



The collection of performance data from four systems in the PigStun project that provide alternatives to high CO<sub>2</sub> stunning

Deliverable D3

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# Executive summary

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In EU pig slaughterhouses two stunning methods are most commonly applied: electrical stunning and carbon dioxide stunning at high concentration. In 2004 and 2020, the European Food Safety Authority (EFSA) concluded that gas stunning of pigs at concentrations above 30% CO<sub>2</sub> is aversive and causes hyperventilation and irritation of the mucous membranes. This is likely to be painful and elicits hyperventilation and gasping before loss of consciousness. For this reason, high concentration CO<sub>2</sub> stunning has been criticized. The general objective of the PigStun project is to encourage EU pig slaughterhouses using high carbon dioxide concentration for stunning pigs to convert to systems with high animal welfare standards, by developing technical specifications for promising alternatives. This deliverable D3 presents four alternatives, describes how they have been tested for several aspects of sustainability and presents potential ways in which they could be implemented in practice. The actual test results will be published in the next deliverable of the project, deliverable D4.

## Task 3.1 Data collection

When developing different stunning alternatives, standardised assessment protocols are needed to determine their impact on e.g. animal welfare. The assessment protocol developed in WP2 is used to assess animal welfare aspects during the different phases for the 4 alternatives. In addition to animal welfare assessments, product quality and technical qualities are also investigated.

The assessment protocol starts from the moment pigs are unloaded at the slaughter plant: the pre-stunning period, assessing animal behaviour and possible stress. Pre-stunning stress will affect animal behaviour, which means that ease of handling at the slaughter plant may affect the stunning process and outcomes. The results from the pre-stunning period are used as possible explanatory information.

To assess improvements of the alternatives to the current high CO<sub>2</sub> practice, it is relevant to assess animal-based indicators during the stunning process itself. The stunning process starts on entry into the stunning system (gondola or raceway) and includes the exposure to the gas or electric stun. The effect of the stunning phase is assessed through indicators of unconsciousness as defined in the assessment protocol (deliverable D2).

The economic performance (including meat quality) is of importance for the acceptability or motivation to change to other stunning systems and is therefore part of the assessment protocol.

The goal of this deliverable is to describe the implementation of the selected alternatives under conditions similar to the commercial situation, and the collection of the pre-selected data. Provision of evidence of implementation of the plan (D2) through photos and videos for each alternative (WP3), and for further use in WP5. The collected data in WP3 will be analysed in WP4 and reported in deliverable D4.

## Task 3.2 Descriptions of the alternative systems at commercial level

In Task 3.2 the four alternatives are described as commercial stunning systems operating levels of 600-800 pigs per hour or in a way that the principal can be translated into commercial operating levels. The four alternatives are at different development or implementation level, and this means the detail of description differs per stunning alternative.

The Optimised CAS (controlled atmosphere stunning) process (previous called 'Multi-phase Box system') is developed up to a level at which the principal of the method is tested, at the experimental facility. A description of how the Optimised CAS process can look like on a commercial scale is presented in 3.2.

For the Inert Gas Retrofit System the initial plan was to use data that was to be collected in a commercial paternoster system by the separately funded “TIGER”-project<sup>1</sup>, and to collect additional data for the PigStun project on the handling phase. However, operational restraints at the slaughterhouse prevented these experiments to be conducted. Therefore, the Tiger project consortium has instead agreed to share the data for Argon stunning and high-concentration CO<sub>2</sub> stunning from the already conducted experiments in a commercial Dip-Lift system in a commercial slaughter plant in Germany.

For Helium, a small-scale stunner has been developed and build that can contain five pigs at the same time. The performance of this prototype system is tested at a small-scale commercial slaughter plant in Germany and results can be extrapolated to a larger set up.

The improved electrical stunning is the only alternative running in a full commercial slaughter plant at approximate 700 pigs per hour.

The data and outcomes of the different phases of WP3 will be analysed under WP4. Animal welfare consequences, and micro- or macro-economic aspects will be described in Deliverable 4. These results will be used to be discussed in a stakeholder meeting planned for November 2024.

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# 1. Introduction

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The PigStun project aims to support EU pig slaughterhouses using high carbon dioxide concentration for stunning pigs to convert to more animal welfare friendly systems, by developing technical specifications for promising alternatives. These could be CAS (controlled atmosphere stunning) systems applying other gas mixtures or improved electrical stunning systems.

Aim of Work Package 3 (WP3), the Implementation phase, is to collect benchmark values, obtained from commercial slaughter plants that use high concentration CO<sub>2</sub> stunning as well as to collect relevant technical and meat quality data from the alternative gas methods under standardised experimental conditions. Next, the promising alternative systems and their settings (as selected in WP2) were implemented under commercial conditions, or near commercial conditions (performance testing). This was done in commercial slaughter plants in Germany (Helium system and the inert gas retrofit system) and The Netherlands (improved electrical stunning system), and at an experimental site in Denmark (Optimised CAS System).

Data were collected following the assessment protocol as described in D2. Collected data are currently analysed in WP4. Performance testing under (semi) commercial conditions or settings is only possible if a commercial plant is able or willing to introduce the alternative in their processing or, when the alternative is developed up to commercial level.

The Optimised CAS process (previously in D2 named "Multi-phase box system") was developed up to a level at which the principal of the method can be tested, which has been done at the experimental facility in Denmark. A description of the Optimised CAS process is presented below.

The Inert Gas Retrofit System was tested in a commercial Dip-Lift System. As such, this system was successfully tested on a commercial system, albeit not a high throughput one. For other slaughterhouses with a Dip-Lift System to switch to this method, the Inert Gas Retrofit System has to be installed inside the pit of the stunner.

For Helium, a small-scale stunner has been developed and build that can contain five pigs at the same time. The performance of this prototype system is tested at a small-scale commercial slaughter plant and results can be extrapolated to a larger set up.

The improved electrical stunning is the only alternative which is already in commercial operation. It is running in a full commercial slaughter plant at approximate 700 pigs per hour.

The scientific, technical, and socio-economic data as pre-defined in WP1 and WP2 were collected. Data collection included the pre-stunning handling phase, the stunning itself and the post stunning part of the process and meat quality aspects. Furthermore, pictures and videos were taken for each alternative stunning method for further use in WP5 (Dissemination phase) and for better understanding of the practical setting of the alternatives. Collected data in WP3 are currently analysed in WP4 and will be reported in D4.

This deliverable D3 describes:

- 1) how the pre-selected data was collected, and
- 2) what the alternatives will look like when scaled up to the commercial situation.

## 2. Task 3.1 The collection of pre-selected data as described in WP2

### Introduction

When developing different stunning alternatives, standardised assessment protocols are needed to determine their impact on e.g. animal welfare. The assessment protocol developed in WP2 is used to assess animal welfare aspects during the different phases for the 4 alternatives (see Figure 1). In addition to animal welfare assessments, product quality and technical qualities are also investigated.

Pre-stunning stress will affect animal behaviour, which means that ease of handling at the slaughter plant may affect the stunning process and outcomes. Therefore, the assessment protocol starts at the pre-stunning period at the slaughter plant assessing animal behaviour and possible stress. The results from the pre-stunning period are used as possible explanatory information.

To assess improvements of the alternatives to the current high CO<sub>2</sub> practice, it is relevant to assess animal-based indicators during the stunning process itself. The stunning process starts on entry into the stunning system (gondola or raceway) and includes the exposure to the gas or electric stun. The effect of the stunning phase is assessed through indicators of unconsciousness as defined in the assessment protocol (D2).

For the acceptability or motivation to change to other stunning systems, the economic performance (including meat quality) is of importance and therefore part of the assessment protocol.

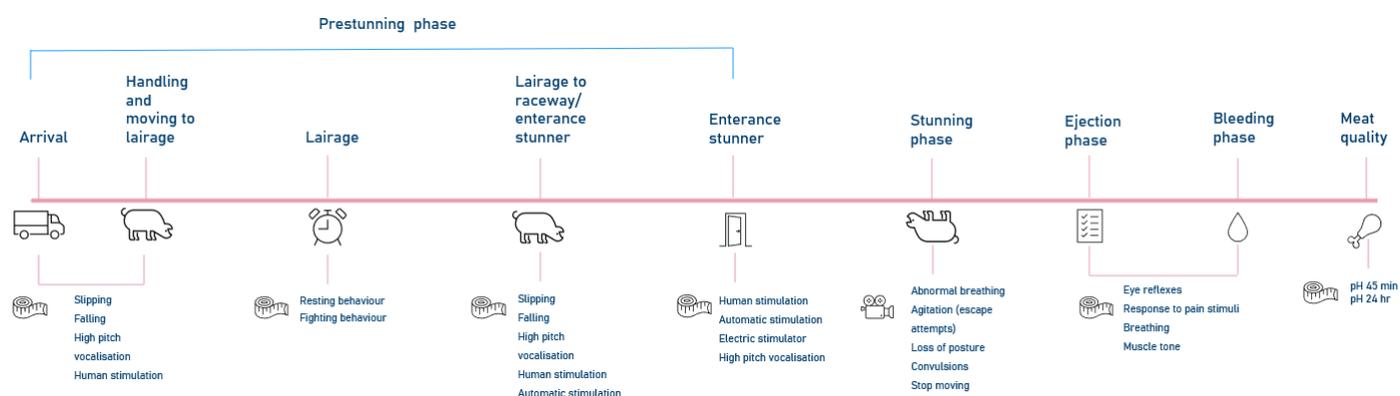


Figure 1. Animal welfare aspects measured during different phases in the slaughter process.

### Arrival & Lairage

The arrival & lairage phase is defined as the moment of entering the slaughter plant (i.e. unloading, moving to lairage and lairage period) until the end of lairage. In this period general behaviour of the animals during handling and lairage will give information on the state of animals which can influence the total process of stunning (see Table 1 for the animal welfare aspects measured).

### Pre-stunning phase

The pre-stunning phase is the phase in which the animals are brought from lairage to the raceways up to the point of stunning. The behaviour of the animals was monitored in the corridor from lairage to the raceway, in the raceway before entering the stunner (see Table 1 for the animal welfare aspects measured).

## Stunning

The period from entering the stunner (i.e. the gondola (CAS) or the conveyor (electrical)) until the moment of leaving the CAS stunner or the moment electrode contact is released. During this period animal-based indicators for aversiveness and for (un)consciousness were assessed, e.g. agitation and escape attempts, loss of posture, convulsions, and cessation of movements (see Table 1 for the animal welfare aspects measured).

## Stunning effectiveness

The effect of stunning was assessed in the period between leaving the stunner and the moment of sticking. During this period animal-based indicators for (un)consciousness were assessed, e.g. breathing, eye reflexes, muscle reactions and the absence of pain reflexes (see Table 1 for the animal welfare aspects measured).

## Bleeding

Bleeding is defined as the period from the moment of sticking until the end of bleed-out resulting in death. During the process, behavioural responses related to (possible) recovery was monitored (see Table 1 for the animal welfare aspects measured).

## Meat quality

Stunning methods might influence the meat quality. For all stunning methods pH at 45 minutes and at 24h post-mortem (PM) were measured as well as temperature development. Furthermore, carcasses were inspected for bloodspots and bleedings or bruises 24hPM and samples were taken for the drip loss analyses and for colour measurements. All meat quality measurements were executed following the SOP's from the EU funded project *mEATquality*, led by Wageningen Research (<https://meatquality.eu/>).

Table 1 shows the animal welfare aspects measured.

Table 1. measurements executed at the slaughter plant to collect data of each phase of the slaughter process.

| Phase in slaughter process | Measurements   |
|----------------------------|--|
| Arrival                    | Slipping or falling<br>Use of human stimulation (stimulation of pigs to move in a certain direction by making sounds or using objects)<br>Presence of high pitch vocalisation  |
| Lairage                    | Resting behaviour (standing, sitting, laying)<br>Fighting behaviour  |
| Pre-stunning phase         | Use of automatic stimulation<br>Use of electric stimulator<br>Group size<br>Presence of high pitch vocalisation<br>Use of human stimulation (stimulation of pigs to move in a certain direction by making sounds or using objects) |

|                 |  |
|-----------------|--|
|                 | Slipping or falling  |
| Stunning        | <p>Correct placement of the electrodes</p> <p>Abnormal breathing</p> <p>Agitation (Escape attempts)</p> <p>Loss of posture</p> <p>Convulsions</p> <p>Stop moving</p> <p>Heart activity</p> |
| Unconsciousness | <p>Eye Reflexes</p> <p>Response to pain</p> <p>Breathing</p> <p>Muscle tone</p>  |
| Bleeding        | <p>Eye Reflexes</p> <p>Response to pain</p> <p>Breathing</p> <p>Muscle tone</p>  |
| Meat quality    | <p>pH 45 min</p> <p>pH 24 hrs</p> <p>Temp 45 min</p> <p>Temp 24 hrs</p> <p>Colour (l a b values)</p> <p>Drip loss</p> <p>PSE</p> <p>Blood spots and brushes</p>                            |

## Data collection for Optimised CAS process and from the alternative gasses under standardised conditions

A pilot scale stunning system was built next to a commercial slaughter location in Denmark to develop and test the Optimised CAS process. In this system data was collected from an optimised CO<sub>2</sub> stunning process. Furthermore, under the standardized experimental conditions relevant technical and meat quality data was collected from the alternative gasses (i.e., argon and helium).

Arrival

All pigs arrived from the same vehicle on the morning of the test at 07.00 am for all gas alternatives. The pigs were observed during unloading by the observer standing at a fixed position next to the unloading ramp, without interfering with the pigs or the personnel, which is marked as a red dot in Figure 2.

On arrival, all animals were checked and cleared by one of the veterinarians at the processing plant before entering the lairage and being used for testing. If pigs did not pass the inspection, they were not included in the test. After unloading, the observer followed the pigs on the route to the lairage, where behavioural observations were scored and reported in the PigStun assessment protocol included in WP2. An employee from the meat processing plant drove the pigs from unloading to the lairage in the tent. From unloading until the pigs arrived at the lairage it took about 15 minutes.

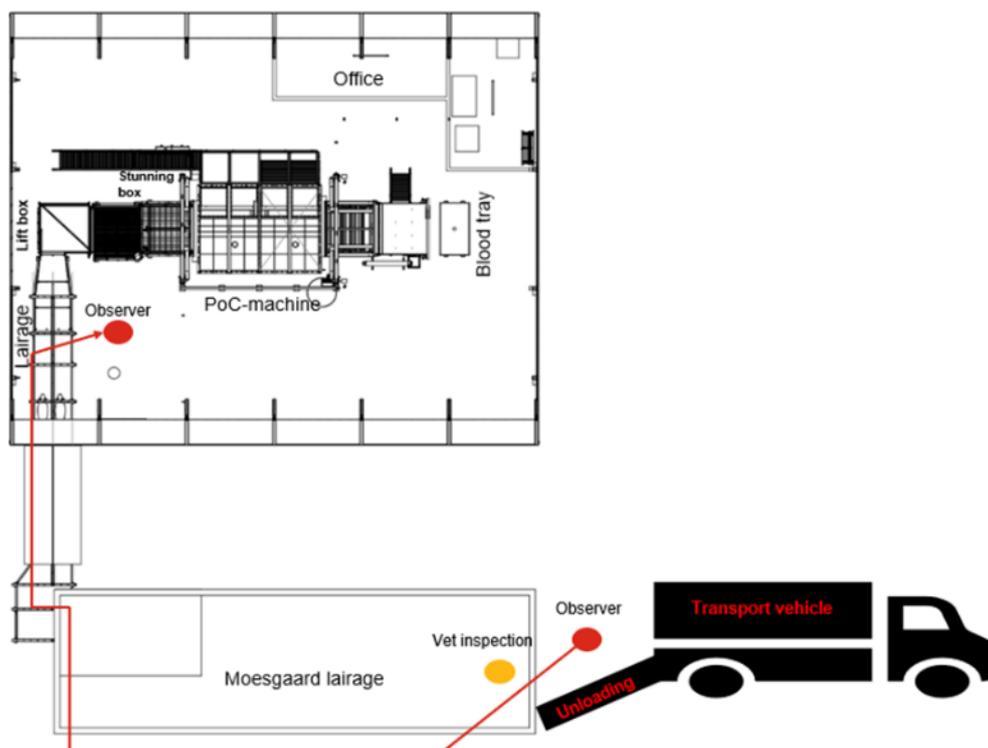


Figure 2. Example of where the observer (red dots) was located and observed the pigs from unloading and during lairage.

The observer counted the number of incidents from unloading and the route to lairage and recorded them on a score list. General information was collected from the slaughter plant and noted:

- o Number of animals on the truck.
- o Unloading time and transport duration.
- o Type of pig (breed, sex, etc.).
- o Weather conditions (pers. Observation).
- o Copy of transport document. Transport documents were available at arrival at the plant. A picture of the transport document was taken on arrival.
- o Control for vital signs/ Correct stunning.

#### Lairage

All pigs ( $n = 9$ ) arriving on the day of testing were kept in the same lairage pen inside the tent located at the processing plant. On arrival at the lairage, 5 of the 9 pigs were randomly picked and fitted with HR (heart rate) belts (Polar H10), which took up to 30 minutes.

After pigs were fitted with HR belts, they were left at least 1 hour in lairage (Figure 3). The observer registered the behaviour of the pigs every 10 minutes during the first hour in lairage according to D2. During lairage a GoPro camera recorded the pigs to be checked afterwards.



Figure 3. All 9 pigs were kept in lairage for 1 hour.

#### HR measurements

During the test, half of the pigs (4-5) per group of 9 were monitored with HR equipment from Polar H10 sensors to register heart activity. The HR was measured starting in lairage (1 hour) and through the whole stunning process.



Figure 4. Pigs provided with a Polar heartrate sensor (left without and right with protection from biting by other pigs).

#### Moving from lairage to stunner (box)

After 1 hour in lairage, a group of 3 pigs was randomly driven into the box lift and then into the stunning box. In each batch 1-2 pigs equipped with an HR belt were present. The stunning box and the top of the PoC machine were equipped with GoPro cameras to follow the pigs in the stunning box

until the end of the stunning phase. See Figure 5 for the location of cameras. The batch rapport was filled out before the box entered the PoC machine. The water bowl in the stunning box was filled with fresh water.

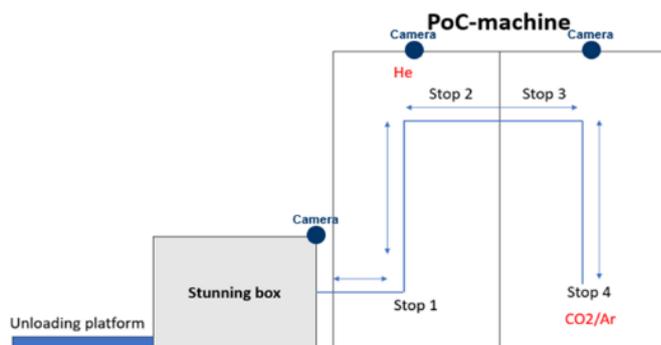


Figure 5. Illustration of the PoC-machine, and location of GoPro cameras.

An A4 sheet test specifications was placed on top of the stunning box, so that it was visible on camera:

- CO<sub>2</sub>, batch x
- Argon, batch x
- Helium, batch x

In the batch report, the following parameters were filled out for each run:

- Number of pigs in the batch
- Gender
- Breed
- Color ID
- Lairage time
- Visible injuries
- Approx. weight
- MNo (tattoo ID)

#### Pre-stunning phase

##### CO<sub>2</sub>

After the 3 pigs were loaded in the stunning box, the box was transported up to Stop 3, where the pigs were held in green light and music for 45 min. After 45 minutes, the box containing 3 pigs was transported downhill until reaching stop 4 where the pigs entered an atmosphere of 88% CO<sub>2</sub>. This part of the stunning phase was also carried out in green light and music. See Figure 6.

##### Argon

After the 3 pigs were loaded in the stunning box, the box was transported up to Stop 3, where the pigs were held in green light and music for 45 min. After 45 minutes, the box containing 3 pigs was transported downhill until reaching stop 4 where the pigs entered an Argon atmosphere ( $\leq 2\%$  residual O<sub>2</sub>). This part of the stunning phase was also carried out in green light and music. See Figure 6.

##### Helium

After the 3 pigs were loaded in the stunning box, the box had a waiting time of 45 minutes in Stop 1 of the PoC-machine in green light and music. After 45 min the box containing 3 pigs was transported upwards until reaching Stop 2 where the pigs entered Helium atmosphere ( $\leq 2\%$  residual  $O_2$ ). This part of the stunning phase was also carried out in green light and music. See Figure 6.



Figure 6. Three pigs in the stunning box for 45 min lairage in green light and music.

### Stunning

During exposure to the gas mixture, behavioural observations were recorded using GoPro video cameras. The videos are analysed by WP4 for signs of respiratory distress, abnormal breathing, and agitated behaviour before the moment of loss of posture (LOP). The moment of LOP was scored for all 3 pigs, from the first blue light (200 mm above the floor of the stunning box) until the pig lost standing posture. After LOP, the number of pigs showing convulsions (excitations) and the timing of cessation (stop) of movements were recorded. Observations were made of all pigs and included in the analysis in WP4. In the pigs equipped with HR belts, the HR was measured and HRV (heart rate variability) will be calculated and analysed in WP4.

\*To prevent possible recovery of consciousness the stunning time was extended for all alternatives compared to commercial settings as in the pilot set up the stun to stick interval is longer than in commercial settings. The stunning time for the different gas alternatives was as followed:  $CO_2$ : 6 min, Argon: 8 min and Helium: 8 min.

### After stunning

For Helium, the box went out backwards, as the door between stops 2 and 3 was blocked to maintain the gas concentration with He with  $\leq 2\%$  residual  $O_2$ . When the box came out of the PoC-machine, the three pigs were pulled out onto the unloading platform. The large blue arrows indicate how the box was transported out of the PoC-machine after the pigs were stunned with Helium (Figure 7).

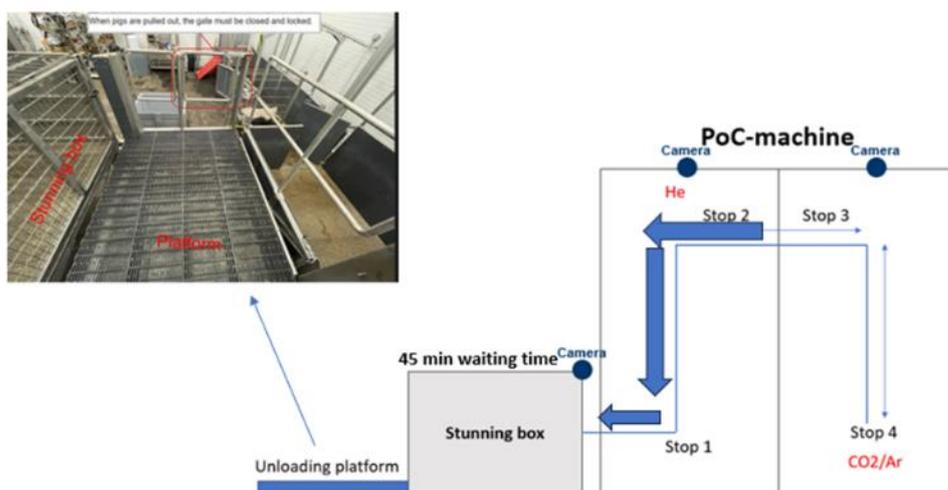


Figure 7. Process after stunning with Helium. Large blue arrows indicate how the stunning box was going out of the PoC-machine after end of stunning.

For Argon and CO<sub>2</sub>, the box went back to stop 3, and out of the PoC-machine as shown in Figure 8.

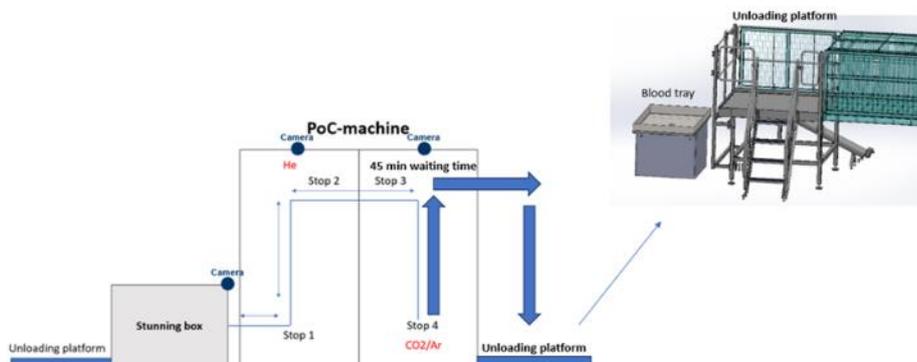


Figure 8. Process after stunning with CO<sub>2</sub> or Argon. Large blue arrows indicate how the stunning box is going out of the PoC-machine after end of stunning.

The HR bands were removed just before the pigs were stabbed for bleeding on the platform. If necessary, the belts were cleaned in water. The pigs were shackled and lifted with a forklift, with a blood container placed under the pigs. The pigs were stabbed while they were tattooed on each side of the ham with an ID number, and both ears were split to identify them on the slaughter line. Plastic bags were placed around the head of each pig to prevent dripping on the way to the slaughter line. From the end of the stunning process, until the pigs were in the slaughter line, it took about 15-20 min. After each test, the blood container and stunning box were cleaned with water.

#### Unconsciousness

The stunning effectiveness was assessed according to the assessment protocol in the period between leaving the stunner and before sticking. During this period, animal-based Indicators for (un)consciousness were assessed, e.g., breathing, eye reflexes (corneal and/or palpebral reflex), muscle reactions, and the absence of pain reflexes.

#### Stun to stick time

The stun to stick interval was timed from when the box exited the PoC machine until the last pig was stuck. The time for each batch was noted.

#### Tattoo of pigs

After leaving the stunning process, during bleeding, the pigs were tattooed with 3-4 digits on the shoulder and back ham (buttock) of the pigs on both halves of the carcass with the tattoo hammer (Figure 9). The first digits that was tattooed indicated the batch number, the second digit the pig number and the third number the type of gas (1= CO<sub>2</sub>, 2= Argon, 3= Helium).

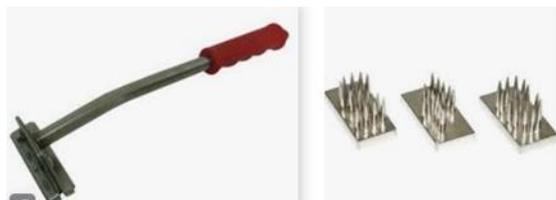


Figure 9. Tattoo hammer which was used to identify the pigs at the meat quality assessment

#### Technical settings (logging of data)

- Temp in the PoC-machine (logs every 5 sec).

- Gas concentrations in the PoC-machine (every sec).
- Batch report with number of animals, duration of stunning, and average gas concentrations for each run.

#### Collection of data

- Video recordings from 3 GoPro cameras per alternative, 1 at lairage, 1 from stunning box and 1 from top of PoC-machine.
- HR data.
- Behavioural observation according to WP2.
- Meat quality.

#### Set-up of the workflow for assessment of the animal behaviour

The moment the pigs arrived on the site, observation of the behaviour during unloading of the pigs to the lairage started according to deliverable D2. The position of the observer can be seen in Figure 10. Furthermore, a camera was installed in the lairage so that the behaviour of the pigs could also be checked afterwards. Subsequently in the stunning phase, the pigs' loss of posture, the amount of sound the pigs produced (vocalization), the excitations and muscle convulsions were recorded using video recordings from 2 angles. Once the pigs left the stunner before sticking and during bleeding pigs were tested for absence of muscle tone, absence of breathing and absence of Corneal reflex as described in the assessment protocol in D2.

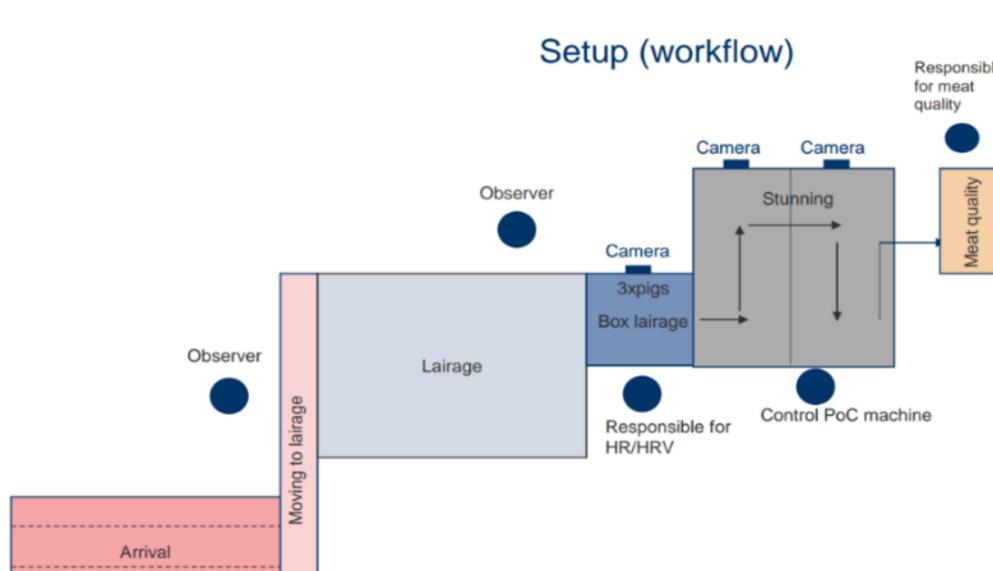


Figure 10. Workflow of the tests from arrival of the pigs in the lairage to leaving the PoC machine after the stunning process. Thereby indicated the position of the observers for analysing the animal behaviour and the position of the GoPro cameras.

#### Stunning effectiveness

##### Muscle tone

Stunned animals will show a general loss of muscle tone after the termination of tonic-clonic convulsions. Loss of muscle tone can be recognized from the completely relaxed legs, floppy ears and tail, and relaxed jaws with protruding tongues. Ineffectively stunned animals and those recovering consciousness will show a righting reflex and attempt to raise their head (EFSA AHAW Panel, 2013).

No righting reflex: no attempt to lift head or stand up.

##### Breathing

Occurrence of apnoea (absence of breathing) or unrhythmic and irregular breathing. Check for ribcage and see if it moves in and out. Absence of breathing movements indicates unconsciousness.

### Eye reflexes

The eye reflexes are examined immediately before stabbing with an ordinary pencil or another tool with a corresponding tip. On the open eye, the cornea is touched quite lightly, holding the pencil at an angle of 45 degrees. The touch has been repeated 3-4 times in quick succession. In case of consciousness, when touching the cornea, the eye will close. To check for the absence of the palpebral reflex the medial canthus of the eye are touched with a finger, cotton swab or pen; blinking should not occur.

### Meat quality measurements

Figure 11 shows the timeline of the sequence of the different meat quality measurements. The meat quality measurements were identical for all 3 gas alternatives and for the test under practical conditions in the slaughter plants.

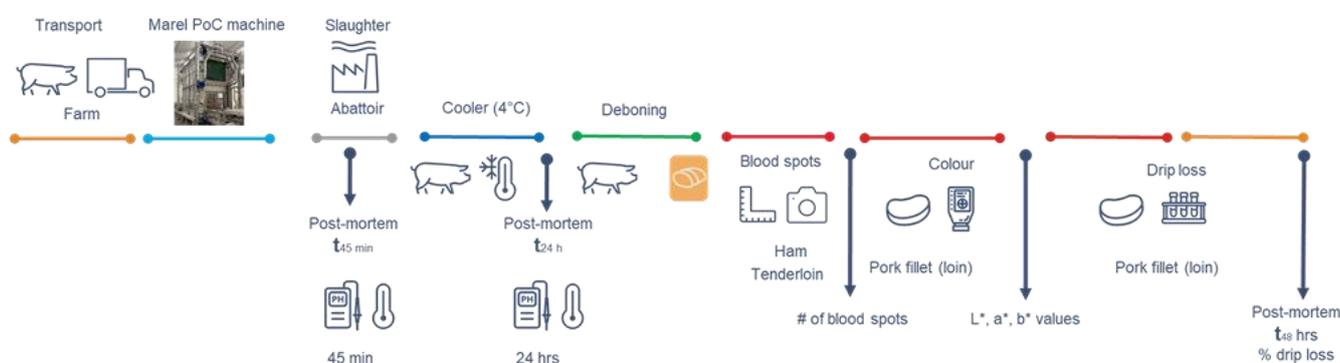


Figure 11. Timeline for the different meat quality measurements.

### pH value

Measuring the pH value is of importance as it directly impacts the quality, safety, and shelf life of the meat product. The pH of meat is linked to tenderness, water holding capacity, colour, juiciness, and flavour. The pH of the meat is measured 45 min and 24 hours after slaughter. The pH measured at 45 min after slaughter reflects how quickly the muscle glycogen is being converted into lactic acid. This phase can indicate the level of stress the animal experienced before and during slaughter. A rapid decline in pH (below 6.0) could suggest stress, leading to Pale Soft Exudative (PSE) meat, which can lead to undesirable traits like softness, discoloration, and spoilage. Next, the pH at this stage can help predict the ultimate quality of the meat, it can serve as an early quality indicator. If the pH drops too quickly it can also result in poor water holding capacity and texture making the meat less juicy and more susceptible for spoilage.

The pH at 24 hours post-mortem, often referred to as the 'ultimate pH' is considered as a key indicator of the meat quality. Ultimate pH influences colour, texture and tenderness, water holding capacity, shelf life and microbial stability and flavour of the meat. The ultimate pH should ideally be in the range of 5.3 to 5.8. Meat in this pH range has a bright, reddish-pink colour which is associated with freshness. A higher pH (>6.0) can lead to dark, firm, and dry (DFD) meat, which appears darker. The darker colour is often perceived as less fresh. The ultimate pH also has an impact on the texture of the meat. Pork meat within the optimal pH range tends to be tender and juicy, whereas pork meat with pH > 6.0 is perceived as tough and pork meat with very low pH is perceived as soft, watery, and less tender. The ultimate pH also has an impact on the ability of the meat to retain water (water holding capacity) which is important for juiciness and cooking performance. Meat with either low pH or high pH leads to final products with undesirable texture, excessive drip loss and reduced juiciness. A pH within the optimal range helps the inhibition of growth of spoilage bacteria. The pH also influences the flavour profile of meat. Properly balanced pH levels contribute to a desirable taste, while pH values either too high or too low can lead to off-flavours.

The pH value ranges from 0 to 14, but in fresh meat the range is narrow, mostly within 5.3 – 5.8. That is why the value up to the second decimal place should be recorded. Moreover, the pH is strongly affected by the temperature and therefore these parameters should be measured simultaneously. The temperature of the sample influences H<sup>+</sup> activity. In the tests a pH electrode with a built-in temperature sensor was used.

The pH and temperature of the pork samples were measured directly in the muscle at the carcass by electrode insertion at fixed post-mortem times. The measurement location was Left and right *longissimus thoracis et lumborum* (LTL) (loin) at the last rib (T<sub>13</sub> – L<sub>1</sub> level) See figures C and D. At 24h post-mortem the electrode was inserted 2cm away from the insertion at 45 minutes. The equipment for pH and temperature measurement is shown in Figure 12 and the detailed position of the pH meter in the *Longissimus thoracis et lumborum* is shown in Figures 13 and 14. The pH and temperature measurements were performed in duplicate for both left and right half of the carcass for both 45 min and 24 h.



Figure 12. Equipment for pH and temperature measurement.



Figure 13. pH measurement in *Longissimus thoracis et lumborum* at the last rib (T<sub>13</sub>-L<sub>1</sub> level)

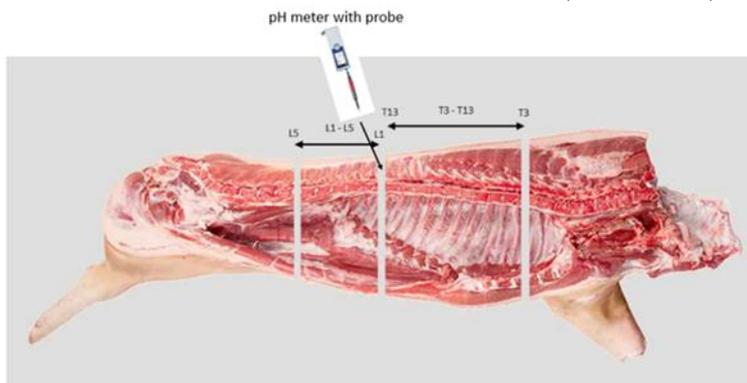


Figure 14. Half pig carcass showing the position T<sub>3</sub> – 13 and L<sub>1</sub> – L<sub>5</sub>. The pH meter indicates where the pH is measured at pH<sub>45</sub> and pH<sub>24</sub>.

### Deboning

To perform meat quality measurements for colour, drip loss and bloodspots, it is necessary to debone the carcasses after 24 hours of cooling to obtain the desired meat parts. Figure 15 shows the different meat cuts that were made, i.e., tenderloin (1), ham (2), shoulder (3), belly (4), and loin (5). The loin (5) was used for the colour and drip loss measurements and the ham (2), and tenderloin (1) were used to determine the number of bloodspots.

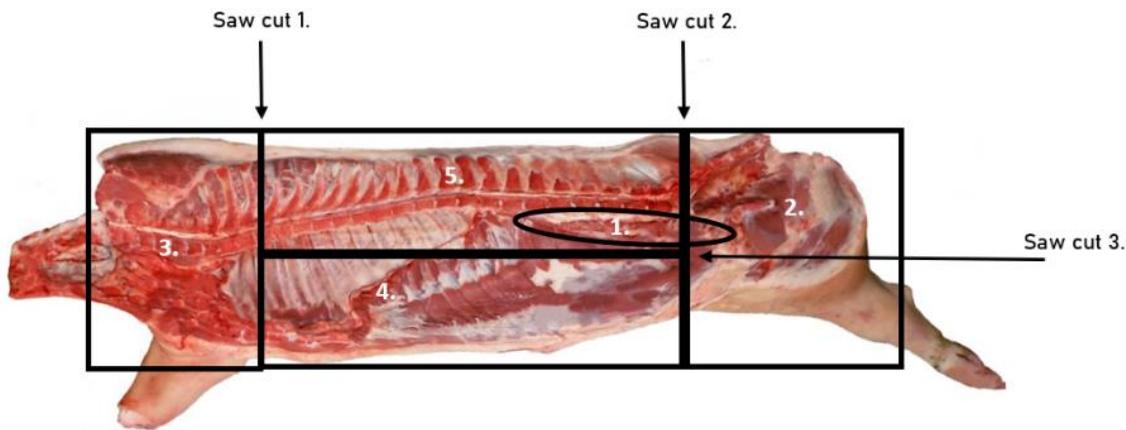


Figure 15. Half pig carcass with different meat cuts showing the position of 1. tenderloin, 2. ham, 3. shoulder, 4. belly and 5. loin. In addition, the different saw cut positions are indicated.

The carcasses were collected from the cooling (Figure 16a) and placed on a cutting table. The first step of the deboning was to remove the tenderloin with a knife (Figure 16b) and trim it for fat and glands, so that bloodspot evaluation could be done.



Figure 16. Carcasses in cooling (left picture 16a) and removing the tenderloin from the carcass (right picture 16b)

Then the carcass was cut with a saw at three positions. Saw cut 1 was made at the point just behind the front shank (20 mm), perpendicular to the longitudinal axis of the carcass and the shoulder part was obtained (Figure 17a). At the point where the tailbone and clavicle meet, perpendicular to the longitudinal axis of the carcass saw cut 2 was made, and the ham was obtained (Figure 17b). The ham was deboned and trimmed for blood spot evaluation. The belly was separated from the loin part by saw cut 3 over the total length of the carcass (Figure 17c). Then the ribs were cut from the loin (Figure 18), so that samples could be prepared for colour and drip loss measurements.



Figure 17. cutting the shoulder (left, picture 17a), the ham (middle, picture 17b) and belly from the loin (right, picture 17c)



Figure 18. Cutting samples for colour and drip loss

### Colour of the meat

Measuring the colour of the meat 24 h post-mortem provides valuable insight into the quality, freshness, and appeal of the meat. The colour is considered a key factor in consumer perception and is often used as an indicator of the meat's overall quality. The colour of the meat can indicate whether the meat is prone to conditions like Pale, Soft and Exudative (PSE) resulting in lighter colour or Dark, Firm, Dry (DFD) resulting in darker colour. Consumers often judge meat quality based on the colour. A bright, reddish pink colour is associated with freshness and high quality, while a deviating colour can hold buyers back. The colour of the meat changes as it ages and undergoes oxidation. Deterioration in colour can indicate the onset of spoilage or oxidation affecting the shelf life. Next, colour of the meat can provide an indication for stability of the process, deviating meat colour could reveal issues during processing, such as improper chilling, handling, or stress on the animal before slaughter. And lastly, colour measurement can be used in combination with pH measurements to validate the quality of the meat. For instance, meat that is too pale might correspond with deviating pH levels, providing a more comprehensive understanding of the meat's condition.

To assess the colour of the meat, the loin was cut from the carcass and two fresh meat steaks were obtained and the L\* (lightness)-, a\* (red/green) and b\* (yellow/blue) values were determined by Hunterlab Miniscan colour meter (Figure 19).



Figure 19. Colour measurement of a steak with Hunterlab Miniscan

The L\*, a\* and b\* values represent different colour aspects :

- L\* value: ranges from 0 (black) to 100 (white), indicating the lightness or darkness of the meat.
- a\* values: positive values indicate red, while negative values indicate green. For meat, higher positive a- value indicates more redness.
- b\* values: positive values indicate yellow, while negative values indicate blue.

L, a, b values are subject to change due to factors such as the breed of the pig, diet, and post-mortem handling.

#### Procedure for the colour measurements

Two steaks, each with a thickness of 2.0 cm were obtained by cutting at the level of rib T<sub>7</sub> – T<sub>9</sub> transverse to the grain (across the fibre direction) (Figure 20). The steaks were placed in a plastic container with a lid and were allowed to bloom for 30 min at 4°-6°C. After blooming for 30 min, the steaks were removed from the container and were placed on a flat surface. The colour of each steak was measured with the Hunterlab Miniscan (Figure P) which was calibrated against a black and white plate using an 8 mm aperture, illuminate D65 (6500 K color temperature) at a standard observation of 10° at five different positions on the exposed surface of the steak. The L\*, a\* and b\* values were recorded on a sheet (Figure N).

The average L\*, a\* and b\* values were calculated from the five readings per steak to obtain the mean and standard deviation for each sample.

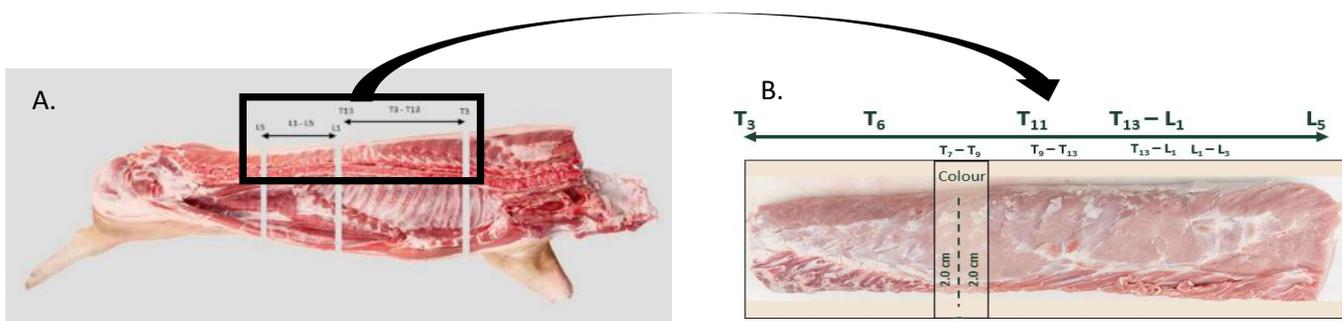


Figure 20. showing in A) half pig carcass showing the positions T<sub>3</sub> – T<sub>13</sub> and L<sub>1</sub> – L<sub>5</sub> and B) the corresponding loin showing position T<sub>7</sub> – T<sub>9</sub> at which the two steaks are cut from the loin for colour measurement

Step-by-step overview of the colour measurement protocol is presented in Figure 21.

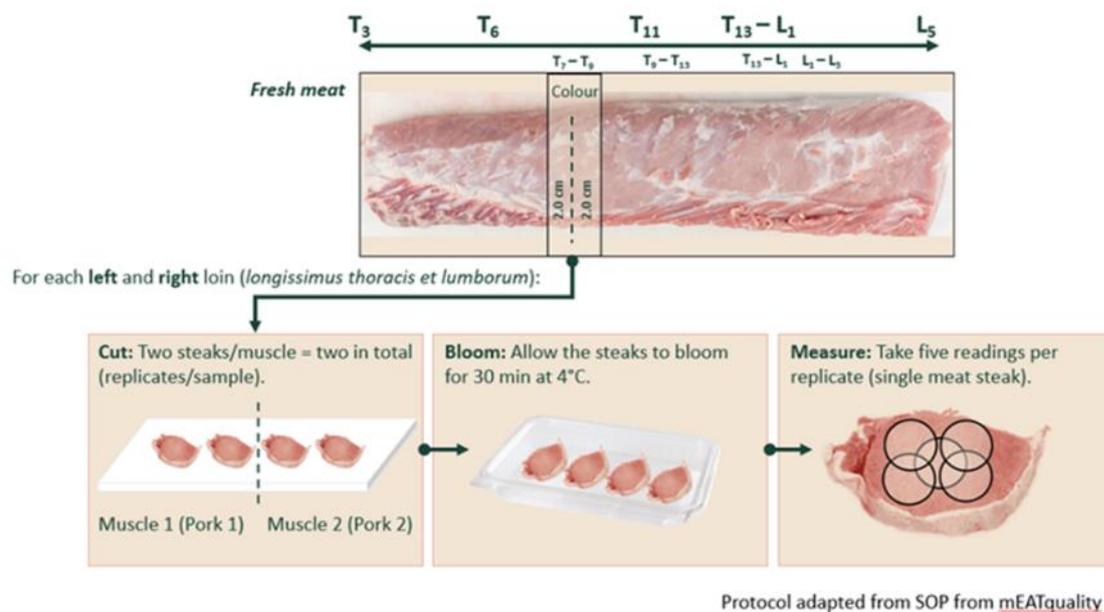


Figure 21. overview steps in the protocol for colour measurements

### Drip loss measurement

Measuring the drip loss of pork meat 24 hours post-mortem is important for several reasons related to the quality, shelf life, and consumer acceptance of the meat. The drip loss is an indicator of water holding capacity (WHC) of meat. It refers to the ability of the meat to retain the moisture. High drip loss indicates poor WHC, which means that the meat will lose more water during storage and cooking. High drip loss is often associated with Pale, Soft and Exudative (PSE) meat. Meat with high drip loss is more prone to spoilage as the exuded moisture provides an ideal environment for microbial growth. Consumers expect pork meat to be juicy and tender, however meat with high drip loss tends to be less juicy with a drier texture. The post-mortem pH of pork meat is closely related to the drip loss with the rate and extent of pH decline determining how much moisture the meat will retain or lose. If the pH of the pork drops too quickly and reaches a pH of 5.4 – 5.6 while the muscle temperature is still high ( $\sim 35^\circ\text{C} \pm 45$  min post-mortem), it leads to denaturation of proteins. This denaturation reduces the muscle fibres' ability to retain water, leading to higher drip loss. Whereas when the pH declines to an optimal range (5.3 to 5.8) at a moderate rate (after 24 hours post-mortem), the proteins in the meat have adequate water retention without excessive exudation. Meat within this pH range is considered high quality, with good water holding capacity, juiciness, and texture.

## Procedure for the drip loss measurement

One steak with a thickness of 2.0 cm was obtained by cutting at the level of rib T<sub>13</sub> – L<sub>1</sub>, by cutting transverse to the grain (across the fibre direction). The samples for drip loss were taken by cutting the meat steak with a 25 mm circular knife. The next step was that the empty container including cap was weighed. Then, per meat steak two samples were taken with the circular knife and the meat cuts were placed in the drip loss container. The cap of the container was closed and the container with the meat was weighed and recorded in the sheet. The containers with meat were then stored for 24 h at 4°C and were then ready to be weighed. After 24 h, the cap was opened, and the piece of meat was taken out of the container with a tweezer. The container with the juice was weighed and the weight was noted. A complete overview of the protocol is given in Figure 22.

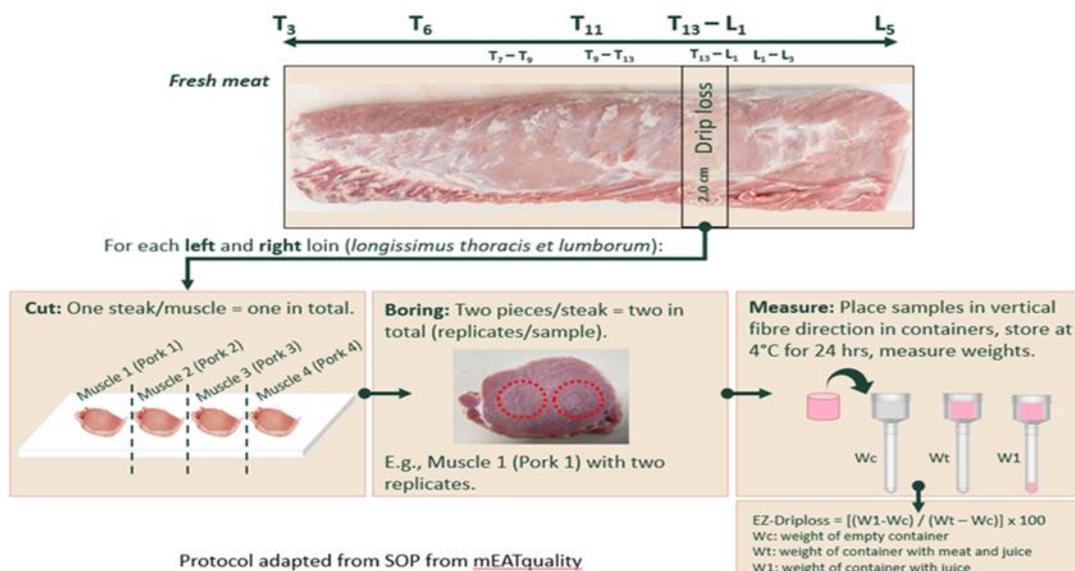


Figure 22. overview of drip loss protocol.

## Weighing after 24 h

The containers were weighed on a scale with 3 decimals and the weight were recorded with 3 decimals in the spreadsheet. The drip loss was calculated according to Equation 1:

$$\% \text{ Driploss} = \left[ \frac{W_1 - W_c}{W_t - W_c} \right] \times 100 \quad (\text{Eq.1})$$

$W_c$  is the weight of the empty drip loss container.

$W_t$  is the weight of the drip loss container with meat and juice.

$W_1$  the weight of the drip loss container with juice.

## Blood spot determination

Blood spot determinations are important for assessing meat quality because they can indicate issues related to animal welfare and processing conditions such as inadequate stunning, improper handling or issues with the bleeding process. The presence of blood spots can also indicate whether the animal experienced significant stress before slaughter. High stress levels can cause capillaries in the muscle tissue to rupture, leading to the appearance of blood spots. Blood spots may also result from inadequate bleeding out during slaughter. Incomplete bleeding can leave residual blood in the meat leading to the formation of spots. Blood spots on the meat have an influence on the visual and textural quality of meat. Blood spots on the meat make it less appealing to consumers. Meat with blood spots is often downgraded or rejected by consumers and retailers due to its unappealing appearance. This can reduce the market value of the meat and lead to economic losses for producers.

Assessment of bleedings was performed as a subjective assessment according to a three-point scale for both the tenderloin and ham. The protocol is based on the paper by Dich-Jørgensen *et al.* (2017).

The ham was scored based on the following 3 categories:

- Multiple point bleedings.
- 5 – 30 spots on the muscle.
- >30 spots on the muscle.

Example of bleedings on the ham is shown in Figure 23.



Figure 23. Ham topside with pinpoint bleedings in the caudal part of the muscle (ruler in cm).

In addition, the dimensions (length x width) of a maximum of five bleedings were measured. Example of a blood spot measurement is shown in Figure 24.

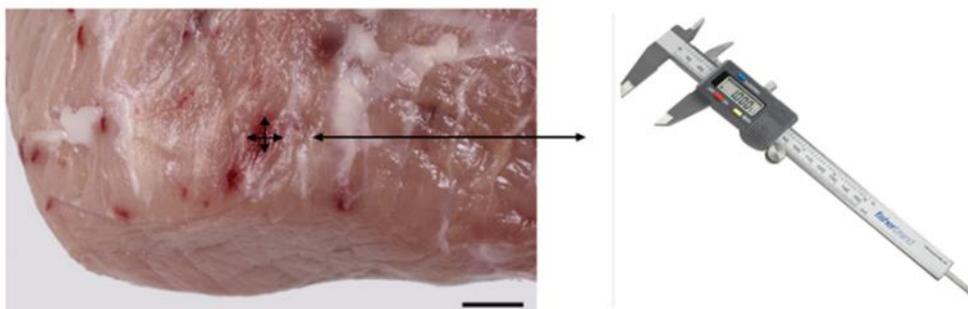


Figure 24. Measuring the dimensions of the blood spot with a caliper.

The percentage of bloodspots was calculated per category according to equation 2:

$$\% \text{ Blood spots} = \left[ \left( \frac{\text{Number of bleedings}}{\text{Total number of hams}} \right) \right] \times 100 \quad (\text{Eq.2})$$

The tenderloin was scored based on the following 3 categories:

- Single circular
- At the tip of the muscle
- At the head of the muscle

Example of a bleeding on the tenderloin is shown in Figure 25. In addition, the dimensions (length x width) of a maximum of five bleedings were measured. Example of a blood spot measurement is shown in Figure 26.



Figure 25. Blood spot (arrow) in the tenderloin tip (A). Bloodspot in the tenderloin head (B). Ruler in cm (Dich-Jørgensen et al., 2017).



Figure 26. Measuring the dimensions of the blood spot with a caliper.

The percentage of bloodspots was calculated per category according to equation 3:

$$\% \text{ Blood spots} = \left[ \left( \frac{\text{Number of bleedings}}{\text{Total number of tenderloins}} \right) \right] \times 100 \quad (\text{Eq.3})$$

In addition to recording and measuring the bleedings, photos were taken of all hams and tenderloins in a photo box with Canon PowerShot SX280 HS (Figure 27). In this way the photos were taken with fixed camera settings and conditioned light. The photos are stored in a database and can still be consulted if necessary.



Figure 27. Photo box for making pictures of ham and tenderloin samples.

## Data collection for Helium Stunning System

Various stunning methods for pigs are well known. CO<sub>2</sub> or argon, already exist or at least are confirmed to be promising. Another gas which is allowed through the European Regulation (EG 1099/2009), as it is an inert gas, is helium. The idea of using helium to stun pigs is not new, but there is only one approach described that tested the effect of helium while stunning pigs in an experimental setting (Machtolf et al., 2013). Apart from this experimental setting there is no commercial stunner installed and approved.

As part of the PigStun project, the first helium stunner was installed and approved, located at the local slaughterhouse in Kulmbach, Germany. Data for the Helium Stunning System was collected at this location. Helium gas was tested as well during the pilot testing in Denmark.

Data has been collected from October 2023 till the time of writing this report (August 2024); i.e. a total of 15 test days from which 5 test days were selected for the purpose of observation with Boris software (data collection). The number of stunned pigs is 1149 with 2.0% of re-stunning rate. These collected numbers covering both phases: pilot and performance tests, while it can explain the high numbers of re-stunned pigs. If comparing just performance testing, re-stunning rate is below 1.0%.

### Arrival & Lairage

The pigs arrived in the morning. They were delivered by farmers from a radius of approx. 50 km around Kulmbach. The unloading was done by the farmers (Figure 28). The pigs were moved into lairage by an employee of the slaughterhouse and were housed in groups of 5-10 pigs for about 2 hours. Due to the short waiting time, pigs were not fed but water was provided *ad libitum*.

The observation where done based on the PigStun assessment protocol (as described in D2). We observed the unloading (6 trucks), the moving to lairage (Figure 29) and the pigs at the resting in the waiting pens (Figure 30) (observation of 4 individual pens – 22 pigs in total).



Figure 28. Arrival and unloading.



Figure 29. Observation during moving to lairage.



Figure 30. Observing pigs in pens during resting.

### Pre-stunning phase

From lairage the pigs were re-grouped prior to entering raceway to stunner. The manual re-grouping is basically to have 2-5 pigs in a stunning group, because up to 5 pigs are allowed per gondola. The re-grouped pigs enter the raceway (Figure 31 and Figure 32) in which they are separated by a vertical pneumatic door. After separation the robot-controlled push door moves the group into the stunner (see Figure 33 and Figure 34). The group is separated on the raceway and is waiting till the previous group is stunned. It is possible to have two separated groups on the raceway at the same time.



Figure 31. Raceway, view to stunner.



Figure 32. Group of pigs waiting in the raceway, separated by robot-controlled push door.

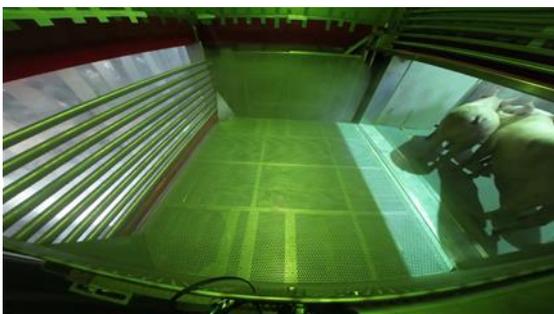


Figure 33. Group of pigs being moved/pushed to the gondola.



Figure 34. Group of pigs inside the gondola; gondola start to ascend in the Helium chamber.

During this process high pitch vocalisation and the automatic stimulation by the push-door was recorded groupwise according to Pigstun assessment protocol.

### Stunning

When the target concentration for the residual oxygen concentration of less than 1% was met, the gondola was lifted up to the top of the tower which took about 40 seconds. When the gondola reached the top of the chamber (which means that pigs are fully exposed to the Helium atmosphere), the 200 seconds exposure time to the Helium stunning starts.

Video recordings were performed during the whole process for live observation on the control screen and for further analysis of the pig's behaviour during stunning afterwards, see Figure 35. Animal behaviour during stunning was analysed using the Boris software following the PigStun ethogram from D2.



Figure 35. Behaviour observation during stunning via portable camera.

### Unconsciousness

Stunning effectiveness or (un)consciousness was tested by response to pain stimuli, eye reflexes- (palpebral and/or corneal reflexes), presence or absence of breathing and muscle tone, see Figures 36 and 37.

Each pig was tested by a veterinarian on the bleeding table just before sticking. In case of a positive response or reflex, the pig was re-stunned by captive bolt before sticking was performed. The stun-stick interval was recorded on group level. Timing of the stun-stick interval started when the first pig was falling on the belt and ended when the last pig of the group was stuck.



Figure 36. Pain stimuli check by nose prick.



Figure 37. Eye reflexes check.

### Bleeding

Bleeding was performed while pigs are on the belt prior to shackling on the slaughter line, see Figure 38. There were two workers to perform the sticking, one on either side of the belt because the pigs falling out of the gondola caused the pigs' heads to be in a random orientation. A third worker was there to check the reflexes again and to shackle the pigs (Figure 39).



Figure 38. Sticking on the belt.



Figure 39. Sticking, reflexes check.

## Meat quality

Meat quality measurements were performed on the left half of 50 carcasses for the following parameters:

- pH 45min
- pH 24h
- Temperature
- Conductivity
- Colour (CIE  $l^*a^*b^*$ )
- Drip Loss
- Blood spots

Colour, pH and temperature measurements were measured in a compatible way to the SOP's described in EU *mEATquality* Horizon 2020 project. The protocol used for blood spots was shared with the PigStun project to allow using it for the pilot testing in Denmark, and the Improved Electrical Stunning System,

The method for drip-loss differs to that of the *mEATquality* project. We used the same meat cut for colour measurement and drip-loss.

Before all the above measurements (except pH 45min), the carcasses were deboned into individual muscle parts, see Figure 40 and Figure 41.



Figure 40. Pork halves prepared for deboning.



Figure 41. Example of deboned muscle parts.

Example of blood spots, colour and drip loss sample preparation, see Figures 42 and 43.

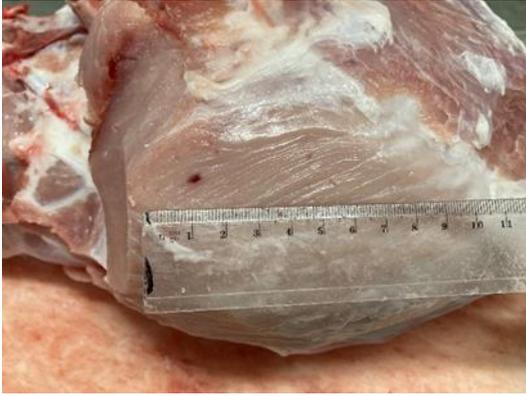


Figure 42. Blood spots recordings for further evaluation.



Figure 43. Sample preparation for colour and drip loss measurement.

## Data collection for Inert Gas Retrofit System

Stunning pigs with high concentrations of CO<sub>2</sub> can lead to aversive reactions, respiratory distress and fear (EFSA, 2020). Due to concerns regarding gas stability, costs, meat quality and stunning effectiveness, alternatives like inert gases and mixtures of inert gases with low concentrations of CO<sub>2</sub> have not been considered market-ready until now. Recently a new gassing system was developed – it allows to achieve stable gas atmospheres of inert gases with residual oxygen levels well below 1 % as an alternative to high concentration CO<sub>2</sub> stunning.

The initial plan was to use data planned to be collected in a commercial paternoster system by the separately funded TIGER-project<sup>2</sup>, and to collect additional data for the PigStun project on the handling phase. However, operational restraints at the slaughterhouse prevented these experiments to be conducted in the context of the TIGER project, including the simultaneous collection of additional data on handling for the PigStun project. Therefore, the Tiger project consortium has agreed to instead share the data for Argon stunning and high-concentration CO<sub>2</sub> stunning from the already concluded experiments in a commercial Dip-Lift system.

The testing of the gassing system was carried out in a commercial slaughterhouse with a Dip-Lift stunner in Germany and was split into two phases:

1. Comparison of gas mixtures: 8 gas mixtures, split into two separate series for Argon and Nitrogen based mixtures, respectively tested along with CO<sub>2</sub> to identify two gas mixtures to proceed to phase 2
2. Estimation of necessary exposure times for Argon and a Nitrogen-Argon mixture and CO<sub>2</sub> as a reference.

Data for parameters that were collected in line or similar to the later defined assessment protocol are shared with the PigStun project from the experiment's *second phase* for Argon and CO<sub>2</sub> conditions. These data were collected between January and March 2023 on 14 days in seven weeks, with measurements on Monday and Tuesday nights (approx. 6pm to 11pm). The inert gas mixtures and CO<sub>2</sub> as a reference were investigated on each measurement day such as to minimize slaughter day effects. The order of the gas mixtures that were measured within each day was shuffled within the constraints of the slaughterhouse. Video recordings of the stunning procedure were used to analyse the aversiveness of the different gas mixtures. Therefore, aversive, and other events were scored by two observers according to an ethogram. Stunning efficiency of the pigs was assessed with animal-based indicators. In total 900 animals were used during the 14 days in the second phase of the testing. The animals in this phase originated

<sup>2</sup> The project is supported (was supported) by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme. With additional financial support from Verband der Fleischwirtschaft e.V., QS Science Funds and Fördergesellschaft für Fleischforschung e.V.

from 8 different suppliers. Within each day, groups of animals from different farms were driven to the stunner in an order aimed to maximize an even distribution of animals from different farms for the different experimental conditions.

### Arrival & Lairage

The aim of the study was to test the new gassing system as close to commercial conditions as possible. As such, procedures were mostly in line with the typical conditions at that slaughterhouse: The animals arrived in the morning, moved into lairage and were housed in groups of 6 to 8 pigs in pens separate from other batches of slaughter pigs with at least 1.2 m<sup>2</sup> space / pig. The pigs were fed when necessary and water was provided *ad libitum*. For this experiment the pigs were slaughtered in the evening before the regular slaughter of the remaining animals.

#### *Deviations from assessment protocol:*

A systematic assessment of the handling of pigs in this phase was not part of the experiment and thus cannot be provided. However, the randomization of conditions was meant to ensure that animals in the Argon and the CO<sub>2</sub> control condition were handled similarly.

### Pre-stunning phase

Pigs were moved by the animal handlers in groups of 2 animals to the stunner. Just before entering the gondola, the animals were individually marked with spray (Figure 44).



Figure 44. marking pigs in lairage.

#### *Deviations from assessment protocol:*

A systematic assessment of the handling of pigs in this phase was not part of the experiment and thus cannot be provided. However, the randomization of conditions was meant to ensure that animals in the Argon and the CO<sub>2</sub> control condition were handled similarly.

### Stunning

Pigs were stunned in groups of two animals. The aim of the second part of the study was the optimization of the exposure time. To do so, a variable exposure time was used and optimized in a way that is similar to the work of Hoenderken (1978). However, to gain a rate of 99.5 % of successful stuns as opposed to the 90% criteria of Hoenderken (1978) and to limit the number of animals, an extra layer of adaption was needed. A high exposure time was used in the beginning to ensure successful stunning. After each successfully stunned gondola (N = 2) the exposure time was reduced by 10 seconds. Upon an insufficient stun, the exposure time was increased by 10 s and the animals needed for

the next reduction was doubled ( $N = N*2$ ), until a maximum of  $N = 200$  animals was reached. The exposure time necessary to reach a target rate of 99.5% successful stuns was then estimated by a binominal model.

Table 2. Gas and gas mixtures used during the second phase of the experiment. The series with 90 % CO<sub>2</sub> was used as a reference.

| Concentration             | CO <sub>2</sub> [%] | Inert gas [%] |                       | Remaining O <sub>2</sub> [%]    |  | # Animals |
|---------------------------|---------------------|---------------|-----------------------|---------------------------------|--|-----------|
|                           |                     | Ar            | (Rem.) N <sub>2</sub> | target at 50cm below top of bit | Approx. at highest reachable point at bottom |           |
| Argon                     | 0                   | 95            | 4                     | 1                               | 0.5  | 300       |
| CO <sub>2</sub> Reference | 90                  | 0             | 8                     | 2                               | 0  | 300       |
| Nitrogen                  | 0                   | >40           | <59                   | 1                               | 0.5  | 300       |

The start time of descend of the gondola as well as the exposure duration, number of animals and the name of their farm of origin were recorded in an experimental database. A unique ID was created for each gondola and animal. Within the gondola two cameras were installed for video recording of the stunning process (Figure 45). The videos from both cameras were time synchronised to allow viewing the animals from two angled simultaneously. For each measurement day, videos of the stunning sequences were re-cut in a randomized order into a new video to reduce potential biases from observers (e.g. shifting criteria) and to ensure that the observers were not aware of which gas atmosphere was used in a given sequence.

The occurrence of aversiveness and other behaviours was scored using an ethogram. The number of animals showing these behaviours as well as the latencies and durations of these behaviours were analysed.

*Deviations from assessment protocol:*

The ethogram used for the Tiger project is more detailed than the one agreed on for the PigStun project but can be made compatible by combining selected scored behaviours for the new PigStun behaviour categories.

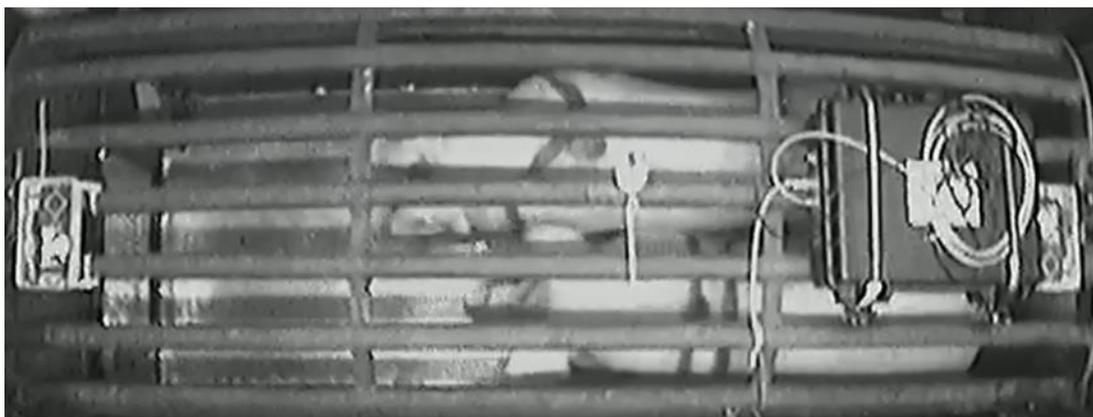


Figure 45. Camera position on the gondola during stunning.

**Unconsciousness**

Unconsciousness of pigs was checked immediately following ejection of the animals from the stunner (Figure 46) as well as continuously during bleeding for at least three minutes after ejection. It was established by checking presence of, if any, eye reflexes, response to pain stimuli, breathing and muscle tone.

In case of clear signs of a likely recovery, animals were subjected to an additional electrical and/or captive bolt stunning.



Figure 46. Checking for signs of consciousness.

### Bleeding

Once it was determined that the pigs were unconscious, they were subjected to sticking (Figure 47) and the time of sticking was recorded in the database, to allow calculation of the stun to stick interval. Signs that were closely and continuously checked during bleeding for at least 3 minutes post ejection from the stunner included breathing, response to pain and positive reflexes.



Figure 47. sticking and checking for signs of consciousness.

### *Deviations from assessment protocol:*

The animals were continuously monitored after bleeding, with one scientist following for each pig for at least 3 minutes after ejection to avoid missing signs of recovery, and not observing at fixed positions, as defined in the assessment protocol.

### Meat quality

We investigated whether Argon influences the quality of meat and examined it on the basis of pH, temperature, drip loss, colour and blood spots. Muscle pH (pH45 and pH24) and temperature (T45 and T24) in *M. longissimus thoracis et lumborum* (LTL) (loin) and *M. Semimembranosus* (ham), respectively were measured 45 mins and 24 hrs post-mortem respectively using a pH meter equipped with an electrode housed in a body with a stainless-steel blade, and a built-in temperature compensation (Figure 48).



Figure 48. Measuring pH.

Blood spots in the *M. semimembranosus* and *M. gracilis* (ham) muscles were assessed and scored on a 4-point scale (0: no blood spots, 1: <5 blood spots, 2: <30 blood spots, 4: > 30 blood spots) in accordance with the established protocol.

Drip loss was evaluated in slices of *M. Semimembranosus* by weighing the meat before and after storage in a modified atmosphere packaging (MAP; 80% O<sub>2</sub>:20% CO<sub>2</sub>) at 4°C for 72 hrs at 300–450 lux (14h/day). For packaging, meat slices were placed in 227 x 178 x 40 mm polypropylene plastic trays on moisture-absorbing pads, and a biaxially oriented polyethylene terephthalate/polypropylene film (< 3 cm<sup>3</sup>/m<sup>2</sup> 24 h bar O<sub>2</sub> permeability; < 8 cm<sup>3</sup>/m<sup>2</sup> 24 h bar CO<sub>2</sub> permeability) was used for sealing. Seventy-two hours post-storage, drip loss was evaluated by weighing the samples a second time.

Colour was measured on the ham approximately 40 hrs post-mortem by using a spectrophotometer (Minolta Chroma Meter CR-400). The aperture of the device was Ø8mm/Ø11mm (measuring area / illuminated area) with a standard illumination of D65 and 10-degree standard observer. All measurements were performed on fresh meat and fine dissection to 2.5 cm thick slices was carried out in the laboratory. The blooming time before the measurement was 30 min. Finally, respective values for L\*, a\* and b\* were recorded and further subjected to the analysis. For each value an average of six measurements per slice were considered.

#### *Deviations from assessment protocol:*

All pH measurements were measured in a way compatible to the SOP's described in EU *mEATquality* Horizon 2020 project. Colour was measured in the ham and with 6 instead of 5 measurement samples. The method for drip-loss also differs to that of the *mEATquality* project. The protocol used for blood spots was shared with the PigStun project to allow using it for the other systems.

In addition, several other tests, e.g. on blood and meat samples, including gene expression, shear force measurement, and sensory testing by an expert panel, were conducted. However, these are not part of the assessment protocol and are thus not included.

## Data collection for Improved Electric stunning process

There is sufficient scientific evidence that electrical stunning can be a safe and effective way of rendering pigs unconscious until sticking and death (e.g. EFSA 2004, 2020). Nevertheless, EFSA (2020) identified five hazards for pig welfare relating to the application of electrical stunning: handling and restraining the pigs, wrong placement of the electrodes, poor electrical contact, too short exposure time and inappropriate electrical parameters. All these hazards are related to or will increase by high throughput rates. To improve these factors, a commercial electrical slaughter

plant improved the lay-out of their slaughterhouse and the whole electrical stunning process. Specific to improve animal welfare throughout the whole electrical stunning process, two main solutions were implemented. First is to reduce the maximum number of animals per stunner per hour and second was a re-designed raceway and entrance of the stunners (More improvements are listed in deliverable D2). Because of all the improvements that were implemented, the improved electrical stunning system at this commercial plant was selected as a promising alternative and tested in this project.

To study possible pig welfare hazards during the electrical stunning process, the handling and restraining of the pigs during arrival and lairage, the pre-stunning phase and during stunning were observed according to deliverable D2. During stunning in one of the four stunners, placement of the electrodes was observed, as well as the exposure time and the electrical parameters. Right after stunning and during bleeding, the unconsciousness of the stunned pigs was observed. Meat quality of the carcasses of 60 pigs was measured at 45 minutes and 24 hours after stunning.

### Arrival & Lairage

Arrival of the pigs took place approximately 6 hours before stunning. During unloading of the pigs, they were moved out of the transport truck by the truck driver, while keeping the compartment groups intact. This resulted in the moving of pigs in groups of 6-8 individuals. Once unloaded, they were moved into lairage, leaving the compartment groups intact (due to European law and Dutch enforcement). During moving of the pigs, handling of the staff and stimulation was observed (Figures 49, 50 and 51). In each pen, a drinking nipple and pen enrichment was present. During lairage the pigs were observed repeatedly every 10 minutes during a fixed period of 2 minutes for the first hour that they were in lairage (as described in deliverable D2). During each two-minute period the number of pigs standing, sitting or lay down (resting behaviour) and the number of fighting interactions or negative social interaction (i.e. mounting) were counted.



Figure 49. Unloading of pigs at slaughter plant.



Figure 50. Pigs moving to lairage.



Figure 51. Pigs in lairage.

## Pre-stunning phase

During the measurement at the electrical stunning facility, they were experimenting with moving transport groups out of the truck towards lairage as well as unloading the whole truck and arranging the pigs in lairage. Even though the results are not well documented, the facility states that there are less fatigued pigs and less pigs that had to be stunned on the unloading deck or in lairage when keeping the pigs in the same small groups in the lairage as they were during transport. Figure 52 show the difference in lairage between small groups (transport groups) and groups set at a certain number despite transport groups (each group has access to water and enrichment material).



Figure 52. Pigs in mixed groups of 36 pigs per pen (left picture) and pigs in stable transport groups of approx. 12 pigs per pen (right).

## Stunning

The behaviour of the animals and of animal handlers when moving pigs from lairage to the stunner area was monitored in the corridor from lairage to the raceway (Figure 53) and in the raceway (see deliverable D2 for more details). Since stress or fear of animals in the route to the stunner can lead to resistance to move or animals trying to return, improvements have been carried out to ease the handling of the pigs in the raceway.



Figure 53. Pigs moving from lairage to raceway.



Figure 54. Observation at the raceway.

These improvements compared to the traditional high throughput electrical stunning system are realized and implemented under commercial slaughter conditions:

- Small groups of pigs are guided from lairage to the raceway (6-8), rather than individuals in a single file
- Workers are in view of the pigs as little as possible (in walkway to raceway and in the raceway), to reduce baulking due to startling responses.
- When entering the stunning system, pigs can choose into which raceway they are walking (left vs right), which reduces the incidence of blocked access.

Once the pigs entered the raceway, handling of the pigs was monitored (Figure 54). There was an extra focus on the use of the electrical simulator. To reduce stress and improve animal welfare adjustments have been made to the use of electrical stimulators:

- The use of a low voltage electrical stimulator
- The use of electrical prods or stimulators is limited (Workers are using brushes instead of electrical prods to stimulate pigs to walk in the raceway)
- Workers are not allowed to hit the pigs with an upper arm swing
- The prods are placed high above the worker, so they must reach for it (and not have them in hand)
- Use is only allowed on the first pig if it is not moving, not on the following pigs
- There is a two second delay on the stimulator

Cameras with artificial intelligence are used to inspect the use of the electrical prods and handling of the animals throughout the pre-stunning process, to record movement and use of the electrical prod. These recordings are used to monitor the use of the electrical prods through artificial intelligence and to provide feedback for workers.

### Stunning

Once the pigs enter the electrical stunner, the placement of the electrodes, the electrical contact, the exposure time and the correct electrical parameters are of uttermost importance. Of the four stunners in function, one stunner (stunner 1) was monitored (Figures 55 and 56). Since one out of two stunners waits for space on the slaughter line, the pigs occasionally stayed a bit longer in the stunner.

Video recordings were performed during the whole process for live observation and to watch them again for further analysis of the pig's behaviour during stunning. Animal behaviour during stunning was analysed using the Boris software, following the PigStun ethogram from deliverable D2.

Approximately 175 pigs pass through the stunner per hour. The exposure time for each pig is 2.5 seconds for head stunning, and 2.4 seconds for chest stunning. The electrical parameters are set at the same standard parameters for each stunner.



Figure 55. Pig in electrical stunner.



Figure 56. Observation of stunning process and of bleeding.

The minimum current to the head is 1.25 A, the minimum voltage to the chest is 75 V. The stunner uses a maximum frequency of 60 Hz, and each slaughter pig is exposed to electricity for a minimum of three seconds.

The correct placement of the electrodes was observed for 60 pigs on three consecutive days through one stunner. Video recordings were made from each of these 180 pigs.

#### Unconsciousness

Once the pigs are stunned, they slide down a slope onto the bleeding table for sticking. During this period, the effectiveness of stunning before sticking was examined by an examiner standing close to the bleeding table. The pigs were observed for the occurrence of eye reflexes and unrhythmic and irregular breathing.

#### Bleeding

Once the pigs are on the slaughter line, they were stuck. Per stunner one worker was responsible for bleeding the animals. Since the area where the pigs are bled is crowded, the animals were observed while being chackled. The pigs were observed for the occurrence of eye reflexes and unrhythmic and irregular breathing, as well as the absence of muscle tone.

#### Meat quality

Meat quality parameters such as bloodspots and bleedings or bruises as well as drip loss analyses, pH and colour measurements were obtained. At 45 minutes after stunning the pH was measured, and at 24 hours the pH, colour, drip loss and the occurrence of bloodspots was measured.

# 3.Task 3.2 Descriptions of the alternative systems at commercial level

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## Introduction

In this chapter the four alternatives are described as (semi) commercial stunning systems that in principle can operate at 600–800 pigs per hour or in a way that the principle can be translated into commercial operating levels. The four alternatives are at different development or implementation level, and this means the detail of description differs per stunning alternative. Based on the WP4 analyses of the data collected in WP3, the descriptions of the systems will be adapted.

## Description of Optimised CAS process on commercial scale

The improved CO<sub>2</sub> stunning method under development focuses on two distinct aspects related to animal welfare risks, 1) Reducing pre-stunning stress in the handling and lairage phase and 2) reducing the negative effects of exposure to high CO<sub>2</sub> in the CAS phase.

This systematic approach is split into two phases:

- Topic 1: Introducing an Automatic walkway whereas the animals are guided moving towards the stunner, with the intention not to touch them;
- Topic 2: Introducing an Optimized Climate to facilitate equal animal treatment to all animals in the gondola at the same time.

The original proposed box-system is not feasible both from an economic as well as from an improvement point of view. Therefore, the company decided to present an envisioned system of an Automated Walkway, in which the animals have to walk themselves in a fully automated and controlled environment. All this to secure continuous and paced movement towards the stunning process, avoiding all human interaction.

A pilot system is already operational since 2021 (Figure 2 above). In this system the cages have a functionality to mimic the Automated Walkway (see figure 57). There will be a lairage period between 30–240 minutes. A minimal time of lairage is needed for planning, stunning quality, and meat quality, hence the open areas in lairage prior to enter the Automated Walkway.

## Description of outlook of optimised CAS process

Based on the findings from the PigStun project, two CO<sub>2</sub>-based solutions are proposed: a backloader system and a tunnel system. These solutions are designed to ensure an enhanced stunning process for pigs with as minimum as possible stress.

Each proposed system utilizes an Automated Walkway, which include guiding plates to move the animals in a continuous flow. This setup ensures that pigs are transported without exerting any effort, thereby minimizing stress.

The entire drive mechanism is controlled by servo systems, which guarantee low acceleration rates and precise movements. The servo systems ensure smooth and accurate translations between the different driveway sections.

This Automated Walkway secures a consistent and gentle handling of the pigs, further contributing to a stress-free environment. This approach not only improves animal welfare but also optimizes the operational efficiency of the stunning process.

## Backloader system

The climate conditions within the Backloader will be optimized to achieve better control over CO<sub>2</sub> and O<sub>2</sub> concentrations. This enhancement will be accomplished through the integration of additional sensors capable of accurately measuring both CO<sub>2</sub> and O<sub>2</sub> levels.

To further refine the control over gas concentrations, a PID regulation system will be implemented. This control system will provide precise regulation of CO<sub>2</sub> levels throughout the entire Backloader system.

The PID controller will continuously adjust the gas concentrations based on real-time sensor data, ensuring that the desired levels of CO<sub>2</sub> and O<sub>2</sub> are maintained. This approach not only enhances the efficiency of the Backloader system but also ensures a stable and controlled environment, thereby improving the overall animal welfare.

By incorporating these advancements, the Backloader system will achieve a higher degree of climate management, leading to improved animal welfare compared to the current Backloader system.

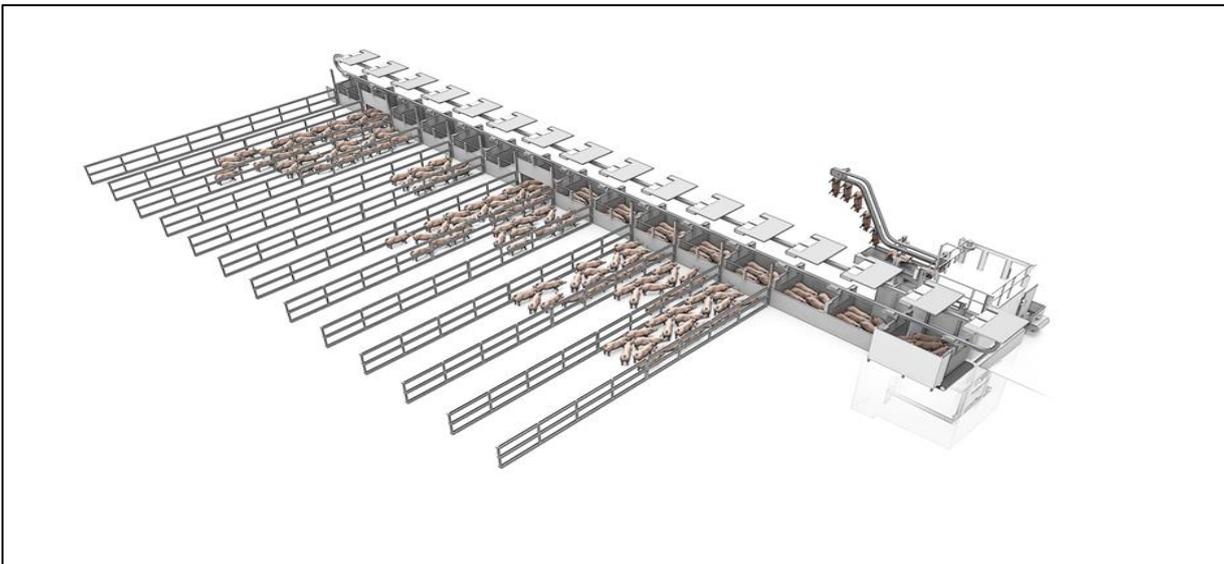


Figure 57. An artist's impression of the Backloader system at commercial scale.

## The Tunnel solution

In the tunnel-based stunning solution, a tunnel is utilized for the stunning process instead of the traditional pit system. This design offers several advantages, including the direct alignment of the tunnel with the driveway, which facilitates the seamless transfer of pigs into the stunner. Additionally, this approach eliminates the need for deep pits within the slaughterhouse, thereby simplifying the infrastructure requirements.

All movements within the tunnel are controlled by servo systems, ensuring low acceleration rates and precise positioning. The servo systems will provide smooth and accurate transitions, enhancing the overall efficiency and welfare standards for the pigs in the stunning process.

The climate within the tunnel is managed by a PID (proportional integral derivative) -regulated ventilation system. This control system ensures that the climate within the tunnel is consistently maintained at optimal levels. The PID controller continuously adjusts the ventilation parameters based on real-time sensor data, ensuring precise control over temperature, humidity, and gas concentrations throughout the entire tunnel.

By integrating these advancements, the tunnel solution not only improves the operational efficiency of the stunning process but also enhances animal welfare by providing a controlled environment with minimal stressors. This approach represents a significant improvement over traditional pit systems, offering both practical and welfare benefits.

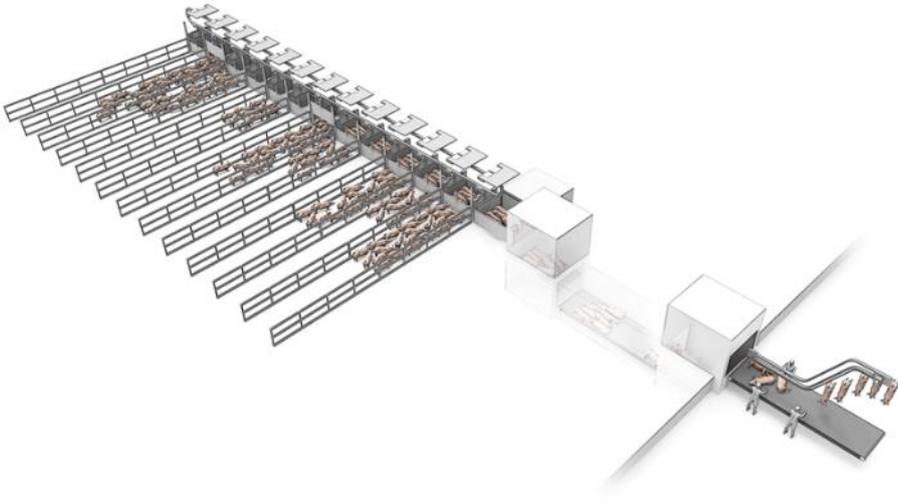


Figure 58. An artist's impression of the Tunnel system at commercial scale.

## Description of Helium Stunning System on commercial scale

The scientific literature suggests that stunning animals with Helium (He) can be an animal-friendly stunning method. In experimental setting at MRI, Machtoft et al. (2013) observed that pigs showed no aversive reactions when subjected to the helium atmosphere (experimental facility; n=40) while animals stunned with CO<sub>2</sub> (dip-lift system; n=40) hyperventilated, revealed retreat, or escape attempts and vocalization. None of the carcasses showed ecchymosis after the helium stunning and the other measured meat quality parameters are as good as they are for CO<sub>2</sub> stunning.

Due to the properties of helium, which is lighter than air, it needs a different built-up of the system than stunning systems using CO<sub>2</sub> or argon. For the use of helium, a tower in which the pigs move up, is required instead of the traditional pit (Figure 59). The helium tower (height 4.0 meters) consists of double walls to recycle the helium and reduce the consumption. The gondola (H0.86 x W1.90 x L2.09) (Figure 60) with pigs (max 5 pigs at the same time) is lifted up to the top of the tower where the helium atmosphere is with the required residual level of O<sub>2</sub>.

The process is program controlled: initial filling with helium before stunning and refilling during stunning to achieve a required residual level of O<sub>2</sub> less than 1%. The system reacts when the level of O<sub>2</sub> at a certain sensor is higher than 0.7%. The operation of the system is fully automatic. At the end of the day the residual helium will be recycled, stored, and reused for the first filling at the next slaughter day. The total amount of helium required for stunning, as litres of helium per pig (e.g., 695,17 L/pig), is recorded in the system for internal review.



Figure 59. Overall view - Helium stunner ("Tower").



Figure 60. Look inside lower part of the tower (gondola).

The control-process screen (Figure 61) is projected on the wall showing remaining time of the stunning period and percentage of residual O<sub>2</sub> at different levels in the tower, so operators can see in which phase the stunning process is and live-video what is happening inside the helium chamber and on the raceway, see Fig. 61.



Figure 61. Control-process screen.

The tests with Helium were performed in a small commercial slaughterhouse, but not at a high throughput rate. The Helium stunning system which we approved in the PigStun trials is an option for small or medium size

slaughterhouses up to a maximum throughput rate of 60 pigs per hour. Because of the extended stunning time up to 200 s, compared to CO<sub>2</sub> stunning this will lead to a reduction in throughput capacity based on process timing and number of pigs per gondola (gondola ascend/descend 60s, stunning duration 200s, loading/unloading, 5 pigs per gondola). In current CO<sub>2</sub> stunning the exposure duration is approximately 3 minutes whereas in helium stunning this will be 5 minutes.

For the implementation of a helium stunning system the arrival area and the lairage doesn't need to be changed and can stay as they are. The biggest investment when changing from CO<sub>2</sub> to helium is the reorganisation of the stunner itself. The stunning system must be changed completely from a pit to a tower and none of the installed technique could be used.

Various scenarios are conceivable to counteract the reduction in slaughter speed in this exact case of small commercial slaughterhouse and if not apply all on high throughput one:

1. the gondola could be enlarged to increase the number of pigs per cycle (for example increased capacity from 5 to 8 per cycle will increase the capacity by 38%)
2. several towers could be built next to one another; this means that the costs of the installation can vary based on difference in capacity (for example 4 towers doubles the capacity)
3. a new concept for the existing system would be conceivable.
4. by skilfully planning the work shifts, slaughter days could be extended and thus the slaughter output per day could be increased
5. in principle, processes within the system and when using the system should be optimised, e.g. the time the gondola needs to go up or down could be reduced, the robot could start leading the pigs earlier.

## Description of Inert Gas Retrofit System on commercial scale

The experiments with the Inert Gas Retrofit System were carried out in a commercial Dip-Lift System. As such, this system was successfully tested on a commercial system, albeit not a high throughput one. During a previous field study using the regular CO<sub>2</sub> stunning system, animals were slaughtered at median of 19.5 gondolas per hour. During the experiments with the Inert Gas Retrofit System, animals were slaughtered at a median rate of 11.1 gondolas per hour when stunned with Argon and 12.7 gondolas per hour in the CO<sub>2</sub> control condition. During the experiments, each gondola was loaded with two animals, to ensure visibility of behaviour and the cameras and adherence to stocking density requirements.

For other slaughterhouses with a Dip-Lift System to switch to this method, the Inert Gas Retrofit System has to be installed inside the pit of the stunner (Figure 62). This can likely be achieved in a single day.

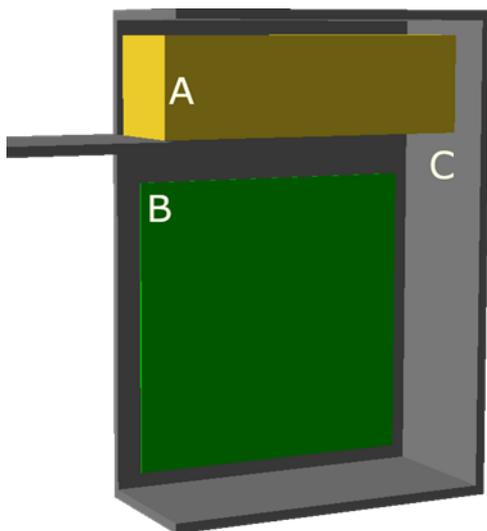


Figure 62. The patented gas delivery system (B) was retrofit into an existing commercial Dip-Lift (C) stunning system, in which a gondola (A) is lowered into a pit with a controlled gas atmosphere.

Additionally, the control unit (alarms for inadequate gas atmospheres and regulation of gas flow) for the Dip-Lift System may have to be adjusted for the use of Argon and control by residual oxygen content. For the easiest user experience with only limited necessary changes, cooperation from the manufacturer of the Dip-Lift System would be necessary. This would also ensure storage of relevant data in a single system. Without this cooperation, the controls for the gas atmosphere would either have to be disabled in the old Dip-Lift System and replaced by a separate solution, or the sensor input to the existing Dip-Lift control would have to be reverse engineered to allow handling of residual oxygen sensors. Again, these changes should be possible to implement in a single day and could be implemented to allow easily switching back to unchanged CO<sub>2</sub> stunning if desired. After these changes, the stunner is ready to be used with Argon or other inert gas mixtures.

Technical tests, to demonstrate that the necessary residual oxygen levels can be reached in a paternoster system, were carried out as part of preparations in the Tiger-project in different scenarios. During the worst-case scenario, the floor of the gondola was covered with plastic foil to imitate lying pigs and bags were used as a simulation for the pigs' volume (Figure 63). Residual oxygen levels of less than 1% were reached at the first and last stop, with oxygen levels at the bottom position being lower than those at the other positions. These tests indicate that the new gassing system can be used to stun pigs with argon in a commercial paternoster system.



Figure 63. Simulation of pigs in a gondola in an existing Dip-Lift CO<sub>2</sub> stunner

The necessary exposure time will increase compared to high concentration CO<sub>2</sub> stunning, leading to a reduction in throughput capacity. The details will be reported as part of the analysis of the test. The best measure to compensate for this reduction in hourly capacity will depend on the specific situation on each slaughterhouse, possible scenarios are:

- Should the slaughterhouse operate in a region with declining demand, the reduction of hourly capacity may still allow for operation within the current working hours
- If working hours can be extended more shifts could be added to achieve a comparable daily capacity
- If the current stunner can be modified to allow more animals (larger gondola or more gondolas in a paternoster system), hourly capacity could be restored by these measures
- If those options are not possible, a second stunner could be installed. For Dip-Lift systems, one of the cases reported in this project does already operate multiple Dip-Lifts in parallel. Multiple Paternoster systems can also be run in parallel.

## Description of Improved Electric stunning process on commercial scale

EFSA (2020) identified five hazards for pig welfare relating to the application of electrical stunning: handling and restraining the pigs, wrong placement of the electrodes, poor electrical contact, too short exposure time and inappropriate electrical parameters. All these hazards are related to or will increase by high throughput rates. To improve these factors, a commercial electrical slaughter plant improved the lay-out of their slaughterhouse and the whole electrical stunning process. Our goal is to clarify which aspects of the electrical stunning process can be improved at facilities and which should be considered when designing or adjusting an electrical stunning facility.

As mentioned, the electrical stunning facility improved their lay-out by expanding the lairage area, adjusting the raceway and incorporated four stunners and two bleeding lines (Figure 64). The lairage area is set up so each pen of pigs can move towards each stunner. However, since the stunners are located in the middle of the lairage area, the pigs moving from the outside pens have to cross a larger distance. The pigs are being led into the walkway towards the raceway and are able to pick one out of two doors towards the stunner. In the raceway the pigs are motivated to walk towards the stunner, where an automatic band moves the pigs into the stunner towards the electrodes.

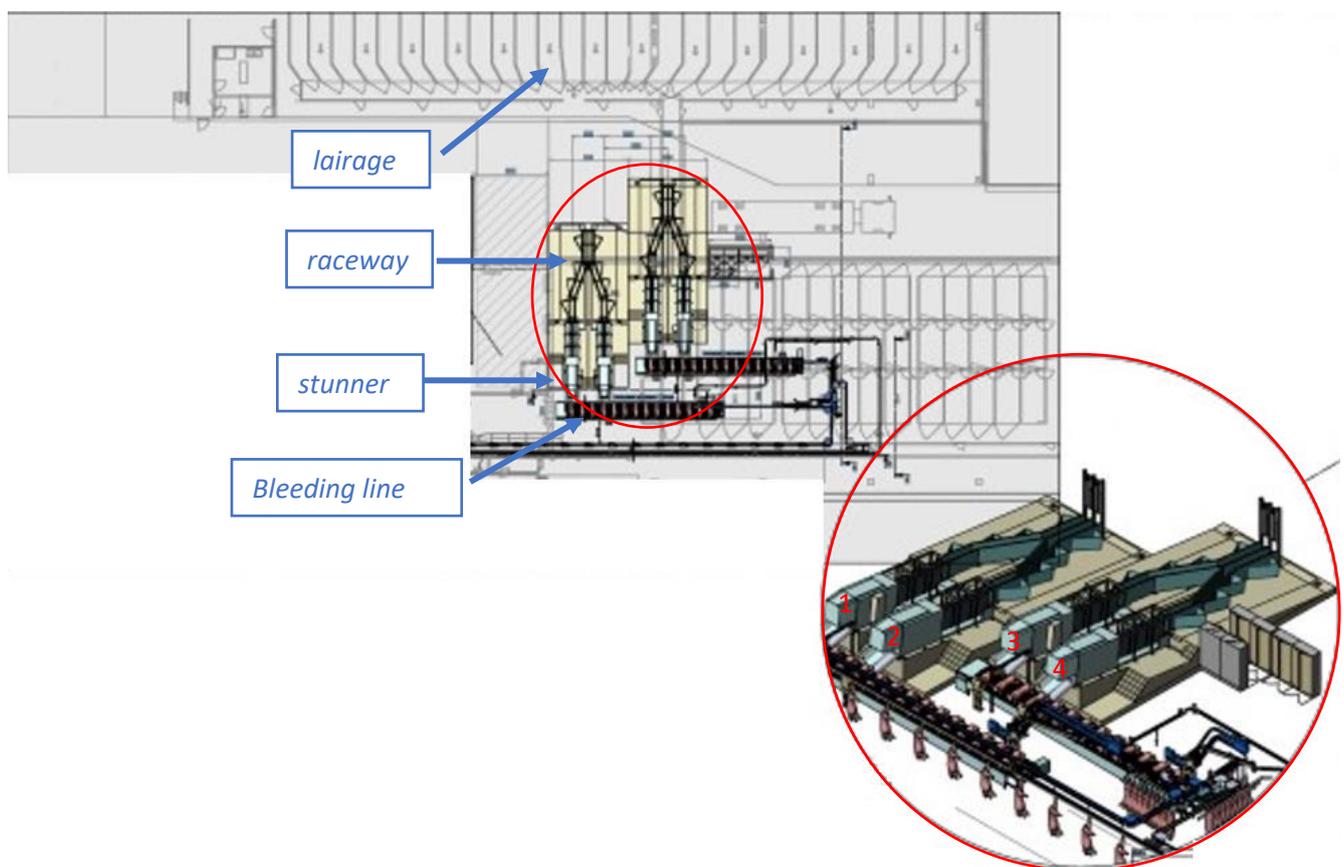


Figure 64. impression of improved electrical stunning set up (source Compaxo).

Two electrical stunners together feed one bleeding table to facilitate very short stun to stick intervals. Sticking is performed while the pigs are still in the tonic phase. This results in a very short stun-stick interval and to a save to apply procedure for personnel. The two bleeding tables come together up to one line where the animals are shackled. This layout gives enough time and space to check for (un)consciousness and dead and to interfere with a back-up stunning procedure if required.

### Stunning

The main animal welfare hazard related to electrical stunning is forcing animals from a group into a single line at high speed. To maintain high speed, there is little room for error, natural movement of the pigs and a short stunning period.

To reduce possible animal welfare hazards at a high slaughter speed, the route to and into the stunner should be optimized and the throughput rate per stunner needs to be reduced. In the commercial setting this resulted in the installation of 4 Midas electrical stunners. This reduces the maximum throughput rate per electrical stunner to on average 75% of the original 650/700 pigs per hour to approximal 175 pigs/hour. Optimal throughput rate per stunner could be 275-350 (according to personal communication of the involved slaughter plant). This reduced throughput rate reduces the force on the animals walking from lairage towards the stunner. Once the pigs are aroused in lairage, they are moved in small groups (6-8 pigs) towards the raceway. Once they left lairage and enter the last part of the walkway before entering the raceway, the walkway has high side walls to limit interaction with workers and keep them out of view (Figure 65). The electrical stunning facility has noted that limited interaction with workers reduces the amount of pigs baulking due to startling responses. Once the group of pigs enters the closed off part of the walkway, a worker enters the walkway behind the pigs and closes the exit using a board to prevent the pigs from walking back.

The stunners operate in a two-by-two set-up (Figure 66). This means that one corridor from lairage to the stunner ends at the raceway to two stunners. At this point pigs are given the opportunity to freely choose if they enter raceway 1 or raceway 2 (see Figure 66). Giving pigs a free choice reduces the number of pigs that need to be stimulated manually or by electrical prods to enter the raceway. A consequence of free choice is that the number of pigs per stunner can differ or fluctuate during operation therefore the set-up of the stunners and bleeding line is designed to cope with these fluctuations.

Pigs moving from the raceway into the stunner need some time to make the step onto the moving conveyor. The reduced throughput rate creates more time for animals to decide resulting in less pressure on the animals and less



Figure 65. pigs walking towards raceway. Note high walls and the double doors at the end.



Figure 66. Entrance of the raceway. Pigs are able to freely choose one of the two doors.



Figure 67. Location of the electrical stimulator above the raceway.

use of the electrical stimulator. Furthermore, the use of the electrical stimulator is discouraged by placing the stimulator above the workers (see figure 67) so that they need to reach to use it. The electrical stimulator itself consists only of a low voltage and education of staff resulted in a proper use of the device. For instance, workers are only allowed to use the electrical stimulator on the first pig who refuses to move, and only in case using a brush does not work sufficiently. This reduces the use of an electric stimulator (low voltage) to a minimum. The use of the stimulator is recorded by video surveillance and there is an automatic detection of correct or incorrect use.

#### Summary of the optimal electrical stunning process

To avoid welfare hazards concerning the electrical stunning process a lot of finetuning is necessary. The commercial electrical stunning facility has been working hard to improve their process and been finetuning the process together with animal welfare organizations. Looking back at their progress compared to their old system, they claim that the change from one to four stunners has been the biggest improvement in the system, for animal welfare and meat quality. However, all adjustments mentioned above have influenced the animal welfare at the facility, such as

movement of small groups and reducing use of the electrical stimulator. Moving pigs from a group into a single line remains the main welfare risk area of electrical stunning, but enhancing all aspects of the process will increase animal welfare.

To optimize other electrical stunning facilities an overview of the optimal electrical system is presented in Table 5, which could be used by other facilities to see factors that might have an impact on their animal welfare and meat quality.

Table 3. Summary of what has been realized at the electrical stunning facility to make it a working alternative.

|                 | Optimal electrical system  |
|-----------------|--|
| Arrival         | <ul style="list-style-type: none"> <li>Moving pigs in transport groups</li> </ul>  |
| Lairage         | <ul style="list-style-type: none"> <li>Minimal interaction with pigs</li> <li>Keeping pigs in transport groups</li> </ul>  |
| Stunning        | <ul style="list-style-type: none"> <li>When entering the stunning system, provide a choosing option for pigs (stunner A vs stunner B), which reduces the incidence of blocked access.</li> <li>Limit interaction with workers &amp; keep them out of view (reduces baulking due to startling responses)</li> <li>Use of multiple stunners (prolonged stunning times and reduced number of pigs that go through a stunner per hour)</li> <li>Limit the use of electrical prods or simulators.               <ul style="list-style-type: none"> <li>Workers use brushes instead of electrical prods to stimulate pigs to walk in the raceway</li> <li>Workers are not allowed to hit the pigs with an upper arm swing</li> <li>The traditional electrical prod is replaced by a low voltage electrical stimulator</li> <li>The prods are placed high above the worker, so they have to reach for it (and not have them in hand)</li> <li>A camera is recording movement and use of the 'electrical prod' through artificial intelligence</li> <li>Use is only allowed on the first pig if it is not moving, not on the following pigs</li> </ul> </li> </ul> |
| Unconsciousness | <ul style="list-style-type: none"> <li>Experienced workers at line</li> <li>Since throughput per stunner per stunner is lower, there is more time for checking unconsciousness and sticking</li> <li>Secondary stunning option easily accessible</li> </ul>  |

# Positioning deliverable D3

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In WP3 of the PigStun project data was collected according to the protocols as described in deliverable D2, at different levels. At the test facilities of Marel in Denmark we collected animal welfare and meat quality data of pigs exposed to CO<sub>2</sub>, Argon or Helium under the same conditions. For improved electrical stunning, the inert gas retrofit system and the helium system data was collected following the same protocol as much as possible under (semi) commercial conditions. Furthermore, benchmark data was collected in 2 commercial high throughput slaughter plants, one using a paternoster CO<sub>2</sub> stunning system and one using a back-loader or dip lift CO<sub>2</sub> system.

A technical description of the four alternative stunning systems is produced (Task 3.2).

The data and outcomes of the different phases of WP3 will be analysed under WP4. Animal welfare consequences, and micro- or macro-economic aspects will be described in Deliverable 4.

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