

Comparison of metabolic, oxidative and inflammatory status of Simmental x Holstein crossbred with parental breeds during peripartum and early lactation period

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SUPPLEMENTARY FILE

Table legend:

Table S1: Least squares means (LSM) and standard errors of estimation (SEE) of biochemical parameters of the three genetic groups at different time points.

Table S2: Least squares means (LSM) and standard errors of estimation (SEE) of oxidative status of the three genetic groups at different time points.

Table S3: Least squares means (LSM) and standard errors of estimation (SEE) of inflammatory markers of the three genetic groups at different time points.

Table S4: Correlation between biochemical, oxidative and inflammatory parameters and milk yield.

Table S5: Heterosis effect for metabolic, inflammatory and oxidative markers.

Table S1

Traits	time point ¹	<i>P</i> -value								
		CR		SI		HO		CR vs. SI	CR vs. HO	SI vs. HO
		LSM	SEE	LSM	SEE	LSM	SEE			
Glucose (mmol/L)	1	3.93	0.23	4.12	0.32	4.05	0.28	ns	ns	ns
	2	3.69	0.22	3.63	0.32	3.40	0.31	ns	ns	ns
	3	3.87	0.22	4.42	0.32	5.52	0.28	ns	0.0007	ns
	4	3.29	0.22	3.70	0.32	3.55	0.31	ns	ns	ns
	5	3.54	0.23	3.90	0.32	3.89	0.29	ns	ns	ns
	6	3.42	0.23	3.85	0.32	4.02	0.29	ns	ns	ns
NEFA (mmol/L)	1	0.26	0.09	0.53	0.12	0.57	0.11	ns	ns	ns
	2	0.38	0.09	0.69	0.13	0.43	0.12	ns	ns	ns
	3	0.64	0.09	0.74	0.13	1.04	0.11	ns	ns	ns
	4	0.82	0.09	0.80	0.13	1.23	0.12	ns	ns	ns
	5	0.80	0.09	0.59	0.13	0.70	0.11	ns	ns	ns
	6	0.42	0.09	0.53	0.13	0.50	0.11	ns	ns	ns
BHB (μ mol/L)	1	318.06	59.46	303.89	70.92	397.09	61.07	ns	ns	ns
	2	305.86	59.49	325.44	71.53	299.08	69.32	ns	ns	ns
	3	411.72	59.46	404.91	70.92	458.62	61.07	ns	ns	ns
	4	683.75	65.89	576.00	72.10	850.12	69.34	ns	ns	ns
	5	577.49	70.71	423.34	72.10	628.37	64.62	ns	ns	ns
	6	424.94	62.29	361.65	71.68	431.75	64.62	ns	ns	ns
Total bilirubin (μ mol/L)	1	3.37	1.00	5.55	1.56	4.16	1.12	ns	ns	ns
	2	3.32	0.95	8.69	1.56	3.21	1.27	ns	ns	ns
	3	8.24	0.92	8.03	1.29	10.81	1.12	ns	ns	ns
	4	6.37	0.93	5.77	1.30	6.39	1.27	ns	ns	ns
	5	4.17	0.92	4.38	1.30	4.28	1.19	ns	ns	ns
	6	3.52	0.97	5.42	1.36	3.63	1.19	ns	ns	ns
ALT (U/L)	1	20.13	1.82	20.52	2.49	21.20	2.16	ns	ns	ns
	2	18.51	1.75	20.52	2.50	21.55	2.45	ns	ns	ns
	3	19.37	1.74	15.28	2.48	15.29	2.16	ns	ns	ns
	4	17.62	1.75	16.86	2.50	16.16	2.45	ns	ns	ns
	5	20.61	1.78	16.61	2.62	20.63	2.28	ns	ns	ns
	6	25.41	1.83	20.74	2.63	25.86	2.45	ns	ns	ns
AST (U/L)	1	64.63	8.71	52.65	11.94	64.36	10.35	ns	ns	ns
	2	61.30	8.40	57.80	11.10	64.04	11.74	ns	ns	ns
	3	90.72	8.35	65.74	12.53	77.90	10.34	ns	ns	ns
	4	100.46	8.40	81.80	11.10	87.65	11.74	ns	ns	ns
	5	81.94	8.54	66.71	12.57	77.20	10.95	ns	ns	ns
	6	84.51	8.75	71.34	12.63	75.63	11.74	ns	ns	ns
ALP (U/L)	1	51.70	12.22	64.02	16.74	40.63	14.51	ns	ns	ns
	2	60.54	11.79	68.94	16.83	50.63	16.46	ns	ns	ns
	3	64.69	11.71	62.66	16.71	41.46	14.51	ns	ns	ns
	4	39.46	11.79	53.07	16.83	30.01	16.46	ns	ns	ns
	5	46.81	11.98	56.47	17.63	29.63	15.35	ns	ns	ns
	6	51.85	12.24	55.32	17.65	28.13	15.35	ns	ns	ns

CK (U/L)	1	118.11	271.61	97.62	391.35	82.44	322.54	ns	ns	ns
	2	129.75	286.26	90.23	378.13	100.62	366.19	ns	ns	ns
	3	817.81	265.71	290.47	415.46	74.14	322.53	ns	ns	ns
	4	150.71	262.04	143.63	374.08	122.83	366.03	ns	ns	ns
	5	170.27	266.20	134.24	372.90	124.75	341.31	ns	ns	ns
	6	169.41	272.13	173.16	392.34	135.87	341.31	ns	ns	ns
Total proteins (g/L)	1	72.09	1.53	72.59	2.09	69.93	1.81	ns	ns	ns
	2	68.03	1.47	71.24	2.10	69.76	2.06	ns	ns	ns
	3	66.99	1.46	65.67	2.19	66.98	1.81	ns	ns	ns
	4	72.30	1.47	74.43	2.10	75.84	2.06	ns	ns	ns
	5	76.53	1.50	75.43	2.09	77.88	1.92	ns	ns	ns
	6	76.37	1.53	71.35	2.11	81.13	1.92	ns	ns	ns
Albumin (g/L)	1	37.48	1.60	38.16	2.19	37.66	1.90	ns	ns	ns
	2	36.90	1.54	37.77	2.20	36.74	2.16	ns	ns	ns
	3	36.02	1.53	35.75	2.19	37.41	1.90	ns	ns	ns
	4	34.53	1.54	37.36	2.20	35.76	2.16	ns	ns	ns
	5	35.96	1.57	33.18	2.20	35.88	2.01	ns	ns	ns
	6	37.62	1.60	33.15	2.20	34.36	2.01	ns	ns	ns
Creatinine (μ mol/L)	1	115.69	4.84	121.31	6.63	90.73	5.75	ns	ns	ns
	2	122.34	4.67	128.65	6.66	89.12	6.52	ns	0.008	0.004
	3	124.09	4.64	121.88	6.62	95.23	5.75	ns	0.013	ns
	4	91.10	4.67	104.83	6.66	77.43	6.52	ns	ns	ns
	5	89.72	4.74	97.65	6.64	75.14	6.08	ns	ns	ns
	6	86.67	4.85	102.49	6.65	71.83	6.08	ns	ns	ns
UREA (mmol/L)	1	4.47	0.38	6.05	0.52	4.97	0.45	ns	ns	ns
	2	4.62	0.36	5.89	0.52	4.77	0.51	ns	ns	ns
	3	4.78	0.36	5.28	0.51	5.45	0.45	ns	ns	ns
	4	6.12	0.36	7.07	0.52	4.30	0.51	ns	ns	0.016
	5	6.40	0.37	6.90	0.52	4.86	0.47	ns	ns	ns
	6	7.46	0.38	7.89	0.52	6.95	0.47	ns	ns	ns

CR: Italian Simmental x Italian Holstein; SI: Italian Simmental; HO: Italian Holstein; NEFA: non-esterified fatty acid; BHB: β -Hydroxybutyrate; ALT: alanine transaminase; AST: aspartame aminotransferase; ALP: alkaline phosphatase; CK: creatine kinase; ¹ time point 1 and 2 correspond to 30 \pm 3 and 15 \pm 3 days before the expected calving; time point 3 corresponds at calving or at the **day post calving**; time point 4, 5 and 6 correspond at 15, 30 and 60 **days post calving**.

Table S2:

Traits	time point ¹	<i>P</i> -value								
		CR		SI		HO		CR vs. SI	CR vs. HO	SI vs. HO
		LSM	SEE	LSM	SEE	LSM	SEE			
d-ROM (U CARR)	1	58.58	7.26	83.10	9.97	68.50	9.11	ns	ns	ns
	2	55.66	7.04	99.70	10.05	40.48	10.69	ns	ns	0.005
	3	63.33	6.95	82.06	9.92	147.49	8.61	ns	<0.0001	0.0003
	4	66.63	7.00	81.96	9.99	166.23	9.77	ns	<0.0001	<0.0001
	5	77.36	7.11	81.38	9.96	156.38	9.11	ns	<0.0001	<0.0001
	6	64.64	7.26	88.50	9.97	167.25	9.11	ns	<0.0001	<0.0001
BAP (μ mol/L)	1	1874.16	764.60	-25.03	1049.02	2510.88	959.12	ns	ns	ns
	2	1642.23	740.45	2105.96	1057.64	1442.26	1125.08	ns	ns	ns
	3	2441.89	731.70	1111.66	1043.86	673.75	906.32	ns	ns	ns
	4	3453.98	736.37	1389.41	1051.22	3264.72	1028.61	ns	ns	ns
	5	3588.39	748.06	4479.81	1047.90	1118.88	959.12	ns	ns	ns
	6	4548.38	764.60	3700.58	1049.02	2031.0	959.12	ns	ns	ns
OSi	1	5.90	10.03	16.35	13.76	6.29	12.58	ns	ns	ns
	2	11.48	9.72	21.99	13.88	1.25	14.76	ns	ns	ns
	3	6.86	9.60	33.96	13.70	32.44	11.89	ns	ns	ns
	4	2.43	9.66	17.30	13.79	89.52	13.50	ns	<0.0001	0.002
	5	5.55	9.82	6.03	13.75	43.17	12.58	ns	ns	ns
	6	4.89	10.03	2.56	13.76	29.16	12.58	ns	ns	ns

CR: Italian Simmental x Italian Holstein; SI: Italian Simmental; HO: Italian Holstein.

d-ROMs: derivatives of reactive oxygen metabolites; BAP: Biological Antioxidant Potential; OSi: d-ROM/BAP x100.

¹ time point 1 and 2 correspond to 30 \pm 3 and 15 \pm 3 days before the expected calving; time point 3 corresponds at calving or at the **day post calving**; time point 4, 5 and 6 correspond at 15-, 30- and 60-**days post calving**.

Table S3

Traits	time point ¹	<i>P</i> -value								
		CR		SI		HO		CR vs. SI	CR vs. HO	SI vs. HO
		LSM	SEE	LSM	SEE	LSM	SEE			
IL-6 (pg/mL)	1	74.53	55.25	142.83	75.80	113.75	69.31	ns	ns	ns
	2	137.66	53.51	177.72	76.43	196.10	81.30	ns	ns	ns
	3	195.19	52.87	130.41	75.43	315.30	65.49	ns	ns	ns
	4	213.65	53.21	255.77	75.96	69.92	74.33	ns	ns	ns
	5	176.78	74.06	202.18	75.72	67.92	69.31	ns	ns	ns
	6	155.09	55.25	190.74	75.80	73.29	69.31	ns	ns	ns
Haptoglobin (mg/mL)	1	0.09	0.01	0.12	0.02	0.07	0.01	ns	ns	ns
	2	0.09	0.01	0.14	0.02	0.08	0.02	ns	ns	ns
	3	0.12	0.01	0.17	0.02	0.13	0.01	ns	ns	ns
	4	0.10	0.01	0.13	0.02	0.13	0.02	ns	ns	ns
	5	0.09	0.01	0.11	0.02	0.09	0.01	ns	ns	ns
	6	0.09	0.01	0.16	0.02	0.11	0.01	ns	ns	ns
SAA (ng/mL)	1	67.91	23.05	39.52	31.62	45.47	28.91	ns	ns	ns
	2	46.16	22.32	45.96	31.88	29.94	33.91	ns	ns	ns
	3	243.78	22.06	269.50	31.47	221.29	27.32	ns	ns	ns
	4	98.12	22.20	102.55	31.69	87.51	31.01	ns	ns	ns
	5	92.07	22.55	76.09	31.59	61.26	28.91	ns	ns	ns
	6	77.21	23.05	118.31	31.62	84.81	28.91	ns	ns	ns

CR: Italian Simmental x Italian Holstein crossbred; SI: Italian Simmental; HO: Italian Holstein; IL-6: interleukin-6 SAA: serum amyloid

A; ¹ time point 1 and 2 correspond to 30±3 and 15±3 days before the expected calving; time point 3 corresponds at calving or at **the day post calving**; time point 4, 5 and 6 correspond at 15-, 30- and 60-days **post calving**.

Table S4

	Time of sampling		
	at 15 day	at 30 day	at 60 day
	milk yield	milk yield	milk yield
Glucose (mmol/L)	-0.21	-0.41	0.23
NEFA (mmol/L)	0.14	0.20	-0.11
BHB ($\mu\text{mol/L}$)	-0.08	0.63**	0.45
Total bil. ($\mu\text{mol/L}$)	0.12	-0.14	-0.61**
ALT(U/L)	0.41	0.35	0.47
AST(U/L)	0.44*	0.34	0.40
ALP(U/L)	0.09	0.29	-0.03
CK(U/L)	-0.04	0.22	0.11
Total protein (g/L)	-0.10	-0.10	0.22
Albumin (g/L)	0.06	0.42	0.75
Creatinine ($\mu\text{mol/L}$)	-0.18	-0.29	-0.47
Urea (mmol/L)	0.23	0.02	-0.31
d-Rom (U CARR)	0.02	-0.05	-0.08
BAP ($\mu\text{mol/L}$)	0.21	0.41	0.24
Osi (d-ROM/BAP x100)	-0.18	-0.09	0.33
IL6 (pg/mL)	0.23	0.10	-0.32
Haptoglobin (mg/mL)	-0.34	-0.41	-0.74***
SAA (mg/mL)	-0.37	-0.38	-0.73***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table S5

Traits	Heterosis (%)
Glucose	-11
NEFA	-20
BHB	0
Total bil.	-17
ALT	5
AST	14
ALP	8
CK	98
Total protein	-2
Albumin	1
Creatinine	7
Urea	-4
d-ROM	-39
BAP	47
OSi	-75
IL-6	-2
Hp	-17
SAA	6.0