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Modelling beef cows' individual response to short nutrient restriction in different lactation stages

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ABSTRACT

Short-term nutrient restrictions can occur naturally in extensive beef cattle production systems due to low feed quality or availability. The aims of the study were to (1) model the curves of milk yield, plasma non-esterified fatty acids (**NEFAs**) and β-hydroxybutyrate (**BHB**) contents of beef cows in response to short nutritional challenges throughout lactation; (2) identify clusters of cows with different response profiles; (3) quantify differences in cows' response between the clusters and lactation stages. Data of BW, body condition score (**BCS**), milk yield, NEFA, and BHB plasma concentration from 31 adult beef cows (626 ± 48 kg at calving) were used to study the effect of 4-day feed restriction repeated over months 2, 3 and 4 of lactation. On each month, all cows received a single diet calculated to meet the requirements of the average cow: 100 % requirements for 4 days (d-4 to d-1, basal period), 55 % requirements on the next 4 days (d0 to d3, restriction period) and 100 % requirements for 4 days (d4 to d7, refeeding period). Natural cubic splines were used to model the response of milk yield, NEFA and BHB to restriction and refeeding in the 3 months. The new response variables [baseline value, peak value, days to peak and to regain baseline, and areas under the curve (**AUC**) during restriction and refeeding] were used to cluster cows according to their metabolic response (**MR**) into two groups: Low MR and High MR. The month of lactation affected all the traits, and basal values decreased as lactation advanced. Cows from both clusters had similar BW and BCS values, but those in the High MR cluster had higher basal milk yield, NEFA and BHB contents, and responded more intensely to restriction, with more marked peaks and AUCs. Reaction times were similar, and baseline values recovered during refeeding in both clusters. Our results suggest that the response was driven by cows' milk potential rather than size or body reserves, and despite high-responding cattle's higher milk yield, they were able to activate metabolic pathways to respond to and recover from the challenge.

Implications

Modelling beef cows' short-term response to feed restriction and refeeding for 3 months of lactation allowed us to identify-two groups of cows with different magnitudes for their responses (milk loss and fat mobilisation). Their coping strategies changed as lactation advanced. Identifying cows which, even with a high milk yield, show a better response is potentially interesting for future breeding programmes.

Introduction

Beef cattle managed under extensive conditions depend on the local availability of feed resources, which vary throughout the year in quality and quantity terms. This results in seasonal mobilisation patterns and the replenishment of body reserves, which might limit animal performance in critical physiological stages (Noya et al., 2019). The fact that cows face perturbations prevents them from fully expressing their production potential, with wide variability in individual coping strategies. In temperate climates, beef herds are housed in the winter (Blanco et al., 2008), and management is often simplified by group-feeding cows with a single diet irrespectively of their individual requirements. In these circumstances, animals' ability to cope with a nutritional challenge is particularly relevant.

This individual variability has been addressed in cows by testing different models to quantify the gap between the potential and disturbed performance that natural or induced perturbations cause (Codrea et al., 2011; Bjerre-Harpøth et al., 2012; Adriaens et al., 2021; De La Torre et al., 2022) as an indicator of not only animals' resilience but also their capacity to be minimally affected by

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perturbations and to rapidly return to the previous state (Berghof et al., 2019). When disturbances happen during lactation, complex homeostatic and homeorhetic mechanisms concur to maintain a physiological equilibrium while redirecting nutrient partitioning towards milk production (Bauman and Currie, 1980). In dairy cows, the major source of milk yield variation in animals lies in their ability to partition nutrients towards the mammary gland (Baumgard et al., 2017). This process is mediated by the somatotropic axis, with increased growth hormone and decreased insulin production in higher-yielding cows, which promotes glucose-sparing mechanisms and the mobilisation of body reserves in peripheral tissue (Knight et al., 2004). Pareek et al. (2007) found differences in this endocrine regulation of nutrient partitioning between dairy and beef breeds in relation to their different milk secretion and body mass accretion potentials.

To ensure adequate nutrient supply for milk production, lipolysis releases non-esterified fatty acids (**NEFAs**) from adipose tissue, which can be oxidised in the liver into ketone bodies like β-hydroxybutyrate (**BHB**) (Bell, 1995). Both metabolites have been proposed to assess the degree and effects of a negative energy balance (**EB**) in ruminants (Kessel et al., 2008; Gross et al., 2011), whereas BW and body condition score (**BCS**) changes are poor indicators in dairy cattle (Pedernera et al., 2008). With feed restriction, negative EB is associated with decreased milk yield and higher NEFA and BHB concentrations, and the magnitude of these effects depends on the lactation stage, and also on restriction severity and duration (Leduc et al., 2021).

The joint analyses of milk yield dynamics and other traits are useful for analysing the drivers of their concomitant changes (Ben Abdelkrim et al., 2021b). Multitrait clustering in different lactation phases has been used to identify distinct strategies to cope with metabolic challenges (Friggens et al., 2016; De Koster et al., 2019). In the long term, this has provided data to characterise dairy cows according to their ability to prioritise nutrient use among different life functions (Ollion et al., 2016), but this approach has not been used in beef cows. Therefore, the objectives of this study were to (1) model beef cows' response of milk yield and plasma NEFA and BHB concentrations to short feed restriction and refeeding in three lactation stages; (2) cluster cows according to their metabolic response (MR); (3) determine differences between groups of cows and lactation stages. We hypothesised that beef cows would respond differently to restriction depending on their potential milk yield, and eventually on their size and fat reserves, and different coping strategies would be elicited as lactation advanced.

Material and methods

Experimental design

This experiment was conducted at the CITA La Garcipollera Research Station (Spain, 42°37′N, 0°30′W, 945 m a.s.l.). It involved 31 Parda de Montaña lactating beef cows [626 ± 47.7 kg BW, 2.8 ± 0.22 BCS and 7.5 ± 2.91 years at calving]. Cow-calf pairs were loose-housed in straw-bedded pens (7 or 8 cows/pen, 10 × 20 m) equipped with individual feeders for forage and ALPRO automatic concentrate feeding stations (Alfa Laval Agri, Tumba, Sweden). Calves were penned in cubicles and allowed to suckle twice daily for 30 min at 0600 h and 1400 h. The study consisted of three feeding periods repeated over the second, third and fourth lactation months. During each lactation month, cows received a diet that was calculated to meet 100 % of their requirements for 4 days (d-4 to d-1, basal period), then, they were restricted for 4 days (d0 to d3, restriction period) with a diet that met only 55 % of their requirements and were returned to the 100 % energy diet for 4 days (d4 to d7, refeeding period). On the first day (d0) of the restriction period, cows were in milk for 31, 58 and 87 (±5.5) days (**DIM**; months 2, 3, and 4 of lactation, respectively) (Fig. 1).

Cows were fed a flat-rate regime during lactation. Diets were calculated by considering the net energy and metabolisable protein requirements for the maintenance and lactation of a standard cow (BW 615 kg, milk yield 8.5 kg/d) using INRA equations (INRA, 2007). During the basal and refeeding periods, all the cows received 8.0 kg of hay (as a fed basis) daily, and only 7.0 kg of hay during the restriction period, offered daily at 0800 h as a single meal in individual feeders. Cows were tied up for approximately 2 h until they finished their ration. The ALPRO feeding stations were programmed to offer 3.0 kg (as fed)/day of concentrate to all the cows during the basal and refeeding periods. The individual intake was recorded daily. Animals had free access to water and mineral blocks.

Measurements, sampling and chemical analyses

Samples of the offered feedstuffs were collected daily to determine their chemical composition and nutritive value (Table 1). All the analyses of feedstuffs were run in duplicate. Official methods were used to determine the contents of DM, ash and CP (Nitrogen analyser, Model NA 2100, CE Instruments, Thermoquest SA, Barcelona, Spain) (Association of Official Analytical Chemists (AOAC), 2000). The methods of Van Soest et al. (1991) were followed to obtain the contents of NDF, ADF and ADL using a fibre analyser (model Ankom 200/220, Ankom, Macedon, NY, USA). In the forage samples, NDF was assayed with heat-stable amylase. Lignin was analysed on ADF residue by the solubilisation of cellulose with sulphuric acid. All the values were corrected for ash-free content. The feed values were calculated from the measured chemical composition of diets using INRA equations (INRA, 2007).

In the 3 months of lactation, during the basal period (d-4 and d-2), the BCS was assessed on a scale from 0 to 5 based on the estimation of fat covering loin, ribs and tailhead. Milk yield was estimated (d-4, d-2 and daily from d1 to d7) by the weigh-suckle-weigh technique (Le Neindre and Dubroeucq, 1973), calculated using the milk consumed by the calf during both daily sucklings. Cows were weighed and bled on the same days at 0700 h, after suckling and before the hay was offered. Blood samples were collected from the coccygeal vein using test tubes with EDTA and heparin (BD Vacutainer, BD, Plymouth, UK) for the NEFA analysis and the BHB analysis, respectively. They were immediately centrifuged (3 500 rpm for 20 min at 4 °C). Plasma was collected and frozen at −20 °C until further analyses. Randox kits (Randox Laboratories ltd, Crumlin, UK) were used to determine the BHB plasma concentration (kinetic enzymatic method, sensitivity: 0.100 mmol/L) and the NEFA concentration (colorimetric method, sensitivity: 0.072 mmol/L). The mean intra- and interassay CVs were 6.8 % and 6.8 % for BHB and 4.0 % and 4.9 % for NEFA, respectively.

Calculations and statistical analysis

The statistical analysis involved three steps:

Step 1: Modelling the individual response. The curve predicted for each trait (milk yield, NEFA, BHB) on the day of the experiment was modelled using natural cubic splines. A natural cubic spline with K knots is represented by K basis functions. Each basis function is a third-degree polynomial specified in the Hermite form. Compared to other splines, a natural cubic confers additional constraints; i.e. function is linear beyond boundary knots. This frees up four degrees of freedom, which can be spent more profitably by sprinkling more knots in the interior region (Perperoglou et al., 2019). Each parameter that defines the natural cubic spline basis with eight knots was estimated for each cow within each month using

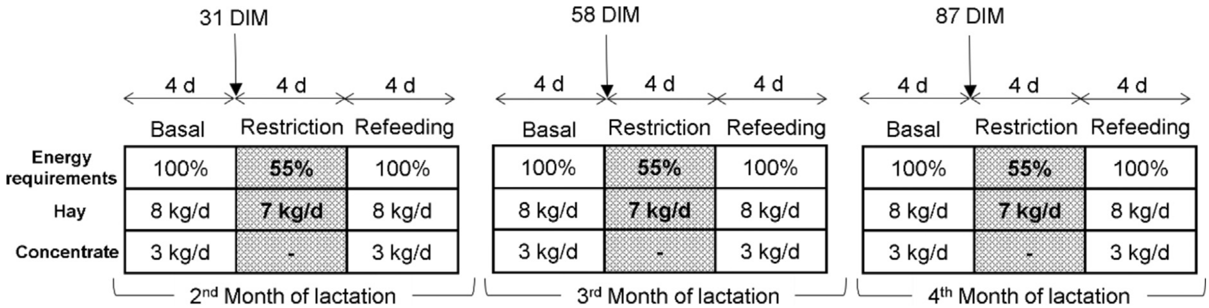

Fig. 1. Schematic representation of the timeline of three short nutritional challenges of the beef cows throughout lactation. DIM: days in milk.

Table 1
Chemical composition and nutritive value (mean ± SD) of the feedstuffs received by the beef cows during each month of lactation.

Item	Month 2	Month 3	Month 4
Hay			
Chemical composition			
DM, g/kg	919 ± 12.1	922 ± 11.7	918 ± 10.5
Ash, g/kg DM	98 ± 12.7	86 ± 24.4	78 ± 3.9
CP, g/kg DM	97 ± 25.7	109 ± 18.3	85 ± 8.1
NDF, g/kg DM	558 ± 59.2	570 ± 52.4	614 ± 21.2
ADF, g/kg DM	334 ± 33.5	324 ± 32.9	333 ± 15.9
Lignin, g/kg DM	41 ± 4.0	35 ± 12.8	28 ± 4.1
Nutritive Value			
Net energy, MJ/kg DM	5.4 ± 0.54	5.5 ± 0.54	5.4 ± 0.54
Metabolisable protein, g PDI/kg DM	81 ± 17.9	79 ± 12.8	59 ± 5.7
Concentrate			
Chemical composition			
DM, g/kg	907 ± 2.4	906 ± 4.0	911 ± 11.1
Ash, g/kg DM	68 ± 1.3	68 ± 1.4	69 ± 2.1
CP, g/kg DM	173 ± 3.5	167 ± 4.7	169 ± 4.2
NDF, g/kg DM	246 ± 17.4	256 ± 23.2	254 ± 18.2
ADF, g/kg DM	102 ± 4.5	114 ± 11.1	120 ± 10.5
Lignin, g/kg DM	25 ± 7.5	29 ± 8.8	33 ± 6.6
Nutritive Value			
Net energy, MJ/kg DM	7.5 ± 0.34	7.3 ± 0.34	7.5 ± 0.34
Metabolisable protein, g PDI/kg DM	123 ± 2.4	119 ± 3.3	120 ± 3.0

a non-linear mixed model with the random effect of the cow. The basal level of each cow within a month was also modelled with a mixed model, which included only the intercept, the linear random regression coefficients and the data from the basal and refeeding periods. Splines were obtained using command ns in the library splines of R (R Development Core Team, 2014). Mixed models were solved using command nlme in library lme4 of R.

The new response variables obtained from the fitted curve for milk yield and plasma metabolites (NEFA and BHB) are depicted in Fig. 2a and 2b, respectively. These response variables were 1) baseline: estimated values without feed restriction according to a linear interpolation from the basal to the refeeding period; 2) peak: the maximum difference between the actual daily value and the baseline value. For milk yield, the peak was the maximum daily milk loss, whereas it was the maximum daily increment compared to baseline values for NEFA and BHB; 3) days to peak: days from the start of restriction until the peak values were reached; 4) area under the curve (**AUC**) during restriction: the estimated total milk loss or the extra NEFA or BHB contents during restriction compared to the baseline values; 5) days to regain baseline: days from the start of restriction until the milk yield, and the NEFA or BHB contents reached the baseline again. 6) AUC during refeeding: the estimated total milk loss or extra NEFA or BHB contents during refeeding until the baseline values were regained.

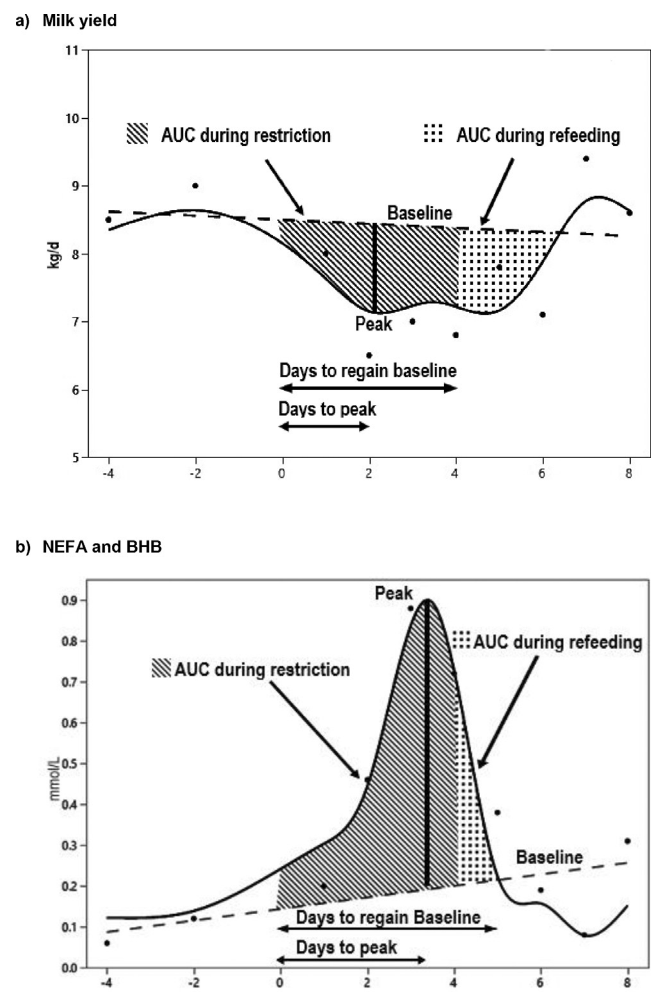

Fig. 2. Schematic representation of the piecewise model for describing the variables of the milk yield (2a) and non-esterified fatty acids and β-hydroxybutyrate (2b) beef cows' response curves to a 4-day restriction and a 4-d refeeding period. AUC: area under the curve; NEFAs: non-esterified fatty acids; BHB: β-hydroxybutyrate.

Step 2: Multivariate analysis. The new response variables obtained in step 1 for each trait, individual cow and month were employed to perform a multivariate analysis using the Factor Mine statistical package of the R software. First of all, a principal component analysis (**PCA** function) was used to identify the variables which accounted for most of the variability in the response among individuals. Then, hierarchical clustering on these principal components (**HCPC** function) was carried out to group the cows with a similar response pattern. The optimum number of clusters was calculated automatically by the algorithm.

Step 3: Effect of cluster and lactation stage on performance and MR. The phenotypic values and the new response variables during the three lactation months were studied according to the clusters obtained in the previous step using the SAS statistical package v 9.4 (SAS Institute Inc., Cary, NC, USA). Mixed linear models (MIXED procedure) were employed after taking cluster, month, and their interaction as fixed effects, and cow as the random effect. The least square means and associated SE were obtained, and multiple comparisons were adjusted with Tukey correction. The Pearson correlations (r) between the response variables were obtained following the CORR procedure. The results were considered significant when $P < 0.05$, and trends were discussed when $0.05 \leq P < 0.10$.

Results

The first three principal components obtained in the PCA accounted for 48 % of the total variance. The first one (Dim 1, 25.6 % of variance) was positively associated with the peaks and AUCs of NEFA, and negatively with the peaks and AUCs of milk yield during restriction (Fig. 3a). The second principal component (Dim 2, 12.5 % of variance) was associated positively with the AUCs of NEFA during both restriction and refeeding, and negatively with peaks and AUCs of milk yield and BHB during restriction. Finally, the third principal component was associated positively with the peak and AUC of milk yield in months 2 and 4, and with days to regain the baseline values of all the traits in month 2, and negatively with peak and AUC of BHB in month 4 (data not shown). The clustering analysis generated two clusters which differed in their MR, named Low MR (n = 16) and High MR (n = 15) (Fig. 3b). The cows in the Low MR cluster had lower energy requirements and a less negative EB and showed a poorer response to restriction in terms of milk yield and plasma NEFA and BHB concentrations. The cows in the High MR cluster showed a stronger response (Fig. 4).

Considering individual DM intake, on average diets met 91 %, 61 % and 93 % of the net energy requirements and 100 %, 58 % and 103 % of the metabolisable protein requirements during the basal, restriction and refeeding periods, respectively. Cow BW and BCS during the basal period did not differ between MR clusters (591 vs 590 kg in the Low MR and the High MR, respectively, $P = 0.91$; 2.80 vs 2.70 BCS points, respectively, $P = 0.18$). Both traits were affected by lactation stage and were higher in month 2 than thereafter (599, 588 and 584 kg in months 2, 3 and 4, respectively, $P < 0.001$; 2.81, 2.73 and 2.71, respectively, $P < 0.001$). The milk yield response to feed restriction and subsequent refeeding according to the MR cluster and the month of lactation is shown in Table 2. The MR cluster affected the baseline values and the response to restriction ($P \leq 0.04$), but not the recovery pattern in the refeeding phase. The High MR cows had a higher baseline milk yield and AUC values during restriction and tended to have greater peak milk loss. The month of lactation affected all the response variables during restriction ($P \leq 0.02$), but not during refeeding. A lower baseline yield was observed in month 4, and peak loss was greater in month 3 than in month 4, with intermediate values in month 2. The peak was reached more quickly, and total milk loss (AUC during restriction) was greater in month 3, with similar values in months 2 and 4.

The response of the plasma NEFA and BHB concentrations is shown in Table 3. For NEFA, the MR cluster affected the baseline values, peak and AUC during restriction ($P \leq 0.001$), with higher values obtained by the High MR cows. No differences were observed in the days to peak or to regain the baseline. All the NEFA response variables were affected by the month of lactation ($P \leq 0.04$). The baseline values were lower in month 4 compared

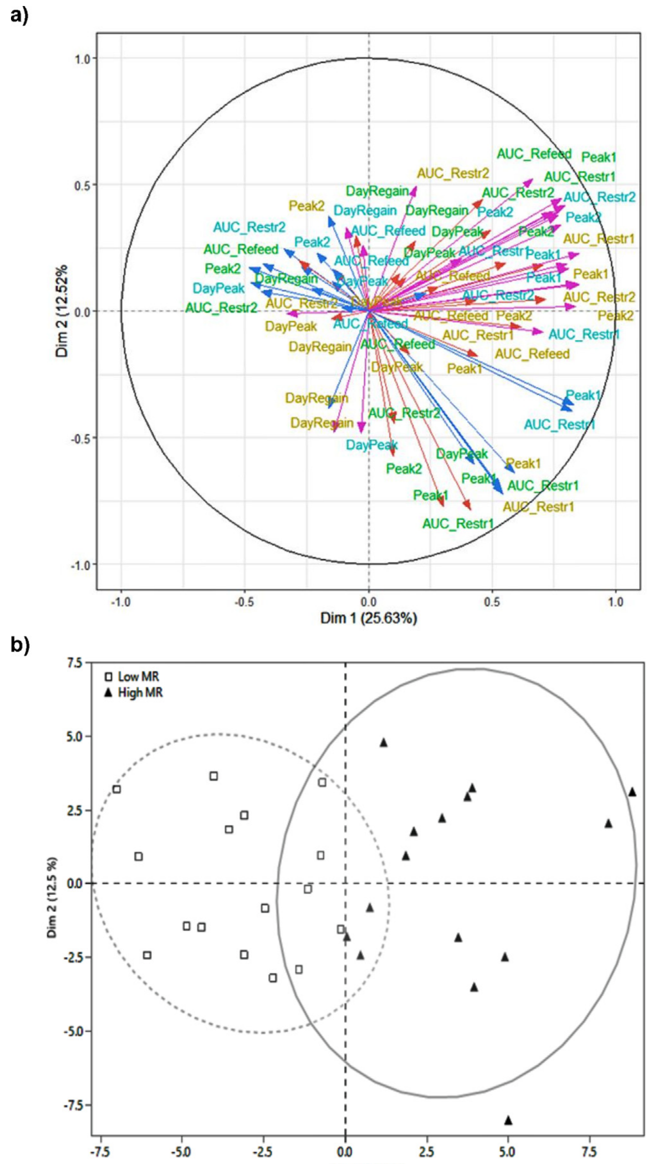

Fig. 3. Variable factor map of the first two Principal Components (3a). Variables related to milk yield (blue arrows), plasma non-esterified fatty acids (pink arrows) and plasma β-hydroxybutyrate (red arrows), and months of lactation 2, 3, and 4 for yellow, green, and blue text labels, respectively. Distribution of the cows into the two generated metabolic response (MR) clusters (3b).

to the other two months. Peak concentrations during restriction decreased significantly from lactation month 2 to lactation month 4 and were reached more quickly in month 4 than in the others. The days to regain baseline were also affected by month, with faster recovery in months 2 and 4 than in month 3. Only the AUC during refeeding was affected by the interaction between the MR cluster and the month of lactation (Fig. 5a). Regarding the BHB response, the baseline values and the AUC during restriction were higher in the High MR than in the Low MR cluster ($P \leq 0.02$). The month of lactation affected both parameters and the AUC during refeeding, which were lower in month 3 ($P \leq 0.03$), and tended to affect the days to regain the baseline ($P = 0.06$). Finally, the peak was affected by the interaction between the MR cluster and the month of lactation ($P = 0.03$), and the differences between the MR clusters were only significant in month 2, but not thereafter. Furthermore, the peak BHB in the Low MR cows remained stable

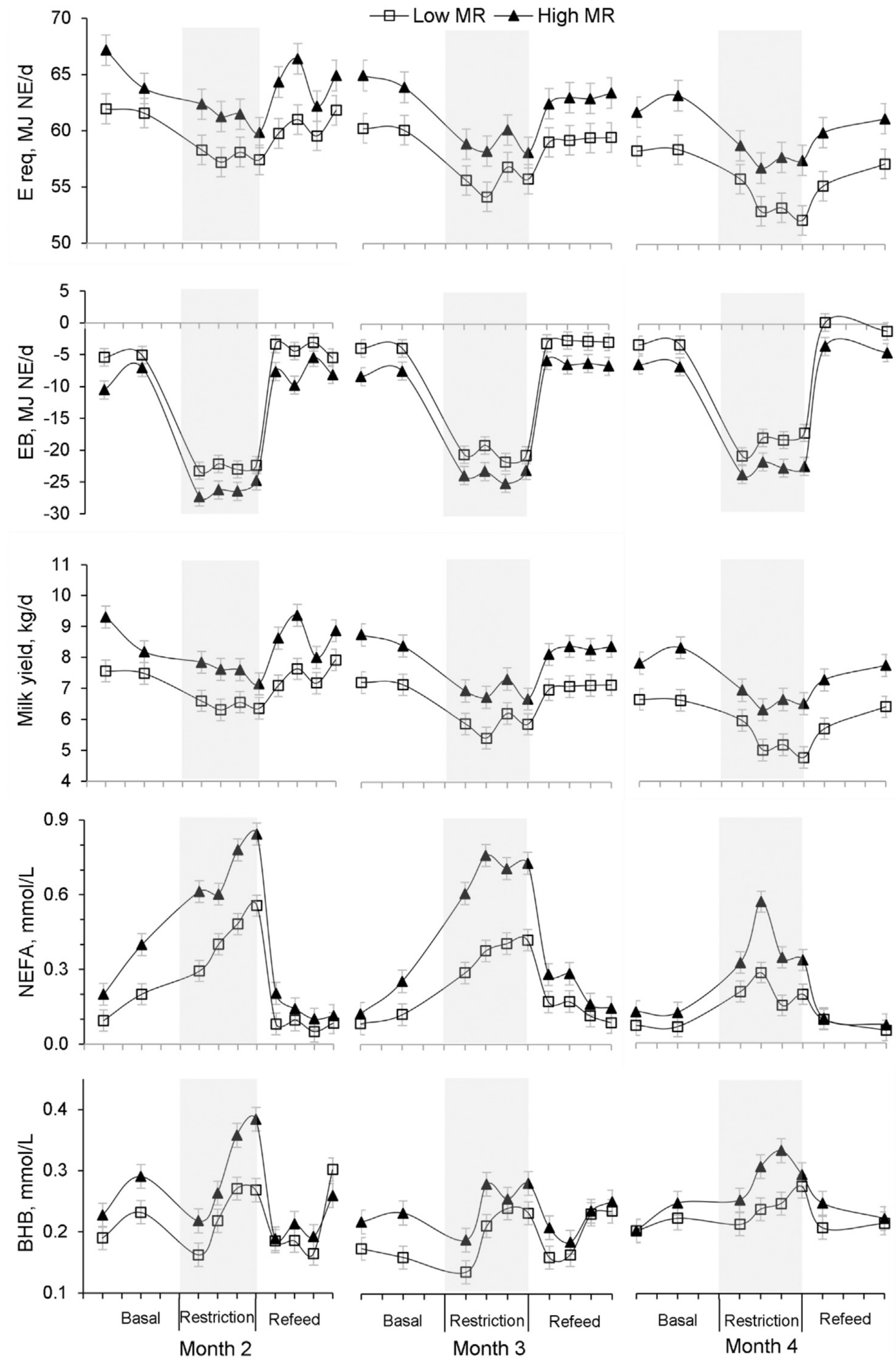

Fig. 4. Energy requirements (E req), energy balance (EB), milk yield, and plasma non-esterified fatty acid (NEFA) and β-hydroxybutyrate (BHB) concentrations of Low and High metabolic response (MR) beef cows during the experiment. Means are plotted, and the vertical bars indicate the SE.

throughout lactation, whereas the values in their High MR counterparts were higher in month 2 than later (Fig. 5b).

The significant correlations among the response variables of the milk yield, NEFA and BHB concentrations, all months considered, are shown in Fig. 6. Within trait, the AUC during restriction correlated strongly with the peak ($P < 0.001$), but not with the days to peak. For milk yield, the baseline values correlated negatively with the peak and AUC during restriction ($P < 0.001$). Milk loss (AUC) during refeeding correlated positively with the peak and AUC during restriction, but negatively with days to peak and to regain baseline ($P < 0.001$). For NEFA, the baseline values correlated positively with the peak and AUCs during restriction and refeeding ($P < 0.001$). The AUC during refeeding correlated strongly with the peak and AUC during restriction, and only moderately with days to peak and regain the baseline ($P < 0.001$). Regarding BHB, the AUC during refeeding correlated positively with the peak and AUC during restriction ($P < 0.001$). In the three traits, the correlations between days to peak and days to regain baseline were not significant. Across traits, the milk yield baseline values correlated moderately with the NEFA peak and AUC during restriction and the BHB baseline values, and weakly with the NEFA baseline values and the BHB peak ($P \leq 0.03$). The NEFA peak correlated weakly

Table 2
Effect of metabolic response (MR) cluster and month of lactation on the milk yield response of beef cows to a 4-day restriction and a 4-day refeeding period.

	MR Cluster (Cl)		Month (M)				*P*-values[1]	
Item	Low MR	High MR	2	3	4	RSD	Cl	M
Baseline, kg/d	6.94^{y}	8.27^{x}	8.10^{a}	7.80^{a}	6.92^{b}	0.584	0.002	0.001
Peak*, kg/d	−1.32	−1.56	−1.45^{ab}	−1.61^{b}	−1.27^{a}	0.463	0.068	0.020
Days to peak, d	2.57	2.63	2.80^{a}	1.78^{b}	3.22^{a}	0.990	0.813	0.001
$AUC^{\dagger}_{restriction}$*, kg	−3.80^{y}	−4.81^{x}	−4.01^{a}	−5.21^{b}	−3.70^{a}	1.656	0.036	0.002
Days to regain baseline, d	5.93	5.74	5.65	5.98	5.87	0.935	0.326	0.376
$AUC^{\dagger}_{refeeding}$*, kg	−0.83	−0.74	−0.68	−0.82	−0.86	0.798	0.644	0.647

Within a variable, least square means with different superscripts ($^{x, y}$) differ between MR clusters with $P < 0.05$; least square means with different superscripts ($^{a, b}$) differ among months with $P < 0.05$.
† area under the curve; *deviation from baseline.
[1] the interaction was not significant for any variable ($P > 0.05$).

Table 3
Effect of metabolic response (MR) cluster and month of lactation on plasma non-esterified fatty acid (NEFA) and β-hydroxybutyrate (BHB) response of beef cows to a 4-day restriction and a 4-day refeeding period.

	MR Cluster (Cl)		Month (M)				*P*-values[1]	
Item	Low MR	High MR	2	3	4	RSD	Cl	M
NEFA								
Baseline, mmol/l	0.09^{y}	0.15^{x}	0.13^{a}	0.15^{a}	0.08^{b}	0.049	0.001	0.001
Peak*, mmol/l	0.26^{y}	0.51^{x}	0.54^{a}	0.38^{b}	0.24^{c}	0.129	0.001	0.001
Days to peak, d	2.94	3.05	3.38^{a}	3.09^{a}	2.51^{b}	0.583	0.453	0.001
$AUC^{\dagger}_{restriction}$*, mmol × d/l	0.68^{y}	1.42^{x}	1.36^{a}	1.17^{a}	0.62^{b}	0.396	0.001	0.001
Days to regain baseline, d	5.74	5.74	5.55^{b}	6.08^{a}	5.59^{b}	0.869	0.991	0.036
$AUC^{\dagger}_{refeeding}$*, mmol × d/l	0.13^{y}	0.21^{x}	0.24^{a}	0.23^{a}	0.04^{b}	0.094	0.001	0.001
BHB								
Baseline, mmol/l	0.220^{y}	0.248^{x}	0.238^{ab}	0.222^{b}	0.243^{a}	0.031	0.024	0.026
Peak, mmol/l	0.07^{y}	0.11^{x}	0.12^{a}	0.07^{b}	0.08^{b}	0.068	0.002	0.003
Days to peak, d	3.20	3.11	3.29	3.08	3.09	0.815	0.574	0.540
$AUC^{\dagger}_{restriction}$*, mmol × d/l	0.04^{y}	0.13^{x}	0.10^{a}	0.02^{b}	0.13^{a}	0.135	0.011	0.006
Days to regain baseline, d	5.30	5.21	4.91	5.29	5.56	1.064	0.662	0.062
$AUC^{\dagger}_{refeeding}$*, mmol × d/l	−0.003	0.01	0.01^{a}	−0.02^{b}	0.02^{a}	0.045	0.175	0.001

Within a variable, least square means with different superscripts ($^{x, y}$) differ between MR clusters with $P < 0.05$; least square means with different superscripts ($^{a, b, c}$) differ among months with $P < 0.05$.
† area under the curve; *deviation from baseline.
[1] the interaction was significant for NEFA $AUC_{refeeding}$ ($P = 0.01$) and BHB Peak ($P = 0.03$).

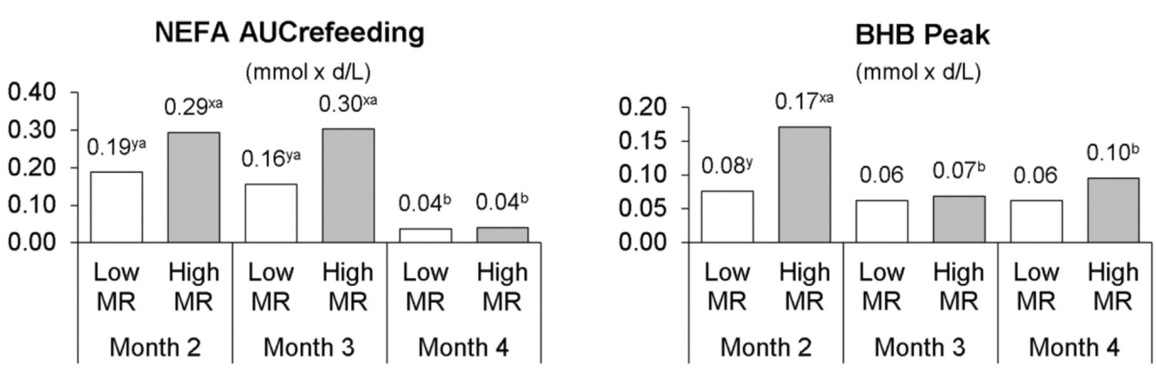

Fig. 5. Effect of the metabolic rate (MR) cluster and month of lactation on non-esterified fatty acids (NEFAs) $AUC_{refeeding}$ (5a) and β-hydroxybutyrate (BHB) peak (5b) in beef cows in response to a 4-d restriction and a 4-d refeeding period. For each response variable, means with different superscripts ($^{x, y}$) differ between MR clusters within month ($P < 0.05$) and with different superscripts ($^{a, b}$) differ among months within MR clusters with ($P < 0.05$). $AUCr_{efeeding}$: area under the curve during the refeeding period.

with the BHB peak and negatively with the milk yield peak ($P \leq 0.03$), but the milk yield and BHB peaks did not correlate. The AUCs of milk yield and NEFA during restriction were negatively correlated ($P \leq 0.003$), but not with those of BHB.

Discussion

Response curves

Different mathematical models have been used to characterise milk yield in dairy cows, from traditional models describing the shape of the lactation curve to individually adjusted polynomial curves based on well-established statistical models (Harder et al., 2019). Fewer modelling studies have been conducted on beef cattle because it is not routinely measured in common practice (Cortés-Lacruz et al., 2017; Sepchat et al., 2017; Sapkota et al., 2020). Animal performance can be affected by perturbations caused by climate, management or diseases, which can compromise both animal nutrition and welfare. Several studies have evaluated the response of ruminant females to natural (Poppe et al., 2020; Adriaens et al., 2021) or induced (Codrea et al., 2011; Friggens et al., 2016; Barreto-Mendes et al., 2022) perturbations, and found wide interindividual variations. They have analysed deviations

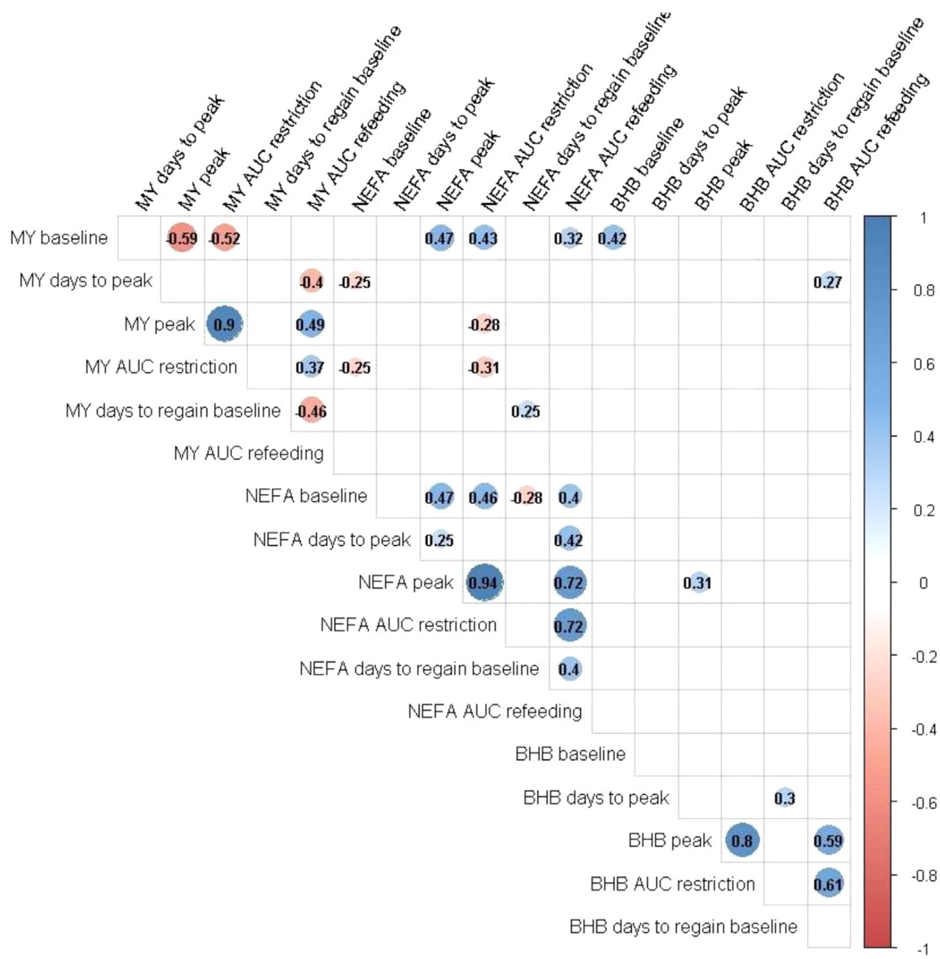

Fig. 6. Significant Pearson correlations between the response variables of milk yield (MY) and the plasma non-esterified fatty acid (NEFA) and β-hydroxybutyrate (BHB) concentrations in beef cows. AUC: area under the curve.

from a theoretical unperturbed lactation curve (Ben Abdelkrim et al., 2021a), which corresponds to the baseline in our study, and they have described the response while conducting challenges and in the recovery phase. Although most studies have modelled milk yield, this methodology could be extrapolated to other biological time-series data (Codrea et al., 2011), which are increasingly available with the rise of in-line measurement technologies. Ben Abdelkrim et al. (2021b) used a similar model to simultaneously predict the dynamics of milk yield and BW response over time, and to explore the relation between them, as we do herein with plasma NEFA and BHB.

Effect of the metabolic response cluster

The clustering analysis identified two distinct groups of cows that differed mainly in terms of their milk yield and NEFA response, and less markedly in their BHB response to nutritional challenges. Both BW and the BCS were similar in the two clusters throughout lactation, which implies that body size or fat reserves did not affect the response, which would be driven mainly by milk yield and the concomitant metabolic effort to sustain it. These findings are similar to those reported by Pedernera et al. (2008), who found that BW and BCS changes did not accurately reflect the extent of mobilisation of dairy cows' body reserves in early lactation. Schuh et al. (2019) reported that the BCS affected reserve mobilisation intensity, with higher NEFA and BHB serum concentrations in the cows with a high BCS, but this was not the case in our study. Breed or parity (Adriaens et al., 2021; Ben Abdelkrim et al., 2021a) can also influence individual responses to perturbations, but they did not differ between the MR clusters.

The size of the response was related to basal performance. All the basal values were higher in the High MR than in the Low MR profile, which coincides with Friggens et al. (2016). At the individual level, significant correlations were observed between the basal values and the response during restriction (peaks and AUCs) for milk yield and NEFA, but not for BHB. Berghof et al. (2019) have also indicated that high-performing animals can be more sensitive to perturbations. Interestingly, these differences were only observed in the magnitude of the response, but not in the time taken to react and recover, which reflects the plasticity of cows' response.

The impact of feed restriction on milk yield can widely range (from −7% to −71 %) depending on restriction severity and duration, and also on the lactation stage (Leduc et al., 2021). Here, the absolute milk loss was higher in the High MR than in the Low MR cows, but peak milk loss in relative terms was 19 % of the basal milk yield for both groups. When comparing Holstein and Montbéliarde cows, with different prechallenge milk yields, Billa et al. (2020) also observed a similar relative response to a 6-day 50 %-feed restriction between them. The MR cluster did not affect the time taken to reach the peak here (mean 2.6 days) or to regain the baseline (5.8 days), which implies that responses were larger, but not faster, in the High MR than in the Low MR cows. Both reaction times were shorter than those observed in natural (Adriaens et al., 2021) or induced (Bjerre-Harpøth et al., 2012) perturbations in dairy cows, which is likely due to the lower milk yield and the associated metabolic load of beef cows.

Homeorhetic controls regulate different metabolic adaptations to support lactation. Of them, growth hormone and insulin are key mediating factors responsible for the partition of nutrients away from body storage and towards the mammary gland (Knight et al., 2004; Baumgard et al., 2017). Although the hormones involved in this partitioning were not herein investigated, we observed significant effects of feed restriction on the plasma metabolites that result from their action, which were more evident in NEFA than in BHB. With poor nutrient supply, cows mobilise adipose tissue by releasing circulating NEFA so they are either converted into milk triglycerides in the udder or oxidised in the liver as an energetic substrate (Bell, 1995). All the NEFA response variables had almost doubled in the High MR than in the Low MR cluster, which denotes that the cows with higher milk yields had greater basal fat mobilisation and were able to further increase lipolysis during the nutritional challenge. Excessive lipid mobilisation can surpass the liver's metabolic capacity to oxidise NEFA, and ketone bodies such as BHB are produced (Mann et al., 2016). Thus, the High MR cows also had a higher BHB peak and AUC during restriction than the Low MR cows. Threshold values of 0.60 mmol NEFA/l (Jorjong et al., 2014) and 1.2 mmol BHB/L (Li et al., 2012) are associated with the risk of clinical ketosis in dairy cows. Regarding NEFA, they were reached only by the High MR cows during the peaks of months 2 and 3, but not by the Low MR cows, and never for BHB, which suggests that circulating NEFA supplied enough energy to meet the metabolic demands induced by nutrient restriction.

The response profiles observed herein suggest that the High MR cows had a higher potential milk yield and were able to efficiently partition more nutrients towards milk synthesis than the Low MR cows. Elgersma et al. (2018) considered that dairy cows with fewer milk yield fluctuations under natural perturbations were more resilient because the minor variance in performance genetically correlated with better health and longevity. Conversely, we can conclude that the High MR cows were able to establish homeorhetic mechanisms in the short term (Bauman and Currie, 1980) with sufficient intensity to ensure that, despite their more negative EB, they continued to display better lactation performance and recovered after the challenge. Ollion et al. (2016) have described different profiles in dairy cows depending on their lactation performance, reproduction and ability to maintain their reserves, the most determinant life functions among which trade-offs have often been identified. They found that milk yield was an important driver of these profiles, as we observed in the present work, but not the only one given the wide individual variability in the strategies to prioritise nutrient allocation to these life functions.

Effect of the lactation stage

Previous studies have analysed the adaptations of lactating ruminants to feed restriction in different phases. Within-animal responses are repeatable between early- and mid-lactation in dairy cows (Gross and Bruckmaier, 2015), between consecutive lactations in dairy goats (Friggens et al., 2016) and between two consecutive feeding challenges of different duration in beef cattle (De la Torre et al., 2022), which indicate that variability may be genetically driven. Here, we clustered cows according to their response throughout lactation and analysed the month of lactation separately, finding a strong effect on most response variables. The general lack of interactions between MR cluster and month confirmed the validity of our approach.

To the best of our knowledge, no comparable studies are available on beef cows in different lactation stages. As stated above, the lactation curves of beef breeds are less well-known than those of dairy cattle. Sepchat et al. (2017) have described slow increases in milk production after calving, which peaked between the first and third lactation months. The curve was flatter than in dairy cows due to the balance between a calf's ability to drink milk and the dam's production potential. A recent meta-analysis by Sapkota et al. (2020) described earlier peak milk yields dairy-beef crosses (4–6 weeks) compared to pure beef cows (5–8 weeks), the latter showing a better persistency. The basal milk yield here was similar in months 2 and 3, which suggests that the peak was reached before week 8, and then decreased in month 4. The basal values agreed with previous observations in multiparous Parda de Montaña cows, as in Blanco et al. (2008), regardless of suckling management, calf sex or supplementation (Cortés-Lacruz et al., 2017).

The impact of feed restriction on milk yield was higher in month 3 than in months 2 and 4, as shown by the greater peak loss (in both absolute and relative terms, 21 % vs 18 %), which was attained more quickly, and the total milk loss. With an induced short-term feed restriction, Bjerre-Harpøth et al. (2012) found a similar milk loss in relation to prechallenge values (30 %) in early-, mid- and late lactation with dairy cows, unlike our results. In response to natural perturbations, effects were severer, developed more quickly and recovered more slowly in early- to mid-lactation than in later stages (Adriaens et al., 2021). Conversely, we found that the lactation stage did not affect the recovery rate during refeeding, as observed by Codrea et al. (2011).

Whereas the milder effect of nutrient restriction in later stages (i.e. in month 4) was supported by the above-mentioned literature, the stronger impact in month 3 than in month 2 was not expected given the similar energy and protein intake. We hypothesise that, as the basal milk yield was similar, but both BW and the BCS were lower in month 3, these beef cows' coping strategies in month 3 were not sufficient to buffer the effect of feed restriction on milk production. The basal NEFA concentrations were similar in months 2 and 3, and were higher than those of month 4, but the peak values of NEFA and BHB decreased steadily, and were reached more quickly for NEFA, as lactation progressed. All this indicates decreasing lipid mobilisation. Apparently, despite the metabolic demand for milk yield still being high in month 3, these beef cows' response to homeorhetic controls was not sufficient to ensure adequate nutrient supply to support milk synthesis under the feed restriction. Baumgard et al. (2017) indicated that when a negative EB occurs, the dairy cows selected for higher milk yield are able to partition more nutrients away from storage and towards mammary utilisation. The opposite would be the case in our study, where that response would be less intense in beef cows with a lower genetic capacity for milk production. This is supported by the findings of Pareek et al. (2007), who compared the response to a metabolic challenge between breeds of different genetic merits for milk yield, and found that dairy cows had lower insulin levels, a lower EB, but greater milk production efficiency than beef cows which, in turn, had a higher potential for body energy and protein accretion.

Regarding the BHB peak, the interaction between month and the MR cluster implied that lipid mobilisation was insufficient only in month 2 for the High MR cows, and the ketogenesis from NEFA resulted in a greater BHB peak in response to feed restriction in early lactation. The higher metabolic load in earlier lactation stages has been described in dairy cows, with natural NEFA peaks 1–2 weeks postpartum and a delayed response in BHB peaks at 2–3 weeks (Kessel et al., 2008; Gross et al., 2011), which decrease thereafter. In Parda de Montaña beef cows fed at 75 % (Alvarez-Rodríguez et al., 2009) or 100 % (Noya et al., 2019) of their requirements, NEFA peaked at 0.27–0.35 mmol/l up to week 5 postpartum and then decreased to reach 0.08 mmol/l in month 4, whereas BHB contents remained constant (approx. 0.20 mmol/l) throughout lactation (Rodríguez-Sánchez et al., 2018).

This effect of month on the basal values could condition the coping strategies which cows apply to face undernutrition in different stages. Bjerre-Harpøth et al. (2012) found decreasing basal NEFA concentrations from early to late lactation, and high BHB contents only in early lactation. When short-term energy deficit was induced, the relative changes in NEFA during restriction increased throughout lactation, while BHB only responded in early lactation. Other studies report that plasma NEFA concentrations are less responsive to feed restriction in late lactation (Carlson et al., 2006; Gross et al., 2011), when even a drastic energy restriction may not increase the BHB concentration if there are not sufficient NEFAs for ketogenesis. According to our results, in a recent review on the effects of feed restriction on dairy cows, Leduc et al. (2021) found that NEFA increased (+14 % to + 3475 %) in most studies, while the effect on BHB was less consistent (+26 % to + 721 % in only 14 of the 23 studies).

Conclusion

Changes in the performance and plasmatic indicators of lipolysis and ketogenesis of beef cows in response to short-term feed restriction can be modelled using spline curves, which allows different MR profiles to be established. The extent, but not the speed, of the individual response was driven primarily by basal milk yield, but adaptation strategies changed as lactation advanced, and as the nutrient demand for milk production and concomitant fat mobilisation decreased. Although long-term performance should also be evaluated, identifying animals that can respond to a nutritional challenge by establishing mechanisms to minimise the impact on their performance is key to develop breeding programmes for enhanced beef cows' resilience.

Ethics approval

The Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) approved all experimental procedures (protocol no. CEEA-03-2018-01), which followed the guidelines of the Directive 2010/63/EU on the protection of animals used for scientific purposes.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declaration of interest

None.

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References

Adriaens, I., van den Brulle, I., D'Anvers, L., Statham, J.M.E., Geerinckx, K., De Vliegher, S., Piepers, S., Aernouts, B., 2021. Milk losses and dynamics during perturbations in dairy cows differ with parity and lactation stage. Journal of Dairy Science 104, 405–418.
Alvarez-Rodríguez, J., Palacio, J., Casasús, I., Revilla, R., Sanz, A., 2009. Performance and nursing behaviour of beef cows with different types of calf management. Animal 3, 871–878.
AOAC, 2000. Official methods of analysis. The Association of Official Analytical Chemists (AOAC), Arlington, VA, USA.
Barreto-Mendes, L., De La Torre, A., Ortigues-Marty, I., Cassar-Malek, I., Pires, J., Blanc, F., 2022. How to approach the resilience of livestock exposed to environmental challenges? Quantification of individual response and recovery by means of differential calculus. Animal - Open Space 1, 100008.
Bauman, D., Currie, B., 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. Journal of Dairy Science 63, 1514–1529.
Baumgard, L.H., Collier, R.J., Bauman, D.E., 2017. A 100-Year Review: Regulation of nutrient partitioning to support lactation. Journal of Dairy Science 100, 10353–10366.
Bell, A., 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science 73, 2804–2819.
Ben Abdelkrim, A., Puillet, L., Gomes, P., Martin, O., 2021a. Lactation curve model with explicit representation of perturbations as a phenotyping tool for dairy livestock precision farming. Animal 15, 100074.
Ben Abdelkrim, A., Tribout, T., Martin, O., Boichard, D., Ducrocq, V., Friggens, N.C., 2021b. Exploring simultaneous perturbation profiles in milk yield and body weight reveals a diversity of animal responses and new opportunities to identify resilience proxies. Journal of Dairy Science 104, 459–470.
Berghof, T.V.L., Poppe, M., Mulder, H., 2019. Opportunities to improve resilience in animal breeding programs. Frontiers in Genetics 9, 692.
Billa, P.A., Faulconnier, Y., Larsen, T., Leroux, C., Pires, J.A.A., 2020. Milk metabolites as noninvasive indicators of nutritional status of mid-lactation Holstein and Montbéliarde cows. Journal of Dairy Science 103, 3133–3146.
Bjerre-Harpøth, V., Friggens, N.C., Thorup, V.M., Larsen, T., Damgaard, B.M., Ingvartsen, K.L., Moyes, K.M., 2012. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. Journal of Dairy Science 95, 2362–2380.
Blanco, M., Villalba, D., Ripoll, G., Sauerwein, H., Casasús, I., 2008. Effects of pre-weaning concentrate feeding on calf performance, carcass and meat quality of autumn-born bull calves weaned at 90 or 150 days of age. Animal 2, 779–789.
Carlson, D.B., Litherland, N.B., Dann, H.M., Woodworth, J.C., Drackley, J.K., 2006. Metabolic effects of abomasal L-carnitine infusion and feed restriction in lactating Holstein cows. Journal of Dairy Science 89, 4819–4834.
Codrea, M., Højsgaard, S., Friggens, N.C., 2011. Differential smoothing of time-series measurements to identify disturbances in performance and quantify animal response characteristics: An example using milk yield profiles in dairy cows. Journal of Animal Science 89, 3089–3098.
Cortés-Lacruz, X., Casasús, I., Revilla, R., Sanz, A., Blanco, M., Villalba, D., 2017. The milk yield of dams and its relation to direct and maternal genetic components of weaning weight in beef cattle. Livestock Science 202, 143–149.
De Koster, J., Salavati, M., Grelet, C., Crowe, M.A., Matthews, E., O'Flaherty, R., Opsomer, G., Foldager, L., Hostens, M., 2019. Prediction of metabolic clusters in early-lactation dairy cows using models based on milk biomarkers. Journal of Dairy Science 102, 2631–2644.
De La Torre, A., Barreto-Mendes, L., Pires, J.A.A., Cassar-Malek, I., Ortigues-Marty, I., Blanc, F., 2022. Exploration of robustness indicators using adaptive responses to short-term feed restriction in suckling primiparous beef cows. Animal 16, 100556.

Elgersma, G.G., de Jong, G., van der Linde, R., Mulder, H.A., 2018. Fluctuations in milk yield are heritable and can be used as a resilience indicator to breed healthy cows. Journal of Dairy Science 101, 1240–1250.
Friggens, N.C., Duvaux-Ponter, C., Etienne, M.P., Mary-Huard, T., Schmidely, P., 2016. Characterizing individual differences in animal responses to a nutritional challenge: Toward improved robustness measures. Journal of Dairy Science 99, 2704–2718.
Gross, J.J., Bruckmaier, R.M., 2015. Repeatability of metabolic responses to a nutrient deficiency in early and mid lactation and implications for robustness of dairy cows. Journal of Dairy Science 98, 8634–8643.
Gross, J., van Dorland, H.A., Bruckmaier, R.M., Schwarz, F.J., 2011. Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. Journal of Dairy Science 94, 1820–1830.
Harder, I., Stamer, E., Junge, W., Thaller, G., 2019. Lactation curves and model evaluation for feed intake and energy balance in dairy cows. Journal of Dairy Science 102, 7204–7216.
INRA, 2007. Alimentation des bovins, ovins et caprins. Besoins des Animaux-Valeur des aliments, Editions Quae, Versailles, France.
Jorjong, S., van Knegsel, A.T.M., Verwaeren, J., Lahoz, M.V., Bruckmaier, R.M., De Baets, B., Kemp, B., Fievez, V., 2014. Milk fatty acids as possible biomarkers to early diagnose elevated concentrations of blood plasma nonesterified fatty acids in dairy cows. Journal of Dairy Science 97, 7054–7064.
Kessel, S., Stroehl, M., Meyer, H.H.D., Hiss-Pesch, S., Sauerwein, H., Schwarz, F., Bruckmaier, R.M., 2008. Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. Journal of Animal Science 86, 2903–2912.
Knight, C., Alamer, M., Sorensen, A., Nevison, I., Flint, D., Vernon, R., 2004. Metabolic safety-margins do not differ between cows of high and low genetic merit for milk production. Journal of Dairy Research 71, 141–153.
Le Neindre, P., Dubroeucq, H., 1973. Observations sur l'estimation de la production laitière des vaches allaitantes per la pesée du veau avant et après la tétée. Annales de Zootechnie 22, 413–422.
Leduc, A., Souchet, S., Gelé, M., Le Provost, F., Boutinaud, M., 2021. Effect of feed restriction on dairy cow milk production: a review. Journal of Animal Science 99, 1–12.
Li, P., Li, X.B., Fu, S.X., Wu, C.C., Wang, X.X., Yu, G.J., Long, M., Wang, Z., Liu, G.W., 2012. Alterations of fatty acid β-oxidation capability in the liver of ketotic cows. Journal of Dairy Science 95, 1759–1766.
Mann, S., Nydam, D.V., Lock, A.L., Overton, T.R., McArt, J.A.A., 2016. Short communication: Association of milk fatty acids with early lactation hyperketonemia and elevated concentration of nonesterified fatty acids. Journal of Dairy Science 99, 5851–5857.
Noya, A., Casasús, I., Achiaga, J., Sanz, A., 2019. Long-term effects of maternal subnutrition in early pregnancy on cow-calf performance, immunological and physiological profiles during the next lactation. Animals 9, 936.
Ollion, E., Ingrand, S., Delaby, L., Trommenschlager, J.M., Colette-Leurent, S., Blanc, F., 2016. Assessing the diversity of trade-offs between life functions in early lactation dairy cows. Livestock Science 183, 98–107.
Pareek, N., Voigt, J., Bellmann, O., Schneider, F., Hammon, H.M., 2007. Energy and nitrogen metabolism and insulin response to glucose challenge in lactating German Holstein and Charolais heifers. Livestock Science 112, 115–122.
Pedernera, M., García, S.C., Horagadoga, A., Barchia, I., Fulkerson, W.J., 2008. Energy balance and reproduction on dairy cows fed to achieve low or high milk production on a pasture-based system. Journal of Dairy Science 91, 3896–3907.
Perperoglou, A., Sauerbrei, W., Abrahamowicz, M., Schmid, M., 2019. A review of spline function procedures in R. BMC Medical Research Methodology 19, 46.
Poppe, M., Veerkamp, R.F., van Pelt, M.L., Mulder, H.A., 2020. Exploration of variance, autocorrelation, and skewness of deviations from lactation curves as resilience indicators for breeding. Journal of Dairy Science 103, 1667–1684.
R Development Core Team, 2014. R: A language and environment for statistical computing (https://www.R-project.org/). R Foundation for Statistical Computing, Vienna, Austria.
Rodríguez-Sánchez, J.A., Sanz, A., Ferrer, J., Casasús, I., 2018. Influence of postweaning feeding management of beef heifers on performance and physiological profiles through rearing and first lactation. Domestic Animal Endocrinology 65, 24–37.
Sapkota, D., Kelly, A.K., Crosson, P., White, R.R., McGee, M., 2020. Quantification of cow milk yield and pre-weaning calf growth response in temperate pasture-based beef suckler systems: A meta-analysis. Livestock Science 241, 104222.
Schuh, K., Sadri, H., Häussler, S., Webb, L.A., Urh, C., Wagner, M., Koch, C., Frahm, J., Dänicke, S., Dusel, G., Sauerwein, H., 2019. Comparison of performance and metabolism from late pregnancy to early lactation in dairy cows with elevated *v.* normal body condition at dry-off. Animal 13, 1478–1488.
Sepchat, B., D'hour, P., Agabriel, J., 2017. Production laitière des vaches allaitantes : caractérisation et étude des principaux facteurs de variation. INRAE Productions Animales 30, 139–152.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597.