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EXECUTIVE SUMMARY

Background	WP2 is about establishing and operating procedures to promote TNA; receive and evaluate TNA proposals, and then establish and monitor TNA projects at infrastructures managed by SmartCow partners.
Objectives	*The objective of Task 2.4 is to ensure that commissioned projects are progressing along agreed timescales and that the TNA services are continuously improved.*
Methods	*Templates including questions for both the TNA users and facility managers to survey user's satisfaction for the reports to the panel were developed. To monitor the progress different reporting periods are defined and questions asked about any delay.*
Results & implications	*Templates are developed for four reporting periods:* *Before starting (report 0)* *At the start of the data collection (report 1)* *Mid-point in the data collection (report 2)* *60 days after the end of data collection, including a final report from the user (report 3).* *In total, 48 proposals were received* *A total of 29 project obtained funding, 11 from round 1, 7 from round 2, 5 from round 3, and 6 from round 4.* *Across the four calls, five projects were withdrawn due to lack of national funding that was needed beyond the SmartCow funding or due to other reasons e.g. practical reason or lack of permits.*

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	In total 24 projects were performed and 9482 cow.weeks have been used which is close to what was expected. *The TNA user was from academia for 9 projects and from private companies for 15 projects.* *The gender balance for the TNA users was female:12, male:12.* *All projects have now handed in their final reports.* *Countries : France (7); UK (2); Germany (2); Austria (2); Switzerland (2); Spain (2); Ireland (2); Norway; Denmark; Netherlands; Bulgaria; Macedonia*

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1 3rd Project Evaluation Report

This is the third project evaluation report including description of the progress in the TNA projects as well as the lessons learned during the process of monitoring and evaluating the commissioned projects. This report includes a description of the process and an overview of all the projects initiated. Furthermore, the report includes a summary of the lessons learnt both during the process and from the reports from the TNA users and facility managers.

2 Process and monitoring of the TNA projects

2.1 The selection process

For each call an initial evaluation of 1st stage proposals for eligibility (participation rules) and feasibility (availability of facilities) was conducted by the Access Management Team – in order to give applicants a rapid response and decision on whether they should work on a full proposal. In the additional fourth call a 1-step procedure was used to secure that experiments could finish within the project period. Applicants were encouraged to maintain contact with Facility Managers whilst they worked up full proposals in order to ensure continued feasibility. Each of the eligible Full Proposals was independently evaluated by one internal reviewer (i.e. from a project partner not involved in the proposed work/facility) and one external reviewer. The Access Management Team allocated reviewers. Proposals were also checked by the Ethics Committee (we had asked reviewers to flag any specific issues, but on this occasion the committee looked at all applications).

Reviewers were provided with a scoring template and this allowed us to score all proposals (out of 100). For the first call, this was done manually, however, for subsequent calls an agreement with Oxford Abstracts was established to keep help track of the proposals and the reviewer comments. Proposals scores were then considered alongside the availability of space at each facility and discussions amongst the Access Management Team identified options for alternative (second preference) facilities in some cases. These options were then discussed with Facility Managers and a final list of approved projects and available facilities drawn up. With some redistribution of capacity between INRA facilities, we were able to accommodate all work.

For the first call out of 13 full applications 11 were funded. Two of the proposals involved a different type of work than was originally envisaged for a specific facility – but both facilities indicated that the work was feasible. For the second call out of 16 full applications evaluated, 6 were agreed for the first choice facility; one was accommodated by offering access at a different site (which will also have to adjust its cow-weeks budget allocated to different facilities) and 9 were not supported. For the third call, 5 project out of 9 proposals were granted. For the final extra fourth call 6 projects out of 10 were granted. In total 5 projects were withdrawn and the net number of projects from the 4 calls were therefore 9, 6, 5, and 4, respectively, in total 24.

2.2 Monitoring of TNA projects

After initiation of the funded projects both the facility manager and the TNA user received a template to fill in with questions related to the performance of the TNA. Thus, concise reports will be returned at start, mid-point and end of each experiment for the access management team to monitor the progress and summaries lessons learnt for future actions both from a TNA user perspective and to develop the actions at the facilities. In order for the access management team to get a start date, all TNA users were asked to send report 0 including some basic information about the project such as start date of the experiment etc. After the first call 11 projects were agreed on, one project was however withdrawn due

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to lack of national funding to cover expenses not covered by SmartCow finances. Table 1, 2, 3 and 4 in the appendix show the projects and the status of the projects from call 1, 2, 3 and 4, respectively. All projects have handed in their final report to SmartCow. Most projects and therefore also the reports have been delayed due to Covid-19.

3 Summary of lesson learnt for selection, operation and monitoring of future projects

The summaries are based on the output from the templates provided by the TNA users and the facility managers, as well as the experience from the selection panel.

3.1 The selection process

TNA processes were reviewed during the annual project meeting in Dumfries (March 2019) and the following changes proposed for the second call:

1. Move to using an online tool for submission and evaluation of proposals (to make the process easier to manage and also improve the process for proposers and reviewers;

2. Greater clarity on the way in which ethical review operates (this will also be facilitated by the online submission and evaluation tool);

3. Provide checklists and templates for use in drawing up agreements for project work.

The Procedural Manual was updated in advance of the second call, mostly to reflect the change to using the online tool for submission and management of applications for the Second Call.

Within the Access Management Team we developed a TNA Term sheet gathering all general principles about implementing a TNA project and explaining both the host facility/organisation and the user(s)' rights and obligations. Once the TNA Proposal is accepted, the term sheet is aimed to help the TNA beneficiary and the facility to plan the TNA project and to agree on important aspects before starting it.

3.2 Monitoring TNA projects

From all the report 0 received it seems clear that the call text was clear. All the applicants, who got funded, found that there were no information missing in the call text at the webpage; they all answered "*no*" to the question *"Are there any information missing in the call text at the webpage?".* Furthermore, we encouraged applicants to contact the facility managers in the answer after the preproposal. This worked out well, since all the applicants were in contact with the facility managers or a scientist related to the facilities in questions during the process of preparing the full proposal.

After the decision, all funded TNA's have had contact with the facilities and some oral communication either by phone, skype or videoconference have been used for all projects. However, in a few cases it has been necessary to include René Baumont and Richard Dewhurst to solve questions and in one case regarding miscommunications between the facilities and the TNA user. Prompted response to questions is mentioned as an important way to support the projects by TNA users.

All projects are finished and have handed in their final report and they seem to have run without any problems, and TNA users are content with the process.

Close contact between the facility and the TNA user has contributed significantly to success according to both TNA users and the facility managers.

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There has been some uncertainly between TNA Users and TNA host about who had the responsibility for submitting the reports during the trial and the final report afterwards. There has therefore been to long delays before especially the final reports were submitted. This has challenged the job of collection the reports.

It is concluded that the granted TNA projects already have provided new knowledge for both TNA users and SmartCow facilities, and a range of TNA projects have been extended with bilateral add-on projects between TNA users and scientists from the host institutions. However, the scientific outcome of a number of projects still awaits to be shared within the SmartCow project, probably due to that data processing and potential publication of these data is still in process in most projects due to the delays induced by Covid-19.

3.3 Covid-19

Covid-19 has had a significant influence on trials. A significant number of granted projects were delayed during 2020 or even postponed to 2021, due to full shutdown of facilities or local restrictions imposing new trials.

Also the planned visits by the users in ongoing trails have been cancelled or postponed. This means that most of the communication between users and hosts have been by e-mail and online meetings, but no projects were cancelled due to covid-19.

4 Projects

In the following detailed information is reported based on the information received from each project from the reports returned at the end of each animal trial (final report).

4.1 Investigating links between beef cattle behaviour, temperament and diet with changes in the rumen microbiome and implications for performance by Gareth Arnott (Queens University Belfast)

The project was planned to start July 2019. However, misunderstanding between the TNA user and the facility manager regarding who has the responsibility for getting the permission from the Ethical committee has led to a delay in obtaining the permission. The project was withdrawn.

4.2 Impact of physically effective fiber concentrations on chewing behavior, rumen microbial protein synthesis, and nitrogen efficiency in cows by Ruth Heering (University of Hohenheim)

Final report by User:

Project aim: The project aims to unravel the processes underlying the effects of an increased dietary physically effective fiber (peNDF) concentration on chewing behavior, rumen fermentation, fractional passage rates, efficiency of microbial protein synthesis, and partitioning in nitrogen excretion in dairy cows. Fostering the inherent ability of ruminants to recycle nitrogen and/or increasing the

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efficiency of rumen microbial protein synthesis would be of ecological and economic benefit. The greater peNDF concentration may increase saliva production and thereby maintain rumen health.

Hypothesis: The hypotheses were the following: (1) At a negative rumen nitrogen balance level, increasing peNDF concentration promotes rumen fermentation and microbial protein synthesis via enhanced nitrogen recycling. (2) A quadratic effect of increasing dietary peNDF concentration on digestibility and performance will be expected.

Results and dissemination: Results will contribute to a better understanding of the interrelation of physical, behavioral, metabolic, and digestive processes in order to promote animal welfare and health, increase nitrogen use efficiency, and mitigate nitrogen emissions from dairy production. They could provide a basis for complementary projects (e.g. at European level) on how different carbohydrates, fiber, and nitrogen sources interplay with the particle size of the diet, animal behavior, and health, as well as emissions from dairy systems. The findings will at first be presented on national and international scientific conferences on ruminant behavior and herbivore nutrition. Results will be published in one research article in open access international peer-reviewed journals and will be part of a PhD thesis. In addition, results may contribute to an environmentally friendlier and more economical use of protein feed resources and thus be interesting to relevant stakeholders such as dairy farmers or feed industry.

Other comments: This SmartCow project was a great opportunity for our working group as it gave us the possibility to work with another research team, which gave rise to expertise exchange. Further, it has gave the PhD student good insight in working with fistulated animals and with another research group during the student's stay in INRA Theix. The visits to INRA before the start of the experiment have helped in the preparations of the experiment so that both parties were in agreement and a smooth start of the experiment could be accomplished. However, as mentioned in the previous progress report, administrative issues related to the TNA agreement, associated partnership agreement, the research stay of Ms. Heering, and the reimbursement of travel costs were labor and time-intensive, partly due to the fact that the procedures and requirements were not fully clear. Maybe this could be organized centrally and supported by better guidance by the SmartCow project.

4.3 From grassland biodiversity to animal's microbial ecosystems and cheese qualities by Joël Berard (ETH)

Final report by User:

Objectives/Hypothesis: Grassland farming systems are increasingly emerging as the strongest future options for ruminant livestock systems. Although the benefits of the grassland-based milk production have been demonstrated, in-depth knowledge is lacking in understanding the underlying mechanisms of interactions between ecosystems, feeds, animals, milk and cheese. This project proposes to study the effect of the botanical diversity of pastures and forage conservation methods on the rumen microbiota, which will affect the microbiota of faeces, litter, teat skin and subsequently of milk and raw milk cheese. We hypothesize that more biodiverse pastures with elevated levels of plant secondary compounds will increase the abundance and diversity of rumen microbial species. This will support the proper functioning of the rumen and therefore have a positive impact on animal health and on compositional and sensory quality of milk and cheese. We rely on one of the major paradigms of ecology, namely that

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stability in natural systems is based on biodiversity and synergy between species or functional groups capable of differential responses. We also assume that different feed conservation methods (drying and ensiling) have a strong impact on rumen ecosystem and consequently also on milk and cheese properties. This project will provide answers to important questions concerning the effects of pasture biodiversity and forage conservation methods on the microbiota of milk and the properties of dairy products. This project will allow to study, at different levels from plant communities to cheese, how farm, milk and cheese microbiota respond to their environment in terms of community structure and orientation of their metabolism.

Materials and methods: Forty-eight dairy cows (24 Holsteins and 24 Montbéliardes) that calved between October 2018 and March 2019 have been divided into 4 balanced lots in terms of breed and parity (16 primiparous - 32 multiparous), milk production measured during the first week of May, calving date and SCC. The 48 cows involved in the trial were kept in the same pen from the beginning of April. Sampling for the "covariate" of the two batches of cows going out to pasture took place on May 6, 9 and 10 when all animals receive a ration of hay and concentrates. Sampling for the "covariate" of the other two lots took place during week 21 when the cows still received the same ration of hay and concentrates. The rest of the trial is split into two periods.

BIODIV test (June): effect of floristic diversity:

The objectives of the BIODIV trial are:

- To study the assembly of microbial communities from the soil to the ripened cheese on two very contrasting plots in terms of plant biodiversity

- To understand the effect of plant biodiversity on the ruminal bio-hydrogenation of grass lipids and on the sensory properties of ripened cheeses.

Twenty-four cows corresponding to two of the 4 groups grazed from May 10 on two plots with very different levels of plant biodiversity; the “Montagne Florac” plot (MF), whose vegetation, particularly diversified, is similar to that of a upland summer semi-natural pasture and a plot of permanent grassland with very little diversity (“Bas Florac” : BF). In both groups, the animals grazed, without any feed supplements other than minerals. On the MF plot, which is later than the BF plot, the cows first grazed at the bottom of the plot and were gradually directed upwards towards the top of the plot where they remained exclusively from 1 June at the latest until 26 June. A part of BF has been mowed on week 18 in order to offer good grazing condition and herbage at a phenological stage comparable to those of the more tardive MF pasture. Cheese samples and production have been carried out in sub-batches of 4 cows (2 Holsteins, 2 Montbeliardes) balanced according to milk production, which will constitute the statistical unit. Cheese production focused on the study of the assembly of microbial communities of the milk from the cow sub-batches and the corresponding mixing milk: during each manufacturing day, the 3 milks from the sub-batches of 4 cows have been processed in parallel as well as the mixing milk from the batch (4 tanks in parallel). Six days of production have been carried out between June 11 and 25, using a technology similar to that of Cantal.

CONSHERB test (July): effect of the grass conservation method:

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The objectives of the CONSHERB trial are:

- To understand the effect of the way the grass is exploited on the ruminal bio-hydrogenation of the grass lipids and on the sensory properties of milk and ripened cheeses.

From May 24, the 24 cows of the two lots remaining indoors will receive feed, either grass silage (brought back from Theix) or hay (Bas Florac from June 2018). From July 1, silage and hay have been replaced respectively by silage and hay made around end of May on the same plot ("Borie Bas"plot). The two grazing cow lots in the BIODIV trial have been remixed on June 26 to 2 groups by balancing the origin (1/2 MF, 1/2 BF) and considering the other grouping criteria. From 26 June, one group (the PAT group) grazed the regrowth of Borie Bas, after mowing for hay and silage. The other group (Ha) was fed indoor with fresh herbage cut on the same "Borie bas" plot. As for the BIODIV test, cheese samples and production have been carried out in sub-batches of 4 cows (2 Holsteins, 2 Montbéliardes) which will constitute the statistical unit. Cheese production focused on studying the effect of herbage exploitation: during each day of production, 4 milks corresponding to a sub-batch of each of the 4 diets have been processed in parallel. Three days of production have been carried out between July 16 and 23, using a technology similar to that of Cantal. For the BIODIV and CONSHERB tests, samples from animals and plots have been taken during the weeks of cheese production. They will be used mainly to describe microbial communities (prokaryotes and eukaryotes) using a high-throughput amplicon sequencing approach. The latter will be analysed on the floor, grass and lying areas in contact with the udder as well as in ruminal fluid (collected by oesophageal tube), faeces (rectal sampling), teat surface (sampled with wipes), mixed milk from each batch (only BIODIV) and each sub-batch and the corresponding refined cheeses. The characterization of these different microbial communities will be completed by analyses of the composition of the forage (botanical composition, nitrogen, walls, tannins and fatty acid profile), ruminal liquid (pH, volatile and total fatty acid profiles), faeces (nitrogen, walls), individual milk (classical biochemical analyses and fatty acid profiles etc), mixed milk (classical biochemical analyses, volatile compounds, fatty acid profiles, sensory analyses) of the corresponding cheeses (classical biochemical analyses, sensory analyses, volatile compounds, colour, rheology).

Expected outcomes, innovation/impact of the results: The most innovative expected outcome is the comprehension of the microbial flux form the environment (pasture, litter, water, soil, teats, etc) to the rumen, the milk and the cheese. This flow is still almost unexplored and its investigation represents a relevant scientific innovation. Furthermore, the effect of the pasture biodiversity level and the exploitation mode of the herbage (fresh grazes or fed indoor, and conserved as hay or as silage) will allow to understand how farming practices can affect the microbial flow. A change in microbial flora in the rumen or in the dairy products is expected as well to change the characteristics of derived dairy product. The comparison of chemical composition and sensory properties of milk and cheese as affected by the microbiological flow will another innovative result. The acquired knowledge about the microbiological link between environment and dairy products will help to understand and highlight the link between a *terroir*, the related farming practices and specific characteristics of dairy products.

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Dissemination plan: One Ph.D. student, Elisa Manzocchi, from ETH Zurich is directly involved in this experiment. The results will be part of her Ph.D thesis. The data obtained will be published in at least two scientific papers in peer-reviewed journals and will be presented at scientific international conferences (e.g. on animal nutrition, animal production, milk processing and food microbiology). The involved research groups and platforms will be in charge of the dissemination of the results via their websites, leaflets and training sessions they organize for students, technicians and farmers. We anticipate strong interest from the public for this type of innovative research at the interface of basic and applied sciences resulting in direct benefits for farmers, consumers and society. Research highlights will also be communicated to stakeholders and lay persons in end-user meetings, in press releases and by interviews in public media. In addition, our research project will be presented during AgroVet-Strickhof Conference to relevant stakeholders. This project will also be presented and discussed in the boards of GIS "Filières Fromagères sous IG" and RMT "Fromages de terroirs" (gathering all French PDO and PGI cheeses) and results will be disseminated through their website and by training sessions.

4.4 PFA effect on methane production by Poulad Pourazad (Delacon)

Final report by User:

The main objective of the project: In ruminants, methane (CH4) production derives from microbial fermentation of feed (carbon dioxide and hydrogen) in the anaerobic environment in the rumen and hindgut. Diet composition and feed intake are the main factors influencing CH4 production in ruminants. Diets with a high forage portion, rich in structural carbohydrates result in a higher CH4 production compared to rations with higher levels of non-structural carbohydrates. Several in vitro studies indicate the inhibitory effect of plant extracts and essential oils (EO) on methane production. However, considerable in vivo studies on the underlying modes of action and on long-term efficacy are missing. Plant extracts and EO have been proposed as substitutes for chemical feed additives for CH4 reduction due to their potential as modifiers of rumen fermentation. These effects may derive from selective reduction of CH4-producing microbes and from antioxidant properties. EO like thyme oil, clove oil and anise oil have shown inhibitory effects on methanogenic Archaea and consequently methane production in the rumen. Tannins are used in ruminant nutrition to increase protein utilization. This effect is obtained though binding of tannins to dietary proteins, then become 'rumen-escape' proteins available in the small intestine. Tannin interaction with proteins, metal ions, and amino acids in ruminants can depress activity of methanogenic microbes in the rumen and thus decrease CH4 emissions. Saponins influence CH4 production and protein metabolism in the rumen by their toxic effect on protozoa.

The hypothesis that are tested: In the planned study a blend of essential oils, plants extract, tannins and saponins will be used to study their effects on performance and methane production in dairy cows. It is intended to study the impact of 3 different prototypes on: DMI, methane production, milk yield and composition. The results of this study could help to get a more detailed insight into the mechanisms of natural feed additives on animal performance and methane production.

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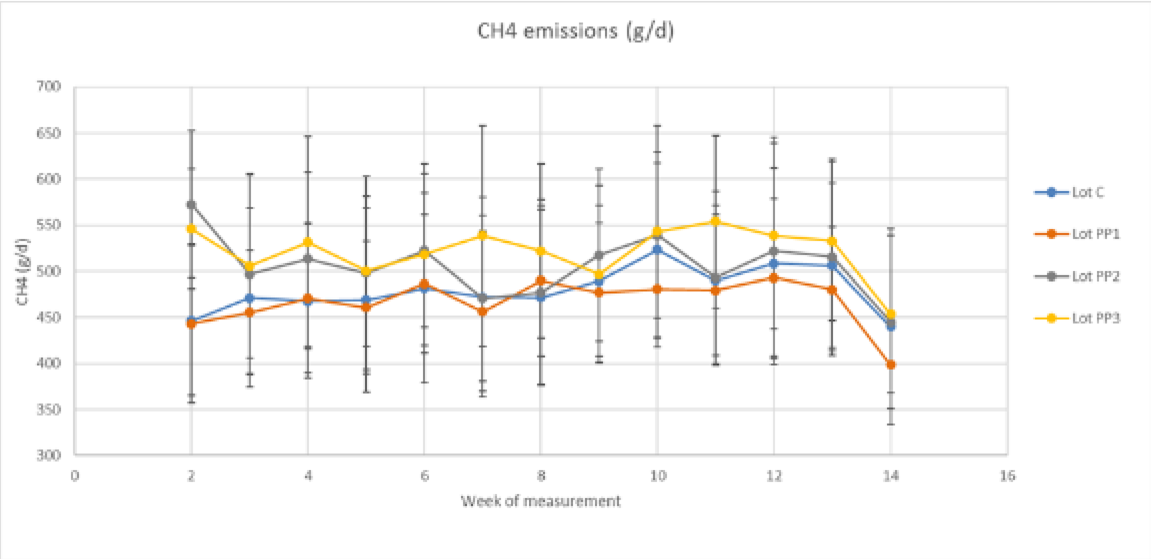
The main scientific outcome, innovation/impact of the results: (i) Animal performances. It has been contractually agreed that Delacon will receive a raw dataset and assume the statistical analysis of the results. Nevertheless, a first analysis performed on the zootechnical dataset (Milk yield and composition, Feed Intake and Body Weight) showed that the different blends of essential oil delivered to the cows through the concentrates have no effect on these parameters. This means, at least, that the tested compounds does not impair milk production and are as palatable as the control concentrate, which does not contain any essential oil. Further analysis has to be carried out by Delacon GmbH to get more details. (ii) Methane emission. It has been contractually agreed that Delacon will receive a raw dataset and assume the statistical analysis of the results. Nevertheless, a visual inspection of daily CH4 emissions data, pooled per week and per treatment, has been performed:



How do you expect to disseminate the results: Although the objective of the proposed collaboration between INRA-Theix and Delacon Biotechnik GmbH represents an initial and essential part of a product development process within Delacon, the company own R&D department is highly interested in publication of trial results in international peer reviewed journals (preferably in Journal of Dairy Science). Moreover, introducing new results to the relevant scientific community, via contributions at international congresses as oral and/or poster presentations, perfectly align with the freshly adapted internal publication strategy of Delacon Biotechnik GmbH. Depending on the results of this collaborative study, the findings are planned to be directly converted into formulations of Delacon future products for ruminants. In this case, diverse information materials will be created to explain the modes of action of these novel products for our customers and for further interested people, considering individual differences in their technical background: basic information will be given on the product site of Delacon's homepage, articles in international semi-technical journals will be created for nutritionists, and a more detailed description of the mode of action will appear within one of the next Delacon Dossiers, as a periodically published booklet on the company's innovations for a readership with high technical know-how (see also: https://www.delacon.com/Tech-Talk/Dossiers).

Any suggestions to improve the TNA procedure: Need to formalize the way to communicate with the user (frequency especially) while writing the agreement between the facility and the partner.

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4.5 Multiple spatially resolved reflection spectroscopy (MSRRS) - carotenoid content of the skin of cows by Martina Jakob (ATB)

Final report from the User:

The main objective of the project: A sensor based on multiple spatially resolved reflection spectroscopy (MSRRS) developed to scan the palm skin of humans was tested on cows. The measurement results of the sensor display the carotenoid content of human skin within a range of 0 to 15. The carotenoid content of human skin provides information about the health status and stress level. The aim of the study was to find out if a similar range is achieved from the skin of cows. Since the skin measured needs to be without hair and non-pigmented, the teats were chosen to be measured.

The hypothesis that are tested: The hypothesis was to see if the sensor gives plausible feedback. If successful, the sensor could be used to develop an early warning system, mainly for inflammatory diseases such as mastitis. This kind of warning system could enable a farmer to react early and hopefully prevent a severe illness, and at the same time reduce the application of antibiotics.

The main scientific outcome, innovation/impact of the results: All trials displayed plausible results. The Scottish cows were measured twice on two teats every day for three weeks in a row. This procedure was repeated four months later due to the Scottish lockdown including the same cows. The repetition per teat was used to see how reliable the measurements are. According to the developer, the variation between two measurements is ± 1. This was mostly achieved for the measurements of the teats in Dumfries. Apart from a general value displaying the carotenoid content of the skin, a value describing the measurement quality was available. For a successful measurement it is necessary, that the sensor is covered completed and no disturbing light falls onto it. Because of the flat shape of the sensor, the teats were gently pressed on it to achieve full coverage of the light emitting area of the sensor. As some teats were fairly thin, this may not always have been successful and therefore may have influenced the measurement quality. Overall, the measurement quality in Dumfries was excellent. 1500 samples (nearly 75 %) achieved the highest quality. A decrease in quality was mostly coupled with a decrease in the value displaying the carotenoid content. The overall sample size was 2080 measurements. The average value for all cows in Scotland was 10.7. The French average value, based on 2467 samples, was 14.1 and the Irish average, based on 468 samples was 9.4. The achieved results show, that the sensor has potential to be used for cows. The carotenoid content of humans and cows seems to be similar, but cows have a slightly higher value. The values of the French sample exceeded the calibrated range of 15. The main limitation therefore is that the sensor is calibrated for human skin. Further trials with contemporaneous blood analysis for the blood carotenoid content of the cows are needed now, to adjust the calibration for cow's skin and check the plausibility. Overall, the dynamics of the carotenoids are little researched for dairy cows. For the French sample a dependency on the fodder was statistically proven with higher values for those cows that were let outside on a paddock grazing. Fresh grass but also sunlight are able to increase the level of carotenoids in the skin. Nutrition is generally the main influencing factor on the carotenoid content in the skin, and it is therefore plausible to observe a raise in the value between fresh grass and silage.

There was only one incidence of mastitis in all samples. The affected cow showed a slight drop in the value (-1), but the day the mastitis was diagnosed was not measured due to the fact that it was a weekend,

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and on weekends, no measurements took place. Therefore the acute phase of the mastitis was missed. Two days after the start of the antibiotic treatment the value was at the same level as it was before the mastitis.

Any other achievements of the visit: The visit was valuable to show and explain the measurement procedure. It was also valuable to the herd and the parlors.

How do you expect to disseminate the results: Parts of the results have already been presented on a conference in September 2020, but the Scottish sample was not included. This is the conference link: https://www.agroscope.admin.ch/agroscope/de/home/aktuell/veranstaltungen/akal2020.htmlIt is planned to publish the whole sample in a peer reviewed journal as soon as possible.

Any suggestions to improve the TNA procedure: The cooperation with the Scottish partner worked really well

4.6 Impact of oscillating supply of essential amino acids on whole-body nitrogen partitioning, mammary gland metabolite utilization, and milk nitrogen efficiency in lactating dairy cows by Rolland Matthieu (Ajinomoto)

Final report from the User:

Objectives: Studies with sheep and beef cattle have shown that infrequent supplementation of protein improves N retention and decreases urinary N losses, presumably by sustained urea recycling to the gut or through ornithine cycle adaptations in relation to labile protein pools in the body. These processes may also help to more efficiently convert protein from human non-edible resources into high quality milk protein. The impact of oscillating amino acid (AA) supply on N dynamics in dairy cattle is unknown. This project aims to evaluate milk yield, N retention and milk N efficiency in response to oscillating supplementation of AA in dairy cattle

Hypotheses: We hypothesize that oscillating AA supply is a dietary strategy that will improve milk N efficiency and reduce urinary N losses, through deposition and an efficient re-utilization of N in labile pools. Upon favorable experimental results, this dietary strategy would help the dairy sector to improve utilization of human non-edible resources and reduce N emissions.

The main scientific outcome, innovation/impact of the results: The outcome/impact is not possible to give within 6 wks after ending of experiment. Facility researcher and user are in contact and will discuss results once sufficient data from lab are available.

Any other achievements of the visit: A planned visit had to be cancelled unfortunately, due to Covid19

How do you expect to disseminate the results: We aim to publish the results in peer-reviewed, open access publication(s) aimed at the dairy production sector at large, with authors from Ajinomoto and

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Wageningen University. We will also endeavor to disseminate the results as soon as practicable in professional journals in Europe. A webinar is envisaged, aimed at dairy nutritionists, dairy industry representatives, universities and policy makers, on the potential of oscillating AA supply to improve N efficiency. In all dissemination of results, we will acknowledge the access provided, and specify that the project received research funding from the European Community's Horizon 2020 Programme**.**

Any suggestions to improve the TNA procedure: Facility manager: Really good procedure in general. To improve, have realistic expectations as to what can be delivered in terms of results only 6 wk after end of trial. User: No additional comment

4.7 Effects of Bacillus probiotic on productivity, health and welfare of dairy cows by Noriko Nakamura (Calpis).

Final report from the User:

Calsporin® is a zootechnical feed additive based on viable spores of Bacillus subtilis C-3102, approved in the EU for several target animal categories, but not yet in dairy cattle. The objective of this study is to evaluate the efficacy of Calsporin® in dairy cows, on feed intake and milk production. Rumen fluid samples and faeces were analysed to gain insights about the effect of fermentation and digestion.

A total of 64 multiparous Holstein Friesian dairy cows were enrolled in a study with a 15 day pre-period and a 105 day study period. After the pre-period, the cows were approximately 60-160 days in lactation and allocated to one of two treatments: T0 Control and T1 Calsporin, 32 cows per treatment. Both groups were balanced for parity, days in lactation and milk yield. Cows in group T1 Calsporin received the Calsporin® product delivering 3 x 10^9 CFU/cow/day. Cows in group T0 Control received an identical diet, without added Calsporin®. Individual feed intake and milk production were registered daily, while milk composition was determined weekly.

The study was executed in the summer period with a period of high outside temperatures between 30-35^{0}C resulting in some heat stress for all animals, with a temporary decrease in feed intake and milk production. Overall, milk yield was 0.7 kg/d higher for cows in the T0 Control group, but after correction for energy, fat or fat and protein content, milk yield was equal for both groups. Total dry matter intake was also equal in both groups and on average 24.5 kg/d. Feed efficiency as expressed by kg of corrected milk per kg of dry matter intake was on average 1.5 kg/kg and did not differ between the two treatments.

Calsporin® in the diet affected the rumen volatile fatty acids concentrations significantly, relatively more acetic acid (+2.1%) and less propionic (-2.7%) and valeric acid (-0.3%) compared to the control diet. Ammonia concentration in rumen fluid did not differ between treatments.

Faecal digestibility was measured by using an inert marker supplemented via the diet. The composition of the faeces and fibre digestibility was influenced by the treatment. Fibre digestibility (NDF, ADF as well as hemicellulose and cellulose) increased significantly by T1 Calsporin treatment, up to 5%.

Microbiota profiling of rumen fluid samples showed differences. Listing of dominant bacteria and archaea at phylum, family and genus levels in samples taken at three time points were influenced by the treatments applied.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N°730924

Based on the taxonomic abundance profiles of the bacterial-dominated microbiome (16S v5v6 region) of the different samples, rumen fluid samples collected at three time points from cows fed the control diet or Calsporin based diet showed significant difference in bacterial community composition for time and treatment. However, the taxonomic abundance profiles of the microbiome dominated by archaea (16S v1v3 region) showed significantly different bacterial community composition only in the treatment.

In conclusion, under the present circumstances, the addition of Calsporin® did not positively nor negatively affect performance in early- to mid-lactating dairy cattle. However, by feeding this feed additive, fibre digestibility improved, and substantial differences occurred in rumen fatty acids and microbiota profile.

4.8 Amino acids requirements in early lactation dairy cows by Lahlou Bahloul (Adisseo)

Final report from the User:

Hypothesis, objectives and results: Combined objective of more cost effective and less polluting dairy farms could be met, without compromising productivity, through a reduced input of dietary protein, with rations balanced for individual essential amino acids (EAA). In recent research, substantial increments in milk yield (MY) and marginal N efficiency were reported with increased supply of digestible protein in early lactation. However, it is not known if this large impact could be obtained by increasing the supply of only a few EAA. Indeed, recommendations for individual EAA were determined mainly from data from mid-lactation cows. Furthermore, the N efficiency would be even higher if MY increases via the supply of targeted EAA rather than supplying all AA. Our hypothesis is that supplementation of targeted EAA in early lactation will increase MY, N efficiency, and decrease metabolic disorder incidence

Our objective was to investigate the effect of continuous abomasal infusion of total AA (TAA) or only essential AA (EAA) in early postpartum dairy cows on performances and metabolism. Nine multiparous Holstein cows were used in a randomized block design with repeated measurements at 5, 15, 29, and 50 days in milk (DIM). At calving day, TAA (n=4; casein profile) or only EAA (n=5; EAA portion of TAA) was initiated. The TAA was graduated with half of full dose at 1 day in milk (DIM), full dose (805 g/d) at 2 to 5 DIM, and followed by daily reductions until 0 g/d at 35 DIM. Cows received the same TMR (NE: 6.85 MJ/kgDM, MP: 102 g/kgDM). Feed intake and milk yield were recorded daily. Milk samples and six sets of tail and mammary venous plasma samples were obtained at sampling days. The DMI did not differ between treatments (P=0.55). Overall, with no treatment × DIM interaction (Trt×d), milk yield was greater with TAA compared with EAA (P<0.01; 47.9 vs 39.3 kg/d, SEM=1.4) as was milk protein yield (P=0.01; 1635 vs 1393 g/d, SEM=50). Milk fat content was lower with TAA compared with EAA (P=0.02; 41 vs 47 g/kg, SEM=1.5), but treatments did not affect milk fat yield (P=0.20). The arterial total EAA concentration was lower (Trt×d=0.01; SEM=0.07) with TAA compared with EAA at 5 (0.97 vs. 1.24 mM) and 15 DIM (0.97 vs 1.13 mM). The arterial total non-EAA concentration was higher (Trt×d<0.01; SEM=0.04) with TAA compared with EAA at 5 DIM (1.35 vs. 1.17mM). Yet, plasma concentration differences across the udder of EAA and non-EAA did not differ between treatments (P>0.88), indicating that the intra-mammary utilization of both EAA and non-EAA was changed. Arterial urea concentration was greater (Trt×d=0.02; SEM=0.21) with TAA compared with EAA at 5 (2.87 vs. 1.75 mM) and 15 DIM (3.06 vs. 1.90 mM) indicating catabolism of some of the AA supplied with TAA in early but not later lactation. Results indicate that some of or all non-EAA are as important as EAA in the early postpartum period. Continued higher milk yield through 50 DIM with TAA after ceasing infusions indicate a carry-over effect.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N°730924

How do you expect to disseminate the results: These results will be published during 2022.

Any suggestions to improve the TNA procedure: no suggestions

4.9 Increased N-utilisation from dairy cows by phase feeding of protein by Nicolaj Ingemann Nielsen (SEGES)

Final report from the User:

The main objective of the project: The objective of this study is to increase feed N-utilisation and milk yield in dairy cows by the targeted use of quantity and quality of feed protein during early lactation.

The hypothesis that are tested: Cows getting high supply of metabolisable protein the first few weeks after calving will have higher milk yield and better N-utilisation than cows fed a common TMR with 17% crude protein.

The main scientific outcome, innovation/impact of the results: The results were not as clear as we had expected. There was tendency towards higher ECM-yield for phase fed cows compared to control, however only in a few weeks and not for the whole trial period of the first 15 weeks of lactation. Therefore the effect of N-utilisation was not that clear either. There is a need for further test of feeding high AAT should happen immediately after calving in contrast to what we did, namely supply the high AAT 4-5 days after calving. Furthermore, there was a surprisingly big difference in milk yield in period '1' between 2 treatments, that was fed the same diet. This difference is despite a lot of effort and work still unexplained.

The results will be a part of the foundation for new feeding and grouping strategies that will increase N-utilisation and milk yield in dairy cows. The results from this trial and other trials will form the basis for implementation of phase feeding on a number of commercial pilot farms in Denmark.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N°730924

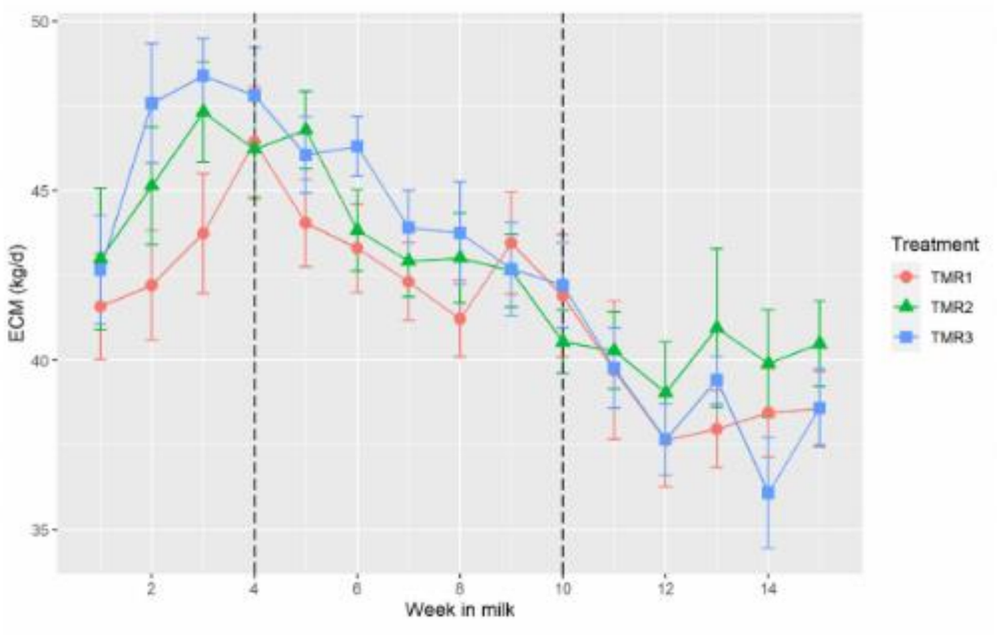

Figure 3. Weakly means of energy corrected milk (ECM) (Sjaunja et al., 1991) for each experimental week over the whole experimental period with ± SE. Dashed lines distinguish between periods 1, 2 and 3. TMR1 has diet 17 AAT (g amino acids absorbed per MJ NEL) in all periods, TMR2 has diet 20 AAT in period 1 and 16 AAT in periods 2 and 3, TMR3 has diet 20 AAT in period 1, diet 17 AAT in period 2 and diet 15 AAT in period 3.

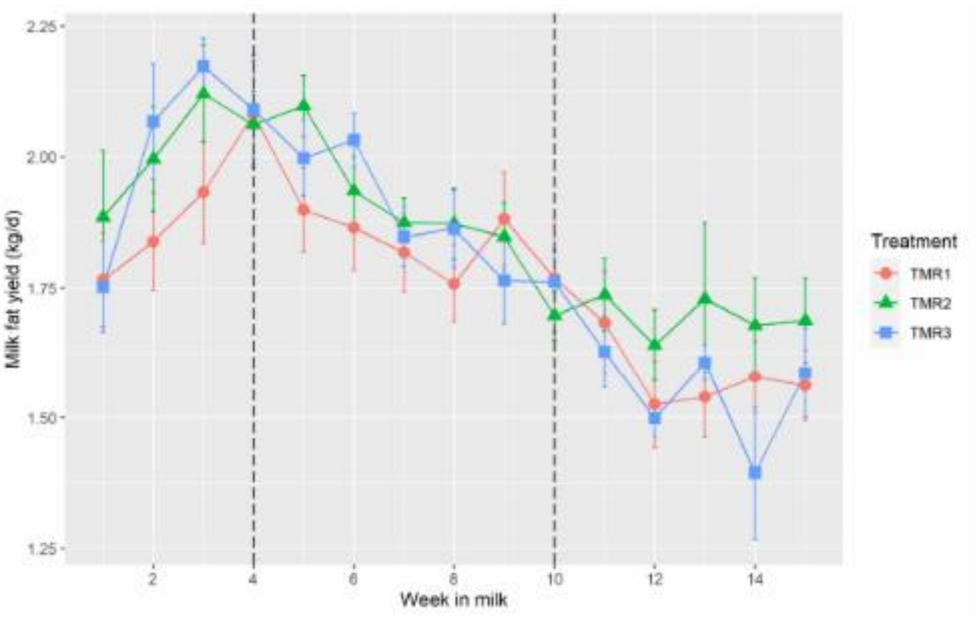

Figure 6. Weekly means of milk fat yield of the treatments with ± SE. Dashed lines distinguish between periods 1, 2 and 3. TMR1 has diet 17 AAT (g amino acids absorbed per MJ NEL) in all periods, TMR2 has diet 20 AAT in period 1 and 16 AAT in periods 2 and 3, TMR3 has diet 20 AAT in period 1, diet 17 AAT in period 2 and diet 15 AAT in period 3.

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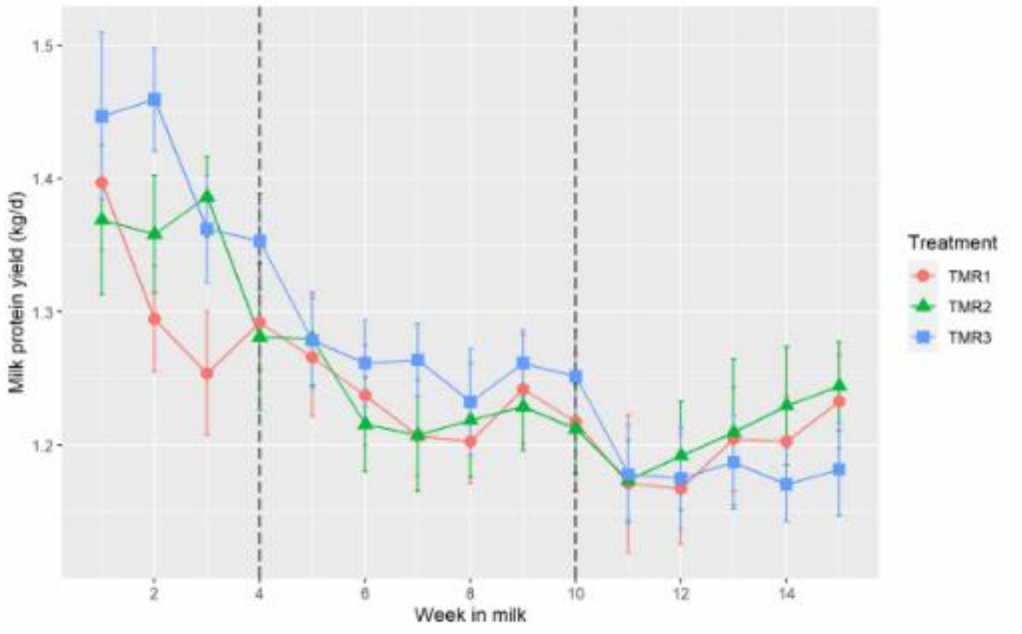

Figure 8. Weekly means of milk protein yield of the treatments with ± SE. Dashed lines distinguish between periods 1, 2 and 3. TMR1 has diet 17 AAT (g amino acids absorbed per MJ NEL) in all periods, TMR2 has diet 20 AAT in period 1 and 16 AAT in periods 2

Any other achievements of the visit: Due to Covid19 there was no visit to Reading University

How do you expect to disseminate the results: Report to dairy-advisors and farmers. Presentations of the main findings at Dairy nutrition seminar in September 2021 or Cattle Congress in February 2022

Any suggestions to improve the TNA procedure: The TNA procedure is a super and fruitful initiative – due to Covid19 we (SEGES) could not see the facility in Reading which was not ideal. However, the setup with TNA is good!

4.10 From feed composition to animal performance by using Near Infrared Spectroscopy by Francisco Maroto (University of Cordoba).

Final report from the User:

The main objective: The objective of this project was to study the feasibility of feed NIR spectra to evaluate animal response, measured as feed intake and milk production.

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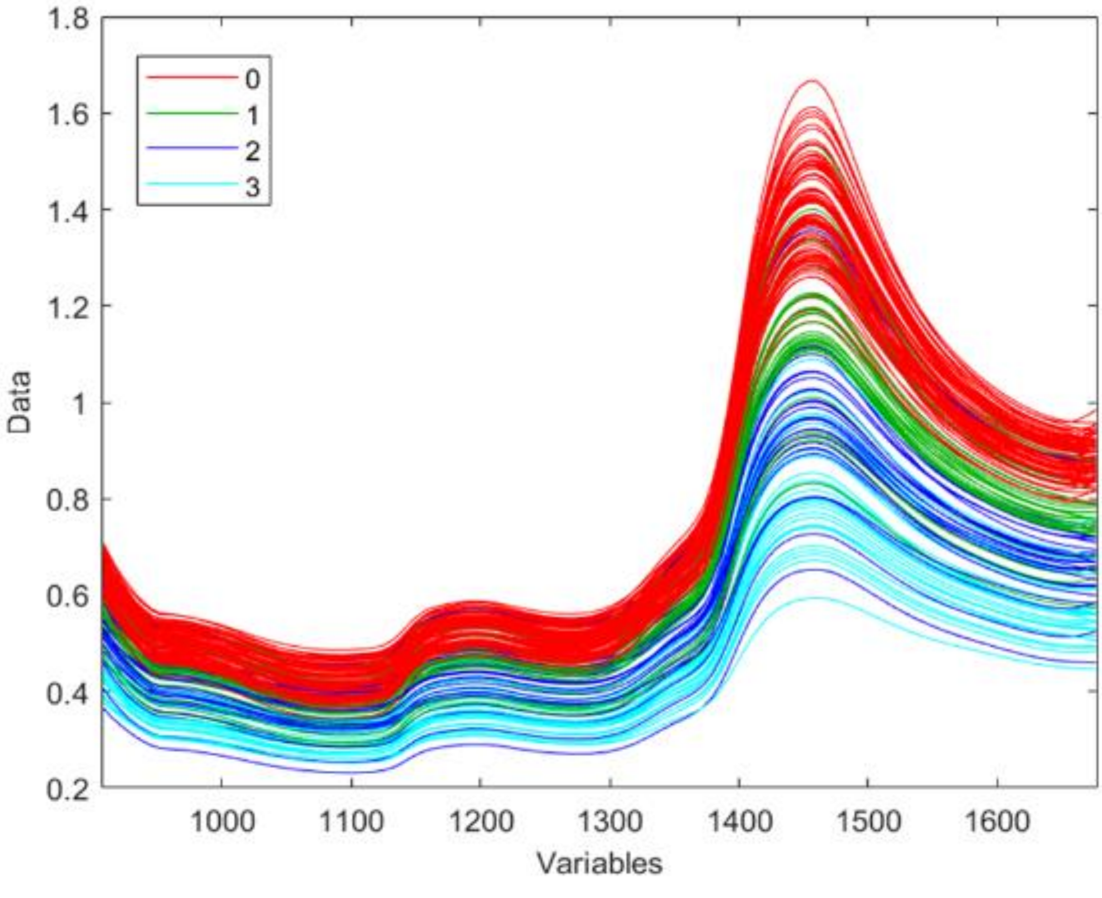
Hypothesis: Near Infrared Spectroscopy (NIRS) has demonstrated to be a precise and cost-efficient tool for the evaluation of feed composition, even in complex matrices like Total Mixed Rations (TMR). Normally, feed composition data are included in feeding models in order to predict animal response. However, it is well known that NIR spectra contain much more information about feed samples than chemical composition, so we hypothesize that feed spectra can be used to directly predict animal response, avoiding prediction errors associated to feeding models.

The mail scientific outcome, innovation/impact of the results: The main scientific outcome of the project is the validation of the possibilities of NIRS technology to directly estimate animal response, in terms of feed intake and milk production. Universal calibrations will not be developed during this project, because of the limited number of cows and rations. However, it can be the first step to raise larger scale projects, which have the potential to highly impact animal feeding in the future. Having real-time information about animal response associated to each diet (not theoretically but measured) has the potential to improve farm profit and reduce livestock environmental impact by means of a better adjustment between diets and animal needs. By the moment, we are still working on data analysis. Some preliminary results are shown below. We planned to have 4 TMRs with variable composition during the experiment, in order to have variability in animal response (needed for NIRS calibrations). For that, we replaced a portion of the high-quality forage in the ration with straw (0, 5, 10 and 15% in the different experimental groups). We can see this variability in the spectral signals of the different diets:



However, in the PCA below, we can see that there is also some overlap between the different types of diets. For example, some samples of diet 1 (5% replaced) are similar to some samples of diet 0 (0% replaced - control) and others to samples of diet 2 (10% replaced).

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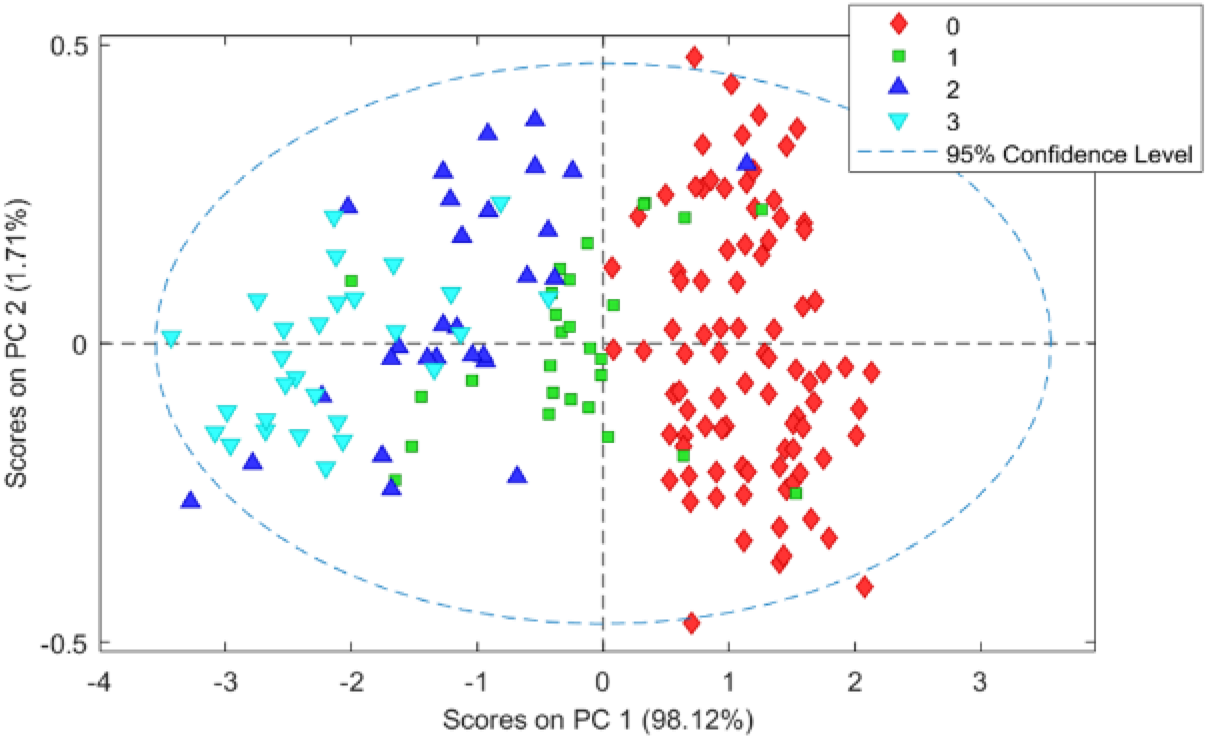
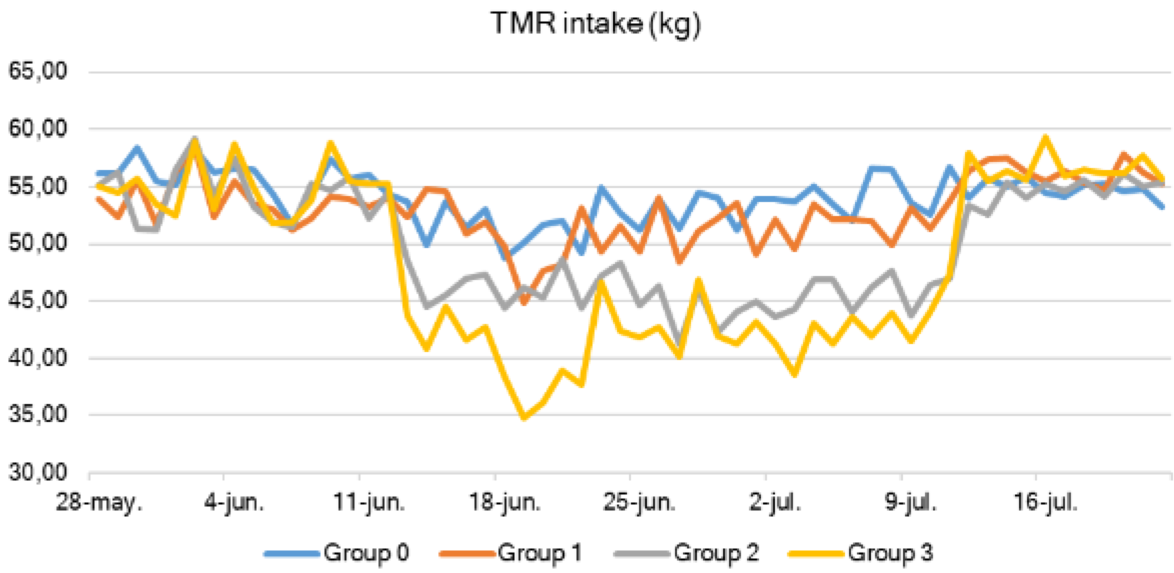


The variability between days for the same diet is important to understand the variability of animal response.

As expected, we also obtained variability in animal response:



At the beginning and the end of the experiment all animals were eating the control diet, and they have a similar TMR intake. During the experimental phase, cows were divided in four groups, three of them eating altered diets. Cows in experimental diets ate less kg of TMR and the change was bigger for the group eating the most altered diet. However, there is an important variability between days in cow intake, even in the control group, and we are currently working on diverse smoothing techniques to obtain an intake value that can be used for NIRS calibrations. On the other hand, some cows had abnormal

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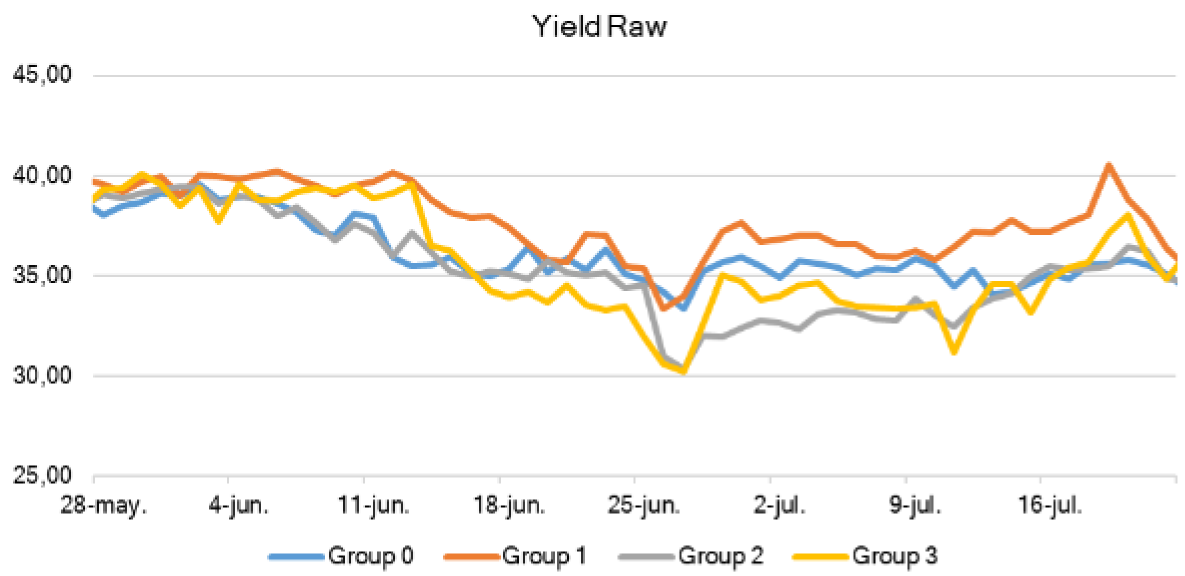
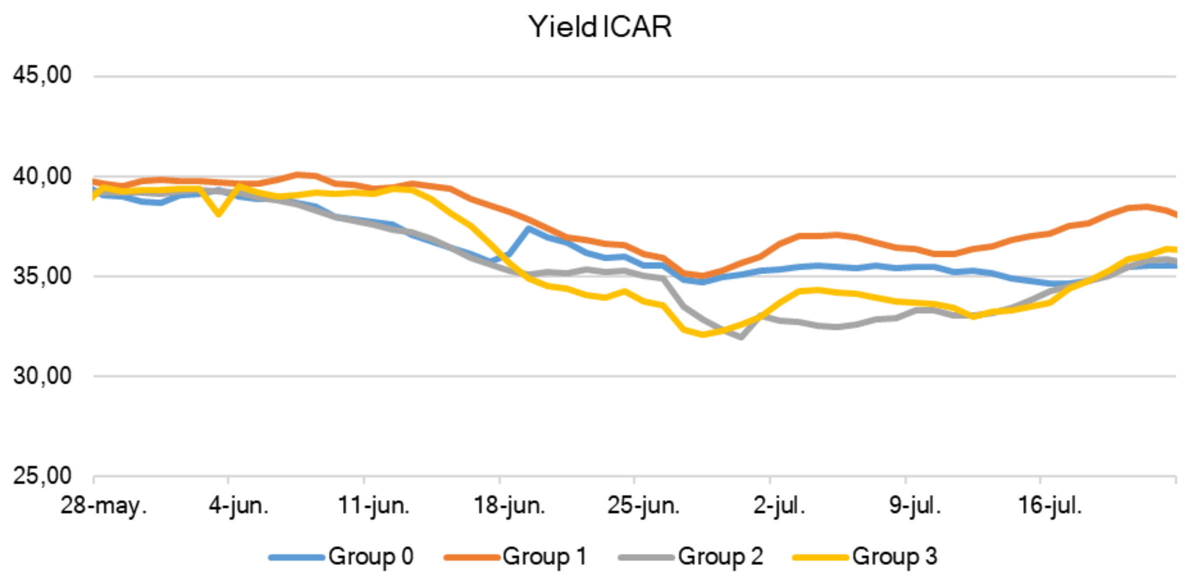
behaviour or health problems during the experiment, and their data must be cleaned before continuing with data analysis. We also had a variable response in milk production, which can be smoothed in different ways (some examples below):





In this case, the original production values were not recovered after the experimental phase (when all cows go back to control diet) so the previous production level need somehow to be included in calibrations. On the other hand, we observed an unexpected decrease in milk production of control group. It is due to the substitution of cows with problems during the experiment and due to an abnormal production response of some cows. For milk production, we are currently analyzing individual cow data in depth.

Any other achievements of the visit: For the user, the collaboration with the host institution (Aarhus University) during the TNA project was very lucrative, not only because of the research experiment itself, but also in terms of training. During the visit to the research farm, the user learnt some issues regarding animal management, data gathering that must be considered for a successful experiment development. This information will be valuable for the user when establishing other experiments involving animals in his own institution.

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How do you expect to disseminate the results: We plan two refereed publications from data obtained in this study. These papers will be published in open journals. Additionally, we will present the main results of the study in at least one scientific meeting, preferably an international congress, such us the EAAP Annual Meeting to be held in Porto (August 2020). On the other hand, a master student is currently working on her master thesis on the basis of the samples and data gathered during the experiment. The results of this thesis will be publicly available. Finally, 172 TMR samples (approx. 0.6 kg each) collected during the experiment has been dried, frozen and stored in the sample bank of the University of Cordoba at -20ºC. These samples, together with their reference data, are available for further studies.

Any suggestions to improve the TNA procedure: In general, the procedure is well organized. It would be nice to have more time for the elaboration of the last report. Data analysis can be a heavy task and more than 30 days would be needed to include preliminary results in the report, especially in the case of Universities, where research tasks share time with teaching activities.

4.11 Improving the nutritive value estimation of multi-species forages for beef cattle by Sophie Herremans (CRA-W)

The project was withdrawn due to lack of national funding being secured.

4.12 Impact of Agolin Ruminant on feed efficiency and methane emissions of finishing beef cattle by Beatrice Zweifel (Agolin)

Final report from the User:

Objective: The objectives of this study were to test the effects of AR on 1) methane emissions from, and 2) feed efficiency of suckler beef steers.

Hypothesis: The hypothesis was that cattle receiving AR will have lower methane yield than control cattle and/or better feed efficiency.

Main Findings: There was no significant difference in methane yield (g CH_4 / kg DMI), digestibility corrected yield (g/kg digestible DM), volume (l/day) or mass (g/day) between treatment groups at the Baseline methane measurement period (ANOVA, $p > 0.05$, see Table 1 for mean values and SEMs). During the treatment period there was a significant effect of Treatment on methane yield ($p<0.05$), methane volume and mass ($p < 0.01$), with values higher in the AR group. However, there was no significant difference in digestibility corrected methane yield.

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Table 1: Effects of treatment and breed on methane volume (litres CH_4/day), mass (g CH_4/day) and yield (gCH_4/kg DMI) on Aberdeen Angus sired and Limousin sired steers. SEM = standard error of the mean. C = Control, AR = treated with Agolin Ruminant.

	Aberdeen Angus X		Limousin X					
	C	AR	C	AR	SEM	Treatment	Breed	Baseline
Baseline								
CH_4 Volume (l/day)	333	340	305	326	13.0	-	-	-
CH_4 Mass (g/day)	218	223	200	214	8.5	-	-	-
CH_4 Yield (g/kg DMI)	25.9	25.1	25.7	27.3	0.89	-	-	-
CH_4 Yield (g/kg Digestible DM)	50.8	47.8	47.7	50.9	1.2	-	-	-
Treatment								
CH_4 Volume (l/day)	267	303	270	276	10.5	$p < 0.01$	ns	$p < 0.01$ *
CH_4 Mass (g/day)	175	199	177	181	6.9	$p < 0.01$	ns	$p < 0.01$ *
CH_4 Yield (g/kg DMI)	21.5	21.7	21.2	24.3	0.77	$P < 0.05$	$P < 0.05$	$p < 0.01$
CH_4 Yield (g/kg Digestible DM)	45.9	46.7	46.4	54.3	1.1	ns	ns	$P < 0.05$

* Significant Treatment x Breed interaction

4.13 An holistic approach on transforming molasses and liquid by-products into more efficient sugar-based liquid feed to increase dairy cattle efficiency by Luiza Fernandes (ED&F Man Liquid Feeds)

This proposal has been withdrawn.

4.14 Sustainable ruminant production: methane emission, microbiome and immune function in dairy cattle by Angela Schwarm (NMBU)

Final report from the User:

The main objective of the project: This project aims to delineate interactions between emissions, microbiome, immune function and production performance of dairy cows. Low methane emissions were associated with higher feed efficiency, but more recently also with less efficient fibre digestion. Accordingly, low methane emitting cows seem to have a decreased immune response, probably lacking the energy to sustain an energetically costly adequate immune response. Furthermore, it is largely unknown how microbial community structure reflects low or high methane emissions.

Several priority areas are addressed: the trade-offs between animal performance and reducing GHG emissions (3.2), understanding the components of feed efficiency and robustness (3.3), animal health (3.4) and comprehensive physiological studies (3.7.2). The results address the objectives of SmartCow by helping to prepare efficient and robust cattle for the future livestock systems. The implications for users could be that selective breeding of low methane emitters may not be sustainable.

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The hypothesis that are tested: It is hypothesized that low and high methane emitting cows (1) differ in quantity and quality of rumen microorganisms. Further it is hypothesized that low emitting cows are characterized by (2) a lower feed conversion and milk production efficiency and (3) a lower immune response than high methane emitters.

The main scientific outcome, innovation/impact of the results: Our project was based on the findings by Meese et al. 2020 in Journal of Dairy Science (https://doi.org/10.3168/jds.2019-17584), reporting early lactating cows with a low immune response cows produce less methane per unit of body weight and per unit of energy-corrected milk compared with medium and high immune-responder cows.

In line with this previous result, our first data evaluation shows a slight positive relation between methane yield and immune response (Figure 1). In our study the proliferation index ranged from 1.43 to 3.83 and the methane emission per unit of dry matter intake ranged from 16.3 to 30.9 g/kg.

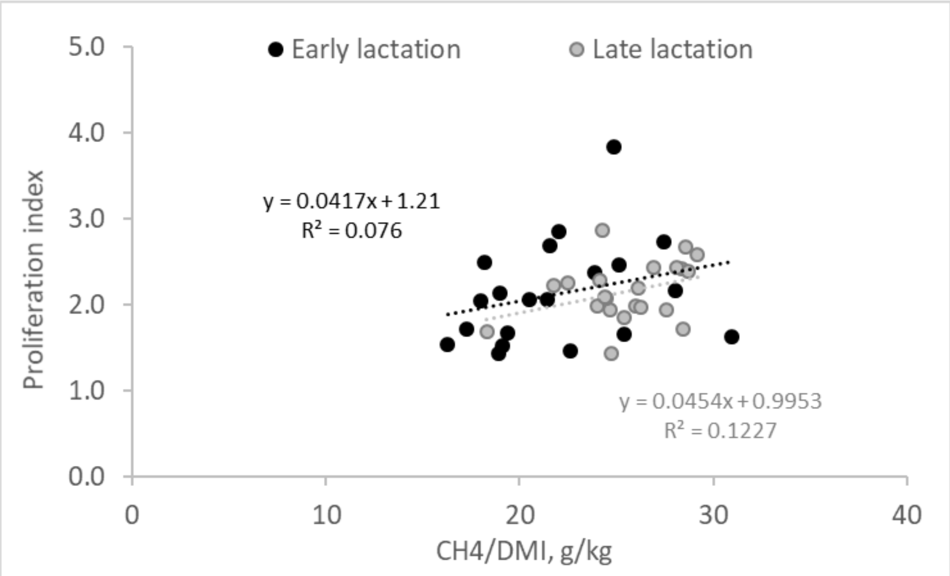

Figure 1. Relationship of methane emitted per unit of dry matter ingested with the proliferation index of peripheral blood mononuclear cells.

In the next step we will categorize the cows retrospectively into groups with low and high methane emissions. This will be done for early and late lactating cows separately. In Meese et al. (2020) we categorized as follows: low responders had a proliferation index (**PI**) of 1.3–1.8, medium responders had a PI of 2.0–2.4, and high responders had a PI of 2.6–4.3. The proportion of low and high responders was defined as 1 SE below and above the mean, respectively. The data analysis will be done by our PhD student Puchun Niu.

Based on this, we will decide to send all or a selection of rumen fluid samples for 16S rRNA sequence analysis by DNAsense (https://dnasense.com/) in Denmark.

The 16S rRNA sequence analysis in rumen fluid will identify the taxonomy and relative abundance of microbial populations. Together with Phil Pope, we will analyse the association with the phenotype of

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the host animal with CowPI (a rumen microbiome focused version of the PICRUSt functional inference software) to predict the function from our 16S data.

Any other achievements of the visit: No visit due to covid-19.

How do you expect to disseminate the results: The findings of the proposed study will result in at least one joint peer-reviewed publication with the host institution. The manuscript will be written, submitted and hopefully published in 2021. The primary dissemination of results will be via open access publication of high standard and scientific meetings such as the Conference on Greenhouse Gas and Animal Agriculture. In addition, the results will be disseminated to colleagues, journalists and the general public through local publications, Twitter communications and the website of the research group (https://twitter.com/AngelaSchwarm; https://www.nmbu.no/ans/angela.schwarm). The data of the study are available from the corresponding author on request after the results have been published. Our PhD student Puchun Niu will be involved in manuscript writing and data analysis and he plans to include this manuscript in his PhD thesis with submission in August 2022.

Any suggestions to improve the TNA procedure: Having an invited presentation at each other Institutions (user/or involved lab member will present at host facility and facility manager/or involved lab member will present at user institution), could be online.

4.15 Detection of reproductive events with smart collars suitable for extensive cattle systems by Ignacio Gomez Maqueda (Digitanimal)

Final report from the User:

Background: The profitability of beef, and especially dairy farms, depends greatly on the reproductive efficiency of cows. Timely and accurate detection of oestrus and calving events are essential for livestock farmers, who are increasingly relying on automated systems based on sensors for the detection of reproductive events. However, these devices are not generally suitable for rangeland systems since they require energy supply and wireless connection. Low-power wide-area network (LPWAN) technology stands out as a suitable alternative for rangeland systems, nevertheless, this technology has some limitations, such as the message size and frequency. The SmartCow TNA project "Detection of calving and oestrus in cattle by smart collars suitable for rangeland systems" carried out at INRAE Le Pin is aimed at developing artificial intelligence algorithms for detecting calving and oestrus events in cattle based on GPS and accelerometer data gathered, processed, and transmitted by smart collars suited for rangeland systems. High frequency data is needed to detect reproductive events; however, energy and connectivity constraints are major obstacles for the implementation of these tools in rangeland systems. Providing smart collars with edge computing capabilities could help to overcome these obstacles. Two independent studies have been performed within this project at INRAE Le Pin facilities, where high quality data has been gathered to develop artificial intelligence algorithms for the detection of reproductive events. The proposed conditions for both studies were:

1) Calving monitoring: 45 smart collars (3D-accelerometers) were placed on pregnant cows 3 to 4 weeks prior to calving. One week before the expected calving date, these cows were moved to calving pens, where they were filmed 24 hours a day to collect behavioral labels

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for the development of artificial intelligence algorithms for calving detection based on accelerometer data.

2) 2) Oestrus monitoring: 80 smart collars (GPS and 3D-accelerometers) were placed on open dairy heifers for 6 weeks. These free-ranging heifers had open access to pasture areas. Some visual observation sessions were arranged for gathering information on the cows' behaviours that could be potential signs of heifers' heat.

The COVID-19 pandemic has had a significant impact on the project. In March 2020, the experiments were interrupted due to the lockdown, restarting in January 2021. Since then, the project tasks have been adapted to the situation. Although the initial project schedule was delayed, the planned tasks have been completed.

Methodology: Every reproductive event that has occurred within any of the two studies (calving and oestrus monitoring) has been assigned to a particular experiment. Each experiment is defined by the following relationship: collar - animal - reproductive event - date, so that each experiment, consisting of the data collected by a smart collar mounted on a cow that was due to have a reproductive event on a specific day, is uniquely identified. The smart collars used in the project have been adapted to the requirements for the detection of reproductive events, mainly focusing on gathering high frequency data. Smart collars with 3-D accelerometers have registered data every 100 ms, while smart collars with GPS have registered data every 2 minutes. The smart collars include a SD card where the data is stored with an associated timestamp that is provided by an internal chronometer.

Oestrus detection: In the oestrus monitoring experiments, smart collars with GPS and 3D-accelerometers have been mounted on open heifers for 6 weeks. These heifers have also been fitted with heat detection collars provided by INRAE Le Pin. Throughout this time, frequent live observations of the cows have been arranged for the detection of specific behaviours that could be potential signs of heat. The confirmed date of heat, as indicated by the heat detection collars, and the visual observation of behaviours have been registered in an Excel file. At the end of the 6-week period, the smart collars were removed from the heifers. Once the collars were removed, the farm manager and his team at INRAE Le Pin retrieved the data from the collars' SD card.

GPS and accelerometer data have been gathered in the oestrus monitoring experiments. However, up to this point, the data analysis has been only focused on the GPS data. First of all, a diagnosis of the device functioning has been performed. After this, the GPS coordinates have been filtered and resampled for obtaining the heifers' locations in fixed, two-minute intervals. Two different approaches have been tested for trying to capture indirectly any signs of heat, such as the heifer restlessness or the heifer standing to be mounted by other heifers. The first approach is based on the analysis of the distances travelled by heifers on different time windows, while the second approach is based on the analysis of the proximity between different heifers. The analysis of the distances travelled by heifers has been performed comparing different time windows. Slicing time windows with an 8-hour length have been used, meaning that for every 8- hour period (0 - 8, 1 - 9, 2 - 10, …) the distance travelled by the heifers has been calculated. Then, this slicing time windows have been compared for different days to check whether there have been any significant differences. On the other hand, the analysis of the proximity between heifers has been tested. In this case, given that the trajectories have been resampled for fixed timestamps, the distance to the closest heifer at every 2-minute interval has been calculated and analyzed.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N°730924

Calving detection: In the calving monitoring experiments, the smart collars have been mounted on pregnant cows (both beef and dairy cows) 3 to 4 weeks before calving. One week before the expected calving date, cows were moved to the calving pens. A few hours after calving, the smart collars were removed from the cows. The farm manager at INRAE Le Pin and his team registered in a Google Drive file all the information required (event datetime, animal ID, …) and retrieved the data from the collars' SD card. IP cameras in the calving pens were used for filming the cows' behaviours 24 hours a day, however, some video recordings have been lost due to technical issues with the IP cameras. Nevertheless, the available recordings have provided high quality reference data for modelling the cows' behaviours using 3-D accelerometers. The video recordings of each experiment have been splitted into 15 minutes long files and stored in a Google Drive folder. BORIS software has been used to label the cows' behaviours based on the available video recordings. BORIS is a free, open-source software for video/audio coding and live observations. This software includes the following functionalities: definition of ethograms and exclusion matrices. Ethograms describe the basic behaviours that aim to be modelled. Exclusion matrices consider mutually exclusive behaviours. These functionalities are provided within BORIS software to facilitate the labelling process. Finally, the generated labels have been exported in xlsx format and uploaded to a Google Drive folder.

The first step in the information analysis process is the diagnosis of the device functioning. The errors found mainly consisted in reboots of the device and corrupt or incomplete records. After fixing the existing errors, a visual exploration of the accelerometer data is made to check if the experiment has been completely captured. If the uninstallation timestamp of the device has not been registered, it is not possible to assume that the reproductive event has been captured. One of the main reasons behind incomplete experiments is a possible battery drain. The next step in the analysis is the synchronization of the accelerometer timestamp with the video recordings. For this, videos have been inspected for characteristic movements of the animal that might be easily recognizable in the accelerometer data, for example, calf licking. Two of these points in the time series are used for the synchronization, one at the beginning of the labelled dataset and the other at the end of it. This method ensures that the accelerometer data is perfectly fitted to the labels. Once the accelerometer time series has been synchronized with the labels, different statistics are calculated in order to proceed with the analysis. For this, an exhaustive search was conducted on different variables used in scientific studies based on accelerometers. In the end, more than 100 statistics or features were calculated based on the raw measurements recorded by the accelerometer. These statistics are computed in different time windows ranging from 5 to 60 seconds. The idea behind this approach is to define an efficient aggregation strategy for smart collars used in rangeland systems.

The data processing sets the stage for extracting quality information from the data itself. This has been done using different data science techniques such as Principal Component Analysis (PCA), feature selection techniques, linear regression, or multiclass classification. Every technique has been used to detect differences between behaviours or to directly estimate the current behaviour of the animal given its computed features in each time window. PCA gives an initial idea of how easily some behaviours are distinguished from others, while feature selection techniques allow to remove those features that do not contain relevant information to reduce the noise input to the models. Linear regression was used to estimate the proportion of behaviours in each time window. As this focus did not offer satisfactory results, we opted for a multiclass classification to estimate animal behaviour. Specifically, decision trees and logistic regression are the classification models used for its simplicity and its ease of export to any future commercial device that estimates animal behaviour in real time.

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Preliminary results:

Oestrus detection: Altogether more than 146 experiments were carried out, detecting more than 90 heat events. The analysis of the distances travelled on sliding time windows suggest that there is not a specific pattern for heat detection shared among all the cows that have been monitored. The distances travelled can vary significantly depending on the available grazing area or the management type. Figure 1 shows the accumulated distance travelled by a cow on 8-hour intervals. In this case, the pattern is easily recognizable. On May 2, the day of the oestrus event, the distance travelled at midnight by the cow was way higher than the other days. However, most of the experiments carried out in the project do not show this clear pattern.

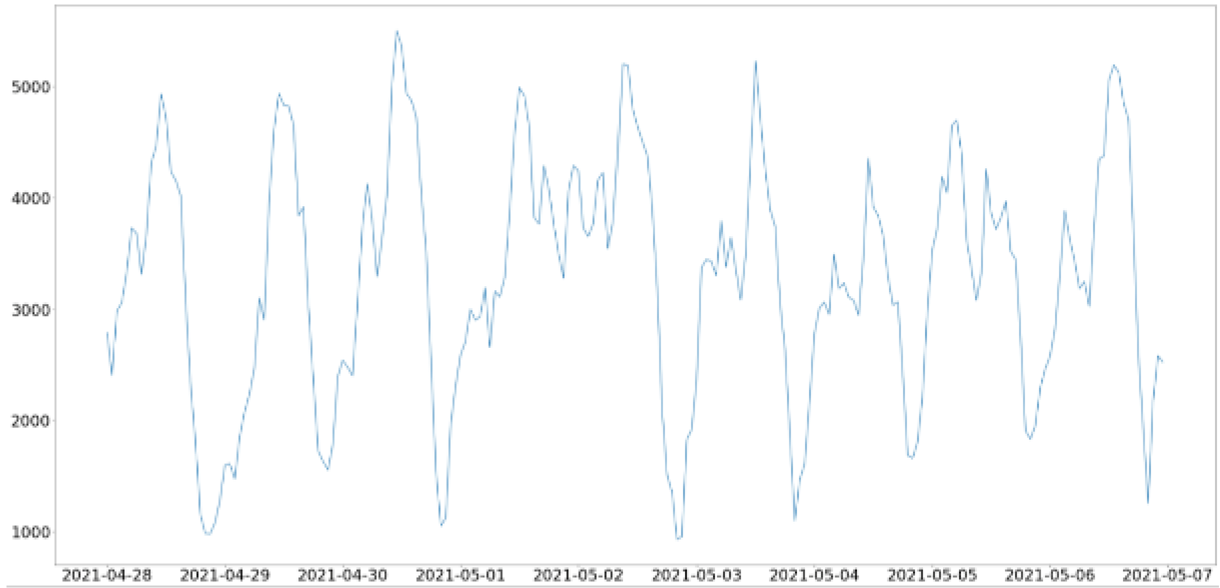

Figure 1. Accumulated distance travelled by a heifer in a time window of 8 hours.

The analysis of the proximity between heifers has not yielded good results. Figure 2 shows the distance between a particular heifer and the closest heifer in every 2-minute interval. The main purpose of this analysis was to try to identify whether the heifer on heat has been closer to other heifers, as an indirect sign of the heifer being mounted by other heifers. However, the complexity of the signal and the randomness of the proximity between heifers makes it difficult to find any patterns for heat detection.

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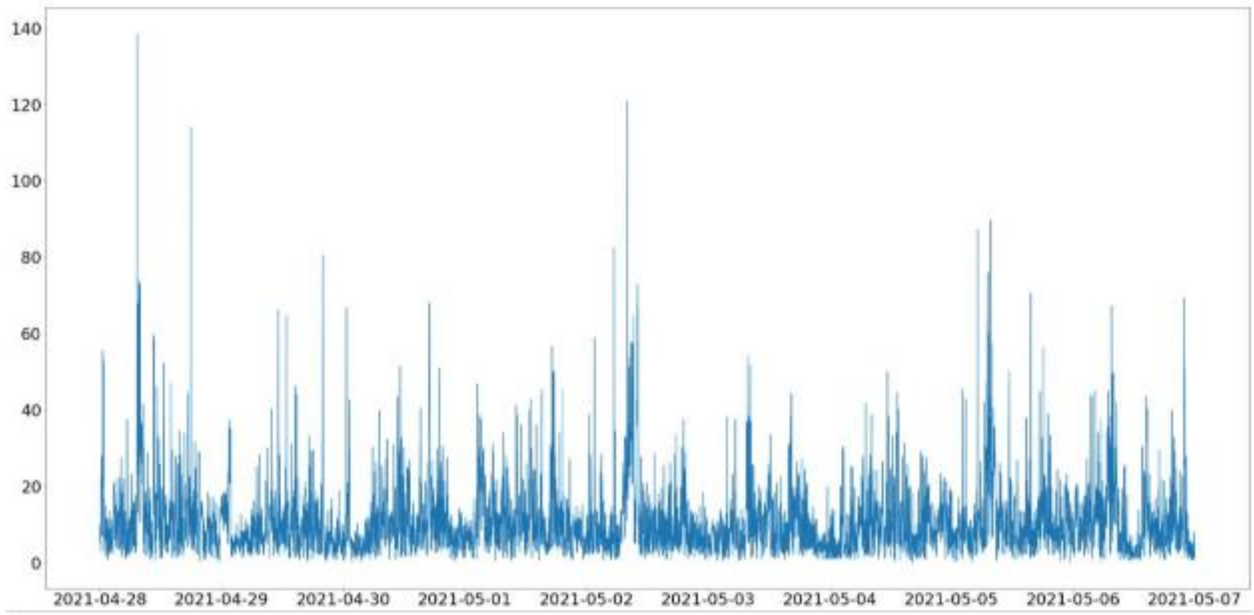

Figure 2. Distance between a heifer and its closest heifer within the herd in a time window of 2 minutes.

Calving detection: In total, 92 calving events were captured in the experiments. Several results and knowledge improvements have been achieved through its analysis depending on the phase of the modelling process. In Figures 3 and 4, PCA is applied to the data obtained from two different experiments, showing in different colours some of the behaviours labelled. The small points of each figure represent each observation recorded while the big ones represent the centroid of the observations for that behaviour. It is clear that, when there are enough observations of the animal lying in a lateral position, this behaviour is clearly differentiated from the rest. Besides, walking and grazing seem to be quite similar with each other while licking the calf, standing up or lying down are more distinguishable.

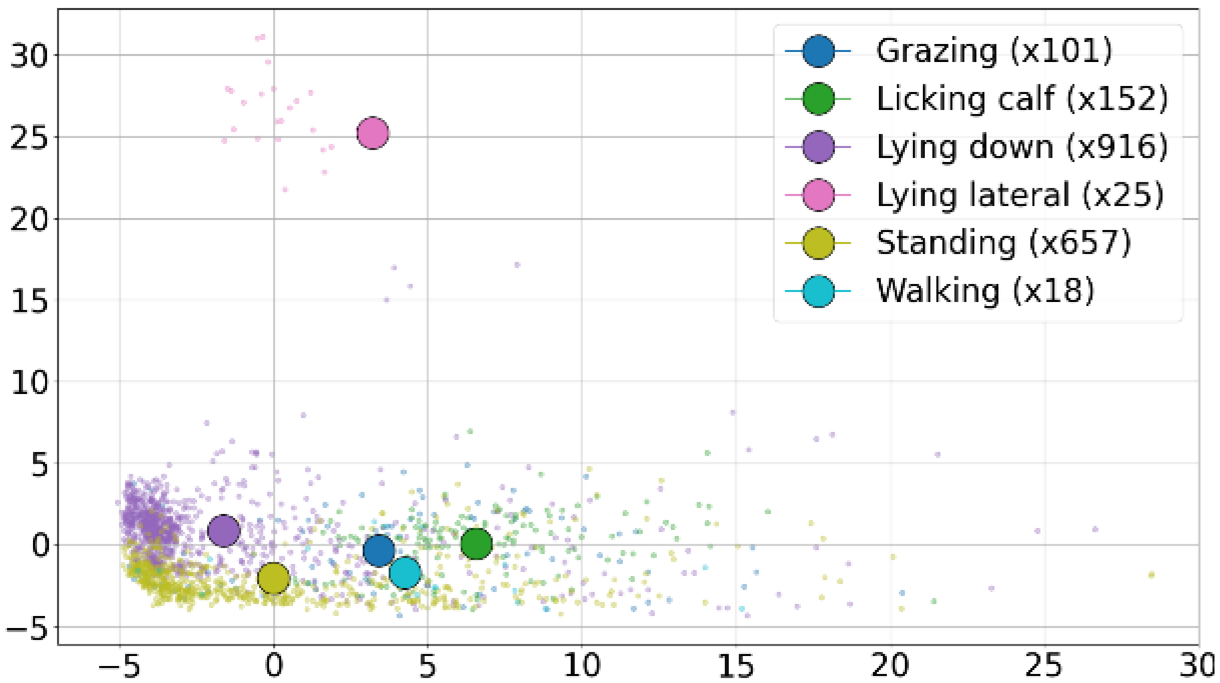

Figure 3. PCA applied to data obtained from a calving experiment. Enough points to distinguish the behaviour lying lateral.

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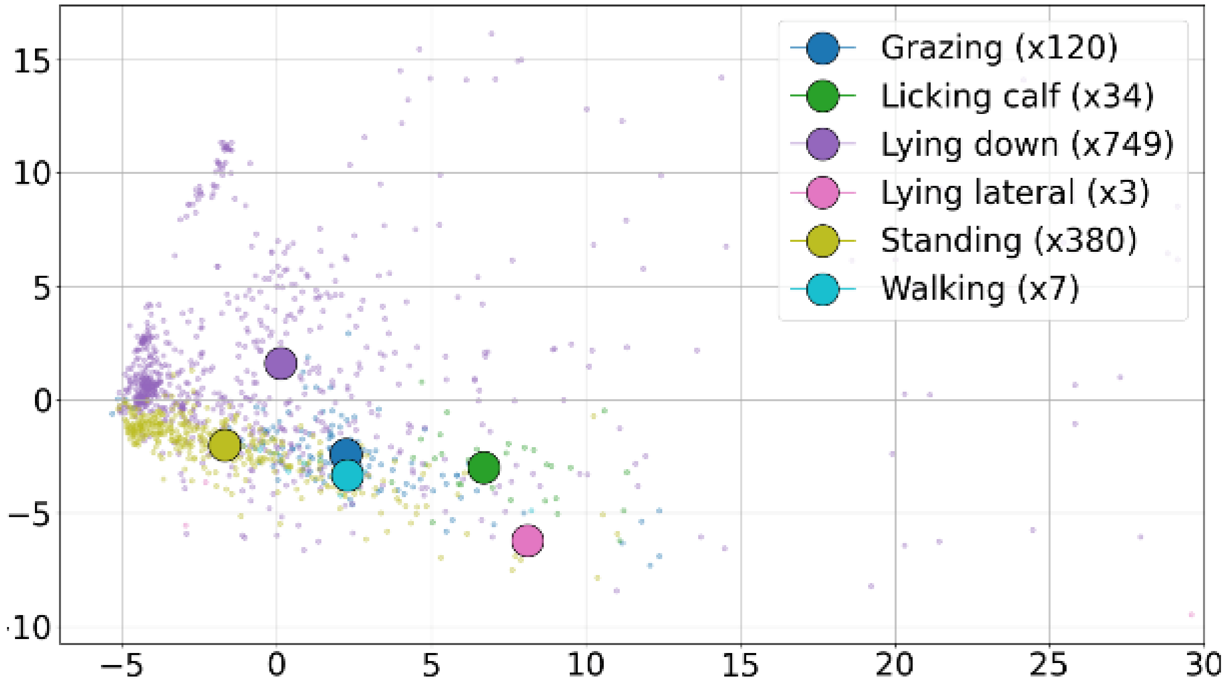

Figure 4. PCA applied to data obtained from a calving experiment. Not enough points to distinguish the behaviour Lying lateral.

For the feature selection, Boruta (Kursa & Rudnicki, 2010) was used following two different approaches: one considering only the windows size used to compute the features (Boruta_1) and other considering the windows size and the behaviour (Boruta_2). That is, in the first approach all the behaviours were used together while in the second each behaviour was isolated and then the features that best classified it were selected. Major improvements were achieved in the multiclass classification when applying any type of Boruta selection. Finally, the tests carried out to classify the labelled behaviours through a multiclass classifier showed different performances depending on the time windows selected. The performance of the different classifiers evaluated has been assessed using a generalization of the Matthews correlation coefficient (MCC) to multiclass problems (Gorodkin, 2004).

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Table 1. Results achieved for the multiclass classification per time window, model and feature selection approach.

Time window (seg)	Model	Features selection	MCC
55	Logistic Regression	Boruta_2	0.861211
50	Logistic Regression	Boruta_2	0.849644
45	Logistic Regression	Boruta_2	0.847411
60	Logistic Regression	Boruta_2	0.833869
15	Decision Tree	Boruta_1	0.815417
35	Logistic Regression	Boruta_1	0.815242
10	Decision Tree	Boruta_2	0.813254
25	Logistic Regression	Boruta_1	0.809285
5	Logistic Regression	Boruta_1	0.809223
40	Logistic Regression	Boruta_2	0.806986
30	Logistic Regression	Boruta_1	0.789571
20	Logistic Regression	Boruta_2	0.789070

Conclusions: The main difference between calving and oestrus experiments lies in the availability of video recordings. The accelerometer data combined with the labels obtained from video recordings in the calving monitoring experiments have proven to be very valuable information for the classification of behaviours, creating a new research line within the Digitanimal team. However, the preliminary results obtained from the analysis of high frequency GPS-data for heat detection suggest that GPS collars are not the best approach. Also, it is important to note that this is an ongoing project and there is still much work to do. The classification of behaviours will facilitate the detection of changes in the state of the animal and, therefore, the detection of reproductive events. Therefore, the classification of behaviours is a first step towards the detection of heat and calving events. Moreover, the outcomes of the SmartCow TNA project will enable the improvement of Digitanimal's experimental and commercial devices. The errors found in the data received from the experiments of this project helped to improve the performance of the smart devices used. Besides, new experiments are being carried out with the models developed estimating the animal behaviour in real time, with the objective of designing new commercial products.

References: Gorodkin, J. (2004). Comparing two K-category assignments by a K-category correlation coefficient. Computational biology and chemistry, 28(5-6), 367-374.

Kursa, M. B., & Rudnicki, W. R. (2010). Feature selection with the Boruta package. J Stat Softw, 36(11), 1-13.

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4.16 The effect of a molasses based liquid feed on in vivo fibre digestion and nitrogen use efficiency by Georgina Chapman (ED&F Man Liquid Products)

Final report from the User:

The main objective of the project: Previous *in vitro* rumen fermentation studies found increased nitrogen efficiency, fibre digestion and improved pH when a molasses based liquid feed was added to a dairy cow diet. The objective of the current study was to investigate the effect of incremental diet inclusion of a molasses based liquid feed (Regumix) in a lactating dairy cow ration on fibre digestibility, nitrogen utilisation and rumen pH. In additon to digestibility measurements, various production parameters were also measured including milk production and composition, dry matter intake and diet sorting. The rationale for this project is based on supporting sustainable farming by increasing the efficiency of home-grown forage utilisation and so reducing the reliance on bought in feeds. The improvement of fibre digestion can allow greater energy extraction from home grown feeds and improving nitrogen retention can lower the environemental impact of animal production by potentially reducing the amount of nitrogen excretion therefore, supporting long term sustainability on farm. If positive results are obtained, this will encourage further research to determine the extent to which molasses based liquid feeds can enable lower protein diets to be fed commercially.

The hypothesis that are tested: The hypothesis is that the treatment diet will improve whole tract fibre digestion and efficiency of nitrogen capture. Consequently, animal health performance will be improved in the treatment group in terms of milk yield, milk components and a more optimum rumen pH maintained. Sorting of feed will also be reduced in the treatment group due to the addition of a molasses based liquid feed.

The main scientific outcome, innovation/impact of the results: Sixteen cows were blocked in groups of four based on milk yield and stage of lactation then randomly assigned to one of the four dietary treatments in a 4 x 4 x 4 Latin Square design balanced for first order carry over effects. Three squares were used for production measurements and the fourth square was used for both production and digestion measures. Treatments periods were four weeks in duration and cows were incrementally adapted to new diets over four days at the beginning of each period.

Measurements for the fourth square consisted of milk yield and composition, diet intake and digestion, and urinary N excretion were obtained using total faecal and urine collection over the last five days of each period. Reticular pH was measured using a wire-less rumen pH bolus. Cows were fed a control diet composed of grass silage, maize silage and concentrate blend. Treatments were three levels of Regumix included to achieve intakes of 0.7, 1.4 and 2.1kg as fed, proportionally diluting the other diet components. This resulted in isonitrogenous and iso energetic diets with differing concentrations of carbohydrate fractions. Regumix is a high protein molasses based liquid feed with a typical dry matter specfication of 68% dry matter, 27% crude protein, 53% sugar and 13.1 ME.

The digestion results found that increasing inclusion of Regumix increased neutral-detergent fibre ($P<0.024$) and acid-detergent fibre ($P<0.088$) digestibility in a quadratic manner, with an increase in

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NDF and ADF digestibility over control of 33 (P<0.052) and 29 (P<0.043) g/kg respectively for the 1.4kg/d Regumix inclusion. N retention increased numerically with increasing Regumix and milk N output numerically decreased, but the effect was not significant. Milk yield and composition, digestion of other diet components and rumen pH were unaffected by Regumix inclusion in this square. In conclusion, adding 1.4kg of Regumix to a lactating dairy cow ration significantly increased fibre digestion without negatively effecting the rumen environment.

The production results were not as expected as dry matter intake decreased numerically with increasing Regumix, although the effect was not significant. Both energy corrected milk yield and fat corrected milk yield significantly decreased over control of 1.05 (P<0.05) and 1.09 (P<0.05) kg respectively for the 1.4kg/d Regumix inclusion. However, the original plan was for the increasing inclusion of Regumix to replace a proportion of the concentrates in the diet and the forage level to remain constant. However, the diets that were formulated proportionally diluted both the forage and concentrate components of the TMR thereby removing an increasing proportion of fibre from the diet with increasing Regumix. This is likely the cause for the different production results compared to other nutrition studies which have found the addition of a molasses based liquid feed to increase milk production, milk fat yield and dry matter intakes.

The digestion results show that the inclusion of a molasses based liquid feed can significantly increase fibre digestion and maintain an optimum rumen pH even when fed at high levels. This is a promising start to highlighting the benefits of a molasses based liquid feed as part of a long-term sustainability strategy on farm and utilizing less carbon expensive by-product feeds. Further research is warranted to investigate further the effects on N utilisation in lower protein diets as a strategy to encourage lower protein diets to be fed commercially without negatively affecting animal performance.

How do you expect to disseminate the results: The findings have been submitted as an abstract to EAAP 2021 and will also be presented at both internal and external conferences around the UK. Further abstract submissions will be made to Total Dairy to take place in October 2021. We work with most agricultural merchants within the UK, these results will be shared with all and passed on to customers. The findings of this study will also be distributed through key agricultural journals and press, including Feed Compounder, British Dairying, Dairy Farmer, Farmers Weekly and Farmers Guardian. The findings will also be communicated globally through our internal company portal to be shared with our international teams, this is including 15 European countries, each will receive a copy of the research results and this can be distributed through our network into the EU farmer base.

4.17 Evaluation of zinc sources in dairy cattle by Valerie Kromm (Animine)

Final report from the User:

Data quality: Data from the last week (week=12) of experiment (except for serum Zn, week=11) were analyzed using proc GLM, SAS software to investigate the linear and quadratic responses of incremental levels on Zn in the experimental diets. Intake and performance data were analyzed in proc MIXED, SAS software and differences between all treatments were controlled for multiple comparisons using the Tukey procedure. Since data for serum zinc and IgA measurements were not distributed normally for measured parameters, data for each treatment were compared to the control level with Wilcoxon rank test using proc NPAR1WAY, SAS software. Significance was declared at p<=0.05 and tendency towards significance was declared at 0.05<p<=0.15. Based on the laboratory analysis of the orts animals actually received an average of 41.1, 58.4, 85.2, and

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110.3 mg/kg DM Zn for groups that were designed to receive 40 (T40), 60 (T60), 90 (T90) and 120 (T120) mg/kg Zn respectively.

DMI, milk production and milk composition and body weight change: Data for DMI, milk production and milk composition and body weight change are presented in table 1. For dry matter intake (kg/d), milk production (kg/d) milk fat and protein content (%), milk fat and protein yield (kg/d) there was no linear or quadratic trend and parameters did not differ significantly amongst treatments. Energy corrected milk (kg/d; ECM) also did not follow a linear or quadratic trend with respect to increase of dietary Zn and ECM was not different for experimental treatments. The difference in feed efficiency (FE; ECM(kg)/DMI(kg)) Body weight change (kg; initial body weight-final body weight, BWC) failed to reach significance for all the treatments and there was no linear or quadratic response to increasing amounts of dietary Zn for FE or BWC.

Table 1. Least square means of DMI, milk production, milk composition and body weight change for dairy cows in the experiment receiving incremental levels of dietary Zn

Parameter	Treatment[1]				*SEM*
	T40	T60	T90	T120	
DMI (kg/d)	25.1	26.9	28.1	27.7	0.68
Milk production (kg/d)	27.2	26.6	30.1	28.4	0.98
Milk fat (%)	3.85	3.88	3.79	3.86	0.06
Milk protein (%)	3.78	3.74	3.75	3.77	0.02
Milk fat (kg/d)	1.04	1.01	1.11	1.07	0.03
Milk protein	1.04	0.97	1.11	1.07	0.04
ECM (kg/d)	29.54	28.22	31.73	30.52	0.93
FE[2]	1.17	1.07	1.15	1.11	0.03
BWC (kg)[3]	43.5	44.5	38.6	53.4	5.24

1: T40= 40mg/kg DM dietary Zn, T60= 60mg/kg DM dietary Zn, T90= 90mg/kg DM dietary Zn, T120= 120mg/kg DM dietary Zn

2: Feed efficiency= ECM(kg)/DMI(kg)

3: Body weight change= initial body weight-final body weight

Serum and Milk IgA and serum Zn: Data for serum and milk IgA and serum Zn concentration are presented in table 2. Serum IgA concentration (ng/L) was the same for T90 and T120 (p>0.48) and strongly tended to be higher for T60 (P=0.06) compared to T40 with no significant linear nor quadratic trend. For milk IgA (ng/ml) content, there was no linear or quadratic trend and milk IgA (ng/ml) did not differ significantly for all the treatments compared to T40. Serum Zn content

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(mg/L) increased linearly with increase in the Zn content of diets (P=0.05) but there was no significant quadratic trend. Serum Zn content strongly tended to be higher for T90 (P=0.05) and tended to be higher for T120 (P=0.13) compared to T40 while the difference between T40 and T60 was not significant (P=0.92).

Table 2. DMI, milk production, milk composition and body weight change for dairy cows in the experiment receiving incremental levels of dietary Zn (Mean±SD).

Parameter	Treatment[1]				*P* value[2]		
	T40	T60	T90	T120	C1	C2	C3
Serum							
IgA	226.5±78.72	288.3±107.67	235.8±127.2	244.7±81.73	0.06	0.87	0.48
Zn	0.82±0.09	0.81±0.133	1.02±0.187	0.95±0.134	0.92	0.05	0.13
Milk							
IgA	153.7±90.67	223.1±185.57	281.5±583.23	170.5±192.54	0.43	0.76	0.62

1: T40= 40mg/kg DM dietary Zn, T60= 60mg/kg DM dietary Zn, T90= 90mg/kg DM dietary Zn, T120= 120mg/kg DM dietary Zn

2: C1=T40 vs. T60, C2=T40 vs. T90, C3=T40 vs. T120

Health events: Mastitis was the most important health issue during the study. In fact, the incidence of mastitis during the study (33%) was unusually very high in comparison with our previous study (9%). The use of a not totally composted bedding material and arriving to the end of the rye-grass silage silo (which usually is mouldier) were the main causes that probably challenged the animals with their health events. Although no statistical analysis was done due to the low number of animals to evaluate health issues, cows in T120 had the lowest number of quarters with mastitis events.

Table 3. Incidence of clinical mastitis in dairy cows feed different doses of zinc supplemented with HiZox® as a feed supplement in the milking parlour. First row indicates the number of animals that had any mastitis event during the study. Then, Q0 means the number of healthy quarters within the mastitic animals, Q1 means the number of quarters that were treated once within the mastitic animals, Q2 means the number of quarters that were treated twice within the mastitic animals, Q3 means the number of quarters that became chronic within the mastitic animals.

	Treatments[1]			
	T1	T2	T3	T4
Animals	7	6	5	2
Q0	15	13	12	5
Q1	5	6	3	1
Q2	5	1	3	1

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Q3	3	4	2	1

[1]T1 = no Zn supplementation; T2 = Zn supplemented to have a final dose of 60 mg/kg DM; T3 = Zn supplemented to have a final dose of 90 mg/kg DM; T4 = Zn supplemented to have a final dose of 120 mg/kg DM

Conclusion: Zinc diet supplementation increased serum Zn concentration when Zn was supplemented at 90 and 120 mg/kg. Although no improvements in performance were observed, data from this study slightly indicated benefits on health performance due the lower number of quarters with mastitis in Zn-supplemented cows compared with non-supplemented animals.

4.18 Investigate the effect of inclusion of seaweed on milk production, feed efficiency and rumen microbiome of dairy cattle by Katerina Theodoridou (Queens University))

Final report from the User:

This study examined the effect of partial replacement (300 g) of corn meal with seaweed (*Ascophyllum nodosum*) on milk composition, efficiency, hematological parameters, rumen microbiome, and milk mineral composition.

The hypothesis tested were that *Ascophyllum nodosum* seaweed was able to replace corn in lactating dairy cows diet without impairing performance and milk iodine content, and is able to alter rumen microbiome by reducing methanogens.

The main scientific outcome, innovation/impact of the results are summarized in the abstract sent for presentation in EAAP and the tables below:

Lactating Holstein cows (n=48) were allocated into two experimental groups, balanced for parity, milk yield, contents of fat, protein, and somatic cell count (SCC), in a randomized block design. Diet treatments were (i) control (CON) and (ii) partial replacement of corn meal with *A. nodosum* (SWD; 330 g/day on dry matter (DM) basis). After a 2-week adaptation, cows consumed the experimental diets for 8 weeks. DM intake, feed, milk, and blood samples were collected daily, weekly, fortnightly and at the end of the trial respectively. Linear mixed models used diet, week, and their interaction as fixed factors, and cow (nested within diet) as random factor; with pre-treatment records as the covariate. Week was excluded from the model for hematological parameters (measured once). Milk yield, basic composition and efficiency parameters were similar between CON and SWD. When compared with CON, cows fed SWD had lower neutrophil concentrations (2.65e9/L vs 2.06e9/L respectively, p=0.009) and a tendency for lower white blood cell count (7.37e9/L vs 6.70e9/L, respectively, p=0.050); although their concentrations were within the normal range for healthy dairy cows in both diets. This study provides evidence that corn meal can be safely partially replaced with *A. nodosum* at 330 g/day, without negative implications to cows' productivity, milk quality and efficiency, while changes in measured hematological parameters did not indicate any potential health risks.

No visit was performed due to COVID-19 situation

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Results have been summarized in an abstract for 2022 EAAP SmartCow session, and Eric Newton from University of Reading is preparing a manuscript for publication including the analysis of milk, and feed samples provided. Also, rumen samples are under analyses for volatile fatty acids, ammonia, microbiome profile.

Methane production will be predicted according to VFA stoichiometry equation below:

Predicted CH_4 (ml) = 22.4 × (0.5 × AA − 0.25 × PA + 0.50 × BA − 0.25 × VA)

where AA, PA, BA, and VA are the production (mmol) of acetate, propionate, butyrate and valerate, and 22.4 is gas volume (ml/mmol gas) (Wolin, M.J. A theoretical rumen fermentation balance. J. Dairy Sci. 1960, 40, 1452–1459)

Table 1: Means, standard error (SE), and ANOVA p-values for the effect of dietary treatment (Control, no seaweed, CON; Seaweed, 330g fresh per day, SWD) on animal diet data, milk production and basic composition, and efficiency parameters.

	Dietary Treatments			**ANOVA p-Values [1]**		
Parameters	**CON**	**SWD**	**SE**	**Diet**	**Week**	**Diet x Week**
Dry Matter Intake (kg/d)	24.54	24.53	0.368	0.979	0.000	0.000
Milk Yield (kg/d)	32.12	32.02	0.312	0.826	0.532	0.430
Milk Fat (g/100g milk)	3.65	3.64	0.060	0.899	0.000	0.528
Milk Protein (g/100g milk)	3.36	3.31	0.021	0.137	0.000	0.466
Milk Lactose (g/100g milk)	4.90	4.89	0.014	0.534	0.000	0.286
Solids non-fat (%)	8.98	8.93	0.027	0.207	0.000	0.318
Milk Urea (mg/L)	224.1	211.4	5.59	0.123	0.000	0.466
Milk SSC (x1000/ml) [2]	152.7	90.6	35.80	0.709	0.012	0.988
Fat:Protein	1.09	1.10	0.0249	0.574	0.008	0.617
ECMY [3]	31.62	31.40	0.7552	0.673	0.001	0.331
Protein Efficiency (g in milk/kg of DMI)	44.04	42.84	0.484	0.096	0.000	0.000
Fat Efficiency (g in milk/kg of DMI)	47.72	46.82	0.733	0.382	0.001	0.000
Feed Efficiency (kg milk/kg of DMI)	1.32	1.32	0.035	0.859	0.000	0.000
ECMY Feed (ECMY/kg of DMI)	1.31	1.27	0.020	0.070	0.000	0.000

[1] Significances were declared at $p < 0.05$.

[2] p-values were generated from the common logarithm of somatic cell count (SCC) values.

[3] Energy Corrected Milk Yield = milk yield (kg) × (0.01 + 0.0122 milk fat (g/kg) + 0.0077 milk protein (g/kg) + 0.053 milk lactose (g/kg))

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Table 2: Means, standard error (SE) and ANOVA p-values for the effect of dietary treatment (Control, no seaweed, CON; Seaweed, 330g fresh per day, SWD) on final blood composition.

	Dietary Treatments			ANOVA p-Values [1]
Parameters	**CON**	**SWD**	**SE**	**Diet**
White Blood Count (10^9/L)	7.37	6.70	0.239	0.050
Neutrophils (10^9/L)	2.65	2.06	1.710	0.009
Lymphocytes (10^9/L)	4.36	4.33	0.203	0.932
Monocytes (10^9/L)	0.13	0.11	0.010	0.372
Eosinophils (10^9/L)	0.24	0.20	0.040	0.333
Basophils (10^9/L)	0.0003	0.0000	0.00035	0.330
Neutrophils (%)	35.9	30.7	2.06	0.060
Lymphocytes (%)	59.3	64.8	2.37	0.085
Monocytes (%)	1.71	1.70	0.146	0.958
Eosinophil (%)	3.083	2.957	0.418	0.650
Basophiles (%)	0.0035	0.0000	0.00345	0.330
RBC (10^12/L)	5.90	5.70	0.117	0.211
Hemoglobin (g/dL)	10.42	10.38	0.158	0.829
Hematocrit (%)	28.84	28.59	0.449	0.681
MCV (fL)	49.14	50.36	0.804	0.212
MCH (pg)	17.78	18.31	0.295	0.146
MCHC (g/dL)	36.18	36.36	0.226	0.527
RDW-CV (%)	22.00	20.96	0.677	0.169
PLT (10^9/L)	246.2	231.1	23.30	0.631
MPV (fL)	6.52	6.56	0.108	0.801

[1] Significances were declared at $p < 0.05$.

4.19 Impact of a phytogenic additive on methane production and performance in cows by Beatrice Zweifel (Agolin)

Final report submitted by the User:

Experimental period: Start: 7. December 2020 End: 31. August 2021

Objective: To investigate whether medium-term (6 weeks) and long-term (14 weeks) administration of the phytogenic additive Agolin Ruminant Liquid at a dose of 1 g/d affected methane production and performance of dairy cows

Hypothesis: The administration of 1 g/d of the phytogenic additive Agolin Ruminant Liquid over 6 or 14 weeks reduces methane production and improves milk yield.

Trial description: Application for ethical approval of the cow experiment was received from the licensing authority State Office for Agriculture, Food Safety and Fishery, Mecklenburg-Western

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Pomerania, Germany, on 5. November 2020 (7221.3-1-066/20). The trial was conducted with 30 clinically healthy, gestating German Holstein cows in lactation no. 1 to 4 and 7, which were beyond 100 days in milk at the start of the study to avoid confounding of methane emission due to differences in lipolysis in early lactation (Bielak et al., 2016). The study was conducted as a randomized complete block design (9 blocks) with staggered entry of cows in the experiment. In the FBN facility 4 respiration chambers are available which allowed measurement of 4 cows at the same time. Each block consisted of 2 or 4 cows, equally distributed to the control (KN, n=15 cows) and treatment (AgolinRuminant Liquid, AR, n=15 cows) groups. Animals within blocks entered the experiment consecutively at intervals of one or more weeks, depending on the availability of the respiration chambers (Figure 1). Cow pairs within blocks, with one cow in control and one in treatment group, were always in the same lactation number and similar in their lactational stage. The mean characteristics of cows in the week prior to the start of the feeding experiment are shown in Table 1.

Table 1: Cow characteristics in the week (experimental week 0) before the start of the experimen (means ± SD).

	Control (KN)		Treatment (AR)	
	Means	SD	Means	SD
Lactation no.	2.2	1.7	2.2	1.7
Days in milk (d)	166.7	35.8	180.3	47.0
Body weight (kg)	601.6	69.6	633.8	86.2
Water consumption (L/d)	71.8	9.1	66.7	12.8
Feed dry matter intake (kg DM/d)	16.2	1.9	16.6	2.3
Rectal temperature (°C)	38.3	0.2	38.3	0.3
Milk (kg/d)	28.3	3.6	27.9	5.4

In the AR group, a liquid Agolin Ruminant supplement (Batch no. 201100, date of production 11/2020, expiration date 04/2022) at a dose of 1 g/cow and day was administered daily in 2 portions mixed in concentrate (Concentrate MF 2000; Vollkraft Mischfutterwerke GmbH, Güstrow, Germany) and given to the AR cows during milking.

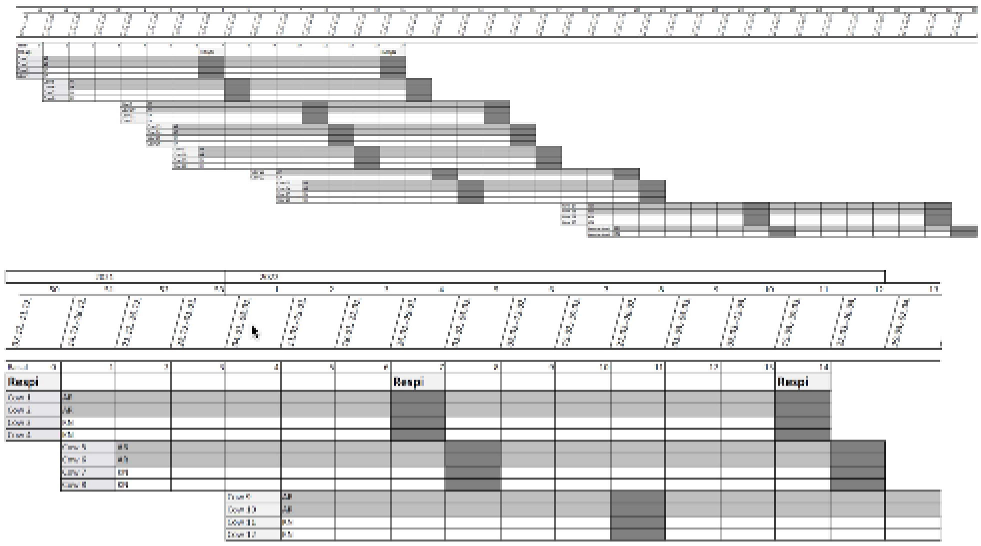

Figure 1: Time schedule of the AgoR-Meth feeding experiment and methane emission measurements (A). Within week of respiration measurement (Respi: 0, 7, 14 weeks) cows continued on their respective diet. Two to four cows were measured at one time and were kept in the respiration chambers from Monday mornings to Wednesday noons (in total 2.5 d/cow) during the respective weeks.

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The Agolin Ruminant (AR) Liquid supplement was stored in a refrigerator at 4°C. The AR/concentrate mixture was prepared fresh weekly. Per cow and day 49 g concentrate pellets were mixed with 1 g Agolin Ruminant Liquid. The total amount needed per calendar week was calculated and prepared (amount depending on number of AR cows in the experiment), divided into two portions per day per cow and stored individually in color-coded Ziplock plastic bags marked with cow numbers in the refrigerator at 4°C. Half of the daily pre-mixed portion per cow was mixed with 100 g untreated concentrate pellets in a bucket, and was given to each AR cow at the morning and evening milkings, respectively, in the milking parlor (approximately 4.30 h and 16.00h) to ensure complete intake of AR. The weight of refusals was determined and recorded. Twenty g of sub-samples of the pre-mix consisting of 49 g concentrate and 1 g Agolin Ruminant Liquid were taken weekly, pooled over one month and stored at -20°C. These pooled samples were send to SYNLAB Analytics & Services GmbH or SGS Analytics Germany GmbH monthly (from April 2021 onwards) for essential oil analysis. The concentration measurement resulted in a mean of 2.2 ± 0.3% of AR compared to the target concentration of 2%. The same concentrate at the same level - but without AR supplement - was given to the KN group. Before and between respiration measurements cows were kept in a free stall barn equipped with weighing troughs. The average composition and nutrient content of standard TMR at FBN for mid- to late lactation cows is shown in Table 2. This TMR was fed to the cows for ad libitum intake continuously during a pre-experimental period of 6 weeks followed for a total of 15 weeks during the experiments (1 basal week 0 + 14 experimental weeks).

Table 2: Average ingredient composition and nutrients and energy contents of the total mixed ration fed to experimental cows.

Ingredients	g/kg DM	Nutrients and Energy	g/kg DM	MJ/kg DM	
Grass silage	211.3	Dry matter	466.1	-	-
Alfalfa hay	23.6	Crude ash	71.4	-	-
Maize silage	412.6	Crude protein	165.7	-	-
Barley straw	2.4	Crude fat	33.8	-	-
Concentrate MF2000	190.5	Crude fibre	160.1	-	-
Maize meal	68.3	Starch	231.0	-	-
Rapeseed extract meal	40.7	Saccharose	33.3	-	-
Soybean extract meal	24.6	Structured fibre (%)	-	-	76.8
Wheat meal	15.3	UCP*	157.7	-	-
Panto Min R 7609	7.2	Ruminal N balance	-	-	1.5
Feed lime	2.0	Metabolizable Energy	-	11.41	-
Soybean oil	1.6	Net Energy Lactation	-	7.04	-

*UCP = Utilizable crude protein

During the experimental period each cow was measured for methane emission 3 times in the respiration chamber: basal measurement (experimental week 0; prior to the start of the Agolin Ruminant Liquid treatment), after 6 weeks on the AR supplemented or KN diet (experimental week 7) and after 13 weeks on the AR supplemented or KN diet (experimental week 14) for 2.5 days each (0.5 days gas equilibration; 2 days gas exchange measurement). The administration of Agolin Ruminant Liquid in the AR group was started on Monday in week 1. Individual feed/dry matter intake, milk yield and methane production measured in respiration chambers was determined as daily mean (weeks 0, 7, 14). Body weight and milk yield in the barn was measured every two weeks (0, 2, 4, 6, 8, 10, 12, 14); backfat thickness was measured by ultrasound in experimental weeks 0, 3, 6, 9, 12, 14. Data were analyzed with repeated measures ANOVA using PROC MIXED (SAS/STAT 9.4; SAS Institute Inc., Cary, NC). The model to analyze data derived from the measurements in the respiration chambers (Agolin Ruminant Liquid intake, methane production, dry matter intake, body weight, milk yield, rectal temperature) contained the fixed

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effects of treatment group (KN, AR), experimental week (0, 7, 14), and the interaction effect between treatment group and experimental week. Body weight, milk yield and backfat thickness over the study (experimental weeks 0 to 14) while cows were kept in the barn was analyzed by a model containing the fixed effects of treatment group (KN, AR), experimental weeks (body weight and milk yield 0, 2, 4, 6, 8, 10, 12, 14; back fat thickness 0, 3, 6, 9, 12, 14), and the interaction effect between treatment group and experimental week. Effects were considered significant at P < 0.05 and least squares means were compared using the Tukey- Kramer test with the SLICE statement for performing a partitioned analysis of the least squares means for the interaction.

Results and Discussion :

Methane production: Cows were in the mid to late phase of their lactation (1-4, 7) during the study. Body weight did not differ between groups within the experimental week as did rectal temperature (Table 3) (F-Test, P>0.8). In both groups body weight was increased between the start and the end of the experiment by approx. 45 kg which is typical for cows in mid lactation (P<0.01). Cows allocated to the AR supplementation group consumed Agolin Ruminant Liquid in an amount very close to the target amount (1 g/d). The AR supplemented cows showed a similar dry matter intake as control cows (F- Test, P>0.8). No difference in milk yield was found between cow groups (P>0.6). Milk yield decreased from experimental week 0 to weeks 7 (AR) and 14 (AR, KN) (P<0.05) (Table 3). Milk yield decreased between experimental weeks 0 and 14 by approx. 3.5 kg/d which is typical for cows in mid lactation over this time period. Methane production did not differ between cow groups (P=0.6) and also not between groups within experimental weeks (P>0.9). Methane yield was 33 L/kg dry matter intake for both groups.

Performance: In the barn, cows in the AR group consumed daily on average of 0.95 g Agolin Ruminant Liquid throughout the total experimental application period. During the 15 experimental weeks (basal week 0, weeks 1-14) back fat thickness (BFT) was affected by experimental week (F-Test, P<0.05) but not by treatment group (F-Test, P=0.4) (Table 4). Within the AR group BFT was higher in EW 12 and 14 compared the EW 0, 6 and 9 (P<0.05) which is in line with the observation of an increasing body weight measured in the respiration chambers during the expeimental period (Table 3). In contrast, body weight did not differ according to treatment group when measured in the barn (F-Test, P=0.13) nor experimental week (F-Test, P=11) while a numerical increase over time could be observed. Milk yield did not differ among the two treatment groups (F-Test, P=0.4). Milk yield was lower in EW 12 and 14 as compared to EW 2 (P<0.05).

Table 3: Cow characteristics, methane emission, Agolin Ruminant Liquid and dry matter intake, and milk yield of cows in experimental weeks 0 (pre-treatment), 7 and 14 (mean ± SE).

Group	EW	Body Weight* (kg)		Rectal Temperature (°C)		ARI (g/d)		Dry matter intake (kg DM/d)		Milk yield** (kg/d)		CH_4 production (L/d)	
Control (KN) (n=15)	0	601.1^{a}	17.0	38.3	0.06	0	-	15.2	0.6	28.2^{a}	1.4	537.0	17.9
	7	618.7^{b}	17.0	38.3	0.06	0	-	15.5	0.6	26.0^{ab}	1.4	530.2	17.9
	14	645.3^{c}	17.0	38.4	0.06	0	-	15.8	0.6	24.8^{b}	1.4	542.9	18.0
Agolin AR (n=15)	0	626.0^{a}	17.0	38.3	0.06	0	-	15.6	0.6	28.1^{a}	1.4	539.1	17.9
	7	647.4^{b}	17.0	38.3	0.06	0.94	0.15	15.3	0.6	25.3^{b}	1.4	548.6	17.9
	14	671.6^{c}	17.0	38.3	0.06	0.98	0.07	17.0	0.6	24.5^{b}	1.4	555.6	17.9

EW – Experimental week
*Body weight; Means with different superscript letters in a colums within Treatment group differ between experimental weeks, Tukey Kramer test P<0.01
ARI – Agolin Ruminant Liquid intake
**Milk yield; Means with different superscript letters in a colums within Treatment group differ between experimental weeks, Tukey-Kramer test P<0.05

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Table 4: Back fat thickness, milk yield and body weight of cows in Agolin Ruminat Liquid supplemented and control cows between 0 and 14 experimental weeks (LSmean ± SE).

Group	EW	BFT			EW	Milk yield		Body weight	
		(mm)				(kg/d)		(kg)	
Control (KN) (n=15)	0	10.4	1.4		0	29.3	1.4	606.9	17.8
	3	10.9	1.4		2	30.0^{a}	1.4	613.3	17.8
	6	11.8	1.4		4	29.9	1.4	611.2	17.8
	9	11.6	1.4		6	29.4	1.4	615.6	18.0
	12	11.7	1.4		8	29.1	1.4	629.9	17.8
	14	12.0	1.4		10	28.3	1.4	624.9	17.8
					12	28.2^{b}	1.4	629.1	17.8
					14	27.8^{b}	1.4	650.9	18.0
Agolin AR (n=15)	0	11.7^{a}	1.4		0	28.6	1.4	631.1	17.8
	3	12.5	1.4		2	29.1	1.4	638.3	17.8
	6	12.3^{a}	1.4		4	28.6	1.5	630.7	17.8
	9	13.0^{a}	1.4		6	28.0	1.5	646.3	17.8
	12	14.1^{b}	1.4		8	26.9	1.4	652.9	17.8
	14	14.2^{b}	1.4		10	26.1	1.4	671.5	17.8
					12	25.5	1.4	670.8	17.8
					14	25.3	1.5	680.5	17.8

EW = Experimental week
BFT = Back fat thickness
^{a,b} Means with different superscript letters in a colums within Treatment group differ between experimental weeks, Tukey-Kramer test P<0.05

Conclusion: Supplementing a phytogenic additive (Agolin Ruminant Liquid) at a target dose of 1 g/d over 6 or 14 weeks to dairy cows did not affect methane production and milk yield. The hypothesis had to be rejected.

References: A Bielak, M Derno, A Tuchscherer, H M Hammon, A Susenbeth, B Kuhla. Body fat mobilization in early lactation influences methane production of dairy cows. Sci Rep 2016, 6:28135. doi: 10.1038/srep28135

4.20 Effects of phytogenic feed additives (PFA) in lactating dairy cows under Heat-stress condition by Poulad Pourazad (Delecon)

Final report from the User:

Aim of the trial: The purpose of this study was to assess the effects of the additive Actifor® Boost HS on dairy cows' milk performances, and milk quality compared with a control diet. Thus, milk yield, milk quality, SCC, behavior, and energy parameters in calorimetric chambers during heat stress challenge.

Keywords: dairy cows, milk performances, behavior, heat stress, calorimetric chamber, scientific trial, Actifor® Boost HS.

Material and methods:

To evaluate the effect of Actifor Boost HS prototypes, we used 2 groups of 10 animals managed during 3 periods (figure 1):

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- \- The period 1: each group (Co = Control and PE = Plant Extract) received a prototype during the transition period of 14 days
- \- The period 2: each group was managed in a climate chamber for 7 days for the cool period (15°C)
- \- The period 3: each group was submitted to a hot temperature (28°C) for 7 days.

Figure 1: Trial design

Period 1 Period 2 Period 3

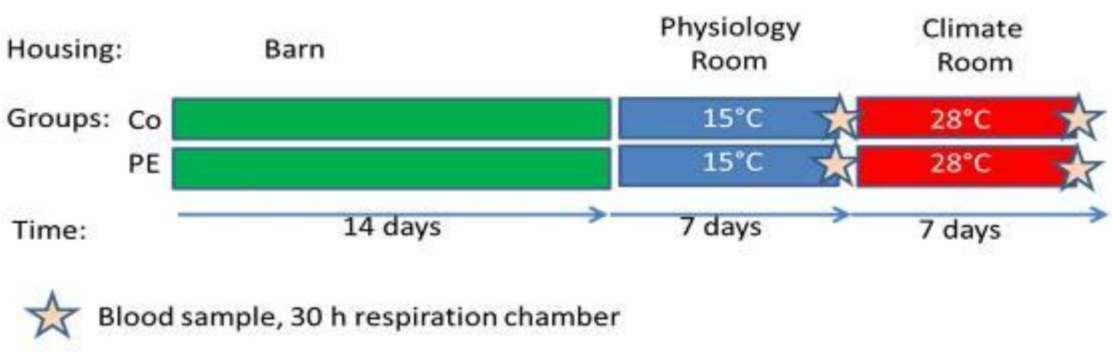

Table 1: Animal characteristics selected

	Co		PE	
	Mean	SD	Mean	SD
Number	10		10	
Parity	3.1	0.9	2.8	0.7
Days in milk	135	54	175	116
Body weight (kg)	655	70	648	70
Milk production 1st day (L/d)	37.5	6.0	34.9	7.6

For the first group (Co=Control), each cow received a product based on wheat semolina and aroma during milking (100 g/cow in the morning and 100 g/cow in the evening). For the second group (PE=Plant extract), each cow received a product based on wheat semolina, aroma, and Actifor® Boost HS prototype 1 during milking (100 g/cow in the morning and 100 g/cow in the evening). Each pair of animals (based on milk production and parity) were introduced every week from the 27th of September 2021 to the 10th of January 2022. (table 1).

Parameters: During each period, the milk production and feed intake are measured daily. (Table 2). Milk quality parameters (fat, protein, somatic cells, milk urea) are measured twice during the 1st period, twice during the 2nd period, and once during the last period. During the chamber periods (periods 2 and 3), the behavior parameters are measured to understand the heat stress effect in the morning and evening: heart rate, breath frequency, and rectal temperature. Just before the 3d period, the metabolic activity of each animal is measured. Concentrations of carbon

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dioxide and dioxygen are measured in the chamber and the airflow is analyzed to estimate the consumption of oxygen and the production of carbon dioxide during respiration. Based on that the metabolic respiratory quotient is calculated: $RQ_{metab} = V_{CO2}/V_{O2}$ Based also on the methane production per head, per day, and the urine nitrogen (assuming 100 g daily Nu), the heat production is calculated with the Brouwer equation (Derno et al, 2019) : Heat production (kJ) = $16.18V_{O2}$ (L) + $5.02V_{CO2}$ (L) – $2.17V_{CH4}$ (L) – 5.99Nu (g). By difference between metabolized energy intake (MEI) and heat production (HP), we can estimate the energy retention (RE = MEI – HP), and by difference with metabolic energy in milk (MEM) the energy used for energy retention in body tissues (RE_{tissue}) : RE_{tissue} = RE – MEM.
Fat and Carbohydrates oxidation are also evaluated based on gas production:

Carbohydrate oxidation (g) : -2.965 V_{O2} (L) + 4.170 V_{CO2} (L) – 2.55 Nu (g). Fat oxidation (g) : 1.718 V_{O2} (L) -1.718 V_{CO2} (L) – 0.315 Nu (g).

Table 2: Parameters measured during this trial

Measures	Period 1 (transition)	Period 2 (Cool period in chamber)	Period 3 (Hot period in chamber)
Dry Matter Intake	Daily	Daily	Daily
Milk production	Daily	Daily	Daily
Milk quality	Weekly	2 times	1 time
Body weight	Weekly	Weekly	Weekly
Confort parameters	No	Daily	Daily
Metabolic parameters	No	1 time at the end of this period	No

Diet: During this whole trial, each cow received a TMR according to the group with the prototype distributed during the 2 milking per day (see tables 3 and 4). Plant extract in the prototype PE is an evolution of Actifor® Boost (called Actifor® Boost HS).

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Table 3: Diet's composition at the feeding table

FEED RATION	CO	PE
Grass silage (kg DM)	5.2	5.2
Corn silage (kg DM)	10.0	10.0
Concentrate (kg RM)	5.0	5.0
Rapeseed cake (kg RM)	1.8	1.8
Soybean cake (kg RM)	1.35	1.35
Wheat (kg RM)	0.9	0.9
Mineral feed (kg RM)	0.18	0.18
Limestone (kg RM)	0.09	0.09
Soya oil (kg RM)	0.05	0.05
Prototype Co (kg RM)	0.2	
Prototype PE (kg RM)		0.2
Total Intake (kg DM)	**24.9**	**24.9**

Table 4: Diet nutrient values

Diet Characteristics	CO	PE
Crude protein (g/kg DM)	171	171
Crute fat (g/kg DM)	32	32
Crude fiber (g/kg DM)	163	163
Crude ash	74	74
Metabolized energy (MJ/kg DM)	11.4	11.4

Climatic chambers: During the cool period in the climatic chambers, the temperature was stabilized at 15.8 °C on average and the hygrometry was 65.7%. The calculated THI (Temperature-Humidity Indice) according to the NRC is 60.0. Below 68, this THI value is in the comfort area. During the hot period in the climatic chamber, the temperature was 27°C and the hygrometry was 48.4%. The calculated THI is 74.2. Between 72 and 75, the THI suggests discomfort for animals. THI = (1.8 × T + 32) (0.55- 0.0055 × RH) × (1.8 × T- 26) where T = air temperature in °C and RH is relative humidity in %.

Statistical analysis: After a first check of the data to remove any aberrant productions or dry matter intake, the parameters are analyzed as :

ANOVA model: X ~ COV + Treatment + Period + Treatment: Period

For the covariable, we considered the 3 first days of the 1st period. The last 12 days are considered as a transition period. (see table 5)

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Table 5: animals characteristics during the 3 first days

Characteristics	Co	PE
Dry matter intake (kg DM/day)		
Average	23.7	23.2
Min	19.6	18.7
Max	31.1	27.2
Milk production (kg/day)		
Average	37.6	35.4
Min	26.3	21.8
Max	44.8	47.0

Results: For the main parameters (feed intake and milk production) measured every day, heat stress has reduced drastically the feed intake by 7.7 kg/head/day of dry matter and milk production by 9.6 kg/head/day significantly. At the same time, the feed efficiency was improved by 0.20 L/kg of dry matter during heat stress. This effect is linked with a short-term effect on feed intake mainly. The Actifor® Boost HS prototype has improved significantly the feed efficiency (milk production per kg dry matter) during the 2 periods but mainly during the heat stress period (+0.24 L/kg dry matter). (Table 6)

Table 6: Effect of heat stress and treatment on milk production and feed intake

	Cool period			Hot period			Proba		
	Co	PE	PE effect	Co	PE	PE effect	Period	Treatment	Interaction
Data Number	10 x 8 (-1)	10 x 8 (-1)		10 x 7	10 x 7				
Milk production (L/d)	34.7	35.2	+0.5	25.9	26.5	+0.6	<0.01	0.263	0.863
Feed intake (kg/d)	24.2	23.1	-1.1	15.7	15.4	-0.3	<0.01	0.059	0.846
Milk production per kg DMI	1.45	1.53	+0.08	1.69	1.77	+0.024	<0.01	0.031	0.888

For the milk quality analysis, heat stress affected all parameters except lactose and somatic cells. Heat stress has increased significantly milk fat and milk urea but has decreased significantly the milk protein. (see table 7). At the same time, heat stress has decreased water intake and increased body weight losses. The prototype Actifor® Boost HS has improved significantly the feed efficiency (energy corrected milk / kg dry matter intake), especially during the heat stress period (by +0.48). Also, this product has numerically reduced milk urea by 14 mg/L during the cool period and by 47 mg/L during the heat stress period.

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Table 7: Effect of heat stress and treatment on milk production and on feed intake

	Cool period			Hot period			Proba		
	Co	PE	PE effect	Co	PE	PE effect	Period	Treatment	Interaction
Data Number	20	20		10	11				
Milk production (kg/d)	35.8	36.5	+0.7	26.6	26.4	-0.2	<0.01	0.83	0.72
Feed intake (kg/d) (DMI)	23.1	22.1	-1.0	15.6	14.1	-1.5	<0.01	0.17	0.98
Energy corrected milk ECM (kg/d)	34.4	34.9	+0.5	25.3	27.2	+1.9	<0.01	0.39	0.60
ECM / DMI	1.50	1.59	+0.09	1.64	2.12	+0.48	<0.01	0.03	0.13
Milk fat %	4.08	3.95	+0.13	4.43	5.24	+0.8	<0.01	0.27	0.29
Milk protein %	3.70	3.65	-0.05	3.51	3.44	-0.07	<0.01	0.44	0.92
Milk lactose %	4.90	4.85	-0.05	4.82	4.78	-0.04	0.12	0.35	0.93
Milk urea (mg/L)	231	217	-14	412	365	-47	<0.01	0.09	0.352
SCC (log)	4.81	5.02	+0.21	4.84	4.97	+0.13	0.71	0.34	0.58
Water intake (kg)	99.0	90.4	-8.6	84.5	81.9	-2.6	0.037	0.30	0.58
Body weight variation (kg)	16.7	12.4	-4.3	-23.1	-24.7	-1.6	<0.01	0.61	0.82

For the behaviour (table 8), all parameters were affected by heat stress: a reduction of heart rate, an increase in breath frequency, and rectal temperature. The prototype Actifor® Boost HS has increased significantly the heart rate and breath frequency compared to control group.

Table 8: Effect of heat stress and treatment on behavior

	Cool period			Hot period			Proba		
	Co	PE	PE effect	Co	PE	PE effect	Period	Treatment	Interaction
Data Number	68	68		70	70				
Heart rate morning (2mn)	155.4	160.2	+3.8	141.1	148.8	+7.7	<0.01	<0.01	0.54
Heart rate afternoon (2 mn)	162.9	168.4	+5.5	147.4	152.7	+5.3	<0.01	0.047	0.97
Breath frequency morning (2 mn)	73.2	74.7	+1.5	158.7	165.2	+6.5	<0.01	0.19	0.41
Breath frequency afternoon (2 mn)	85.4	79.9	-5.5	157.2	169.2	+12	<0.01	<0.01	0.32
Rectal temperature morning (°C)	38.2	38.2	=	39.6	39.7	+0.1	<0.01	0.91	0.49
Rectal temperature afternoon (°C)	38.4	38.4	=	40.0	39.9	-0.1	<0.01	0.68	0.48

In table 9, due to a low number of data, it's impossible to see a significant effect of the prototype Actifor® Boost HS. Heat production, metabolic respiratory quotient, and fat and carbohydrate oxidation are similar between the two groups. The metabolic respiratory quotient (RQ) mirrors the whole animal metabolism including feed nutrient degradation in the rumen: for carbohydrates oxidation, RQ is 1 while for fat oxidation this level will be 0.71. It's common to see a value below 1 in dairy cows. The negative value for body retention has shown body weight losses in dairy cows during this trial.

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Table 9: Effect of heat stress and treatment on behavior

	Cool period			Proba
	Co	PE	PE effect	
Data Number	9	9		
Heat production (KJ / BW^0.75)	1202	1212	+10	0.794
Produced CO2 (L)	8063	7903	-160	0.598
Consumed O2 (L)	7347	7277	-70	0.798
Methane (L)	598.6	590.9	-7.7	0.789
Metabolic RQ	0.96	0.95	-0.01	0.467
Carbohydrate oxidation (g/d)	8262	7817	-445	0.450
Fat oxidation (g/d)	286.4	416.3	+129.9	0.521
Metabolized energy intake (MJ/day)	253.6	239.6	-14.07	
Heat production (MJ/day)	156.7	115.3	-1.45	
Energy retention (MJ/day)	96.9	84.3	-12.6	
Milk energy retention (MJ/day)	114.5	114.9	+0.38	
Body energy retention (MJ/day)	-17.6	-30.6	-13.0	
Standing time (mn)	883.7	974.3	+90.6	0.342
Position changes (from laying to standing of from standing to laying)	26.89	21.44	-5.45	0.386

Conclusions: Unfortunately, selected dairy cows didn't have exactly the same performance and the same feed intake at the beginning of this trial. The heat stress has shown a reduction of feed intake and performance but a positive effect on feed efficiency. This effect is probably because the heat stress period was very short (1 week only). Actifor® Boost HS increased significantly the feed efficiency during heat stress (by 30%) with, in parallel, a reduction of milk urea (-11%). Milk production was also reduced during heat stress but not significantly (+ 7.5% = +1.9 L/day). The data for the behavior have shown a significant increase in breath frequency and rectal temperature during the heat stress period. However, the heart rate decreased during this period: is it because of the short period of heat stress, or is it due to the stress during the cool period in the chamber. Actifor® Boost HS has increased heart rate and breath frequency significantly showing that this product has no impact on behavior.

4.21 Novel trace mineral source by Denise Cardoso (Animine)

Final report from the User:

Experiments conducted *in vitro* have provided evidence for ruminal responses (e.g., acetate:propionate ratio) to Animine's proprietary potentiated zinc oxide (Fellner et al., 2021, Applied Animal Science 37: 27–32), compared with other sources of Zn. Whether these responses are relevant *in vivo* is not known. The objective of this study was to determine the effect of a mix of Animine proprietary sources of Zn and Mn on cow performance (milk yield and composition, body weight and condition score) and – potentially - rumen function (pH and VFA concentrations) and total tract digestibility in lactating dairy cows.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N°730924

Hypothesis: Providing Zn and Mn as Animine proprietary sources rather than sulphates will increase ruminal acetate:propionate ratio, increase total tract NDF digestibility and increase yields of milk protein and fat.

Material and Methods The experimental design was based on that used by Faulkner and Weiss (2017, J. Dairy Sci. 100: 5358–5367) to compare hydroxy trace minerals with sulphates in lactating dairy cows. Those authors used 18 cows in a split-plot Latin Square to compare two sources of Zn, Mn and Cu at two levels of dietary fibre, using 28d periods. In our experiment, two sources of Zn and Mn were evaluated using a 2 x 2 crossover design, with 28d periods and 24 lactating Holstein cows less than 150 days in milk arranged in 12 blocks (pairs) according to parity (primiparous or multiparous) and stage of lactation. We did not include Cu due to the known high Cu status of the SRUC herd (based on forage Cu concentrations and liver Cu measured in cull cows). Within pairs, one cow was allocated at random to either treatment sequence AB or treatment sequence BA and the second cow was allocated to the treatment sequence not followed by the first cow.

Treatments were:

A. Control. Zn and Mn provided as sulphates.
B. Animine. Zn and Mn provided as proprietary products 'HiZox', 'CoRouge' and 'ManGrin' (Animine SA, France)

In both cases, diets were formulated to supply 75% of the Zn and Mn permitted by EU (and UK) feed legislation. Cows were housed in a single group in a cubicle (free-stall) barn and offered the same total mixed ration (TMR) for *ad libitum* intake via electronically operated Hokofarm feeding stations (Insentec, Netherlands). Cows had free access to fresh drinking water at all times. Trace minerals were provided within manufactured premixes designed for use within TMR. The TMR was formulated using the 'Feed into Milk' system to 12.4 MJ ME/kg DM and (as % DM) 16.6, 33.1 and 14.9 CP, NDF and starch, respectively. Forages were grass silage and whole crop wheat silage at 38.0 and 17.6% of DM, respectively. A manufactured vitamin/mineral premix was included at 0.7% of diet DM (targeting an intake of 150g/cow/d). Premixes contained 10250mg/kg Zn as zinc sulphate monohydrate (treatment A) or as zinc oxide (treatment B), and 8800mg/kg Mn as manganese sulphate monohydrate (treatment A) or as manganese (II) oxide (treatment B).

Dry matter intake was calculated daily for each cow from individual meals recorded by the Hokofarm feeding equipment. Milk yield was recorded at each milking (cows were milked twice daily at 04:00 and 16:00) and concentrations of fat, crude protein, lactose, urea, somatic cells and major fatty acids in milk were determined by mid-infrared spectroscopy on samples collected during the last three days of each period. Cows were weighed when entering the milking parlour and Body condition score (BCS) was estimated by two operatives at the end of each period. Rumen contents were sampled by naso-oesophageal intubation on one occasion per period for analysis of pH, VFA and ammonia-N, with sub-samples retained (at -80 C) for possible microbiome analysis. Faecal samples were collected at intervals on the last two days of each period to permit later calculation of total tract digestibility (using acid insoluble ash as an internal marker), depending on cow production results. Data were analysed by analysis of variance (Genstat 18, VSN International Ltd.), fitting terms for block, period, treatment and their interactions.

The main scientific outcome, innovation/impact of the results: On average, premixes contained more Mn (+13%) and less Zn (-5.6%) than targeted. Premix B (test) contained 5% more Mn and 9% more Zn than premix A (control). Overall levels of cow performance met expectations: dry matter intake close to 24kg/cow/d and mean milk yield of 40.1kg/d. Treatment had no effect

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(P>0.1) on any measure of cow performance (dry matter intake, milk yield, milk composition, body weight or body weight change). Due to a technical problem with the gas chromatograph, ruminal VFA concentration data are considered unreliable. Due to a change in laboratory staff, a repeat analysis of stored back-up samples has not yet been completed. No decision has yet been made concerning analysis (not funded through Smart Cow) of faecal samples for calculation of total tract digestibility. Lack of response to treatment might be due to (a) imprecise delivery of targeted concentrations of Zn and Mn, (b) insufficient experimental power (replication was based on the precedent offered by Faulkner and Weiss, 2017), or (c) targeted concentrations of Zn and Mn were insufficient to cause, *in vivo*, effects previously observed *in vitro*.

How do you expect to disseminate the results: Results may be reported at conferences such as RRR (France) or ADSA (USA).

4.22 Strengthen Laser Methane m-g device measurement protocol to estimate methane emission of dairy cow ruminants directly in any commercial farm by Raphael Bore (Idele)

Final report from the User:

Introduction: The laser methane detector (LMD), a hand held open path laser measuring device. Its measurements are based on infrared absorption spectroscopy using a semiconductor laser as a collimated excitation source. It employs second harmonic detection of wavelength modulation spectroscopy to establish methane concentration (Iseki, 2004). The LMD is manufactured by Tokyo Gas Engineering Solutions, Ltd. and was originally developed for the detection of gas leaks, and therefore, discriminate between high CH4 concentrations and the low background concentration in the atmosphere (Crowcon, 2017). Since its use in livestock methane determination was first introduced by Chagunda et al (2009), different models of this device have been developed but using the same technology, namely tunable diode laser absorption spectroscopy. The LaserMethaneMini® is the one currently in circulation. Although different studies have so far employed the LMD to measure methane in both cattle and goats, some of the underlaying assumption in the measuring techniques which were proposed in earlier stuies (e.g. Chagunda et al, 2009; Chagunda et al., 2013; chagunda 2015) although based on sound biological knowledge, have not been fully tested.

The main objective of the project: The current study aimed at validating some of the major measuring assumption of the LMD in a systematic and robust manner. Specifically, the study investigated the effect of distance between the animal and LMD, monitoring angle, presence of adjacent animals on the reliability of the laser measurements and then identify the best combination to ensure a repeatable and reliable measure to quantify methane release by the animals. The hypotesis that are tested: Laser Methane m-g device measure methane concentration in ppm*m. Thus, we suppose that distance between the device and the animal should affect the concentration measured and should be corrected or fixed. When animal breath or belch, a cloud of air is exhaled and the methane concentration measured should also be affect by the size of cloud crossed by the laser beam. Thus, monitoring angle during the measurement should also affect the measurement. Finally, presence of adjacent animals should contaminate the concentration of methane measured if cloud of air exhaled by another animal is interfering with the cloud of air exhaled by the animal measured. In this study, we have tested the impact of these 3 factors on the

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reliability of the laser measurements and identified a protocol to ensure a repeatable and reliable measure to quantify methane release by the animals.

Study description: Study has involved 20 lactating dairy cows of different age, number of lactation and milk production feeding with a unique ration established to cover the needs of dairy cows in the context usually encountered on the Dairy Research Centre of the Scotland Rural College. In total, 720 LMm-g measurements (36 with each animal) of 4 minutes have been spread out over 20 days. The 12 combinations of distance from device to nostrils (2 and 3 meters), monitoring angle (45° and 90°) and distance between adjacent animals (0 gap, 1 animal width, 2 animal width between animals)) have been performed on each animal and repeated 3 times on the same day to assess the potential changes in methane emissions in relation to rumen fill (Figure 1). In practice, 3 different combinations measurements have been run 3 times on 4 animals each day (cf. table 2). Each combination factors was randomly selected for each animal per day and per measurement moment (Morning during feeding, Midday and Evening after milking). The four animals for each measurement moment have been choose randomly as described in the table 2. There is some practical considerations in this experimental design, for each measurement the presence order of adjacent animals was taken into account to better manage the experiments by operators (0 fence, 1 fence and 2 fences).

Table 1 : Example of experimental plan for measurements of day 1.

Order	Moment	Cow	Measurement 1			Measurement 2			Measurement 3		
			Distances	Angles	gap	Distances	Angles	gap	Distances	Angles	gap
4	Morning	1	3	45	0	2	45	1	2	45	2
3	Morning	2	2	45	0	2	90	1	3	45	2
2	Morning	3	3	90	0	3	45	1	2	90	2
1	Morning	4	2	45	0	2	90	1	2	45	2
4	Midday	1	3	45	0	2	45	1	2	45	2
1	Midday	2	2	45	0	2	90	1	3	45	2
3	Midday	3	3	90	0	3	45	1	2	90	2
2	Midday	4	2	45	0	2	90	1	2	45	2
1	Evening	1	3	45	0	2	45	1	2	45	2
3	Evening	2	2	45	0	2	90	1	3	45	2
2	Evening	3	3	90	0	3	45	1	2	90	2
4	Evening	4	2	45	0	2	90	1	2	45	2

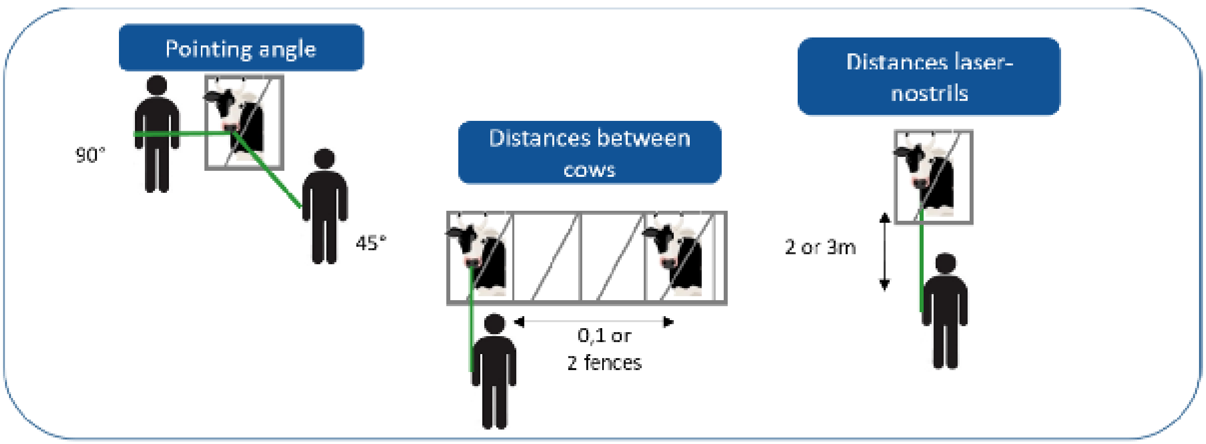

Figure 1 : Description of factors and modalities tested

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Laser Methane m-g measurement have been resumed accordingly to Chagunda and Sorg who are using the mean of all pic of the laser signal PMEAN. All effects have been analyzed with mixed model method taking into account the repeated measurements. Response are transformed in log(PMEAN).

The main outcome: Methane concentration measured are between 0 and 12 187 ppm*m (Table 2).

Min.	Max.	Mean	Median	SD
0	12187	80.2	50	118.7

Methane concentrations are similarly distributed according to factors and modalities (Figure 2): distance (2 or 3 meters), angle (45 and 90°) and proximity of neighbors (0 = 0 meter between cows, 1 = 1 meter between cows and 2 = 2 meters between cows)

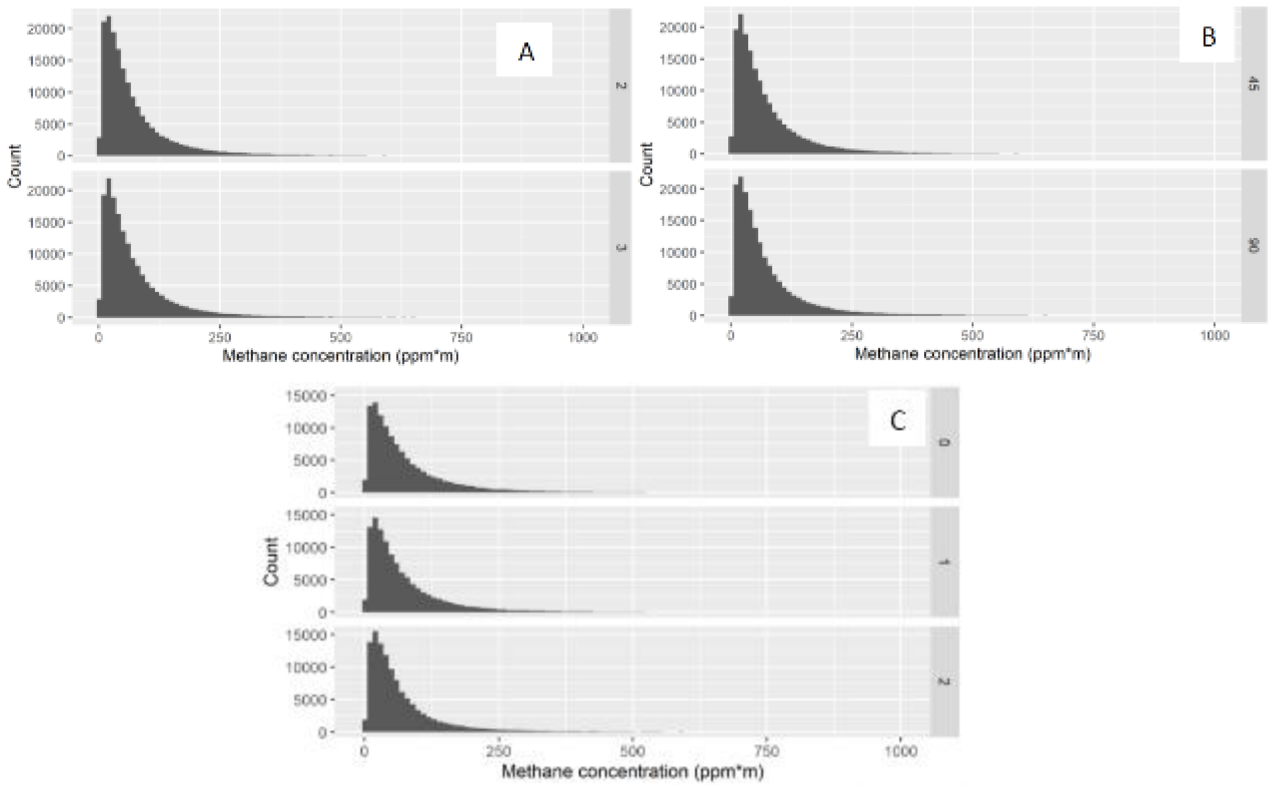

*Figure 2: Distribution of methane concentration (in ppm*m) measured (A : Distance; B : Angle; C : Presence of neighbors)*

Statistical analysis of PMEANs: Following boxplots report that PMEAN's seems really similar according to factors (distance, angle, moment of the day or presence of neighbours) and modalities which average value around 120 ppm*m.

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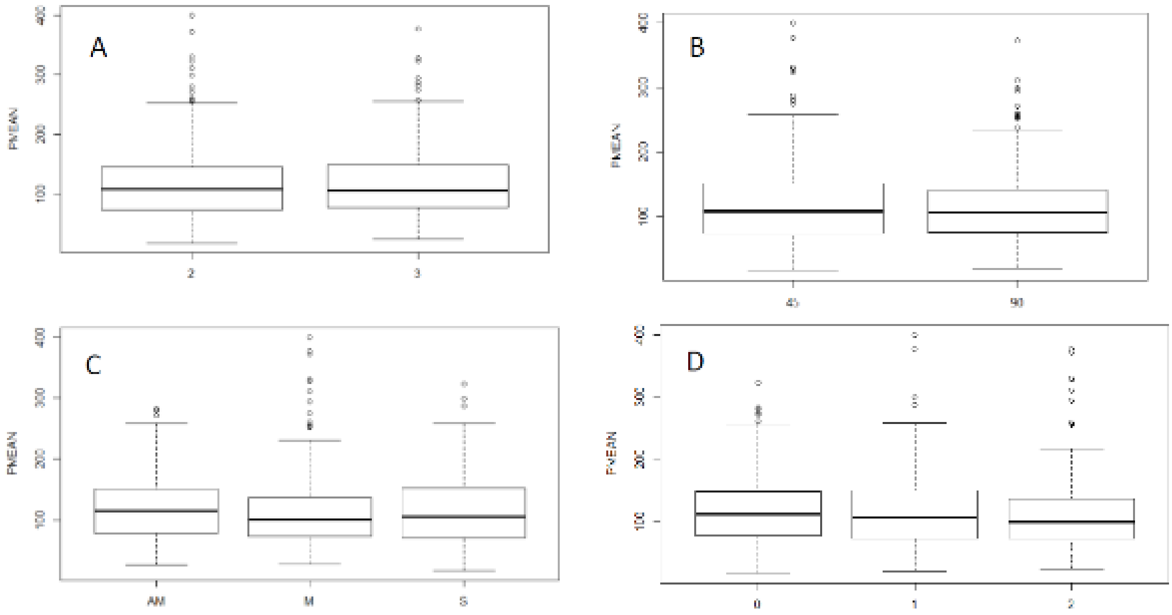

Figure 3 : Distributions of PMEAN according to factors and modalities (A : Distance; B : Angle; C : Moment of the day; D : Presence of neighbors)

Statistical analysis showed no significant difference between PMEAN for each factors.

```
Response: log(PMEAN)
               Chisq Df Pr(>Chisq)
(Intercept) 4428.5420  1     <2e-16 ***
Dist           0.2762  1     0.5992
Ang            0.7542  1     0.3851
Gap            2.9451  2     0.2293
```

According to this trial, we can conclude than position of operator during measurement did not affect the PMEAN value calculated from the 4 minutes laser measurement usually used on peer-reviewed scientific paper about laser.
Other variables are in study to be sure that the analysis did not miss some kind of difference between modalities of factors.

How do you expect to disseminate: Two scientific articles in peer reviewed international journals and one technical papers will be published which titles will be:
- Effect of measuring position on the variation and accuracy on LMD in dairy cattle
- Effect of diurnal patterns on the variation in LMD measured methane.
- Using LMD on farm conditions

4.23 Consequences of the grazed pasture diversity on annual variability of nutritional value and technological properties of milk, and nutritional status of Holstein-Friesian and Jersey Holstein-Friesian crossbred dairy cows (GRAMIQS for GRAzing – MIlk –QualitieS) by Anne Boudon (INRAE)

Final report submitted by the User:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N°730924

Main objective and hypotheses that were tested: In a context of increasing societal issues around animal production, a significant part of consumers asks for products coming from more sustainable and ethical production systems. In Europe and specifically in France and in Ireland, grazing dairy systems benefit of a good image and more and more, dairy companies segment their collection rounds to offer milk specifically from these systems. A consequence is a higher seasonal variability of milk composition at the scale of the collection round that cannot be compensated by the diversity of systems. The reasons for this seasonal variability are multiple. The effect of the breed on milk composition is relatively well known.

The objective of the study was to characterize the variability of milk fine composition and technological properties in grazing dairy systems, as well as that of the nutritional status of cows of two breeds. Our hypothesis is that the effects of the grazed species on milk composition and functionality annual variability, as well as that of the interaction of the plant species and cow breed, can be important and need a better characterization.

The project compared the performances of perennial ryegrass (PRG) only, PRG and white clover (PRG-WC), or diverse multispecies (MSS) swards and Holstein-Friesian and Jersey Holstein-Friesian crossbred cattle on dairy system performance, milk composition and functionality and biomarkers of nutritional status of the animal within intensive pasture-based grazing systems.

The main scientific outcome, innovation/impact of the results

Context of the study: feed allowance and pasture composition: Herbage, milk and blood were sampled 3 times on 8 cows per breed and sward. The dates were for period 1, April 13th and cow average stage of lactation was 70 days in milk (DIM), for period 2, May 18th (105 DIM) and for period 3, June 28th (146 DIM). Herbage allowance was on average 12 kg/cow/during the experiment. Herbage allowance and consequently dry matter intake decreased in period 2 for the three compared swards, because of low height and high density in the paddock. However, sward mineral composition differed between swards, with higher Ca, P and Zn contents in MSS than in PRG or PRG-WC ($P<0.05$). It was also numerically the case for Cu, Fe, K, S, and Mg. The higher Ca, P and Zn in MSS was related to the high content of Plantain and Chicory in those elements, especially in the last period when the contribution of these species to the MSS sward was high. The cows were also supplemented with 1 kg of concentrate. The contribution of the concentrate was major for Cu, Zn and Mg supplies because the concentrate contents in those elements was more than 10 times higher than the pasture mineral contents. Concentrate Cu and Zn contents were even 30 to 70 times higher than those of pasture. For some modalities of sward and period, the contribution of the concentrate was major for Mn.

Mineral status of cows: The plasma mineral contents of cows was not affected by the breed but it was systematically affected by the period with higher Ca, K, Mg, Na, P, S, Cu, and Zn in the last period than in both first periods. Plasma inorganic P only increased in the last period with the MSS sward and plasma Fe content was lower in the last period. The increase of plasma Mg, P and Zn was also higher with MSS sward than for PRG and PRG-WC, this tended to be significant also for plasma Ca. For most elements, the plasma mineral contents in the first period were lower than the considered physiological range (Boudon et al. 2018). It remained true to a lesser extent during the second period. Only plasma Cu contents were higher than the physiological range.

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The effect of period was confounded with the effect of lactation stage (69 days in lactation in period 1 in April and 145 days in lactation in period 3 in June), herbage allowance, and botanical and chemical pasture composition. This made the dissociation between the stage of lactation and the seasonal evolution of sward difficult to dissociate. The low plasma contents of many minerals at the period 1 could be expected considering it is the first sampling point after calving. However, the average of lactation at period 1, i.e. 70 ± 8.8 DIM was too important to consider that these low plasma contents could be linked to *peripartum* troubles. It can be considered that from a combination of relatively high milk production with limited herbage allowance, the mineral supplies could have been suboptimal for some elements. However, we did not observe a clear single link between herbage mineral content and animal mineral status indicating that interaction between elements will have to be carefully considered.

Milk production and composition: There was no effect of breed on milk yield, lactose content and SCC, but milk fat and protein contents and yields were significantly higher with JFX as in Auldist et al (2004) and Poulsen et al (2015). Milk citrate was also higher as in Poulsen et al (2015) and milk chlorine content was lower with JFX. Except with PRG, cheese making aptitude was improved with JFX: RCT tended to be shorter (p=0.094) and firmness (a30) tended to be higher (p=0.079) as in Auldist et al (2004) and Poulsen et al (2015). At standardized pH, RCT depends on colloidal Ca / casein ratio or more simply from the sole content of colloidal calcium (Hurtaud et al, 2001). Breed had no effect on heat stability of milk. Plasma NEFA tended to be higher (respectively p=0.057) and plasma glucose was higher with JFX. The sward did not affected on milk yield and most parameters of milk composition. Only milk urea content was higher with MSS and firmness of curd measured as a30 were higher with PRGCW and MSS. Period significantly affected almost all the measured parameters. Milk yield, milk fat and lactose contents decreased from April to June. Milk urea largely decreased in May and increased after. However, this trends were sensibly different according to the sward. Milk yield linearly decreased from April to June with MSS while it decreased from April to May with PRG and PRGWC and stabilized, or even increased after. Milk lactose had an inverse behavior: it's quite stable with MSSS and largely increased in May with PRG and PRGSW. Plasma urea decreased from April to May with the 3 swards, after stabilized with PRG and PRGWC but increased with MSS. SCC largely increased in May and decreased after. Milk heat stability increased from April to June whatever the sward, whereas milk RCT decreased in April whereas curd firmness increased during the same month.

Analyses of milk mineral contents are still in progress at this stage.

Any other achievements of the visit: Unfortunately, because of the sanitary evolution, the visit from French researchers to Moorepark facilities was not possible during the experiment. Videoconferences will be organized for the finalization of the results. Some results remains to be analyzed at the time of this report for the first publication, especially the milk mineral content. Other results, related to vitamins milk composition, or the use of lactose as a bio indicator of energy status of cows will be treated later which will allow a long term collaboration between our organisations.

How do you expect to disseminate the results: Data from this study will be published in several papers in A rank international journals (Grass and Forage Science, Journal of Dairy Research or Journal of Dairy Science). The first paper will be related to the variability of milk fine composition and functionality in relation mineral status of cows. Another one should be related to vitamin and antioxidant status of cows and a last one to the use of lactose as a bio indicator of energy status of cows. This will be accompanied by quick diffusion of the results in an international scientific

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meetings (EAAP 2022 Porto). An objective will be also to include the data of herbage composition in the INRAE databasis of forage composition that is free of access on (http://www.inration.fr). Given the important interest of some dairy companies for the topics of the variability of milk composition in grazing system, results will be disseminated during annual meeting of French milk inter profession (3R 2022) but also in international congress related to dairy processing (ADSA 2023).

Any suggestions to improve the TNA procedure: Depending on the nature of the project, a deadline of 30 days after the end of the experimental phase is very short to achieve all laboratory analyses and give a good overview of the main results of the project. A latitude on this delay at the submission of the project will allow to better respect the deadlines.

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4.24 Is animal-to-variation in feed efficiency mostly driven by differences in appetite in beef cattle? by Krum Vladimirov Nedelkov (Trakia University) / Gonzalo Cantalapiedra (INRAE)

Final report from the User:

Introduction: Improving animal feed efficiency (FE) may contribute to the sustainability of the beef cattle sector. Beyond well-defined nutritional strategies, the improvement of FE could also be achieved through genetic selection. Alternative definitions of FE are used within animal breeding programmes, with growth-related (feed conversion efficiency [FCE] and residual gain [RG]) or intake-related (residual feed intake [RFI]) indices the most prevalent in the literature (Cantalapiedra-Hijar et al., 2018). Previous research has pointed to appetite as one of the most important true determinants underlying between-animal differences in RFI in beef cattle (Lines et al., 2014). However, more research is required to confirm this, and to determine whether it applies to other measures of FE.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N°730924

The main objective of the project: To compare the feed efficiency ranking under ad-libitum and restricted feeding conditions on the same sixty young bulls with analysis of blood parameters, reflecting energy and N metabolism, and rumen microbiota composition under both feeding conditions.

The hypothesis that are tested:

i) Variation in residual feed intake observed during ad-libitum conditions would almost disappear when the same animals are restricted and fed at similar feeding levels
ii) Some biological mechanisms are related to feed efficiency ranking only because they co-vary with feed intake
iii) Appetite is the major determinant of feed intake when measured as residual feed intake but not as feed conversion efficiency or residual gain.

Material and methods: Sixty Charolais crossbred young bulls (427 ± 20.4 kg body weight, BW) were tested twice for FE in a crossover design with 2 test periods of 70-d each. After an initial adaptation period of 3 weeks, animals were blocked on BW, and within block randomly allocated to one of two groups of 30 individuals. For the first 70-d test period, one group was assigned to restricted (R1) and the other group to ad libitum (A1) feeding levels. For the second 70-d test period, the groups switched feeding level and group R1 was this time fed ad libitum (A2) and group A1 was restricted (R2). Animals were offered a grass silage and barley-based concentrate total mixed ration on a 50:50 dry matter (DM) basis. Feed restriction level was chosen at 1.45 % BW for all restricted animals, a level promoting a theoretical average daily gain (ADG) of 0.5-0.6 kg according to INRA (2018). Backfat depth measurements were conducted by ultrasound at the beginning and end of each test period. The RFI and RG values (kg/d) were calculated for each condition (R1, A1, A2, R2) independently, as the difference between observed and predicted DMI or BW gain (kg/d), respectively. The DM intake and BW gain were predicted from a linear model including the block effect, mean metabolic body weight (MMBW), changes in ultrasound backfat depth, and either ADG for RFI or DM intake for RG calculations, respectively. The FCE (kg/kg) was calculated as the observed ADG (kg/d) by unit of observed DMI (kg/d).

Main preliminary results: DM intake was 6.70 and 8.55 kg/d for animals undergoing feed restriction in the first (R1) and second (R2) period, respectively. Corresponding values for ad-libitum animals were 9.55 (A1) and 10.50 (A2) kg/d. This represented a mean feeding level of 1.42% and 1.85% BW for the restricted and ad-libitum groups, respectively. The ADG for R1, R2, A1 and A2 groups was 0.74, 0.92, 1.59 and 1.49 kg, respectively. For RFI, the between-animal variability (coefficient of variation, CV) was, on average, 6 times higher for the ad-libitum (4.5% CV) compared to the restricted (0.7% CV) feeding group, in both test periods. In contrast, with RG and FCE, the CV almost twice as high in the restricted (15% and 23%, respectively) compared with the ad-libitum (8% and 13%, respectively) feeding group during the first test period, but was similar (approximately14%) for both feeding groups in the second test period. Spearman correlations (rs) between the two feeding periods (A1 vs. R2 or R1 vs. A2) for RFI, FCE and RG measured on the same animal were low and not statistically significant ($-0.25 \leq rs \leq 0.14$; $P>0.05$). Within-condition, the RFI ranking was correlated or tended to be correlated with RG ($P<0.05$) and FCE ($P<0.10$) in ad-libitum but not in restricted feeding levels ($P>0.05$). In contrast FCE and RG were highly correlated with each other ($0.63 \leq rs \leq 0.82$; $P<0.05$) within each feeding condition (R1, A1, A2 and R2).

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Impact of the results: Restricting the feeding level of young bulls severely decreased the observed variability in residual feed intake (RFI) compared with animals fed ad-libitum, regardless if feed restriction preceded or followed ad-libitum feeding level. However, for feed conversion efficiency (FCE) and residual gain (RG) this pattern was the opposite, such that between-animal variability almost doubled when feed restriction preceded, but did not differ when followed by ad-libitum feeding level. Our findings support the concept that appetite is a primary determinant of RFI variation in bulls but not of variation in growth-related traits such as FCE or RG. These results also suggest that feed efficiency measured under restricted feeding situations may be a different animal trait compared with that measured under ad-libitum feeding conditions.

Any other achievements of the visit: Unfortunately, no visits were planned because of the sanitary context.

How do you expect to disseminate the results?: A first abstract (Cantalapiedra-Hijar et al. 2022) with preliminary results has been submitted to the ISEP congress (12-15 September, Spain). Ongoing analysis on plasma metabolome and isotopes will be finished before summer 2022 and the submission of a first paper reporting performance and metabolic data is planned before the end of 2022 to an open access journal. A second paper could be also proposed based on actions and results concerning the analysis of rumen microbiota composition on these same animals.

Any suggestions to improve the TNA procedure: Nothing to suggest, because communication was good and expected outcomes were achieved.

4.25 The use of sustained release boluses with essential oils to improve the productivity of dairy cows in forage based systems by Jamie Bennison (Agrimin)

Withdrawn

4.26 Effects of live yeast supplementation on dairy cows by Tanja Kotevska (Greenagro dooel)

Final report from the User:

This study evaluated yeast supplementation in dairy cows diets.

The hypothesis was that yeast supplementation could improve rumen pH, regulate milk fat:protein ratio, and feed efficiency.

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To assess this objective and to validate the hypothesis two groups of cows were done (n=18) one group with yeast supplementation in the concentrate feed delivered in the milking parlour twice a day, and the other without yeast supplementation. Animals were followed for 70 days after calving. During this period individual DM intake, milk yield and quality, body weight were recorded, and blood samples and BCS at 7, 28 and 70 day of study were obtained. Rumen pH was monitored in 7 animals per treatment throughout the study.

The experimental part was completed and finished the 28th April. The final dataset is being prepared to be delivered to GreenAgro.

Mid-term performance analysis is reported in the following table:

YEAST FEEDING TO DAIRY COWS

Control: n=13 (Parity: 2.00±1.47) Yeast: n=12 (Parity: 2.17±1.40)

Table 1. Lactation Performance

Parameter	Control	Yeast	Treatment	DIM	Treatment x DIM
BW, kg	691±3	693±3	0.6900	0.0001	0.6434
BCS	2.92±0.07	2.99±0.07	0.1342	0.0001	0.6554
DMI, kg	20.9±0.2	20.6±0.2	0.0236	0.0001	0.6167
DMI, % of BW	3.05±0.03	2.99±0.03	0.0009	0.0001	0.6863
Milk Yield, kg	39.2±0.3	39.1±0.3	0.1120	0.0001	0.9971
FCR (MY/DMI)	1.89±0.05	1.96±0.06	0.2561	0.0019	0.7029
Fat, %	4.14±0.09	3.81±0.10	0.0002	0.0001	0.9880
Fat, kg/d	1.55±0.06	1.47±0.05	0.0562	0.0216	0.3140
Protein, %	3.02±0.04	3.07±0.06	0.4324	0.0001	0.9884
Protein, kg/d	1.14±0.04	1.20±0.04	0.0088	0.0002	0.2330
Fat:Protein	1.37±0.03	1.25±0.03	0.0002	0.0001	0.9701
Lactose, %	4.93±0.02	4.86±0.04	0.0428	0.0096	0.7945
Lactose, kg/d	1.87±0.07	1.90±0.06	0.4598	0.0001	0.6850
Non-Solid Fat, %	8.76±0.04	8.72±0.08	0.3833	0.0001	0.8449
Urea, mg/L	182±5	177±6	0.3538	0.0058	0.5897
SCC, 10^3 per ml	165±28	240±56	0.0946	0.1676	0.2812

4.27 Evaluating Signal Strength of Implanted RFID (ESSIR)by Alastair McMahon (Chordata)

Withdrawn

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N°730924

4.28 Magnesium butyrate in periparturient dairy cows: effect on rumen redevelopment and potential impact on ruminal nutrient absorption by Joan Edwards (Palital Feed Additives)

Final report from the User:

The main objective of the project: The original objective of the proposal was to verify the proposed ruminal mode of action of magnesium butyrate (Rumen-Ready®) using rumen fistulated periparturient cows fed with or without magnesium butyrate for three weeks prior to calving. This involved key measurements of rumen wall surface area. However, due to a limited amount of remaining SmartCow budget this was not possible. Instead, a similar trial approach was planned using intact animals (n=6 per treatment), in conjunction with stomach tubing (one time point per sampling day) and rumen bolus devices, in order to gain mechanistic insights of how magnesium butyrate influenced the rumen, feed intake, performance and blood based markers.

The hypothesis that are tested: It was hypothesized that due to magnesium butyrate increasing the rumen wall surface area prepartum, resulting in the rumen being more effective in absorbing VFA postpartum and having an optimal rumen pH. Associated with this a higher milk yield was anticipated during early lactation without an increase in feed intake – i.e. the increased milk is associated with improved feed efficiency as a consequence of a more optimal rumen pH and VFA absorption.

In the experiment, before (day (d)-21, d-10) and after (d+0.5 to 1.5, d+7, d+14, d+21, d+28) calving, parameters from the rumen (i.e. VFA molar proportion and microbiota) and blood (i.e. NEFA, BHB, glucose, haptoglobin) will be assessed along with feed intake (d-21 to +28), rumen pH (d-21 to +28) and colostrum/milk yield and composition (d0 to +28). Rumen microbiota samples were collected for potential metagenomic sequencing in order to assess the impact of the magnesium butyrate treatment on the rumen microbiota.

The main scientific outcome, innovation/impact of the results: The main scientific outcome of the study is not known yet as the data is not fully available or analyzed at this point. Due to one of the cows (control group) developing ketosis during early lactation, this animal will have to be removed from the postpartum dataset. As a consequence of two cows (both control group) calving early, the feed intake and rumen pH data will only be analyzed from d-14 to +28, unlike the planned d-21 to+28.

It is anticipated that the trial findings will generate novel insights into the value of feeding of magnesium butyrate to prepartum cows, and the benefits of stimulating earlier rumen redevelopment. This is important as previously published studies looking at the value of butyrate for transition cows failed to find positive effects.

Any other achievements of the visit: As well as facilitating the planning of the experiment, the visit also enabled the range of dairy cow facilities and capabilities at DKC to be fully appreciated and valuable in-person meetings with the researchers involved. This helps build a strong foundation for planning future research projects at Aarhus.

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How do you expect to disseminate the results: It is anticipated that the results will be disseminated via a peer reviewed publication as well as related activities by Palital in terms of social media, webinars and articles in dairy industry journals.

Any suggestions to improve the TNA procedure: I would suggest that the users be asked to indicate how they intend to handle the data processing and statistical analysis if this is not being funded as part of the project. I am now finding the execution of the statistical analysis of the trial data challenging as I had not given this enough thought/attention earlier.

4.29 Amino acid nutrition by Lahlou Bahloul (Adisseo SA)

Amino acid nutrition affects both dairy cow productivity (e.g., milk protein yield) and the environmental sustainability (e.g., nitrogen use efficiency, NUE) of milk production. More precise diet formulation to optimise the profile of essential amino acids in metabolisable protein (MP) may permit use of lower protein diets without loss of milk protein production, thereby increasing NUE.

While considerable research is available documenting responses to improved amino acid nutrition (through the use of rumen-protected amino acids), responses of high-yielding cows offered low protein diets based on extensively fermented grass silage (a common production system in NW Europe) are poorly characterised. Our objective was to fill this knowledge gap.

Hypotheses: 1. Compared with the current commercial diet (based on extensively fermented grass silage), reducing dietary CP and MP without optimising amino acid profile will reduce productivity (milk protein yield) and have no effect on environmental sustainability (NUE). 2. Optimising the amino acid profile of a diet containing lower than current commercial CP and MP will improve productivity (milk protein yield) and improve environmental sustainability (NUE).

Material and Methods: Three treatment diets were compared using a randomised complete block design, using 63 multiparous lactating Holstein dairy cows less than 150 days in milk. Cows were first arranged into three blocks (cohorts) of 21 based on calving date. Each cohort occupied the experimental facility consecutively for 35d. Cohorts 1 and 2 completed the study before 30 April 2022 (i.e., within the Smart Cow project). Cohort 3 cows are scheduled to be on experiment in May and June 2022, supported by additional funding directly from Adisseo SA.

Treatments were:

A. Total mixed ration (TMR) formulated to meet ME and MP requirements according to the Feed into Milk model (Thomas, 2004) (as a diet descriptor, CP = *circa* 17.5% CP in DM).

B. Lower protein TMR, formulated to meet ME requirements and 0.95 of MP requirements (diet is expected to be *ca.* 16% CP in DM).

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C. Amino acid optimised TMR, formulated to meet ME requirements and requirements for metabolisable methionine and lysine. This diet will be isonitrogenous with diet B.

63 multiparous lactating Holstein dairy cows less than 150 days in milk were first arranged into three blocks (cohorts) of 21 based on calving date. Each cohort occupied the experimental facility consecutively. Within each cohort, cows were arranged into seven blocks of three according to stage of lactation and milk protein yield. Within each block of three, cows were allocated at random to treatment and offered treatment diets for 35d.

Diet ingredients were grass silage, whole crop wheat silage, dried sugar beet pulp, rapeseed meal, rumen-protected rapeseed meal, maize distillers' grains, rumen-inert fat, vitamin/mineral premix and a source of rumen-protected methionine.

Cows were milked twice daily, feed intake was recorded daily (from visits of individual cows to Hokofarm feed bins) and milk composition (fat, crude protein, lactose, urea, somatic cells and major fatty acids) was measured by mid-infrared spectroscopy in samples collected throughout the 35d experimental period. Cows were weighed automatically on entry to the milking parlour and Body Condition Score was recorded at the beginning and end of the experimental period.

The main scientific outcome, innovation/impact of the results: Results are presented for cohorts 1 and 2, pending complete data and analysis for cohort 3.

Feed analysis

For cohort 1, concentrations of CP in TMR (Table 3) were 4, 4, and 3% less than targeted for TMR A, B and C, respectively (Table 1). Measured CP in TMR was similarly below target for cohort 2.

Table 1. Analysis of total mixed rations

	Cohort 1			Cohort 2		
	TMR A	TMR B	TMR C	TMR A	TMR B	TMR C
DM (g/kg)	450	454	447	435	429	428
CP (g/kg DM)	174	155	157	174	151	155
NDF (g/kg DM)	314	345	341	355	375	371
Starch (g/kg DM)	182	168	160	156	170	167
Ash (g/kg DM	77	80	80	74	71	73

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Oil (g/kg DM)	50	52	53	52	52	51

These shortfalls in CP can be attributed to lower than expected CP concentrations in blends and grass silage (data not shown).

Cow performance

Mean results across cohorts 1 and 2 are presented: differences between cohorts will be evaluated when full results are available for cohort 3.

Treatment groups did not differ in the stage of lactation of cows at enrolment to the study (103, 107 and 113 days after calving for treatments A, B and C, respectively (P>0.05)).

Treatment had no effect on DMI (Table 4). However, cows on treatment A were lighter than those on treatments B and C, such that DMI as % BW was lowest for treatment B and highest for treatment A.

Milk yield tended to be lower for the two low protein treatments, B and C, than treatment A, although the difference was numerically large (4.7kg/d). Consistent with this, milk urea concentration was lower for treatments B and C than treatment A.

Milk macro-composition (lactose, fat and crude protein concentrations) was not affected by treatment, while milk urea was significantly lower for the two low protein treatments, B and C, than treatment A.

Largely as a consequence of differences in milk yield, yields of lactose, fat and protein all fell when the concentration of dietary protein was reduced. This fall in N output compensated for the reduction in N intake, such that NUE was not affected by the reduction in dietary CP.

No cow performance variable was different between treatments B and C.

Table 2. Cow performance.

	A	B	C	Sem	P
DMI (kg/d)	23.7	23.5	23.7	0.59	0.945
DMI (% BW)	3.46[a]	3.17[b]	3.35[ab]	0.082	0.057
Milk yield (kg/d)	39.5	34.8	34.7	1.53	0.053
Milk lactose (%)	4.61	4.51	4.49	0.041	0.103
Milk fat (%)	4.27	4.18	4.23	0.149	0.908
Milk crude protein (%)	3.19	3.16	3.19	0.079	0.957
Milk urea (%)	0.0155[a]	0.0062[b]	0.0073[b]	0.00199	<0.001
Somatic cell count (Kcell/ml)	30	105	104	39.1	0.310
Milk lactose (g/d)	1822[a]	1566[b]	1556[b]	71.4	0.020
Milk fat (g/d)	1680[a]	1450[b]	1436[b]	62.8	0.016
Milk protein (g/d)	1255[a]	1096[b]	1077[b]	40.9	0.008

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NUE	0.299	0.298	0.286	0.086	0.477
Body weight (kg)	686[a]	743[b]	711[ab]	12.7	0.012
Body weight change (kg/d)	0.064	0.150	0.047	0.1163	0.799
Body Condition Score	*Data not yet evaluated*				
BCS change (units/d)	*Data not yet evaluated*				

Discussion

Lowering the concentration of dietary CP (treatment B versus treatment A) reduced milk protein yield but did not increase NUE.

Responses of milk protein yield to MP are curvilinear, with the apparent efficiency of utilisation of MP for milk production approaching 0.64-0.67 at low protein intakes (well below estimated requirements) and declining to typically 0.15 or lower as protein intake increases.

In this experiment, across all diets, measured dietary CP was ca. 7-8g/kg DM lower than target levels, placing the diets on a steeper part of the response curve than planned. Thus, the decline in milk protein yield is expected to be higher, and the gain in NUE lower, than anticipated.

Using formulated values for MP concentration (Table 2) and treatment means for milk protein yield (Table 3), and assuming no difference in the partitioning of MP, the calculated marginal efficiency of utilisation of MP for milk protein production is actually greater than 1. Body weight change was not different between treatments, so a major contribution to MP supply from mobilised body protein is unlikely. Perhaps the most likely explanation is that the difference between treatments in MP was greater than the 4g/kg DM (108-104) estimated when diets were formulated. The contribution of MP from digestible undegradable protein (DUP) may have been less than planned. Further calculations of the N economy for individual cows is warranted.

Adding methionine in the form of Metasmart Dry did not increase milk protein yield or NUE (treatment C versus treatment B). It is possible that other nutrients (such as metabolisable lysine or histidine) were limiting to milk protein synthesis. The possibility of degradation of the active molecule in Metasmart (HMBi) under the damp conditions of the TMR is currently being investigated.

How do you expect to disseminate the results? Results may be reported at animal science conferences such as RRR (France), BSAS (UK) or ADSA (USA).

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5 Appendix

Table 1. Overview of projects agreed on from the first call (Arnott and Herremans were both withdrawn)

Applicant	Country	Title	Facility	Requested cow-weeks
Gareth Arnott (Queens University Belfast)	United Kingdom	Investigating links between beef cattle behaviour, temperament and diet with changes in the rumen microbiome and implications for performance	Teagasc Grange	960
Ruth Heering (University of Hohenheim)	Germany	Impact of physically effective fiber concentrations on chewing behavior, rumen microbial 4 protein synthesis, and nitrogen efficiency in cows	INRA Theix	48
Joël Berard (ETH)	Switzerland	From grassland biodiversity to animal's microbial ecosystems and cheese qualities	INRA Marcenat	576
Poulad Pourazad (Delacon)	Austria	PFA effect on methane production	INRA Marcenat	784
Martina Jakob (FBN)	Germany	Multiple spatially resolved reflection spectroscopy (MSRRS) - carotenoid content of the skin of cows.	SRUC Dairy INRA Le Pin Teagasc Moorepark	100 80 56
Rolland Matthieu (Ajinomoto)	France	Impact of oscillating supply of essential amino acids on whole-body nitrogen partitioning, mammary gland metabolite utilization, and milk nitrogen efficiency in lactating dairy cows	Carus	68
Toshihiro Marubashi/Noriko Nakamura (Calpis)	United Kingdom/Japan	Effects of Bacillus probiotic on productivity, health and welfare of dairy cows	WUR Dairy campus	1008
Lahlou Bahloul (Adisseo)	France	Essential amino acid supplementation…	Aarhus (AU2)	64

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Nicolaj Ingemann Nielsen (SEGES)	Denmark	Increased N-utilisation from dairy cows by phase feeding of protein	Reading CEDAR	540
Francisco Maroto (University of Cordoba)	Spain	From feed composition to animal response by u sing Near Infrared Spectroscopy	Aarhus (AU1)	480
Sophie Herremans (CRA-W)	Belgium	Improving the nutritive value estimation of multi-species forages for beef cattle	Teagasc Moorepark	16

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Table 2. Overview of projects agreed on from the second call (Fernandes was withdrawn)

Applicant	Country	Title	Facility	Requested cow-weeks
Beatrice Zweifel (Agolin Ireland)	Ireland	Impact of Agolin Ruminant on feed efficiency and methane emissions of finishing beef cattle	SRUC Beef Centre 2 SRUC Beef Centre 1	48 720
Luiza Fernandes (ED&F MAN Liquid Feeds)	Spain	An holistic approach on transforming molasses and liquid by-products intor more efficient sugar-based liquid feed to increase dairy cattle efficiency	FBN EFC -Barn	720
Angela Schwarm (Norwegian University of Life Science	Norway	Sustainable ruminant production: Methane emission, microbiome and immune function in dairy cattle	FBN EFC Resp-Charm	32
Ignacio Gomez Maqueda (Digitanimal)	Spain	Detection of reproductive events with smart collars suitable for extensive cattle systems	INRA PEB Le Pin	758
Georgina Chapman (ED&F Man Liquid Products)	Ireland	The effect of a molasses based liquid feed on in vivo fibre digestion and nitrogen use efficiency	UREAD CEDAR	64
Valérie Kromm (Animine) / Stéphane Durosoy	France	Evaluation of zinc sources in dairy cattle	IRTA EVAM	720
Katerina Theodoridou (Queen's University Belfast)	UK	Investigate the effect of Inclusion of seaweed on milk production, feed efficiency and rumen microbiome, of dairy cattle	IRTA EVAM	480

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Table 3. Overview of projects agreed on from the third call.

Applicant	Country	Title	Facility	Requested cow-weeks
Beatrice Zweifel (Agolin)	Switzerland	Impact of a phytogenic additive on methane production and performance in dairy cows	FBN	180 barn/90 chambers
Poulad Pourazad (Delacon) / Thierry Aubert	Austria	Effects of phytogenic feed additives (PFA) in lactating dairy cows under Heat-stress condition.	FBN	20 chambers/ 40 physiol
Denise Cardoso / Stéphane Durosoy (Animine)	France	Novel trace mineral source	SRUC dairy	192
Raphaël Boré (Idele)	France	Strengthen Laser Methane m-g (LMm-g) device measurement protocol to estimate methane emission of dairy cow ruminants directly in any commercial farm.	IRTA	100
Anne Boudon (Inrae)	France	Consequences of the grazed pasture diversity on annual variability of nutritional value and technological properties of milk, and nutritional status of Holstein-Friesian and Jersey Holstein-Friesian crossbred dairy cows (GRAMIQS / GRAzing – MIlk –QualitieS)	Teagasc Moorepark	576

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Table 4. Overview of projects agreed on from the fourth call (McMahon and Bennison were withdrawn)

Applicant	Country	Title	Facility	Requested cow-weeks
Krum Vladimirov Nedelkov (Trakia University) / Gonzalo Cantalapiedra (INRAE)	Bulgaria/France	Is animal-to-animal variation in feed efficiency mostly driven by differences in appetite in beef cattle?	Teagasc Grange	1100
Jamie Bennison (Agrimin)	UK	The use of sustained release boluses with essential oils to improve the productivity of dairy cows in forage based systems	Teagasc Moorepark	600
Tanja Kotevska (Greenagro dooel)	Macedonia	Effects of live yeast supplementation on dairy cows	IRTA EVAM	288
Alastair McMahon (Chordata)	UK	Evaluating Signal Strength of Implanted RFID (ESSIR)	WUR	48
Joan Edwards (Palital Feed Additives)	NL	Magnesium butyrate in periparturient dairy cows: effect on rumen redevelopment and potential impact on ruminal nutrient absorption	Aarhus (AU2)	60
Lahlou Bahloul (Adisseo SA)	FR	Amino acid nutrition	SRUC Dairy Centre	210

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