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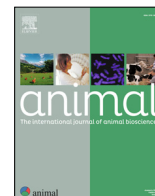
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Short communication: Variability of response to feed restriction in lactating Charolais cows



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ABSTRACT

Involuntary temporary feed restriction on commercial cattle is likely to become more frequent with forage shortages in the context of climate change. If general consequences of feed restriction have been the subject of an abundant scientific literature, focus on the inter-individual variability of response is scarce. Here, we explore the response profile in terms of BW, body condition score, milk production, calf weight and cyclicity resumption of 293 lactations from 169 Charolais cows during a winter feed restriction in early lactation and its subsequent recovery at grazing using a principal component analysis followed by a hierarchical clustering on principal component. Results show a very continuous range of response profiles that was divided into three clusters: one with light animals having an intermediate response in terms of milk production and body maintenance, one with animals prioritising body maintenance and cyclicity resumption over milk production and calf weight, and the last one with animals prioritising milk production and calf weight over the rest. Among the animals performing more than one lactation, 57% remain in the same cluster on two successive lactations. This work highlights that an average group response to feed restriction may hide various resilience individual profiles. Further studies are required to determine the existence of a genetic component as well as the consequences of not taking this phenomenon into consideration with the regular use of feed restriction in commercial farms.

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Implications

Involuntary temporary feed restriction due to forage shortages is likely to become more frequent in the context of climate change. If an abundant literature exists on average breed or group response to restriction, between animals variability of response is more rarely studied. This study highlights the existence of a large variability in response profiles within Charolais lactating dams with some females prioritising their own maintenance while others prioritise their milk production. Not taking this phenomenon into consideration in commercial farms could have consequences that need to be evaluated by further studies.

Introduction

Feed restriction is regularly and deliberately used on commercial cattle farms to prepare females for calving, to facilitate dry off or to achieve compensatory growth in young animals. It may

also occur involuntarily due to limitations in forage resources, a situation that is likely to become more frequent in the context of climate change (Giridhar and Samireddypalle, 2015). Therefore, the consequences of feed restriction have been the subject of an abundant scientific literature. On lactating females from suckling breeds, an impact has been shown on BW, body condition score (BCS), milk production, calf weight and cyclicity resumption, this impact varying with environmental factors such as lactation rank or duration and intensity of restriction (de la Torre et al., 2010; Martin et al., 2022). Different profiles have also been observed depending on the breed: Salers dams were found to prioritise the maintenance of milk production over their own body reserves, while the opposite was observed in Limousine cows (D'Hour et al., 1995). We recently studied the effect of feed restriction on a group of Charolais lactating females (592 lactations, half restricted, half *ad libitum*) and showed that the difference between the restricted and the unrestricted females was of similar intensity for all traits, suggesting a balanced response for that breed (Martin et al., 2022). However, this previous study, similarly to the literature, focused on the restriction effect on the group scale and did not explore the possible variability of individual responses within

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the group. Therefore, this new study is interested in the existing variability of individual profiles of response to restriction after all already known environmental effects have been corrected.

Material and methods

Experimental animals

The animals were enrolled in an experiment that was performed at two experimental farms belonging to the French National Research Institute for Agriculture, Food, and the Environment (INRAE, formerly INRA): Le Pin (Farm 1, 198 females) and Bourges (Farm 2, 142 females). Animals used in this study consisted in the LOW group from the experiment described in [Martin et al. \(2022\)](#). The females were 169 purebred Charolais cows that calved for the first time between 2014 and 2018. At Farm 1, whenever possible, the cows were retained and studied for their second and third lactations. The total number of lactations was 293.

Briefly, for these animals, the experiment consisted in two periods: a winter restriction period that lasted from ten days after calving (between November and February, with primiparous females calving first) to the start of the grazing season (generally in mid-April depending on the weather) and a spring recovery period, from the first day on grass until mid-July. During the restriction period, the animals were kept indoors in pens of 10–20 individuals on straw litter and fed 3 FU (forage units, 1 FU = 7.12 MJ ([INRA, 2018](#))) less than their theoretical needs. The distributed ration was composed of low-quality grass silage, 0.5 kg canola, 200 g mineral and vitamin supplementation, and enough straw to fill the intake capacity. Estimates of each individual's theoretical needs were based on recommendations from the INRAE nutrition system ([INRA, 2018](#)) and were updated every year. On average, restricted cows were fed 6.5 FU. During the spring recovery period, the animals had an *ad libitum* access to high-quality pastures. On average, the winter period lasted 85 days (SD 28 days, minimum duration of 40 days) and the spring period 89 days (SD 11 days, minimum duration of 60 days).

Phenotypes

Multiple phenotypes were recorded during both the restriction and recovery periods. The weights of cows and calves, as well as each cow's BCS (on a scale of 0–5; [Agabriel et al., 1986](#)), were recorded twice at both the start and end of each period, and again every-two weeks within each period. For BCS, scores were assigned by two trained technicians on each farm and the average score was used.

The milk production of each dam was measured indirectly—three times during lactation, using calf weight before and after suckling following the method described in [Martin et al. \(2022\)](#). Shortly, the calf was separated from its dam the evening before measurement. The next morning, the calf was weighed, reunited with its dam for suckling, and weighed again immediately afterwards before being separated once again. A second suckling event was recorded in the same way in the evening, and then, the calf and dam were returned to their pen together. Milk production was estimated by adding together the morning and evening weight gains of the calf. The first series of measurements was recorded midway through the winter period (on average, 35 days after the start of winter, SD 10 days), the second series at the end of the winter period (on average, 80 days after the start, SD 24 days), and the third midway through the spring period (on average, 133 days after the start of winter, or 48 days from the start of spring, SD 30 days).

Starting from 30 days after calving, trained technicians collected a blood sample from each dam every 10 days and sent it to the INRAE PRC Unit (Nouzilly, France) for the determination of progesterone levels. Two successive positive samples were deemed to indicate the resumption of cyclicity. Animals were sampled until cyclicity resumed.

A total of 20 traits were defined. For dam weight, we quantified initial BW at the start of restriction (**BWi**); BW at the end of the winter period, i.e. the end of restriction (**BWrest**); BW at the end of the spring period, i.e. the end of recovery (**BWrec**); the average daily difference during the winter period (**ADDWrest**); and the average daily difference during the spring period (**ADDWrec**). The five BCS traits corresponded exactly to the weight traits: **BCSi**, **BCSrest**, **BCSrec**, **ADDBCSrest**, and **ADDBCSrec**. For calf weight, we considered weight at the start of restriction (**CWi**), at the end of restriction (**CWrest**), and at the end of the recovery period (**CWrec**); the average daily gain during restriction (**ADGrest**) and during recovery (**ADGrec**); and the weight at 150 days of age estimated by regression (**CW150**). The three assessments of milk production were designated Milk1, Milk2, and Milk3, and resumption of cyclicity (in days after calving) was recorded as **Res_cycl**.

Statistical analyses

Firstly, phenotypes were corrected by all significant environmental effects detected in [Martin et al. \(2022\)](#) with single trait linear models using Proc GLM in SAS/STAT® software ([SAS institute Inc., 2008](#)). The possible fixed effects were the contemporary group (farm-year), the parity (number of lactations), the duration of restriction and/or recovery period, the calf sex, the age of calf, whether the calf was from a single or twin calving, and all significant interactions. For milk-related traits, the effect of the lactation stage was also included by grouping all cows within the same 10-day milk interval.

Then, the residuals of the 20 traits obtained from these linear models were used as performances in a principal component analysis (**PCA**) followed by a hierarchical clustering on principal component (**HCPC**), performed using the FactoMineR package of the R Software ([Lê et al., 2008](#)). Principal component analysis was first implemented in order to reduce dimensionality by eliminating low variance dimensions before clustering. Hierarchical clustering on principle components was then used to identify the cluster of cows with similar responses to the feed challenge.

Finally, to better describe the differences among the clusters, the same linear models as before were re-done with the addition of the cluster into the fixed effects and least-squares means for each cluster for each trait were predicted.

Results and discussion

Results of the PCA are presented in [Fig. 1](#), with (1a) showing the projection of the 20 variables on the two first dimensions, (1b) showing the projection of the 20 variables on dimensions 2 and 3 and (1c) showing the projection of the individuals (or rather lactation-individuals as some individuals perform more than one lactation) on the two first dimensions with each colour representing a different cluster. It is important to remind that this PCA was performed on residuals from linear models for the 20 traits and that, therefore, environmental effects such as lactation rank were corrected before and do not influence the results of both the PCA and the HCPC. The first dimension of the PCA explains a quarter of the total variance, the first three dimensions represent half of the total variance, and the first five dimensions correspond to two-thirds of the total variance. Based on [Fig. 1a](#)), the first dimension seems to be linked mostly to milk production and calf weight

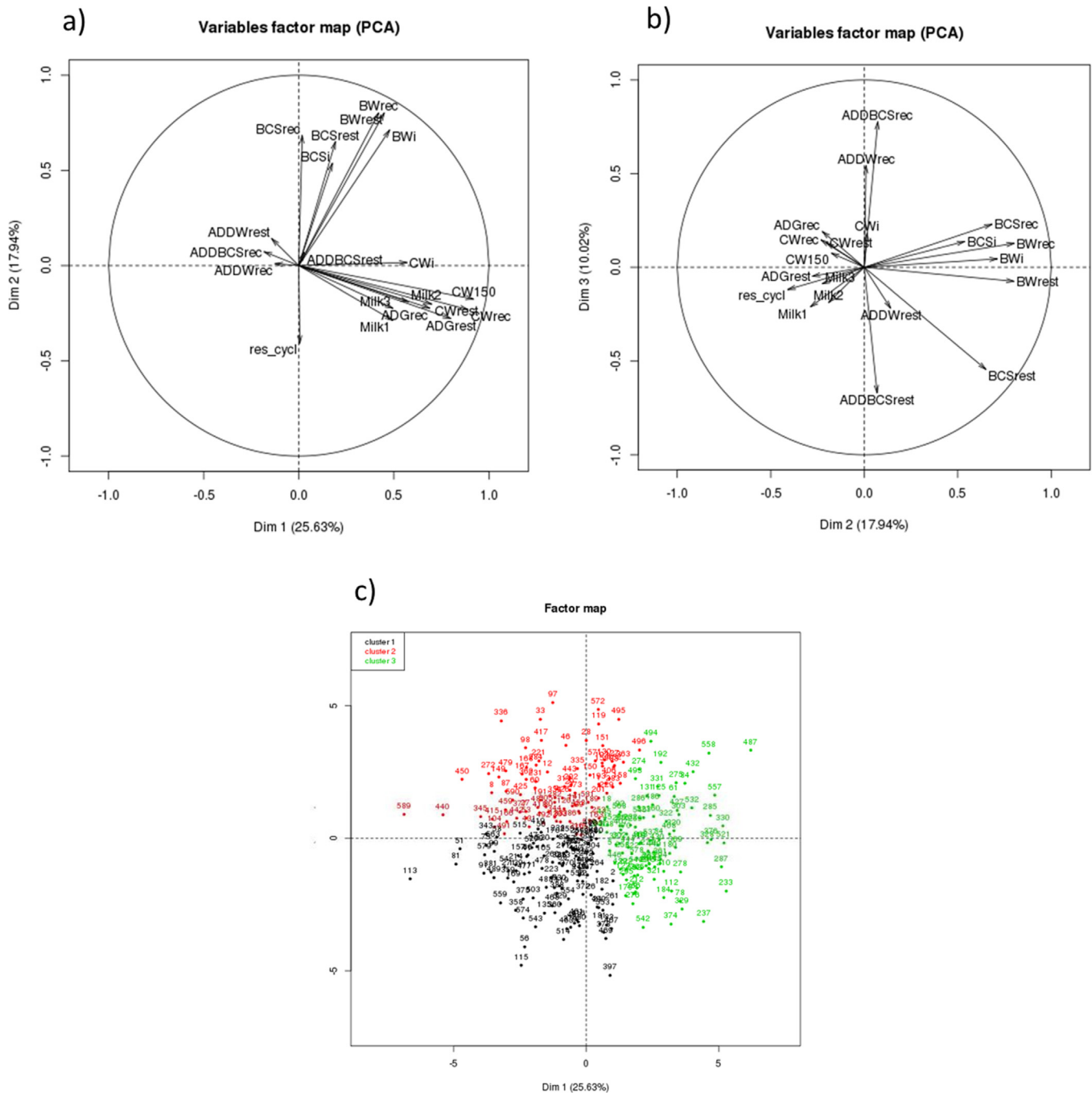


Fig. 1. Variables factor map of the principal component analysis (PCA) on the 20 considered traits presenting (a) the first two dimensions and (b) dimensions 2 and 3 with (c) the projection of each considered lactation coloured by the cluster defined by the hierarchical clustering (cluster 1 = black, cluster 2 = red and cluster 3 = green) on the lactating Charolais cattle With **ADDBCSrec**: Average daily difference in body condition score during the spring (recovery) period, **ADDBCSrest**: Average daily difference in body condition score during the winter (restriction) period, **ADDWrec**: Average daily difference in BW during the spring (recovery) period, **ADDWrest**: Average daily difference in BW during the winter (restriction) period, **ADGrec**: Average daily gain in calf weight during the spring period, **ADGrest**: Average daily gain in calf weight during the winter period, **BCSI**: Initial body condition score (at the start of the winter period), **BCSrec**: Body condition score at the end of the spring (recovery) period, **BCSrest**: Body condition score at the end of the winter (restriction) period, **BWi**: Initial BW, **BWrec**: BW at the end of the spring period, **BWrest**: BW at the end of the winter period, **CW150**: Calf weight at 150 days, **CWi**: Initial calf weight, **CWrec**: Calf weight at the end of the spring period, **CWrest**: Calf weight at the end of the winter period, **Milk1**: milk production at the first milk assessment, **Milk2**: milk production at the second milk assessment, **Milk3**: milk production at the third milk assessment, and **Res_cycl**: resumption of cyclicity.

variables, while the second dimension is mostly linked to the dam’s weight and BCS and cyclicity resumption. Changes in dam’s weight and BCS have almost no influence on these two first dimensions, but are linked, with the BCS during recovery, to the third dimension, changes during restriction and recovery being in opposition (Fig. 1b). Groups of variables observed here are the reflect of

correlations between traits estimated in Martin et al. (2022), such as for example, the opposition between cyclicity resumption and BCS, meaning that the highest the BCS is, the shortest will be the resumption.

Based on this PCA, the HCPC was performed on the first 10 dimensions (90% of variance). Even though the distribution of the

projected lactations on the two first dimensions looks like a continuum, the HCPC allows the determination of clusters, the smoothest division being three clusters. As presented in Fig. 1c), cluster 3 (green) is separated from the two others on the first dimension while cluster 1 (black) and cluster 2 (red) are divided following the second dimension. The first cluster corresponds to 111 lactations, cluster 2 to 89 lactations and cluster 3 to 93 lactations. These three clusters describe different response profiles to the restriction based on the 20 considered traits, as presented in Table 1. The first cluster contains animals with the smallest BW and BCS, both initially and during the entire experiment (restriction and recovery), and intermediate milk production and calf weight and the longest cyclicality resumption. On the contrary, the cluster 2 contains the animals with the highest BW and BCS, a limited weight loss during the restriction, the quickest cyclicality resumption and the lowest milk production and calf weight. Animals from cluster 3 present intermediate BW and BCS, a long (but not longest) cyclicality resumption and the highest milk production and calf weight. It is therefore interesting to note that despite the average response of the entire group being very balanced (Martin et al., 2022), animals from cluster 2 seem to prioritise themselves like what was observed on the Limousine breed while animals from cluster 3 seem to prioritise their calf's growth like what was observed on the Salers breed (D'Hour et al., 1995).

Being interested in the variability of individual response to a perturbation is not very frequent in cattle publications. However, when studied, it appears clearly that all the animals do not react exactly the same. Recently, Charton et al. (2019) used an HCPC in

Table 1

Comparison of the cluster corrected means for each of the 20 considered traits in the Charolais cows.

Cluster	1 (n = 111)	2 (n = 89)	3 (n = 93)
BWi (kg)	647 (a)	730 (b)	734 (b)
BWrest (kg)	605 (a)	696 (b)	684 (b)
BWrec (kg)	657 (a)	746 (b)	731 (c)
ADDWrest (kg/d)	-0.52 (a)	-0.43 (a)	-0.67 (b)
ADDWrec (kg/d)	0.57 (a)	0.56 (a, b)	0.50 (b)
BCSi (point)	2.3 (a)	2.6 (b)	2.5 (c)
BCSrest (point)	1.8 (a)	2.3 (b)	2.1 (c)
BCSrec (point)	2.3 (a)	2.8 (b)	2.5 (c)
ADDBCSrest ((point/d) * 100)	-5.5 (a)	-4.2 (a)	-4.3 (a)
ADDBCSrec ((point/d) * 100)	6.1 (a)	5.8 (a)	5.1 (a)
CWi (kg)	50.8 (a)	51.6 (a)	56.6 (b)
CWrest (kg)	109.2 (a)	101.3 (b)	126.7 (c)
CWrec (kg)	217.5 (a)	206.8 (b)	246.6 (c)
ADGrest (kg/d)	0.80 (a)	0.72 (b)	0.96 (c)
ADGrec (kg/d)	1.14 (a)	1.07 (b)	1.25 (c)
CW150 (kg)	179.3 (a)	172.0 (b)	205.2 (c)
Milk1 (kg)	6.7 (a)	5.7 (b)	7.5 (c)
Milk2 (kg)	5.8 (a)	5.2 (b)	6.7 (c)
Milk3 (kg)	6.3 (a)	5.6 (b)	7.1 (c)
res_cycl (d)	109 (a)	85 (b)	102 (a)

A different letter into brackets means that the cluster means are significantly different.

With **ADDBCSrec**: Average daily difference in body condition score during the spring (recovery) period, **ADDBCSrest**: Average daily difference in body condition score during the winter (restriction) period, **ADDWrec**: Average daily difference in BW during the spring (recovery) period, **ADDWrest**: Average daily difference in BW during the winter (restriction) period, **ADGrec**: Average daily gain in calf weight during the spring period, **ADGrest**: Average daily gain in calf weight during the winter period, **BCSi**: Initial body condition score (at the start of the winter period), **BCSrec**: Body condition score at the end of the spring (recovery) period, **BCSrest**: Body condition score at the end of the winter (restriction) period, **BWi**: Initial BW, **BWrec**: BW at the end of the spring period, **BWrest**: BW at the end of the winter period, **CW150**: Calf weight at 150 days, **CWi**: Initial calf weight, **CWrec**: Calf weight at the end of the spring period, **CWrest**: Calf weight at the end of the winter period, **Milk1**: milk production at the first milk assessment, **Milk2**: milk production at the second milk assessment, **Milk3**: milk production at the third milk assessment, and **Res_cycl**: resumption of cyclicality.

order to characterise response profiles to once-daily milking (instead of twice). Their study, based on raw performances, aimed to determine also which factors influenced the response profile. Here, we based our study on residuals from linear models in order to correct for the effects of external factors and focus on the true inter-individuals variability. It is interesting to note that among the animals performing more than one lactation, 57% remain in the same cluster on two successive lactations (similar percentage between lactations 1 and 2 and between lactations 2 and 3), and only 16 of the 47 animals (34%) performing three lactations remain in the same cluster for the three lactations. One can indeed wonder if the observed variability and repeatability could have, at least partially, a genetic determinism. This question will be explored in a future study.

Based on the results of this study, it appears clearly that some inter-individual variability exists in the response profile to feed restriction. Further studies are required to determine the possible genetic influence and consequences of not taking this phenomenon into consideration with the regular use of feed restriction in commercial farms.

Ethics approval

Throughout the course of the experiment, all animals were handled with care in line with INRAE's ethics policy and in compliance with the guidelines on animal research issued by the French Ministry of Agriculture (<https://www.legifrance.gouv.fr/eli/decret/2013/2/1/2013-118/jo/texte>).

Data and model availability statement

None of the data or the models were deposited in an official repository. Data are confidential.

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Declaration of interest

None.

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