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Opinion paper: How can we achieve standards and common guidelines for experimental studies with cattle?

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Introduction

Livestock farming is confronted with substantial global issues, such as emissions of greenhouse gases and related climate change, environmental pollution and declining biodiversity, the achievement of high animal welfare standards and consumer acceptance, and not least sustainable production and food security. Research including experiments with animals is essential to find the best solutions for these challenges, to study their contribution to global impacts and their welfare. There are currently many similar studies performed in different countries, though they differ in the experimental techniques used and standards applied. These differences reflect, among others, different agro-environmental contexts, different legislation, or established traditions. Besides, protocols may differ between research infrastructures due to varying expertise and equipment. These differences make the results difficult to compare and generalise. If guidelines were harmonised and follow established standards, many datasets generated around the world could be better compared and more effectively combined, to produce new knowledge e.g. from meta-analyses. Harmonisation of guidelines would also minimise duplication of effort, save resources, and allow more information to be extracted from each study. Moreover, following commonly agreed and comprehensive guidelines will ensure that methods are updated to reflect new knowledge and promote high-quality experimental research. This requires common awareness amongst animal scientists, but would help to reduce the number of animals used in research by reducing the number of experiments as well as by reducing the number of animals per experiment, thereby following the Replacement, Reduction, and Refinement (**3 R**) principles. It is essential that common standards and guidelines for measurements and data recording are developed, comprehensively applied, continuously updated and cited in scientific publications. These standards and guidelines should preferably be published in openly accessible resources that can be readily and easily updated. In this paper, we discuss initiatives to achieve harmonisation of guidelines and protocols across research institutions conducting experiments with cattle and how these guidelines can be useful to research.

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Standardising scientific experiments with cattle

The International Committee for Animal Recording (**ICAR**) develops global standards for animal identification, performance and health recording in farm animal production. For the cattle sector, ICAR published guidelines related to the measurement of milk yield, assessment of functional traits (e.g. fertility, claw and udder health, lameness) and more recently feed intake and methane emissions in the context of genetic evaluations (ICAR, 2021). The ICAR guidelines provide a reference tool for practitioners and scientific facilities. They do not include common protocols used with cattle, e.g. for the measurement of emissions or different metabolic, digestive, anatomic and behavioural traits. We have recently started to assemble this information. Numerous scientists, both from within and outside the Horizon 2020 infrastructure project SmartCow (https://www.smartcow.eu), compiled and critically evaluated existing protocols regarding differences, similarities, state-of-the-art, and general applicability for scientists working on cattle. The guidelines were, if necessary, extended to achieve harmonisation and standardisation based on the most recently established, innovative and non-intrusive methods and current best practice. The defined cross-border standards for each guideline include, where meaningful, instructions for experimental planning (e.g. statistical power analysis, repetitions, experimental duration), ethical concerns, calibration of instruments, automated measurements, manual recording of clinical aspects, environmental impacts, and, where applicable, data processing, calculation, and formatting.

Common guidelines for experiments with cattle

The guidelines assembled by the SmartCow consortium followed the standard requirements described above and were published by Publisso as an open-access living handbook entitled "Methods in cattle physiology and behaviour research – Recommendations from the SmartCow consortium" (Danesh Mesgaran et al., 2021). The book provides guidelines for routine and specific recording methods for the main metabolic, digestive, anatomical and behavioural traits in cattle. Besides a foreword, the book includes eight major sections. Among them, the section on "Ethical aspects" proposes a pragmatic approach to guide researchers in their decision to undertake an experiment, balancing issues between constraints on the cattle and expected benefits of an experiment, and in the implementation of the 3R principles for animal experimentation.

The section on "Feed and water intake" emphasises the importance of obtaining reliable measurements at the individual level, both for housed and grazing conditions. Manual and automated standard operating procedures are introduced, ranging from feeding total mixed rations or partial mixed rations, to pasture-based feeding or a combination thereof. The "Body characteristics" section provides guidelines for manual and automated measurement of BW and body condition score, ultrasound-assisted back fat thickness measurement, determination of mammary gland volume, and various body temperature recording methods, including those using thermal cameras. It highlights modern methods that allow rapid, frequent, and reliable measurements of key body traits for the investigation of nutritional and reproductive physiology and management. A special section is dedicated to techniques used with calves, namely the measurement of milk or milk replacer intake, monitoring of rumen and mammary parenchyma development, and the assessment of body anatomy and composition using dual-energy X-ray absorptiometry (**DXA**) or magnetic resonance imaging (**MRI**).

The guidelines assessing parameters related to "Digestive tract physiology" focus on methods that do not require surgically modified animals, as their access is not universal and is increasingly restricted, even in research facilities. They include rumen fluid sampling via oral stomach tubing method, monitoring of rumen pH using probes, marker techniques to measure passage rate and digesta retention time, and the performance of nutrient digestibility and balance studies with beef and dairy cattle, using total collections of faeces and urine as the reference method.

The study of animal behaviour benefits from recent technological developments that have led to an increase in the number of sensors and devices for measuring an increasing array of animal parameters. A comprehensive section about "Behavioural physiology" introduces the reader to a checklist to validate sensor output for the recording of cattle behaviour. It provides guidelines and examples for the performance of behavioural tests, measurement of rumination activity, lying, standing, and eating behaviour, as well as for detection and scoring of lameness. In the section on "Reproductive assessment", guidelines for visual heat detection, the use of an automated heat detection system via vocalisation, and for pregnancy examination are provided.

The last section of the book focuses on "Gas exchange and methane emission measurements". Respiratory chambers, originally developed to study heat production of animals, allow the measurement of enteric methane emissions of animals, and are considered as the gold standard reference method. The section details the procedures for operating a respiration chamber facility, emphasising the importance of gas recovery tests. It also describes methane emission measurements using alternative widely used techniques such as the GreenFeed system or the sulphur hexafluoride (**SF_6**) tracer technique that can be performed in a wide range of feeding conditions, including outdoors on pasture.

Implementation of ontologies

All guidelines of the living handbook implement Animal Trait Ontology for Livestock (**ATOL**), Environment Ontology for Livestock (**EOL**) or Animal Health Ontology for Livestock (**AHOL**) numbers (Le Bail et al., 2014; Golik et al., 2012), which are summarised under: https://sicpa-web.cati.inrae.fr/ontologies/visualisation/ontologie/. These ontologies allow the unambiguous definition of phenotypic traits and thereby serve as a reference tool for users. ATOL, EOL and AHOL are key to unifying research methodologies and make the results obtained from experiments with cattle more interoperable.

Implications and future steps

With the book on "Methods in cattle physiology and behaviour research", the SmartCow consortium aims to contribute to the achievement of standards and common guidelines for experimental studies with cattle. Two more chapters entitled "Guidelines to apply for ethical approval of animal experiments" and "Validation of eating duration using an automatic feeding system" are currently in preparation and will be added to the book soon. Researchers interested in adding a new method to the book should contact the corresponding author of this paper for further information and publication conditions. This invitation is explicitly directed to all scientists beyond the SmartCow consortium. Updates on existing guidelines and inclusion of further guidelines into the existing book are highly desirable and encouraged. Because each new guideline and each updated version of a guideline will be assigned to a single digital object identification (e.g. doi) number, authors can reference the version they used in scientific publications. The progressive implementation of the guidelines in

the experimentation with cattle and referencing of the guidelines in scientific publications are encouraged. Referencing standardised, comprehensively described and commonly agreed guidelines would also make an extensive description in the "Material and Method" part of traditional scientific publications unnecessary, avoid many repetitions within and across scientific journals, and reduce apparent plagiarism among authors. In addition, as the improvement of methods is a continuous process, even minor refinements to methods can be published in the updatable, living resource that the SmartCow consortium has established. By contrast, traditional journals often publish improvement to methods only when major changes are made. For this purpose, the book described above has been designed as an open-access living handbook, which means that it is open to new contributions from the SmartCow consortium, as well as external ones.

Ethics approval

All ethical standards have been met.

Data and model availability statement

Not applicable.

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B.K. drafted the first version of the manuscript. All other authors worked on the further improvement of the first draft. All authors have read and agreed to the final version of the manuscript before submission.

Declaration of interest

None.

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References

Danesh Mesgaran, S., Baumont, R., Munksgaard, L., Humphries, D., Kennedy, E., Dijkstra, J., Dewhurst, R., Ferguson, H., Terré, M., Kuhla B., 2021. Methods in cattle physiology and behaviour research – Recommendations from the SmartCow consortium. Retrieved on 13 May 2022 from https://books.publisso.de/en/publisso_gold/publishing/books/overview/53.

Golik, W., Dameron, O., Bugeon, J., Fatet, A., Hue, I., Hurtaud, C., Reichstadt, M., Meunier-Salaün, M.C., Vernet, J., Joret, L., Papazian, F., Nédellec, C., Le Bail, P.-Y., 2012. ATOL: The multi-species livestock trait ontology. In: Dodero, J.M., Palomo-Duarte, M., Karampiperis, P. (Eds.), Metadata and Semantics Research. MTSR 2012. Communications in Computer and Information Science. Springer, Berlin, Heidelberg, Germany, pp. 289–300.

ICAR, 2021. ICAR guidelines. Retrieved on 13 May 2022 from https://www.icar.org/index.php/icar-recording-guidelines/.

Le Bail, P.Y., Bugeon, J., Dameron, O., Fatet, A., Golik, W., Hocquette, J.F., Hurtaud, C., Hue, I., Jondreville, C., Joret, L., Meunier-Salaun, M.C., Vernet, J., Nédellec, C., Reichstadt, M., Chemineau, P., 2014. ATOL : une ontologie générique pour le phénotypage des animaux d'élevage. INRA Productions Animales 27, 195–208 https://doi.org/10.20870/productions-animales.2014.27.3.3067.