Improving research quality and collaboration by developing standard operating procedures

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Disclaimer: the PIGWEB project has received funding from European Union's Horizon 2020 research and innovation program under Grant Agreement No 101004770.

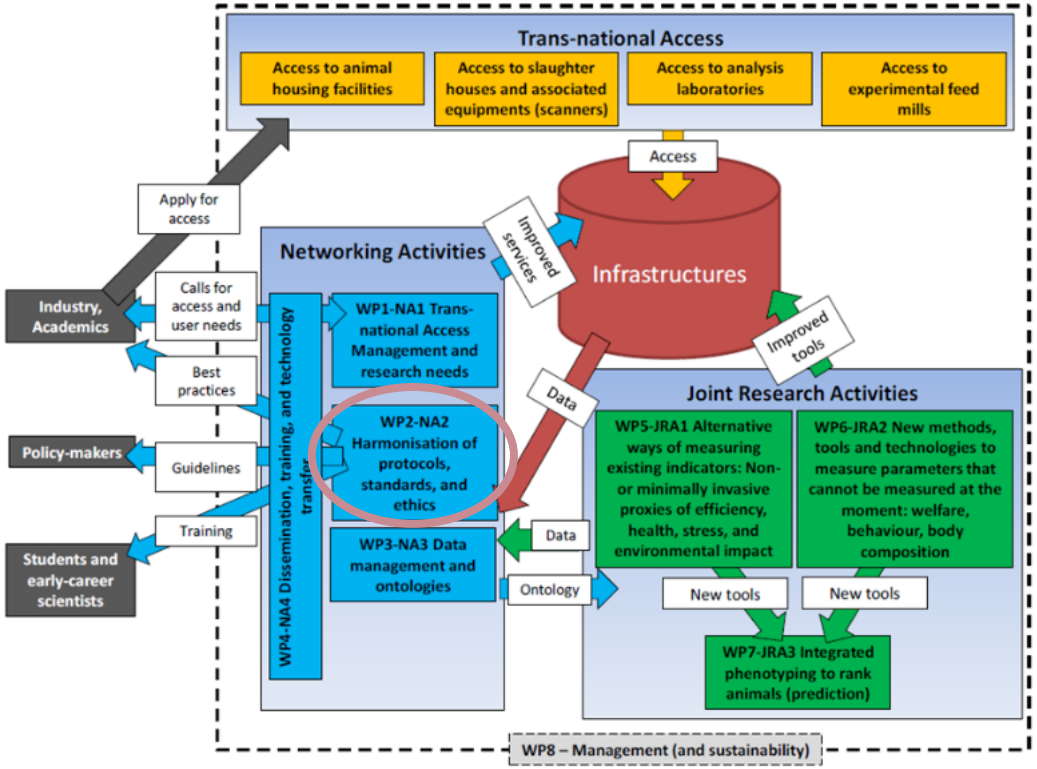
04.09.2024

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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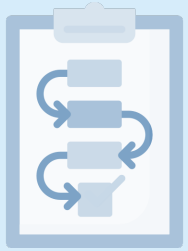
Tasks of the work package 2

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 ?

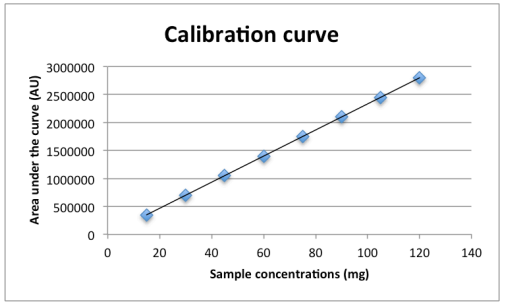
A Standard Operating Procedure (**SOP**) is a **step-by-step guide** to performing a **specific** experimental **procedure**
= how a sample is taken (like following a cooking recipe)

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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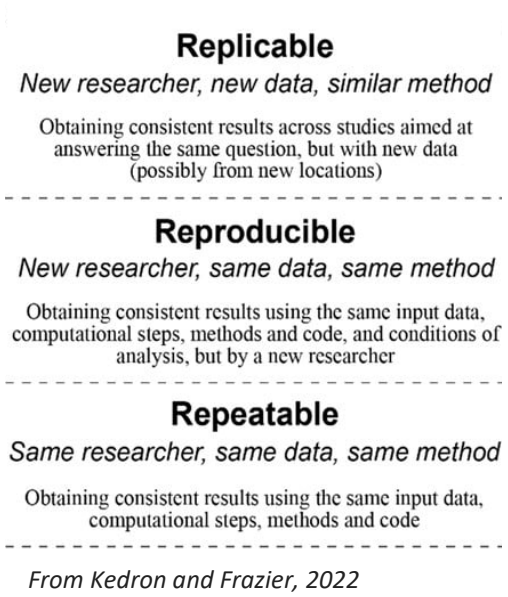
Benefits of using SOPs

Improve research quality

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

MORE ROBUST DATA



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 body and carcass composition	Urine collection	Saliva sampling and salivary cortisol measurement
Agroscope (CH)	×	×	×	×	
AU (DK)	×	×		×	×
EV ILVO (BE)		×			
FBN (DE)	×			×	×
INRAE (FR)	×		×		×
IRTA (ES)			×		×
MATE (HU)		×		×	
Medicopus (HU)	×		×		
SLU (SE)	×	×			
WU (NL)	×	×			×

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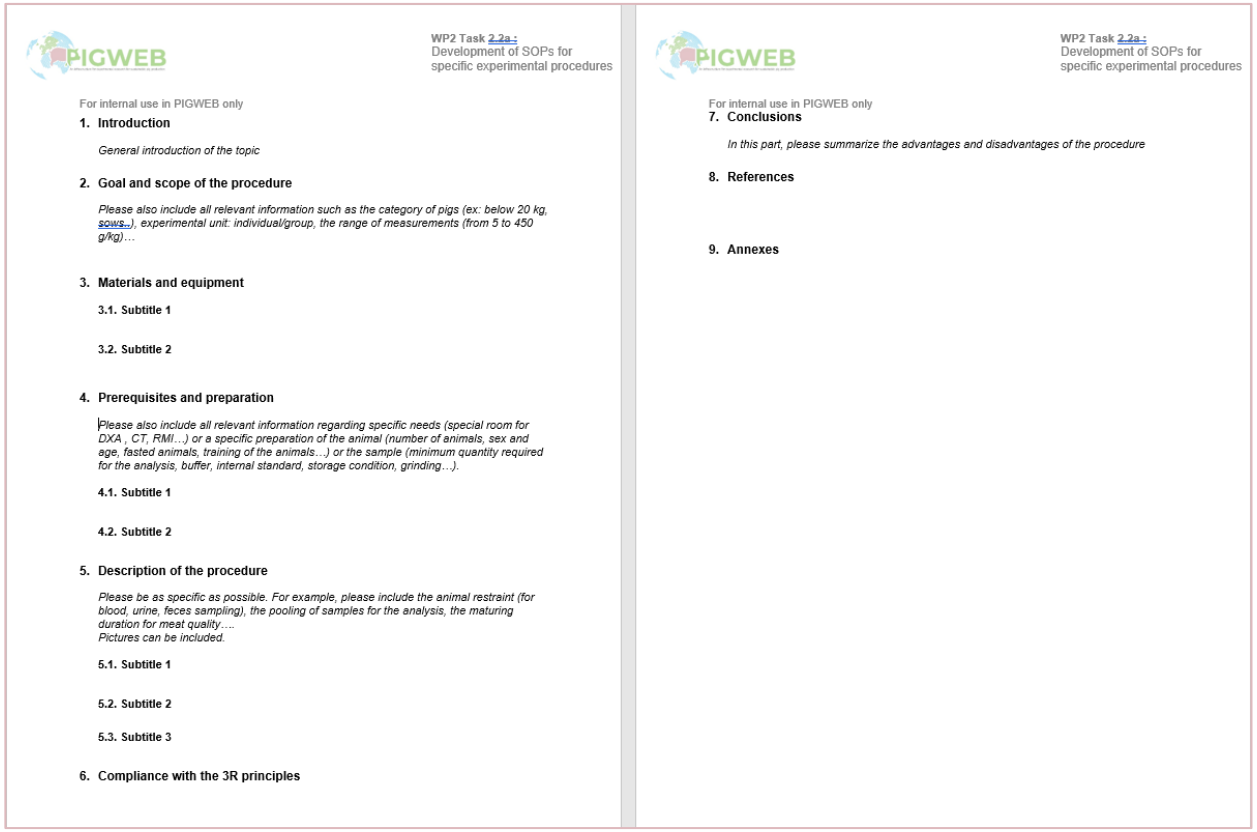
How did we proceed in PIGWEB?

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



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

For internal use in PIGWEB only

1. **Introduction**

General introduction of the topic

2. **Goal and scope of the procedure**

Please also include all relevant information such as the category of pigs (ex: below 20 kg, sows..), experimental unit: individual/group, the range of measurements (from 5 to 450 g/kg)…

3. **Materials and equipment**

3.1. Subtitle 1

3.2. Subtitle 2

4. **Prerequisites and preparation**

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…).

4.1. Subtitle 1

4.2. Subtitle 2

5. **Description of the procedure**

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.

5.1. Subtitle 1

5.2. Subtitle 2

5.3. Subtitle 3

6. **Compliance with the 3R principles**

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

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7. **Conclusions**

In this part, please summarize the advantages and disadvantages of the procedure

8. **References**

9. **Annexes**

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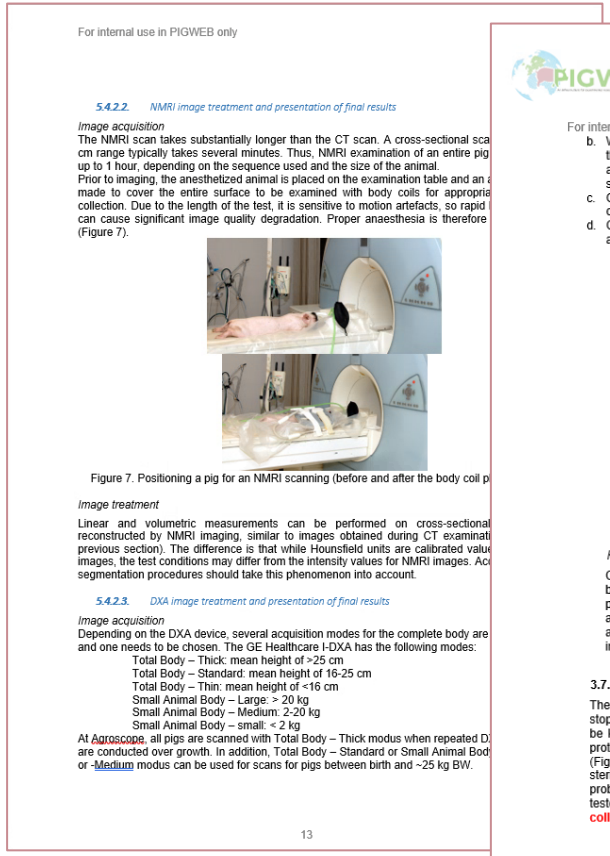
How did we proceed in PIGWEB?

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 sca
cm 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 a
made to cover the entire surface to be examined with body coils for appropria
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 p

Image treatment

Linear and volumetric measurements can be performed on cross-sectional
reconstructed by NMRI imaging, similar to images obtained during CT examinati
previous section). The difference is that while Hounsfield units are calibrated value
images, the test conditions may differ from the intensity values for NMRI images. Ac
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
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 modus when repeated D
are conducted over growth. In addition, Total Body – Standard or Small Animal Bod
or -Medium modus can be used for scans for pigs between birth and ~25 kg BW.

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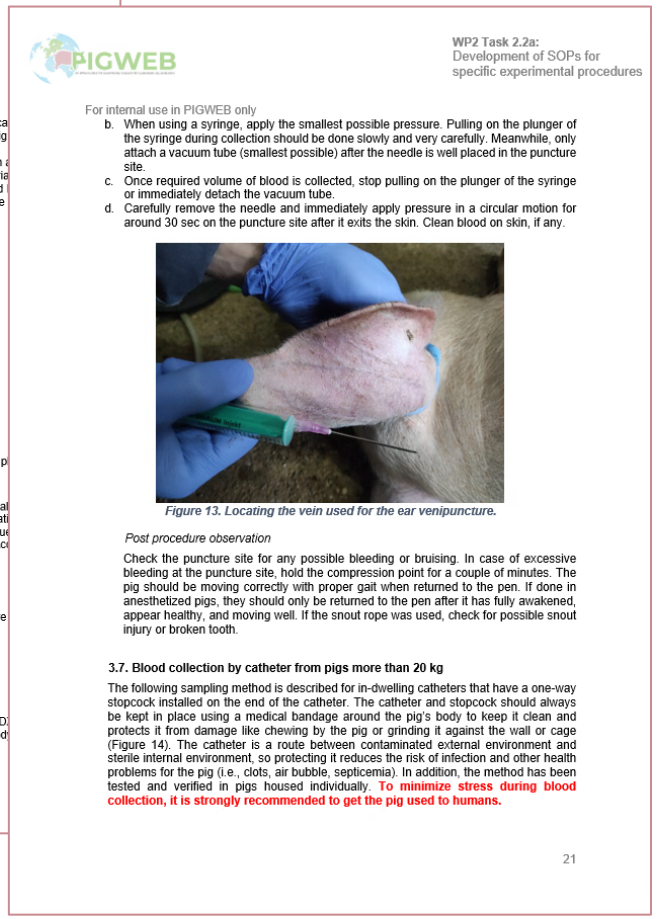
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WP2 Task 2.2a:
Development of SOPs for
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b. When using a syringe, apply the smallest possible pressure. Pulling on the plunger of the syringe during collection should be done slowly and very carefully. 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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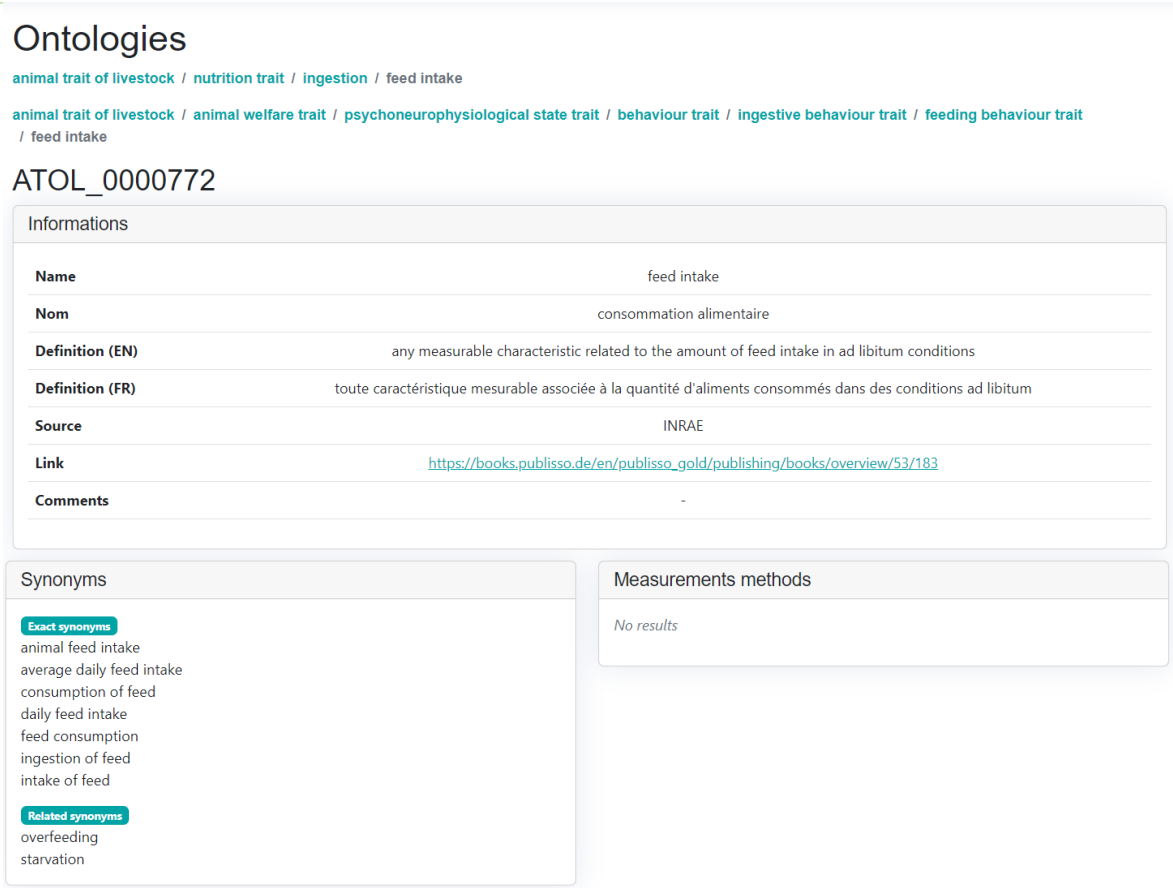
What remains to be done?

- 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.



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Conclusion

Start writing your own SOPs

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Thank you to all the contibutors

Ludovic Brossard, Nathalie Le-Floc'h, Hélène Quesnel

Albert Brun, Maria Font-i-Furnols, Marina Gispert

Ulrike Gimsa, Liza R. Moscovice, Quentin L. Sciascia, Andreas Vernunft

Carolien De Cuyper, Sophie Goethals, Sam Millet

Tamas Donkó, Örs Petneházy

Maria Eskildsen, Helle Nygaard Laerke

Emma Ivarsson, Axel Sannö

Giuseppe Bee, Francis Eugenio, Marion Girard, Claudia Kasper, Catherine Ollagnier, Patrick Schlegel

Sonja de Vries , Bjorge Laurensen, Nikkie van der Wielen, Tamme Zandstra

Virág Ács, Annamária Tischler

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Thank you for your attention