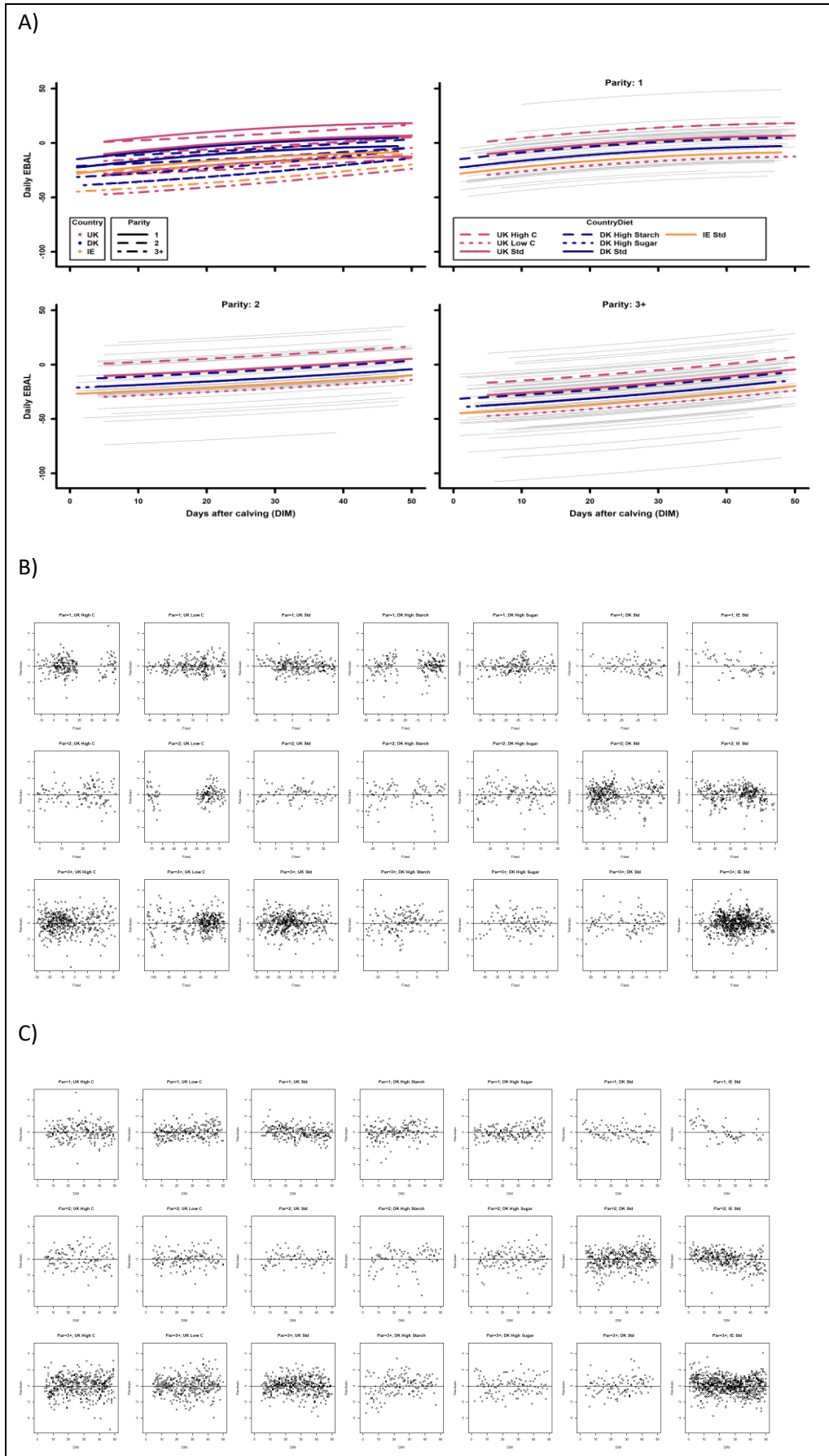


**Between and within-herd variation in blood and milk biomarkers in Holstein cows in
early lactation**

M.A. Krogh, M. Hostens, M. Salavati, C. Grelet, M.T. Sorensen, D.C. Wathes, C.P. Ferris, C. Marchitelli, F. Signorelli, F. Napolitano, F. Becker, T. Larsen, E. Matthews, F. Carter, A. Vanlierde, G. Opsomer, N. Gengler, F. Dehareng, M.A. Crowe, K.L. Ingvarlsen and L. Foldager

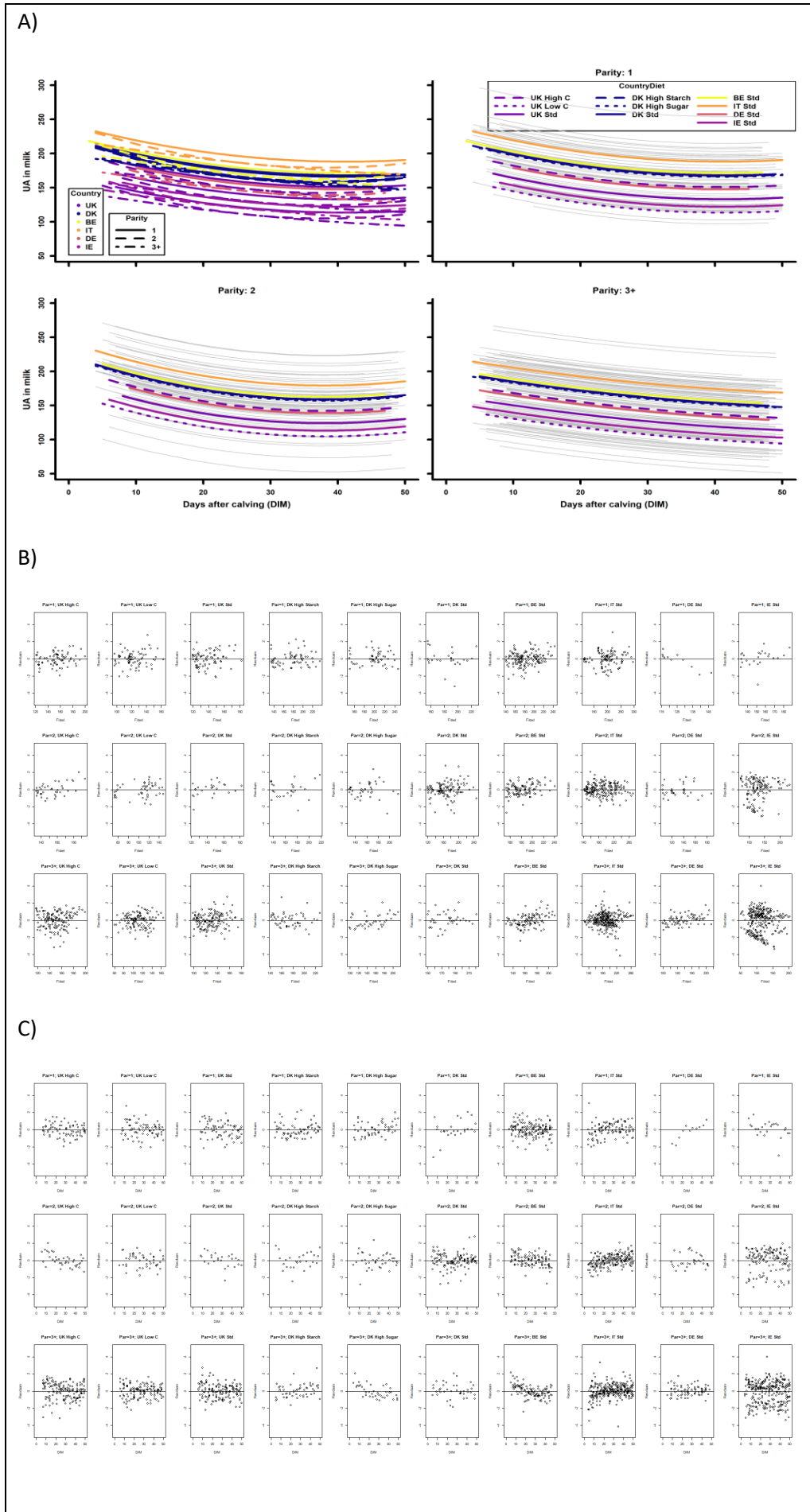
Animal Journal

Supplementary Figure S1 (Energy Balance)



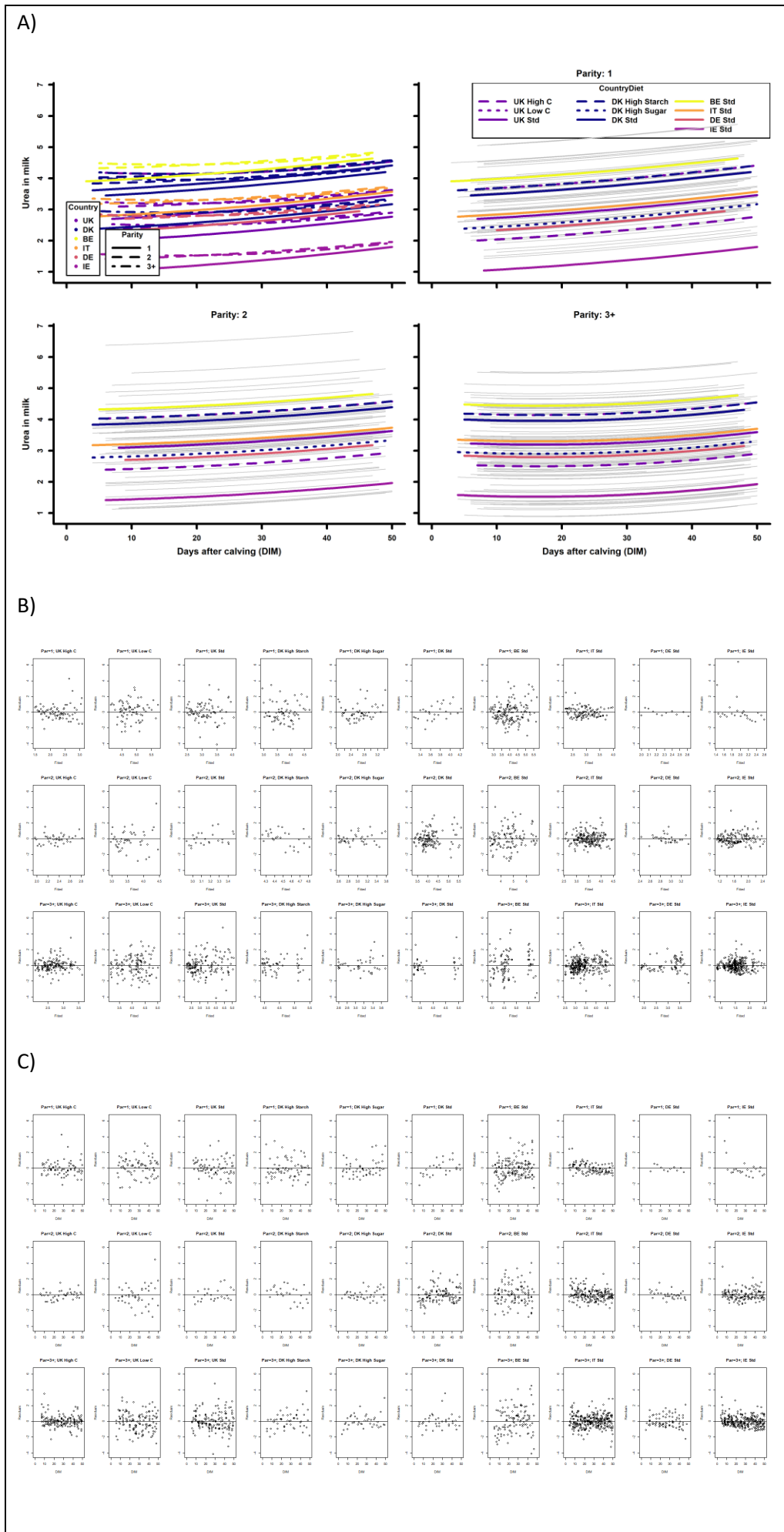
Supplementary Figure S1 Energy balance (EBAL, in MJ/day) over the study period (1 to 50 days-in-milk (DIM)) divided on herd/diet and parity for the three research farms (UK, DK and IE) where individual Holstein dairy cow feed intakes were recorded daily, and appropriate feed sampling undertaken. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM², and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). The upper left panel can be used to compare across herd/diets and parities, whereas the other three panels show the separate results for each parity group. **Plot B)** Fitted vs. Residual plots. Several deviant patterns are observed. Funnel-shapes (Par1-UK-low C). Trends (Par1-DK-Std and Par1, IE-Std). **Plot C)** DIM of sampling vs. residuals plots of the analysis of Energy balance. A trend appears in Par2-IE-Std. Multiple negative outliers in Par2-DK-HighStarch and Par3-DK-HighStarch.

Supplementary Figure S2 (Uric Acid)



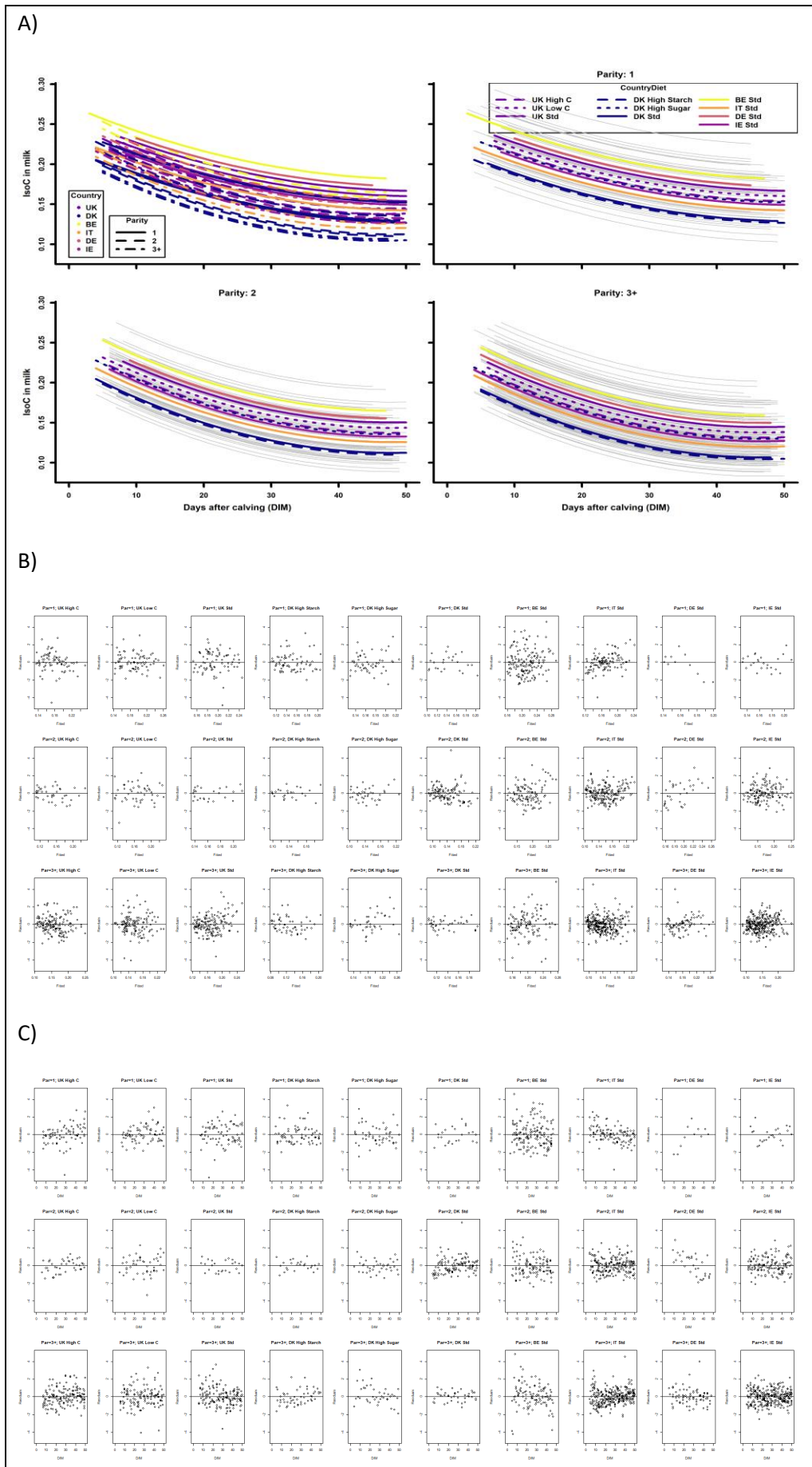
Supplementary Figure S2 Uric Acid (UA in μM) over the study period (1 to 50 days-in-milk (DIM)) divided on herd/diet and parity. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM², and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). The upper left panel can be used to compare across herd/diets and parities, whereas the other three panels show the separate results for each parity group. **Plot B)** Fitted vs. Residual plots for the analysis of Uric Acid. An extreme outlier >20 was removed. Funnel patterns can be seen in Par3+IT-Std and Par3+IT-Std. and there more residuals below -2 than above +2. **Plot C)** DIM of sampling vs. residuals plots of the analysis of Uric Acid. An extreme outlier >20 was removed. Possible trend in Par3+BE-Std and Par3+DK-High Sugar.

Supplementary Figure S3 (urea)



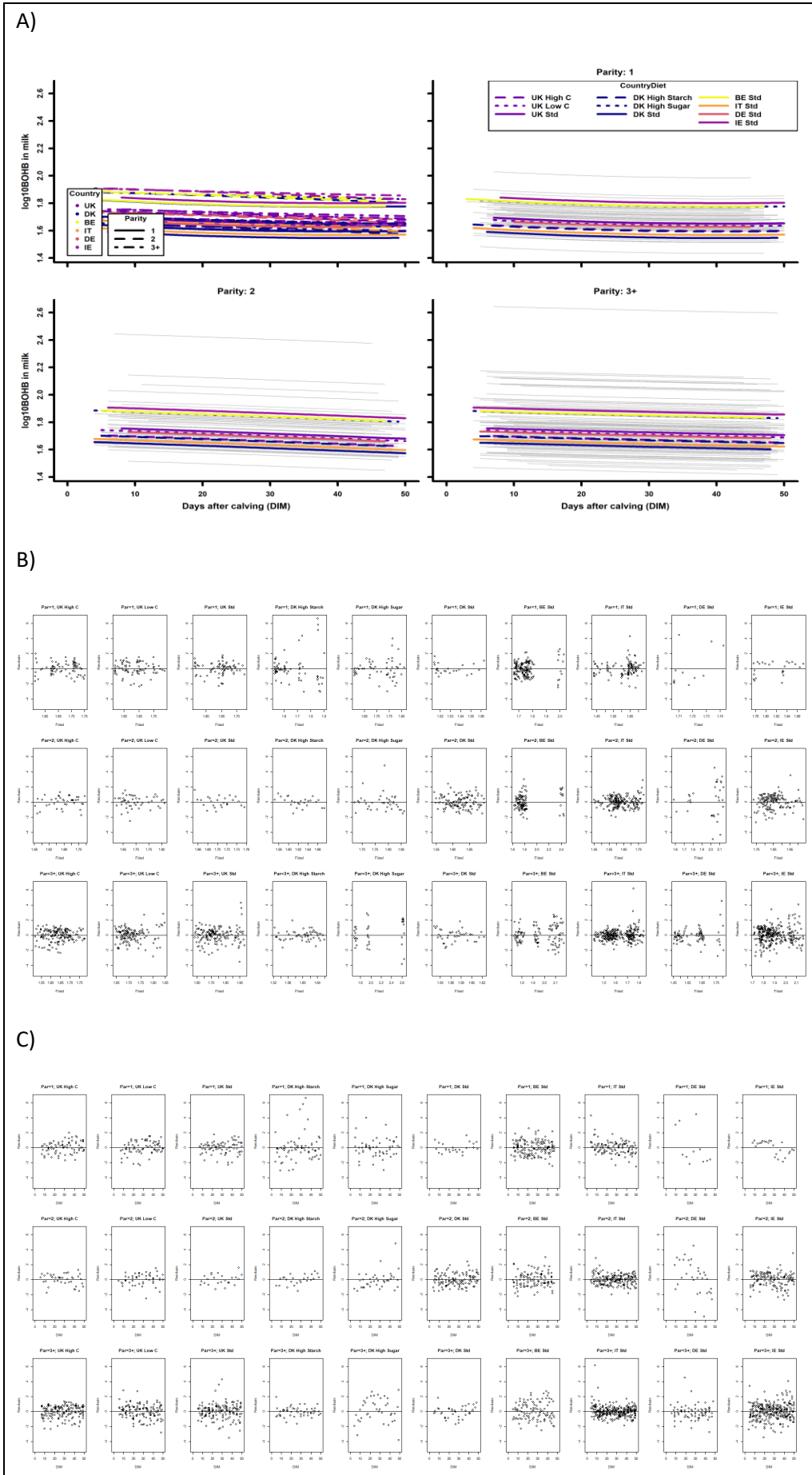
Supplementary Figure S3 Urea (mM) over the study period (1 to 50 days-in-milk (DIM)) divided on herd/diet and parity. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM², and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). The upper left panel can be used to compare across herd/diets and parities, whereas the other three panels show the separate results for each parity group. The panels demonstrate substantial differences between different herds/diets. **Plot B)** Fitted vs. Residual plots for the analysis of Urea (mM) The plots show some differences between different herds/diets within parity (e.i. Par1-BE-Std and Par1-IT-Std), but also what seems to be larger residuals for increasing parity. **Plot C)** DIM of sampling vs. residuals plots of the analysis of Urea. The plots show tendencies for funnel shapes (Par1-UK-Std) and trends (Par1-DK-Std).

Supplementary Figure S4 (Isocitrate)



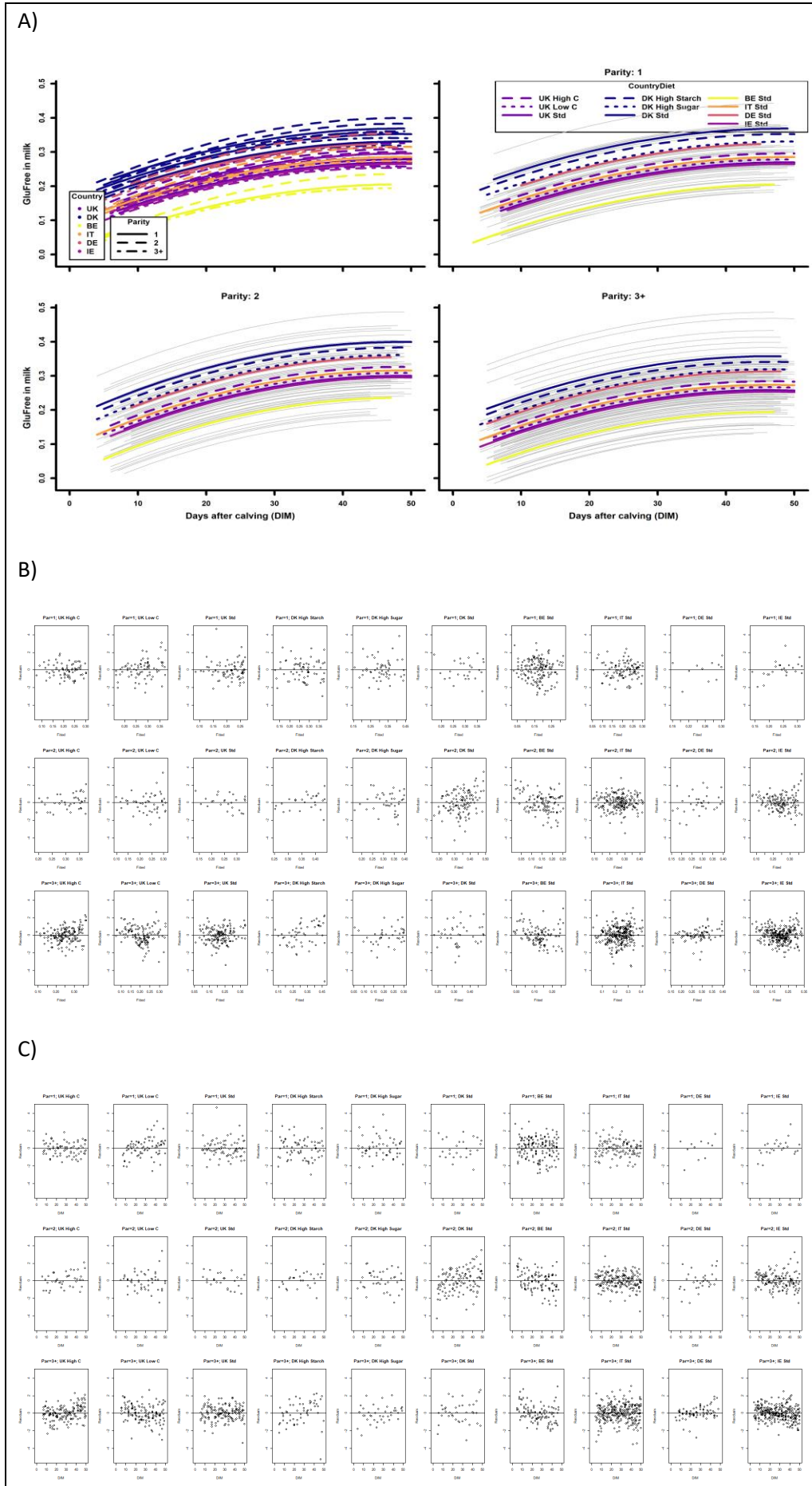
Supplementary Figure S4 Isocitrate (IsoC in mM) over the study period (1 to 50 days-in-milk (DIM)) divided on herd/diet and parity. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM², and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). The upper left panel can be used to compare across herd/diets and parities, whereas the other three panels show the separate results for each parity group. **Plot B)** Fitted vs. Residual plots for the analysis of Isocitrate. The plots several outliers (Par1-BE-Std) and differences in variation in different herd/diets (Par3+-DK-Std vs Par3+-IT-Std). **Plot C)** DIM of sampling vs. residuals plots of the analysis of Isocitrate. Trend patterns appear in Par1-UK-High C, Par2-UK-High C and Par2-DE-Std.

Supplementary Figure S5



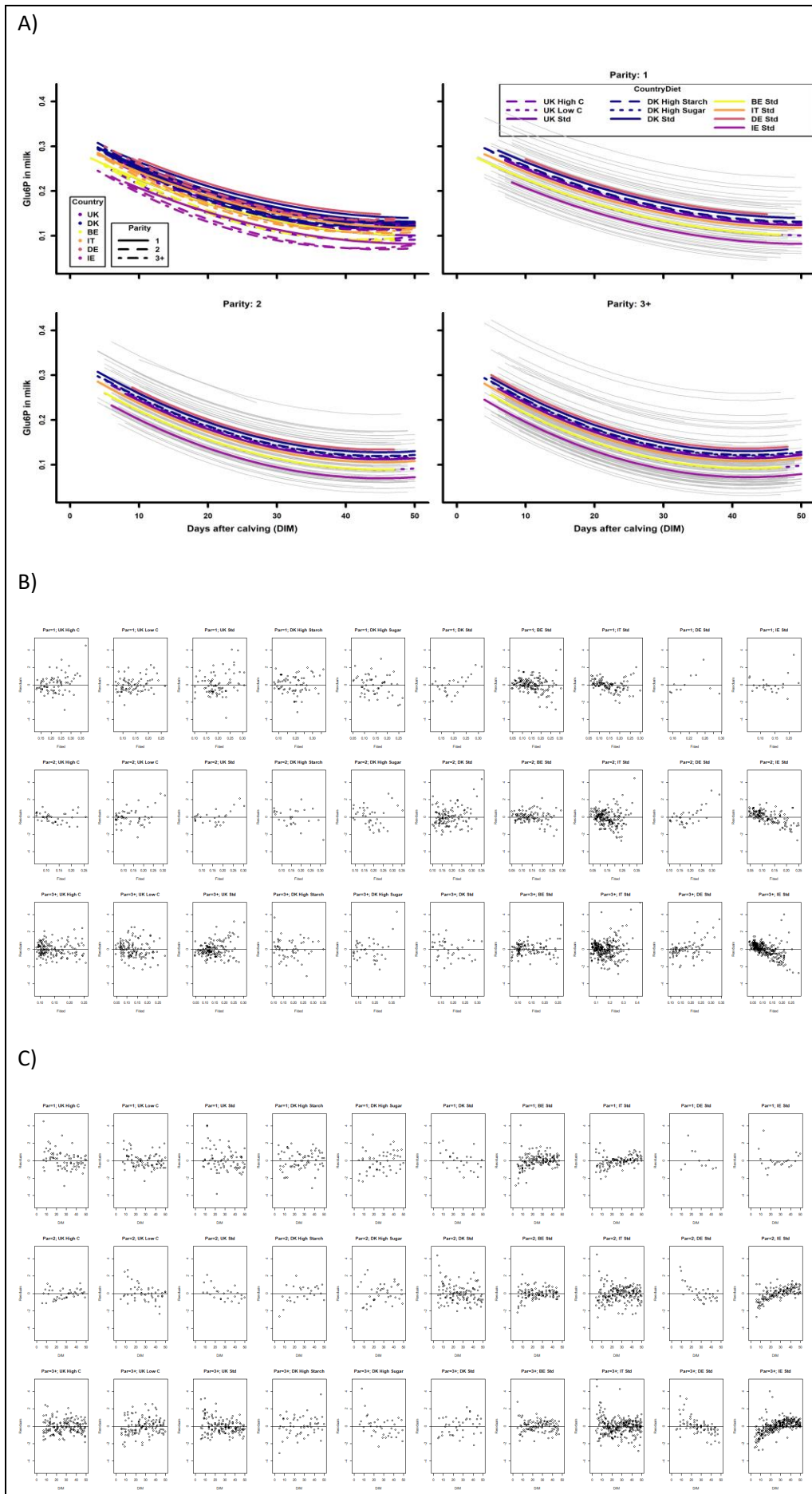
Supplementary Figure S5 $\log_{10}(\beta\text{-hydroxybutyrate})$ (BHB in μM) over the study period (1 to 50 DIM) divided on herd/diet and parity. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM2, and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). The upper left panel can be used to compare across herd/diets and parities, whereas the other three panels show the separate results for each parity group. **Plot B)** Fitted vs. Residual plots for the analysis of $\log_{10}(\beta\text{-hydroxybutyrate})$. Multiple outlier in Par1-DK-High Starch and at least one in Par3+IT-Std and Par3+UK-Std. There seems to be differences in variance between the different herds within parity groups. **Plot C)** DIM of sampling vs. residuals plots of the analysis of $\log_{10}(\beta\text{-hydroxybutyrate})$. The plots show no apparent, systematic difference over the 50 DIM but some difference in variance between herds within parity group (e.i. Par2+IT-Std and Par2-DE-Std).

Supplementary Figure S6 (Glucose)



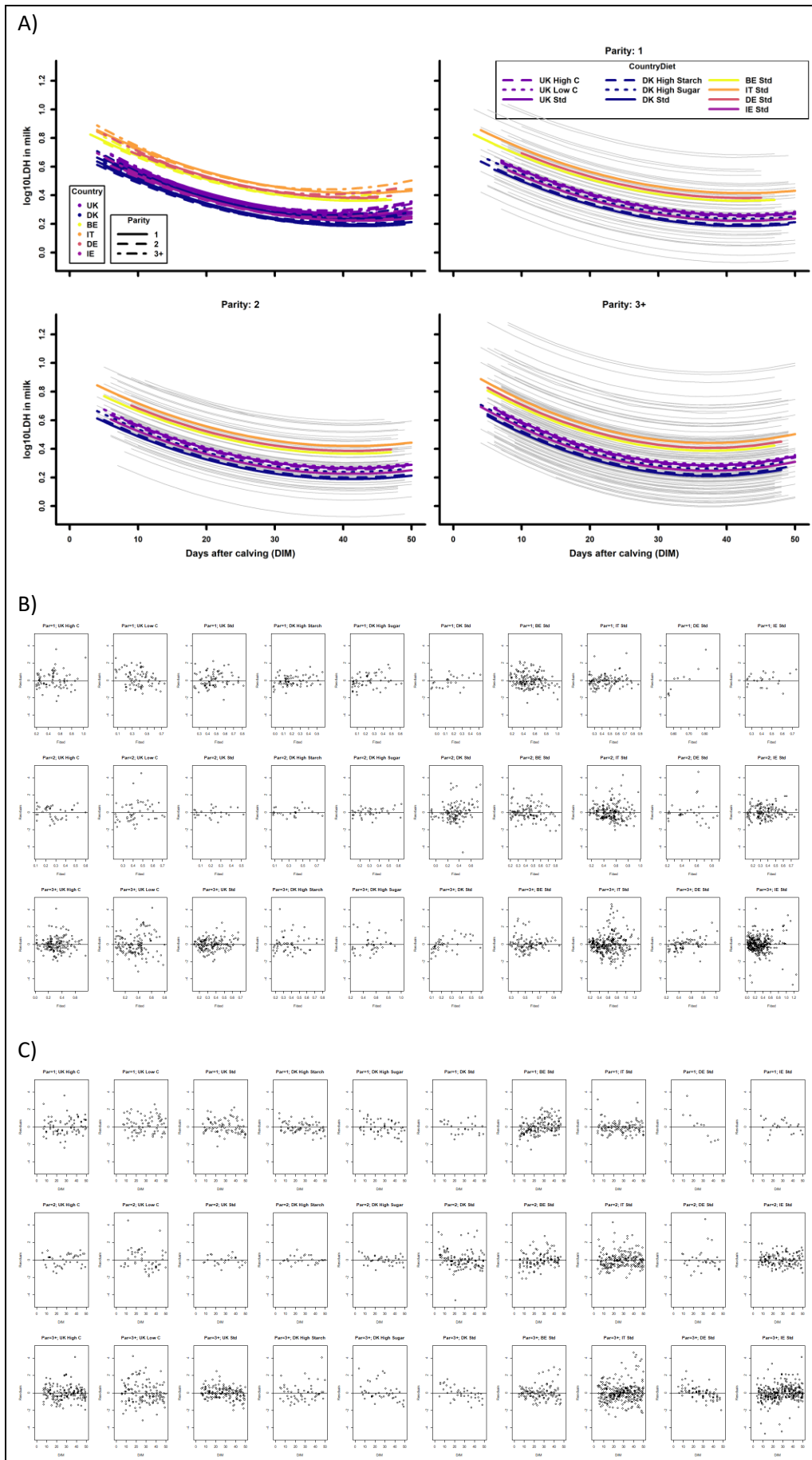
Supplementary Figure S6 Glucose (GluFree in mM) over the study period (1 to 50 days-in-milk (DIM)) divided on herd/diet and parity. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM2, and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). The upper left panel can be used to compare across herd/diets and parities, whereas the other three panels show the separate results for each parity group. **Plot B)** Fitted vs. Residual plots for the analysis of Glucose (mM) over the study period (1 to 50 DIM) divided on herd/diet and parity. Trends appears in Par2-DK-Std and Par2-Be-Std and several plots show individual outliers (Par3+DK-High Stach). **Plot C)** DIM of sampling vs. residuals plots of the analysis of Glucose (mM) over the study period (1 to 50 DIM) divided on herd/diet and parity. A trend appear in the Par3+DK-High Starch.

Supplementary Figure S7 (Glucose-6-phosphate)



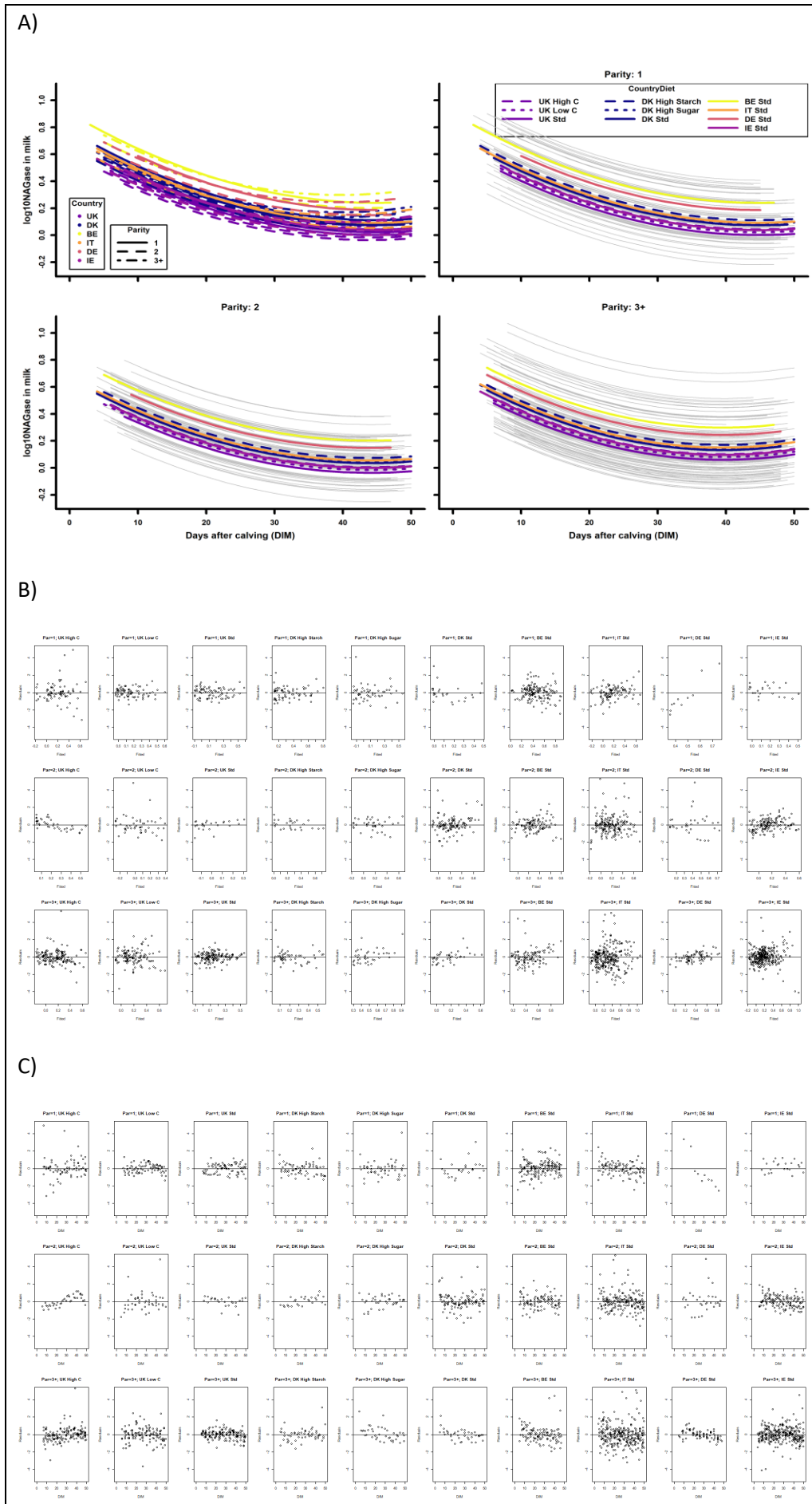
Supplementary Figure S7 Glucose-6-phosphate (Glu6P in mM) over the study period (1 to 50 day-in-milk (DIM)) divided on herd/diet and parity. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM2, and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). **Plot B)** Fitted vs. Residual plots for the analysis of Glucose-6-phosphate (mM) over the study period (1 to 50 DIM) divided on herd/diet and parity. Three extreme outliers are not shown on the plots. Besides outliers trends are seen for Par2-EI-Std and Par3-EI-Std. **Plot C)** DIM of sampling vs. residuals plots of the analysis of Glucose-6-phosphate (mM) over the study period (1 to 50 DIM) divided on herd/diet and parity. Three extreme outliers are not shown on the plots. Trend shapes are seen in Par2-EI-Std and Par3-EI-Std.

Supplementary Figure S8 (Lactate dehydrogenase)



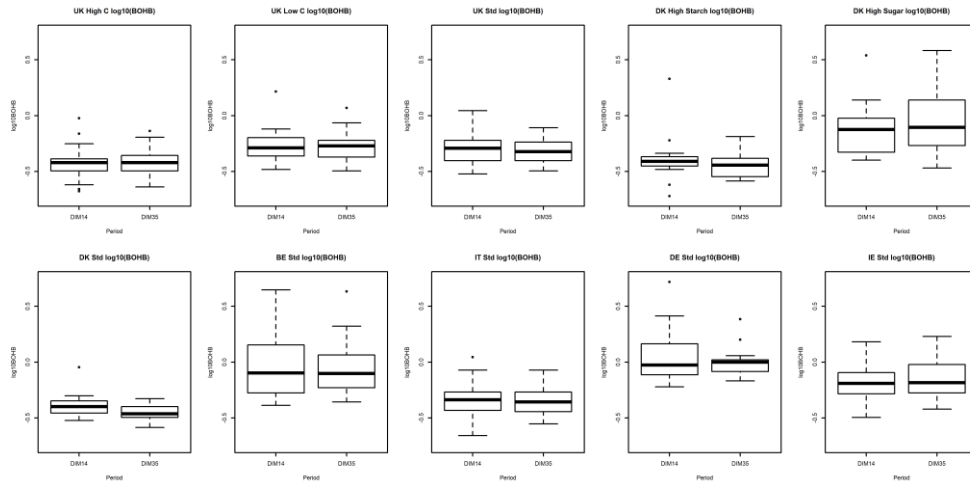
Supplementary Figure S8 Log_{10} (Lactate dehydrogenase) (log_{10} (LDH) in units/L) over the study period (1 to 50 days-in-milk (DIM)) divided on herd/diet and parity. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM2, and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). The upper left panel can be used to compare across herd/diets and parities, whereas the other three panels show the separate results for each parity group. **Plot B)** Fitted vs. Residual plots for the analysis of Log_{10} (Lactate dehydrogenase) over the study period (1 to 50 DIM) divided on herd/diet and parity. One extreme outlier in Par1-UK-Low C has been removed. The plots show no apparent systematic difference with different herd/diets or parity, but a few outlier observations are present (Par 3+, EI). **Plot C)** DIM of sampling vs. residuals plots of the analysis of Log_{10} (Lactate dehydrogenase) over the study period (1 to 50 DIM) divided on herd/diet and parity. One extreme outlier in Par1-UK-Low C has been removed. A trend can be seen in Par3+DE-Std.

Supplementary Figure S9 (*N*-acetyl- β -D-glucosaminidase)



Supplementary Figure S9 \log_{10} (N-acetyl- β -D-glucosaminidase) (NAGase in Units/L) over the study period (1 to 50 days-in-milk (DIM)) divided on herd/diet and parity. Random 2nd order polynomial regression was used with a random intercept for herd/diet (three different diets for each of UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)) and cow. Fixed effects included parity (Par) with first (1), second (2), and third or more (3+), DIM and DIM2, and all two-way interactions. The model was fitted by the 'lme' function from the 'nlme' package in R. **Plot A)** The thin grey lines shown in three of the panels are the predictions for each cow, while the coloured lines are herd/diet and parity specific (see legends in the two upper panels). The upper left panel can be used to compare across herd/diets and parities, whereas the other three panels show the separate results for each parity group. **Plot B)** Fitted vs. Residual plots for the analysis of NAGase (Units/L) over the study period (1 to 50 DIM) divided on herd/diet and parity. Five outliers above 5 are not shown. The plots show some difference in variation within parity (e.g. Par3+IT-Std vs. Par3+UK-Std) as well as several outliers in different plots. **Plot C)** DIM of sampling vs. residuals plots of the analysis of NAGase (Units/L) over the study period (1 to 50 DIM) divided on herd/diet and parity. A trend is seen in Par2-UK-High C.

Supplementary Figure S10 (β -hydroxybutyrate)



Supplementary Figure S10 Boxplot of blood β -hydroxybutyrate (BOHB) in Holstein dairy cows for the two different time points (14 and 35 days-in-milk (DIM)) in the different herds/diet combinations (UK (High Concentrate (High C), Low Concentrate (Low C) and Standard (Std)), and DK (High Starch, High Sugar and Std), and one for IE (Std)).

Supplementary Table S1. Summary statistics for energy balance (EBAL, in MJ/day) of Holstein dairy cows over the study period (1 to 50 days-in-milk (DIM)) for the three research farms where individual cow intakes were recorded daily, and appropriate feed sampling undertaken

Herd	Diet	Mean (SD)	Median (Q1 ; Q3)	Min ; Max	N _{cows}	N _{samples}
UK	Low C	-29.9 (33.2)	-25.3 (-45.4 ; -9.16)	-166 ; 51.6	20	738
	Standard C	-9.31 (24.9)	-7.76 (-24.2 ; 8.96)	-92.7 ; 57.3	20	778
	High C	3.1 (28.6)	2.43 (-13.9 ; 19.5)	-110 ; 139	21	793
	Pooled	-11.6 (32.0)	-9.75 (-29.7 ; 7.78)	-166 ; 139	61	2309
DK	High starch	-8.85 (27.5)	-3.31 (-26.6 ; 10.5)	-110 ; 48.9	11	417
	High sugar	-17.1 (23.4)	-17.6 (-32.9 ; -0.58)	-89.3 ; 46.9	10	408
	Standard	-16.1 (24.7)	-16.4 (-32.7 ; -1.39)	-104 ; 69.2	14	568
	Pooled	-14.2 (25.4)	-14.1 (-31.8 ; 3.56)	-110 ; 69.2	35	1393
IE	Standard	-29.1 (25.4)	-27.2 (-46.2 ; -11.1)	-117 ; 50.8	36	1180
All	Pooled	-16.6 (29.6)	-15.5 (-34.8 ; 2.71)	-166 ; 139	132	4882

Q1: first quartile, Q3: third quartile, C: concentrate.

Supplementary Table S2. Summary statistics for energy balance (EBAL, in MJ/day) over the study period (1 to 50 days-in-milk (DIM)) divided on herds and parity for the three research farms where individual Holstein dairy cow intakes were recorded daily, and appropriate feed sampling undertaken

Herd	Parity	Mean (SD)	Median (Q1 ; Q3) ¹	Min ; Max	N _{cows}	N _{samples}
UK	1	2.03 (23.1)	1.76 (-12.5 ; 15.7)	-66.9 ; 139	18	685
	2	-2.76 (33.9)	1.63 (-21.0 ; 19.8)	-126 ; 78.7	9	349
	3+	-21.4 (32.3)	-19.2 (-39.4 ; -1.42)	-166 ; 83.6	34	1275
DK	1	-17.8 (22.1)	-17.7 (-32.8 ; -2.18)	-110 ; 36.9	11	430
	2	-9.67 (25.9)	-10.7 (-25.6 ; 8.79)	.89.3 ; 69.2	14	593
	3+	-17.3 (27.1)	-17.8 (-37.0 ; 3.25)	-104 ; 48.9	10	370
IE	1	5.63 (16.5)	3.26 (-4.77 ; 15.8)	-35.3 ; 50.8	2	62
	2	-25.2 (20.7)	-22.0 (-39.2 ; -9.87)	-103 ; 20.0	11	336
	3+	-33.5 (25.5)	-33.1 (-50.6 ; -16.3)	-117 ; 50.7	23	782

¹ Q1: first quartile, Q3: third quartile.

Supplementary Table S3 Summary statistics of milk metabolites and enzymes in Holstein dairy cows at different days-in-milk (DIM) intervals

Measure ¹	Period	Mean (sd)	Median (Q1 ; Q3) ²	Min ; Max	N _{cows}	N _{samples}
Glucose-6-phosphate (mM)	DIM 1-7	0.26 (0.12)	0.23 (0.18 ; 0.29)	0.11 ; 0.81	135	142
	DIM 8-14	0.23 (0.09)	0.21 (0.16 ; 0.28)	0.07 ; 0.93	219	424
	DIM 15-21	0.18 (0.06)	0.16 (0.13 ; 0.22)	0.04 ; 0.39	218	428
	DIM 22-28	0.14 (0.05)	0.14 (0.11 ; 0.17)	0.03 ; 0.38	221	435
	DIM 29-35	0.12 (0.04)	0.12 (0.09 ; 0.15)	0.002 ; 0.28	221	438
	DIM 36-42	0.11 (0.04)	0.11 (0.08 ; 0.14)	0.0003 ; 0.28	219	437
	DIM 43-50	0.10 (0.04)	0.10 (0.08 ; 0.13)	0.01 ; 0.27	219	450
	DIM 1-50	0.15 (0.08)	0.14 (0.10 ; 0.19)	0.0003 ; 0.93	223	2754
Glucose (mM)	DIM 1-7	0.13 (0.07)	0.13 (0.09 ; 0.17)	0.001 ; 0.35	135	142
	DIM 8-14	0.17 (0.08)	0.17 (0.12 ; 0.21)	0.005 ; 0.44	219	424
	DIM 15-21	0.21 (0.09)	0.20 (0.15 ; 0.26)	0.02 ; 0.53	218	428
	DIM 22-28	0.24 (0.09)	0.23 (0.18 ; 0.29)	0.0005 ; 0.52	221	435
	DIM 29-35	0.27 (0.09)	0.26 (0.20 ; 0.33)	0.006 ; 0.64	221	438
	DIM 36-42	0.28 (0.10)	0.27 (0.21 ; 0.34)	0.02 ; 0.62	219	437
	DIM 43-50	0.29 (0.10)	0.29 (0.22 ; 0.34)	0.03 ; 0.69	219	450
	DIM 1-50	0.24 (0.10)	0.23 (0.17 ; 0.30)	0.0005 ; 0.69	223	2754
BHB (μM)	DIM 1-7	64.3 (35.1)	52.2 (42.7 ; 75.3)	20.0 ; 238	135	142
	DIM 8-14	64.9 (56.8)	50.1 (40.4 ; 72.9)	20.0 ; 701	219	424
	DIM 15-21	65.7 (71.5)	50.0 (39.0 ; 67.5)	20.0 ; 858	218	428
	DIM 22-28	67.1 (75.9)	50.5 (39.0 ; 69.5)	20.0 ; 896	221	435
	DIM 29-35	65.0 (75.5)	50.8 (37.7 ; 65.0)	19.6 ; 819	221	437
	DIM 36-42	60.1 (55.6)	48.3 (37.3 ; 62.0)	19.7 ; 735	219	436
	DIM 43-50	56.8 (42.6)	47.5 (38.0 ; 61.5)	19.5 ; 523	218	446
	DIM 1-50	63.3 (63.0)	50.0 (38.4 ; 66.5)	19.5 ; 896	223	2748
Isocitrate (mM)	DIM 1-7	0.21 (0.05)	0.21 (0.18 ; 0.24)	0.09 ; 0.42	135	142
	DIM 8-14	0.20 (0.05)	0.20 (0.17 ; 0.23)	0.09 ; 0.44	219	424
	DIM 15-21	0.18 (0.05)	0.17 (0.15 ; 0.20)	0.04 ; 0.33	218	429
	DIM 22-28	0.16 (0.05)	0.16 (0.13 ; 0.19)	0.01 ; 0.34	221	435

Urea (mM)	DIM 29-35	0.15 (0.04)	0.15 (0.12 ; 0.18)	0.01 ; 0.32	221	437
	DIM 36-42	0.14 (0.04)	0.14 (0.12 ; 0.16)	0.02 ; 0.31	219	436
	DIM 43-50	0.14 (0.04)	0.13 (0.11 ; 0.16)	0.0007 ; 0.25	218	446
	DIM 1-50	0.16 (0.05)	0.16 (0.13 ; 0.20)	0.0007 ; 0.44	223	2749
	DIM 1-7	3.15 (1.11)	3.08 (2.41 ; 3.84)	0.79 ; 6.09	133	140
	DIM 8-14	3.12 (1.26)	3.04 (2.31 ; 3.79)	0.56 ; 7.99	219	425
	DIM 15-21	3.14 (1.38)	3.04 (2.21 ; 4.00)	0.47 ; 8.30	218	428
	DIM 22-28	3.09 (1.37)	2.94 (2.10 ; 4.03)	0.12 ; 7.78	221	433
	DIM 29-35	3.29 (1.46)	3.13 (2.23 ; 4.11)	0.08 ; 8.33	221	434
	DIM 36-42	3.41 (1.34)	3.31 (2.44 ; 4.21)	0.29 ; 7.49	219	436
	DIM 43-50	3.38 (1.44)	3.24 (2.40 ; 4.19)	0.70 ; 8.53	219	445
	DIM 1-50	3.23 (1.37)	3.11 (2.29 ; 4.04)	0.08 ; 8.53	223	2741
	DIM 1-7	189 (58.2)	195 (159 ; 225)	10.2 ; 349	135	142
	DIM 8-14	177 (60.2)	182 (143 ; 215)	9.19 ; 343	219	424
	DIM 15-21	163 (51.1)	166 (133 ; 195)	5.04 ; 326	218	429
Uric acid (μ M)	DIM 22-28	152 (54.5)	156 (123 ; 186)	2.36 ; 300	221	435
	DIM 29-35	152 (67.1)	148 (122 ; 188)	4.85 ; 1080	221	438
	DIM 36-42	145 (51.2)	144 (115 ; 179)	6.08 ; 272	219	436
	DIM 43-50	142 (53.5)	142 (112 ; 176)	5.91 ; 312	219	450
	DIM 1-50	157 (58.2)	158 (124 ; 193)	2.36 ; 1080	223	2754

¹ M: molar (mol/l), BHB: β -hydroxybutyrate.

² Q1: first quartile, Q3: third quartile.

Supplementary Table S4 Summary statistics of milk enzymes in Holstein dairy cows at different days-in-milk (DIM) intervals

Measure ¹	Period	Mean (SD)	Median (Q1 ; Q3) ¹	Min ; Max	N _{cows}	N _{samples}
N-acetyl- β -D-glucosaminidase (units/l)	DIM 1-7	4.46 (2.63)	3.90 (2.76 ; 5.18)	1.32 ; 18.1	134	141
	DIM 8-14	3.13 (2.07)	2.60 (1.95 ; 3.63)	0.89 ; 25.1	219	424
	DIM 15-21	2.29 (1.66)	1.92 (1.44 ; 2.52)	0.36 ; 17.0	218	429
	DIM 22-28	1.80 (1.09)	1.53 (1.18 ; 2.06)	0.22 ; 11.2	221	435
	DIM 29-35	1.61 (1.04)	1.37 (1.05 ; 1.80)	0.41 ; 11.4	221	438
	DIM 36-42	1.59 (2.45)	1.22 (0.97 ; 1.68)	0.36 ; 48.1	219	437
	DIM 43-50	1.61 (2.29)	1.57 (0.91 ; 1.57)	0.29 ; 32.6	219	450
	DIM 1-50	2.12 (2.05)	1.62 (1.15 ; 2.40)	0.22 ; 48.1	223	2754
Lactate dehydrogenase (units/l)	DIM 1-7	6.22 (5.04)	5.00 (3.34 ; 6.90)	1.61 ; 32.4	134	141
	DIM 8-14	4.65 (3.31)	3.79 (2.75 ; 5.38)	1.32 ; 30.5	219	424
	DIM 15-21	3.51 (3.94)	2.65 (1.89 ; 3.79)	0.42 ; 46.0	218	429
	DIM 22-28	2.92 (2.43)	2.22 (1.62 ; 3.37)	0.27 ; 26.1	221	435
	DIM 29-35	2.72 (2.47)	2.10 (1.60 ; 2.97)	0.0007 ; 29.3	221	438
	DIM 36-42	2.52 (2.53)	1.91 (1.45 ; 2.66)	0.62 ; 32.8	219	437
	DIM 43-50	2.75 (3.56)	1.92 (1.41 ; 2.64)	0.55 ; 32.6	219	450
	DIM 1-50	3.33 (3.36)	2.40 (1.69 ; 3.73)	0.0007 ; 46.0	223	2754

¹ Q1: first quartile, Q3: third quartile.

Supplementary Table S5 Summary statistics of serum IGF-1, plasma metabolites, and index of physiological imbalance (PI-index)¹ in Holstein dairy cows at different days-in-milk (DIM)

Measure ²	Period	Mean (SD)	Median (Q1 ; Q3) ³	Min ; Max	N
Glucose (mM)	DIM 14	3.38 (0.49)	3.40 (3.10 ; 3.70)	1.70 ; 4.51	224
	DIM 35	3.54 (0.51)	3.60 (3.28 ; 3.90)	1.93 ; 4.70	223
Urea (mM)	DIM 14	3.15 (1.15)	3.1 (2.3 ; 3.8)	1.0 ; 6.7	224
	DIM 35	3.47 (1.23)	3.4 (2.4 ; 4.3)	0.9 ; 6.9	223
Cholesterol (mM)	DIM 14	3.03 (0.71)	2.98 (2.54 ; 3.43)	1.52 ; 5.05	224
	DIM 35	4.58 (1.04)	4.58 (3.75 ; 5.31)	1.90 ; 7.33	223
Fructosamine (μM)	DIM 14	252.4 (18.2)	250.9 (240.4 ; 264.4)	200.1 ; 294.8	221
	DIM 35	254.4 (18.2)	254.1 (241.4 ; 267.4)	192.8 ; 298.8	219
BHB (mM)	DIM 14	0.71 (0.60)	0.53 (0.40 ; 0.76)	0.19 ; 5.23	224
	DIM 35	0.65 (0.48)	0.52 (0.39 ; 0.74)	0.23 ; 4.29	223
NEFA (μekv/l)	DIM 14	733.8 (445.9)	616.5 (446.7 ; 923.9)	95.8 ; 2757	224
	DIM 35	469.6 (335.2)	385.4 (227.7 ; 626.6)	26.1 ; 2254	223
IGF-1 (ng/ml)	DIM 14	93.1 (66.7)	75.5 (43.1 ; 130.1)	6.62 ; 435.6	227
	DIM 35	110.9 (70.7)	94.9 (57.0 ; 139.8)	12.6 ; 399.5	225
PI-index ¹	DIM 14	0.54 (2.18)	0.43 (-0.86 ; 1.67)	-4.42 ; 8.31	224
	DIM 35	-0.54 (2.21)	-0.76 (-2.02 ; 0.65)	-5.74 ; 6.72	223

¹ PI-index = (NEFA + BHB – glucose), calculated from standardised plasma metabolites.

² M: molar (mol/l), BHB: β-hydroxybutyrate, NEFA: non-esterified fatty acids

³ Q1: first quartile, Q3: third quartile