Introduction

1

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Abstract

A standard operating procedure (SOP) is a step-by-step instruction manual describing how to perform a specific work routine. The primary objective of SOPs in a research setting is to enhance research quality (replicability, reproducibility and repeatability) by establishing standardised ways of performing specific experimental procedures. SOPs serve as a valuable structure for internal communication and sharing of best practices (i.e. training documents to guide new users), and they facilitate the preparation of an experiment and the subsequent publication process, since they provide all information about the equipment needed and how to carry out the procedure in detail. The use of SOPs also makes it easier to collaborate between research institutes, and the generation of more robust and reliable data facilitates its use and re-use for comparison between studies, such as in meta-analyses. This book includes nine SOPs for specific experimental procedures developed within the PIGWEB project. The topics cover both common procedures performed at most experimental facilities, such as blood sampling, and more novel and minimally invasive research methods, such as saliva sampling. The broader goals of this open-access volume are to disseminate current gold standards for pig research while emphasising the importance of future innovations to promote better animal welfare.

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Standard operating procedure · SOP · Research quality · Pigs

Recent years have seen growing public concern regarding the quality of scientific research in general and especially the reproducibility of results (e.g., Calnan et al. 2024). This also applies to research in the animal sciences. Researchers and working groups around the globe have come up with a range of behavioural, physiological and cognitive indicators of animal welfare, health and nutrition over the years, and a variety of methods for how to assess them in farm animals. However, more often than not, research results are inconsistent across studies (Loss et al. 2021). A potential indicator that was useful for detecting differences between two treatments in one study might not be affected by the same (or similar) treatments in another study. This leads one to question whether the indicator itself is valid or whether the hypothesis regarding the treatment effects on animal welfare, health and nutrition is false.

One possible explanation for inconsistent results in animal research is a lack of standardisation or documentation across labs and other research facilities regarding experimental procedures, analytical approaches or investigated populations. The variability in experimental details can roughly be separated into three main areas: (1) animals, (2) housing and management and (3) methods and execution of experimental procedures (as in how/when sampling is carried out, equipment and type of materials used, calibration of technical devices, handling of samples, etc.). Regarding animals, different breeds differ in production performance and may have varying susceptibility or resilience to stress (Oster et al. 2025; Laghouaouta et al. 2024). Also, within breeds, there are often differences between sexes (van den Broeke et al. 2020) or during ontogenetic development (Prunier et al. 2020). Regarding housing and management, there are significant differences in legislation and regulation between countries and/or regions, for example, regarding the use of farrowing crates vs free farrowing (EFSA AHAW Panel 2022). Differences between production schemes (e.g. conventional vs organic vs welfare labels) or feeding practices (e.g. differences in feeding frequency, group or individual feeding and provision of roughage/organic enrichment) can also introduce variation in results. Considering pig research specifically, there is currently a large variation in animals, management and housing conditions across different pig research facilities, which cannot be completely standardised. To a certain degree, this can be seen as a strength since it represents the even larger variation across farms in different countries, where, ideally, results from research would eventually be put into practice. However, regarding how actual sampling is performed in practice and how samples are measured, more can be done to standardise the methods used and how experimental procedures are executed within and across research facilities, to improve research quality and reproducibility.

A standard operating procedure (SOP) is a step-by-step instruction manual describing how to perform a specific work routine. The primary objective of SOPs in a research setting is to enhance research quality (replicability, reproducibility and repeatability). By establishing a systematic way of performing a task, SOPs ensure that the task is done consistently by all involved persons (Manghani 2011), be it within or across research facilities. Development and implementation of SOPs for specific experimental practices is therefore one way of creating more standardised ways of performing research in order to improve the consistency of research methodologies and enhance the reliability and credibility of research findings. The use of SOPs also makes it easier to collaborate between research institutes, and the generation of more robust and reliable data facilitates its use and reuse for comparison between studies, such as in meta-analyses.

Apart from improving the quality of research, the use of SOPs also improves transparency within an organisation, serves as a valuable structure for internal communication and facilitates the sharing of best practices (Amare 2012). An SOP can also contribute to efficiency by serving as a training document to guide new users (e.g. new colleagues or students) through the process outlined in the SOP (Akyar 2012). For researchers, having an SOP can also facilitate both the preparation of an experiment and the subsequent publication process, since it provides all information needed to report how specific procedures were carried out. Introducing SOPs in animal research is also in line with the current implementations of the 3R principles (replace-reduce-refine), as it should help to reduce the number of experiments required by reducing avoidable variability in experimental designs. Furthermore, it should also help with the refinement of methodologies, especially those used across many research groups, by defining best practices to minimise animal stress.

In laboratory research environments and commercial labs, the use of standards from the International Organization for Standardization (ISO) for specific analyses is already routine. However, within many research farms and other facilities designated for research on farm animals, SOPs are currently lacking. In a large collaborative project called PIGWEB (funded by the European Union's Horizon 2020 research and innovation program under Grant Agreement No 101004770), which aimed to connect pig researchers across Europe, we set out to address this lack of SOPs within the leading European pig research infrastructures. The PIGWEB consortium comprises 16 partners from 10 European countries, including 11 research institutions working on pig welfare, health and nutrition. We set a goal of encouraging specialists across a range of institutes and areas of pig research to share and harmonise their existing SOPs as well as to develop new SOPs where needed, for previously undocumented or emerging methods that are important to their own research. This edited volume is the result of those efforts. In general, small teams of pig researchers using the same method in different facilities prepared the initial drafts. These drafts were then internally reviewed by other PIGWEB consortium members who were not specialists in the techniques, allowing them to assess how easy the SOPs were to follow. In addition, every SOP was externally reviewed by researchers outside of the PIGWEB consortium. The final SOPs presented in this

book are the outcome of this two-stage review process. Each SOP represents the agreed-upon best practices for carrying out a given procedure with pigs. In addition to emphasising easy-to-follow instructions, the SOPs introduce innovative and minimally invasive techniques whenever possible and emphasise in each protocol best practices for pig welfare, as well as for research purposes. Some of the SOPs have already been used in a multi-lab study (Reimert et al. 2026) in order to assess their applicability. By promoting the dissemination of SOPs within and across pig research facilities, our broader goal is to boost the quality of research by achieving a higher consistency of research methodologies across institutions and having guidelines available to train the next generation of researchers.

This book includes nine SOPs for specific experimental procedures. The topics cover both common procedures performed at most experimental facilities, such as blood, faecal and urine sampling, and more novel and minimally invasive research methods, such as saliva sampling and thermography for physiological assessments, near-infrared scanning/spectroscopy (NIR) to determine ileal digestibility and image analysis to determine body and carcass composition. In addition to the specific experimental procedures presented in this book, PIGWEB has also been promoting the development of SOPs for basic management routines and recording of standard traits in experimental pig research facilities. Such SOPs must, however, be adapted to the specific housing conditions, existing equipment and legislations governing each facility. The results of this work can be found on the PIGWEB website (www.pigweb.eu) and at the Social Science Research Network (SSRN, Westin and Wallenbeck 2024). An example template to be used for the development of internal SOPs for basic management routines is attached in ESM 1.

Several of the SOPs presented in this book emphasise the importance of habituating the pigs to the described sampling techniques and give examples of how this can be done. However, we also want to mention the potential for the use of positive reinforcement training (PRT), also known as clicker training, in pig research. PRT is a growing field within animal research and has successfully been applied in pigs for a range of procedures including the following: (1) intravenous blood sampling without restraint (Fiderer et al. 2024), (2) sling-training (Yang et al. 2021), (3) ultrasound examination (Rydén et al. 2019), (4) urine sampling (Rydén et al. 2019) and (5) blood sampling by catheters without restraint (Rydén et al. 2019). Implementing PRT also provides the animals with cognitive enrichment, which further improves their welfare (Sørensen 2010). Although not the focus of these SOPs, we urge interested readers to inform themselves about PRT and implement such training whenever possible in their own pig husbandry and research procedures.

The huge societal demand for improvements to farm animal welfare and health, and for valid, reliable biomarkers to measure these aspects, should be a motivating factor for all farm animal researchers. This demand can only be met if we improve the quality of our research, raise the standards for best practices, and continue to develop innovative practices reflecting how animals should be farmed in the future.

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