Microscopic differential cell count and specific mastitis pathogens in cow milk from compost-bedded pack barns and cubicle barns

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SUPPLEMENTARY FILE

Supplementary Materials and Methods

Statistical models

For statistical analyses of the quasi Gaussian distributed percentages of cell fractions for macrophages, lymphocytes, PMN, segmented and banded neutrophils, the linear mixed model included the fixed effects for the lactation number (1 or 2), for the lactation stage classes (0-100 days, 101-200 days, 201-300 or >300 days after calving), for the farming system (compost or cubicle), for classes for the daily production level in milk yield at the recording date (< 25 kg, 25.1-30 kg, 30.1-35 kg, 35.1-40 kg, > 40 kg), for the herd-test-date at milk sampling and for the person counting the cells in the laboratory, for the interaction between the system with milk yield classes, for the interaction between the system with lactation stage classes, and for the interactions between the system with SCC classes. For the separation of the system effect from remaining farm management effects, a hierarchical design was considered, implying to nesting the system effect nested within in herd-test-day effect. A further fixed effect was the individual udder quarter. Due to the repeated measurements within cow (512 cows), the cow effect was treated as random. For the analyses of binary mastitis pathogens (major pathogens, minor pathogens, CNS, COR, AER, *Aesculin hydrolyzing streptococci*), we considered the same fixed and random effects as defined for the linear mixed model. The only difference is the consideration of a logit-link function in the generalized linear mixed model, implying the exclusion of a random error term.

Supplementary Table S1

Descriptive statistics for the differential somatic cell counts and mastitis pathogens per udder quarter

Traits[1]	Mean	Min	Max	SD
Cell fractions (in relation to the Total sum of all cell counts)				
Macrophages	0.292	0.000	0.980	0.208
Lymphocytes	0.608	0.000	1.000	0.246
PMN	0.100	0.000	0.971	0.143
Banded neutrophils	0.005	0.000	0.130	0.013
Segmented neutrophils	0.095	0.000	0.951	0.140
Mastitis pathogens (in prevalences)				
Negative samples	0.514	0.000	1.000	0.500
CNS	0.274	0.000	1.000	0.446
COR	0.183	0.000	1.000	0.387
AER	0.035	0.000	1.000	0.184
Aesculin hydrolyzing streptococci	0.015	0.000	1.000	0.122
Minor pathogens	0.407	0.000	1.000	0.491
Major pathogens	0.030	0.000	1.000	0.171

[1]Polymorphonuclear neutrophils (PMN), *coagulase-negative staphylococci* (CNS), *Corynebacterium sp.* (COR), *Aerobic bacilli* (AER), minor pathogens (including CNS and COR), major pathogens (including *Aerococcus sp., Aesculin hydrolyzing streptococci, Candida krusei, Enterococcus sp., Escherichia Coli, Lactococcus sp., Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberis, mold fungus* and *Proteus sp.*)

Supplementary Table S2

Least squares means (LSM) and corresponding standard errors (SE) for the differential somatic cell counts and prevalences of specific mastitis pathogens in the housing systems compost bedded pack barn (compost) and conventional cubicle barn (cubicle) and *P*-Values for the LSM differences in the two farming systems

	compost		cubicle		
Traits	LSM [%]	SE [%]	LSM [%]	SE [%]	p-value
Cell fractions (in relation to the Total sum of all cell counts)					
Macrophages	29.700	0.800	27.500	0.869	0.0533
Lymphocytes	56.300	0.988	59.400	1.075	0.0307
PMN	13.900	0.582	13.100	0.630	0.3291
Segmented neutrophils	13.100	0.570	12.300	0.616	0.3153
Banded neutrophils	0.795	0.054	0.801	0.058	0.9409
Mastitis pathogens (in prevalences)					
Negative samples	51.100	2.850	33.900	2.920	<0.0001
CNS	18.600	1.900	36.700	2.720	<0.0001
COR	10.600	1.630	12.800	2.020	0.3939
AER	0.004	2.349	0.001	0.704	0.9989
AESC	1.130	0.460	0.000	0.043	0.9945
Minor	35.000	2.500	54.100	2.920	<0.0001
Major	2.925	0.748	0.153	23.323	0.9845

[1]Polymorphonuclear neutrophils (PMN), *coagulase-negative staphylococci* (CNS), *Corynebacterium sp.* (COR), *Aerobic bacilli* (AER), minor pathogens (including CNS and COR), major pathogens (including *Aerococcus sp., Aesculin hydrolyzing streptococci, Candida krusei, Enterococcus sp., Escherichia Coli, Lactococcus sp., Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberis, mold fungus* and *Proteus sp.*)

Supplementary Table S3

Least squares means (standard errors) for the differential somatic cell fractions lymphocytes, macrophages and polymorphonuclear leucocytes (PMN) from the two production systems compost-bedded pack barns (compost) and conventional cubicle barns (cubicle) depending on lactation stage, cow productivity in milk yield per day and the total somatic cell counts in 1000ml/l.

Trait	Lactation stage (in days)	compost	cubicle
Macrophages	< 100	0.243 (0.011)	0.230 (0.015)
	101-200	0.291 (0.017)	0.276 (0.014)
	201-300	0.290 (0.018)	0.275 (0.013)
	>300	0.330 (0.019)	0.289 (0.016)
Lymphocytes	< 100	0.679 (0.016)	0.682 (0.020)
	101-200	0.608 (0.023)	0.628 (0.019)
	201-300	0.603 (0.024)	0.622 (0.017)
	>300	0.510 (0.025)	0.554 (0.021)
PMN	< 100	0.079 (0.010)	0.088 (0.013)
	101-200	0.101 (0.015)	0.097 (0.012)
	201-300	0.107 (0.015)	0.103 (0.011)
	>300	0.159 (0.016)	0.157 (0.013)
	Milk yield (in kg per day)		
Macrophages	< 25	0.322 (0.018)	0.298 (0.018)
	26-30	0.297 (0.016)	0.255 (0.016)
	31-35	0.278 (0.016)	0.267 (0.014)
	36-40	0.253 (0.019)	0.270 (0.017)
	> 40	0.273 (0.017)	0.257 (0.016)
Lymphocytes	< 25	0.544 (0.024)	0.578 (0.024)
	26-30	0.586 (0.022)	0.646 (0.021)
	31-35	0.620 (0.021)	0.620 (0.019)
	36-40	0.675 (0.026)	0.626 (0.023)
	> 40	0.612 (0.023)	0.631 (0.021)
PMN	< 25	0.134 (0.015)	0.124 (0.016)
	26-30	0.117 (0.014)	0.099 (0.013)
	31-35	0.102 (0.013)	0.113 (0.012)
	36-40	0.072 (0.016)	0.104 (0.015)
	> 40	0.115 (0.015)	0.112 (0014)
	Total somatic cell count (in 1000ml/l)		
Macrophages	<13	0.251 (0.011)	0.232 (0.013)
	13-25	0.282 (0.012)	0.258 (0.012)
	26-50	0.297 (0.014)	0.265 (0.012)
	51-100	0.301 (0.016)	0.286 (0.013)

	101-200	0.314 (0.018)	0.297 (0.015)
	>200	0.341 (0.016)	0.303 (0.015)
Lymphocytes	<13	0.715 (0.013)	0.720 (0.016)
	13-25	0.668 (0.015)	0.679 (0.014)
	26-50	0.613 (0.017)	0.652 (0.015)
	51-100	0.563 (0.019)	0.600 (0.016)
	101-200	0.483 (0.021)	0.536 (0.018)
	>200	0.337 (0.020)	0.402 (0.018)
PMN	<13	0.036 (0.008)	0.048 (0.010)
	13-25	0.050 (0.009)	0.064 (0.009)
	26-50	0.090 (0.011)	0.083 (0.009)
	51-100	0.136 (0.012)	0.115 (0.010)
	101-200	0.204 (0.014)	0.167 (0.012)
	>200	0.319 (0.012)	0.293 (0.012)

[1]Polymorphonuclear neutrophils (PMN),

Supplementary Table S4

Least squares means (standard errors) for prevalences of cultural negative samples, major pathogens, minor pathogens, *Coagulase-negative staphylococci* (CNS) and *Corynebacterium sp.* (COR) from the two production systems compost-bedded pack barns (compost) and conventional cubicle barns (cubicle) depending on lactation stage, cow productivity in milk yield per day and the total somatic cell counts in 1000ml/l.

Trait	Lactation stage (in days)	compost	cubicle
Negative samples	< 100	0.716 (0.033)	0.506 (0.050)
	101-200	0.595 (0.055)	0.488 (0.046)
	201-300	0.533 (0.060)	0.381 (0.041)
	>300	0.493 (0.063)	0.342 (0.048)
Major	< 100	0.018 (0.007)	0.001 (0.229)
	101-200	0.025 (0.013)	0.003 (0.498)
	201-300	0.035 (0.016)	0.003 (0.512)
	>300	0.083 (0.033)	0.002 (0.370)
Minor	< 100	0.201 (0.027)	0.425 (0.047)
	101-200	0.337 (0.050)	0.435 (0.044)
	201-300	0.348 (0.053)	0.497 (0.044)
	>300	0.340 (0.058)	0.580 (0.049)
CNS	< 100	0.133 (0.021)	0.328 (0.042)
	101-200	0.192 (0.038)	0.324 (0.039)
	201-300	0.189 (0.038)	0.313 (0.035)
	>300	0.162 (0.039)	0.358 (0.045)
COR	< 100	0.051 (0.013)	0.102 (0.027)
	101-200	0.104 (0.031)	0.125 (0.029)
	201-300	0.122 (0.037)	0.157 (0.031)
	>300	0.162 (0.051)	0.165 (0.039)
	Milk yield (in kg per day)		
Negative samples	< 25	0.551 (0.059)	0.423 (0.062)
	26-30	0.623 (0.053)	0.490 (0.053)
	31-35	0.537 (0.052)	0.412 (0.047)
	36-40	0.603 (0.063)	0.384 (0.054)
	> 40	0.632 (0.055)	0.412 (0.052)
Major	< 25	0.060 (0.023)	0.001 (0.086)
	26-30	0.053 (0.021)	0.002 (0.245)
	31-35	0.031 (0.014)	0.004 (0.422)
	36-40	0.019 (0.013)	0.002 (0.187)
	> 40	0.017 (0.010)	0.004 (0.451)
Minor	< 25	0.305 (0.051)	0.482 (0.061)
	26-30	0.246 (0.043)	0.442 (0.051)
	31-35	0.352 (0.047)	0.498 (0.046)

	36-40	0.326 (0.058)	0.519 (0.055)
	> 40	0.265 (0.047)	0.477 (0.052)
CNS	< 25	0.180 (0.038)	0.301 (0.051)
	26-30	0.151 (0.032)	0.343 (0.046)
	31-35	0.185 (0.034)	0.368 (0.043)
	36-40	0.165 (0.041)	0.343 (0.049)
	> 40	0.134 (0.033)	0.292 (0.044)
COR	< 25	0.083 (0.029)	0.123 (0.036)
	26-30	0.086 (0.027)	0.095 (0.026)
	31-35	0.129 (0.033)	0.102 (0.026)
	36-40	0.110 (0.038)	0.157 (0.042)
	> 40	0.097 (0.030)	0.207 (0.048)
	Total somatic cell count (in 1000ml/l)		
Negative samples	<13	0.808 (0.028)	0.716 (0.041)
	13-25	0.658 (0.043)	0.589 (0.043)
	26-50	0.595 (0.055)	0.413 (0.047)
	51-100	0.527 (0.067)	0.276 (0.044)
	101-200	0.262 (0.062)	0.194 (0.043)
	>200	0.219 (0.050)	0.093 (0.026)
Major	<13	0.011 (0.006)	0.001 (0.132)
	13-25	0.023 (0.011)	0.001 (0.090)
	26-50	0.022 (0.013)	0.000 (0.064)
	51-100	0.024 (0.016)	0.001 (0.220)
	101-200	0.026 (0.017)	0.002 (0.343)
	>200	0.163 (0.050)	0.017 (2.487)
Minor	<13	0.123 (0.021)	0.222 (0.035)
	13-25	0.219 (0.035)	0.340 (0.039)
	26-50	0.325 (0.049)	0.517 (0.046)
	51-100	0.397 (0.064)	0.642 (0.048)
	101-200	0.639 (0.066)	0.691 (0.053)
	>200	0.528 (0.063)	0.769 (0.045)
CNS	<13	0.065 (0.015)	0.161 (0.028)
	13-25	0.094 (0.022)	0.213 (0.030)
	26-50	0.123 (0.030)	0.280 (0.038)
	51-100	0.206 (0.050)	0.453 (0.050)
	101-200	0.507 (0.070)	0.561 (0.058)
	>200	0.347 (0.059)	0.596 (0.056)
COR	<13	0.044 (0.013)	0.084 (0.021)
	13-25	0.114 (0.028)	0.134 (0.028)
	26-50	0.164 (0.041)	0.210 (0.041)
	51-100	0.116 (0.038)	0.163 (0.038)
	101-200	0.115 (0.040)	0.081 (0.027)
	>200	0.122 (0.038)	0.124 (0.036)

[1]*coagulase-negative staphylococci* (CNS), *Corynebacterium sp.* (COR), minor pathogens (including CNS and COR), major pathogens (including *Aerococcus sp., Aesculin hydrolyzing streptococci, Candida krusei, Enterococcus sp., Escherichia Coli, Lactococcus sp., Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberis, mold fungus* and *Proteus sp.*)