Supplementary Material

Observations on the effects of non-maternal adult contact on the behavioural patterns of pre-weaned dairy heifers

Laura Field[A,], Lauren M. Hemsworth[A], Ellen Jongman[A], Ian Hunt[B], and Megan Verdon[B]*

[A]Animal Welfare Science Centre, University of Melbourne, Corner Flemington Road and Park Drive, Parkville, Vic. 3052, Australia.

[B]Tasmanian Institute of Agriculture, University of Tasmania, Private Bag 3523, Burnie, Tas. 7320, Australia.

[*]Correspondence to: Laura Field Animal Welfare Science Centre, University of Melbourne, Corner Flemington Road and Park Drive, Parkville, Vic. 3052, Australia Email: lfield@student.unimelb.edu.au

Supplemental Files

Supplementary I: Ethogram[1]

Category	Behaviour	Description
Lying	Lying	Lying in sternal or lateral recumbency.
Upright	Standing	Animal remains stationary with body elevated from the floor and weight supported by two or more limbs, and not partaking in any other activity (i.e. grazing or drinking).
	Grazing	Grazing or browsing at a walk or while standing.
	Self-grooming	Licking, rubbing and/or scratching own body, including with hind hoof, in a standing or lying position.
Exploratory	Explore Environment	Interacting with (i.e. sniffing, licking, touching, chewing, rubbing) or standing in contact with any fabricated part of the environment, including water trough, gates, fences and shelter structure. This may include consuming grain from concentrate trough located within shelter.
	Explore Cow[2]	Calf sniffs (up to 10cm away) or nibbles cow (but not suckling or grooming) in investigation, including cow's tail, or follows behind cow in locomotion.
Locomotion	Locomotion	Walking, trotting or galloping more than two steps with forelegs, forwards or backwards and not engaged in any other activity (e.g. grazing, exploratory behaviour, bucking, kicking).
	Solitary Play	Comprising locomotor play (i.e. gallop with, or: leap, jump, buck, kick out).
Social Interaction (calf-calf)	Push	The initiating animal uses the head or some part of the body in an attempt to butt, push or physically displace the receiving animal.
	Mount	Fore legs are on the front, side or back of another calf (mock sexual behaviour)
	Fight	Initiator standing in front of receiver, two calves standing head to head and/or lightly pushing their heads against the head/neck of the other
	Kick	Striking or aiming to strike another animal on any body part with one or both hind limbs.
	Cross sucking	Sucking any part of another calf's body, particularly ears and navel/udder region, and/or forcibly nudging another calf's udder region.
	Allogrooming	Licking, rubbing, scratching and/or otherwise manipulating with no other definition the face or body of another calf.
Social Interaction (Cow-calf)[2]	Receive Agonistic	Calf receives agonistic behaviour from a cow, including pushing (cow attempts to butt, push and/or physically displace calf), fighting (cow and calf standing head to head and/or lightly pushing their heads against the head/neck of the other) and/or kicking (kicking in the direction of a calf or lifting of the hind leg as a kicking threat), and/or chasing (approach at faster than a walk, head down in threat, causing retreat)
	Non-nutritive suckling/attempt	Calf's nose reaches under cow's udder or between her back legs, forcibly nudges the udder region, nibbles at udder net or displaces net to latch onto teat.
	Groom Cow	Licking, rubbing, nibbling, scratching and/or otherwise manipulating with no other definition the face or body of a cow.
	Receive Grooming	Calf receives grooming from one of the three 'nanny cows'; grooming as the cow licking, rubbing, nibbling, scratching and/or otherwise manipulating with no other definition the face or body of the calf.
	Proximity	The number of calves in +S groups observed in proximity to each of the cows (within one adult cow's body length) at each time point.

[1]Developed with reference to Kerr & Woodgush (1987b), MackKay et al. (2014) & Webb et al. (2014). [2]Only recorded in groups of calves housed with cows

Supplementary II – Summary of replicate:treatment interaction in model[1]

Quartile	Measure	Behaviour			
		Lying	Standing	Grazing	Locomotion
1	*df*	1	1	1	1
	Sum Sq	0.014	0.001	0.017	7.92E-05
	F-value	0.426	0.176	2.100	0.030
	p-value	0.526	0.682	0.171	0.865
	r-squared	0.627	0.844	0.299	0.755
	Adjusted r-squared	0.512	0.796	0.083	0.680
2	*df*	1	1	1	1
	Sum Sq	0.122	0.007	0.014	0.002
	F-value	7.332	4.366	3.449	3.398
	p-value	**0.019****	*0.059**	*0.088**	*0.090**
	r-squared	0.630	0.699	0.528	0.638
	Adjusted r-squared	0.507	0.598	0.371	0.518
3	*df*	1	1	1	1
	Sum Sq	0.006	0.001	0.000	0.000
	F-value	0.236	0.435	0.052	0.556
	p-value	0.635	0.521	0.823	0.469
	r-squared	0.511	0.652	0.327	0.564
	Adjusted r-squared	0.361	0.545	0.120	0.430
4	*df*	1	1	1	1
	Sum Sq	0.514	0.010	0.149	0.019
	F-value	13.982	3.964	4.198	4.200
	p-value	**0.002****	*0.068**	*0.061**	*0.061**
	r-squared	0.708	0.301	0.484	0.265
	Adjusted r-squared	0.618	0.085	0.325	0.039

[1] y-variables are raw values, x-variables are week 1 dummy variable, replicate, treatment and replicate:treatment interactions
** denotes p<0.05; * denotes a tendency at 1>p>0.05. *n*=17 or 18 for all measures.

Supplementary III – Regression summary for lying behaviour[1]

Quartile	Measure	First Week	Replicate	Treatment	Replicate:Treatment
1	df	1	1	1	1
	Sum Sq	0.675	0.000	0.005	0.014
	F-value	21.277	0.003	0.150	0.426
	p-value	**0.000****	0.960	0.705	0.525
	r-squared	0.610	0.611	0.615	0.627
	Adjusted r-squared	0.586	0.559	0.532	0.512
2	Sum Sq	0.159	0.011	0.047	0.122
	F-value	9.603	0.670	2.858	7.331
	p-value	**0.009****	0.429	0.117	**0.019****
	r-squared	0.296	0.316	0.404	0.630
	Adjusted r-squared	0.249	0.219	0.267	0.507
3	Sum Sq	0.157	0.118	0.036	0.006
	F-value	6.758	5.076	1.527	0.236
	p-value	**0.022****	*0.042**	0.238	0.635
	r-squared	0.254	0.445	0.502	0.511
	Adjusted r-squared	0.207	0.371	0.396	0.361
4	Sum Sq	0.481	0.132	0.033	0.514
	F-value	13.071	3.594	0.898	13.982
	p-value	**0.003****	*0.080**	0.360	**0.002****
	r-squared	0.293	0.374	0.394	0.708
	Adjusted r-squared	0.249	0.291	0.264	0.618

[1] y-variables are raw values, x-variables are week 1 dummy variable, replicate, treatment and replicate:treatment interactions. For all regressions, $n=17$ or 18.. All regression output is reported to the third decimal place.
** denotes p<0.05; * denotes a tendency at 1>p>0.05.

Supplementary IV– Regression summary for standing behaviour[1]

Quartile	Measure	Variable			
		First Week	**Replicate**	**Treatment**	**Replicate:Treatment**
1	Sum Sq	0.209	0.000	0.013	0.001
	F-value	66.251	0.000	3.978	0.176
	p-value	**0.000****	0.987	*0.068**	0.682
	r-squared	0.794	0.794	0.842	0.844
	Adjusted r-squared	0.781	0.767	0.808	0.796
2	Sum Sq	0.028	0.005	0.004	0.007
	F-value	17.484	3.441	2.508	4.366
	p-value	**0.001****	*0.088**	0.139	*0.059**
	r-squared	0.439	0.526	0.589	0.698
	Adjusted r-squared	0.402	0.458	0.494	0.598
3	Sum Sq	0.023	0.001	0.003	0.001
	F-value	20.216	0.848	2.861	0.435
	p-value	**0.001****	0.374	0.115	0.521
	r-squared	0.541	0.564	0.640	0.652
	Adjusted r-squared	0.512	0.506	0.563	0.545
4	Sum Sq	0.002	0.000	0.002	0.010
	F-value	0.707	0.020	0.896	3.964
	p-value	0.416	0.888	0.361	*0.068**
	r-squared	0.038	0.039	0.087	0.301
	Adjusted r-squared	-0.022	-0.089	-0.108	0.085

[1] y-variables are raw values, x-variables are week 1 dummy variable, replicate, treatment and replicate:treatment interactions. For all regressions, *n*=17 or 18. All regression output is reported to the third decimal place.
** denotes p<0.05; * denotes a tendency at 1>p>0.05.

Supplementary V – Regression summary for grazing behaviour[1]

Variable

Quartile	Measure	First Week	Replicate	Treatment	Replicate:Treatment
1	Sum Sq	0.009	0.008	0.010	0.016
	F-value	1.188	1.013	1.244	2.100
	p-value	0.295	0.332	0.285	0.171
	r-squared	0.064	0.119	0.186	0.299
	Adjusted r-squared	0.006	0.001	0.011	0.083
2	Sum Sq	0.032	0.000	0.008	0.014
	F-value	8.015	0.032	1.945	3.449
	p-value	**0.015****	0.861	0.188	*0.088**
	r-squared	0.315	0.316	0.393	0.528
	Adjusted r-squared	0.269	0.219	0.253	0.371
3	Sum Sq	0.013	0.028	0.006	0.000
	F-value	1.780	3.676	0.799	0.052
	p-value	0.205	*0.077**	0.388	0.823
	r-squared	0.092	0.283	0.324	0.327
	Adjusted r-squared	0.035	0.187	0.179	0.119
4	Sum Sq	0.208	0.044	0.031	0.149
	F-value	5.875	1.233	0.888	4.198
	p-value	**0.031****	0.287	0.363	*0.061**
	r-squared	0.233	0.282	0.317	0.484
	Adjusted r-squared	0.185	0.186	0.171	0.325

[1] y-variables are raw values, x-variables are week 1 dummy variable, replicate, treatment and replicate:treatment interactions. For all regressions, n=17 or 18. All regression output is reported to the third decimal place.
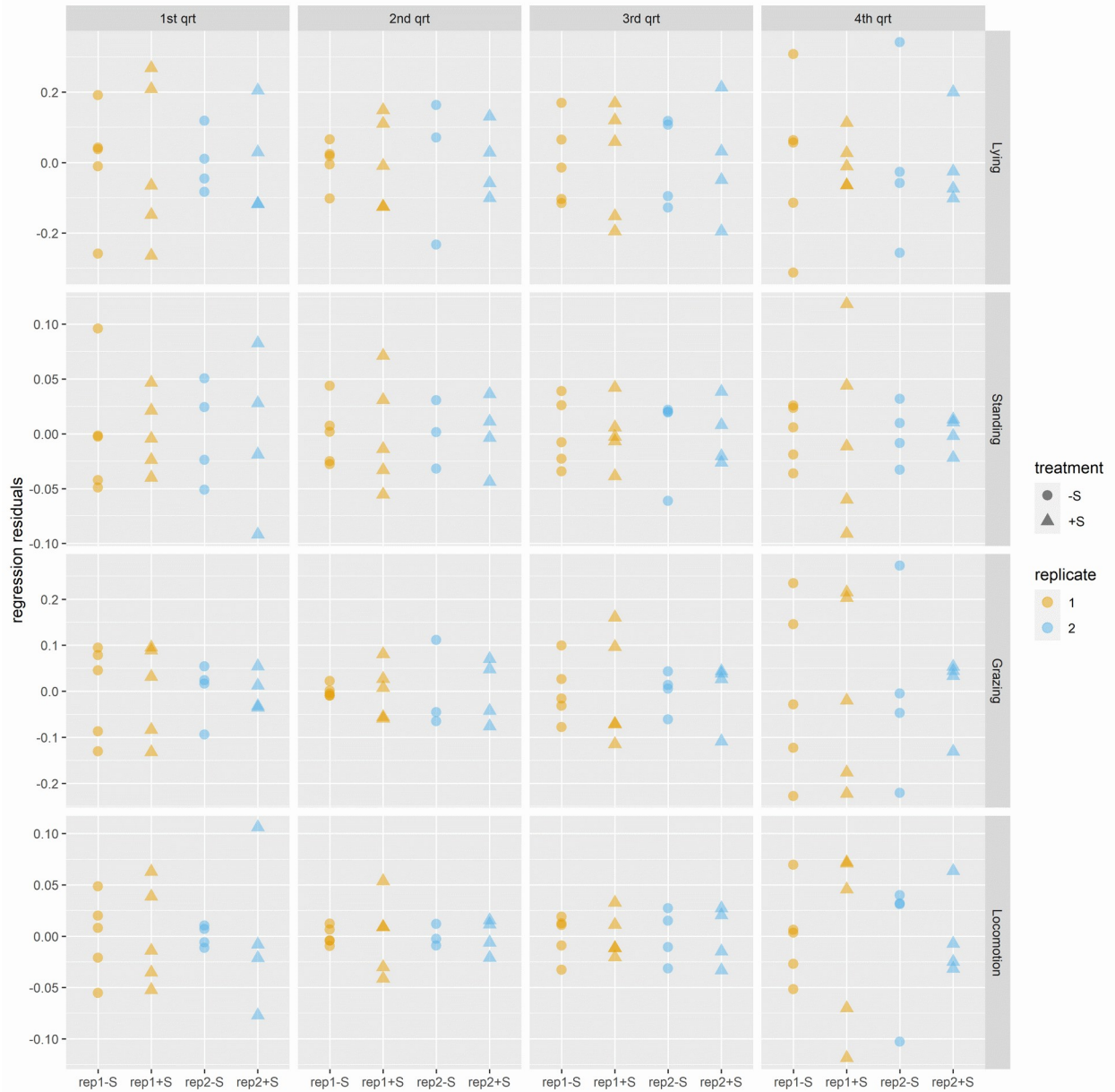** denotes $p<0.05$; * denotes a tendency at $1>p>0.05$.

Supplementary VI – Regression summary for locomotion behaviour[1]

Variable

Quartile	Measure	First Week	Replicate	Treatment	Replicate:Treatment
1	Sum Sq	0.103	0.002	0.000	0.000
	F-value	39.293	0.704	0.080	0.030
	p-value	**0.000****	0.417	0.781	0.864
	r-squared	0.740	0.753	0.755	0.755
	Adjusted r-squared	0.724	0.720	0.702	0.680
2	Sum Sq	0.009	0.001	0.001	0.002
	F-value	15.085	1.342	1.348	3.398
	p-value	**0.002****	0.269	0.268	*0.090**
	r-squared	0.455	0.495	0.536	0.638
	Adjusted r-squared	0.418	0.423	0.429	0.518
3	Sum Sq	0.008	0.002	0.000	0.000
	F-value	12.319	3.912	0.046	0.556
	p-value	**0.004****	*0.070**	0.834	0.469
	r-squared	0.413	0.544	0.546	0.564
	Adjusted r-squared	0.376	0.483	0.448	0.430
4	Sum Sq	0.000	0.002	0.000	0.019
	F-value	0.045	0.433	0.003	4.200
	p-value	0.836	0.522	0.960	*0.061**
	r-squared	0.003	0.027	0.027	0.265
	Adjusted r-squared	-0.060	-0.103	-0.181	0.039

[1] y-variables are raw values, x-variables are week 1 dummy variable, replicate, treatment and replicate:treatment interactions. For all regressions, *n*=17 or 18. All regression output is reported to the third decimal place.
** denotes p<0.05; * denotes a tendency at 1>p>0.05.
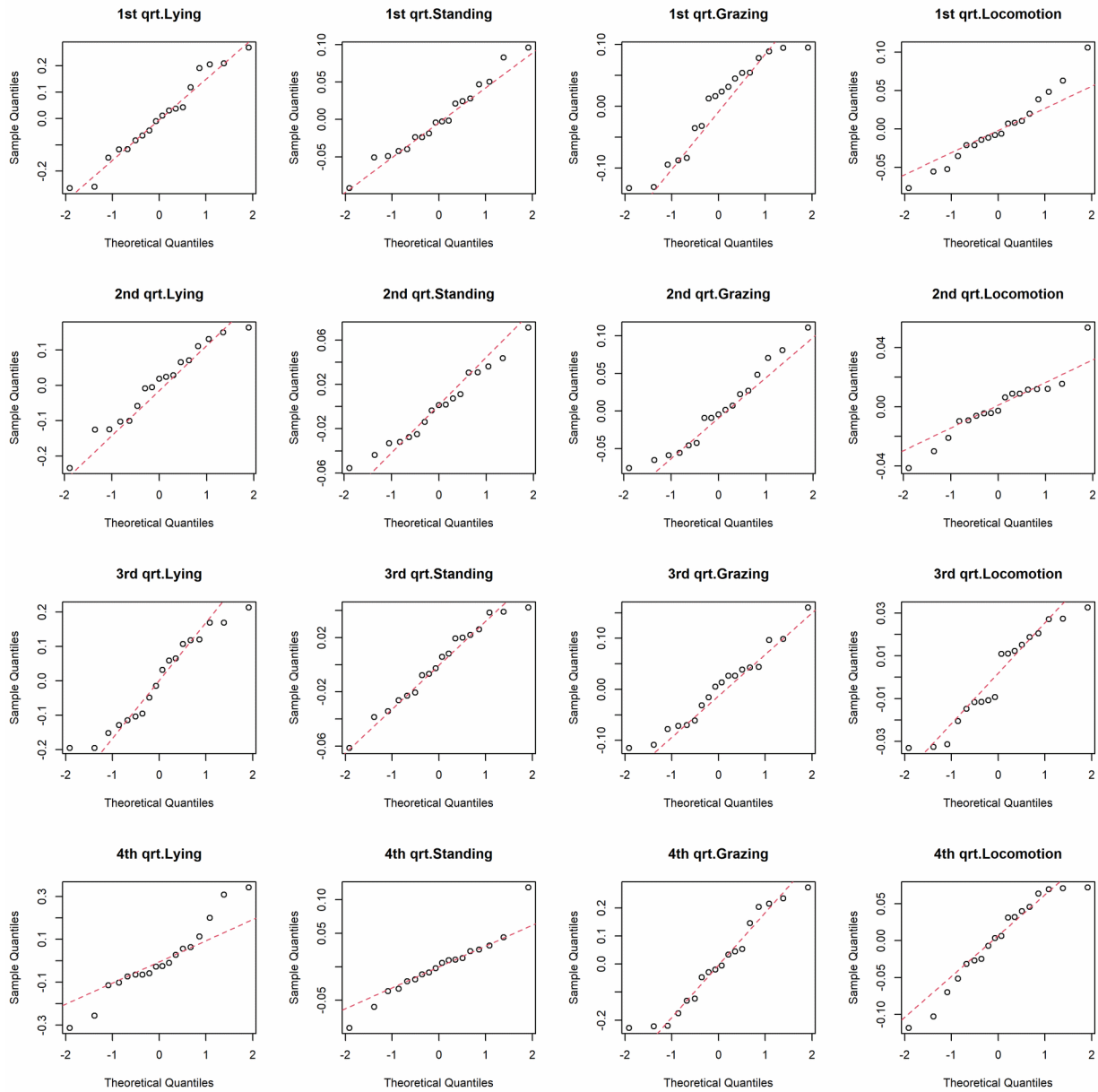
Supplementary VII: Plots of residuals versus replicate structure[1]

[1]An absence of clear patterns indicates a random effects model is unnecessary (nor could we find evidence for significant autocorrelation across weeks for any group).

Supplementary VIII: QQplots against a standard normal distribution[1]

[1]A straight line indicates approximate normality