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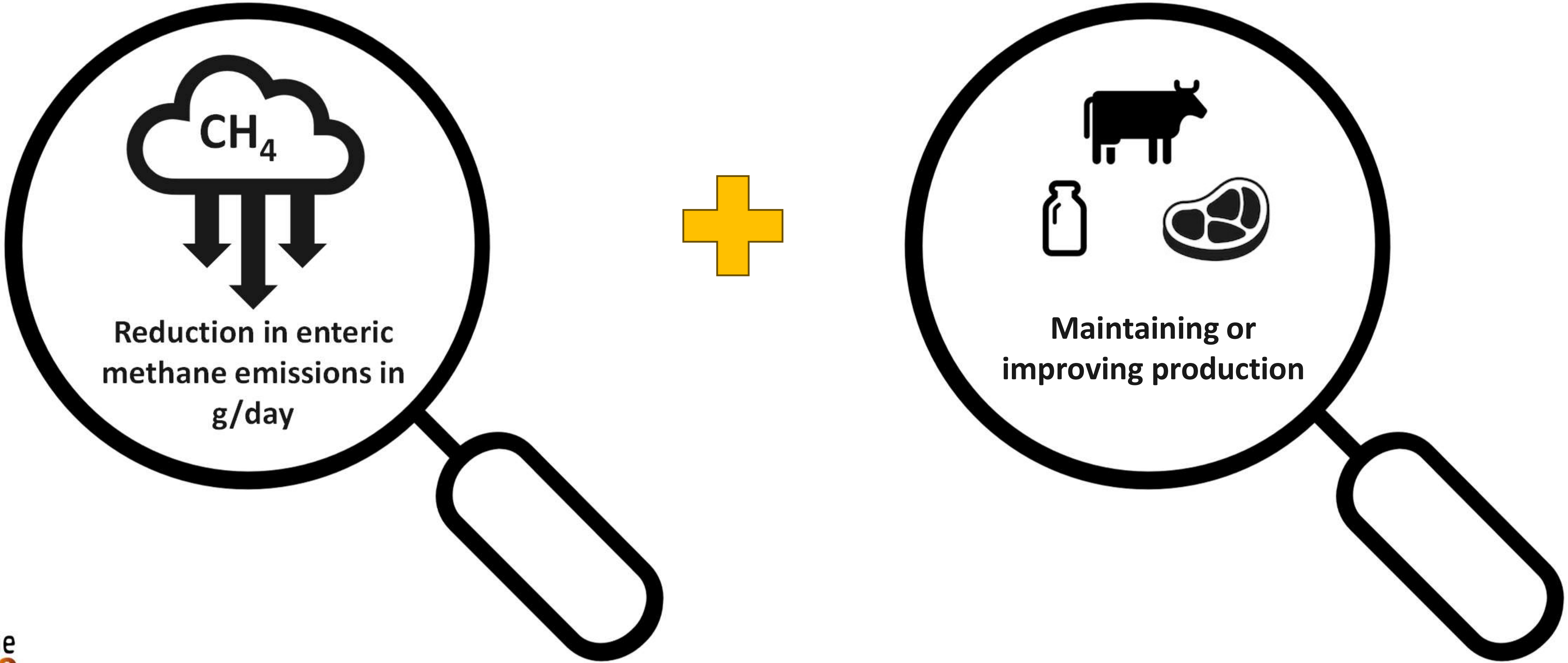
Examples of zootechnical experiments on enteric methane emissions using GreenFeed in cattle

Raphaël Boré

Bertrand Deroche

Feed additives lever to reduce enteric methane emissions

Choice of feed additives lever



Our process to study feed additive lever

1. Identifying an additive and learn about it
2. Establish reduction assumptions and performances impacts
3. Building the experimental design
4. Selecting animals and allocating them
5. Carry out the experiment
6. Cleaning datasets
7. Running statistics analysis
8. Data Valorization

Step 1 : Identifying an additive and learn about it

Literature review

Discussion with the additive supplier

In vitro studies only ?
In vivo studies ? Conditions (diet, animals …), doses, …

Step 2 : Establish CH_4 reduction assumptions and performance impacts

For a precise dose : Mean difference between treatment and control
+ standard deviation

Step 3 : Building the experimental design

The aim of the study

What is my question? ➡ Depending on information collected during discussion with manufacturer + literature review

Examples

Does the **ADDITIVE A decrease** the CH4 production?

Is there any "**dose effect**" of **ADDITIVE A** on the CH4 production?

- Does the **ADDITIVE A** and/or the **ADDITIVE B decrease** the CH4 production?
- Does the **ADDITIVE A** have a **stronger effect** on CH4 production than **ADDITIVE B**?
- Does the **combination ADDITIVE A+B** have a **synergic effect** on CH4 production?

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Step 3 : Building the experimental design

The lots

What lots will I be looking at to answer my question?

Examples

Control
VS
Additive A

Control
VS
Additive A Dose 1
VS
Additive A Dose 2

Control
VS
Additive A
VS
Additive B
VS
Additive A VS Additive B
VS
Additive A+B
VS

Step 3 : Building the experimental design

Experimental design and duration period

★ Essential

	Pre-experiment period (5 weeks)		Experimental period (9 weeks, including 1 adaptation week)		Post treatment period
Control	GreenFeed Adaptation 3 weeks ★	Standard feeding 2 weeks ★	Standard feeding ★		Post treatment 2 weeks
Additive A	GreenFeed Adaptation 3 weeks	Standard feeding 2 weeks	Adaptation 1 week	Experimental feeding	Post treatment 2 weeks

Covariate (Standard feeding, 2 weeks)

Effect of the product maintained after distribution ? (Post treatment period)

Measurement time with GreenFeed per period, depends on:

- The maximum number of visits configure (see “GreenFeed parameters”)
- The minimum number of visits to get a valid CH4 value: between 20 and 50 visits collected during 7 to 14 days (Martin et al., 2020)

Step 3 : Building the experimental design

Number of individuals

- Type of animals (calf, dairy cows, steers…)
- Number of animals needed to make the desired difference significant (workforce calculation)

Parameter	Example value
Significance p-value threshold	5 %
Expected difference	10 % (50 g CH_4/day)
Expected population standard deviation	60 g CH_4/day

Step 3 : Building the experimental design

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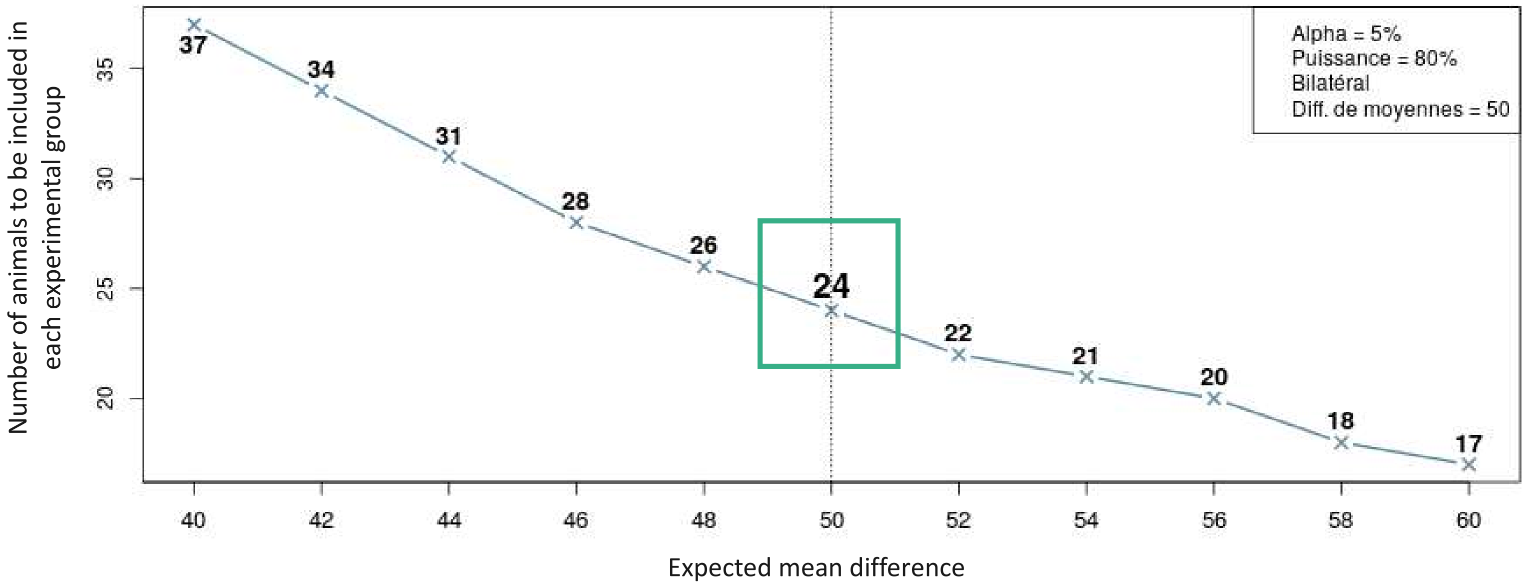
Parameter	Example value
Significance p-value threshold	5 %
Expected difference	10 % (50 g CH_4/day)
Expected population standard deviation	60 g CH_4/day

Number of animals to be included in each group based on the expected difference in means



Step 3 : Building the experimental design

GreenFeed description

Gold standard

Respiratory chamber

SF6

RESEARCH → **FARM**

✚✚✚ **Precision** ✚

✚ **Large scale measurement** ✚✚✚

Proxys
(e.g. milk infrared)
&
Tools
(e.g. laser methane detector)

GreenFeed (C-lock Inc)

Automatic concentrate dispenser with CH_4 measurement sensors.

Good prediction quality with the Chamber (R^2 = 0,79) *(Bayat et al., 2025)*

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Step 3 : Building the experimental design

GreenFeed position : First action to ensure good attendance

Indoor conditions

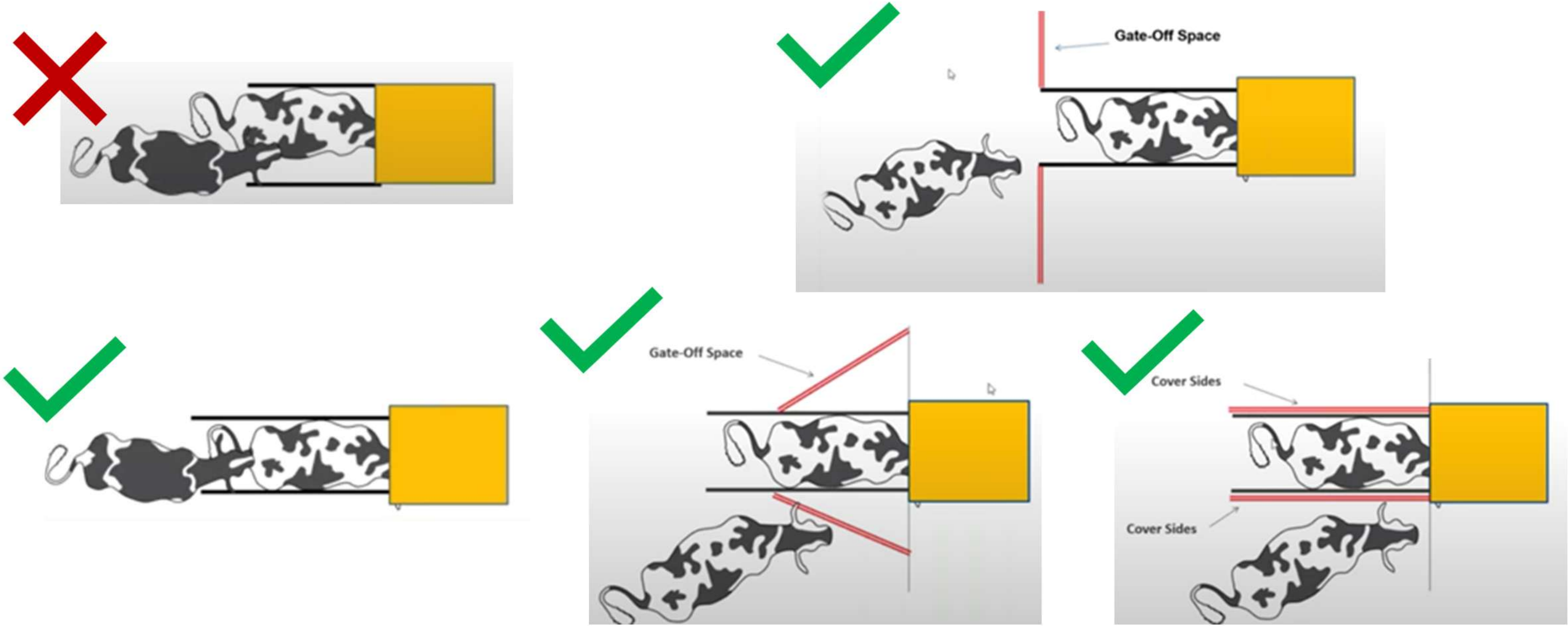
- **Install the GreenFeed in a location that is easily accessible and safe to animals AND operators**
- **Check that it does not interfere with operations (feeding, mulching, animal movement).**
- **Do not place multiple GreenFeed side by side to prevent animals from moving from one to the other too quickly.**
- **Protect GreenFeed against damage (material, animals)**
- **Isolation of the animal visiting GreenFeed from other one**

- **Strong light contrast between the animals' living area and the location of the GreenFeed**
- **A lot of dust**
- **Steps just before the animal enters the GreenFeed**

Step 3 : Building the experimental design

GreenFeed position

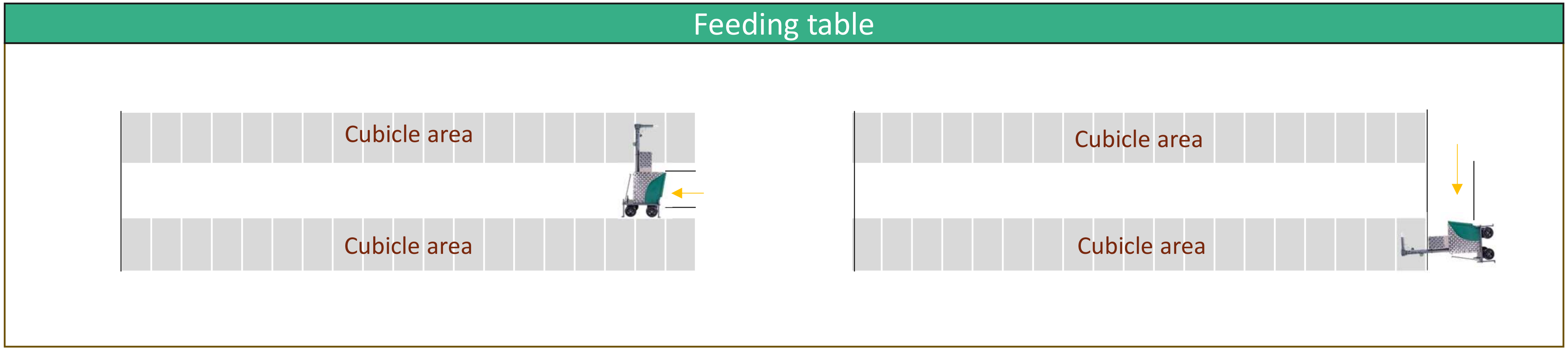
C-lock Inc scheme



Step 3 : Building the experimental design

GreenFeed position : First action to ensure good attendance

Example in dairy cows



Step 3 : Building the experimental design

GreenFeed position : First action to ensure good attendance

Example in dairy cows

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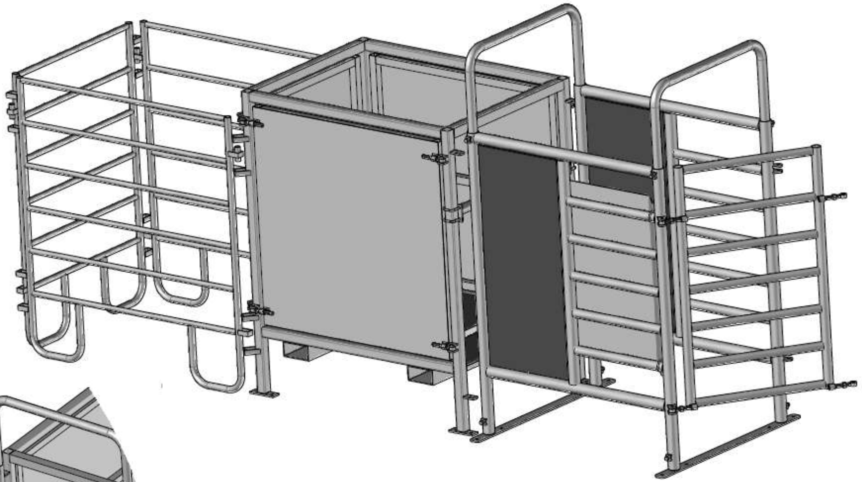
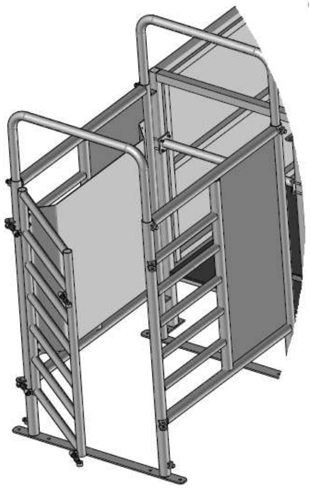
Step 3 : Building the experimental design

GreenFeed position : First action to ensure good attendance

Example in beef cattle





Step 3 : Building the experimental design

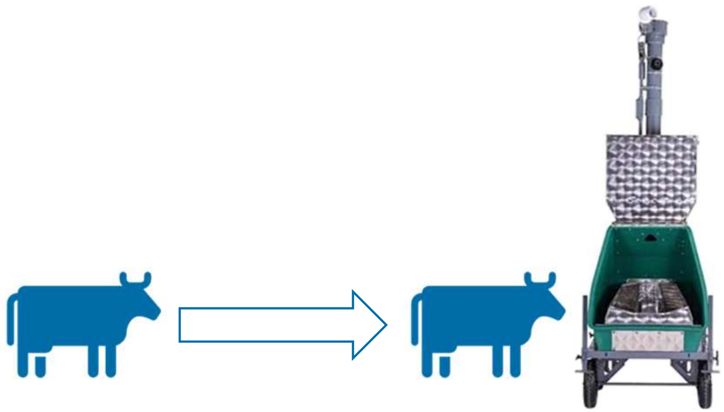
GreenFeed parameters : 2nd action to ensure good attendance

Example

Computer configuration

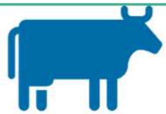
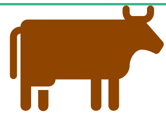
	Duration visit (min)	Drop dispense interval (sec)	Minimum time between feeding periods (hour)	Maximum drop per feeding period	Max feeding periods
GreenFeed adaptation	3 3 3	15 20 25	1 2 3	12 9 7	4 4 4
Animals adapted	3	30	4	6	4

Attracting / handling animals



Step 3 : Building the experimental design

Draw up a list of measures to be implemented

Measurement		
Animal characteristics (age, gender, parity …)	Once per trial	Once per trial
Dry matter intake (kg DM/day) [**Individual** or collective]	Every day	Every day
Chemical component and feed value of feedstuff	Every day	3 times per week
Enteric methane emission – GreenFeed	Every day	Every day
Milk yield (kg)	Each milking	-
Milk : composition (protein, fat, somatic cells, lactose, urea) + Mid-infrared spectra	Twice a week	-
Live weight	At least once a month	Once a month
Body condition score	Twice a month	Depending on animal type (Once a month)
Carcass (weight, yield, fat cover)	-	Depending on animal type

Step 3 : Building the experimental design

The protocol

Iterative process

- Write the protocol
- Identify every constraints and their impact on the trial
  (management, ethical, equipment, animal, additive supplier …)
- Talk to a statistics expert to check the feasibility of statistical analysis of your trial
- Modify previous steps

Step 4 : Selecting animals and allocating them

Data collection (15 days)

Identifying order of allocation criteria

Identifying pairs of animals

Checking the consistency of animal pairs

Conducting a lottery to assign animals on each groups

Step 4 : Selecting animals and allocating them

Allocation criteria in each sector

Criteria
Calving date
Milk yield
Protein content
Fat content
Intake (if data available)
Live weight

Depending on animal type and trial

Criteria	Calves	Cow	Growth / finishing animals
Liveweight	X	X	X
Age	X	X	X
Gender (if different)	X		X
Intake (if data available)		X	X
Other	Genetic type Breed	Body condition score Parity Physiological stage	Farm of origin Morphology (e.g. muscle type)

Prioritise criteria according to their importance !

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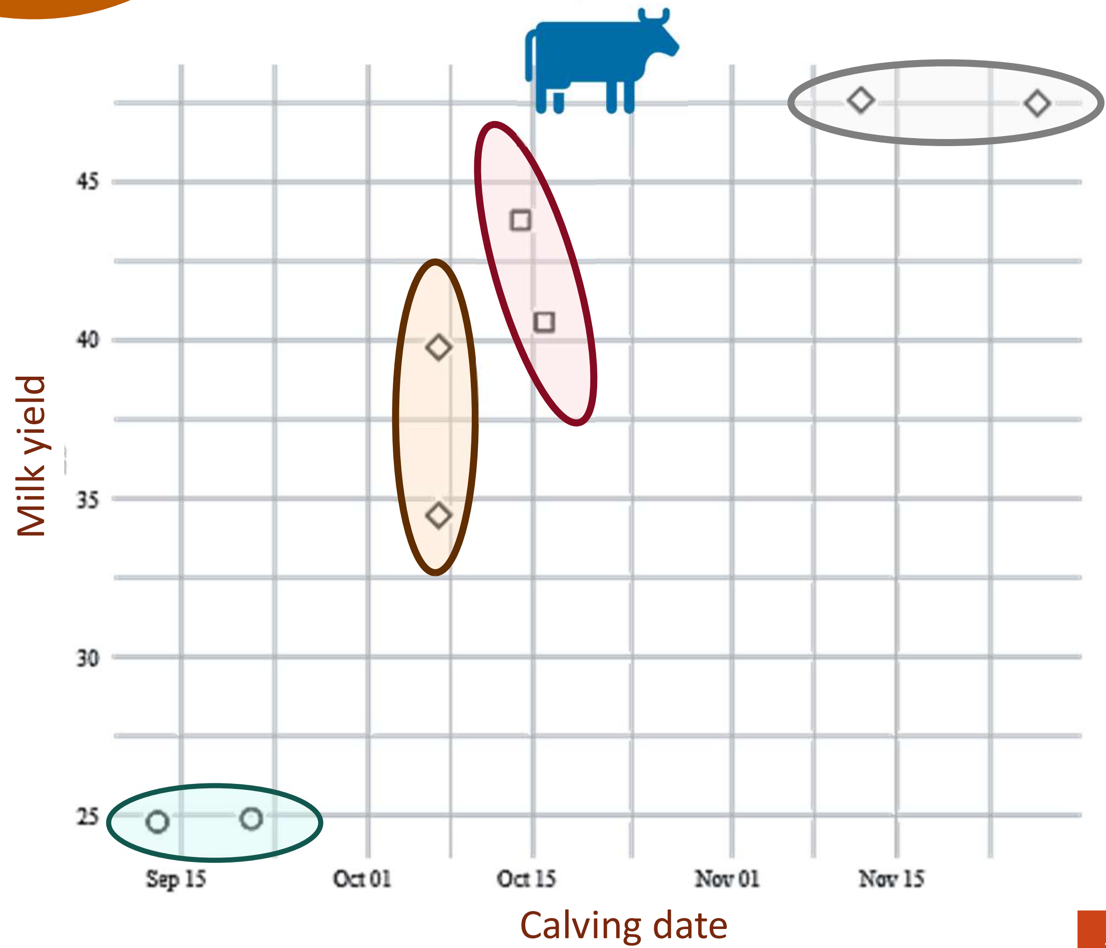
Step 4 : Selecting animals and allocating them

Example of allocation method in dairy cow (pairing animals)

Animal	Number of lactation	Calving date	Milk Yield	Fat content	Protein content	Live weight
1559	1	13/09/20	24,8	42,9	35,8	627
1540	1	21/09/20	24,9	44,5	34,3	664
1442	2	14/10/20	43,8	41,4	32,2	650
1428	2	16/10/20	40,6	39,9	33,7	661
1249	3	27/11/20	47,5	46,6	35,3	776
903	6	12/11/20	47,6	41,5	33,8	826
1190	3	07/10/20	34,5	38,3	33,3	682
1354	3	07/10/20	39,8	40,5	33,0	751



Step 4 : Selecting animals and allocating them

Random distribution of animals within their experiment group

Animal	Number of lactation	Calving date	Milk Yield	Fat content	Protein content	Live weight
1559	1	13/09/20	24,8	42,9	35,8	627
1540	1	21/09/20	24,9	44,5	34,3	664
1442	2	14/10/20	43,8	41,4	32,2	650
1428	2	16/10/20	40,6	39,9	33,7	661
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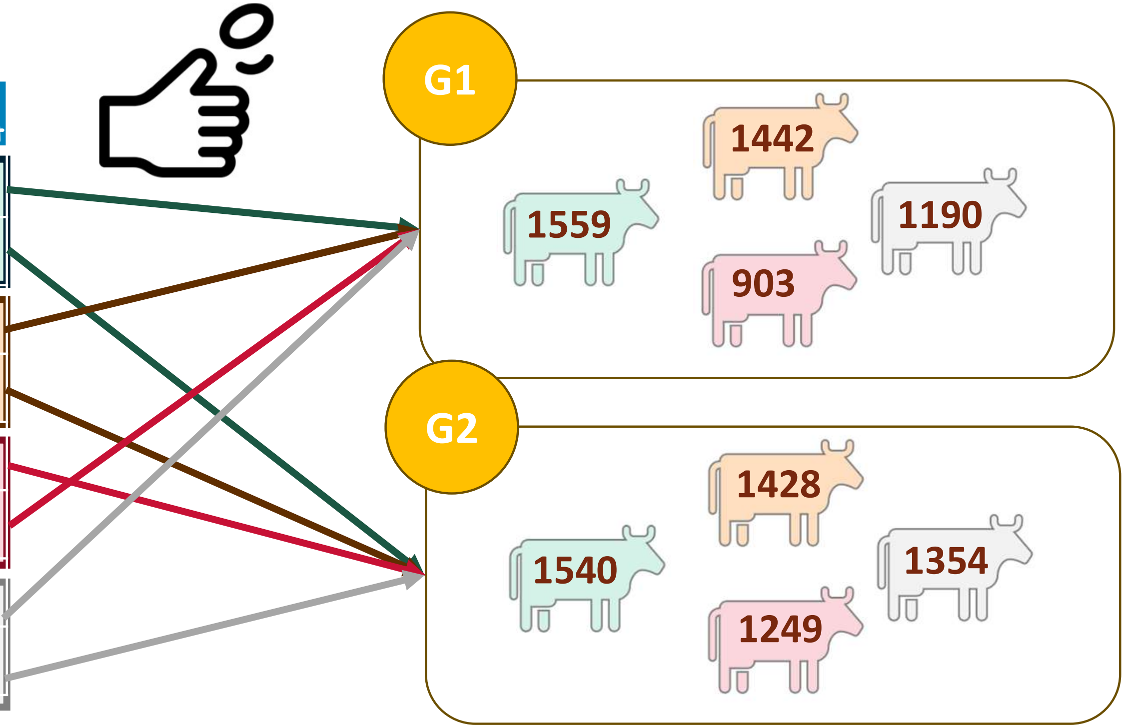
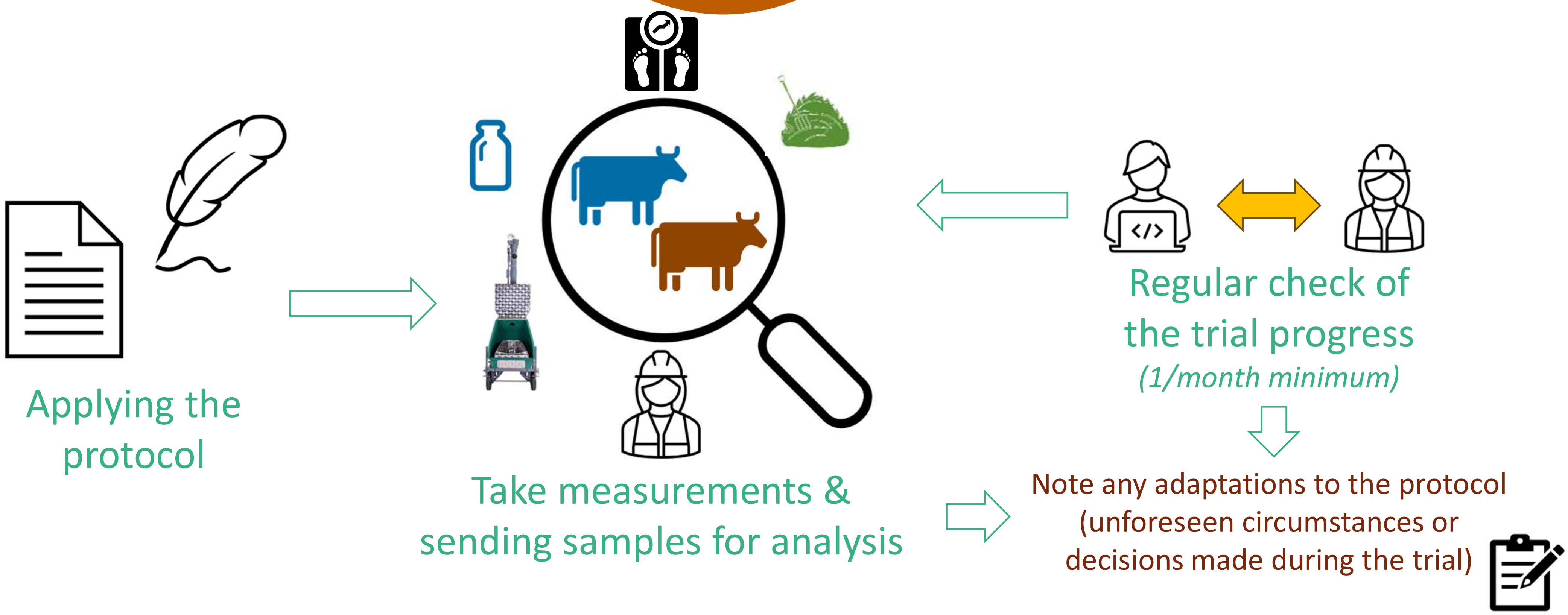


G1 almost = to G2 in average

Step 5 : Carry out the experiment



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Step 6 : Cleanning datasets

CHECK each measurement each day for each animals and **REMOVE** sensors errors, disease events …

PLOTING

NOTES **(writing during experiment by technicians in charge of experiment)**

MERGE each measurement inside a clean database for analysis.

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Step 6 : Cleaning datasets

FOCUS GreenFeed

GreenDA
GreenFeed® Data Analyzer

Jeon et al., (in progress)

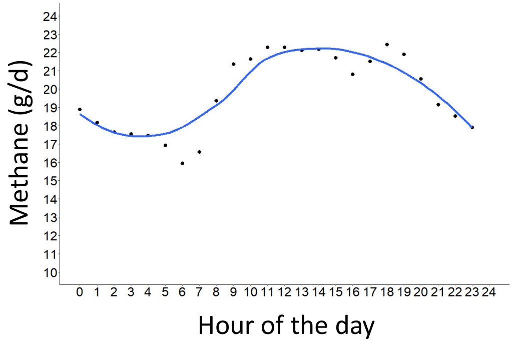
1

- Animals with less than 20 visits
- Visit duration < 2min
- Airflow < 27 L/s
- Outliers CH4 and CO2 (SD Method)

Manafiazar et al., 2017

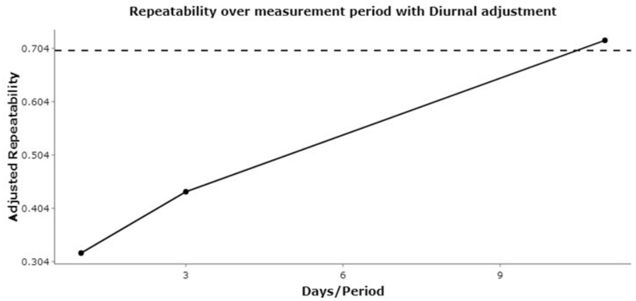
2

Adjustement for diurnal variations



3

Calculation of CH4 repeatability : determine the minimum of days needed to average CH4



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Step 7 : Running statistics analysis

1 **Descriptive analysis**
(mean, standard deviation, box plot ...)

2 **STATISTIC MODEL = ANOVA**

Example of model carried out :

$$CH_4\,(Experiment)$$
$$=$$
$$\underbrace{CH_4\,(Pre\ Experiment) + Group * Parity}_{\text{Fixed effect}} + \underbrace{Animal\ in\ his\ pair}_{\text{Random effect}}$$

Fixed effect **Random effect**

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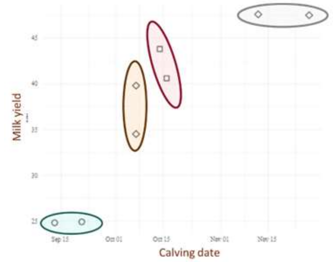
Step 8 : Data valorization

- Interpretation of data
  - Is the data in accordance with literature ?
  - Could I answer to the aim of the study ?
  - Possibly: discuss with the additive supplier

- Write your report, your article … for a congress !

Key points to success

- Confirm the expected reduction in methane emission → calculate number of individuals needed to be sure to have enough statistical power to make the expected difference statistically significant
- Give careful thought to the experimental design to best answer the question posed
- Properly prepare the installation and configuration of GreenFeed to ensure effective data collection.
- Make the allocation as uniform as possible between lots (and pairs of animals).
- Define every measures, identify every constraints and modify your protocol accordingly
- Carry out the experiment and check it regularly
- Check your data and clean them before using the adapted statistical analysis



Finally …
take a breath !

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Photo credit : https://www.istockphoto.com/fr/illustrations/vache-meugle

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Thanks for your attention

FINANCEURS

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