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Responses of selected plasma metabolites to a two-day nutritional challenge of goats divergently selected for functional longevity

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ABSTRACT

Understanding the extent to which genetics \times environment plays a role in shaping individual strategies to environmental challenges is of considerable interest for future selection of more resilient animals. Accordingly, the objective of this study was to evaluate the metabolic responses to a nutritional challenge of goats divergently selected for functional longevity based on plasma metabolites and the repeatability of these responses across 2 experimental farms and years. We carried out 6 different experimental trials from years 2018 to 2022 (4 trials on site Bourges (2018–21) and 2 trials (2021–22) on site Grignon) in which 267 first kidding goats, daughters of Alpine bucks divergently selected for functional longevity, longevity plus ($n = 137$), and longevity minus ($n = 130$), were exposed to a 2-d nutritional challenge in early lactation. The experiments consisted of a 5 or 7-d control period (pre-challenge) on a standard lactation diet followed by a 2-d nutritional challenge with straw-only feeding and then a 7 or 10-d recovery period on a standard lactation diet, for site Bourges and Grignon, respectively. During the challenge plasma metabolite composition was recorded daily. Linear mixed-effects models were used to analyze all traits, considering the individual as a random effect and the 2x2 treatments (i.e., genetic line and year nested in site) and litter size as fixed effects. The linear mixed-effects model using a piecewise arrangement was used to analyze the response/recovery profiles to the nutritional challenge. Random parameters estimated for each individual, using the mixed-effects models without the fixed effects of genetic line, were used in a Sparse Partial Least Square Discriminant Analysis (sPLS-DA) to compare the goat metabolism response to the challenge on a multivariate scale. The plasma metabolites, glucose, β -hydroxybutyrate (BHB), and nonesterified fatty

acids (NEFA), and urea concentrations responded to the 2-d nutritional challenge. Selection for functional longevity did not affect plasma glucose, NEFA, BHB, and urea response/recoveries to a 2-d nutritional challenge. However, site, trial, and litter size affected these responses. Moreover, the plasma metabolites seem not to fully recover to prechallenge levels after the recovery phase. The sPLS-DA analysis did not discriminate between the 2 longevity lines. We observed meaningful between-individuals' variability in plasma BHB, especially on the prechallenge and rate of response and rate of recovery from the 2-d nutritional challenge (CV = 26.2%, 36.1%, and 41.2%, repeatability = 0.749, 0.322, and 0.741, respectively). Plasma NEFA recovery from challenge also demonstrated high between-individuals' variability (CV = 16.4%, repeatability = 0.323). Selection for functional longevity did not affect plasma metabolites responses to a 2-d nutritional challenge in dairy goats. Plasma NEFA and BHB response/recovery presented high between-individuals' variability, indicating individual adaptive characteristics to nutritional challenges not related to the environmental conditions but to inherent individual characteristics.

Key words: resilience, energy metabolism, adaptive capacities, divergent selection, biomarkers

INTRODUCTION

The functional longevity of farm animals relies in part on their capacity to overcome or adapt to environmental challenges (Saviotto et al., 2013). The longer the animal stays functional in the herd, the more efficient the herd becomes because the non-productive young phase of life gets diluted by a longer productive lifespan. Dairy goats can have a long productive life, with some individuals reaching up to 8 lactation cycles in the Alpine breed. However, the average lactation number is 3.22 ± 1.9 parities (Arnal et al., 2018). Also, it has been suggested that animals with higher resilience have a longer productive lifespan because they are less likely to fail when facing environmental challenges

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(Poppe et al., 2020). In this context, it is likely that the goat's ability to cope with environmental disturbances is an underlying component of its ability to stay in the herd for a long time and increase herd efficiency.

Among the adaptive strategies to cope with environmental disturbances, the increase of lipomobilization and protein catabolism during short-term nutritional challenges have been described (Ndibualonji et al., 1997; Friggens et al., 2016; Billa et al., 2020). These strategies are both relevant to guarantee energy for basal functions, for offspring, and to alleviate oxidative stress during undernutrition. Therefore, it is likely that the ability of a goat to cope with environmental disturbances is related to energy metabolism and body reserves dynamics (Saviotto et al., 2015). Friggens et al. (2016) have demonstrated the diversity of individual metabolic responses to environmental challenge under experimental conditions using plasma metabolites related to body reserves mobilization. This suggests a genetic role on individual adaptive strategies to environmental disturbances. One way to assess this role is to select lines of individuals divergent on functional longevity and challenge them against environmental disturbances. However, studies evaluating this are scarce, even if the approach is promising (Gindri et al., 2022a; b).

Some studies have demonstrated environmental effects on metabolism of energy and body reserves dynamics, suggesting an environmental role on individuals' adaptive strategies to environmental disturbances (Hales and Barker, 2001; Cameron et al., 2005). Moreover, recent studies have demonstrated that individuals divergently selected for functional longevity present different body reserve mobilization dynamics during the early lactation (Ithurbide et al., 2022), and clusters of adaptive types to a nutritional challenge are associated with longevity (Ithurbide et al., 2023). Therefore, understanding the extent to which genetics \times environment plays a role in shaping these individual strategies is of considerable interest for future selection of more resilient animals. Accordingly, the objective of this study was to evaluate the metabolic responses to a nutritional challenge of goats divergently selected for functional longevity, and the repeatability of responses across 2 experimental farms and years.

MATERIALS AND METHODS

Experiment, animals, diet, and treatments

All procedures performed on animals were approved by the Ethics Committee on Animal Experimentation and the French Ministry of Higher Education, Research and Innovation (APAFIS#8613–2017012013585646

V4 and APAFIS#24314–2019120915403741, for sites Bourges and Grignon, respectively). Six experiments were carried out, with the objective to measure time-series measurements of plasma metabolites linked to nutritional status and body reserves mobilization, in dairy goats exposed to a 2-d nutritional challenge in early lactation (38 ± 8 d in milk corresponding to the body reserve mobilization phase of lactation). The experiments consisted of a 5 or 7-d control period (hereafter called pre-challenge) followed by a 2-d nutritional challenge with straw-only feeding and then a 7 or 10-d recovery from challenge, for the sites of Bourges and Grignon, respectively. The 2-d challenge was used because it had previously been tested in goats (Friggens et al., 2016). It induced significant perturbations in performance and metabolism and has been demonstrated to be useful in previous studies for characterizing individual differences in animal responses to a nutritional challenge (Friggens et al., 2016). It fits well with equivalent studies done in dairy cattle (4-d challenge with a 60% straw dilution of the diet) (Bjerre-Harpøth et al., 2012). We used a group of 267 first kidding goats, daughters of Alpine bucks divergently selected for functional longevity (**LGV**), longevity plus (LGV +; $n = 137$), and longevity minus (LGV -; $n = 130$). Considering our study focused on understanding and identifying proxies that can be used to elaborate strategies for future selection of more resilient individuals based on plasma metabolites linked to body reserves, the beginning of first lactation is the highly recommended period to identify differences in body reserve dynamics during early life.

The experiments were carried out in 2 different experimental INRAE farms, Bourges (coordinates: 47.059, 2.510) and Grignon (coordinates: 48.840, 1.951), France. The 2 locations are close, around 200 km apart, with a semi-oceanic climate. In Bourges, experiments took place in 2018 ($n = 21$ LGV- and 13 LGV+), 2019 ($n = 35$ LGV- and 27 LGV+), 2020 ($n = 21$ LGV- and 29 LGV+) and 2021 ($n = 21$ LGV- and 28 LGV+). In Grignon, experiments took place in 2021 ($n = 17$ LGV- and 18 LGV+) and 2022 ($n = 15$ LGV- and 22 LGV+). Average characteristics of animals during pre-challenge for each year and site are presented in Table 1. In Grignon, goats were fed with a total mixed ration (TMR) given ad libitum 2 times a day, whereas in Bourges goats had hay available ad libitum and access to an automatic concentrate feeder. In Grignon, the standard TMR (on a DM basis) consisted of 23% of hay, 29% dried lucerne, 27% beet pulp, 20% lactation concentrate (Fluivialac (Agralys Aliment, Châteaudun, France)), 0.50% minerals, and 0.50% bicarbonate. In Bourges, the diet was composed of alfalfa hay plus a standard lactation concentrate. The 2 lines were created at the INRAE experimental facility with the sci-

entific input of GenPhyse in close collaboration with Capgènes, the French AI center for goats. The average estimated lifespan of the 35 bucks that sired the LGV- and LGV+ lines is of 909 (± 651) and of 1071 (± 722) days, respectively (Ithurbide et al., 2022). Moreover, Ithurbide et al., (2022) compared the observed survival of the 2 lines with a cox model that allowed to estimate the life expectancy, i.e., LGV- of 787 d and LGV+ 830 d. The average effect of line over all life stages was significant ($P = 0.005$), with the LGV+ having a decreased risk of culling (hazard ratio 0.63; CI = 0.465; 0.864).

Blood samples were collected from jugular veins daily, during the whole experiment (13 d for Bourges and 17 d for Grignon), using evacuated tubes containing EDTA (1.95 mg/mL; Terumo Europe NV, Leuven, Belgium). Samples were centrifuged for 10 min at $3,000 \times g$ at 4°C , and the plasma was conserved at -20°C until analysis for urea (mM), glucose (mM), β -hydroxybutyrate (BHB; mM), and nonesterified fatty acids (NEFA; $\mu\text{mol/L}$). For the plasma samples recorded at INRAE farm Bourges, the plasma samples were analyzed using an automatic analyzer (Arena 20XT, Thermo Fisher Scientific, Cergy Pontoise, France) with commercial kits for glucose (glucose oxidase method; mM), BHB (d- β -hydroxybutyrate-dehydrogenase method; mM), urea (glutamate dehydrogenase method; Thermo Electron SAS, Courtaboeuf Cedex, France; mM), and NEFA (acylCoA synthase method; Fujifilm Wako Chemicals Europe GmbH, Neuss, Germany; $\mu\text{mol/L}$). For the

plasma samples recorded at INRAE farm Grignon, the plasma samples were analyzed using a Cobas Mira-Analyzer (Roche, Mannheim, Germany) with commercial kits for urea (11489364216), glucose (GL364), NEFA (FA115), and BHB (RB1007).

Statistical analysis

The plasma metabolite concentrations, recorded daily throughout the whole experiment, were first analyzed using a piecewise approach, in which the response trait was represented by different functions over specific time intervals according to biological responses to the challenge, as proposed and validated by Friggens et al. (2016). That is, we decomposed the response trait into 3 different phases throughout the experiments. The first phase was the pre-challenge, during which the response variable was not perturbed. For this phase, the piecewise model has the overall intercept of the model (V1). The second phase was the response to the 2-d challenge, during which the individuals are subjected to the 2-d nutritional challenge. For this phase, the piecewise model has the parameter V2 which gives the linear rate of response per unit time from the time the challenge starts. The third phase was the recovery from challenge, during which individuals were no longer subjected to the 2-d nutritional challenge. For this phase, the piecewise model has the parameters V3 and V4. As proposed by Friggens et al. (2016), the recovery phase is nonlinear, and a quadratic model can represent this phase. The main reasons for using the piecewise model to explore this kind of experimental data are: first the simplicity; second, the possibility of considering all experimental days in the model parameterization (given the limited number of days recorded in each phase, especially during the response to challenge, it would not be statistically credible to fit a model for each stage separately); and, third the possibility of extracting features that can be easily comparable across individuals, traits, and studies (i.e., pre- and post-challenge levels, rates of response and recovery).

The piecewise approach is based on adding effects. For this, the time variable was expressed as days from the challenge and segmented into 2 time-variables that represent the periods of response and recovery from the challenge and used in the model as regressors for V2, V3, and V4. For this, we fitted the following piecewise mixed-effects model:

$$Y_{ijkl} = V1_{i^{*j+h}} + V2^{*}T_{2i^{*j+h}} + V3^{*}T_{3i^{*j+h}} + V4^{*}T_{3i^{*j+h}}^2 + V1_k + V2^{*}T_{2k} + V3^{*}T_{3k} + V4^{*}T_{3k}^2 + e_{ijkl} \quad [1]$$

Table 1. Description of the six experiments in the data set used to access dairy goats' metabolic responses during nutritional challenge

| | n | DIM ¹ , days | BW ² , kg | MY ² , kg |
|---------|----|-------------------------|----------------------|----------------------|
| Bourges | | | | |
| 2018 | | | | |
| LGV- | 21 | 41.13 \pm 7.22 | 41.8 \pm 4.99 | 2.65 \pm 0.42 |
| LGV+ | 13 | 40.31 \pm 7.37 | 41.4 \pm 4.77 | 2.72 \pm 0.46 |
| 2019 | | | | |
| LGV- | 35 | 41.88 \pm 16.34 | 44.4 \pm 8.68 | 3.05 \pm 0.72 |
| LGV+ | 27 | 43.44 \pm 16.92 | 44.2 \pm 6.29 | 2.78 \pm 0.54 |
| 2020 | | | | |
| LGV- | 21 | NA | 45.2 \pm 6.69 | 3.21 \pm 0.75 |
| LGV+ | 29 | NA | 45.6 \pm 6.91 | 3.27 \pm 0.60 |
| 2021 | | | | |
| LGV- | 21 | NA | 43.0 \pm 6.07 | 2.92 \pm 0.62 |
| LGV+ | 28 | NA | 45.9 \pm 5.08 | 2.90 \pm 0.55 |
| Grignon | | | | |
| 2021 | | | | |
| LGV- | 17 | 37.00 \pm 5.47 | 51.15 \pm 6.64 | 3.28 \pm 0.44 |
| LGV+ | 18 | 36.39 \pm 5.51 | 46.18 \pm 5.79 | 3.01 \pm 0.5 |
| 2022 | | | | |
| LGV- | 15 | 40.07 \pm 7.37 | 49.03 \pm 8.8 | 2.57 \pm 0.84 |
| LGV+ | 22 | 39.73 \pm 6.94 | 45.20 \pm 5.61 | 2.73 \pm 0.67 |

¹DIM: Days in milk at the beginning of the pre-challenge period.

²BW and MY during the prechallenge, 5 or 7 d before the feed restriction, for Bourges and Grignon sites, respectively.

where Y_{ijkl} is the dependent variable, V_1 is the model intercept, T_2 , and T_3 are the 2 time-variables that represent the periods during the response to challenge (T_2) and recovery from challenge (T_3). V_2 , V_3 , and V_4 are the regression coefficients for response and recovery. $i*j+h$ are the fixed effects of genetic line i , year nested in site (site/year) j , and litter size (i.e., single or multiple) h , k is the random effect of animal estimated for V_1 , V_2 , V_3 , and V_4 that are assumed to be $\sim iidN(0, \sigma_k^2)$, and e_{ijkl} is the residual error ($e_{ijkl} \sim N(0, R)$, with R as the heterogenous autoregressive of order 1 error covariance structure, used to correct for lack of independence in the residual and to correct for heterogeneity of variance. The interaction genetic line and site/year was tested in the model as a fixed effect. The piecewise models were fitted using the *lme* function and variance components of all evaluated plasma metabolites were recorded using *VarCorr* function of *nlme* package (Pinheiro and Bates, 2000) of software R (R Core Team, 2022). Statistical significance was set at $P \leq 0.05$.

Contrasts on the models' parameters, using general hypothesis testing, function *glht* of package *multcomp* (Hothorn et al., 2008) of software R (R Core Team, 2022) were used to evaluate differences between sites within year 2021 and among years within site. Contrasts were also used to compare the stabilization period i.e., post-challenge after recovery (**V5**), to prechallenge level (**V1**). **V5** is derived from the other coefficients as shown in eqn 2. The contrasts to compare post-challenge level to prechallenge level (**V5-V1**) were set according to the following equation:

$$V5-V1 = (V1 + V2 \times 2 + V3 \times 4 + V4 \times 4^2) - V1 \quad [2]$$

The previously described piecewise mixed-effects model was also fitted for all plasma metabolites without considering the fixed effect of genetic line on all piecewise parameters (V_1 , V_2 , V_3 , and V_4). The resulting 4 individual random parameters of all the metabolites were then integrated in a Sparse Partial Least Square Discriminant Analysis (sPLS-DA) (Lê Cao et al., 2011) to compare the goats metabolic response to the challenge on a multivariate scale. The sPLS-DA seeks the components, built as linear composition of the individual parameters, that best predict the longevity line of the goats. We chose the number of components using a 5-fold cross validation on a model that comprised all variables and then tuned the number of variables to select on each component. The variable selection on each component was run using lasso penalization. We finally assessed the quality of the prediction of the model by

calculating a balanced error rate of the model within a 5-fold cross validation.

RESULTS

The plasma metabolites, glucose, NEFA, BHB, and urea concentrations responded to the 2-d nutritional challenge ($P < 0.001$; Figure 1). Glucose concentration decreased, while NEFA and BHB increased during the nutritional challenge. Plasma urea concentrations decreased during the nutritional challenge for site Bourges, regardless of year, and increased for the experiments run at the site Grignon. On average, none of the metabolites returned to the pre-challenge level 2 weeks after the nutritional challenge ($P < 0.01$).

The interaction genetic line and (site/year) was not significant for all of the evaluated traits ($P \geq 0.21$). The evaluated plasma metabolites suggested that the selection for functional longevity does not affect plasma metabolites responses and recoveries to a 2-d nutritional challenge ($P \geq 0.18$; Table 2). However, site, year, and litter size seem to affect these responses (Table 2). The responses and recoveries of all plasma metabolites were different between sites within year 2021 ($P \leq 0.02$; Table 2). In general, the responses and recoveries of plasma glucose, NEFA, and BHB were similar among years within the site Grignon but different among years within the site Bourges (Table 2). Goats that had carried one fetus presented a faster plasma glucose response to challenge and a quicker and shaper recovery from challenge than goats that had carried multiple fetus ($P \leq 0.054$; Table 2). However, the opposite was observed for the other evaluated plasma metabolites (i.e., NEFA, BHB, and urea; $P \leq 0.049$; Table 2). Moreover, the plasma metabolites seem not to fully recover to prechallenge levels after the recovery phase ($P < 0.01$). Plasma glucose and urea post-challenge levels were above prechallenge levels and the opposite was observed for plasma NEFA and BHB.

When the individual response and recovery metabolic profiles were studied taking into account their interconnections using the multivariate approach, the sPLS-DA analysis was also not able to discriminate between the 2 longevity lines (52.2% of balanced error rate; Figure 2). This result was obtained even if the sPLS-DA analysis summarized almost 50% of the total between-individuals' variability into the 2 selected components. Plasma BHB response and recovery to the 2-d nutritional challenge were the traits selected for the first selected component, that explained 29% of total between-individuals' variability. This indicates that plasma BHB have the highest between-individuals' variability when compared with other traits in our data set. Plasma NEFA response and recovery also demon-

strated high variability among individuals as shown by their great contribution to the second selected component.

The results of sPLS-DA analysis (Figure 3) also corroborate the results of the variance components from the piecewise mixed-effects models. We observed meaningful between-individuals' variability in plasma BHB, especially on the prechallenge, and rate of response and rate of recovery from the 2-d nutritional challenge (CV = 26.2%, 36.1%, and 41.2%, repeatability = 0.749, 0.322, and 0.741, respectively; Table 3; Figure 4). Plasma NEFA recovery from challenge also demonstrated high between-individuals' variability (CV = 16.4%, repeatability = 0.323; Table 3). These results suggest

plasma metabolites related to body reserves dynamics and liver fatty acid oxidation are highly variable among individuals especially during the phase of recovery from a 2-d nutritional challenge.

DISCUSSION

The adaptive capacity of animals to overcome environmental challenges relies on their capacity to adjust metabolism. The use of body lipid reserves is one of the key strategies of domestic ruminants to cope with feed shortage situations (Chilliard et al., 2000). Our results showed an increase in blood NEFA concentration during the nutritional challenge and a drop in

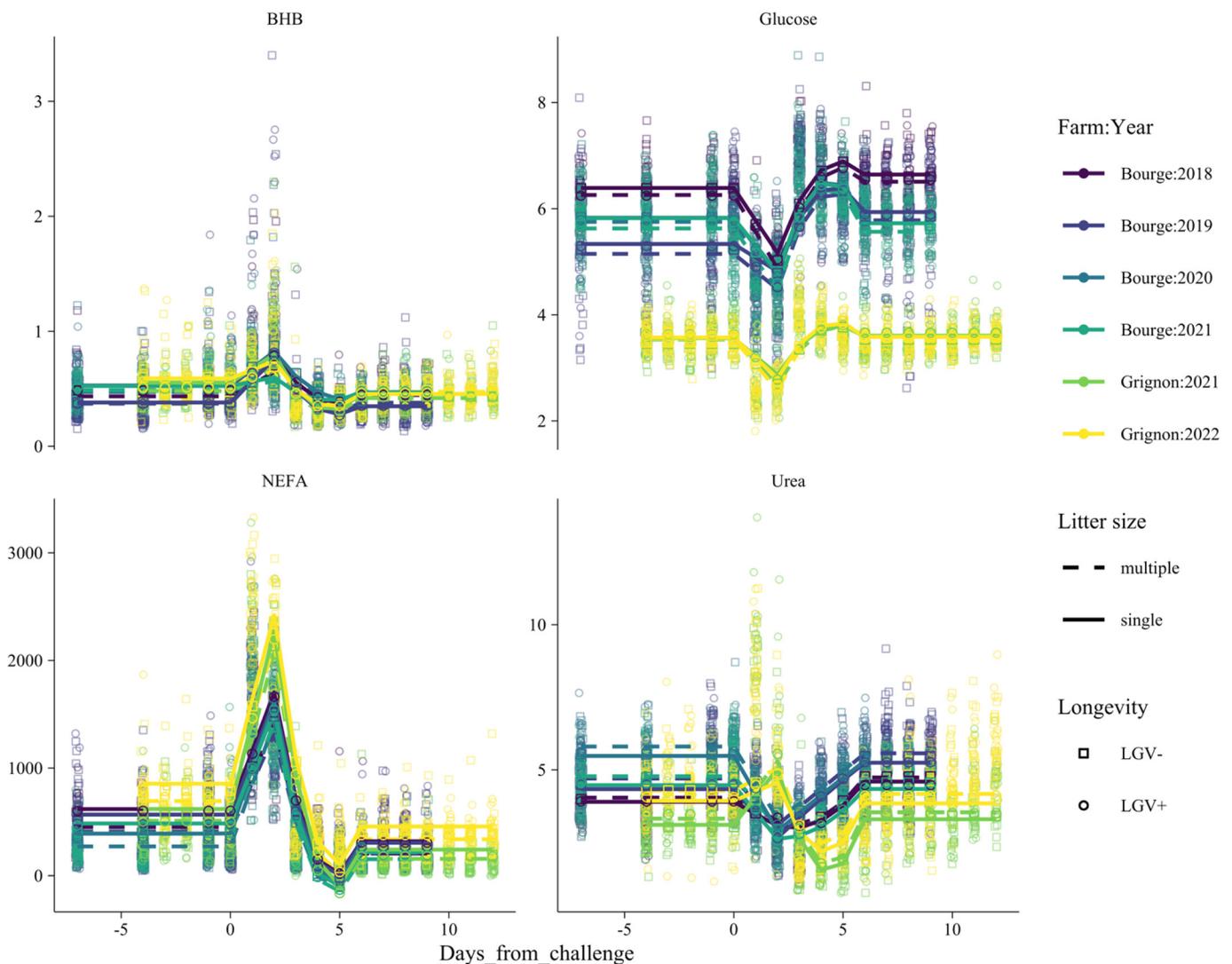


Figure 1. Plasma metabolites concentration average time trends (Glucose (mM), nonesterified fatty acids (NEFA; $\mu\text{mol/L}$), Urea (mM), and B-hydroxybutyrate (BHB; mM)) following nutritional challenge of first lactation Alpine goats from 2 divergent genetic lines, according to lifespan duration, longevity plus (LGV+) and longevity minus (LGV-) evaluated across 6 years in 2 different sites (Bourges and Grignon). Symbol and line represent observed and predicted trajectory from the fixed part of the fitted mixed-effects model only, respectively.

Table 2. Plasma metabolite average values for pre-challenge level, rates of response during challenge, and recovery post-challenge for a two-day nutritional challenge in first lactation Alpine goats from two divergent lines, according to functional lifespan duration, longevity plus (LGV +) and longevity minus (LGV -) and evaluated across six years in two different sites (Bourges and Grignon)

| Trait | LGV - | LGV + | Bourges | | | | | | Grignon | | | | SEM | Genetic line | Litter size | Site/year | P-value | |
|---|--------|--------------------|---------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|--------------|-------------|-----------|---------|------|
| | | | 2018 | 2019 | 2020 | 2021 | 2021 | 2021 | 2021 | 2022 | 2022 | 2022 | | | | | | 2022 |
| Glucose | | | | | | | | | | | | | | | | | | |
| Pre-challenge level (mM) | 5.01 | 5.07 | 6.34 ^a | 5.23 ^c | 5.79 ^b | 5.74 ^{Ab} | 3.57 ^{Ba} | 0.0786 | 0.180 | 0.012 | <0.001 | |
| Rate of response (mM/d) | -0.471 | -0.516 | -0.668 ^b | -0.311 ^b | -0.594 ^a | -0.555 ^{Aa} | -0.396 ^{Ba} | 0.0591 | 0.219 | 0.017 | <0.001 | |
| Rate of recovery (mM/d) | 1.24 | 1.18 | 1.23 ^b | 1.32 ^b | 1.58 ^a | 1.54 ^{Ab} | 0.73 ^{Ba} | 0.1151 | 0.422 | 0.020 | <0.001 | |
| Rate of deceleration in recovery (mM/d ²) | -0.241 | -0.223 | -0.209 ^b | -0.252 ^b | -0.322 ^a | -0.320 ^{Aa} | -0.130 ^{Ba} | 0.0250 | 0.285 | 0.054 | <0.001 | |
| NEFA | | | | | | | | | | | | | | | | | | |
| Pre-challenge level (μmol/L) | 527 | 519 | 536 ^a | 490 ^{ab} | 329 ^c | 441 ^{Bb} | 569 ^{Ab} | 36.74 | 0.718 | <0.001 | <0.001 | |
| Rate of response (μmol/L/d) | 613 | 611 | 510 ^b | 492 ^b | 589 ^a | 517 ^{Bb} | 803 ^{Aa} | 35.36 | 0.951 | 0.037 | <0.001 | |
| Rate of recovery (μmol/L/g) | -1274 | -1281 | -1136 ^a | -1084 ^a | -1165 ^a | -1159 ^{Ba} | -1565 ^{Aa} | 71.20 | 0.881 | 0.010 | <0.001 | |
| Rate of deceleration in recovery (μmol/L/d ²) | 225 | 228 | 203 ^a | 195 ^a | 212 ^a | 209 ^{Ba} | 268 ^{Aa} | 14.98 | 0.775 | 0.027 | <0.001 | |
| BHB | | | | | | | | | | | | | | | | | | |
| Pre-challenge level (mM) | 0.495 | 0.480 | 0.481 ^a | 0.370 ^b | 0.500 ^a | 0.512 ^{Aa} | 0.0262 | 0.391 | 0.005 | <0.001 | |
| Rate of response (mM/d) | 0.100 | 0.113 ^a | 0.119 ^{ab} | 0.188 ^a | 0.135 ^b | 0.032 ^{Bc} | 0.103 ^{Aa} | 0.0278 | 0.502 | 0.719 | <0.001 | |
| Rate of recovery (mM/d) | -0.248 | -0.266 | -0.262 ^a | -0.323 ^a | -0.222 ^{ab} | -0.142 ^{Bb} | -0.325 ^{Aa} | 0.0433 | 0.538 | 0.202 | <0.001 | |
| Rate of deceleration in recovery (mM/d ²) | 0.0455 | 0.0494 | 0.0491 ^a | 0.0565 ^a | 0.0333 ^{ab} | 0.0293 ^{Bb} | 0.0634 ^{Aa} | 0.00741 | 0.427 | 0.291 | <0.001 | |
| Urea | | | | | | | | | | | | | | | | | | |
| Pre-challenge level (mM) | 4.34 | 4.35 | 3.98 ^c | 4.51 ^b | 5.63 ^a | 4.62 ^{Ab} | 3.20 ^{Bb} | 0.1272 | 0.905 | 0.001 | <0.001 | |
| Rate of response (mM/d) | -0.362 | -0.330 | -0.403 ^c | -0.816 ^b | -1.276 ^a | -0.905 ^{Bb} | 0.975 ^{Aa} | 0.0947 | 0.609 | 0.493 | <0.001 | |
| Rate of recovery (mM/d) | -0.685 | -0.784 | -0.246 ^b | 0.657 ^a | 0.587 ^a | -0.066 ^{Bb} | -3.055 ^{Aa} | 0.1732 | 0.384 | 0.358 | <0.001 | |
| Rate of deceleration in recovery (mM/d ²) | 0.233 | 0.249 | 0.155 ^a | -0.007 ^b | 0.001 ^b | 0.120 ^{Ba} | 0.655 ^{Aa} | 0.0368 | 0.508 | 0.437 | <0.001 | |

^{AaBbC} Upper-case letters mean differences between sites within year 2021 and lower-case letters mean differences among years within site ($P \leq 0.05$).

The time trends were estimated using a piecewise mixed-effects model considering the fixed effects of genetic line, litter size (i.e., single or multiple), year nested in site (site/year) and the random effect of individual.

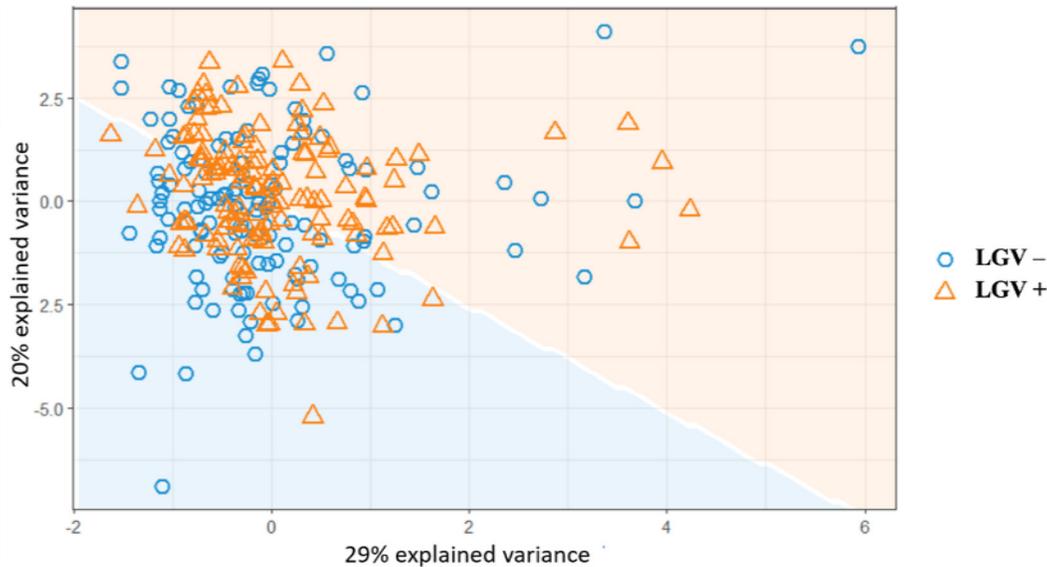


Figure 2. Scores plot from the Sparse Partial Least Square Discriminant Analysis applied to the random parameters from the piecewise mixed-effects model, adjusted for the effect of litter size and year nested in site, to discriminate 2 lines of goats selected for extreme functional longevity, longevity plus (LGV+) and longevity minus (LGV-), evaluated across 6 years in 2 different sites (Bourges and Grignon)

blood glucose concentration. This indicates an increase of lipolysis during the 2-d nutritional challenge, along with a reduction of fatty acid re-esterification and reduction in glycerol 3-phosphate synthesis from glucose (Dunshea et al., 1990; Forest et al., 2003). During feed shortage, glucose becomes a scarce metabolite for survival and maintaining lactose synthesis and milk production (Bauman, 2000). Along with this, BHB also increased during the nutritional challenge demonstrating incomplete β -oxidation of mobilized NEFA by the liver, which is used as an oxidable substrate in specific tissues to spare blood glucose (Heitmann et al., 1987). Similar results were also demonstrated by studies with dairy cows and dairy goats during early, mid, and late lactation (Bjerre-Harpøth et al., 2012; Friggens et al., 2016; Billa et al., 2020).

However, in disagreement with our hypothesis of observing different metabolic responses and recoveries between genetic lines, plasma NEFA, BHB, and glucose responses to the nutritional challenge were not different between genetic lines. This was found both for the univariate analysis of the piecewise shapes of response and recovery for each plasma metabolite, and for the multivariate analysis across all plasma metabolites. Thus, although the literature indicates that body energy dynamics are central to adaptive capacity, the use of goats from lines divergently selected on functional longevity did not provide the expected contrast in resilience profiles. Physiological reasons for this and the influence of other factors, such as uncontrolled variability induced

by the difference in management and location between farms, in this study are discussed below.

A study comparing Barbarine and Lacaune ewes constantly fed with low energy supply for 22 weeks demonstrated that barbary ewes, a breed adapted to harsh environments, presented a better ability to maintain a moderate rate of fat mobilization and decrease energy expenditure and keep their higher level of body fatness than Lacaune ewes during same feed restriction (Atti et al., 2004). These findings suggest that there is a distinction to be made between short-term and long-term responses to changes in environmental conditions. In this context, the 2-d nutritional challenge may not be long enough to differentiate the mechanisms related to longer-term body energy dynamics in individuals divergently selected for functional longevity. Monaghan (2008) proposed 3 levels of phenotypic changes triggered by the environment during development changes. Monaghan proposed that within a certain level of environmental change, organisms may be able to adjust their phenotype such that fitness is maintained (plasticity). Outside this range of environmental change, fitness declines as the development of an optimal phenotype is constrained and phenotypic adjustments may mitigate the negative effects on fitness, and trade-offs between traits or across life-history stages may occur. Beyond this 'mitigation zone' pathologies develop, and fitness drops dramatically. In the present study, with the 2 d of nutritional challenge, the focus was on short-term metabolic resilience which may only be one aspect underpinning differences in functional longevity.

Not only body lipids but also body proteins have a role in metabolic adaptations during nutritional deficit. Body protein contributes by providing energy precursors for gluconeogenesis thereby sparing body lipid reserves and alleviating the toxic effects of lipid metabolites during the nutritional challenge. Dairy cows after 2 d of starvation demonstrated increased muscle protein catabolism by increasing blood concentration of 3-methylhistidine (indicator of muscle protein catabolism in cattle) and urea (Ndibualonji et al., 1997). Increased arterial urea concentration during feed restriction has also been demonstrated by other studies with 1–3 d of fasting in dairy cows (Eisemann and Nienaber, 1990; Toerien and Cant, 2007; Girard et al., 2019; Pires et al., 2019). The increase of blood urea during feed restriction can also be related to an increase in urea recycling to the rumen by liver synthesis (Silanikove, 2000). Even with its high cost (7.1–25% of liver oxygen consump-

tion and endogenous amino acids), urea recycling is beneficial to feed efficiency and metabolizable protein supply during feed shortage situations (Huntington and Reynolds, 1987; McBride and Kelly, 1990; Westwood et al., 1998). On the other hand, some studies with dairy cattle and goats have demonstrated a decrease in blood urea concentration during feed restriction. This has been attributed to a lower ammonia load in the rumen due to reduced nitrogen intake (Velez and Donkin, 2005; Billa et al., 2020). In our results, urea responses to the 2 d nutritional challenge were not different between genetic lines, demonstrating that urea metabolism in response to a 2 d nutritional challenge is not different among goats divergently selected for functional longevity. However, the overall urea response and recovery profiles were markedly affected by the site/year effect.

Our results indicated significant differences in metabolic responses across sites and years but with no interaction with genetic line. The statistical model incorporated site and year effects to assess the variability of responses across diverse environments, aiming to determine the suitability of metabolites as biomarkers on farms, and also to reduce statistical noise. This effort aimed to focus on the impacts of genetic lines and litter size rather than extraneous factors. However, we acknowledge the potential presence of unmodeled complex interactions in terms of farm and year effects. Below, we explore potential reasons for the observed variation across different sites and years.

Both climates are semi oceanic, and the locations are close enough (200km) to suggest that the weather is the main explanation of differences between those sites. In Grignon, the individuals were fed ad libitum TMR, 2 times a day, whereas in Bourges they had ad libitum lucerne hay plus concentrates supplied with an automatic feeder. The TMR involves shorter particle size that can impact microbiota activity, and sometimes increase the risk of acidosis even if no clinical acidosis occurred during the experiments. The frequency of the ingestion also has an impact on microbiota activity and rumen pH and could be different depending on the TMR vs automatic feeder. The type of forage could also explain the higher pre and post challenge plasma urea concentrations observed in Bourges compared with Grignon. During challenge, the decrease of nitrogen intake resulted in a strong plasma urea decrease in Bourges, whereas the plasma urea increased in Grignon, linked with the catabolism of body proteins (Hossaini-Hilali et al., 1993; Pascottini et al., 2019; Ansia et al., 2020). The within farms year differences could be linked to hay quality fluctuations, health status fluctuations, or other unrecording events. Moreover, a study has demonstrated a relationship between the environmental conditions, where individuals are raised, and ad-

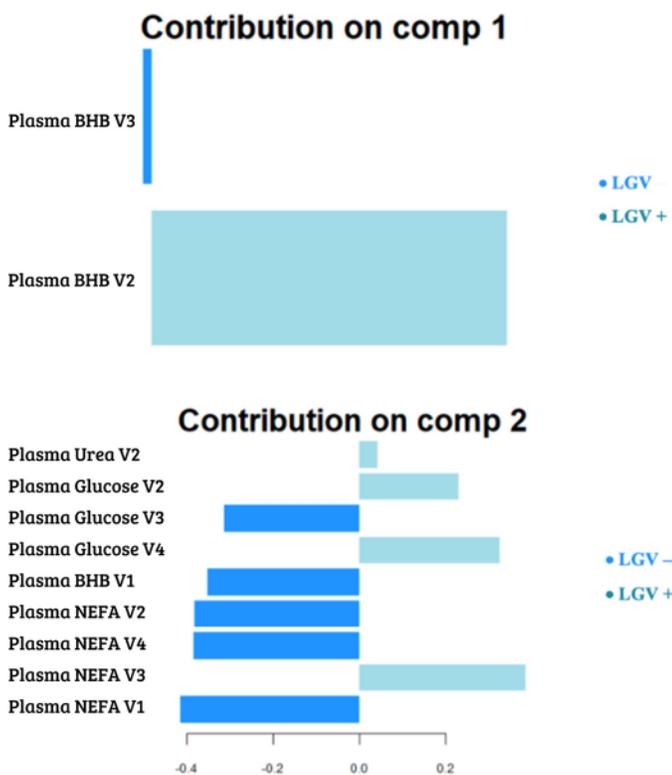


Figure 3. Loading plot of the first 2 principal components (**comp**) from the Sparse Partial Least Square Discriminant Analysis applied to the random parameters from the piecewise mixed-effects model, adjusted for the effect of litter size and year nested in site, to discriminate 2 lines of goats selected for extreme functional longevity, longevity plus (**LGV+**) and longevity minus (**LGV-**), evaluated across 6 years in 2 different sites (Bourges and Grignon). Colors indicate the line in which the median is maximum for each variable (Glucose (mM), nonesterified fatty acids (**NEFA**; $\mu\text{mol/L}$), Urea (mM), and B-hydroxybutyrate (**BHB**; mM) Pre-challenge level (**V1**), Rate of response (**V2**), Rate of recovery (**V3**), Rate of deceleration in recovery (**V4**).

aptative response to environmental challenges during adulthood (Cameron et al., 2005). Rats, deprived of energy during fetal life, showed a pattern of development that favors energy conservation, including high rates of fat accumulation, and an increased capacity for both gluconeogenesis and basal lipolysis in adulthood. In addition, Friggens et al. (2016), evaluated the response profiles of dairy goats to nutritional challenge and demonstrated significant between-individuals' variability in their physiologic responses and recovery. These studies suggest that individual strategies in response to environmental challenges may be impacted by inherent individual characteristics, due to genetics, and in response to early life programming.

Regarding litter size, our results demonstrated plasma glucose and NEFA responses and recoveries to 2-d nutritional challenge was affected by litter size during the previous gestation. Females who had carried one single fetus presented quicker and sharper plasma NEFA recovery from challenge during the subsequent lactation than females who had carried more than one fetus. The opposite was observed for plasma glucose. Litter size has been related to body condition score and plasma glucose and NEFA levels in sheep (González-García et al., 2014, 2015). In general, females who had

carried one single fetus manifested higher body condition score than females who had carried more than one fetus, even after weaning (González-García et al., 2014, 2015). Several studies have demonstrated that the lipolytic potential is regulated according to physiological needs and by the amount of lipid stored; Thin and fat ewes have shown the lipid mobilization during lactation is clearly dependent on initial fatness when they are severely underfed (Cowan et al., 1982; Gilson et al., 1996; Atti and Bocquier, 1999; Pulina et al., 2012). This was also observed in dairy cows (Chilliard, 1992; Pires et al., 2013; Roche et al., 2015).

Even though body energy dynamics, as indicated by plasma NEFA, were not different between genetic lines subjected to a short-term nutritional challenge, literature suggests that there may be other adaptations of goats divergently selected for functional longevity to cope with nutritional disturbances. In rabbits, increased ability for resource acquisition under a constrained environment has been related to longer functional longevity (Savietto et al., 2015). Genetic studies have also found that dairy sheep selected for different levels of somatic cell count had different levels of adipose tissue mobilization (plasma NEFA), fatty acid utilization for energy production (plasma BHB), and fat-to-protein milk

Table 3. Summary statistics for between-individuals' (Indiv.) variability in time trends (variance components of piecewise mixed-effects models considering the fixed effects of genetic line, litter size, and year nested in site) following two-days nutritional challenge of first lactation Alpine goats from two divergent lines, according to functional lifespan duration, longevity plus and longevity minus evaluated across six years in two different sites (Bourges and Grignon)

| Trait | Indiv. variance | Indiv. StdDev | Estim. Param ¹ | Indiv. CV ² | Repeatability ³ |
|---------------------------------|-----------------|---------------|---------------------------|------------------------|----------------------------|
| Glucose | | | | | |
| Pre-challenge level | 0.0867 | 0.294 | 5.04 | 5.84 | 0.173 |
| Rate of response | 0.0084 | 0.092 | -0.493 | 18.6 | 0.0199 |
| Rate of recovery _r | 0.0789 | 0.281 | 1.21 | 23.2 | 0.160 |
| Rate of deceleration in recover | 0.0033 | 0.0572 | -0.232 | 24.7 | 0.00785 |
| Residual | 0.414 | 0.643 | | | |
| NEFA | | | | | |
| Pre-challenge level | 15249 | 123 | 523 | 23.6 | 0.141 |
| Rate of response | 9933 | 100 | 612 | 16.3 | 0.0969 |
| Rate of recovery _r | 44127 | 210 | -1277 | 16.4 | 0.323 |
| Rate of deceleration in recover | 1397 | 37.4 | 226 | 16.5 | 0.0149 |
| Residual | 92558 | 304 | | | |
| BHB | | | | | |
| Pre-challenge level | 0.0160 | 0.126 | 0.482 | 26.2 | 0.749 |
| Rate of response | 0.00254 | 0.050 | 0.140 | 36.1 | 0.322 |
| Rate of recovery _r | 0.0154 | 0.124 | -0.300 | 41.2 | 0.741 |
| Rate of deceleration in recover | 0.000439 | 0.0210 | 0.0544 | 38.5 | 0.0757 |
| Residual | 0.00754 | 0.0869 | | | |
| Urea | | | | | |
| Pre-challenge level | 0.272 | 0.521 | 4.34 | 12.0 | 0.399 |
| Rate of response | 0.0241 | 0.155 | -0.346 | 44.9 | 0.0556 |
| Rate of recovery _r | 0.227 | 0.476 | -0.734 | 64.9 | 0.357 |
| Rate of deceleration in recover | 0.00955 | 0.0977 | 0.241 | 40.6 | 0.0228 |
| Residual | 0.409 | 0.639 | | | |

¹mean across treatments.

²Coefficients of variance (%) were calculated as standard deviation of variance components divided by the respective mean value of the estimated fixed effects across line and diet.

³Repeatability = $\left[\frac{\sigma_{individuals}^2}{\left(\sigma_{individuals}^2 + \sigma_{residual}^2 \right)} \right]$.

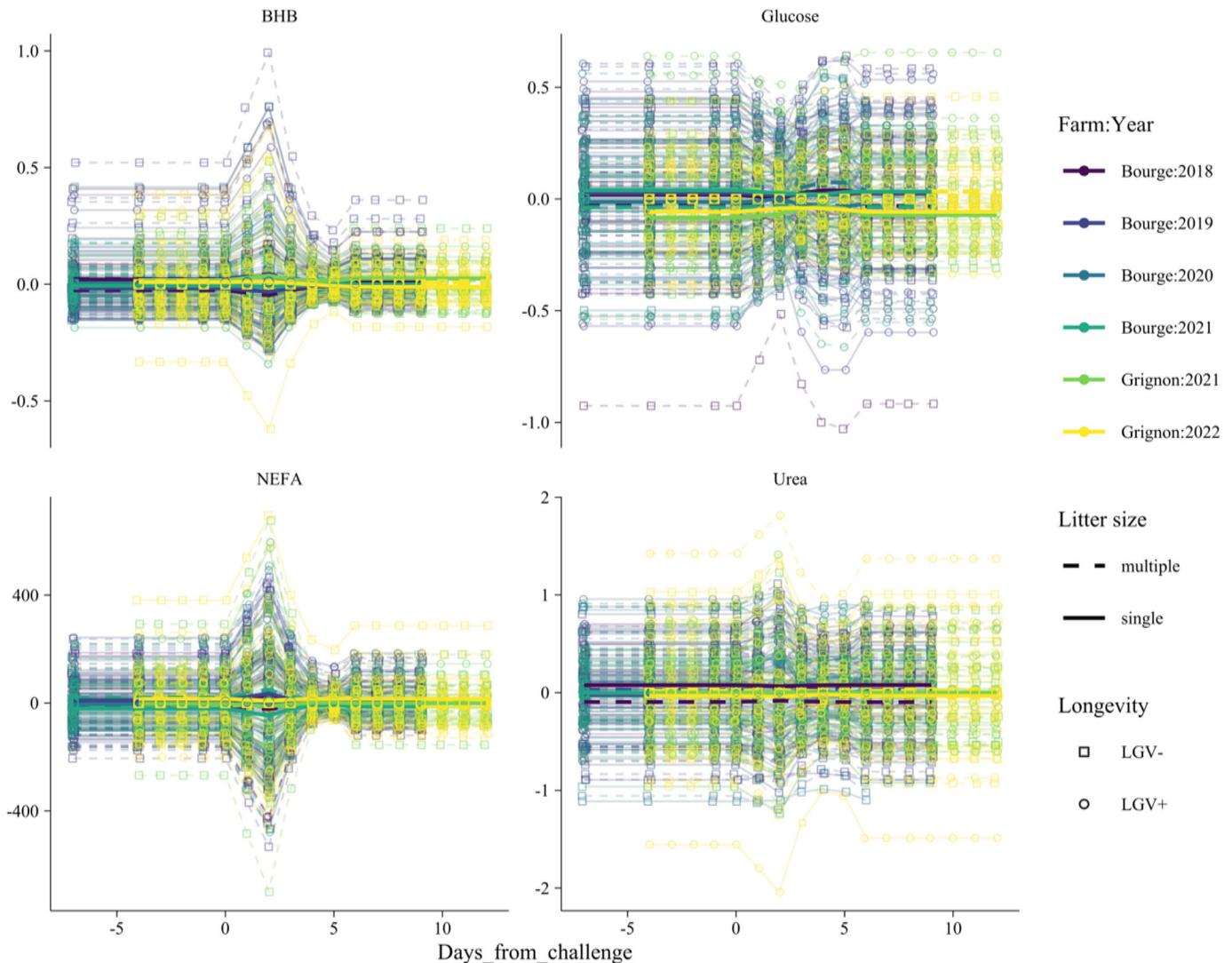


Figure 4. Plasma metabolites concentration deviations time trends (Glucose (mM), nonesterified fatty acids (NEFA; $\mu\text{mol/L}$), Urea (mM), and B-hydroxybutyrate (BHB; mM)) of the individuals (thin lines; random effects) throughout the nutritional challenge across year nested in site (site/year), genetic lines, and litter size estimated by the piecewise mixed effects model. The thick lines represent the individual deviations averaged by (site/year), genetic line, and litter size. These are first lactation Alpine goats from 2 divergent lines, selected for extreme functional longevity, longevity plus (LGV+) and longevity minus (LGV-), evaluated across 6 years in 2 different sites (Bourges and Grignon)

ratio (Bouvier-Muller et al., 2018) This suggests that body energy dynamics are linked with udder infection, an important underlying component of functional longevity. Therefore, other metabolic adaptations, rather than those related to body energy dynamics, may occur during a short-term nutritional challenge, which may be related to functional longevity. This more nuanced view of the links between short-term adaptive capacity and functional longevity is reflected in the high, and meaningful, between-individuals' variability for plasma NEFA and BHB responses and recoveries from the 2-d nutritional challenge found in the present study. This diversity of plasma BHB and NEFA concentration

within a group of animals under similar housing and feeding conditions has also been observed in dairy cows (van Dorland et al., 2009; Weber et al., 2013).

The between-individuals' variability in short-term adaptive capacity is of interest. Friggens et al. (2016), evaluating the response and recovery profiles of dairy goats to nutritional challenge, also found significant between-individuals' variability in their plasma metabolite response and recovery. Recent multivariate studies have shown how this individual variance in metabolic responses can be used to characterize differences in resilience (Abdelkrim et al., 2023) and thereby assess the

contribution of this resilience to functional longevity (Ithurbide et al., 2023).

CONCLUSIONS

Response and recovery profiles of plasma metabolites to a 2-d nutritional challenge in dairy goats were not different between animals selected to be divergent on functional longevity. There were significant effects of site and year within site, and an effect of litter size. The litter size effect on the responses and recoveries seems dependent on nutritional status (and body reserve level) before challenge. Plasma NEFA and BHB response and recovery presented high between-individuals' variability, indicating individual adaptative characteristics to nutritional challenges are more related to inherent individual characteristics than to the environmental conditions.

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