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

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

EXECUTIVE SUMMARY

Background	In the frame of SmartCow project, FBN has provided access to its Research Installation "EFC" through Trans National Access (TNA).
Objectives	This Deliverable aims at describing the TNA provided by FBN during the SmartCow project.
Methods	The Deliverable is composed of a table summarising the TNA provided by the Research Installation (RI) and by the reports of activities provided by the TNA users who accessed this RI.

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Table of contents

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

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

1 TNA provided

Name of the TNA project	Name of TNA user	Organisation of TNA user	Country of TNA user	Installation from the RI	Start date	End date	Number of units of access provided
Impact of a phytogenic additive on methane production and performance in cows	Katrin Spengler, Arnaud Jouve	Agolin (Ireland) Ltd.	Ireland	(1)Barn, (2)Resp Cham	07/12/2020	31/08/2021	270 cow.weeks
Metabolic responses in lactating dairy cows supplemented with phytogenic feed additives under heat-stress condition	Poulad Pourazad and Theirry Aubert	Delacon Biotechnik GmbH	Austria	(2)Resp Cham (3) ExpPhysRoom	27/09/2021	08/02/2022	80 cow.weeks
Sustainable ruminant production: Methane emission, microbiome and immune function in dairy cattle	Angela Schwarm, Puchun Niu	Norwegian University of Life Sciences	Norway	(2)RespCham	01/03/2020	15/12/2020	32 cow.weeks

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2 Final reports of each TNA provided

2.1 TNA 1

The aim was 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. It was hypothesized that the administration of 1 g/d of the phytogenic additive Agolin Ruminant Liquid over 6 or 14 week reduces methane production and improves milk yield.

The trial was conducted with 30 clinically healthy, gestating German Holstein cows (Control, KN, n=15 cows; Treatment AgolinRuminant Liquid, AR, n=15 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. The study was conducted as a randomized complete block design (9 blocks) with staggered entry of cows in the experiment. Mean cow characteristics in the week prior to the start of the study were similar in the 2 groups in regard to lactation no. (2.2), days in milk (173 d), body weight (618 kg), feed dry matter consumption (16.4 kg), rectal temperature (38.3 °C), and milk yield (28.1 kg/d). 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.

The Agolin Ruminant (AR) Liquid supplement was stored in a refrigerator at 4oC. 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 4oC. 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 standard TMR at FBN for mid- to late lactation cows based on grass and maize silage (crude protein 16.6 g/kg dry matter; 7.04 MJ NEL/kg dry matter) 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). 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

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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).
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 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.

Cows were in the mid to late phase of their lactation during the study. Body weight did not differ between groups within the experimental weeks as did rectal temperature (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 in a physiological manner from experimental week 0 to weeks 7 (AR) and 14 (AR, KN) (P<0.05). 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.
In the barn, cows in the AR group consumed daily 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). Within the AR group BFT was higher in EW 12 and 14 compared to 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 experimental period. In contrast, body weight did not differ by group when measured in the barn (F-Test, P=0.13) nor experimental week (F-Test, P=0.11) while a numerical increase over time could be observed. Milk yield did not differ among the groups (F-Test, P=0.4). Milk yield was lower in EW 12 and 14 as compared to EW 2 (P<0.05).

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

Visits: A planned visit had to be cancelled unfortunately, due to Covid19.

Dissemination plan: No plan for publication is made.

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2.2 TNA 2

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.
To evaluate the effect of Actifor Boost HS prototypes, we used 2 groups of 10 animals managed during 3 periods:
- 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.

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).

During each period, the milk production and feed intake were measured daily. Milk quality parameters (fat, protein, somatic cells, milk urea) were 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 behaviour 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 was measured. Concentrations of carbon dioxide and dioxygen are measured in the chamber and the airflow is analysed to estimate the consumption of oxygen and the production of carbon dioxide during respiration. Based on that the metabolic respiratory quotient was calculated: RQmetab = VCO2/VO2. Based also on the methane production per head, per day, and the urine nitrogen (assuming 100 g daily Nu), the heat production was calculated using the Brouwer equation. By difference between metabolized energy intake (MEI) and heat production (HP), we estimated the energy retention (RE = MEI – HP), and by difference with metabolic energy in milk (MEM) the energy used for energy retention in body tissues (REtissue): REtissue = RE – MEM.

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

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 Index) 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.

The following parameters were analyzed as:
ANOVA model: X ~ COV + Treatment + Period + Treatment: Period
For the covariable, we considered the 3 first days of the 1st period.

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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).

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. 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. For the behavior, 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. The metabolic RQ was 0.71 and the negative value for body retention indicated weight losses in dairy cows during this trial.

Outcome/impact: 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: If this finding is due to the short period of heat stress, or due to general stress in the chamber remains to be elucidated. Actifor® Boost HS has increased heart rate and breath frequency significantly showing that this product has no impact on behavior.

Visits: No visit due to Covid19 restrictions.

Dissemination plan: No plan for publication is made**.**

2.3 TNA 3

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

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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.

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.

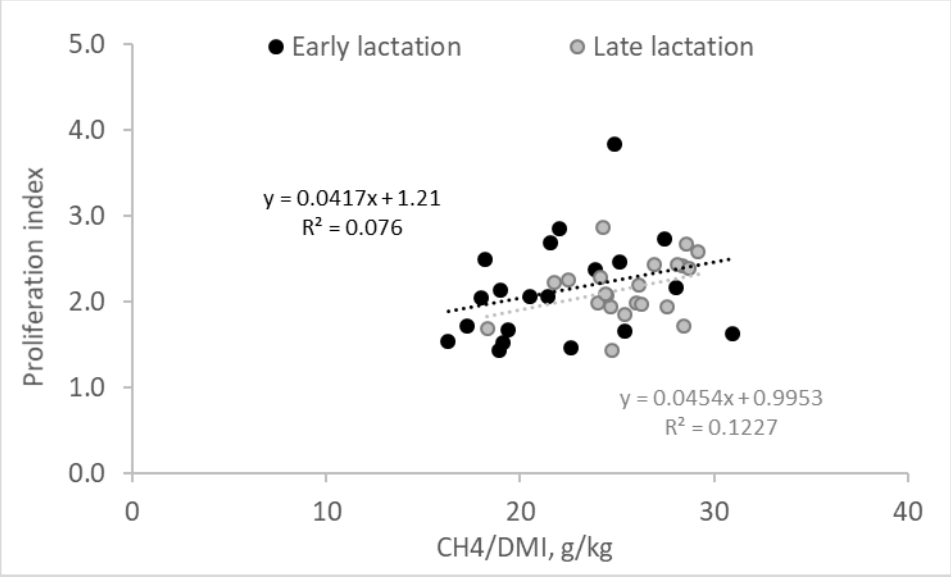

Figure 1. Relationship of methane emitted per unit of dry matter ingested with the proliferation index of peripheral blood mononuclear cells.

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

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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 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.

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