

Ethics in experiments on animals

Refinement

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Basic principle: application of the 3R rule

REPLACE animal models

- Need to use animals?
- Are there alternative methods?

REDUCE the number of animals to the minimum

- Experimental scheme?
- Statistical analyses?

REFINE (improve) procedures and methods

- Housing conditions of animals in experimentation?
 - Animals' monitoring?
 - *Management of suffering, if it exists?*
 - *End points?*
- } *Already discussed*

Constraints imposed on animals & regulations relative to experimental animals

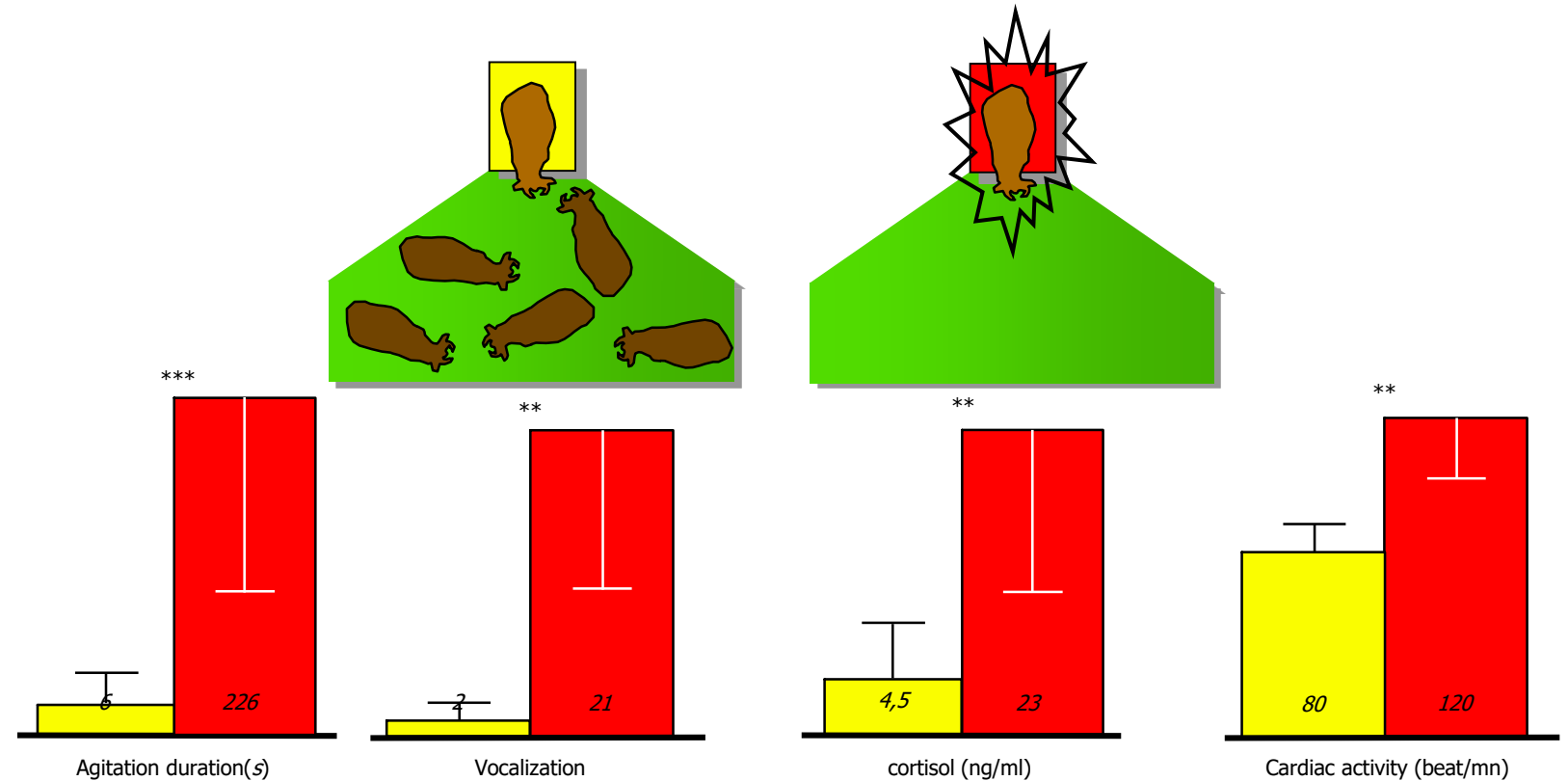
Constraints imposed on animals (ruminants) used for experimental purposes

- **Indoor breeding** (0 pasture): time spent by a cow looking for food and ingestion on pasture 6 to 12h / day VS in a stable 4h
- **Containment:** reduction of movement and "play" activities (galloping, jumping, pawing)
- **Noise pollution:** metallic noise, machine noise, etc.....
- **Social instability:** allotment and re-allotment that disrupt the hierarchical structure and social affinities

Constraints imposed on animals used for experimental purposes

Social isolation: gregarious animals

Constraints can induce abnormal behavior:



Housing and enrichment

Housing Animals, except those which are naturally solitary, shall be socially housed in stable groups of compatible individuals.

In cases where single housing is allowed in accordance with article 33(3) the duration shall be limited to the minimum period necessary and visual, auditory, olfactory and/or tactile contact shall be maintained.

The introduction or re-introduction of animals to established groups shall be carefully monitored to avoid problems of incompatibility and disrupted social relationships

Table 7.1.

Cattle

Body weight (kg)	Minimum enclosure size (m ²)	Minimum floor area/ animal (m ² /animal)	Trough space for ad-libitum feeding of polled cattle (m/animal)	Trough space for restricted feeding of polled cattle (m/animal)	Date referred to in Article 33(2)
up to 100	2,50	2,30	0,10	0,30	1 January 2017
over 100 to 200	4,25	3,40	0,15	0,50	
over 200 to 400	6,00	4,80	0,18	0,60	
over 400 to 600	9,00	7,50	0,21	0,70	
over 600 to 800	11,00	8,75	0,24	0,80	
over 800	16,00	10,00	0,30	1,00	

Housing and enrichment

Sheep and goats

Body weight (kg)	Minimum enclosure size (m ²)	Minimum floor area/animal (m ² /animal)	Minimum partition height (m)	Trough space for ad-libitum feeding (m/animal)	Trough space for restricted feeding (m/animal)	Date referred to in Article 33(2)
less than 20	1,0	0,7	1,0	0,10	0,25	1 January 2017
over 20 to 35	1,5	1,0	1,2	0,10	0,30	
over 35 to 60	2,0	1,5	1,2	0,12	0,40	
over 60	3,0	1,8	1,5	0,12	0,50	

Regulations for the accommodation of animals

- Minimum surface
 - Food ...
 - ...
- and
- enrichment

Enrichment

Definition of 'environmental enrichment'

« Behavioural ecology » approach

Newberry (1995)

*« An environmental enrichment is an **improvement in the biological functioning** of captive animals resulting from modifications to their environments »*

Proofs of biological functioning improvements:

- Increased lifetime reproductive success
- Increased inclusive fitness*
- Improved health

➡ Approach based on capacity of the individual to pass on his genes (or those of his close relatives) and on his health

« Cognitive ethology » approach

Anses – Animal welfare working group (2013)

[Report on environmental enrichment for pigs by the provision of manipulable materials] & Boissy et al (2007)

*« [...] environment is to be enriched for the **behavioural benefit** of the animals. This enrichment reduces the expression of inappropriate behaviour and stimulates positive emotions and cognitive functions »*

➡ Approach based on the individual and on his behaviours, to minimise negative emotions et maximise positive ones

* Inclusive fitness = the ability of an individual organism to pass on its genes to the next generation, taking into account the shared genes passed on by the organism's close relatives

Animal needs and expectations to be fulfilled on farm and during experiments!

Enrichment ➡ Improve **animal welfare**



“The welfare of an animal is its positive
mental and physical state as related to
the fulfilment of its **physiological
and behavioural needs**

in addition to its **expectations**

This state can vary depending on the animal's
perception of a given situation”

(Anses, 2018)



- Ingestion (food and water)
- Evacuation (urine & faeces)
- Thermoregulation
- Health
- Reproduction/maternal behaviour
- Body care/grooming
- Locomotion
- Rest
- Social relationship
- Exploration (including foraging/grazing...)
- Play
- Safety
- ...



Related to event anticipation

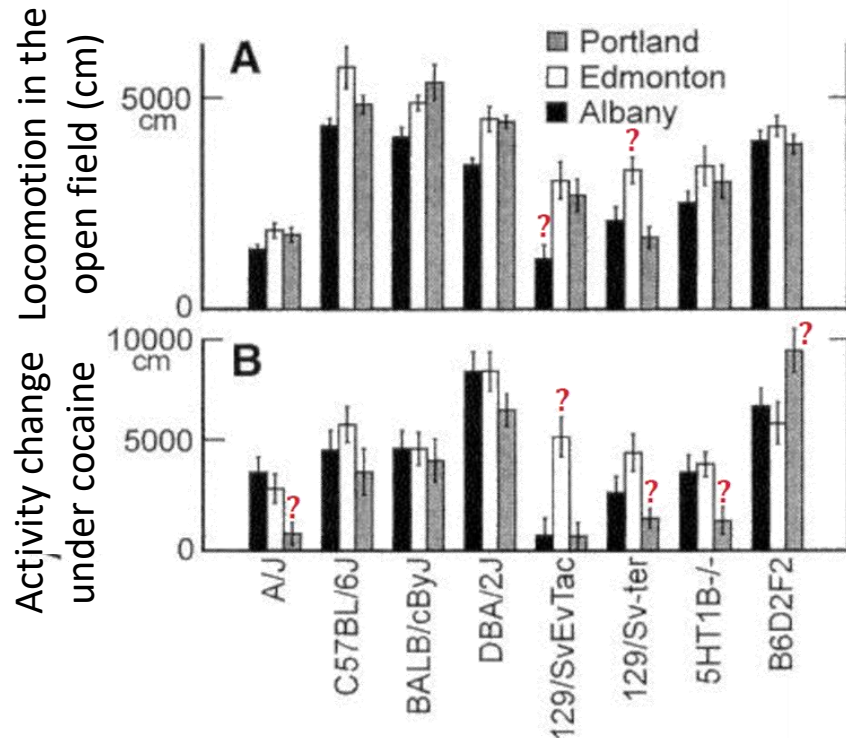
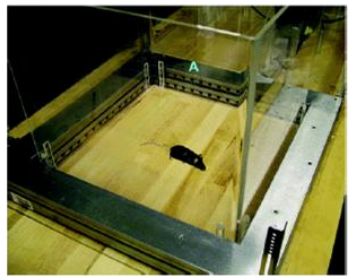
Why enrich the environment of experimental animals?

For both animals AND scientists

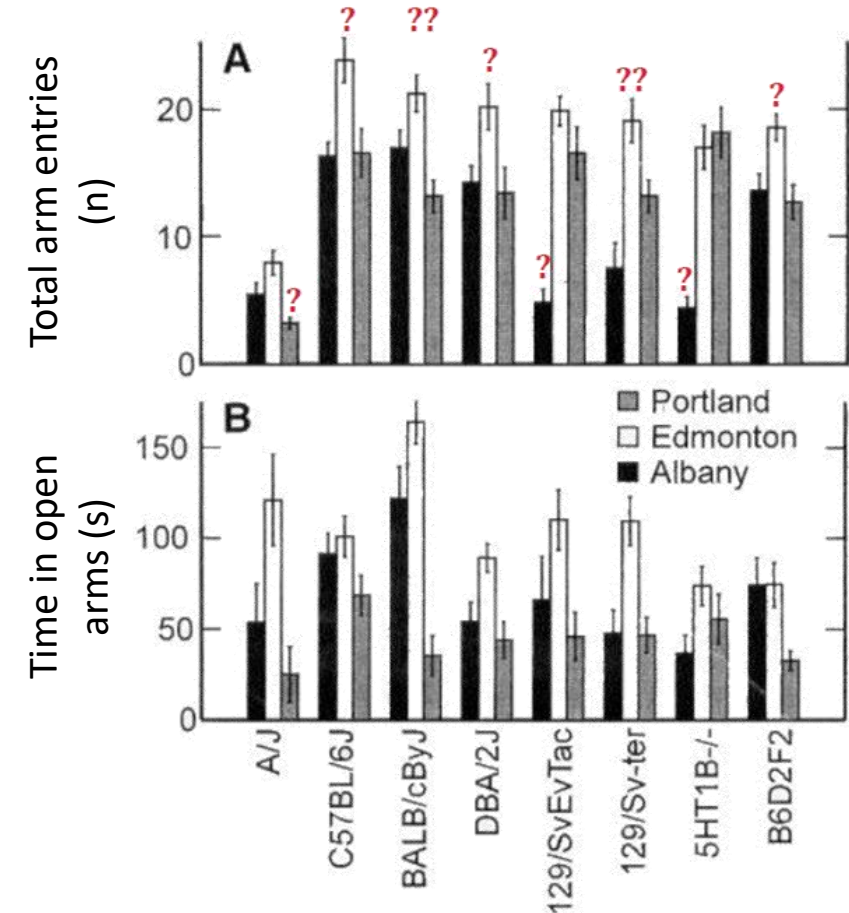
Enrichment: ethics & results' reliability

Comparison of 8 mice strains' reactivity in 3 different labs

Open field test



T maze test

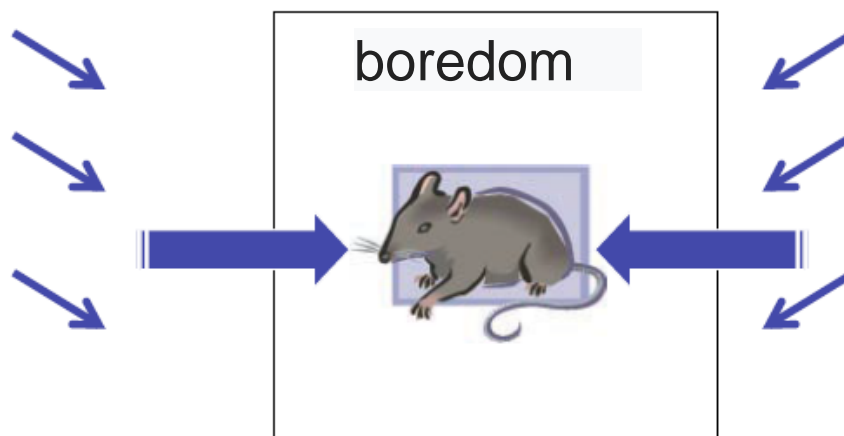
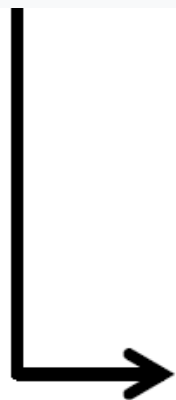


(Crabbe, Wahlsten & Dudek, 1999)

➡ WHY SUCH A VARIABILITY BETWEEN LABS & EXPERIMENTS?

how to explain such experimental variability

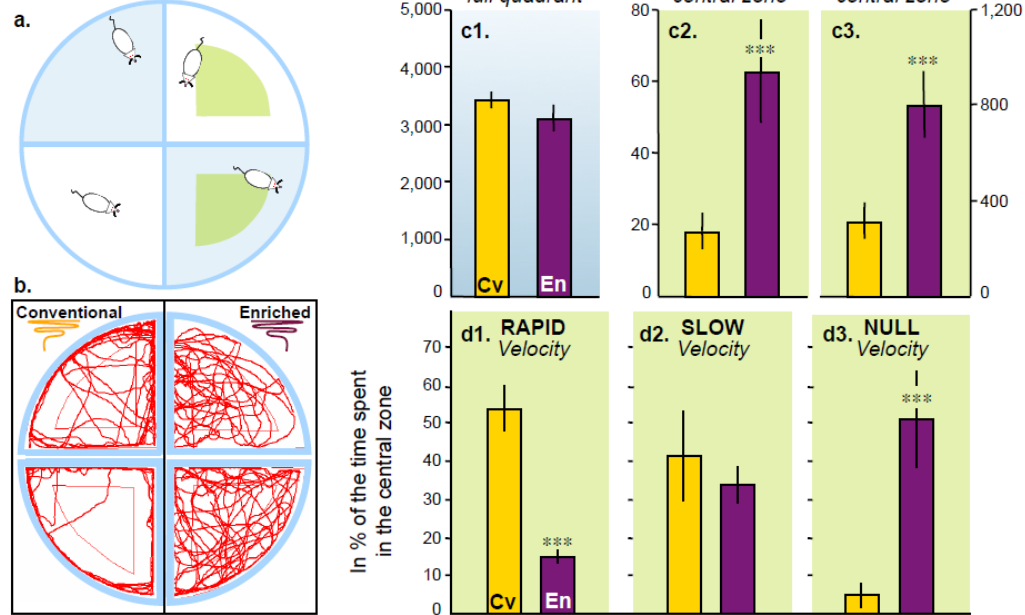
insignificant and
random events



random results

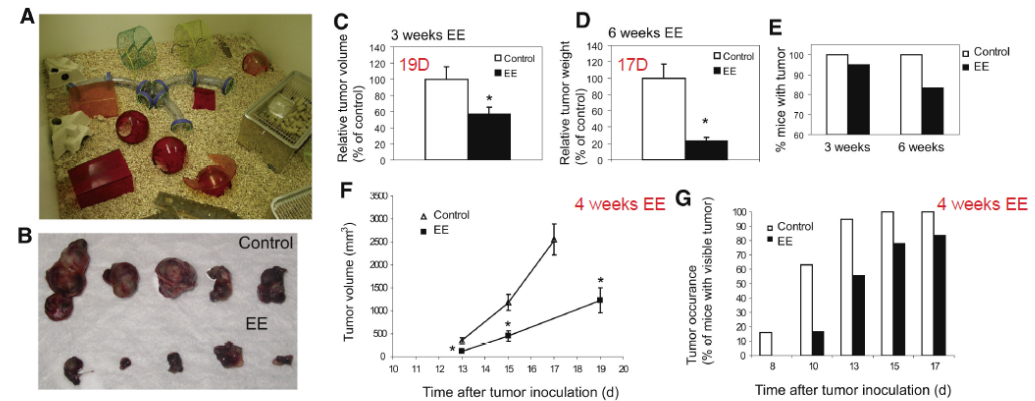
- ❖ new objective: bring the interest back inside the cage, trying to maximize the significance of the elements introduced

Water Exploration Test (WET)



Cao et al., 2010, Cell, 142:52-64

Tumor dissection 17 days after inoculation



Which enrichments for farm & experimental animals?

Between verified enrichments and promising leads

5 categories of enrichment

Five non-exclusive **categories of environmental enrichment** can be defined

(Bloomsith et al., 1991 ; Mandel et al., 2016) :

- **Occupational** : enrichment favouring the expression of new activities allowing the development of the animal's locomotor activity and/or the use of its cognitive abilities (for example, offering the possibility for the animal to control its environment, mobilizing its memorisation and learning capacities, etc.)
- **Physical** : improving comfort or increasing complexity of the animal's living environment by, for instance, providing objects and substrates or creating refuge areas
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Occupational enrichment: Access to outdoor exercise area / pasture

➔ Locomotion, exploration and play needs

To stimulate physical activity (walk, trot, gallop, jump...)

(Veissier et al, 2008; Castro et al, 2011...)

and play behaviour (especially on young animals)

(Calves: Held and Spinka, 2011)

➔ Health need

since inactivity may lead to
lesions or joint problems



E.g. Blanc Bleu Belge bulls in the selection center



(Keil et al, 2006)

Occupational enrichment: balls

Ball on the floor

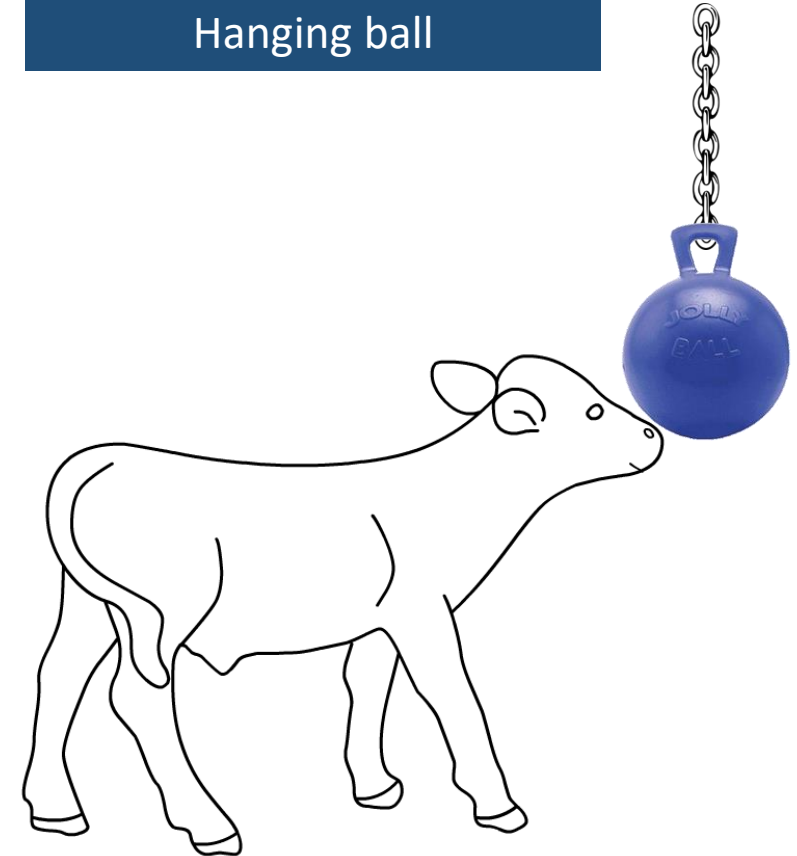


(Horses: Jorgensen et al, 2011)



(Lambs: Chapagain et al, 2014)

Hanging ball



(Calves: Bulens et al, 2014)

➡ Play and exploration needs

Occupational enrichment: ropes & chains

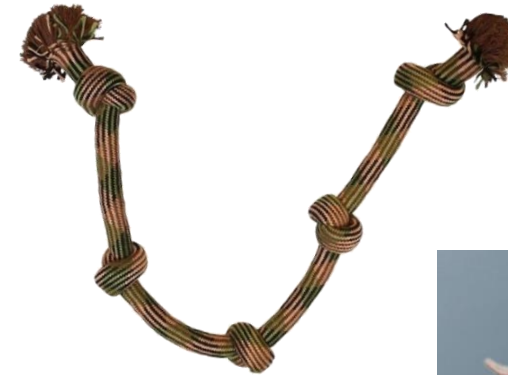


➡ favouring **oral activities**:
exploration need & sucking need
for young animals

E.g. hanging chain



E.g. rope toys for dogs



E.g. chewing disc



(Lambs: Chapagain et al, 2014)

Occupational enrichment: cognitive enrichment

(Coulon et al, 2011; Bailey et al, 2006;
Wredle et al, 2006; Webb et al, 2014;
Gaillard et al, 2014...)

Cognitive abilities of domestic animals are yet demonstrated:

- Memory (human and congener face recognition, spatial memory)
- Learning capacity
- Positive anticipation
- Environment control
- Problem solving
- ...

Providing animals with more opportunity to use their cognitive abilities has been suggested to be an important component of animal welfare
(Carlstead and Shepherdson, 2000)

➔ Exploration and play needs

E.g. a lever that heifers have to press to obtain a food reward (Hagen and Broom, 2004)
E.g. ringtone to alert the ewes of the feed distribution 1 min. before it occurs → anticipation
(Coulon et al, 2013)

Enrichments favouring use and development of cognitive abilities

Not too easy to solve
(to present an interest for the animal)
BUT must remain feasible
(→ discouragement)



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Physical enrichment: increased comfort

➔ Resting and health needs

- Mattresses for cubicles
- Bedding material
- ...

*(Tuytens et al, 2005;
Fregonesi et al, 2007;
Norrington et al, 2008...)*



Wooden chips
Straw



Miscanthus



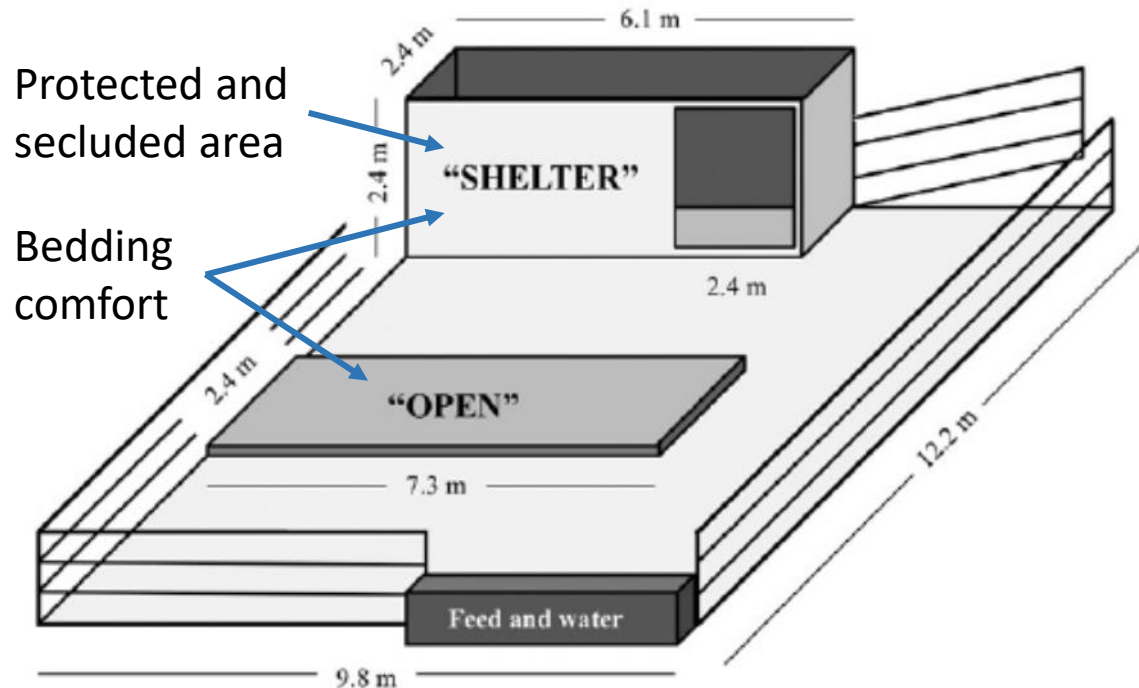
Mattresses with
caoutchouc



Waterbed

Physical enrichment: increased comfort & higher complexity

➡ Rest, safety and social (isolation) needs

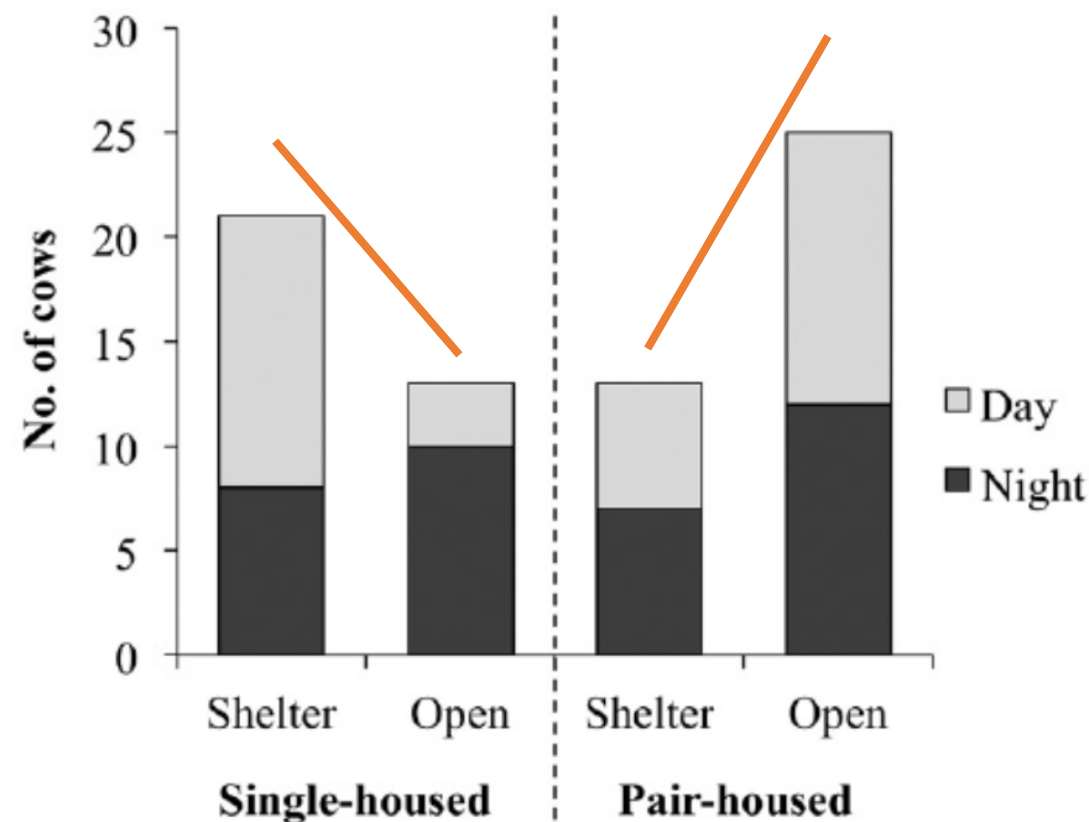


Design of maternity pens with a shelter and an open area for calving. Both areas were fitted with a mattress and a layer of sawdust bedding. The shelter was built using 1.2 by 2.4 m plywood partitions. Four maternity pens were used; in 2 pens the sides of the sheltered and open areas were reversed.

➡ Preferences vary **between cows**, depends on **social context** (isolation need) and **daytime** (night vs. day)

Authors also showed that preferences depends on **physiological state** with a preference for isolated area (shelter) near to calving whereas cows prefer be with the other cow around 10 to 8 hours before calving

No. of calvings in the different areas

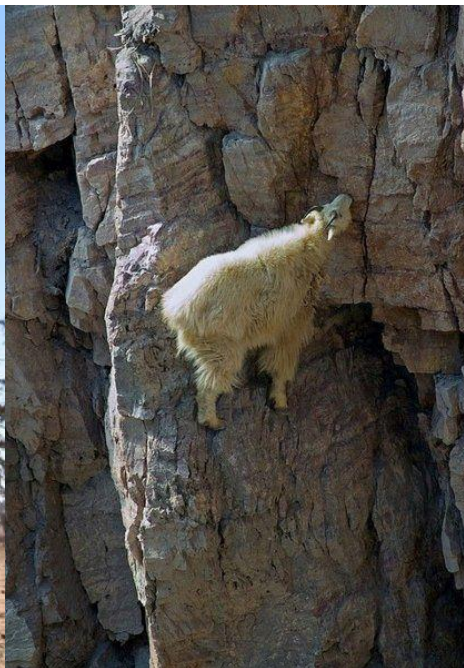


Number of pair-housed (n = 38) and single-housed (n = 34) cows that calved in the sheltered or open area during the day (0800 to 1959 h) and at night (2000 to 0759 h).

Physical enrichment: higher complexity

- Platforms

➔ Exploration, play, locomotion and safety needs
(depending on the species)



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Sensorial enrichment



= stimulation designed to trigger one or more of an animal's senses (*Wells, 2009; Bloomsmith et al, 1991*)

- **Audition** → e.g. music (classical, slow...)
- **Olfaction** → e.g. by the diffusion of odours in the environment
- **Taste** → e.g. by adding flavourings to the hay
- **Sight** → e.g. mirrors, images of conspecifics, images of landscape

Especially for isolated animals
- **Touch** → e.g. brush

*Music: Dairy cows: Uetake et al (1997),
Wredle et al (2004), Kiyici et al (2013)
Dogs: Bowman et al (2015)
Horses: Stachurska et al (2015)*

*Prenatal **olfactive** enrichment:
Rats: Pedersen and Blass (1987)
Rabbits: Coureaud et al (2003)
Post-natal **olfactive** enrichment:
Cows: Wilson et al (2002)*

*Images & mirrors: Piller et al (1999),
Coulon et al (2011),
Landscape: Haskell et al (2013)*

*Brushes: DeVries et al (2007), Mandel
et al (2013), Gutmann (2010), Georg
and Totschek (2001), Hoyer (2013),
Newby et al (2013)...*

➡ Exploration, body care & grooming needs, may also facilitate ingestion need

Sensorial enrichment: brushes



➔ Maintenance need:
grooming, scratching and rubbing
complexity

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Nutritional enrichment: Feed diversity

= presenting varied or novel food types
or changing the method of food delivery (*Bloomsmith et al, 1991*)

➔ Ingestion and exploration needs

Feed diversity through the nature of the offered foodstuffs
(*Newberry, 1995*)

- + different textures
 - + different tastes
 - risk to not control the nutritional value of ingested food
(due to animal preferences)
- ➔ in relation to **palatability**



Nutritional enrichment: Feed delivery

= presenting varied or novel food types
or changing the method of food delivery (*Bloomsmith et al, 1991*)

➔ Ingestion and exploration needs + sucking need for calves...

Calf milk ingestion:



By sucking the cow
(dam or foster cow)



With a rubber nipple



In a bucket, without nipple

*De Passillé (2001),
Jensen and Weary (2013)...*

Nutritional enrichment: Feed delivery

Other examples of possible enrichments modifying feed delivery...



5 categories of enrichment

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Social enrichment: Rear animals in group

➡ Social need



Individual
housing



Small group housing



Large group housing



Social enrichment: Rear animals in STABLE group

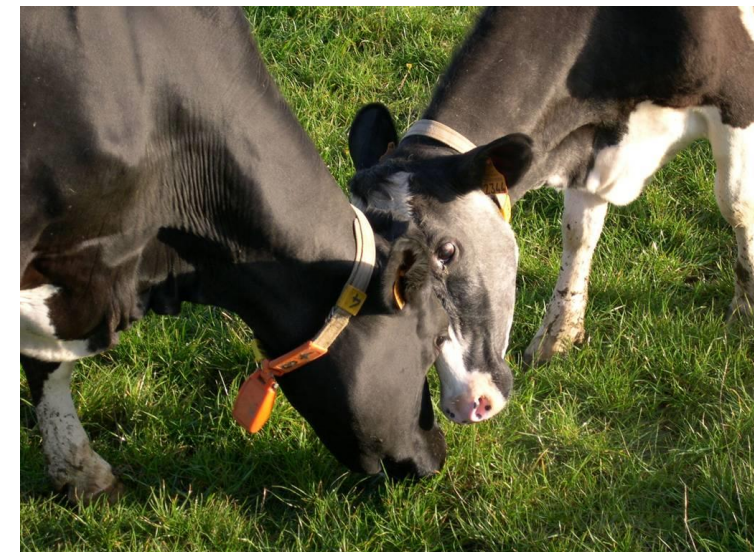
➡ Social and safety needs

Cattle are gregarious animals, with a strong and stable hierarchy within the same group:

- ➔ At the beginning, some 'fighting' behaviours to establish the hierarchy within the group
- ➔ Then few 'severe' agonistic behaviours (fight, headbutts...), replaced by 'moderate' (like threat-avoidance, i.e. without physical contact) since the hierarchy is well established

BUT each time the composition of the group is modified, the hierarchy is to be re-established through an increase of fights and headbutts = generating stress (*von Keyserlingk et al, 2008*)

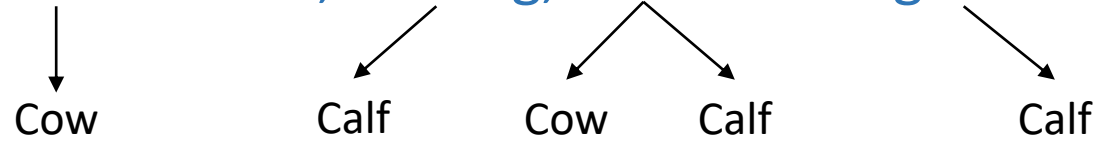
In addition, familiarity between cows increases with the cohabitation duration, thus favouring allogrooming behaviours (*Mandel et al, 2016*)



➔ Rear the ruminants in stable groups = a 'plus' for animal welfare

Social enrichment: Nursing calves by cows

➡ Maternal behaviour, sucking, social and ingestion needs



Current projects:

Agenäs et al – SLU (**Sweden**)

Pomies et al – Inrae (**France**)



Social enrichment: Contacts with humans

➔ Social need



Gentle interactions with human (*Waiblinger et al, 2004*)

- During ‘normal’ farm activity: stroking, giving small amounts of concentrates out of the hand, speaking in a soothing way...
- During stressful/aversive events (like veterinary procedure)

E.g. stroking the cows at the neck and head + speaking to them in a soothing way



improve human-animal relationship
+ “calming down” the animals during stressful event

5 categories of enrichment → to be combined

Different enrichments for:

- different **needs** and **expectations**
- different **individuals**
- evolutive **physiological state**
- changing **contexts** (climatic conditions, social context...)
- ...

5 categories of enrichment → to be combined

E.g. project Inrae on dairy goats



Exploration



Climbing



Grooming



Evaluation of enrichment efficacy?

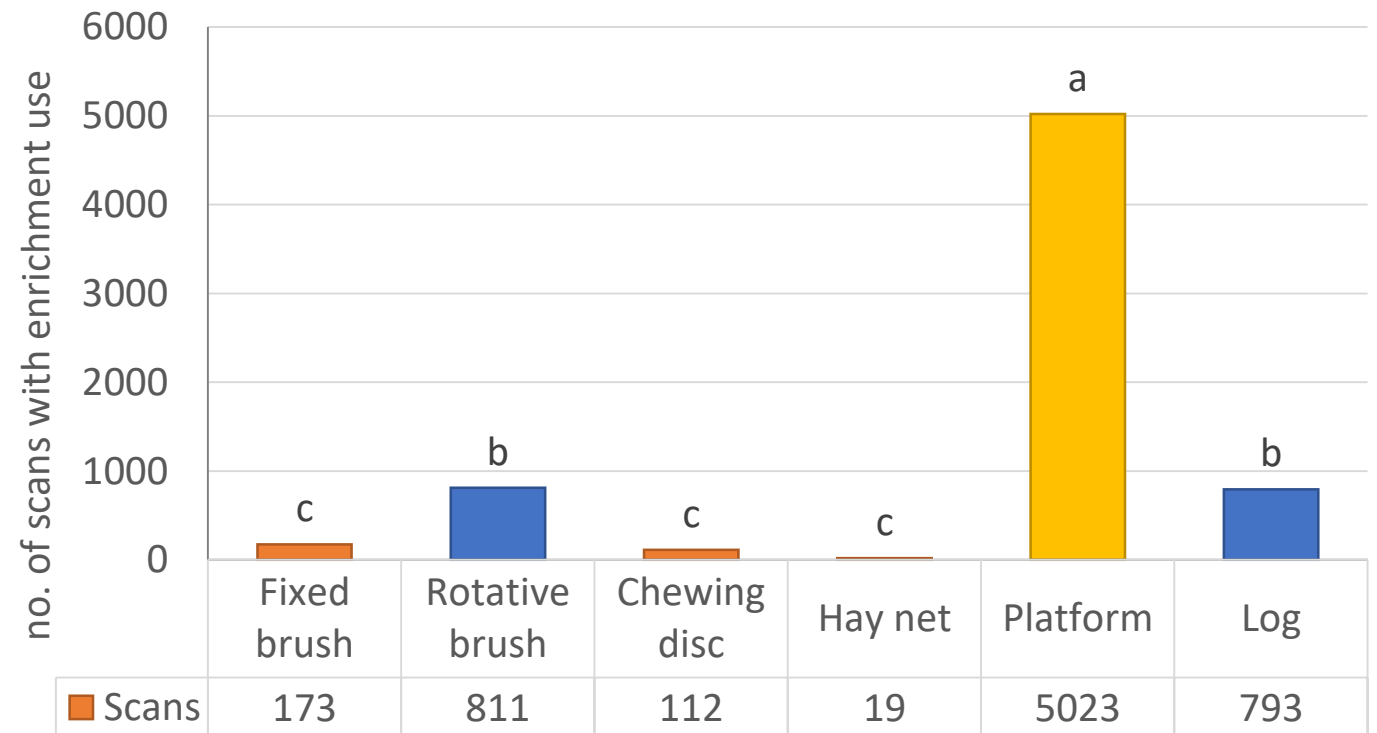
- 1- How animals use the enrichment (preferences, use duration, use frequency...)?
- 2- What is the impact of enrichment on animal welfare?

1- How animals use the enrichment? preferences, use duration, use frequency...



- Group A: 15 goats & Group B: 14 goats
→ 29 goats
- Scan Sampling (every 5 min)
between 7h and 21h15 × 6 days
→ 988 scans per goat
- A total of 28 652 scan records
with 6 931 scan records corresponding
to enrichments' use (24,2 %)

E.g. project Inrae on dairy goats



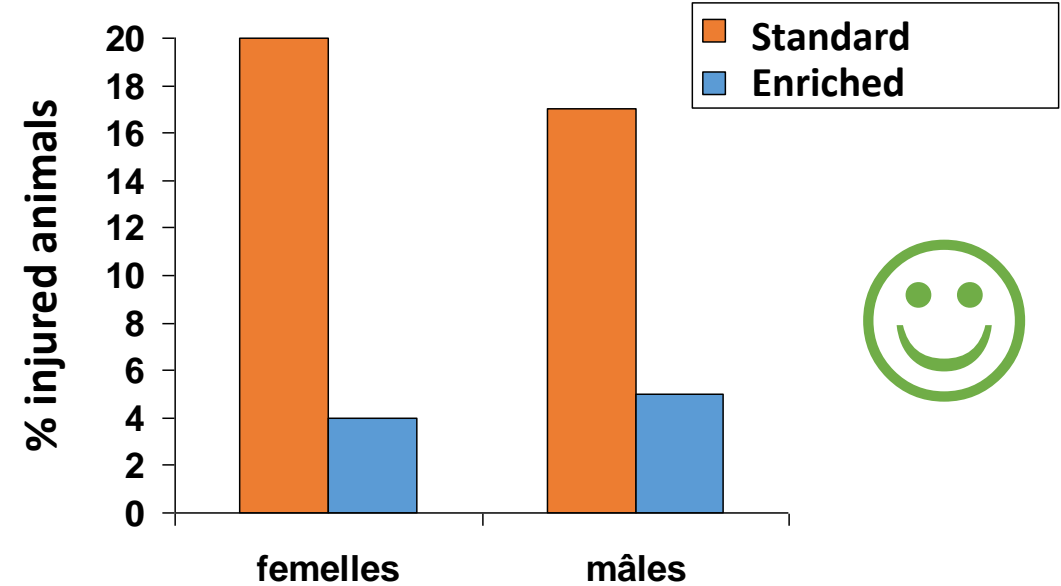
2- What is the impact of enrichment on animal welfare?



Numerous turkeys
+ high density



Increased **agonistic**
behaviours and **injuries**
due to **pricking**







Classical solution:
beak trimming



- Experimental solution
(*Mirabito & Michel, 2003*):
Combining several enrichments
- Platforms + access ramp
 - Hanging objects
 - Straw provision within a rack



2- What is the impact of enrichment on animal welfare?

Veal calves	Alone	In pairs
Respiratory disease		+
		
Time spent at feeder		+
No. of visits at feeder		+
Age to start ingesting concentrates	Later	
Development of social skills	-	
Reactivity when meeting unfamiliar calves	More reactive	
Ability to cope with environmental stressors	-	
Reactivity when restrained for blood sampling	More reactive	
		

2- What is the impact of enrichment on animal welfare? Example of platforms

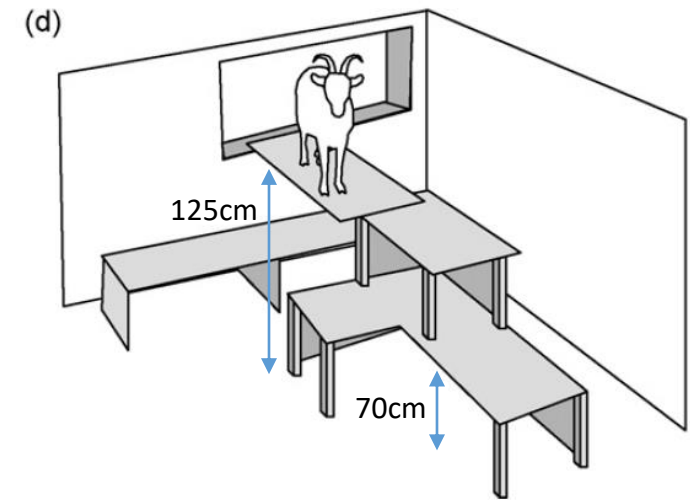
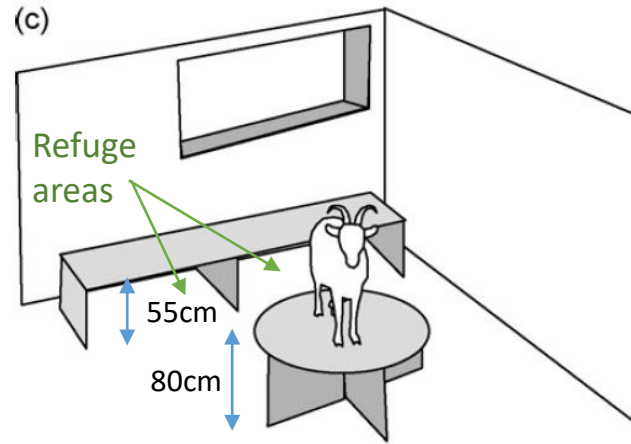
GOATS



E.g. *Aschwanden et al. (2009)*

Sheltered areas allowing the goats to lie down “hidden” from the other + Platforms = positive for

- **Feeding** (with longer feeding bouts),
- **Rest** (resting bouts less interrupted by displacements)
- **Social interactions** : less agonistic behaviours



RABBITS



E.g. *Trocino et al (2019)*

- Platforms =
- Increased behavioural repertoire
 - Increased locomotion
 - No effect on production
 - More injuries

Animal monitoring especially during experiment

Close monitoring of experimental animals

- Daily monitoring of each animal by caretakers



Detect early as possible **problems**



Necessary to check that predefined
end-points are not reached



Early detection of problems/disorders

Modification of animals' behaviour

- Use of Precision Livestock Technologies to detect subtle changes in behaviour due to sickness

Automatic recording systems (CowView system)

× algorithms (*Wagner, Veissier et al, 2020*)

→ identification of abnormal behaviours (e.g. modification of circadian rhythm)

→ detection of subclinic disorders

Performances

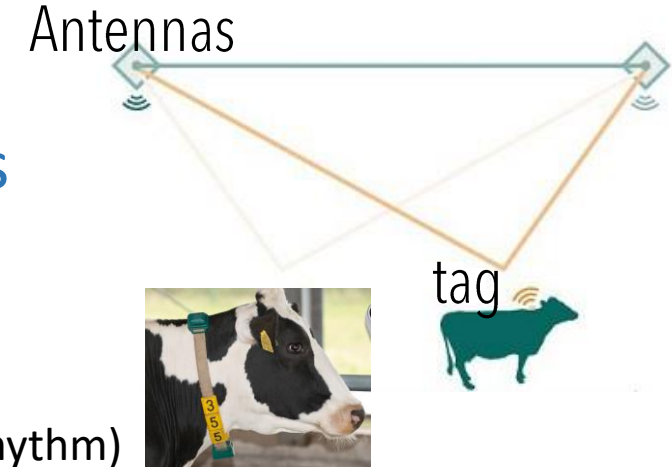
% false positive: 20%

% detection of something happening:

60 – 100 %

can be 90-100% in case of a health problem

Early detection: 1 day before caretakers for mastitis



Examples of detected events	% of detected events on 4 farms			
	1	2	3	4
Calving	100	-	-	99.4
Oestrus	95.1	85.7	69.2	91.4
Lameness	100	93.8	-	98.2
Mastitis	100	-	-	87.5
Ruminal acidosis	-	69	-	-
Other disease	80	75	-	90.9

Early detection of problems/disorders

Modification of animals' behaviour

- Modification of enrichment use
as indicator of cows' stress and disease



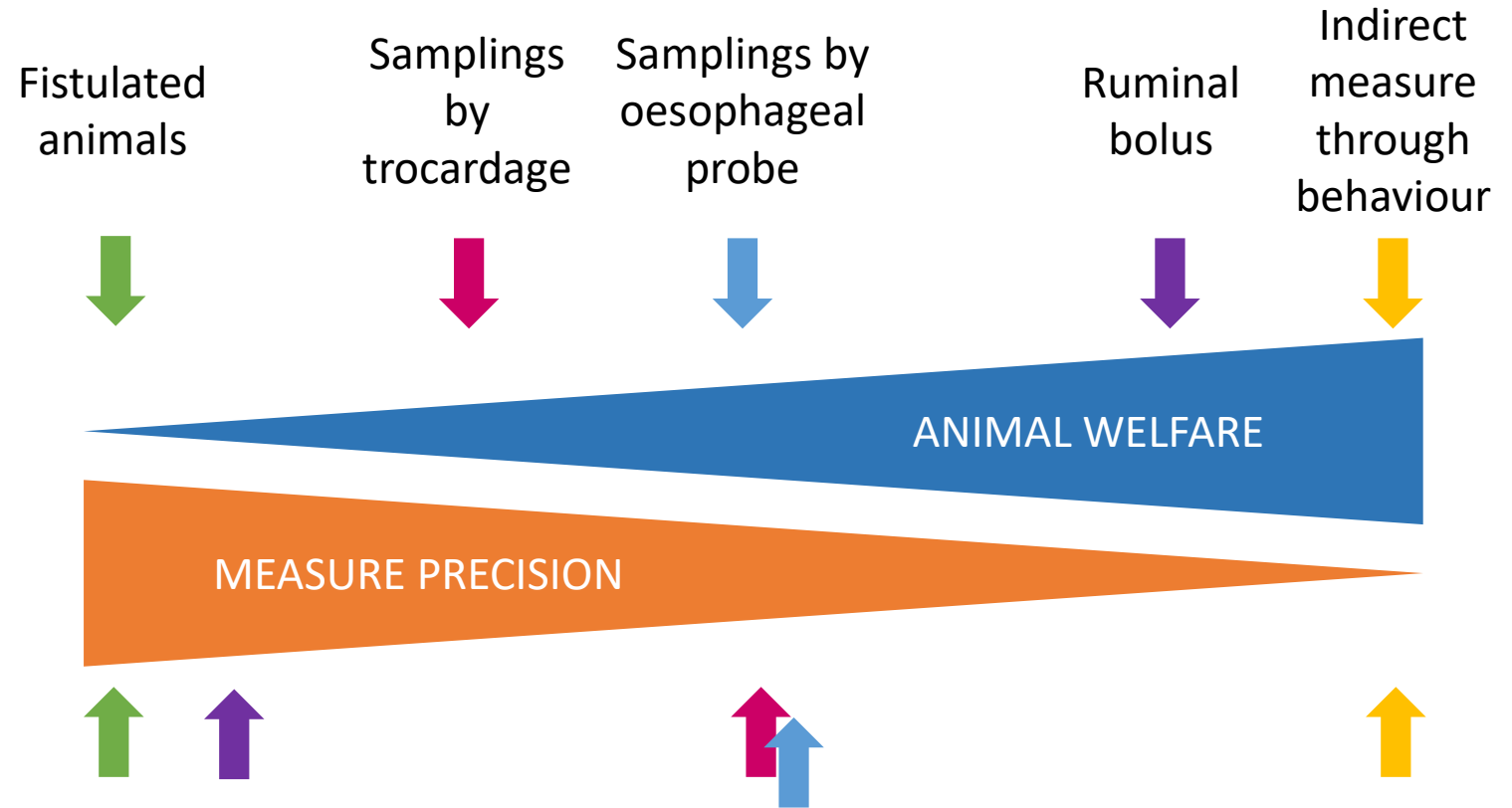
Brush use	Control	Heat wave	Metritis
No. events/d	4.5	-0.062	
Duration/d	88 s	/THI unit	44 s

→ During thermic stress or metritis,
cows use less the automatic rotative brush

Refinement of experimental procedures

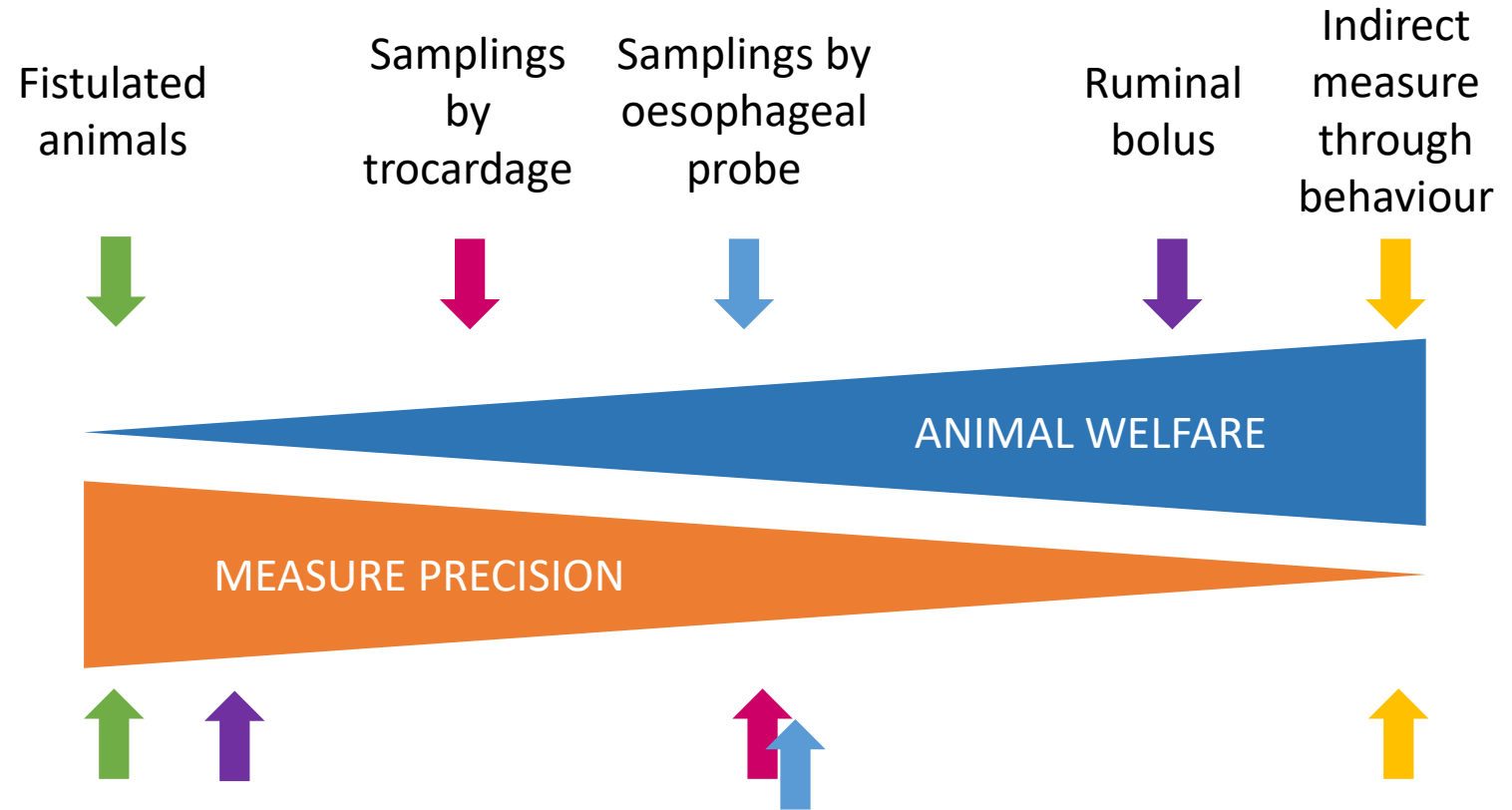
Choice of the most convenient experimental procedure

E.g. ruminal pH



Choice of the most convenient experimental procedure

E.g. ruminal pH



Which procedure to answer scientific question while minimising impact on animal welfare?

If the scientific question requires a pH kinetic with measure every 15 minutes → the **ruminal bolus** is probably to be chosen since indirect measure through behaviour modification would not be sufficiently precise

