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ABSTRACT

The aim of this study is built in two phases: to quantify the ability of novel milk metabolites to measure between-animal variability in response and recovery profiles to a short-term nutritional challenge, then to derive a resilience index from the relationship between these individual variations. At two different stages of lactation, sixteen lactating dairy goats were exposed to a 2-d underfeeding challenge. The first challenge was in late lactation, and the second was carried out on the same goats early in the following lactation. During the entire experiment period, samples were taken at each milking for milk metabolite measures. For each metabolite, the response profile of each goat was characterised using a piecewise model for describing the dynamic pattern of response and recovery profiles after the challenge relative to the start of the nutritional challenge. Cluster Analysis identified three types of response/recovery profiles per metabolite. Using cluster membership, multiple correspondence analyses (**MCAs**) were performed to further characterise response profile types across animals and metabolites. This MCA analysis identified three groups of animals. Further, discriminant path analysis was able to separate these groups of multivariate response/recovery profile type based on threshold levels of three milk metabolites: β-hydroxybutyrate, free glucose and uric acid. Further analyses were done to explore the possibility of developing an index of resilience from milk metabolite measures. Different types of performance response to short-term nutritional challenge can be distinguished using multivariate analyses of a panel of milk metabolites.

Implications

This study proposes a non-invasive methodology to combine and characterise the different milk metabolite responses into the nutritional challenges and identify a gradient of animal behaviour. Detection and analysis of these patterns can help reveal the resilience of the animal and assessing the effects of a nutritional challenge on milk metabolites could provide parameters for quantifying and understanding how animals cope with their environment and thus better manage them.

Introduction

As a consequence of climate change, the scarcity of feed resources and the concomitant pressures of achieving global food security, livestock systems will be increasingly exposed to environmental perturbations. Thus, there is a need for livestock with improved resilience, i.e. the capacity of an animal to adapt favourably to environmental disturbances (Knap and Doeschl-Wilson, 2020; Friggens et al., 2021). In this context, resilience (not to be confused with animal robustness that combines high production potential with resilience to external stressors (Berghof et al. 2019)), here described as the pattern of response to and recovery from a perturbation, is an increasingly important characteristic on farmed animal (Friggens et al., 2017). Indeed, recent studies have shown that there is a correlation between the degree of perturbation of milk yield curves through lactation (expressed as the variability of milk yield) and frequencies of health events such as mastitis and ketosis, as well as with productive longevity (Poppe et al., 2020).

However, resilience is difficult to measure. This is in part because it involves capturing dynamic features, such as rates of response and recovery from a perturbation, and that requires high-frequency repeated measures (Ben Abdelkrim et al., 2021). It is also in large

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part because the full response to a perturbation is expressed across multiple measures and thus requires a multivariate approach to better characterise resilience. Sadoul et al. (2015 and 2017), clearly showed the multivariate nature of resilience across physiological and behavioural responses in rainbow trout. They also showed that there was variability between animals in the relative weight of the different components within the overall response to perturbation. Similar results have been found in ruminants (Friggens et al., 2016; Billa et al., 2020), which has led to the notion of multivariate indexes for describing animal health status (Bramley et al., 2008; Foldager et al., 2020). However, to date, appropriate methodologies for sequentially filtering, combining, and then extracting the key information from multiple measures of response/recovery remain to be clearly described in the livestock domain.

Milk metabolite measures are attractive candidates for an improved phenotyping of resilience as the requisite samples can be readily obtained on-farm, are non-invasive, and could be integrated into automated on-farm biomarker systems, examples of which have been commercialised (e.g., Herd Navigator System™, DeLaval International, Tumba, Sweden & Lattec I/S, Hillerød, Denmark). Accordingly, the aim of this study was to quantify the ability of milk metabolite measures to capture variability in the response and recovery profiles to a short-term nutritional challenge by applying multivariate statistical methods to profile shapes. Finally, this study explores the possibility of developing an index of resilience from milk metabolite measures.

Material and methods

Animals and challenge design

At two different stages of lactation, sixteen primiparous lactating dairy goats were exposed to an underfeeding challenge consisting of: a 7-d control phase on a standard total mixed ration (**TMR**) fed *ad libitum*, followed by 2 d of straw-only feeding, and a 10-d recovery phase on the TMR fed *ad libitum*. Prior to the start of each challenge period, the goats had received the standard TMR for at least 15 d. The first challenge was in late lactation (mean of days in milk (**DIM**) = 249), and the second was carried out on the same goats early in the following lactation (mean DIM = 28). The TMR (20% chopped hay, 30% chopped dried alfalfa, 30% sugar beet pulp, and 20% commercial dairy concentrate) and straw were distributed twice daily, shortly after milking (at 0700 a.m. and at 0300 p.m.). The goats were housed in individual pens. The experiment is described in greater detail, together with the performance and blood metabolite results in Friggens et al. (2016).

All procedures were conducted in accordance with the French legislation on controlling experiments/procedures of live animals and the European Convention for the protection of vertebrates used for experimental purposes or for other scientific purposes (European Directive 86/609).

Milk metabolite sampling analyses

Throughout the experiment, proportional milk samples were taken individually at each morning and afternoon milking. In addition to standard analysis for milk fat (**MFC**) and milk protein (**MPC**) (Fossomatic, Hillerød, Denmark), AM and PM milk were analysed separately for β-hydroxybutyrate (**BHB**), glucose-6-phosphate (**G6P**), galactose (**GAL**), free glucose (**F_G**), uric acid (**UA**), lactate dehydrogenase (**LDH**), triacylglycerol (**TAG**), isocitrate (**Isocit**), cholesterol (**Chol**) and urea (**U**). Milk urea was analysed using flow injection analysis (Nielsen et al., 2005) using a FIAstar 5000 Analyzer (Foss Tecator AB, Höganäs, Sweden). Enzymatic-fluorometric methods were used to analyse TAG and minor milk constituents: BHB (Larsen and Nielsen, 2005), LDH activity (Larsen, 2005), UA (Larsen and Moyes, 2010), TAG (Larsen et al., 2011), Chol (Larsen, 2012), Isocit (Larsen, 2014), F_G and G6P (Larsen, 2015). GAL in milk was analysed by an analogous procedure to Isocit, using *b*-galactose dehydrogenase (EC 1.1.1.48) to start the fluorometric determination.

Statistical analyses

All statistical analyses were performed using R (RCore team, R Foundation for Statistical computing, 2018, R: A language and environment Statistical Computing, Version 3.14.0, Vienna, Austria, http://www.r-project.org).

For statistical analysis, daily metabolite concentrations in milk were calculated with am and pm values weighted according to milk yield at those milking. During each challenge sequence (pre-, during, and postchallenge) and for each goat, the individual response profiles of the different milk metabolites were characterised over the whole period. To describe the relationship between the prechallenge, response to challenge and postchallenge, a piecewise model with four parameters was used as described by (Friggens et al., 2016). Briefly, this model consisted of two steps. In the first step, for each milk metabolite and each lactation stage, the time-series measures were characterised separately using the following model:

$$y_t = V_1 * I_{t\leq 0} + V_2 * T * I_{(0<t\leq 2)} + (V_3 * T + V_4 * t * 2) * I_{(2<t\leq 4)} + V_5 * I_{(t>4)} + E_t$$

where y_t is the milk metabolite measured at time t (the start of the challenge was designated to be $t = 0$).V_1, V_2, V_3,V_4 and V_5 correspond at the prechallenge level, the linear slope of the response during challenge, the linear component of the recovery, the quadratic component of the recovery and the postchallenge level, respectively. It must be emphasised that the V_5 results from the combination of V_1toV_4. To compute and process these coefficients, a dummy variable I is used with value 1 if the time condition is true and 0 otherwise. E_t is the error term and assumed to be Gaussian and independent. In a second step, within the lactation stage, clustering analysis was carried out based on the parameters $V_1, \ldots, V_5$ to identify the goats with a similar response profile in milk using the expectation maximisation algorithm (Dempster et al., 1977) and a fixed cluster number of 3 (see (Friggens et al., 2016). These metabolite response profiles presented a fairly large variability, not only at the level of these three clusters but also at the level of the three phases of challenges. Within each lactation stage, the construction of a matrix where the average time-courses of the different milk metabolites during the prechallenge, challenge and postchallenge phases were replaced by qualitative variables indicating the concentration profiles. For example, H.M.L. represent the individuals with the highest concentration profile for a given metabolite during the prechallenge, the medium concentration profile during the challenge and the lower concentration profile during postchallenge phase. Multiple correspondence analyses (**MCAs**), which is an extension of Correspondence Analysis (CA, Benzécri, 1973) for more than two variables (Greenacre and Blasius, 2006), were performed on this matrix. This used all milk metabolites listed above except TAG, which was considered to be an alternative measure of MFC. The MCA provides a way to visualise, across the full panel of metabolite, associations between different response-recovery profile shapes. It also allows visualisation of the position of the different goats within these multivariate associations.

Visualisation of the relationships among metabolite profiles in the first and second axes resulting from the MCA (which accounted for the greatest proportion of variance) highlighted a grouping of the different goats studied (see results). To confirm this grouping,

a Gower distance matrix (Pavoine et al., 2009) was calculated, and the partition around medoids clustering algorithm (Kaufman and Rousseeuw, 2005) was performed to find the optimal number of clusters. Intrinsic clustering quality was analysed using the silhouette coefficient as suggested in Rousseeuw (1987) and Pollard and van der Laan (2002). On the basis of the results obtained from the clustering analysis, a decision tree was constructed by using the classification and regression trees method (Struyf and Džeroski, 2005). The objective was to predict which metabolites were most involved in the discrimination between goats in the different identified groups. The trees were pruned to minimise the cross-validated error. This decision tree procedure was also carried out on the following quantitative variables from the piecewise model; V_1 which characterises the prechallenge metabolite concentrations, and V_2 which characterises the amplitude of the response to the challenge.

In order to explore the extent to which the variation in milk metabolite profiles may be linked to variation in performance, the above analyses approaches were also conducted on the following panel of performance measures: DM intake, milk yield, TAG and milk protein content reported by Friggens et al. (2016). This allowed characterisation of the relation between milk metabolite profiles and overall performance profiles using the same approach basis. Then, Partial Least Square regression (Wold et al., 2001) was carried out in order to determine a relation between predictor variables (milk metabolites) and the performance variables. This procedure consists of generating linear combinations of predictors, via principal component rotation, in order to best explain variance in the dependent variable (Carrascal et al., 2009). A combined performance variable was created by fitting the overall trend of the relationship between the first two axes of the MCA on DMI, MY, TAG, and MPC profiles, using a cubic spline. The cubic spline (that best represents animal performance) was chosen as the *'x'* variable, and the five first MCA dimensions of the milk metabolite values were selected as individual *'y'* variables. Pearson's correlation coefficients between the predicted values and the observed values were also calculated, to determine the predictive ability of the model.

Response profiles index

Exploratory analysis to construct a response profiles index was carried out using as a basis the two first dimensions of the MCA, from the milk metabolite profiles. This index combines the multivariate responses of the different milk metabolites into one measure. The first step was to fit the overall trend of the relationship between the first two axes using a cubic spline. Thereafter, the response profiles index was calculated by multiplying the fitted values obtained by the smoothing spline and the eigenvalues percentage of variance of each axis. In order to simplify the scale, and for convenience, it was linearly transformed into a 0–10 range. To be able to interpret this index, the performance profile categories were projected in the same planes as supplementary variables.

Results and discussion

General milk metabolite responses to the nutritional challenge

The average time-courses of the different milk metabolites, reflecting different facets of energy metabolism, during the prechallenge, challenge and postchallenge phases are shown in Fig. 1 and Table 1, for both the late- and the early-lactation challenge periods. Similar results have been reported in dairy cows. Indeed, Billa et al. (2020) recently reported similar time trends of milk concentration of G6P, F_G and Isocit when 18 cows underwent 6 d of feed restriction. Increased milk Isocit and G6P, and decreased milk Glucose, concentrations during feed restriction are coherent with previous observations in starved goats (Chaiyabutr et al., 1981).

The rapid mobilisation of body fat induces a reduction of fatty acid synthesis (Peaker et al., 1981) and could explain increased isocitrate during the challenge. Indeed, the increase in Isocit concentration is strongly correlated with decreased synthesis of fatty acids (Garnsworthy et al., 2006). The increase in milk G6P content, which is used for galactose synthesis and NADPH and ATP production, may reflect a change in this pathway in mammary epithelial cells during challenge, or alternatively a decrease in plasma insulin and low de novo FA synthesis (Billa et al., 2020).

The UA increase we observed may be due to the marked drop in dietary protein intake during the challenge. The straw-only feeding shifts the balance of dietary protein towards microbial protein synthesis. It is also possible that milk UA may reflect the intra-rumen changes due to the dietary perturbation since it has been suggested as an indicator of microbial protein synthesis (Larsen and Moyes, 2010).

BHB in milk generally reflects dietary energy shortfalls coupled with increased lipid mobilisation associated with glucose shortage to oxidise NEFA (Klein et al., 2013). However, experimental feed restrictions in literature show quite contrasted results for effects on milk BHB. Billa et al. (2020) found a significant decrease in BHB followed by an increase after refeeding in late lactation but found an increase in early lactation during the feed restriction. Bjerre-Harpoth et al. (2012) also reported an increase in early-lactation cows after 96 h of feed restriction. Thus, the net changes in milk BHB will be affected both by the impact of diet on BHB and the effects of lipid mobilisation on metabolism. Increase in BHB in challenge conditions may also reflect body lipid mobilisation associated with lower glucose (see Friggens et al., 2016; Leduc et al., 2021).

Milk LDH concentrations are generally related to mammary infections (Larsen, 2005; Chagunda et al., 2006; Nyman et al., 2014). In goats, LDH is a reliable biomarker of udder inflammatory processes but parity and lactation stage might influence its concentration (Stuhr et al., 2013). As the challenge applied in the present study was nutritional, it was not expected to impact udder health status directly. However, Foldager et al. (2020) also found an increased LDH milk concentration in cows with physiological imbalance. Two scenarios are possible: the first is a local upregulation of LDH synthesis in order to increase ATP flux from Glucose as LDH is a common enzyme found in all glycolytic pathways. The second is that feed restriction induced increases in permeability of mammary cell junctions allowing more plasmatic LDH to flow to mammary gland.

Bjerre-Harpoth et al. (2012) found an increase in plasma cholesterol during feed restriction. Gross et al. (2015) found that cholesterol milk concentration was not affected by a 3 weeks of feed restriction starting at 100 DIM whereas plasma concentration was increased. One hypothesis is that the milk concentration of cholesterol was not affected during feed restriction because it occurred in mid-lactation. Indeed, this study concluded that cholesterol metabolism was impacted by the stage of lactation.

Descriptive analysis of response-recovery profiles using univariate clustering

One of the aims of this paper is to present a sequential methodology for moving from univariate description of each milk metabolite separately, to a multivariate description. This allows an improved description of variation between animals in their overall ability to cope with perturbations. The first step in this sequence was to characterise individual profile shapes into clusters. For each

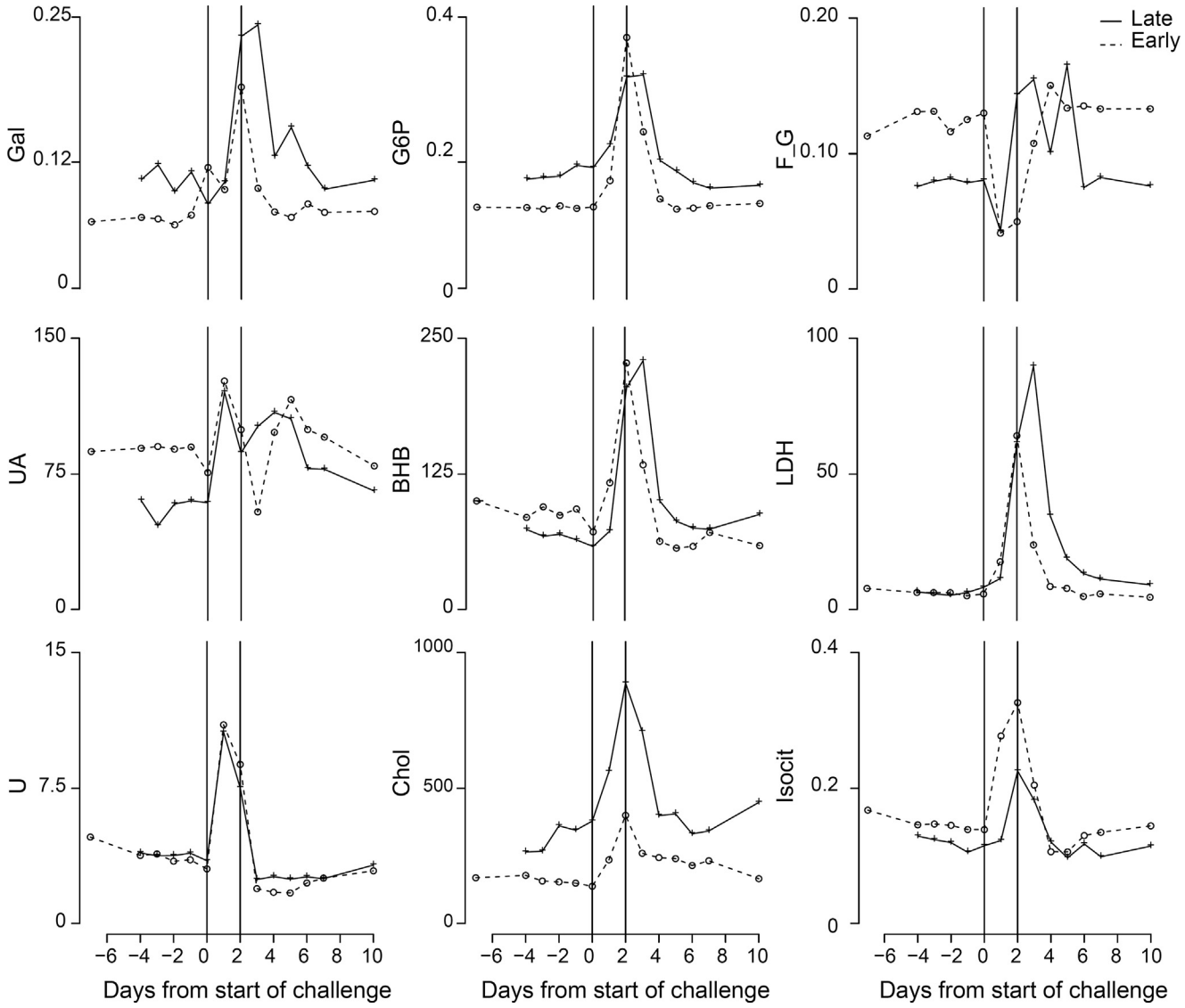

Fig. 1. The average time trends of the different goat milk metabolites analysed through the prechallenge (before day 0), challenge (day 0–2), and postchallenge (day > 2) phases in both late (solid line) and the following early-lactation (dashed line) periods. GAL: galactose, G6P: glucose-6-phosphate, F_G: free glucose, UA: uric acid, BHB: β-hydroxybutyrate, LDH: lactate dehydrogenase, U: urea, Chol: cholesterol, TAG: triacylglycerol, and Isocit: isocitrate.

Table 1
Average amplitudes of response (i.e. the change relative to the prechallenge level) of goat milk metabolites to a short-term nutritional challenge in late lactation and in the following early lactation. Average levels pre- and postchallenge are also given (SD in parentheses).

	Late			Early		
Item	Prechallenge	Amplitude of response	Postchallenge	Prechallenge	Amplitude of response	Postchallenge
GAL (mmol/L)	0.10 (0.01)	0.24	0.113 (0.02)	0.06 (0.003)	0.18	0.07 (0.005)
G6P (mmol/L)	0.17 (0.01)	0.31	0.157 (0.01)	0.12 (0.002)	0.37	0.12 (0.004)
F_G (mmol/L)	0.08 (0.002)	0.15	0.100 (0.04)	0.12 (0.008)	0.15	0.13 (0.001)
UA (μmol/L)	56.10 (6.69)	120.18	81.48 (16.88)	88.95 (1.10)	126.29	97.47 (15.10)
BHB (μmol/L)	68.55 (4.00)	229.23	79.33 (6.29)	91.57 (6.14)	227.12	61.01 (6.61)
LDH (U/l)	5.88 (0.59)	89.67	13.03 (4.17)	6.23 (0.97)	64.01	5.63 (1.48)
U (mmol/L)	3.82 (0.08)	10.59	2.684 (0.37)	3.87 (0.53)	11.00	2.33 (0.51)
Chol (μmol/L)	308.41 (50.86)	886.83	380.59 (54.65)	160.76 (11.83)	398.87	212.29 (33.21)
TAG (mmol/L)	32.07 (7.01)	61.86	35.36 (7.91)	37.63 (3.38)	61.29	38.72 (3.16)
Isocit (mmol/L)	0.12 (0.01)	0.22	0.107 (0.01)	0.15 (0.01)	0.33	0.13 (0.02)

Abbreviations: GAL = galactose, G6P = glucose-6-phosphate, F_G = free glucose, UA = uric acid, BHB = β-hydroxybutyrate, LDH = lactate dehydrogenase, U = urea, Chol = cholesterol, TAG = triacylglycerol, and Isoci = isocitrate.

milk metabolite, clustering was carried out to identify types of response-recovery profile within each lactation stage. Because a preliminary clustering with no limit on the number of clusters showed that the most frequent number of clusters identified for each metabolite was 3, the number of clusters was then fixed at three for all metabolites to facilitate subsequent analyses (the results of clustering with no fixed cluster number are shown in Supplementary Table S1). Examples of response-recovery profile

types are shown in Fig. 2 (all the types of response-recovery profiles for all metabolites are shown in Supplementary Fig. S1a–c).

This permitted analysis of the extent to which cluster type was reproducible for individual goats between late lactation and early lactation. Within metabolite, each of the three profiles was coded using the 3-letter nomenclature of each profile type (e.g. Fig. 2c). As shown in Table 2, 22 different profiles were noted (10 profiles present in both periods, four present only in late-lactation and eight present only in early-lactation).

This increased individual variation in response profiles to nutritional challenges in early lactation has been reported by Friggens et al. (2007), Moyes et al. (2009) and Bjerre-Harpoth et al. (2012) and reflects the diversity of response pathways that are fully expressed when facing the severe physiological imbalance that occurs during early lactation.

Multivariate analysis of response-recovery clusters

To characterise integrated response profile types across metabolites, we used MCA on the cluster classes for all the traits (Fig. 3; for further details concerning the correlations between variables and axis and contribution of the variable categories for the construction of the axes, see Supplementary Figs. S2 and S3).

The simultaneous projection of individuals and of the different response profiles onto the first two dimensions of the MCA explained 47.9 and 36.5% of the total variability in late (Fig. 3a) and early lactation (Fig. 3b), respectively. Visual inspection of the MCA for the late-lactation response/recovery profiles showed the presence of three groups of individuals. These three groups were separated principally by the variables F_G, LDH, BHB, UA and G6P. The MCA biplot concerning the early-lactation challenge also showed three groups but less well separated. The variables which contributed to the separation between these early-lactation groups were F_G, LDH, Isocit and BHB.

F_G and LDH appear to be the most discriminant metabolites for determining the membership of all clusters. That agrees with the results of Foldager et al. (2020) who found that F_G and LDH were the most important metabolites to distinguish physiologically imbalanced cows from normal ones at 35 DIM of second lactation. Different F_G profiles may reflect different challenge intensities (for lower vs higher milk yield levels) but also different abilities to buffer the challenge via the mobilisation of body reserves.

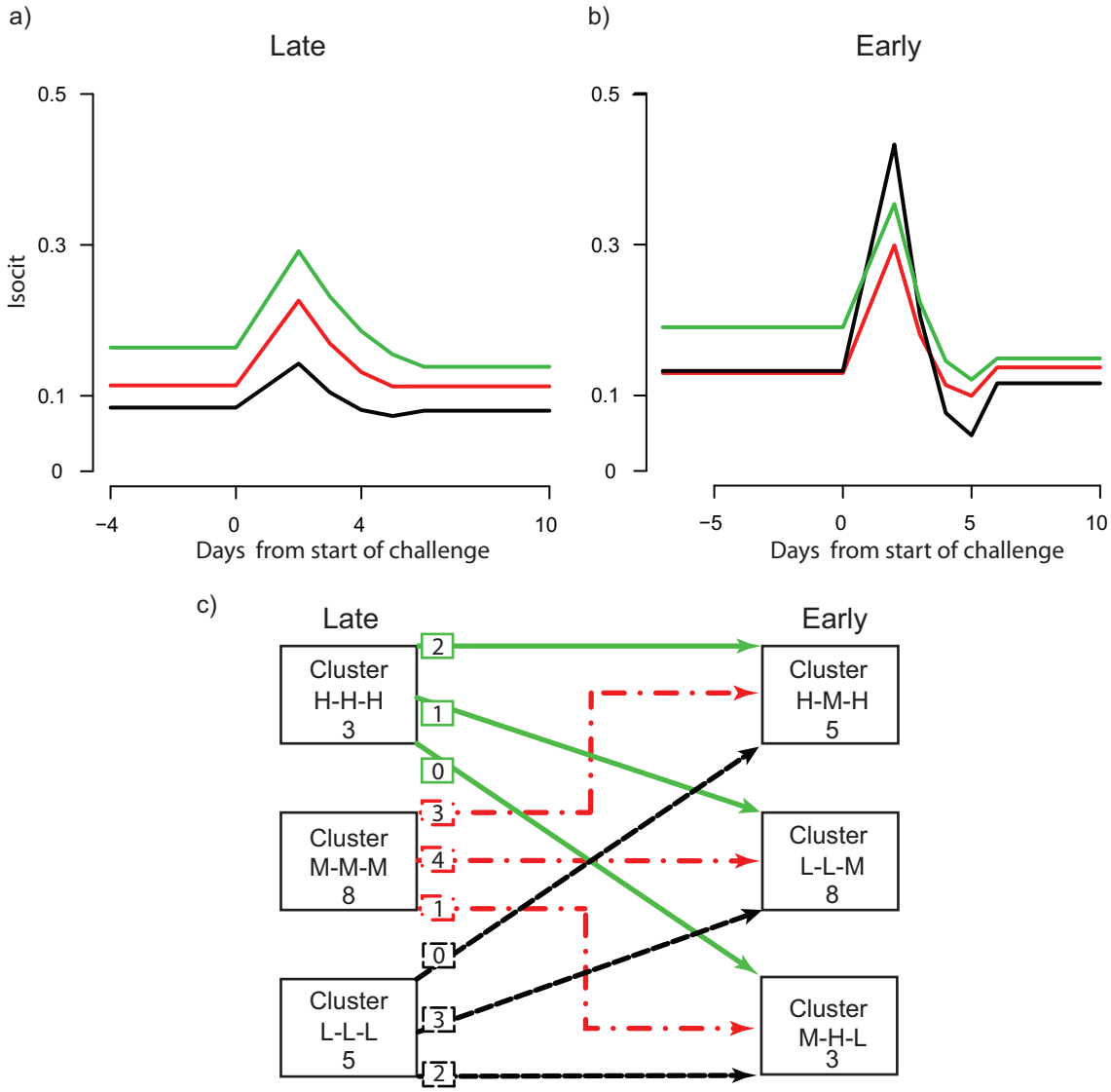

Fig. 2. Classes of response-recovery profile for Isocitrate (Isocit) in (a) late and following (b) early lactation and (c) visualisation of numbers of goats shifting between the three predefined clusters (arrow) with H: high, M: medium and L: low, which indicates the concentration level of the metabolite at each phase (prechallenge, challenge, postchallenge) of the challenge.

Table 2
The different response profiles detected in goats in late and in following early lactation (in parentheses the number of goats who expressed this kind of profiles for a given metabolite).

LPreC[1]	LC[2]	LPostC[3]	PN[4]	Late	Early
H	H	H	H.H.H.	14 (5: UA; 3: Isocit; 4: GAL; 2: G6P)	4 (UA)
H	H	M	H.H.M.	0	0
H	H	L	H.H.L.	0	1 (TAG)
H	M	H	H.M.H.	10 (5: LDH; 4: F_G; 1: Chol)	22 (3: U; 5: Isocit; 2: GAL; 9: G6P; 3: Chol)
H	M	M	H.M.M.	3 (1: U; 2: TAG)	5 (BHB)
H	M	L	H.M.L.	0	5 (LDH)
H	L	H	H.L.H.	0	1 (F_G)
H	L	M	H.L.M.	9 (BHB)	0
H	L	L	H.L.L.	0	0
M	H	H	M.M.H.	5 (BHB)	0
M	H	M	M.M.M.	20 (5: UA; 8: Isocit; GAL: 7)	5 (F_G)
M	H	L	M.L.M.	0	20 (4: UA; 9: LDH; 7: Chol)
M	M	H	M.H.M.	7 (1: TAG; 6: Chol)	10 (3: U; 1: GAL; 6: G6P)
M	M	M	M.H.L.	0	3 (Isocit)
M	M	L	M.H.H.	10 (2: U; 8: TAG)	8 (BHB)
M	L	H	M.L.H.	0	0
M	L	M	M.L.M.	0	0
M	L	L	M.L.L.	24 (9: LDH; 5: G6P; 10: F_G)	0
L	H	H	L.H.H.	0	2 (LDH)
L	H	M	L.H.M.	2 (LDH)	0
L	H	L	L.H.L.	2 (BHB)	16 (10: F_G; 6: Chol)
L	M	H	L.M.H.	2 (F_G)	12 (TAG)
L	M	M	L.M.M.	9 (G6P)	3 (TAG)
L	M	L	L.M.L.	0	8 (UA)
L	L	H	L.L.H.	0	0
L	L	M	L.L.M.	0	8 (Isocit)
L	L	L	L.L.L.	43 (13: U; 6: UA; 5: GAL; 5: TAG; 5: Isocit; 9: Chol)	27 (10: U; 13: GAL; 1: G6P; 3: BHB)

Abbreviations: H = high, M = Medium, L = Low, GAL = galactose, G6P = glucose-6-phosphate, F_G = free glucose, UA = uric acid, BHB = β-hydroxybutyrate, LDH = lactate dehydrogenase, U = urea, Chol = cholesterol, TAG = triacylglycerol, and Isoci = isocitrate.
[1] Levels of metabolite concentration during the prechallenge.
[2] Levels of metabolite concentration during the challenge.
[3] Levels of metabolite concentration during the postchallenge.
[4] Profiles name.

When considered from the perspective of finding candidate biomarkers of different response types, it is of interest to know which panel of the milk metabolites are key for distinguishing the groupings of integrated response-recovery types. Accordingly, a classification tree was constructed using the information from the MCA on which metabolites accounted for most of the variation between groups. In late lactation, only two metabolites (BHB and F_G) were necessary to correctly assign goats to the appropriate cluster (Fig. 4a). Likewise, in early lactation, only two metabolites were needed, these were LDH and F_G (Fig. 4b). Whilst it would be unwise to give too much importance to these decision tree results, given that they are based on only 16 individuals, if these findings hold when tested on a larger population, they would suggest that it should be possible to phenotype metabolic response types using a limited set of non-invasive milk measures.

Relation between milk metabolite profiles and performance profiles

In order to frame these results in the context of differences in resilience, these findings need to be related to performance and recognised indicators of resilience. Accordingly, and given recent literature showing a relationship between milk yield responses and indicators of resilience (Adriaens et al., 2020; Poppe et al., 2020), we examined the relationship between the multivariate measures of milk metabolite profiles and the performance responses of the same goats. The aforementioned literature suggests that animals with the greatest responses in milk (and intake) would be the least resilient. In order to establish the link between milk metabolites and animal performance using a comparable performance dataset, the above multivariate clustering analyses were repeated on the MY, DMI, MPC and MFC. The plots for the first and the second dimension are shown in supplementary Fig. S4. In late lactation, these plots showed a performance gradient in the first dimension. This gradient was also noted in early lactation but less markedly. Focusing on the two extremes of these dimensions, it is possible to establish links between groups determined separately by the MCA on metabolites and the MCA on the performance. This was further explored by capturing the overall trend in the milk measures MCA using a cubic spline (Fig. 5).

The spline curve was used in order to summarise into one continuous variable the information of the two first components of the milk metabolites MCA. The magnitude of correlations between Partial Least Square predicted values and the "observed" values of the performance spline (Supplementary Fig. S5) was 0.78 for the late period and 0.70 for the early period. These correlations clearly show the link between the model to summarise animal performance and the variation in metabolite profiles. The underlying interpretation is that there is a continuum of metabolic pathways that can be linked to a continuous value of resilience. It should be noted that the term "resilience" is used here specifically to mean "resilience in performance to a nutritional challenge".

A response profiles index was then calculated from the milk metabolites. Interestingly, the shape of this index allows two distinct points in the multivariate space representing the distribution of milk metabolite profiles, in other words, two supposed sets of metabolic pathways, to get the same "resilience value". Conceptually, it is generally considered that a given level of resilience can be achieved by different combinations of its underlying component mechanisms (Kitano, 2004; Bateson and Gluckman, 2012).

The response profiles index was then projected on the same plane as the MCA for the performance data in order to check to what extent the proposed response profiles index fits with the performance profiles (Fig. 5). The response profiles index was created solely from the milk metabolites MCA. Fig. 5 shows the numerical

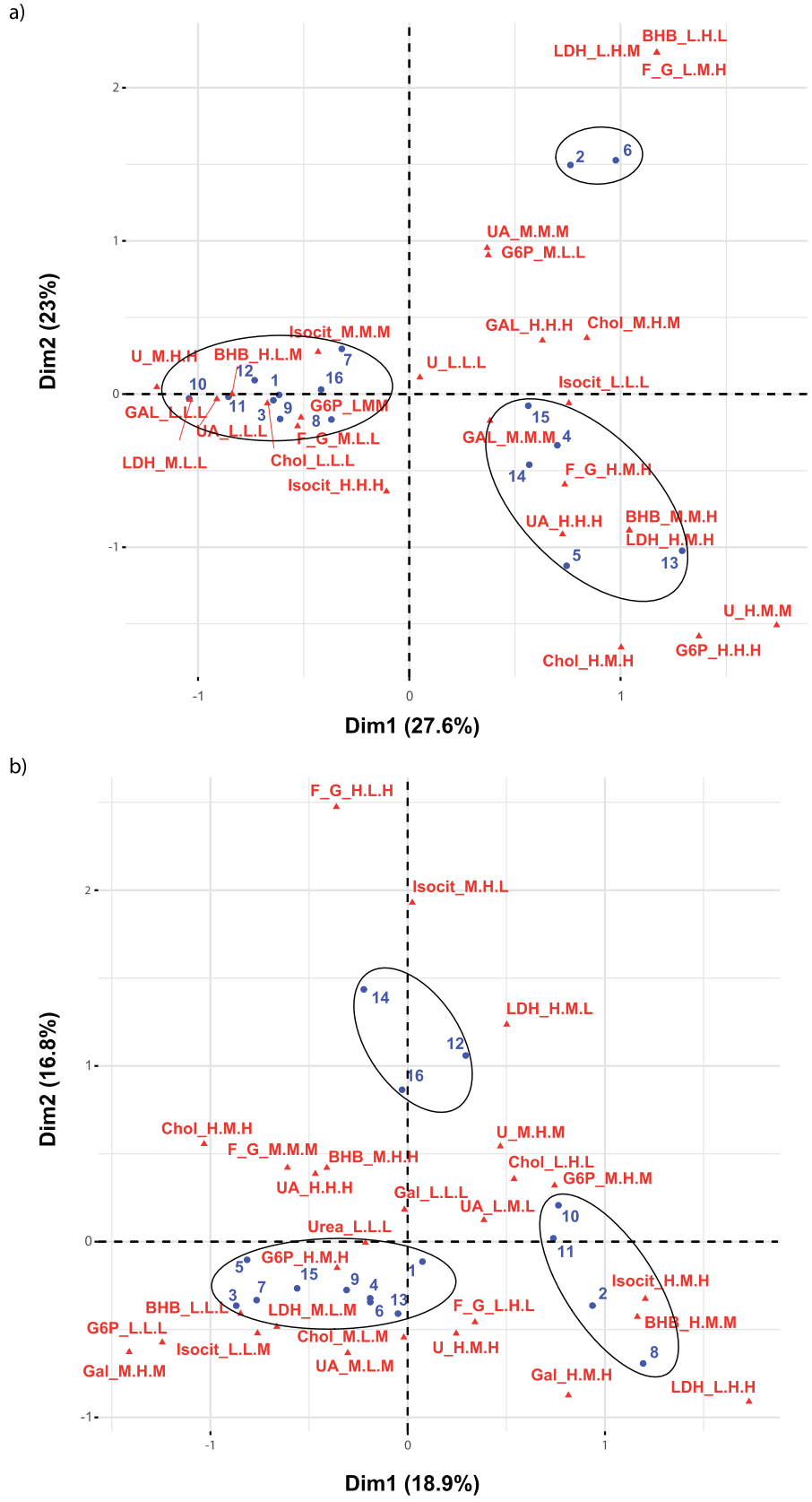

Fig. 3. Biplot of the first two dimensions of multiple correspondence analyses of profile classes across all measures carried out on goats in late lactation (a) and in early lactation (b) with H = high, M = medium and L = low, GAL = galactose, G6P = glucose-6-phosphate, F_G = free glucose, UA = uric acid, BHB = β-hydroxybutyrate, LDH = lactate dehydrogenase, U = urea, Chol = cholesterol, TAG = triacylglycerol, and Isocit = isocitrate.

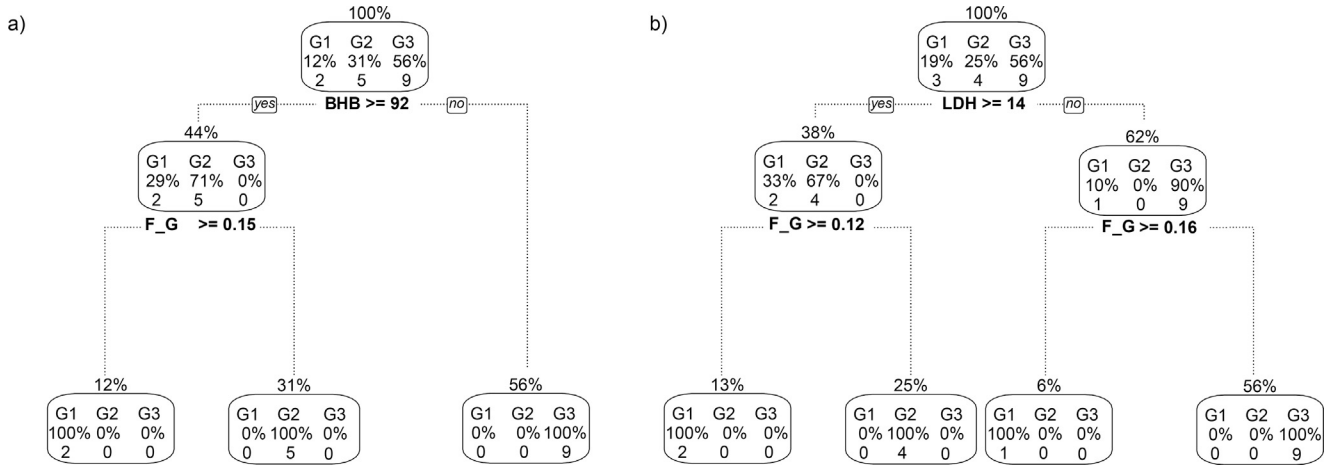

Fig. 4. Decision trees built using the best variables selected to separate the goats of different groups in late (a) and early lactation (b). The membership of the three groups (G1, G2, and G3) is shown by percentage, and number, of goats. Threshold values are shown in bold with BHB = β-hydroxybutyrate, LDH = lactate dehydrogenase, and F_G = free glucose.

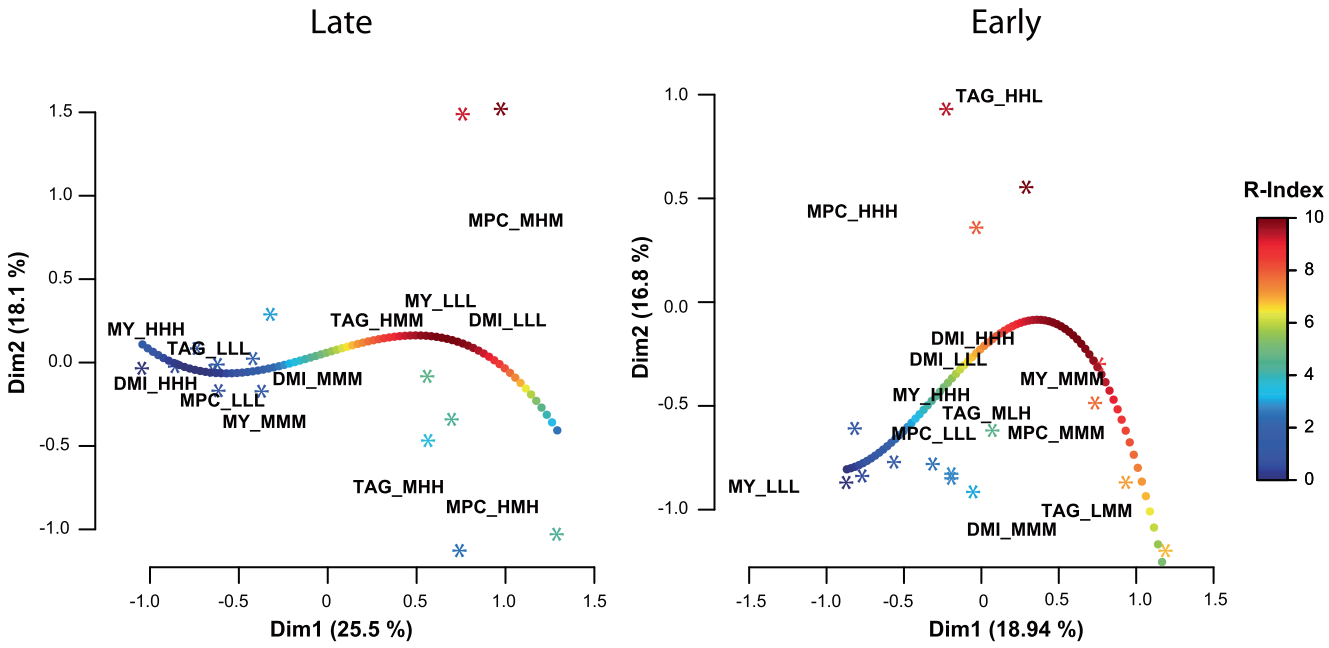

Fig. 5. The smoothed trend in goat milk metabolite profiles (R -index) overlayed on the multiple correspondence analyses (MCAs) for performance measure profiles, with value for these profiles indicate as measure-profile code (e.g. TAG-HHL) with H = high, M = medium and L = low. Individual goat position in positions in the performance MCA is shown as asterisks shaded according to their R-index. DMI = DM intake, MY = milk yield, MPC = milk protein content and TAG = triacylglycerol.

relation between the response profiles index, which describes the variation of metabolites profiles of goats using the two first dimensions of the MCA, and the MCA-score for the performance variables.

Comparing the distribution of goats, metabolite profiles, performance profiles and the representation of the response profiles index on the same plane highlights a relationship between milk metabolites derived response profiles index and the performance profiles. During the late-lactation period, the horizontal element of the response profiles index trend is strongly associated with milk yield and DMI response-recovery profiles, with decreasing performance levels from left to right. The vertical element of the response profiles index was more associated with the different profiles in milk protein and milk fat. The goats forming the first group (most to the left) take response profiles index values ranging from 2.06 to 4.22, with values ranging from 8.80 to 9.32 for the second group, and from 3.32 to 5.41 for the third group. The results shown in Fig. 5 for late lactation suggest a link between profiles of milk metabolites during a nutritional challenge and the ability of the goat to deal with the challenge.

The associations between the response profiles index and the performance profiles were not so clear in the early-lactation period. In the early-lactation period, relative differences in response between profiles in both milk yield and DMI were less than in late lactation, which perhaps explains the weaker link between these performance measures and the R-index in early lactation. Conversely, differences in milk fat content were greater in early than late lactation, and this is reflected in the stronger association of the milk composition measures with changing R-index in early lactation. The differences between early and late lactation in the relationship between the R-index and performance profiles are in line with what would be expected from considerations of the homeorhetic changes in nutritional physiology through lactation.

This step, projecting the milk metabolite index onto the performance MCA, allows us to interpret the value of the index created, relative to the overall performance profile, both in qualitative terms (what are the biological underlying mechanism?) and in quantitative terms (whether a high, medium, or low index should be preferred in term of resilience). It suggests that there is information on animal adaptation mechanisms that can be obtained from non-invasive milk measures. Supporting this idea, Grelet et al. (2019) and Foldager et al. (2020) concluded that a set of milk metabolites and enzymes close to the one used in the present study was predictive for the physiological imbalance of the cows. In addition, several studies have focused on the characterisation of lactation curves in order to index the resilience value of individual animals (Berghof et al., 2019; Poppe et al., 2020). Our results strongly support the assumption of considering milk profiles as a potential index of resilience (Adriaens et al., 2020;Poppe et al., 2020).

Conclusions

This study proposes a combination of methodology (nutritional challenge × statistical approach of animal data) to extract from a non-invasive approach (milk), different patterns of aggregated response to build a response index in goats. Although the number of animals was small, the use of multivariate tools made it possible to appreciate the diversity of the forms of responses to this challenge. Through the use of MCA and clustering methods, the identification of an expression gradient of these reactions which define themselves in three groups has been possible. These methodologies also allowed the identification of non-invasive biomarkers from milk, which makes it possible to differentiate the three groups of profiles. Based on these data, a response index has been developed which may be used as an indicator of resilience.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100727.

Ethics approval

Animals were cared for and handled in accordance with the French legislation on animal experimentation and European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes (European Directive 86/609).

Data and model availability statement

The data/models were not deposited in an official repository. The data/models that support the study findings are available from the authors upon request.

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Nicolas Friggens: Supervision, Writing – Review and Editing.

Declaration of interest

None.

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