

1 **Comparison of the feeding behaviour of primiparous and**  
2 **multiparous Jersey and Holstein cows kept under equal conditions**  
3 **throughout lactation**

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7 **Supplementary File**

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10 **Supplementary Methods**

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12 *Animals*

13 A total of 334 cows (116 Danish Jersey and 218 Danish Holstein cows) housed in the same  
14 barn at the Danish Cattle Research Centre (Foulum, Denmark) were included in this study.  
15 Data were collected between 4 January, 2018 and 30 April, 2019. Because feed composition  
16 impacts the feeding behaviour of cows (Grant and Ferraretto 2018; Coons *et al.* 2019) and to  
17 keep environmental conditions as constant as possible, only data from cows fed the standard  
18 partially mixed ration (PMR) at the research facility were included in this analysis. Mean  
19 parity was  $1.90 \pm 1.16$  for Holstein cows and  $2.14 \pm 1.32$  for Jersey cows. For both breeds,  
20 parity ranged from one to eight lactations. The group composition was dynamic, with cows  
21 entering and leaving the experiment, depending on their expected calving dates. Cows that  
22 received veterinary treatment during lactation were not excluded from the study unless they  
23 were moved to a sick pen. Number of treatments per group was similar over the whole time  
24 period with 524, 546 and 437 for groups 1, 2 and 3, respectively. An ethical approval was not

25 needed as the study was performed according to European and Danish laws and current  
26 guidelines for the ethical use of animals in research.

27

### 28 *Feeding behaviour*

29 All cows were allowed to feed on PMR ad libitum and were fed up to 3 kg of concentrate per  
30 day in the milking robot during milking. Silage and concentrate samples were collected every  
31 week. PMR samples were pooled over the course of the study to obtain the average. PMR  
32 was composed of an average of  $6.51 \pm 0.04$  MJ /kg dry matter (DM), 26.81% corn silage,  
33 28.67% grass–clover silage, 0.60%, horse beans, 6.87% barley, 0.50% spring barley straw,  
34 and 35.49% wheat and mineral mix. The concentrate contained an average of 18.2% crude  
35 protein and 10.2% crude fibre.

36 Time intervals between visits were calculated for each cow from the stop time of the previous  
37 visit and the start time of the subsequent visit. To determine if an interval was part of a meal,  
38 we used a simple approach to estimate a minimum interbout interval as the following. Time  
39 intervals measured in seconds were put in 1-min bins for the whole experimental period.  
40 Then, the average bin frequency was plotted against minutes. The x-axis was log-  
41 transformed to delineate the break point clearly for this curve and, consequently, the  
42 threshold for meals (i.e. minimum interbout interval). The minimum interbout interval criterion  
43 was set at the break point of 3 min, and time intervals shorter than this were deleted.

44

### 45 *Data handling*

46 To investigate the effect of breed and parity on feeding behaviour, a grand total of 69,398  
47 feeding behaviour recordings were analysed utilising SAS 9.4 (SAS Institute Inc., Cary, NC,  
48 USA). The experimental unit was the individual cow with feeding behaviour records obtained  
49 from 218 individual Danish Holstein and 116 Danish Jersey cows consisting of a daily  
50 average of 108 and 57 cows, respectively. However, data from 15 Holstein cows (8 at first  
51 parity, 1 at second parity, 6 at higher parities) and 6 Jerseys cows (3 at first parity, 2 at  
52 second parity, 1 at a higher parity) were excluded from the analyses as less than 14 days of

53 records were available within a parity. The cows were grouped according to breed and parity  
54 (first, second and later parity).

55 After visual inspection of the outcomes, data collected before day 15 after calving was not  
56 included in the analysis. To avoid decreasing numbers of animals, and to exclude any effects  
57 of special handling of cows at the end of pregnancy, any measurements exceeding 252 days  
58 from calving were omitted from the analysis. In addition, during a period of autumn of 2018,  
59 many cows were enrolled in other experiments. To retain reasonably high numbers of cows  
60 within days and similar levels across days, this led to the exclusion of 63 dates. After  
61 exclusions, data from 419 dates recorded from a total of 211 Holstein cows and 112 Jersey  
62 cows remained available for the analysis. Some cows were included from more than one  
63 parity. The total number of cows at first, second and later parities was 130, 79 and 83 for  
64 Holstein cows, respectively, and 68, 50 and 37 for Jersey cows, respectively.

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#### 66 *Statistical analysis*

67 The overall effects of breed and parity group, as well as their interaction, were analysed by  
68 linear mixed effects models using the MIXED procedure in SAS. Daily recordings of each  
69 variable and individual animal were averaged over lactation week. Week in lactation was  
70 included in the model as a covariate. These averages were then log-transformed (natural  
71 logarithm) to fulfil the assumption of normal distributed residuals. Based on Akaike's  
72 information criterion, first order autoregressive (**AR1**) residual covariance structure was  
73 chosen to account for correlation among repeated measurements from each cow within  
74 parity. The results are reported as least square means with 95% confidence intervals, both  
75 on the log-transformed and exponentially back-transformed scale. The confidence intervals  
76 and P values for differences were adjusted with the Tukey-Kramer method at a significance  
77 level of 5%, i.e., (adjusted)  $P < 0.05$  was considered statistically significant.

78 Model 1 to analyse the effect of breed and parity on eating time per visit as well as eating  
79 rate was the following:

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$$Y_{ijkl} = B_i + P_j + BP_{ij} + (\beta_{B_i} + \beta_{P_j})w_{ijkl} + \varepsilon_{ijkl}$$

82

83 Here,  $Y_{ijkl}$  is the natural logarithm of the response variable,  $B_i$  is Breed effect (i = Holstein,  
84 Jersey),  $P_j$  is the Parity effect (j = 1, 2, 3),  $BP_{ij}$  is the interaction effect of breed and parity,  $\beta_{B_i}$   
85 is the breed-specific slope parameter for weeks in lactation,  $\beta_{P_j}$  is the parity-specific slope  
86 parameter for weeks in lactation,  $w_{ijkl}$  and  $\varepsilon_{ijkl} \sim N(0, \sigma^2)$  are the random residuals where  $l$   
87 index the repeated measures over weeks for cow  $k = 1, \dots, n_{ij}$ . The AR(1) covariance means  
88 that  $cov(\varepsilon_{ijkl}, \varepsilon_{ijkm}) = \sigma^2 \rho^{|l-m|}$ .

89 To analyse the effect of breed and parity on between meal intervals, model 1 was used  
90 although excluding the weeks in lactation covariate as it was not significant ( $P > 0.05$ ).

91 To analyse the effect of breed and parity on eating time per day and number of visits per day,  
92 a second order polynomial was used for weeks in milk to better fit nonlinear changes during  
93 lactation. This resulted in the following model 2:

94

95

$$Y_{ijkl} = B_i + P_j + BP_{ij} + (\beta_{B_i} + \beta_{P_j})w_{ijkl} + (\gamma_{B_i} + \gamma_{P_j})w_{ijkl}^2 + \varepsilon_{ijkl}$$

96

97 In addition to the parameters described for model 1,  $\gamma_{B_i}$  and  $\gamma_{P_j}$  are the breed- and parity-  
98 specific parameters for weeks in lactation squared,  $w_{ijkl}^2$ .

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103 Supplemental Figures

104 Supplemental Figure S1 Average eating rate vs. weeks in milk for Jersey and Holstein  
105 cows at each parity. Daily records were averaged for each week in milk and each  
106 animal, and smoothed lines were drawn through the scatter of points against weeks  
107 in milk.

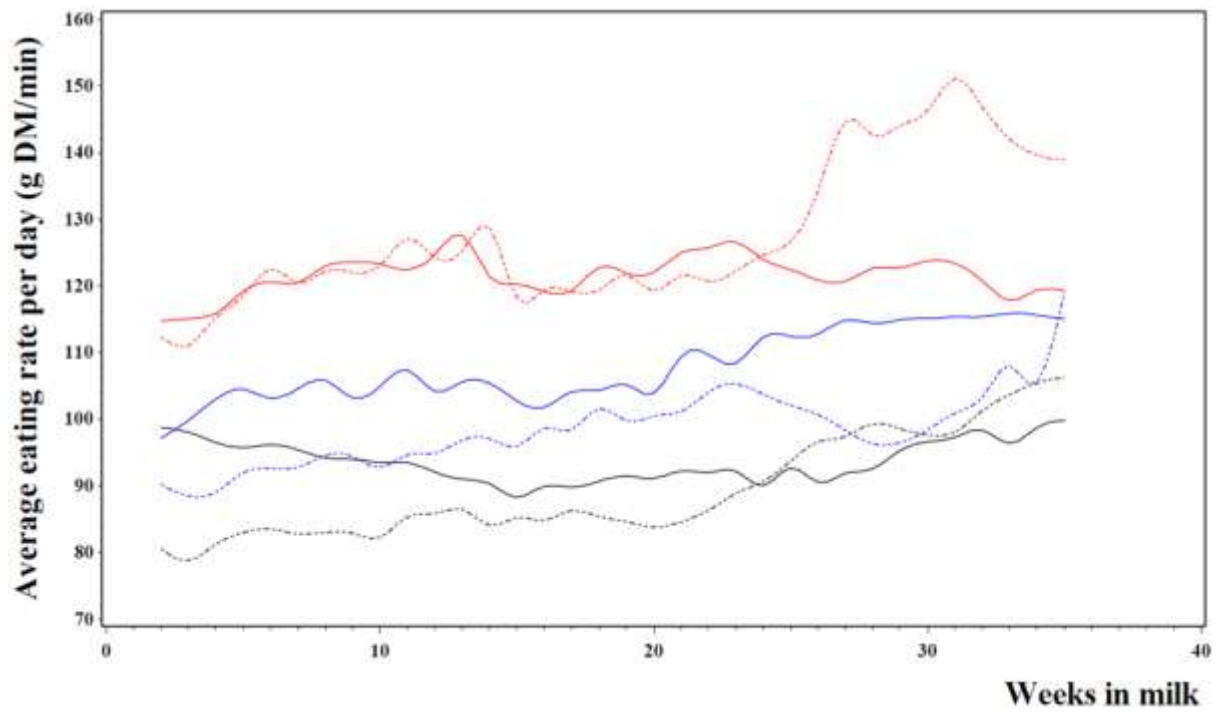
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109 Supplemental Figure S2 Average eating time per day (A) and average eating time per visit  
110 (B), vs. weeks in milk for Jersey and Holstein cows at each parity. Daily records were  
111 averaged for each week in milk and each animal. These averages were then log-transformed  
112 (natural logarithm), and smoothed lines were drawn through the scatter of points against  
113 weeks in milk.

114

115 Supplemental Figure S3 Average eating rate (A) and average number of visits per day (B),  
116 vs. weeks in milk for Jersey and Holstein cows at each parity. Daily records were averaged  
117 for each week in milk and each animal. These averages were then log-transformed (natural  
118 logarithm), and smoothed lines were drawn through the scatter of points against weeks in  
119 milk.

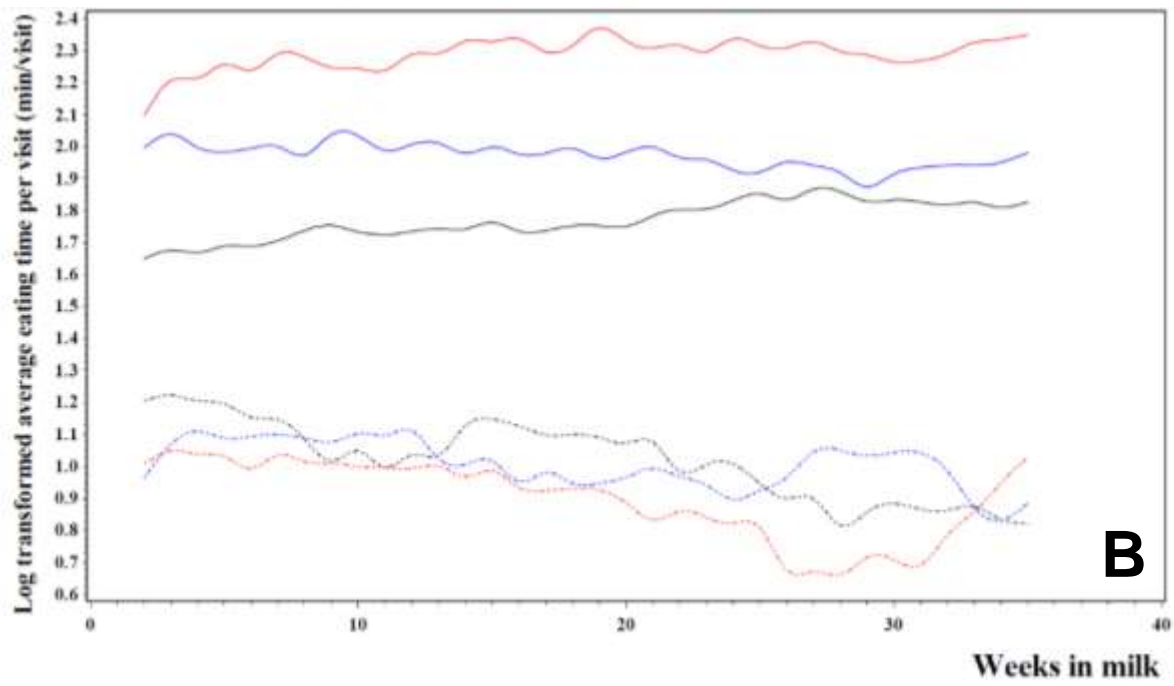
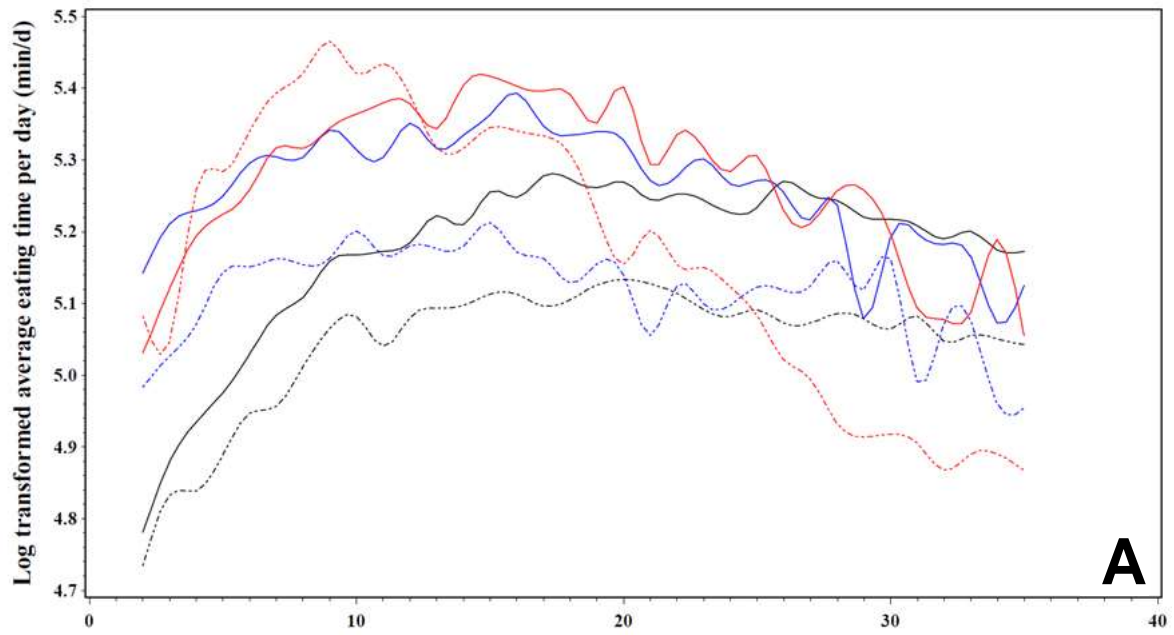
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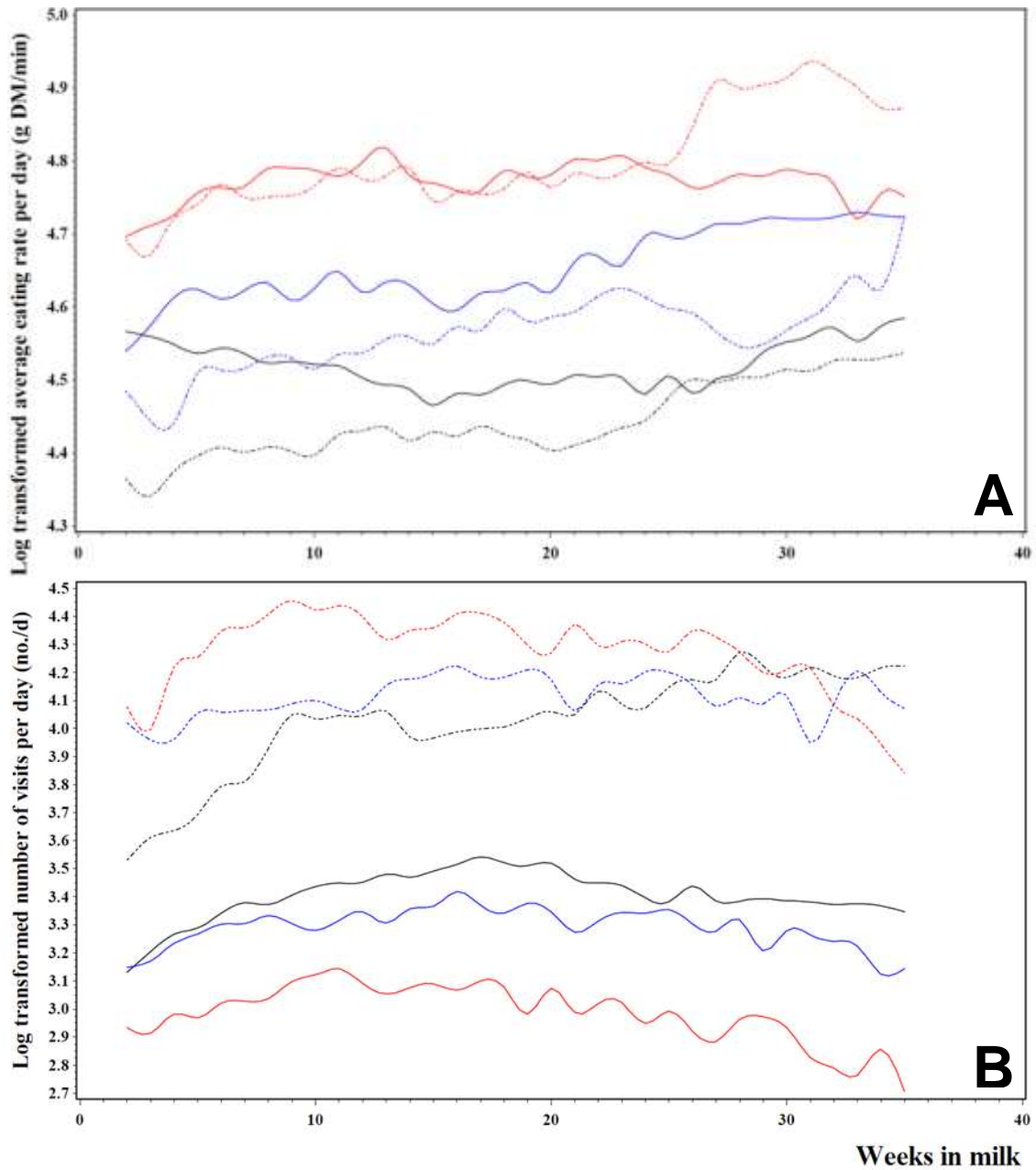
122 Supplemental figure S1

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124 Supplemental figure S2

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126 Supplemental figure S3

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128 Supplementary references

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