COMPARISON OF A FLOUROMETRIC AND A FAT-CONTENT-BASED METHOD TO MEASURE CARRY-OVER IN MILKING SYSTEMS

February 10, 2018

Christian Ammon
Leibniz-institute for Agricultural Engineering and Bioeconomy (ATB)
Sandra Rose-Meierhöfer
University of Applied Sciences, Department of Agricultural Machinery

NATIONAL DHIA · Quality Certification Services Inc. · ICAR THE GLOBAL STANDARD FOR LIVESTOCK DATA

Carryover – A Significant Cause for Concern

- Carryover is comingling of milk between cows before the sample is tested

- Carryover occurs:
  - **Sample collection**
  - Cross-contamination on the dairy
  - Cross-contamination in laboratory

- Two effects of carryover:
  - **Contamination**
  - Dilution

Historical Look at Carry-Over Research

- First exploratory study 1997 NL – CRV & Animal Health service
- Measuring left overs by water flushing and analyzing COD (chemical oxygen demand)

- **Scope:**
  - Recorder jars, Electronic milk meters, Effect of sampling routine by technician (careful vs not)
- **Results milk meters**
  - Carry-over related to milk yield (7 vs 20kg : from 3,7% to 1.3%)
  - Non careful procedure : doubling carry over : (7 vs 20kg: from 7.0% to 2.5%)
- **Results recorder jars**
  - Carry-over related to milk yield (7 vs 20kg : from 2,0% to 1.6%)
  - No careful procedure : doubling carry over : (7 vs 20kg: from 3,7% to 2.8%)
- **Conclusion:**
  - Carry over affected by milk volume, measurement system, sampling volume and sampling procedure
  - No major issue when careful operation procedure for sampling is applied

High Carryover Concern in Sampler

In the Sampler

2ml in 25-30ml sample vial
Min. carryover estimate – 8-12%

2ml in 80ml sample vial
Min. carryover estimate – 2-3%

In the Meter Flask

1.5 lbs. milk remaining in flask
Subsequent cow yield of 40lb (18.1kg)

Min. carryover estimate – 3.8%

Testing for Carryover in Recording & Sampling Devices

- There is no ‘reference method’ for carry-over determination in ICAR Guidelines
- ICAR Test Centres in France, Germany & Netherlands need a reproducible, low cost protocol for determination of carry-over
- Lovendahl proposed a colorimetric method (previously presented)
- Alternative procedure proposed by Phillippe Trossat, MASC using whole milk and skim milk

Testing for Carryover in Recording & Sampling Devices

- Recording & Sampling Devices SC commissioned study to develop a ‘standard’ and ‘reproducible’ method
- Comparison of alternative methods for determination
- Experimentation and validation under way

Colorimetric Determination of Carry-Over

Lovendahl

Fluorescence based tracers

- Direct method –
- Based on dilution of colour intensity

Colorimetric Determination of Carry-Over

Lovendahl

Protocol

- **Phantom cows**: Yellow and White
- 8 Kg "**yellow**" **milk**, samples before and after
- 8 Kg "**white**" **milk**, sample: how yellow is after ?
- Replicate 6 x

Replicate	*Run*	*Liquid*	*Volume (Kg)*	*Samples*
1	1_1	Yellow	5	A **B** C
	1_2	White	5	A **B** C
2	2_1	Yellow	5	A **B** C
	2_2	White	5	A **B** C
	2_3	White	5	A **B** C
3	3_1	Yellow	8	A **B** C
	3_2	White	8	A **B** C
4	4_1	Yellow	8	A **B** C
	Wash	No		
	4_2	White	8	A **B** C

Colorimetric Determination of Carry-Over

Lovendahl

Equipment results

Equipment	Volume (N)	CO%, AY73	CO%, 4MeU	Average
1.A AMS Standard	5 (1)	8.4	8.5	8.5
	8 (3)	3.3	2.8	3.1
1.B	8 (6)	6.7	7.2	7.0
2 AMS Unadjusted	5 (2)	17.0	16.9	17.0
	8 (1)	20.0	19.7	19.9
3 AMS Well adjusted	6 (1)	3.1	4.6	3.9
	8 (6)	2.1	2.5	2.3
4 AMS Modified	6 (2)	10.5	10.0	10.3
	8 (3)	11.2	11.8	11.5
5 Conventional	8 (6)	3.3	3.7	3.5

- Un-adjusted and modified: C-O up to 20% ☹
- Well adjusted: 2 – 5%, similar to conventional
- Conventional: 3.5%

Challenges with the Colorimetric Determination of Carry-Over

- Cost of equipment and labor
- Repeatability between ICAR Test Centres is unknown but would include different flourometers, variations in technique
- Hard to adapt by device manufacturers for internal design & development
- Laboratories require more precise information regarding possible carry-over in milk samples for animal health tests

Proposed Method

Analyze at raw and skimmed milk according the following sequence (at least 2 consecutives sequences)

“Raw 1 (R1) - Raw 2 (R2) - Skimmed 1 (S1) - Skimmed 2 (S2)”

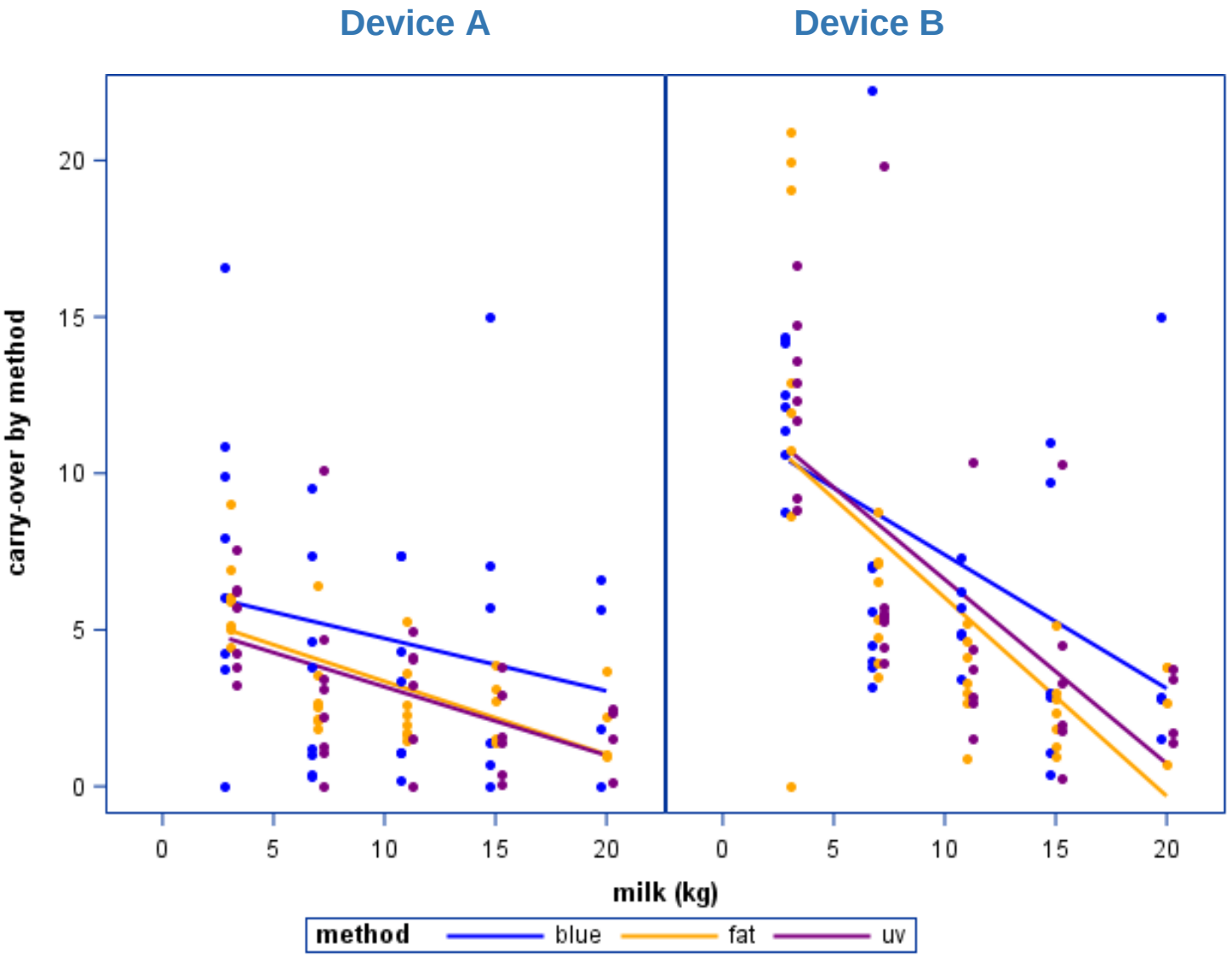
Carry over (%) = ((ΣS1- Σ S2))/((Σ R2- Σ S2)) "x 100"

The carry over must be calculated on several sequences. These sequences are performed with different milk yields, for instance: 5, 10, 20 and 30 kg.

Example Calculation of Carry-Over Using Milk Fat Concentration

Sequence	Milk	Yield (kg)	Fat Content (%)	Carry-Over
1.1	Raw 1	5	4.24	=((0.75+0.78)-(0.51+0.53))/((4.26+4.26)-(0.51+0.53)) x 100 = **6.55 %**
1.2	Raw 2	5	4.26	
1.3	Skimmed 1	5	0.75	
1.4	Skimmed 2	5	0.51	
1.5	Raw 1	5	4.21	
1.6	Raw 2	5	4.26	
1.7	Skimmed 1	5	0.78	
1.8	Skimmed 2	5	0.53	
2.1	Raw 1	10	4.20	=((0.72+0.70)-(0.52+0.54))/((4.27+4.25)-(0.52+0.54)) x 100 = **4.83 %**
2.2	Raw 2	10	4.27	
2.3	Skimmed 1	10	0.72	
2.4	Skimmed 2	10	0.52	
2.5	Raw 1	10	4.21	
2.6	Raw 2	10	4.25	
2.7	Skimmed 1	10	0.70	
2.8	Skimmed 2	10	0.54	
3.1	Raw 1	20		
…				

Initial Results from Comparison of Carry-Over Determination Methods



THE GLOBAL STANDARD FOR LIVESTOCK DATA

Initial Results from Comparison of Carry-Over Determination Methods

- Carry-over varies between type of device and within a specific devices
- Operational techniques vary between milk recording technicians
- Carry-over is dependent on milk volume but not uniform in its dependency
- Carry-over in AMS more variable due to design, maintenance, and sampling tray

Goals and Next Steps of the RSD-SC on Carry-Over Testing

- ICAR Test Centre workshop to review procedures and determine a standard protocol for carry-over determination
- There would not be specific ICAR guidelines for carry-over level in RSD but levels will be reported
- Development of best practices for device usability for milk sampling for specific tests
- MROs must reinforce proper sampling procedures as these are only minimal estimates of carry-over