

# Supplemental tables and figures for J. Dairy Sci. article 2024-25488: Production and metabolic responses of Montbéliarde and Holstein cows during periparturient period and a sequential feed restriction challenge

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# SUPPLEMENTAL TABLES AND FIGURES FOR THE FOLLOWING PAPER PUBLISHED IN THE JOURNAL OF DAIRY SCENCE

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# Production and metabolic responses of Montbéliarde and Holstein cows during

# periparturient period and a sequential feed restriction challenge

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#### INTERPRETIVE SUMMARY

**Production and metabolic responses of Montbéliarde and Holstein cows during periparturient period and a sequential feed restriction challenge.** Dairy cows face challenges derived from climate change, including variations in feed availability and quality. We studied the responses and resilience of Montbéliarde and Holstein cows during early lactation and to a sequence of four short partial feed restrictions, each followed by a period of normal feeding. Holstein cows produced more milk and used more fat reserves during early lactation compared to Montbéliarde cows. These differences continued during the feed restrictions, especially during the first two. Cows were able to respond to and recover from short restrictions, demonstrating resilience in both breeds.

Supplemental Table S1. Prepartum intake, energy balance, BW and BCS of Holstein (HOLS) and Montbéliarde (MONT) multiparous cows.

	- D1	Period	<i>P</i> -v	value (wk -4	to calving	)
	Breed	wk -4 to calving	Breed	Week	Breed × week	Parity <sup>2</sup>
DMI (kg/d)	HOLS MONT	15.2 (14.4, 15.9) 13.2 (12.6, 13.9)	< 0.001	< 0.001	0.54	NS
Energy balance (MJ/d)	HOLS MONT	31.2(27.2, 35.4)20.0(16.2, 23.7)	< 0.001	< 0.001	0.47	NS
Body weight (kg)	HOLS MONT	778 (753, 803) 748 (726, 770)	0.07	< 0.001	0.86	< 0.01
BCS (0 to 5 scale)	HOLS MONT	3.07(2.87, 3.27)3.32(3.14, 3.50)	0.07	< 0.01	0.44	NS

<sup>1</sup> Values are LSM and (95% CI) <sup>2</sup> 2 vs 3 or 4 lactations. Parity (second or third and greater) effects were tested and included in the model whenever *P*-value  $\leq 0.10$ .

Supplemental Table S2. Milk and milk component yield of Holstein (HOLS) and Montbéliarde (MONT) multiparous cows from calving to 10 wks of lactation and from 18 to 22 weeks of lactation<sup>1</sup>.

		Period		<i>P</i> -value (from calving to wk 10)					Period		<i>P</i> -value (from wk 18 to $22$ ) <sup>5</sup>		
	Breed HOLS MONT	Breed		alving to wk 10 <sup>2</sup>	Breed	Breed Week $\begin{array}{c} Breed \times \\ Week \end{array}$ Parity <sup>4</sup>		From	From week 18 to $22^3$		Week	Breed × Week	
Milk yield (kg/d)	HOLS MONT	37.0 30.7	(35.2, 38.7) (29.1, 32.3)	< 0.001	< 0.001	0.35	0.03	31.8 28.0	(30.3, 33.2) (26.7, 29.2)	< 0.001	< 0.001	0.62	
Fat yield (kg/d)	HOLS MONT	1.463 1.228	(1.392, 1.534) (1.164, 1.292)	< 0.001	< 0.001	0.11	< 0.01	1.282 1.168	(1.225, 1.340) (1.116, 1.220)	< 0.01	< 0.001	0.37	
Protein yield (kg/d)	HOLS MONT	1.146 1.039	(1.092, 1.200) (990, 1.087)	< 0.01	< 0.001	0.03	0.05	1.060 0.997	(1.015, 1.105) (0.957, 1.038)	0.04	< 0.001	0.65	
Lactose yield (kg/d)	HOLS MONT	1.820 1.520	(1.729, 1.911) (1.438, 1.602)	< 0.001	< 0.001	0.79	0.07	1.532 1.359	(1.459, 1.605) (1.293, 1.425)	0.001	< 0.001	0.28	

<sup>1</sup> Values are LSM and (95% CI) <sup>2</sup> 40 cows (22 MONT and 18 HOLS). <sup>3</sup> 38 cows (21 MONT and 17 HOLS).

<sup>4</sup> 2 vs 3 or 4 lactations. Parity (second or third and greater) effects were tested and included in the model whenever *P*-value  $\leq 0.10$ . <sup>5</sup>Parity effects (2 vs 3 or 4 lactations) were not significant for all variables during mid-lactation period, except for a trend observed for milk fat yield (*P* = 0.07).

**Supplemental Table S3**. Milk and milk component yield, PDI balance, energy conversion efficiency and BW in Holstein (HOLS) and Montbéliarde (MONT) multiparous cows subjected to a nutritional challenge composed of a sequence of four 4-d periods of feed restriction<sup>1,2</sup>.

						Period					P-v	alue <sup>3</sup>
	Breed	Before	FR1	Recovery 1, 10 d	FR2	Recovery 2, 3 d	FR3	Recovery 3, 3 d	FR4	Recovery 4, 10 d	Breed	Breed × Period
Milk yield (kg/d)	HOLS MONT	a 37.3 <sup>y</sup> (35.8, 38.8) 31.9 <sup>z</sup> (30.6, 33.2)	cde 30.1 <sup>y</sup> (28.6, 31.6) 26.3 <sup>z</sup> (25, 27.6)	b 32.9 <sup>y</sup> (31.4, 34.4) 28.3 <sup>z</sup> (27, 29.6)	def 29.4 <sup>y</sup> (27.9, 30.9) 25.3 <sup>z</sup> (24, 26.6)	cd 30.5 <sup>y</sup> (29, 32) 26.2 <sup>z</sup> (24.9, 27.5)	fg 27.8 <sup>y</sup> (26.3, 29.4) 25.4 <sup>z</sup> (24.1, 26.7)	ef 28.8 <sup>y</sup> (27.3, 30.3) 25.5 <sup>z</sup> (24.2, 26.8)	g 26.9 <sup>y</sup> (25.4, 28.4) 24.2 <sup>z</sup> (22.9, 25.5)	c 31.2 <sup>y</sup> (29.7, 32.7) 27.3 <sup>z</sup> (26, 28.6)	< 0.001	0.001
Fat yield (kg/d)	HOLS MONT	a $1.463^{y}$ (1.400, 1.522) $1.315^{z}$ (1.2561, 1.368)	b 1.293 <sup>y</sup> (1.234, 1.352) 1.168 <sup>z</sup> (1.114, 1.221)	bc 1.247 <sup>y</sup> (1.194, 1.306) 1.134 <sup>z</sup> (1.081, 1.188)	c 1.207 <sup>y</sup> (1.148, 1.266) 1.105 <sup>z</sup> (1.052, 1.158)	de 1.116 <sup>y</sup> (1.056, 1.175) 1.012 <sup>z</sup> (0.959, 1.066)	d 1.133 (1.074, 1.193) 1.059 (1.005, 1.112)	e 1.053 (0.994, 1.112) 0.998 (0.945, 1.051)	de 1.086 (1.026, 1.145) 1.043 (0.989, 1.096)	c 1.195 <sup>y</sup> (1.136, 1.254) 1.101 <sup>z</sup> (1.047, 1.154)	< 0.01	0.02
Protein yield (kg/d)	HOLS MONT	a 1.175 (1.126, 1.225) 1.125 (1.082, 1.167)	cd 0.894 (0.844, 0.944) 0.869 (0.826, 0.911)	b 1.021 (0.971, 1.070) 0.965 (0.922, 1.007)	cde 0.883 (0.833, 0.933) 0.832 (0.789, 0.874)	c 0.914 (0.864, 0.964) 0.865 (0.822, 0.907)	de 0.840 (0.790, 0.890) 0.846 (0.804, 0.889)	cd 0.884 (0.834, 0.934) 0.857 (0.815, 0.899)	e 0.829 (0.779, 0.879) 0.808 (0.765, 0.850)	b 1.006 (0.956, 1.056) 0.952 (0.910, 0.995)	0.18	0.35
Lactose yield (kg/d)	HOLS MONT	a $1.870^{\text{y}}$ (1.795, 1.946) $1.610^{\text{z}}$ (1.542, 1.678)	c 1.486 <sup>y</sup> (1.410, 1.562) 1.298 <sup>z</sup> (1.230, 1.366)	b 1.615 <sup>y</sup> (1.539, 1.691) 1.386 <sup>z</sup> (1.310, 1.455)	de 1.426 <sup>y</sup> (1.350, 1.502) 1.233 <sup>z</sup> (1.165, 1.302)	cd 1.476 <sup>y</sup> (1.400, 1.552) 1.273 <sup>z</sup> (1.205, 1.342)	e 1.353 <sup>y</sup> (1.278, 1.429) 1.241 <sup>z</sup> (1.173, 1.310)	e 1.373 <sup>y</sup> (1.297, 1.449) 1.216 <sup>z</sup> (1.147, 1.284)	f 1.298 <sup>y</sup> (1.222, 1.374) 1.169 <sup>z</sup> (1.100, 1.237)	c 1.502 <sup>y</sup> (1.426, 1.577) 1.318 <sup>z</sup> (1.250, 1.387)	< 0.001	< 0.01
PDI balance (g/d) <sup>4</sup>	HOLS MONT	521 <sup>y</sup> (379, 662) 309 <sup>z</sup> (181, 437)	-547 (-611, -484) -567 (-624, -509)	551 (409, 693) 451 (323, 579)	-511 (-575, -447) -513 (-570, -455)	721 <sup>y</sup> (579, 863) 511 <sup>z</sup> (383, 639)	-396 <sup>y</sup> (-459, -332) -490 <sup>z</sup> (-548, -433)	974 <sup>y</sup> (832, 1116) 671 <sup>z</sup> (543, 799)	-367 (-430, -303) -405 (-463, -348)	693 (552, 835) 525 (396, 653)	<0.01	<.01
ECE (MJ secreted / MJ intake) <sup>5</sup>	HOLS MONT	c 0.61 (0.57, 0.65) 0.61 (0.58, 0.64)	a 1.09 (1.04, 1.14) 1.04 (1.00, 1.09)	d 0.57 (0.54, 0.6) 0.56 (0.53, 0.58)	a 1.03 (0.99, 1.07) 1.00 (0.97, 1.03)	e 0.52 (0.49, 0.54) 0.53 (0.50, 0.55)	b 0.94 (0.90, 0.97) 0.95 (0.91, 0.98)	e 0.47 (0.43, 0.50) 0.51 (0.47, 0.54)	b 0.91 (0.86, 0.96) 0.91 (0.86, 0.95)	e 0.52 (0.50, 0.54) 0.53 (0.51, 0.55)	0.81	0.46

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		a	e	ab	ef	с	fg	d	g	b		
Body	UOLS	695	653	689	647	676	642	668	636	683		
weight	HOLS	(668, 722)	(626, 680)	(662, 717)	(620, 674)	(648, 703)	(615, 670)	(641, 696)	(609, 664)	(656, 710)	0.26	< 0.01
(kg)	MONT	705	666	702	668	691	662	685	662	697	0.50	< 0.01
	MONT	(681, 730)	(642, 691)	(677, 726)	(643, 692)	(666, 715)	(638, 687)	(660, 710)	(638, 687)	(672, 721)		

<sup>1</sup> Each feed restriction (FR1, FR2, FR3 and FR4) consisted of 4 d of restricted feed allowance to meet 50% of individual NE<sub>L</sub> requirements that were calculated before the challenge period (INRA, 2007). The first feed restriction period (**FR1**) was initiated at 87 ± 9 DIM. FR1 was followed by 10 days of measurements at ad libitum intake (**Recovery 1**), FR2, FR3 and FR4 were separated by 3 days of ad libitum intake (**Recovery 2** and **Recovery 3**), 10 d of measurements at ad libitum intake were recorded after FR4 (**Recovery 4**).

<sup>2</sup> Values are LSM and (95% CI), 40 cows (22 MONT and 18 HOLS).

<sup>3</sup> Period effect significant for all variables (P < 0.001), parity effects (2 vs 3 or 4 lactations) significant for fat yield (P = 0.04) and lactose yield (P = 0.02). A Trend for a covariate effect of DIM at start of the challenge was observed for milk yield (P = 0.08).

<sup>4</sup> Balance of protein truly digestible in the small intestine (INRA, 2007)

<sup>5</sup> Energy conversion efficiency expressed as MJ NE<sub>L</sub> secreted / MJ NE<sub>L</sub> intake.

y, z Breed means not sharing a common superscript differ within the period ( $P \le 0.05$ )

a – g Period means (main period effects) not sharing a common superscript differ ( $P \le 0.05$ )

						Period					<i>P</i> -v	value <sup>3</sup>
	Breed	Before	FR1	Recovery 1	FR2	Recovery 2	FR3	Recovery 3	FR4	Recovery 4	Breed	$\begin{array}{c} \text{Breed} \times \\ \text{Period} \end{array}$
		a	b	а					b			
Inculin	HOLS	9.2	4.3	9.1					4.6			
$(\mu U/mL)^4$	1 110L5	(6.7, 12.5)	(3.1, 5.8)	(6.7, 12.4)					(3.4, 6.2)		0.12	0.53
	MONT	7.8	2.8	8.2			3.4		0.15	0.55		
	MONT	(5.9, 10.4)	(2.1, 3.7)	(6.2, 10.8)					(2.5, 4.5)			
		a	b	а					b			
		136	98	140					117			
IGF-1	HOLS	(118, 154)	(80, 116)	(122, 158)					(99, 135)			
(ng/dL) <sup>4</sup>		153	111	154					117		0.29	0.34
	MONT	(137, 170)	(95, 128)	(138, 170)					(100, 133)			

**Supplemental Table S4.** Plasma insulin and IGF-1 concentrations in Holstein (HOLS) and Montbéliarde (MONT) multiparous cows subjected to a nutritional challenge composed of a sequence of four 4-d periods of feed restriction<sup>1,2</sup>.

<sup>1</sup> Each feed restriction period (FR1, FR2, FR3 and FR4) consisted of 4 d of restricted feed allowance to meet 50% of individual NE<sub>L</sub> requirements that were calculated before the challenge period (INRA, 2007). The first feed restriction period (**FR1**) was started at 87 ± 9 DIM. FR1 was followed by 10 at ad libitum intake (**Recovery 1**), FR2, FR3 and FR4 were separated by 3 days of ad libitum intake (**Recovery 2** and **Recovery 3**), and ad libitum intake after FR4 (**Recovery 4**).

<sup>2</sup> Values are LSM and (95% CI); 40 cows (22 MONT and 18 HOLS).

<sup>3</sup> Period effect were significant for all variables (P < 0.001), parity effects (2 vs 3 or 4 lactations) were not significant.

<sup>4</sup> Analyzed in samples collected before and at the end of FR1, before FR2, and at the of FR4.

a, b: Period means (main period effects) not sharing a common superscript differ ( $P \le 0.05$ ).

**Supplemental Figure S1.** Dry matter intake (DMI), energy balance, energy-corrected milk yield (ECM) and percentage change in ECM in multiparous Holstein (HOLS; n = 18) and Montbéliarde (MONT; n = 22) cows subjected to a nutritional challenge composed of a sequence of four 4-d periods of partial feed restriction. Each feed restriction period (FR1, FR2, FR3 and FR4) consisted of 4 d of restricted feed allowance to meet 50% of individual NE<sub>L</sub> requirements (INRA, 2007) that were calculated before the challenge. The first feed restriction period (FR1) was initiated at  $87 \pm 9$  DIM, and was followed by 10 days of measurements at ad libitum intake (Ad.lib.); FR2, FR3 and FR4 were separated by 3 days of ad libitum intake, and 10 d of intake and production measurements were recorded at ad libitum intake after FR4. Daily data was analyzed using cow means calculated for each variable within each period of the nutritional challenge. Values are LSM and bars are 95% CI. *P*-values of fixed effects in the statistical models are presented in Table 4. \* Breed means differ within the period ( $P \le 0.05$ ). a – e Period means (main period effects) not sharing a common superscript differ ( $P \le 0.05$ ).





☑ HOLS ■ MONT

HOLS MONT





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**Supplemental Figure S2.** Plasma nonesterified fatty acid (NEFA), glucose, urea and bilirubin concentrations in multiparous Holstein (HOLS; n = 18) and Montbéliarde (MONT; n = 22) cows subjected to a nutritional challenge composed of a sequence of four 4-d periods of partial feed restriction. Each feed restriction period (FR1, FR2, FR3 and FR4) consisted of 4 d of restricted feed allowance to meet 50% of individual NE<sub>L</sub> requirements (INRA, 2007) that were calculated before the challenge. The first feed restriction period (FR1) was initiated at  $87 \pm 9$  DIM, and was followed by 10 days of measurements at ad libitum intake (Ad.lib.); FR2, FR3 and FR4 were separated by 3 days of ad libitum intake. Data was analyzed using cow means calculated for each variable within each period of the nutritional challenge. The period 'pre-chall.' corresponds to samples collected on d -4, -3 and 0 relative to FR1; The period FR1 correspond to samples collected on d 1, 2, 3, 4 of FR1; The periods FR2, FR3 and FR4 correspond to samples collected on d 2 and d 4 of FR2, FR3 and FR4, respectively. Samples were collected on d 1, 2, 3, 7 and 10 d after refeeding at ad libitum intake after FR1, and 3 d after refeeding at ad libitum intake following FR2, FR3, and FR4. Values are LSM and bars are 95% CI. *P*-values of fixed effects in the statistical models are presented in Table 5. \* Breed means differ within the period ( $P \le 0.05$ ). a – d Period means (main period effects) not sharing a common superscript differ ( $P \le 0.05$ ).





HOLS MONT



HOLS MONT



HOLS MONT

Supplemental Figure S3. Heatmap illustrating Spearman rank correlations between selected variables recorded during early lactation and the same variables recorded during each period of the nutritional challenge im multiparous cows. The analyses were conducted separately for A) Holstein (n = 18) and **B**) Montbéliarde (n = 22) cows. The variables studied by correlation were energy corrected milk yield (ECM), dry matter intake (DMI), energy balance (EB), and plasma NEFA concentrations. Individual cow means were calculated separately during early lactation and each period of the nutritional challenge. The nutritional challenge was composed of a sequence of four 4-d periods of partial feed restriction. Each feed restriction period (FR1, FR2, FR3 and FR4) consisted of 4 d of restricted feed allowance to meet 50% of individual NE<sub>L</sub> requirements (INRA, 2007) that were calculated individually before the nutritional challenge. The first feed restriction period (FR1) was initiated at  $87 \pm 9$ DIM, and was followed by 10 days of measurements at ad libitum intake (Recovery 1); FR2, FR3 and FR4 were separated by 3 days of ad libitum intake (Recovery 2 and Recovery 3), 10 d of intake and production measurements were recorded at ad libitum intake after FR4 (Recovery 4). Early lactation data for ECM, DMI and EB were recorded weekly from calving until wk 10 of lactation. Plasma NEFA concentrations were analyzed on wk 1, 2, 3, 4, 6 and 8 of lactation. During the sequential nutritional challenge, daily records were used for ECM, DMI and EB. For plasma NEFA concentrations, the "pre.challenge" is the average concentration of d -4, -3 and 0 relative to FR1; 'FR1' is the average of d 1, 2, 3, 4 of FR1; 'FR2', 'FR3' and 'FR4' are averages of d 2 and d 4. 'Recovery 1' is the average of concentration of d 5, 6, 7, 8, and 11 and 14 relative to FR1 initiation (i.e. 1, 2, 3, 7 and 10 d after refeeding at ad libitum intake post-FR1); 'Recovery 2', 'Recovery 3' and 'Recovery 4' are concentrations 3 d after refeeding at ad libitum intake following the end of the preceding FR. Nonsignificant correlations (P > 0.05) are crossed by an '×'.

### Supplemental Figure S3.



## Supplemental Figure S3.

