

Fifteen months of using litter from recycled manure as bedding for dairy cows: a focus on *Klebsiella pneumoniae*, *Klebsiella variicola* and *Clostridium perfringens*

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Introduction

In recent years, for economical purposes and to improve well-being conditions of cows, several dairy producers have adopted litter made from recycled manure solids (RMS). However, its use raises several safety issues, particularly with respect to mastitis and other infections. We previously observed cases of mastitis caused by *K. pneumoniae* and *K. variicola*, and two deadly infections from *C. perfringens* from cows on RMS.

Objective

Assess the populations of *Klebsiella pneumoniae*, *Klebsiella variicola* and *Clostridium perfringens* and study their relationship with somatic cell count data (SCC) and mastitis incidence in dairy cows on RMS.

Methods

The use of animals was approved by the CRSAD Animal Ethics Committee (CPA-CRSAD) and all procedures were performed according to the guidelines of the Canadian Council on Animal Care. The trial was conducted at CRSAD, Deschambault, Quebec, Canada.

A herd of Holstein cows, distributed in two barns of about 40 lactating cows, was used. RMS was used in one barn, and wood-chip litter (WCL), in the other. RMS was generated by roller presses separation followed by conditioning at 55°C for 24h. Clean and soiled (used) RMS and WCL samples were collected for 15 months. Similarly, material samples before conditioning were also collected, and all samples were analyzed by viability-qPCR. Milk samples from cows with mastitis and somatic cell count data (SCC) were analyzed.

Data were analyzed using a linear model including treatment, month and their interaction if significant as fixed effects. Comparisons between pairs were made using Student's test. Data from mastitis incidence were analyzed by categorical analysis and Pearson's chi-square tests were used for frequency comparison.

Table 1. Treatments

Treatments	Description
WCL	Wood-chip litter
RMS	Recycled manure solids litter

Results

Total and living contents of *K. pneumoniae*, *K. variicola*, *C. perfringens* and *Klebsiella spp* are higher in the RMS than the WCL, and composting 24h at 55°C does not ameliorate their levels in RMS.

Table 2. Effects of treatments and months on levels of *K. pneumoniae*, *K. variicola*, *C. perfringens*, *Klebsiella spp* and general bacteria of the WCL and RMS.

Bacteria		Treatments					Std E	Months			E Std	Effects (p values)		
		WCL		RMS				AU2018	NO2018	FE2019		TRT	Month	Month
		clean	used	BeComp	clean	used								
<i>C. perfringens</i>	total	0.00 ^b	-0.01 ^b	2.16 ^a	1.87 ^a	1.78 ^a	0.44	1.21	1.56	0.71	0.40	0.0085	0.2652	0.8296
	alive	0.00 ^b	0.02 ^b	1.96 ^a	2.19 ^a	1.77 ^a	0.48	1.32	1.19	0.34	0.38	0.0053	0.1205	0.5290
<i>K. pneumoniae</i>	total	0.00 ^c	2.27 ^b	2.51 ^b	2.25 ^b	3.84 ^a	0.36	2.81 ^a	2.61 ^a	1.10 ^b	0.27	<.0001	<.0001	0.0545
	alive	-0.19 ^d	1.21 ^c	2.79 ^{ab}	2.19 ^{bc}	3.66 ^a	0.42	2.22 ^{ab}	2.52 ^a	1.63 ^b	0.34	<.0001	0.0905	0.2125
<i>K. variicola</i>	total	0.00 ^d	2.06 ^c	3.52 ^b	4.19 ^a	3.63 ^{ab}	0.29	2.59 ^{ab}	3.15 ^a	2.30 ^b	0.22	<.0001	0.0823	0.0024
	alive	0.02 ^d	2.09 ^c	2.79 ^{bc}	3.70 ^a	3.11 ^{ab}	0.35	1.63	2.82	2.52	0.28	<.0001	0.1501	0.0001
<i>Klebsiella spp.</i>	total	0.00 ^{cd}	-0.83 ^d	0.97 ^{bc}	2.22 ^a	1.67 ^{ab}	0.52	1.14	1.04	0.23	0.39	0.0005	0.1369	0.0179
	alive	-1.91 ^c	-0.71 ^{bc}	0.80 ^{ab}	2.12 ^a	1.07 ^a	0.59	1.90	2.88	1.77	0.46	0.0016	0.1269	0.1590
General bacteria	total	0.00 ^c	1.43 ^b	3.25 ^a	3.36 ^a	3.44 ^a	0.26	1.75 ^b	2.63 ^a	2.51 ^a	0.20	<.0001	0.0095	0.3917
	alive	-0.54 ^c	1.23 ^{bc}	2.07 ^{ab}	3.03 ^a	2.83 ^{ab}	0.58	1.06 ^b	3.05 ^a	2.68 ^a	0.46	0.0072	0.0364	0.2930

The differences in the log₁₀ of the least squares means compared to the clean WCL are presented. Treatments not connected by the same letter are significantly different, according to a value of p < 0.05. BeComp, material before composter; Std E, standard error; WCL, wood-chip litter; RMS, litter made from recycled manure; TRT, treatments.

RMS shows no effect on somatic cell count (SCC)

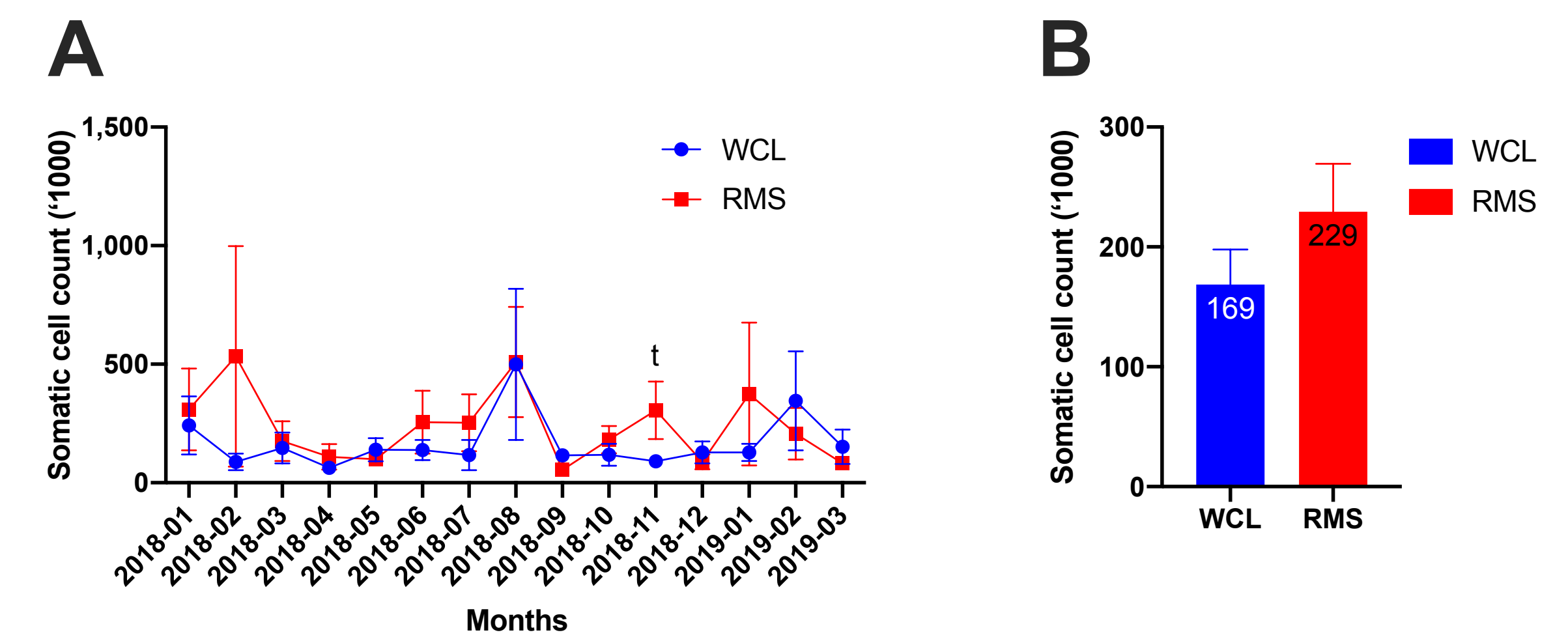


Figure 1. Somatic cell count (SCC) of cow's milk on WCL and RMS. (A) SCC every month and (B) average over 15 months. WCL, wood-chip litter; RMS, litter made from recycled manure.

RMS increases mastitis incidence in dairy cows

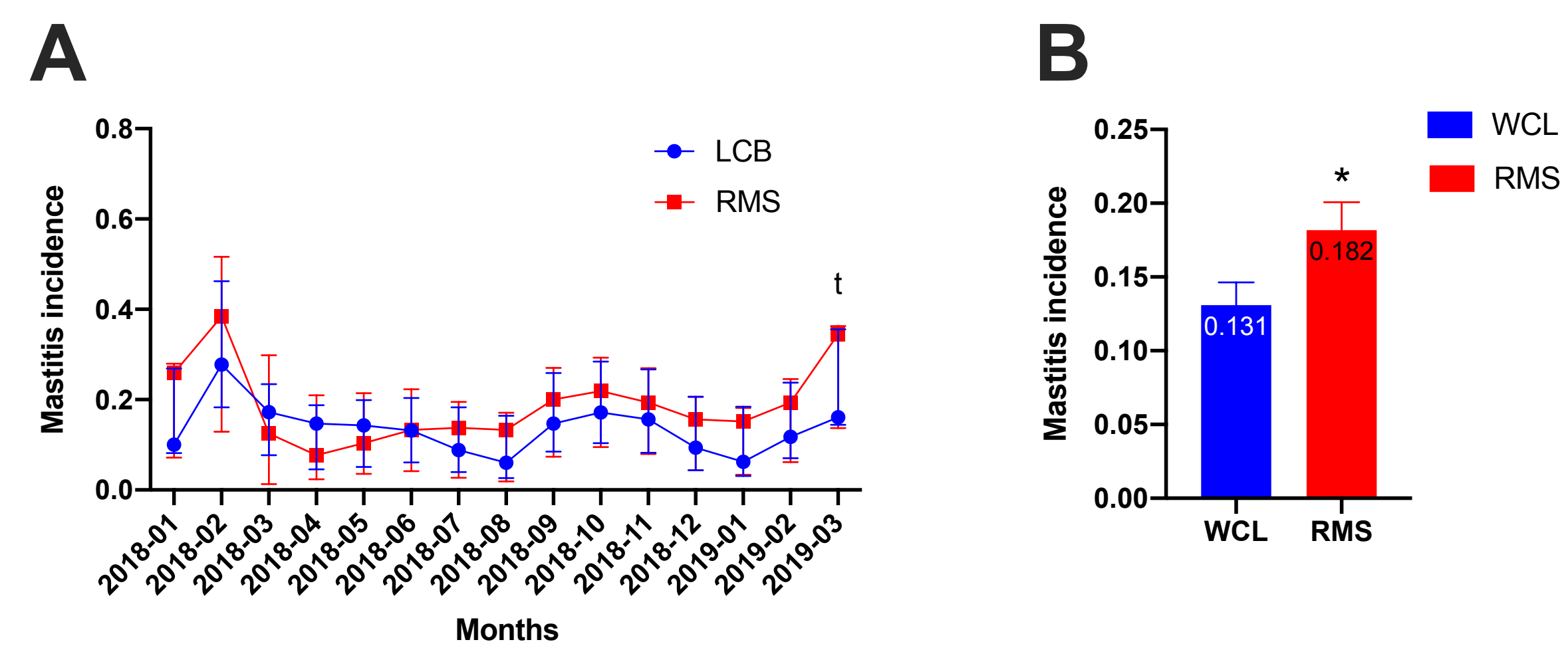


Figure 2. Mastitis incidence of cows on WCL and RMS. (A) Mastitis incidence every month and (B) average over 15 months. WCL, wood-chip litter; RMS, litter made from recycled manure.

Conclusion

- Levels of *K. pneumoniae*, *K. variicola*, *C. perfringens* and *Klebsiella spp* levels were 1.6-4.2 log₁₀ higher in the RMS compared to the WCL.
- RMS had no significant effect on SCC, but increased mastitis incidence (+39%) compared to WCL.
- Milk analysis revealed that higher levels of *K. pneumoniae* found in RMS could contribute to the increase in mastitis cases caused by this pathogen.
- Data reported from this study are not favorable to the use of an RMS conditioned for 24h at 55°C with dairy cows, apropos the microbiological aspect. However, these results must be confirmed by other studies.

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