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An added aroma changes the behaviour of domestic pigs in a novel situation aimed for stunning

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ABSTRACT

The currently most widespread stunning method for pigs is high concentration of carbon dioxide (CO_2), but this method is under scrutiny due to animal welfare deficits. One alternative method currently under development with potential to replace CO_2 is nitrogen (N_2) filled high expansion foam. While N_2 does not elicit the same aversive behaviour as CO_2, it is currently not known if the high expansion foam itself may be frightening to the pigs. Means to alleviate fear reactions to foam could consist of diverting pigs' attention using aromas (an odour in combination with taste) of interest to them. The current study aimed to investigate if an added aroma (vanilla aroma) affected pigs' avoidance and exploratory behaviour when exposed to air-filled high expansion foam in a foam box. The study included 50 pigs (30 females, 20 males) of 14–16 weeks of age, of different crosses between Yorkshire, Hampshire and Duroc. The pigs were randomly assigned to either the treatment with air-filled foam with added aroma or the control treatment with no added aroma, which they were exposed to in a foam box. The results showed that pigs with the aromatised foam expressed significantly more exploration behaviour directed towards the foam and box walls, but not towards the floor or the lid. The aromatised foam also resulted in a higher activity level of the pigs. In contrast to the hypotheses, pigs with aromatised foam expressed more avoidance behaviour, and escape attempts were exclusively observed when pigs were exposed to aromatised foam. Slipping events and frequency of vocalisations did not differ between pigs with and without aroma added to the foam. This study shows that an added aroma (taste and odour in combination) increased pigs' exploration behaviour towards the foam, and thus could serve as a distraction for the pigs when inside a confined foam box. The results emphasise the importance of taking foam aroma into consideration in the further development of the N_2-filled high expansion as a stunning method. The potential anxiety- or stress-reducing effect of any added aroma needs further investigation, and future studies should focus on assessing various types of aromas, and/or odours, as well as different concentrations of the compounds to determine which solution is most efficient in alleviating pig stress and anxiety.

1. Introduction

It is currently debated if high concentration carbon dioxide (CO_2) stunning should be banned, but banning CO_2 as a stunning method requires alternatives, and there is thus an urgent need for research on alternative stunning methods. 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 2004, the European Food Safety Authority (EFSA) was already discussing that stunning of pigs by CO_2 is an inadequate practice, and that focus should be put on development of better methods for large-scale stunning and slaughtering of pigs (EFSA, 2004). In pigs, respiratory distress has been demonstrated when the concentration of CO_2 surpasses 30% (Raj and Gregory, 1996) and even hungry pigs (fasted for 24 hours) would not enter a box with apples if filled with 90% CO_2 (Raj and Gregory, 1995). In 2020, a review showed that since EFSA concluded a need of further research of alternative stunning methods in 2004, only 15 new scientific articles on the subject had been published (Sindhøj et al., 2021). These 15 articles included different gas mixtures combining CO_2 and other gases such as argon or N_2 in varying concentrations, hence were not focusing on methods without CO_2. A

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method currently developed in small scale, with potential to be an alternative to CO_2 in commercial slaughter is nitrogen (N_2)-filled high expansion foam. Studies have shown that N_2 does not elicit the same aversive behaviour in pigs as CO_2 (Llonch et al., 2012), although it needs further research (Atkinson et al., (2015). N_2 exposure at extremely low oxygen levels in humans has shown to cause unconsciousness in seconds and without warning MineArc (28 April, (2023). N_2 makes up a large portion of the atmospheric air, which makes it relatively cheap to produce. However, the density of N_2 is similar to air, which makes it harder to contain compared to CO_2 (Dalmau et al., 2010). This is the reason why high expansion foam has been used to deliver N_2 when tested as a method of stunning. The foam thus 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). At present, knowledge about the functionality of N_2 in high expansion foam is insufficient to allow for approval for slaughter even if N2 in itself (and other inert gases) is approved for stunning of pigs within the EU (Council Regulation (EC) no 1099/2009). In Swedish national legislation, inert gases such as N_2 are not approved as a method to stun and euthanize pigs (SJVFS 2020:22) as more knowledge about the method is needed and a proven commercially feasible system for N_2 stunning is lacking. Although EU has not yet approved the use of high expansion N_2 foam, the method is commercially available for culling of sick piglets on farm. Hence, the use of N_2 for stunning purposes is not novel, but adding high expansion foam to facilitate the stunning process with N_2 is new and in need of further research. The foam eliminates oxygen on average 2.7 times faster than pure N_2 gas (Lindahl et al., 2020), thus the foam substantially decreases the time until initiation of unconsciousness. A proof of concept for the N_2 foam method for stunning young pigs has been provided by Lindahl et al. (2020). The method effectively stunned the pigs and all pigs were in deep unconsciousness or dead after being in the box for five minutes after foam start. The stunning function in N_2 stunning is hypoxia, in contrast to CO_2 where the stunning function is a combination of hypoxia and hypercapnia. Both N_2 and CO_2 stunning are reversible, meaning that the pigs can regain consciousness, but depending on the exposure time they can also be irreversible. Further comparisons between stunning methods, as well as description of current state of development and implementation are described by Wallgren et al. (2021). It is crucial to understand how pigs perceive high expansion foam and how it may affect the level of stress when pigs are covered in foam, in the process of evaluating the method for stunning.

1.1. Pig behaviour when exposed to foam as indicator of emotional state

In gas stunning situations, behavioural indicators of aversion to the situation include; backing away, head shaking, and escape attempts (EFSA, 2004). Research on pig behaviour when exposed to 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 when it contains air (Lindahl et al., 2020; Nilsson, 2021). Furthermore, in a study comparing pigs' responses to foam containing N_2 to foam containing atmospheric air, the number of escape attempts was similar in both groups (Lindahl et al., 2020). Collectively, this indicates that N_2 is not in itself aversive to the pigs. The interest shown by pigs toward 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.

Although pigs are vocal animals, research about vocal communication in pigs is limited. Pigs communicate through continuous series of grunts of different length which can be heard in a range of situations, for instance when greeting, during social isolation, as a response to fear or anticipation (Kiley, 1972). If heard while for example rooting or foraging, these grunts can be interpreted as positional signals between individuals (Houpt, 1998) to facilitate group cohesion. Barks are mostly heard when pigs are startled or in frustration-like situations, whereas squeals or screams are heard when pigs are in pain or in a fear-eliciting situation (Kiley, 1972). Vocalisations may thus be used as indicators of emotional states of pigs.

Another mean to evaluate emotional state in pigs while exposed to foam is exploration behaviour. In wild or free roaming pigs, most of the active time is spent rooting or grazing, and another large proportion is spent moving and exploring the environment (Stolba and Woodgush, 1989; Studnitz et al., 2007). Pigs are highly motivated to explore their environment (Studnitz et al., 2003; Boissy et al., 2007) and the driver for this behaviour is thought to be positive emotional states. Increased exploration can therefore serve as an indicator for positive emotional states in pigs (Zupan et al., 2016). Behaviours that are specifically anxiety or fear induced are freezing, high-pitch vocalisation, defecation and escape attempts (Fraser, 1974; Reimert et al., 2013). 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.

1.2. Pig olfaction and aromas as distraction

Pigs are thought to have a well-developed and sensitive olfactory apparatus, and pigs use this sensory modality in their daily activities (for detailed review see: Schild and Rørvang, 2023). Sniffing and rooting are among the most common natural pig behaviours (Wood-Gush and Vestergaard, 1989; Day et al., 1995; Studnitz et al., 2007), where the sense of olfaction plays a crucial role in locating food (Stolba and Woodgush, 1989; Studnitz et al., 2007). Pigs are able to distinguish between individuals based on only the smell of their urine (Meese and Baldwin, 1975; Mendl et al., 2002). Pigs also have glands in the facial region, which emit odours, and which have an important role in mating behaviour and when interacting with unknown or known individuals (Stolba and Woodgush, 1989). Olfaction, and information contained in odours, thus seem important to pigs and to communication among pigs. It is therefore somewhat surprising that relatively little research has been done on the sense of olfaction, olfactory behaviour in pigs, or the use of odours in practical management of pigs (Schild and Rørvang, 2023). Studies focussing on olfactory enrichment in pig production are limited. The few studies that exist show that pigs prefer enrichment that have an added olfactory component. One study found that pigs preferred to interact with ropes with added garlic oil compared to ropes with neutral odour (Blackie and de Sousa, 2019). Another study showed that objects with added odours were preferred as enrichment by pigs of all ages (Van de Weerd et al., 2003). Studies comparing different odours indicated that pigs preferred odours of natural origin over artificial odours (Nowicki et al., 2015). This could explain why another study that enriched the pigs' environment with objects with added artificial rum or banana odour did not find any increased interest in these odourised objects (Machado et al., 2017). In a recent study, odours of natural origin have been shown to be of interest to pigs (Rørvang et al., 2023), and such odours could potentially serve as a distractor when pigs are exposed to potentially aversive situations such as a stunning box and high expansion foam. In the studies by Blackie and de Sousa (2019), Machado et al. (2017), Nowicki et al. (2015) and Van de Weerd et al. (2003) the olfactory component was added onto enrichment materials which the pigs had physical access to (combined odour and taste termed *aroma*), whereas Rørvang et al. (2023) assessed purely olfactory enrichment (i.e., no taste confound), as pigs only had access to sniff the volatiles of the odour. An odour is a particular smell or aroma associated with a specific item or substance, which can be pleasant or unpleasant. The term smell, on the other hand, is used to describe the sensing of an odour that is experienced when the animal detects the particular odour. This is a neurobiological process that involves the detection of volatile organic compounds (chemicals) in the air by the olfactory system, which then stimulate the olfactory bulb located in the brain triggering the interpretation of the odour by the brain (Schild and Rørvang, 2023). The term taste refers to the sensing of a taste compound by the taste receptors of the mouth (usually located in the tongue). Once the sensation of a taste and a smell is collectively experienced, this is referred to as a flavour. A compound stimulating a combined flavour experience is termed an aroma. In order to be efficient in alleviating stress, the added compound needs to have a pleasant and interesting taste and smell to the pigs when added to the foam, and it can be hypothesised that the attenuating effects could be larger if using both a pleasant smell and taste. Addition of an aroma to high expansion foam thus seem a viable mean to alleviate stress to divert pigs' attention, due to a combined smell and taste of interest to pigs.

The current study aimed to investigate if an added aroma of natural origin affected the behavioural response of pigs when exposed to air-filled, high expansion foam in a stunning box. The overall hypothesis was that behaviour indicative of aversion from being covered in foam in a foam box would be reduced if the foam had an added aroma. The hypotheses were that 1) pigs would show more exploratory behaviour towards the foam and box interior when the foam had an added aroma compared to the original foam detergent, and 2) pigs with aromatised foam would show less avoidance behaviour toward the foam and being in the box due to the distracting aroma.

2. Materials and methods

2.1. Ethical considerations

This experiment was conducted under the ethical permit for basic research and education at Lövsta, with registration number 5.8.18–06784 (approval date 2020–06–26). Humane endpoints were pre-defined in collaboration with the veterinarian and the head of research at the research centre. All procedures in the experiment were conducted in agreement with the ethical guidelines proposed by the Ethical Committee of the ISAE (International Society of Applied Ethology) (Sherwin et al., 2003) and met the ARRIVE guidelines (Kilkenny et al., 2010).

2.2. The experimental venue and general animal management

This study took place over three experiment days, 11th, 18th and 19th of October 2021, at the pig research facility at the research centre Lövsta, Swedish University of Agricultural Sciences, Uppsala. The pig production at Lövsta is a SPF (specific pathogen free) integrated farrowing to finish production with about 110 sows. The housing and management procedures in the Lövsta herd are described in SLU (2017). The 48 pigs included in this study were between 14 and 16 weeks old (104 ± 4.8 and 109 ± 2.3 days old in the Aroma (N=29) and Control (N=19) groups respectively) when entering the study. At 9 weeks of age, they weighed on average 29.2 ± 4.48 and 27.7 ± 4.31 kg in the Aroma and Control groups respectively. The pigs were different crosses between Yorkshire, Hampshire and Duroc. In total, 30 females and 18 males were randomly selected from the 10 pens in one section of the stable (4 or 5 pigs per pen), with pigs of a suitable size relative to the size of the foam box used (see 2.3. below). In the pens, every second pig in ascending order of identification number, were assigned the treatment with foam with added aroma, and the other half of the pigs were assigned to the treatment with no added aroma. For both treatment groups, the exposure to foam/aromatised foam was their first foam exposure.

2.3. The experimental equipment

The foam agent and box used were developed and produced by the Dutch company Anoxia BV. The inner dimensions of the box were 110 × 92 × 67 cm, and the vertical box walls were opaque (Fig. 1). In one of the short vertical box sides, a gate allowed the pigs access to the box. The floor and lid were made of transparent polycarbonate. The floor was taped with clear anti-slip tape, which was replaced after day two of the study. On the underside of the floor, red tape divided the box into four equally sized sections to enable assessment of pig movement while inside the box. To produce the air filled foam, two 50-litre bottles (200 bar) with compressed air were used. 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 (Fig. 1). For foam production, a 3% solution of foam agent (Anoxia Hi-Ex foam mild, Anoxia BV, Putten, Netherlands) and tap water was used. For the experiments with added aroma, 360 ml Dr Oetker vaniljarom [vanilla aroma] (Dr. Oetker, Gothenburg, Sweden, sugar contents: 94–96%) was added per 20 litres of foam solution. The choice of vanilla aroma was based on previous findings (preliminary results at that time) that this odour is detectable by pigs, and in comparison with 11 other odours the only odour known to

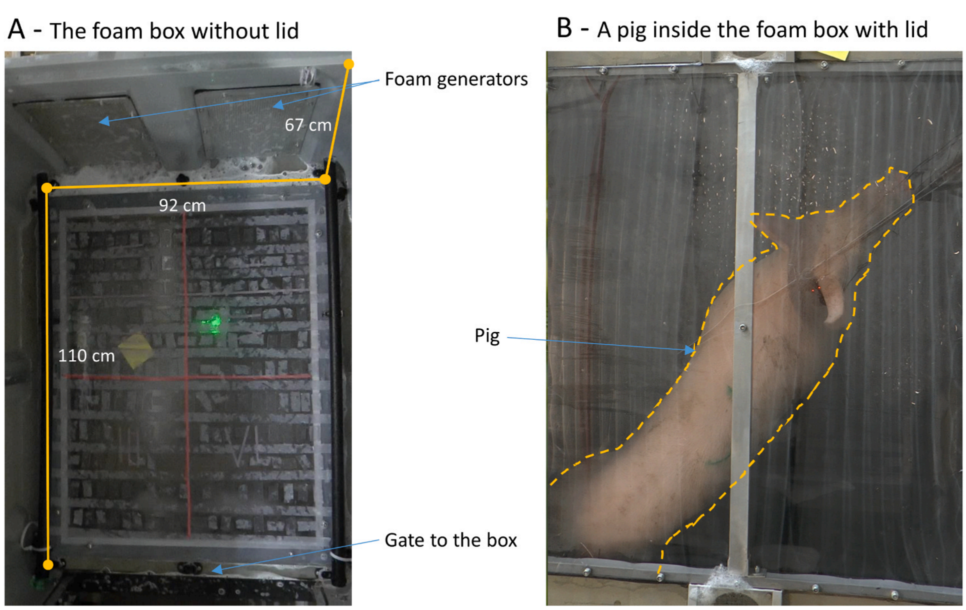

Fig. 1. The foam box. A: View of the foam box from above with the lid open. The transparent floor and clear anti-slip tape are shown. The red tape dividing the floor into four sections is enhanced to be seen in this photo. The foam generators are shown, and along the floor the gas jet pulse system is illustrated (black pipes). B: View of the foam box from above with a pig inside and the lid closed. The pig is highlighted by the orange dashed marking. Foam generators are in the top of the image (in front of the pig's head), and the gate is located at the back (behind the pig's rear), as in A.

have a sweet and hence presumably pleasant taste for pigs (Rørvang et al., 2023). On two sides along the floor of the box, a gas jet pulse system was placed, which was used to destroy the foam (Fig. 1).

Two video cameras were installed to record the experiment digitally. 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, connected to the camera filming from above, was placed inside the box. A digital stopwatch was used to keep track of the time each pig spent inside the box.

2.4. The experimental procedure

All procedures of the experiment were conducted by two experimenters. The two experimenters were veterinary students in their last year of veterinary education, who had experience with handling of pigs, but was not known to the pigs beforehand. Both students were supervised by an experienced supervisor with certified training in ethical handling of animals in research. On the day of the experiment, the pigs to be tested were moved in groups of five pen-mates from their home pen in a separate section of the stable into a new pen (waiting pen) inside the section of the stable where the experiment took place. When inside the waiting pen, pigs were given at least 15 minutes to acclimatize before the first pig was moved from the pen to the foam box. One pig at a time, in ascending order of identification number, was moved to the foam box by negative reinforcement i.e., applying pressure to the hind of the pig until it moved forward, thereby releasing the pressure. The foam box and the waiting pen were separated by an area where equipment was stored. Thus, while one pig was inside the box, the pen mates could not see the pig, but hear and potentially smell. Due to time constraints, it was not possible to also record the behaviour of the companion pigs. When the pig was in the foam 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 activated for a short time. The purpose of the air pulse destroying the foam is to create an even atmosphere in the whole box, destroying potential air pockets (which is relevant for stunning purposes when the foam is filled with N_2), and to increase visibility related to the monitoring of the pig in the box. The duration of the filling of the box with foam varied. The time until the pig was fully covered in foam depended on how much the pig moved and the position of the pig inside the box. The box was 100% filled with foam after 80–120 seconds. Three minutes after the pig had entered the foam box, the video cameras were turned off and the pig was let out and reunited with the pen-mates. The foam box was subsequently cleaned with water before the next pig was moved to the foam box. When all five pigs in one group had gone through this procedure, they were moved back to their home pen.

In total, 29 pigs were exposed to foam with added vanilla aroma (17 females and 12 males), and 19 pigs went through the above experiment without any added aroma to the foam (13 females and 6 males). To reduce the risk of aroma condemnation, and to avoid contamination of aroma from previous aroma tests, the box was ventilated between tests. The tests were thus performed on three different days; 14 of the pigs exposed to foam with added aroma were tested 11th of October 2021. Seven days after, on the 18th of October 2021, the 19 pigs in the control group (no added aroma to foam) were tested. Before control pigs were tested, careful washing of equipment and pig barn interior was done, and thorough ventilation of the entire pig barn was carried out throughout the elapsed 7 days. The remaining 15 pigs from the treatment group exposed to foam with added aroma were tested the day following the control, 19th of October 2021.

2.5. Behavioural recordings

All video recordings were analysed, and registration of behaviour was done for a total of 12 ten-second intervals (120 seconds) per pig and day, three of which were the 30 seconds before the foam generators started and nine of which were the 90 seconds after the foam generators were started. When pigs are stunned with N_2 foam, the time interesting to assess pig reactions is until they are unconscious. In previous studies where pigs were stunned with N_2 foam, all pigs were unconsciousness by 120 seconds, thus the additional time in the foam pen was not included in this study. Moreover, as the aim of this study was to evaluate pigs' responses to the aromatised foam, the behaviour of the pigs after the foam was destroyed was not relevant. The extraction of behaviour from the videos were performed by one observer who also participated in the practical experiments. When the observer analysed the videos, the treatment of the specific pig in each video was not known.

In total, 14 different behaviours were registered. These behaviours and corresponding definitions are presented in the table below (Table 1), and all behaviours were registered as number of times the behaviour was observed during each 10-second interval, except for activity which was registered as number of lines (i.e. red tape on floor) crossed during each 10-second interval.

2.6. Statistical analyses

Statistical analyses were performed using Statistical Analysis Software (SAS) version 9.4 (SAS Institute Inc., 2012). Response variables were assessed for normal distribution using Proc Univariate and descriptive statistics were analysed using Proc Freq and Proc Means.

As there were no significant interactions between treatment and 10 second intervals in the initial analyses, data was summarised for the entire 120 second test for each pig and further analyses were performed with pig as the statistical unit.

Differences between the aroma and control treatments were analysed with general linear models using Proc GLM for the normally distributed variables Explore wall, Explore floor, Explore lid, Explore foam, Avoid foam, Grunt vocalisation and Activity. The non-normally distributed variables (Slip and High pitched vocalisation) were transformed into

Table 1
Definitions of all behaviours recorded in the experiment. Behaviour term and definition. All behaviours were registered as number of times the behaviour was observed during each 10-second interval, except for activity which was registered as number of lines (i.e. red tape on floor) crossed during each 10-second interval.

Behaviour	Definition
Stand	Standing position with all four hooves on the floor
Sit[a]	Sitting position with weight on the front hooves, one or both buttocks in contact with the floor
Lying[a]	Lying position with one side or the belly in contact with the floor
Behaviour	**Definition**
Slip[a]	One or more hooves sliding fast and uncontrolled over the floor
Explore wall[a]	Snout in physical contact with the wall
Explore floor[a]	Snout in physical contact with the floor
Explore lid[a]	Snout in physical contact with the lid; with ≤ 50% of the body covered in foam
Explore foam[a]	Snout in contact with the foam, or active movement while snout is placed inside the foam
Avoid foam	The pig is actively moving its' snout away from the foam i.e., stretching snout upwards, towards lid or is jumping over the foam; while >50% of the body is covered in foam
Escape attempt[b]	Kicking with front or back legs, jumping or pushing snout, body or head against the lid
Grunt vocalisation[b]	Grunts
High pitched vocalisation[b]	Screams or squeals
Activity[b]	Number of lines on the floor crossed with both front legs
Defecation[a]	

[a] Adapted from Lindahl et al., (2020)
[b] Adapted from Söderquist et al., (2023)

binomial variables (i.e. the pig performed or did not perform the behaviour during the test) and differences between aroma and control treatments were analysed with generalised linear models using Proc GLIMMIX (using a binomial distribution and a logit link). Multiple comparisons were adjusted for using the Tukey-Kramer method. Development of the statistical model was made by stepwise backward selection, starting from a full model including interactions. Age at test day and 9 weeks weight of the pigs were included as covariates in the development of the statistical model, but was not significant for any of the response variables, thus not included in the final model. All variables were analysed with a model including the fixed effects of treatment (aroma or control; n = 29 and 19 respectively), sex (male or female; n = 18 and 30 respectively), Dam breed (Yorkshire, Yorkshire*Hampshire, Yorkshire*Duroc; n = 20, 18 and 10 respectively) and Sire breed (Yorkshire, Hampshire or Duroc; n = 5, 29 and 14 respectively), with pig as the statistical unit. In the final models, Dam breed had a significant effect ($P < 0.05$) on the variables Explore floor, Explore foam and Activity and Sire breed had significant effect on the variables Explore foam and Activity.

The variation in body position (Standing, Sitting and Lying down), Defecation and Escape attempts was too low to perform any further analyses beyond descriptive statistics.

3. Results

The pigs were in upright standing body position during the test in the foam box (99.7% of intervals). None of the pigs laid down during the test and sitting was rare (sitting was observed in 6 pigs, and in total 9 events of sitting were recorded, 1.5% of intervals). Defecation was observed in 4 pigs, 2 pigs per treatment.

Escape attempts were directed towards the lid of the foam box, never towards walls or the door of the box. Escape attempts were only performed by pigs in the aroma treatment (31.0% of the pigs) and never by pigs in the control treatment. Due to the low variation among the pigs in the control treatment, differences between treatments could not be analysed in a generalised linear model, but a chi square test gave a chi square value of 7.26 and a P-value of 0.0071.

There were no significant differences between treatments for the binomial variables Slip ($F_{df} = 0.06_{41}$, $P > 0.1$) and High pitch vocalisation ($F_{df} = 0.57_{41}$, $P > 0.1$) analysed with generalised linear models.

Differences between aroma and control treatments for the normally distributed variables are presented in Table 2.

4. Discussion

This study investigated pigs´behavioural reactions to aromatised air-filled high expansion foam, when pigs were kept inside a foam box built for stunning. The main hypothesis of the study was partially confirmed as pigs with aromatised foam expressed significantly more exploration behaviour directed towards the foam and box walls, but not towards the floor or the lid. In contrast to the hypotheses, pigs exposed to aromatised foam expressed more avoidance behaviour, and escape attempts were exclusively observed when pigs were exposed to aromatised foam. Furthermore, aromatised foam resulted in a higher activity level of the pigs, which could be related to the increased exploration behaviour as well as the increased avoidance and escape attempts. Slipping events and frequency of vocalisations did not differ between pigs exposed to foam with or without added aroma.

Table 2
Differences in pig behaviour between the aroma and control treatment (number of performed events during the 120 sec observation period). Least square means (LSM) and standard error (SE) are given for both treatments, as well as F-statistics with corresponding P-values.

Response variable	*Aroma*		*Control*			
	LSM	SE	LSM	SE	*F*	*P*
Explore wall	6.0	0.52	2.4	0.67	23.36	0.0001
Explore floor	6.2	0.64	5.9	0.84	0.12	0.73
Explore lid	1.8	0.45	1.2	0.59	0.90	0.35
Explore foam	6.5	0.40	4.0	0.53	18.96	0.0001
Avoid foam	3.5	0.37	1.9	0.48	8.60	0.0055
Vocalisation - grunts	14.4	2.40	11.4	3.13	0.76	0.39
Activity	6.5	0.40	4.0	0.53	18.96	0.0001

4.1. The effect of aromatised foam on pig exploration behaviour

It was hypothesised that pigs exposed to the aromatised foam would increase exploration of both foam and box wall, lid and floor as the added aroma was assumed to trigger a general motivation to explore the environment (Studnitz et al., 2007) and the aromatised foam. This hypothesis was supported in connection to exploration of the foam and to the box wall, and this may be explained by the foam coverage of the floor, and lid, and the fact that the foam itself may be more interesting to pigs than the box interior. In addition, the measure of activity was significantly higher in the treatment with added aroma. This could partly be related to the higher frequency of exploration as the pigs are moving around when exploring the box and foam. The greater interest in exploring the foam therefore seems directly linked to the added aroma, indicating that vanilla aroma increased pigs' interest in exploring the foam. This finding is in line with previous studies, investigating aroma added to enrichment objects where conclusions were increased interest and interaction with the aromatised objects (Van de Weerd et al., 2003; Nowicki et al., 2015; Blackie and de Sousa, 2019). The finding is, on the other hand, somewhat contradicting to recent results from Rørvang et al. (2023), where vanilla odours was of the least interest to the pigs (measured as total sniffing duration) compared with 11 other natural odours. This, however, opens an opportunity to investigate other odours (and aromas) in high expansion foam, as other compounds might have a greater stress alleviating effect due to a higher perceived interest by the pigs (Rørvang et al., 2023). Future studies should therefore focus on testing other odours, and aromas, investigating different concentrations in the aims of elucidating the best and most stress reducing solution as well as validation of effects of foam exploration on the effective states of pigs.

4.2. The effect of aromatised foam on activity

Increased activity has also been noted in other studies on pig behaviour when exposed to foam (Thurehult, 2019; Söderquist, 2020). In the study by Nilsson (2021), the observations were done on sick or injured piglets, which inevitably affects the activity level of the pigs. In the Nilsson (2021) study, pigs were additionally exposed to N_2-filled foam, and hence the animals lost consciousness (i.e., collapsed) during the experiment and therefore the change in activity will differ to this study. Lindahl et al. (2020) investigated healthy pigs, and pigs were either exposed to N_2-filled, or air-filled foam, making the reactions by pigs exposed to air filled foam comparable to this study. Lastly, the pigs used in the Lindahl et al. (2020) study were slightly younger (9 weeks of age) compared to the pigs in this study (14–16 weeks of age), and hence the size of the pigs likely also affected their activity while inside the box as a smaller body size would allow more movement. In the current study, pig weight was adjusted for in the statistical model. However, it was not the weight on the day of the experiment that was used, but the pig weight some weeks earlier (9 weeks of age). It should be noted that the weight of the pigs at the day of the experiment would be more appropriate to adjust for.

It has been noted in earlier studies that increased activity is related to slower filling of the box (Lindahl et al., 2020; Söderquist et al., 2023) and thus possibly a prolonged stunning process. Moreover, adding the vanilla aroma to the foam solution could change the solution and possibly make the foam less stable. These aspects need to be further investigated if aroma is to be added to the foam solution in the future.

4.3. Behavioural indicators of negative emotional states

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 aroma. These findings however need to be interpreted with caution, as the low variance in the variable did not allow for full analysis. The occurrence of high pitch vocalisations, another indicator of negative emotional state, did not differ significantly between the treatments which could indicate that the level of negative emotional state was the same in both treatments. Vocalisation in the form of grunts were registered in both groups of pigs exposed to foam with and without aroma, and the occurrence of grunts did not differ between the groups. As grunts are present in a range of situations (during greeting, social isolation, and as a response to fear or anticipation (Kiley, 1972)), which may both have a positive and a negative relation, it is not possible to evaluate the valence of the grunts performed in this study. However, since no difference between groups was found, the aroma treatment did not seem to have any effect on the occurrence of grunts. On the other hand, avoidance behaviour, which is a clear indicator of negative emotional valence towards the foam, was higher for pigs exposed to aromatised foam, and since avoidance behaviour likely precedes an escape attempt this result adds further support to the power of the detected difference between treatments. In addition, the higher activity level found in the pigs exposed to aromatised foam may also be an indicator of increased arousal or anxiety. To further investigate if these results are valid, future studies should include a larger sample size, and it may also be interesting to add further measurements of emotional valence such as a more detailed analysis of vocalisations (e.g. Briefer et al., 2022). Previous studies on escape attempts have noted an increase of the frequency of the behaviour over time as foam level increases (Lindahl et al., 2020; Söderquist, 2020). This is thought to be explained by the foam increasingly covering the pigs' head, something that in this study occurred later because of the bigger size of the pigs (Lindahl et al., 2020). Escape attempts in the study by Lindahl et al. (2020) was higher than in the current study with 80% of the pigs exposed to air filled foam showing escape attempts, and as many as 60% of the pigs in one interval expressing escape attempts. In comparison to both treatments in the current study, the numbers reported in Lindahl et al. (2020) are much higher. The relatively low occurrence of escape attempts in this study could therefore be related to the bigger size of the pigs compared with previous studies.

4.4. Practical implications

If the method of stunning pigs through gas filled foam becomes an approved method for slaughter, it is important to take the aroma of the foam into consideration, as aroma may affect the pigs' reaction to the foam. In earlier studies with N_2-filled foam, the period from foam generators starting to loss of posture (i.e., first sign of loss of consciousness) is quite short, a maximum of 54–76 seconds has been recorded (Lindahl et al., 2020). By improving the foam production capacity, the time to loss of posture could most likely be further shortened, but any measures to lower the stress and discomfort during this period would be beneficial to safeguard the welfare of the pig. It is necessary to validate suitable aromas to ensure that their effects on pigs' levels of stress is as intended. To safeguard validity of the potentially stress reducing behavioural effects or aroma distraction, future studies should also include physiological measures of stress such as cortisol and heart rate, and neurobiological measures of stress such as non-invasive telemetric electroencephalograms. Lastly, aspects of potential effect on the end-product (i.e., the meat), should also be considered to ensure quality and food safety.

5. Conclusion

The addition of vanilla aroma to air-filled high expansion foam resulted in higher activity and more exploration behaviour in pigs, which indicate an increased interest in the foam compared to exposure to foam with no added aroma. Added vanilla aroma, however, also increased avoidance behaviour and escape attempts and therefore the current study does not yield any uniform conclusion about the overall effect of the foam on pigs' experience of being exposed to and covered in high expansion foam. The study however provides evidence that an added aroma increases pigs' exploration behaviour towards the foam, and may thus serve as a distraction for the pigs when inside a foam box. Taken together, the results of this study show that the aroma of the foam affects pigs' responses to the foam, which emphasise the importance of taking foam aroma into consideration in further development of the high expansion N_2 foam stunning method. Further studies are needed to evaluate the impact of adding an aroma to high expansion foam, and future studies should focus on assessing other types of aromas, or odours, as well as different concentrations of the compounds to determine which solution is most efficient in reducing pig avoidance behaviour.

CRediT authorship contribution statement

Author contributions according to CRediT in the order authors appear on the paper. **MVR:** Validation, investigation, writing - original draft, writing - review and editing, visualisation. **MB:** Conceptualisation, methodology, Investigation, data curation, writing – review and editing. **CL:** Conceptualisation, methodology, and writing – review and editing, and supervision. **AW:** Conceptualisation, methodology, validation, formal analysis, investigation, writing – review and editing, supervision, project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All data supporting the conclusions in the article will be made readily available upon request to AW: anna.wallenbeck@slu.se.

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