

Improving research quality and collaboration by developing standard operating procedures

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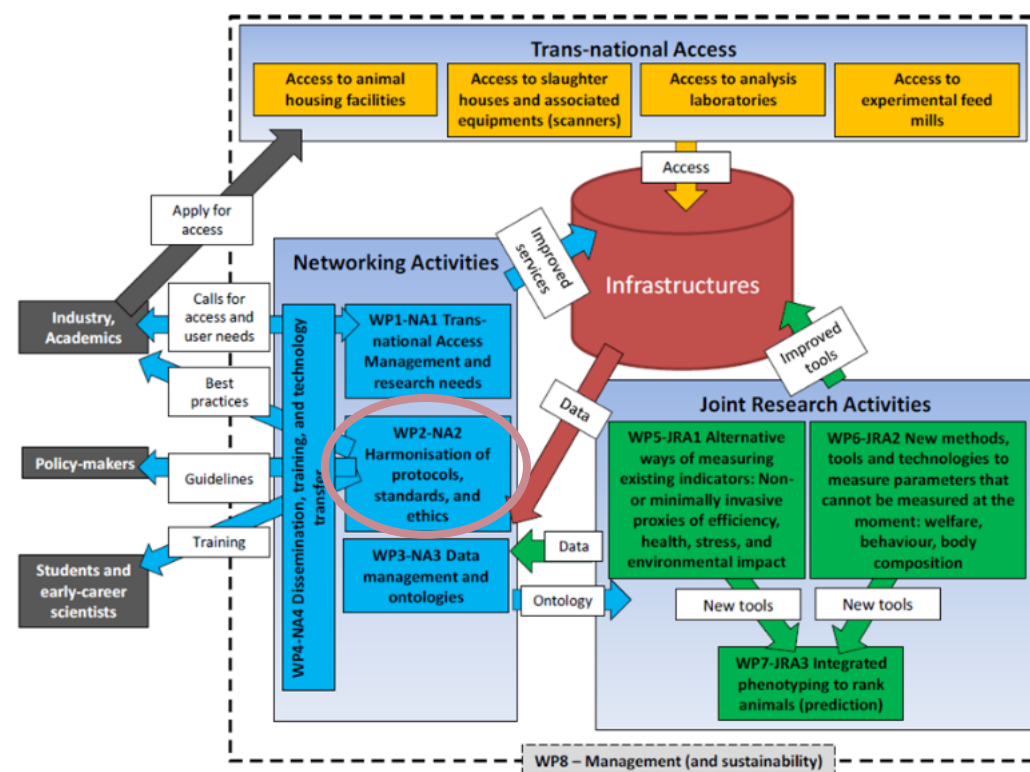
The PIGWEB project

An infrastructure for experimental research for sustainable pig production

The aim of the project is to strengthen the pig research community by providing and facilitating access to research infrastructures, reinforcing a culture of cooperation between the research community and industrial and societal stakeholders, and improving and integrating the services provided by the research infrastructures.



More info about PIGWEB



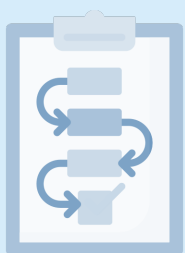
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Tasks of the work package 2

The objective of WP2 is to harmonise protocols, develop standard operating procedures (SOPs), and to promote the use of standards and guidelines to ensure a high quality of expertise and ethics in research conducted at the research infrastructures of the network and beyond.

Task 2.1 Harmonisation of basic management and protocols for recording of standard traits in the experimental facilities

- EAAP : Wallenbeck *et al.*, 2023.
Development of protocols for standard management and recording in pig research facilities



Task 2.2 Development of SOPs for specific experimental procedures and improving quality of experimental design

- De Cuyper *et al.*
 - **Guidelines for designing pig trials on performance, digestibility, meat and carcass quality**
 - **Identifying key pitfalls in experimental design of pig trials: a group discussion**
- Ampe *et al.* **Common mistakes in experimental design and analysis of pig trials: an overview**
- The development of SOPs

Task 2.3 Procedures and practices concerning ethical standards for pig experiments

- Session 78: Franchi *et al.* **Conceptualisation of “animal discomfort” using the domesticated pig (*Sus scrofa*) as model**
- Policy paper. Boswijk. **Harmonization of pig research**



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What is an SOP and why is it important ?

A Standard Operating Procedure (**SOP**) is a **step-by-step guide** to performing a **specific** experimental **procedure** = how a sample is taken (like following a cooking recipe)

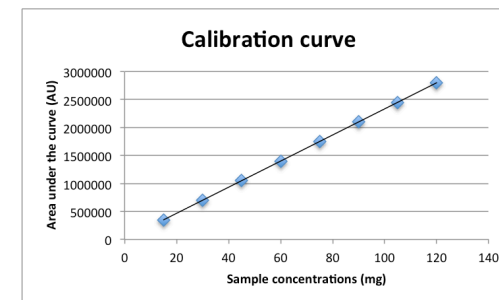
Pig research infrastructures: ↗ collaboration → need for robust data collection to **share** and **re-use** them

standardisation of
analytical methods

International
Organization for
Standardization



reporting of appropriate
validations (calibration curves,
internal standard ...)



From
<https://theory.labster.com/hplc-calibration-curve/>

? **standardisation of specific experimental procedures** → can affect the quality of results

Different sampling methods may involve different degrees of invasiveness and have differing impacts on behaviour and emotional states, which may ultimately affect the results.



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Benefits of using SOPs



Improve research quality

- ↗ Repeatability, Reproducibility and replicability
- Better comparison of results between studies (meta-analysis)



MORE ROBUST DATA

Replicable

New researcher, new data, similar method

Obtaining consistent results across studies aimed at answering the same question, but with new data (possibly from new locations)

Reproducible

New researcher, same data, same method

Obtaining consistent results using the same input data, computational steps, methods and code, and conditions of analysis, but by a new researcher

Repeatable

Same researcher, same data, same method

Obtaining consistent results using the same input data, computational steps, methods and code

From Kedron and Frazier, 2022



Contribute to the 3Rs principles

- reduce the number of animal used in a study
- Appreciated by ethic committee in our ethical application



Save time

- help researchers to prepare an experiment (material needed and animals)
- Can be shared with new students or colleagues



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How did we proceed in PIGWEB?



1) agreement on specific experimental procedures → methods to collect common biological samples (blood, faeces, urine, saliva) and specific measurements (carcass and body composition)



2) Each partner was asked to indicate which procedures they would contribute to and 5 working groups were formed with one person acting as group coordinator.



3) Each working group had to define the method(s) to be standardised.

SOPs Partners	Blood sampling techniques	Faeces collection and digestibility measurements	Image analysis for the assessment of the body and carcass composition	Urine collection	Saliva sampling and salivary cortisol measurement
Agroscope (CH)	x	x	x	x	
AU (DK)	x	x		x	x
EV ILVO (BE)		x			
FBN (DE)	x			x	x
INRAE (FR)	x		x		x
IRTA (ES)			x		x
MATE (HU)		x		x	
Medicopus (HU)	x		x		
SLU (SE)	x	x			
WU (NL)	x	x			x



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How did we proceed in PIGWEB?



4) Collection of the specific experimental procedures used in the different infrastructures



Many of us did not have written procedures



A general template was created:

- Introduction, goal, materials and equipment
- Prerequisites and preparation of the animals
- Description of the procedure
- Consideration of the 3Rs and a focus on promoting animal welfare

 WP2 Task 2.2a Development of SOPs for specific experimental procedures	 WP2 Task 2.2a Development of SOPs for specific experimental procedures
For internal use in PIGWEB only 1. Introduction <i>General introduction of the topic</i> 2. Goal and scope of the procedure <i>Please also include all relevant information such as the category of pigs (ex: below 20 kg, <u>sows</u>), experimental unit: individual/group, the range of measurements (from 5 to 450 g/kg)...</i> 3. Materials and equipment 3.1. Subtitle 1 3.2. Subtitle 2 4. Prerequisites and preparation <i>Please also include all relevant information regarding specific needs (special room for DXA, CT, RMI...) or a specific preparation of the animal (number of animals, sex and age, fasted animals, training of the animals...) or the sample (minimum quantity required for the analysis, buffer, internal standard, storage condition, grinding...).</i> 4.1. Subtitle 1 4.2. Subtitle 2 5. Description of the procedure <i>Please be as specific as possible. For example, please include the animal restraint (for blood, urine, feces sampling), the pooling of samples for the analysis, the maturing duration for meat quality... Pictures can be included.</i> 5.1. Subtitle 1 5.2. Subtitle 2 5.3. Subtitle 3 6. Compliance with the 3R principles	For internal use in PIGWEB only 7. Conclusions <i>In this part, please summarize the advantages and disadvantages of the procedure</i> 8. References 9. Annexes



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How did we proceed in PIGWEB?



5) The coordinator of each working group compiled all the information received into a single document to produce the SOPs. The use of the template by the partners facilitated the process.



6) Each working group exchanged information in many meetings to define the best practices.



7) Production of 7 SOPs that have been reviewed by scientific experts outside the PIGWEB project



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5.4.2.2. NMRI image treatment and presentation of final results

Image acquisition
The NMRI scan takes substantially longer than the CT scan. A cross-sectional scan range typically takes several minutes. Thus, NMRI examination of an entire pig up to 1 hour, depending on the sequence used and the size of the animal. Prior to imaging, the anesthetized animal is placed on the examination table and an X-ray made to cover the entire surface to be examined with body coils for appropriate collection. Due to the length of the test, it is sensitive to motion artefacts, so rapid can cause significant image quality degradation. Proper anaesthesia is therefore (Figure 7).



Figure 7. Positioning a pig for an NMRI scanning (before and after the body coil placement).

Image treatment
Linear and volumetric measurements can be performed on cross-sectional images reconstructed by NMRI imaging, similar to images obtained during CT examination (previous section). The difference is that while Hounsfield units are calibrated values, the test conditions may differ from the intensity values for NMRI images. Accurate segmentation procedures should take this phenomenon into account.

5.4.2.3. DXA image treatment and presentation of final results

Image acquisition
Depending on the DXA device, several acquisition modes for the complete body are available and one needs to be chosen. The GE Healthcare i-DXA has the following modes:
Total Body – Thick: mean height of >25 cm
Total Body – Standard: mean height of 16-25 cm
Total Body – Thin: mean height of <16 cm
Small Animal Body – Large: > 20 kg
Small Animal Body – Medium: 2-20 kg
Small Animal Body – small: < 2 kg
At Agroscope, all pigs are scanned with Total Body – Thick mode when repeated DXA are conducted over growth. In addition, Total Body – Standard or Small Animal Body – Medium mode can be used for scans for pigs between birth and ~25 kg BW.

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WP2 Task 2.2a:
Development of SOPs for specific experimental procedures

PIGWEB

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- b. When using a syringe, apply the smallest possible pressure. Pulling on the plunger of the syringe during collection should be done slowly and very carefully. Meanwhile, only attach a vacuum tube (smallest possible) after the needle is well placed in the puncture site.
- c. Once required volume of blood is collected, stop pulling on the plunger of the syringe or immediately detach the vacuum tube.
- d. Carefully remove the needle and immediately apply pressure in a circular motion for around 30 sec on the puncture site after it exits the skin. Clean blood on skin, if any.



Figure 13. Locating the vein used for the ear venipuncture.

Post procedure observation
Check the puncture site for any possible bleeding or bruising. In case of excessive bleeding at the puncture site, hold the compression point for a couple of minutes. The pig should be moving correctly with proper gait when returned to the pen. If done in anesthetized pigs, they should only be returned to the pen after it has fully awakened, appear healthy, and moving well. If the snout rope was used, check for possible snout injury or broken tooth.

3.7. Blood collection by catheter from pigs more than 20 kg
The following sampling method is described for in-dwelling catheters that have a one-way stopcock installed on the end of the catheter. The catheter and stopcock should always be kept in place using a medical bandage around the pig's body to keep it clean and protects it from damage like chewing by the pig or grinding it against the wall or cage (Figure 14). The catheter is a route between contaminated external environment and sterile internal environment, so protecting it reduces the risk of infection and other health problems for the pig (i.e., clots, air bubble, septicemia). In addition, the method has been tested and verified in pigs housed individually. **To minimize stress during blood collection, it is strongly recommended to get the pig used to humans.**

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Limitations in the harmonisation

- Challenging because we have different facilities, different equipment
- How accurate should we be ?
 - To ensure that the procedures are used by as many people/institutions as possible, we need to find a compromise.
- Differences in ethics between the country/institutes
 - Task 2.3: see policy paper. Boswijk Heleen.



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PIGWEB
HARMONIZATION OF PIG RESEARCH
JUNE 2024

PIGWEB aims to contribute to the goals of the European Green Deal and the 'Farm to Fork' strategy, by strengthening the pig research community and developing solutions for sustainable pig production systems. One challenge is variation in transposition of the *European Directive* on the protection of animals used for scientific purposes into national legislation. This variation can hinder scientific progress and raise ethical concerns. To harmonize pig research, we propose three key recommendations.

KEY RECOMMENDATIONS

- Make a coordinated effort to create a more harmonized legislation for pig research. Improve informing citizens about pig farming and research.
- Increase harmonization of training and education by specifying learning goals and competencies of researchers, caretakers and assessors.
- Define task and scope of ethical evaluation, standardize 'tools' for ethical decision making, including the harm-benefit analysis.

INTRODUCTION

National legislation on pig experimentation varies widely across Europe, creating significant challenges for animal researchers at universities, public research institutions and companies. The lack of harmonization in legislation not only causes confusion and constitutes an administrative burden but can also raise ethical concerns and hamper scientific progress.

In PIGWEB, an EU-funded research project, surveys were conducted to gather information on national legislation and procedures concerning ethical considerations and authorization on animal (pig) research.

With the results of these surveys, guidance is offered for future EU-wide regulations that would lead to harmonization of the situation across the EU and avoid potential 'shopping' for locations with fewer ethical constraints towards pig experimentation.

1 Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Text with EEA relevance (europa.eu)

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What remains to be done?

- Production of 3 additional SOPs
 - Inclusion of ontologies → collaboration with WP3
- Ontologies represent a set of concepts that facilitate standardisation of terminology within specific domains of interest (Hulsegge et al., 2012)*
- Publication of all the SOPs in an Open Access e-book.

Ontologies

[animal trait of livestock](#) / [nutrition trait](#) / [ingestion](#) / [feed intake](#)

[animal trait of livestock](#) / [animal welfare trait](#) / [psychoneurophysiological state trait](#) / [behaviour trait](#) / [ingestive behaviour trait](#) / [feeding behaviour trait](#) / [feed intake](#)

ATOL_0000772

Informations	
Name	feed intake
Nom	consommation alimentaire
Definition (EN)	any measurable characteristic related to the amount of feed intake in ad libitum conditions
Definition (FR)	toute caractéristique mesurable associée à la quantité d'aliments consommés dans des conditions ad libitum
Source	INRAE
Link	https://books.publisso.de/en/publisso_gold/publishing/books/overview/53/183
Comments	-

Synonyms	Measurements methods
Exact synonyms - animal feed intake - average daily feed intake - consumption of feed - daily feed intake - feed consumption - ingestion of feed - intake of feed Related synonyms - overfeeding - starvation	No results



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Conclusion

Start writing your own SOPs



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Thank you to all the contributors



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Thank you for your attention

