Euthanizing single or pairs of sick, injured or weak piglets with nitrogen foam

– effects on animal welfare

Avlivning av en eller två sjuka, skadade eller svaga smågrisar med kvävgasskum – effekt på djurvälfärd

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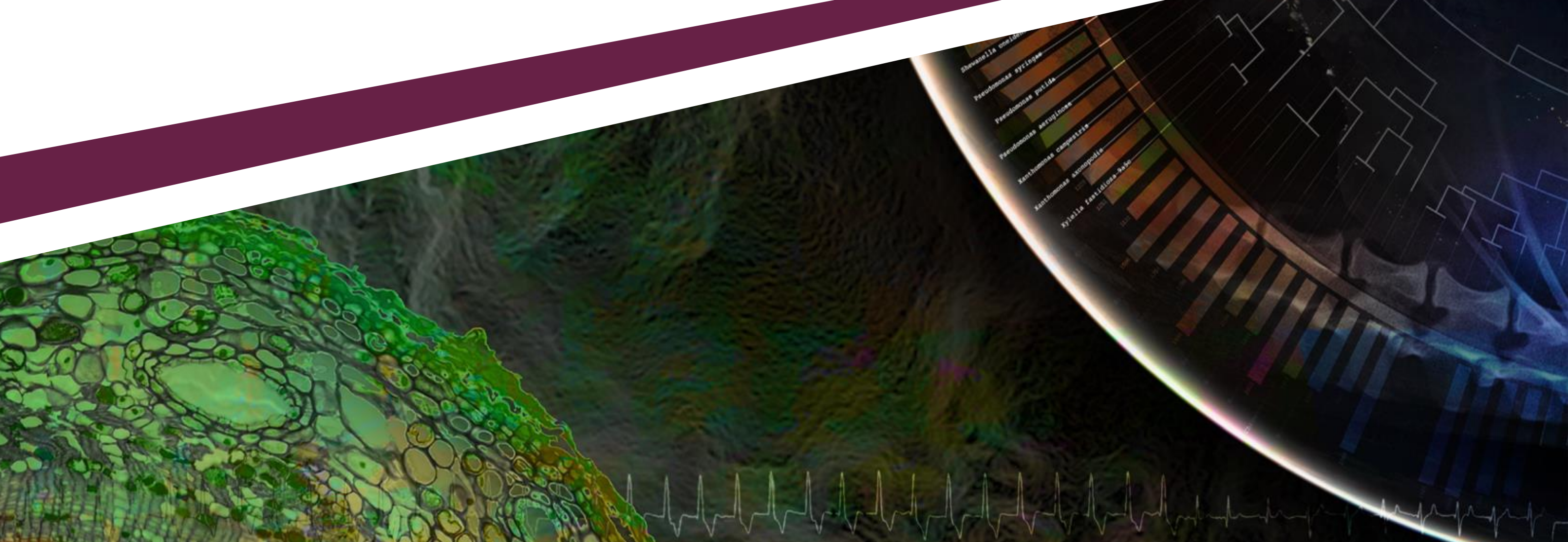

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Abstract

In pig production, it is inevitable having to euthanize some piglets due to sickness, injuries or weakness. The methods available for euthanizing piglets that are 0-42 days are blunt force trauma and captive bolt followed by sticking. These are physical methods that could induce psychological stress in the farmer and prolong the piglets' suffering if the farmer is reluctant to use the available method. The aim of this thesis was to evaluate the effectiveness of nitrogen foam for euthanizing single and pairs of piglets that are sick, injured or weak. In addition, welfare aspects were assessed to evaluate if nitrogen foam could be a more humane euthanasia method compared to traditional methods. A total of 21 crossbreed piglets of Yorkshire*Dutch Yorkshire dams and Hampshire sires were used in this study. Age ranged from 4–38 days with a weight ranging from 1–12 kg. The piglets were either euthanized individually (treatment 1) or with a companion piglet (treatment 2). The initial response, when exposed to the foam, was for piglets to flinch and/or retreat, followed by exploration of the foam. The exploration of foam at the beginning of foam production indicated that the piglets were not fearful of the foam itself and that the nitrogen gas did not cause discomfort. The explorative behaviour decreased as foam levels began to increase and instead the piglets started to avoid the foam by raising their snout above foam level. When the piglets began to be covered with foam, escape attempts increased. There was a significant difference in vocalisation between treatments, where single piglets vocalized more than pairs, but no differences were seen in activity or escape attempts. All piglets were successfully euthanized and no reflexes or regaining of consciousness was seen at observations after 12 minutes of being submerged in foam. Approximately half of the piglets had no heartbeats when taken out of the box and presumed to be already dead. The results prove nitrogen foam to effectively euthanize pairs of piglets and induce moderate levels of aversive behaviours. Nitrogen foam euthanasia could be an important alternative for on-farm euthanasia, especially for small piglets over 14 days. However, research on how the aversiveness towards the foam can be reduced is needed before the method can be recommended for on-farm use.

Sammanfattning

Inom grisproduktionen är det oundvikligt att behöva avliva vissa smågrisar på grund av sjukdom, skador eller svaghet. Metoderna som används är slag mot bakhuvudet och penetrerande bultpistol följt av avblodning, vilket är fysiska metoder som kan framkalla psykologisk stress hos utövaren och även kan förlänga smågrisarnas lidande om djurskötaren är ovillig att använda de tillgängliga metoderna. Syftet med avhandlingen var att utvärdera effektiviteten av kvävgasskum för att avliva sjuka, skadade eller svaga smågrisar ensamma eller i par. Välfärdsaspekter bedömdes för att utvärdera om kvävgasskum kan vara en mer human avlivningsmetod jämfört med traditionella metoder. Totalt användes 21 korsningsgrisar av Yorkshire*x*Dutch Yorkshiresuggor och Hampshiregaltar i studien. Åldern varierade mellan 4–38 dagar med en vikt mellan 1–12 kg. Smågrisarna avlivades antingen individuellt (behandling 1) eller med en annan artfrände (behandling 2). Initialt ryckte smågrisarna till och/eller backade när skumgeneratorn startade, följt av undersökning av skummet med trynet. Utforskningen av skummet i början av skumproduktionen indikerade att smågrisarna inte var rädda för själva skummet och att kvävgasen inte orsakade obehag. Det utforskande beteendet minskade när skumnivåerna ökade och i stället började smågrisarna att undvika skummet genom att höja trynet över skumnivån. När smågrisarna började täckas med skum ökade flyktförsöken. Det var en signifikant skillnad i vokalisering mellan behandlingar där ensamma smågrisar grymtade mer än par, men inga skillnader sågs i aktivitet eller flyktförsök. Alla smågrisar avlivades framgångsrikt och inga reflexer eller andra tecken på medvetande sågs vid kontroll efter att smågrisarna varit i täckta av skum i 12 minuter. Ungefär hälften av smågrisarna hade inga hjärtslag efter de togs ut ur boxen och var antagligen redan döda. Resultaten visar att kvävgasskum effektivt avlivar par av smågrisar och framkallar måttligt aversivt beteende. Avlivning med kvävgasskum kan vara ett möjligt alternativ till traditionella metoder på gårdar, särskilt för små smågrisar över 14 dagar. Dock krävs studier på hur grisarnas aversiva reaktion på skummet kan minskas innan metoden kan rekommenderas för användning på gårdar.

Populärvetenskaplig sammanfattning

Efterfrågan på svenskt griskött och förväntningarna på hög djurvälfärd ökar i Sverige, vilket ställer krav på att grisarna ska behandlas bra både under produktionstiden och vid avlivning. Det är oundvikligt att lantbrukare behöver avliva smågrisar på grund av att de är sjuka, skadade eller svaga under uppfödningsperioden och därför behövs utveckling av humana avlivningsmetoder som kan utföras på gård.

I dagsläget används slag mot huvudet som avlivningsmetod för smågrisar som är under 14 dagar och som väger mindre än 5 kg och bultpistol följt av avblodning för smågrisar som är äldre eller större än så. Dessa metoder kan anses osmakliga för konsumenter och obehagliga för lantbrukaren att utföra. Metoderna är inte heller optimala ur ett djurvälfärdsperspektiv då effektiviteten är starkt beroende av utförarens skicklighet.

Kvävgas har föreslagits vara en alternativ bedövnings- eller avlivningsmetod. Det finns ett fåtal tidigare studier som undersökt kvävgas bundet i skum som bedövningsmetod för grisar. Där har forskarna sett att kvävgasskummet effektivt trycker undan syret i en sluten låda vilket skapar en stabil, näst intill syrefri miljö som bedövar grisarna genom syrebrist.

Syftet med den här studien var att undersöka hur effektiv kvävgasskum är som avlivningsmetod för en eller två sjuka, skadade eller svaga smågrisar och även undersökta potentiella välfärdsaspekter.

Studien utfördes på Lövsta forskningscentrum i Uppsala där 21 smågrisar avlivades med kvävgas-skum i en låda. Smågrisarna avlivades antingen ensamma eller i par. Experimentet spelades in och beteendeobservationer utfördes i efterhand för att undersöka hur små-grisarna reagerade på kvävgas-skummet.

Resultatet från studien visade att smågrisarna till en början blev skrämda när skumgeneratorn startade men sedan började utforska skummet. När skumnivån i lådan började bli hög försökte de undvika skummet och fly undan det.

Det fanns inga större skillnader mellan smågrisarnas beteenden i de två behandlingarna. Smågrisar som avlivades i par vokaliserade mindre än smågrisar som avlivades ensamma, men inga skillnader i t.ex. flyktförsök sågs.

Alla smågrisarna avlivades effektivt utan att visa några tecken på att återfå medvetandet när de tagits ur lådan. Kvävgasskum bedöms därför vara en effektiv avlivningsmetod. Dock krävs fler studier på hur man kan minska smågrisarnas upplevda stress vid höga skumnivåer

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1. Introduction

In 2019, the production of pork meat in Sweden amounted to approximately 240 000 ton and constituted 16 % of the total value of the animal production (Öberg 2020). Since 2014, and especially after the drought in 2018 and the pandemic 2020, the demand for domestic pork meat has increased at the same time as the production of pork meat has decreased (Öberg 2020). Animal production has been under harsh scrutiny in the media recently with reports of animal cruelty and neglect which has led to outcries among consumers, demanding a more humane production and slaughter. A humane euthanasia method should induce a rapid loss of consciousness followed by brain death as well as minimize the distress experienced by the animal before loss of consciousness (AVMA et al. 2020). To meet the demands of the consumers, the animal production systems need to shift towards more sustainable and humane, management systems.

A management aspect that is unpleasant for both the society to witness and the farmer to exert, is the killing of piglets that are sick, injured or weak. In pig production, having to euthanize piglets is inevitable especially during the first few days after birth. In Sweden, the average piglet mortality from birth to weaning was 17.7 % year 2019 (Gård & Djurhälsan n.d.). Euthanasia is most often performed by applying manual blunt force trauma to the piglet's head, which is an accepted method of euthanasia of single piglets up to 14 days old according to the Swedish Board of Agriculture's regulations and general advice on slaughter and other killing of animals (9 cap. 8 § SJVFS 2020:22 case no. L 22). In the European Council Regulation 1099/2009 the limiting factor for using blunt force trauma is weight and states that piglets up to 5 kg can be euthanized with this method. This means that piglets that are either over 14 days or that are under 14 days but weighing more than 5 kg cannot be euthanized with blunt force trauma. Blunt force trauma is usually performed by striking the piglets head against the floor or wall or with a hard object such as a hammer (Dalla Costa et al. 2019). This method is considered to be a fast, effective and cheap euthanasia method if performed correctly (Dalla Costa et al.

2020). However, this method relies upon the farmers' ability to perform it accurately, if unconsciousness is not achieved, the piglet risks suffering pain and fear (Dalla Costa et al. 2020). Manual euthanasia methods such as blunt force trauma can also be unpleasant and cause psychological stress for the farmer and studies have found that stock people using this technique would prefer another method (Rault et al. 2017; Dalla Costa et al. 2019). Further, the unpleasantness of performing the method could make farmers reluctant to cull piglets which prolongs the animal´s suffering (Rault et al. 2017).

For piglets over 14 days, blunt force trauma is no longer permitted as a euthanasia method, instead, a captive bolt gun (7 cap. 13 § SJVFS 2020:22 case no. L 22) is most often used as a stunning method followed by sticking. This method is however not ideal and European Food Safety and Authority (EFSA) has identified welfare hazards with the captive bolt. It requires the animal to be restrained which induces pain and fear, and there is a risk of insufficient stunning due to incorrect shooting position or inappropriate cartridge or power (EFSA AHAW Panel et al. 2020b). An insufficient stunning could be due to staff being improperly trained, fatigue, poor restraint or faulty bolt gun, which leads to severe pain and fear for the piglet. Piglets that are approximately 14 days old are often too small for the usage of a captive bolt but are too old for blunt force trauma which leaves no good option for euthanasia. Therefore, an alternative method is needed.

Controlled atmosphere stunning (CAS) could be a more humane euthanasia method for pigs since it enables pigs to remain in groups when stunned and requires no restraint (Raj & Gregory 1996; Steiner et al. 2019). Carbon dioxide (CO_2) is commonly used as a stunning method in the abattoir but is usually not used on farms. Carbon dioxide has been criticized for inducing aversive behaviour before unconsciousness (Raj & Gregory 1996; Steiner et al. 2019; EFSA AHAW Panel et al. 2020b). When exposed to high concentration CO_2, unconsciousness is induced by metabolic acidosis which lowers the pH levels in the cerebrospinal fluid and inhibits spontaneous brain activity (Mota-Rojas et al. 2012). CO_2 binds to chemoreceptors and irritant receptors in the mucous membranes, which causes respiratory distress, expressed through air-hunger and breathlessness (Steiner et al. 2019; EFSA AHAW Panel et al. 2020a). For these reasons, EFSA stated in 2004 that CO_2 euthanasia should be phased out and is therefore, not considered as a viable CAS alternative for euthanizing piglets on-farm in this study.

Instead, EFSA (2004; 2020a) recommend future scientific research to evaluate the welfare implications of using non-aversive gasses which induce hypoxia to stun or euthanize pigs. Inert gasses are an approved euthanasia method for pigs according to the European Council Regulation 1099/2009 but are not approved in Swedish regulations. The advantage of inert gasses is that they do not react to other substances, are odour-, taste- and colourless and most of all, do not irritate the mucus membrane or airway passages (Raj & Gregory 1995; Dalmau et al. 2010). Nitrogen is an inert gas that naturally occurs abundantly in the atmosphere and is, therefore, readily accessible and cheap to produce. Mammals also have no intrapulmonary chemoreceptors and irritant receptors for high concentrations of nitrogen (Manning & Schwartzstein 1995; Dalmau et al. 2010). Because of these properties, nitrogen is a potential alternative for euthanasia of piglets on-farm.

A high concentration of nitrogen creates an anoxic atmosphere which induces hypoxia in mammals. Studies have shown that nitrogen induces less aversive behaviour than CO_2 (Llonch et al. 2012a; b; c; Detotto et al. 2019). However, nitrogen has a lower density than atmospheric air which makes it challenging to contain (Dalmau et al. 2010). When pure nitrogen gas was supplied to a pit for 90 minutes, only a 6 % oxygen level could be achieved 60 cm above the floor, which is insufficient to effectively stun an animal (Dalmau et al. 2010). A doctoral thesis by Pöhlmann (2018) used high-expansion foam filled with nitrogen to stun slaughter-ready pigs. The study showed that exposure to nitrogen foam for 3.5 minutes did not result in secure unconsciousness or insensibility. This could be due to pigs breaking the bubbles, making it difficult to submerge the pig in foam and allowing mixing of oxygen (Sindhøj et al. 2021). Therefore, a closed container system is needed to contain the gas and sustain an oxygen level below 2 % (Steiner et al. 2019; Sindhøj et al. 2021). Lindahl et al. (2020) used high-expansion foam filled with nitrogen to quickly create a stable anoxic environment (0.02 % oxygen) in a closed top box to stun 9 weeks old pigs. The foam effectively purged the box of oxygen and avoided mixing with oxygen. No differences of aversive behaviour were found comparing the responses to nitrogen-filled foam to air-filled foam, where the pigs avoided putting the snout in the foam and performed some escaped attempts in both treatments as the foam level increased. In contrast, at the start of foam production, the pigs initially explored the foam. This indicates that the aversive behaviour was performed as a response to being covered with foam rather than to the nitrogen (Lindahl et al. 2020). After 5 minutes in the anoxic box, the pigs were taken out and either assessed as being in deep unconsciousness or dead.

Previous studies have studied the effectiveness of nitrogen foam on single pigs, but no study has been found to investigate the effectiveness of the method when euthanising several pigs. Lindahl et al. (2020) proposed group treatments with nitrogen foam as potential future research in order to evaluate its effectiveness. In a thesis by Söderquist (2020), 9-weeks pigs were exposed to air-filled foam either alone or together with a familiar or an unfamiliar conspecific. The results showed that pairs of pigs expressed fewer escape attempts than pigs that were alone. Pigs in the foam treatment also spent more time close to and in physical contact with each other compared to the control treatment in three different intervals. Söderquist (2020) concludes that this indicates a positive effect of a companion pig by reducing the aversive behaviour towards the foam.

On-farm, several piglets might need to be euthanised at the same time and it would be economically unsustainable to euthanize each piglet one at a time with nitrogen foam, as it would take up too much of the farmer’s time. Further, it could lead to welfare problems if injured or sick animals could not be euthanized at once and would have to wait. This is also true in the slaughter process where several pigs need to be stunned together to have a sufficient flow of pigs. When euthanizing pigs together, several welfare aspects need to be considered. If the pigs differ in weight or health, they could lose unconsciousness and start convulsions at different times which could possibly affect the other pig physically or psychologically. These aspects need to be evaluated before nitrogen foam can be approved as a euthanasia method in Sweden.

Since EFSA:s first recommendation in 2004, very little research has been done evaluating alternative euthanasia methods (Sindhøj et al. 2021). The aim of this thesis is therefore to investigate the effectiveness and aversiveness of using nitrogen-filled foam as a euthanasia method for single or pairs of sick, injured or weak piglets. The results of this study will contribute to important knowledge on nitrogen foam to base further studies on and help to evaluate if this may be a more humane euthanasia method.

The following hypotheses have been stated based on previous research and knowledge:

- Piglets euthanized together will show fewer escape attempts than piglets euthanized alone, since the piglets will have a calming effect on each other.

- The time until loss of posture occurs will be longer when euthanizing piglets together compared to alone, since it is expected to take longer before the foam presses the oxygen out of the box, due to animals breaking the foam when moving.
- The nitrogen foam method will be effective in consistently euthanising the piglets in both treatments.

To answer these hypotheses, the following questions have been formulated:
- How does the euthanasia process function when euthanizing single or pairs piglets with nitrogen foam?
- Is the same euthanasia quality observed when pairs of piglets are euthanised together as observed when euthanizing a single piglet?
- How is the piglet's behaviour affected and how do the piglets affect each other when placed together in the euthanasia box?
- Is 12 minutes of exposure to nitrogen foam enough time to ensure the piglets die and do not regain consciousness?

2. Material and Method

2.1. Ethical Permit

This animal experiment has been approved by the ethical committee in Uppsala, Sweden (ref.no. 5.8.18-01572/2020). The experiment was performed in accordance with the ethical permit and the 3 R:s.

2.2. Animals and Housing

The study was conducted in the pig facilities of the Swedish Livestock Research Centre, Swedish University of Agricultural Sciences, in Uppsala, Sweden. The pig farm is conventional with integrated production and approximately 100 sows with planned farrowing biweekly. The herd is Specific Pathogen Free. The piglets are born at the research facility in farrowing pens, where feed and water are provided ad libitum. The pens are cleaned and enriched with chopped straw daily. At birth all piglets are weighed, sex is recorded, and all piglets are given an individual ear tattoo. After five days, ear tags and iron injections are given, and weight is recorded again. The piglets are weaned after five weeks and kept in the farrowing pen for five more weeks. If necessary, cross-fostering is implemented.

A total of 21 crossbreed piglets of Yorkshire×Dutch Yorkshire dams and Hampshire sires were used in this study. Age ranged from 4-38 days with a weight ranging from 1-12 kg (table 1). Piglets in this study had been destined for euthanasia according to the farm's standard protocol due to injuries, sickness or for production efficiency reasons. Any piglet that was in acute distress or pain was euthanised by the staff with standard methods. The piglets were born between January and April year 2021. The piglets were either euthanized alone (treatment 1) or with another conspecific (treatment 2). In treatments with two piglets, they were always unknown to each other. A total of 15 batches of piglets were conducted, 9 batches of single treatments and 6 batches of pair treatments.

Table 1. Mean value and standard deviation of age in days and weight in kilogram at culling within treatments

Variable	Treatment	Number of piglets	Mean	StDev
Age at culling (days)	1	9	14.7	9.71
	2	12	19.1	8.63
Weight at culling (kg)	1	9	3.0	2.59
	2	12	4.3	2.83

2.3. Test Equipment

The euthanasia box used in the experiments was produced by the Dutch company Anoxia, with some additional modifications made by the Research Institutes of Sweden (RISE). It was equipped with two rectangular foam generators on one short side of the box, opposite to the door, and a gas jet pulse system running along the long sides of the box to break the bubbles. Attached to the foam generators were two 50-litre bottles of compressed nitrogen (200 bar; AirLiquide gas AB, Uppsala, Sweden), reduced to 5 bar pressure per bottle. The foam was produced using water and a 3 % foam agent (Anoxia hi-ex foam mild). In order to monitor the box's environment, it was equipped with an oxygen sensor with a flow-through fluorescence-based electrode, obtained from SST-Sensing (UK), connected to a sampling pump. From this sensor, oxygen level, temperature, atmospheric pressure and time was logged and stored on an SD card. The sensor was moisture sensitive which meant it could only be started after the jet pulse system had broken the bubbles, and it took approximately 10 s before the reading stabilized.

The box had an openable lid and measured 115 cm * 95 cm * 68 cm, but was divided in half to suit the size of the piglets used in this study. This was done by placing a divider in the middle of the box, which ensured that the foam filled the part with the piglets first and then filled the other side. The area available to the piglets was therefore 57.2 cm * 95 cm * 68 cm. Red masking tape divided the floor into four equally sized squares to describe the piglet's position and movement. Transparent anti-slip tape lined the floor to minimize the risk of piglets slipping. The lid and the floor of the box were made of transparent plexiglass, which enabled cameras to be placed above and below the box. The cameras allowed recordings to be made of every batch of piglets in the experiment when exposed to the foam. A Garmin Virb Ultra 30 camera recorded below the box and a Panasonic HC-X920 camera recorded from above which also recorded sounds from inside the box with a microphone.

Figure 1. The euthanasia box interior with foam generators on the right side where the piglets were placed. Along the sides are the black jet pulse system and the floor is marked with red tape into four squares.

2.4. Experimental procedure

The piglets were taken from the farrowing pen, weighed, marked with a permanent pen (one of the piglets in treatment 2) and then put into the euthanasia box. Regardless of treatment, the procedure was the same except for single piglets not being marked. The piglets were allowed to acclimatise to the environment in the box for 2 minutes before the foam generators were started.

The foam generators filled the half of the box with the piglets first (see figure 1), then the foam spilt over to the other half until it filled the box completely. When the box was completely filled with foam, the foam production was stopped, and the jet pulse system was started for approximately 2 seconds to break the bubbles. The oxygen sensors were started, and oxygen levels were logged until the box was opened. After the piglets had remained in the box for 12 minutes in total from the start of the foam production, the lid was opened, and the piglets taken out. Immediately after taking the piglet out, an assessment of consciousness was performed following a protocol, and a stethoscope was used for detecting heartbeats and ensuring death was achieved. In case heartbeats were noted, a five-minute silence was used for ensuring that the last heartbeat was recorded and the piglets were dead. The box was cleaned thoroughly with water after every batch to minimize odour contamination.

2.4.1. Euthanasia quality assessment

Immediately after the lid was opened and the piglets were taken out, the euthanasia quality was assessed following a standardized procedure, see below. If no reflexes or signs of consciousness were seen, the piglets were declared dead 5 minutes after the last heartbeat was recorded.

The following procedure was used for assessing consciousness, which is a modified version from Lindahl et al. (2020):

1. Corneal reflex: touching the pig's cornea and checking for any movement of the eyelid (blinking).
2. Pain reflex: pricking the inner snout of the pig with a sharp-pointed metal stick and checking for any withdrawal response.
3. Gag reflex: sticking one or two fingers down the throat of the pig and checking for any movement.
4. Any kicks, body convulsions or other movements were noted.
5. Any gasping or breathing and opening/closing of the mouth was noted.

2.4.2. Behaviour registrations

Behaviour registrations of the piglets were performed analysing the video recordings according to the definitions stated in the ethogram (table 2) after the experiment was completed. One observer analysed both video recordings, from above and below the box, and listened to the vocalisation recorded by the microphone. Both video recordings were synchronized using the start of foam production to avoid registration of the same behaviour in different intervals. Due to the nature of the experiment, the observer could not be blinded to which treatment, single piglet or pair, the piglets were subjected to. The video from below was mainly used for assessing the movement of the piglet, snout position and closeness to conspecific. The video recording from above was primarily used for assessing the vocalisation, escape attempts and other behaviours not visible from below.

Behaviours were observed continuously for two minutes in total with 10 seconds intervals. Observations started 30 seconds before foam production and continued for 90 seconds after foam start. This resulted in 12 intervals in total, of which the first 3 intervals were before foam production started and interval 4-12 was after the foam production started. The social behaviours (close to conspecific, social behaviour, agonistic behaviour, climbing on conspecific) was assessed only for the treatments of two piglets. For both treatments, the time from the start of foam production to the loss of posture (LOP) was recorded as well as time to last observed muscular contraction, last severe muscular convulsion and last heartbeat.

Figure 2. View from the camera recording from above. Two piglets can be seen inside the box exploring the floor.

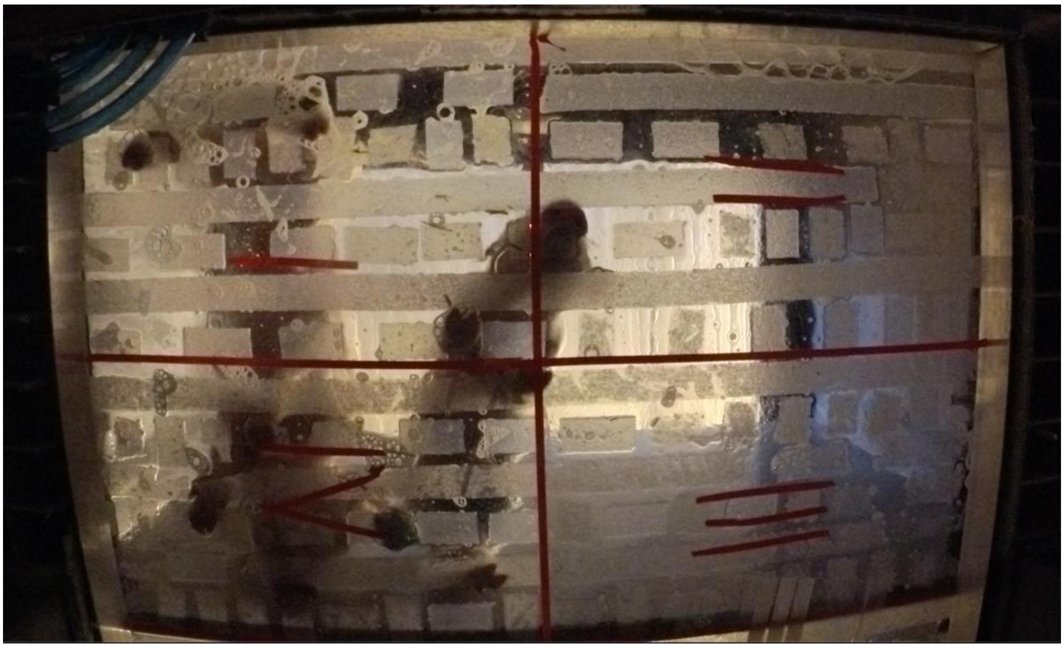

Figure 3. View from the camera recording from below the box. The same two piglets as above (figure 2) can be seen exploring the floor.

Table 2. Ethogram describing the assessed behaviours, their definitions and registration.

Behaviour	Definition	Registration
Sit	In a sitting position, on one or both buttocks, with support from front hooves on the floor but not back hooves	No. of times observed within each 10 s interval
Lay down	In a laying position with either the side or the belly in contact with the floor	No. of times observed within each 10 s interval
Stand	In a standing position with all four hooves in contact with the floor	No. of times observed within each 10 s interval
Slip	One or more hooves slip at a fast phase across the floor	No. of times observed within each 10 s interval

Behaviour	Definition	Registration
Flinch	A sudden involuntary movement or jump	No. of times observed within each 10 s interval
Retreat	Movement backwards away from sound or foam	No. of times observed within each 10 s interval
Shake	Shakes body	No. of times observed within each 10 s interval
Explore floor	Snout touches floor or air-pipes	No. of times observed within each 10 s interval
Explore wall	Snout touches wall	No. of times observed within each 10 s interval
Explore foam	Snout intentionally touches the foam	No. of times observed within each 10 s interval
Avoid foam	The pig intentionally avoids touching the foam with snout	No. of times observed within each 10 s interval
Escape attempt wall	Kicks with front or hind legs, jumps or pushes the wall	No. of times observed within each 10 s interval
V-grunt	Pig grunts	No. of times observed within each 10 s interval

Behaviour	Definition	Registration
V-squeal	Pig squeals	No. of times observed within each 10 s interval
V-scream	Pig screams.	No. of times observed within each 10 s interval
Defecate	Self-explanatory	No. of times observed within each 10 s interval
Activity	Number of lines on the floor crossed with both front legs	No. of times observed within each 10 s interval
Position in box	Square number 1, 2, 3 or 4 where both front legs are positioned	No. of squares
Loss of posture	Loses control over the body and falls to the floor, including the head, without getting back up.	Time of observation
Convulsion	Uncontrolled muscle contractions after LOP	Qualitative description of the whole process
Last severe convulsion	The last time after LOP the body moves violently, screams or squeals loudly or has muscle convulsions over a majority of its body.	Time of final observation
Last convulsion	Last time uncontrolled muscle contraction is detected after LOP	Time of final observation
Gagging	Involuntary gasping after LOP	No. of times observed within each 10 s interval

Behaviour	Definition	Registration
Gasping	Deep inhale with a wide-open mouth, may involve stretching of the neck	No. of times observed within each 10 s interval
Climbing on conspecific	One or more hooves touches conspecifics side or back or lays head on top of conspecific	No. of times observed within each 10 s interval
Agonistic behaviour	Displace conspecific by pushing or ramming him/her with head, and/or bite or chase conspecific in a non-playful manner	No. of times observed within each 10 s interval
Close to conspecific	Two or more legs placed in the same box as a conspecific stand with two or more of his/her legs or one front leg is a maximum of 10 centimetres apart from a conspecific leg.	Focal sampling with continuous recording using 10 s intervals, recorded in seconds and times expressed
Social behaviour	Interact with a conspecific in an apparent non-aggressive manner, pushing conspecific with head or shoulder, move in synchronisation, or engage in gentle snout contact	Focal sampling with continuous recording using 10 s intervals, recorded in seconds and times expressed
Foam cover	Percentage of pig´s body covered in foam during at least half the interval or more	0 %, <0 %, 50 %, >50 %, 100 %
Escape foam	The pig tries to escape the foam by jumping up over it.	No. of times observed within each 10 s interval

2.5. Statistical analyses

Data from behaviour registrations were collected in Microsoft Excel 2021 and edited before statistical analyses were performed in Minitab version 19 (Minitab, LCC, 2020). A 95 % confidential interval was used for significant results, and p-values between 0.05-0.10 was considered as a tendency for significance.

Variables that could not be considered normally distributed were converted to binary variables (0-1, the behaviour was or was not seen during the 10 s interval) which included all escape attempts, explorative behaviours, avoiding foam, slip, flinch, retreat and gagging. Non-normally distributed variables were analysed with a Chi-Square test to detect differences between treatments in each interval and graphically described as proportions of pigs performing the behaviour.

Variables that were considered normally distributed was kept as continuous (counts of the number of times the behaviour was performed in each interval) and included all vocalisations, activity, time close to and social behaviour towards conspecific. Normally distributed data were analysed with a Two-sample t-test to detect differences in the mean between treatments in each interval and graphically described using the mean of pigs performing the behaviour. Vocalisations registered in treatment 2 was divided with two and only one pig included in the analysis since it was not possible to determine which pig vocalized.

Data that was registered in time (LOP, last convulsion, last severe convulsion, time to fill box half and completely) was analysed with a Two-sample t-test to detect differences between treatments and described with descriptive analyses.

Position in box, stand, sit and laying down was only used for describing the movements of the pigs. These variables were registered as counts, except for position in box which was registered as which square the pig was in and to which it moved. They were analysed with a Two-sample t-test and plotted with a line plot comparing the two treatments. Shake, defecation, slip and gasping was not included in statistical analyses due to too few registrations. Neither was climbing on conspecific or agonistic behaviour in treatment 2 due to too few registrations.

3. Results

3.1. Box filling time

There was a significant difference in time for filling the box half (t=-5.03 p =0.000) and fully (t=-5.19 p =0.000) between treatments where it took longer to fill the box with foam in treatment 2. The average time for filling the box half was 47 s (N=9 SD=7.30 range 40-63 s) and for filling the box fully 97 s (N=9 SD=7.62 range 89-108 s) for treatment 1. The average time in treatment 2 for filling the box half was 65 s (N=12 SD=8.60 range 54-75 s) and for filling the box fully was 113 s (N=12 SD=5.87 range 104-122 s,). In interval 6, 83 % of piglets in treatment 1 and treatment 2 were covered with foam and from interval 8 all piglets were covered with foam, see figure 4. The oxygen levels were monitored during the experiments and were below 2 % in all batches.

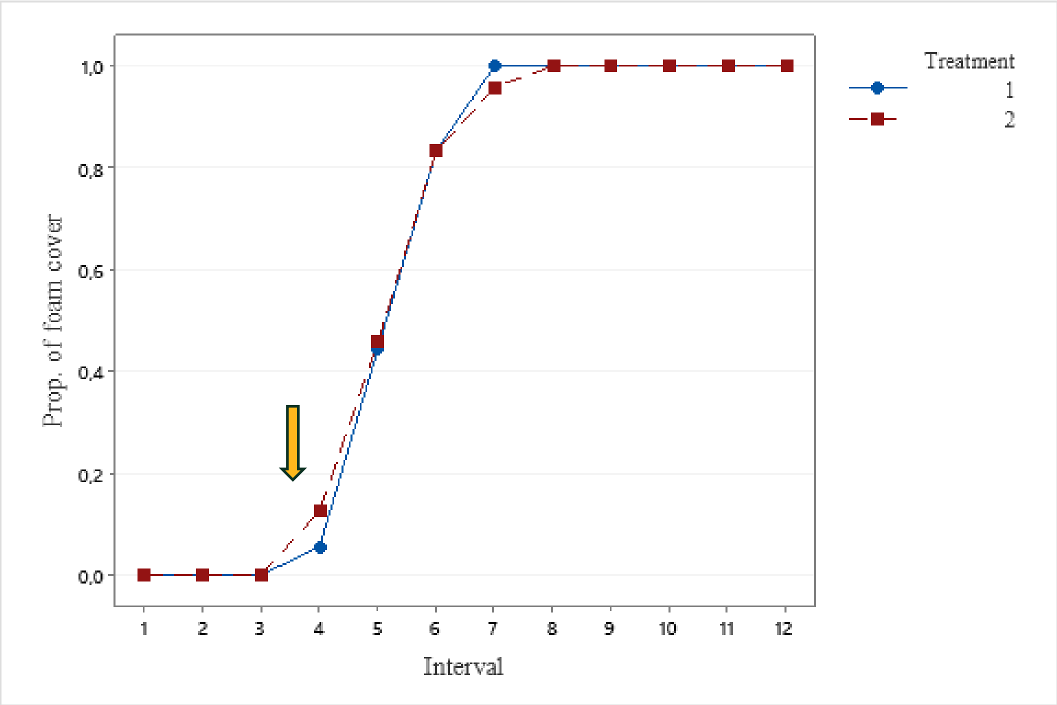

Figure 4. The proportion of foam cover defined as the proportion of the piglet´s body covered in foam in each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

3.2. Loss of posture and convulsions

There were no statistically significant differences between treatments for time to LOP (t=1.45 p=0.174) and last convulsion (t=0.64 p=0.540). The average time from foam start to LOP was 38 s (*SD*=8.86) in treatment 1 and 33 s (*SD*=6.24) in treatment 2, see table 3. The average difference in time to LOP between the piglets in the pairs in treatment 2 was 8 s (*SD*=3.5 *range*=3-13). After losing their posture, all piglets showed vigorous convulsions, often moving the legs in a running manner, stretching the torso and/or neck and a few rolled over. After a few seconds, the movements became more irregular and then turned into sporadic twitches and/or gagging. There was a numerical difference in average time from foam start to last severe convulsion between treatments, 102 s for treatment 1 and 61 s for treatment 2, but no statistical difference could be found (t=1.87 p=0.111). Of the 21 piglets, 11 had no heartbeat when taken out of the box, and 10 piglets had heartbeats when taken out of the box. For the piglets that had heartbeats, the average time from foam start to the last heartbeat was 15 minutes (*SD*=1.40).

Table 3. Average time in seconds from the start of foam production to LOP, last severe convulsion, last convulsion and last heartbeat for treatment 1 and 2.

Variable	Treatment	N	Mean	StDev	Minimum	Maximum
LOP	1	8	38.0	8.86	31	54
	2	12	32.8	6.24	23	42
Last severe	1	7	101.7	56.30	60	218
convulsion	2	12	61.3	13.74	39	88
Last	1	9	187.9	74.90	100	355
convulsion	2	12	170.9	33.15	123	219
Last	1	6	14.9	1.69	13	18
heartbeat	2	4	15.6	0.88	14	16

3.3. Piglets' movements

There were no significant differences in activity between treatments in total over all intervals (($M_{treat\ 1}$=0.9 $SD_{treat\ 1}$=1.18; $M_{treat\ 2}$=0.7 $SD_{treat\ 2}$=1.11) t=1.51 p=0.132)). After foam production started at interval 4, an increase in activity could be seen, which cumulated in interval 6 and then quickly decreased (figure 5). The mean activity for both treatments in the 30 s after foam production started (interval 4-6) is significantly higher (t=-5.77 p=0.000) than the 30 s before foam production (interval 1-3), 20 (SD=1.26) and 0.8 (SD=0.94) lines crossed respectively. Piglets in treatment 2 tended to have a lower activity in the third 30 s interval (interval 7-9) compared to piglets in treatment 1, 0.2 (SD=0.58) and 0.6 (SD=1.0) respectively (t=1.89 p=0.066).

The decrease in activity is due to pigs losing their posture in interval 8, seen in figure 6 which presents the proportion of pigs laying down. The activity seen in interval 8 and 9 (figure 5) is due to one pig falling and standing up as well as moving between squares which results in registrations of activity as well as laying down. From interval 10 and forward, no pig showed any activity and were all lying down. However, all pigs had convulsions after LOP which is not registered in the activity variable.

In interval 4 when foam generators started, 48 % of all piglets flinched and 38 % of all piglets retreated. Retreat was also registered in interval 5 (24 %) and interval 6 (14 %), and one pig also retreated before foam generators started in interval 2. At least once, 52 % (N=11) of the piglets slipped during the experiment. This happened mainly in interval 4 and 6, see figure 7. There was a tendency for piglets in treatment 2 to slip more in interval 4 than piglets in treatment 1 (X^2=3.71 p=0.054).

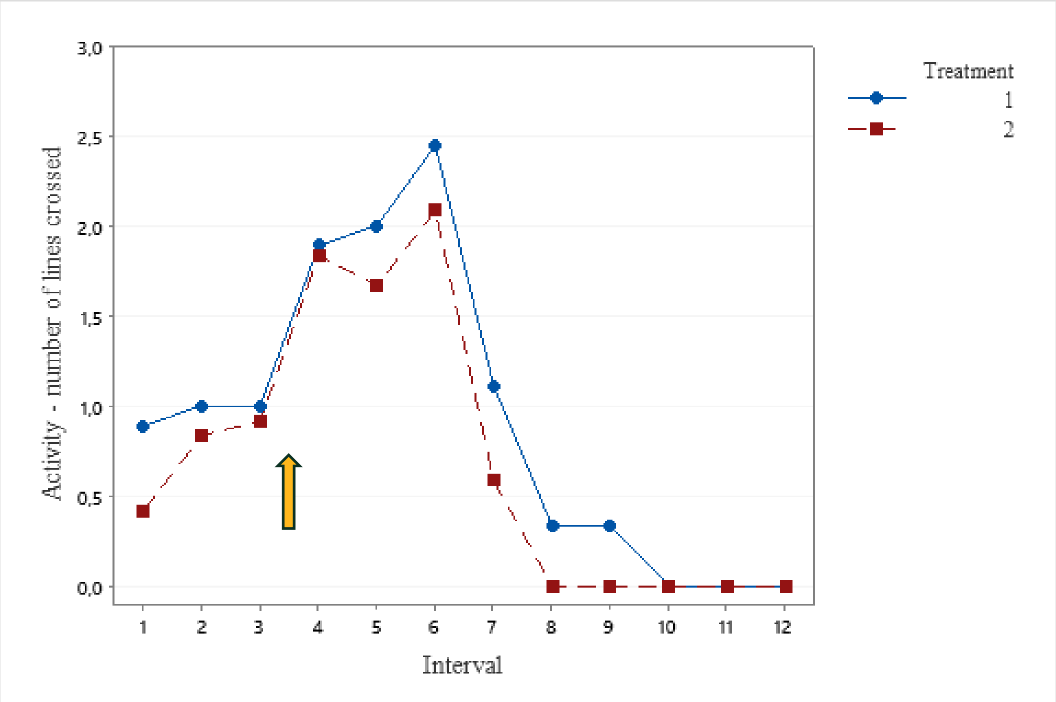

Figure 5. Movement within the box defined as the mean number of lines crossed during each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

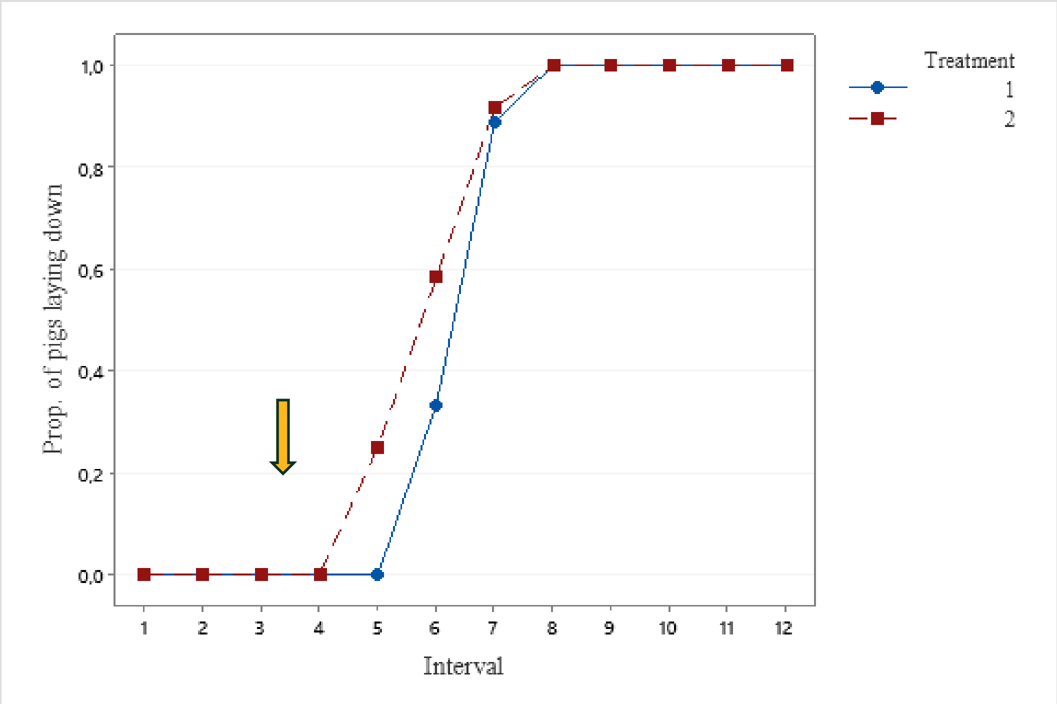

Figure 6. The proportion of piglets laying down at least once in each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

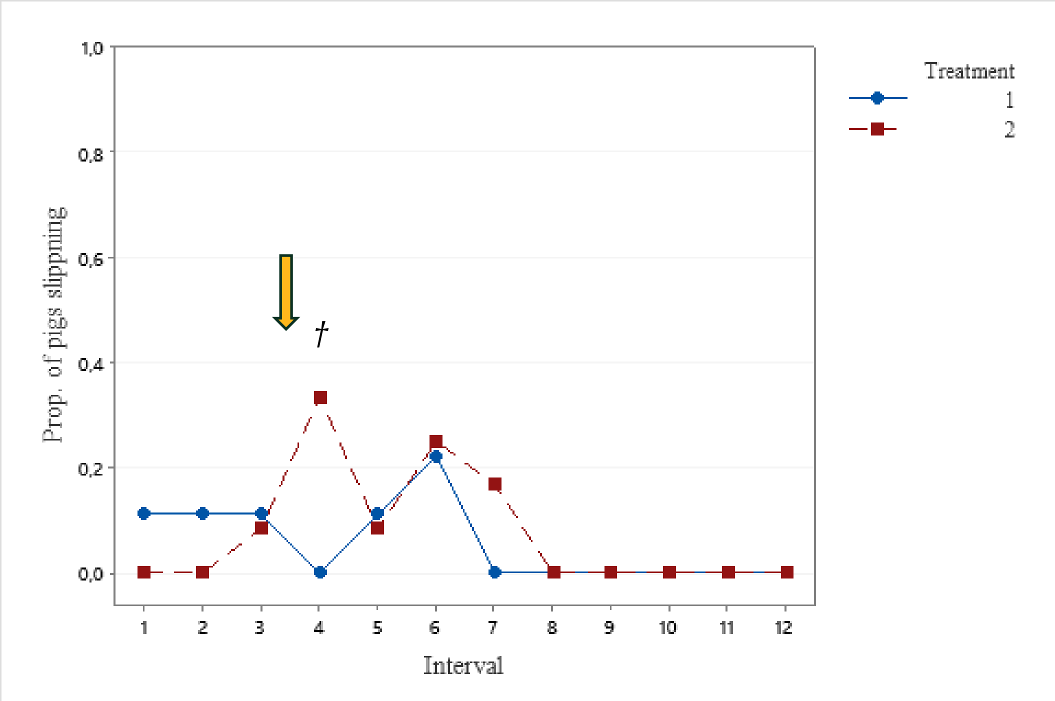

Figure 7. The proportion of piglets slipping at least once during each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production. A tendency, 0.05<p<0.1, for difference between treatments is marked with an †.

3.4. Explorative behaviours

A significant difference in exploring the floor of the box was found between treatments in total over all intervals (X^2=4.15 p=0.042), a higher proportion of piglets in treatment 1 explored the floor compared to piglets in treatment 2. Specifically, in interval 4 (X^2=8.42 p=0.004) and interval 5 (X^2=4.67 p=0.031), more piglets explored the floor in treatment 1 compared to treatment 2 (figure 8). In both treatments, the largest proportion of piglets explored the floor at the beginning of the observations (interval 1-3) and declined after foam production started in interval 4-6 (X^2=19.92 p=0.000).

No significant difference in explorative behaviour towards the wall was found between treatments in total during all intervals (X^2=2.40 p=0.121), see figure 9. The explorative behaviour towards the wall follows a similar pattern to exploring the floor, see figure 8 and 9. The highest proportion of piglets in both treatments explored the wall 30 s before foam production started (interval 1-3) and declined compared to the 30s (interval 4-6) after foam generators started (X^2=16.95 p=0.000).

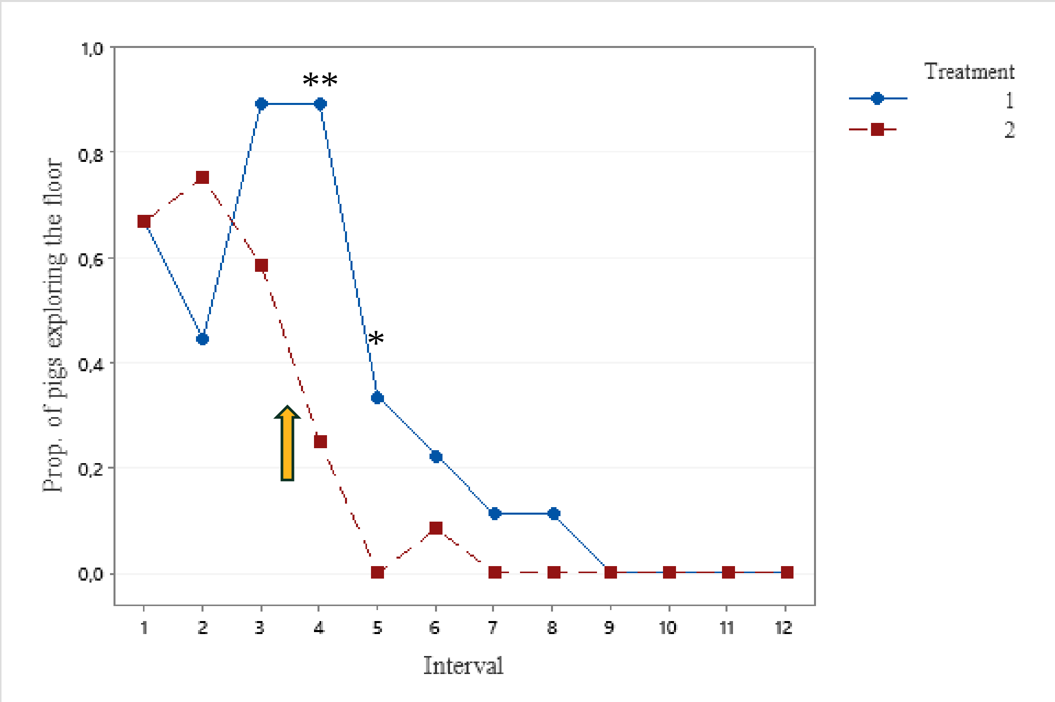

*Figure 8. The proportion of pigs exploring the floor at least once during each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production. Significant differences between treatments in an interval where 0,001<p<0.01 is marked with *.*

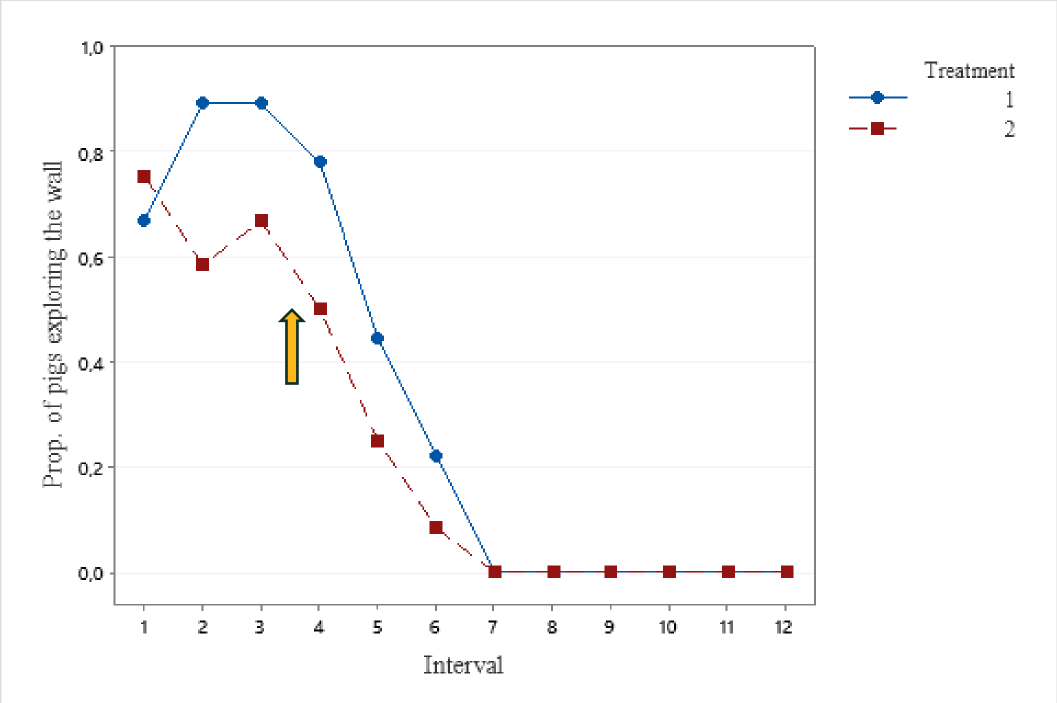

Figure 9. The proportion of pigs exploring the wall at least once during each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

Explorative behaviour towards the foam and avoidance of the foam followed similar patterns across both treatments. No significant difference in the total proportion of piglets exploring the foam (X^2=0.06 p=0.814) or avoiding the foam (X^2=0.44 p=0.506) during all intervals was found. The piglets initially explored the foam (figure 10) with 67 % of the piglets in treatment 1 and 56 % in treatment 2 exploring the foam and no piglet avoiding the foam (figure 11) in interval 4. The explorative behaviour towards the foam decreased as the foam started to fill the box, see figure 10. After the initial response, the proportion of piglets exploring the foam started to decrease and the avoidance of foam increased, see figure 11. There was a numerical difference in the proportion of piglets avoiding the foam in interval 6: with 78 % of piglets in treatment 1 avoiding the foam at least once compared to 50 % in treatment 2 (X^2=1.68 p=0.195).

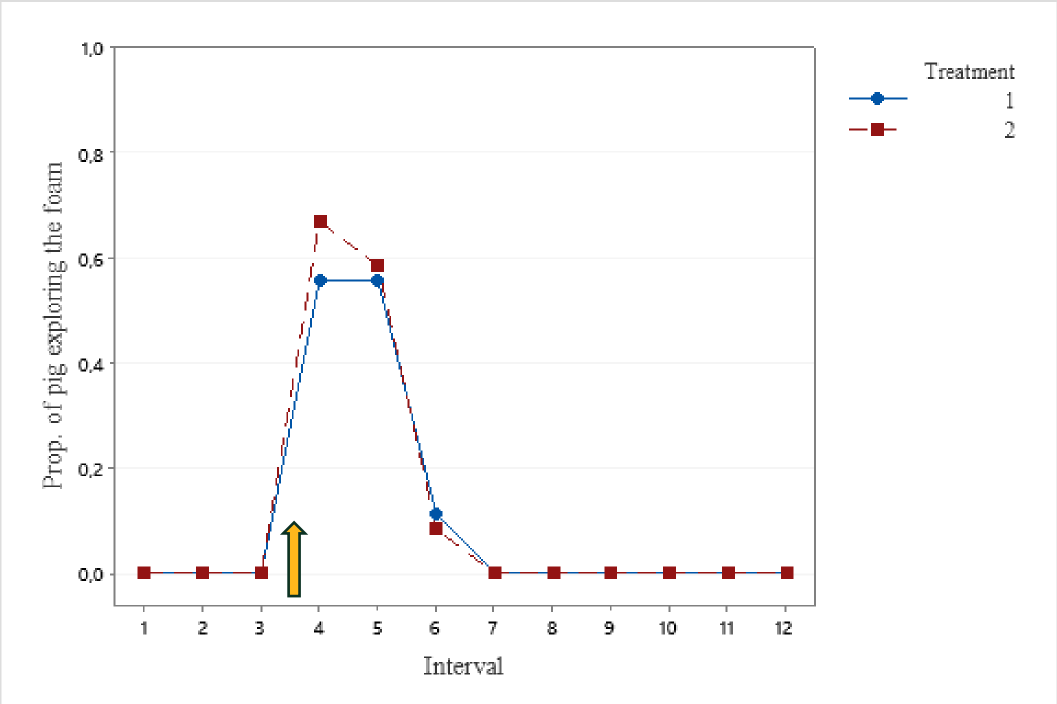

Figure 10. The proportion of pigs exploring the foam at least once during each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

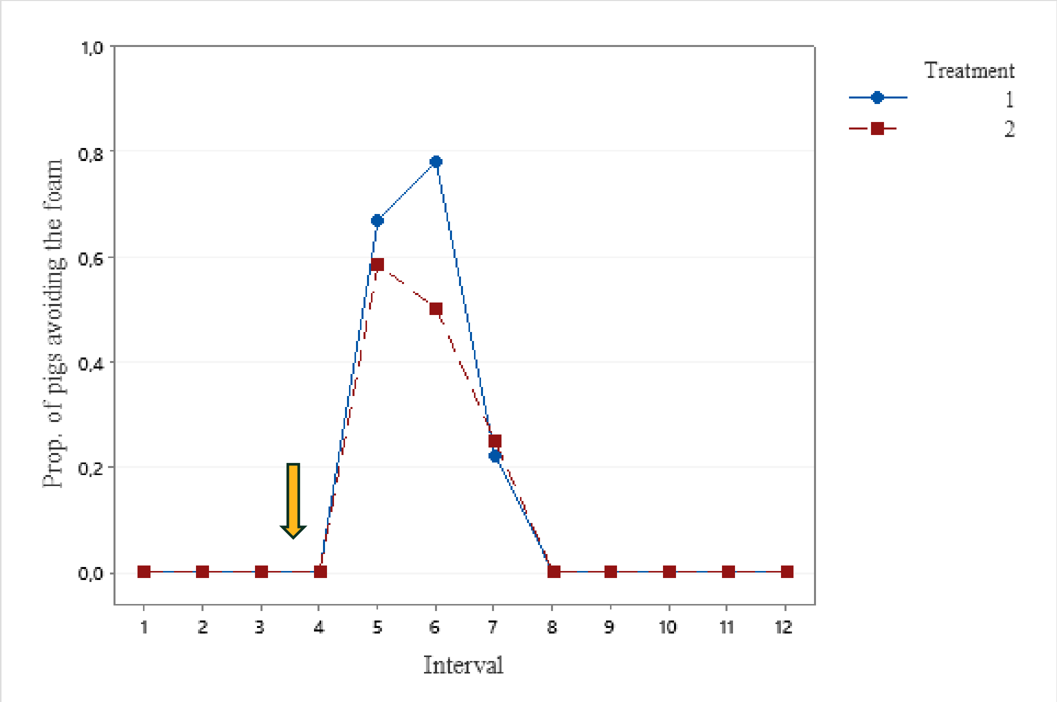

Figure 11. The proportion of pigs avoiding the foam at least once during each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

3.5. Escape attempts

There were no significant differences between treatments in total proportions of piglets performing escape attempts at the wall (X^2=2.59 p=0.107) or in the foam (X^2=0.00 p=0.952). Most escape attempts occurred in interval 6 and 7, see figure 12 and 13, which was the intervals when most piglets began to be covered in foam (figure 4). In interval 6: 33 % of piglets in treatment 1 performed escape attempts at the wall and 25 % of piglets in treatment 2: 44 % of piglets in treatment 1 performed escape attempts away from the foam and 58 % of piglets in treatment 2. In interval 7: 44 % of piglets in treatment 1 performed escape attempts at the wall and 42 % of piglets in treatment 2: 44 % of piglets in treatment 1 performed escape attempts away from the foam and 50 % of piglets in treatment 2. The sharp drop in escape attempts in interval 8 is due to pigs losing their posture and falling, seen in figure 6.

Significantly more escape attempts at the wall were performed in interval 7 compared to 8 (X^2=11.46 p=0.001); interval 7 compared to 5 (X^2=4.20 p=0.040); interval 7 compared to 4 (X^2=11.46 p=0.001). Significantly more escape attempts at the wall were performed in interval 6 compared to 8 (X^2=7.00 p=0.008); interval 6 compared to 4 (X^2=7.00 p=0.008).

Significantly more escape attempts away from the foam were performed in interval 7 compared to 8 (X^2=9.98 p=0.002); interval 7 compared to 5 (X^2=3.86 p=0.050); interval 7 compared to 4 (X^2=13.13 p=0.000). Significantly more escape attempts were performed away from the foam in interval 6 compared to 8 (X^2=11.67 p=0.001); interval 6 compared to 5 (X^2=5.08 p=0.024); interval 6 compared to 4 (X^2=14.90 p=0.000).

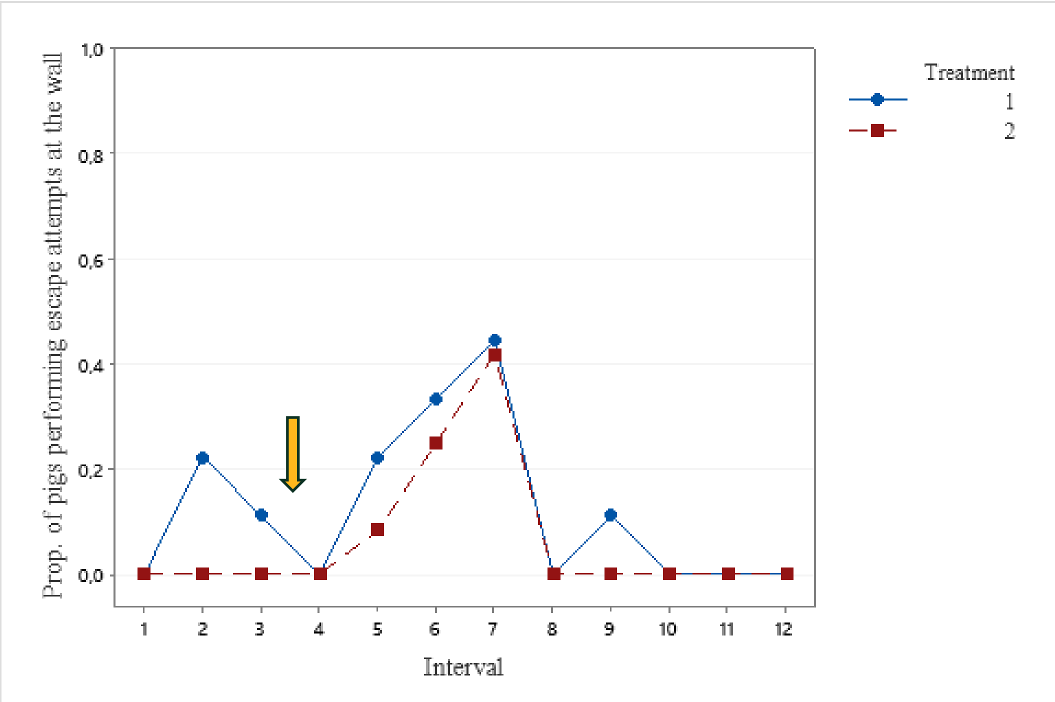

Figure 12. Proportions of piglets performing escape attempts at the wall at least once during each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

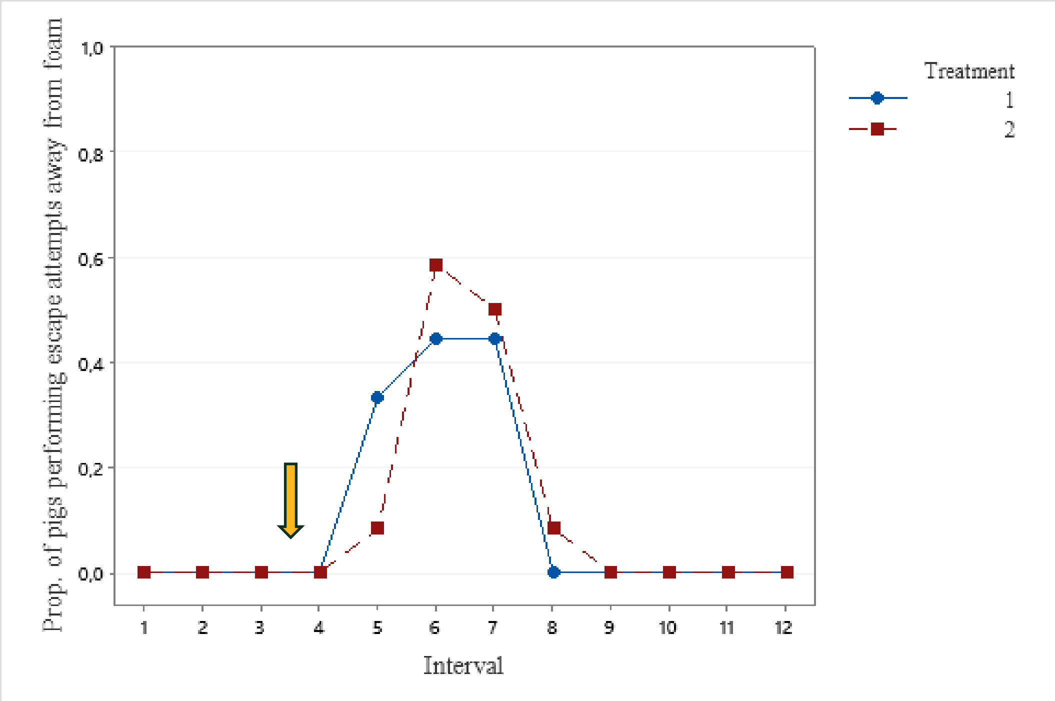

Figure 13. Proportions of piglets performing escape attempts away from the foam at least once during each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

3.6. Vocalisations

There was a significant difference in occurrence of grunts between treatments in total over all intervals (t=2.63 p=0.009), where piglets in treatment 1 (M=2.7 SD=4.37) grunted more than piglets in treatment 2 (M=1.4 SD=1.82). In interval 2, piglets in treatment 1 (M=7.1 SD=5.44) tended to grunt more than piglets in treatment 2 (M=3.6 SD=1.56) (t=1.83 p=0.100). During the first 30 s (interval 1-3) piglets in treatment 1 (M=7.3 SD=5.96) grunted more than piglets in treatment 2 (M=3.8 SD=1.70) (t=2.87 p=0.007). Piglets in treatment 1 (M=3.0 SD=2.71) also grunted more during the 30 s after foam production started (interval 4-6) than piglets in treatment 2 (M=1.6 SD=0.98) (t=2.32 p=0.026).

There was a sharp drop in vocalization in interval 4 when foam production started, see figure 14 and 15. Total grunts from both treatments in interval 4 (M=2.1 SD=2.76) was significantly lower than interval 1 (M=5.9 SD=5.03; t=2.57 p=0.018), interval 2 (M=5.7 SD=4.58; t=2.61 p=0.016) and interval 3 (M=6.1

SD=5.70; *t*=2.44 *p*=0.024). During the first 30 s (interval 1-3) the piglets grunted more frequently in total registrations from both treatments, than during the 30 s after foam production started (interval 4-6), 5.9 (*SD*=5.01) and 2.4 (*SD*=2.27) times respectively (*t*=4.23 *p*=0.000).

No difference in total squeals ((*M1*=0.3 *SD1*=1.13; *M2*=0.2 *SD2*=0.68) *t*=0.72 *p*=0.471) or screams ((*M1*=0.3 *SD1*=0.98; *M2*=0.2 *SD2*=0.47) *t*=0.60 *p*=0.553) were found between treatments. No differences were found in intervals between treatments for squeals and screams. Squeals occurred in higher frequency in total registrations from both treatments in the first 30 s compared to the 30 s after foam production started, 0.9 (*SD*=1.75) and 0.1 (*SD*=0.34) times respectively (*t*=3.09 *p*=0.003). Screams occurred in a higher frequency in the third 30 s interval (7-9) compared to the 30 s interval when foam production started (interval 4-6), 0.9 (*SD*=1.35) and 0.0 (*SD*=0.21) times respectively (*t*=-4.24 *p*=0.000), see figure 16. Piglets screamed more in total registrations from both treatments in interval 8 (*M*=1.5 *SD*=1.56) compared to interval 9 (*M*=0.6 *SD*=0.62) (*t*=2.15 *p*=0.045) but not compared to interval 7 (*M*=0.7 *SD*=1.54) (*t*=-1.47 *p*=0.153).

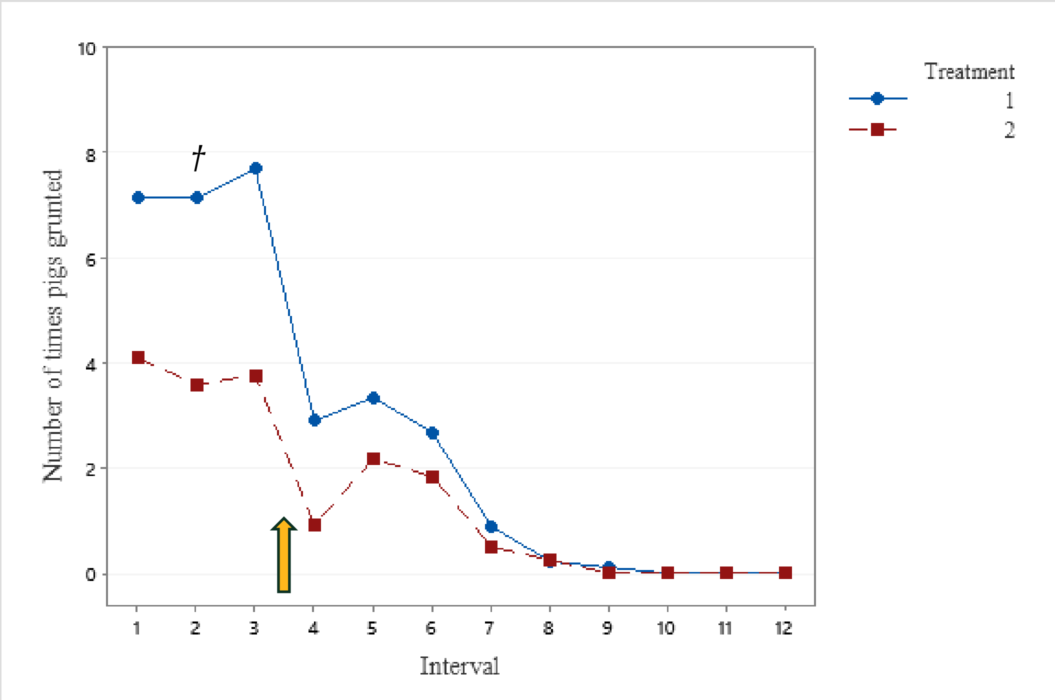

Figure 14. Mean number of times piglets grunted in each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production. A tendency, 0.05<p<0.1, for difference between treatments is marked with an †.

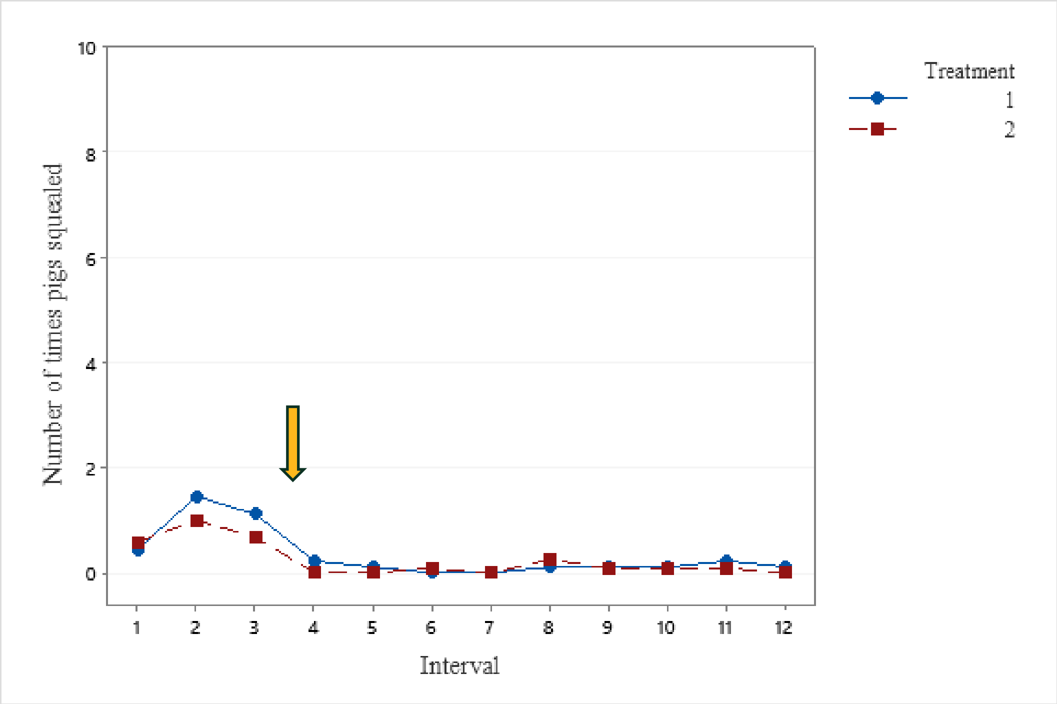

Figure 15. Mean number of times piglets squealed in each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

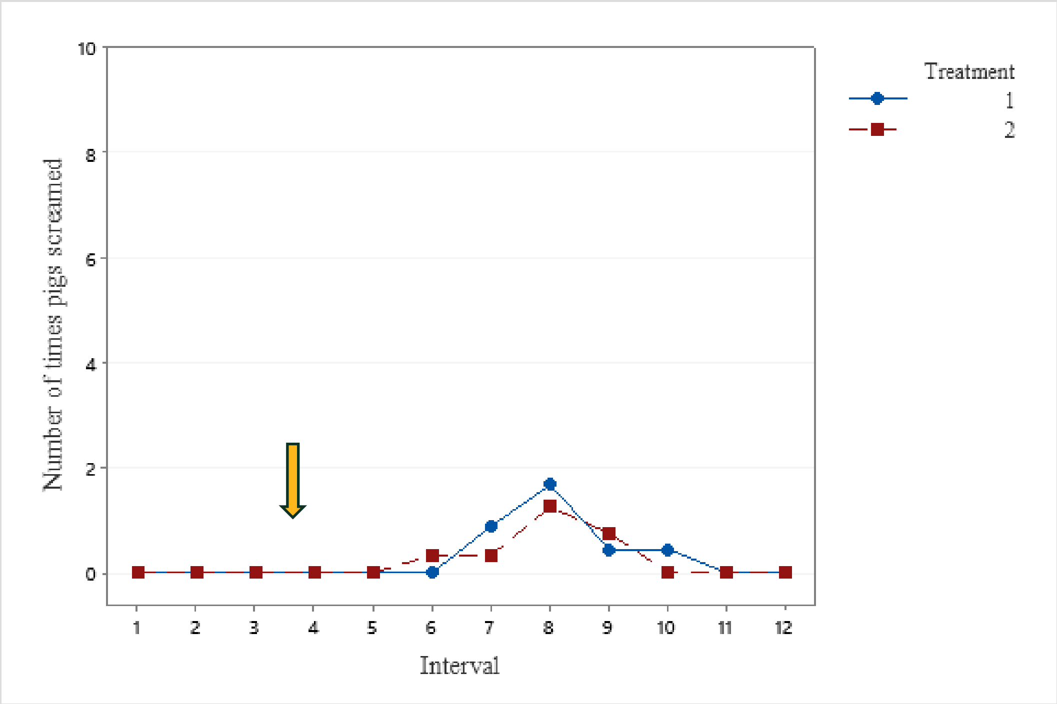

Figure 16. Mean number of times piglets screamed in each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

3.7. Social behaviours

Piglets in treatment 2 spent in average 7.7 s (*SD*=2.47) close to each other in the first 30 s (interval 1-3), 5.5 s (*SD*=2.90) in the 30 s after foam production started (interval 4-6) and 0.2 s (*SD*=0.93) in interval 7-9, see figure 17. Piglets spent more time close in the first 30 s (interval 1-3) compared to the 30 s after foam production started (interval 4-6) (t=3.41 p=0.001) and compared to interval 7-9 (t=16.98 p=0.000). There was a significant difference in average time spent close to each other between interval 3 and 4, 7.9 s (*SD*=1.88) and 5.6 s (*SD*=2.71) respectively (t=2.45 p=0.024).

The average time piglets in treatment 2 spent expressing social behaviour was 3.4 s (*SD*=3.32) in the first 30 s (interval 1-3), 3.0 s (*SD*=3.43) in the 30 s after foam production started (interval 4-6) and 0.2 s (*SD*=0.74) in interval 7-9, see figure 18. There was no significant difference in time expressing social behaviour in the first 30 s compared to the 30 s after foam production (t=0.52 p=0.602) started but there was a significant difference compared to interval 7-9 (t=5.69 p=0.000).

No agonistic behaviours were recorded but 42 % of piglets did at least once climb on the companion piglet when trying to avoid or escape the foam in interval 4, 5 and 6.

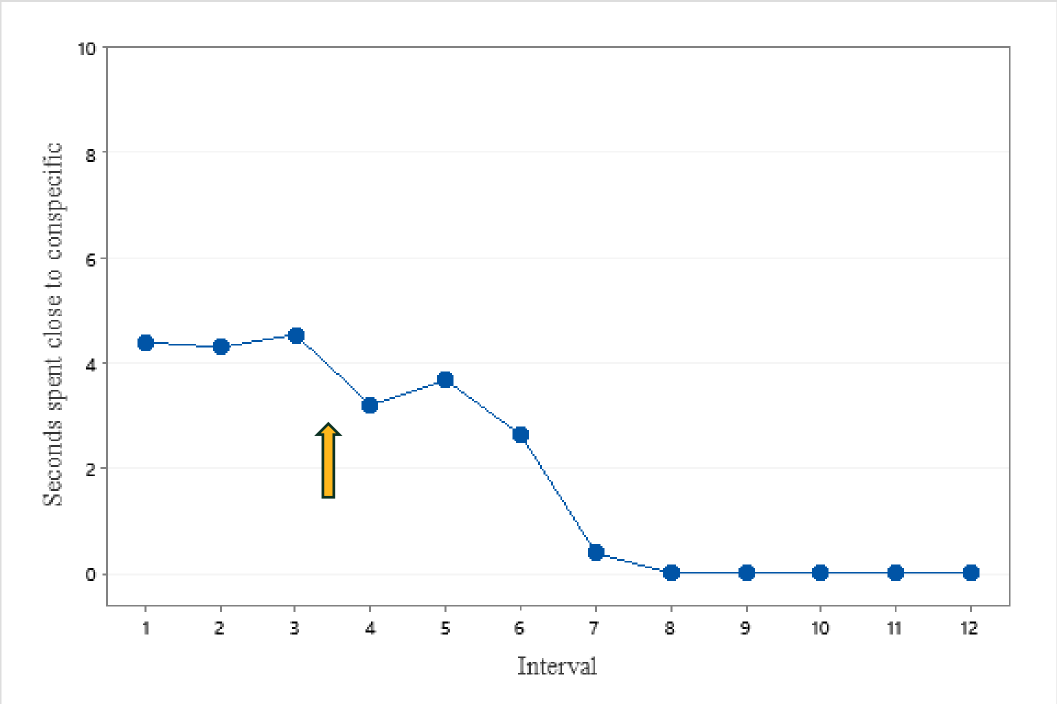

Figure 17. Mean time in seconds piglets spent close to each other in each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

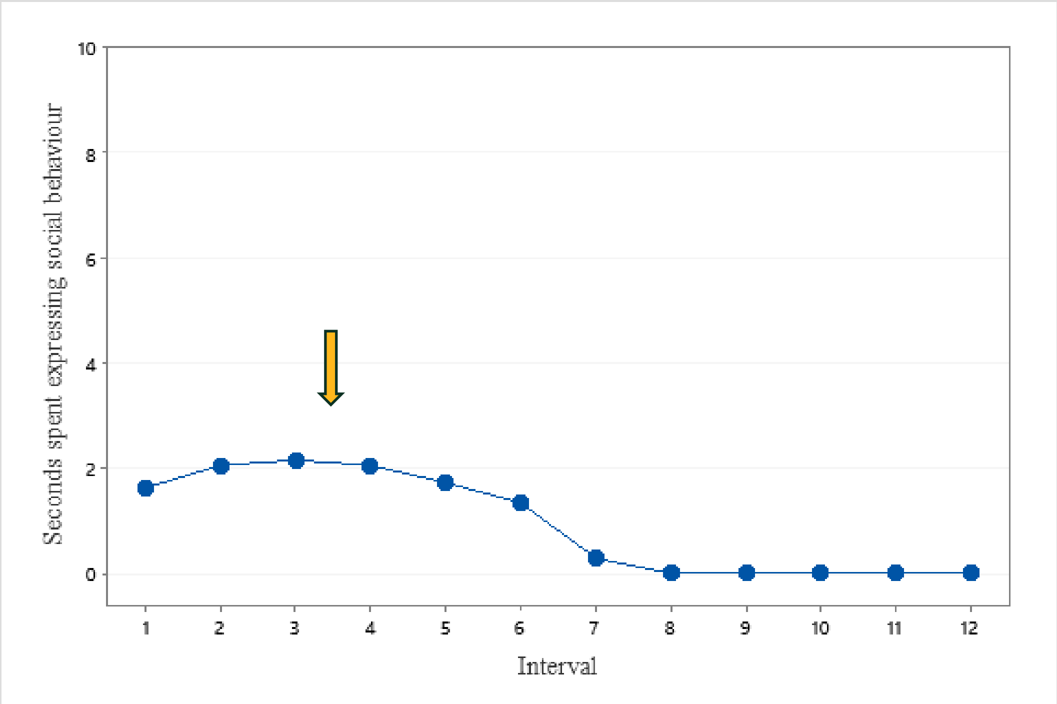

Figure 18. Mean time in seconds piglets spent expressing social behaviour from each other in each interval. Each interval represents 10 s, and the orange arrow marks the start of foam production.

4. Discussion

4.1. Effectiveness of nitrogen foam

No piglet showed any sign of regained consciousness after being taken out from the box. Heartbeats were detected in 10 of 21 piglets, which means that 11 piglets were presumed to be already dead when taken out of the box. This indicates that 12 minutes submerge in nitrogen foam is enough to ensure the piglets die and do not regain consciousness. In addition, it supports the hypothesis made that nitrogen foam is an effective euthanasia method for sick, injured or weak piglets. There was a numerical difference in time to LOP between treatments, where treatment 1 had longer time to LOP than treatment 2, 38 s and 33 s, respectively (table 3). However, no significant difference could be proven with the analyses performed. With a greater sample size and more detailed analyses, it is possible that a significant difference could be found. The numerical longer time for LOP to occur in treatment 1 is in contrast with the hypothesis made that it would take longer for piglets in treatment 2 to LOP since it would take longer time to fill the box with foam. The time to fill the box with foam, both half and completely, took significantly longer in treatment 2 compared to treatment 1 but this does not seem to have prolonged the time to LOP. The shorter time to LOP for piglets in treatment 2 could possibly be explained by a greater number of bubbles being broken and releasing nitrogen, which lowered the oxygen level quicker in the box compared to treatment 1. This is unfortunately not possible to evaluate in this study since the oxygen sensors started after the box was filled with foam due to the oxygen sensor's moisture sensitivity. The possibility to monitor the oxygen level from the start of foam production would enable differences to be found between single or pair treatments and is recommended to be included in future studies.

LOP is often used in behavioural studies as the first sign of loss of consciousness (Raj & Gregory 1996), but an animal may lose its posture without simultaneously losing consciousness (Steiner et al. 2019). When using CO_2 loss of consciousness has been found to occur approximately 10 s after LOP in slaughter ready pigs (Verhoeven et al. 2016). It is not possible to conclude exactly when in the euthanasia process the piglets in this study lost their consciousness since no brain monitoring was performed and these piglets were much smaller than the pigs in Verhoeven et al. (2016) study. To fully evaluate the welfare impact of nitrogen foam euthanasia it is crucial to know when the piglets lose their consciousness, since a conscious animal can experience distress and pain (Steiner et al. 2019). It is, therefore, possible that the piglets in this study were conscious when the convulsions started since convulsions started almost immediately after LOP occurred. This could be a welfare issue. Therefore, further studies should include brain monitoring data to assess the time of unconsciousness and brain death. Though, Steiner et al. (2019) highlight the importance of identifying the correct markers for unconsciousness and brain death before secure assessments can be achieved. Brain monitoring could also enable future studies to evaluate the effect on stress levels foam exposure has before the onset of unconsciousness which further reveals the welfare impacts (Murrell & Johnson 2006).

4.2. Behavioural response to foam exposure

The initial response when starting the foam generators was for the piglets to flinch and/or retreat, followed by exploration of the foam. The exploration of foam at the beginning of foam production indicates that the piglets were not fearful of the foam itself and that the nitrogen gas did not cause discomfort. The explorative behaviour decreased as foam levels began to increase and instead the piglets started to avoid the foam by raising their snout above foam level. When the piglets were covered in foam, escape attempts increased. A similar behaviour pattern has been found in previous studies, where pigs initially were startled by the foam generators and then began exploring the foam, followed by avoidance and escape attempts (Brattlund Hellgren 2019; Lindahl et al. 2020; Wallenbeck et al. 2020). Similar to previous studies, a sharp drop in the number of grunts at the onset of foam generators in interval 4 was found (figure 14). Additionally, flinch only happened when foam generators were started, which indicates that the noise from the foam generators startled the piglets.

The escape attempts seem to have been performed as a response to rising foam levels and not the nitrogen gas. This is supported by the fact that the peak in escape attempts (figure 12 and 13) coincides with piglets being covered with foam (figure 4). In interval 6, 80 % of the piglets' bodies were covered in foam, and in interval 7, over 90 % were covered which is the intervals when most escape attempts were performed. The same conclusion was drawn in Lindahl et al. (2020) and Brattlund Hellgren (2019) as there was no difference in escape attempts between treatments with nitrogen or air-filled foam. In Söderquist (2020), no registration of flinch or retreat behaviours was made, but an increase in activity and a sharp drop in vocalisation was seen at the onset of foam generators as well as escape attempts when foam levels increased. This indicates that the pigs were startled at the beginning of the foam production and performed escape attempts as a response to being covered in foam (Söderquist 2020). The relationship between foam cover and escape attempts could be tested in statistical analyses but was not performed in this study due to limited time.

In the study by Llonch et al. (2012a), gasping was seen in pigs during exposure to 70-85 % nitrogen mixed with CO_2, and the authors conclude that all gas mixtures would be somewhat aversive compared to air. Respiratory distress as seen with CO_2 is considered to have a pronounced negative effect on animal welfare (Beausoleil & Mellor 2015). In this study with pure nitrogen, no gasping was recorded which suggests that neither the gas nor foam mixture caused any respiratory distress. However, retreat behaviours occurred over multiple intervals indicating that the behaviour could have been a response to either the foam and/or nitrogen gas. Though, because of the behaviour responses discussed in this paragraph, it is hypothesised that the piglets react to the novelty of foam exposure and not towards the nitrogen.

4.3. Indications of social buffering

Studies have found pigs to seek social proximity and physical contact during stress (Geverink et al. 1998; Herskin & Jensen 2000), which was seen both before and after being exposed to foam, such as snout contact and moving in synchronization (figure 18). In contrast to the results in Söderquist (2020) study, where pigs spent more time close to each other after foam exposure, pigs in this study spent less time close to each other after foam exposure compared to before (figure 17). This could be due to the difference in the definition of close to conspecific and the age difference of pigs between that study and this, or as Söderquist (2020) also states, the social proximity could be a coincidence of piglets trying to avoid the foam. In this study, piglets that were unfamiliar to each other were used which could have influenced the result as a familiar companion seems to have a more pronounced buffering effect than an unfamiliar (Kikusui et al. 2006; Kanitz et al. 2014). This could explain why piglets in this study did not express more social behaviours or moved closer to each other during foam exposure.

Isolation of piglets has been found to induce stress behaviours in piglets, such as vocalisation, activity and jumping/escape attempts (Herskin & Jensen 2000; Kanitz et al. 2009). Piglets in treatment 1 vocalized more (grunts) than piglets in treatment 2 in the 30 s before and 30 s after foam production started (figure 14). This could indicate that piglets in treatment 2 were less stressed than piglets in treatment 1 (Fraser 1974; Kanitz et al. 2009; Herskin et al. 2011). However, no difference in activity or escape attempts was found between treatments suggesting the social support in treatment 2 was not enough to reduce the occurrence of these stress behaviours.

The emotional state of a companion in a stressful event has been found to affect social buffering (Kikusui et al. 2006; Rault 2012). Where the effect of social buffering is diminished if the companion pig is itself stressed. Therefore, it is possible that the piglets in treatment 2 were influenced by one another so that if one piglet became stressed the other one became stressed as well. Furthermore, behaviours as climbing on the companion piglet could induce stress and escape behaviours in the other piglet. Since both piglets were taken from their home box and placed in a novel environment with an unfamiliar companion, as well as having an injury or being sick or weak, the social buffering may have been reduced. This could explain why a greater social buffering was not seen in the results between treatments. From an animal welfare perspective, a familiar companion pig would probably have the most positive effect when exposed to nitrogen foam (Kikusui et al. 2006; Rault 2012; Söderquist 2020). However, the companion pig did not aggravate the stress behaviours performed by piglets in treatment 2 and no aggressive behaviours were seen. There is a possibility that if a higher number of animals would have been used in the study, more differences and statistically significant results could have been detected. Since no monitoring of brain activity, blood or tissue samples were taken, it is possible that physiological effects of social buffering were missed. Future studies should include some methods to measure physiological stress responses e.g., plasma markers such as cortisol and norepinephrine (Murrell & Johnson 2006).

4.4. Welfare aspects

EFSA (2020b) identified four main welfare consequences when euthanizing pigs on farm: pain, fear, impeded movement and respiratory distress. Piglets in this study displayed some aversive behaviour, mostly when foam began covering their heads, by running and performing escape attempts, which is an indication of fear (EFSA AHAW Panel et al. 2020b). Some of the piglets were injured, and when covered with foam the fear could trigger them to perform escape attempts and/or slip, which could momentarily increase their pain before LOP. Slipping is an indication of impeded movement (EFSA AHAW Panel et al. 2020b). The piglets in this study mostly slipped when foam generators started in interval 4, probably as a response to the unexpected noise. The foam seemed to make the plexiglass floor slippery even though anti-slip tape was used designed for wet floors. This resulted in piglets slipping in interval 6, causing more distress, and possibly increasing other stress behaviours, which Lindahl et al. (2020) acknowledged as a welfare threat as well. Plexiglass floor was needed in this study to be able to record from below, but when commercially used, the floor should consist of an effective anti-slip material to increase the welfare of the animals.

Before foam production started, piglets squealed which is seen as an indication of distress or heighten excitement compared to low grunts (Marchant et al. 2001). This is probably due to being taken from the home box and placed in a novel environment (Fraser 1974; EFSA AHAW Panel et al. 2020b) which is inevitable when using this method. However, by minimizing the time until foam generators start, this distress could be reduced and will most likely be shorter when used on farms compared to the 2 minutes used in this study.

There is a possibility that piglets lose their posture at different times when euthanized together compared to being euthanized alone. The average difference in time was 7.5 s. This may affect the piglets negatively in that the piglet still standing could become more stressed when the companion piglet falls and starts to convulse and vocalize. The piglet that loses its posture first risks getting stepped on by the standing piglet and also risks kicking the standing piglet when starting to convulse. It is therefore recommended by EFSA (2020b) to make sure that the animals have sufficient floor space and should be able to lay down at the same time. Neonatal animals seem to be more resistant to hypoxia, which means it could take longer for LOP and unconsciousness to occur when using nitrogen compared to e.g. CO_2 (AVMA et al. 2020). The relationship between age and time to LOP was not examined in this study but an effect of age has been seen in other studies on CO_2 and argon euthanasia, where neonates take longer to LOP with argon but not with CO_2 (Sadler et al. 2014a; b; Sutherland et al. 2017). Logically it would be the size of the piglet when it is no longer neonatal, that determines how fast it is covered with foam and inhales the high concentration of nitrogen. This should be considered when euthanizing two or more piglets at the same time and precaution should be made to ensure as homogenous groups as possible.

The piglets exhibited aversive behaviour as retreating, avoiding the foam and escape attempts, raising the question of how great the welfare benefits are compared to traditional euthanasia methods. With nitrogen foam, the piglets do not need to be restrained and can be euthanized together which is not possible with a captive bolt or blunt force trauma. All three methods often require the piglets to be moved from the home pen, but nitrogen foam probably has the longest time from removal from pen to loss of consciousness which is a welfare disadvantage. Blunt force trauma is an effective and fast method when performed correctly, but because it is an entirely manual method there is a risk of unsuccessful concussion and therefore, EFSA (2020b) do not recommend using this method for on-farm euthanasia. The Swedish regulation state 14 days as a threshold (7 cap. 13 § SJVFS 2020:22 case no. L 22) and not 5 kg as the EU regulations (EC 1099/2009) for blunt force trauma use which is a cause for this void of good alternatives for these piglets. For piglets over 14 days that are small or piglets over 14 days that weigh more than 5 kg, the captive bolt cannot be used. Nitrogen foam could enable those piglets to be euthanized at the correct time and improving animal welfare by ending the piglets suffering. Since there is a desire among farmers to use other methods than the traditional (Dalla Costa et al. 2019), nitrogen foam could be an alternative since it is less physical and should therefore induce less psychological distress in the performer. Additionally, nitrogen foam offers a standardised euthanasia method that relies less on the skill of the performer. This is positive from a welfare perspective since the human factor is one of the major welfare hazards when euthanizing animals on-farm (EFSA AHAW Panel et al. 2020b).

There are not enough studies to determine the full welfare complications of nitrogen foam euthanasia, but with further research it is a possible alternative euthanasia method for piglets on farms. Especially as an alternative for small piglets over 14 days since no good method is available today. For farmers who are not comfortable with using blunt force trauma on piglets up to 14 days, nitrogen foam could be a less physical method. Further studies should investigate if the aversiveness towards the foam can be reduced since it seems to induce the most stress behaviours. Evaluating different filling paces, faster, slower and possibly pre-filled box, might affect the piglet's behaviours and hopefully reduce the experienced stress.

4.5. Method consideration

The behaviour observations occurred for two minutes, divided into 12 10 s-intervals and was continuously observed. The same observer, the author, looked at all recordings, which eliminates the risk of different perceptions of behaviours. Video recording enabled the observer to pause and play the video backwards so that all behaviours was correctly observed. However, in some cases, the piglet was not visible on the recordings due to the edges of the box not being made of plexiglass. This could mean some behaviours were missed and are underrepresented in the results. There were also some technical problems with the foam generators that caused two batches of piglets to have to be removed from the box when the foam production did not start. These piglets had then already been in the box for two minutes when placed in the box again, and this may have affected their behaviour. The technical problems also caused the foam production to start at slightly different times after turning it on. This may explain some of the differences seen in time to fill the box with foam and thereby affecting the time to LOP.

Different types of behavioural recordings were used in this study for different variables depending on what question was aimed to answer. For variables where the time until the behaviour occurred was the aim, as for LOP, last convulsion, last severe convulsion and last heartbeat, time recording was used. For the other variables, frequency recording was used where the number of times a behaviour was performed was recorded. All variables, except activity, vocalisation, time close to conspecific and time expressing social behaviour, were converted to partial interval recording where the behaviours were marked with a 0 or 1, depending on if the behaviour was seen during the 10 s intervals or not. This was done since too few registrations were made for the variables to be considered normally distributed. This could have been done from the beginning to save time, but since only a few studies have been performed on the subject, it was difficult to beforehand determine what behaviours should be recorded in what way. In future studies, it should be possible to merge the variables "explore floor" and "explore wall" and merge the escape attempts to get fewer variables, since whether or not the behaviour occurs is the most interesting and not in what way.

4.6. Conclusion

Nitrogen foam effectively works as a euthanasia method for euthanizing single and pairs of sick, injured or weak piglets, and could provide an alternative method for euthanizing piglets on-farm. The novelty of foam seems to induce moderate aversive behaviours and more research is needed to be able to evaluate the full welfare complications and possible methods to reduce aversiveness.

5. Societal and ethical aspects

This study was planned and performed using the 3 R:s: replacement, reduction and refinement. These principles do not only aim to minimize the use of animals in research but to improve the animal's welfare in studies that require animals. Since the aim of this study was to evaluate the behavioural response and effectiveness of nitrogen foam as a euthanasia method for piglets, it was not possible to use another technique or research method to replace the piglets. By only using piglets that were destined for euthanasia by the farmers' standard protocol, the number of piglets used was naturally reduced and refined so that no healthy piglet was culled for the sake of the study, but still enough to get statistically significant results. Additionally, the piglets were kept in their regular box with the sow and siblings just up to the experiment and was minimally handled by experienced personnel.

All piglets were weighed, and a few marked with a regular permanent pen before the experiment, which should only induce mild discomfort. Being placed in the novel euthanasia box would be considered to induce some stress since pigs are social animals (Steiner et al. 2019) The piglets placed alone in the box probably experienced the most stress. This was, however, only for a few minutes until LOP occurred, the stress induced by isolation was therefore considered to be necessary for the experimental purpose. The time until foam generators started was 2 minutes, allowing the piglets to acclimatize to the environment. However, it seemed by the observers like the piglets did not relax during these minutes since they did not lay down and vocalized quite frequently. In future studies, it should be possible to decrease this to a shorter time in order to reduce the stress piglets may experience during social isolation without adversely affecting the results.

The overall aim of the project on nitrogen foam is to promote animal welfare and working conditions when euthanizing piglets on farm. This study aimed to evaluate the effectiveness of nitrogen foam as a euthanasia method for single or pairs of sick, injured or weak piglets, and if it could be more humane compared to traditional euthanasia methods. By developing equipment that meets the characteristics of nitrogen, the problems seen in Pöhlmann (2018) was solved. The effectiveness of nitrogen foam when euthanizing two piglets of various sizes was proven to be reliable with no piglet regaining consciousness after 12 minutes submerged in foam. Since the LOP was numerically lower in pairs of piglets, it seems likely to assume that euthanizing more than two piglets at once should be possible and still achieve an effective euthanasia. At farms, the farmers should be able to leave the piglets when submerge in foam and not stand around and wait since that would take up too much of the farmers' time. The euthanasia box should be equipped with oxygen sensors that alert if oxygen levels increase, to ensure that the piglets do not regain consciousness.

Evaluating if nitrogen foam could be a more humane euthanasia method is a more multifaceted question which this study was not able to answer completely. This study complies with previous research on nitrogen foam, where some aversive behaviours toward the foam were seen (Brattlund Hellgren 2019; Lindahl et al. 2020; Söderquist 2020; Wallenbeck et al. 2020). The stress behaviours are milder than those seen when CO_2 is used, without pigs expressing respiratory distress. Therefore, it seems to be possible for nitrogen foam to be implemented at abattoirs and replacing CO_2 as a more humane stunning method. Though, before it can be practical implemented, research on the time required for effective stunning of slaughter-ready pigs and time until the last severe convulsion needs to be determined for securing an effective and safe stunning process as well as possible implications the foam might have on meat and carcass quality. Nitrogen foam has been proven to be effective on pigs from 0 to 9 weeks and could therefore play an important role as an alternative to traditional methods as blunt force trauma and captive bolt. The most important function nitrogen foam could have on farms is probably to fill the existing gap of viable euthanasia methods for small piglets over 14 days. Since nitrogen is cheap to produce, and the box is a one-time investment, the economical aspect should be fairly reasonable. However, this method is still more expensive than traditional methods which require little or no resources.

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