Friend or foam?

Improved methods for stunning of pigs with foam; effects of repetition and scent on pig reaction to foam

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Förbättrade metoder för bedövning av gris med skum; effekter av upprepning och lukt på grisars reaktioner till skum

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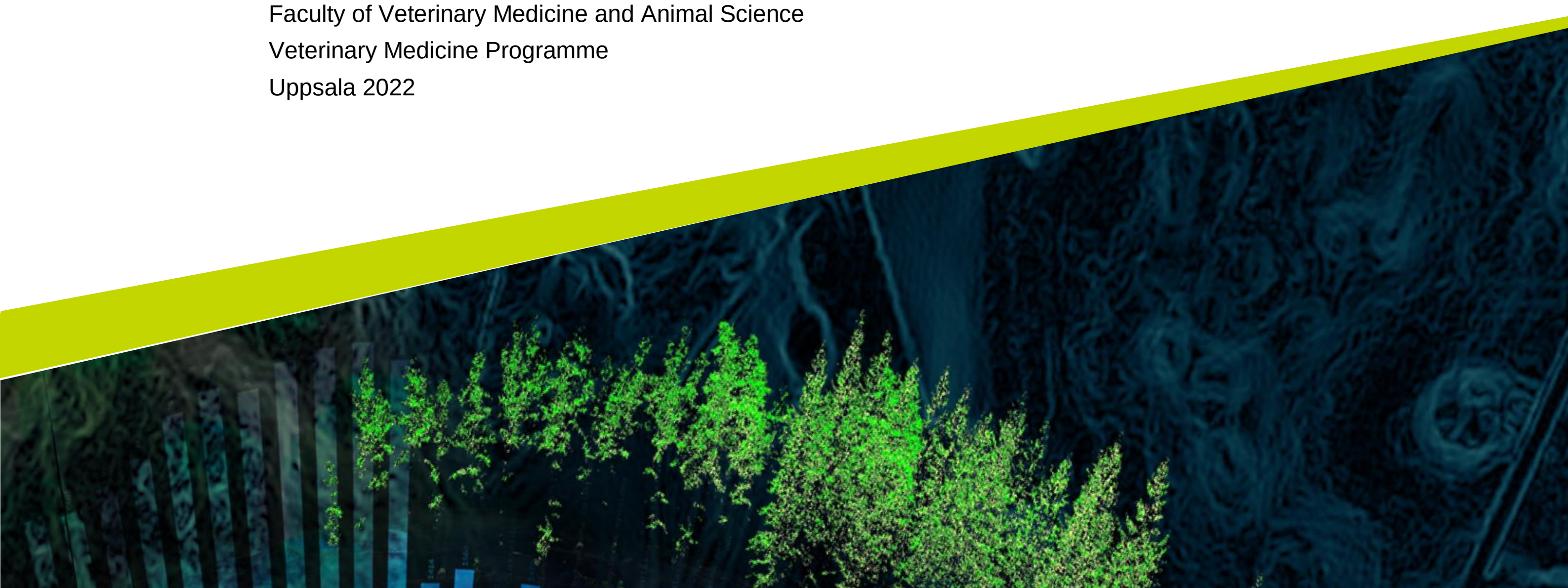

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Abstract

There are several methods available for stunning of pigs before slaughter, but the most frequently used at larger abattoirs are electricity and CO_2. There are advantages and disadvantages with both methods. As there is no method that does not have major animal welfare drawbacks. EFSA (European Food Safety Authority) concluded in 2004 that research on new and developed methods for stunning of pigs before slaughter needs to be prioritised. However, research in the area is lacking.

One method that has been researched is stunning with nitrogen filled high expansion foam. With this method it is possible to use a gas (e.g., nitrogen) that is not thought to be as aversive to pigs as carbon dioxide, but that is difficult to use in free form due to having similar density as air. The foam keeps the gas from mixing with air and purges the space from air helping to create an anoxic environment in a shorter time. When this method has been studied, questions have arisen on how aversive the foam itself is to the pigs, and whether or not it is possible to reduce this aversiveness by, for example, adding a scent to the foam or by exposing the pigs to foam on repeated occasions.

The aims of this study were to investigate if the pigs' behaviour were altered when scent was added to the foam, and also if repeated exposure to foam would affect the pigs' behaviour.

In total, 50 pigs with an age of 14-16 weeks were included in this study. There were 30 pigs in the group with a vanilla scent added to the air-filled foam, and 20 pigs in the group that was exposed to air-filled foam without an added scent on three consecutive days. The observations from the first exposure to foam in the group with 20 pigs in the repeated study were also used as a control group to the study with an added scent.

The result from the experiment with foam with an added scent showed that there was a larger proportion of pigs exploring the foam when it had an added vanilla scent. There was also an increased interest in that group to explore the wall, as well as increased activity. No effects could be shown on number of escape attempts.

The result from the experiment where pigs were exposed to foam on three consecutive days showed a larger proportion of pigs vocalising, both in forms of grunts and screams/squeals, on day three than on day one. There were also more escape attempts on day three than on day one. Furthermore, increased exploration of the walls and decreased exploration of the floor was seen from day one to three.

In conclusion the results of this study indicate that it is possible to increase the pigs' interest in the foam by adding a scent to the foam. If foam is to be given an added scent in the future more research is needed, both in terms of relevant scent and concentration as well as assuring it has no effect on the meat for the consumer. The results also indicate that the foam is not highly aversive to pigs, as pig avoidance behaviour towards the foam does not greatly increase when exposed to it repeatedly. Number of escape attempts increased slightly however, and a future study with a larger number of pigs might reveal more information. The increased vocalisation could either indicate a return to more normal levels of vocalisations because of adaption to the environment but could also mean an increased reactivity to the situation. It is also possible that increased vocalisation comes from knowing that that they will soon be let out and therefore try to communicate with their box mates. Further studies would be needed to confirm stress levels in the pigs in these situations.

Keywords: pig, behaviour, stunning, foam, smell, scent, fear assessment, animal welfare

Sammanfattning

Det finns flera metoder för bedövning av gris vid slakt, där de vanligast använda på större slakterier är koldioxid (CO_2) och elektricitet. Med båda metoderna finns både för- och nackdelar. Eftersom det inte finns någon bedövningsmetod som är helt fri från djurvälfärdsmässiga nackdelar menade EFSA (European Food Safety Authority) redan 2004 att forskning på nya och utvecklade bedövningsmetoder av gris inför slakt är önskvärt och bör prioriteras. Trots detta har väldigt få studier gjorts inom ämnet sedan dess.

En metod som har studerats är bedövning via ett högexpansivt skum innehållande inerta gaser. En gas som studerats i denna kontext är kvävgas, vilken inte verkar ge upphov till lika kraftigt aversivt beteende hos grisar som koldioxid gör. Skummet gör så att gasen inte blandas med luft vilket annars sker då luft och kvävgas har liknande densitet. Skummet trycker även undan luften så att utrymmet snabbare bli syrefritt, vilket är nödvändigt då bedövning med inerta gaser sker genom syrebrist. I studier kring denna metod har frågetecken uppkommit kring hur obehagligt grisarna upplever skummet, och om det skulle gå att minska det obehaget genom tillsats av till exempel en lukt eller genom att grisarna utsätts för skum vid upprepade tillfällen.

Målet med denna studie var att studera om grisarnas beteende ändras om de utsätts för ett luftfyllt skum som är luktsatt, och om deras beteende ändras om de utsätts för icke luktsatt, luftfyllt, skum vid upprepade tillfällen.

Totalt ingick 50 grisar mellan 14 och 16 veckors ålder i studien. Dessa var fördelade som följer: 30 grisar utsattes för luftfyllt skum med tillsatt vaniljlukt vid ett tillfälle, och 20 grisar utsattes för ett luftfyllt skum utan tillsatt lukt under tre på varandra efterföljande dagar. Observationerna från det första tillfället med de 20 grisarna i den upprepade studien användes som kontrollgrupp för studien med luktsatt skum.

Resultatet av studien med luktsatt skum visar att grisarna utforskade det luktsatta skummet mer och att även utforskandet av väggarna i boxen ökade i frekvens. Aktiviteten ökade också i denna grupp men ingen ökning av flyktförsök sågs.

Resultatet av studien där grisar utsattes för skum vid upprepade tillfällen visade att grisarna vokaliserade mer, både genom grymtningar och skrik, och uppvisade fler flyktbeteenden på dag tre än på dag ett. Det var även ett ökat intresse av att utforska väggen, men minskat intresse av att utforska golv på dag tre jämfört med dag ett.

Sammanfattningsvis indikerar resultaten i den här studien att det är möjligt att öka grisarnas intresse för skummet genom att tillsätta en lukt till det. Om skummet ska luktsättas i framtiden behövs mer forskning, både på vilka lukter och koncentrationer som vore relevanta samt för att säkerställa att det inte har några effekter på köttet för konsumenten. Resultaten tyder också på att skummet inte är väldigt aversivt eftersom undvikandet av skum inte ökar kraftigt när grisarna utsätts för skum vid upprepade tillfällen. Antalet flyktförsök ökade dock något, men då siffrorna var så låga skulle en studie med fler grisar krävas för att säkerställa relevansen. Att vokaliseringen ökade kan tyda på en normalisering vokaliseringsnivån till följd av tillvänjning av miljön men skulle också kunna tyda på en ökad reaktivitet till situationen. Det är också möjligt att den ökade vokaliseringen kommer till följd av att grisarna förstår att de snart blir utsläppta och därför försöker kommunicera mer med grisarna utanför lådan. Vidare studier skulle krävas för att undersöka stressnivåerna hos grisar i denna typ av situationer.

Nyckelord: gris, beteende, bedövning, skum, lukt, doft, rädslobedömning, djurvälfärd

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Abbreviations

CO_2	Carbon dioxide
EFSA	European Food Safety Authority
N_2	Nitrogen gas

Introduction

In 2004, the European Food Safety Authority (EFSA) was already discussing that the stunning of pigs by carbon dioxide (CO_2) is an inadequate practice, and that focus should be put on development of new, better methods for large-scale stunning and slaughtering of pigs (EFSA 2004).

In humans, breathing in even low levels (7.5%) of CO_2 is associated with increased feelings of anxiety and fear (Bailey *et al.* 2005). The feeling being described in an older study as “horrible”, “unbearable” and “a feeling of impending death” (Sechzer *et al.* 1960). In pigs, it has been demonstrated that they show signs of respiratory distress if the concentration of CO_2 is greater than 30% (Raj & Gregory 1996), and that even after fasting for 24 hours they are not willing to enter a box with apples if the box is filled with 90% CO_2 (Raj & Gregory 1995). It could thus be argued that pigs at least have feelings or sensations similar to those experienced by humans exposed to increased levels of CO_2.

In 2020, a review study showed that since EFSA’s conclusion in 2004 about a need of further research of alternative stunning methods, only 15 new scientific articles had been published on the subject (Sindhøj *et al*. 2021). This is less than one study per year. These 15 articles on new methods included different gas mixtures combining CO_2 and other gases such as argon or nitrogen in varying concentrations, hence not focusing on methods without CO_2.

A method that has been studied as an alternative to CO_2 is nitrogen filled high expansion foam. Studies have shown that nitrogen does not seem to give the same aversive behaviour in pigs as CO_2 (Llonch *et al.* 2012a), but as nitrogen is lighter than air it is more difficult to contain in free form in a room. The foam prevents the gas from mixing with air and pushes the air out of a stunning container quickly, thereby creating an anoxic environment (Lindahl *et al.* 2020). There are presently not enough studies on the foam to allow this to be an approved method for abattoirs, even if N_2 in itself is approved for use within the EU (Council Regulation (EC) No 1099/2009). However, N_2 is not approved in Sweden (SJVFS 2020:22). The method using foam is commercially available for culling of sick piglets on farms in some countries such as the Netherlands, Belgium, and Spain (HEFT 2021).

The aim of this study was to increase the knowledge about the stunning method using gas filled high expansion foam for pigs. More specifically the aims were to investigate if the foam is unpleasant, and if so, to find out if it is possible to make it less so by adding a scent to the foam or by repeatedly exposing the pigs to air filled high expansion foam.

Hypotheses and constructed questions

Based on current knowledge and previous studies, the following hypotheses were constructed:

- The pigs will show more investigating behaviours for a longer period when the foam has an added scent compared to when the foam has the original foam detergent scent.
- Repeated exposure to foam will decrease aversive behaviour towards the foam and behaviours related to stress in pigs.

To prove these hypotheses, the following questions have been constructed:

- Do the pigs’ responses to foam change when the foam has an added scent?
- Do the pigs’ responses to the foam change when they are repeatedly exposed it?

Literature review

Pig Behaviours

To be able to evaluate and interpret pigs' reactions in abattoirs, there is a need to understand what different behaviours pigs show and why. To observe an animal's behaviour is not overly complicated, but to interpret motivations for performing different behaviour certainly is. There are different aspects that need to be taken into consideration when studying animal behaviour. One is to study the species' origin and how the behaviour has evolved in the species. The ancestor of the domestic pig (*Sus scrofa domesticus*) is the wild boar (*Sus scrofa*). The wild boar originally lived mainly in forests and are omnivores, spending a major part of their active time searching and rooting for feed (Blasetti *et al.* 1988). This explains why the pigs' senses are developed the way they are, and in extension why certain behaviours are seen in the domestic pig. Much of the communication between conspecifics are through olfaction and vocalisation, and they seem to rely less on visionary cues, which makes sense if living in a forest with a restricted field of vision (Houpt 1998; Jensen 2006).

Research of communication through vocalisation are relatively limited considering pigs are very vocal, something one only must enter a stable of pigs to realise. Communication through vocalisation in pigs often consists of a continuous series of grunts of different length which can be heard in most situations, e.g., in greeting, isolation, fear or anticipation (Kiley 1972). If heard while for example rooting or foraging, these grunts can be interpreted as some sort of positional signal between individuals (Houpt 1998; Jensen 2006). Grunts also play a very important role in the communication between sow and piglets at feeding time. Barks are mostly heard when pigs are being startled or in frustration-type situations, whereas squeals or screams are heard when they are in pain or in a fearful situation (Kiley 1972). Overall, it also seems that the energy of vocalisation, i.e., the duration and pitch, is related to the level of excitement, either positive or negative, in the pig.

A considerable part of the normal behaviour repertoire in pigs is communication and interaction between individuals. They often live in family groups consisting of sows and their female offspring (Blasetti *et al.* 1988). They prefer to rest and sleep near each other, and when foraging and rooting they stay in the same general area. Pigs therefore can be considered as highly social, which needs to be considered when evaluating their behaviours and reactions to stimuli. There is research to support that if pigs have a companion when taken away from the sow and other littermates, the number of anxiety or fear related behaviours are significantly lower, and if the companion is known these behaviours are even fewer (Kanitz *et al.* 2014). In an earlier study exposing pigs to high expansion foam, it was seen that when they had a companion, their general activity levels were lower and less escape attempts were seen (Söderquist 2020). However, this could not be seen in the most recent study on euthanisation of piglets with high expansion foam (Nilsson 2021). The only difference seen between pairs of piglets and single piglets in that study were less frequent vocalisation in the form of grunts when the piglets were tested in pairs. This could somewhat be explained by the familiarity of the paired individuals, and possibly also by the age differences in these studies. In scientific experiments on pigs, it is common that they are being studied and handled individually, separated from their herd, which may lead to a higher frequency of behaviours connected to anxiety and fear than if they had been studied in pairs or groups.

Rooting and exploring

In wild or free roaming pigs, most of the active time is spent rooting or grazing, and another large portion is spent moving and exploring the environment (Stolba and Wood-Gush 1989 see (Studnitz *et al.* 2007)). Rooting seems to be the preferred way for pigs to explore their environment, even if exploration appears to be the main goal and can be reached in other ways than rooting if they cannot perform the behaviour (Studnitz *et al.* 2003).

Fear and anxiety

Behaviours that have been observed in situations which are thought to be anxiety or fear inducing are freezing, high-pitch vocalisation, defecation and escape attempts (Fraser 1974; Reimert *et al.* 2013). Furthermore, when exposed to a fearful or novel stimulus backing up or turning away is commonly seen (Dodman 1977; Dalmau *et al.* 2009). These behaviours have also been shown to be less apparent when the pigs were treated with anxiolytics (Dalmau *et al.* 2009), thus supporting the interpretation that these behaviours are anxiety or fear induced.

One way to correlate behaviour to feeling or emotional state is to study physical parameters such as heart rate, blood pressure and levels of norepinephrine or other hormones. This is not always practically possible, but when it is there is much to gain from that information. For example, in many studies frequent defecation has been shown to correlate with higher concentration of norepinephrine in the urine

(Smulders *et al.* 2006), which increases the likelihood of this behaviour being related to stress. However, higher levels of norepinephrine can also be related to other factors than stress.

Something else to consider when correlating behaviours to a specific emotional state is that pigs show significant individual variations in how they react to a certain stimuli or situation (Fraser 1974).

Pigs' sense of smell

Previous experiments of pigs' behaviour when exposed to high expansion foam have shown that they likely experience stress in these situations (Lindahl *et al.* 2020; Nilsson 2021; Söderquist 2020). A hypothesis of this thesis is that this reaction might be reduced if the foam has a different scent, as this might act as a distraction by attracting attention. But although pigs are thought to have a very good sense of smell, as studies have indicated that it is at least as good as that of a dog (Jensen 2006), it is relatively unstudied. So, what do we know about pigs' sense of smell?

It is shown that the area of the olfactory organ in a pig is about 30-45 times bigger than that of a human (Jensen 2006). Since the number of receptors per millimetre is the same in both species, one could argue that the possibility to register smells would be at least 30-45 times better in a pig than in a human. For example, it has been shown that pigs have the capacity to distinguish between individuals just from the smell of their urine (Meese *et al*. 1975; Mendl *et al.* 2002). They also have several glands in the face which emits smells, and which are seen to have an important role in mating behaviour as well as when interacting with other unknown or known individuals (Jensen 2006). In general, however, there is relatively little research on the olfactory sense in pigs, although the pigs' general behavioural patterns indicates that olfaction play an important role in their communication.

Considering that olfaction is often thought to be one of the most important senses to pigs, there has been relatively few studies considering olfactory enrichment in pig production. Some of the research that has been done, however, have shown that pigs tend to prefer objects or forms of enrichment that have an added olfactory component of some kind. One study found that pigs preferred to interact with ropes with added garlic oil compared to ropes with neutral scent (Blackie & de Sousa 2019). Another study showed that objects with added scents were preferred as enrichment by pigs of all ages (Van de Weerd *et al.* 2003). There have been studies in which different scents were compared, and the results indicated that the pigs preferred naturally scented objects over artificially scented ones (Nowicki *et al.* 2015). This might also be the reason why a study that enriched the pigs' environment with objects with added artificial rum or banana scent did not find any increased interest in the scented objects (Machado *et al.* 2017). The scent may not be pleasant or interesting enough to attract the pigs' attention. It is, of course, difficult to evaluate whether an object has a distinct or interesting scent for a pig or not, considering that they have such a supreme sense of smell compared to humans. The objects used as controls in the above-mentioned studies, which supposedly have a neutral scent or described as un-scented, most likely smell something to a pig which complicates the interpretation of their behavioural response.

Approved stunning methods for pigs at slaughter

All animals in Sweden must be stunned before slaughter (SFS 2018:1192), and legislation regulates what methods are approved for different breeds of animals (SJVFS 2020:22). The methods allowed for stunning of pigs in Sweden are captive bolt guns, shotguns, electricity, and CO_2. A new method is going to be compared to old ones both in terms of animal-based factors such as animal welfare and safety as well as more practically based factors such as economy and applicability. It is therefore necessary to give a short background to the methods mostly used today, with their advantages and disadvantages. As the objective of this thesis is to focus on the stunning methods' implication on animal welfare in the early part of the stunning process, that is the part of today's stunning methods that mainly will be discussed.

The majority of the pigs in Sweden are stunned by controlled atmosphere stunning (CAS) with the use of CO_2. The same goes for the bigger abattoirs in Germany (Tönnies 2022; Vion 2020), which is the largest European producer of pig meat. To give a sense of the numbers of animals affected by this, about 2.5 million pigs are slaughtered in Sweden each year according to The Swedish Board of Agriculture (Jordbruksverket 2021). That is about 246 000 tons of pig meat each year which makes up about 1% of Europe's yearly production (Eurostat 2021). In comparison, Germany produces around 22 % of the total annual production in Europe.

Carbon dioxide (CO_2)

When pigs are stunned by CO_2, they are being moved groupwise into a box and lowered into a shaft with increasing percentage of CO_2. This increase in CO_2 leads to a chemical reaction which lowers the pH at a cellular level and disables normal neurological function (Mota-Rojas *et al.* 2012). This leads to unconsciousness after a period of up to about 30 seconds (EFSA 2004), during which the pigs show clear signs of respiratory distress through vocalization, gasping and escape attempts

(Velarde *et al*. 2007; Wallgren *et al.* 2021). Stunning with CO_2 has both advantages and disadvantages from an animal welfare point of view. The main disadvantage is the respiratory distress that stems from breathing in a high percentage of CO_2 and the main advantages are the possibility to handle pigs in groups as well as not needing to restrain them at any point.

Why stunning of pigs with CO_2 has become one of the most used methods today can partly be explained by the relatively low levels of stress associated with handling and driving the pigs up until stunning (Wallgren *et al.* 2021). This is positive from an animal welfare point of view as well as for the processors, also enabling a higher pace in the abattoirs. By stunning with CO_2, pigs can be moved in the same small group all the way into the stunning box, and therefore there is never any need for individual handling or separation of individuals. As mentioned earlier, pigs are social creatures (Blasetti *et al.* 1988) that do not like being separated from their group (Kanitz *et al*. 2014), which is why this method of moving pigs in an abattoir is preferable.

Electricity

In Europe, electricity is one of the most common methods of stunning pigs (EFSA 2004). It works by passing a strong current of electricity through the animals' head which, if the electrodes are placed correctly, leads to an epileptiform seizure which causes immediate unconsciousness (McKinstry & Anil 2004). If the electrodes are placed on either side of the chest the method can also be used to induce cardiac arrest (EFSA 2004). For the method to work properly, the animal needs to be restrained to make sure the placement of the electrodes is correct, which can be extremely stressful for the pigs. This method also requires a skilled operator to work well, as different pigs will need slightly different placements of the electrodes. There are also completely automated systems for both restraining and placement of electrodes, but these can also lead to an inaccurate placement of electrodes and therefore to an ineffective stunning.

The main reason as for why this is not the most common method in Sweden is probably that to deliver the currency in a proper way the pig needs to be properly restrained, which is thought to cause too much stress. An alternative is that the electrodes are placed on the pig when it is not restrained, but this takes a skilled and fast operator and is always a risk and stress-factor both to the person and to the animals, hence is not applicable in large scale abattoirs (Wallgren *et al.* 2021). When this method is used at large scale abattoirs in other countries it is always used together with some form of stricter restraining method, which is correlated to stress in the animals.

Captive bolt gun

This method works through causing damage to the brain and brain stem as the bolt penetrates the skull and causes immediate, irreversible unconsciousness (Wallgren *et al.* 2021). In Sweden, captive bolt guns are only used in small abattoirs or for euthanasia at the farm. The reason for this being the need to handle animals individually as well as firm restraint to make sure this method is carried out right (EFSA 2004), both of which are stressful when it comes to pigs. This method also needs a great deal of skill in the operator to become fully safe and lower the risk of need to re-stun, as the correct area of which to apply the gun for effective stunning is very small in pigs. In larger animals, such as older sows or boars, the thickness of the scull can also become a problem as a normal captive bolt gun sometimes is not powerful enough.

Alternative stunning methods – studied but not (yet) commercially available

As mentioned in the introduction, there has been limited research in the field of new stunning methods (Sindhøj *et al.* 2021), even though today's methods have been criticized from an animal welfare point of view (EFSA 2004). When it comes to stunning of pigs compared to other animals, it is somewhat harder to develop a new method as it not only has to meet the normal stunning criteria such as workers' health and safety, reliability, and general practicality, but also needs to include the pigs' need for handling and driving in a group. This rules out stunning by electricity or captive bolt as it is performed today, and leaves CAS as the main method, with the differences potentially lying in what gases or gas mixtures are being used. Inert gases are allowed for stunning in the EU (Council regulation (EC) No 1099/2009), but practical issues such as availability and economic aspects and technical issues such as the density of gases causing them to mix with air has yet to be overcome.

Studies have been conducted regarding different gases and gas mixtures for stunning purposes. Argon (Dalmau *et al.* 2010b) and helium (MacHtolf *et al.* 2013) are two inert gases that have been evaluated as possible alternatives to CO_2. Both these gases have been studied for pig stunning purposes with promising results but the main drawback for both are the economic aspects as they are more expensive than CO_2. Argon could work in today's abattoirs as it is since, like CO_2, it is heavier than air. Helium, however, has a lower density than air and would need a completely new system to work.

To get around both the problem of the aversiveness seen during stunning with high concentrations of CO_2, as well as the high cost of the above-mentioned gases, there have been studies on different mixtures of gas. The studies that have been conducted have mainly shown that if the gas mixtures contain CO_2 in any higher percentage (>30%) it will still be aversive to the pigs (Llonch *et al.* 2012a).

Nitrogen gas (N_2) has also been studied and so far, it has been found to at least be less aversive than CO_2 to pigs (Llonch *et al.* 2012b) although it needs further research (Atkinson *et al*. 2015). It also makes up a large portion of the atmospheric air, which makes it relatively cheap to produce. The main problem with N_2 is that it is slightly lighter than air, which makes it harder to contain than CO_2 (Dalmau *et al.* 2010a). This is the reason why high expansion foam has been used to deliver N_2 when used as a method of stunning. The foam then serves two purposes; it helps achieve an anoxic environment faster as well as filling up the space with N_2 without it escaping or mixing with air (Lindahl *et al.* 2020).

The very limited research concerning high expansion foam has shown that pigs explore the foam with their snout; both when the foam contains N_2 (Lindahl *et al*. 2020) and not (Lindahl *et al.* 2020; Nilsson 2021). Furthermore, in a study comparing foam containing N_2 to foam containing air, the number of escape attempts was similar in both groups (Lindahl *et al.* 2020). This all indicates that N_2 is not in itself aversive to the pigs. The interest pigs show towards the foam generally ends when they are covered in it to a greater extent, after which they show more escape attempts as well as more behaviours with their head and snout above the foam. In some studies, however, this is not interpreted by the authors to be a reaction of panic as much as a reaction to a novel experience of being covered in foam (Lindahl *et al.* 2020; Nilsson 2021). To summarize, the method of nitrogen filled high expansion foam for stunning of pigs might not be free from aversive behaviour. However, it might be a viable alternative to CO_2 if it is established that the pigs' do not experience pain or show signs of suffocation. One physical sign related to suffocation is gasping, something that is seen when pigs are stunned with CO_2 (Velarde *et al.* 2007), but which has not been seen in studies using N_2 for stunning (Lindahl *et al.* 2020; Llonch *et al.* 2012a).

Lastly there have been some studies done in the field of low atmosphere pressure stunning (LAPS) where anoxia is reached through lowering the pressure in the box in which the animals are kept (Bouwsema & Lines 2019). However, this method was shown to induce aversive behaviours in the pigs such as shakes, head tilts, grimacing and escape attempts. It is possible that these reactions are partly because of physical pain as low pressure is known to cause pain in the ears in humans, and some evidence of ruptured ear drums have been found after studies of LAPS (McKeegan *et al.* 2020 through Grandin 2021). It seems therefore that this probably is not a viable alternative and further research is most likely not going to change the negative aspects already found.

Material and Methods

Ethical permit

This experiment falls under the ethical permit for basic research and education at Lövsta, with registration number 5.8.18-06784; *Undervisning och klinisk träning på nötkreatur, grisar samt höns vid Lövsta lantbruksforskning i Sveriges lantbruks-universitets djurrelaterade grund-, och forskarutbildningar, samt specialist-, fort- och vidareutbildning av teknisk och akademisk personal. Insamling av basinformation. [Education and clinical practice on cattle, pigs and poultry at Lövsta research facility in animal-related programs of all levels at Swedish University of Agricultural Sciences, also for further education of technical and academical personnel. Collecting of basic information]*.

The animals

This study took place over 4 consecutive days, 18-21st of October 2021, plus a pilot study on the 11th of October at the pig research facility at Lövsta belonging to SLU (Swedish University of Agricultural Sciences). The pig production at Lövsta is SPF (specific pathogen free) and integrated with about 110 sows of the breed Yorkshire. Pregnant sows are moved to the farrowing pens about a week before the planned farrowing date, and the production is planned so that approximately 10 sows farrow every second week. The piglets are kept in the farrowing pen for around ten weeks, of which the first five weeks are together with the sow. At birth the pigs are gendered, weighed, and get an identification tattoo in the ear. At 5 days they get an iron injection as well as an ear tag for individual identification and at two weeks they get their second injection of iron. At two and four to five weeks they are weighed and at about ten weeks they are moved to the area for growing pigs and weighed again. The piglets get feed made for piglets from about two weeks of age.

The 50 pigs used in this study were between 14 and 16 weeks old (106 ±7 days) when entering the study. At 9 weeks of age they weighed on average 28.6 kg, ranging between 20.2 kg and 40.2 kg. The pigs were different crosses between Yorkshire, Hampshire and Duroc. In total there were 30 females and 20 males. The pigs were randomly chosen from a section of the stable with pigs of a suitable size for this study (mainly, fitted in to the foam box reasonably well). Three pens containing at least ten pigs were randomly selected. In the pens, every second pig – in ascending order of identification number, were chosen for the group with foam with added scent and the other half of the pigs were chosen for the treatment with repeated visits to the foam box. The group with repeated visits included five more pigs belonging to a randomly chosen pen in the same section of the stable. The pilot study included fifteen pigs belonging to three randomly chosen pens in the same section of the stable.

The material

The foam agent and box used were developed and produced by the Dutch company Anoxia. The box's outer dimensions were 120x100x85cm, and inner dimensions were 110x92x67cm (figure 1). The floor and lid were made of polycarbonate and were transparent. The floor was taped with clear anti-slip tape on the inside and was re-applied after day two of the study. On the outside red tape divided the box into four equally sized sections. Two 50-litre bottles with compressed air, at 200 bar, were used, and the pressure was reduced to 5 bar per bottle. These were connected to two flat, high-capacity foam generators connected to one side of the box (figure 1). For foam production, a 3% solution of foam agent (Hi-Ex foam mild) and water was used. For the experiments with added scent, 360 ml *Dr Oetker vaniljarom* [vanilla aroma] was added per 20 litres of foam solution. On two sides along the floor there was a gas jet pulse system to destroy the foam (figure 1).

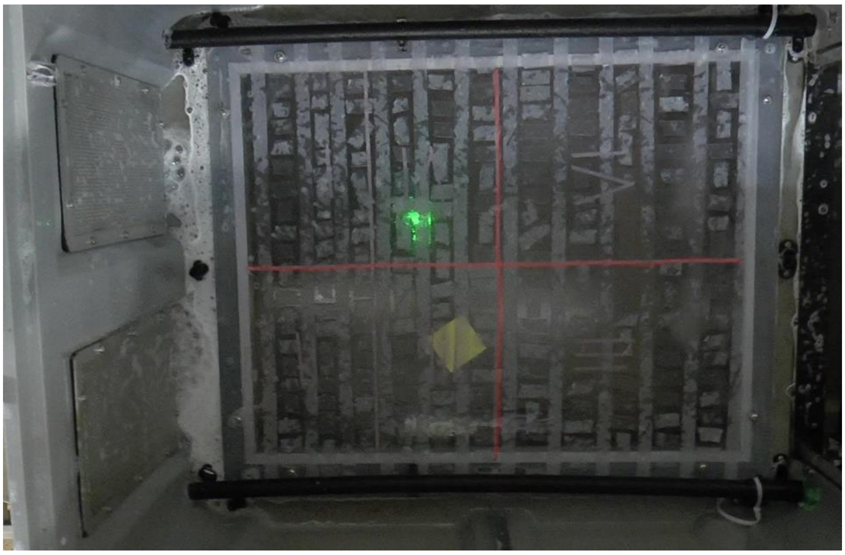

Figure 1. The foam box from above, with lid open. The transparent floor and clear anti-slip tape are seen. The red tape dividing the floor into four sections is enhanced to be seen in this photo. On the left side, the two, flat foam generators are seen, and along the floor the gas jet pulse system (black pipes).

To record the animals on video, two cameras were used. A Garmin Virb Ultra was placed in a stand in the culvert under the box, and a Panasonic HC-x920 was placed in a stand above the box. A microphone was placed inside the box and connected to the camera filming from above. A digital stopwatch was used to keep track of the time each pig spent inside the box.

Method studying pigs in the foam box

The pigs were moved from their original section of the stable and pen in groups of five pen-mates to an empty pen in the section of the stable where the experiment took place. They were given at least 15 minutes to acclimatize before the first pig was moved to the foam box. One pig at a time, in ascending order of identification number, was moved to the foam box. Some force (i.e., pushing) was often needed to make the pig enter the foam box. When the pig was in the box, the lid and door was closed and cameras as well as the stopwatch were started. After one minute in the box, the foam generator was started, and when the box was completely filled with foam the generator was turned off. About five seconds later, an air pulse which destroyed the foam was turned on for a short time. The time it took for the box to be filled with foam varied, mostly depending on how much the pig moved or where it was standing in the box. Three minutes after the pig entered the box, the video cameras were turned off and the pig was let out and taken back to the pen-mates. The foam box was cleaned with water before the next pig was moved to the box. When all five pigs in one group had gone through this procedure they were taken back to their original section of the stable.

All video recordings were analysed, and registration of behaviour was done for a total of 12 ten second intervals per pig and day, three of which were before the foam generators started and nine of which were after the foam generators were started.

The experiments were performed by the same two persons on all days, and the analysing of videos were done by one person.

Study with foam with added scent

In total, 30 pigs, 17 females and 13 males, went through the above explained experiment, and were exposed to foam with added vanilla scent, on one occasion. Out of these 30 pigs, 15 went through the experiment on the pilot study day (see page 22), and 15 went through the experiment on the first of the four consecutive days.

Study with repeated exposure to foam

In total, 20 pigs, 13 females and 7 males, were exposed to foam without an added scent. They went through the above explained experiment on three occasions, on three consecutive days. The collected data from these 20 pigs on day one also worked as a control to the study of how pigs react to foam with added scent.

Behaviour recording

In total, 22 behaviours were registered. These are presented in the table below (table 1).

Table 1. Definitions of behaviours observed and how these were registered.

Behaviour	**Definition**	**Registration**
Stand	Standing position with all four hooves on the floor	Number of times the behaviour is observed during a 10 second interval
Sit	Sitting position with weight on the front hooves, one or both buttocks in contact with the floor	Number of times the behaviour is observed during a 10 second interval
Lay down	Laying position with one side or the belly in contact with the floor	Number of times the behaviour is observed during a 10 second interval

Behaviour	Definition	Registration
Slip	One or more hooves sliding fast and uncontrolled over the floor	Number of times the behaviour is observed during a 10 second interval
Explore wall	Snout in contact with the wall	Number of times the behaviour is observed during a 10 second interval
Explore floor	Snout in contact with the floor	Number of times the behaviour is observed during a 10 second interval
Explore lid	Snout in contact with the lid; with ≤50% of the body covered in foam	Number of times the behaviour is observed during a 10 second interval
Explore foam	Snout in contact with the foam, or active movement against the foam.	Number of times the behaviour is observed during a 10 second interval
Avoid foam	Pig is actively trying to get its' snout away from the foam or is jumping over it; with >50% of the body covered in foam.	Number of times the behaviour is observed during a 10 second interval
Escape attempt through lid	Kicking with front or back legs, jumping or pushing against the lid	Number of times the behaviour is observed during a 10 second interval
Escape attempt through door	Kicking with front or back legs, jumping or pushing against the door	Number of times the behaviour is observed during a 10 second interval
Escape attempt through wall	Kicking with front or back legs, jumping or pushing against the wall	Number of times the behaviour is observed during a 10 second interval
Vocalisation - grunts	Grunts	Number of times the behaviour is observed during a 10 second interval
Vocalisation – screams	Screams or squeals	Number of times the behaviour is observed during a 10 second interval
Defecation		Number of times the behaviour is observed during a 10 second interval

Behaviour	Definition	Registration
Activity	Number of lines on the floor crossed with both front legs	Number of lines crossed during a 10 second interval
Backing	Movement backwards (from noise or foam)	Number of times the behaviour is observed during a 10 second interval
Shaking	Rapid shaking of the body and/or head	Number of times the behaviour is observed during a 10 second interval
Startled	One rapid flinch through the body	Number of times the behaviour is observed during a 10 second interval
Freezing	No movement of hooves, with body and head fixed	Registered when duration >5 seconds
Positioned towards the door	Both front legs positioned in square 3 or 4 (towards the door)	Occurring at least once within a 10 second interval
Foam coverage	Percentage of the pig covered by foam	0, <50%, 50%, >50%, 100%

Statistical analysis

All behaviours were compiled in Microsoft Excel (Office16), and thereafter the statistical analyses were performed using Minitab version 19 (Minitab, LCC, 2020).

As no behaviours were normally distributed, the behaviours which occurred multiple times for a pig within an interval were converted to binary variables (1 or 0, i.e., the behaviour did (1) or did not (0) occur during a 10 second interval for a pig). The statistical unit analysed was 10 second interval per pig. Pairwise differences for the binary behavioural variables between treatment (scent or no scent) or day (1, 2, 3) were analysed with 2 proportions z-test on both day and on interval level. The P-value was determined with Fisher's exact test.

Rarely observed behaviours (less than 10 times in total) were described as proportion of pigs showing the behaviour but were not further analysed. These behaviours were startle, shaking, backing up and defecation (described more in detail in table 1).

Some behaviours were never observed in any pig during any interval, and these were lying down and escape attempts through door or wall (described more in detail in table 1) and were thus not further analysed.

The 15 pigs used in the pilot study were exposed to foam with added scent. The behavioural response of these 15 pigs corresponded to the behaviour of the 15 pigs in the same treatment, on the first of the four consecutive days, when assessed ocularly in histograms. Therefore all 30 pigs that were exposed to foam with added scent were as one group in the further statistical analysis.

Missing observations

Due to technical issues with the cameras, some 10 second intervals are missing for some pigs. In total 9 intervals from one pig were missing completely, with both the upper and lower camera shutting off at the same time. Thus, this information is missing in the data. This occurred in the treatment with no added scent, which also is day 1 of the repeated study.

For the intervals where only one camera was shut down, and as there were no normally distributed behaviours and they were converted into binary variables, it was still possible on all occasions to observe if studied behaviours happened or not in each interval even if the video recording from underneath was missing. Thus this information is still included in the data. The lower camera shut off at four other occasions leaving out 25 other intervals from that camera. The intervals missing belonged to one pig in the treatment with added scent (3 intervals), two pigs in the pilot study with added scent (14 intervals) and one pig on day three of the repeated study (8 intervals).

The only behaviour that was not possible to register when the lower camera was shut off was "placement in the box", therefore there are in total 34 intervals in which this behaviour is not studied.

Results

The results are presented separately for the two sub-studies; added scent and repeated exposure to foam.

Foam with added scent

The proportion of pigs covered by foam ≥50% during each interval are presented in figure 2. There was a significant difference between the two treatments in interval 7, but no overall difference. That is, no general difference between the two treatments on how fast the pigs became covered by foam, nor a difference within any other intervals.

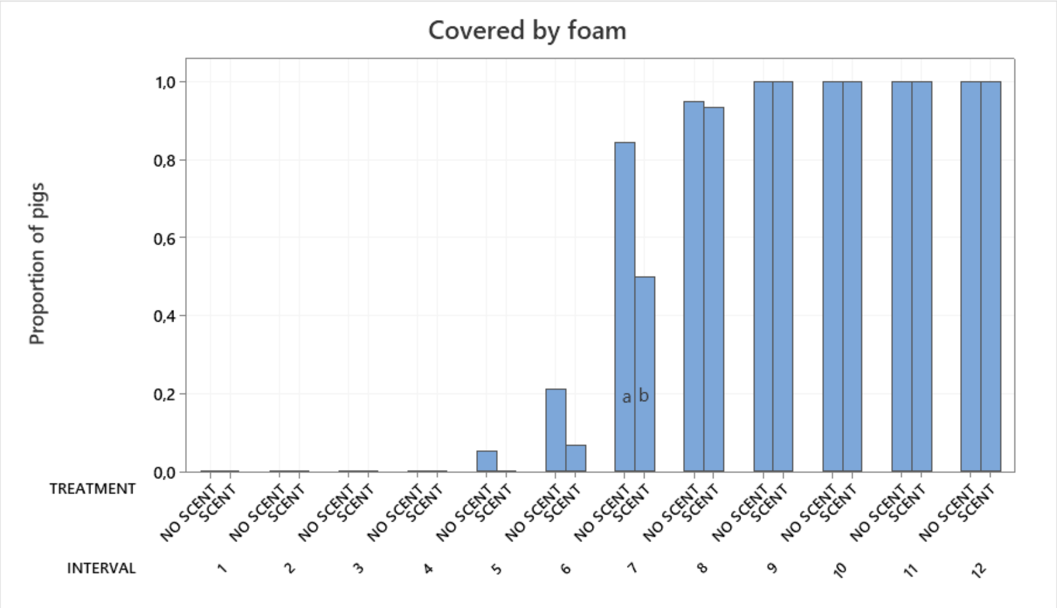

Figure 2. Proportion of pigs ≥50% covered by foam in each interval on the Y-axis, and the interval 1-12 as well as the treatments on the X-axis. In total 30 pigs in the treatment scent, and 20 pigs in the treatment no scent.
The letters a & b indicate significant pairwise difference using 2 proportions test and Fisher's exact of a P-value <0.05 In interval 7 the P-value was 0.018 and the Z-value was 2.76.

In the overall comparison (all intervals included) between the treatments there were significant (P<0.05) differences between treatments for the behaviours: activity, freezing, escape attempt, exploring wall, exploring foam, and sitting. The exact P-values of these differences as well as the proportions of pig interval in which each behaviour was seen in the "scent" and "no added scent" treatments are presented in table 2.

Table 2. Proportion of pig intervals in which each behaviour was shown in the two treatments. In total 30 pigs in treatment scent and 20 pigs in treatment no added scent, with 12 10-second intervals per pig.
The letters a and b indicate a significant difference (P<0.05) in behaviour between treatments using 2 proportions test and Fisher's exact. All Z-values and P-values using 2 proportions test and Fisher's exact are included.

Behaviour	Scent	No added scent	Z-value	P-value
Activity	0.583[a]	0.489[b]	-2.25	0.028
Freeze	0.078[a]	0.186[b]	3.71	<0.001
Squeals/Screams	0.228	0.212	-0.45	0.686
Grunts	0.675	0.610	-1.60	0.113
Escape attempt through lid	0.064[a]	0.000[b]	-4.96	<0.001
Explore lid	0.142	0.134	-0.26	0.903
Avoid foam	0.258	0.195	-1.83	0.090
Explore wall	0.436[a]	0.247[b]	-4.91	<0.001
Explore floor	0.472	0.481	0.20	0.866
Explore foam	0.425[a]	0.242[b]	-4.76	<0.001
Slip	0.028	0.048	1.20	0.255
Stand	0.994	1.000	1.42	0.519
Sit	0.017[a]	0.050[b]	2.14	0.026

There were some low frequent occurring behaviours that were observed in less than 10 intervals. These behaviours are presented below and without statistical testing as the proportions were very low.

- Startle was observed in 1/30 pigs in treatment scent, and in the treatment with no added scent it was observed in 3/20 pigs. It was observed either at start of foam production or at start of air pulse destroying the foam.
- Shake was observed in 1/30 pigs at two different intervals in treatment scent, and in the treatment without added scent it was not observed in any of the pigs.
- Backing was observed in 3/30 pigs and in one of these pigs at two different intervals in treatment scent, and in the treatment without added scent it was observed in 5/30 pigs.
- Defecation was observed in 2/30 pigs in treatment scent, and in the treatment without added scent it was observed in 2/20 pigs.

There were also some behaviours that did not occur at all, these are presented below.

- Lay down
- Escape attempt through wall
- Escape attempt through door

In figures 2-4, the results of the behaviours that showed statistical significances in at least one interval are presented. The behaviours whose graphs showed a random pattern when ocularly examined where not included.

Explore wall

The behaviour “explore wall” was defined as the pig having its’s snout against the wall. This behaviour had a higher overall occurrence in the treatment scent (table 2) and had a higher numerical occurrence in all intervals, with significant differences in intervals 1, 4, 5 and 7 (figure 3).

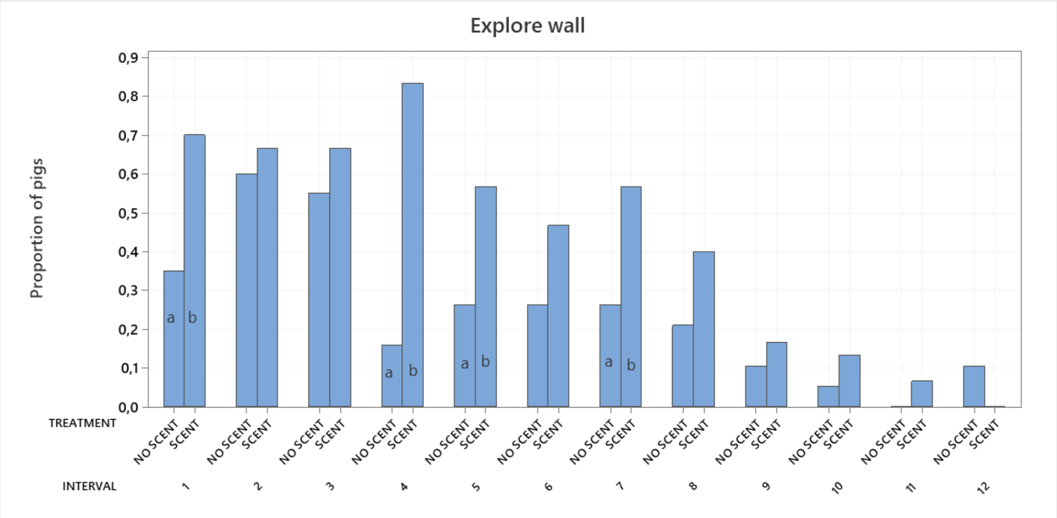

Figure 3. The behaviour “explore wall”, with the proportion of pigs displaying the behaviour on the Y-axis, and the interval 1-12 as well as treatment on the X-axis.
The letters a & b indicate significant pairwise differences using 2 proportions test and Fisher’s exact of a P-value <0.05.

Explore foam

The behaviour "explore foam" was defined as the pig having its' snout in contact with the foam when it was optional, that is when the snout/head was moving into the foam with perceived intention. This behaviour had a higher overall occurrence in the treatment with added scent (table 2) and had a higher numerical occurrence in all intervals except interval 4, with significant differences in interval 7 and tendencies to a difference (P<0.1) shown in interval 6 and 10 (figure 4).

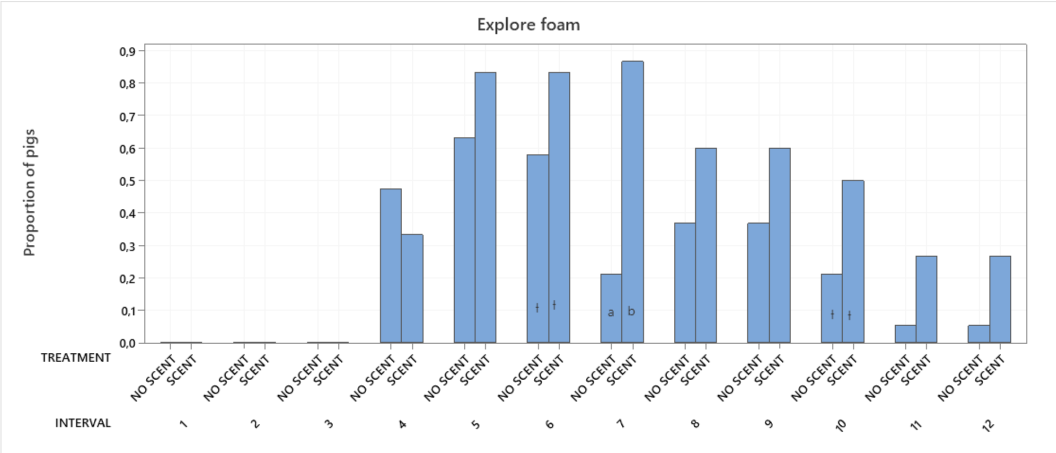

Figure 4. The behaviour "explore foam", with the proportion of pig displaying the behaviour on the Y-axis, and the interval 1-12 as well as treatment on the X-axis.
The letters a & b indicate significant pairwise differences using 2 proportions test and Fisher's exact of a P-value <0.05, and the crosses indicate tendencies to a difference using the same method but with a P-value <0.1.

Activity

The behaviour "activity" was defined as when both front legs crossed one of the lines taped on the box floor. This behaviour had a higher overall occurrence in the treatment scent, and a higher numerical occurrence in all intervals except interval 6 and 7, with a significant difference seen in interval 11 (figure 5).

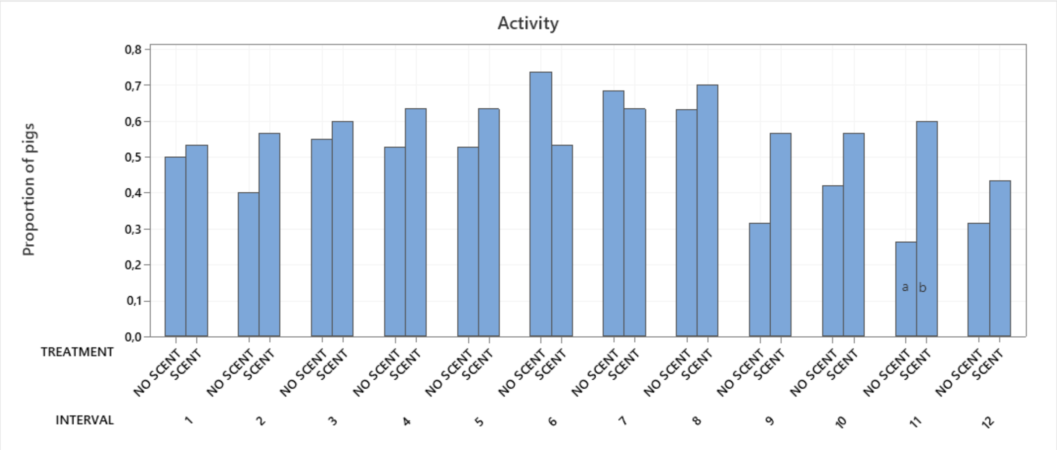

Figure 5. Activity, i.e., taped lines on the floor crossed, with the proportion of pig displaying the behaviour on the Y-axis, and the interval 1-12 as well as treatment on the X-axis.
The letters a & b indicate significant pairwise differences using 2 proportions test and Fisher's exact of a P-value <0.05.

Repeated exposure to foam

The proportion of pigs covered by foam to ≥50% during each interval are displayed in figure 6. There were no significant differences, overall or within any specific interval, between the days (figure 6).

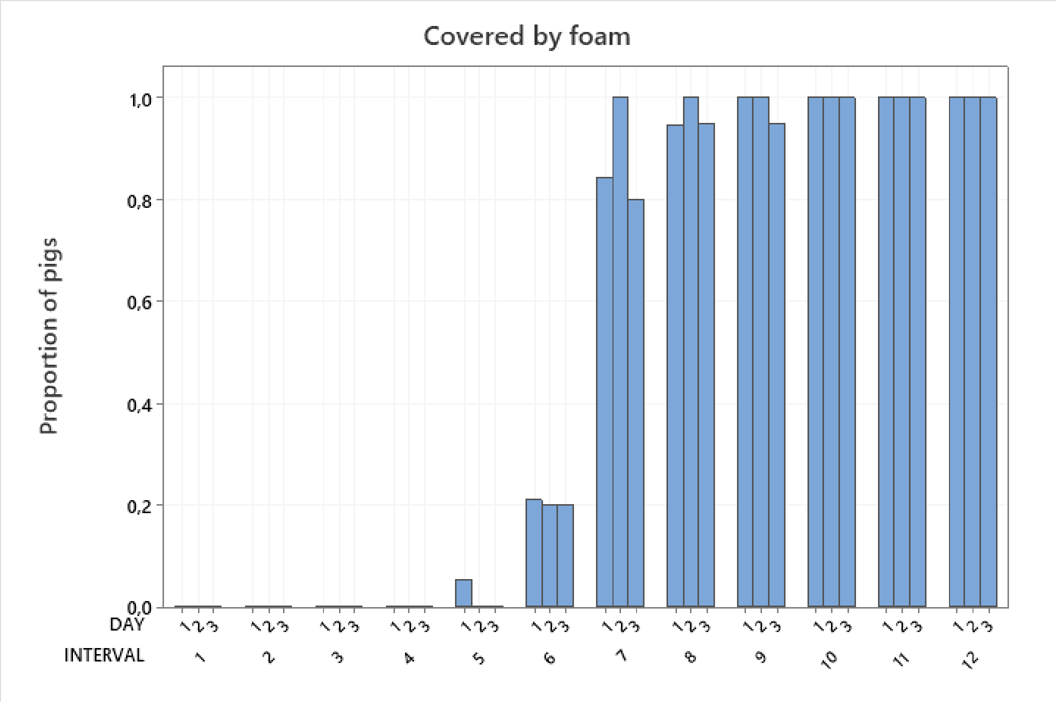

Figure 6. Proportion of pigs ≥50% covered by foam in each interval on the Y-axis, and the interval 1-12 as well as the days on the X-axis. There were no significant differences between any interval nor overall.

In the comparison between the three days there were significant ($P<0.05$) differences between individual days in the behaviours: squeals/screams, grunts, escape attempt, exploring wall, exploring floor, slipping, sitting and positioned towards the door. There was also a tendency to a difference in the behaviour avoiding foam. The exact P-values of these differences, between which days they were observed as well as the proportions of pigs performing each behaviour on each day are displayed in table

Table 3. Proportion of pigs showing each behaviour on each day; day 1,2 and 3. In total 20 pigs on each day, with 12 10-second intervals per pig. The letters a, b and c indicate a pairwise significance using 2 proportions test and Fisher's exact. The Z-values and P-values using 2 proportions test and Fisher's exact are included for all pairwise tests between the days.

Behaviour	**Day 1**	**Day 2**	**Day 3**	**Z-value**			**P-value**		
				Between day 1-2	*Between day 1-3*	*Between day 2-3*	*Pairwise difference day 1-2*	*Pairwise difference day 1-3*	*Pairwise difference day 2-3*
Activity	0.489	0.550	0.458	-1.32	0.67	2.02	0.186	0.519	0.055
Freeze	0.195	0.158	0.154	0.80	0.92	0.13	0.465	0.391	1.000
Squeals/screams	0.212[a]	0.296[b]	0.317[b]	-2.10	-2.59	-0.50	0.044	0.012	0.692
Grunts	0.610[a]	0.788[b]	0.821[b]	-4.26	-5.19	-0.92	<0.001	<0.001	0.421

Escape attempt through lid	0.000[a]	0.008[ab]	0.025[b]	-1.42	-2.48	-1.43	0.499	0.030	0.285
Explore lid	0.134	0.097	0.083	1.45	1.77	0.32	0.148	0.102	0.872
Explore wall	0.247[a]	0.458[b]	0.692[c]	-4.93	-10.81	-5.32	<0.001	<0.001	<0.001
Explore floor	0.481[a]	0.496[a]	0.371[b]	-0.33	2.42	2.79	0.782	0.020	0.007
Explore foam	0.242	0.200	0.179	1.11	1.69	0.58	0.317	0.113	0.642
Avoid foam	0.195	0.267	0.196	-1.86	-0.03	1.85	0.080	1.000	0.083
Slip	0.048[a]	0.138[b]	0.050[a]	-3.42	-0.12	3.33	0.001	1.000	0.001
Stand	1.000	0.996	0.996	1.00	1.00	0.00	1.000	1.000	1.000
Sit	0.013[a]	0.058[b]	0.017[a]	-2.69	-0.33	2.42	0.011	1.000	0.028
Positioned towards the door	0.632[a]	0.733[ab]	0.776[b]	-2.37	-3.43	-1.08	0.023	0.001	0.288

There were some behaviours that were shown in less than 10 intervals. These are presented below and without statistical testing as the proportions were very low.

- Startle was observed in 3/20 pigs on day one and 2/20 pigs on day three.
- Backing was observed in 5/20 pigs on day one.
- Defecation was observed in 2/20 pigs on day one, 2/20 pigs on day two and 2/20 pigs on day three.

There were also some behaviours that did not occur at all, these are presented below.

- Lying down
- Escape attempt through door
- Escape attempt through wall
- Shaking

Explore floor

The behaviour “explore floor” was defined as the pig having its’s snout against the floor. This behaviour had a lower overall occurrence on day three than day one (table 3) and had a lower numerical occurrence on day three than day one in all intervals but the three last (10-12). There were significant differences in interval 2, 4 and 9. There were significant differences between day two and three in interval 2 and 4, and between day one and three in interval 9 (figure 7).

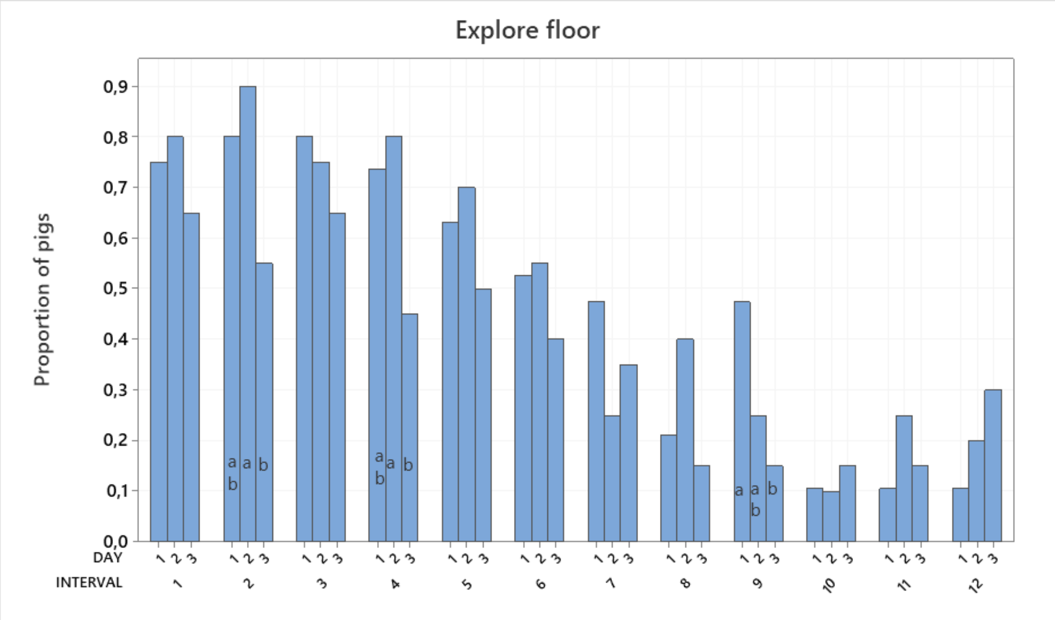

Figure 7. The behaviour “explore floor”, with the proportion of pig displaying the behaviour on the Y-axis, and the interval 1-12 as well as the day on the X-axis.
The letters a & b indicate significant pairwise differences using 2 proportions test and Fisher’s exact of a P-value <0.05.

Explore wall

The behaviour "explore wall" was defined as the pig having its's snout against the wall. This behaviour had a higher overall occurrence on day three than one (table 3) and had a higher numerical occurrence in all intervals. There were significant differences in interval 1, 4, 5, 6, 7, 8, 9, 10 and 11 (figure 8).

The significant differences were seen between day one and three in all intervals mentioned above. Between day one and two significant differences were seen in interval 4, 6, 7 and 9, and between day two and three significant differences were seen in interval 8 and 10 (figure 6).

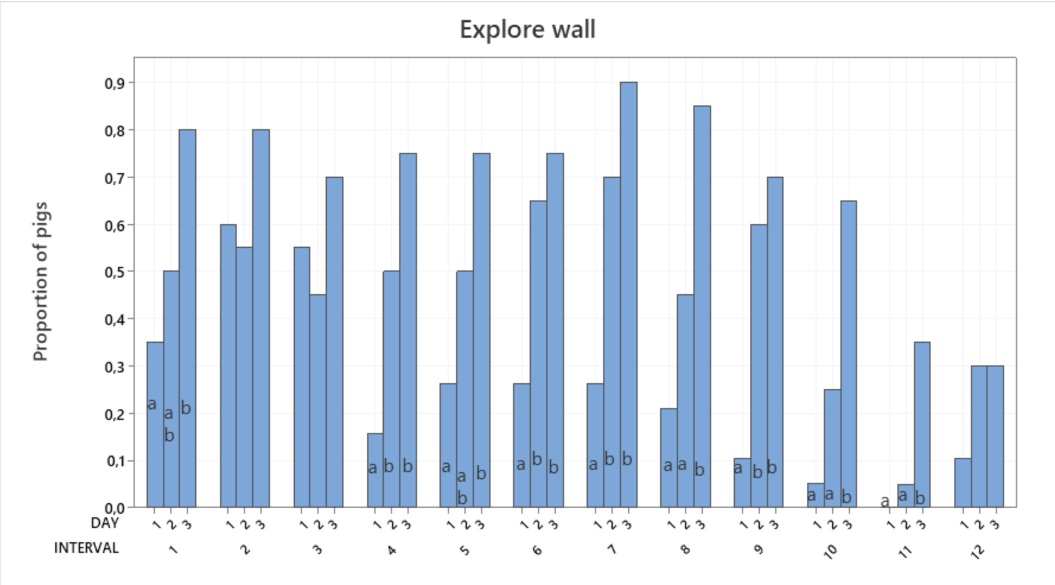

Figure 8. The behaviour "explore wall" is displayed, with the proportion of pig displaying the behaviour on the Y-axis, and the interval 1-12 as well as the day on the X-axis.
The letters a & b indicate significant pairwise differences using 2 proportions test and Fisher's exact of a P-value <0.05.

Grunts

This behaviour had a higher overall occurrence on day three than day one (table 3) and had a higher numerical occurrence in all intervals but the last. There were significant differences in interval 4, 5, 6, 9 and 10 (figure 7).

The significant differences were seen between day one and three in interval 4, 5, 6 and 9, and between day one and two significant differences were seen in interval 4, 5 and 10. No significant differences were found between day two and three (figure 9).

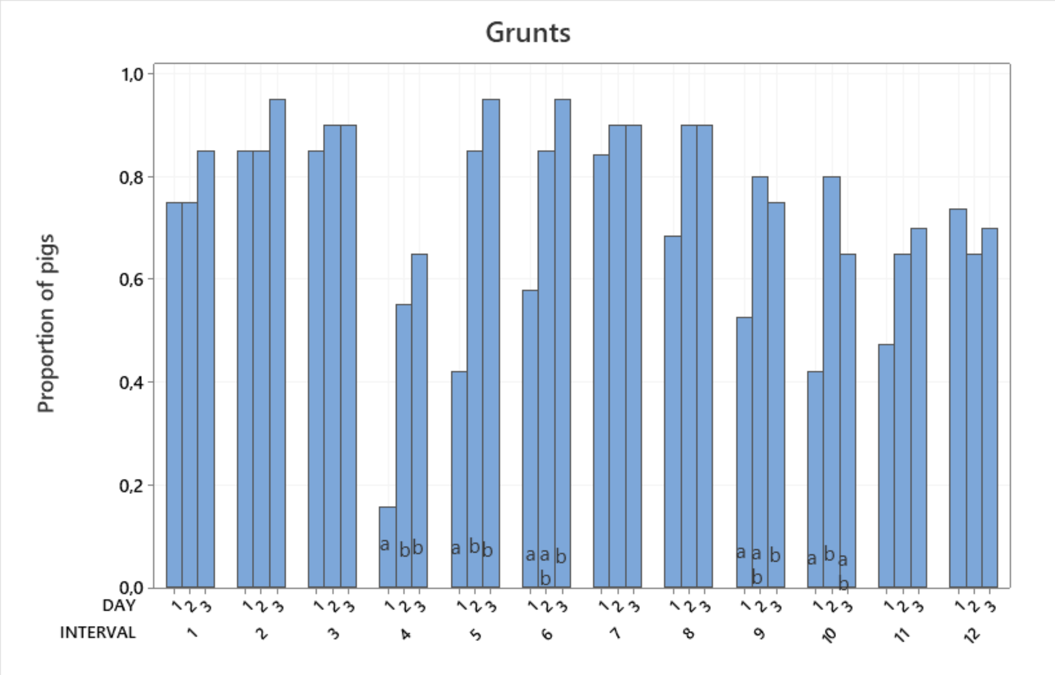

Figure 9. The behaviour "grunts" is displayed, with the proportion of pig displaying the behaviour on the Y-axis, and the interval 1-12 as well as the day on the X-axis.
The letters a & b indicate significant pairwise differences using 2 proportions test and Fisher's exact of a P-value <0.05.

Squeals/Screams

Vocalisation in form of squeals and screams had a higher overall occurrence on day three than day one (table 3) and had a higher numerical occurrence in interval 1, 4, 5, 6, 7, 8, 9 and 10. The pigs performed the behaviour more frequently on day one than day three in interval 3, 11 and 12 (figure 10).

There were significant differences in interval 4, 5, 6 and 12. Between day one and three in all intervals mentioned above, there were no significant differences observed between day one and two or between day two and three.

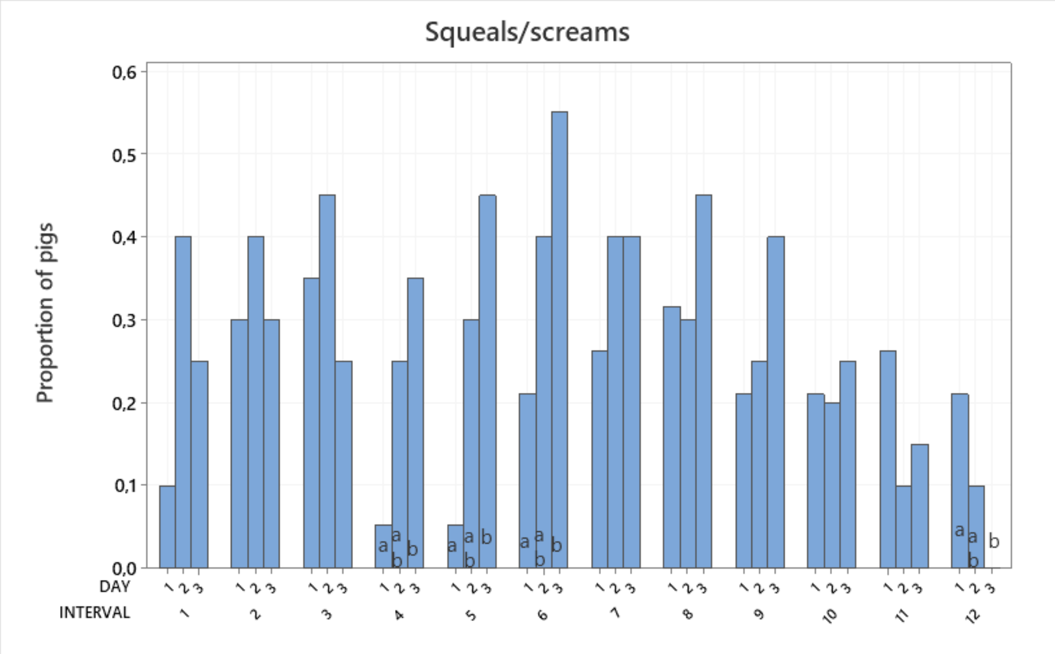

Figure 10. The behaviour "squeal/scream" is displayed, with the proportion of pig displaying the behaviour on the Y-axis, and the interval 1-12 as well as the day on the X-axis.
The letters a & b indicate significant pairwise differences using 2 proportions test and Fisher's exact of a P-value <0.05.

Positioned towards the door

The proportion of pigs facing towards the door at some point during an interval had a significantly higher overall occurrence on day three than day one (table 3). There were no significant differences within any single interval, but a significant differ-rence observed between day one and three.

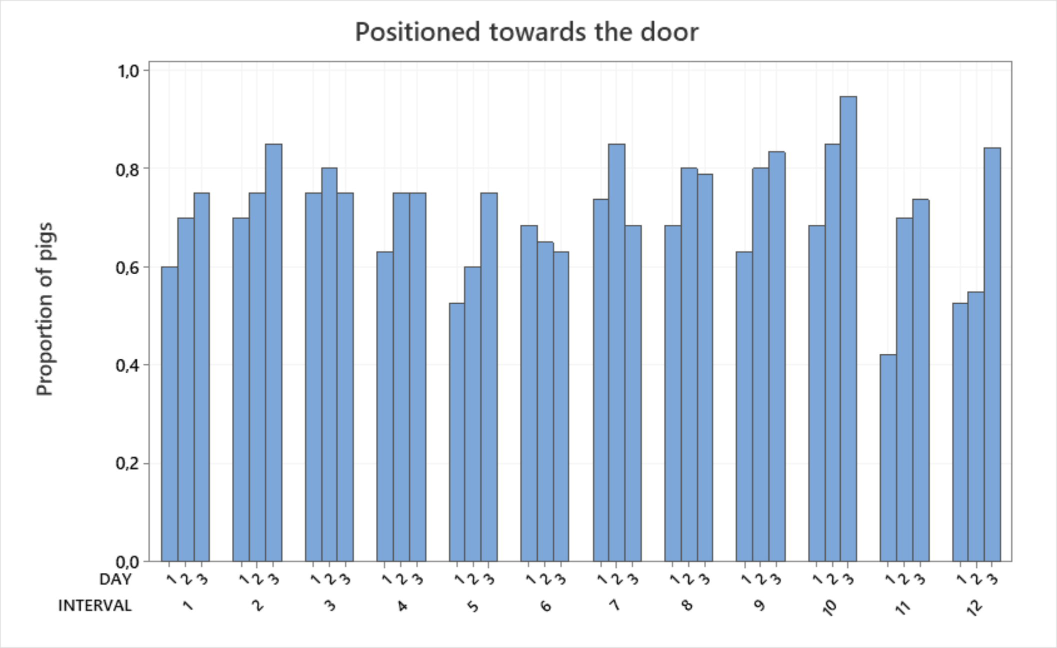

Figure 11. The behaviour "position in the box" is displayed, with the proportion of pigs facing the door on the Y-axis, and the interval 1-12 as well as the day on the X-axis.

Discussion

The following discussion is divided into sections. In the first section a discussion of the results of the study of foam with or without added scent is presented. Thereafter, the results of the study with repeated exposure to foam is discussed, followed by a discussion regarding low-frequency behaviours and behaviours not observed at all. The discussion section also includes a method discussion and a conclusion summarising both studies.

Foam with added scent versus foam with no added scent

In the treatment "foam with added scent" the process to fill up the box with foam was slightly slower than for the pigs in the control treatment. As the pigs in the treatment with added scent generally were more active and explored the foam more, it is likely that they simply destroyed more of the foam, as it is noted in earlier studies that increased activity is related to slower filling of the box (Lindahl *et al.* 2020). However, another explanation may be that adding the vanilla aroma to the foam solution changed the solution and possibly made the foam less stable. This is an aspect that needs to be further investigated if scent is to be added to the foam solution in the future.

Exploratory behaviour

In the treatment "foam with added scent", there was a larger proportion of pigs displaying exploratory behaviour, as both exploration of foam and wall had significantly higher overall occurrence. Both types of exploratory behaviour became less common with time (over intervals), which is in line with previous studies (Thurehult 2019; Lindahl *et al.* 2020; Nilsson 2021). The greater interest in exploring the foam seems to be directly related to the added scent, indicating that the scent made the foam more interesting to the pigs and therefore worthy of exploration. This finding is supported by previous studies, which concluded that added scent to enrichment objects increased the pigs' interest and interaction with the objects (Van de Weerd *et al.* 2003; Nowicki *et al.* 2015; Blackie & de Sousa 2019).

The increased interest in exploring the wall when the foam had an added scent could also be related to the scent of the foam, as the whole box would probably smell more interesting when the foam has an added scent. It could also be that increased exploring of foam could lead to pigs exploring everything else as well, simply because it triggers the exploration behaviour generally and exploring the environment is natural to pigs (Studnitz *et al.* 2007). That an increase in exploration of the floor is not seen could be because the floor is quickly covered by foam, and the foam itself might be more interesting than the floor. The results show that increased activity could be associated to a longer time until complete filling of the box with foam. These results are in line with an earlier study (Lindahl *et al.* 2020) and is further discussed under "activity and freezing behaviour" below. This needs to be further researched to ensure that when the foam is filled with gas and used for stunning purposes, scent-induced increased activity does not significantly increase the time until loss of posture and/or unconsciousness.

If the method of stunning pigs through gas filled foam becomes an approved method in abattoirs, a scent could quite possibly be used to decrease the pigs' distress through creating a distraction. In earlier studies with nitrogen filled foam, the period from foam generators starting to loss of posture is quite short, a maximum of 54 to 76 seconds has been noted (Lindahl *et al.* 2020; Nilsson 2021). By improving the foam production, the time to loss of posture could most likely be further shortened, thus the scent could possible work as enough of a distraction for most of that time. It would of course then be necessary to ensure that the distraction coming from an added scent actually lowers the pigs' levels of stress.

Activity and freezing behaviour

The measure of activity was significantly higher overall in the treatment with added scent. This is likely related to the higher frequency of exploration as the pigs are moving around more when exploring the box and foam. In the treatment with added scent, the proportion of pigs being active did not decrease over time as much as in the group without added scent. This behaviour is hard to compare directly to earlier studies since it has been described differently. In two studies it was described as number of lines crossed instead of proportion of pigs crossing any line at all (Thurehult 2019; Söderquist 2020). And in two studies performed with nitrogen filled foam the pigs lost their posture and/or consciousness during the experiment (Lindahl *et al*. 2020; Nilsson 2021), and therefore the change in activity over time will differ to this study. Either way it would be hard to compare as the pigs in those earlier studies have been younger and thus smaller, while the pigs in this study did not have much room to move around in the box.

On the opposite scale of moving around because of exploratory behaviour, there is freezing behaviour, where the pigs freeze in place with their hooves and keep body and head fixed. Freezing is a behaviour that has not been analysed in this type of study before. Even though it was not seen in a large proportion of pigs in this study, there was a significant difference over all between the two treatments, and more frequently seen in the treatment with no added scent. This is a type of behaviour that has been seen in studies with anxiety or fear inducing stimuli (Fraser 1974; Reimert *et al*. 2013) and it seems possible that where some pigs react to stress or fear in an explosive way with escape attempts or increased activity, other pigs freeze for periods of time. This behaviour was mainly observed in the later intervals of the trial, which further strengthens the connection to it being induced by stress or anxiety from being almost completely covered by foam. Since this behaviour was only seen in a limited number of pig intervals however (18.6% in treatment "no added scent" and 7.8% in treatment "scent"), it is hard to know how to interpret it. It would be an interesting behaviour to continue studying in further research on pig behaviour during exposure to foam, and then also relate it to other indicators of stress (e.g. stress hormones in the blood) to support the interpretation.

Escape attempts

Generally, there were very few escape attempts performed (at most 10% of the pigs in any single interval), but still a significant difference between the treatments was found, with a larger proportion of pigs showing escape attempts in the treatment with added scent. Had the study included a larger number of pigs it would perhaps have been possible to draw conclusions from this behaviour, as it could turn out to be important. In some previous studies, escape attempts have been seen to increase with time spent in the foam (Lindahl *et al.* 2020; Söderquist 2020), which was not seen in this study. In earlier studies the increase in escape attempts have been thought to be related to the foam covering the pigs' heads, something that in this study occurred later because of the bigger size of the pigs. That the pigs in this study did not show escape attempt to any great extent could therefore be more related to their size than to the sensation of the foam covering the head.

Sitting and slipping

The behaviour sitting has a similar problem in interpretation as the escape attempts, as there were very low proportions of pigs displaying the behaviour in both treatments (0.017-0.05). It is therefore not possible to draw any conclusions from this result even if there were significant differences between the treatments. However, sitting was usually seen in combination with slipping, in that the pigs slipped and ended up in a sitting position and then stayed there for a shorter or longer period. Both slipping and sitting was seen more in the treatment without scent, and those trials were conducted the last day before the floor of the box was re-taped with new anti-slip tape, which might have played a big part.

Pigs exposed to foam on repeated days

Exploratory behaviour

The proportion of pigs exploring the wall increased with repeated exposure, whereas the exploration of the floor was less seen on day three than on day one. This might be because the floor could initially be more interesting, it is wet and smells of the foam solution, whereas on day two and three the floor has already been explored and therefore the wall becomes more interesting. As seen in earlier studies, the exploration of the floor decreases over time (over intervals during treatment) (Thurehult 2019; Lindahl *et al*. 2020; Nilsson 2021), but results from the current study also showed a slight numerical increase on day two and three on the last two intervals. This could be random but could also indicate that the pigs find the foam less frightening with time and will explore the floor even when it is covered by foam.

Activity

There was a slight decrease in the proportion of pig intervals where activity was observed between days two and three. As there was also a decrease in exploration of the floor, these behaviours could be related to each other. When the pig explores the floor less, it might move around less. However, as there is an increase in the exploration of the walls which could be thought to lead to increased activity this theory is not conclusive. On day three it might be likely that the pigs have already moved around in the box enough to know where it is most interesting or less frightening to stand. The pigs might also have realised or learned what is going to happen and know that they will soon be let out. The latter theory is somewhat supported by the fact that they are facing the door to a greater extent, especially in the last two intervals, on day three than one.

Vocalisation in the form of grunts and squeals/screams

The proportion of pigs grunting increased between day one and three. There was also a clear drop in the behaviour as foam generators were started in the beginning of interval four, which is similar to what has been observed in earlier studies (Söderquist 2020; Nilsson 2021). Grunting is seen as a way for pigs to communicate and it is usually a more or less continuous activity that happens while for example exploring the environment (Kiley 1972; Houpt 1998; Jensen 2006). That more pigs were grunting on day two and three could therefore indicate that they are more comfortable in the box even when it is filling up with foam, and that they are resuming their normal behavioural pattern. It could also indicate an anticipation of soon being let out to their box-mates again.

The proportion of pigs squealing or screaming also increased from day one to three. This could of course indicate that the pigs are more anxious (Kiley 1972), but it might also mean that they are getting more comfortable in the situation as a whole and therefore are more comfortable making noise. Since both grunting and more high frequency vocalisation is heard, it is likely that this is connected to the anticipation of being let out and that this excitement is thus communicated to their box-mates who the pig know is outside the box. The actual increase was between day one and two, and day three stays the same so it does not get more and more intense as the days go on. What is interesting is that on day one, there is a sharp numerical drop in proportion of pigs squealing or screaming when foam production starts (interval 4), which has also been found in previous studies (Lindahl *et al.* 2020; Söderquist 2020; Nilsson 2021). The drop in proportion of pigs squealing or screaming at start of foam production is not seen on day two and three which indicates that the pigs get used to the sudden sound of the foam generators.

Escape attempts and avoiding foam

There was an increase in escape attempts from day one to day three. As the total number is very small however, (0 pigs on day 1 and 6/20 pigs on day 3, that is in 2.5% of the intervals as no pig was seen performing an escape attempt on more than one occasion on the same day) it is not possible to draw any major conclusions from this result. It is nevertheless an increase and so it would be interesting keep track of this behaviour if studies with a larger number of pigs are performed.

There was also a very slight (not significant) increase in the behaviour of avoiding foam from day one to two (proportion of pig intervals where the behaviour was seen increasing from 0.195 to 0.267), but this difference is also so small that this is difficult to discuss further. Furthermore, there is no difference in numbers between day one and three.

It is possible of course that the changes in both these behaviours stem from the pigs being more stressed when exposed to foam after having experienced it before. They know what is going to happen and might try to escape or avoid the foam if they know that it is going to be uncomfortable. The increase of the total number of pigs displaying escape attempts could point to this, and a higher reactivity due to sensitisation could be a possible cause.

Sitting and slipping

There was an increase in proportion of pigs slipping and sitting on day two. The relationship between these two behaviours is explained further in the discussion about foam with added scent. What is odd is that the floor was re-taped with anti-slip tape between day one and two in this study, and therefore was expected to be less slippery on day two. There is no good explanation to the increase.

Rarely observed behaviours

The behaviours observed very seldom were startling, shaking, backing up and defecation. Because of how seldom these behaviours were seen it was not possible to analyse them further. It is however interesting that all these behaviours, which are thought to be related to stress or fear in pigs (Dalmau *et al.* 2009; Reimert *et al.* 2013), are observed in such small numbers. In an earlier study, startling is seen in 60% of the pigs (Lindahl *et al.* 2020), whereas in this study it is only seen in at most 15% of the pigs on day one in the repeated study.

Possibly as backing up was only observed on day one, it is an indication of fear that disappears on repeated days as the pigs get used to the sound from the foam generators and air pulse. But as it was only registered in a total of 8/50 individuals (16%) on the first day, that is a very weak indicator.

A behaviour that was noted, but not often observed on the videos, was defecation. It was often seen before the start of the treatment, or in such places as were not seen from the cameras (the walls, or sides of the floor). There were many individuals that defecated at some time between being put in the box and taken out of it, even if few of these were caught on camera during the studied intervals. Defecation has been correlated to stress in pigs (Smulders *et al*. 2006), so that so many pigs defecate at some point during the trial seems reasonable. However, in future research it might be better to look at if the pig has defecated at all during the time in the box or not, and not count this in certain intervals or times only visible through the cameras. It would of course make it impossible to know if a certain part of the experience gives rise to a higher frequency of defecation, but it might still give more information about the experience as a whole.

Method consideration

The same person, the author, analysed all the video recordings and registered what behaviours occurred, which minimizes the risk of perceiving behaviours differently between pigs or treatments. A disadvantage with this approach was that it was not a blinded study and could therefore be a source of bias, but as the author did not have any stake or self-interest in the outcome of the study the risk was found acceptable. It was not always possible to see what the pig was doing with its' head due to condensation on the lid, the box shifting position when the pig entered it or the head being on the edges of the box, which were not transparent. However, as the same person did all observations, and the conditions were similar in all trials, it should still be possible to compare the observations between treatments.

Considering the statistical analysis there is a risk of mass significance due to the number of statistical tests performed. However, the perceived relevance of the results is not only based on statistically significant differences, but also on the trends of observed behaviours. It would have been possible to further lower the risk of mass significance through advanced statistical analyses, but in this study there was no time for that.

The pigs used in this study were older, and therefore larger, than the ones used in previous studies, but the same test box was used. This most likely influenced the behaviours the pigs showed. Escape attempts via door or wall were not observed, and all escape attempts seen were interpreted as directed towards the lid of the box. It is possible that escape attempts towards the top part of the door or wall, where the head and snout would be due to the size of the pigs, were interpreted as escape attempts towards the lid. In previous studies, escape attempts have usually been grouped together regardless of where they are directed (Lindahl *et al.* 2020; Söderquist 2020; Thurehult 2019), as the motivator for the behaviour is likely the same and the main objective was to compare the frequency of escape attempts.

Another behaviour, which was not observed in the study, is lying down. This is most likely because the pigs were not relaxed enough to perform this behaviour. It was also noted that they did spend very little time, if any, lying down in the "temporary pen" together with their pen mates as well. In total, it therefore seems likely that the pigs are simply not relaxed in the new section of the stable or in the foam box, which seems reasonable but still differs from one earlier study where lying down was seen (Lindahl *et al.* 2020).

There was a difference in how fast the box was filling up with foam, mostly having to do with how the pigs were standing and how much they were moving. This affected how much time each pig spent completely covered by foam, and also when the air pulse destroying the foam was started. As the air pulse destroying the foam made a different noise and occurred in different intervals for different pigs it is possible that behaviours showed in the latter intervals are affected by this.

In choosing what scent that was to be added to the foam solution in the treatment "scent" consideration was taken into account of what type of scent was thought to induce as little irritation in the face of the pig. Vanilla aroma approved for human consumption was then decided to be the kindest alternative possible at this time. As far as deciding on a concentration, vanilla aroma was added to the foam solution until the vanilla scent could be detected and the chemical "scent" of the foam solution was less strong. For future research it would be interesting to try different scents and concentrations and carry out studies where pigs would choose from these.

Lastly, the floor of the box has to be transparent to be able to study behaviour, but this also makes the floor slippery without application of anti-slip tape. However, as the tape wears off over time, the floor gets more slippery day by day. Thus, it is necessary to re-apply tape after a certain number of pigs, depending on how much the pigs are moving around, but still, that is a factor that will differ between days which could have influence on pig behaviour.

Conclusion

When the foam had an added scent, the pigs showed significantly more exploratory behaviour indicating an increased interest to the foam throughout the whole trial. Therefore, it can be concluded that adding a scent to the foam solution may be a way to distract the pigs and possibly to make the experience less uncomfortable for the pigs. There was an increase in the number of escape attempts in treatment "scent", but as this was in very small numbers it is harder to evaluate and makes the results less clear overall. Adding a scent to foam would need further research on other types of scent and concentration to determine the best scent to be added and to make sure that it does not affect the end product e.g., quality or food safety aspects. It also needs further research to ensure that the change in behaviour seen is actually correlated to a lower level of stress.

When the pigs were repeatedly exposed to foam, exploration of the wall and vocalisations became more frequent with exposure occasions. Their behaviours did not indicate that pigs got a very increased fear or stress reaction when repeatedly exposed to foam, but neither did their stress related behaviours decrease. The most clearly stress-related behaviour such as escape attempts and avoiding of foam was seen in too low numbers to draw many conclusions from. An increase in vocalisation overall could also have different explanations, from anticipation of soon being let out, to increased anxiety. This is why further studies would be needed to evaluate the levels of anxiety in this situation. A larger number of pigs on an increased number of days would probably yield more conclusive results.

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Populärvetenskaplig sammanfattning

Alla djur som slaktas i Sverige måste bedövas innan avblodning, och beroende på djurslag finns olika metoder tillgängliga. För gris är de vanligaste bedövningsmetoderna koldioxid och elektricitet. Vid elektrisk bedövning placeras två elektroder på vardera sida av grisens huvud och en stark ström leds igenom vilket orsakar ett epilepsilikt anfall som leder till medvetslöshet. Den största nackdelen med denna metod är att grisarna behöver drivas en och en fram till bedövningen, vilket är problematiskt då grisar är sociala djur som föredrar att röra sig i flock. Det krävs också någon form av fasthållning av grisarna för att elektroderna ska kunna placeras korrekt, vilket också bidrar till mycket stress för grisarna och för personalen som hanterar dem. Vid bedövning med koldioxid sänks grisarna gruppvis ner i ett schakt med ökande koldioxidkoncentrationer, vilket efter en period på upp till 30 sekunder ger en sänkning av pH-värdet i centrala nervsystemet vilket ger upphov till medvetslöshet. Den största nackdelen med denna metod är att grisarna uppvisar kraftigt obehag när de sänks ner i koldioxiden, de flämtar efter luft, skriker och försöker fly. I studier på människa har exponering för höga koncentrationer koldioxid gett upphov till starka reaktioner av rädsla och oro. Redan år 2004 fastslog av EFSA (European Food Safety Authority) att båda dessa metoder har omfattande brister ur djurvälfärdssynpunkt, och att nya studier inom ämnet behövs för att hitta alternativ – men trots detta har endast ett fåtal nya studier publicerats inom ämnet sedan dess.

En viktig del av utvecklandet av nya bedövningsmetoder är att utgå ifrån djurens normala beteenden. Grisar är som tidigare nämnts sociala djur som vill röra sig i grupp, varför metoder som underlättar drivning och bedövning i grupp är att föredra för att minska stress för både människor och djur på slakteriet. Den typ av nya metoder som främst undersöks i dagsläget är därför bedövning med gas av olika slag, eftersom det möjliggör just gruppbedövning. I EU är inerta gaser så som argon, helium och kvävgas redan godkända för bedövning av gris. Dock finns det inte några alternativa kommersiella bedövningssystem tillgängliga, detta på grund av begränsade studier inom ämnet samt både tekniska och ekonomiska utmaningar kvar att lösa. Både argon och helium anses vara för dyra för att fungera i stor skala, men kvävgas är billigt, lättillgängligt och inerta gaser har i studier inte visat sig ge upphov till samma kraftiga obehag hos grisar som koldioxid. Vid användning av inerta gaser sker bedövning genom syrebrist, vilket kräver en snabb ersättning av luft med gas. Problemet med kvävgas är att den har en något lägre densitet än luft och därför lätt blandas med luften om gasen används i fri form, varför det blir svårt att håla en stabil atmosfär med tillräckligt låg syrenivå. En möjlig lösning är att binda gasen i ett högexpansivt skum som därmed hindrar kvävgasen att blandas med luft samt trycker undan luften och därmed också syret snabbare, vilket resulterar i en snabbare bedövning.

I studier som gjorts med skum, både när det varit fyllt med kvävgas och med vanlig luft, har frågor uppkommit kring hur grisarna upplever skummet i sig, då det observerats vissa aversiva (undanflyende) beteenden i samband med exponeringen. Det har diskuterats om grisarnas reaktioner i studierna hänger ihop med att skummet upplevs som skrämmande eller om reaktionerna framförallt har att göra med det faktum att det är en helt ny situation för dem. I och med det har det även uppkommit frågor kring om skummet går att göra mindre obehagligt. För att vidare undersöka de aspekterna var denna studies huvudfrågeställningar om grisarnas beteenden förändras om skummet är luktsatt samt om grisarna kan komma att vänja sig vid skummet om de utsätts för det vid upprepade tillfällen.

I studien användes totalt 50 grisar; 30 grisar utsattes för ett luftfyllt skum med en tillsatt vaniljlukt, och 20 grisar utsattes under tre på varandra efterföljande dagar för ett luftfyllt skum som inte hade någon tillsatt lukt. Dock har skummet i sig en viss kemisk lukt. Dag ett för grisarna i den upprepade studien användes som en kontrollgrupp i frågeställningen kring hur grisarna reagerade på luktsatt skum. Grisarna fick gå in i en försöksbox som sedan stängdes och efter en minut började fyllas med ett luftfyllt skum. Skumproduktionen fortsatte tills boxen var fylld och sedan stängdes den av och skummet blåstes sönder med en luftpuls. Totalt spenderade grisarna 3 minuter i försöksboxen innan de därefter släpptes ut och fick gå tillbaka till sin grupp. All tid i boxen filmades, båda ovanifrån och underifrån, och av dessa filmer analyserades totalt 2 minuter per gris med avseende på vissa utvalda beteenden.

I studien med luktsatt skum utforskade grisarna skummet och väggarna i boxen mer om skummet var luktsatt än om det inte var det. De förflyttade sig även mer i boxen och hade färre perioder när de stod helt stilla. Totalt sett kan deras beteenden tolkas som att de tyckte skummet blev mer intressant när det var luktsatt, eftersom de utforskade det mer och att det även ledde till att de utforskade boxen mer i övrigt också. Att de rörde på sig mer bedöms hänga samman med ökningen av det utforskande beteendet. Det fanns även en liten ökning hos gruppen med luktsatt skum i antalet flyktförsök som uppvisades, men det var väldigt få tillfällen totalt och är därför svårt att analysera vidare.

I studien där grisarna exponerades för skum under tre dagar i rad visade resultaten att grisarna utforskade väggarna mer för varje tillfälle, men att de utforskade golvet mindre. Resultaten visade också att grisarna vokaliserade mer, både skrik och grymtningar blev mer frekventa från dag ett till dag tre. Resultatet från denna del av studien är något mer svårtolkat. Att de utforskar väggarna mer, men golvet mindre kan handla om att intresset skiftar, på dag två och tre har de kanske redan utforskat golvet klart. Att vokaliserandet både i form av grymtningar och skrik ökar kan ha olika förklaringar. Det kan handla om en normalisering av vokaliserings-beteendet, då grisar normalt sett kommunicerar kontinuerligt med varandra via ljud. Detta skulle i så fall också kunna hänga ihop med en förväntan på att snart bli utsläppta till sina kompisar. Men ökad vokalisering skulle också kunna tyda på en ökad oro i situationen. Det skulle helt enkelt krävas vidare studier för att utreda hur grisarna upplever skum vid upprepade tillfällen.

För att summera, visar studien med luktsatt skum att grisarna tycker det är mer intressant med ett skum som luktar vanilj. De undersöker både det och omgivningen i boxen i övrigt mer. Det ökade intresset verkar inte vara huvudsakligen skräckfyllt, utan luktsättningen på skummet skulle snarare kunna ses som en fungerande ”distraktion”, även om det fanns en ökning i antalet flyktförsök var det ett väldigt begränsat antal totalt sett. Fler studier skulle behöva göras för att utreda om det som tolkas som distraktion faktiskt innebär minskade stressnivåer för grisarna och undersöka om det finns andra lukter som skulle kunna vara ännu mer intressanta. Vidare behövs studier när skummet är fyllt med gas för att se så att luktsättningen fungerar bra även då. Resultaten i den upprepade studien kan tolkas som att grisarna inte upplever skummet som väldigt obehagligt eftersom de inte verkar få en ökad stressreaktion när de vid upprepade tillfällen utsätts för skum. Å andra sidan finns det inte heller tydliga tecken på att stressen minskar vid upprepade tillfällen i kontakt med skummet. Resultaten är helt enkelt något mer svårtolkade i den del-studien. För att vidare undersöka hur grisarna upplever skummet skulle en studie med fler upprepningar i skumboxen kunna göras för att se om en större förändring i beteende skulle ske över tid. Men även en studie där grisarna får välja om de vill gå in i luftfyllt skum eller inte, och om de kan lockas in med till exempel äpplen, skulle vara av intresse för att få vidare kunskaper om hur skummet upplevs av grisarna.