Received: 22 November 2021 | Accepted: 10 August 2022

DOI: 10.1111/jpn.13766

ORIGINAL ARTICLE

Ruminants

Journal of Animal Physiology and Animal Nutrition WILEY

Energy, protein and redox metabolism underlying adaptive responses in New Zealand versus North American Holstein cows in pasture-based dairy systems

Ezequiel Jorge-Smeding[1] | Mariana Carriquiry[1] | Alberto Casal[2] | Diego Armand-Ugon[3] | Mauricio Mastrogiovanni[4] | Andrés Trotschansky[4] | Alejandro Mendoza[3] | Ana L. Astessiano[1]

[1]Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay

[2]Departamento de Producción Animal y Pasturas, Estación Experimental Mario A. Cassinoni, Facultad de Agronomía, Universidad de la República, Paysandú, Uruguay

[3]Programa de Producción de Leche, Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay

[4]Departamento de Bioquímica y Centro de Investigaciones Biomédicas (CEINBIO), Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Correspondence

Ana L. Astessiano, Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Avda. Eugenio Garzón 780, 12900 Montevideo, República Oriental del Uruguay.
Email: lauaste@gmail.com

Abstract

This study explored the metabolic adaptions to grazing conditions of two Holstein genetic strains (GS; North American, NAH; New Zealand, NZH) in two feeding strategies (FS; restricted, P30, vs. maximised, PMAX, grazing). Four groups (NAH-P30, NZH-P30, NAH-PMAX and NZH-PMAX; $n = 10$ cows each) were compared between −45 and 180 days in milk (DIM). NZH cows had lower ($p = 0.02$) fat and protein corrected milk (FPCM) yield and a tendency for lower ($p = 0.09$) body condition score concomitantly with a trend ($p < 0.07$) for higher average plasma insulin and lower ($p = 0.01$) 3-methylhistidine (3MH) at −45 DIM than NAH. Plasma glucose tended to be affected by the triple interaction GS × FS × DIM ($p = 0.06$) as it was similar between NAH-P30 and NZH-P30, but higher ($p \leq 0.02$) for NZH-PMAX than NAH-PMAX except at 21 DIM. The physiological imbalance index was affected by the GS × FS interaction ($p < 0.01$) as it was lower ($p < 0.01$) only for NZH-PMAX versus NAH-PMAX. NZH cows had higher ($p = 0.01$) plasma thiobarbituric acid reactive substances at −45 DIM and tended to have higher protein carbonyls ($p = 0.10$) and superoxide dismutase (SOD) activity ($p = 0.06$) on average, and had higher ($p < 0.01$) α-tocopherol during mid-lactation than NAH Regarding the FS, FPCM was similar ($p = 0.12$) among them, but PMAX cows had higher ($p < 0.01$) plasma non-esterified fatty acids and 3MH, and lower insulin ($p < 0.01$) than P30 at 100 DIM. PMAX cows showed higher average SOD activity ($p = 0.01$) and plasma α-tocopherol at 100 and 180 DIM ($p < 0.01$). Under grazing, NZH cows can have a better energy status and lower muscle mobilisation but a higher redox reactivity. Maximising grazing can worsen energy status and muscle mobilisation while improving antioxidant response with no effect on FPCM.

KEYWORDS

genetic strain, metabolic adaptation, muscle mobilisation, oxidative stress, pasture-based dairy system

1 | INTRODUCTION

Major physiological challenges for dairy cows rely upon the need to cope with increased nutrient requirements (Bell & Bauman, 1997), and facing the physiological imbalance during negative energy balance (NEB) at early lactation stage (Moyes et al., 2013). The magnitude and duration of this catabolic state, in which dairy cows experience peripheral tissues mobilisation, are strongly affected by the feeding strategy and body reserves at parturition (Meikle et al., 2013). During the last decades grazing dairy systems—especially those which maximise grazing—are becoming more relevant as they reduce feed cost and offer benefits for animal welfare (Dillon et al., 2006) and environmental care (Basset-Mens et al., 2009). However, it has been indicated that grazing dairy cows are not able to express their potential dry matter intake (DMI) as the ability of grazing is constrained by the ingestive behaviour, bite mass, bite rate and grazing time which can be determined by herbage mass, sward structure, pasture quality and supplementation strategy (Baudracco et al., 2010; Kolver & Muller, 1998; Méndez et al., 2020). In consequence, cows with high genetic merit for milk yield usually show a deeper and longer NEB of early lactation when compared to confined dairy cows in total mixed ration (TMR) based-systems (Astessiano et al., 2015; Baudracco et al., 2010; Meikle et al., 2013). Thus, the metabolic challenge of the onset of lactation is usually exacerbated in grazing systems.

It has been proposed the cow genotype should be selected according to the production system in order to achieve reasonable milk yield goals without compromising animal reproduction, welfare, and metabolic status (Delaby et al., 2009; Horn et al., 2014). During several decades, the genetic selection in United States's Holstein (NAH) led to high individual milk yields and decreased reproductive efficiency (Brito et al., 2021; Lucy, 2001). Despite reproductive traits were introduced in the genetic selection index in the early 2000's leading to improved reproductive efficiency during these last 20 years, the reproductive performance in North American Holstein is still not optimal being the improved pregnancy rates are mostly due to environmental rather than genetic effects (Brito et al., 2021; VanRaden et al., 2004). In contrast, New Zealand's selection strategy has been focused on milk solid yield of New Zealand Holstein (NZH) cows in grazing systems (Harris & Kolver, 2001) and several studies reported better reproductive performance for NZH than NAH genetic strain as reviewed by Baudracco et al. (2010). In fact, as reviewed by Rodríguez-Bermúdez et al. (2017), NZH strain has been shown to be better adapted to pastoral conditions than NAH strain. Indeed, when compared to NZH under grazing conditions, NAH cows presented higher DMI per unit of metabolic live weight (LW), increased energy and nutrient partitioning towards milk production sustained by a higher loss of body reserves during NEB, and a stronger uncoupling of the somatotropic axis, and increased insulin resistance (Chagas et al., 2009; Lucy et al., 2009; Talmón et al., 2022). Despite it is well known that also muscle catabolism and oxidative stress take part within the homeorhetic responses when nutritional requirements cannot be fulfilled by feed intake (Pedernera et al., 2010; van der Drift et al., 2012), the specific role of amino on the metabolic homeorhetic adaptions to challenging situations are recently gaining scientific attention in dairy physiology and nutrition (e.g., Liang et al., 2021; Webb et al., 2020). In this sense, through a metabolomic approach we have recently observed several differences, including higher plasma concentrations of branched-chain amino acids in NAH versus NZH cows suggesting that specific amino and protein metabolic changes should underly metabolic adaptive differences between these genetic strains (GS) (Jorge-Smeding, Carriquiry, Cantalapiedra-Hijar, et al., 2021). However, the possible roles of amino and protein metabolism underlying the metabolic adaptive responses of NAH and NZH to grazing conditions have not been studied yet. Thus, this work aimed to assess the effect of Holstein genetic strain (NAH vs. NZH) combined with different grazing-based feeding strategies (FS) on cows' protein and redox metabolism in relationship with energy and lipid homeorhetic changes across lactation. We hypothesised that NAH cows have a lower adaptive capacity to face grazing systems constraints which lead to more acute changes in energy and protein metabolism (enhanced catabolic state) associated with higher oxidative stress because of its higher milk yield when compared with NZH cows under grazing conditions. The metabolic differences between NAH and NZH are expected to be more evident when grazing activity is maximised.

2 | MATERIALS AND METHODS

2.1 | Experimental design and treatments

The experiment was located at the Experimental Research Station 'La Estanzuela' (34°20' S, 57°40' W) belonging to the National Institute of Agronomic Research of Uruguay. Forty out of 120 fall-calving multiparous dairy cows of two Holstein GS (NAH, $n = 20$; NZH, $n = 20$) were randomly selected from a larger grazing experiment. Within each genetic strain, cows were paired by parity (3.1 ± 0.9 lactations) and calving date (5/8/2018 ± 18 days), and randomly allocated to one of the two FS (P30 vs. PMAX) in a 2 × 2 factorial arrangement leading to 4 experimental groups: NAH in P30 (NAH-P30, $n = 10$), NZH in P30 (NZH-P30, $n = 10$), NAH in PMAX (NAH-PMAX, $n = 10$) and NZH in PMAX (NZH-PMAX, $n = 10$). Previous to calving, NAH cows had a LW of 626 ± 15 kg and a body condition score (BCS) of 3.19 ± 0.04, while NZH had a LW of 537 ± 15 kg and a BCS of 3.29 ± 0.04. At least 87.5% of each cow's ancestors (three generations) had an American (USA or Canada) or New Zealand proved origin for NAH and NZH GS, respectively (Mejoramiento y Control Lechero Uruguayo; https://www.geneticalechera.com.uy/). The 305-days expected milk yield was 7500 and 5500 kg, and the economic and productive breeding index, which includes milk yield, fat, protein, fertility and udder health (Rivero et al., 2012), was 108 ± 3 and 125 ± 2 (mean ± SE) on average for NAH and NZH cows, respectively. The NAH cows had an expected progeny difference of +34 ± 41 kg, +0.08 ± 0.03% and +0.02 ± 0.02% for milk yield, milk fat

content, and milk protein content, respectively, compared to the national herd. The NZH cows had an expected progeny difference of −141 ± 40 kg, +0.13 ± 0.03%, and +0.14 ± 0.01%, for milk yield, milk fat and milk protein content, respectively, compared to the national herd.

During the dry period and prepartum all cows stayed outdoors and were offered the same diet through a TMR (comprising maize silage, oat haylage, barley straw, soybean meal, soybean hulls, urea, CaCo3, commercial mineral and vitamin supplement); the average chemical composition is presented in Table 1. At calving, cows were assigned to the FS previously stated, and grazed annual (*Avena byzantine* or *Lolium multiflorum*) and perennial (*Dactylis glomerate* + *Medicago sativa* or *Festuca arundinacea)* pastures on a daily rotational grazing system. Daily paddocks with free access to water were allocated to cows of each group (GS × FS) after milking. Herbage mass (above 5 cm to ground level) was estimated weekly by measuring sward height using a pasture metre (C-Dax pasture metre; C-Dax) and a calibrated equation. Both FS aimed to maximise yearling milk production per surface unit of the milking platform (i.e., the total area assigned to lactating cows exclusively) through maximising pasture production and high stocking rates while similar use of concentrates per cow and year. FS were designed to achieve the predicted DMI according to (NRC, 2001), and mainly differed in the strategy of pastures use through different proportions of grazed versus harvested herbage included in the diet leading to differences in annual grazing activity (Stirling et al., 2021). The P30 feeding strategy was conducted to reach, on average during lactation, one-third of estimated DMI from directly grazed pastures, while in PMAX the objective was to maximise pasture DM intake per ha according to the weekly pasture growth rate of the grazing platform. On average during lactation, P30 comprised 35% and 65% (relative to cows' DMI) of directly grazed pastures and TMR, respectively. The TMR was made up of conserved forages and concentrate (forage-to-concentrate ratio 50:50 ± 9%, mean ± SD; DM basis; Table 1), and it was offered once a day after morning milking. Herbage allowance was adjusted weekly to offer one-third of the estimated DM intake, and excess growth rate was mechanically harvested as haylage to maintain the same average pasture stock as in PMAX and to avoid a reduction in pasture quality. In PMAX, herbage allowance (kg DM/cow·day; Table 1) was adjusted weekly on the basis of pasture growth rate in the grazing platform and stocking rate, to keep a pasture stock of 665 ± 312 (kg DM/ha). In addition, a commercial concentrate (Table 1) was offered twice a day at the

TABLE 1 Pasture characteristics and average diet composition (mean ± SD) according to feeding strategy

	P30				PMAX			
	−45 DIM	21 DIM	100 DIM	180 DIM	−45 DIM	21 DIM	100 DIM	180 DIM
a. Pasture characteristics								
Herbage mass (kg DM/ha)[a]	-	2964 ± 50	2695 ± 73	1620 ± 611	-	2790 ± 467	2549 ± 64	1563 ± 293
Herbage height (cm)	-	23.6 ± 1.9	19.1 ± 0.9	15.2 ± 4.4	-	24.5 ± 2.3	19.3 ± 0.5	14.9 ± 2.1
Herbage allowance (kg DM/cow·day)	-	11.9 ± 0.3	13.9 ± 3.1	9.7 ± 1.3	-	17.5 ± 1.6	21.8 ± 0.8	21.2 ± 1.0
b. Diet composition								
Pasture (%)[b]	-	35.6 ± 2.5	35.6 ± 2.9	32.5 ± 0.4	-	40.9 ± 0.3	37.8 ± 3.8	55.3 ± 1.6
Concentrate (%)[c]	-	-	-	-	-	36.3 ± 1.8	38.3 ± 0.6	44.7 ± 1.6
Forage reserves (%)[c]	-	-	-	-	-	22.8 ± 3.0	23.9 ± 3.2	0.0 ± 0.0
Total mixed ration (%)[c,d]	100.0 ± 0.0	64.4 ± 2.5	64.4 ± 2.9	67.5 ± 0.8	100.0 ± 0.0	-	-	-
DM (%)[e]	52.9 ± 0.0	47.5 ± 1.8	49.0 ± 0.2	59.2 ± 0.2	52.9 ± 0.0	50.6 ± 3.5	48.7 ± 9.3	52.1 ± 1.0
CP(%)[e,f]	14.0 ± 0.0	17.8 ± 1.1	19.4 ± 0.1	17.2 ± 0.1	14.0 ± 0.0	15.2 ± 2.6	17.1 ± 3.4	20.8 ± 0.1
NDF (%)[e,f]	49.2 ± 0.0	39.2 ± 0.8	38.2 ± 0.3	39.5 ± 0.1	49.2 ± 0.0	37.4 ± 0.5	38.1 ± 1.2	39.3 ± 0.4
ADF (%)[e,f]	33.2 ± 0.0	25.8 ± 0.5	24.4 ± 1.4	25.0 ± 0.1	33.2 ± 0.0	22.5 ± 1.4	24.1 ± 2.1	19.9 ± 0.3
ENL (MJ/kg DM)[e,f,g]	5.8 ± 0.0	6.2 ± 0.1	6.6 ± 0.1	6.4 ± 0.1	5.8 ± 0.0	6.2 ± 0.1	6.8 ± 0.4	7.1 ± 0.1

Abbreviations: ADF, acid detergent fibre; CP, crude protein; DIM, days in milk; DM, dry matter; ENL, net energy of lactation; NDF, neutral detergent fiber; P30, feeding strategy P30; PMAX, feeding strategy with maximised grazing activity; SD, standard desviation given by variability within feeding strategy during the 5 days of milk recording in each experimental period.

[a]Estimated by C-Dax pasture metre (C-Dax, Turitea, New Zealand);

[b]Estimated by the difference between offered and refused herbage mass at ground level;

[c]Estimated by the difference between the feed offered and refused;

[d]Diet composition at −45 DIM correspond to the offered diet in a unique herd;

[e]Estimated as average composition based on diet composition and nutritional quality of each aliment;

[f]Expressed on DM basis;

[g]According to NRC (2001).

milking parlour. Whenever pasture allowance was considered restrictive to maintain the target DM intake, or in rainy conditions, forage reserves (a mix of corn silage and pasture haylage; 73:27 ± 6% on a DM basis, respectively) were offered in a feeding parlour immediately before the afternoon milking. On average during lactation, PMAX comprised 45% of grazed pastures, 40% of concentrate and 15% of conserved forage. The FS had similar nutritional value (Table 1). Specific details on chemical composition of individual feeds can be found in Suppl. Table 1, while greater details on grazing management can be found in previously reported data for this experiment (Stirling et al., 2021; Talmón et al., 2020).

2.2 | Animal measurements and sampling

Cows were milked twice a day at 0400 and 1400 h. Milk yield was measured daily using an automated recording system (Dairy Plan; GEA Farm Technologies), and milk samples preserved with potassium dichromate (Lactopol®; Grupo Benzo) were collected every 14 days to determine fat, protein and milk urea (MUN) concentration by a milk analyser (Combi FOSS FT+; Foss Electric; HillerhØd). Cow LW and BCS were measured every 14 days from −45 to 180± 18 DIM. LW was measured using an electronic scale (model AD-4406; A&D Weighing), and BCS was measured by two trained operators using the Edmonson's scale (1−5, Edmonson et al., 1989). At −45, 21, 100 and 180 ± 18 DIM blood samples were taken by coccygeal venipuncture using 10 ml heparinized Vacutest® tubes (Vacutest Kima). Plasma samples were immediately harvested by centrifuging at 4000*g* for 12 min, aliquoted, and stored at −80°C until analysis.

2.3 | Metabolites, hormones and enzymatic activities determinations

Plasma concentrations of glucose, non-esterified fatty acids (NEFA), β-hidroxybutyrate (BHB), cholesterol, urea (MUN), total protein, and albumin were quantified by spectrophotometry using a 96-well microplate reader (Multiskan FC, Thermo Fisher Scientific) and commercial kits (Biosystems SA, Barcelona, Spain for glucose, cholesterol, urea, total protein, and albumin; and Randox Laboratories, Crumlin, UK for NEFA and BHB). For all assays, the intra-assay and inter-assay coefficients of variation for low and high controls were <16.5% and 12.0%, respectively. Plasma concentrations of 3-methylhistidine (3MH) were quantified after derivatization with fluorescamine according to Houweling et al. (2012) using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Insulin concentrations were determined by radioimmunoassay in a single assay (DIAsource Immuno Assays). Intra-assay coefficients of variation were 3.7% and 9.4% for the low and high controls, respectively.

Lipid and protein oxidation biomarkers were assessed determining plasma concentrations of thiobarbituric acid reactive species (TBARS) and protein carbonyls, respectively. Concentrations of TBARS were measured using a colorimetric method at 532 nm according to (Wernicki et al., 2006) using a 96-well microplate reader (Multiskan FC; Thermo Fisher Scientific). The concentration of malondialdehyde was calculated using its extinction coefficient (156,000 mol/cm; adjusted for the path length of the solution in the well). Protein carbonyls concentration was determined through absorbance of 2,4-dinitrophenylhydrazine (DNPH) derived carbonyls at 380 nm as previously reported (Ceci et al., 2015) using a 96-well microplate reader (Multiskan FC, Thermo Fisher Scientific Inc). Concentrations were calculated using the DNPH molar extinction coefficient (22,000 mol/cm; adjusted for the path length of the solution in the well). Results were expressed as nmol of DNPH/mg of total protein. The antioxidant system was assessed by enzyme activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in plasma as well as plasma concentration of α-tocopherol (vitamin E). The activity of SOD and GPx were measured spectrophotometrically by kinetic assays using commercial kits (Randox Laboratories Ltda) in a Varioskan Flash microplate reader (Thermo Fisher Scientific) according to manufacturer's protocol. For GPx activity determination plasma samples were not diluted while SOD protocol had a minor modification: prior to apply the manufacturer's protocol, 30 µl of chloroform and 50 µl of ethanol were added to 100 µl plasma samples, mixed and vortexed for 1 min. Subsequently, samples were centrifuged at 17,000*g* for 1 min and 4°C. Supernatant was recovered and 9 µl diluted with 0.01 M phosphate solution (pH = 7.0) in a 25:75 relationship (vol/vol, sample:-diluent). Results were expressed as mU of activity per mg of total protein measured in plasma. Plasma α-tocopherol (vitamin E) concentration was determined using a reversed-phase HPLC method using an 1100 Agilent quaternary pump HPLC (Agilent Technologies) with a fluorescent detector as previously reported (Schweigert et al., 2003).

2.4 | Calculations and statistical analyses

Fat and protein corrected milk (FPCM) yield was calculated as previously reported (Østergaard et al., 2003) according to the following equation (1):

$$\text{FPCM} = [0.383(\%\text{fat}) + 0.242(\%\text{protein}) + 0.7832]/[3.14] \cdot [\text{MY}] \quad (1)$$

where MY is milk yield (kg/day).

Insulin sensitivity was estimated by the *Revised Quantitative Insulin Sensitivity Check Index* (RQUICKI) (Holtenius & Holtenius, 2007), while the risk of developing production metabolic diseases was estimated by the *Physiological Imbalance Index* (PI) (Moyes et al., 2013), calculated according to equations 2 and 3, respectively:

$$\text{RQUICKI} = (\log[\text{glucose}] + \log[\text{insulin}] + \log[\text{NEFA}]) \quad (2)$$

$$\text{PI} = \ln[\text{NEFA}] + \ln[\text{BHB}] - [\text{glucose}] \quad (3)$$

Where glucose concentration is in mg/dl, insulin is in µU/ml and NEFA is in mmol/L in equation 2, while in equaytion 3 [NEFA] is expressed in mEq/L, (BHB] in mmol/L and [glucose] in mmol/L.

Data were analysed as repeated measures with a mixed model using the MIXED procedure (SAS®; University Edition; SAS Institute). The model included the genetic strain, feeding strategy, DIM and its interactions as fixed effects, and the cow as a random effect. Calving date and days in pregnancy were tested as covariates and were removed because of lack of significance ($p > 0.1$, in all cases). Spatial power was chosen for covariance structure due to unequally spaced sampling periods (Littell et al., 2006). For all results, means were considered to differ when $p \leq 0.05$, and trends were identified when $0.05 < p \leq 0.10$. Least squares means were compared using Tukey's test. Correlation analysis was done in R (www.r-project.org) using the *hlmisc* package. In order to integrate the different metabolic variables measured in the current study, the metabolic trajectory was semi-quantitatively assessed through principal component analysis (PCA) as previously reported (Zhang, Sun, Sun, Jiao, & Wang, 2013) on the basis of plasma metabolites, hormones and enzymatic activity described above of each cow ($n = 40$, 10 cows per group) at each sampling time (4 sampling dates) using R software (www.r-project.org). In brief, the PCA integrates the original variables by combining them in latent variables containing most of the variation (Wehrens, 2011). Then, by plotting each sample (each animal at each time point) according to its score values (score plots), the metabolic trajectory of each group can be addressed through the pattern described by the centroid of each ellipse (95% confidence of each group at each time point).

3 | RESULTS

3.1 | Milk yield, milk composition, LW and BCS

FPCM yield was higher ($p = 0.02$) for NAH than in NZH cows (32.3 vs. 28.9 ± 1.0 kg/day, respectively) and decreased ($p < 0.04$) from 21 to 180 DIM for all cows (Figure 1a), and it was similar ($p = 0.12$) between FS (29.5 vs. 31.7 ± 0.9 kg/day, for P30 and PMAX, respectively, Table 2). Cow LW was higher ($p < 0.01$) for NAH than NZH cows (Table 2, Figure 1b) and was affected by the triple interaction between GS, FS and DIM ($p < 0.01$), as LW decreased (−10% on average, $p \leq 0.02$) in all cows from −45 to 21 DIM except for NZH-PMAX which showed no significative changes at this time (517 vs. 497 ± 20 kg, for −45 vs. 21 DIM, respectively, $p = 0.32$). Then, all cows increased ($p < 0.01$) by 10% their LW from 21 to 100 DIM, and remained unchanged until 180 DIM. In contrast, cow BCS tended to be higher ($p = 0.09$) for NZH than NAH cows (2.71 vs. 2.65 ± 0.06, respectively) on average, and was affected by DIM ($p = 0.01$), as it decreased ($p < 0.01$) from −45 to 100 DIM, remaining then unchanged until 180 DIM in all cows (Figure 1c).

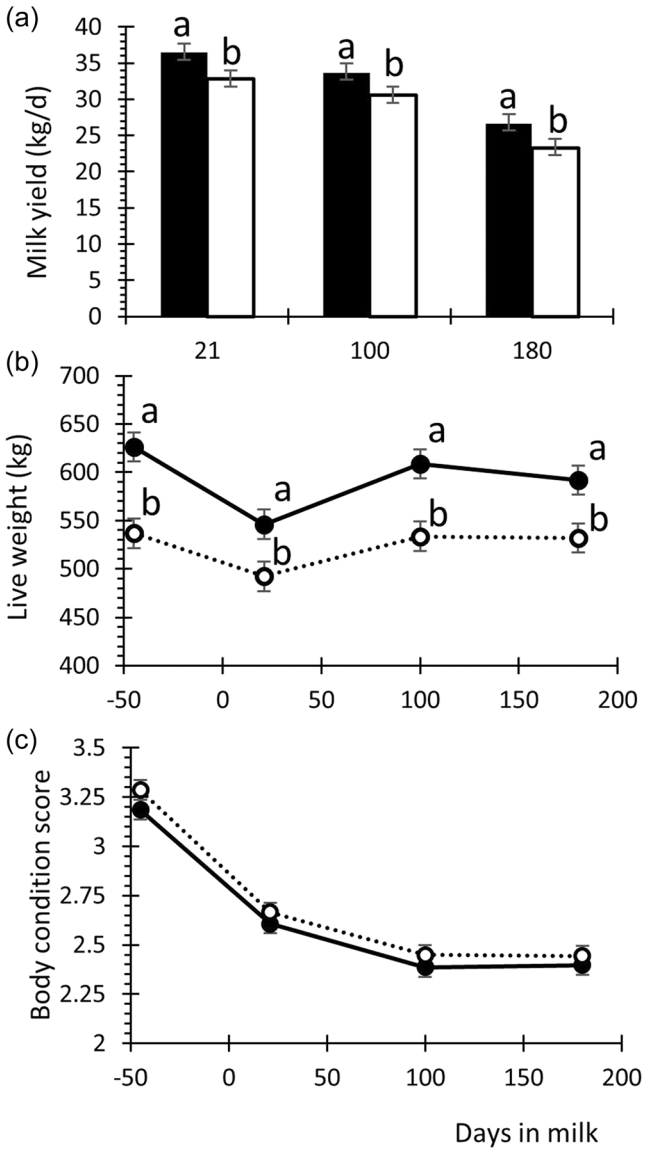

FIGURE 1 Fat and protein corrected milk yield (a), live weight (b) and body condition for North American and New Zealand cows (full black: NAH; empty: NZH) across lactation (days in milk). Significative differences ($p \leq 0.05$) between genetic strains at any given time are denoted by different letters.

3.2 | Energy and protein metabolism

Plasma NEFA concentrations were similar between GS, and it differed between FS in a DIM-dependent manner ($p = 0.02$ for the interaction FS × DIM; Table 2; Figure 2a) as it increased ($p < 0.01$) in both FS from −45 to 21 DIM, but then it decreased faster in P30 than PMAX cows, reaching lower ($p < 0.01$) concentrations in P30 than in PMAX at 100 DIM (Figure 2a). Plasma BHB was only affected by DIM ($p < 0.01$), increasing from −45 to 21 DIM and decreasing ($p < 0.01$) at 100 DIM in all cows regardless the GS and FS (Table 2; Figure 2b). Cholesterol concentrations were higher ($p < 0.01$) for NZH than NAH cows on average. Additionally, it differed between FS in a DIM-dependent manner ($p < 0.01$ for the interaction FS × DIM) as it increased from 21 to 180 DIM only for PMAX cows, determining higher ($p \leq 0.03$) concentrations for PMAX than P30 at 100 and 180 DIM (Figure 2c).

TABLE 2 Productive performance and metabolic variables according to genetic strain and feeding strategy interaction

	Average mean					*p* value					
	P30		PMAX								
	NAH	NZH	NAH	NZH	SEM	GS	FS	DIM	GS × FS	GS × DIM	FS × DIM
a. Productive perfomance											
FPCM (kg/day)	31.6	27.4	33.0	30.4	2.01	0.02	0.12	<0.01	0.59	0.95	0.10
LW (kg)	598	524	589	523	14.5	<0.01	0.63	0.06	0.66	<0.01	0.16
BCS	2.67	2.74	2.62	2.68	0.060	0.09	0.19	<0.01	0.83	0.90	0.16
b. Energy metabolism											
NEFA (mmol/L)	0.198	0.226	0.253	0.239	0.0501	0.84	0.29	<0.01	0.52	0.44	0.02
BHB (mmol/L)	0.300	0.277	0.300	0.291	0.0352	0.52	0.78	<0.01	0.76	0.37	0.37
Cholesterol (mmol/L)	2.80	3.42	3.26	4.37	0.280	<0.01	<0.01	0.04	0.21	0.48	<0.01
Glucose (mmol/L)	3.26	3.38	2.99	3.83	0.270	0.01	0.64	0.02	0.07	0.23	0.02
Insulin (μIU/ml)	8.05	8.64	7.01	7.96	0.600	0.07	<0.01	<0.01	0.69	0.19	<0.01
c. Protein metabolism											
MUN (mg/100 ml)	18.9	20.5	17.2	20.2	0.60	<0.01	0.03	<0.01	0.13	0.04	0.69
PUN (mmol/L)	4.21	5.33	4.45	4.65	0.331	0.01	0.33	0.29	0.06	0.15	<0.01
3MH (mmol/L)	3.92	3.60	4.68	4.04	0.400	0.09	0.03	<0.01	0.56	0.04	<0.01
Total protein (g/L)	63.3	63.4	64.4	63.6	3.61	0.90	0.80	0.18	0.88	0.64	0.58
Albumin (g/L)	26.6	25.2	27.2	26.2	1.21	0.16	0.38	0.42	0.84	0.04	0.49
d. Metabolic indexes											
RQUICKI	0.56	0.53	0.54	0.52	0.040	0.20	0.44	<0.01	0.87	0.26	0.03
PI index	$-0.17^{a,b}$	0.17^{b}	0.85^{c}	-0.27^{a}	0.292	0.06	0.16	0.02	<0.01	0.20	<0.01

Note: Variables were not affected by the triple interaction between GS, FS and DIM except FPCM ($p < 0.01$), LW ($p < 0.01$), glucose ($p = 0.06$) and insulin ($p = 0.06$). Different superscript letters mean significant differences ($p < 0.05$) among groups according to Tukey test.

Abbreviations: BCS, body condition score; BHB, β-hidroxybutyrate; DIM, days in milk; FPCM, fat and protein corrected milk; FS, feeding strategy; GS, genetic strain; LW, live weight; MUN, milk urea nitrogen; NAH, North American Holstein; NEFA, non-esterified acids; NZH, New Zealand Holstein; P30, feeding strategy P30; PI index, Physiological imbalance index; calculated as PI = ln[NEFA] + ln[BHB]−[glucose]. Where, NEFA concentration is in mEq/L,while BHB and glucose concentrations are in mmol/L PMAX, feeding strategy with maximised grazing activity; PUN, plasma urea; 3MH, 3-methylhistidine; RQUICKI, Revised Quantitative Insulin Sensitivity Check Index, calculated as RQUICKI = 1/(log[glucose]+log[insulin]+log [NEFA]); Where, glucose concentration is in mg/dl, insulin is in μUI/ml and NEFA is in mmol/L (Holtenius and Holtenius, 2007).

Plasma glucose was higher ($p = 0.01$) for NZH than NAH on average, and it differed between FS in a DIM-dependent manner ($p = 0.02$ for the interaction FS×DIM) as it was higher ($p = 0.01$) for PMAX than P30 only during the prepartum. Additionally, plasma glucose tended to be affected ($p = 0.06$) by the triple interaction GS × FS × DIM as it was similar between NAH-P30 and NZH- P30 along the experiment, but it was higher ($p \leq 0.02$ in all cases) for NZH-PMAX than NAH-PMAX except at 21 DIM (Figure 3a). Moreover, while NZH-PMAX cows increased ($p < 0.01$) their plasma glucose from 21 to 180 DIM, in NAH-PMAX it decreased constantly from −45 to 180 DIM ($p < 0.01$). Plasma insulin tended to be higher ($p = 0.07$) for NZH than NAH cows on average, and it differed between FS in a DIM-dependent manner ($p < 0.01$ for the interaction FS × DIM) as it decreased in both FS from −45 to 21 DIM increasing then at 100 DIM in all cows but reaching higher ($p < 0.01$) concentrations in P30 than in PMAX cows (Table 2; Figure 3b).

The RQUICKI was similar between GS, and it differed between FS in a DIM-dependent manner ($p < 0.03$ for the interaction FS × DIM) as in P30 it was low from −45 to 21 DIM increasing ($p < 0.01$) then until 180 DIM, while in PMAX it decreased ($p < 0.01$) from −45 to 100 DIM increasing ($p < 0.01$) then at 180 DIM (Table 2; Figure 3c). This determined that RQUICKI was higher ($p < 0.01$) for P30 than PMAX only at 100 DIM. The PI index tended to be lower ($p = 0.06$) for NZH than NAH cows on average (Table 2) and it differed between GS in a FS-dependent manner ($p < 0.01$ for the interaction GS × FS) as it was the higher ($p < 0.01$) for NAH-PMAX than NZH-PMAX with intermediate values for NAH and NZH in P30 (Table 2). Additionally, the PI differed between FS in a DIM-dependent manner ($p < 0.01$) as it increased ($p < 0.01$) from −45 to 21 DIM and decreased ($p < 0.01$) then after calving in all cows but it decreased deeper for PMAX cows which reached lower values than P30 only at 100 DIM (Table 2; Figures 3g, 3h).

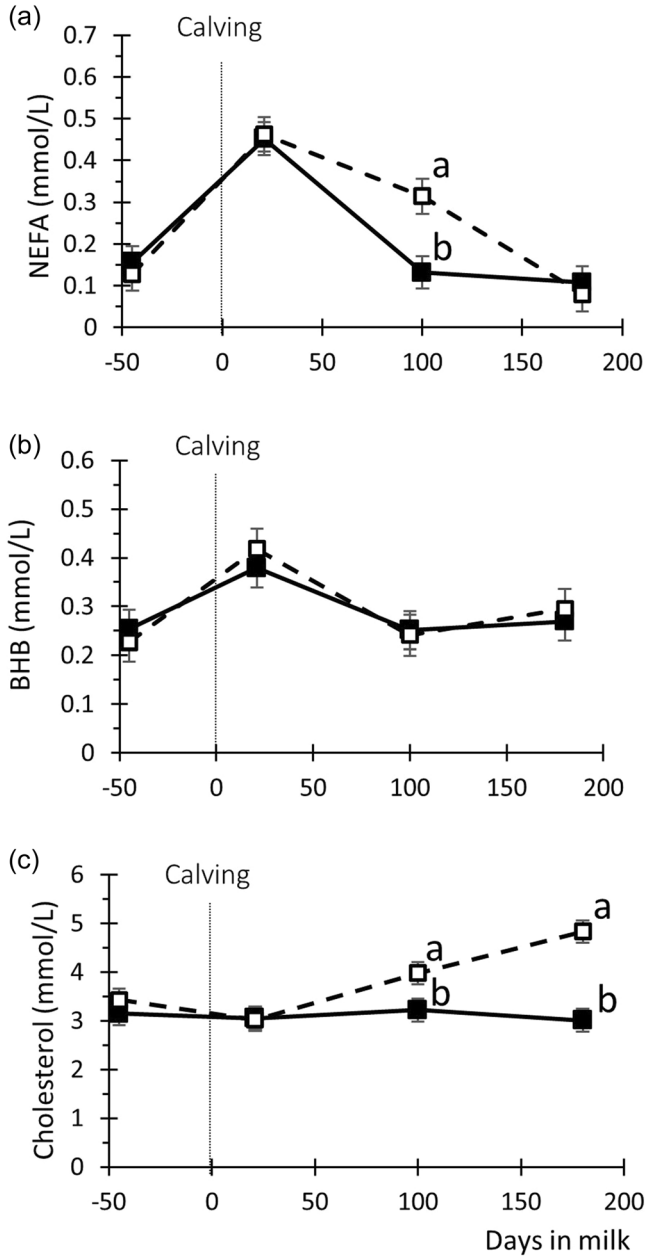

FIGURE 2 Plasma concentrations of non-esterified fatty acids (a, NEFA), β-hydroxybutyrate (b, BHB) and cholesterol (c) for cows fed different feeding strategies (FS) (full black: P30; empty: PMAX) across lactation. Significant differences ($p \leq 0.05$) between FS at any given time are denoted by different letters. BHB, β-hidroxybutyrate.

Concentrations of MUN were higher ($p = 0.03$) for P30 than PMAX on average, and it differed between GS in a DIM-dependent manner ($p = 0.04$ for the interaction GS × DIM) as it was similar between GS at 21 DIM increasing ($p < 0.01$) then from 100 to 180 DIM in all cows but being higher for NZH than NAH at 100 and 180 DIM (Figure 4a). Concentrations of PUN were higher ($p < 0.01$) for NZH than NAH cows on average. Additionally, PUN differed between FS in a DIM-dependent manner ($p < 0.01$ for the interaction FS × DIM) as it increased ($p < 0.01$) from −45 to 100 DIM and decreased ($p < 0.01$) then at 180 DIM for P30 cows, while in PMAX it remained low and unchanged from −45 to 100 DIM and increased ($p < 0.01$) then (Figure 4d). This determined that PUN was higher ($p < 0.01$) at 100 DIM but lower ($p < 0.01$) at 180 DIM for P30 than PMAX cows (Figure 4d). Concentrations of 3-MH changed across time being the highest ($p < 0.01$) at −45 DIM in all cows. Additionally, it differed between GS in a DIM-dependent manner ($p = 0.04$ for the interaction GS × DIM) as it was higher ($p < 0.01$) for NAH than NZH cows only during the prepartum (−45 DIM; Figure 4e). The 3-MH also differed between FS in a DIM-dependent manner ($p < 0.01$ for the interaction FS × DIM) as it was higher ($p < 0.01$) for PMAX than P30 cows only at 100 DIM (Figure 4f). Plasma total protein concentrations were not affected by GS, FS and DIM nor their interactions (Table 2). Albumin differed between GS in a DIM-dependent manner ($p = 0.04$ for the interaction GS × DIM) as it was higher ($p = 0.04$) for NZH than NAH cows only at 100 DIM.

3.3 | Oxidative metabolism compounds and enzymes

Plasmatic TBARS levels tended ($p = 0.06$) to be affected by the interaction GS × DIM as it was higher ($p = 0.01$) for NZH than NAH cows during the prepartum (Figure 5a). Plasma protein carbonyls tended to be higher ($p = 0.10$) for NZH than NAH cows on average, and it also tended to differ between FS in a DIM-dependent manner ($p = 0.09$ for the interaction FS × DIM) as it remained unchanged from −45 to 180 DIM for P30 while it decreased ($p < 0.01$) from 21 to 180 DIM in PMAX. This determined higher ($p = 0.04$) protein carbonyls for P30 than PMAX only at 180 DIM (Figure 5d). Plasma GPx activity was not affected by GS, FS DIM nor their interactions (Table 3; Figures 6a, 6b). In contrast, SOD activity was higher ($p = 0.01$) for PMAX than P30 on average and it also tended to differ between GS in a DIM-dependent manner ($p = 0.10$ for the interaction GS × DIM) as it decreased ($p = 0.03$) from −45 to 21 in NAH cows, while in NZH it remained unchanged until 21 DIM decreasing ($p = 0.01$) then from 21 to 180 DIM (Figure 6a). Additionally, SOD activity tended to differ between FS in a DIM-dependent manner ($p = 0.10$ for the interaction FS × DIM) as it decreased earlier in lactation for P30 than PMAX cows, determining lower ($p = 0.02$) SOD activity for P30 than PMAX at 21 DIM (Figure 6b). Plasma α-tocopherol tended to differ between GS in a DIM-dependent manner ($p = 0.10$ for the interaction GS×DIM) as its increased ($p = 0.03$) from −45 to 100 DIM only in NZH cows (Figure 6c). In addition, it differed between FS in a DIM-dependent manner ($p = 0.05$ for the interaction FS × DIM) as it was higher ($p < 0.01$) for PMAX than P30 only at 100 and 180 DIM (Figure 6d).

3.4 | Correlation analysis

Correlation analysis showed some moderate correlations between different parameters, the FPCM yield had a positive correlation with plasma NEFA and BHB ($r \geq 0.20$, $p < 0.03$) and PI index ($r = 0.31$,

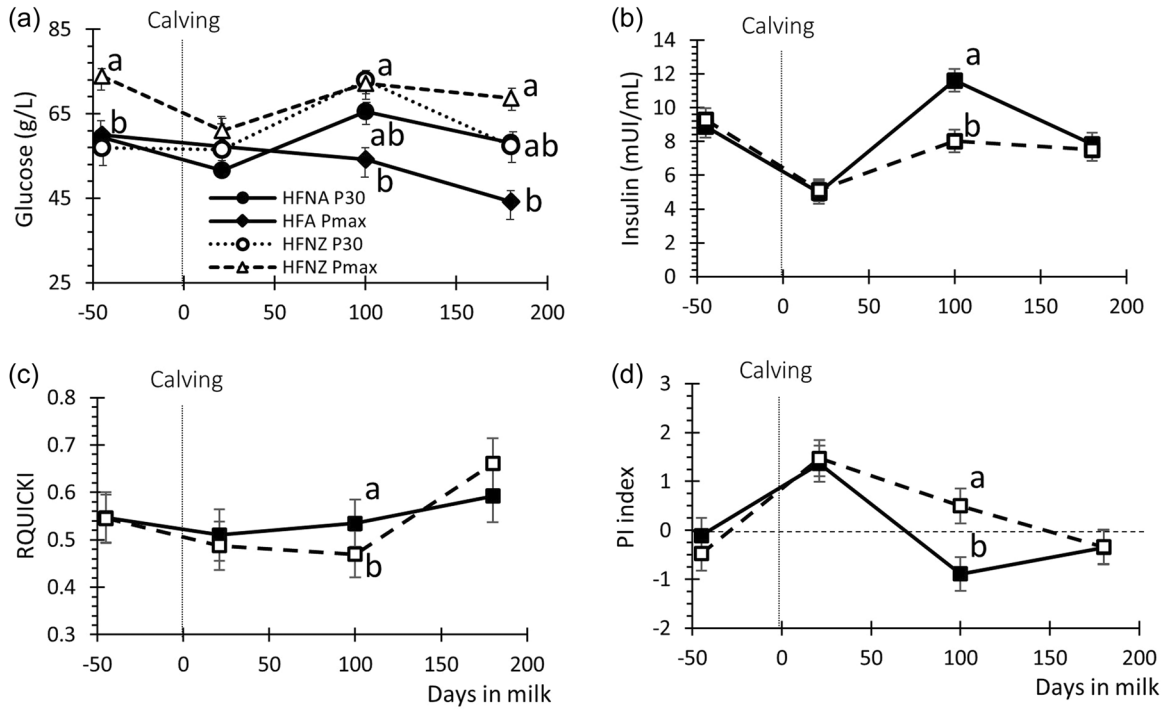

FIGURE 3 Plasma concentrations of glucose (a), insulin (b) and metabolic indexes RQUICKI (c) and PI (d). In panel a, glucose concentrations are presented according to the triple interaction between genetic strain, feeding strategy and days in milk (DIM). For panel b–d, data is presented according to the interaction between different feeding strategy (full black: P30; empty: PMAX) and DIM. Significative differences ($p \leq 0.05$) between groups at any given time are depicted with different letters.

$p < 0.01$). The plasma concentration 3-MH had a positive correlation with BCS ($r = 0.41$, $p < 0.01$), and negative with plasma NEFA, BHB ($r = -0.20$, $p = 0.02$ in both cases) and albumin ($r = -0.34$, $p < 0.01$). Plasma cholesterol was negatively correlated with BCS, and positively with α-tocopherol ($r = 0.36$, $p < 0.01$). Plasma carbonyls, SOD and GPx were positively correlated between them ($r \geq 0.38$, $p < 0.01$ in all cases).

3.5 | Metabolic trajectory analysis

Metabolic trajectory analysis by PCA showed the more evident metabolic changes likely occurred between −45 and 21 DIM in all cows as indicated by the longer distance between their respective ellipses in the score plot (Figure 7). Specifically, in PMAX, the score plots revealed some overlap between −45, 21, 100 and 180 data for NZH cows, while for NAH data regarding −45 and 21 DIM was clearly separated with data at 100 and 180 being likely closer to 21 than −45 DIM (Figures 7b, 7d).

4 | DISCUSSION

Several studies reported until now have demonstrated a significant effect of the interaction genotype × environment on productive, reproductive and metabolic parameters when comparing NAH versus NZH under different feeding strategies (Chagas et al., 2009; Lucy et al., 2009; Roche et al., 2006; Stirling et al., 2021). Physiological studies have addressed differences between these GS at the level of glucose metabolism and adipose tissue mobilisation sustained by differences in somatotropic uncoupling and insulin resistance during the transition (Chagas et al., 2009; Lucy et al., 2009). As further discussed, in the current work, the results suggested that also protein and redox metabolism are involved in metabolic adaptions to lactation differing between NAH and NZH cows under grazing conditions as NZH cows mobilised lower muscle protein and likely had more reactive redox responses.

4.1 | Genetic strain

The higher FPCM yield together with the trend for a lower BCS observed for NAH cows throughout the experiment are in agreement with the genetic selection focused on individual milk yields applied during decades on this genetic strain (Brito et al., 2021; Harris & Kolver, 2001; Horan et al., 2005). The higher milk production in NAH cows should be sustained by a higher DMI in particular with the advance of lactation as previously reported (Sheahan et al., 2011). In fact, data obtained in the same animals at the individual level of the cow revealed that metabolic energy intake (MEI), expressed as $kJ/LW^{0.75}$, was similar between GS at 115 DIM, but it was higher for NAH than NZH at 192 DIM (Talmón et al., 2020). In addition, feed intake estimated at the herd level indicated higher DMI for NAH than

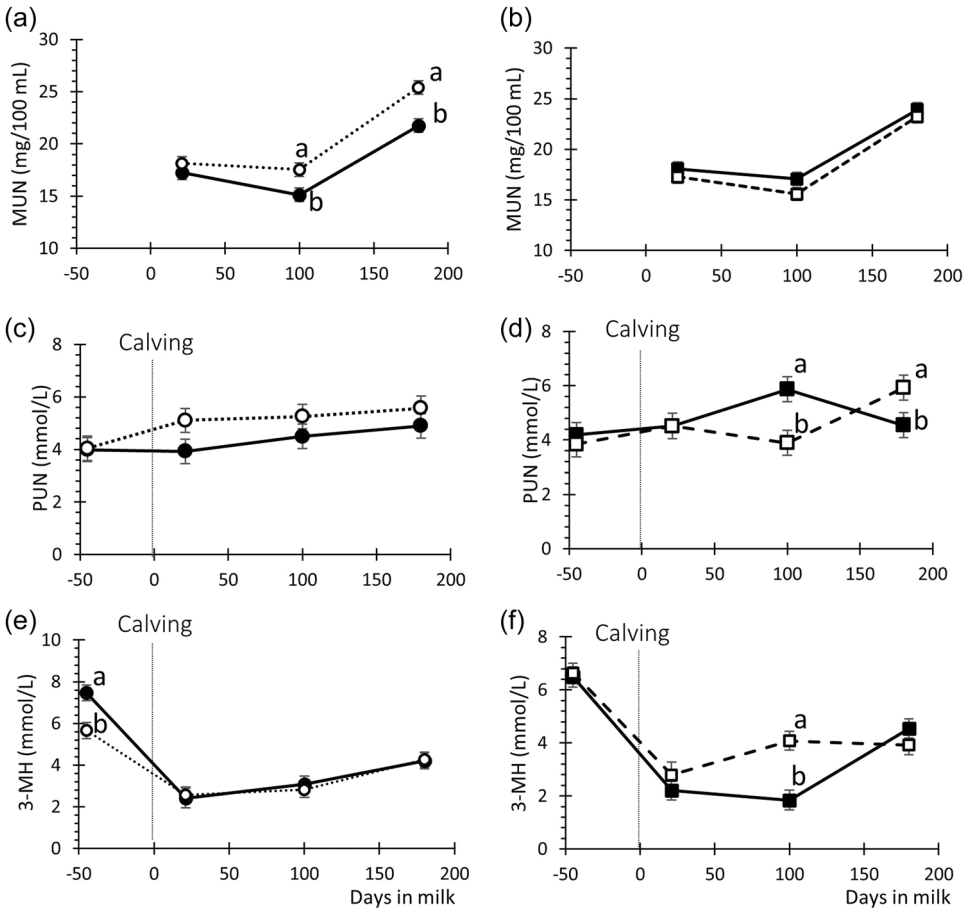

FIGURE 4 Concentrations of milk urea nitrogen (MUN), plasma urea (PUN) and 3-methyilhistidine (3MH) for North-American and New Zealand (a, c and e, respectively) and different feeding strategies (FS) (b, d and f, respectively) across lactation. Genetic strains (GS) are depicted by circles (full black: NAH; empty: NZH), and FS by squares (full black: P30; empty: PMAX). Significant differences ($p \leq 0.05$) between GS or FS at any given time are denoted by different letters. NAH, North American Holstein; NZH, New Zealand Holstein.

NZH cows along the lactation for the current experiment (Stirling et al., 2021).

In disagreement with previously reported data comparing NAH versus NZH dairy cows, adipose tissue mobilisation did not seem to have differed as BCS changes, and plasma concentrations of NEFA and BHB across time were similar between GS. However, the higher FPCM yield in NAH cows probably led to a worse metabolic status compared to NZH cows as denoted by the lower glucose and insulin observed in the NAH cows (Bjerre-Harpøth et al., 2012). These differences were exacerbated in the PMAX, especially for glucose which was always higher for NZH than NAH except at 21 DIM, indicating more challenging conditions for the NAH cows as grazing activity was increased (Billa et al., 2020; Kolver et al., 2000). Furthermore, according to the PI index, the NAH cows displayed a higher physiological imbalance compared with NZH cows, suggesting that NAH cows had a higher risk of metabolic diseases (Moyes et al., 2013), especially when grazing activity was maximised (PMAX). Interestingly, plasma cholesterol recovery after calving, which has been associated with shorter intervals from calving to conception (Reist et al., 2003), was greater for NZH than NAH cows. In this sense, for the current experiment on the basis of 2 years data, Stirling et al. (2021) reported lower services per conception and a higher pregnancy rate to first service for NZH than NAH cows, especially in PMAX. Taken together, our results would suggest these GS displayed different homeorhetic and homoeostatic responses that were more evident as grazing activity was increased possibly associated with the reduced capacity of feed intake of NAH versus NZH cows when grazing is maximised. Interestingly, the energy intake (per unit of metabolic LW) was higher for NAH versus NZH in mid lactation in a pasture-based feeding strategy with supplement, (Talmón et al., 2020), but lower when these strains were compared under grazing with no supplementation (Talmón et al., 2020). Indeed, previous studies demonstrated that in pasture-based diets with increasing levels of concentrates, the substitution rate is lower for NAH than NZH cows meaning that NAH cows have a better ability of get an improved diet as the diet include lower levels of grazed pastures (Baudracco et al., 2010).

Interestingly, the elevated concentrations of 3-MH previous to calving indicated that all cows were mobilising labile protein at that time (Houweling et al., 2012) possibly due to a state of amino acids

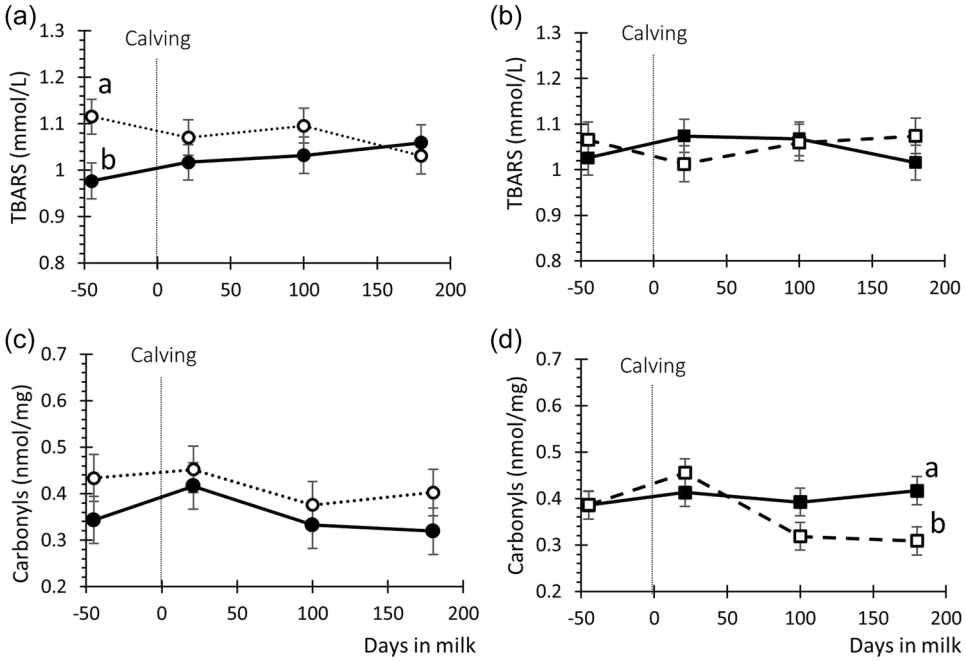

FIGURE 5 Plasma concentrations of thiobarbituric acid reactive species (TBARS) and protein carbonyls for North-American and New Zealand cows (a, c) and different feeding strategies (FS) (b, d, respectively) across lactation. Genetic strains (GS) are depicted by circles (full black: NAH; empty: NZH), and FS by squares (full black: P30; empty: PMAX). Significant differences ($p \leq 0.05$) between GS at any given time are denoted by different letters. NAH, North American Holstein; NZH, New Zealand Holstein.

TABLE 3 Oxidative metabolism variables according to the interaction between genetic strain and feeding strategy

	Average mean					*p* value					
	P30		Pmax								
	NAH	NZH	NAH	NZH	SEM	GS	FS	DIM	GS × FS	GS × DIM	FS × DIM
TBARS (mmol MDA/L)	1.02	1.07	1.02	1.08	0.06	0.17	0.87	0.86	0.87	0.06	0.16
Carbonyls (nmol DNPH/mg TP)	0.39	0.42	0.32	0.41	0.15	0.10	0.33	0.20	0.38	0.64	0.09
SOD (mU/mg TP)	48.2	49.9	75.9	71.8	1.2	0.60	0.01	0.04	0.16	0.10	0.10
GPx (mU/mg TP)	2.66	2.36	2.55	2.39	0.28	0.83	0.76	0.27	0.42	0.22	0.83
α-Tocopherol (μg/ml)	2.24	1.99	2.8	2.88	0.46	0.79	0.03	0.01	0.62	0.10	0.05

Note: Different letters mean significant differences ($p \leq 0.05$) according to Tukey test. Variables were not affected by the triple interaction between GS, FS and DIM ($p > 0.10$ in all cases)

Abbreviations: α-ToDIM, days in milk; DNPH, Dinitrophenylhydrazine; FS, feeding strategy; GPx, glutathione peroxidase; GS, genetic strain; MDA, malondialdehyde; NAH, Northa American Holstein; NZH, New Zealand Holstein; P30, feeding strategy P30; PMAX, feeding strategy with maximised grazing activity; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive species; TP, total protein.

deficiency previous to the massive mobilisation of adipose tissue in early lactation (van der Drift et al., 2012). However, despite all cows were likely mobilising labile protein at −45 DIM, the higher concentrations observed in the NAH cows indicated that skeletal muscle catabolism was probably enhanced in these cows compared to NZH cows. Considering that all cows were offered a TMR ration *ad libitum* during the dry off and prepartum period, enhanced muscle catabolism in the NAH should not be associated with nutrient ingestion but with their lower circulating insulin leading to a weaker inhibition of the proteolysis in the peripheral tissue (van der Drift et al., 2012). In addition, the higher PUN and MUN observed in the NZH cows might be indicative of a lower nitrogen use efficiency, probably due to greater nitrogen absorption as a consequence of higher DMI per unit of LW when compared to NAH cows (Talmón et al., 2022). These results may be related to frame differences (Aikman et al., 2008) as lower nitrogen use efficiency in lower-sized cows (Kauffman & St-Pierre, 2001) have been related to a better distribution of the ingestion across the day, and differences in ruminal daily kinetics as a consequence of relative lower mouth and bite sizes (Aikman et al., 2008). In agreement with our previous findings of different amino acid plasma profile for the current NAH versus NZH cows at early lactation (Jorge-Smeding et al., 2021),

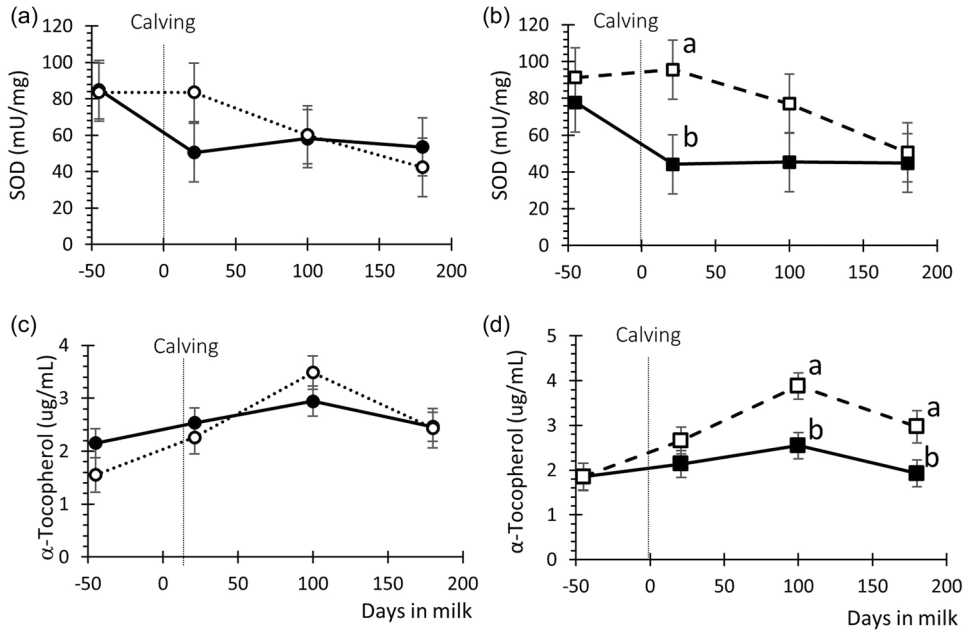

FIGURE 6 Plasma activity of superoxide dismutase (SOD) and plasma concentration of α-tocopherol for North-American and New Zealand cows (a, c) and different feeding strategies (FS) (b, d) across lactation. Genetic strains (GS) are depicted by circles (full black: NAH; empty: NZH), and FS by squares (full black: P30; empty: PMAX). Significant differences ($p \leq 0.05$) between GS at any given time are denoted by different letters.

these results indicate that not only glucose and lipid metabolism can be affected by the Holstein GS, but protein metabolism could also be comprised within the metabolic adaptations, at least when comparing NAH versus NZH cows under grazing conditions.

Redox variables responses seemed to suggest that higher oxidative damage joint with enhanced antioxidant responses could be related with adaptive differences among different genotypes. In the present work, higher TBARS concentrations previous to calving, together with the trend for higher plasma carbonyls across lactation observed in NZH cows, could suggest a higher ROS production in these animals and thus a higher oxidative load in these animals (Ceci et al., 2015; Laubenthal et al., 2017). In this sense, the higher adiposity of NZH (as revealed by their tendency for a higher BCS) could be associated with increased lipid oxidative damage (Laubenthal et al., 2017). However, in the current study the NZH likely mobilised adipose tissue in a similar rate than their NAH counterparts while other studies suggested that plasma TBARS are more related with adipose mobilisation (and so BCS change) rather than the adiposity level itself (Bernabucci et al., 2005). On the other hand, higher oxidative damage of lipids and proteins accounting for increased ROS production could be hypothetically associated with a higher immune activation in the NZH cows during transition since ROS are secreted by neutrophils and phagocytes during the immune response leading to oxidative stress that can be properly managed by the animal (Celi & Gabai, 2015). In this sense it interesting to note that NZH cows seemed to have not only greater oxidative damage, but also tended to have a high SOD activity for longer during lactation, and had a higher recovery of α-tocopherol during mid-lactation than NAH cows. Although the differences among GS were weak, our results might suggest that NZH had a higher redox reactivity given by increased oxidative damage and antioxidant response. Interestingly, previous studies reported increased GPx and SOD activity in a local-breed of cows adapted to mountain grazing compared to non-adapted breeds and it was suggested that a more reactive redox metabolism (higher oxidative damage and antioxidant response) could constitute a structural mechanism of adaptation in cattle subjected to challenging conditions (Marco-Ramell et al., 2012). However, these results need to be confirmed as the observed differences between GS were consistent but not strong, possibly due to the low number of animals. Thus, new studies are warranted to properly establish if higher redox reactivity is associated with a differential adaptive capacity among distinct genotypes (breeds, GS).

4.2 | Feeding strategy

The higher FPCM yield observed in PMAX cows on average during early lactation, could be associated with the higher consumption of pasture in detriment of forage reserves and concentrates leading to a slightly better nutritional value (higher content of metabolisable energy and protein) in PMAX than P30 (Stirling et al., 2021). However, PMAX cows decreased their milk yield to similar production levels as P30 cows at mid-lactation, and this was associated with higher plasma NEFA, and lower insulin and PUN concentrations, suggesting a decrease in DMI in the PMAX relative to P30 cows (Billa et al., 2020; Cavestany et al., 2009).

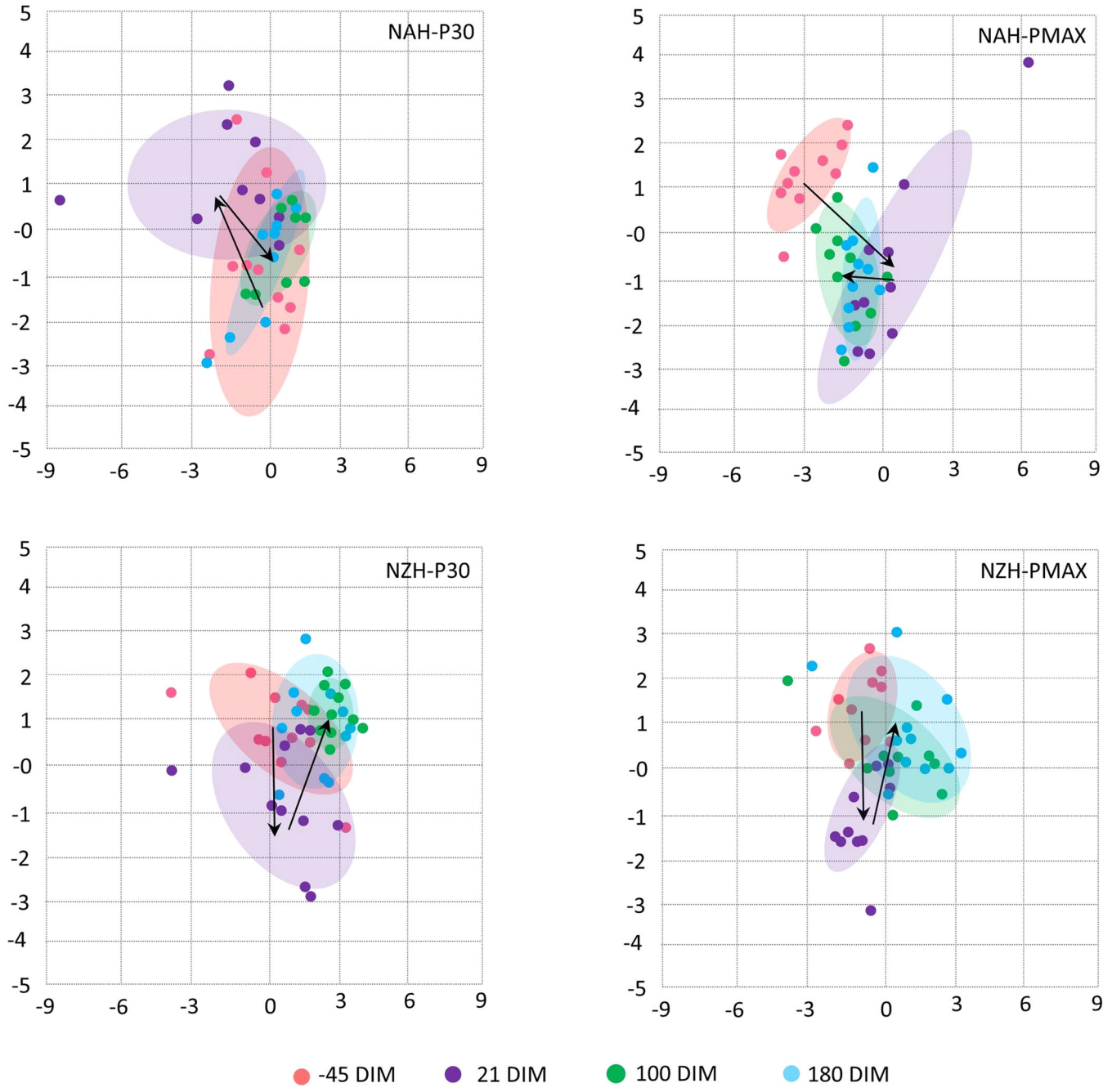

FIGURE 7 Metabolic trajectory for North-American cows in P30 (a) and PMAX (b), and New Zealand cows in P30 (c) and PMAX (d) according to score plots of principal component analysis. The ellipses indicate 95% confidence. [Color figure can be viewed at wileyonlinelibrary.com]

Furthermore, the increase of 3-MH observed in PMAX cows at this time would indicate a new stage of labile protein catabolism (van der Drift et al., 2012). Despite labile protein mobilisation has been postulated to be circumscribed to the onset of lactation, in grazing conditions DMI is very constrained in winter due to grass shortfalls (Fariña & Chilibroste, 2019), and it could determine muscle mobilisation in mid-lactation cows triggered by a nutrient deficit. Interestingly, PMAX cows had a higher recovery of plasma cholesterol during mid lactation which is likely in agreement with previous reported data showing increasing plasma cholesterol when comparing increasing pasture allowance in the diet (Meikle et al., 2013). However, more studies are needed to properly address this aspect.

The lower plasma carbonyls at 100 and 180 DIM indicated that protein oxidation decreased with the advance of lactation for PMAX cows (Tsiplakou et al., 2017), possibly associated with the higher activity of SOD after calving in these cows, leading to a more effective counteraction of pro-oxidants. In fact, plasma carbonyls were positively correlated with GPx and SOD activities, and negatively with the α-tocopherol concentrations, which

pointed out that higher oxidative load was associated with enhanced antioxidant response (Pedernera et al., 2010). It has been widely suggested that increased GPx activity after calving would be the result of an activated state of gene expression given by the increased oxidative load (Gessner et al., 2013). Moreover, the higher recovery of α-tocopherol in PMAX cows at mid-lactation could be a consequence of lower oxidative damage at this time and, thus, reduced waste of exogenous antioxidants due to higher antioxidant response during early lactation (higher SOD activity) compared with P30. However, higher α-tocopherol recovery could be also related to a higher intake of α-tocopherol as pasture intake was higher for PMAX than P30 cows in mid-to-late-lactation (Kay et al., 2005).

4.3 | Integrated metabolic trajectories

The PCA score plots reflected the great metabolic load associated with the onset of lactation as the greatest displacement were observed between −45 and 21 DIM as denoted in the score plot by the longer distances between −45 and 21 DIM ellipses, compared to 100 and 180 DIM. Regarding the interaction between the GS and FS, it is worth to note that in PMAX the NAH cows during lactation seemed to had a slower progression toward their initial state at −45 DIM compared with NZH cows which showed some overlap across time, even between −45 and 21 DIM ellipses. This could suggest that NAH cows had a weaker ability of metabolically recover after calving which could be associated with a lower metabolic resilience (Friggens et al., 2017). Interestingly, despite the results obtained through PCA are semiquantitative, they seem to agree with preliminary results of a quantitative analysis of the metabolic trajectories of the current cows also pointing out a higher metabolic perturbation after calving for the NAH than NZH, in particular when compared in PMAX (Jorge-Smeding, Carriquiry, Naya, et al., 2021). Despite the semiquantitative character of the PCA, our results suggest this analysis could be an easy to use and interpret tool for integrating metabolic variables in addition to classical analysis of variance applied separately to each variable.

5 | CONCLUSIONS

When compared to NAH, the NZH cows developed better metabolic responses to cope with grazing conditions as suggested by their better energy status (glucose, insulin and cholesterol) and lower risk of developing metabolic disorder as suggested by their lower PI, especially when compared in PMAX. Furthermore, metabolic adaptive differences between NAH and NZH grazing cows seemed to comprise not only energy but also lower muscle catabolism and higher redox sensibility in the NZH cows. Despite it was assessed by a semiquantitative approach, our results suggest that NZH had a higher metabolic recovery after calving when compared with NAH, particularly when grazing activity was maximised (PMAX). Regarding the FS, the higher inclusion of grazed pastures in the diet led to similar milk yield but it likely determined a worse energy status and increased muscle catabolism in mid-lactation during winter. Also, maximising grazing likely improved the redox metabolism leading to lower oxidative damage of proteins and enhanced antioxidant status. More studies are warranted in order to better establish the role of protein and redox metabolism in relationship with the metabolic adaptive capacity of cows to pasture-based dairy system.

AUTHOR CONTRIBUTIONS

Ezequiel Jorge-Smeding formulated the research question and animal sampling, carried out laboratory analyses, performed data curation, data analysis and writing of the original draft and revised versions. Mariana Carriquiry and Ana L. Astessiano participated in the formulation of the research question, earned the funding and supervised the data analyses, interpretation and the reviewing-and-editing process. Diego Armand-Ugon participated during the field experiment in sampling and productive data management. Alberto Casal participated in field sampling and laboratory analyses. Mauricio Mastrogiovanni and Andrés Trotschansky participated in laboratory analyses. Alejandro Mendoza conceptualised, conceived and conducted the field experimental platform. All authors were involved in reviewing the original draught and approved the manuscript.

ACKNOWLEDGEMENTS

The authors thank the staff of the Experimental Station 'La Estanzuela' from Instituto Nacional de Investigación Agropecuaria (Uruguay) for their help during the fieldwork and sample collection. This research was partially funded by the CSIC I + D Research Programme (Udelar) awarded to M. Carriquiry, and by the Agencia Nacional de Investigación e Innovación (Uruguay) through the graduate scholarship awarded to E. Jorge-Smeding (POS_NAC_2017_1_141119). This study was carried out in compliance with the ARRIVE guidelines, and the European Union (EU) standards on the protection of animals used for scientific purposes. All procedures were approved by the Ethics Committee on Animal Experimentation of INIA (form #INIA 2017.2).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Ezequiel Jorge-Smeding http://orcid.org/0000-0002-3964-8619

REFERENCES

Aikman, P. C., Reynolds, C. K., & Beever, D. E. (2008). Diet digestibility, rate of passage, and eating and rumination behavior of Jersey and Holstein cows. *Journal of Dairy Science*, *91*(3), 1103–1114. https://doi.org/10.3168/jds.2007-0724

Astessiano, A. L., Meikle, A., Fajardo, M., Gil, J., Mattiauda, D. A., Chilibroste, P., & Carriquiry, M. (2015). Metabolic and endocrine profiles and hepatic gene expression of Holstein cows fed total mixed ration or pasture with different grazing strategies during early lactation. *Acta Veterinaria Scandinavica*, *57*(1), 70. https://doi.org/10.1186/s13028-015-0163-6

Basset-Mens, C., Ledgard, S., & Boyes, M. (2009). Eco-efficiency of intensification scenarios for milk production in New Zealand.

Ecological Economics, *68*(6), 1615–1625. https://doi.org/10.1016/j.ecolecon.2007.11.017

Baudracco, J., Lopez-Villalobos, N., Holmes, C., & Macdonald, K. (2010). Effects of stocking rate, supplementation, genotype and their interactions on grazing dairy systems: A review. *New Zealand Journal of Agricultural Research*, *53*(2), 109–133. https://doi.org/10.1080/00288231003777665

Bell, A. W. & Bauman, D. E. (1997). Adaptations of lugcose metabolism during pregnancy and lactation. *Journal of Mammary Gland Biology and Neoplasia*, *2*(3), 265–278. https://doi.org/10.1023/A:1026336505343

Bernabucci, U., Ronchi, B., Lacetera, N., & 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*(6), 2017–2026. https://doi.org/10.3168/jds.S0022-0302(05)72878-2

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*(4), 3133–3146. https://doi.org/10.3168/jds.2019-17466

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*(5), 2362–2380. https://doi.org/10.3168/jds.2011-4419

Brito, L. F., Bedere, N., Douhard, F., Oliveira, H. R., Arnal, M., Peñagaricano, F., Schinckel, A. P., Baes, C. F., & Miglior, F. (2021). Review: Genetic selection of high-yielding dairy cattle toward sustainable farming systems in a rapidly changing world. *Animal*, *15*, 100292. https://doi.org/10.1016/j.animal.2021.100292

Cavestany, D., Kulcsár, M., Crespi, D., Chilliard, Y., La Manna, A., Balogh, O., Keresztes, M., Delavaud, C., Huszenicza, G., & Meikle, A. (2009). Effect of prepartum energetic son productive and reproductive characteristics, and metabolic and hormonal profiles in dairy cows under grazing conditions. *Reproduction in Domestic Animals*, *44*(4), 663–671. https://doi.org/10.1111/j.1439-0531.2007.01044.x

Ceci, R., Duranti, G., Sgrò, P., Sansone, M., Guidetti, L., Baldari, C., Sabatini, S., & Di Luigi, L. (2015). Effects of tadalafil administration on plasma markers of exercise-induced muscle damage, IL6 and antioxidant status capacity. *European Journal of Applied Physiology*, *115*(3), 531–539. https://doi.org/10.1007/s00421-014-3040-5

Celi, P., & Gabai, G. (2015). Oxidant/antioxidant balance in animal nutrition and health: The role of protein oxidation. *Frontiers in Veterinary Science*, *2*, 48. https://doi.org/10.3389/fvets.2015.00048

Chagas, L. M., Lucy, M. C., Back, P. J., Blache, D., Lee, J. M., Gore, P. J., Sheahan, A. J., & Roche, J. R. (2009). Insulin resistance in divergent strains of Holstein-Friesian dairy cows offered fresh pasture and increasing amounts of concentrate in early lactation. *Journal of Dairy Science*, *92*(1), 216–222. https://doi.org/10.3168/jds.2008-1329

Delaby, L., Faverdin, P., Michel, G., Disenhaus, C., & Peyraud, J. L. (2009). Effect of different feeding strategies on lactation performance of Holstein and Normande dairy cows. *Animal*, *3*(6), 891–905. https://doi.org/10.1017/S1751731109004212

Dillon, P., Berry, D. P., Evans, R. D., Buckley, F., & Horan, B. (2006). Consequences of genetic selection for increased milk production in European seasonal pasture based systems of milk production. *Livestock Science*, *99*(2–3), 141–158. https://doi.org/10.1016/j.livprodsci.2005.06.011

Edmonson, A. J., Lean, I. J., Weaver, L. D., Farver, T., & Webster, G. (1989). A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science*, *72*(1), 68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0

Fariña, S. R., & Chilibroste, P. (2019). Opportunities and challenges for the growth of milk production from pasture: The case of farm systems in Uruguay. *Agricultural Systems*, *176*, 102631. https://doi.org/10.1016/j.agsy.2019.05.001

Friggens, N. C., Blanc, F., Berry, D. P., & Puillet, L. (2017). Review: Deciphering animal robustness. A synthesis to facilitate its use in livestock breeding and management. *Animal*, *11*(12), 2237–2251. https://doi.org/10.1017/S175173111700088X

Gessner, D. K., Schlegel, G., Keller, J., Schwarz, F. J., Ringseis, R., & Eder, K. (2013). Expression of target genes of nuclear factor E2-related factor 2 in the liver of dairy cows in the transition period and at different stages of lactation. *Journal of Dairy Science*, *96*(2), 1038–1043. https://doi.org/10.3168/jds.2012-5967

Harris, B. L., & Kolver, E. S. (2001). Review of holsteinization on intensive pastoral dairy farming in New Zealand. *Journal of Dairy Science*, *84*, E56–E61. https://doi.org/10.3168/jds.S0022-0302(01)70197-X

Holtenius, P., & Holtenius, K. (2007). A model to estimate insulin sensitivity in dairy cows. *Acta Veterinaria Scandinavica*, *49*(1), 29. https://doi.org/10.1186/1751-0147-49-29

Horan, B., Dillon, P., Faverdin, P., Delaby, L., Buckley, F., & Rath, M. (2005). The interaction of strain of Holstein-Friesian cows and pasture-based feed systems on milk yield, body weight, and body condition score. *Journal of Dairy Science*, *88*(3), 1231–1243. https://doi.org/10.3168/jds.S0022-0302(05)72790-9

Horn, M., Steinwidder, A., Starz, W., Pfister, R., & Zollitsch, W. (2014). Interactions between calving season and cattle breed in a seasonal Alpine organic and low-input dairy system. *Livestock Science*, *160*, 141–150. https://doi.org/10.1016/j.livsci.2013.11.014

Houweling, M., van der Drift, S. G. A., Jorritsma, R., & Tielens, A. G. M. (2012). Technical note: Quantification of plasma 1- and 3-methylhistidine in dairy cows by high-performance liquid chromatography–tandem mass spectrometry. *Journal of Dairy Science*, *95*(6), 3125–3130. https://doi.org/10.3168/jds.2011-4769

Jorge-Smeding, E., Carriquiry, M., Cantalapiedra-Hijar, G., Mendoza, A., & Astessiano, A. L. (2021). Plasma concentrations of branched-chain amino acids differ with Holstein genetic strain in pasture-based dairy systems. *Scientific Reports*, *11*(1), 22414. https://doi.org/10.1038/s41598-021-01564-0

Jorge-Smeding, E., Carriquiry, M., Naya, H., Mendoza, A., & Astessiano, A. L. (2021). A new integrative mathematic approach to study metabolic trajectories of grazing dairy cows. *Book of Abstracts* (625). Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-918-3

Kauffman, A. J., & St-Pierre, N. R. (2001). The Relationship of Milk Urea Nitrogen to urine nitrogen excretion in Holstein and Jersey cows. *Journal of Dairy Science*, *84*(10), 2284–2294. https://doi.org/10.3168/jds.S0022-0302(01)74675-9

Kay, J. K., Roche, J. R., Kolver, E. S., Thomson, N. A., & Baumgard, L. H. (2005). A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows. *Journal of Dairy Research*, *72*(3), 322–332. https://doi.org/10.1017/S0022029905000944

Kolver, E. S., & Muller, L. D. (1998). Performance and nutrient Intake of high producing Holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science*, *81*(5), 1403–1411. https://doi.org/10.3168/jds.S0022-0302(98)75704-2

Kolver, E. S., Napper, A. R., Copeman, P. A. J., & Muller, L. D. (2000). A comparison of New Zealand and overseas Holstein Friesian heifers. *Proceedings of the New Zealand Society of Animal Production*, *60*, 265–269.

Laubenthal, L., Ruda, L., Sultana, N., Winkler, J., Rehage, J., Meyer, U., Dänicke, S., Sauerwein, H., & Häussler, S. (2017). Effect of increasing body condition on oxidative stress and mitochondrial biogenesis in subcutaneous adipose tissue depot of nonlactating dairy cows. *Journal of Dairy Science*, *100*(6), 4976–4986. https://doi.org/10.3168/jds.2016-12356

Liang, Y., Cardoso, F. F., Parys, C., Cardoso, F. C., & Loor, J. J. (2021). Branched-chain amino acid supplementation alters the abundance of mechanistic target of rapamycin and insulin signaling proteins in subcutaneous adipose explants from lactating Holstein cows. *Animals: An Open Access Journal from MDPI*, *11*(9), 2714. https://doi.org/10.3390/ani11092714

Littell, R. C., Milliken, G., Stroup, W., Wolfinger, R., & Schabenberger, O. (2006). *SAS for mixed models* (2nd ed.). Cary, N.C: SAS Institute, Inc.

Lucy, M. C. (2001). Reproductive loss in high-producing dairy cattle: Where will it end? *Journal of Dairy Science*, *84*(6), 1277–1293. https://doi.org/10.3168/jds.S0022-0302(01)70158-0

Lucy, M. C., Verkerk, G. A., Whyte, B. E., Macdonald, K. A., Burton, L., Cursons, R. T., Roche, J. R., & Holmes, C. W. (2009). Somatotropic axis components and nutrient partitioning in genetically diverse dairy cows managed under different feed allowances in a pasture system. *Journal of Dairy Science*, *92*(2), 526–539. https://doi.org/10.3168/jds.2008-1421

Marco-Ramell, A., Arroyo, L., Saco, Y., García-Heredia, A., Camps, J., Fina, M., Piedrafita, J., & Bassols, A. (2012). Proteomic analysis reveals oxidative stress response as the main adaptative physiological mechanism in cows under different production systems. *Journal of Proteomics*, *75*(14), 4399–4411. https://doi.org/10.1016/j.jprot.2012.04.002

Meikle, A., de Lourdes Adrien, M., Mattiauda, D. A., & Chilibroste, P. (2013). Effect of sward condition on metabolic endocrinology during the early postpartum period in primiparous grazing dairy cows and its association with productive and reproductive performance. *Animal Feed Science and Technology*, *186*(3–4), 139–147. https://doi.org/10.1016/j.anifeedsci.2013.10.003

Méndez, M. N., Chilibroste, P., & Aguerre, M. (2020). Pasture dry matter intake per cow in intensive dairy production systems: Effects of grazing and feeding management. *Animal*, *14*(4), 846–853. https://doi.org/10.1017/S1751731119002349

Moyes, K. M., Larsen, T., & Ingvartsen, K. L. (2013). Generation of an index for physiological imbalance and its use as a predictor of primary disease in dairy cows during early lactation. *Journal of Dairy Science*, *96*(4), 2161–2170. https://doi.org/10.3168/jds.2012-5646

NRC. (2001). *Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001* (p. 9825). National Academies Press. https://doi.org/10.17226/9825

Østergaard, S., Sørensen, J. T., & Houe, H. (2003). A stochastic model simulating milk fever in a dairy herd. *Preventive Veterinary Medicine*, *58*(3–4), 125–143. https://doi.org/10.1016/S0167-5877(03)00049-7

Pedernera, M., Celi, P., García, S. C., Salvin, H. E., Barchia, I., & Fulkerson, W. J. (2010). Effect of diet, energy balance and milk production on oxidative stress in early-lactating dairy cows grazing pasture. *The Veterinary Journal*, *186*(3), 352–357. https://doi.org/10.1016/j.tvjl.2009.09.003

Rivero, M. J., Urioste, J. I., Astigarraga, L., & Rovere, G. (2012). Objetivos de selección para un sistema productive lechero basado en pastures, utilizando un modelo de simulación bioeconómico. *Archivos Latinoamericanos de Producción Animal*, *20*(1–2), 37–46.

Roche, J. R., Berry, D. P., & Kolver, E. S. (2006). Holstein-Friesian strain and feed effects on milk production, body weight, and body condition score profiles in grazing dairy cows. *Journal of Dairy Science*, *89*(9), 3532–3543. https://doi.org/10.3168/jds.S0022-0302(06)72393-1

Rodríguez-Bermúdez, R., Miranda, M., Orjales, I., Rey-Crespo, F., Muñoz, N., & López-Alonso, M. (2017). Holstein-Friesian milk performance in organic farming in North Spain: Comparison with other systems and breeds. *Spanish Journal of Agricultural Research*, *15*(1), e0601. https://doi.org/10.5424/sjar/2017151-10037

Schweigert, F. J., Klingner, J., Hurtienne, A., & Zunft, H. J. (2003). Vitamin A, carotenoid and vitamin E plasma concentrations in children from Laos in relation to sex and growth failure. *Nutrition Journal*, *2*(1), 17. https://doi.org/10.1186/1475-2891-2-17

Sheahan, A. J., Kolver, E. S., & Roche, J. R. (2011). Genetic strain and diet effects on grazing behavior, pasture intake, and milk production. *Journal of Dairy Science*, *94*(7), 3583–3591. https://doi.org/10.3168/jds.2010-4089

Stirling, S., Delaby, L., Mendoza, A., & Fariña, S. (2021). Intensification strategies for temperate hot-summer grazing dairy systems in South America: Effects of feeding strategy and cow genotype. *Journal of Dairy Science*, *104*(12), 12647–12663. https://doi.org/10.3168/jds.2021-20507

Talmón, D., Garcia-Roche, M., Mendoza, A., Mattiauda, D. A., & Carriquiry, M. (2020). Energy partitioning and energy efficiency of two Holstein genotypes under a mixed pasture-based system during mid and late lactation. *Livestock Science*, *239*, 104166. https://doi.org/10.1016/j.livsci.2020.104166

Talmón, D., Mendoza, A., & Carriquiry, M. (2022). Holstein strain affects energy and feed efficiency in a grazing dairy system. *Animal Production Science*, *62(8)*, 765–773. https://doi.org/10.1071/AN20587

Tsiplakou, E., Chatzikonstantinou, M., Mitsiopoulou, C., Karaiskou, C., Mavrommatis, A., Sotirakoglou, K., Labrou, N., & Zervas, G. (2017). Effect of soya bean and fish oil inclusion in diets on milk and plasma enzymes from sheep and goat related to oxidation. *Journal of Animal Physiology and Animal Nutrition*, *101*(4), 733–742. https://doi.org/10.1111/jpn.12516

van der Drift, S. G. A., Houweling, M., Schonewille, J. T., Tielens, A. G. M., & Jorritsma, R. (2012). Protein and fat mobilization and associations with serum β-hydroxybutyrate concentrations in dairy cows. *Journal of Dairy Science*, *95*(9), 4911–4920. https://doi.org/10.3168/jds.2011-4771

VanRaden, P. M., Sanders, A. H., Tooker, M. E., Miller, R. H., Norman, H. D., Kuhn, M. T., & Wiggans, G. R. (2004). Development of a national genetic evaluation for cow fertility. *Journal of Dairy Science*, *87*(7), 2285–2292. https://doi.org/10.3168/jds.S0022-0302(04)70049-1

Webb, L. A., Sadri, H., Schuh, K., Egert, S., Stehle, P., Meyer, I., Koch, C., Dusel, G., & Sauerwein, H. (2020). Branched-chain amino acids: Abundance of their transporters and metabolizing enzymes in adipose tissue, skeletal muscle, and liver of dairy cows at high or normal body condition. *Journal of Dairy Science*, *103*(3), 2847–2863. https://doi.org/10.3168/jds.2019-17147

Wehrens, R. (2011). *Chemometrics with R*. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-17841-2

Wernicki, A., Urban-Chmiel, R., Puchalski, A., & Tokarzewsky, S. (2006). Evaluation of plasma cortisol and TBARS levels in calves after short term transportation. *Revue Médecine Véterinaire*, *157*(1), 30–34.

Zhang, A., Sun, H., Sun, W., Jiao, G., & Wang, X. (2013). Trajectory analysis of metabolomics profiling in liver injured rats using ultra-performance liquid chromatography coupled with mass spectrometry. *Analytical Methods*, *5*(19), 5294. https://doi.org/10.1039/c3ay40578h

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Jorge-Smeding, E., Carriquiry, M., Casal, A., Armand-Ugon, D., Mastrogiovanni, M., Trotschansky, A., Mendoza, A., & Astessiano, A. L. (2022). Energy, protein and redox metabolism underlying adaptive responses in New Zealand versus North American Holstein cows in pasture-based dairy systems. *Journal of Animal Physiology and Animal Nutrition*, 1–15. https://doi.org/10.1111/jpn.13766