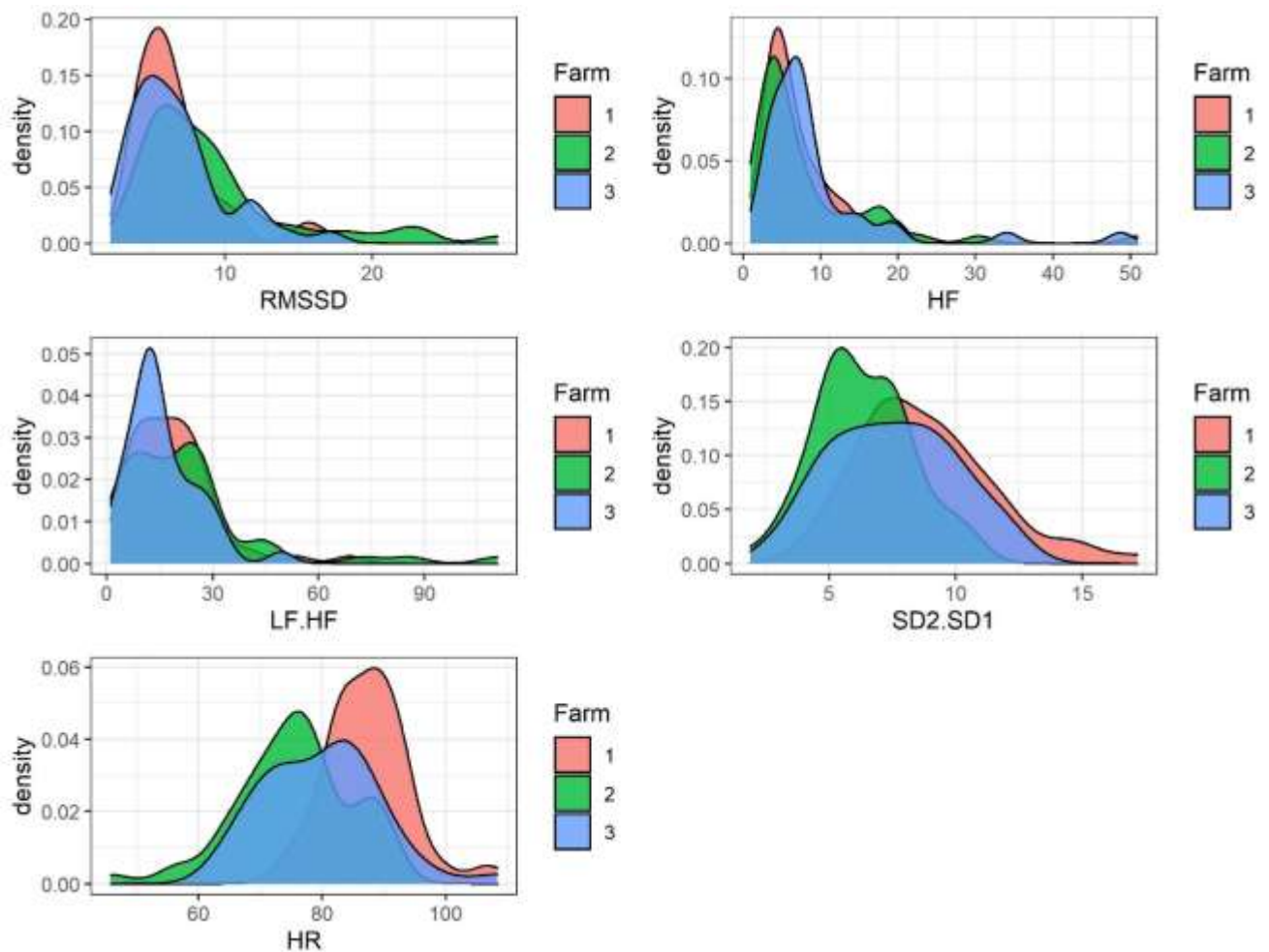**Associations between cow-level parameters and heart rate variability as a marker of the**

physiological stress response in dairy cows

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SUPPLEMENTARY FILE

Supplementary Figure S1. Density plots divided by farm for each of the HRV parameters reported (HR: Heart rate, RMSSD: Root mean square of the successive differences, HF: High frequency band, LF.HF: Ratio of Low Frequency to High Frequency power, SD2.SD1: Ratio of Poincaré plot standard deviation along the line of identity (SD2) to Poincaré plot standard deviation perpendicular the line of identity (SD1)).

Supplementary Table S1: Overview of the production and management system of the three farms enrolled in the study (between August 2017 and July 2018). Production data are extracted from the relevant milk recording database reports generated for each farm (Cattle Information Services and Dairy Comp 305).

	Farm 1	Farm 2	Farm 3
Number of cows	550	54	850
Number of milking/d	3x	2x	3x
Milk yield (305d milk yield and l/cow/d)	9824 - 32	10373 - 32.6	12880 - 39.5
Butterfat % (average - range)	3.82 (3.60 - 4.24)	3.9 (3.17-4.15)	3.77 (3.60 - 4.09)
Protein % (average - range)	3.09 (2.98 - 3.22)	3.27 (3.10-3.43)	3.17 (3.06 - 3.32)
Milking parlor	24/48 Herringbone swingover	8/16 Herringbone swingover	50-point rotary
Type of free stalls	Rubber mattresses topped with ground down recycled wood	Rubber mats and sawdust	Deep oat husks and lime
Number of stalls	Primiparous - 140 Multiparous high yield - 168 Multiparous mid yield - 123	Whole milking herd - 67	Primiparous - 167 Multiparous high yield - 200 Multiparous mid yield -240

Milking groups - stocking rate (based on number of cubicles)	Post-partum - 94% Primiparous - 92% Multiparous high yield - 95% Multiparous mid yield - 86%	Post-partum - 50% Rest of the herd - 81%	Post-partum - 95% Primiparous - 120% Multiparous high yield - 139% Multiparous mid yield - 103%
Feeding system	TMR (grass silage based)	Partial Mixed Ration (grass silage based with in parlor concentrate)	TMR (grass silage based)
Milking groups - feeding space (m/cow)	Post-partum - 0.71 Primiparous - 0.64 Multiparous high yield - 0.64 Multiparous mid yield - 0.55	Post-partum - 1.0 Rest of the herd - 0.78	Post-partum - 0.60 Primiparous - 0.40 Multiparous high yield - 0.54 Multiparous mid yield - 0.56
Dry cow management	Far-off dry cows: outdoor between May-Sep Close-up cows: indoors on straw	Far-off dry cows: outdoor between May-Sep Close-up cows: indoors on straw	Indoor all-year round

Supplementary Table S2: Overview of the key performance indicators (KPIs) of the three farms enrolled in the study (between August 2017 and July 2018) extracted from the relevant milk recording database reports generated for each farm (Cattle Information Services and Dairy Comp 305).

	Farm 1	Farm 2	Farm 3
Herd profile	1st lactation: 34% 2nd lactation: 27% 3rd or more: 39%	1st lactation: 29% 2nd lactation: 29% 3rd or more: 43%	1st lactation: 39% 2nd lactation: 29% 3rd or more: 32%
21-day Pregnancy Rate	22%	12%	23%
% animals pregnant by 100 DIM	58%	41%	59%
Monthly Milk recording SCC (average - range)	175 (135-203)	111 (49-231)	169 (129-198)
Clinical mastitis incidence (cases/100 cows/year)	28	52	36
Lameness performance (based on monthly mobility scoring, 0 to 3, by LV)	7% (score 2 or 3)	37% (score 2 or 3)	4% (score 2 or 3)
Transition disease index	10%	44%	12%
Death Rate	5.1%	12.9%	6.2%
Culling Rate	28%	13%	38%
Age at 1st calving (average)	25 months	26 months	23 months

Supplementary Table S3. Summary of HRV parameters calculated from IBI measurements and the biological relevance of each parameter. Modified from von Borell *et al.,* 2007.

Parameter	Unit	Description	Relevance	Association with ANS	Relationship to stress
HR	Beats/ min	Heart rate	Frequency of beats	Influenced by both ANS branches	Positive
RMSSD	ms	Root mean square of the successive differences	Statistical measure of short-term variability	Reflects PSNS (vagal) influence	Negative
HF	Hz	High frequency band	Measures short-term variability	Reflects PSNS influence	Negative
LF/HF	%	Ratio of Low Frequency to High Frequency power	LF power may reflect SNS activity while HF power may reflect the PNS	Indicator of relative activity within the two branches of the ANS. High values, SNS dominance, low values PSNS dominance	Positive
SD2/SD1	%	Ratio of Poincaré plot standard deviation along the line of identity (SD2) to Poincaré plot standard deviation perpendicular the line of identity (SD1)	Long-term variability and the unpredictability of the RR time series	Reflects sympatho-vagal balance	Negative