

Use of approved cefazolin based intramammary dry goat treatment and its effects on the milk microbiota

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21 **Supplementary figures and table legends**

22 **Supplementary Fig. S1:** Somatic cell counts between groups (CG=Not Treated; TG=Treated) over time
23 (T1, dry-off; T2, clostrum; T3, 5-10 DIM).

24 **Supplementary Fig. S2:** Pairwise contrasts between phylum abundances (CSS-normalised counts)
25 along timepoints. Red cells indicate significant contrasts (p-value <0.05). These results are irrespective of
26 the experimental group (CG, TG).

27 **Supplementary Table S1:** Detailed composition with the relative proportions of each phylum in all
28 groups (CG and TG) and timepoints (T1, T2 and T3)

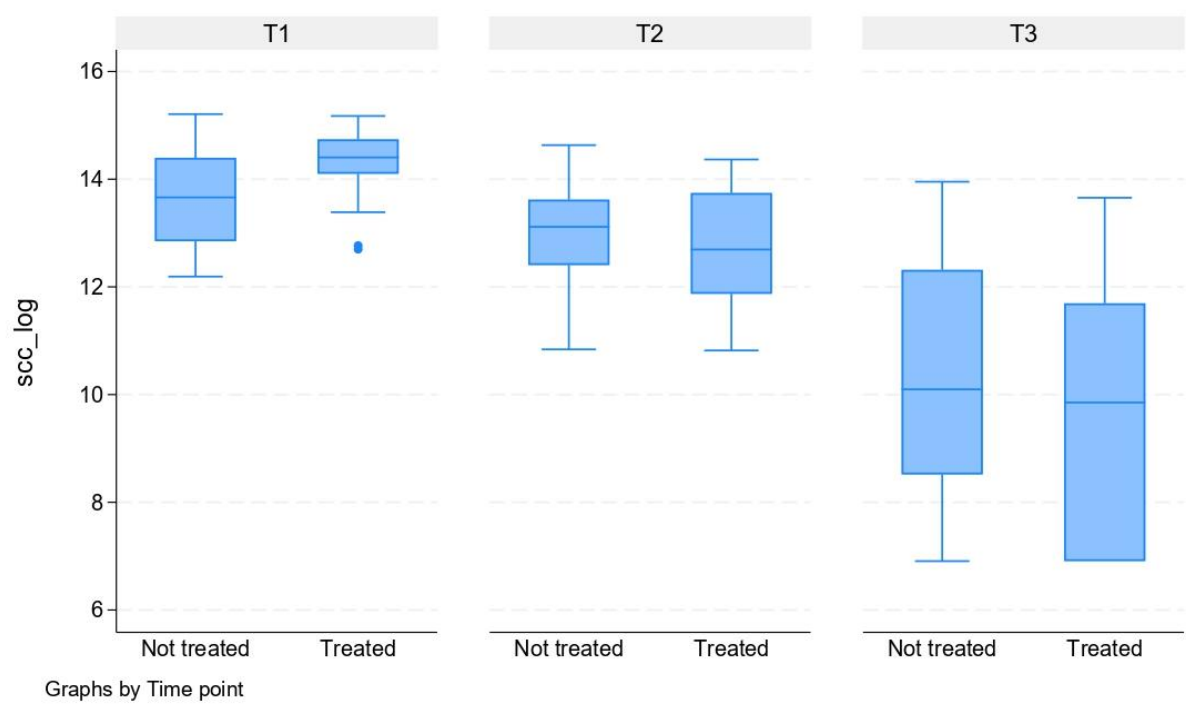
29 **Supplementary Table S2:** Results from the comparison between treatment groups within the timepoint
30 (equation (2)).

31 **Supplementary Table S3:** Results from comparing healthy and infected milk samples at T1; p-values for
32 the alpha and beta diversity indices were obtained from model (1).

33 **Supplementary Table S4:** Linear regression model results of alpha diversity indices with both timepoint
34 and treatment effects. The baseline is T1 for the time effect and the control group for the treatment effect.

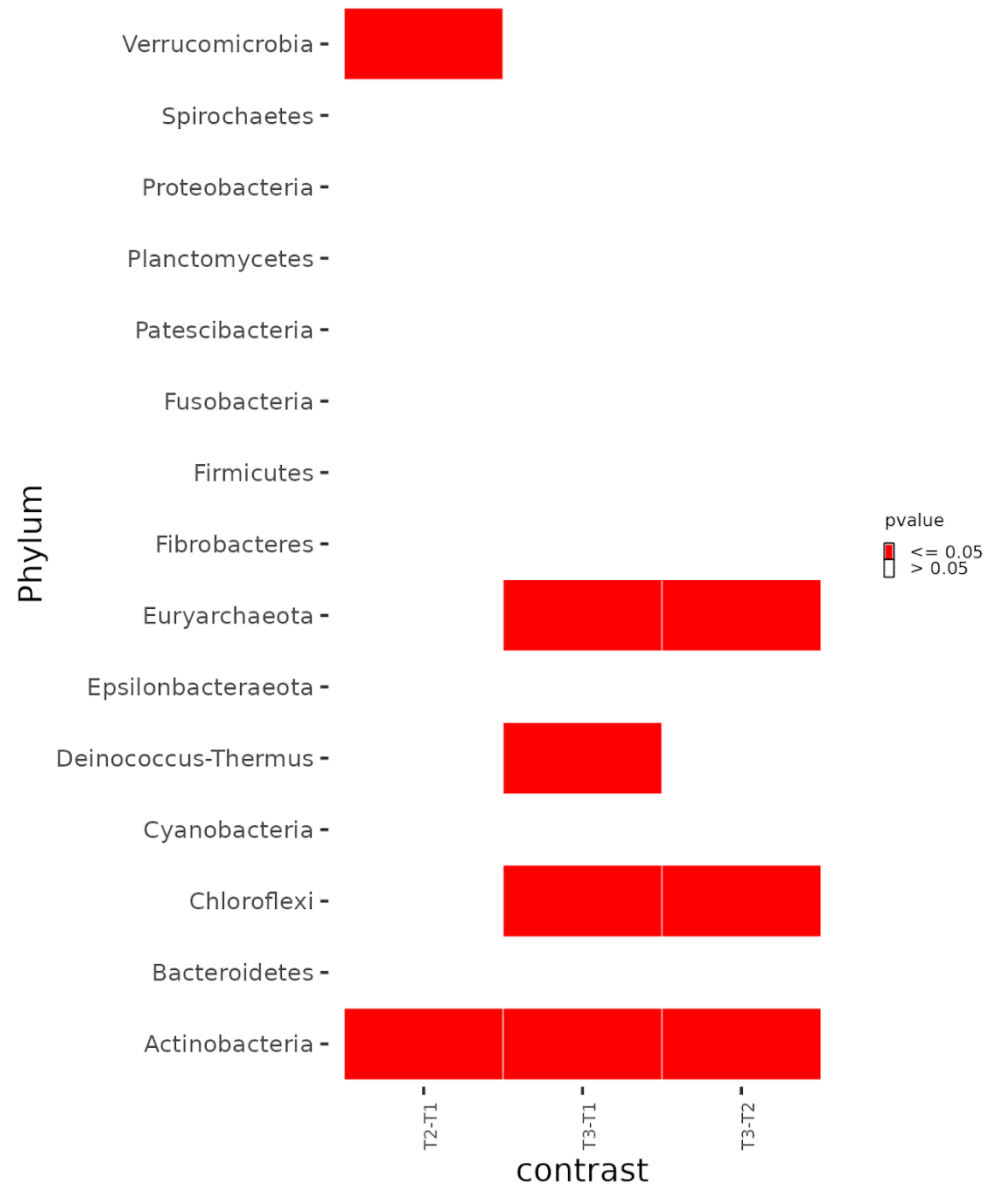
35 **Supplementary Table S5:** Results from permutational analysis of variance (PERMANOVA).

36 **Supplementary Fig. S1** Somatic cell counts between groups (CG=Not Treated; TG=Treated) over time
37 (T1, dry-off; T2, clostrum; T3, 5-10 DIM).



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Supplementary Fig. S2 Pairwise contrasts between phylum abundances (CSS-normalised counts) along timepoints. Red cells indicate significant contrasts (p-value <0.05). These results are irrespective of the experimental group (CG, TG).



44 **Supplementary Table S1** Detailed composition with the relative proportions of each phylum in all
45 groups (CG and TG) and timepoints (T1, T2 and T3)

Treatment	Timepoint	Phylum	Avg
CG	T1	Actinobacteria	0.31947
TG	T1	Actinobacteria	0.27436
CG	T2	Actinobacteria	0.27903
TG	T2	Actinobacteria	0.31192
CG	T3	Actinobacteria	0.23870
TG	T3	Actinobacteria	0.31701
CG	T1	Bacteroidetes	0.01918
TG	T1	Bacteroidetes	0.05606
CG	T2	Bacteroidetes	0.03627
TG	T2	Bacteroidetes	0.01222
CG	T3	Bacteroidetes	0.04658
TG	T3	Bacteroidetes	0.04560
CG	T1	Chloroflexi	0.00000
TG	T1	Chloroflexi	0.00002
CG	T2	Chloroflexi	0.00079
TG	T2	Chloroflexi	0.00197
CG	T3	Chloroflexi	0.00052
TG	T3	Chloroflexi	0.00513
CG	T1	Cyanobacteria	0.00242
TG	T1	Cyanobacteria	0.00245
CG	T2	Cyanobacteria	0.00180
TG	T2	Cyanobacteria	0.00326
CG	T3	Cyanobacteria	0.00320
TG	T3	Cyanobacteria	0.00331
CG	T1	Deinococcus-Thermus	0.00000
TG	T1	Deinococcus-Thermus	0.00000
CG	T2	Deinococcus-Thermus	0.00000
TG	T2	Deinococcus-Thermus	0.00001
CG	T3	Deinococcus-Thermus	0.00028
TG	T3	Deinococcus-Thermus	0.00034

Treatment	Timepoint	Phylum	Avg
CG	T1	Epsilonbacteraeota	0.00000
TG	T1	Epsilonbacteraeota	0.00016
CG	T2	Epsilonbacteraeota	0.00012
TG	T2	Epsilonbacteraeota	0.00009
CG	T3	Epsilonbacteraeota	0.00018
TG	T3	Epsilonbacteraeota	0.00000
CG	T1	Euryarchaeota	0.00224
TG	T1	Euryarchaeota	0.00125
CG	T2	Euryarchaeota	0.00050
TG	T2	Euryarchaeota	0.00052
CG	T3	Euryarchaeota	0.00276
TG	T3	Euryarchaeota	0.00289
CG	T1	Fibrobacteres	0.00000
TG	T1	Fibrobacteres	0.00000
CG	T2	Fibrobacteres	0.00003
TG	T2	Fibrobacteres	0.00000
CG	T3	Fibrobacteres	0.00022
TG	T3	Fibrobacteres	0.00009
CG	T1	Firmicutes	0.38826
TG	T1	Firmicutes	0.44336
CG	T2	Firmicutes	0.50345
TG	T2	Firmicutes	0.44001
CG	T3	Firmicutes	0.41308
TG	T3	Firmicutes	0.30958
CG	T1	Fusobacteria	0.00000
TG	T1	Fusobacteria	0.00016
CG	T2	Fusobacteria	0.00000
TG	T2	Fusobacteria	0.00000
CG	T3	Fusobacteria	0.00401
TG	T3	Fusobacteria	0.00000
CG	T1	Patescibacteria	0.00045
TG	T1	Patescibacteria	0.00019
CG	T2	Patescibacteria	0.00051

Treatment	Timepoint	Phylum	Avg
TG	T2	Patescibacteria	0.00220
CG	T3	Patescibacteria	0.00033
TG	T3	Patescibacteria	0.00024
CG	T1	Planctomycetes	0.00023
TG	T1	Planctomycetes	0.00012
CG	T2	Planctomycetes	0.00000
TG	T2	Planctomycetes	0.00022
CG	T3	Planctomycetes	0.00036
TG	T3	Planctomycetes	0.00038
CG	T1	Proteobacteria	0.24875
TG	T1	Proteobacteria	0.21279
CG	T2	Proteobacteria	0.15650
TG	T2	Proteobacteria	0.21730
CG	T3	Proteobacteria	0.28484
TG	T3	Proteobacteria	0.31360
CG	T1	Spirochaetes	0.00050
TG	T1	Spirochaetes	0.00050
CG	T2	Spirochaetes	0.00013
TG	T2	Spirochaetes	0.00009
CG	T3	Spirochaetes	0.00057
TG	T3	Spirochaetes	0.00007
CG	T1	Verrucomicrobia	0.01850
TG	T1	Verrucomicrobia	0.00858
CG	T2	Verrucomicrobia	0.02087
TG	T2	Verrucomicrobia	0.01019
CG	T3	Verrucomicrobia	0.00436
TG	T3	Verrucomicrobia	0.00179

47 **Supplementary Table S2** Results from the comparison between treatment groups within the timepoint
48 (equation (2)).

Genus	Treatment	Timepoint	Avg	Std	Estimate	Std. error	Statistic	p value
<i>Acinetobacter</i>	Treated	T1	0.665	6.958	0.4544243432	0.2096786925	2.167241401	0.03031710672
<i>Acinetobacter</i>	Treated	T2	0.271	2.466	-6.247805088	1.864389731	-3.3511261	0.0008193153708
<i>Atopostipes</i>	Treated	T3	0.266	1.993	-1.066410302	0.3615808512	-2.949299718	0.003256018181
<i>Bacillus</i>	Treated	T2	0.914	3.683	0.8863862374	0.4349261337	2.038015582	0.04348702256
<i>Bacteroides</i>	Treated	T2	0.333	1.722	-0.8697720218	0.3093346742	-2.811750814	0.005002103793
<i>Christensenellaceae</i> <i>R-7 group</i>	Treated	T1	0.174	0.784	-0.3265492801	0.150753001	-2.166121258	0.03068108736
<i>Christensenellaceae</i> <i>R-7 group</i>	Treated	T3	0.963	4.815	-1.016195447	0.4854036593	-2.093505947	0.03668622351
<i>Flavobacterium</i>	Treated	T3	1.529	7.305	1.338675652	0.6263070253	2.137411203	0.0334530341
<i>Iamia</i>	Treated	T3	2.395	7.578	2.238197403	0.7250614787	3.086907068	0.002284893309
<i>Kocuria</i>	Treated	T2	1.019	3.59	0.6489190517	0.3182403556	2.039084737	0.04226293208
<i>Mannheimia</i>	Treated	T1	0.1	0.36	0.09982190155	0.04994824602	1.998506645	0.04832295674
<i>Mannheimia</i>	Treated	T2	1.426	3.361	1.118891794	0.5181768544	2.159285551	0.03348752335
<i>Methylobacterium</i>	Treated	T2	0.012	0.141	-0.3691043821	0.1177106498	-3.135692334	0.001901061953
<i>Neisseria</i>	Treated	T3	0.887	2.738	0.7968158039	0.3723258023	2.140103638	0.03459723458
<i>Nocardioide</i> s	Treated	T1	0.195	1.103	0.1951123037	0.08178428593	2.385694286	0.01756047811
<i>Nocardioide</i> s	Treated	T3	1.427	6.752	1.196423785	0.4936244575	2.423753052	0.01582347563
<i>Novosphingobium</i>	Treated	T1	1.101	4.334	0.8889969228	0.3933761149	2.259915865	0.02466075923
<i>Novosphingobium</i>	Treated	T2	0.184	1.014	0.1837394628	0.09258663502	1.984513885	0.04839754987
<i>Paracoccus</i>	Treated	T1	0.477	2.521	0.3959202874	0.1901797883	2.081821054	0.0380608742
<i>Paracoccus</i>	Treated	T3	2.029	6.797	1.659763651	0.5011462908	3.311934422	0.001014618437
<i>Proteiniphilum</i>	Treated	T2	0.242	1.004	0.2424669481	0.1183222127	2.049209041	0.04236420865
<i>Proteiniphilum</i>	Treated	T3	1.558	5.107	1.44029787	0.5637095694	2.555035338	0.0115327242
<i>Pseudofulvimonas</i>	Treated	T3	1.386	4.899	1.291492096	0.5429364276	2.378716974	0.01853024609
<i>Ruminiclostridium</i> 9	Treated	T2	0.209	0.908	-1.147169849	0.5711359722	-2.008575725	0.04656212616
<i>Ruminococcaceae</i> <i>NK4A214 group</i>	Treated	T3	0.026	0.231	-1.015630126	0.3956883588	-2.566742497	0.01116544975
<i>Ruminococcaceae</i> <i>UCG-005</i>	Treated	T3	0.621	3.257	-1.684859002	0.506995348	-3.32322379	0.0009177799662

Genus	Treatment	Timepoint	Avg	Std	Estimate	Std. error	Statistic	p value
<i>Ruminococcus 1</i>	Treated	T1	0.229	1.226	0.1623123297	0.07822155154	2.07503337	0.03843146486
<i>Ruminococcus 1</i>	Treated	T3	0.589	3.318	-0.8720151892	0.4431951052	-1.967565027	0.04957601623
<i>Stenotrophomonas</i>	Treated	T1	0.166	0.517	-0.7549442742	0.286116291	-2.638592411	0.008745548892
<i>Tessaracoccus</i>	Treated	T3	2.221	9.189	2.030253132	0.8763203264	2.316793381	0.02144336092
<i>Tetrasphaera</i>	Treated	T3	9.324	31.335	9.217823092	4.187337056	2.201356845	0.02983830734
<i>Treponema 2</i>	Treated	T3	0.081	0.618	-0.9104546384	0.4298144093	-2.118250619	0.03566999067
<i>Turicibacter</i>	Treated	T2	0.604	2.153	0.5630960716	0.2237905036	2.516175005	0.01272833822
<i>Tyzzarella 4</i>	Treated	T3	0.403	2.236	-1.489893989	0.7480213166	-1.991780122	0.04891931553
<i>[Eubacterium] coprostanoligenes group</i>	Treated	T2	0.437	2.084	-0.8330071465	0.4134847232	-2.014601991	0.04467734189

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50 **Supplementary Table S3** Results from comparing healthy and infected milk samples at T1; p-values for
51 the alpha and beta diversity indices were obtained from model (1).

Metric	Timepoint	p value
ACE	T1	0.1368167
Chao1	T1	0.1293970
Fisher	T1	0.1386017
InvSimpson	T1	0.8551761
Observed	T1	0.1362772
Shannon	T1	0.9548349
Simpson	T1	0.4808464
Bray-Curtis distances	T1	0.4375624

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53 **Supplementary Table S4** Linear regression model results of alpha diversity indices with both timepoint
54 and treatment effects. The baseline is T1 for the time effect and the control group for the treatment effect.

Metric	Term	Estimate	Std. error	Statistic	p value	Avg	Diff
ACE	T2	-363.433	57.093	-6.366	2.27E-09	686.727	-362.523
ACE	T3	-392.431	54.551	-7.194	2.85E-11	657.200	-392.050
ACE	Treated	-41.871	45.616	-0.918	3.60E-01	780.432	-37.055
Chao1	T2	-365.823	56.042	-6.528	9.85E-10	674.389	-364.965

Metric	Term	Estimate	Std. error	Statistic	p value	Avg	Diff
Chao1	T3	-391.998	53.547	-7.321	1.43E-11	647.714	-391.639
Chao1	Treated	-39.489	44.776	-0.882	3.79E-01	771.182	-34.638
Fisher	T2	-95.333	11.146	-8.553	1.34E-14	102.918	-95.198
Fisher	T3	-80.941	10.650	-7.600	3.05E-12	117.232	-80.884
Fisher	Treated	-6.188	8.906	-0.695	4.88E-01	137.919	-4.902
InvSimpson	T2	-9.981	1.487	-6.713	3.74E-10	3.860	-9.985
InvSimpson	T3	-9.555	1.421	-6.726	3.49E-10	4.287	-9.557
InvSimpson	Treated	0.207	1.188	0.174	8.62E-01	7.581	0.340
Observed	T2	-322.411	46.716	-6.901	1.38E-10	539.022	-321.632
Observed	T3	-334.961	44.637	-7.504	5.20E-12	526.018	-334.636
Observed	Treated	-35.841	37.325	-0.960	3.38E-01	627.573	-31.555
Shannon	T2	-2.007	0.177	-11.331	7.41E-22	2.065	-2.006
Shannon	T3	-1.404	0.169	-8.295	5.94E-14	2.668	-1.404
Shannon	Treated	-0.041	0.142	-0.287	7.74E-01	2.957	-0.013
Simpson	T2	-0.353	0.036	-9.816	7.51E-18	0.514	-0.353
Simpson	T3	-0.244	0.034	-7.095	4.87E-11	0.623	-0.244
Simpson	Treated	-0.001	0.029	-0.019	9.84E-01	0.675	0.004

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56 **Supplementary Table S5** Results from permutational analysis of variance (PERMANOVA).

Set	Term	Df	Sum of Sqs	R2	F	Pr(>F)
All	Timepoint	2	5.55623	0.20251	18.992	0.0009990
Al	Treatment	1	0.08523	0.00311	0.583	0.7582418
Al	Residual	149	21.79585	0.79439		
Al	Total	152	27.43731	1.00000		
T1	Treatment	1	0.07079	0.01047	0.529	0.9420579
T1	Residual	50	6.68877	0.98953		
T1	Total	51	6.75956	1.00000		
T2	Treatment	1	0.13873	0.01957	0.878	0.4685315
T2	Residual	44	6.95077	0.98043		
T2	Total	45	7.08950	1.00000		
T3	Treatment	1	0.05350	0.00666	0.355	0.9710290
T3	Residual	53	7.97852	0.99334		

Set	Term	Df	Sum of Sqs	R2	F	Pr(>F)
T3	Total	54	8.03202	1.00000		