GenTORE

Genomic management Tools to Optimise Resilience and Efficiency

Grant agreement n°: 727213

H2020 - Research and Innovation Action

D2.3
Report on the MultiSite experiment

Due date: 12/20

Actual submission date: 12/20

Project start date: 1st June 2017 **Duration:** 60 months

Workpackage concerned: WP2

Concerned workpackage leader: Didier Boichard

Lead Beneficiary: INRAE

Dissemination level:

- **X PU:** Public (must be available on the website)
- ☐ **CO:** Confidential, only for members of the consortium (including the Commission Services)
- ☐ **CI:** Classified, as referred to in Commission Decision 2001/844/EC

Outline

Objectives

There is a growing need to be able to breed and manage bovine livestock so as to achieve optimum levels of resilience and efficiency. However, it is generally believed that the most efficient animals are also more prone to poor fertility and to infectious and non-infectious diseases. This would mean that their resilience, *i.e.* their ability to postpone culling, is decreased. This assumption remains to be confirmed when efficiency is not simply production level and when it is assessed on the long term. Answering that question is crucial to define conditions to improve both efficiency and resilience simultaneously. A critical objective of GenTORE is therefore to assess the relationship between efficiency and resilience over different time horizons and over a broad range of animals and production systems.

However very little data exist to address this question. Most efficiency data are collected in experimental or specialized facilities, on rather short periods, and usually on young animals. In growing animals, efficiency data can rarely be associated to length of productive life, fertility, disease or any resilience information due to their limited length of productive life. In dairy cows, efficiency data are recorded during a few months of lactation at most and, again, can rarely be associated to resilience traits, whereas in suckling cows this kind of data are nearly inexistant. In contrast, commercial farms can provide rich information to characterize resilience (longevity, reproduction, health, production…) on a large scale but efficiency is nearly impossible to characterize in these farms beyond production level, at least with conventional methods based on feed intake recording.

The main goal of this multi-site experiment was therefore to generate information on both efficiency and resilience, in order to study their interplay. A second goal was to validate potential proxy measures of resilience proposed in other tasks and WP, across a range of conditions. For that aim, the different experiments were not simply replicates of the same protocol. In contrast they were designed to cover a broad range of conditions and systems. They differed in the kind and breed of animals (including beef and dairy), methods to assess efficiency, methods to assess resilience, periods and duration of measures. The wide range of conditions will allow to quantify the sensitivity of correlations between resilience and efficiency. This experiment has used key breed-environment combinations across the bovine livestock production spectrum, with experiments in 3 contrasting types of environment: indoors feeding, enriched grazing (maritime environments), and mountain grazing including both organic and conventional systems. An important effort was dedicated to beef cattle because this information was especially lacking for these animals. In some experiments, efficiency and resilience were characterized simultaneously whereas in others, efficiency was characterized first and resilience later. As far as possible, common features were defined for the different components of this multi-site experiment. In the first year of the GenTORE project, a document was jointly elaborated, describing these common features and the minimum common traits to measure. This document is presented in Annex 1. Different resilience criteria were used, including the most conventional ones such as ability to recalve, production, health, but an important effort was dedicated to the response to perturbations, as a proxy of resilience. Nutritional challenges were chosen as a method of choice to induce perturbations, as they were shown to be useful to characterize adaptation response. They were variable in number, length, and intensity. These data will help to determine to what extent productive lifespan and other aspects of resilience can be predicted by early life traits describing responses to these challenges. They will also help to characterize the relationship with efficiency.

This experiment measured efficiency and resilience components using well-established reference methods (feed intake, liveweight and liveweight change, body fatness (so-called body condition score), milk production, health and fertility indicators) and also non-conventional high-throughput potentially proxy measures from sensor devices. A common accelerometer sensor system was chosen, from the Medria company, and was used in different facilities providing high-frequency time-series data over the production phase, *i.e.* relatively long timescales. All animals were also genotyped with 50k SNP chips to facilitate data reusability in other workpackages.

Six experiments were carried out:

- Long-term (3 lactations) experiment in Charolais cows, with characterization of feed efficiency as heifer, and study of response to a long nutritional challenge (several months) and a recovery period, repeated over three lactations This experiment was carried out by INRAE-GABI and le Pin experimental facility.
- Two studies of the response to repeated short nutritional challenges in beef cows and their calves, carried out by INRAE-UMRH and CITA. The protocols were coordinated between both partners. In one of these studies, cows were divergently selected for residual feed intake.
- One study of the response to repeated short nutritional challenges in dairy cows, carried out by INRAE-UMRH.
- One experiment in two organic commercial dairy farms carried out by FIBL. Although measures were more limited than in experimental facilities, nutritional challenges were simulated according to the quality of the grazed grass during the paddock rotation.
- At LfL, the objective was rather different. The study aimed at reestimating energy content of modern cattle. Indeed, these values, obtained a long time ago, are very important for animal feeding recommendations.

In this document, we report these experiments and their main conclusions. Due to COVID19, several of these experiments were not fully completed and analyzed at the date of this deliverable. Six to nine months are still necessary to finalize the lab work, the data analyses and the scientific papers. Therefore, this report presents the results obtained at month 42 of the project, *ie* in November 2020.

Description of the experiments

Experiment of INRAE GABI - le Pin

Partners :

- UMR GABI, INRAE Jouy-en-Josas = Gilles Renand, Pauline Martin, Aurélie Vinet
- Unité Expérimentale du Pin https://doi.org/10.15454/1.5483257052131956E12
- Unité Expérimentale de Bourges https://doi.org/10.15454/1.5483259352597417E12

General Description

In the current context of economic and environmental difficulties in suckling beef production, the ability of cows to valorise roughage and to cope with food restrictions is a key issue. Food challenge is a way to test the abilities of the animals to maintain their production through a food restriction period and a subsequent recovery, and therefore to measure resilience. We performed such a challenge during the critical period of early lactation on females that had been previously characterized for their efficiency in transformation of roughage through a feed intake experiment when they were heifers. This experiment was performed in full compliance with the national legislation on animal care.

This experiment was a large and long-term experiment. It started with the characterization of feed efficiency of the heifers. Then the response to the food challenge was investigated during the first three lactations of the females in order to identify the most efficient cows throughout their whole career. In order to obtain sufficient power, four annual batches of females were studied.

Between 2011 and 2015, 613 heifers were previously bred in INRAE Experimental Units in le Pin and Bourges, with 283 and 330 heifers, respectively. At 22 months of age, their feed intake was recorded during 12 weeks with a roughage diet distributed *ad libitum.* During this step, they were transferred to feed intake recording stations equipped with individual troughs with automatically opening gates. The diet included hay supplemented with one kg concentrate at le Pin, and grass silage at Bourges. After 4 weeks of adaptation, feed intake was individually recorded during 12 weeks.

From 2014 to 2020, 161 females among those bred at le Pin were engaged in a nutritional challenge experiment from their first lactation up to the end of their third lactation. In addition, in Bourges, 142 cows were also challenged from 2015 to 2017, during their first lactation only. Altogether, a total of 592 suckling periods from 340 cows and their calves were studied.

The challenge was divided into two periods. During the first part, named « Restriction Period », the herd was split into two groups: a HIGH group that was fed 20% above its theoretical needs, and a LOW group that was fed 30% below its theoretical needs, according to the INRAE Feeding System for Ruminants. During the second period, named « Recovery Period », both cows groups were treated together with *ad libitum* feeding.

Alltogether, this experiment lasted 9 years to characterize feed efficiency at heifer step during 5 years and resilience in 1st to 3rd lactations during 7 years. GenTORE contributed to the funding to the last 4 years of this large experiment in Le Pin experimental farm.

Detailed protocol

Feed efficiency step :

Experimental females were born over two periods, in fall or winter, and were weaned at an average age of 32 weeks. At the age of 22 months their intake was controlled for 12 weeks with roughages distributed *ad libitum*. They were transferred to feed intake recording stations equipped with individual troughs and automatically opening gates. They were fed *ad libitum* with hay supplemented with one kilo concentrate at le Pin. In Bourges, the diet comprised only grass silage. After 4 weeks of adaptation, individual feed offers and refusals were recorded for 12 weeks.

Heifers' intake:

Offered forage was weighted every day, refusals were weighted three times a week on monday, wesdenay and friday. Refusals were weighed on the morning, before the daily distribution of feed. So, refusals weighted on monday morning correspond to total refusals of friday, saturday and sunday, refusals weighted on wednesday correspond to total refusals of monday and tuesday, and so on.

Feed Intake data uploaded in the GenTORE Sharepoint was summarised in one observation per animal per week and consist of the daily average of the feed quantity offered minus refusals of the week.

Heifers' growth:

At the beginning and at the end of the feed intake measurement period, heifers were also weighed on 2 consecutive days to establish initial and final body weights. They were also weighed every second week during the 12 weeks of feed intake measurement.

From 23 to 27 months of age and for 9 weeks, all heifers were mated with AI sires. After calving, those suckling a calf were engaged in the nutritional challenge experiment.

Principle of the food challenge :

The Restriction Period lasted 90 days on average, from 10 days *post partum* to the day the animals were released on pasture (in April or May, depending on the year). Calvings were distributed between November and March, with primiparous cows calving first. During the Restriction Period, the herd was split into two groups: the HIGH group was fed 20% above its needs, while the LOW group was fed 30% below its needs according to the INRAE Feeding System for Ruminants. For example, for a primiparous of 700 kg producing 7 kg milk per day, needs are 9.5 FU (5.6 FU for maintenance, 3.2 FU for milk production, 0.7 FU for growth). Then, energy intake was 6.5 FU for LOW groups and 11.5 FU for HIGH groups. Those theoretical needs were updated every week taking to account the average weight of cows and their milk production. During this period, cows were group-fed, stuck in feedgates after distribution to ensure feed access for all cows.

The Recovery Period lasted 12 weeks and started when then animals returned to pasture until July. During this period, both groups were conducted together with *ad libitum* grazing. All cows in experiment were lactating, suckling their natural calf (N=583 lactations) or an adopted calf (N=9 lactations).

During the Recovery Period, cows were bred between April and July with natural service bulls. As a result of these matings, cows that calved and had a lactation were engaged in a second, and then a third nutritional challenge. For these subsequent challenges the principle was exactly the same as in first lactation, except that the Restriction Period was shorter because multiparous cows calved later than primiparous cows. Theoritical needs were calculated for each contemporary x treatment (HIGH *vs* LOW) groups. During their three lactations, the cows remained in their initial group.

Growth of the cows and their calves:

Body weights (BW) of cows in nutritional challenge and those of their suckled calves were recorded monthly during the Restriction and the Recovery periods, as well as at the beginning of the Restriction period, and at the beginning and end of Recovery Period. During the Restriction Period, calves were exclusively fed by suckling.

In the TREATMENT file uploaded in the GenTORE database, the « stress » (S) group corresponds to the « LOW » treatment and the « control » (C) group corresponds to the « HIGH » treatment.

Results (preliminary)

Heifers' ingestion :

To assess the feed efficiency of the heifers, the residual feed intake (RFI) was calculated as the difference between the recorded dry matter intake (DMI) and that predicted by multiple regression accounting for mid-test weight (MW) and average daily gain (ADG), as well as some identified environmental effects (µ) depending on the batches. In other words, RFI was the error term in the following model:

$$DMI = \mathrm{\mu} + \mathrm{\alpha}.\,\mathrm{MW}^{0.75} + \mathrm{\beta}.\,\mathrm{ADG} + \mathrm{RFI}$$

Descriptive statistics of performances measured on heifers are shown in Table 1.1, genetic parameters estimates are given in Table 1.2.

Table 1.1 : Mean, standard deviations and coefficient of variation of performances recorded on heifers.

Phénotypes	Unit	N	Mean	SD	CV* (%)
MW	Kg	569	521	46	9
DMI	kg / day	569	8.50	1.04	12
ADG	g / day	569	681	168	25
RFI	kg / day	569	0	0.83	10

**for RFI: CV = Standard Deviation/DMI*

Heritabilities of MSI and RFI are low and do not exceed 0.13 (table 1.2). However, these coefficients are close to those estimated in a previous experiment with 498 heifers also controlled with a grass silage diet, but younger, at 15 months of age (Vinet et al., 2008). In the literature, heritability estimates for MSI and RFI averaged 0.40 and 0.33 (Berry and Crowley, 2013), but mostly measured on younger calves in intensive growth phase and fed with higher energy diets. There are no similar studies on older heifers. However, there are four studies conducted on adult cows that show that the heritability of RFI is low in this type of animal, from 0.03 to 0.23 (Fan et al., 1996; Archer et al., 2002; Freetly et al., 2020; Martin et al., 2019). Differences in maintenance requirements account for the main part of the energy intake and probably the major part of the differences in RFI between these adult cows. One could think that maintenance needs, such as RFI, are not very heritable and that most of the differences in intake between two-year-old heifers and adult cows are not of genetic origin. In their study, however, Archer et al. (2002) and Freetly et al. (2020) showed high genetic correlations (+0.98 and +0.41, respectively) between RFI of adult cows and that of heifers measured just after weaning. These genetic relationships support the existence of a genetic determinism of maintenance needs that would be expressed throughout the life of the females.

Table 1.2 : Genetic parameters of feed efficiency measured on heifers

	MW	DMI	ADG	RFI
Heritability	0.30 ±0.11	0.12 ±0.09	0.01 ±0.08	0.13 ±0.10
MW		0.25 ±0.22	NE	-0.28 ± 0.29
DMI	0.50		NE	0.62 ± 0.27
ADG	0.33	0.33		NE
RFI	0.00	0.84	0.00	

Genetic correlations ± standard error above the diagonal, phenotypic correlations below the diagonal ; NE : not estimable due to low h^2

Food challenge :

The descriptive statistics of performances of cows and calves during nutritional challenge according the experimental treatment, HIGH *vs* LOW, are given in Table 1.3.

Effect of nutritional challenge on performances of cows and calves

The effect of parity on performances is shown in Table 1.4, and the effect of the nutritional challenge is shown in Table 1.5. The cow weight at parturition does not differ significantly between the two groups. For primiparous cows, this result is quite logical, as they did not start any restriction yet. Regarding multiparous cows, this result shows that they are no longer affected by previous year restriction. After the restriction period, cows of the HIGH group are on average 59 kg heavier than those of the LOW group. This results from a weight loss of 8 kg for the LOW group and a weight gain of 51 kg for HIGH group.

During the recovery period, both groups gain weight, and at the end, the difference of weights between cows from HIGH and LOW groups is 31 kg. This means that during the recovery period, the cows of LOW group partially compensate for the lack of weight gain accumulated during the restriction period but this compensation is not complete. The difference observed in our study during the restriction period is a bit higher than those found in a similar nutritional challenge in Charolais cows (De La Torre et al., 2010). In their experiment, at the end of restriction, there was a difference of 52 kg between primiparous of HIGH and LOW group and a difference of 25 kg for multiparous. This suggests the existence of an interaction between groups and parity, interaction that is not significant in our study. The difference in the results can be due to the restriction not being exactly under the same conditions. Indeed, in the experiment of De La Torre et al. (2010), animals were complemented with high-density pellets while in our project cows are only fed with forage, diets were calculated according to weight at parturition and theoretical needs were updated every week. This challenge is also characterized by longer restriction periods and shorter recovery periods. For example, in their experiment, restriction period lasted on average 125 days for primiparous cows and 132 days for multiparous cows, while in our experiment the average duration is 113 days for primiparous and 70 days for multiparous cows.

Table 1.3. Descriptive statistics of performances of cows and calves in nutritional challenge.

	HIGH group			LOW group		
	n	Mean	SD	n	Mean	SD
Cows' performances						
BW at parturition	266	664.2	71.6	257	667.4	69.0
BW at the end of restriction (kg)	270	693.0	83.3	259	638.4	70.1
BW at the end of recovery (kg)	269	715.9	74.3	262	682.2	70.7
Calves' performances						
Calf BW at birth (kg)	269	44.7	7.2	263	44.3	6.8
Mean calf AGE at the end of restriction (d)	273	98.8	27.4	263	98.5	27.4
Mean calf BW at the end of restriction (kg)	266	139.4	31.8	258	121.7	23.6
Age adjusted calf BW at the end of restriction (kg)	272	141.4	22.2	263	126.9	20.7
BW at 120d (kg)	273	169.6	22.3	263	157.5	21.9
Mean calf AGE at the end of recovery (d)	273	185.5	31.9	263	185.4	32.9
Mean calf BW at the end of recovery (kg)	270	242.2	45.7	258	222.4	38.9
Age adjusted calf weight at the end of recovery (kg)	273	234.7	43.8	263	217.5	38.4
BW at 210d (kg)	273	257.8	36.5	263	240.4	35.5

In our experiment, most of the cows are still growing. This growth is then also to be taken into account and it is difficult to quantify body reserves loss. In body mobilization periods, cows lose lipids first and proteins in a second step, while bone mass remains constant (Blanc et al., 2006). In a growing cow, bone structure increases and muscles fix proteins. Then, weight change does not only represent loss of body reserves. It is also worth noting that live weight, as used here, includes digestive content which varies between animals and according to feed intake (with roughage, about a 6 kg digestive content variation for 1 kg dry matter intake variation per day). The digestive content represents on average 15% of the live weight (Martin, Sauvant, 2003). Animals of different feed groups have different diets with different digestive fillings. The rations given to LOW group were designed to fill the rumen the same way as in the HIGH group. As it was not possible to measure the digestive content, we applied standard rules to obtain the « real » weight.

Table 1.4. Effect of parity (Least Square Means).

	Parity		
	1	2	3
Cows' performances			
BW at parturition (kg)	654.2	695.8	735.5
BW at the end of restriction (kg)	654.1	681.1	724.9
BW at the end of recovery (kg)	677.5	713.3	735.3
Calves' performances			
BW at birth (kg)	41.3	47.9	51.5
BW at the end of restriction (kg)	138.8	111.4	102.0
BW at 120d (kg)	159.0	175.5	174.5
BW at the end of recovery (kg)	234.8	203.4	190.4
BW at 210d (kg)	244.2	267.0	264.3

Table 1.5. Effect of feed group (Least Square Means).

	HIGH group	LOW group	HIGH-LOW Difference
Cows' performances			
BW at parturition (kg)	695.4	694.8	0.6 (ns)
BW at the end of restriction (kg)	715.9	657.5	58.4
BW at the end of recovery (kg)	724.5	692.9	31.6
Calves' performances			
BW at birth (kg)	47.3	46.8	0.5
BW at the end of restriction (kg)	125.6	109.2	16.4
BW at 120d (kg)	178.7	160.6	18.1
BW at the end of recovery (kg)	218.1	201.0	17.1
BW at 210d (kg)	267.2	249.6	17.6

ns : no significant effect of the feed group.

In order to compare calves growth, weights at 120 and 210 days were calculated by linear regressions for all calves with at least 2 measures during the challenge. The group does not affect calf weight at birth (Table 1.5). Again, the primiparous cows have never been restricted before, but for the multiparous cows, we could think that the restriction experienced before conception could have had an effect. At the end of restriction, the average age of the calves is 98 days and the average weights of the two groups differ by 16.4 kg. From birth to 3 months of age, they are expected to eat 6 to 8 kg milk and 0 to 2 kg hay per day (Dufrasne, 2011). From the end of restriction to 210 days of lactation, the group effect remained significant and the gap between HIGH and LOW groups is steady, at about 18 kg. This means that calves of LOW group do not compensate for their previous lack in growth, but this difference no longer increases once the dams are back to the same unrestricted diet. This result is in agreement with a previous study where calves restricted during their first 3 months hardly ever catch up their delay in growth (Dufrasne, 2011).

Priorities in biological functions

To compare the evolution of different variables during challenge, we expressed them in the same unit. Therefore, we converted the difference of results between HIGH and LOW groups at the end of restriction and at the end of recovery HIGH group in standard deviation at the beginning of challenge (Table 1.6). To limit the impact of the sprawl of parturitions between primiparous and multiparous cows, the differences in calves' BW were calculated at 120 and 210 days.

Table 1.6. Difference between HIGH and LOW group, expessed in standard deviation units.

Trait	Differences at the end of restriction	Differences at the end of recovery
Cow BW	0.70	0.43
Calf BW	0.82 (at 120 d)	0.49 (at 210 d)

Cows weights show that even if those traits show a rebound effect, the LOW group do not get back to the level it would have had without restriction. Consequently, the adaptation of this trait to restriction seems to be flexible but not elastic as cows do not recover the weight they would have if they did not experience restriction. However, since restriction does not have a significant effect on weigths at later calvings, we can conclude that those traits have an elastic path but the recovery period is not long enough in our experiment to witness the complete rebound. Calf weight models show that from the end of restriction to 210 days of lactation, the feed group effect remains significant and the gap between HIGH and LOW groups is steady, showing that calves of LOW group do not recover at all their late in growth in the recovery period.

In cow weight models, at the end of restriction period, interaction between feed group and parity effects is significant. The average weight difference between the two feed groups decreases as parity increases, from 64 kg in first lactation to 42 kg in third lactation. The same tendency appeared in a previous food challenge (De La Torre et al., 2010) as at the end of restriction, the difference between groups HIGH and LOW was 39 in primiparous cows and only 8 kg in multiparous cows. It shows that restriction does not have the same impact on weight according to parity. As the cows remained in the same feed group throughout their three years of challenge, this interaction could show a plasticity in adaptation to food restriction, cows being less impacted by a restriction if previous restrictions occurred before. However, we cannot ignore the fact that growth of the animals decreases with parity and that it may also have an effect. Moreover, the animals from the LOW group that were actually able to perform their three lactations were those that could deal better with restriction while the ones that coped less well were those unable to reproduce. This unvoluntary selection may have increased the average performance level of the LOW group with lactation rank. This hypothesis needs to be further investigated.

In calf weights, interaction between lactation rank effect and weight is significant at the end of restriction. It shows that primiparous have the biggest gap between HIGH and LOW groups (22 kg of calf weight difference) and this gap goes on decreasing when lactation rank increases. It may be partly due that primiparous cows calve before multiparous cows, so their restriction period is longer. At 120 days, the interaction between feed group and parity is significant but not anymore at the end of recovery period and at 210 days of lactation. There is no evolution of difference between HIGH and LOW groups calf weights from the end of restriction to 210 days of age through the different lactations, so if there is any plasticity in calf weights, it is not perceptible here.

Papers

Renand et al., 2020. Précocité et efficience alimentaire des génisses de renouvellement en races à viande. In Rencontres autour de la Recherche sur les Ruminants, December 3-4 2020, Paris.

Allart, 2019. Etude de la capacité adaptative des vaches charolaises à une restriction alimentaire. Report on Engineer Internship.

Situation regarding Covid and expected date of final results.

Due to Covid, the last year of the feeding challenge could not be achieved. That concerns only the food challenge of the cows of the last cohort (born in 2015) in their third lactation. Therefore, the experiment is slightly truncated but it does not alter its overall informativity.

The complete analysis of this experiment, including the relationship between feed efficiency and response to challenge, will be carried out in 2021, with at least one expected publication.

Data

All data were uploaded on the WP2.3 common repository at url https://sites.inra.fr/site/gentore-wp2/efficiency in directory GABI/exp5.

Conclusion

Our results show a low heritability of the feed efficiency of heifers fed with rough forages as well as a good capacity of Charolais cows to mobilize their body reserves to feed the calf, elucidating the ability of this breed to favor the growth of the offspring and the dam. Due to these low heritability estimates, genetic correlations remain hard to estimate. Complete results, including the relationship between feed efficiency with forage-based diets and the resilience of females throughout their career, will be available within a year.

Partners:

INRAE UMR Herbivores, 63122 Saint-Genès Champanelle, France

INRAE, HerbiPole, Low Mountain Ruminant Farming Systems Experiment Facility

General Description

To study the relationship between resilience and efficiency in beef cows, two experiments were performed at the HerbiPole, INRAE Low Mountain Ruminant Farming Systems Facility (https://doi.org/10.15454/1.5572318050509348E12). These two experiments were performed in full compliance with the national legislation on animal care.

While experimental methods exist to evaluate feed efficiency in animals, no standard procedures are available to assess resilience of different types of ruminants. For dairy cows several recent studies used short-term feed restriction (from 2 to 6 days) to induce marked changes in terms of production and metabolism and followed those changes over time (Bjerre-Harpoth, 2012; Friggens et al., 2016; Billa et al, 2019). For beef cows, the ability of such experimental methods to induce quantifiable productive and metabolic responses and their relevance to assess resilience and its between-animal variability needed to first be tested.

A first experiment was performed during the winter season 2018 (GENTORE2018, from 2018/03/12 to 2018/07/08). Its objective was strictly methodological. It was to test in beef cows the ability of a short-term feed restriction to induce marked and quantifiable changes of production and metabolism. The second experiment was realised during the winter season 2019 (GENTORE2019, 2018/12/11 to 2019/06/23). Its objectives were to characterize and quantify the adaptive responses of beef cows exposed to i/ a single short-term feed restriction (assessment of resilience) and ii/ three repeated short-term feed restrictions (assessment of robustness), and to address the relationship between resilience and feed efficiency. For this purpose, the cows used in GENTORE2019 were phenotyped for feed efficiency at 3 different physiological stages (as heifers, at early and at mid-lactation during their first lactation).

GENTORE2018 Experiment:

The experiment GENTORE2018 is an observational experiment with a single group of primiparous Charolais beef cows (n=13) undergoing a control (55 days, *ie* from calving to d54 post-partum) followed by an experimental period (49 days) during these cows were subjected to two short-term feed restrictions (FR), each followed by a recovery period (Recov). As for the FR treatments, cows underwent two short-term feed restrictions that differed only by their duration. Cows were first subjected to a 4-day FR (FR4, from d54 to d58 post-partum), and subsequently to a 10 day FR (FR10, from d75 to d85 post-partum). The recoveries periods lasted 17 and 18 days each: Recov4 (from d58 to d75), and Recov10 (from d85 to d104).

During the control and the recoveries periods, cows received an ad libitum hay diet supplemented with a concentrate to meet 100% of their net energy and metabolisable protein. During the FR periods, cows were feed restricted at 50 % of their energy requirements with a hay diet supplemented with concentrate. During the whole experiment, the proportion of concentrate in the diet remained constant at 16 %.

The cows (39 ± 2 months old; 680 ± 42 kg at calving) were housed in free-stalls equipped with individual feed bunks and automatic gates. Water and salt-block were available ad libitum. The feeding treatments and all measures were performed at individual level. Figure 2.1 illustrates the experimental design.

All measures were performed from calving (d0) until the end of the experimental period (d104). Figure 2.2 illustrates the frequency of the measures performed. Briefly, daily amounts of feed offered and refused were individually recorded as well as their dry matter contents (60°C, 72h). The total dry matter intake (DMI, kg of DM) was computed for each cow as daily amount of DM offered minus DM refused. Body weights were regularly recorded at the frequency reported in Figure 2.2 at 13h with no prior feed withdrawal. Body condition score (BCS) was assessed once a week during the entire experiment by the two same assessors on a 0-5 scale (Agabriel et al, 1986). Milk production was measured by the weigh-suckle-weigh method (Le Neindre et al, 1973). The individual daily milk production (kg/d) was estimated as the sum of the milk drunk by the calf at the evening and the following morning suckling. Blood samples were collected from coccygeal vessels at the tail before the morning feeding; days are reported in Figure 2.2. Blood was sampled into EDTA evacuated blood tubes and immediately centrifuged at 1500G during 20 minutes. Plasma was then stored at -20°C until analysis of NEFA, β-OH butyrate, glucose and urea using an automatic analyzer (ARENA 20XT, Thermo-Fischer Scientific).

The static and the dynamic responses of beef cows to FR were analysed. As planned, DMI was reduced by half (7.30 kg DM) during both FR and intake returned immediately to pre-restriction levels (13.8 kg DM) at the end of FR periods. Cows lost on average 25 and 28 kg of BW in FR4 and FR10 respectively, which represents a BW loss of 3.6 to 4.1 % in comparison to the pre-restriction period. No change in BCS was observed during the entire experiment. The milk production averaged 6.6 kg/d during the first 50 days post-partum. Milk loss amounted to 11% (FR4) to 15% (FR10) in comparison to pre-restriction periods. The milk loss during FR4 was fully recovered during Recov4 [5.6 kg/d (recov4) vs. 5.8 kg/d (FR4)] whereas recovery was incomplete after FR10 [4.4 (recov10) vs 4.8 kg/d (FR10), P<0.0001)]. Among the plasma metabolites measured, only plasma NEFA concentrations showed highly significant changes (Figure 2.3A). FR4 and FR10 periods induced a 2.5 to 3.4 times increase in concentrations in comparison to pre-restriction.

To quantify deviations in responses occurring during FR and recoveries, a functional data analysis (FDA, Codrea et al, 2011) was performed using data from the two variables that showed the most important and significant changes, milk production (MY) and plasma NEFA concentrations. Briefly, a reference trajectory for both variables was defined for each cow. It represented the expected adaptive trajectory in absence of FR and recovery periods. A cow is then considered as its own control. For plasma NEFA concentrations, the reference trajectory is assumed to be a straight horizontal line whereas for milk variable, the reference trajectory was obtained by applying the same FDA algorithm on MY outside FR and recoveries. Reference and adaptive trajectories for milk and plasma NEFA concentrations were used to calculate new additional variables, such as rate of response or recovery, amplitude of deviation and different time features, to describe the dynamic changes. Baseline value of milk yield observed averaged 6.5 and 5.8 before FR4 and FR10, respectively, and amounted at the lowest 5.6 and 4.4 kg/d. The milk loss did not differ whatever the FR duration (-0.18 and -0.22 kg/d in FR4 and FR10 respectively) and averaged less than 1 kg/d. Similarly, the rate of recovery did not differ among both FR and averaged 0.11 and 0.15 kg/d. For plasma NEFA dynamic responses, a 3 to 4.4 fold increase was observed during FR periods. The amplitude of deviation was higher in FR10 than in FR4 (0.24 vs 0.18 mmol/l, P<0.01, paired-Wilcoxon test). When FR were stopped, plasma NEFA concentrations returned to their initial values at the same rate for both Recov4 and Recov10 (-0.06 mmol/l/d) within 3 ± 1 days. Significant and positive correlations were shown between both FR duration for several variables extracted from FDA of NEFA and MY showing that high responders cows in FR4 remained the same in FR10.

To conclude, this first experiment showed that short-term feed restriction can be used as an experimental model to induce marked changes in productive and metabolic traits in beef cows and assess resilience. We assumed that a 50% feed restriction is nevertheless necessary. The recovery of productive and metabolic functions, assessed through the milk production and the plasma NEFA concentrations, to pre-restriction levels occurred within 3 days. Dynamics of milk and plasma NEFA concentrations were the most responsive variables to FR. FDA applied to both milk and NEFA variables was also a relevant framework to analyze dynamics of response to and recovery from FR. Each cow being its own control, these analyses accounted for individual variability. Further research is necessary to test the relevance of some variables extracted from NEFA and milk dynamics as proxies for animal resilience before they can be used to target animals for selective management and breeding strategies. This GENTORE2018 experiment contributed to define a relevant framework to study resilience in beef cows.

The results of this experiment were presented as a short communication (De La Torre et al., 2020) and a poster presentation (Mendes LB et al., 2020) at the 71st EAAP annual meeting (Virtual meeting, 1-4th December 2020). An original paper is also in preparation and should be submitted, after approval by the GenTORE dissemination committee, in early 2021.

GENTORE2019 Experiment:

The GENTORE2019 experiment studied the efficiency x resilience interactions. Based on the above results, a 4-day short-term feed restriction at 50 % of energy requirements was chosen to challenge beef cows and evaluate their resilience. Robustness of animals was then tested by applying 3 successive 4-day FR spaced by 3 days of recovery. Beef cows included in the 2019 experiment had previously been phenotyped for feed efficiency (residual feed intake, RFI) over 12 weeks when they were heifers. To evaluate the repeatability of animal feed efficiency, two additional 8-week periods of feed efficiency phenotyping were performed, from calving to day 55 post-partum and from day 96 to day 150 post-partum, respectively. Figure 2.4 illustrates the experimental design of the GENTORE2019 experiment that included 22 primiparous beef cows (695 ± 55 kg, 34 ± 1.9 months old).

Similar measures to those of the GENTORE2018 experiment were performed. Milk samples on 8 beef cows at 21 different times along the experiment were taken to measure milk composition and metabolic status. Moreover, each cow was equipped with a Medria AXEL® sensor placed on a collar around the neck. The sensor records position (standing or lying) as well as 5 behaviors: eating, ruminating, resting, over activity and unidentified activity which refers to all other activities besides those previously cited. A large part of the analyses has been completed, but plasma and milk metabolites remain to be analysed.

A call for a post-doctoral fellowship position (1 year) is currently open to start in the beginning of 2021.

Data

All data were uploaded on the common repository at url https://sites.inra.fr/site/gentore-wp2/efficiency, in directory INRA-UMRH/exp6.

Situation regarding Covid and expected date for final results.

There has been a 6-month delay in the laboratory analyses due to the COVID-19 situation and lockdown. The original article based on first results obtained in 2018 is expected to be submitted in early 2021. A second original article included results of GENTORE 2019 is planned to be submitted at the end of 2021.

References

Agabriel J, Giraud JM and Petit M 1986. Détermination et utilisation de la note d'état d'engraissement en élevage allaitant. Bulletin Technique CRZV Theix, INRA, 66, 43-50.

Billa, P-A, Faulconnier, Y., Larsen, T., Leroux, C., Pires, JAA. Milk metabolites as noninvasive indicators of nutritional status of mid-lactation Holstein and Montbeliarde cows. J. Dairy Sci., 103(4), 3133-3146. https://doi.org/10.3168/jds.2019-17466

Bjerre-Harpøth, V., N.C. Friggens, V.M. Thorup, T. Larsen, B.M. Damgaard, K.L. Ingvartsen, and K.M. Moyes. 2012. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. J. Dairy Sci. 95:2362–2380. DOI: 10.3168/jds.2011-4419

Codrea, M.C., S. Hojsgaard, and N.C. Friggens. 2011. Differential smoothing of time-series measurements to identify disturbances in performance and quantify animal response characteristics: An example using milk yield profiles in dairy cows. J. Anim. Sci. 89:3089–3098. http://doi:10.2527/jas.2010-3753

De La Torre, A., Mendes, LB., Pires J., Sepchat B., Cassar-Malek I., Ortigues-Marty I. and F. Blanc. 2020. Increasing duration of feed restriction: performance ranking and variability of beef cows' response. 71st EAAP annual Meeting, Virtual, 1-4 december 2020, pp. 281.

Friggens, N.C., C. Duvaux-Ponter, M.P. Etienne, T. Mary-Huard, and P. Schmidely. 2016. Characterizing individual differences in animal responses to a nutritional challenge: Toward improved robustness measures. J. Dairy Sci. 99:2704–2718. http://dx.doi.org/10.3168/jds.2015-10162

https://doi.org/10.1051/animres:19730402

INRA 2007. Nutrition of cattle, Sheep and Goats: Animal Needs and Feed Values. Quae Editions, Paris, France. https://hal.archives-ouvertes.fr/hal-00896064

Le Neindre, P., Dubroeucq, H., 1973. Observations sur l'estimation de la production laitière des vaches allaitantes par la pesée du veau avant et après tétée. Ann. Zoot. 22, 413-422.

Mendes L.B., De La Torre A., Pires J., Cassar-Malek I., Ortigues-Marty I. and Blanc F. 2020. Functional data analysis on MY and NEFA responses of beef cows exposed to feed restrictions. 71st EAAP annual Meeting, Virtual, 1-4 december 2020, pp.371.

Figure 2.1: Schematic representation of the sequence of feed treatments applied to beef cows during GENTORE2018 experiment

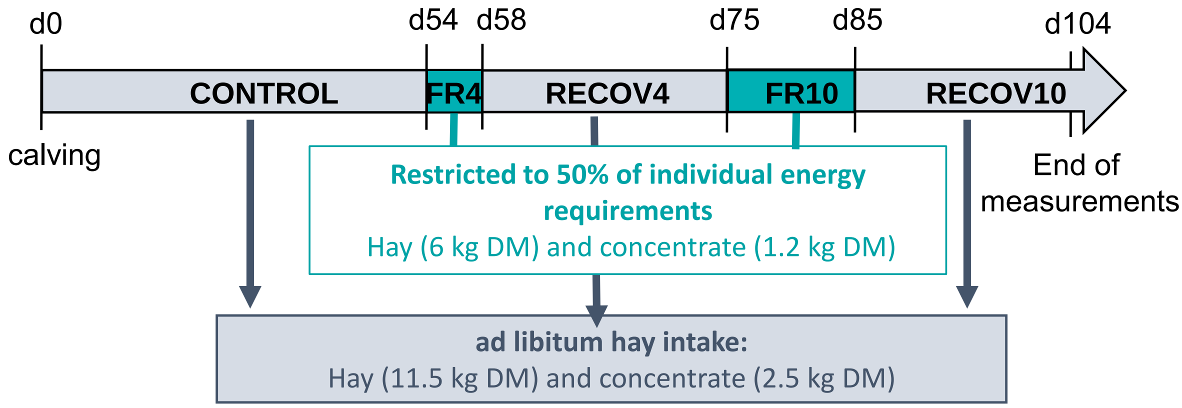


Figure 2.2: Measures and sampling time line

	Control			FR4				RECOV4																
	0-49	50	51	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
BW	x	x	x			x	x				x			x				x			x	x		
BCS	x	x				x					x											x		
MY	x	x		x	x	x	x	x	x	x	x		x		x			x		x		x		
Blood	x	x	x	x	x	x	x	x	x	x	x	x	x		x			x		x		x		

	FR10											RECOV10															
	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	104
BW			x	x					x						x	x			x					x	x	x	x
BCS			x						x						x				x						x		
MY	x	x	x	x	x				x		x	x	x	x	x		x		x			x		x		x	x
Blood	x	x	x	x	x			x		x	x	x	x	x	x		x		x			x		x		x	x

Figure 2.3: Changes in milk yield (kg/d) of suckling Charolais primiparous beef cows over time, during a control (d-6 to d0), feed restriction [FR4 (d0 to d4) and FR10 (d21 to d31] and recovery periods [(Recov4, d5 to d20) and Recov10, d32 to d48)].

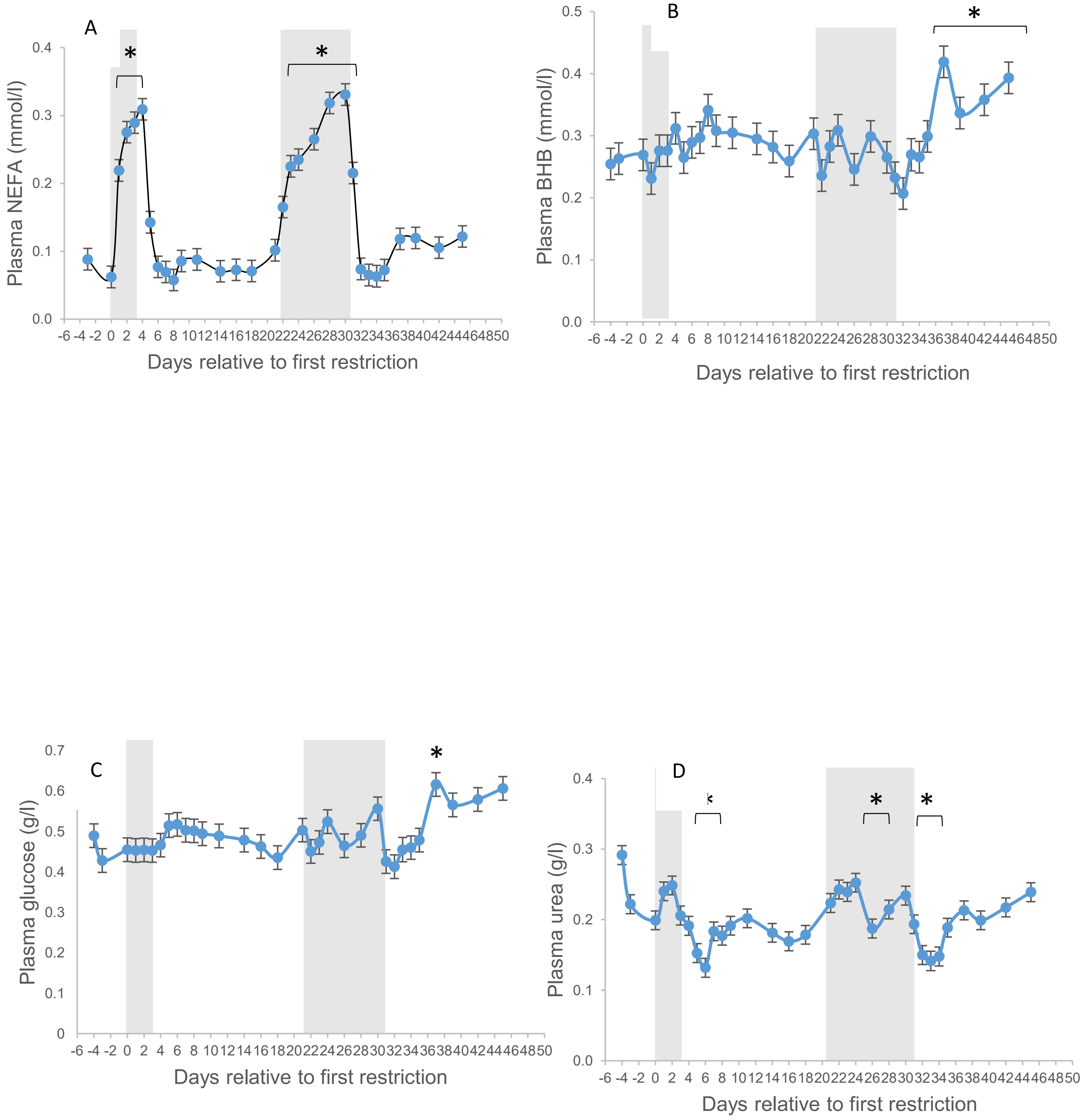
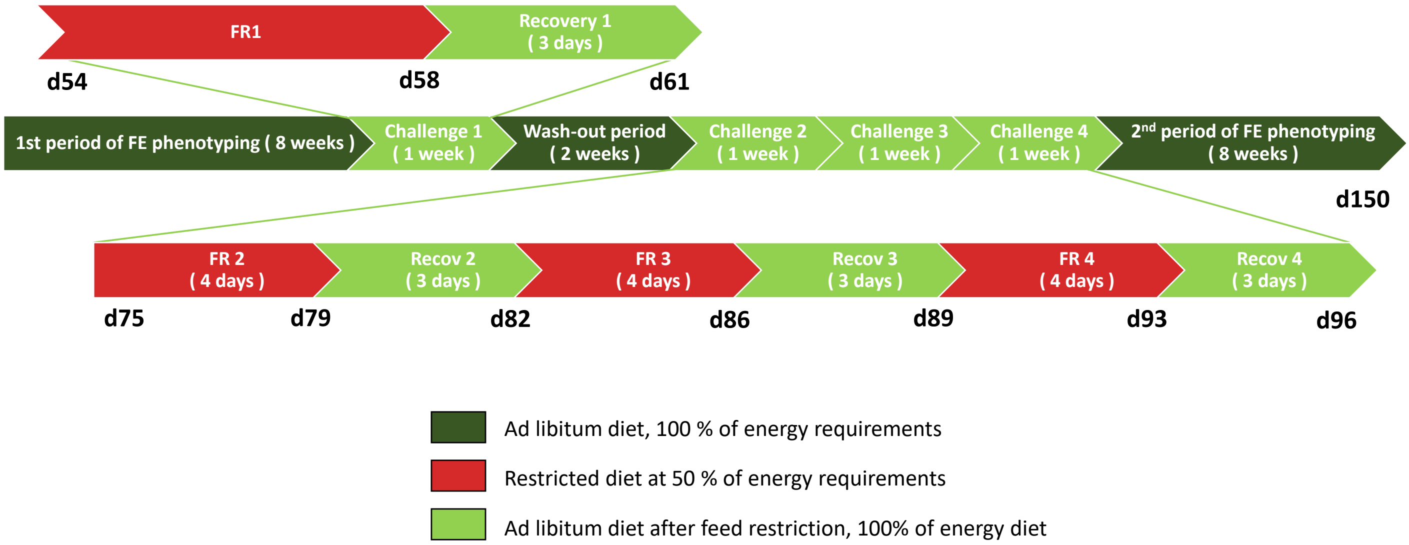


Figure 2.4: Schematic representation of the sequence of feed treatments applied to beef cows during GENTORE2019 experiment



UMRH – Herbipôle – Dairy experiment

Partners:

INRAE UMR Herbivores, 63122 Saint-Genès Champanelle, France

INRAE, HerbiPole, Low Mountain Ruminant Farming Systems Experiment Facility

General Description

The objective was to study the relationship between resilience and efficiency in Holstein and Montbéliarde cows. The experiment was performed in compliance with legislation on animal experimentation at the Herbipole experimental unit of INRAE (https://doi.org/10.15454/1.5572318050509348E12).

Experimental methods exist to evaluate feed efficiency in farm animals, but no standard procedures are available to assess animal resilience. For dairy cows several recent studies employed short-term feed restrictions (from 2 to 6 days) to induce marked changes in production and metabolism indicators, and assess animal responses over the time (Bjerre-Harpoth, 2012; Friggens et al., 2016; Billa et al, 2019). The **objective** was to study indicators of feed efficiency of in early and midlactation Holstein and Montbéliarde, and their responses to nutritional challenges, here used as proxies for resilience, and to assess potential interactions between efficiency and resilience.

Detailed description of Experiment:

Protocol and timeline

From September 2019 to mid-May 2020, an experiment was conducted at INRAE Herbipôle experimental farm involving 40 multiparous dairy cows, from 4 wks prior to expected calving until 22 wks of lactation (22 Montbéliarde and 18 Holstein completed the experimental protocol). Feed intake, milk production and composition, body weight (BW) and body condition score (BCS) were recorded, and blood and milk samples were collected to phenotype RFI and metabolic adaptations during early lactation (wks -4 to 11 ±2).

Cows underwent 4 nutritional challenges, each lasting 4 d during which feed allowance was restricted to meet 50% of Net Energy Lactation (NEL) requirements. The first nutritional challenge was initiated on wk 12 (±2) of lactation and was followed by 10 days of ad libitum intake to phenotype the recovery. Subsequently, cows were subjected to a series of three consecutive nutritional challenges (4 d at 50% of NEL requirements), each separated by 3 days at ad libitum intake, followed by 10 days to phenotype the recovery from the 3 challenges. Intake milk production and composition were recorded daily, and plasma and milk samples for laboratory analyses were collected daily during restrictions.

Datasets and Preliminary Analyses and Results

We initiated preliminary statistical analyses of animal responses during early lactation (DMI, BW, milk yield and composition, energy balance) using repeated measures analyses (Proc Mixed) and feed efficiency indicators (eg. residual feed intake, RFI) by multiple regressions (Proc GLM of SAS).

A significant effect of lactation week was for all variables, as expected. Dry matter intake did not differ significantly during th first 12 weeks of lactation (Figure 3.1). Breed effects were observed for milk yield (Figure 3.2), milk energy secretion, and energy balance. A breed by time interaction was observed for BW ($P < 0.05$; Figure 3.3). Preliminary RFI calculations indicate a range of -1.5 to + 1.3 kg of DMI/day for Holstein cows, and a range of -2.4 to +2.4 for Montbéliarde cows during the first 10 weeks of lactation (Figure 3.4).

Laboratory analyses of plasma metabolites (glucose, BHB, NEFA, urea; 1547 samples) are ongoing. Plasma NEFA during early lactation indicate moderate mobilization of adipose reserves. A marked plasma NEFA concentration response was observed during the first feed restriction (Figure 3.5). These results validate the feed restriction model, and we expect to find plasma NEFA responses to the restrictions 2, 3 and 4.

Conclusions and Perspectives

Preliminary analyses show breed differences for milk production and BW during early lactation, metabolic responses to the first restriction period and a good range of RFI during early lactation. Early lactation Holstein cows produced more milk and exported more energy in milk than Montbéliarde cows. Because feed intake did not differ between the two breeds, Holstein cows relied on body reserves to support lactation a greater extent than Montbéliarde cows during the same period, as suggested by BW changes and plasma NEFA.

Laboratory and statistical analyses are ongoing.

Impact of COVID

When Covid-19 lockdown in France was declared, a group of 25 cows were initiating restriction 2 (out of 4). The experimental protocol proceeded unchanged (including of daily intake and production measurements) but the frequency of blood and milk sampling was limited to 3 per week (at d 0, 2 and 4 of restriction).

For the same reason, the midlactation RFI measurements (performed after nutritional challenges) were limited to 4 weeks (wk 18 to 22±2 of lactation) for all cows in the study.

The complete analysis is planned in 2021.

Data

All data were uploaded on the WP2.3 common repository at url https://sites.inra.fr/site/gentore-wp2/efficiency, in directory INRA-UMRH/exp5.

Figure 3.1: Dry matter intake observed in early lactation Holstein (H) and Montbéliarde (M) cows. Significant week effect was observed. Values are least square means obtained from SAS repeated measures analyses.

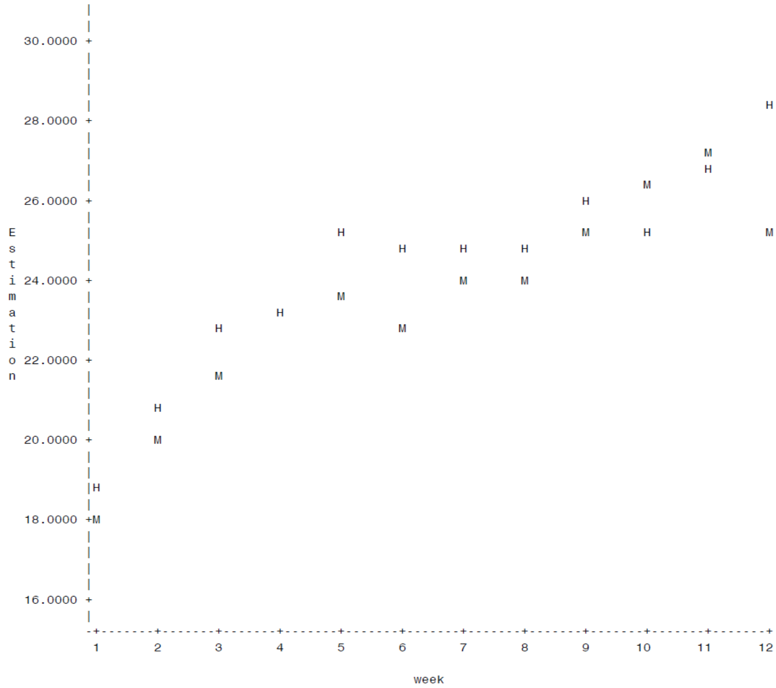


Figure 3.2: Milk yield (kg/d) observed in early lactation Holstein (H) and Montbéliarde (M) cows. Significant breed and week effects were observed. Values are least square means obtained from SAS repeated measures analyses.

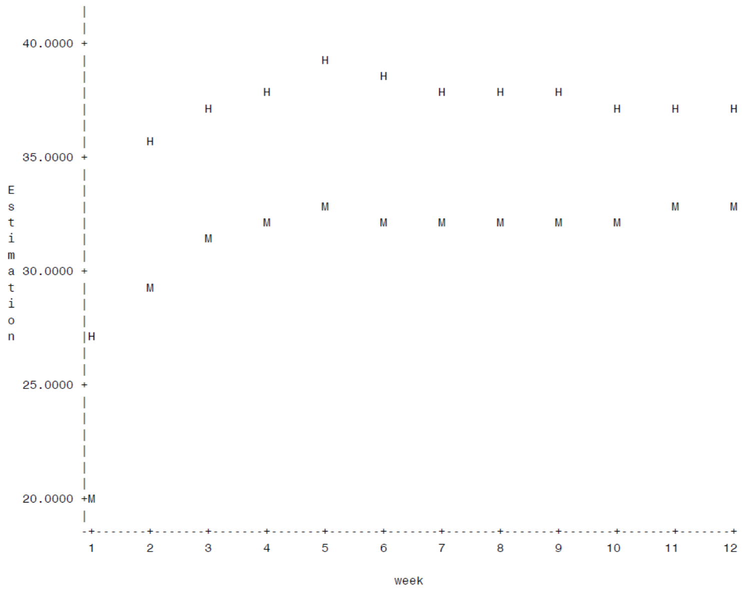


Figure 3.3: Body weight observed in early lactation Holstein (H) and Montbéliarde (M) cows. Significant week effect and breed x week interaction were observed. Values are least square means obtained from SAS repeated measures analyses.

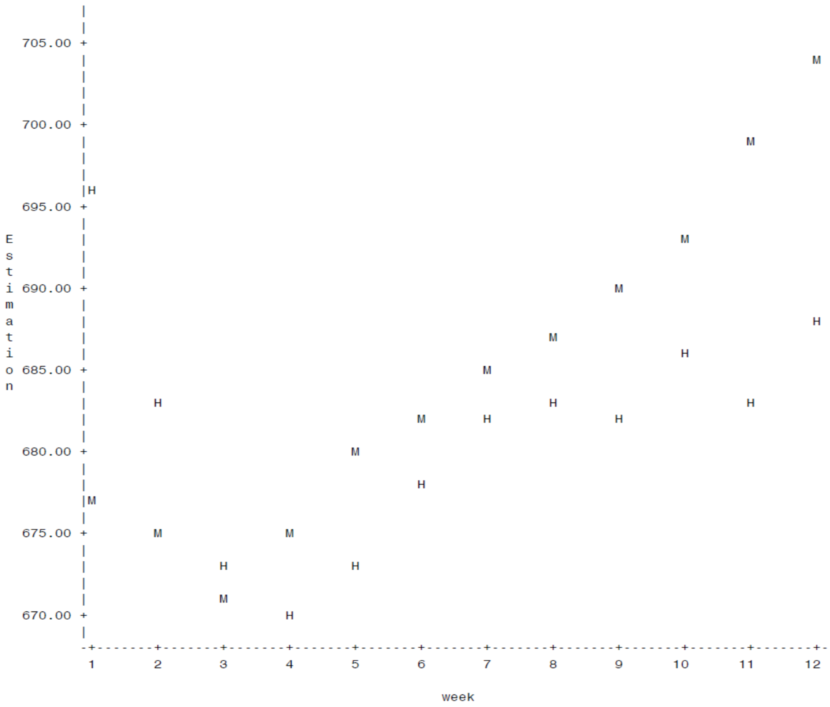


Figure 3.4. Preliminary residual feed intake (RFI) results obtained from multiple regression analysis where observed dry matter intake was the independent variable, and breed, milk energy secretion, body weight and body weigh changes were included as explanatory variables. Calculated using individual means of DMI, milk energy secretion and BW from weeks 1 to 10 of lactation.

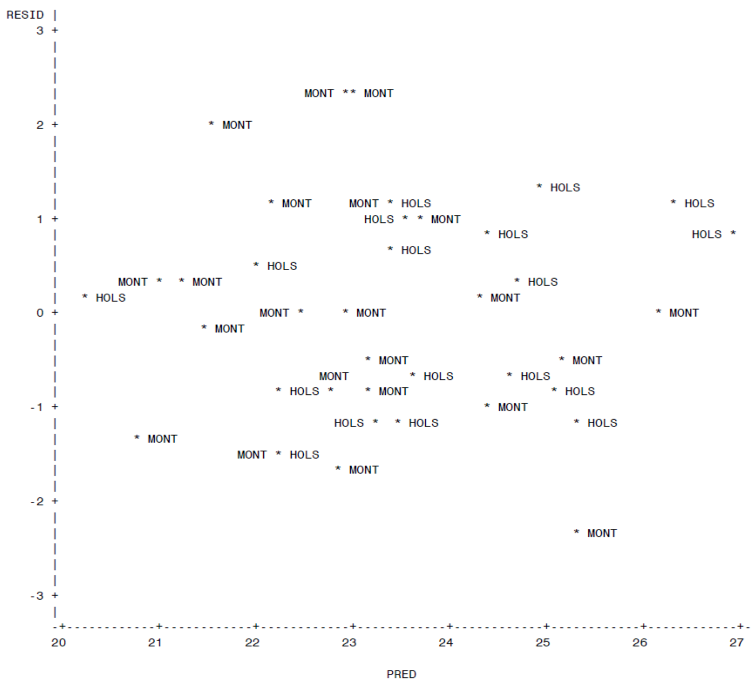
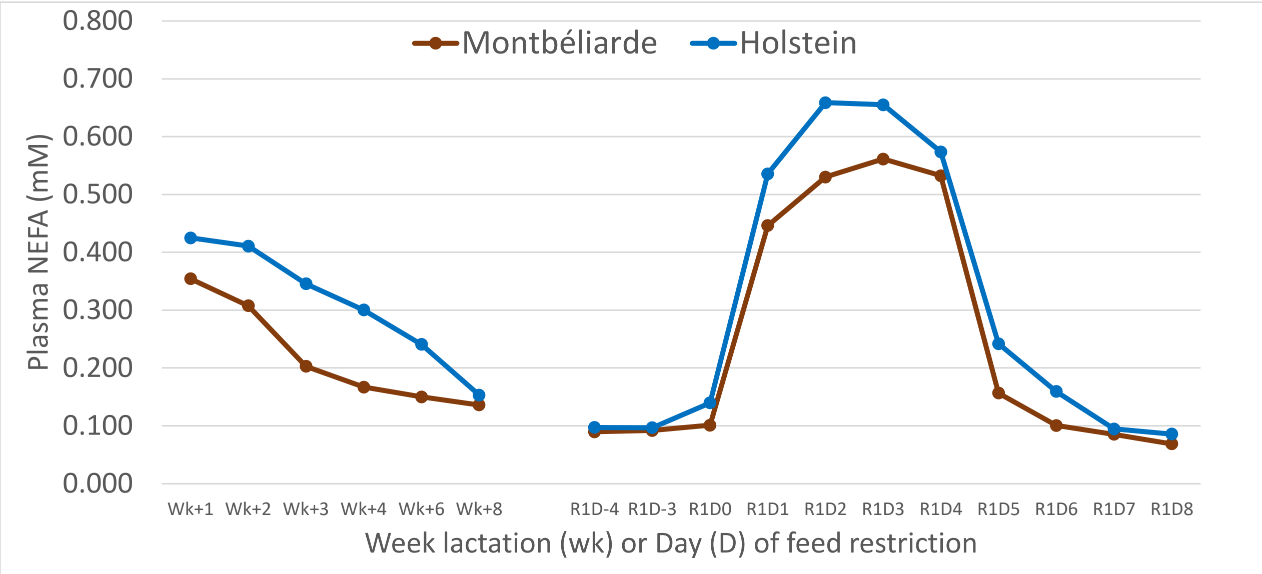


Figure 3.5. Plasma NEFA concentrations during early lactation (wk+1 to wk+8) and in response to 4 days of partial feed restriction (R1D1 to R1D4). During feed restriction period, TMR intake was limited to meet 50% of NE_L requirements. Values are simple means.



CITA Experiment (2018-2019)

Partner
Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Spain.

OVERALL DESCRIPTION

Dates
Field experiment conducted during 2018 and 2019, laboratory determinations 2019 and 2020, results under analysis.

General objective
The aim of this experiment was to study the strategies with which suckler cows cope with short nutritional challenges during lactation, and determine the effect of their energy balance and the stage of lactation on their individual performance and metabolic response. For that purpose, 31 lactating Parda de Montaña cows and their calves were subjected to five short nutritional challenges repeated over lactation. The first three challenges consisted in a 4-day diet restriction (55% energy requirements) in months 2, 3 and 4 post-calving, which were compared in order to evaluate cow strategies to cope with undernutrition with advancing lactation. In month 4 post-calving, three consecutive challenges (4-day restriction, 3-day refeeding) were applied, in order to analyse their cumulative effect at the end of lactation. The ultimate objective is to determine the components of resilience of suckler cows throughout lactation.

DETAILED PROTOCOL

The experiment was conducted at CITA La Garcipollera Research Station in the Pyrenees mountain area (Spain, 42°37' N, 0°30' W, 945 m a.s.l.)., and all experimental procedures were approved by the Animal Ethics Committee of CITA, following the guidelines of the Directive 2010/63 EU on the protection of animals used for experimental and other specific purposes (EU, 2010).

Animal management, diets and experimental design

The study was conducted with 31 multiparous Parda de Montaña beef cows [626 ± 48 kg body weight (BW), 2.8 ± 0.2 body condition score (BCS) and 7.5 ± 2.9 years at calving], and was performed from calving until the weaning of calves at 120 days of age. After calving, cows and their calves were randomly allocated in pens (7 or 8 cow-calf pairs/pen, 10x20 m) equipped with individual feeders for forage and automatic stations ALPRO (Alfa Laval Agri, Tumba, Sweden) for the concentrate. Calves were penned in straw-bedded cubicles adjacent to their dams and allowed to suckle their dams twice daily during 30 minutes at 06:00 and 14:00.

The cows were fed at a flat rate, all cows receiving the same amount of feed. The diets were calculated considering the net energy requirements for maintenance and lactation (INRA, 2007) for a standard cow of 615 kg BW with a milk production of 8.5 kg/d. All the cows received a diet formulated to meet 100% of the standard cow energy requirements throughout lactation, except for the five 4-day restriction periods applied (Figure 4.1). The first three challenges aimed at determining the effect of stage of lactation on the individual response to undernutrition, and consisted in a 4-day diet restriction

(55% energy requirements) in months 2, 3 and 4 post-calving, when the cows were 31, 58 and 87 (± 5.5) days in milk (DIM) at the start of challenge 1, 2 and 3, respectively. During the fourth month, three consecutive challenges were applied in order to analyse their cumulative effect at the end of lactation, when the cows were 87, 94 and 101 (± 5.5) DIM in challenge 3, 4 and 5, respectively.

Figure 4.1. Experimental design and sampling days in each challenge.

month	month 1				month 2				month 3				month 4			
week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
challenge					**ch1**				**ch2**				**ch3**	**ch4**	**ch5**	
diet		100%			↑		100%		↑		100%		↑	↑	↑	
					4 d RESTR 55% + 5 d REC 100%				4 d RESTR 55% + 5 d REC 100%				4 d RESTR 55% + 3 d REC 100%	4 d RESTR 55% + 3 d REC 100%	4 d RESTR 55% + 3 d REC 100%	

	challenge 1 & 2												
day	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8
diet	100%				55%				100%				
period	basal				restriction				refeeding				

	challenge 3											challenge 4							challenge 5						
day	-4	-3	-2	-1	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6
diet	100%				55%				100%			55%				100%			55%				100%		
	basal				restriction				refeeding			restriction				refeeding			restriction				refeeding		

The first two challenges (challenges 1 and 2) involved three consecutive periods, considering day 0 as the first day of restriction. The cows first received a diet that met 100% of the standard cow requirements (day -4 to -1, basal period), then 55% of those requirements during four consecutive days (day 0 to 3, restriction period) and finally 100% again during the following 5 days (day 4 to 8, refeeding period). The following three challenges (challenges 3, 4 and 5) were applied consecutively, and consisted of one common basal period with a 100% diet (day -4 to -1, basal period) followed by three weeks in which cows received a 55% diet during four days (day 0 to 3, restriction period) and then a 100% diet in the following 3 days (day 4 to 6, refeeding period).

The diets fed to each cow daily consisted of 8 kg hay (920 g dry matter (DM)/kg, 85.4 g ash/kg DM, 94.4 g crude protein (CP)/kg DM, 584 g neutral detergent fiber (NDF)/kg DM, 330 g acid detergent fiber (ADF)/kg DM, 5.4 MJ net energy (NE)/kg DM) and 3.0 kg concentrate (887 g DM/kg, 68.5 g ash/kg DM, 168 g CP/kg DM, 253 g NDF/kg DM, 114 g ADF/kg DM, 7.5 MJ NE/kg DM) on an as fed basis during the basal and refeeding periods, and 7 kg hay during the restriction period. The animals had free access to water and mineral blocks throughout the experiment.

Measurements

Samples of the feedstuffs were collected daily during the different periods and lyophilized in a Genesis Freeze Dryer 25 (Hucoa Erlöss, SA/Thermo Fisher Scientific, Madrid, Spain) to determine their chemical composition. Daily, the orts of the hay of the previous day were collected individually at 8:00 am and then the hay was offered in a single meal in individual troughs. The cows were tied up for approximately 2 h until they finished their ration. The individual hay intake was calculated as the difference between the quantity offered and the amount refused. The ALPRO feeding stations were programmed to offer 3 kg /day to all the cows in the basal and refeeding periods. The individual intake of concentrate was recorded daily.

The individual performance and metabolic profile of cows were recorded twice during the basal period in all challenges (days -2 and -4 during in challenges 1, 2 and 3), three times during the restriction period (days 1, 2 and 3 in all challenges) and daily during the refeeding period (days 4, 5, 6, 7 and 8 in challenges 1 and 2; days 4, 5 and 6 in challenges 3, 4 and 5), resulting in 40 sampling days per cow.

Cows and calves were weighed on an electronic scale at calving and thereafter at 7:00 on all the sampling days of the study. Body condition score was recorded at calving, on day -2 and the last day of each refeeding period (day 8 in challenges 1 and 2, day 6 in challenges 3 to 5) in a 1-5 scale by a trained person, based on estimation of fat covering the ribs, loin and tailhead (Lowman et al., 1976). Subcutaneous fat thickness was measured by ultrasound scanning, with a multifrequency probe (7.5 MHz, Aloka SSD-900; Aloka Madrid, Spain), in the sacral area (BFT), at the P8 rump site (P8) and at the 13th thoracic vertebra (T13) on day -2 and on the last day of each restriction and each refeeding period.

Milk yield was estimated on all the sampling days of the study by the weigh-suckle-weigh technique of the calf (Le Neindre and Dubroeucq, 1973), where the daily milk yield is the sum of the milk consumed in both sucklings. After the morning suckling, milk samples were manually collected from each dam. A 50-ml sample was collected to determine the milk composition, added with sodium azide (PanReac, Barcelona, Spain) as preservative and refrigerated at 4 °C until the analysis; to determine the fatty acid composition a second 10-ml sample was collected, lyophilized and kept at -20 °C until analysis.

Cows were blood sampled on all the sampling days to determine their metabolic profile. The blood samples were collected from the coccygeal vein at 07:00, after suckling and before the hay was offered. Heparinized tubes (BD Vacutainer Becton-Dickenson and Company, Plymouth, UK) were used for β-hydroxyburyrate (BOHB) and malondialdehyde (MDA) determinations, and tubes that contained EDTA (BD Vacutainer Becton-Dickenson and Company, Plymouth, UK) for the analysis of glucose, non-esterified fatty acids (NEFA) and urea concentrations. Immediately after collection, the blood samples were centrifuged at 3500 rpm for 20 min at 4 °C; the plasma was collected and frozen at -20 °C until further analyses. An extra blood sample per cow was collected at the end of the study into EDTA-coated tubes and frozen at -20 °C for genotyping, using the BovineSNP50 (50 K, Illumina Inc., San Diego) Beadchip, which included 54,609 SNPs.

Fecal samples were collected on all sampling days in order to analyze individual diet digestibility. Rectal samples were collected and freeze-dried to determine their chemical composition.

Animal behavior was recorded throughout the study, but only data collected on the sampling days were retained. All cows were equipped with a collar continuously recording animal activity via a Medria® AXEL accelerometer sensor and data logger. Ruminal temperature was recorded every 5 min by Medria® reticulorumen thermoboluses.

In order to study the onset of ovarian cyclicity after calving, blood was collected by coccygeal venipuncture into heparinized tubes (BD Vacutainer Becton-Dickenson and Company, Plymouth, UK) every 10 days during lactation. Blood samples were centrifuged at 3500 rpm for 20 min at 4 °C immediately after collection. Additionally, the ovaries were examined at the start and end of challenge 2 (days -2 and 8) by ultrasonography (Aloka SSD-500V, Aloka, Madrid, Spain), using a linear-array 7.5 MHz transducer. The presence, number and diameters of the dominant follicles and the corpus luteum were assessed by considering the average between measurements of their two perpendicular axes.

Analyses

Feedstuffs

The chemical composition of the feedstuffs was analysed in duplicate. Briefly, the content of DM and ash were determined according to the AOAC methods (AOAC, 2000). The N content was determined following the Dumas Procedure with a nitrogen analyser (Model NA 2100, CE Instruments, Thermoquest SA, Barcelona, Spain). NDF, ADF and ADL were analysed following the sequential procedure of Van Soest et al. (1991) with the Ankom 200/220 fibre analyser (Ankom Technology Corporation, Fairport, NY, USA). The fatty acids of the freeze-dried feedstuffs were extracted and methylated as proposed by Skuhija and Palmquist (1988). The determination of fatty acid metyl esters (FAMEs) was carried out with a gas chromatograph (Bruker 436 Scion gas, Massachusetts, USA) equipped with a cyanopropyl capillary column (BR-2560, 100 m x 0.25 mm ID x 0.20 µm thickness, Bruker) with flame ionization detector and Compass CDS software. The identification of FAMEs in the feedstuffs was performed using the GLC-532, GLC-401, GLC-643, GLC-642, GLC-463 standard references and the relative retention times observed in the bibliography (Kramer et al., 1997, 2002, Shingfield et al., 2003, Yoshinaga et al., 2013, de la Fuente et al., 2015). Fatty acid quantification was performed as described in UNE-EN 12966-4 Official Method (2015) and expressed as a percentage of the total amount of identified FAME. The gross energy was determined by bomb calorimetry (Model Parr 1341, Parr instrument company, Moline, Illinois, USA).

Milk

In milk samples, fat, protein and urea contents were analysed with an infrared scan (Milkoscan 4000, Foss Electric Ltd., Hillerød, Denmark). The FAME of the freeze-dried milk samples were obtained as described by Kramer et al. (1997). The determination was similar to that of the FAMEs of feedstuffs, using a BR-2560, 200 m x 0.25 mm ID x 0.20 µm thickness, Bruker, cyanopropyl capillary column, and the C18:1 t11, C19:0, C23:0 as standard references for milk (Nu-Chek-Prep Inc., Elysian, Minnesota, USA).

Plasmatic profiles

Concentrations of glucose (enzymatic-colorimetric method, sensitivity: 0.06 mmol/L) and urea (kinetic method, sensitivity: 0.056 mmol/L) were determined in plasma with an automatic analyser (Gernon, RAL S.A, Barcelona, Spain). Plasma BOHB (kinetic enzymatic method, sensitivity: 0.100 mmol/L) and NEFA (colorimetric method, sensitivity: 0.072mmol/L) were determined using Randox kits (Randox Laboratories Ltd, Country Antrim, UK). The oxidative status was determined using malondialdehyde (MDA) as a biomarker of lipid peroxidation. Malondyaldehyde was analyzed by liquid chromatography (Milford, Massachusetts, USA) equipped with a silica-based bonded period column (Acquity UPLC HSS PFP, 100 mm × 2.1 mm × 1.8 µm, Waters), an absorbance detector (Acquity UPLC Photodiode Array PDA eλ detector, Waters) and a fluorescence detector (2475 Multi λ Fluorescence Detector, Waters). The quantification of MDA was done by fluorescence detection at ʎexcitation = 530 nm and ʎemission = 550 nm following the chromatographic conditions described in (Bertolín et al., 2019). The mean intra- and inter-assay coefficients of variation were 4.6% and 7.3% respectively.

Plasma progesterone concentration (ELISA test, sensitivity: 0.27 ng/mL) was measured using a specific kit for cattle (Ridgeway Science, Lydney, UK). The mean intra-assay and inter-assay coefficients of variation were 8.0% and 10.4%, respectively. The onset of luteal activity in cows after calving was considered when progesterone concentration was >1 ng/mL. If cows had not ovulated prior to the end of lactation (d 120), the interval to first ovulation after calving was regarded as this date.

Calculations

The gross energy content of feedstuffs was used to calculate their net energy (NE) content using the INRA system (INRA, 2007). The individual energy balance (EB) was estimated by calculating the difference between inputs (NE intake) and outputs (NE for maintenance and NE for lactation) (INRA, 2007). The NE intake was estimated from the individual intake and energy contents of the feedstuffs.

The NE for maintenance was calculated from the individual metabolic weight, and NE for production was obtained using the milk yield, fat and protein contents in milk.

The apparent whole-tract dry matter digestibility of the diet was calculated using the daily known diet intake and the total fecal output estimated using the indigestible acid insoluble ash of the feedstuffs and the faecal samples as an internal marker.

The milk fatty acids were grouped according to their saturation degree into SFA, MUFA and PUFA, and by their origin as de novo synthesis (C4:0 - C15:1), mixed origin (C16:0 - C16:1) and mobilization (≥ C17:0). Four milk FA ratios previously related to energy balance and metabolic profile were calculated (C18:1 cis-9 to even short-medium-chain fatty acids (eSMCFA), C18:1 cis-9 to C14:0, C18:1 cis-9 to C15:0 and C17:0 to C15:0). eSMCFA is even short-medium-chain fatty acids and results from the sum of C4:0, C6:0, C8:0, C10:0, C12:0.

The raw dataset concerning the animal behaviour during the experiment was accessed online and provided information at 5-min intervals on the position of the animal (standing up or lying down) and on five different activities (“ingestion”, “rumination”, “rest”, “over-activity” and “other”). For each 5-minute period only the single most dominant activity was selected among the five recorded; the resulting 288 5-min periods per 24 hours were summarized into a single daily amount of time (minutes) devoted to each activity.

Statistical Analyses

Preliminary statistical analyses have been conducted using SAS statistical package v 9.4 (SAS Institute Inc., Cary, NC, USA), using different subsets of data.

The metabolic and production data of the cows have been studied in a first data subset, which included data from the first three challenges. In this subset, the response of suckler cows to a dietary restriction and a subsequent refeeding has been compared in different stages of lactation. The challenge number reflected the stage of lactation, given that challenges 1, 2 and 3 were conducted in months 2, 3 and 4 post-calving, respectively. The response to dietary changes was considered using two different time effects within challenge: the feeding period (basal, restriction, refeeding) and the day (-2 to 8 d). Mixed models for repeated measures (MIXED procedure) were used, considering challenge number (1, 2 and 3) and time (either feeding period or day) and their interaction as fixed effects, and the cow as the random effect.

The changes in milk composition and fatty acid profile in response to a dietary restriction and a subsequent refeeding have been analysed in a second subset of data which included records obtained during challenge 2. Additionally, to take into account differences in energy balance that may arise from feeding all cows at a flat rate despite their individual characteristics, the cows were classified according to their BW and BCS at calving and BW, BCS, milk yield and energy balance at day 30 of lactation, using the k-means clustering method. Two clusters were obtained, with the cows in the first cluster being classified as Balanced (n=15) and those on the second cluster as Imbalanced (n=16). An analysis of variance was performed on the classifying variables, using a general linear model (GLM procedure) and considering the cluster as fixed effect. According to pairwise comparisons of performance traits at day 30 in both clusters, Balanced cows were lighter than Imbalanced cows (563 vs. 633 kg, respectively, S.E.M. (standard error of the mean) 4.12, $p < 0.001$), had a lower milk yield (7.5 vs. 8.6 kg/d, respectively, S.E.M. 0.17, $p = 0.03$) and were in a less negative Energy balance (-3.5 vs. -10.0 MJ NE/d, respectively, S.E.M. 0.77, $p < 0.001$). Within this challenge 2, mixed models for repeated measures (MIXED procedure) included the energy balance cluster (Balanced and Imbalanced), time (feeding period or day) and their interaction as fixed effects, and the cow as the random effect.

In all mixed models the degrees of freedom were adjusted with the Kenward-Roger correction to take into account-missing values. Covariance structure was selected on the basis of the lowest Akaike and

Bayesian information criteria. The least square means and associated standard errors were obtained and multiple comparisons adjusted with the Tukey correction. The relationships among variables were studied with Spearman rank correlations (r). The p-value for significance was established at $p<0.05$ and trends were discussed when $0.05 \le p < 0.10$.

Further analyses will be carried out in a third data subset, where cow metabolic and production performance will be analysed in the three consecutive challenges conducted in month 4 post-calving (4-day restriction, 3-day refeeding). Here, the cumulative effect of several restriction-refeeding periods at the end of lactation will be determined.

PRELIMINARY RESULTS

Cow DMI was constant across challenges but differed among feeding periods, being greater in the basal and refeeding periods than in the restriction period ($p < 0.001$). Accordingly, energy and protein intake were lower during the restriction than in the basal and refeeding periods (59.3, 34.8 and 59.6 MJ NE/day during the basal, restriction and refeeding periods, respectively; and 1145, 608 and 1145 g protein intake/d, respectively; $p < 0.001$).

Cow BW, calf gains, and the milk yield and composition according to challenge number and feeding period are shown on Table 4.1. The change of milk yield after restriction and refeeding as compared to basal values per challenge is presented in Figure 4.2.

Cow BW was affected by the interaction between challenge number and feeding period, because it dropped immediately on the first day of restriction and kept decreasing during the refeeding period in challenge 1 but not in the others. Basal cow BW decreased significantly from challenge 1 to challenge 3.

Calf gains decreased significantly during the restriction except for challenge 3, and during the refeeding period they had intermediate gains, regained basal values or decreased further in challenges 1, 2 and 3, respectively ($p < 0.001$).

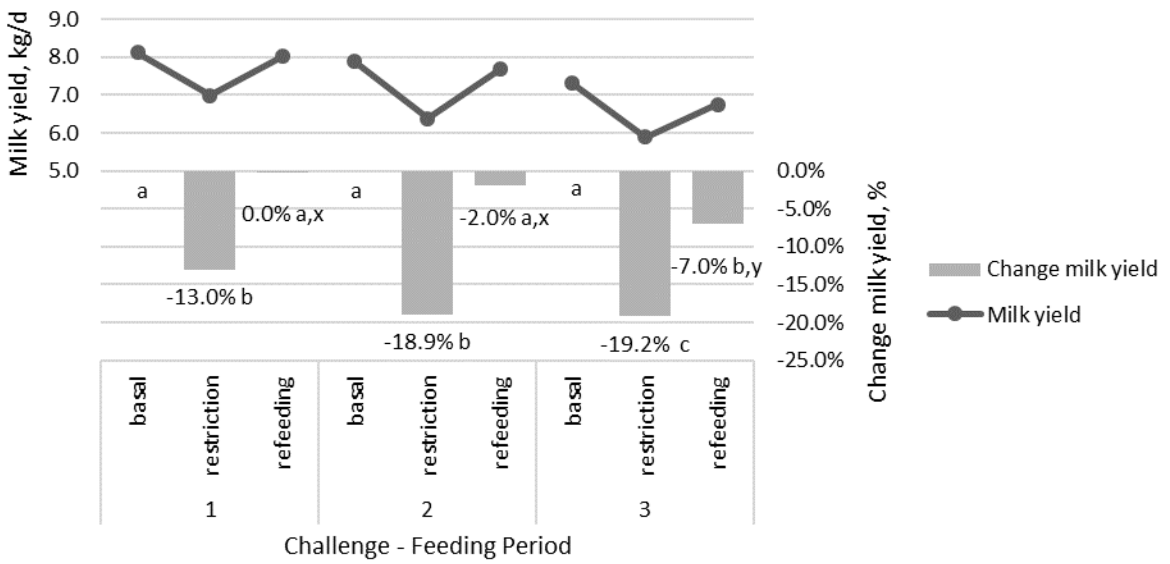
Milk yield decreased significantly throughout lactation ($p < 0.001$), and it was significantly lower during the restriction period in all the challenges. A trend was observed ($p = 0.06$) towards a reduced recovery after refeeding as lactation advanced, because basal milk yields were regained in challenges 1 and 2 but not in challenge 3 (Figure 4.2).

Table 4.1. Effect of challenge number (Ch) and feeding period (P) on the performance of the cows and calves and the milk yield and composition.

	Challenge						p-values			
Variable	1		2		3		R.S.D.	Ch	P	Ch × P
Cow BW, kg							7.34	< 0.001	< 0.001	< 0.001
Basal	599	a,x	588	a,y	585	a,z				
Restriction	585	b,x	577	b,y	575	b,y				
Refeeding	579	c	576	b	577	b				
Calf ADG, kg/d							0.46	0.11	<0.001	<0.001
Basal	1.058	a	0.94	a	1.07	a				
Restriction	0.525	b	0.48	b	0.8	a				
Refeeding	0.808	ab,x	1.02	a,x	0.17	b,y				
Milk yield, kg/d							0.65	< 0.001	< 0.001	0.14
Basal	8.11	a,x	7.86	a,x	7.31	a,y				
Restriction	6.99	b,x	6.37	b,y	5.89	c,y				
Refeeding	8.01	a,x	7.67	a,x	6.75	b,y				
Milk fat content, g/100 g milk							0.86	0.044	0.012	0.004
Basal	37.1	a,x	45.5	y	43.9	y				
Restriction	42.9	b	47.1		44.3					
Refeeding	47.6	b	44.5		45.5					
Milk protein content, g/100 g milk							0.11	< 0.001	< 0.001	< 0.001
Basal	31.8	a,x	29.6	a,y	29.7	y				
Restriction	29.6	b,y	28.5	b,x	29.3	x				
Refeeding	29.9	b	29.8	a	29.1					

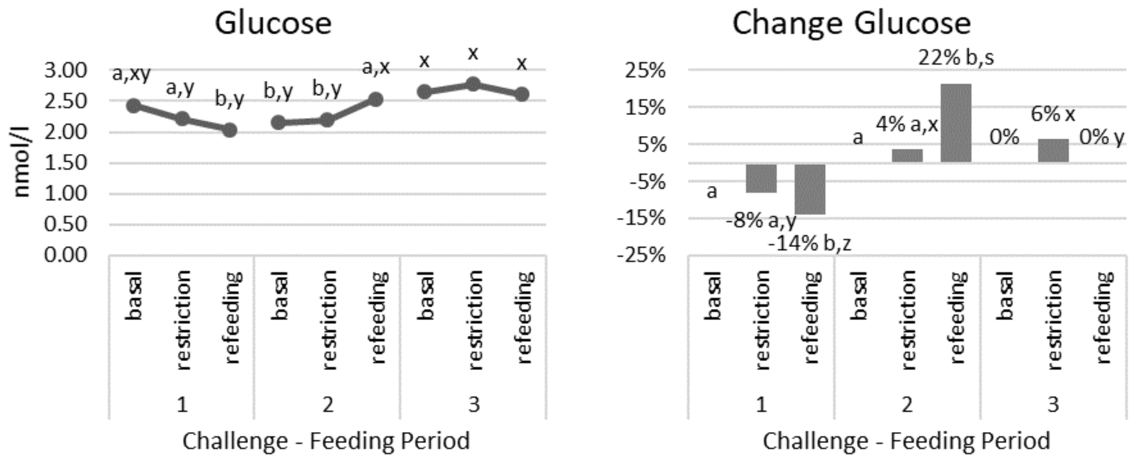
For each trait, LS means in the same column with different letter (a,b,c) differ among feeding periods, and LS means in the same row with different letters (x,y,z) differ among challenges ($p < 0.05$)

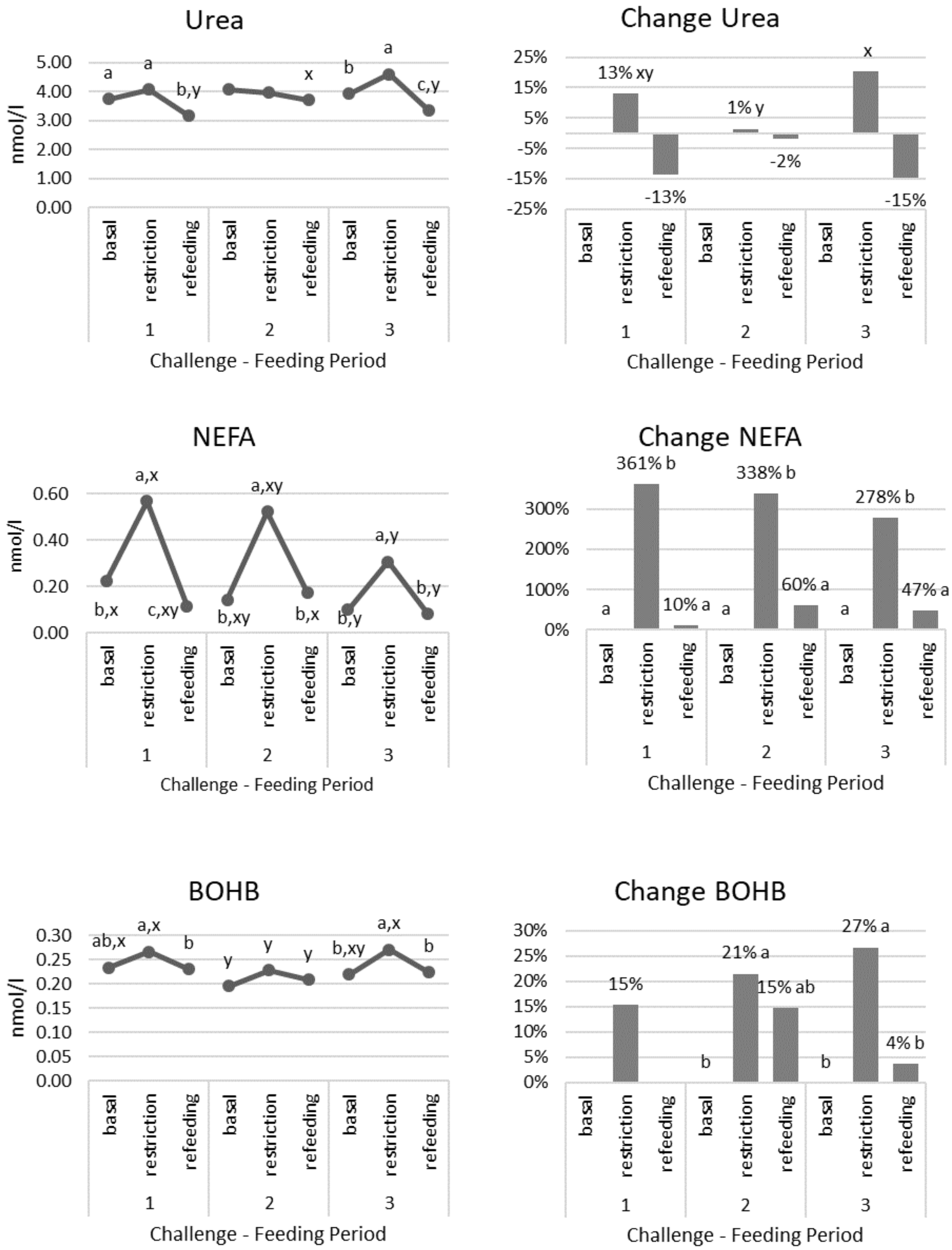
Figure 4.2. Effect of challenge number on the relative change in milk yield after restriction and refeeding as compared to basal values (%).

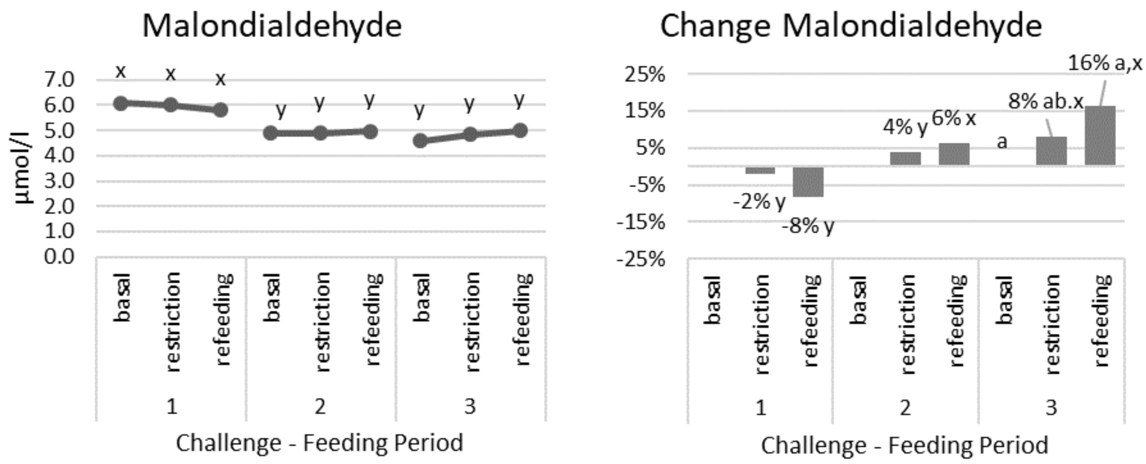


LS means with different letters (a,b,c) indicate differences among feeding periods within challenge and (x,y) denote differences among challenges within feeding period($p < 0.05$) for the variable Change in milk yield.

Figure 4.3. Effect of challenge number (Ch) and feeding period (P) on the cow concentration of plasma metabolites, and on their relative change after restriction and refeeding as compared to basal values (%).




Urea
nmol/l
5.00
4.00
3.00
2.00
1.00
0.00
a
a
b,y
x
b
a
c,y
basal
restriction
refeeding
1
2
3
Challenge - Feeding Period
Change Urea
25%
15%
5%
-5%
-15%
-25%
13% xy
-13%
1% y
-2%
x
-15%
Challenge - Feeding Period
NEFA
nmol/l
0.60
0.40
0.20
0.00
a,x
b,x
c,xy
a,xy
b,xy
b,x
a,y
b,y
b,y
Challenge - Feeding Period
Change NEFA
300%
200%
100%
0%
361% b
10% a
338% b
60% a
278% b
47% a
a
a
a
Challenge - Feeding Period
BOHB
nmol/l
0.30
0.25
0.20
0.15
0.10
0.05
0.00
ab,x
a,x
b
y
y
y
b,xy
a,x
b
Challenge - Feeding Period
Change BOHB
30%
25%
20%
15%
10%
5%
0%
15%
21% a
15% ab
b
27% a
4% b
b
Challenge - Feeding Period



LS means with different letters (a,b,c) indicate differences among feeding periods within challenge and (x,y,z) denote differences among challenges within feeding period ($p < 0.05$)

An interaction was observed between challenge number and feeding period for most metabolites ($p < 0.001$) except for BOHB. Glucose concentrations were lower in early lactation (challenges 1 and 2) than in challenge 3, but responded differently to the feeding management in the three challenges. Urea increased with restriction in challenge 3 and decreased at refeeding (challenges 1 and 3), but remained unchanged in challenge 2. The concentrations of NEFA increased by three-fold during the restriction period, and then basal values were regained during the refeeding phase but were even lower than basal in challenge 1. A strong negative correlation was found between plasmatic NEFA and energy intake ($r = -0.66$, $p<0.001$). The relative change of NEFA as compared to basal values in the restriction and refeeding periods was negatively correlated with that of milk yield ($r = -0.42$, $p<0.001$). Plasmatic concentrations of BOHB were lower at the end of lactation (0.16 nmol/l in challenge 3) than in the first two challenges (0.30 and 0.28 nmol/l in challenge 1 and 3, respectively, $p<0.001$), and were always affected by a dietary restriction (3.90, 4.21 and 3.40 28 nmol/l in the basal, restriction and feeding period, respectively, $p<0.001$). BOHB was positively correlated with plasma urea ($r = 0.52$, $p<0.001$), and its relative in the restriction and refeeding periods as compared to basal values was positively correlated with that of glucose ($r = 0.57$, $p<0.001$). Finally, MDA was higher at the start of lactation (5.98, 4.92 and 4.81 μmol/l in challenge 1, 2 and 3, respectively, $p<0.001$), but did not differ among feeding periods. Plasma MDA concentration was positively correlated with plasma urea concentration ($r = 0.67$, $p<0.001$), BOHB concentration ($r = 0.46$, $p<0.001$) and milk protein content ($r = 0.49$, $p<0.001$)

These results indicate that some metabolites respond immediately to diet changes, particularly NEFA and BOHB, and therefore they are accurate short-term indicators of lipid mobilization of suckler cows under a negative energy balance. In the case of the other metabolites, preliminary analyses on a daily basis elicited some differences within feeding periods that were not significant in the between-period analyses (see Orquera et al., 2019).

When lipid mobilization was assessed through body condition scored and subcutaneous fat measures by ultrasonography, neither BCS nor BFT were affected by challenge or feeding period. Subcutaneous thickness measured both at P8 and T13 decreased from throughout lactation ($p<0.001$) and P8 also decreased from the basal to the refeeding period ($p<0.01$). Hence, except for P8, external measurements of fat thickness only reflected changes in the long term, and therefore would not be adequate indicators of short-term differences in energy balance (Orquera et al., 2020).

The milk FA profile was analysed during challenge 2, considering the effects of the energy balance cluster and the feeding period. Their effects on the individual fatty acid contents and the sums according to their saturation and origin are shown in Table 4.2.

Regarding the major individual fatty acids, the milk contents of C14:0 and C16:0 were lower during the restriction than in the basal and refeeding periods (p<0.001), and additionally C16:0 tended to be higher in Balanced than in Imbalanced cows (p = 0.09). Both of them had a positive correlation with energy balance (r=0.70 and 0.62 for C14:0 and C16:0, respectively, p<0.001, respectively), and C14:0 had a negative correlation with NEFA plasma content (r= -0.58, p<0.001). The C18:0 milk content was lower during the refeeding phase than the basal and restriction periods (p<0.001), and finally, the C18:1 cis-9 milk content was different by EB cluster and period, with higher concentrations in Imbalanced than in Balanced cows (p = 0.002) and during the restriction period than in the basal and refeeding periods (p<0.001), which reflects the different mobilization of body fat, where C18:1 cis-9 is predominant.

When the FA were analysed according to their saturation degree, the SFA and MUFA were different by EB cluster and feeding period (p<0.05 and p<0.001 respectively), and PUFA only by feeding period (p<0.01). The milk FA profile of Balanced cows had higher SFA and lower MUFA content than that of their Imbalanced counterparts, whereas the PUFA contents were similar in both EB clusters. Regarding the feeding period, SFA content decreased during the restriction period (p<0.001), MUFA and PUFA increased when feed was restricted, but only MUFA recovered the basal values during the refeeding.

According to their origin, the de novo FA (C4:0 - C15:1) contents were affected by EB cluster, with greater values in Balanced cows (p = 0.04), and by feeding period, with lower contents when feed was restricted (p<0.001). Mixed origin FA (C16:0 - C16:1) only tended to be higher in Balanced cows (p = 0.09) and were lower during the restriction than in the other periods (p<0.001). Finally, the mobilization FA (≥ C17:0) were higher in Imbalanced than in Balanced cows (p = 0.02) and during the restriction period than the rest (p<0.001). The individual EB was highly correlated with the milk contents of de novo and mixed origin FA (r = 0.68 and 0.60, respectively, p<0.001), whereas a negative correlation was observed with those of mobilization FA (r = -0.71, p<0.001). The de novo and mobilization FA presented correlations of different sign with NEFA plasma concentration (r = -0.60 and 0.53, p<0.001, respectively).

When analysed on a daily basis, preliminary results indicate that the milk FA profile responded immediately to changes in the energy balance and/or the diet. Basal values were always regained by the end of the refeeding phase and even a "rebound effect" after restriction and refeeding was observed for some traits (see Casasús et al., 2020).

Table 4.2. Effect of energy balance (EB) cluster and feeding period (P) on the concentration of the major fatty acids (FA) in the milk and the FA according to the saturation, origin and FA ratios.

	EB cluster		P				p-values[3]	
	Balanced	Imbalanced	Basal	Restriction	Refeeding	R.S.D.[2]	EB	P
Individual FA, g/100 g ID[1] FAME								
C14:0	8.9	8.4	9.8x	6.2y	9.8x	1.16	0.1	< 0.001
C16:0	26.7	25.9	27.3x	24.1y	27.4x	1.49	0.09	< 0.001
C18:0	10.6	11	11.6x	11.4x	9.4y	1.14	0.31	< 0.001
C18:1 cis-9	24.1b	26.1a	22.3x	30.2y	22.9x	2.55	0.002	< 0.001
FA according to saturation, g/100 g identified FAME								
SFA	61.9a	60.3b	64.7x	55.6z	63.0y	2.95	0.04	< 0.001
MUFA	32.9b	34.6a	30.8y	38.8x	31.7y	2.6	0.01	< 0.001
PUFA	5.2	5.1	4.5y	5.6x	5.4x	0.66	0.46	< 0.001
FA according to origin, g/100 g identified FAME								
De novo	22.1a	20.8b	23.4x	16.8y	24.1x	2.41	0.04	< 0.001
Mixed origin	29.1	28.2	29.5x	26.7y	29.8x	1.48	0.09	< 0.001
Mobilization	48.8b	51.0a	47.2y	56.5x	46.1y	3.52	0.02	< 0.001

Within a trait, LS means with different letter (a,b) indicate differences between EB clusters and (x,y,z) denote differences among feeding periods (p<0.05)

CONCLUSIONS

These preliminary results indicate that the patterns with which beef cows cope with short but severe nutritional challenges change throughout lactation, and so does their ability to regain basal performance and metabolic status after a refeeding period. Some traits respond immediately to changes in the diet, but the individual factors influencing the magnitude of the response remain to be analyzed.

SITUATION REGARDING COVID AND EXPECTED DATE OF FINAL RESULTS

Lockdown first and then working from home due to COVID-19 has delayed the latest field work pending in WP2, laboratory analyses and the preparation of papers. The two first issues have been solved by hiring a technician to support the extra field/lab work, with no change of budget concerning personnel costs. The analysis of results has been concomitantly delayed, but still some preliminary results have been presented at different virtual meetings this year (ADSA, EAAP). Manuscripts are being prepared for publication but the expected submission dates have been postponed. The final results are expected at the end of the project.

A training day for farmers and vets at La Garcipollera research farm had been programmed for late march, where the preliminary results of task 2.3 should have been presented. The activity had to be cancelled, but will be rescheduled when the situation changes.

DATA

All data were uploaded on the WP2.3 common repository at url https://sites.inra.fr/site/gentore-wp2/efficiency, in directories CITA/exp.7 and CITA/exp.8.

PUBLICATIONS

Preliminary results have been presented at GenTORE annual meetings and in several scientific meetings:

- Orquera K., Blanco, M., Bertolín J.R., Ferrer, J., Casasús, I. (2019). Respuesta productiva y metabólica de vacas nodrizas ante una subnutrición breve e intensa al inicio de la lactación. XVIII Jornadas sobre producción animal AIDA, Zaragoza, 7-8 mayo. J. A. A. M. Blanco, A. Bernués, J.H. Calvo, M.A. Latorre, S. Lobón, D. Martín, J. Palacio, G. Ripoll. 194-196.
- Casasús I., Orquera, K., Bertolín J.R., Ferrer, J., Blanco, M. (2019). Performance and oxidative status and of beef cows facing short nutritional challenges during lactation. 70th Annual Meeting EAAP (European Federation of Animal Science), Ghent (Belgium), 26-30 Agosto. (Ed.). Wageningen Academic Publishers. Book of abstracts No. 25: 617. https://doi.org/10.3920/978-90-8686-890-2
- Casasús I., Bertolín J. R., Orquera K., Ferrer J., Blanco M. (2020). Milk fatty acid profiles of beef cows in response to a short feed restriction during lactation. Annual Meeting of the American Dairy Science Association, Virtual Annual Meeting, 21-24 June. Journal of Dairy Science, 103 (Suppl. 1): 188.
- Orquera K., Ortigues, I., Thollon, N., Casasús, I., Sepchat, B., De la Torre, A. (2020). The effect of routine management practices on the behaviour of beef cows according to their feeding management. 71st Annual Meeting of European Federation of Animal Science, Virtual meeting, December 1-4.
- Orquera K., Ripoll, G., Blanco, M., Ferrer, J., Bertolín, J.R., Casasús, I. (2020). Indicators of body fat mobilization of lactating beef cows under short nutritional challenges. 71st Annual Meeting of European Federation of Animal Science. Virtual meeting, December 1-4.

Manuscripts under preparation

- Performance and milk fatty acid profile of suckler cows differing in energy balance during a restriction-realimentation period. Orquera K., Blanco, M., Bertolín, J.R., Ferrer, J., Casasús, I. In preparation for Journal of Dairy Science, expected submission December 2020.
- Effect of energy balance and stage of lactation on the performance and metabolic response of suckler cows to cope with short nutritional challenges. Orquera et al., expected submission June 2021.
- Effect of consecutive short nutritional challenges on the coping strategies of suckler cows in late lactation. Orquera et al., expected submission December 2021.

Experiment at FiBL

Partners:

- FIBL, Frick, Switzerland (Florian Leiber, Anna Bieber, Florian Moser, Anet Spengler-Neff)
- Two commercial farms with Fleckvieh cattle

Objectives.

The experiment conducted at FiBL had three main targets: a) to assess the suitability of chewing sensor-halters (RumiWatch) to characterize the intake and rumination behavior of dairy cows, b) the empirical assessment of reactions in chewing behavior (and its correlation with milk yields and composition) to changes in the feeding regime or feed offer (“challenges”), and c) to investigate the correlations between chewing behavior variables and efficiency estimates in order to understand, whether or not the former are suitable to predict the latter. The frame of the investigation was grassland-based zero concentrate organic dairy production with the double purpose breed “Swiss Fleckvieh”.

Methods.

We included two Swiss organic dairy farms with >40 cows of Swiss Fleckvieh each. Each of the farms was visited four times during one year. For respectively nine days, all cows were equipped with sensor halters and their jaw movement behavior was recorded 24/7 with a resolution of 1 min, summarized to averages per hour. The sensor data were transformed to “eating”, “ruminating” and “idling” by respective algorithms (Rombach et al., 2018). From these data, we calculated rumination and eating time, rumination intensity and speed, and the frequency of activity changes. Feed intake was aimed to be estimated based on sensor data with existing regressions (Rombach et al., 2018). Always in the middle of the ten days periods, the feed change happened, which was either a change of pastures, or the change from pasture to winter feeding or vice versa.

We included only lactating cows. Due to this and due to occasional data gaps in the sensor data, the effective number of cows per farm visit in the final dataset varied between 21 and 42. Cows were weighed, BCS was assessed once before and once after the feeding change; and milk was quantified and analysed (for protein, fat, lactose) twice before and after the challenge. For all cows, lactation data such as lactation yield, calving intervals and age at first calving were included. All feed stuffs provided to the animals were collected and analysed for nutrient proximates always before and after the challenge.

Two approaches were used to evaluate the data.

In a first approach we did not consider the feed change (challenge), but counted instead the respective two periods (before and after the challenge) as separate farm visits, thereby increasing the N of farm visits to 8 per farm. With this dataset we calculated the general influence of feed quality on chewing sensor variables and the correlation of the latter with efficiency estimates. Efficiency estimates used body weight, milk yields and dry matter intake, estimated according to De Souza et al. (2019), since intake estimation by chewing sensor data did unfortunately not result in plausible datasets. The whole methodology and results of this first approach is described in the manuscript draft in annex.

In a second approach we focus on the response of the animals' behavior and milk yield performance to the feed challenges. Here, we are analyzing, whether consistent differences exist between cows regarding their behavioral challenge response, and to which degree the response correlates to milk yields, efficiency estimates, constitutional and fertility traits and breeding values according to the herdbook. The first step was the empirical evaluation of the responses to the challenge. The following steps are the development of ranking models for the cows and regression calculation between the abovementioned variables and the herdbook data. These procedures are ongoing.

Preliminary results.

The results of the first approach are in the manuscript draft added to the annex. In brief, the influence of feed quality descriptors on rumination and eating behavior was found to be high, while the correlation of the latter with efficiency estimates was not significant and the explanatory power for variance was low. This means that the data do not provide evidence for the possibility to predict efficiency of dairy cows from chewing sensor parameters. However, as a side effect of the study, the data give a clear picture of the development of chewing sensor parameters in the course of lactation and in reaction to feed quality in roughage-based feeding regimes.

The preliminary results of the second approach showed strong responses of eating (Figure 5.1) and ruminating (Figure 5.2) to the feed challenge, but at the same time strong interactions with cows, indicating that individual cows responded with activity shifts in different directions. The same appeared true for milk yield (Figure 5.3) and composition (Figure 5.4). The still running data evaluation is now dedicated to rank the cows according to their response patterns and to correlate the rankings with health and fertility indicators as well as herdbook data (breeding values).

Valorisation of the results.

Two hypotheses underlying this experiment could not be verified: dry matter intake estimation based on sensor data did not lead to plausible results, and predictability of efficiency estimates (generated with other models than sensor data based) by sensor variables was very low.

On the other hand, the sensor data showed clear and plausible development curves during lactation, and clear responses to feed quality and changes in feeding regimes. The latter were individually different between cows and are currently evaluated for their suitability for animal characterization and correlations related to other important traits and breeding values.

Data

All data were uploaded on the WP2.3 common repository at url https://sites.inra.fr/site/gentore-wp2/efficiency in directory FIBL/exp0.

Article

The following article, to be submitted for publication, is presented in Annex 1

Bieber A, Moser F, Spengler-Neff A, Leiber A. The influence of days in milk and feeding on foraging behavior of dairy cows and the ability to predict efficiency of dairy cows based on sensor data in roughage-based feeding systems.

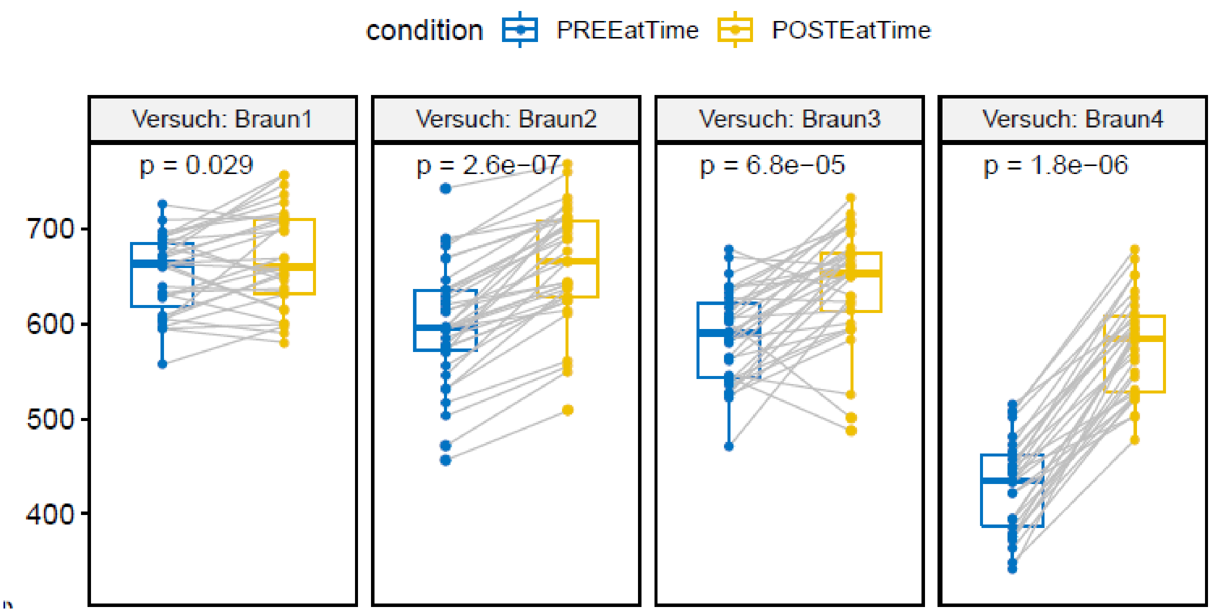


Figure 5.1. Eating time [min/day] in response to different feeding regimes during 4 farm visits (between “PRE” and “POST” at each visit, feeding regime was changed).

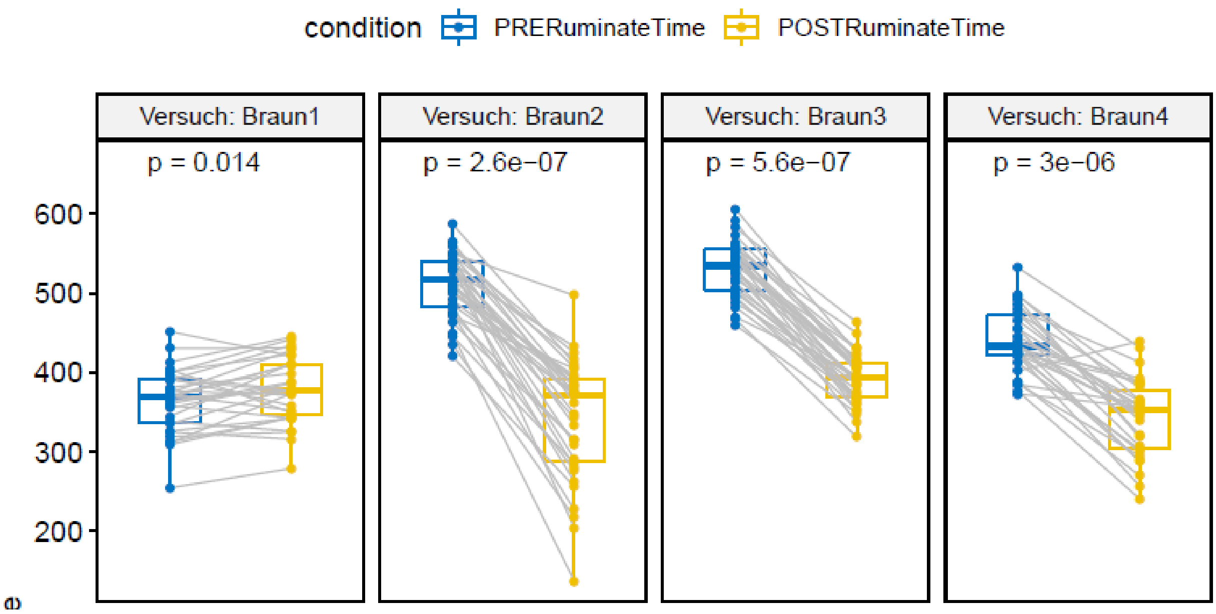


Figure 5.2. Ruminating time [min/day] in response to different feeding regimes during 4 farm visits (between "PRE" and "POST" at each visit, feeding regime was changed).

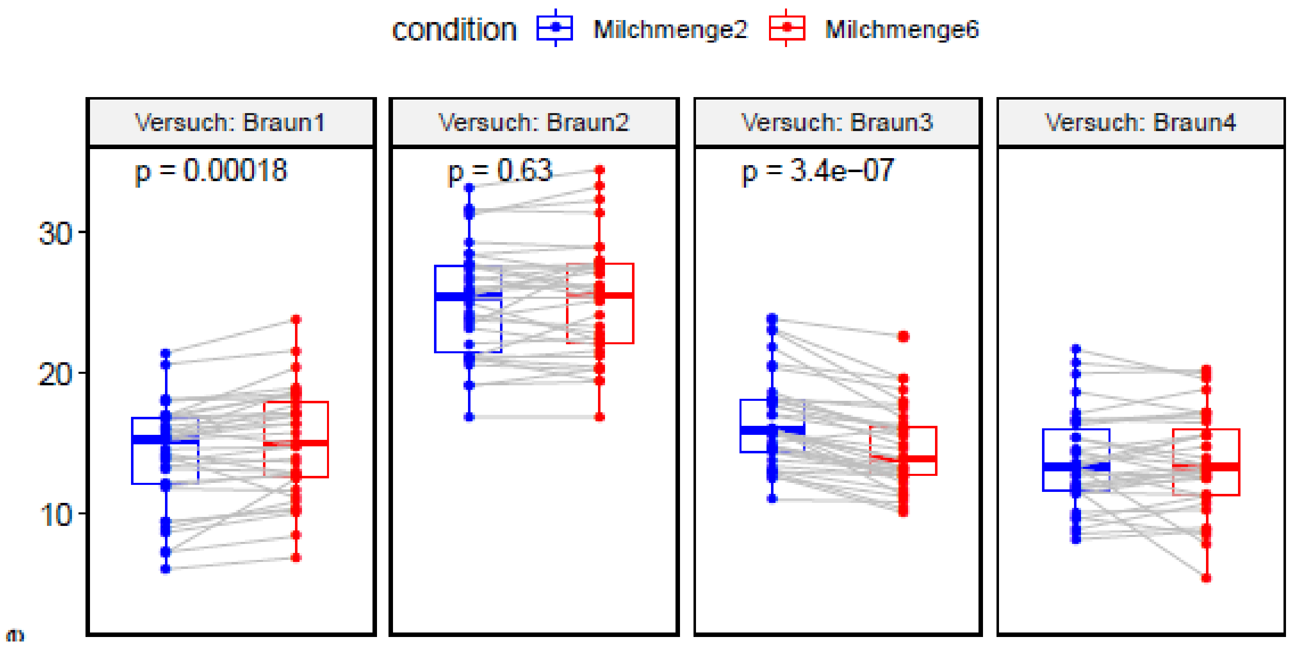


Figure 5.3. Daily milk yield [kg] in response to different feeding regimes during 4 farm visits (between "PRE" and "POST" at each visit, feeding regime was changed).

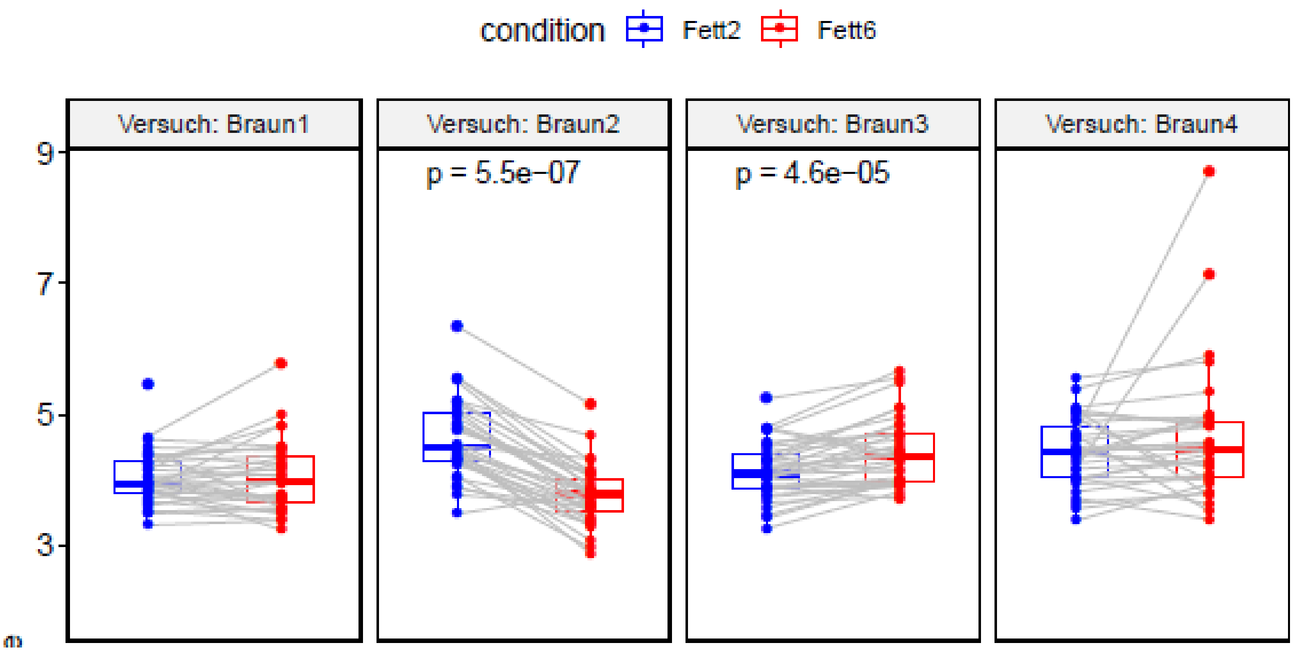


Figure 5.4. Milk fat concentration [g/100g] in response to different feeding regimes during 4 farm visits (between “PRE” and “POST” at each visit, feeding regime was changed).

Experiment at LfL

Influence of dietary energy concentration and body weight at slaughter on carcass composition and beef cuts of modern type Fleckvieh (German Simmental) bulls

<u>**Partner**</u>:
Bavarian State Research Center for Agriculture (LfL), Vöttinger Straße 38, 85354 Freising, Germany

<u>**General objectives and strategy of the experiment**</u>

Data on chemical composition and nutrient retention (especially body fat) are crucial for factorial requirement derivation in modern breeds. Because of high labour input no actual data are available for Simmental bulls, except of data on 30 Simmental cows dissected and analyzed about 10 years ago. It may be expected that efficiency of nutrient retention has been changed over the last decades. Besides the fundamental importance of nutrient retention of cattle for fattening from dual purpose breeding this may be a crosslink for evaluation of the correlation in growing and mature animals of a breed. For this reason, a feeding trial with consecutive serial slaughter trial was conducted. The animals were slaughtered at different stages of growth and analyzed for carcass composition, carcass quarters, beef cuts and their tissue compositions as well as chemical body composition in order to evaluate nutrient and energy retention in Simmental bulls of the actual genotype.

The feeding trial lasted from July 2017 to April 2019. The trial involved a total of 72 Fleckvieh calves. Calves were reared up to a body weight of about 200 kg at the LfL`s experimental farm Karolinenfeld (Theodor-Mayer-Weg 25, 83059 Kolbermoor, Germany). The fattening period was conducted at LfL`s experimental farm Grub (Prof.-Zorn-Straße 19, 85586 Poing/Grub, Germany). Slaughtering and dissection of animals was conducted at LfL`s slaughterhouse in Grub between September 2017 and April 2019. Chemical analyses were performed by LfL`s Dept. of Quality Assurance and Analysis (Prof.-Duerrwaechter-Platz 3, 85586 Poing/Grub, Germany).

Methods

Calf rearing

The experiment was conducted at the Bavarian State Research Center for Agriculture (LfL) according to European guidelines for animal experiments (Directive 2010/63/EU) and was approved by the ethics committee of the Ethics of Animal Experiments of LfL. For the study, 72 male Fleckvieh calves (German Simmental; age: 42d ±9, body weight (BW): 80kg ±6) were randomly purchased from cattle farms in Bavaria, Southern Germany. The calves were randomly assigned to deep litter calf pens and fed with restricted amounts of milk replacer and ad libitum total mixed rations (TMR) according to Table 6.1 over a period of 6 weeks. The TMR for the period after weaning (8 weeks) was adjusted weekly and supplemented with brewer's yeast, 110g per calf and day. The feed intake of each animal group was recorded daily and individual milk replacer intake was recorded by automatic calf feeders. Calves' BW was determined with a calf scale every second week.

Fattening period

For the fattening period, starting with an average BW of 225kg ±29 and age of 154d ±15, the bulls were randomly assigned to six beef pens housing 12 animals each. The pens were equipped with straw litter on sloped floors, automatic manure scrapers and automatic feed intake monitoring systems. The individual feed intake was recorded daily, and BW was determined using a cattle scale in four-week intervals. Three pens each were allocated to a normal energy (NE) and a high energy (HE) ration with 11.6 and 12.4 MJ ME/kg DM, respectively. Crude protein contents per kg DM remained constant in both diets, while the HE group was fed lower amounts of maize silage and more feedstuffs with higher energy density than the NE group. The compositions of NE and HE TMRs are illustrated in Table 6.2.

Feed analysis

The individual feed components were sampled and analyzed individually, while concentrates and TMRs were sampled weekly and pooled for a four-week period. The analysis was performed using methods of VDLUFA (2012) for dry matter (DM, method 3.1), crude ash (CA, method 8.1), crude protein (CP, method 4.1.2), sugar (method 7.1.1) and neutral detergent fiber (aNDFom, method 6.5.1) determination. Additionally, by methods of the Commission Regulation (EC) No 152/2009, the content of crude fat (CF, method 152-H) and starch (method 152-L) was determined. The DM of maize silages was corrected for losses by oven drying according to Weißbach and Kuhla (1995) and the content of metabolizable energy (ME) was calculated from the individual analyses (GfE 2008; DLG 2011). The crude nutrient and energy content of the TMRs was calculated by their compositions and the crude nutrient and energy contents of the individual feed components.

Slaughtering and meat cutting

During the feeding trial, 5 target live weights were set for slaughtering animals from both feeding groups: 120kg (4+4 animals), 200kg (5+5 animals), 400kg (9+9 animals), 600kg (9+9 animals), and 780kg (9+9 animals), respectively. The bulls were separated from the group 20 hours prior to slaughter, weighed and fasted from the TMR by feeding a hay and water diet for ad libitum intake in an isolation box. Slaughtering and dissection took place at the LfL Research Abattoir in Grub, Germany. The bulls were transported to the abattoir (distance 500m), inspected by a veterinarian (ante-mortem inspection), weighed and held in lairage with free access to water until slaughter. Slaughtering was carried out in compliance with the Commission Regulation (EC) No 1099/2009. The bull's final live weights were determined after stunning with a cattle gun and prior to bleeding. Skinning, evisceration, carcass halving and trimming were carried out according to European standards (Commission Regulation (EC) No 1249/2008). Post-mortem inspection was performed by a veterinarian during the slaughtering process. Dressed carcasses (carcasses without inner organs, hanging tender, suet and body cavity fat) were weighed and chilled for 20 hours at 4°C.

During slaughtering, the bulls' empty body weights were determined as final live weight minus the contents of urinary bladder and contents of the gastrointestinal tract (GIT) and the whole empty body was dissected to body tissues with fractions hide, blood, organs, empty GIT, body fat, muscle, tendon and bone. Blood was collected and weighed quantitatively during bleeding. After skinning, the hide was weighed, divided along the dorsal line and the right half of the hide was cut to pieces of approximately 10 cm^2 and stored at -18 °C for further processing. During evisceration, the organs (brain, spinal cord, eyes, tongue, heart, lung, diaphragm, liver with gall bladder, spleen, pancreas, kidneys, urinary bladder, testicles, penis) were collected, fat trimmed and weighed without fat trimmings. In the same way, fat tissue was manually removed from the GIT and afterwards, the GIT including its contents was weighed, emptied, washed, hung up to drain and weighed again as empty GIT. The right side of the carcass was dissected to muscle, tendon, fat and bone tissues. Body fat (body cavity fat, carcass fat), muscles (head, carcass, tail), tendons (feet, carcass) and bones (head, carcass, feet, tail) were weighed and bones of the right side of the body were stored at -18 °C. The gall bladder and urinary bladder were emptied and the organs, combined with the blood, became ground in a meat grinder (FW 114, K+G Wetter GmbH, Germany) and sampled as one batch. Likewise, body fat, muscle, tendons and GIT became ground separately in a meat grinder. Individual tissue samples were taken, and all samples were stored at -18 °C for further analysis.

Later, the hide portions were homogenized using a bowl cutter (bowl volume 65l, Krämer & Grebe, Germany) and the bones were crushed using a bone crusher (FX-300, Zhengzhou Fusion Machinery Equipment Co., Ltd, China). After processing, hide and bone tissues were sampled separately and the samples stored at -18 °C.

More details on carcass dissection are given in Honig et al. (2020).

Chemical analysis of body tissues

The frozen tissue samples were thawed in a refrigerator at 4°C for 48h and thereafter homogenized in a knife mill (Grindomix GM 200, Retsch, Germany) at 5000 rpm for 1:30 min, except for hide tissue, which was already suitable for analysis after bowl cutting. Body tissues were analyzed individually using methods of VDLUFA (2012) for crude fat (method 5.1.1), crude protein (method 4.1.2), crude ash (method 8.1) and loss on drying (method 3.5). The energy content of each tissue was calculated based on studies of Böhme & Gädeken (1980), which determined the energy contents of fat and protein with 39,0 kJ/g and 22,6 kJ/g, respectively.

Rib eye area and meat quality analysis

A sample of the Longissimus thoracis (LT) muscle (9^{th} and 10^{th} rib cut) was used for meat quality analysis. Muscle pH was measured using a portable pH meter (testo 205, Testo SE & Co. KGaA, Germany) 1 hour, 24 hours and 14 days after slaughtering. The rib eye area of the 9th rib was measured by digital image analysis and intramuscular fat (IMF) content was measured using petroleum ether in a Soxhlet extraction apparatus. Meat color was measured in CIELAB color space (L*: lightness, a*: redness, b*: yellowness), using a portable spectrophotometer (CM-508i, Minolta Camera CO., LTD., Japan). Ageing loss was recorded after storing the muscle sample from the 10th rib for 14d at 4°C, cooking loss was determined after heating the 2.5cm thick, stored sample in 70°C warm water up to a meat core temperature of 70°C. The shear force was measured after storing the cooked sample for 24h at 4°C, using the Warner-Bratzler method (2519-1kN, Instron GmbH, Germany).

Statistical analysis

Statistical analysis was performed using the Proc Mixed procedure of SAS (Version 9.4, SAS Institut, Cary, NC, USA) and the Kenward-Roger method to provide corrected degrees of freedom. The linear model included a two-way ANOVA with interaction (feed energy, weight group, feed energy x weight group) and residuals. Differences between groups were tested using the PDIFF option with effects stated as significant when $p<0.05$. Results are shown as LS Means (LSM) and standard error of mean (SEM).

Results and discussion

Daily feed, energy and nutrient intake

As a consequence of feeding varying energy concentrations during the fattening period, HE treated bulls showed in all stages of the finishing period a higher daily DM, sugar, starch and energy intake than the NE animal group, while NE fed bulls showed a higher daily aNDFom intake (Table 6.3). Higher feed intake in the HE treatment group was most likely a result of higher concentrates proportion of the diet. Similar conclusions were drawn by Steen & Kilpatrick (2000), who fed varying amounts of concentrates to Simmental crossbred steers. Although crude protein contents of the NE and HE TMRs were identical, crude protein intake differed between the treatment groups because of the higher daily DM intake of HE fed bulls. However, crude protein intake of bulls in both treatment groups exceeded the bull's crude protein requirements (GfE 1995) and therefore did not limit growth.

Fattening performance

Feeding varying amounts of concentrates alters the daily weight gain, as was previously described by Slabbert et al. (1992) and Steen & Kilpatrick (2000). Further studies with Fleckvieh bulls fed with high energy diets found daily live weight gains of 1210 g/d for the fattening period in a weight range from 200-650kg (Schwarz et al. 1992) and peak live weight gain of 1536 g/d in a weight range 205-363kg (Schwarz & Kirchgessner 1990). In the current study, the high energy ration led to average daily weight gains of 1699 and 1792g/d for the NE and HE treatment group, respectively ($p<0.1$). Peak live weight gains, with significantly higher gains of the HE treatment group, were reached in between 400-600kg with 1753 and 1910 g/d in NE and HE treatment group, respectively (Table 6.3). Hence, bulls fed with high energy rations reached the target weight in shorter time.

While the target weights in the present study were kept comparable for NE and HE treatment groups, the slaughter ages of bulls in weight groups 600 and 780kg differed between treatment groups (Table 6.4). The average slaughter age of HE bulls at 600kg was 9 days less than those of NE bulls at the same weight. Likewise, HE bulls with final live weights of 780kg were slaughtered 21 days earlier ($p<0.05$) than NE bulls with the same final weights. Comparisons with former studies indicated an approximately 500g/d higher daily weight gain of HE fed modern type Fleckvieh bulls than high energy fed bulls of past decades (Schwarz et al. 1992; Dannenberger et al. 2006). Thus, present-day Fleckvieh bulls grow faster and reach final live weights of 600kg approximately 130 days earlier than past decade bulls of the same breed when fed with high concentrates rations. The following results illustrate how the accelerated growth rates affected slaughter traits and carcass composition of modern type Fleckvieh bulls.

Dressing percentage and carcass tissues

The present data showed no significant effects of dietary energy concentration on dressing percentage, carcass composition and meat quality traits of NE and HE treatment groups. Hence, the combined results of both animal groups are shown.

With increasing body weight, the dressing percentage increased from 52.2% to 59.7% ($p<0.05$) in bulls with 120kg and 780kg final weights, respectively (Table 6.5). Comparison with previous studies showed that the dressing percentages of growing bulls in the present study were approximately 2% lower than in past decades Fleckvieh bulls with 650kg final weight (Otto et al. 1994), but 3-4% higher than present Simmental bulls, steers and heifers (Coyne et al. 2019; Sami et al. 2004; Terler et al. 2016). Hence, as a late maturing breed, modern type Fleckvieh converted feed energy efficiently into lean carcass growth.

Comparing the lowest and highest weight groups with 120 and 780kg, carcass muscle percentage decreased by 4% ($p<0.05$) while percentage of fat tissue increased by 13.6% ($p<0.05$; Table 6.5). During the growth period, the percentage of bone tissue in the chilled carcasses decreased from 23.1% in 120 kg bulls to 13.2% in 780kg bulls ($p<0.05$). However, percentage of tendon did not vary between weight groups 120 and 780kg with 6.0% and 5.9%, respectively. These changes in carcass tissue composition of growing Fleckvieh bulls are in line with the results of Augustini et al. (1992) and Keane (2011) which concluded that growth alters the carcass composition of beef cattle. However, present data could not confirm former studies by Augustini et al. (1992), who observed differences in muscle and fat deposition between growing Fleckvieh bulls of a restricted and ad libitum feeding group.

In comparison, early maturing Hereford bulls featured lower dressing percentage (Bartoň et al. 2006; Manninen et al. 2011; Pesonen et al. 2013) while studies comparing Hereford and Simmental breeds indicate higher carcass fat proportion in Hereford and higher meat proportion in Simmental cattle (Mandell et al. 1998; Bartoň et al. 2006). The same effect could be observed in comparison to high yielding dairy cattle breeds like Holstein Friesian. This breed showed lower dressing percentage (Pfuhl et al. 2007; Keane 2011; Geuder et al. 2012) and carcass muscle tissue (Keane 2011; Geuder et al. 2012), but higher carcass fat tissue percentage (Keane 2011; Geuder et al. 2012) than Fleckvieh bulls. Overall, our data prove that late maturity leads to carcasses with a relatively high amount of muscle but low amount of fat, even in high weight groups. More details on Carcass quarters and tissues are presented by Honig et al. (2020).

Longissimus thoracis of modern type Fleckvieh bulls showed larger rib eye area, but similar IMF content and meat lightness as in previous studies on Fleckvieh bulls (Table 6.6). Meat quality traits were characterized by higher ageing and cooking loss, but better tenderness compared to previous studies. Fattening Fleckvieh bulls to high final live weights of 780kg had only a limited effect on IMF, but increased rib eye area and intensified meat color at consistently good tenderness.

Body tissue composition and deposition

Since there were only minor effects of dietary energy concentration on empty body tissue composition in normal and high energy treatment groups, the combined results of both animal groups are shown (Fig. 1). Muscle and tendon percentage of empty body weight, with average of 42.9 % ±0.5 and 4.2 % ±0.1, respectively, did not vary between weight groups. During growth, the percentage of blood, organs, GI tract, and bone decreased ($p<0.05$; blood: 6.0-4.0 % ±0.1; organs: 7.2-5.7 % ±0.1; GI tract:

7.4-3.9 % ±0.2; bone: 19.0-11.1 % ±0.2), while hide and body fat percentage increased ($p<0.05$) from 9.2 to 10.5 % ±0.2 and 3.7 to 18.5 % ±0.6, respectively.

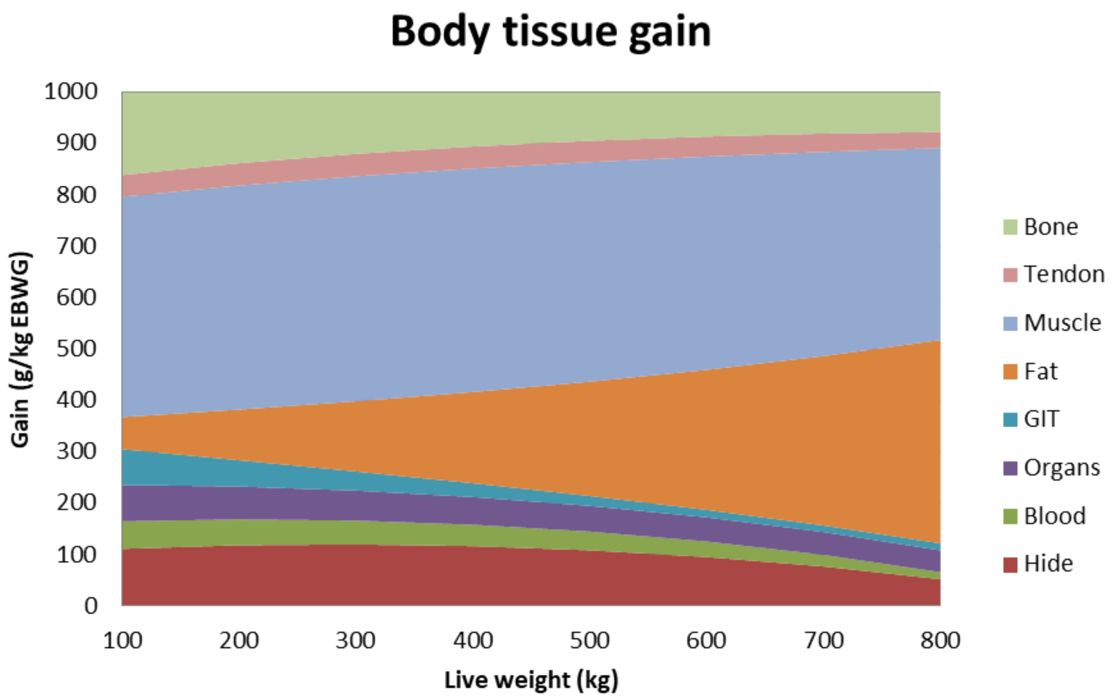


Figure 1: Body tissue gain of Fleckvieh bulls in dependence of live weight

Contents and deposition of chemical components

Since there were only minor effects of dietary energy concentration on nutrient contents in normal and high energy treatment groups, the combined results on chemical body composition of both animal groups are shown. During growth, the percentage of crude protein, crude ash and water decreased ($p<0.05$; crude protein: 20.6-19.1 % ±0.2; crude ash: 4.8-4.4 % ±0.1; water: 68.4-55.3 % ±0.5), while the crude fat percentage increased ($p<0.05$) from 6.2 to 21.3% ±0.7. The development of body nutrient gain in dependence of live weight is shown in Fig. 2

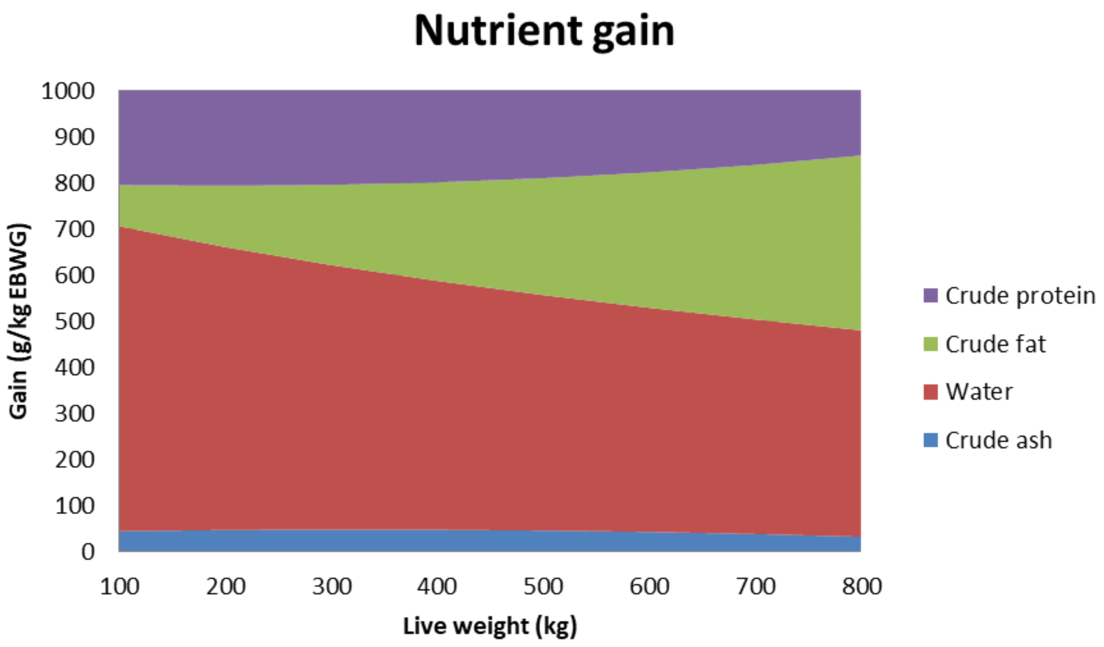


Figure 2: Body nutrient gain in dependence of live weight

Papers, situation regarding Covid and expected date of final results

Peer reviewed papers on experiment:
Honig, A.C., Inhuber, V., Spiekers, H., Windisch, W., Götz, K.-U., Ettle, T. (2020): Influence of dietary energy concentration and body weight at slaughter on carcass tissue composition and beef cuts of modern type Fleckvieh (German Simmental) bulls. Meat Science, 169, 108209 (https://doi.org/10.1016/j.meatsci.2020.108209).

Covid resulted in a temporary lockdown of our laboratory and consequently in a delay of chemical analyses. Moreover, staff and researchers worked temporarily from home office what hampered communication and complicated data processing. However, data for a second paper on body nutrient composition and retention is prepared and a respective manuscript should be submitted soon. Time point of acceptance and publication can hardly be estimated.

Conclusion

Comparison with former studies indicates that modern type Fleckvieh bulls grow faster than bulls of past decades and the present study shows that feeding high energy rations shortens the fattening period for a high target weight as 780kg. Since late maturing cattle breeds are efficient in exploiting high energy diets, only minor effects of the dietary energy concentration on carcass weights and the tissue compositions of carcass, quarters and cuts in NE and HE treatment groups were observed. The characteristics of a late maturing cattle breed became obvious during growth, when bulls produced large, lean carcasses with high muscle and low fat content. Percentage of fat in the carcasses increased primarily at the expense of bone and subsidiary muscle tissue, while the tendon content remained unchanged. Meat quality traits like IMF, meat color and tenderness increased in high final weight groups. Hence, fattening Fleckvieh bulls to high final weights as 780kg can be recommended. In summary, modern type Fleckvieh bulls meet the needs of meat markets which target high production rates of lean beef.

The empty body compositions of modern type Fleckvieh bulls corresponded widely to literature data from past decades (Schulz et al., 1974). During growth, the amount of body fat increased mainly at the expense of bone tissue. A decrease of muscle tissue in higher weight classes could not be observed. Variations in dietary energy concentrations within margins found under practical conditions did not alter the body composition to a relevant extent.

Variations in dietary energy concentrations within margins found under practical conditions did not alter the body nutrient composition to a relevant extent. The body nutrient contents of modern type Fleckvieh bulls corresponded widely to literature data from past decades (Kirchgessner et al., 1993). During growth, the amount of crude fat increased mainly at the expense of body water. Furthermore, modern bulls showed a 600 g higher daily weight gain during the fattening period and thus had a higher nutrient accretion than bulls in former studies (Schwarz et al., 1992). In summary, modern type Fleckvieh bulls feature a higher growth potential and can be fattened up to 780 kg final live weight.

Data

All data were uploaded on the WP2.3 common repository at url https://sites.inra.fr/site/gentore-wp2/efficiency in directory LFL/exp10.

Acknowledgement

The authors are grateful to staff of LfL Research Farm in Grub for care and management of the experimental animals, to staff of LfL Research Abattoir for slaughtering and carcass processing and to LfL Department of Quality Assurance and Analytics for conducting the chemical analyses. Furthermore, the authors are grateful to M. Pickl and G. Fleischmann of the LfL Institute for Animal Breeding for scientific documentation during slaughtering and carcass dissection.

References

Augustini, C., Branscheid, W., Schwarz, F. J., & Kirchgeßner, M. (1992). Growth specific alterations of the carcass quality of fattening cattle of German Simmentals. 2. Influence of feeding intensity and slaughter weight on the coarse tissue composition of young bull carcasses. Fleischwirtschaft, 72 (12), 1706-1711.

Bartoň, L., Řehák, D., Teslík, V., Bureš, D., & Zahrádková, R. (2006). Effect of breed on growth performance and carcass composition of Aberdeen Angus, Charolais, Hereford and Simmental bulls. Czech Journal of Animal Science, 51, 47-53.

Berg, R. T., & Butterfield, R. M. (1976). New concepts of cattle growth. Sydney University Press, Sydney, Australia.

Commission Regulation (EC) No 1249/2008 (2008) laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcases and the reporting of prices thereof. Official Journal of the European Union, L 337, 3-30.

Commission Regulation (EC) No 152/2009 (2009) laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union, L 54, 1-130.

Commission Regulation (EC) No 1099/2009 (2009) on the protection of animals at the time of killing. Official Journal of the European Union, L 303, 1-30.

Coyne, J. M., Evans, R. D., & Berry, D. P. (2019). Dressing percentage and the differential between live weight and carcass weight in cattle are influenced by both genetic and non-genetic factors. Journal of Animal Science, 97, 1501-1512.

Dannenberger, D., Nuernberg, K., Nuernberg, G., & Ender, K. (2006). Carcass- and meat quality of pasture vs concentrate fed German Simmental and German Holstein bulls. Archives Animal Breeding, 49, 315–328.

Directive 2010/63/EU (2010) on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276, 33-79.

DLG (1985). DLG-Schnittführung für die Zerlegung der Schlachtkörper von Rind, Kalb, Schwein und Schaf. Deutsche Landwirtschafts-Gesellschaft e.V., Frankfurt am Main, Germany.

DLG (2011). Stellungnahme des DLG-Arbeitskreises Futter und Fütterung. Leitfaden zur Berechnung des Energiegehaltes bei Einzel- und Mischfuttermitteln für die Schweine- und Rinderfütterung. https://www.dlg.org/fileadmin/downloads/fachinfos/futtermittel/Stellungnahme_Energieberechnung_Rind_Schwein.pdf.

Geuder, U., Pickl, M., Scheidler, M., Schuster, M., & Götz, K.-U. (2012): Growth performance, carcass traits and meat quality of Bavarian cattle breeds. Züchtungskunde, 84, 485-499.

GfE (1995). Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere, Nr. 6, Empfehlungen zur Energie- und Nährstoffversorgung der Mastrinder. Ausschuß für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie, DLG-Verlag-GmbH, Frankfurt am Main, Germany.

GfE (2008). Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology: New equations for predicting metabolisable energy of grass and maize products for ruminants. Gesellschaft für Ernährungsphysiologie, Proceedings of the Society of Nutrition Physiology, 17, 191-198.

Huuskonen, A., Pesonen, M., Kämäräinen, H., & Kauppinen, R. (2013). A comparison of purebred Holstein-Friesian and Holstein-Friesian x beef breed bulls for beef production and carcass traits. Agricultural and Food Science, 22, 262-271.

Irshad, A., Kandeepan, G., Kumar, S., Ashish Kumar, A., Vishnuraj, M. R., & Shukla, V. (2013). Factors Influencing Carcass Composition of Livestock: a Review. Journal of Animal Production Advances, 3, 177-186.

Keane, M. G. (2011). Relative Tissue Growth Patterns and Carcass Composition in Beef Cattle. Teagasc, Grange Beef Research Centre Occasional Series No. 7.

Kirchgessner M., Schwarz F.J., Otto R., Reimann W., Heindl U. (1993): Journal of Animal Physiology and Animal Nutrition. 70: 266-277.

Mandell, I. B., Gullett, E. A., Wilton, J. W., Allen, O. B., & Kemp, R. A. (1998). Effects of breed and dietary energy content within breed on growth performance, carcass and chemical composition and beef quality in Hereford and Simmental steers. Canadian Journal of Animal Science, 78, 533-541.

Manninen, M., Honkavaara, M., Jauhiainen, L., Nykänen, A., & Heikkilä, A.-M. (2011). Effects of grass-red clover silage digestibility and concentrate protein concentration on performance, carcass value, eating quality and economy of finishing Hereford bulls reared in cold conditions. Agricultural and Food Science, 20, 151-168.

Murray, A. C. (1989): Factors affecting beef color at time of grading. Canadian Journal of Animal Science, 69, 347-355.

Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J., Scollan, N. D., Wood, J. D., Nute, G. R., & Richardson, R. I. (2005). Effect of a grass-based and a concentrate feeding system on meat

quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. Livestock Production Science, 94, 137-147.
Obuz, E., Dikeman, M. E., Grobbel, J. P., Stephens, J. W., & Loughin, T. M. (2004). Beef longissimus lumborum, biceps femoris, and deep pectoralis Warner-Bratzler shear force is affected differently by endpoint temperature, cooking method, and USDA quality grade. Meat Science, 68, 243-248.
Otto, R., Heindl, U., Augustini, C., Schwarz, F. J., Reimann, W., & Kirchgessner, M. (1994).
Influence of live mass and feeding intensity on non carcass parts of bulls, heifers and steers (German simmental). Fleischwirtschaft, 74 (7), 779-783.
Page, J. K., Wulf, D. M., & Schwotzer, T. R. (2001). A survey of beef muscle color and pH. American Society of Animal Science, 79, 678-687.
Papaleo Mazzucco, J., Goszczynski, D. E., Ripoli, M. V., Melucci, L. M., Pardo, A. M., Colatto, E., Rogberg-Muñoz, A., Mezzadra, C. A., Depetris, G. J., Giovambattista, G., & Villarreal, E. L. (2016). Growth, carcass and meat quality traits in beef from Angus, Hereford and cross-breed grazing steers, and their association with SNPs in genes related to fat deposition metabolism. Meat Science, 114, 121-129.
Pesonen, M., Honkavaara, M., Kämäräinen, H., Tolonen, T., Jaakkola, M., Virtanen, V., & Huuskonen, A. (2013). Effects of concentrate level and rapeseed meal supplementation on performance, carcass characteristics, meat quality and valuable cuts of Hereford and Charolais bulls offered grass silage-barley-based rations. Agricultural and Food Science, 22, 151-167.
Pfuhl, R., Bellmann, O., Kühn, C., Teuscher, F., Ender, K., & Wegner, J. (2007). Beef versus dairy cattle: a comparison of feed conversion, carcass composition, and meat quality. Archives Animal Breeding, 50, 59-70.
Sami, A. S., Augustini, C., & Schwarz F. J. (2004). Effects of feeding intensity and time on feed on performance, carcass characteristics and meat quality of Simmental bulls. Meat Science, 67, 195-201.
Schulz E., Oslage H.J., Daenicke R. (1974) Untersuchungen über die Zusammensetzung der Körpersubstanz sowie den Stoff- und Energieansatz bei wachsenden Mastbullen. Fortschritte in der Tierphysiologie und Tierernährung, Heft 4
Schwarz, F. J., & Kirchgessner, M. (1990). Fattening characteristics of Simmental bulls, steers and heifers. Züchtungskunde, 62 (5), 384-396.
Schwarz, F. J., & Kirchgessner, M. (1991). Nutritional influences on beef quality. Landwirtschaft Schweiz, Band 4, 325-329.
Schwarz, F. J., Kirchgeßner, M., Augustini, C., & Branscheid, W. (1992). Growth specific alterations of carcass quality of fattening cattle of German Simmentals. 1. Growth rate of bulls, steers and heifers fed with different feeding intensity. Fleischwirtschaft, 72 (11), 1584-1589.
Slabbert, N., Campher, J. P. Shelby, T., Leeuw, K.-J., & Kühn, G. P. (1992). The influence of dietary energy concentration and feed intake level on feedlot steers. 2. Feed intake, live mass-gain, gut fill, carcass gain and visual and physical carcass measurements. South African Journal of Animal Science, 22 (4), 107-114.
Steen, R. W. J., & Kilpatrick, D. J. (2000). The effects of the ratio of grass silage to concentrates in the diet and restricted dry matter intake on the performance and carcass composition of beef cattle. Livestock Production Science, 62, 181-192.
Terler, G., Velik, M., Kitzer, R., & Kaufmann, J. (2016): Effects of high final weights on fattening performance, carcass traits and meat quality of bulls. 43. Viehwirtschaftliche Fachtagung 2016, 1-10.

Van der Westhuizen, J. (2013). Making sense out of maturity types in beef cattle. SA Stud Book and Animal Improvement Association, October 2013, 97-99. http://www.studbook.co.za/images/photos/sa%20graan-okt%202013_83.pdf

VDLUFA (2012). Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln, VDLUFA-Verlag, Darmstadt. Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten e.V..

Weißbach, F., & Kuhla, S. (1995). Stoffverluste bei der Bestimmung des Trockenmassegehaltes von Silagen und Grünfutter – entstehende Fehler und Möglichkeiten der Korrektur. Übersichten zur Tierernährung, 23, 189-214.

Yancey, J. W. S., Apple, J. K., & Wharton, M. D. (2016). Cookery method and endpoint temperature can affect the Warner-Bratzler shear force, cooking loss, and internal cooked color of beef semimembranosus and infraspinatus steaks. Journal of Animal Science, 94, 4434-4446.

Table 6.1: Composition, crude nutrient and energy contents of feedstuffs fed during calf rearing

Feedstuff	**Composition**		**Crude nutrients**					
	While milk feeding %DM	**After weaning** %DM	**DM** g/kg	**CA** g/kg DM	**CP** g/kg DM	**CF** g/kg DM	**aNDFom** g/kg DM	**ME** MJ/kg DM
Calf milk replacer (120 g/L)	5 L/d	-	961	69	210	191	0	16.6
Maize silage	-	63.6%	443	29	78	43	392	11.6
Hay	30.0%	3.7%	852	61	140	20	629	8.5
Molasses	14.3%	1.9%	775	209	108	0	0	10.9
Barley	17.7%	1.2%	879	23	98	20	154	13.0
Maize grain	11.1%	7.1%	888	14	98	42	82	13.3
Rapeseed meal	13.4%	14.2%	889	90	360	45	363	11.6
Pressed beet pulp	11.1%	6.2%	902	80	96	7	418	11.6
Soybean oil	0.3%	0.8%	999	0	0	999	0	30.6
Minerals, 26% Ca, 2% P	1.7%	1.1%	981	981	0	0	0	0
Calcium Carbonate	0.4%	0.3%	997	997	0	0	0	0
Brewer’s yeast	-	110 g/d	928	59	280	25	431	12.4

Table 6.2: Composition, crude nutrient and energy contents of feedstuffs fed for the fattening period

Feedstuff	**Composition**		**Crude nutrients**					
	Normal energy %DM	**High energy** %DM	**DM** g/kg	**CA** g/kg DM	**CP** g/kg DM	**CF** g/kg DM	**aNDFom** g/kg DM	**ME** MJ/kg DM
Maize silage	80.0%	40.0%	355	31	77	34	336	11.8
Wheat	0.5%	15.5%	888	21	169	16	132	13.3
Maize grain	-	20.6%	889	13	91	41	94	13.3
Rapeseed meal	16.4%	16.7%	891	85	380	39	337	11.7
Pressed beet pulp	0.9%	5.5%	893	96	89	4	408	11.5
Feed grade urea 46,5% N	0.5%	-	990	0	2906	0	0	0
Minerals 26% Ca, 2% P	0.8%	0.8%	981	981	0	0	0	0
Calcium Carbonate, cattle salt	0.8%	0.8%	994	994	0	0	0	0

Table 6.3: Daily feed, nutrient, energy intake and weight gain of bulls in normal and high energy treatment groups in different weight ranges

Feed intake/ fattening performance	**Weight range**								**SEM**	**p-value**		
	80-120kg	**120-200kg**	**200-400kg**		**400-600kg**		**600-780kg**			feed	weight	feed x weight
			NE	HE	NE	HE	NE	HE				
	n = 72	n = 64	n = 27	n = 27	n = 18	n = 18	n = 9	n = 9				
DM (kg/d)	1.92	4.38	7.03^{A}	7.75^{B}	9.47^{A}	10.66^{B}	10.72^{A}	11.35^{B}	0.09	<0.0001	<0.0001	<0.0001
CP (g/d)	321	647	1001^{A}	1107^{B}	1372^{A}	1546^{B}	1538^{A}	1665^{B}	11.91	<0.0001	<0.0001	<0.0001
aNDFom (g/d)	450	1464	2274^{A}	1973^{B}	3116^{A}	2701^{B}	3526^{A}	2759^{B}	28.87	<0.0001	<0.0001	<0.0001
Starch (g/d)	349	1245	2233^{A}	3276^{B}	2802^{A}	4419^{B}	3208^{A}	4769^{B}	36.68	<0.0001	<0.0001	<0.0001
Sugar (g/d)	358	229	208^{A}	286^{B}	257^{A}	357^{B}	292^{A}	398^{B}	4.20	<0.0001	<0.0001	<0.0001
ME (MJ/d)	24.6	51.4	82.1^{A}	96.1^{B}	110.1^{A}	132.3^{B}	124.3^{A}	141.7^{B}	1.02	<0.0001	<0.0001	<0.0001
Daily weight gain (g/d)	980	1452	1717^{A}	1841^{B}	1753^{A}	1910^{B}	1500	1521	27.07	0.0139	<0.0001	0.0407

Means within a weight range sharing the same superscript are not significantly different

Table 6.4: Animal performance of bulls in different treatment of energy density and slaughter groups

Animal performance	Slaughter group								SEM	p-value		
	120kg	200kg	400kg		600kg		780kg			feed	weight	feed x weight
			NE	HE	NE	HE	NE	HE				
	n = 8	n = 10	n = 9	n = 9	n = 9	n = 9	n = 9	n = 9				
Slaughter age (d)	94	147	271	271	375	366	502^{A}	481^{B}	5.50	0.1744	<0.0001	0.5561
Final live weight (kg)	121	200	399	401	595	595	777	784	4.05	0.6334	<0.0001	0.9597
Warm carcass weight (kg)	63	105	228	226	345	352	469	462	2.84	0.9257	<0.0001	0.3701
Cold carcass weight (kg)	61	102	224	220	339	346	463	456	2.74	0.9357	<0.0001	0.2304

Means within a slaughter group with different superscripts differ significantly

Table 6.5: Average dressing percentage and carcass tissue composition of bulls in different slaughter groups

Carcass	Slaughter group					SEM	p-value		
	120kg	200kg	400kg	600kg	780kg		feed	weight	feed x weight
	n = 8	n = 10	n = 18	n = 18	n = 18				
Dressing percentage	52.2^{A}	52.7^{A}	56.7^{B}	58.5^{C}	59.7^{D}	0.44	0.4784	<0.0001	0.0510
Muscle	67.5^{A}	67.8^{A}	67.2^{A}	65.3^{B}	63.5^{C}	0.52	0.6566	<0.0001	0.7231
Tendon	6.0^{AB}	5.9^{B}	6.4^{A}	6.1^{AB}	5.9^{B}	0.15	0.2388	0.0683	0.5784
Fat	2.7^{A}	6.0^{B}	9.1^{C}	12.7^{D}	16.3^{E}	0.56	0.9888	<0.0001	0.6179
Bone	23.1^{A}	19.6^{B}	16.5^{C}	15.0^{D}	13.2^{E}	0.22	0.8211	<0.0001	0.4754

Means within a row sharing the same superscript are not significantly different

Table 6.6: Rib eye area and meat quality traits of bulls in different slaughter groups

Rib eye area and meat quality traits	Slaughter group					SEM	p-value		
	120kg n = 8	**200kg** n = 10	**400kg** n = 18	**600kg** n = 18	**780kg** n = 18		feed	weight	feed x weight
Rib eye area (cm^2)	20.7^{A}	30.2^{B}	53.8^{C}	74.2^{D}	87.0^{E}	2.31	0.6361	<0.0001	0.5515
pH, 1h	6.8	6.8	6.8	6.8	6.8	0.05	0.2869	0.9418	0.1543
pH, 24h	5.6^{A}	5.6^{A}	5.5^{B}	5.4^{B}	5.4^{B}	0.02	0.2659	0.4179	0.6458
pH, 14d	5.6	5.6	5.6	5.5	5.5	0.02	0.4962	0.4537	0.4183
IMF (%)	0.5^{A}	0.7^{A}	1.3^{B}	2.4^{C}	3.3^{D}	0.20	0.4108	<0.0001	0.7271
Ageing loss, 14d (%)	4.9^{AB}	5.8^{A}	4.6^{B}	4.8^{B}	5.1^{AB}	0.31	0.2410	0.0775	0.5646
Cooking loss (%)	26.0^{A}	27.4^{A}	29.7^{B}	30.6^{B}	30.7^{B}	0.76	0.3539	0.0002	0.9576
Shear force (N)	61.9^{A}	86.5^{B}	49.2^{C}	42.1^{C}	46.0^{C}	3.79	0.7912	<0.0001	0.4233
Meat color									
L*	42.2^{A}	42.0^{A}	37.2^{B}	36.9^{B}	34.6^{C}	0.56	0.4147	<0.0001	0.1774
a*	5.8^{A}	4.6^{A}	8.9^{B}	11.0^{C}	13.2^{D}	0.49	0.6723	<0.0001	0.8162
b*	4.5^{A}	3.6^{AB}	3.3^{B}	4.6^{A}	4.2^{A}	0.37	0.6269	0.0341	0.2603

Means within a row sharing the same superscript are not significantly different

Gentore Data Repository for Experimental Data

The data corresponding to the 6 experiments carried out in Gentore WP2.3 task were shared and uploaded in a common repository. The details are provided in this chapter.

Server and data management

Sharepoint address: https://sites.inrae.fr/site/gentore-wp2/efficiency

Logical arborescence: The main directory "efficiency" has a partner subdirectory /$partner/$exp with different datasets and files : $dataset/$table_$yyyymmdd.csv

with

- $partner= CITA, FIBL, INRAGABI, INRAUMRH, LFL
- Dataset=exp3, exp5, … The list of directories is given below for each partner. A dataset corresponds to one experiment.
- Tables : see list. Different versions of each table may exist. They differ according to date, only the most recent one is valid.

Example: /efficiency/INRA-GABI/EXP5/ID_20201102.csv, FEEDINTAKE_20201102.csv, FEEDDIET_20201102.csv, WEIGHT_20201102.csv, etc

At /efficiency/$partner/ level, the **metadata_description_$date.csv** file describes each $dataset directory with the following information.

- One row per dataset (for task 2.1 or 2.3) ; delimiter=';' ;
- Information: partner (see above);dataset (eg, EXP5);breed (eg, Charolais);sex (M or F);beef or dairy (B or D);growing or lactating (G or L)
- Example: /efficiency/inragabi/metadata_description.csv could contain : INRAGABI;EXP5;Charolais;M,B;G;

__Assumptions__: either original or recodified, each animal ID is unique within organization. Possible duplicates across organizations will be considered as different.

Nota:

- A table may be optional or mandatory. If mandatory, the table must exist for each partner bringing information. If optional, the table may exist if the partner wants to bring information.
- The mandatory table ID cannot be empty: all animals present in any other table of the same experiment have a row in the ID table.
- The other mandatory tables contain information only if this information exists. Therefore, these tables must exist but may be empty

- The mandatory table FEEDDIET includes as many rows as diets specified in FEEDINTAKE table.
- In a row of a table, some variables are mandatory, some other may be absent if not available. For instance, birth weight may be missing in the ID mandatory table.
- For some tables, all variables in a row are mandatory. It means that if a variable is missing, the row does not exist.

AUTHORIZATIONS and RULES

All partners providing data have

- Read/write access to their data (their directory)
- Read access to the entire Efficiency directories of all partners. Use of these data requires prior agreement of the data owners.

Inrae has a full access for data management purpose only (data quality control, production of summary statistics)

RULE : Data providers have a complete priority (in terms of date and topic study) to publish on their own data. They are aware of and agree with all use of their own data. They are associated to all common valorizations using their data.

Detailed description of Tables

17 tables are defined

Seven tables are mandatory: ID, FEEDINTAKE, FEEDDIET, WEIGHT, GROUP, TREATMENT, PARITY

One additional table is mandatory for dairy lactating animals: MILK

Nine Tables are optional, ie are present if the partner provides this information: WATER, BACKFAT, METABOLIC, CARCASS, BCS, DISEASE, CULLING, INS, INDIRECT_MILK

Note that the Reference Information section, at the end of this document, provides some codification to use in the tables.

1. Identification: ID table (example: ID_20201102) - MANDATORY

- 1 row per animal; delimiter=';' ; missing information=NULL (ie, no value between two ';'). Missing values are possible only for optional variables)
- Information
    - Organization (mandatory)
    - Dataset (mandatory)
    - Animal ID (national or anonymous Id, mandatory, unique per organization)
    - Sex (M or F, mandatory)
    - Birthdate (YYYYMMDD, optional)
    - Sire Id (optional)

- Dam Id (optional)
- Herd of birth (optional)
- Birth weight (kg, optional)

Example:
INRAGABI;Charolais;FR1234567890;M;20100320;FR0024681357;FR0013572468;FR61000001;46;
INRAGABI; Charolais;GABI0001;M;;;;;;

2. Groups: GROUP table (example: GROUP_20201102) - MANDATORY

- This table described the allocation of the animals in the experimental groups
- 1 row per animal (and, if groups change, per period); delimiter=';' ;
- Information
  - Animal ID (mandatory)
  - Group (free nomenclature, eg (T vs C, or 1 2 3 …) (mandatory)
  - Date of entrance in the group (YYYYMMDD, mandatory)
  - Date of exit from the group (YYYYMMDD, optional if no change)

3. Treatments: TREATMENT table (example: TREATMENT_20201102) - MANDATORY

- This table described the successive experimental treatments in each group. Two possibilities to provide the information : per animal (if dates vary according to animals), or per group (if dates are defined by groups)
- 1 row per change; delimiter=';' ;
- Information
  - 'G' for groups, or 'A' for animals (mandatory)
  - Group ID or Animal ID (mandatory), according to first information
  - Type of treatment : S for stress, C for control (mandatory)
  - Date of start of the treatment (YYYYMMDD, mandatory)

4. Parity: PARITY table (example: PARITY_20201102) – MANDATORY for lactating cows

- 1 row per animal; delimiter=';' ;
- Information
  - Animal ID (mandatory)
  - Date de calving (YYYYMMDD, mandatory)
  - Parity number (mandatory)
  - Calving condition (1-5, optional)
  - Calf birth weight (kg, optional)
  - Sex of calf (M/F, optional)
  - Next drying-off date (YYYYMMDD, optional)
  - Estimated/Observed drying-off (E/O, optional)

5. **Weights: WEIGHT table (example: WEIGHT_20201102) - MANDATORY**

- 1 row per measure ; delimiter=';' ; all variables mandatory
- In case of suckling cows, calf weight can be provided here, and the relationship dam-calf is described in the ID table, with one row for the calf (with dam known) and one row for the dam.
- Information
    - Animal ID
    - Date de record (YYYYMMDD)
    - Body weight (kg)

6. **Feed intake: FEEDINTAKE table (example: FEEDINTAKE_20201102) – MANDATORY for the recorded periods**

- 1 row per measure; delimiter=';' ; all variables mandatory
- Information
    - Animal ID
    - Date de record (YYYYMMDD)
    - Daily feed intake (hg)
    - Feed diet Id
-

7. **Feed diet: FEEDDIET table (example: FEEDDIET_20201102) - MANDATORY**

- 1 row per diet ; delimiter=';' ; missing information=NULL
- Information
    - Feed diet Id (mandatory)
    - Dry matter (%, mandatory)
    - Energy (kJ/kg DM, mandatory)
    - Neutral detergent fiber (NDF, %, optional)
    - Acid detergent fiber (ADF, %, optional)
    - Crude Protein (CP, %, optional)
    - Water-soluble carbohydrates (%, optional)
    - Starch (%, optional)
    - Ether extract (%, optional)
    - Ash (%, optional)

8. **Body Condition Score: BCS table (example: BCS_20201102) - OPTIONAL**

- 1 row per record ; delimiter=';' ;
- Information
    - Animal ID (mandatory)
    - Date de record (YYYYMMDD, mandatory)

- BCS (1-5, mandatory)

9. Milk production: MILK table (example: MILK_20201102) – MANDATORY for lactating animals

- 1 row per test-day ; delimiter=';' ;
- Milk yield always present, milk composition when available
- Information
  - Animal ID (mandatory)
  - Date de record (YYYYMMDD, mandatory)
  - Number of Milkings per day (mandatory)
  - Milk yield per day (kg, mandatory)
  - Fat (g/kg, optional)
  - Protein (g/kg, optional)
  - SCC (x1000, optional)
  - Lactose (g/kg, optional)
  - BHB (optional)
  - Acetone (optional)
  - Urea (optional)

- Example:
  - FR0134579246;20170320;2;27.1;40.5;32.6;123;49.1;;;;
  - FR0134579246;20170321;2;25.9;;;;;;;

10. Water Intake: WATER table (example: WATER_20201102) – OPTIONAL

- 1 row per measure ; delimiter=';' ; all variables mandatory
- Information
  - Animal ID
  - Date de record (YYYYMMDD)
  - Water intake (hg)

11. Backfat: BACKFAT table (example: BACKFAT_20201102) – OPTIONAL

- 1 row per measure ; delimiter=';' ; all variables mandatory
- Information
  - Animal ID
  - Date de record (YYYYMMDD)
  - Backfat thickness (mm)

12. Carcass parameters at slaughtering: CARCASS table (example: CARCASS_20201102) – OPTIONAL

- 1 row per animal with information; delimiter=';' ;

- Information
    - Animal ID
    - Date de record (YYYYMMDD, mandatory)
    - Carcass weight (kg)
    - %meat (%)
    - %fat (%)
    - %bones (%)

13. Disease: DISEASE table (example: DISEASE_20201102) - OPTIONAL

- 1 row per event ; delimiter=';' ;
- Information
    - Animal ID (mandatory)
    - Date de record (YYYYMMDD, mandatory)
    - Disease code (1= mastitis, 2= ketosis, 3=metritis, 4= cystic ovaries, 5=retained placenta, 6= displaced abomasum, 7= milk fever, 8=acidosis, 9= respiratory problem, 10=diarrhea, 11= claw disease, 12=lameness, 13=other) - mandatory

14. Insemination: INS table (example: INS_20201102) – OPTIONAL

- For reproducing animals
- 1 row per animal ; delimiter=';' ;
- Information
    - Animal ID
    - Date de insemination (YYYYMMDD, mandatory)
    - Sexed semen (Y/N, optional)
    - Hormonal treatment (Y/N, optional)
    - Crossbred insemination (Y/N, optional)

15. Culling: CULLING table (example: CULLING_20201102) - OPTIONAL

- 1 row per culled animal; delimiter=';' ;
- Information
    - Animal ID (mandatory)
    - Date de culling (YYYYMMDD, mandatory)
    - Reason of culling (optional, codes in Reference Information section)

16. Indirect milk production: INDIRECT_MILK table (example: INDIRECT_MILK_20201102)

- 1 row per measure ; delimiter=';' ;
- Information

- Animal ID
- Date de record (YYYYMMDD, mandatory)
- Calf Id (mandatory)
- Milk yield (kg, mandatory)
- Milk composition (Y/N) – if yes, information present in MILK table

17. Blood metabolic parameters: METABOLIC table (example: METABOLIC_20201102) – OPTIONAL

- 1 row per measurement; delimiter=';' ;
- Information (all optional except Animal and Date.
  - Animal ID (mandatory)
  - Date de record (YYYYMMDD, mandatory)
  - Urea (mM)
  - NEFA (mM)
  - Glucose (mM)
  - BHB (mM)
  - Leptin (nM)
  - Na (mM)
  - Cl (mM)
  - Creatinin (mM)

18. Sensor data: SENSOR table (example: SENSOR_20201102) - OPTIONAL

- This table described the percentage of time in the different activities, per animal and day, as assessed by the accelerometer.
- 1 row per animal and day; delimiter=';' ;
- Information
  - Animal ID (mandatory)
  - Date of measurement (YYYYMMDD, mandatory)
  - % of standing position
  - % of intake time
  - % of rumination time
  - % of rest time
  - % of time dedicated to other activities
  - % of laying time
  - % of overactivity

Reference Information

List of partners: CITA, FIBL, INRAGABI, INRAUMRH, LFL, UDL

List of breeds and codes:

ANG=Angus and crossbreds,

CHA: Charolais,

HOL: Holstein,

JER=Jersey,

LIM=Limousin and crossbreds,

MON=Montbeliarde,

PAM=Parda Montaña

SIM: Simmental

Birth condition score: 1-5

Body condition score: 1-5

Codes of disease:

1= mastitis
2= ketosis
3=metritis
4= cystic ovaries
5=retained placenta
6= displaced abomasum
7= milk fever
8=acidosis
9= respiratory problem
10=diarrhea
11= claw disease
12=lameness
13=other

Codes of culling

1 – Sale for production

2 - Herd turnover (no specific reason but replacement by another cow)

3 – Low milk production

4 – Slaughtering for beef value

5 - Dead on the farm

6 – Reproduction

7 - Dystocia/obstetrics

8 - Mammary gland

9 – Locomotor

10 - Metabolic/digestive

11 - Respiratory

12 – Accident

13 - Infectious disease

14 - Other or Unknown

Dates: all with YYYYMMDD format

Weights in kg (with no decimal for animals, one decimal digit for feed, milk, water)

Milk composition:

Fat, protein, lactose in g/kg

SCC in thousand/ml

BHB, acetone in mMol/L

urea mg/dL

Blood Metabolites: Urea, NEFA, Glucose, BHB, Leptin, Na, Cl, Creatinin in mMol/L

Diet composition: Dry matter, NDF, ADF, CP, carbohydrates, ether extract, ash in %

Summary statistics of the multisite experiments carried out within GenTORE WP2.3 (table 7.1)

Experiments	CITA 2018	CITA 2019	FIBL	LfL	INRAE-GABI	INRAE-UMRH-D	INRAE-UMRH-B
Directory	CITA/exp7	CITA/exp8	FIBL/exp0	LFL/exp10	GABI/exp5	UMRH/exp5	UMRH/exp6
Breed(s)	Parda	Parda	Fleckvieh	Simmental	Charolaise	Holstein, Montbéliarde	Charolaise
Type of animals	Cows + Calves	Cows + Calves	Dairy Cows	Bulls	Cows + Calves	Dairy Cows	Cows + Calves
Duration of experiment	1 lactation	1 lactation	1 lactation	Growth	3 lactations	1 lactation	1 lactation
#, duration of challenges / lact	4 x 4 days	4 x 4 days	1-3 x 8 days	no	1 x ~3 months	4 x 4 days	2-5 x 4-10 days
# animals	16 cows, 16 calves	15 cows, 15 calves	98	72	340 cows, 592 calves	40	35
# weights	1472	645	534	1728	19188	5828	4074
# intake records	2752	4012	534	19472	13729	5939	9617
# milk records	656	301	534	-	-	6285	2423
# BCS	160	90	528	-	-	1143	622
# Carcass data	-	-	-	72	-	-	-
# Metabolic data	Yes	Yes	No	No	No	Yes	Yes
# Sensor data	Yes	Yes	Yes	No	Year 2019	Yes	Yes

Conclusion

Six experiments have been carried out in the framework of WP2.3. In addition, two of them were repeated two consecutive years with slightly different characteristics. All of them but one addressed the question of the resilience of beef or dairy cows, through the response to and recovery after a nutritional challenge. In beef cows, they also include the response of calves. According to a common protocol designed during the first year of the project, many measurements were made in a similar way, in order to facilitate the future meta-analyses. Accordingly, the experiments included feed intake measurements before or during the period of resilience study. Different nutritional challenges were defined, rather short in most experiements, but in the larger experiment they were long (3 months) and repeated over three lactations. In one case, the experiment was carried in 2 commercial farms. Accelerometers were used to determine if the information provided by these sensors could be used to predict resilience.

One of these experiments was very different. It involved growing bulls and aimed at estimating the body composition and energy content of bulls at different ages, and feed with different diets. Indeed, these values are very important to elaborate feeding strategies but, because of the high workload required to estimate them precisely through dissection, available data are very old and may not reflect the current situation after dozens of years of selection.

At the date when this report is written, all these experiments on animals are fully terminated, but the corresponding lab work is not yet completed in some cases. Indeed, because of Covid, the experimental work was stopped or strongly reduced during several months. Except in Le Pin where the long-term protocol had to be stopped 4 months earlier than expected, all other experiments were carried out with all measurements and samples. But the lab work has often been delayed. In all situations, the data analyses have to be postponed and most of them will be carried out in 2021. Only the first papers, corresponding to the earliest analyses, have been produced, many others are expected by the end of next year.

The preliminary results show a large variability of individual response to nutritional challenges. Plasticity seems to increase with age, it is higher in cows than in calves, and higher for older cows than for primiparous. Short term response is always quite strong, as shown by high AGNE levels. Recovery is easier after shorter challenges. Repeatability of response is rather high, showing a strong individual component in this trait. The relationship between efficiency and resilience will be studied in 2021 on these data.

All data are stored in a common repository with those collected in task2.1 and 2.2, on a standardized format. They will be a unique resource for task 2.4 which requires good datasets with both feed efficiency and resilience information. They are also available for WP4. Hypotheses derived from WP3 about sensor information will also be tested on these data.

Annex1. Common Experimental Protocol

This protocol was designed in 2017-18 by the different partners involved in task 2.3. It resulted in Milstone MS8, delivered at month 12.

GenTORE WP2 Task 3:

Multi-Site experiment to validate RFI and resilience measures

Common Protocol

version 101218

Objectives:

- ***To provide strong datasets in beef and dairy for testing the improved methodologies for estimating efficiency and resilience (RFI, etc) developed in WP2.1 and 2.2***
- ***To provide strong datasets in beef and dairy for testing the proxy measures for efficiency and resilience developed in WP3***

This document describes the core elements of the experiment protocol, and lists for each site the local variants, for:

- Experimental design overview
- Challenge description
- Measures of
    - Intake
    - Feed characterization
    - Milk Yield
    - Milk composition
    - Body weight and body condition score
    - Reproduction
    - Health

    - Activity
    - Temperature
    - Other automated measures
    - Other (non-automated) measures
- Data storage
- Feed formulation

Experimental Design Overview:

This experiment will measure efficiency and resilience components using both:

- well-established reference methods (intake, liveweight, body fatness, milk production, MIR, health and fertility indicators)
- proxy measures being developed in conjunction with WP3 (activity, temperature, automated body measures

Measures will be made over relatively long timescales (1 year/ 1 production phase) to provide data on the evolution of efficiency measures through time.

The experiments will also include a short-term nutritional challenge, which we have previously shown to be a useful method for characterizing between-animal variation in adaptive response (e.g. Bjerre-Harpoth et al 2012). These short-term environmental perturbations will also provide a strong test of the ability of the proxy measures to track this resilience component, including a detailed monitoring of reproduction parameters.

To ensure that the data from the different sites can be properly combined, all experimental sites will be characterized in terms of their local environment (collaboration with WP1) and all animals will be genotyped (collaboration with WP4).

Site	CITA	FiBL	INRA-Pin	INRA-Herb1	INRA-Herb2	LfL
Breed	Parda de Montaña	Brown Swiss or Fleckvieh	Charolais	Charolais	Holstein and Montbéliarde	Fleckvieh
Number	32 (16 x 2 periods)	100	200 cows (+140 with only 1st lact)	34 Primiparous	24 Hols + 24 Mont, Multiparous	72 males
Age	Adults (>4.5 yr)	24-72 mo	Adult (parity 1 – 3)	Adult	Adult	1.5 - 15 mo
Feeding type	Hay + concentrate	Pasture	TMR	Hay + low concentrate	TMR	TMR
Housing	Indoors	Alpine and subalpine	Indoors freestall	Indoors	Indoors	Indoors
Purpose (efficiency or resilience)	Resilience	Resilience	Resilience	Resilience	Resilience	Efficiency
Challenge type (energy, protein…)	Nutritional, mid lactation	Change in feeding regime	Nutritional, early lactation, during 3-4 months	Nutritional, at 6-8 weeks postpartum (+ Sequence of 3 nutr. challenges mid lactation)	Nutritional, at 6-8 weeks postpartum (+ Sequence of 3 nutr. challenges mid lactation)	No challenge
Challenge timepoints	1st m pp_100% 2nd m pp_50% 3rd m pp_100% Weaning 150 d	To adapt to the rotations in the commercial farms	Start 10 days after calving till grazing period (mid April)			
End	End of May 2019		Summer 2020	Summer 2019	Summer 2020	Finished
Key contact	icasasus@aragon.es	Anna.bieber@fibl.org	Rachel.lefebvre@inrae.fr	anne.de-la-torre-capitan@inrae.fr	jose.pires@inrae.fr	Thomas.Ettle@LfL.Bayern.de
Local protocol	GenTORE_t2.3_CITA		Procedure_ChallengeAlimentairePrimiparesG2	AQ830	AQ835	
Data format	*.xls	.csv files	.txt or .csv files			.xlsx.; ASCI
Other particularities						

Challenge Description:

Key characteristics of the challenge are: A pre-period of at least 7 days on a standard regime, a challenge period (4 days for a nutritional challenge), and a post-period of at least 14 days on the standard regime.

Throughout the pre-, challenge, and pots- periods measures should be made with a sufficiently high frequency to allow characterization of the amplitude of the response and the rate of recovery for individual animals, e.g. daily measures of performance, milk and blood composition in a nutritional challenge.

The challenge must be extreme enough to create significant changes in the key measures (e.g. diluting the ration with 60% straw). The site-specific details of the challenges (type of challenge, duration, frequency of measures, stage of growth/lactation, etc.) are:

- CITA: Experiment to determine components of efficiency and specially RESILIENCE in **Parda de Montaña** cows during the first 3 months of lactation (repeated over two calving seasons, around October and February). The cows will be fed according to their requirements during the first month, we will **challenge them with a strong undernutrition** (restricted diet to meet **100% or 50% requirements**) **in the second month** (medium term challenge, 1 mo) and analyze **recovery in the third month**. We will work with **individually fed** cows, **16 calf-dam pairs** per calving season (from around 60 Parda multiparous cows calving in October-November 2018, around 40 calving in February-March 2019).
  We cannot classify them according to previously recorded RFI, but we can compare adult cows (>4.5 yr) of two sizes (based on height at withers, to be measured next April, large vs. small), two potential milk yields (based on previous recordings and pedigree, high vs. low), or two different BCS at calving (fat vs. moderate). (still to decide, with the number of cows expected to calve in the proposed intervals, we can expect to have two very distinct groups of 8 cows of either category).
  Comments: we can extend the first phenotyping phase for more than a month, and change the duration and severity of the restriction. Depending on results obtained at the preliminary experiment to be conducted by INRA Herbipole-1 with beef cows (3 very short nutritional challenges in mid lactation).
  From now on, frequency of measures consider a 1-mo challenge, but if we decide on 3 short nutritional challenges we could change frequency.
- INRA-Pin: **200** lactating **Charolaise** cows from parity 1 to 3 (over 6 years, in 2 experimental farms). **Group-fed** (stuck in feedgates after distribution to insure ingestion for all cows). **Indoor nutritional challenge** (6 vs 11 FU, with a diluted ration + restricted quantity for the low group and adlib ration for the high group), starting 10 days after calving, **lasting 3-4 months** (until grazing at mid-April) and with a **recovery period of 12 weeks at grazing**.
- INRA-Herb: Nutritional challenges yet to be defined (short term expe in course to optimize challenge) → results to discuss with CITA and annex to add to this document
- LfL: At least **72** Male **Fleckvieh** calves are purchased at a commercial market at an age of about **6 weeks** (~80kg BW). Calves are reared at an experimental farm of LfL using a standard total mixed ration (TMR) based on hay, concentrates, and molasses over a **period of 6 wk** (end BW ~120 kg). During this phase, animals additionally will be fed milk replacer (~ 25 kg / animal / 6wk). After **weaning** (challenge), bulls remain at this exp. station for further **8 wk**

(standard TMR, based on maize silage, concentrates, and hay). Animals are **group-fed** throughout this 14-wk period (housed in straw-bedded boxes, **no** individual feed intake). After this 14-wk period, animals are transported to the LfL exp. station Grub, and therefore to a **new environment**. Here the bulls are fed TMR based on maize silage, concentrates, and straw calculated to have 11.2 (group 1) or 12.0 (group 2) MJ ME/kg DM. After an adaptation period of about 2 wk, **daily individual feed intake** is available.
Groups of bulls are slaughtered at different BW (120 (n=8), 200 (n=10), 400 (n= 2x9), 600 (n= 2x9), and 750-780 (n= 2x9) kg BW, respectively). The bodies of the bulls are dissected to fractions of meat, fat and tendons & bones, totally homogenized (1 half of carcass), and analyzed for fat, protein, ash, water, and minerals.

Intake measures:

Daily dry matter intakes (amount offered – amount refused) of all feed components should be recorded for individual animals on at least 4 days per week, ideally throughout the experiment.
For indoors and adlib feeding, amounts refused should always be greater than 5% of amount offered.
Weekly DM values are needed to convert to DMI.
The site-specific details of intake measurements are:

- CITA: diet based on meadow hay (auto-weighing stall) + concentrate (ALPRO system). **Hay intake recorded 4 days per week** during 3 mo post partum, **concentrate recorded daily**. Since hay will not be fed ad libitum, no refusals are expected, but if existing will be weighed.
- INRA-Pin: daily **total offered** amount **per group** (no individual intake measures); since 2017, **grass height** before and after 3 days of grazing.
- INRA-Herb: **individual** intake measured at least 4d/week; and DMI measured before, during and after nutritional challenges. Offered feed and refusals will be weighed and analyzed for DM content to calculate DMI 4 days per week, daily before and during challenges and during recovery periods.
- LfL: For the rearing period (80-200 kg BW), daily **DM intake per group** is available. Intake of feedstuffs is calculated from total intake and composition of the TMR.
  From 220 kg BW until end of the experiment, DM intake is available daily for **individuals** (automatically controlled feeding troughs). Intake of feedstuffs is calculated from total intake and composition of the TMR.

Feed formulation:

Description of composition of the ration

- CITA: meadow hay + concentrate. Diet to be determined after analysis of available feedstuffs. The restriction will be applied reducing concentrate or concentrate+forage in a similar proportion (to be decided, depending on fill value of the hay).
- INRA-Pin: TMR composed of grass silage, hay, straw, rapeseed cake, mineral supplements (+ concentrate and pulp for higher ration)
- INRA-Herb: Charolais: hay and low concentrate offered separately.

Dairy cows: lactation TMR composed of preserved corn and grass silage, concentrate and mineral supplements.

- LfL: 80-120 kg BW: TMR based on hay, concentrates and molasses + milk replacer.
  120-200 kg BW: TMR based on maize silage, concentrates and hay
  220-end of trial: TMR based on maize silage and concentrates (high energy group 40:60 % of DM, low energy group: 80:20 % of DM)

Feed characterisation:

All roughages should be sampled at least once per month. All concentrate feeds should be sampled at least once per 3 months (or per batch if more frequent). In the case of total mixed rations (TMR), it is not necessary to sample the TMR components separately if representative samples of TMR are taken. Proximate analyses for DM, NDF, ADF, CP, water-soluble carbohydrates, ether extract and ash are required.

- CITA: Weekly samples of forage and concentrates, pooled monthly and analyzed for nutrient composition (DM, NDF, ADF, CP, water-soluble carbohydrates, ether extract and ash)
- INRA-Pin: each components of the TMR is analyzed separately: 3 (hay) or 1 (dry components) /week, once at collection for silage
- INRA-Herb: Feed and/or TMR samples will be collected **weekly**, pooled and analyzed for nutrient composition. Diet energy and Protein truly Digestible in the small Intestine (PDI), energy balance will be estimated (Inra, 2017)
- LfL: Maize silage: monthly pooled samples. Hay: 2 samples/rearing period. Straw: 2 samples/fattening period. Compound feed (concentrates): monthly pooled samples.
  DM, NDF, ADF, starch, sugar, EE, ash available.

Milk Yield:

For dairy breeds: Daily yield (frequency, time or interval between milking, type of parlor/AMS)
For beef breeds: Method to estimate milking performance as calf growth: birth, weaning and intermediate weights, weight before/after suckling …

- CITA: calf weighed weekly through the experiment (+ weaning). Milk yield estimated by weigh-suckle-weigh every two weeks.
- INRA-Pin: milk yield estimated by calf weight before and after suckling, 2 times in 24 hours (2 times during challenge, and once at 1 month of grazing). Calf growth estimated through calf weight (1 before challenge and monthly during challenge and recovery period)
- INRA-Herb: Charolais: Weight- Suckle –Weight method, once a week during regular lactation, twice during each period (4 d) of feed restriction.
  Dairy: Yield recorded twice a day
- LfL: not applicable

Milk Composition:

Component: fat, protein percentage (or yield), SCC, urea, lactose, other (FA, Protein, BHB, acetone …)
Sampling method, frequency, time
Analysis method

- CITA: We could add machine milking for milk composition (1/wk) → relevant in case of a long challenge
- INRA-Pin : None
- INRA-Herb: Charolais: None
  Dairy: Composition of 4 milkings per week. Daily before, during and after each restriction period (exact no. of days yet to define)
- LfL: not applicable

Body Weight (BW) and Body condition score (BCS):

BW (as frequently as possible): type of platform, frequency, timing, type of data (individual measures, 24h mean …)
BCS (1/week is ideal): type of measure (sensor or visual), measurement unit, frequency, timing
Fatness ultrasound: part of the animal, measurement unit, frequency, timing

- CITA: cows weighed **once weekly** on a walk-on scale (+ weaning).
  BCS (0–5 scale; Lowman et al. 1976) assessed **monthly**.
  Subcutaneous fat thickness **monthly** by ultrasound at P8 rump site and at the 13th thoracic vertebra.
  Measures taken at 0800 before feeding.
- INRA-Pin : BW: individual measures in kg, at calving, once before challenge and **monthly** during challenge and recovery period, at the beginning of the afternoon
  BCS: individual measures, visual method (0 to 5 point scale), at calving, once before challenge and **monthly** during challenge and recovery period, at the beginning of the afternoon
  Fatness ultrasound: rump, 12th rib and 4th lumbar vertebra (in mm); **3 times**: before challenge, end of challenge/beginning of grazing, end of recovery period (12 weeks), in the morning
- INRA-Herb: Charolais: Body weight and body condition score (BCS, 6-point scale, 0 to 5 scale; Bazin et al, 1984) recorded **weekly**, one day before restriction and on the last day of restriction, or at equivalent timepoints for control. BW will be recorded in 2 consecutive days during the first week of lactation. Ultrasound backfat thickness, at least one before and after each restriction period.
  Dairy Cows: BW will be recorded automatically **at every milking**; BCS **weekly**.
- LfL: During the rearing period, animals are weighed once **every 2 wk** (platform: data scales and Baumann), at **weaning** and **2 wks later**. From 220-780 kg BW, animals are weighed once **every 4 wk** (Texas trading. platform: Iconix).
  Back fat thickness is measured using an ultrasound device 1 day before slaughter (with a high reliability at lower BW) at the connecting line from *tuber coxae* to *tuber ischiadicum*. Moreover, for animals with bodyweight greater than about 400kg BFT is measured every 12 weeks. Bioelectrical impedance analysis (BIA) is conducted before slaughtering.
  No BCS

NB: Presently, all bulls have weights of > 350 kg (thus, they are weaned), about 30 bulls are already slaughtered.

Reproductive measures:

Reproductive period: season, duration
Estrus detection: frequency, type of detection (sensor, visual, bull …)
Mating: natural/insemination, AI rules
Cyclicity/pregnancy detection: method (progesterone, ultrasound …), frequency, blood/milk, period (in animal life)
Use of synchronization methods: type, timing

- CITA: Cows will be synchronized to be inseminated at the end of the restriction period (around day 60). Until the start of the synchronization protocol, blood collected twice a week for analysis of Progesterone (pp anestrous length). Additionally, cows equipped with ALPRO collars for estrous detection.
- INRA-Pin: Apr-July, no estrus detection, natural mating with visual detection, blood progesterone assay for post-partum cyclicity resumption (every 10 days, from 4 weeks post-partum until a positive result), gestation confirmation with ultrasound after 40 DCC
- INRA-Herb: Not budgeted initially
- LfL: not applicable

Health measures:

Health disorders: See the proposal table at the end of the document, with/without gravity indicator, diagnosis method
Health indicators: type (Leptin, NEFA…), frequency, period (in animal life)

- CITA: blood collected once a week during 3 mo pp to determine β-hydroxybutyrate, glucose, cholesterol, NEFA, urea, IGF-I. Health disorders are not expected (eg. cows needing a caesarea will not be used for the experiment) but will be recorded according to the table if existing.
- INRA-Pin: Leptin and NEFA dosage (at calving, 1 before challenge and monthly during challenge and recovery period), some health events are recorded.
- INRA-Herb: Clinical health events are recorded (table to share with partners).
  Charolais: caudal vein puncture before morning feeding, once a week during regular lactation, before challenge, daily during feed restriction and recovery (x times)
  Dairy: Jugular/caudal (yet do define) before morning feeding, once a week during regular lactation, before challenge, daily during feed restriction and recovery (x times)
  Plasma indicators (dairy and beef): NEFA, Glucose, Urea, B-OH, insulin, IGF-1, leptin (in selected samples). Eventually, indicators of inflammation.
  Milk indicators (dairy cows): Fatty acid composition, minor metabolites, eventually transcriptomics and proteomics (in selected samples)
  Other: yet to be decided

- LfL: Health events and injuries are recorded in categories respiratory disease, claw disease, accidental, digestive diseases. No gravity or severity indicator, visual diagnosis.

Activity measures:

In the purpose of evaluating proxies for intake and resilience, ear or neck mounted accelerometers will be used. Their outputs should allow a minima daily time-budgets of eating time, ruminating and daily activity levels. Lying/standing/walking are also desirable.
The site-specific details (accelerometer make, position of accelerometer, measurement frequency, and outputs, interest for Medria sensors, etc) are:

- CITA: none; If available, we would try Medria sensors (16 needed for October) and boluses but we did not consider them in the budget → contact Medria
- INRA-Pin: none; interest in sensors if useful for the end of the experiment
- INRA-Herb: Charolais: possibly on 8 cows measured by Rumiwatch (daily time-budgets of eating, ruminating and physical activities (lying, standing, walking))
- LfL: As an indicator of activity, visits/recordings of access to the weighing troughs can be analyzed (220-780 kg BW).
  About 30 animals are already slaughtered. So it seems not to make sense to measure activity for the other animals.

Temperature measures:

In the purpose of evaluating proxies for intake and resilience, rumen temperature boluses will be used. Their outputs should allow a minima daily values of average temperature, daily range, and number of drinking events. The site-specific details (make, measurement frequency, and outputs, etc) are:

- CITA: interested in 32 boluses → contact Medria
- INRA-Pin: none
- INRA-Herb: interested in 48 boluses for dairy cows → contact Medria
- LfL: none

Other monitoring technologies:

All other automated measures, e.g. in-line milk measures, should be described, including measurement frequency and panel of measures made.

- CITA: none
- INRA-Pin: none
- INRA-Herb: none
- LfL: none

Other measures:

All other measures (non-automated and not specific to the challenge protocol): metabolic profiles from blood samples, genotyping … should be described, including measurement frequency and panel of measures made.

- CITA: See description at “Health measures” section; samples available for genotyping if relevant
- INRA-Pin: genotyping (50k Illumina BeadChip, Labogena)
- INRA-Herb: See description at “Health measures” section; genotyping (EuroG10k, Illumina BeadChip, Labogena)
- LfL: Slaughter criteria (carcass grading, dressing percentage), carcass dissection, chemical body composition; genotypes available as 43k custom SNP-Panel of Fleckvieh (based on SNPs of Bovine SNP50 BeadChip from Illumina)

Data Storage:

Information of data file: type of files, ideal and available resolution level for each measure

- CITA: database of the experimental farm (*.dbf) and ad hoc files (*.xls).
- INRA-Pin: INRA bovine database (manual or automatic recording), txt or csv files, genotypes in phase format (txt file), raw data available for all measures
- INRA-Herb: Unit database for animal production data. PI and UMRH for all data.
- LfL: xlsx file including daily DM and nutrient intake and other measures (BW, BFT …) in the frequency given above can be provided. Information on slaughter criteria and body composition can be provided as a separate file (.xlsx). Data are stored at an Lfl own server. Genotypes could preferably be delivered in Interbull 705 exchange format.

Proposal for health event recording

Health event family	disorder	code	description
A - Reproductive events	Retained placenta	1	benign : no hyperthermia, no appetite loss, no production loss
		2	general state affected: hyperthermia, appetite loss, production loss
	Metritis	3	flux before 21 days post-partum, without other sign
		4	flux after 21 days post-partum, without other sign
		5	putrid flux, general state affected
	Ovarian disorder	6	Cystic disorder

		7	anestrus
		8	irregular ovarian activity
	Hormonal treatment	9	prostaglandin, synchronization…
	Surgery	10	cesarean, episiotomy..
	Other	11	cystitis, urinary infection, prolapsus,…
B – Locomotion disorders	Lameness	1	trimming without lameness
		2	supporting leg lameness
		3	swinging leg lameness
	Claw disease	4	corkscrew claw
		5	dermatitis
		6	double sole
		7	heel horn erosion
		8	horn fissure
		9	interdigital hyperplasia
		10	sole hemorrhage
		11	ulcer
		12	white line disease
		13	other
	Accident	15	quartering, luxation, fracture…
C - Udder disorders	Mastitis	1	lump, general state not affected
		2	hot, painful quarter, general state not affected
		3	general state affected: hyperthermia, appetite loss, production loss
	Edema	4	accumulation of serous fluid in the udder tissue
	Injury	5	
	Other	6	atrophy, eczema, …
D - Metabolic disorders	weight loss, appetite loss, production loss, general state degradation	1	ketosis
		2	acidosis
		3	displaced abomasum
		4	diarrhea enteritis, without fever

		5	diarrhea enteritis, with fever
		6	occlusion
		7	no identified reason, no hyperthermia
		8	no identified reason, with hyperthermia
		9	foreign body
	Milk fever	10	preventive treatment
		11	suspicion of milk fever
		12	lying cow, standing after treatment
		13	lying cow, no standing after treatment
	Other	14	poisoning, salmonellosis …
E - Parasitism			coccidiosis, ascaridiosis, cryptosporidiosis, trematode, ringworm …
F - Respiratory troubles			Rhinitis, Sinusitis, Laryngitis, pneumonia, …
G - Genetic defects			malformation, arthrogryposis, BLAD …
H - Regulated diseases			BSE, TB, …
I - Other			behavior, …
J – Calf disorders			Breath frequency, Breath intensity, Breath noise, Heart frequency, Vitality, Posture, Body condition, Nose flow, cough, Bulbus position, Episcleral vessels, Ears, Umbilicus, Joints, Inner Temperature, Faeces consistency, Faeces colour, Feaces impurity

Annex2. Draft about FIBL experiment

Manuscript draft foreseen for publication. Confidential and preliminary

The influence of days in milk and feeding on foraging behavior of dairy cows and the ability to predict efficiency of dairy cows based on sensor data in roughage-based feeding systems

Anna Bieber, Florian Moser, Anet Spengler-Neff, Florian Leiber

Abstract

To be developed

Introduction

Over the last 50 years milk yield per dairy cow has more than doubled due to improvements in animal husbandry and breeding. However, at animal level this achievements were accompanied by impaired robustness (e.g. Pritchard et al. 2013, Oltenacu and Broom, 2010, Knaus 2009, Rauw et al., 1998). Moreover, high production levels in dairy cattle were achieved by feeding higher shares of cereals from arable land to livestock, therewith inflicting feed versus food competition. There is a growing awareness that, globally, it may be more efficient to consume cereals directly by humans than to consume them in the form of animal protein produced from cereals (van Zanten et al., 2016). Simulations studies at global level show that environment would highly benefit from feeding livestock with grassland and by-products from food production instead of food-competing feed (Schader et al., 2015). Reducing food-competing feedstuffs would lead to a decrease of monogastric husbandry systems in favour of ruminants, but would also require fundamental changes in nutritional patterns through reduction of protein from animal origin in human diets to be a viable option (Schader et al., 2015).

For dairy systems this means that we have to identify animals that are efficient and stay healthy and fertile in grassland based feeding systems, as a contribution to more sustainable food supply.

In recent years, sensors to measure eating and rumination behaviour in cattle have been developed and validated (e.g. Pereira et al., 2018, Rombach et al., 2018, Zehner et al.,2017, Büchel and Sundrum, 2014, Oudshoorn et al., 2013). However, little research has been done in cows fed on pure roughage based rations (Leiber et al., 2015).and on how changing roughage supply in terms of varying quality and quantity might be linked to the efficiency of cows.

Therefore, this study aims at a) investigating how pure roughage feeding and lactation status influence parameters describing foraging behaviour traits in dairy cows obtained by a sensor system, and b) to study the relationship between foraging behaviour and efficiency of concentrate free fed dairy cows.

Materials and Methods

Data collection

We conducted on-farm trials with Swiss Fleckvieh cattle herds on two Swiss organic dairy farms. Each farm herd was visited eight times between August 2017 and April 2019, to investigate foraging behavior in association with feeding and lactation status, and to assess how foraging parameters contribute to describe the cow`s efficiency. The experimental setup of the different runs is shown in Table 1. We equipped up to 45 cows of each herd with RumiWatch® (RW) holsters for three days per run, on eight runs in total. RumiWatch holster are noseband pressure sensors for measurement of eating and ruminating behavior developed by Itin and Hoch GmbH (Liestal, Switzerland; Zehner et al. 2017). After the cows were holstered, they followed their daily routine on the farm. We excluded cows that had reliable data for less than two complete days from further analyses (for details see the section on data validation). We collected samples of the actual feeding, e.g. hay, grass silage, cut grass, lucerne, pasture, for each run. Samples were collected on pastures by cutting five plots of 25 cm^2 on a homogenous sward, they were mixed and analysed as one sample. From hay, silage or cut grass we collected five samples filling a 1 liter-bag from different places of the piles in the barn. They were also mixed and analysed as one sample. Dry matter content was assessed based on the principle of water evaporation out of raw feed. We therefore dried the samples for 24h at 60°C, measuring the sample weight prior and after drying. The dry matter content is expressed in percentage.

Dried samples were milled (Cutting Mill SM100, Retsch GmbH, 42781 Haan, Germany) and analysed with the infrared spectroscopy method (NIRFlex N-500 equipped with a NIRflex Solids measuring cell, Büchi Labortechnik AG, 9230 Flawil, Switzerland) for energy content (MJ NEL), crude protein (CP), ash and crude fibre. Absorbable protein at the duodenum (APD) was calculated.

Additionally, milk samples taken of the total morning/evening milking on day 2 or day 3 of each run were analyzed for milk content through infrared spectroscopy (Milko-Scan-FT ™, FOSS Hilleroed, Denmark) by Suisslab (Zollikofen, Switzerland). After the trials, we removed the holsters and weighted each cowand scored her body condition using the method described by Spengler Neff et al. (2015).

We used TinyTag loggers located on a pasture close to the barn and close the places the cows spent their days, respectively to measure relative humidity and temperature in °C.

Data validation

RumiWatch data

We converted raw data recorded by the RumiWatch holsters using the RumiWatch Converter V.0.7.3.36 to gain average values for 1-hour time intervals. If a cow had spent less than 40 minutes of two consecutive hours with either ruminating or eating, the data of the corresponding 24 hours (defined as time slot from 2pm to 2pm) was excluded for this cow. If we had to exclude more than 24h of data from a cow, we excluded that cow for the current run. We used the variables rumination time (min/d), eating time (min/d) and number of activity changes (n/d), i.e. number of changes between the activities rumination, eating and idling, as descriptors of foraging behaviour, which we validated to the following threshold values 240-795, 220-720 and 55-380, respectively. The two parameters ruminate chews per minute (average of 24h) and ruminate chews per bolus (average of 24h) were highly correlated with ruminate time (min/d) and therefore excluded from analysis (Supplementary Figure S1). A descriptive summary of foraging behaviour parameters and cow characteristics by farm in the validated data set is shown in Table 2.

Feed quality

Because many of the nutritional factors analyzed in the feed were highly correlated, and to reduce the dimensions of the data, we performed a Principal Component Analysis (PCA) using the "prcomp" function in R (v 3.6.2, R Core Team 2020). To assess which components should be included in subsequent analysis we used the broken-stick method, "bstick", implemented in the "vegan" package (v 2.5-6, Dixon 2003).

Definition of efficiency parameters

We calculated the energy corrected milk yield (ECM in kg/d) with a standard of 4 % milk fat, 3.2 % milk protein and 4.8 % milk lactose applying formula [1] from Agroscope (2017). We estimated dry matter intake (DMI) in kg per day using formula [2] proposed by DeSouza et al. (2019). We had to discard the attempt to apply the formula proposed by Rombach et al. (2019) which uses RumiWatch parameters to estimate DMI to our data, as we partly obtained unrealistically low DMI estimates. We calculated the energy corrected average daily milk yield of the total lactation (DMY_TL) for the respective lactation of each cow.

Finally, we calculated two different efficiency parameters, the metabolic milk production efficiency (MPE) calculated as MPE = DMY_TL / (body weight$^{0.75}$), and Efficiency = DMY_TL / DMI$_{DeSouza}$.

$$\mathrm{ECM}\ [\mathrm{kg}] = \frac{(\mathrm{MilkFat}\ [\%] * 0.38 + \mathrm{MilkProtein}\ [\%] * 0.24 + \mathrm{MilkLactose}\ [\%] * 0.17) * \mathrm{DMY}\ (\mathrm{kg})}{3.14} \qquad [1]$$

$$\begin{aligned}\mathrm{DMI}_{DeSouza} = {} & ((3.7 + \mathrm{Parity} * 5.7) + 0.305 \\ & * (0.0929 * \mathrm{MilkFat}\ [\%] * \mathrm{DMY}\ [\mathrm{kg}] + 0.0563 * \mathrm{MilkProtein}\ [\%] * \mathrm{DMY}\ [\mathrm{kg}] \\ & + 0.0395 * \mathrm{MilkLactose} * \mathrm{DMY}\ [\mathrm{kg}]) + 0.022 * \mathrm{Body\ weight}\ [\mathrm{kg}] + (-0.689 \\ & + \mathrm{Parity} * (-1.87)) * \mathrm{BCS}) * (1 - (0.212 + \mathrm{Parity} * 0.136) * \mathrm{e}^{-0.053 * DIM}))\end{aligned} \qquad [2]$$

where DMY = daily milk yield in kg, MilkFat= milk fat content in %, MilkProtein= milk protein content in %, MilkLactose = milk lactose content in %, Parity = a two-level categorical variable indicating whether a cow is primiparous (0) or multiparous (1), BCS = body condition score of the cow on a scale of 1 to 5 with 0.25 intervals, and DIM = days in milk. Data was limited to cows with a lactation status of maximal 305 days in milk.

Statistical analysis

All statistical analysis were performed in R (v 3.6.2, R Core Team 2020). We checked the closeness between continuous variables by calculating Pearson correlation coefficients (Figure S1). We conducted multiple pairwise comparisons as Tukey contrasts using the glht function in the “multcomp” package (Hothorn et al., 2008) to test mean differences in chemical composition (Table 3), as well as mean differences in foraging behaviour traits, ECM, body weight, BCS and efficiency parameters (Figure 3) between the feeding levels “hay”, “mix” and “pasture”, respectively.

We investigated the effect of days in milk (DIM) and feed quality scores on foraging parameters, ECM, body weight, and BCS applying linear mixed effect models in the “lme4” package in R (v 1.1-21, Bates et al., 2015). DIM, feed quality score and lactation class (levels: 1^{st}, 2^{nd} or 3^{rd} lactation onwards) were fixed and cow nested within farms were random effects. To assess the variance explained by the models we extracted pseudo marginal and conditional R squared estimates with the “MuMIn” package (v 1.43.15, Barton, 2019).

Since the explaining variables had a close non-linear relationship with DIM, we modeled the relationship between DIM and our variables as smooth splines with a smoothing parameter (spar) of 0.95 and subtracted this smoothed estimate from each individuals’ respective value. Smooth spline fits were generated for each farm separately. To regain meaningful estimates we thereafter added the mean variable values calculated over both farms to obtain DIM-corrected values for ruminate time, eating time, number of activity changes, ECM, body weight, and BCS, labeled with “corrected”.

We also corrected the behavioral traits for the effects of forage quality scores. Finally, we applied mixed models in “lme4” with efficiency traits as dependent variables and corrected behavioral traits and lactation class (levels: 1^{st}, 2^{nd} or 3^{rd} lactation onwards) as fixed effects and cow nested within farm as random effect.

Statistical significance was determined at $P < 0.05$, with tendency at $P > 0.05$ and < 0.1 in all analyses.

Results

Influence of days in milk

All of the variables of interest for this study, significantly changed with the days in milk (Figure 2, Table 4). While rumination time showed a smoothed U-shape where the early and the late days in milk showed the highest rumination time, eating time had an inverse U shape, with peak eating times in mid lactation (Figure 2, A). The variation of activity changes across DIM was small but statistically significant.

As shown in Figure 2 (B), body weight and BCS decreased in early lactation, but started to increase around 200 DIM. While dry matter intake (DMI) after DeSouza et al. (2019) showed an increase at the beginning of the lactation, which leveled out after approximately 50 DIM, this relationship being accounted for in formula 2, ECM clearly showed a negative relationship with DIM as also reflected in the negative correlation coefficients (Figure S1). We therefore used DIM corrected values hereafter.

Feed quality

The PCA on our feed analyses shown in Figure1 revealed, that most of the variation (88.4 %) was explained by one single component (PC1, Eigenvalue =3.53). PC1 was composed of dry matter content (%), fiber content (g/kg DM), net energy used for lactation (NEL in MJ/kg DM) and protein content (g/kg DM), with a contribution to PC1 of 25.7, 25.5, 24.6, and 24.2, respectively. The broken stick approach revealed that PC1 was the only component explaining more of the variation than expected by chance.

Based on this PCA, we created two variables to describe feeding. First, we derived the feed quality scores from PC1, where a positive feed quality score reflects fibre and dry matter rich feeding, whereas negative feed quality scores indicate protein and energy rich feeding (Figure 1). Second, we identified three main types of feeding (Table1): a fibre rich hay-based diet (“hay”), a protein- and energy-rich feeding, associated with pasture-only based diet (“pasture), and an intermediate feeding characterized by pasture-based diet with additional feed of hay, grass or silage in the barn (“mix”).

Results of feed analysis show that the three feeding levels differ significantly regarding their chemical properties (Table 3).

Impact of feed quality score and feeding on foraging behavior and efficiency parameters

Rumination and eating time differed significantly by the feed quality score and by feeding, while number of activity changes did not (Table 4, Figure 3 A-F). Rumination time increased with the amount of fibre in feed (positive feed quality score values), while eating time showed the inverse pattern, namely an increase in eating time with more protein rich feed (negative feed quality score values).
The feed quality score had a significant influence on mean ECM (Table 4), the latter tended to increase with more protein rich feed (Figure 3G), and was higher in mix and pasture feeding than hay (Figure 3J). By contrast, mean body weight slightly increased with more fibre rich feed rations (Figure 3 H) and was highest in hay fed cows (Figure 3 K). Although the feed quality score showed a small but statistically significant influence on the BCS (Table 4), the mean BCS values did not differ depending on the feeding (Figure 3L).
Mean MPE_TL was lower in hay compared to mix and pasture, but efficiency did not differ significantly between feeding (Figures 3 M-P).

Impact of foraging behavior traits and lactation class on efficiency parameters

The three behavioral traits ruminate time, eating time and number of activity changes, all corrected for days in milk, feed quality score and lactation class, had no significant impact on the variation of the efficiency parameters, efficiency and MPE_LT. By contrast, the lactation class showed higher efficiency values for animals of 2nd lactation and 3rd lactation onwards in both traits. The marginal R squared values representing the variation explained by the fixed factors were 0.095 and 0.229, for the efficiency and MPE model, respectively, and the conditional R squared values 0.604 and 0.790, representing the variation explained by the whole model (Table 5).

Discussion

To be developed.

Tables

Table 1. Experimental setup showing the eight runs per farm, starting date, feeding, Temperature Humidity Index (THI) and number of cows involved

Run	Starting date	Feeding	THI	N_{cows}
Farm1_a	29.08.2017	Pasture	63.8	39
Farm1_b	03.09.2017	Pasture	62.1	39
Farm1_c	06.04.2018	Pasture + grass silage	54.6	34
Farm1_d	11.04.2018	Pasture	56.7	34
Farm1_e	23.08.2018	Pasture + grass	65.0	39
Farm1_f	28.08.2018	Pasture + lucerne	59.2	39
Farm1_g	10.10.2018	Pasture	NA	35
Farm1_h	15.10.2018	Pasture + hay	NA	35
Farm2_a	05.11.2017	Pasture + hay	43.4	42
Farm2_b	10.11.2017	Pasture +hay	40.9	42
Farm2_c	21.03.2018	Hay	46.3	27
Farm2_d	26.03.2018	Pasture + hay	42.8	27
Farm2_e	16.11.2018	Pasture + hay	35.2	30
Farm2_f	21.11.2018	Hay	39.0	30
Farm2_g	16.04.2019	Pasture + hay	59.0	21
Farm2_h	21.04.2019	Pasture	56.1	21

THI= Temperature humidity index

Table 2. Descriptive summary of foraging behavior parameters and cow characteristics by farm

	Farm 1 (n=294)			Farm 2 (n= 240)		
Variable	Mean ± SD	Median	Min - Max	Mean ± SD	Median	Min - Max
Eating time (min/d)	609.9 ± 88.8	628.6	341.3 – 768.3	538.3 ± 71.7	542.3	325.6 – 714.2
Ruminate time (min/d)	413.1 ± 79.4	399.5	240.5 – 606.7	490.4 ± 71.9	486.9	324.5 – 666.5
Ruminate chews per minute (avg. of 24h)	43.2 ± 6.8	42.5	26.6 – 67.5	50.3 ± 7.0	49.8	31.2 – 71.4
Ruminate chews per bolus (avg. of 24h)	34.5 ± 6.2	34.2	18.7 – 55.9	43.7 ± 6.8	43.8	28.3 – 60.7
Activity changes (n/d)	121.9 ± 20.0	120.0	77.3 – 183.0	133.1 ± 26.5	128.7	79.3 – 220.3
Body weight (kg)	581.8 ± 61.1	583	456 – 787	648.4 ± 75.4	658	441 – 810
Body condition score	2.90 ± 0.29	2.75	2.25 – 3.75	3.21 ± 0.34	3.25	2.50 – 4.00
ECM (kg/d)	16.6 ± 5.7	15.1	6.7 – 37.0	21.3 ± 5.7	21.4	5.2 – 33.0
Milk protein content (%)	3.37 ± 0.30	3.34	2.76 – 5.12	3.70 ± 0.50	3.72	2.77 – 5.40
Milk fat content (%)	4.34 ± 0.59	4.29	2.96 – 6.35	4.44 ± 0.79	4.49	1.88 – 6.98
Milk lactose content (%)	4.73 ± 0.15	4.73	4.27 – 5.48	4.67 ± 0.17	4.69	3.78 – 5.13
Days in milk	182.5 ± 76.1	210.5	3 – 300	148.7 ± 108.6	161	3 – 308
Lactation number	3.5 ± 2.8	2	1 – 12	3.9 ± 2.4	3.5	1 – 10
DMY_LT (kg/d)	19.6 ± 3.2	19.7	12.6 – 26.3	20.8 ± 2.9	20.9	14.4 – 27.7

ECM= energy corrected milk yield, DMY_LT= average daily milk yield of total lactation, calculated from energy corrected milk yield of total lactation divided by days in milk of total lactation

Table 3. Results of feed analysis in hay, mixed ratios and on pasture regarding dry matter content (%), crude protein content (g/kg DM), ash content (g/ kg DM), crude fibre content (g/ kg DM), energy content (MJ/kg DM) and absorbable protein at the duodenum (APD, (g/ kg DM)).

Feeding		DM (%)	CP (g/kg DM)	Ash (g/kg DM)	CF (g/kg DM)	NEL (MJ/kg DM)	APD (g/ kg DM)
Hay	Mean ± SD	$89.7^{a} \pm 1.0$	$124.8^{c} \pm 7.9$	$91.8^{c} \pm 3.5$	$279.8^{a} \pm 2.7$	$5.2^{c} \pm 0.0$	$79.2^{c} \pm 5.5$
(n=57)	Min - Max	88.8 – 90.7	117.4 – 133.0	88.5 – 95.4	277.1 – 282.2	5.2 – 5.2	74.0 – 85.0
Mix	Mean ± SD	$32.4^{b} \pm 17.7$	$178.0^{b} \pm 19.6$	$110.4^{b} \pm 24.8$	$236.6^{b} \pm 18.0$	$5.8^{b} \pm 0.2$	$100.2^{b} \pm 5.1$
(n=274)	Min - Max	11.9 – 67.3	149.0 – 204.8	72.2 – 141.8	206.9 – 263.8	5.5 – 6.2	92.5 – 107.8
Pasture	Mean ± SD	$15.4^{c} \pm 3.4$	$207.5^{a} \pm 15.8$	$113.6^{a} \pm 10.2$	$215.0^{c} \pm 11.9$	$6.2^{a} \pm 0.2$	$108.9^{a} \pm 2.8$
(n=203)	Min - Max	9.3 – 20.4	181.0 – 228.3	96.1 – 130.8	195.3 – 226.6	5.8 – 6.5	104.0 – 111.8

DM= dry matter, CP= crude protein, CF= crude fibre, NEL= Net Energy Lactation, APD= Absorbable protein at the duodenum. Different superscript letters within column represent significant differences at P< 0.05 between feeding levels based on mean comparisons by Tukey contrasts.

Table 4. Influence of days in milk (DIM) and the feed quality score on foraging behavior traits, daily energy corrected milk yield (ECM), body weight, and Body Condition Score (BCS) in Swiss organic dairy cows ($N_{observations}$=534, N_{cows} =102, N_{farms}=2)

	Intercept		DIM			Feed quality score		
Response variable	Estimate	SE	Estimate	SE	*P*	Estimate	SE	*P*
Rumination time (min/d)	465.9	19.3	-0.13	0.03	**<0.001**	29.1	1.5	**<0.001**
Eating time (min/d)	650.6	31.3	-0.33	0.03	**<0.001**	-14.9	1.9	**<0.001**
Activity Changes (n/d)	146.7	7.3	-0.05	0.01	**<0.001**	-0.4	0.5	0.434
ECM (kg/d)	23.7	1.8	-0.05	0.001	**<0.001**	-0.6	0.07	**<0.001**
Body weight (kg)	523.4	26.5	0.14	0.01	**<0.001**	5.0	0.7	**<0.001**
BCS	3.18	0.2	-0.0003	0.0003	**<0.05**	0.02	0.006	**<0.01**

P values show t-test results using Satterthwaite`s method in linear mixed models fit by REML with days in milk, feed quality score, and lactation class (1st, 2nd and 3rd lactation onwards) as fixed and cow nested within farm as random effects, R^2m= marginal R squared, i.e. proportion of variance explained by the fixed effects, R^2c= conditional R squared, i.e. proportion of variance explained by the fixed and random effects.

Table 5. Influence of foraging behavior traits corrected for days in milk, feed quality score and lactation class on milk production efficiency (MPE) and efficiency calculated on the basis of total lactation data in Swiss organic dairy cows ($N_{observations}$=534, N_{cows} =102, N_{farms}=2)

	Intercept			Ruminate time corrected			Eating time corrected			Activity changes corrected				
Response variable	Estimate	SE	*P*	Estimate	SE	*P*	Estimate	SE	*P*	Estimate	SE	*P*	R^2 m	R^2 c
MPE_TL	0.1409	0.00985	***	$-1.163e^{-05}$	$8.735e^{-06}$	0.184	$1.067e^{-05}$	$9.182e^{-06}$	0.246	$3.447e^{-05}$	$3.066e^{-05}$	0.257	0.229	0.790
Efficiency_TL	0.9824	0.06643	***	$-3.544e^{-05}$	$6.081e^{-05}$	0.560	$8.164e^{-06}$	$6.354e^{-05}$	0.898	$2.723e^{-04}$	$2.103e^{-04}$	0.196	0.095	0.604

P values show t-test results using Satterthwaite`s method in linear mixed models fit by REML with the corrected behavioural traits ruminate time, eating time and number of activity changes and lactation class (3 levels : 1st, 2nd and 3rd lactation onwards) as fixed effects and cow nested within farm as random effect, R^2m= marginal R squared, i.e. proportion of variance explained by the fixed effects, R^2c= conditional R squared, i.e. proportion of variance explained by the fixed and random effects. †$P < 0.10$, ***$P < 0.001$.

Figures

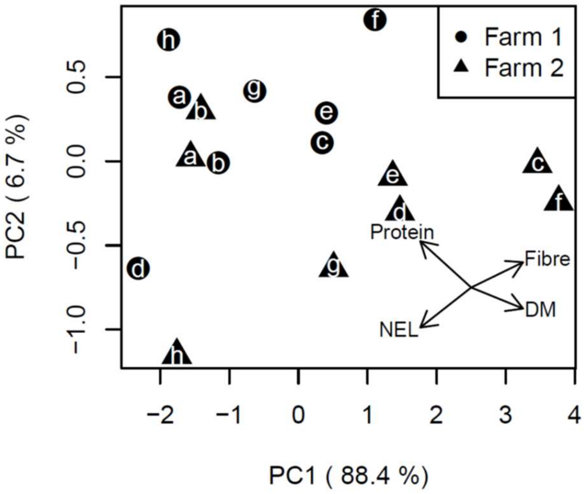


Figure 1: Result of the principal component analysis (PCA) on feed quality parameters. The PCA revealed that PC1 explains more than 88% of the variance in the data, reflecting a gradient from energy- and protein rich feed (PC1 < 0) to fibre rich feed (PC1 > 0). The arrows in the lower right indicate the loadings of the different parameters on the first two components (DM = dry matter, NEL = net energy lactation), and the shape of the symbols represent the two different farms. The letters in the symbols reflect the eight different trials (a - k) on each farm to enable the link to Table 2.

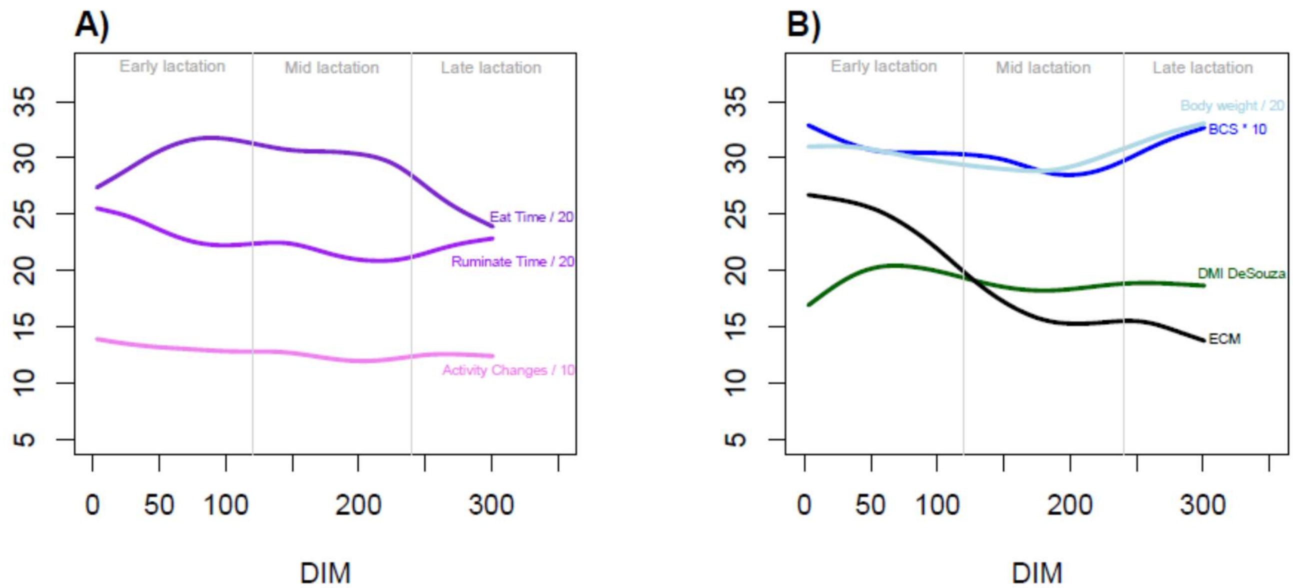


Figure 2: Influence of days in milk (DIM) on A) the forage behavior parameters rumination time (min/d) (lila), eating time (min/d) (purple), and number of activity changes (n/d) (violet), and on B) the efficiency related parameters body weight (lightblue), body condition score (BCS, royalblue), dry matter intake (DMI) based on the formula of DeSouza et al. (2019) (darkgreen), energy corrected milk

yield (ECM, black). Lines represent the smooth splines between DMI and the corresponding variable. To make them comparable, the different variables have been multiplied or divided by the values added to the labels. Vertical lines represent 120-day intervals of DIM.

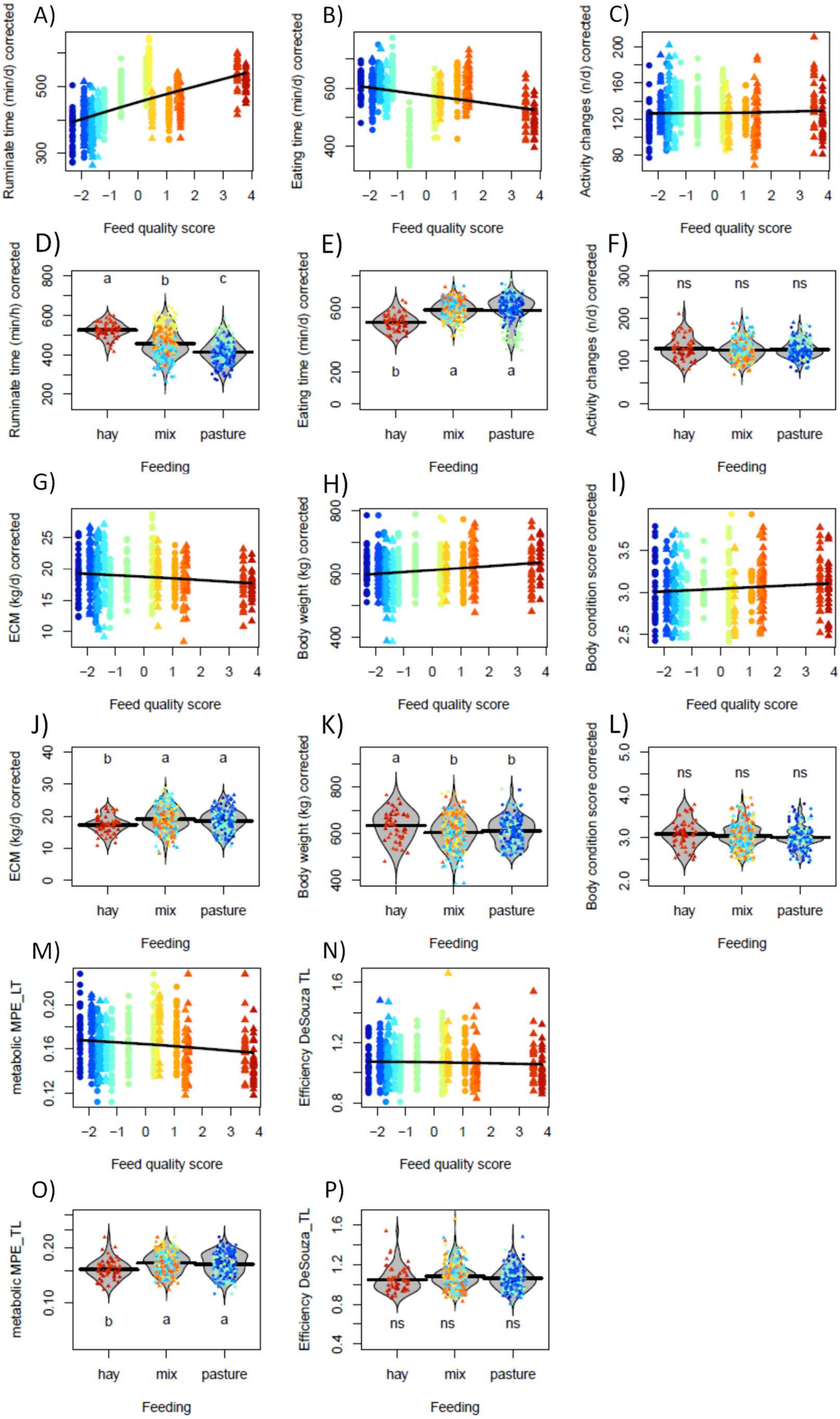


Figure 3: Relationship between the different foraging behavior and efficiency parameters and the feed quality score (A, B, C, G, H, I, M, N), and feeding (levels: hay, mix, pasture) (D, E, F, J, K, L, O, P) of Swiss dairy cows. Feed quality scores were derived from a principal component analysis where negative

values represent protein and energy rich feed and positive values feed high in fibre and dry matter (Figure 1). Levels of feeding characterize three different types of cow diets, i.e. cows fed with pure hay (“hay”), those fed on the pasture plus hay, silage or grass provided in the barn (“mix”), and those only fed on the pasture (“pasture”). Each dot represents a cow, the shape of symbols represent the two different farms (dots = farm1, triangles = farm2) and colors represent the feed quality scores. The bean plots represent the density smooth within feeding levels, where the horizontal line shows the mean value. Different letters represent significant differences between feeding levels based on mean comparisons by Tukey contrasts at $P < 0.05$. ns=not significant

Appendix

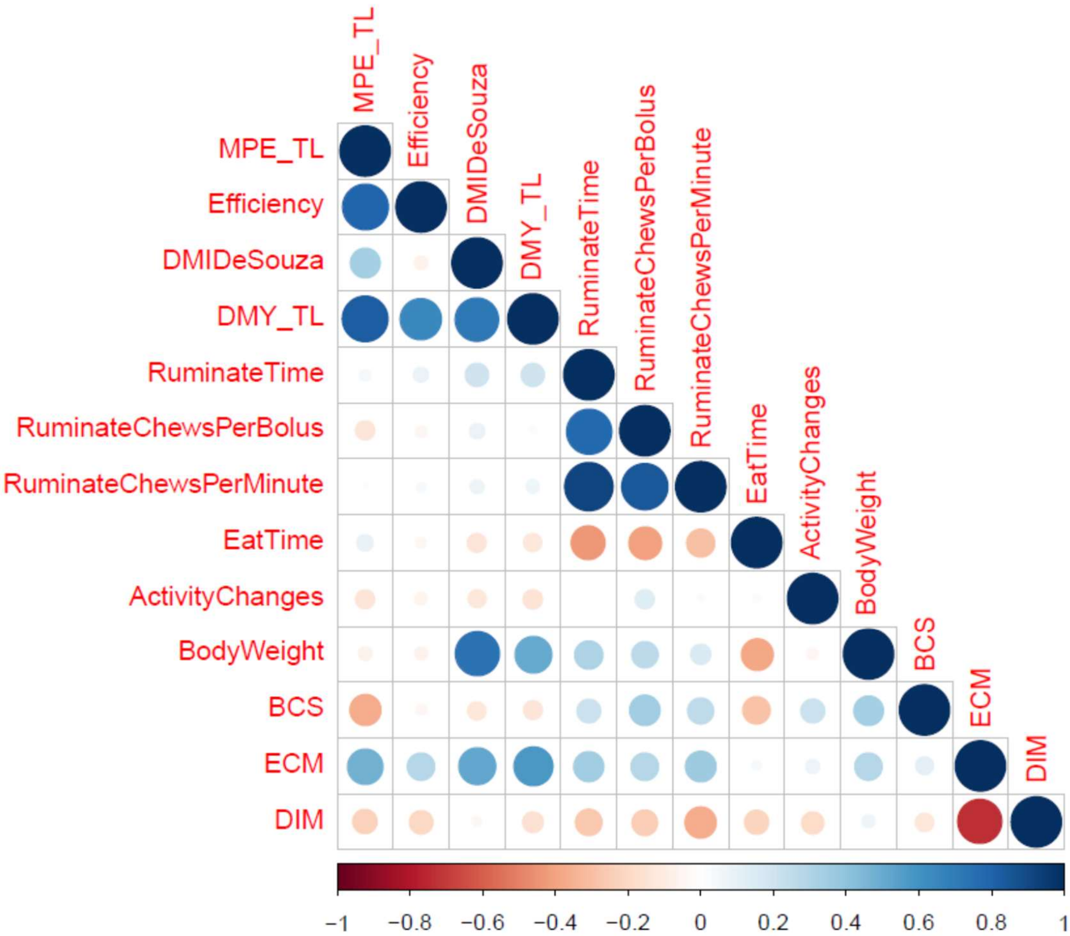


Figure S1: Correlations between efficiency parameters (i.e. MPE_TL= metabolic milk production efficiency of total lactation, DMIDeSouza= dry matter intake calculated after DeSouza et al. (2019) in kg, Efficiency = DMY_TL/DMIDeSouza, DMY_TL= average daily energy corrected milk yield of total lactation), foraging behavior parameters measured by RumiWatch holsters (i.e. ruminate time (min/d), ruminate chews per bolus (avg. in 24h), ruminate chews per minute (avg. in 24h), eating time (min/d), number of activity changes (n/d)), as well as body weight (kg), body condition score (BCS), energy corrected milk yield (ECM in kg), and days in milk (DIM). Red dots represent negative and blue dots represent positive correlation coefficients. The larger the dots and the more intense the color, the stronger the correlation.

References

Agroscope (2017). Feeding Recommendations for Ruminants- Green Book (in German). Chapter 7: Feeding recommendations for dairy cows by Jans F., J. Kessler, A. Münger, F. Schori and P. Schlegel. Online Version. http://www.agroscope.ch/gruenes-buch. Accessed March 23, 2020.

Barton, K (2019). MuMIn: Multi-Model Inference. R package version 1.43.15. https://CRAN.R-project.org/package=MuMIn

Bates, D., M. Maechler, B. M. Bolker and S. C. Walker (2015). "Fitting Linear Mixed-Effects Models Using lme4." Journal of Statistical Software **67**(1): 1-48. http://dx.doi.org/10.18637/jss.v067.i01.

Büchel, S. and A. Sundrum (2014). Technical Note: Evaluation of a new system for measuring feeding behaviour of dairy cows. Computers and Electronics in Agriculture **108**: 12-16.

DeSouza, R. A., R. J. Tempelman, S. S. Allen and M. J. VandeHaar (2019). "Updating predictions of dry matter intake of lactating dairy cows." Journal of Dairy Science **102**: 7948-7960.

Dixon, P. (2003). "VEGAN, a package of R functions for community ecology." Journal of Vegetation Science **14**(6): 927-930.

Hothorn, T., Bretz, F. and Westfall, P. (2008). Simultaneous Inference in General Parametric Models. Biometrical Journal **50**(3): 346-363.

Knaus, W. (2009). Dairy cows trapped between performance demands and adaptability. Journal of the Science of Food and Agriculture **89**: 1107-1114. http://dx.doi.org/10.1002/jsfa.3575

Leiber, F., J.K. Probst, N. Zehner, and A. Spengler Neff (2015). Fress- und Wiederkäuverhalten von Milchkühen bei verschiedenen Fütterungsregimes. Agrarforschung Schweiz **6 (10)**: 462-469.

Oltenacu, P.A., and D.M. Broom (2010). The impact of genetic selection for increased milk yield on the welfare of dairy cows. Animal Welfare **19**:39-49.

Oudshoorn, F.W., C. Cornou, A.L.F. Hellwing, H.H. Hansen, L. Munksgaard, P. Lund and T. Kristensen (2013). Estimation of grass intake on pasture for dairy cows using tightly and lossely mounted di- and tri - axial accelerometers combined with bite count. Coumputeres and Electronics in Agriculture **99**: 227-235.

Pereira, G.M., B.J. Heins, and M.I. Endres (2018). Technical note: Validation of an ear-tag accelerometer sensor to determine rumination, eating, and activity behaviors of grazing dairy cattle. Journal of Dairy Science **101**: 2492-2495.

Pritchard, T., M. Coffey, R. Mrode, and E. Wall (2013). Genetic parameters for production, health, fertility and longevity traits in dairy cows. Animal **7**: 34-46. https://doi.org/10.1017/S1751731112001401

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rauw, W.M.; E. Kanis, E.N. Noordhuizen-Stassen, and F.J. Grommers (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. Livestock Production Science **56**: 15-33. https://doi.org/10.1016/S0301-6226(98)00147-X.

Rombach, M., A. Munger, J. Niederhauser, K. H. Sudekum and F. Schori (2018). Evaluation and validation of an automatic jaw movement recorder (RumiWatch) for ingestive and rumination behaviors of dairy cows during grazing and supplementation. Journal of Dairy Science **101**(3): 2463-2475.

Rombach, M., K. H. Sudekum, A. Munger and F. Schori (2019). Herbage dry matter intake estimation of grazing dairy cows based on animal, behavioral, environmental, and feed variables. Journal of Dairy Science **102**(4): 2985-2999.

Schader, C., A. Muller, N. El-Hage Scialabba, J. Hecht, A. Isensee, K.-H. Erb, P. Smith, H.P.S. Makkar, P. Klocke, F. Leiber, P. Schwegler, M. Stolze and U. Niggli (2015). Impacts of feeding less food-competing feedstuff to livestock on global food system sustainablity. Journal of the Royal

Society Interface 12: 20150891. https://royalsocietypublishing.org/doi/10.1098/rsif.2015.0891

Spengler Neff, A., C. Notz, S. Ivemeyer, and M. Walkenhorst (2015). Body Condition Scoring, Forschungsinstitut für biologischen Landbau (FiBL), Frick, Switzerland. 2nd Edition. https://shop.fibl.org/chde/1650-bsc.html

van Zanten, H.H.E, H. Mollenhorst, C.W. Klootwijk, C.E van Middelaar, and I.J.M. de Boer (2016). Global food supply: land use efficiency of livestock systems. The International Journal of Life Cycle Assessment **21**: 747-758.

Wei, T., V. Simko, M. Levy, Y. Xie, Y. Jin and J. Zemla (2017). "Package ‘corrplot’." Statistician **56**: 316-324.

Zehner, N., C. Umstätter, J.J. Niederhauser, and M. Schick. (2017). System specification and validation of a noseband pressure sensor for measurement of ruminating and eating behavior in stable-fed cows. Computers and Electronics in Agriculture **136**: 31-41.