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Beef cows' performance and metabolic response to short nutritional challenges in different months of lactation

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ABSTRACT

Lactating cows can react to changes in nutrient availability with a range of behavioural and physiological mechanisms, which may differ among lactation stages. We investigated the effects of short feed restriction and refeeding periods on beef cows' performance and metabolic status in different months of lactation. For this, Parda de Montaña beef cows [$n = 31$; 626 ± 47.7 kg body weight (BW)] were subjected to short nutritional restriction and refeeding cycles, which were repeated in months 2, 3 and 4 of lactation. Each month, cows were consecutively fed a diet to meet 100% of their energy and protein requirements during a 4-day basal period, 55% during a 4-day restriction period, and again 100% during a 4-day refeeding period. The performance (energy balance, BW, milk yield and composition) and plasma metabolite concentrations (glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), urea and malondialdehyde) were measured daily. Most of the traits were significantly affected by the interaction between feeding period and lactation month. Feed restriction induced milk yield loss, decreased milk protein and increased milk urea contents to different extents. The plasma NEFA concentrations rose with restriction in months 2, 3 and 4 but BHB and urea concentrations increased only in month 4. Most of these metabolites lowered to basal values during refeeding. These results suggest that beef cows use different adaptation strategies to cope with nutritional challenges as lactation advances, body fat mobilisation predominates in early lactation and protein catabolism prevails at later stages.

1. Introduction

Beef cows managed in temperate grassland systems depend very much on forage availability and quality during the grazing season, and also in the winter when they are usually group-fed preserved forages. Under these conditions, they face a dynamically changing nutrient supply, which can be inadequate to meet their requirements during some key physiological periods (Mulliniks and Beard, 2019). Projected climate changes, including more frequent extreme weather events, will further affect the quantity and nutritive value of the feed available throughout the production cycle (Henry et al., 2018). To successfully cope with these challenges, effective strategies need to be developed at both the animal and farm levels (Blanc et al., 2006).

Lactating cows respond to limiting nutritional environments with the mobilisation of body tissues and a range of behavioural and physiological mechanisms that involve modifications in nutrient allocation towards the different metabolic functions, whose priority differs depending on lactation stage (Bjerre-Harpøth et al., 2012; Murrieta et al., 2010). In order to disentangle the mechanisms that determine this metabolic flexibility in response to environmental change, the nutritional perturbations involving both short- and long-term feed restriction-refeeding cycles have been widely studied in dairy cows (Abdelatty et al., 2017; Gross et al., 2011a; Pires et al., 2019). In beef cattle, several papers have assessed cows' performance and metabolic response to long-term underfeeding (Alvarez-Rodríguez et al., 2009; Fiems et al., 2015), but adaptation to short-term nutrient restrictions has only been recently considered (De La Torre et al., 2022; Orquera-Arguero et al., 2022). Animals' ability to respond to and recover after short-term disturbances, defined as resilience (Friggens et al., 2022), is key for their performance in variable environments.

In dairy cows, the adaptive response to underfeeding usually implies reduced milk yield, and milk composition may, or may not, be affected depending on the length and intensity of restriction, among other factors (Boutinaud et al., 2019; Kvidera et al., 2017; Leduc et al., 2021). In order

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to overcome the negative energy balance (EB), cows will mobilise their body reserves, including both fat and protein. The mobilisation of body fat releases non-esterified fatty acids (NEFA) into the blood stream, which can be oxidised in the liver into ketone bodies, such as β-hydroxybutyrate (BHB), as energy fuel (Bell, 1995). Complementary, NEFA can be esterified to triglycerides and accumulate in the liver, or taken up by the mammary gland, where they account for a significant fraction of milk fat synthesis. When the oxidative metabolism is altered, excessive reactive oxygen species (ROS) production leads to oxidative stress (Abuelo et al., 2015), for which malondialdehyde (MDA), a degradation product of lipid peroxidation, has been proposed as a biomarker (Castillo et al., 2006). The catabolism of the protein mainly from the skeletal muscle yields glucogenic amino acids, and affects plasma glucose and urea concentrations (Ingvartsen et al., 2003). In *ad libitum*-fed dairy cows, body protein catabolism starts in the transition period (from 3 weeks before calving) and extends up to 5 weeks after calving, while fat reserves are mobilised up to 12 weeks postpartum, when feed intake matches milk yield requirements and endocrine status limits mobilisation (Sadri et al., 2023). This period can be shorter in lower milk-yielding breeds (Jorge-Smeding et al., 2021). When faced with temporary nutrient restriction, lactation stage plays a key role in the physiological adaptive response because the priority and requirements of the mammary gland change as lactation evolves by modifying the allocation of nutrients to milk synthesis (Boutinaud et al., 2019; Gross and Bruckmaier, 2019). Furthermore when cows are refed, the post-challenge recovery rate can be faster in later lactation stages (Bjerre-Harpøth et al., 2012). This information is not available in beef cows, where the influence of lactation stage on nutrient allocation may differ from that of dairy cows due to their lower milk yield and different feeding management because they are rarely fed to appetite and are often placed in limited nutrient environments (Mulliniks and Beard, 2019).

The aim of this experiment was to determine lactating beef cows' response to short-term feed restriction and refeeding periods in three different months of lactation both on the productive and physiological levels. We hypothesised that cows would respond to nutritional perturbations by reducing their milk yield and modifying their lipid and protein metabolism differently as lactation progressed.

2. Material and methods

The Animal Ethics Committee of the research centre approved all the experimental procedures (protocol no CEEA-03-2018-01), which followed the EU Directive 2010/63 guidelines on the protection of animals used for experimental and other specific purposes.

2.1. Animal management, experimental and diet design

The experiment was conducted at CITA La Garcipollera Research Station in the Pyrenees mountain area (Spain, 42°37′ N, 0°30′ W, 945 m a.s.l.) using 31 lactating Parda de Montaña beef cows [body weight (BW) (mean ± SD): 626 ± 47.7 kg; body condition score (BCS): 2.8 ± 0.22 (0–5 scale); age: 7.5 ± 2.91 yr]. Cows were randomly allocated in pens (7 or 8 cows/pen, 10 × 20 m) equipped with individual feeders for forage and automatic feeding stations (ALPRO, Alfa Laval Agri, Tumba, Sweden) for concentrate. Calves were stocked in straw-bedded cubicles adjacent to their dams. They were allowed to suckle their dams daily for two 30-min periods at 06:00 h and 14:00 h. All the cows received the same ration, which was composed of different quantities of hay and concentrate. The chemical composition and nutritive value of feedstuffs are presented in Table 1 (for detailed information see Orquera-Arguero et al., 2022). Diets were calculated by considering the net energy and metabolisable protein requirements for the maintenance and lactation (INRA, 2007) of a standard cow with a BW of 615 kg and a milk yield of 8.5 kg/d. From calving to the end of the experiment all the cows were fed a diet that met 100% standard cow energy and protein requirements, except for 3 restriction periods when they were fed a diet to meet 55% standard cow energy and protein requirements. The experiment consisted of three consecutive 4-day feeding periods, which were repeated over months 2, 3 and 4. Every month, the trial started with 4 days on which cows had access to the abovementioned diet, which met 100% of their requirements (basal period). For the next 4 days, they were fed a diet that met 55% requirements (restriction period). On the last 4 days, once again they received the formulated diet to meet their 100% requirements (refeeding period). On the first day of restriction periods, cows were in milk for 31 (month 2), 58 (month 3), and 87 (month 4) days.

Table 1
Chemical composition and nutritive value (mean ± standard deviation) of the feedstuffs offered to the beef cows.

	Hay	Concentrate
Chemical composition		
Dry matter (DM), g/kg	920 ± 10.9	908 ± 6.7
Ash, g/kg DM	87.5 ± 17.3	68.3 ± 1.6
Crude protein, g/kg DM	97.1 ± 20.5	170 ± 4.7
Neutral detergent fibre, g/kg DM	581 ± 51.0	252 ± 19.2
Acid detergent fibre, g/kg DM	330 ± 27.3	112 ± 11.5
Lignin, g/kg DM	34.9 ± 9.30	29.3 ± 8.10
Nutritive Value		
Net energy, MJ/kg DM	5.4 ± 0.13	7.4 ± 0.36
Metabolizable protein, g PDI[1] /kg DM	73 ± 12.1	121 ± 2.9

[1] true protein digestible in the small intestine.

The diet fed to meet 100% energy and protein requirements was composed of 7.4 kg dry matter (DM) hay and 2.7 kg DM concentrate. During restriction, cows received 6.4 kg DM hay to meet 55% of their energy and protein requirements. Throughout the experiment, water and mineral blocks were supplied *ad libitum*. Hay was offered daily as a single meal at 08:00 h in individual feeders with cows tied up for approximately 2 h until they had finished their ration. The ALPRO feeding stations were programmed to offer concentrate to all the cows during the basal and refeeding periods. The individual hay and concentrate intakes were recorded daily.

2.2. Measurements and samplings

All the cow measurements were taken daily in the morning before hay-feeding, and during each feeding period (basal, restriction, refeeding) in experiment months 2, 3 and 4. Cows were weighed on an electronic scale. Milk yield was estimated by the weight-suckle-weight technique of the calf (Le Neindre and Dubroeucq, 1973) as the sum of the milk consumed in both sucklings. After the morning suckling, a composite 50-mL milk sample was manually collected per cow from all four teats, after discarding 3 streams of milk per teat. After calf removal, cows were administered an intramuscular injection of oxytocin (40 UI, Facilpart, Laboratorios Syva, León, Spain) 5 min before the manual extraction to facilitate the letdown of residual milk. Milk samples were preserved with sodium azide (PanReac, Barcelona, Spain) and refrigerated at 4 °C until further analyses. Cow blood samples were collected from the coccygeal vein in heparinised tubes (BD Vacutainer Becton-Dickenson and Company, Plymouth, UK) to determine BHB and MDA, and in tubes containing EDTA (BD Vacutainer Becton-Dickenson and Company) to analyse glucose, NEFA and urea concentrations. Immediately after collection, blood samples were centrifuged at 3500 rpm for 20 min at 4 °C, and plasma was frozen at −20 °C until further analyses.

2.3. Chemical analyses

In milk samples, lactose, fat, protein and urea contents, and somatic cell count, were determined with an infrared scan (Milkoscan 7 RM, Foss Electric Ltd., Hillerød, Denmark). Randox kits (Randox Laboratories Ltd., Country Antrim, UK) were employed to determine the plasma

concentrations of NEFA (colorimetric method, sensitivity: 0.072 mmol/L) and BHB (kinetic enzymatic method, sensitivity: 0.100 mmol/L). An automatic analyser (Gernon, RAL S.A., Barcelona, Spain) was used to measure the plasma concentrations of glucose (enzymatic-colorimetric method, sensitivity: 0.06 mmol/L) and urea (kinetic method, sensitivity: 0.056 mmol/L). The mean intra- and interassay coefficients were for NEFA: 4.0% and 4.9%, BHB: 6.8% and 6.8%; glucose: 2.2% and 2.4%; urea: 4.4% and 5.5%.

The plasma concentration of MDA, used as an indicator of oxidative status, was determined by liquid chromatography as described in Bertolín et al. (2019). An Acquity UPLC H-Class liquid chromatograph (Waters, Milford, Massachusetts, USA), equipped with a silica-based bonded phase column (Acquity UPLC HSS PFP, 100 mm × 2.1 mm × 1.8 μm, Waters), an absorbance detector (Acquity UPLC Photodiode Array PDA eλ detector, Waters) and a fluorescence detector (2475 Multi λ Fluorescence Detector, Waters), were utilised. The intra- and interassay coefficients of variation were 4.6% and 7.3% for MDA, respectively.

2.4. Calculations and statistical analyses

The INRA system (INRA, 2007) was used to estimate the individual EB as the difference between inputs (net energy (NE) intake) and outputs (NE for maintenance and NE for lactation). The NE intake was estimated from the individual DM intake (DMI) and feedstuffs' energy contents. The NE for maintenance was calculated from the individual metabolic BW, and the NE for production was obtained using the milk yield, fat, and protein contents in milk.

Statistical analyses were performed by the SAS statistical package v 9.4 (SAS Institute Inc., Cary, NC, USA) and the R software. Normal data distribution was assessed with the Shapiro-Wilk test ($P > 0.05$). Normality could not be confirmed for the somatic cell count values. Therefore, analyses were run on the log-transformed data. Parameters were analysed with mixed models by taking feeding period (basal, restriction, refeeding), lactation month (months 2, 3 and 4), and their interaction, as fixed effects, and cow as the random effect. Degrees of freedom were adjusted with the Kenward-Roger correction. The least square means and associated standard errors were obtained and multiple comparisons were adjusted with Tukey correction. The Pearson's correlations between variables were obtained and presented on heatmaps for all the data and separately per feeding period using the CORRPLOT package of R (R Development Core Team, 2021). The level of significance for all the tests was $P < 0.05$ and trends were discussed when $0.05 \leq P < 0.10$.

3. Results

The interaction between feeding period and lactation month affected all the parameters ($P < 0.05$ to $P < 0.001$), except milk yield, which only tended to be affected by this interaction ($P < 0.10$) and somatic cell count ($P > 0.05$). For each parameter, the basal values during the three lactation months, and then the effects of restriction and refeeding during the three lactation months are presented.

3.1. Cow performance

On average, 91%, 61% and 93% of the net energy requirements and 100%, 58% and 103% of the metabolisable protein requirements were met during the basal, restriction and refeeding periods, respectively. Cows' EB, BW, milk yield and milk composition are depicted in Fig. 1 according to feeding period and lactation month. The calculated basal EB improved progressively from month 2 to month 4 ($P < 0.01$). According to the experimental design, cows' EB was more negative during restriction than during the basal period in the three lactation months ($P < 0.001$). During refeeding, the EB returned to basal values in lactation months 2 and 3, but went even higher, close to a neutral EB, in lactation month 4 ($P < 0.001$). Basal BW decreased between months 2 and 4 ($P < 0.001$). BW diminished with restriction in the three lactation months (by −2.3%, −2.0% and − 1.7% in months 2, 3 and 4, respectively). During refeeding, BW lowered by a further 1% in month 2 ($P < 0.001$), but remained unchanged in months 3 and 4 ($P > 0.05$).

The basal milk yield was higher in months 2 and 3 than in month 4 ($P < 0.05$ to $P < 0.001$). Milk yield decreased with restriction in the three lactation months by −14%, −19% and − 20% in months 2, 3 and 4, respectively ($P < 0.001$). Milk yield increased during refeeding and reached the basal values in months 2 and 3, but stayed below the basal values in lactation month 4 (by −8%; $P = 0.03$). Regarding milk composition in the basal phase, lactose, fat and urea contents were not affected by lactation month ($P > 0.05$), whereas protein content was higher in month 2 than in the subsequent months ($P < 0.001$), and somatic cell counts were lower in month 2 than thereafter (99, 135 and 131 × 10^3 cells/mL in months 2, 3 and 4, respectively, $P < 0.05$).

Feed restriction did not affect milk lactose in month 2, but lowered in months 3 and 4 (by −1.9 and − 1.5%, respectively) and then increased during refeeding in the three lactation months ($P < 0.001$). Milk fat content was similar regardless of feeding periods ($P > 0.05$). Protein content lowered with restriction in months 2 and 3 (by −5% and − 4%, respectively; $P < 0.001$), but was not affected in month 4 ($P > 0.05$). It remained stable during refeeding in months 2 and 4, but increased to reach the basal values in month 3. Milk urea content increased during restriction in the three months by +8%, +21%, and + 37% in months 2, 3 and 4, respectively ($P < 0.05$), and decreased during refeeding, even below the basal values in month 2 and to the basal values in months 3 and 4 ($P < 0.001$). The highest somatic cell counts were obtained during refeeding (128, 159 and 186 × 10^3 cells/mL in the basal, restriction and refeeding period, respectively, $P < 0.05$).

3.2. Plasma metabolic profile

The plasma concentrations of NEFA, BHB, glucose, urea and MDA are presented in Fig. 2. Lactation month did not affect the basal concentrations of BHB and urea ($P > 0.05$), but affected those of NEFA, glucose and MDA ($P < 0.001$). The basal NEFA concentrations were higher in month 2 than in month 4 ($P < 0.001$). The basal glucose concentrations were lower in month 3 than in months 2 and 4 ($P \leq 0.001$). The basal MDA concentrations were higher in month 2 than in the subsequent months ($P < 0.001$).

Regarding the effect of feeding period, NEFA concentrations increased to different extents due to restriction in the three months (by +157%, +269% and + 212% in months 2, 3 and 4, respectively; $P < 0.001$), whereas refeeding lowered NEFA concentrations to below the basal value in month 2 ($P < 0.001$) and to basal values in months 3 and 4. The BHB concentration rose with restriction in the three months, but only significantly in month 4, by +14% ($P = 0.11$), +17% ($P = 0.11$) and + 23% ($P < 0.001$) in months 2, 3 and 4, respectively. During refeeding, BHB decreased and reached basal values in months 2 and 4. Glucose concentration dropped during restriction in month 2 ($P = 0.01$), with no changes thereafter ($P > 0.05$). During refeeding, it decreased in month 2 ($P < 0.001$), increased in month 3 ($P < 0.001$) and remained unchanged in month 4 ($P > 0.05$). The urea concentration rose significantly during restriction, but only in lactation month 4 (by +18%; $P < 0.001$), and lowered during refeeding below the basal values in months 2 and 4 ($P < 0.01$). The MDA concentration did not change with restriction and was only affected by refeeding in month 4, with higher values than during the basal period ($P = 0.03$).

The significant overall correlations with $r \geq 0.25$ between the performance parameters and plasma metabolites are shown in Fig. 3, whereas the correlations during each feeding period are depicted in Suppl. Fig. 1. The overall correlations were weak ($r = 0.25$ to 0.39) or moderate ($r = 0.40$ to 0.59), but were strong within feeding periods ($r = 0.60$ to 0.79) and very strong ($r \geq 0.80$) ($P < 0.001$). BW correlated positively with milk yield and negatively with the EB. Milk urea content

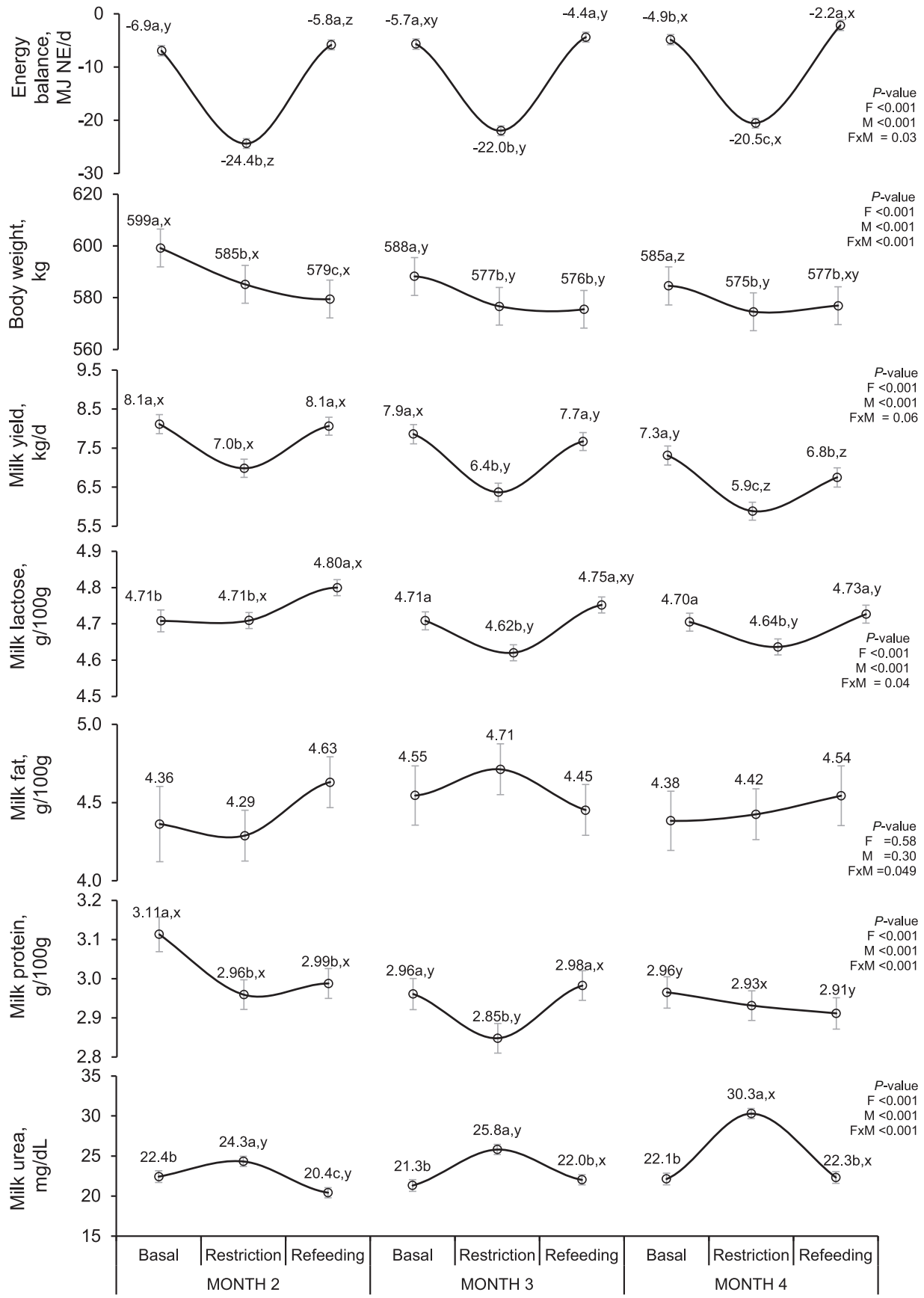

Fig. 1. Effect of feeding period (F: Basal, Restriction, Refeeding) and lactation month (M: 2, 3, 4) on energy balance (EB), BW, milk yield and milk composition. Within a parameter and month, the means with a different letter (a,b,c) indicate differences due to feeding period ($P < 0.05$). Within a parameter and feeding period, the means with a different letter (x,y,z) denote differences due to lactation month ($P < 0.05$).

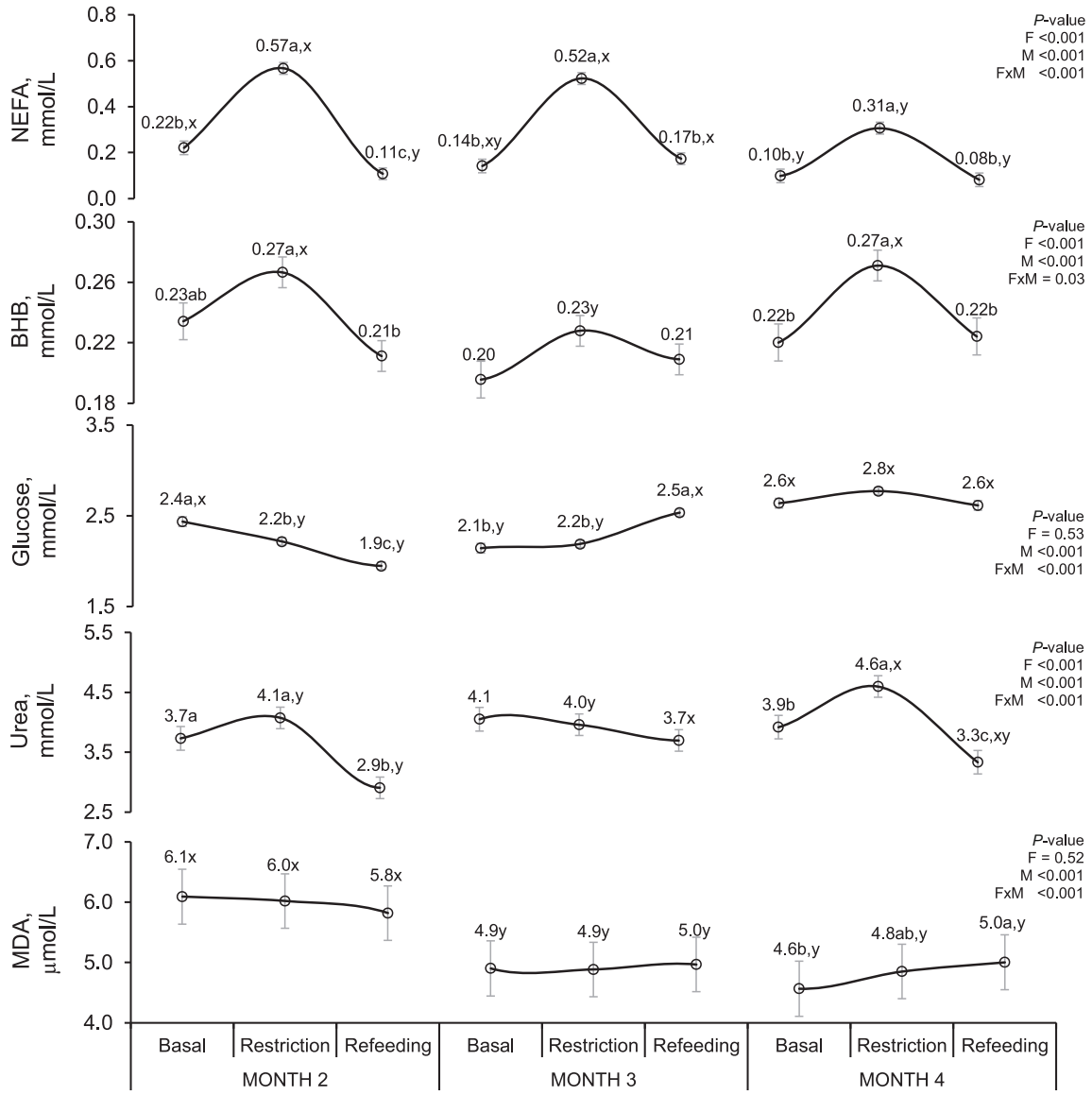

Fig. 2. Effect of feeding period (F: Basal, Restriction, Refeeding) and lactation month (M: 2, 3, 4) on plasma concentrations of non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), glucose, urea and malondialdehyde (MDA).
Within a parameter and month, the means with a different letter (a,b,c) indicate differences due to feeding period ($P < 0.05$). Within a parameter and feeding period, the means with a different letters (x,y,z) denote differences due to lactation month ($P < 0.05$).

correlated negatively with milk yield and the EB ($P < 0.001$). Within the basal, restriction and refeeding periods, correlations were moderate between BW and the EB ($r = -0.52$ to -0.64), and were very strong between milk yield and the EB ($r = -0.87$ to -0.93). The plasma NEFA concentration correlated negatively with the EB and milk protein content, and positively with milk urea content ($P < 0.001$). The BHB concentration correlated negatively with the EB and positively with milk urea and the plasma concentrations of glucose, urea and MDA ($P < 0.001$). The plasma urea concentration correlated negatively with the EB and positively with milk urea content and plasma glucose concentration ($P < 0.001$). Within feeding periods, the plasma urea concentration correlated positively with BW, milk yield and milk protein during the basal period and with milk protein during the refeeding period ($P < 0.001$). The plasma MDA concentration correlated positively with BW, milk yield, milk protein content and plasma urea concentration, and negatively with the EB ($P < 0.001$).

4. Discussion

In the present experiment, restriction implied reductions of −36% in DMI, −42% in net energy intake and − 47% in protein intake on average. The restriction herein applied could be considered moderate according to the review by Leduc et al. (2021) because the reduction in DMI was <50%. Basal cow performance and some plasma metabolites differed among the three lactation months, as did their patterns of response to restriction and refeeding. This scenario suggests a change in the metabolic priority of different biological functions as lactation advanced.

4.1. Cow performance

BW loss between months 2 and 4 agrees with previous experiments with lactating Parda de Montaña cows (Blanco et al., 2009). Beef cows are rarely fed according to their theoretical requirements (Blanc et al., 2006). During lactation, they have to rely on the mobilisation of their body reserves to produce milk. In the present experiment, BW was only

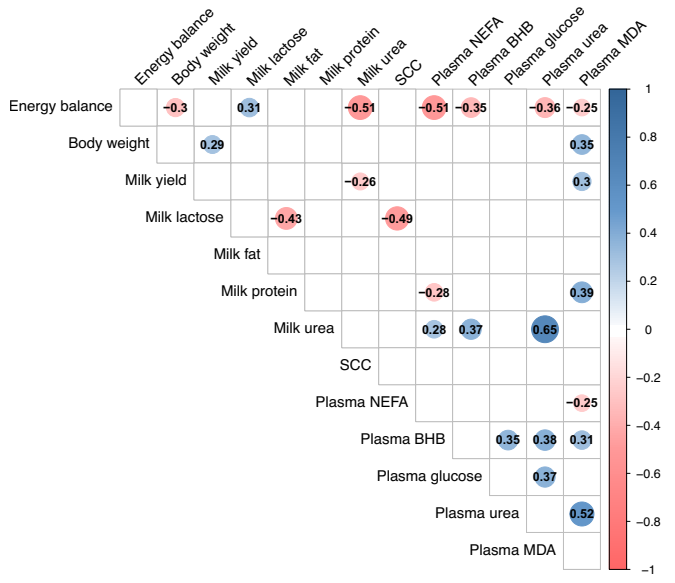

Fig. 3. Significant Pearson's rank correlations[1] between cow performance and plasma metabolites in all the lactation months.
[1]Only the significant correlations ($P < 0.05$) are presented and the correlations between equal variables are omitted. SCC: somatic cell count; NEFA: non-esterified fatty acids; BHB: β-hydroxybutyrate; MDA: malondialdehyde.

mildly affected by a short feed restriction, similarly to the −4 to −5% BW loss reported after a 4-day 50% DMI restriction in beef cows (De La Torre et al., 2022) and dairy cows (Ferraretto et al., 2014; Kvidera et al., 2017). This BW loss could be linked with the decrease in DMI, gut fill loss and mobilisation of body reserves (Gross et al., 2011a; Laeger et al., 2012). Incomplete BW recovery in the 4-day refeeding phase implies that a longer recovery period is needed; *e.g.* 10 days in beef cows after a similar restriction to that herein applied (De La Torre et al., 2022) or at least 1 to 2 weeks in dairy cows with severer restrictions that cause greater BW loss (−10%; Billa et al., 2020; Pires et al., 2019).

The lower basal milk yield values with progressing lactation agree with previous data on Parda de Montaña cows (Casasús et al., 2004; Dervishi et al., 2017), and suggest that the peak milk yield had already been reached at the start of the experiment, in month 2, as described by Sapkota et al. (2020) for beef cows. In the present study, the reduced milk yield caused by feed restriction falls in line with those reported by other studies of comparable lengths and restriction severities in beef cows (−12%; De La Torre et al., 2022) and dairy cows (−13 to −20% in Abdelatty et al., 2017; Laeger et al., 2012; Nielsen et al., 2003). The milk loss magnitude was lower in month 2, when cows displayed the most negative EB, than thereafter. Several homeorhetic mechanisms involved in nutrient partitioning regulation concur to maintain milk yield during feed restriction periods or metabolic imbalance, *e.g.* decreased glucose use, increased body lipids use and the mobilisation of protein reserves as energy sources (Bauman and Currie, 1980; Ingvartsen et al., 2003). However, these regulation processes are stage-dependent and the adaptive response diminishes with advancing lactation (Blanc et al., 2006). Our results indicate that the metabolic priority of the mammary gland in feed-restricted beef cows decreased after month 2. This would be supported by the shift in nutrient partitioning away from the udder towards subcutaneous adipose tissue, as observed on 60 d postpartum in beef cows by Murrieta et al. (2010). The milk yield response to refeeding was fast, with full recovery occurring within 4 days in months 2 and 3, but not in month 4. The lower milk synthesis priority in this later stage may increase the necessary recovery time. A quick response to refeeding has also been reported in low-producing beef cows (2 days for full recovery; De La Torre et al., 2022), but more days are required for full recovery with high-producing dairy cows in early lactation (7 to 8 days; Bjerre-Harpøth et al., 2012; Pires et al., 2019).

Concerning milk composition, the basal milk protein and lactose contents were similar, but fat content was higher than those previously reported in Parda de Montaña cows with a similar milk yield (Casasús et al., 2004; Dervishi et al., 2017). This difference was probably related to the sampling method. In this study, milk samples were manually obtained after calves had suckled (alveolar milk). In the above-mentioned studies, they were collected by machine milking before calves had access to their dam (cisternal milk). The fat concentration in cisternal milk is lower than in alveolar milk, whereas milk protein content is minimally affected (Sarikaya et al., 2005). The basal milk composition was similar in the three months, except for the higher protein content in early lactation. In dairy cows, lactose regulates milk osmolality and generally remains constant throughout lactation, while milk fat and protein tend to decrease from peak lactation in response to improved nutritional status and lower milk yield (Gross and Bruckmaier, 2019). All this was confirmed in our experiment for lactose and protein, but not for fat. This was probably due to the smaller differences in the EB and milk yield among months here than those observed in high-producing dairy cows. Furthermore, the stable basal milk urea throughout lactation agrees with the results reported in beef cows in the first three months of lactation (Wiseman et al., 2019) and in early-, mid- and late-lactating dairy cows (Bjerre-Harpøth et al., 2012).

Milk composition was affected by nutritional perturbation to different extents. Lactose content lowered with restriction and increased during refeeding, which agrees with previous reports in dairy cows that only needed 2 days to recover basal values after restriction had ended (Bjerre-Harpøth et al., 2012; Hervé et al., 2019; Sigl et al., 2013). The negative correlation herein observed between lactose content and somatic cell count has been associated with inflammatory reactions in milk secretory cells (Cinar et al., 2015). However in our study, somatic cell count was always below the threshold for subclinical mastitis (200×10^3 cells/mL; Dervishi et al., 2017).

Milk fat originates from either dietary or mobilisation fatty acids, which are taken up from the bloodstream, or by *de novo* synthesis in the mammary gland (Chilliard et al., 2000). Here milk fat content was not affected by feed restriction, which is consistent with previous results in dairy cows restricted at 50–60% during 4–5 days with 10–22% milk yield loss (Abdelatty et al., 2017; Carlson et al., 2006; Gross et al., 2011a). Other experiments with 30–50% milk loss report increases in milk fat content during feed restriction (Agenäs et al., 2003; Bjerre-Harpøth et al., 2012), which are associated with an increment in the long-chain fatty acids that arise from body fat mobilisation (Gross et al., 2011b). Apparently fat mobilisation and the concurrent rise in circulating NEFA would not have been enough to increase milk fat content in our study, but could have made the proportion of long *vs.* short- and medium-chain fatty acids higher, as observed by Orquera-Arguero et al. (2023).

Milk protein may decrease with feed restriction, but changes in milk urea depend on the nature of restriction (Leduc et al., 2021) given the influence by feed intake, but also by urea transfer from blood to milk, and *vice versa* (Spek et al., 2016). Here we observed reductions in milk protein (in months 2 and 3) and increments in milk urea contents in response to simultaneous reduction in dietary energy and protein supply. These findings agree with other experiments with 50% nutritional restriction, *e.g.* -7% milk protein and + 21% milk urea content in Carlson et al. (2006), −5.6% milk protein in Gross et al. (2011a). The higher milk urea content during restriction, especially in month 4, and its negative correlation with the EB suggests that protein catabolism took place in this phase to compensate for reduced energy intake, and this adaptation mechanism was more intense in later lactation stages. Body protein mobilisation to obtain glucose as an energy substrate increases circulating urea, which can be diffused from the blood stream to mammary glands (Spek et al., 2016). When restriction ended, basal values were regained after four refeeding days in most cases, except for milk protein in month 2. This suggests quicker recovery than that observed in high-producing dairy cows (Billa et al., 2020; Bjerre-

Harpøth et al., 2012; Pires et al., 2019).

4.2. Plasma metabolic profile

Plasma metabolites have commonly been used as indicators of energy, protein and oxidative status (Castillo et al., 2006; van Knegsel et al., 2007). The basal values herein observed were similar to those reported in lactating Parda de Montaña cows fed their 100% requirements in the case of NEFA, BHB and urea (Alvarez-Rodríguez et al., 2009), but were lower than those of glucose (Rodríguez-Sánchez et al., 2018). The fact that basal NEFA decreased from month 2 to month 4 indicates that the lipid mobilisation needed to support the energy demand for milk yield decreased throughout lactation, as shown in dairy cattle (Gross et al., 2011a; Jorge-Smeding et al., 2021). Basal BHB remained stable, as noted by Rodríguez-Sánchez et al. (2018) in beef cows, but were unlike the results of Bruckmaier and Gross (2017) in Holstein cows, where BHB peaked between 2 and 3 weeks postpartum and decreased thereafter, which suggest more metabolic stress for dairy cows in early lactation.

Feed restriction in months 2 and 3 increased the plasma NEFA concentrations to >2-fold their basal values, which came close to the compromised metabolic status threshold in dairy cows (0.57–0.60 mmol/L; Ospina et al., 2010), but induced a milder response in month 4. This supports the high priority of nutrient partitioning towards the mammary gland in response to reduced energy supply in earlier lactation stages, when body fat is largely mobilised and NEFA are released to provide energy for milk synthesis. Orquera-Arguero et al. (2022) observed wide variability in this response among beef cows, with more marked increments in cows' BW and milk yield. Plasma BHB responded to reduced nutrient intake to a much lesser degree (+15 to 20%), and only significantly so in month 4, and remained far below the risk threshold for subclinical ketosis (>1.2 mmol/L) (Benedet et al., 2019). The greater increments in NEFA than in BHB concentrations in response to reduced feed supply agree with previous studies with similar restrictions in dairy cows (Kvidera et al., 2017; Moyes et al., 2009; Pires et al., 2019), but they did not even change in Charolais cows with lower milk yield BHB (De La Torre et al., 2022). Both metabolites reacted quickly to refeeding, and basal values had recovered within 4 days, which agrees with other studies in beef (De La Torre et al., 2022) and dairy cattle (Abdelatty et al., 2017; Gross et al., 2011a), regardless of lactation stage (Billa et al., 2020; Bjerre-Harpøth et al., 2012).

The response of plasma glucose to diet changes was not consistent across lactation stages in the present study because it only decreased with restriction in month 2. The stronger effect on early lactation has been ascribed by Bjerre-Harpøth et al. (2012) to greater physiological imbalance, and could be driven by higher mammary glucose uptake for lactose synthesis (Gross et al., 2011a). In beef cows, no relevant changes were observed when feed was reduced at 54 or 75 days from calving (De La Torre et al., 2022). The literature reports conflicting results on the effect of moderate feed restrictions on glycaemia, which may decrease or remain stable, and has been considered a poor indicator of energy status in cows because gluconeogenesis can balance its concentration (Leduc et al., 2021).

Plasma urea is influenced by a wide variety of interrelated factors, such as dietary protein intake and muscle tissue breakdown when energy supply is insufficient (Puppel and Kuczyńska, 2016). Protein mobilisation from skeletal muscle releases glucogenic amino acids, which are used to supply glucose (Ingvartsen et al., 2003) and to generate urea during the process (Agenäs et al., 2006). The concentrations herein noted fell within the range reported for adequately nourished cows (1.8 to 7 mmol/L; Agenäs et al., 2006), and basal values remained stable throughout lactation, as observed by Bjerre-Harpøth et al. (2012) in early-, mid- and late-lactating cows. The lack of effect of feed restriction in months 2 and 3 agrees with previous reports in beef (De La Torre et al., 2015) or dairy cows (Hervé et al., 2019; Laeger et al., 2012), although other authors have found reduced blood urea in feed-restricted cows (Kvidera et al., 2017). The fact that restriction elicited a rise in the plasma urea concentration in month 4, when protein intake did not differ from previous months, implies that a certain degree of protein catabolism took place during restriction. This resulted in stable glycaemia in this month, as observed by Fiems et al. (2007) in energy-restricted beef cows. Apparently in late lactation, cows rely less on the mobilisation of fat reserves and more on the mobilisation of lean mass as a strategy to cope with a short-term nutritional challenge.

The metabolic adaptation to a negative EB can intensify the NEFA oxidation processes in the liver, and can result in both increased ROS production and oxidative stress developing (Turk et al., 2008), which occur with an imbalance between ROS production and antioxidant availability (van Knegsel et al., 2014). The values obtained in the present experiment are far below the concentrations reported by Castillo et al. (2006) for Holstein cows, which lowered from 69 to 29 μmol/L in the 8 first weeks of lactation. In our case, the higher MDA concentrations in early lactation (month 2) than thereafter, as observed by Castillo et al. (2006) in dairy cows, are likely the consequence of the higher plasma NEFA concentrations available for oxidation (Abuelo et al., 2015; Shi et al., 2015), with which they correlated.

5. Conclusions

Short-term restriction-refeeding periods resulted in both productive and metabolic adaptations in lactating beef cows. The most relevant responses to feed restriction were a drop in milk yield and an increase in the plasma NEFA concentrations, although their magnitude of change decreased as lactation advanced. In early postpartum, the mobilisation of fat reserves partially buffered the impact of a moderate feed restriction on milk yield. In later stages, when priority for milk production decreased, body protein reserves were also mobilised and longer recovery times were needed to compensate for a less effective response. Our results show that beef cows use different metabolic strategies to face nutritional perturbations depending on lactation stage.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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