Behavioural response in pigs at gas stunning with foam

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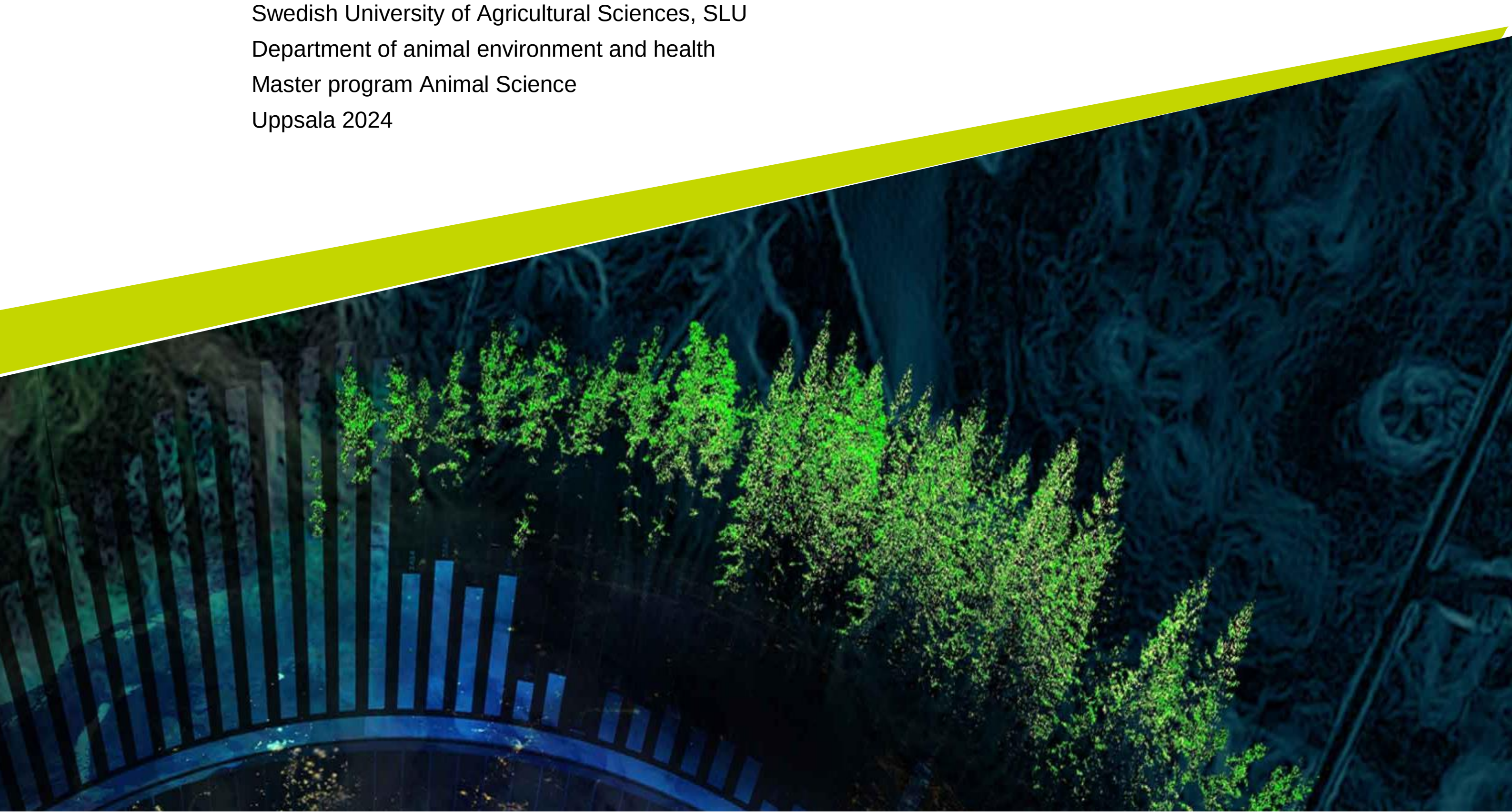

Behavioural response in pigs at gas stunning in foam.

Beteenderespons hos grisar vid gasbedövning i skum.

Madeleine Persson

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Abstract

There are several stunning methods used for the commercial slaughter of pigs. The two most common are electric stunning and stunning with carbon dioxide gas. It has been shown that there are several problems related to animal welfare in the stunning methods used today in commercial slaughter. This master thesis was conducted as a part of a broader study aiming to evaluate the use of a new method for gas stunning with high-expansion foam. This study aimed to investigate the differences in pigs' immediate reaction to high-expansion foam when stunned with nitrogen, argon or carbon dioxide.

The study included a total of 36 pigs, divided into three groups (n=12 per group). The three groups were exposed to three different gas stunning treatments; nitrogen, argon, or carbon dioxide in foam. An ethogram consisting of 12 behaviours was conducted for video-based recording of behaviours. The observations started at the initiation of the foam in the stunning box and continued until the foam filled the entire stunning box.

The result show that the pigs expressed higher frequencies of exploration behaviours when exposed to nitrogen- and argon high-expansion foam, while they expressed higher frequencies of escape attempts when exposed to carbon dioxide high-expansion foam. These findings suggest potential advantages of using nitrogen or argon for stunning pigs with high-expansion foam from an animal welfare point of view. Understanding the behavioural response can contribute to refining stunning techniques, emphasizing the importance of considering the wellbeing of animals during stunning in development and implementation of slaughter practices.

Keywords: Stunning methods, Slaughter, Animal welfare, Carbon dioxide, High-expansion foam, Nitrogen, Argon.

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1. Introduction

1.1 Background

Stunning before slaughter is required in the European Union (EU) regulations (Regulation (EC) No 1099/2009). During slaughter, the regulations state that pain, distress, or suffering should be avoided by using the best practices and methods permitted under the regulation. The animals must be unconscious during the entire bleeding process to prevent unnecessary suffering (EFSA 2004).

A good stunning method should in an effective way disrupt the normal state of neurons or the neurotransmitter regulatory mechanisms in pig's brain and there by induce a pathological brain state that is incompatible with the persistence of consciousness and sensibility. According to EFSA (2004), good and effective stunning of pigs should include a long-lasting depolarized neuronal state, which renders the animal unconscious and insensible.

From an animal welfare perspective, the slaughter and stunning procedure should induce death without causing avoidable fear, anxiety, suffering, distress, or pain (Steiner et al. 2019). An ideal slaughtering process needs to consider the total “stun-to-stick-time”. The “stun-to-stick-time” refers to the time interval from stunning until induced bleeding process. This process is done to minimize the potential suffering that an animal might experience during a slaughter process (Faucitano & Schaefer 2008). The stun-to-stick-time depends on which stunning method that is used, and for gas stunning, the maximum interval will also differ with exposure time. A shorter “stun-to-stick-time” is considered to be more effective and better from an animal welfare perspective (Faucitano & Schaefer 2008).

The most common stunning method for pigs used in commercial slaughter in Europe is carbon dioxide (CO_2), but electrical stunning is also a commonly used stunning method (EFSA 2004; Steiner et al. 2019). The existing stunning methods are suboptimal in terms of animal welfare, as studies indicate that some stunning methods, such as CO_2, can cause pain and distress in the animal (Dalmau et al.

2010b; EFSA 2004; Steiner et al. 2019). Therefore, there is a need for alternative stunning techniques.

1.2 Species specific behaviour of the pig

The domestic pig (*Sus scrofa domesticus)* originates from the wild boar (*Sus scrofa)*. The wild boar is characterized as a herd animal that thrives in flocks and has social behaviour adapted for its natural habitat (D'Eath & Turner 2009). Over time, through domestication and selective breeding, the domestic pig has evolved. Through studies, information has been gathered that shows that the domestic pig's behavioural response corresponds to the wild boars (D'Eath & Turner 2009).

Pigs are curious animals and have strong exploratory behaviour that can have various motivations. By sniffing, rooting, and biting things they become familiar with the surroundings. The exploratory behaviour during foraging becomes stronger when the pig has a limited amount of feed available (Studnitz et al. 2007; Jensen 2017). The foraging behaviour in pigs does not need to be linked to hunger, pigs prefer to search for food rather than have it provided, indicating that exploration may not only be motivated by immediate needs (Studnitz et al. 2007; Jensen 2017). The pig's curiosity could come from boredom but could also be a way to reduce uncertainty about an unfamiliar environment, increasing the chances of survival (Studnitz et al. 2007; Jensen 2017).

When the pig experiences fear both physical and behavioural responses can be triggered. Pigs' fear may express itself in a variety of ways involving movements, head position, facial expressions, vocalizations, and odors (Forkman et al. 2007). It can also affect the pig's activity level, where mild fear can stimulate the activity and strong fear can lead to passivity (Forkman et al. 2007).

1.3 Electrical stunning

The concept of electrical stunning is that a strong current of electricity passes through the pig's head causing a form of epileptiform seizure which leads to immediate unconsciousness (Anil & McKinstry 1998; Grandin 2013). Electrical stunning can also be used to induce cardiac arrest if the electrodes are placed on either side of the pig´s chest (EFSA 2004). This method requires the pigs to be restrained so the placement of the electrodes is correctly done. Electrical stunning is a cost-effective method and has a rapid incubation time (EFSA 2004). The risk with the method is that it can cause distress for the pig because of social isolation because the method requires one by one stunning (Brandt & Aaslyng 2015; Meyer

2015). Electrical stunning can also lead to seizures and brief unconsciousness before the completion of the bleeding process (EFSA 2004). This can be mistakenly perceived as adequate stunning, which can lead to two distinct issues. Firstly, there is a risk of initiating the bleeding process while the pig is still conscious, as the pig may be mistakenly assumed to be adequately stunned. Secondly, there is a risk that the pig might regain consciousness during the bleeding process (Anil & McKinstry 1998; EFSA 2004; Meyer 2015).

1.4 Carbon dioxide

The most common commercial stunning method for pigs is with carbon dioxide (CO_2) (EFSA 2004; Steiner et al. 2019). The stunning method is done in groups, which reduces the risk of causing a stressful situation caused by social isolation compared to electrical stunning (EFSA 2004). The CO_2 method is carried out by lowering a group of pigs down to a pre-filled shaft with a high concentration of CO_2 (Dalmau et al., 2010).

All methods that include stunning by changes in ambient gas concentrations leading to unconsciousness are called controlled atmosphere stunning (CAS). Often gas mixtures that are heavier than air, such as CO_2, are preferable for stunning because it is easier to contain in a pit (EFSA 2004; Steiner et al. 2019).

The carbon dioxide gas is an acidic gas, and studies show that pigs exposed to a concentration of 70% or higher will show aversive behaviour such as gasping and vocalization (Mota-Rojas et al. 2012; Sindhøj et al. 2021). This is because the gas induces respiratory and metabolic acidosis which induces respiratory distress in the pig (Mota-Rojas et al. 2012; Sindhøj et al. 2021). According to The European Council regulation No. 1099/2009, the minimum concentration of carbon dioxide for stunning pigs shall be 80%. The stunning is achieved through a neuronal function caused by hypercapnic hypoxia which lowers the pH of the bloodstream and, consequently, the cerebrospinal fluid, resulting in its anesthetic impact (Sindhøj et al. 2021). It is shown that gasping observed during high-concentration CO_2 stunning is an indicator of the feeling of suffocation before unconsciousness (Dalmau et al. 2010b).

1.5 Alternative gas stunning methods

The primary focus of research as alternatives to carbon dioxide has been two gases, nitrogen and argon, both which are non-aversive gases (Dalmau et al. 2010a; Llonch et al. 2012; Lindahl et al. 2020). Argon (Ar) is easy to contain in free form without the risk of the gas diluting in the air due to its higher density compared to ambient air (Raj & Gregory 1995). Argon gas is odorless and tasteless, and studies have shown that argon in high concentrations causes less distress for the animal compared to carbon dioxide (Raj & Gregory 1995). Argon is an effective gas for stunning pigs. However, it has been indicated that pigs require a longer exposure time to Argon compared to CO_2 to achieve the same results (Sindhøj et al. 2020). Argon is extracted from the atmospheric air but only occurs in small amounts which makes this gas more expensive for commercial use than CO_2 (Raj & Gregory 1995; Dalmau et al. 2010a).

Nitrogen holds the potential to become an alternative method for stunning pigs in the future. The atmospheric air contains of 78% of nitrogen which makes this gas available for a low production cost (Sindhøj et al. 2020). Nitrogen is a gas that is easily available and has a lower density than atmospheric air, which means that the gas is harder to contain in free form compared to argon or carbon dioxide (Llonch et al. 2012; Lindahl et al. 2020). The nitrogen gas can be captured in foam bubbles and used in a high-expansion foam system (Steiner et al. 2019; Lindahl et al. 2020). According to Lindahl et al. (2020), stunning pigs with N_2-filled high-expansion foam that is used in a closed container has been shown to be an effective method. The gas is inert and does not cause respiratory distress as CO_2 tends to do (Lindahl et al. 2020).

The high-expansion foam efficiently expels air during filling, preventing prolonged exposure to declining oxygen levels (Lindahl et al. 2020). This N_2 foam approach, similar to CO_2 stunning, could offer benefits such as group stunning and minimal handling of animals. Nonetheless, the foam itself may induce stress and hinder visual observation of the stunning process (Lindahl et al. 2020; Sindhøj et al. 2021). A study done on poultry showed effective and rapid euthanasia with N_2 high-expansion foam, resulting in minimal aversive responses (McKeegan et al. 2013).

High-expansion foam filled with N_2 in a sealed container offers a viable method to swiftly remove oxygen and establish a lasting anoxic environment (Lindahl et al. 2020). Recent research has been focusing on the potential of stunning pigs and poultry with the high-expansion foam technique where the results have shown different results. A study done by Pöhlmann (2018) showed a notable percentage of inadequately stunned pigs and the results also showed a high number of aversive behaviours in the pigs exposed to high-expansion foam with nitrogen treatments. In contrast, another study showed high effectiveness of nitrogen filled high-expansion foam in rapidly reducing oxygen concentration and the pigs were evaluated as adequately stunned pigs with no aversive behaviours observed (Lindahl et al. 2020). In the study by Pöhlmann (2018) the container used had a open-top container which allowed the nitrogen to mix with air. In the study by

Lindahl et al. (2020) they used a closed-top container which prevented the nitrogen from mixing with the air. The different results in this studies can be explained by technical challenges, rather than indicating ineffectiveness in nitrogen-filled high-expansion foam as a stunning method (Sindhøj et al. 2020).

An ideal stunning method should uphold fundamental animal welfare principles where the objective should be to utilize the beneficial aspects of CO_2 stunning, enabling group stunning and still maintaining a high throughput rate while minimizing aversive experiences and ensuring an irreversible state of unconsciousness before the sticking process (EFSA 2004).

To get a clearer picture of how the high-expansion foam with nitrogen effects the pig, it is essential to conduct further research on the behaviour of the pigs when in contact with the foam.

2. Hypothesis and aim

The overall aim of this master thesis project was to study the behavioural responses in pigs at gas stunning with high-expansion foam. The specific aim was to study differences in pigs' immediate reaction to the foam when stunned with nitrogen, argon or carbon dioxide gas.

The following hypotheses were developed in accordance with current understanding and previous studies:

- The pigs show more avoidance and escape behaviours when exposed to gas filled foam with carbon dioxide compared to argon or nitrogen filled foam.

- Compared to the other two treatments, the high-expansion foam with carbon dioxide gas-treatment will result in less interaction of the pigs with the foam.

3. Material and methods

3.1 The primary study and ethical permit

This study was performed at the Swedish University of Agricultural Sciences' pig research facility at Lövsta and is part of a broader Formas-funded study. The research aims to investigate and evaluate the behavioural and physiological responses of pigs subjected to stunning using argon, nitrogen, and carbon dioxide high-expansion foam (Decision number: FR-2020/0008; dnr: 2020-02554). The primary study consists of two parallel studies, one focusing on behavioural responses (non-restrained pigs) and the other on the physiological aspects of loss of consciousness (restrained pigs). The current study examines the behavioural reactions of pigs during stunning with foam using video recordings from the study focusing on behavioural responses. Approval for the study was granted by the Uppsala Animal Ethics Committee under permit number 5.8.18 – 13402/2021.

3.2 Equipment

During the trial, controlled atmosphere stunning (CAS) with a high expansion foam system was used. The animals used in the trial were stunned in a box where the atmospheric air was controlled. The two boxes used in the trial were modified C1 systems from a Swedish company called High Expansion Foam Technology AB (HEFT; Figure 1). The measurements of the boxes were L1200×W800×H910 mm, with a 0.78m^2 floor area. The two boxes had two foam generators each that were placed in the diagonal corners placed in floor level inside the boxes and transparent floor to allow video recording from below (Figure 2). The high expansion foam system operates according to the principles of controlled atmosphere stunning.

Figure 1. Picture of the outside of the box (Lindahl 2023, unpublished).

Figure 2. Picture of the inside of the box.

The pigs in used in the study were moved to a temporary pen close to the experimental setup, where they were given a minimum of 10 minutes to acclimatise. An animal designated for stunning was positioned within the container, wherein an anoxic atmosphere was established by infusing the container with high-expansion foam. The foam bubbles, which incorporated either N_2, Ar, or CO_2 gas, effectively displace the air within the container, achieving a gas composition of nearly 100% and reducing the oxygen concentration to below 1%. This process ensures a controlled stunning atmosphere for the animal. Once the box was completely saturated with foam, a burst of gas was introduced to rupture the bubbles. This action enhances visibility within the enclosure and, crucially, upholds the anoxic environment throughout the stunning process.

For the video recordings, GoPro 7 black was used. The cameras were placed above and below the containers for recording. There was also a microphone placed inside the container.

3.3 Animals

In the study, crossbred pigs raised at SLU's research facility were used. The research facility is a particular integrated, pathogen-free pig production. The pigs were a mix of modern production lines (table 1), the dams being Yorkshire (Y) or Landrace and Yorkshire mixes (LY), and the sires were either Hampshire (H) or Duroc (D). They were weaned at five weeks of age and then remained in the farrowing pens for another five weeks before being moved to the finishing unit.

Table 1. The number of the pigs of different crossbreeds used in the experiments.

Breed	**Number**
LYD	15
LYH	6
YH	15

A total of 36 pigs, 18 of each gender (females and immune-castrated males), were used in the study, divided into three treatments (n=12). The pigs originated from 9 different litters. Across each treatment, an equal number of pigs were included, ensuring a balanced representation of both genders.

The total age of the pigs during the test day was approximately 12 weeks (86.5 ± 2.90 days, Mean$\pm$Std). The age of pigs in the N_2 treatment was 86.5 ± 2.93 days, for the Ar treatment the age was 86.5 ± 2.93 days, and for the CO_2 the age was 86.5 ± 2.93 days. The pigs were weighed at birth, after 9 weeks, and on the test day, the different weights are presented in Table 2. From the same litters, equal numbers of immune-castrated males and females were used in each treatment.

Table 2. The weights (kg) of the pigs at birth, 9 weeks of age, and on the test day per treatment (mean±Std).

Weighing time	**N_2 (n=12)**	**Ar (n=12)**	**CO_2 (n=12)**	**Total (n=36)**
Birth weight	1.7±0.32	1.4±0.32	1.5±0.370	1.5±0.35
Weight 9w	30.7±3.61	28.9±9.19	29.8±3.73	29.5±3.86
Weight test day	52.2±5.79	47.6±9.20	50.2±6.70	49.9±7.30

3.4 Behavioural study

The behavioural study was conducted on 36 pigs where 12 different behaviours were observed, presented below (*table 3*). The behavoiurs were observed either by "Duration of event" or "Point event". "Duration of event" refers to the period during which a behaviour was observed, starting from its initiation until the pig stopped showing the specific behaviour. "Point event" refers to that the behaviour was registered each time it occurred. Initially, a pilot study was conducted with a total of six pigs, and the ethogram was subsequently refined based on the experience of the pilot study.

The data collection was done through behavioural recording by using the software BORIS v.8.23 (Friard & Gamba 2016). The video footage that was used for the study was mainly the videos from above, while the video footage from below was used if some of the behaviours needed to be confirmed from the video footage from below. Observations started when the foam came out of the foam generators that were placed in diagonal corners inside the box. The observations stopped when the foam reached the roof of the box, and the pigs no longer were visible due to loss of posture. The observations were conducted by an individual who, throughout the study, remained unaware of the specific gas treatments to which the pigs were exposed.

Table 3. Behaviours observed, the definition of behaviours and how they were registered.

Behaviour	Definition	Registration
Touches foam with snout	Positions the snout in the foam without dipping the entire head.	Duration event

Places the whole head under foam	Sticks the entire head into the foam	Duration event
Turns the whole body around 360°	Rotates the body around the entire box, ending up with the head where it was initially.	Point event
Turns body 180°	Turns the entire body from one side of the box to the other (i.e., facing the opposite direction)	Point event
Turns body 90°	Turns the whole body 90 degrees.	Point event
Moves the head up and down	The head is moving constantly up and down.	Point event
Moves the head from side to side	The head moves from side to side in a constant movement.	Point event
Moves the rear part from side to side	Rear part moves from side to side, front part of the body is placed in the same position during the movement.	Point event
Moves front part side to side	Front part of the body moves from side to side, rear part of the body is placed in the same position during the movement.	Point event
Pressing the rear part against the wall	Pressing the rear part of the body against the wall so that the back curves	Duration event
Jumps	Jumps so that one or both front legs don't touch the floor	Point event
Avoid foam	Stretches the head up from the foam to avoid it.	Duration event

3.5 Statistical analyses

The data that were collected from the behaviour registration in BORIS was put into Microsoft Excel version 16.78.3. Data editing was performed, descriptive statistics were calculated and visualised in Microsoft Excel version 16.78.3 and Minitab version 19.2020.1.0. Statistical analyses were performed in SAS version 9.4 (2021; Cary, NC, USA). The statistical analysis was performed on each of the different response variables included in the ethogram (*table 3*). First, the normal distribution of the variables was examined ocularly with help of histograms in MiniTab. All the different behaviours were approximately normally distributed except for "Turns the whole body around 360 degrees" and "Moves the rear part from side to side". Because of the low accurence of these two behaviours, variables were only presented with descriptive statistics. The behaviours that were approximately distributed were analyzed further.

Which gas treatment the pigs were subjected to, N_2, Ar, or CO_2 are presented as treatment. For the different test days, the variable test day is represented. The study was conducted for a total of five days. On the first day, three pigs were stunned, on the second day six pigs were stunned, on the remaining days 9 pigs were stunned each day. Each breed of pigs was represented as the variable breed (*table 1*). To represent either female or immune-castrated males the variable sex was used. The weight of the pigs during the test day the variable weight test day was used.

The differences in behavioural responses, including the count of events and their durations, across various gas treatments, were investigated using general linear models implemented through proc GLM in SAS. This analysis was conducted using the following model:

Model : y = Treatment + Sex + Breed + Weight test day + Test day + e
Where treatment (N2, Ar or CO2), test day (1, 2, 3, 4 or 5), breed (LYD, LYH, YH) and sex (female or immune-castrated males) were included as fixed effects and weight test day as a continuous covariate.

The results obtained from these general linear models are reported as Least Square Means (LSM) along with their corresponding Standard Errors (SE).

4. Results

The differences in performed behaviours between gas treatments from the statistical analyses are presented in Table 4. The mean values and standard deviations of "Duration foam start to first foam touch" for the N_2 treatment was 9.7±12.38 sec, for the Ar 7.3±10.91sec and 7.3±5.98 sec for the CO_2 treatment.

Table 4. Differences in performed behaviours between gas treatment. Presented as least square mean (LSM) and standard error (SE) for number of times the pigs performed the behaviours during the observation period. N = 36 pigs. Different letters in the same row indicate pairwise differences between treatments of p<0.05.

	N_2		Ar		CO_2		
	LSM	SE	LSM	SE	LSM	SE	P-value
Duration of events (seconds per observed minutes)							
Touches foam with snout	5.03	1.248	4.37	1.314	3.84	1.242	0.757
Places the whole head under foam	1.36a	0.792	3.62b	0.828	0.16a	0.786	0.008
Pressing the rear part against the wall	12.28	3.936	11.38	4.140	12.65	3.924	0.967
Avoid foam	5.28a	1.614	6.23a	1.698	11.79b	1.608	0.007
Number of events per observed minute							
Turns body 180°	1.02a	0.228	0.98a	0.240	0.25b	0.228	0.022
Turns body 90°	0.52	0.342	-0.14	0.354	0.49	0.336	0.264
Moves the head up and down	0.58	0.306	0.66	0.318	1.15	0.306	0.283
Moves the head from side to side	2.03	0.420	1.50	0.444	1.63	0.420	0.622
Moves front part side to side	0.60	0.300	0.96	0.312	0.72	0.294	0.640
Jumps	1.11a	0.456	1.61a	0.480	3.16b	0.450	0.004

The effects of breed, sex, test day, and weight in the model were not significant for all variables, except for four behaviours that showed significance in gas treatments. These behaviours were “Places the whole head under foam”, “Avoid foam”, “Turns body 180°”, and “Jumps” (Table 4). The behaviour “Turns body 90°” showed significant differences between sex, test day, and weight test day.

When comparing the three different treatments it is shown that the pigs tended to touch the foam in an earlier stage when exposed to CO_2 and Ar compared to N_2 (Figure 3). However, there was no statistically significant difference between treatments.

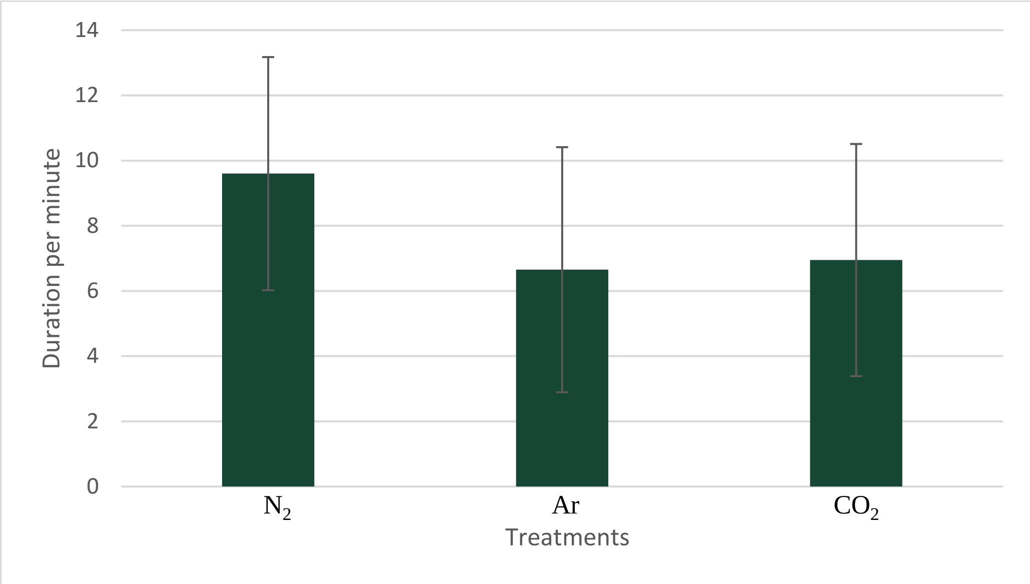

Figure 3. Differences between the three foam treatments N2, Ar and CO2 for "Duration foam start to foam touch" pigs per treatment (n=12). LSM ± SE.

Of the pigs observed, 53% (19 pigs of 36) performed the behaviour "Places the whole head under foam". There were significant differences between treatments N_2 and Ar, and between CO_2 and Ar (Figure 4). This difference shows that the pigs exposed to treatment Ar, spent longer time with their head under the foam compared to the other two treatments.

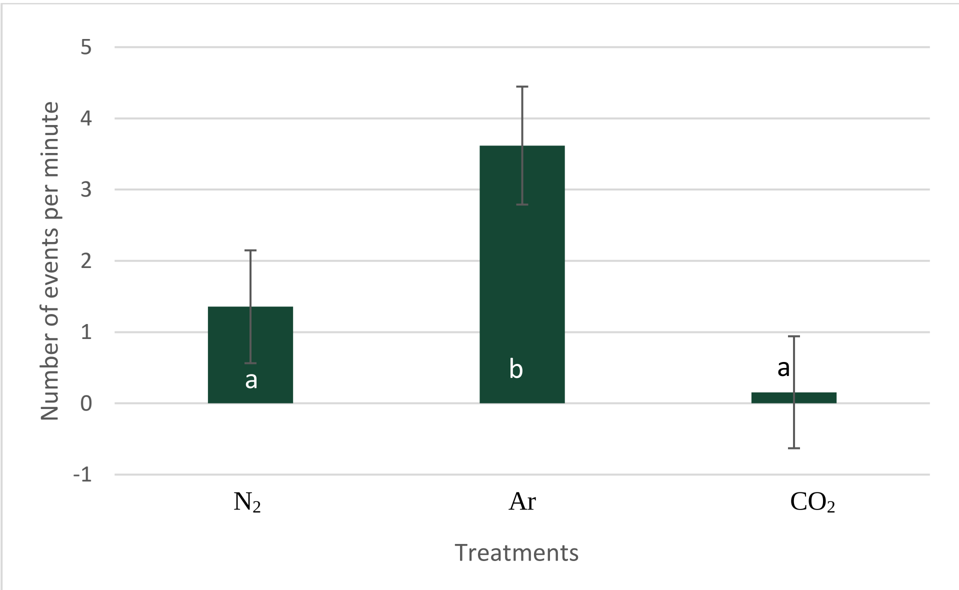

Figure 4. Differences between the three different foam treatments for the behaviour "Places the whole head under foam", pigs per treatment (n=12). LSM ± SE. Different letters indicate pairwise differences between treatments of p<0.05.

For the behaviour "Turns body 180°", 50% (18 of 36) of the pigs performed the behaviour. The results show a statistically significant difference between treatment

N_2 and CO_2, as well as between treatment Ar and CO_2 (Figure 5). The results show that the pigs turned 180° less times when exposed to CO_2 compared to the other two treatments.

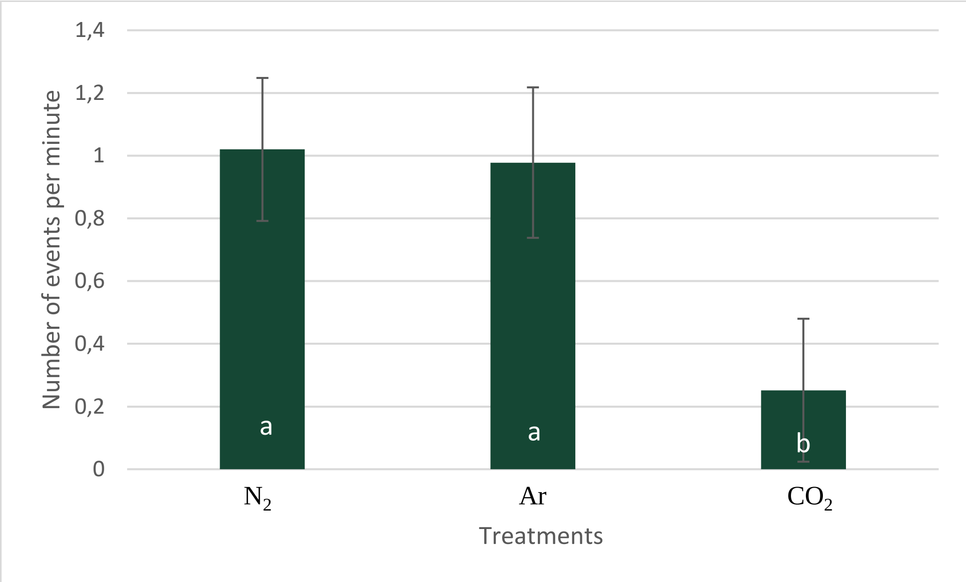

Figure 5. Differences between the three different foam treatments for the behaviour "Turns body 180°", pigs per treatment (n=12). LSM ± SE. Different letters indicate pairwise differences between treatments of p<0.05.

The percentage of pigs exhibiting the behaviour "Jumps" was 86% (31 of 36) of the pigs included in the study. There was a significant difference between the treatment CO_2 compared to the other two gas treatments (Figure 6), indicating a higher frequency of "Jumps" performed among pigs in the CO_2 treatment compared to pigs in the other two treatments.

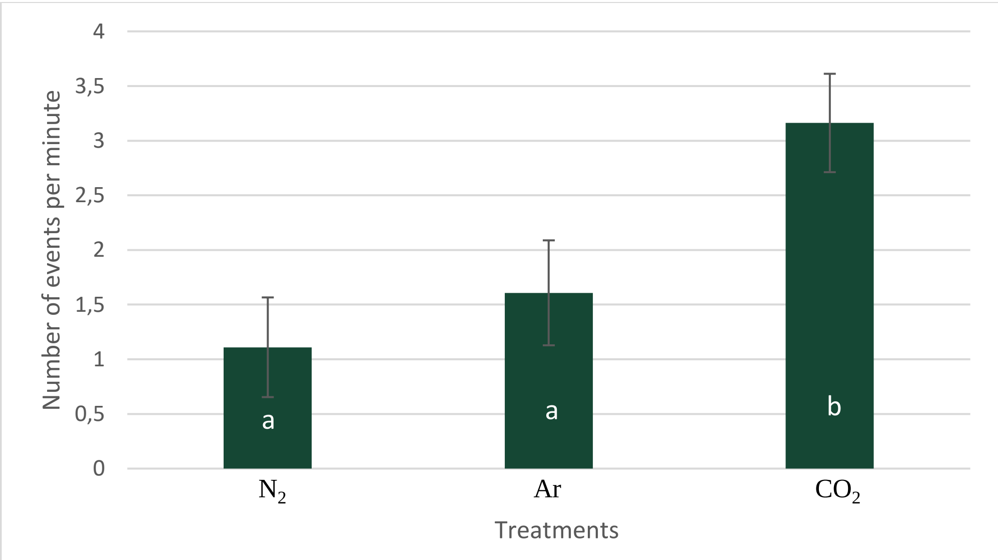

Figure 6. Differences between the three different foam treatments for the behaviour "Jumps", pigs per treatment (n=12). LSM ± SE. Different letters indicate pairwise differences between treatments of p<0.05.

The behaviour "Avoid foam" was performed by 94% (34 of 36) of the pigs in the study. The differences between gas treatments and the behaviour "Avoid foam" indicates in a longer duration of the behaviour "Avoid foam" among pigs exposed to CO_2 compared to pigs exposed to N_2 or Ar (Figure 7).

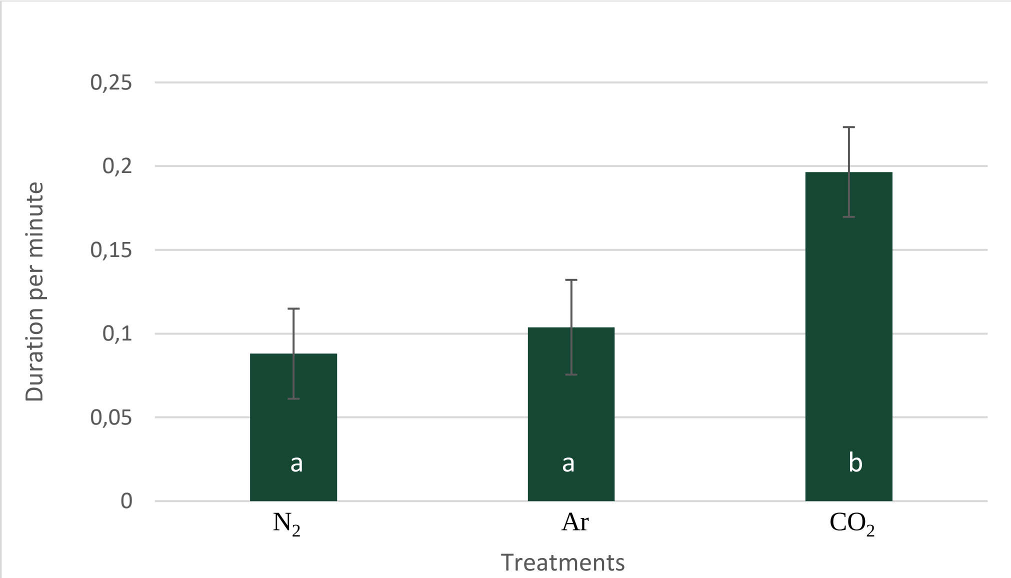

Figure 7. Differences between the three different foam treatments for the behaviour "Avoid foam", pigs per treatment (n=12). LSM ± SE. Different letters indicate pairwise differences between treatments of p<0.05.

There is a comparison between the three gas treatments and the duration from foam start to the first registration of the behaviour "Jumps" (Figure 8). Shown by the results it goes faster for the pigs exposed to CO_2 to perform the behaviour compared with the other two treatments.

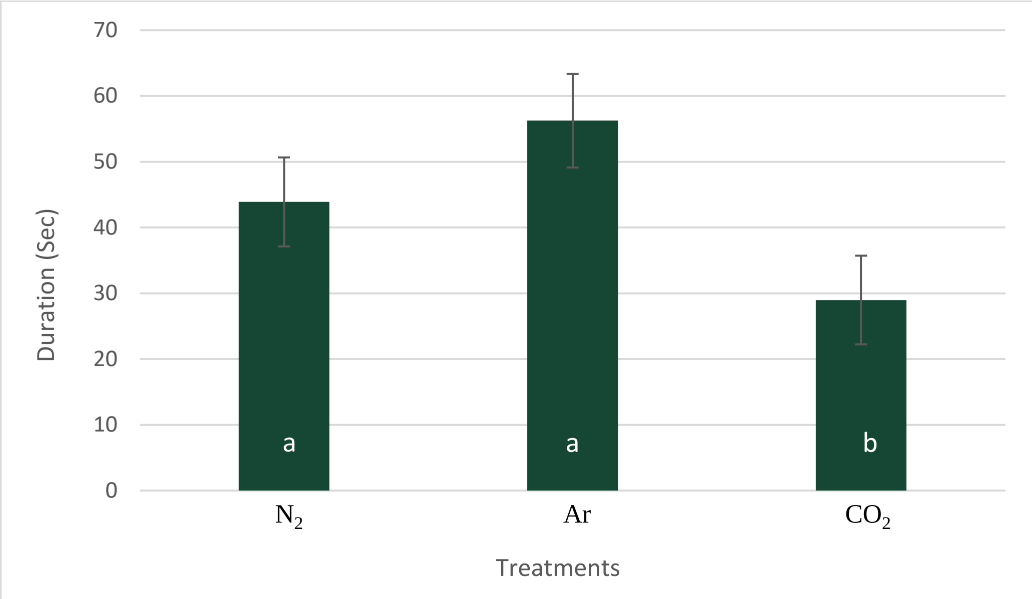

Figure 8. Differences between the three different foam treatments for duration from foam start to first performed jump, pigs per treatment (n=12). LSM ± SE. Different letters indicate pairwise differences between treatments of p<0.05.

Duration from foam start to first registered event of behaviour avoid foam was looked at (Figure 9). The results show that it took longer time for the pigs to do the avoidance behaviour when exposed to N_2 or Ar compared to when they were exposed to CO_2.

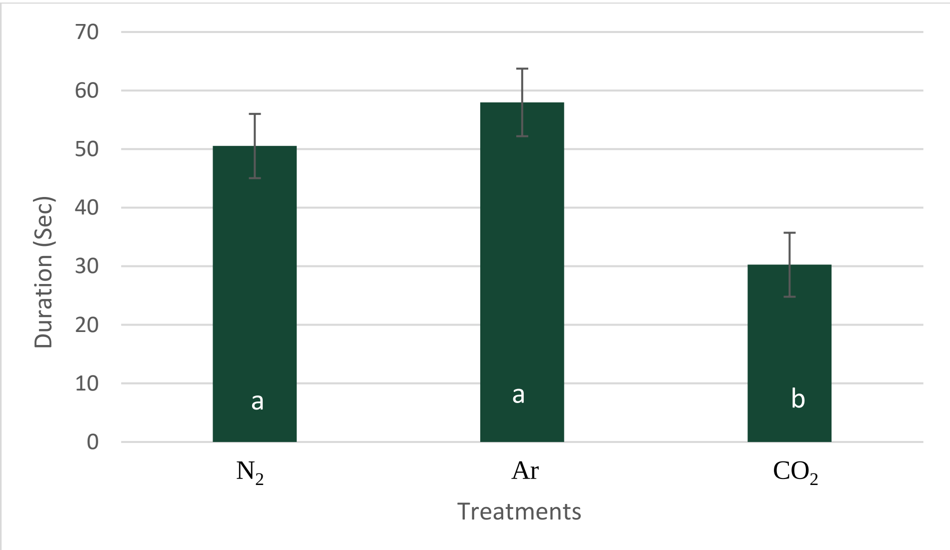

Figure 9. Differences between the three different foam treatments for duration from foam start to first performed avoid foam, pigs per treatment (n=12). LSM ± SE. Different letters indicate pairwise differences between treatments of p<0.05.

5. Discussion

This study aimed to investigate the behavioural response of pigs at gas stunning with high-expansion foam. The primary focus was to study the differences in pigs' immediate reaction to the foam when exposed to nitrogen, argon, and carbon dioxide gas. The key findings of this study that will be elaborated in the discussion include an earlier and higher performance of the escape behaviours "Jumps" and "Avoid foam", in the CO_2 treatments. Additionally, exploratory behaviours were more frequently observed in N_2 and Ar treatments.

The duration from the initiation of foam production until the pigs exhibited contact with the foam, shows no clear differences between the three gas treatments. Although the differences between treatments were small, the graphical results in Figure 3 illustrate an extended duration for pigs to engage with the foam when exposed to N_2 compared to the other two treatments. Although the variance in response times among the different gas treatments appears inconspicuous, the result is noteworthy.

The differences in duration from foam start to first touch of foam between the three different treatments may be explained by the inherent freezing behaviour exhibited by pigs when introduced to a novel situation. Blackshaw et al. (1998) describes that the initial response of pigs to unfamiliar situations involves a temporary period of immobility that proceeds with avoidant behaviour. The freeze response is assumed to occur because the pig needs to orient itself and then subsequently evaluate whether to keep the freezing position or if the pig should avoid the situation thru escape behaviours (Blackshaw et al. 1998). The results in this study may indicate that the pig's response may have a stronger connection to the natural behaviour in unfamiliar situations rather than to different gas treatments.

The behaviour "Puts whole head under foam" was performed more frequently among pigs in the N_2 and Ar treatments compared to CO_2. The trend of pigs to put the whole head under the foam, particularly as a reaction when exposed to argon, may be due to the lower aversiveness associated with this gas, as indicated by Dalmau et al. (2010b). Dalmau et al. (2010b) reported that a lower number of pigs showed escape attempts and gasping when exposed to 90% argon compared to the pigs exposed to a mixture of N_2 and CO_2. Dalmau et al (2010b) results agree with Raj & Gregory (1995), who similarly showed in their study that a concentration of 90% argon by volume in atmospheric air is non-aversive for slaughter pigs. The pigs did not express the behaviour "puts whole head under foam" to the same extent as with the N_2 treatments. Lindahl et al. (2020) observed exploratory behaviours in pigs at foam start and concluded that there was no difference between N_2 and air treatment. The Lindahl et al. (2020) study also observed that when the foam level increased, the pigs actively tried to keep their head above the foam to prevent contact with their eyes and snout. In this study, it is shown that the pigs tended to put their head under the foam in the different treatments in the beginning and middle of the observations. This indicates in exploratory behaviours in the pigs and that they seemed to be courios about the new environement. It is also shown that the pigs tended to stretch their head and snout away from the foam as the foam level increased and started to cover a bigger part of the pig. That the pigs stretches their head and snout away from the foam when the foam level increased indicates that the pigs have a feeling of escape behaviours and that the foam increases a feeling of discomfort in the pigs.

The behaviours that represented movement in the box did not show any significant differences between gas treatments in the result except for "Turns body 180°", where pigs in the N_2 and Ar treatments performed this behaviour to a larger extent than pigs in the CO_2 treatment. Even though there were no clear differences in the movement behaviours, a significant part of the pigs in the study, independent of what treatment they were exposed to, showed a considerable amount of movement. Movements in pigs may potentially be driven by the urge of the pigs to perform exploratory behaviour (Studnitz et al. 2007; Jensen 2017). Given that pigs are curious animals, the increased frequency of movements in the N_2 and Ar treatments may imply that the animals did not experience distress or discomfort. Instead, their choice to explore the environment suggests a level of comfort or curiosity, contrasting with the more restrained response observed in the CO_2 treatment (Wood-Gush & Vestergaard 1989). External stimuli, such as encountering and investigating something new can stimulate this curiosity (Wood-Gush & Vestergaard 1989). The low occurrence of the behaviour in the CO_2 treatment may indicate a freezing or discomfort reaction. As mentioned earlier, pigs tend to restrict their movements when they are exposed to novel situations (Blackshaw et al. 1998).

Due to the movement performed by the pigs after the initiation of foam generation, it could be observed that some foam got destroyed. The movement and the consequently destroyed foam would likely increase if this technique was applied with multiple pigs in the box simultaneously. That could result in an increased amount of foam getting destroyed and consequently prolonging the time for the foam to fill the box. This thought aligns with findings from a prior study by Lindahl et al. (2020), where responses of pigs exposed to a similar stunning technique were evaluated. In that study, it was observed that pig movements led to destroyed foam bubbles in the box. The authors claimed that the N_2 that was realized from the foam bubbles got mixed with the air and then reduced the O_2 concentration (Lindahl et al. 2020). In the study they also discuss the potential for further development of the stunning technique to enhance foam production capacity, thereby extending the time required to fill the box. With an increased foam filling capacity, it could be possible to further reduce the time to loss of posture and unconsciousness (Lindahl et al. 2020).

The avoidance related behaviours, specifically “Jumps” and “Avoid foam”, were most prevalent in the CO_2 treatment. Additionally, a noteworthy observation was the shorter duration from the initiation of foam to the performed behaviours. In contrast, pigs exposed to N_2 and Ar, exhibited a longer duration before showing the behaviours “Jumps” and “Avoid foam”, suggesting a comparatively lower aversive response to these gases.

Llonch et al. (2012) compared the aversion to gas mixtures with varying quantities of N_2 and CO_2, they found that aversion to CO_2 gas mixtures was higher than that to atmospheric air. However, the aversion was lower in mixes with N_2 and CO_2 compared to high-concentration CO_2 gas. Furthermore, Dalmau et al. (2010b), demonstrated that a higher concentration of CO_2 correlated with increased aversion, as evidenced by escape and retreat attempts. While aversion was also observed in pigs exposed to Ar, it was lower compared to gas mixtures with N_2 and CO_2 (Dalmau et al. 2010b). The same study also showed that more escape attempts occurred after a more prolonged exposure time to gas mixtures with Ar and CO_2 combined, compared to a gas mixture with 80 to 90% CO_2 (Dalmau et al. 2010b). It is important to note that the observations in the current study ceased when the foam covered the animal and reached the top of the box. Previous studies have shown an association between discomfort and escape attempts when the foam covered the pig's head and snout (Lindahl et al. 2020; Söderquist et al. 2023). In this study the behaviours “Jumps” and “Avoid foam”, comparable to the studies mentioned above, escape behaviours was observed to be performed primarily when the foam began to rise and cover the pig’s snout and head.

The number of pigs studied in this master thesis was in total only 36 pigs divided into three treatments, resulting in a sample size of 12 pigs per treatment. Due to the limited sample size, the identification of statistically significant differences in observed behaviours was constrained. To achieve a more comprehensive understanding and potential for better statistical results an increase in the number of pigs within the study would be desirable. However, it is important to consider the responsible use of animals in scientific research. That involves the 3Rs, aiming to minimize the number of animals used, reduce potential harm, and refine methodologies to enhance animal welfare. Therefore, it is crucial to consider the responsible use of animals (European Commission, 2019).

Behaviours that were not observed in greater occurrence and did not get analysed were “Turns the whole body around 360 degrees” and “Moves the rear part from side to side”. A reason for the low occurrence of “Turns the whole body around 360 degrees” and “Moves the rear part from side to side” could be explained by a low motivation of the pigs to perform these two behaviours.

When the video material was analysed in this study there were some challenges. The presence of illuminating lamps introduced undesired glare into the camera, obstructing the clarity of the recorded footage. This glare created visual barriers, making it difficult to see and identify specific behaviours exhibited by the pigs. There were also inconsistencies in the positioning of the camera across the different video recordings. The variation in camera placement resulted in differing perspectives and angles, impacting the overall visibility of certain behaviours. Because of these factors, it could affect the reliability of the behavioural data obtained. A consistent approach was maintained throughout the assessment of all videos, and the observation was done under consistent evaluation carried out by the same person. During the data collection, the observer also remained blinded to the treatments that the pigs were exposed to. This ensures a degree of comparability among the videos.

Commercial slaughter has several challenges considering animal welfare, especially the use of carbon dioxide. EFSA (2004), reported in 2004 that carbon dioxide is contributing to animal welfare issues and therefore there is a high need for further research to develop new methods. Even though there is animal welfare issues with this stunning method it is at the same time beneficial for animal welfare when it comes to the minimal handling of the pigs (Steiner et al 2019). Even though EFSA (2004) considers carbon dioxide as a stunning method to be a big welfare problem, the method is still one of the most common commercial methods used.

Stunning with nitrogen in high-expansion foam systems can be a promising technique for better animal welfare related to different stunning methods. By evaluating new stunning methods for pigs an ideal situation would be to find a method that can reduce pain, distress, and anxiety. Finding a more ideal stunning method would not only be better from an ethical perspective but it would also have a wider impact on the regulations and consumer expectations. A stunning method that won’t cause aversive behaviours in pigs would promote sustainable and responsible handling of pigs at slaughter.

There is a need to find new stunning methods and techniques that can be used in commercial slaughter. This study can be a part of the work towards better animal welfare in pigs when stunned at slaughter. The results from this master thesis can be a help in evaluating nitrogen, argon, and carbon dioxide as stunning gases for pigs in high-expansion foam.

There is a need for further research on this stunning technique to be able to use it in commercial slaughter. Further research should focus on evaluating how well this technique could work in commercial use. Further research could also focus on meat quality and whether meat quality can be affected negatively or positively by stunning with high-expansion foam.

6. Conclusion

In this master thesis, there is support that different gas treatments affect the pig's behaviour response to the gas-filled foam differently. It is shown that pigs show a higher frequency of escape behaviours when exposed to carbon dioxide compared to nitrogen and argon. These findings alines with other studies that have investigated stunning of pigs in high-expansion foam. It can also be concluded that pigs have a higher motivation to explore the foam and show more movement when exposed to nitrogen and argon compared to carbon dioxide. These results indicate that carbon dioxide has a higher negative impact on the welfare of the pigs during stunning with high-expansion foam compared to nitrogen or argon.

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Popular science summary

There are specific regulations that mandate stunning before slaughter of animals. The regulations focus on using the best methods to prevent animals from feeling pain or distress during the slaughter process. Stunning is supposed to ensure that animals remain unconscious during the entire bleeding process and to minimize unnecessary suffering. It is crucial that the stunning and slaughter method used induces death without causing avoidable fear, anxiety, suffering, distress, or pain.

Carbon dioxide is one of the most common stunning methods for stunning pigs in commercial slaughter across Europe, followed by electrical stunning. The existing stunning methods are suggested to cause pain and distress and therefore the need for alternative techniques that prioritize animal welfare is needed.

This master thesis is part of a broader study to evaluate behavioural and physiological reactions of pigs when stunned using nitrogen, argon, and carbon dioxide high-expansion foam. The main aim of this project was to study the differences in pigs' immediate reaction to the high-expansion foam when stunned with nitrogen, argon, and carbon dioxide gas.

The study was done on a total of 36 pigs divided into three different treatments. The pigs were stunned individually in a specific stunning box. The pigs were exposed to either nitrogen-, argon-, or carbon dioxide high-expansion foam in the different treatments.

The results of the study show a difference in movement behaviours and escape behaviours between the three treatments. The conclusion is that pigs show a higher frequency of escape behaviours when they are exposed to carbon dioxide. It can also be concluded that the pigs had a higher motivation to explore and show movement behaviours when they were exposed to nitrogen and argon compared to carbon dioxide. This indicated that nitrogen and argon are better from an animal welfare perspective because of the findings between the different gases.

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