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an integrated infrastructure for increased research capability and innovation in the European cattle sector

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of data collection, including a final report from the user (report 3).* *Across the three calls, three projects were withdrawn due to lack of national funding that was needed beyond the SmartCow funding* *All report 0 from the 11 TNA users from the first call were received. Six projects are finished and the final reports are delivered, and three projects are running, and two have been withdrawn* *In the second call 7 projects were agreed, two projects have been postponed to 2021, one project has been withdrawn and 4 projects have started in 2020* *In the third call 5 projects were agreed, and all projects have submitted their initial report.* *The proposals from the additional fourth call are currently being evaluated.*

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1 2nd Project Evaluation Report

This is the second 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 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. Finally, the report includes a detailed description of the status of all projects.

2 Process and monitoring of the TNA projects

2.1 The selection process

For the first three calls 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 accomodated 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.

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

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about the project such as start date of the experiment etc. All TNA users from call one have submitted report 0, for call 2 4 out of 7, as 2 projects have been postponed until 2021 and 1 project has been withdrawn, and for call three all projects have submitted the report 0. After the first call 11 projects were agreed on, one project was however withdrawn due to lack of national funding to cover expenses not covered by SmartCow finances. Table 1, 2, and 3 in the appendix show the projects and the status of the projects from call 1, 2 and 3, respectively at the end of 2020. A total of 6 projects are complete and all the reports returned to SmartCow. Some reports 1, 2 and 3 have been delivered, due to postponement due to Covid-19 and since many projects are still in the initiating phase.

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); and 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

Since only a few projects were finished at the end of 2019, and the majority were planned to start in in 2020 but are delayed due to Covid-19, the experiences and the output from the reports from the TNA users and facility managers is so far limited. However, from report (0) 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 and should be kept in the procedure, 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. The projects that are finished and have reached the date for their final report have

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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.
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 most 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.

3.3 Covid-19

Covid-19 has had a significant influence on both planned and ongoing trials. A significant number of granted projects have been 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 and for now no projects have yet been 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 when projects were starting up (report 0) at the beginning (report 1), midterm (report 2) and end of each animal trial (report 3+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. Thus initiation of this project is still in process and other cows have to be allocated to this project. The project has now been 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 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.

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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 abundancy 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 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

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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:

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

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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.

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,

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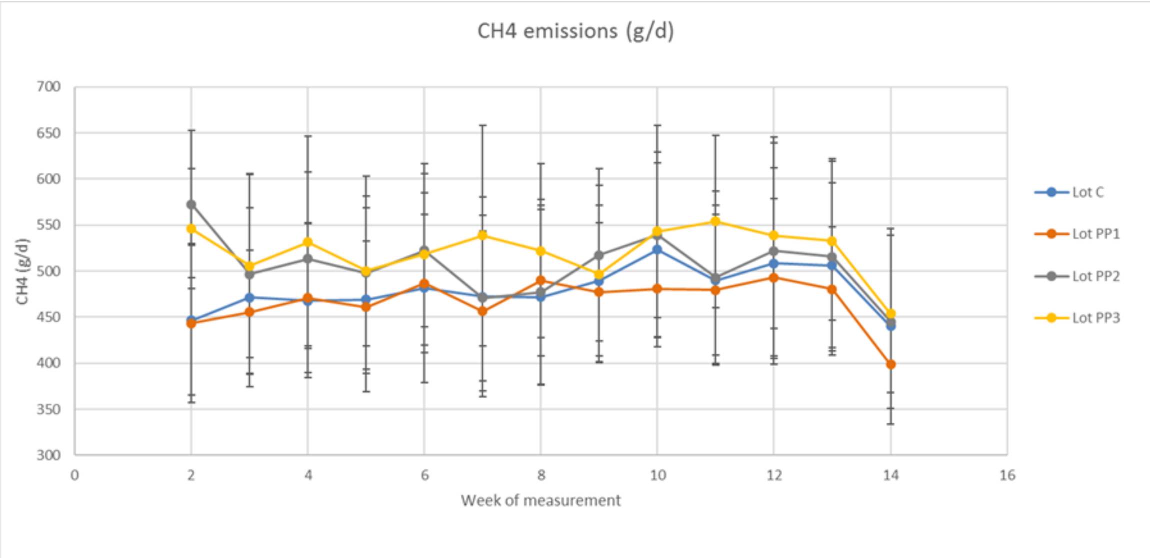
at least, that the tested compounds does not impair milk production and are as palatable as the control concentrate, which does not contain any essential oil. Further analysis has to be carried out by Delacon GmbH to get more details. (ii) Methane emission. It has been contractually agreed that Delacon 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 other achievements of the visit: -.

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, and on weekends, no measurements took place. Therefore the acute phase of the

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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.html
It 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 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.

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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).

The trial was originally planned to run in the period from May 18th until August 6th, but was postponed and will run in the period from June 18th 2020 until March 31st 2021, and report 0 and report 1 have been submitted. The trial includes 896 cow weeks at the WUR-DLO Dairy Campus Installation and a detailed protocol for the experiment as well as a written contract have been provided. The project has been approved by the ethical committee.

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

The project is still running with a contract between the TNA user and AU that goes beyond the funding from SmartCow. A scientist from AU is involved in the projects and will be co-author on the publication(s) about the results. The project aims to determine whether high protein requirements of early lactation dairy cows can be satisfied with an increased supply of only the essential amino acids (EAA). Recent research has indicated substantial increases in milk yield (MY) and marginal N efficiency with an increased supply of digestible protein in early lactation. However, it is not known if this large impact could be obtained by increasing only the supply of EAA. Indeed, previous research with mid-lactation cows indicates only supplemental EAA are needed. Moreover, N efficiency would also be significantly higher if MY increases were attainable with only targeted EAA rather than supplying all AA. We hypothesis that supplementation of targeted EAA in early lactation will increase MY, N efficiency, and ultimately prove to decrease incidence of metabolic disorders. This project is a first step towards identifying the AA most needed in early lactation and for determining whether or not the non-EAA are needed to induce the large MY responses observed in previous experiments. The expected outcome will allow for significant changes in protein and AA feeding recommendations for cows in early lactation, a critical period for establishing subsequent lactation and reproductive performance and reducing incidence of post-calving metabolic disorders. Cows are allocated into four blocks and randomly allocated to one of two treatments within block. Cows are blocked according to lactation number and breeding value for milk yield (Y-index). One cow died in connection with calving and one cow had a poor temperament, which made it difficult to take blood samples. Thus an extra bach of cows is running in the beginning of January. At the day of calving, the abomasal infusion device will be placed and infusion initiated as soon as possible after calving. Experimental treatments will infusion of either all amino acids (**TAA**) or only essential amino acids (**EAA**) where the non-essential will be removed from the infusion mix. At maximal infusion the daily infused amounts will be targeted to 8050 g/d.

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

The experiment was originally planned to start in November 2019 at Reading University, but because more cows are calving in the beginning of 2020, it was decided to postpone the start of the experiment to February 2020. The trial finished in September 2020 and included 540 cow weeks. Report 0+1+2 have been submitted. Visits have not been possible due to Covid-19.

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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.

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:

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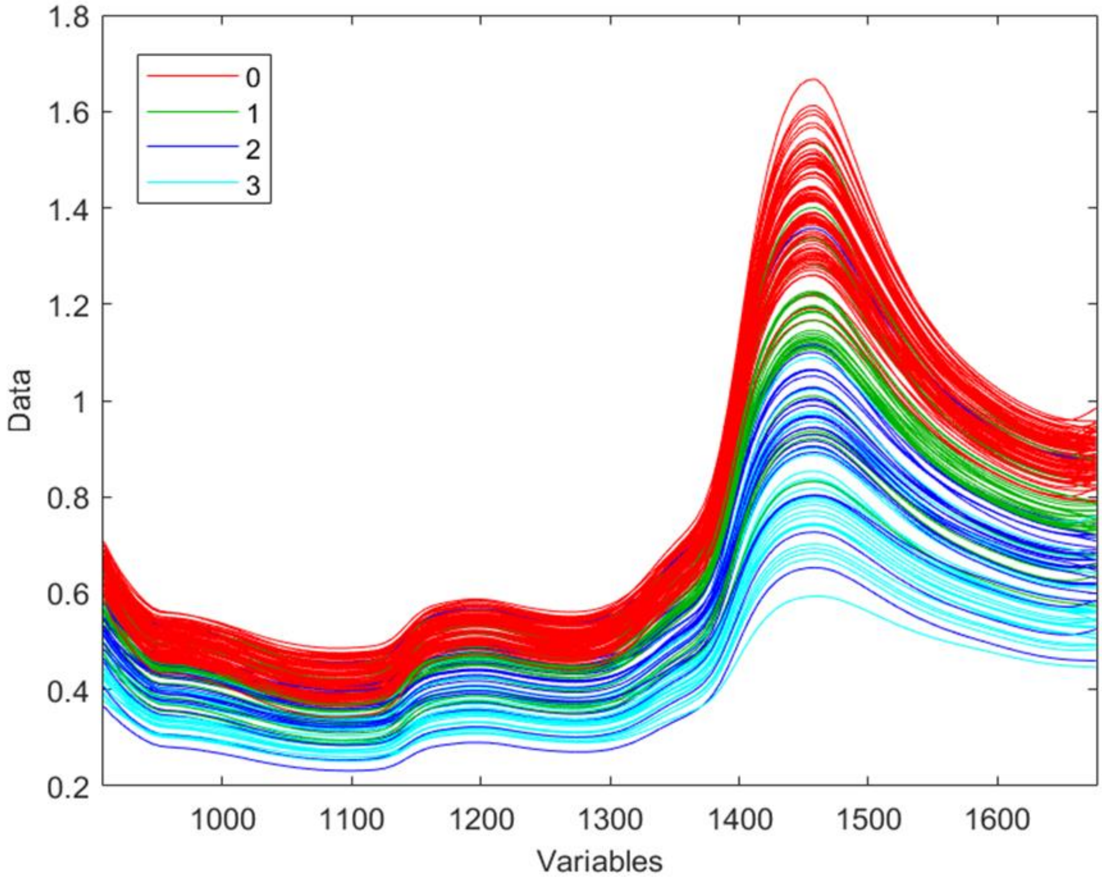

However, in the PCA below, we can see that there is also some overlap between the different types of diets. For example, some samples of diet 1 (5% replaced) are similar to some samples of diet 0 (0% replaced - control) and others to samples of diet 2 (10% replaced).

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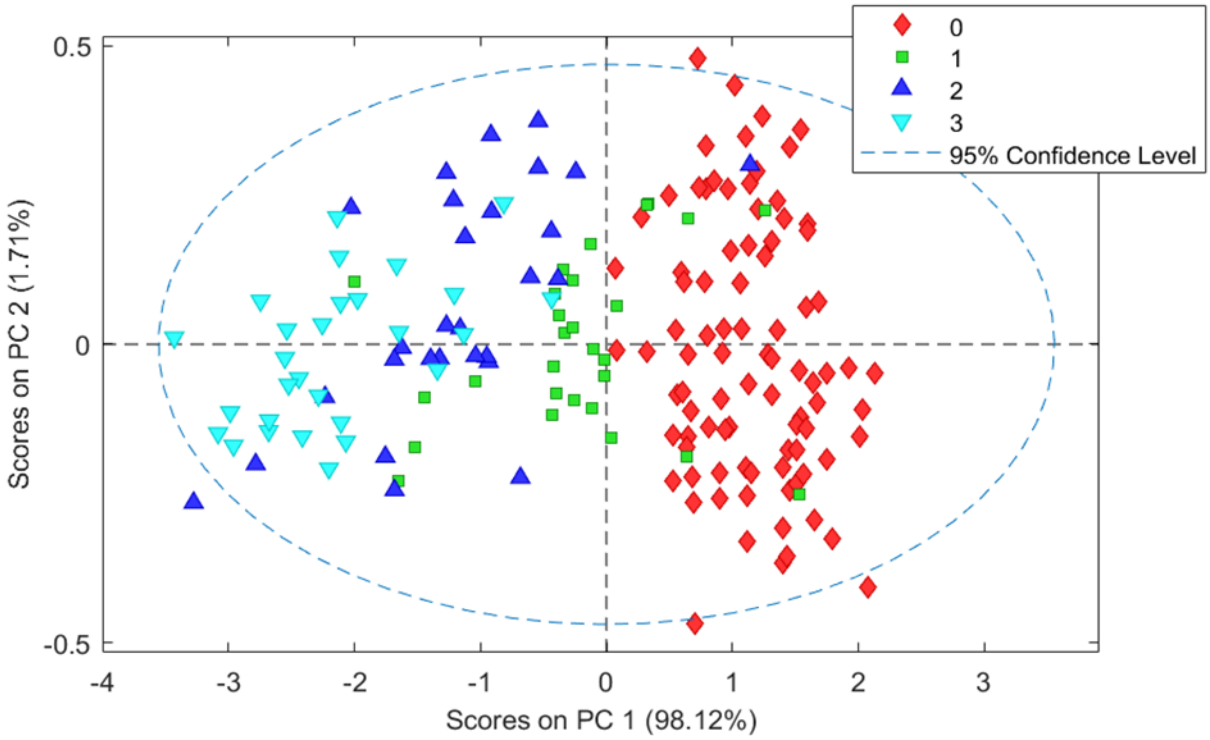
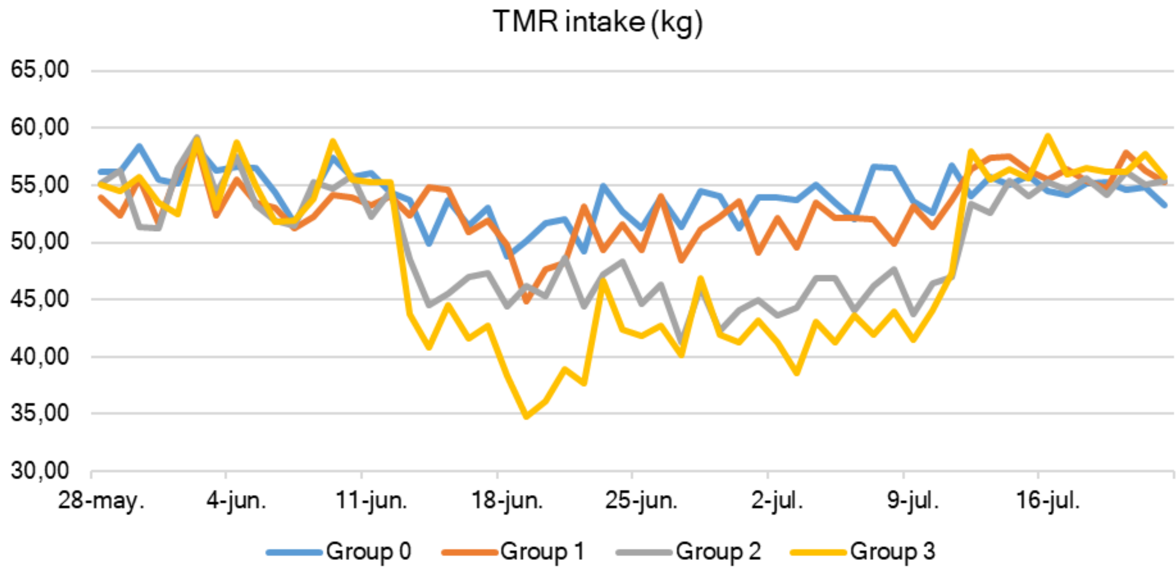


The variability between days for the same diet is important to understand the variability of animal response.
As expected, we also obtained variability in animal response:



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 behaviors 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):

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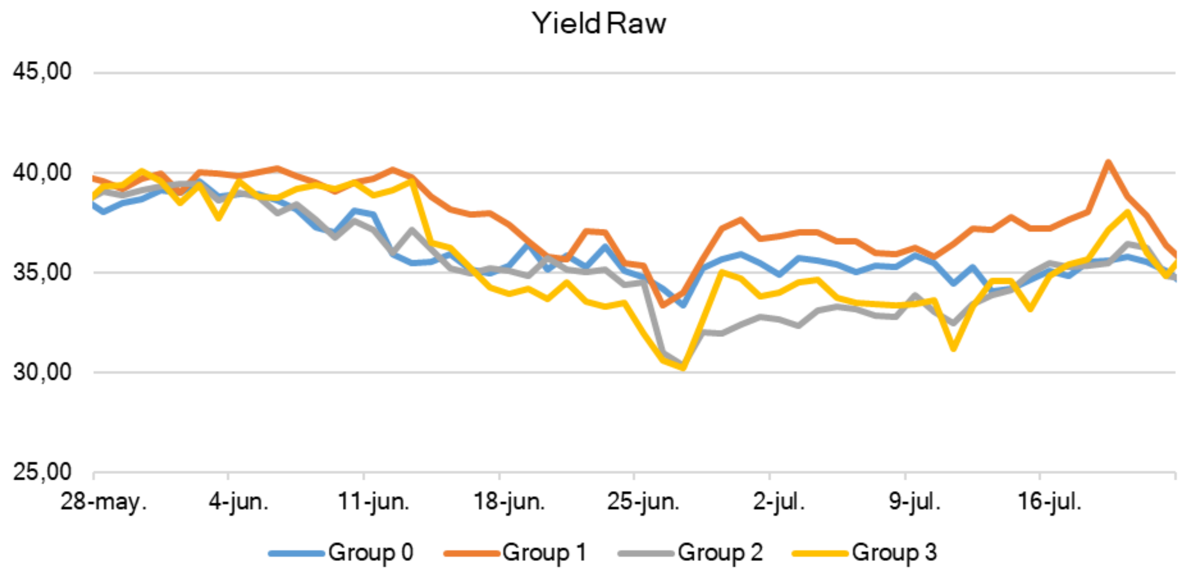

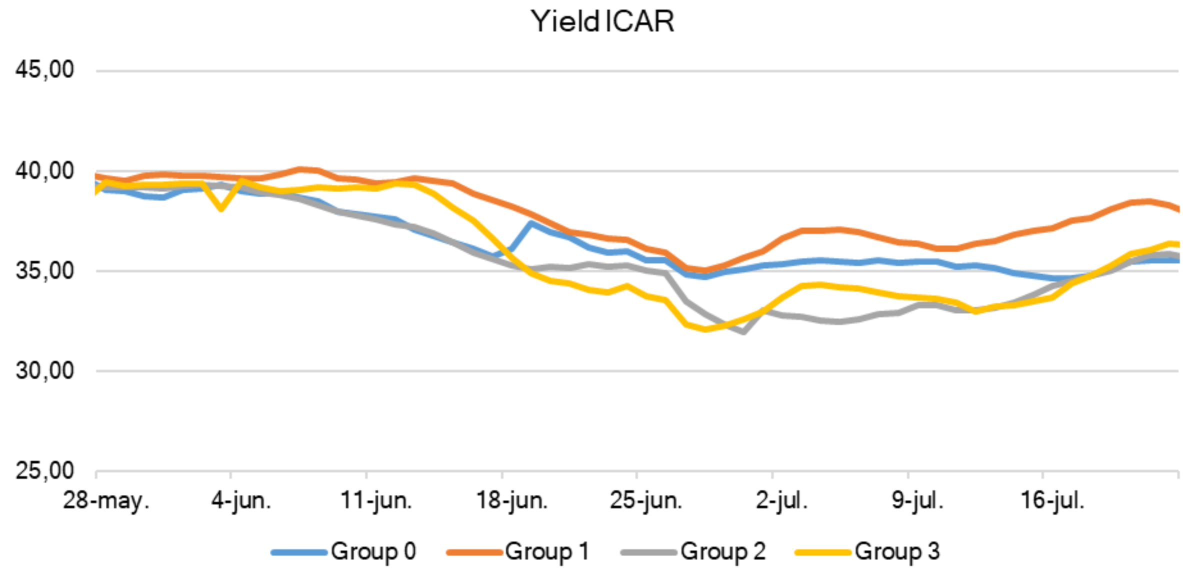

In this case, the original production values were not recovered after the experimental phase (when all cows go back to control diet) so the previous production level need somehow to be included in calibrations. On the other hand, we observed an unexpected decrease in milk production of control group. 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

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successful experiment development. This information will be valuable for the user when establishing other experiments involving animals in his own institution.

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)

The current project is in collaboration with SRUC and includes a total of 48 cow weeks. The trial was originally planned for July 2020- December 2020, but has been postponed due to covid-19. The original plan was to measure methane emissions from cattle 168 days after introduction of the feed additive. However, due to COVID-19 related delays to the trial beginning and consequently the greater initial body weight of the animals this has been shortened to 98 days. This will not impact on the validity of the experimental results. Despite initial delays, the trial is now running smoothly and on track for the experimental work to be completed on 24th December 2020. Reports 0+1+2 have been submitted.

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)

This project is in collaboration with FBN and includes 32 cow weeks. The trial started in January 2020 and finishes in December 2020. At the time for report 2, 21 of 32 cow weeks were completed. Visits have been challenging due to covid-19. Reports 0+1+2 have been submitted.

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

This project has been postponed until 2021 due to Covid-19.

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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)

The study includes 94 cow weeks to complete and is in collaboration with Reading University. Reports 0+1+2 have been submitted. The trial was planned to be conducted in the period from March to July 2020. The project is delayed by a few weeks from the intended start date due to restructuring of site activities due to covid 19 restrictions. Some miscommunication before trial began, thought 1 x IBC of product would be enough for the trial but 2 were needed. New blend had to be created and delivered. Visits have been challenging due to covid-19.

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

This project is in collaboration with IRTA and includes 720 cow weeks. The trial runs from September 22nd 2020 until February 28th 2021, and reports 0+1 have been submitted.

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))

This project has been postponed until 2021 due to Covid-19.

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

This study is from call 3 and in collaboration with FBN and includes 270 cow weeks. The animal trial is planned for December 2020 to August 2021. A detailed protocol has been provided and ethical approval was granted at November 5th 2020. No visits are currently planned due to covid-19. Reports 0+1 have been submitted.

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

This study is from call 3 and is in collaboration with FBN and is expected to run from June/July 2021 until January 2022. The project is still under negotiation between SmartCow, User and FBN-Farm manager. Report 0 has been submitted.

4.21 Copper(I) oxide as source of copper for lactating dairy cows by Denise Cardoso (Animine)

This project is from call 3 and is in collaboration with SRUC and includes the use of 400 cow weeks. Report 0 has been submitted. The trial is intended to start in February 2021 and finish in October 2021. The protocol has been intensively discussed between the parties to make sure all details were covered, and protocol and associated SOPs are still in development.

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)

This study is funded in call 3 and is in collaboration with IRTA. The expected number of cow weeks is 100, and the trial is expected to run from January 2021 until March 2021. Report 0 has been submitted.

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

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)

This study is from call 3 and in collaboration with Teagasc, and report 0 has been submitted. A first meeting was hold in November and a first draft of the protocol was provided by the user. The preliminary time slot for the trial is March 15th 2021 – July 15th 2021. A detailed protocol of the experiment has been provided.

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

5 Appendix

Table 1. Overview of projects agreed on from the first call. Status at the end of 2020.

Applicant	Country	Title	Facility	Requested cow-weeks	Comments	Report 0	Report 1	Report 2	Report 3	Final report
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		Withdrawn	Withdrawn	Withdrawn	Withdrawn	Withdrawn
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		X	X	X	X	X
Joël Berard (ETH)	Switzerland	From grassland biodiversity to animal's microbial ecosystems and cheese qualities	INRA Marcenat	560		X	X	X	X	X
Poulad Pourazad (Delacon)	Austria	PFA effect on methane production	INRA Marcenat	810		X	X	X	X	X
Martina Jakob (FBN)	Germany	Multiple spatially resolved reflection spectroscopy (MSRRS) - carotenoid content of the skin of cows.	SRUC Dairy INRA Le Pin	100 100		X	X	X	X	X
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		X	X	X	X	X
Noriko Nakamura (Calpis)	United Kingdom	Effects of Bacillus probiotic on productivity, health and welfare of dairy cows	WUR Dairy campus	896	Postponed from May 2020 - August 2020 to June 2020 - March 2021.	X	X			
Lahlou Bahloul (Adisseo)	France	Essential amino acid supplementation…	Aarhus (AU2)	64	Experiment finalised					
Nicolaj Ingemann Nielsen (SEGES)	Denmark	Increased N-utilisation from dairy cows by phase feeding of protein	Reading CEDAR	540 (different study type)	Postponed from November 2019-April 2020 to February 2020-September 2020	X	X	X		
Francisco Maroto (University of Cordoba)	Spain	From feed composition to animal response by u sing Near Infrared Spectroscopy	Aarhus (AU1)	480		X	X	X	X	X
Sophie Herremans (CRA-W)	Belgium	Improving the nutritive value estimation of multi-species forages for beef cattle	Teagasc Moorepark	16 (different study type)		Withdrawn	Withdrawn	Withdrawn	Withdrawn	Withdrawn

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Table 2. Overview of projects agreed on from the second call. Status at the end of 2020.

Applicant	Country	Title	Facility	Requested cow-weeks	Comments	Report 0	Report 1	Report 2	Report 3	Final report
Beatrice Zweifel (Agolin Ireland)	Ireland	Impact of Agolin Ruminant on feed efficiency and methane emissions of finishing beef cattle	SRUC Beef Centre 2	48	Postponed from March 2020-October 2020 to July 2020-December 2020	X	X	X		
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		**Withdrawn**	**Withdrawn**	**Withdrawn**	**Withdrawn**	**Withdrawn**
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	January 2020-December 2020	X	X	X		
Ignacio Gomez Maqueda (Digitanimal)	Spain	Detection of reproductive events with smart collars suitable for extensive cattle systems	INRA PEB Le Pin	720	Postponed until 2021					
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	March 2020-July 2020, but delayed a few weeks	X	X	X		
Valérie Kromm (Animine)	France	Evaluation of zinc sources in dairy cattle	IRTA EVAM	720	September 2020-February 2021	X	X			
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	Postponed until 2021					

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Table 3. Overview of projects agreed on from the third call. Status at the end of 2020.

Applicant	Country	Title	Facility	Requested cow-weeks	Comments	Report 0	Report 1	Report 2	Report 3	Final report
Beatrice Zweifel (Agolin)	Switzerland	Impact of a phytogenic additive on methane production and performance in dairy cows	FBN	180 barn/90 chambers	December 2020-August 2021	X	X			
Poulad Pourazad (Delacon)	Austria	Effects of phytogenic feed additives (PFA) in lactating dairy cows under Heat-stress condition.	FBN	20 chambers/ 40 physiol	Still exploring whether this is possible at FBN - June 2021-January 2022	X				
D. Cardoso (Animine)	France	Copper(I) oxide as source of copper for lactating dairy cows	SRUC dairy	408	February 2021-October 2021	X	X			
Raphael Bore (Idele)	France	Strengthen Laser Methane m-g (LMm-g) device measurement protocol to estimate methane emission of dairy cow ruminants	IRTA	100	January 2021-March 2021	X				
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	March 2021-July 2021	X				

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