

## Research Article

\*These authors contributed equally to this work.

**Cite this article:** De Matteis G *et al* (2021). Comparison of metabolic, oxidative and inflammatory status of Simmental × Holstein crossbred with parental breeds during the peripartal and early lactation periods. *Journal of Dairy Research* **88**, 253–260. <https://doi.org/10.1017/S0022029921000650>

Received: 18 January 2021

Revised: 13 June 2021

Accepted: 21 June 2021

First published online: 18 August 2021

### Keywords:

Crossbred; inflammatory response; metabolic profile; oxidative stress; peripartum

### Author for correspondence:

Giovanna De Matteis,

Email: [giovanna.dematteis@crea.gov.it](mailto:giovanna.dematteis@crea.gov.it)

# Comparison of metabolic, oxidative and inflammatory status of Simmental × Holstein crossbred with parental breeds during the peripartal and early lactation periods

Giovanna De Matteis<sup>1,\*</sup>, Maria Carmela Scatà<sup>1,\*</sup>, Gennaro Catillo<sup>1</sup>, Francesco Grandoni<sup>1</sup>, Emanuela Rossi<sup>1</sup>, David Meo Zilio<sup>1</sup>, Alessandra Crisà<sup>1</sup>, Vincenzo Lopreiato<sup>2</sup>, Erminio Trevisi<sup>2</sup> and Vittoria Lucia Barile<sup>1</sup>

<sup>1</sup>Council for Agricultural Research and Economics, CREA Research Centre for Animal Production and Aquaculture, Via Salaria 31, 00015 Monterotondo, RM, Italy and <sup>2</sup>Department of Animal Sciences, Food and Nutrition, Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy

## Abstract

The aim of the research reported in this paper was to evaluate plasma concentrations of energy, oxidative and inflammatory biomarkers of Simmental (sire) × Holstein (dam) crossbred cows, in comparison with the two parental breeds during the peripartal and early lactation periods and to estimate the effects of heterosis for these traits. Thirty-three animals, managed under the same conditions, 8 Simmental (SI), 9 Holstein (HO) and 16 crossbred (CR) cows were enrolled in this study. Glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), total protein, albumin, creatinine and urea were determined in blood sampled at six different time points (30 ± 3 and 15 ± 3 d before the expected calving date, at calving and 15, 30 and 60 d after calving). Furthermore, derived reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), interleukin-6 (IL-6), haptoglobin (Hp) and serum amyloid A protein (SAA) were determined to evaluate inflammatory and oxidative status. Results showed that the CR group had significantly lower average values of glucose and NEFA when compared to HO group; significantly lower values of urea than SI group and significantly higher values of creatinine than HO. Furthermore, CR cows showed the lowest average value of d-ROMs with respect to SI and HO parental breeds. Finally, the average value of haptoglobin was significantly lower in CR and HO groups, when compared to SI group. As for the heterosis we found the highest (positive) percentage for CK (98%) and BAP (47%) and the lowest (negative) percentage for OSi (−75%) and d-ROMs (−39%). A negative percentage was also found for the glucose (−11%) and NEFA (−20%) toward the Simmental parental breed. Our results suggest a different response among the three genetic groups during the peripartal and early lactation periods. In particular, CR and SI cows seem more adaptable regarding energy metabolism and oxidative status. Heterosis led to a positive effect on those parameters in Simmental (sire) × Holstein (dam) crossbred cows F1 population (50% Simmental and 50% Holstein).

High-yielding dairy cows are subjected to several challenges during the transition period, with metabolic stress and inflammatory condition contributing to the increased incidence and severity of diseases (Sordillo *et al.*, 2009; Trevisi *et al.*, 2012). This period is characterized by 5 critical events: reduction of immune competences; negative energy balance resulting in mobilization of adipose and muscle tissue; hypocalcemia due to demand of the mammary gland for milk synthesis; an overt systemic inflammatory response around calving and oxidative stress due to increased production of pro-oxidant molecules (Trevisi and Minuti, 2018).

Holstein is a breed selected for high production efficiency and in general, is today recognized as the breed with the highest milk production in the world. Selection for high production efficiency has often been associated with decreased longevity, increased culling rate and a sharp decrease in fertility in Holstein cows (Hare *et al.*, 2006; Miller *et al.*, 2008; Norman *et al.*, 2009) together with undesired side effects on animal welfare (Oltenacu and Broom, 2010). A recent study of genome-wide association between longevity traits and single nucleotide polymorphisms identified the presence of two suggestive quantitative trait loci (QTLs) which included genes with biological functions related to fertility, reproductive disorders, heat stress and cow welfare (Steri *et al.*, 2019). Furthermore, increased inbreeding has been associated with fitness problems including enhanced risk of disease occurrence and reduced survival (Sorensen *et al.*, 2006; McParland *et al.*, 2007). Crossbreeding is a practice that may

© The Author(s), 2021. Published by Cambridge University Press on behalf of Hannah Dairy Research Foundation. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.



CAMBRIDGE  
UNIVERSITY PRESS

help to overcome these problems. This breeding strategy in dairy production is used to increase the economic profit by improving health, fertility, longevity or milk components, through the benefits due to the effect of heterosis, which reduces the likelihood of inbreeding depression by increasing heterozygosity. The main contribution of crossbreeding in livestock is the increase of efficiency of the production system due to breeds having their genetic merits in different traits so obtaining benefits of heterosis. Crossbred animals are more robust and economically efficient compared to the parental breeds (Sørensen *et al.*, 2008).

Heterosis occurs when unrelated breeds are crossed. Its effect is opposite to the inbreeding depression. In animal breeding this is usually expressed as mid-parent heterosis or the superiority of the F1 cross over the mean performance of the two parents (Falconer, 1960). Deviations from the mid-parent value can be positive or negative but are mostly found to be beneficial.

Crossbreeding between Simmental (dairy type) and Holstein has been performed for a long time, mostly in Europe. Several studies have demonstrated that relative crossbreds had higher reproductive performance and a better ability to restore immune homeostasis after calving than Holstein cows (Knob *et al.*, 2016, 2020; Puppel *et al.*, 2018; Scatà *et al.*, 2020). Knob *et al.* (2016) highlighted improved fertility traits (lower calving interval, higher conception rate and shorter calving to first service interval) and improved longevity in Holstein × Simmental F1 population respect to Holstein purebred. Moreover, Lopreiato and coworkers showed different metabolic adaptation during the transition period and different expression of genes involved in immune adaptation and inflammatory response, between Simmental and Holstein breeds (Lopreiato *et al.*, 2019, 2020). Recently, Knob *et al.* (2021) published a study on a combination of metabolic traits and some body condition traits with early performance characteristics during the transition period in a crisscross breeding program of Holstein and Simmental. The research showed that after calving, Simmental and R1-Simmental cows were able to deal with a negative energy balance to a better extent than purebred Holstein and the other crossbred lines.

With this study we wanted to investigate the changes in energy metabolism, oxidative stress and inflammation biomarkers during the peripartur and early lactation periods in Italian Simmental (SI), Italian Holstein (HO) and their cross (CR) in order to evaluate the response of the F1 crossbred population in comparison to the parental breeds. To the best of our knowledge it is the first time that, beside the energy metabolism parameters, oxidative status and inflammatory response in Simmental × Holstein crossbred cows are reported.

## Materials and methods

### Animal management

Thirty-three cows, from 30 d before the expected calving date until 60 d post actual calving, were included in this study: 8 Italian Simmental (SI), 9 Italian Holstein (HO) and 16 Italian Simmental (sire) × Italian Holstein (dam) crossbred (CR). The animals were housed at the Council for Agricultural Research and Economics (CREA) – Research Centre for Animal Production and Aquaculture. Cows were mixed parity including primiparous, all kept under the same management conditions and fed *ad libitum* once a day with a total mixed ration based on sorghum silage, hay and concentrates (Table 1). Cows were milked twice a day in a milking parlor with automatic milk

recorders and data were acquired and stored by the ALPRO™ herd management system (DeLaval International AB). The Body Condition Score (BCS) was evaluated monthly by a trained operator using a 5-point scale (1 = emaciated, 5 = obese) according to the method described by Edmonson *et al.* (1989). Cows were subjected to routine veterinary examinations to exclude animals with clinical signs of diseases such as endometritis, mastitis or metabolic disorders. Only healthy animals were included in the data analysis. The health status was also evaluated through the inflammatory markers: interleukin-6 (IL-6), haptoglobin (Hp) and serum amyloid A protein (SAA).

### Blood sample collection and biomarker analysis

The experimental procedure was carried out in compliance with the European Directive 2010/63/UE and the Italian regulation D. Lgs n. 26/2014 (Health Ministry authorization n 529/2017-PR). Blood samples were collected from the jugular vein into lithium-heparin BD Vacutainer® (Beckton Dickinson, Plymouth, UK) before the morning feeding. Six different time points were chosen: 30 ± 3 and 15 ± 3 d before the expected calving date, calving day or if not possible the day after, 15, 30, and 60 d after calving. Samples were centrifuged at 3000 g for 15 min at 4 °C and plasma collected and divided into aliquots for storage at –20°C until analysis.

Plasma samples were assayed by a clinical chemistry analyzer (ILAB 650, Instrumentation Laboratory, Lexington, MA, USA) using commercial kits for albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, creatine kinase (CK), creatinine, alkaline phosphatase (ALP), glucose, total protein, and urea (all Instrumentation Laboratory spa, Milan, Italy) as well as β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) (FUJIFILM Wako Chemicals Europe GmbH, Germany).

The amount of free oxygen radicals and the concentration of antioxidants in plasma samples were determined using the derived reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), d-ROMs and BAP tests respectively (Diacron, Grosseto, Italy), modified for a microplate procedure using the same proportion of reagents as the standard method. Briefly, for the d-ROM test, the end-point mode was used and a mix solution of 300 µl of R2 (acidic buffer), 3 µl of R1 (chromogenic mixture) and 1.5 µl of plasma sample was incubated for 90 min at 37°C. Finally, the absorbance at 546 nm was registered by the Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA, USA). The results were expressed in arbitrary Carratelli Units (U CARR), where 1 (U CARR) is equivalent to the oxidizing power of 0.8 mg H<sub>2</sub>O<sub>2</sub>/l. For the BAP test, a mix solution of 300 µl of R1 (chromogenic mixture), 15 µl of R2 (ferric ions solutions, Fe<sup>3+</sup>) and 3 µl of plasma sample was incubated for 5 min at 37 °C. Then, the absorbance at 505 nm was registered by the same microplate photometer. Results of the test were expressed as µmol of ferric ions reducing antioxidants per litre of sample. The degree of oxidative stress was expressed as an Oxidative Status index (OSi) where d-ROMs/BAP × 100 = OSi (Celi, 2011; Fiore *et al.*, 2019).

IL-6 was determined in plasma samples using a bovine ELISA kit (OKEH03748-Aviva Systems Biology, Corp., San Diego, CA, USA) according to the manufacturer's instruction. Hp and SAA concentrations were determined in plasma samples using the commercial tests PHASE™ RANGE Haptoglobin kit (Tridelta Development Ltd., Ireland) and PHASE™ RANGE Multispecies

**Table 1.** Ingredient and nutrient composition of dry and lactation diets.

Item	Dry	Lactation
Ingredient (% DM)		
Sorghum silage	32.38	21.49
Barley grain	11.80	20.04
Polyphyte meadow hay	21.60	19.66
Soybean meal	2.42	17.16
Corn grain	2.33	16.50
Triticale grain	11.83	–
Wheat straw	15.97	–
Fat	–	1.28
Buffer	–	2.48
Minerals and vitamins	1.67	1.39
Nutrient composition (% DM)		
CP	11.25	16.66
NSC	24.96	34.93
Ether extract	1.83	3.25
NDF	52.30	35.08
Forage NDF	45.86	24.76
ADF	33.72	22.02
ADL	4.88	3.35
NE <sup>a</sup> , Milk FU/kg of DM	0.76	0.95

NSC, non-structural carbohydrates.

<sup>a</sup>The net energy of each feedstuff, expressed as milk FU/kg DM, was determined using the chemical composition and digestibility of the organic matter (INRA, 1988).

SAA ELISA kit (Tridelata Development Ltd., Ireland) according to manufacturer's instructions. All the samples were analyzed in duplicate. The O.D. absorbance was read at 450 nm with the same microplate photometer.

### Statistical analysis

The statistical analysis of the metabolic, oxidative and inflammatory parameters was carried out by the PROC MIXED procedure (SAS Inst. Inc., Cary, NC, USA, release 9.4) using a linear model that included genetic group, parity and interaction time × genetic group and time × parity as factor:

$$y = M + B + P + T \times B + T \times P + \text{error}$$

where  $y$  is the observation vector for each trait;  $M$  is the total average;  $B$  is the breed (3 levels): HO, SI, CR;  $P$  is the parity (2 levels): primiparous, multiparous;  $T$  is the time of sampling (6 levels): –30, –15, 0, 15, 30, 60 d; error represents random effects of the residuals.

The traits of the milk production at 15, 30 and 60 d after the calving, in correspondence with blood sampling, were analyzed using the same model described above with the fixed effect time of sampling ( $T$ ) with 3 levels (15, 30 and 60 d). The traits related to the production during lactation, such as 305-d milk yield, % protein, % fat and the BCS parameter were analyzed using the linear model with two fixed effect: breed ( $B$ ) and parity ( $P$ ).

The statistical significance of all traits and least-squares means were determined using Tukey's test available in the MIXED procedure (LSMEANS/ADJUST = TUKEY), with a probability level of  $P < 0.05$ . Tukey's test was used due to unbalanced data in the different groups, as described by Kramer (1956). In order to verify whether there were any relationships between the productive traits (milk production at 15, 30 and 60 d after the calving) and the metabolic, oxidative and inflammatory parameters, the correlation coefficients were estimated using the PROC CORR procedure of the SAS software.

The estimation of the effect due to heterosis was carried out as the difference between the estimated average values of each single trait of the crossbreed and the half-sum of the estimated average values of the two pure parental breeds:

$$\text{Heterosis (in\%)} = \frac{[(\text{CR}(1/2 \times \text{HO} + 1/2 \times \text{SI})) / (1/2 \times \text{HO} + 1/2 \times \text{SI})] \times 100}$$

### Results

Milk production over the first two months of lactation is shown in Table 2. Even though no significant differences in daily milk yield were detected at the experimental time points among the three genetic groups, the 305-d milk yield of CR was intermediate between HO and SI (Table 2). For milk components, the fat percentage was significantly higher in SI than in HO ( $P < 0.05$ ) and the protein percentage was higher in SI than in CR or HO ( $P < 0.05$ ;  $P = 0.01$ , respectively). The average BCS was 3.2, 3.2 and 2.9 for CR, SI and HO groups, respectively. SI and CR had higher BCS values than HO ( $P < 0.05$ ;  $P = 0.01$ ).

Significant differences were found in the mean values of plasma concentration of metabolites, oxidative status and inflammatory response between the three genetic groups, considering all the sampling times (Table 3). In particular, the CR group showed lower average values of glucose ( $P = 0.001$ ) and NEFA ( $P = 0.05$ ) compared to HO group, lower values of urea ( $P < 0.01$ ) than SI group and an intermediate and significantly different value of creatinine when compared to HO ( $P < 0.001$ ). The Hp value was lower in CR ( $P < 0.001$ ) as well as in HO group ( $P < 0.001$ ) compared to SI group. The most relevant differences between the three groups were found for the oxidative status: CR cows showed the lowest average value of d-ROMs respect to SI and HO (both  $P < 0.001$ ). The oxidative status index (OSi) was lower in the CR group than in HO group ( $P < 0.001$ ).

The differences between the three genetic groups at different time points are showed in Figs. 1 and 2 and online Supplementary Tables S1–S3. Significantly lower glucose concentrations were found at calving in CR ( $P < 0.001$ ) compared to HO (Fig. 1a). Interestingly, CR and SI groups showed higher and statistically different creatinine values respect to HO group at –15 d (both  $P < 0.01$ ), while at calving the only significant difference was between CR vs. HO ( $P < 0.05$ ) (Fig. 1b). The urea was statistically lower in HO than SI group, but only at +15 d (Fig. 1c). The trend of other metabolic parameters was similar and no significant differences were found at any time points (online Supplementary Table S1).

A lower concentration of oxidative stress biomarkers was found in CR and SI cows compared to HO group that showed an increase of d-ROMs at calving (online Supplementary Table S2; Fig. 2a). Significantly lower OSi values were obtained

**Table 2.** Least-squares means (LSM) and standard errors of estimation (SEE) of the productive traits

	CR		SI		HO	
	LSM	SEE	LSM	SEE	LSM	SEE
Milk yield (kg/d)						
d 15	27.5	3.36	27.3	3.13	26.6	3.14
d 30	31.1	3.65	29.4	3.13	30.8	3.56
d 60	31.3	4.41	23.4	3.32	31.7	3.56
305-d milk yield	7179	1131	6466	1749	7499	806
Fat (%)	4.2 <sup>ab</sup>	0.13	4.5 <sup>b</sup>	0.15	4.0 <sup>a</sup>	0.14
Protein (%)	3.2 <sup>a</sup>	0.08	3.5 <sup>b</sup>	0.09	3.2 <sup>a</sup>	0.06
BCS	3.2 <sup>a</sup>	0.17	3.2 <sup>a</sup>	0.24	2.9 <sup>b</sup>	0.20

CR, Italian Simmental × Italian Holstein crossbred; SI, Italian Simmental; HO, Italian Holstein; BCS, body condition score

<sup>a, b</sup> Within the same row indicate significant differences between groups ( $P < 0.05$ ).

**Table 3.** Least-squares means (LSM) and standard errors of estimation (SEE) of biochemical, oxidative and inflammatory parameters of the three genetic groups

Traits	CR		SI		HO		P-value		
	LSM	SEE	LSM	SEE	LSM	SEE	CR vs. SI	CR vs. HO	SI vs. HO
Glucose (mmol/l)	3.62	0.09	3.94	0.13	4.17	0.12	ns	0.001	ns
NEFA (mmol/l)	0.56	0.04	0.65	0.05	0.75	0.05	ns	0.05	ns
BHB (μmol/l)	456.27	25.73	399.21	29.21	510.84	26.58	ns	ns	0.02
Total bil. (μmol/l)	4.84	0.39	6.31	0.57	5.42	0.49	ns	ns	ns
ALT (U/l)	20.28	0.73	18.42	1.04	20.12	0.95	ns	ns	ns
AST (U/l)	80.43	3.48	66.18	5.02	74.46	4.56	ns	ns	ns
ALP (U/l)	52.51	4.88	60.08	6.97	36.75	6.31	ns	ns	0.037
CK (U/l)	259.34	110.54	154.89	158.26	106.78	140.35	ns	ns	ns
Total protein (g/l)	72.05	0.61	72.95	0.86	73.59	0.79	ns	ns	ns
Albumin (g/l)	36.42	0.64	35.90	0.90	36.30	0.83	ns	ns	ns
Creatinine (μmol/l)	105.08	1.93	112.80	2.71	83.24	2.50	ns	<0.0001	<0.0001
Urea (mmol/l)	5.64	0.15	6.52	0.21	5.22	0.19	0.005	ns	<0.0001
d-ROM (U CARR)	64.37	2.90	86.12	4.07	124.39	3.85	0.0002	<0.0001	<0.0001
BAP (μmol/l)	2924.84	305.27	2127.07	428.57	1850.75	405.00	ns	ns	ns
OSi (d-ROM/BAP × 100)	6.19	4.00	16.37	5.62	33.64	5.31	ns	0.0002	ns
IL-6 (pg/ml)	158.82	22.06	183.28	30.97	139.38	29.27	ns	ns	ns
Hp (mg/ml)	0.10	0.00	0.14	0.07	0.10	0.01	<0.0001	ns	0.0002
SAA (ng/ml)	104.21	9.20	108.65	12.92	88.38	12.21	ns	ns	ns

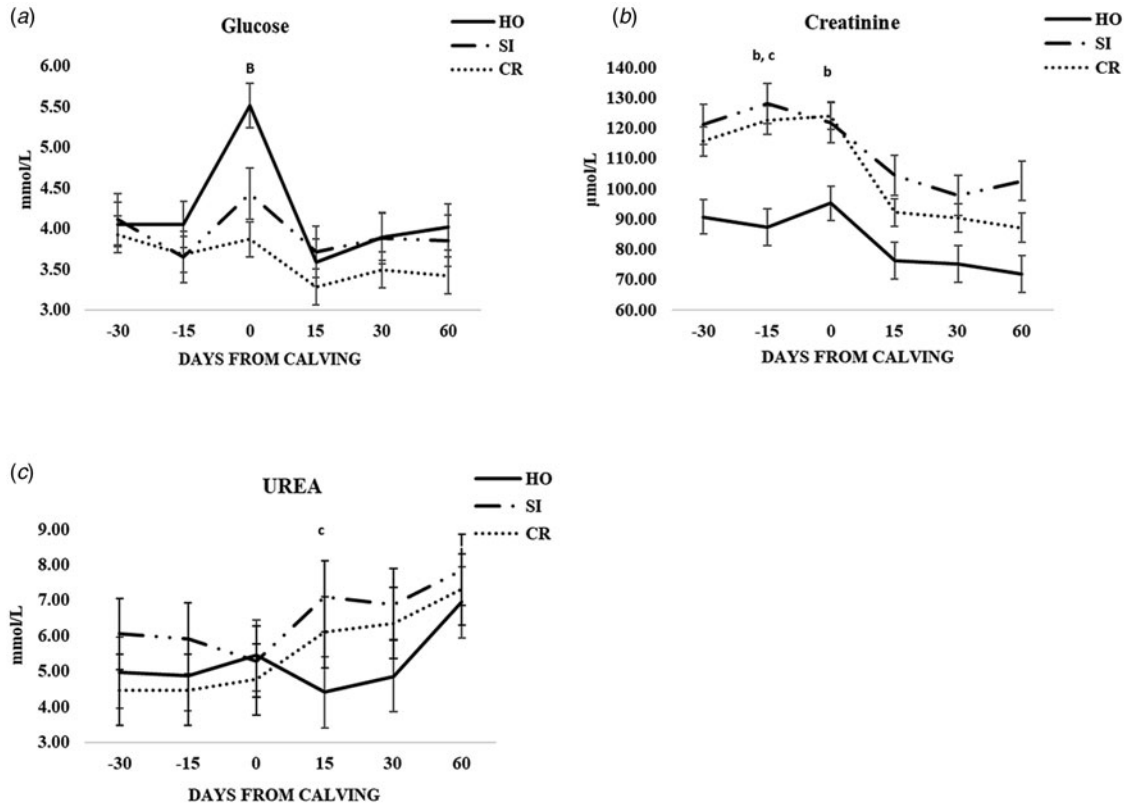
CR, Italian Simmental × Italian Holstein crossbred; SI, Italian Simmental; HO, Italian Holstein; NEFA, non-esterified fatty acid; BHB, β-Hydroxybutyrate; Total bil., total bilirubin; ALT, alanine transaminase; AST, aspartame aminotransferase; ALP, alkaline phosphatase; CK, creatine kinase; d-ROMs, derivatives of reactive oxygen metabolites; BAP, Biological Antioxidant Potential; OSi, Oxidative Status index; IL-6, interleukin-6; Hp, haptoglobin; SAA, serum amyloid A.

for CR and SI at +15 d ( $P < 0.001$  and  $P < 0.01$ , respectively) (online Supplementary Table S2; Fig. 2b). No meaningful differences were observed for the inflammatory markers IL-6, Hp and SAA at any time point (online Supplementary Table S3).

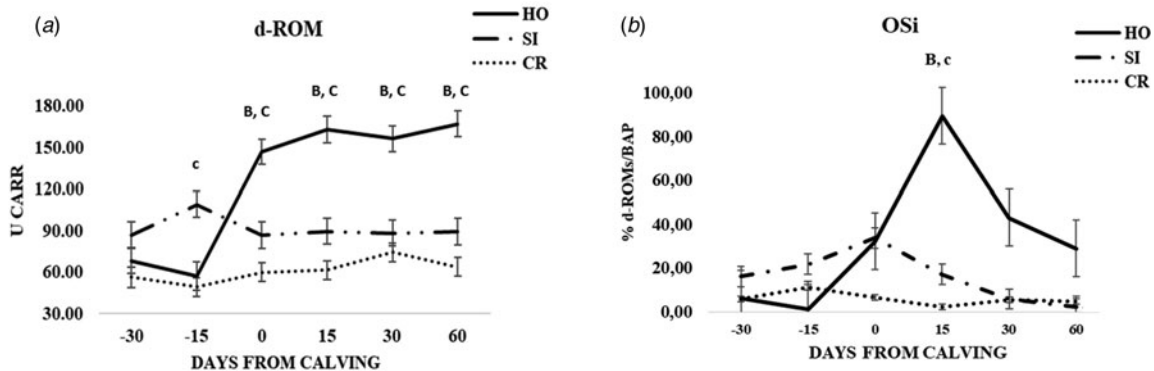
Online Supplementary Table S4 shows the correlations between biochemical, inflammatory and oxidative parameters and milk yield at 15, 30 and 60 d after calving. Haptoglobin and SAA were negatively correlated with milk yield at 60 d ( $r = -0.74$ ,  $r = -0.73$ ,  $P < 0.001$ ), when cows typically reach peak

production. A weaker but significant negative correlation was obtained with total bilirubin in the same lactation period. BHB is positively correlated ( $r = 0.63$ ) to production in the first lactation period at 15 d.

As for the effect of heterosis, positive percentage was found for ALT (5%), AST (14%), ALP (8%), CK (98%), creatinine (7%), BAP (47%) and SAA (6%). On the other hand, negative percentage was found for glucose (-11%), NEFA (-20%), total bilirubin (-17%), urea (-4%), d-ROMs (-39%), OSi (-75%) and Hp



**Fig. 1.** Patterns of variation of glucose (a), creatinine (b) and urea (c) in the three groups of cows (CR, Italian Simmental × Italian Holstein; SI, Italian Simmental; HO, Italian Holstein), at different days from calving (time point) during the observation period. Significant differences ( $P < 0.05$ ) are indicated at each time point as: a = CR vs. SI; b = CR vs. HO; c = SI vs. HO. Significant differences ( $P < 0.001$ ) are indicated at each time point as A = CR vs. SI; B = CR vs. HO; C = SI vs. HO.



**Fig. 2.** Patterns of variation of d-ROM (a), OSi (b) in the three groups of cows (CR, Italian Simmental × Italian Holstein; SI, Italian Simmental; HO, Italian Holstein), at different days from calving (time point) during the observation period. Significant differences ( $P < 0.05$ ) are indicated at each time point as: a = CR vs. SI; b = CR vs. HO; c = SI vs. HO. Significant differences ( $P < 0.001$ ) are indicated at each time point as A = CR vs. SI; B = CR vs. HO; C = SI vs. HO.

(−17%). Values close to zero were found for total protein (−2%), albumin (1%), BHB (0%) and IL-6 (−2%), (online Supplementary Table S5).

**Discussion**

The transition period (3 weeks before to 3 weeks after parturition) is notoriously a critical period for lactating cows as it is characterized by physiological and metabolic changes that affect immune

function and increase susceptibility to postpartum diseases (Goff and Horst, 1997). High-yielding dairy cows are subjected to many challenges during this period and, in addition, parturition leads to a systemic inflammatory state (Trevisi *et al.*, 2012; Bradford *et al.*, 2015). In the first third of the lactation period, high-producing cows enter a state of negative energy balance during which they mobilize body reserves and lose muscle body mass (Weber *et al.*, 2013). This phenomenon could be related to the milk production and to some genetic characteristics selected for

high milk yield in Holstein cows. As reported by Curone *et al.* (2018), Holstein Friesian cows showed a more severe fat mobilization and systemic inflammatory response postpartum in comparison with Rendena cows (a breed adapted to hard climatic and pasture conditions of the Italian alpine mountain range). Furthermore, Petrerá *et al.* (2015) showed different glucose, NEFA and BHB concentration values between Italian Friesian and Modenese cows (a minor Italian breed cows selected for milk yield in the area of Parmesan cheese) during the peripartum period. These studies indicate a different ability of cattle breeds to cope with metabolic stress around the calving period and suggest that the differences might depend on the different genetic merit for milk production.

Previous studies that compared the performance of Holstein purebred with its respective crossbred with Normande, Montbéliarde, Scandinavian Red or Jersey, demonstrated reduced somatic cell score, enhanced first-service conception rate, increased solids in milk and shorter calving interval in crossbreds (Heins and Hansen, 2012; Heins *et al.*, 2012; Coffey *et al.*, 2016). Moreover, Knob *et al.* (2016) highlighted better reproductive performance and higher survival rate in Holstein  $\times$  Simmental crossbred cows than in Holstein cows.

Few studies have investigated the metabolic profile in crossbred cows. Mendonça *et al.* (2014) found no differences in peripartum metabolic status between Holstein pure breed and Montbéliarde-sired crossbred. Our study has shown significant differences for energy profile between HO and CR cows. We found lower levels of NEFA and glucose (mainly at calving time) in CR and in SI when compared to HO cows. Different level of BHB were found between the two pure breeds in agreement with Lopreiato *et al.* (2019). Recently, Knob *et al.* (2021) compared the crossbred generations following the F1 in a two-breed rotational system with the parental dairy cattle breeds Holstein and Simmental. They showed that Simmental cows had lower NEFA values while BHBA and glucose did not differ among genetic groups. Generally, Simmental and R1-Simmental cows seemed to deal better with a negative energy balance after calving than purebred Holstein and the other crossbred lines.

In the weeks immediately after calving, the energy demand for synthesis and secretion of milk is not fully covered by feed intake, particularly in high-yielding dairy cows, thus resulting in a negative energy balance and mobilization of adipose depots (mainly sub-cutaneous). The development of a negative energy balance increases lipid mobilization as NEFA and the subsequent accumulation of BHB in the blood, as a result of the incomplete NEFA oxidation in the  $\beta$ -oxidation pathway (Wankhade *et al.*, 2017). In this context it is noteworthy to highlight that, although similar between SI and CR cows at d + 15 after calving, NEFA were much higher in HO cows, that showed average values near to the threshold of ketosis (around 1.2 mmol/l) for such a milk production (about 26–27 kg/d). Moreover, SI cows showed lower values of BHB leading to the hypothesis that the liver was able to pack NEFA into VLDL (very low-density lipoprotein) to a greater extent and to lower the level of BHB as a consequence of its higher oxidation (Radostits *et al.*, 2000). This mechanism could also explain the higher milk fat content in early lactation stage, in spite of a lower NEFA mobilization. This speculation could be further supported by the fact that there was no difference in energy requirements after calving, as indirectly shown by NEFA levels, between SI and CR cows, at least in the first 15 d of lactation. Indeed, BHB is the final biomarker of NEFA catabolism in liver and it is not directly linked to lipolysis of fat depots for

the energy requirements for milk production, while levels of NEFA, the first product of the lipolysis, are. In order to meet the energy demand for lactation, in the first period after parturition, production of glucose increases through the stimulation of hepatic gluconeogenesis but, despite this mechanism, the greater glucose demand after parturition for milk production leads to a decrease in plasma concentrations, particularly in high yielding cows (Weber *et al.*, 2013). In our study the trend of glucose reflected the data reported in literature (Trevisi *et al.*, 2012; Knob *et al.*, 2021) showing an increase just before calving and a decrease in the first weeks of lactation. Differently from Knob *et al.* (2021), who found glucose levels not significantly different among Holstein, Simmental and their crosses, in our study the CR group showed a lower average value. At the time of calving we found a particular increase of glucose in HO with respect to the other breeds. This finding could be related to the higher value of granulocyte, neutrophil and monocyte proportions at calving found in the HO compared to SI and CR in our previous work (Scatà *et al.*, 2020). Indeed, glucose is required by phagocytic cells for proliferation, survival and differentiation, and has been shown to be the preferred metabolic fuel during inflammation for activated PMN, macrophages and lymphocytes (Ingvarsen and Moyén, 2013). Furthermore, Holstein cows were shown to display an overt systemic inflammatory response related to the transition period, even without signs of microbial infections and/or otherwise determined pathology (Sordillo *et al.*, 2009; Trevisi *et al.*, 2012).

The elevated NEFA level in plasma as observed in HO cows has previously been correlated with risk factor for proinflammatory periparturient diseases such as mastitis and metritis (Bernabucci *et al.*, 2005; Trevisi *et al.*, 2012) and has been linked with early lactation diseases and altered immune competence (Erdmann *et al.*, 2018).

In our previous study involving the same groups of cows, we found that CR and SI showed a better ability to restore immune homeostasis after calving with respect to HO. This last group showed altered levels of cellular immunological traits at calving and in early lactation (Scatà *et al.*, 2020). Furthermore, in the same study, after the evaluation of heterosis for cellular immunological traits we identified an improved activation of humoral immune response and a better ability to restore immune homeostasis after calving in SI  $\times$  HO crossbred cows with respect to parental breeds. In the present study, the F1 derived population seems to follow the Simmental parental breed as shown by heterosis values for the glucose (–11%), NEFA (–20%) and BHB (5.5%). The heterosis related to these parameters could indicate a possible ameliorative effect in F1 group. Based on these results, CR and SI showed lower energy requirement (lower energy waste) and more efficient mobilization of body reserves and oxidation of fatty acids in the liver.

In agreement with Lopreiato *et al.* (2019) we observed higher values of creatinine and urea in SI as well as in CR compared with HO group. The higher urea production in SI and CR cows could be supported by mobilization of muscle protein confirmed by the higher levels of creatinine throughout the whole observation period. Creatinine is an important indicator of body muscle mass and, supporting observations by Pires *et al.* (2013) and Osorio *et al.* (2014), we found a decrease in concentration around and beyond parturition in all groups. In our experimental population SI and CR cows had higher BCS compared to HO cows, in agreement with Knob *et al.* (2016) who found a higher BCS in HO  $\times$  SI crossbred than HO and similarly to Mendonça *et al.* (2014) who

reported lower values in HO than in Montbéliarde-sired crossbred cows.

In contrast to a previous study of Lopreiato *et al.* (2019), all cows used in the present study had access to the same diet. We hypothesized that a greater flux of amino acids from muscle catabolism to the liver, which are in turn catabolised, may occur in SI or CR cows rather than in HO cows. This condition could also explain the better postpartum energy balance in SI and CR cows. However, a different efficiency in the rumen utilization of protein and energy between these two breeds and their crossbred cannot be excluded.

The oxidant capacity of plasma measured with the d-ROMs test was significantly lower in CR group compared to each parental breeds. Changes in d-ROMs in HO cows during the study were in agreement with previous reports that showed an increase in oxidant species after parturition (Bionaz *et al.*, 2007; Trevisi *et al.*, 2009; Abuelo *et al.*, 2013). Furthermore, the oxidative status was assessed using an arbitrary index (OSi) obtained from the ratio between d-ROMs and BAP. Recently, Invernizzi *et al.* (2019) observed significant differences in d-ROMs and OSi parameters across the periparturient period, meaning that they may be considered useful biomarkers for oxidative stress.

Like them, we found increased values of d-ROMs and OSi in HO group at calving. These results indicate that HO group underwent a greater oxidative stress condition from calving to early lactation compared to SI and CR groups and that the increased d-ROMs concentration is not, or not fully, justified by a higher milk production. Furthermore, the absence of differences within the groups in SAA, an acute phase proteins (APP) indirect marker of infections and/or inflammation (Ceciliani *et al.*, 2012), suggest that the differences in oxidative markers in HO cows could be linked more to stress related to calving than to subclinical conditions.

The evaluation of d-ROMs and OSi values and the results suggested a better balance between oxidants and antioxidants during the periparturient and early lactation periods for CR and SI and a better reaction to calving stress. Moreover, we found a high positive percentage of heterosis for BAP value (47%) and markedly negative values for OSi (−75%) and d-ROMs (−39%) in F1 derived population toward SI parental breed. In general, these findings shed light on the different metabolic adaptation during periparturient period and early lactation by the three genetic groups.

Haptoglobin, one of the APPs, has been used as a marker of systemic inflammation in transition dairy cows, as it is elevated immediately after parturition (Bionaz *et al.*, 2007; Huzzey *et al.*, 2009). Lopreiato *et al.* (2019) found higher concentration of Hp after parturition both in SI and HO cows. We observed higher mean average of Hp in SI compared to HO and CR cows with higher levels at calving. It remains unknown why SI has a higher Hp level than the other two groups. Perhaps this increment could be a specific response of SI cows to the applied managing and feeding conditions that were not tailored for that breed or due to the acute trauma of tissue at calving (Trevisi and Minuti, 2018). Moreover, we found a negative correlation of Hp and SAA with milk yield at 60 d of lactation. Since these two parameters are indicators of an inflammatory state, when their value is lower the cow shows a better health condition which is expressed with a higher production.

In conclusion, our data suggest that CR and SI cows are less sensitive to periparturient and early lactation stresses, although this conclusion needs to be validated in higher numbers of animals. These more resilient animals face more efficiently the negative energy metabolism and the inflammatory and oxidative status

giving a better adaptive response. Furthermore, this study showed that the use of crossbreeding programs focusing on Holstein cows bred with Simmental sire, does not significantly impair milk production and produces animals that are more resistant to early lactation stressors.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029921000650>

**Acknowledgments.** This research was funded by the Italian Ministry of Agriculture – MiPAAF – Directorate General for Rural Development (DISR IV) within the Research Project IMMA ‘Identification of immunological markers for disease resistance’- (Grant number: D.M. 16850/7303/2016). The authors gratefully acknowledge: Nicolò Pazzaglia, DVM and Mr. Carlo Cesare Riccioni for blood samples collection and for skillful technical assistance with animals.

## References

- Abuelo A, Hernández J, Benedito JL and Castillo C (2013) Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. *Animal* 7, 1374–1378.
- Bernabucci U, Ronchi B, Lacetera N and Nardone A (2005) Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *Journal of Dairy Science* 88, 2017–2026.
- Bionaz M, Trevisi E, Calamari L, Librandi F, Ferrari A and Bertoni G (2007) Plasma paraoxonase, inflammatory conditions, liver functionality and health problems in transition dairy cows. *Journal of Dairy Science* 90, 1740–1750.
- Bradford BJ, Yuan K, Farney JK, Mamedova LK and Carpenter AJ (2015) Invited review: inflammation during the transition to lactation: new adventures with an old flame. *Journal of Dairy Science* 98, 6631–6650.
- Ceciliani F, Ceron JJ, Eckersall PD and Sauerwein H (2012) Acute phase proteins in ruminants. *Journal of Proteomics* 75, 4207–4231.
- Celi P (2011) Oxidative stress in ruminants. In Mandelker L and Vajdovich P (eds), *Studies on Veterinary Medicine*, vol. 5. New York: Humana Press, pp. 191–231.
- Coffey EL, Horan B, Evans RD and Berry DP (2016) Milk production and fertility performance of Holstein, Friesian, and Jersey purebred cows and their respective crosses in seasonal calving commercial farms. *Journal of Dairy Science* 99, 5681–5689.
- Curone G, Filipe J, Cremonesi P, Trevisi E, Amadori M, Pollera C, Castiglioni B, Turin L, Tedde V, Vigo D, Moroni P, Minuti A, Bronzo V, Addis MF and Riva F (2018) What we have lost: mastitis resistance traits in Holstein Friesians and in a local cattle breed. *Research in Veterinary Science* 116, 88–98.
- Edmonson AJ, Lean IJ, Weaver LD, Farver T and Webster G (1989) A body condition scoring for Holstein dairy cows. *Journal of Dairy Science* 72, 68–78.
- Erdmann S, Mohr E, Derno M, Tuchscherer A, Schäff C, Börner S, Kautzsch U, Kuhla B, Hammon H and Röntgen M (2018) Indices of heart rate variability as potential early markers of metabolic stress and compromised regulatory capacity in dried-off high-yielding dairy cows. *Animal* 12, 1451–1461.
- Falconer DS (1960) *Introduction to Quantitative Genetics*. Edinburgh: Oliver and Boyd Ltd, pp. 1–140. ISBN 058244764X.
- Fiore F, Spissu N, Sechi S and Cocco R (2019) Evaluation of oxidative stress in dairy cows with left displacement of Abomasum. *Animals* 13, 966.
- Goff JP and Horst RL (1997) Physiological changes at parturition and their relationship to metabolic disorders. *Journal of Dairy Science* 80, 1260–1268.
- Hare E, Norman HD and Wright JR (2006) Survival rates and productive herd life of dairy cattle in the United States. *Journal of Dairy Science* 89, 3713–3720.
- Heins BJ and Hansen LB (2012) Short communication: fertility, somatic cell score, and production of Normande × Holstein, Montbéliarde × Holstein, and Scandinavian Red × Holstein crossbreds versus pure Holsteins during their first 5 lactations. *Journal of Dairy Science* 95, 918–924.

- Heins BJ, Hansen LB, Hazel AR, Seykora AJ, Johnson DG and Linn J (2012) Short communication: Jersey × Holstein crossbreds compared with pure Holsteins for body weight, body condition score, fertility, and survival during the first three lactations. *Journal of Dairy Science* **95**, 4130–4135.
- Huzzey JM, Duffield TF, LeBlanc SJ, Veira DM, Weary DM and von Keyserlingk MAG (2009) Short communication: haptoglobin as an early indicator of metritis. *Journal of Dairy Science* **92**, 621–625.
- Ingvartsen KL and Moyes K (2013) Nutrition, immune function and health of dairy cattle. *Animal* **7**(suppl. 1), 112–122.
- INRA (1988) *Alimentation des Bovins, Ovins et Caprins*. Paris: INRA Editions, p. 471.
- Invernizzi G, Koutsouli P, Savoini G, Mariani E, Rebucci R, Baldi A and Politis I (2019) Oxidative indices as metabolic stress predictors in periparturient dairy cows. *Italian Journal of Animal Science* **18**, 1356–1360.
- Knob DA, Alessio DRM, Thaler Neto A and Mozzaquatro FD (2016) Reproductive performance and survival of Holstein and Holstein × Simmental crossbred cows. *Tropical Animal Health and Production* **48**, 1409–1413.
- Knob DA, Scholz AM, Alessio DRM, Bergamaschi Mendes BP, Perazzoli L, Kappes R and Thaler Neto A (2020) Reproductive and productive performance, udder health, and conformation traits of purebred Holstein, F1, and R1 crossbred Holstein × Simmental cows. *Tropical Animal Health and Production* **52**, 1639–1647.
- Knob DA, Thaler Neto A, Schweizer H, Weigand AC, Kappes R and Scholz AM (2021) Energy balance indicators during the transition period and early lactation of purebred Holstein and Simmental cows and their crosses. *Animals* **11**, 309–328.
- Kramer CY (1956) Extension of multiple range tests to group means with unequal number of replications. *Biometrics* **12**, 307–310.
- Lopreato V, Minuti A, Trimboli F, Britti D, Morittu VM, Piccioli Cappelli F, Loor JJ and Trevisi E (2019) Immunometabolic status and productive performance differences between periparturient Simmental and Holstein dairy cows in response to pegbovigrastim. *Journal of Dairy Science* **102**, 9312–9327.
- Lopreato V, Minuti A, Morittu VM, Britti D, Piccioli Cappelli F, Loor JJ and Trevisi E (2020) Short communication: inflammation, migration, and cell-cell interaction-related gene network expression in leukocytes is enhanced in Simmental compared with Holstein dairy cows after calving. *Journal of Dairy Science* **103**, 1908–1913.
- McParland S, Kearney JF, Rath M and Berry DP (2007) Inbreeding effects on Milk production, calving performance, fertility, and conformation in Irish Holstein-Friesians. *Journal of Dairy Science* **90**, 4411–4419.
- Mendonça LGD, Abade CC, da Silva E M, Litherland NB, Hansen LB, Hansen WP and Chebel RC (2014) Comparison of peripartum metabolic status and postpartum health of Holstein and Montbéliarde-sired crossbred dairy cows. *Journal of Dairy Science* **97**, 805–818.
- Miller RH, Kuhn MT, Norman HD and Wright JR (2008) Death losses for lactation cows in herds enrolled in Dairy Herd Improvement test plans. *Journal of Dairy Science* **91**, 3710–3715.
- Norman HD, Wright JR, Hubbard SM, Miller RH and Hutchison JL (2009) Reproductive status of Holstein and Jersey cows in the United States. *Journal of Dairy Science* **92**, 3517–3528.
- Oltenucu PA and Broom DM (2010) The impact of genetic selection for increased milk yield on the welfare of dairy cows. *Animal Welfare* **19**, 39–49.
- Osorio J, Trevisi E, Ji P, Drackley J, Luchini D, Bertoni G and Loor J (2014) Biomarkers of inflammation, metabolism, and oxidative stress in blood, liver, and milk reveal a better immunometabolic status in periparturient cows supplemented with Smartamine M or MetaSmart. *Journal of Dairy Science* **97**, 7437–7450.
- Petrera F, Napolitano F, Dal Prà A and Abeni F (2015) Plasma parameters related to energy and lipid metabolism in periparturient Modenese and Italian Friesian cows. *Journal of Animal Physiology and Animal Nutrition* **99**, 962–973.
- Pires JA, Delavaud C, Faulconnier Y, Pomiès D and Chilliard Y (2013) Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient Holstein-Friesian cows. *Journal of Dairy Science* **96**, 6423–6439.
- Puppel K, Bogusz E, Golebiewski M, Nalecz-Tarwacka T, Kuczynska B, Slosarz J, Budzinski A, Solarczyk P, Kunowska-Slosarz M and Przysucha T (2018) Effect of dairy cow crossbreeding on selected performance traits and quality of milk in first generation crossbreds. *Journal of Food Science* **83**, 229–236.
- Radostits O, Gay C, Hinchcliff K and Constable PD (2000) *Fatty Liver in Cattle (Fat Cow Syndrome, Pregnancy Toxemia in Cattle)*. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*. Philadelphia: W.B. Saunders Company, pp. 1671–1678.
- Scatà MC, Grandoni F, Barile VL, Catillo G and De Matteis G (2020) Simmental × Holstein crossbred: comparison of immunological traits with parental breeds during peripartum and early lactation period. *Journal of Dairy Research* **6**, 1–5.
- Sordillo LM, Contreras GA and Aitken SL (2009) Metabolic factors affecting the inflammatory response of periparturient dairy cows. *Animal Health Research Reviews* **10**, 53–63.
- Sørensen AC, Madsen P, Sørensen MK and Berg P (2006) Udder health shows inbreeding depression in Danish Holsteins. *Journal of Dairy Science* **89**, 4077–4082.
- Sørensen MK, Norberg E, Pedersen J and Christensen LG (2008) Invited review: crossbreeding in dairy cattle: a Danish Perspective. *Journal of Dairy Science* **91**, 4116–4128.
- Steri R, Muioli B, Catillo G, Galli A and Buttazzoni L (2019) Genome-wide association study for longevity in the Holstein cattle population. *Animal* **13**, 1350–1357.
- Trevisi E and Minuti A (2018) Assessment of the innate response in the periparturient cow. *Research in Veterinary Science* **116**, 47–54.
- Trevisi E, Amadori M, Bakudila AM and Bertoni G (2009) Metabolic changes in dairy cows induced by oral, low-dose interferon-alpha treatment. *Journal of Animal Science* **87**, 3020–3029.
- Trevisi E, Amadori M, Cogrossi S, Razzuoli E and Bertoni E (2012) Metabolic stress and inflammatory response in high yielding, periparturient dairy cows. *Research in Veterinary Science* **93**, 695–704.
- Wankhede PR, Manimaran A, Kumaresan A, Jeyakumar S, Ramesha KP, Sejian V, Rajendran D and Varghese MR (2017) Metabolic and immunological changes in transition dairy cows: a review. *Veterinary World* **10**, 1367–1377.
- Weber C, Hametner C, Tuchscherer A, Losand B, Kanitz E, Otten W, Singh SP, Bruckmaier RM, Becker F, Kanitz W and Hammon HM (2013) Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. *Journal of Dairy Science* **96**, 165–180.