

Ethics in experiments on animals

Training session

INRAE, France, 22-23 September 2020

Organizers: Véronique Deiss and Isabelle Veissier

Programme – Day 1

- 8h30 Welcome and presentation of SmartCow - R Beaumont
- 8h45 Tour de table 1 – Who are you? What experience on animals have you contribute to?
Did this experience question you and how?
- 9h45 Balancing the issues and the 3Rs approach – I Veissier
- 10h45 Break*
- 11h15 Welfare, stress, pain – V Deiss & A De Boyer
- 12h15 Lunch break*
- 13h30 Endpoints – V Deiss
- 14h00 Adjustment of animal numbers in experimentation – P Gasqui
- 14h30 Break*
- 15h00 Tour de table 2 - How do these concepts influence your perception of your own work ?
- 16h00 Presentation of home work

Programme – Day 2

8h30 Individual exercise: Evaluation of harms and Benefits

10h Plenary exercise: balancing issues

12h Lunch break

13h30 Refinement – V Deiss, R Botreau, P Faure & F Fournier

15h Break

15h30 Alternatives to experiments – I Veissier

16h Discussion: What did you learn?

Tour de table 1

Who are you?

What experience on animals have you contribute to?

Did this experience question you and how?

8h45 – 9h45

Background

Debates about experiments on alive animals:

- Consequentialist approach: An experiment on alive animals is morally acceptable if the knowledge it aims to produce can result in an overall benefit. In other words, it is acceptable if the constraints imposed on some animals are outBalanced by the larger Benefits expected for others.
- Animal rights approach: Animals are sentient beings, they have a right not to be used by others.

Most people lye between these two extremes, considering that experiments on at least some animals are necessary to gain scientific knowledge on the condition that no alternatives are possible and unnecessary suffering is avoided.

What is an important knowledge? Wat is unnecessary suffering?

Aim of the training: Being able to make explicit all arguments for or against an experiment in order to decide if it is acceptable or not.

The 3Rs approach & balancing issues

I Veissier

The 3 Rs

Russel and Burch, 1959. *The Principles of Humane Experimental Technique*

- ***Replacement:*** methods which avoid or replace the use of animals in research
- ***Reduction:*** use of methods that enable researchers to obtain comparable levels of information from fewer animals, or to obtain more information from the same number of animals
- ***Refinement:*** use of methods that alleviate or minimize potential pain, suffering or distress, and enhance animal welfare for the animals used.

Replacement

Objective: To use alternatives to animal experiments

- In vitro measurements
- Re-use of data
- Use 'routine' data, e.g. spontaneous diseases or data from routine monitoring of animals (PLF)
- ...

Addressed on Day 2 afternoon, as a brainstorming activity

Replacement – Example

Digestibility studies in cows

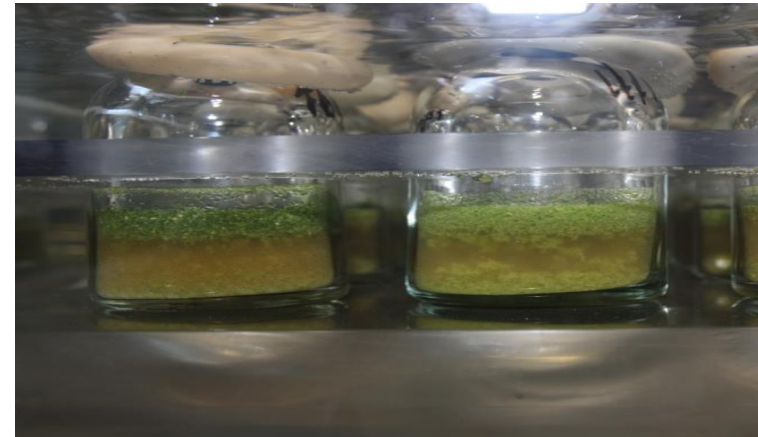
In vivo fermentation



Cows are equipped with a ruminal cannula. Small bags of feeds are loaded in the rumen and removed at certain times



In vitro fermentation



Batch fermenters containing forage substrate and buffered rumen fluid

→ allows a screening of feeds or compounds; still a final checking on few animals is necessary

Reduction

Objective: To use as few animals as possible in an experiment while ensuring obtaining interpretable results

- Determine the minimum number of animals according to the variability in the population of the trait measured and the expected difference between treatments
- Run successive experiments until obtaining a significant difference
- Reduce the variability by using homogeneous animals
- Use adequate statistical models, e.g.:
 - Include covariates in variance-covariance analyses to reduce the errors (denominator in F calculation)
 - If you expect results in one specific direction (e.g. Treatment A reduces CH₄ emissions) then use unilateral statistical tests

Reduction – Example

Use of adequate statistical models

The milk yield of **control cows** is compared to that of cows **supplemented with RO** for 9 weeks

No statistical difference on Wk0 ($P = 0.62$)

Statistical models to analyse effect of diet:

Model 1:

1 factor = diet

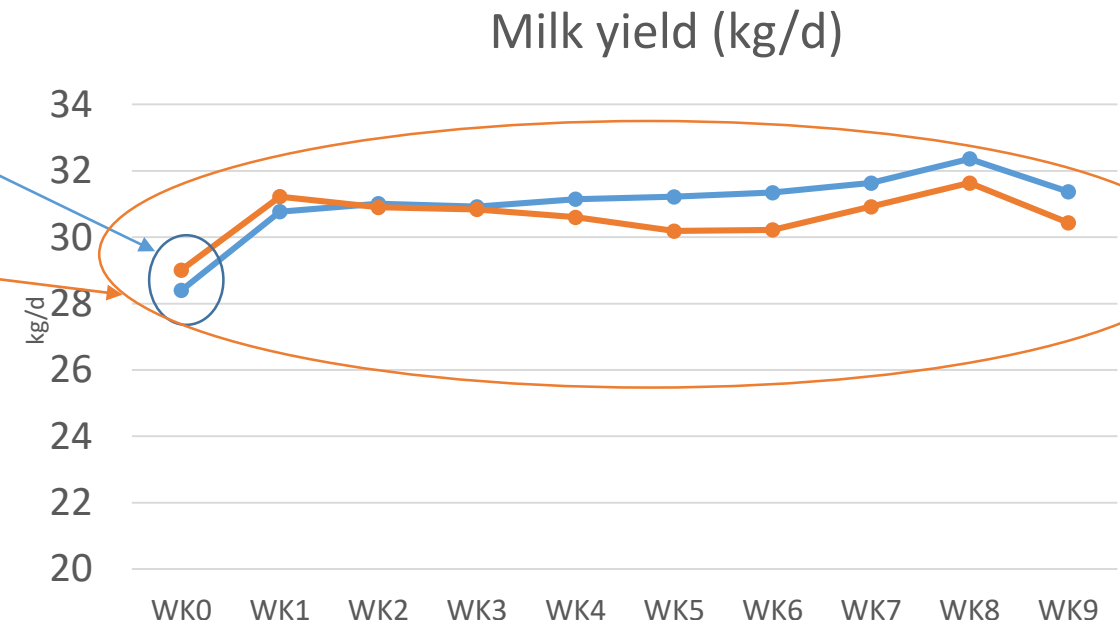
Repeated data: subject = cow; repetition = week

→ $P(\text{diet}) = 0.65 \rightarrow \text{NS}$

Model 2:

$P(\text{covar}) < 0.0001$

→ $P(\text{diet}) = 0.02 \rightarrow \text{significant !}$



Refinement

Objective: To reduce the constraints imposed on animals

- Handling of animals with care
- Non-invasive sampling technics (e.g. cortisol in milk, saliva, hair,...)
- Use pain killers
- Define endpoints (=if suffering is above a given threshold then the experiment is stopped)
- Ensure animals are accommodated and cared adequately, even out of experiments
- ...

Assessment of stress and pain & Endpoints: see next 2 presentations

Refinement – Example

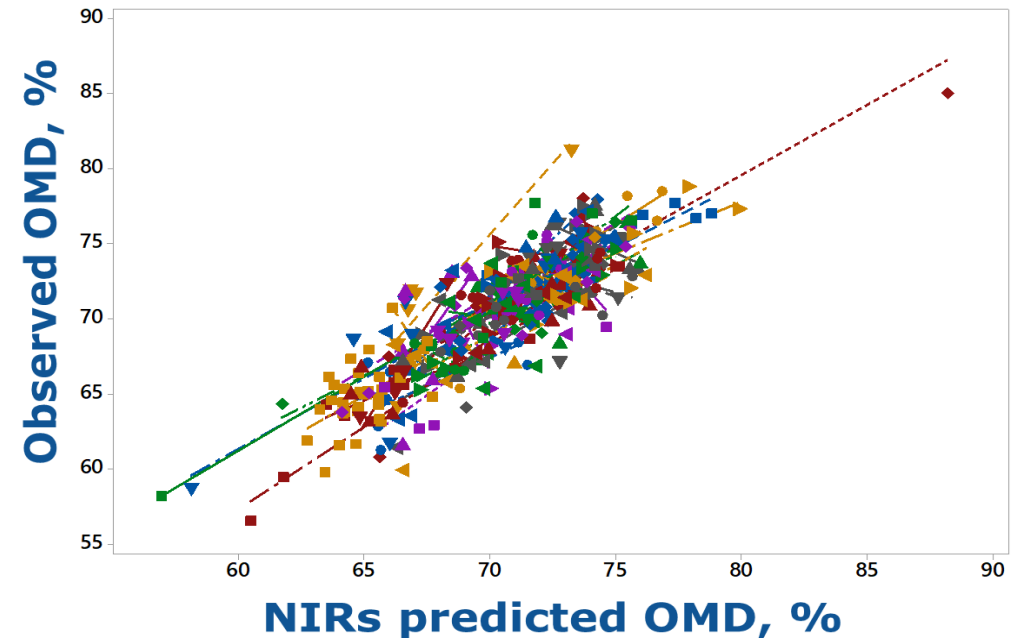
Use of proxies of feed efficiency

Collection of urine and faeces in metabolic crates for 2 weeks



Animals are restrained to be able to collect all urine and faeces separately

NIRS (Near infrared Spectrometry) on faeces in normal environment



Animals stay in their normal environment;
Faeces collection requires only short handling

Additional “Rs”

- Rigour
 - A study must be published. If not, it is a waste of resources
 - Poor experimental design is a major cause of rejection
- Reproducibility
 - Reproducibility crisis: Many scientific results are not reproduced because of poor statistical analyses, incorrect data interpretation, poor design...
 - It is essential to report precisely the conditions in which results were obtained
 - ARRIVE guidelines: framework to describe methods : study design, sample size, procedures, stats,... <https://arriveguidelines.org/>

3 Rs are best fulfilled when there is an institution strategy:

coordination of projects to avoid repetition and allow sharing of experimental design

“3 Rs culture”

Is this enough to apply the 3 Rs?

- 3 Rs → harm on animals is reduced
- But is the remaining harm worth the expected Benefits from the results?

How to Balance the issues ?

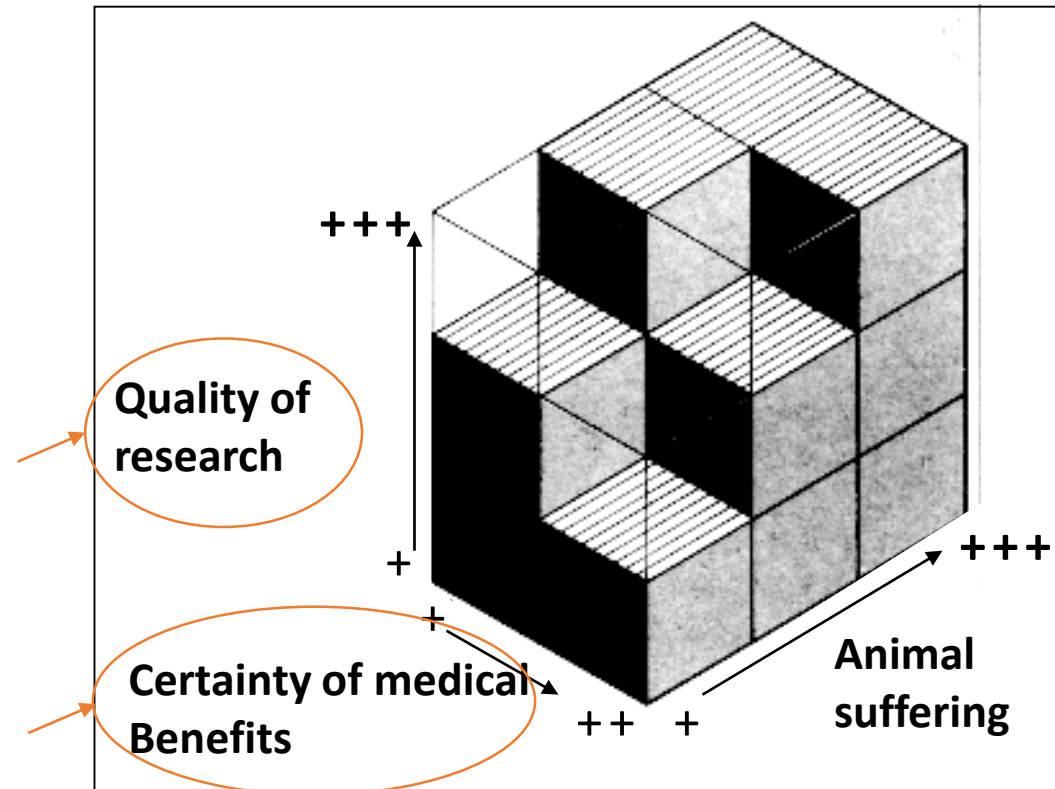
Ethics in experiments : A matter of balancing issues

There are several tools to help decide if an experiment is acceptable

Ex. Bateson, 1986

What is “quality of research” ?
Importance of the expected results?

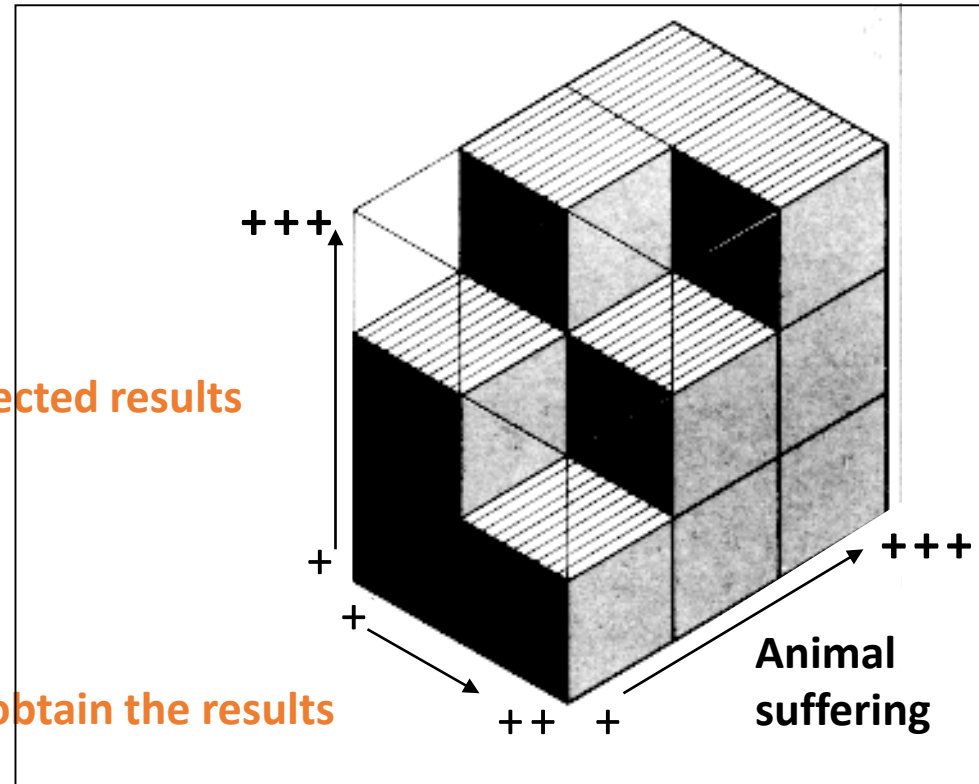
Is this a subset of quality of the research
or a separate criterion?



Adapted from Bateson, 1986

Benefits from the expected results

Probability to obtain the results



How to assess the potential benefits of results?

The Australian National Health and Medical Research Council (1997) requires to justify experiments on animals according to 5 objectives

- understanding humans or animals,
- maintaining or improving the health and well-being of humans and animals,
- improving breeding techniques,
- ecology
- education

However: In some cases, the links between the expected results and one or several of these objectives can be easily estimated beforehand

How to assess the potential benefits of results?

In some cases, the links between the expected results and one or several objectives can be easily estimated beforehand but in most cases it cannot be so

There are examples where a piece of knowledge was produced from so-called sky-blue research and lead to important applications that had not been foreseen

Grimm et al 2017: *“Whether practical Benefits are realized is
(a) impossible to predict and
(b) exceeds the scope and responsibility of researchers”*

Rather the contribution of the research to a disciplinary field should be assessed

→ Both the **scientific value** and the **societal value** of a research should be estimated

Balacing issues – checklist proposed

Issue	Outcome
A- Is there an institutionnal / personal strategy to commit with the 3 Rs approach (including checking that no other studies are carried at the same time with a similar approach, re-use of collected data, meta-analysis of published work,...)	YES / NO
B- How the 3 Rs were taken into account in the current work?	
- Have alternatives to the use of animals been sought and adequately assessed?	YES / NO
- Is the number of animals minimum (incl. reliability of how this was calculated)?	YES / NO
- Has the constraints on animals been minimised?	YES / NO
C- How are the (remaining) constraints on animals, taking into account the sensitivity of the species, the number of animals and the procedures applied (including conditions before experiment)?	Low – Moderate - High
D- What is the importance of the expected results?	
- What knowledge is planned to be brought for a given research program or a discipline?	detail
- Can that knowledge benefit to society?	Very likely – Maybe - Unlikely
E- Are the expected results likely to be obtained	
- Is the protocol described in precise terms?	If not, ask for more information
- Do the experimental design and the statistical models ensure to obtain exploitable results?	YES/NO*

* NO may lead to rejection or to requesting changes

- Questions ?
- Reactions?
- Discussion ?

Tour de table 2

How do these concepts influence your perception of your own work ?

15 h – 16 h

Practical work

4 protocols

Individual work: before Day 2 10:00 you need to assess

- The potential harm to animals (3-4 persons / protocol)
- The expected Benefits and the likelihood of obtaining them (3-4 other persons/ protocol)

Then during Day 2 10:00-12:00 plenary exercise, for each protocol

- Those who analysed the protocol will report on their assessment
- Another 3-4 persons will Balance the issues
- The whole group will take a decision

Practical work: organisation

	Protocol 1	Protocol 2	Protocol 3	Protocol 4
Moise	Harm	Balance	Benefits	
Maria		Harm	Balance	Benefits
GUILLERMO	Benefits		Harm	Balance
GOKA	Balance	Benefits		Harm
Sonia	Harm	Balance	Benefits	
Malam Abulbashar		Harm	Balance	Benefits
JORDI	Benefits		Harm	Balance
Pascal	Balance	Benefits		Harm
Juliette	Harm	Balance	Benefits	
Marta		Harm	Balance	Benefits
Somsy	Benefits		Harm	Balance
Ivelina	Balance	Benefits		Harm
Nadya	Harm	Balance	Benefits	
ALLICE 2		Harm	Balance	Benefits