some answers. As for the limitation of number sample, the limiting criterion is fat percentage for P3 to P5. With 22% of cows not sampled, it is not possible to propose a protocol as P3. Limiting the sampling period during milk recording has a consequence: breeders would be forced to "push cows to the AMS" before the end of the sampling period.

Adjustment for a single sampled milking can be a promising alternative. To maximize statistical rating of the analysis for P6, we chose to consider each sampled milking of the test-day as independent. It can create a slight bias, sampled milking stemming from a limited number of cows. Unfortunately, results are not as high as expected for fat percentage in some specific cases as for short milking interval. With an average correlation of 0.852 for fat percentage, the range is equivalent to those obtained by Hand *et al.*, 2006 (0.856) in a similar protocol.

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