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Nose-clip use in semi-free ranging pigs reduces rooting without disrupting affiliative behaviour or causing prolonged stress

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ABSTRACT

Domestic pigs (*Sus scrofa*) raised under natural conditions can show their complete behavioural repertoire. However, rooting behaviour can have a great impact on the environment. In the context of the promotion of farm animal welfare and environmental concerns, this study investigated the potential of nose-clips as a less invasive alternative to nose-rings for the management of rooting behaviour of free-ranging pigs. We collected behavioural data and salivary cortisol levels on two groups: an experimental group (n = 17) with nose-clips and a control group (n = 17) without nose-clips. After the nose-clipping, we observed a temporary increase in anxiety-related behaviour and cortisol levels during the 1st week, followed by a return to pre-application levels in the following weeks. We found a temporary decrease in affiliative interactions involving the nose during the 1st week after the application of nose-clips, whereas no differences in affiliative interactions without nose contact and aggression levels were observed. Moreover, nose-clips effectively reduced destructive excavation behaviours, without leading to a simultaneous increase in other exploratory behaviours. In conclusion, our findings show that nose-clips could be a solution that mitigates destructive rooting while preserving social interactions and animal welfare. Further research is essential to consolidate these findings and assess the long-term implications of this approach.

Implications

This study shows that nose-clipping to pigs raised in natural habitats reduces invasive rooting but does not chronically increase anxiety or significantly alter behavioural dynamics. This approach could offer a solution for farmers to rear pigs outdoors, balancing animal husbandry with ecological conservation. This research highlights the use of non-invasive anxiety indicators as reliable stress markers, as anxiety-related behaviours reflected salivary cortisol trends around nose-clipping. Notably, the nose-clipping did not increase negative behaviours in the long run nor diminished positive ones, emphasising the need to consider not only negative (aggression/anxiety) but also positive emotional behaviours (affiliation) in animal welfare assessment.

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Introduction

In recent years, many efforts have been made to increase physical, social, and emotional welfare of farm animals. Although with some variations, the classical standards of animal welfare are based on the so-called five freedoms, including (1) freedom from hunger and thirst; (2) freedom from discomfort; (3) freedom from pain, injury, and disease; (4) freedom to express natural behaviour; and (5) freedom from fear and distress (e.g., McCausland, 2014). Over the years, it has been realised that ensuring the minimum requirements is not enough (Miller and Chinnadurai, 2023). In this respect, the focus has been on enabling the animals to freely interact with their environment and effectively manage challenging situations (Dantzer, 2002; Puppe et al., 2007). From an animal welfare perspective, it is crucial to provide animals with the opportunity not only to minimise negative experiences but also to enhance positive ones (Edgar et al., 2013).

Negative experiences can elevate individual anxiety, detrimentally impacting animal welfare. Anxiety is an affective state characterised by tension and/or agitation, often expressed via self-

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directed behaviour (e.g. scratching; Troisi, 2002). Anxiety can be connected to stress, as it often manifests behaviourally alongside a physiological stress response (Barros and Tomaz, 2002; Troisi, 2002; Bourin et al., 2007). Indeed, anxiety-related behaviours appear often linked to the cortisol-mediated stress response, the intensity and duration of which depends on the stressor (Beerda et al., 2000, Thompson, 2011; Kubota et al., 2014; Rinaldi, 2019). Acute stress - depending on the occurrence of specific eliciting stressors - occurs over minutes or hours, whereas chronic stress - depending on long-term aversive conditions - occurs on a time scale from weeks to years (Epel et al., 2018). Several studies have noted that stress can elevate both aggression and subsequent anxiety-related behaviours in both human and non-human animals (Rodgers et al., 1997; Ferrari et al., 1998; Sandi and Haller, 2015). Conversely, positive social interactions, including affiliative behaviours, play a crucial role in promoting and enhancing animal health and welfare status (Rault, 2012, Mellor, 2015a, Rault, 2019).

Domestic pigs are animals with complex socio-emotional interactions (Marino and Colvin, 2015). In pigs, exploration, rooting and foraging are highly motivated behaviours and their performance is intrinsically rewarding (Stolba and Wood-Gush, 1989, Boissy et al., 2007). Preventing pigs from engaging in these behaviours is associated with a number of negative experiences such as frustration, boredom, anger and stress (Panksepp, 2005, Mellor, 2015b, Kells, 2022). Instead, providing opportunities for pigs to perform this kind of behaviour can alleviate some negative experiences and thus improve their welfare (Kells, 2022). Moreover, an adequate social environment can improve the welfare of pigs (Goumon et al., 2020). Affiliative interactions are considered positive social behaviour in the domestic pig (Blackshaw and Hagelsø, 1990; Camerlink et al., 2016) and it has been observed that such interactions represent an effective mechanism to buffer anxiety after stressful events (Norscia et al., 2021b).

When domestic pigs are raised in free or semi-free ranging conditions, they often exhibit social behavioural patterns akin to their wild counterparts, such as wild boars (Jensen, 1986; Stolba and Wood-Gush, 1989). Pigs can (i) exhibit anxiety-related behaviours including scratching, head/body rubbing, yawning, and vacuum chewing, particularly following stressful events (Norscia et al., 2021b), (ii) show emotional contagion, and (iii) freely choose their affiliative partners, with affiliation serving as an anxiety reducer (Cordoni et al., 2023; Norscia et al., 2021a; Norscia et al., 2021b).

While extensive farming practices can be beneficial for pig welfare, raising pigs in a natural environment poses challenges for farmers. Pigs may spend a significant proportion (about 52%) of their activity budget engaging in rooting and foraging behaviours (Stolba and Wood-Gush, 1989). The motivation to root increases not only when pigs are searching for food but also when they are exposed to new areas (Horrell, 1992; Studnitz and Jensen, 2002; Studnitz et al., 2007). However, the impact of pig rooting activity on the paddock is considerable and it leads to substantial depletion of the vegetation cover (Edge et al., 2005). For this reason, nose-rings have been employed to reduce the environmental impact of rooting (Horrell et al., 2000, 2001). Nose-rings can cause discomfort when the nose contacts a hard surface and therefore promote less impactful exploratory behaviours, such as sniffing and grazing (Studnitz et al., 2003a, b). Nevertheless, the practice of nose-ringing has raised many ethical concerns with suggestions that it causes distress and negative consequences for animal welfare (Farm Animal Welfare Council, 1996), which would go against the above–mentioned five freedom standards. To address this issue, Farm Animal Welfare Council (1996) recommends assessing the motivational state underlying the need for rooting, and addressing the frustration that arises from preventing rooting.

Thus, raising pigs in natural habitats while avoiding environmental depletion poses a 'farming conundrum,' as natural habitats promote the freedom to express natural behaviour but the resulting environmental impact may induce farmers and policy makers to opt in favour of indoor facilities, thus ultimately reducing this freedom. The solution to this puzzling issue is yet to be determined. In this study, we aim to explore if pig sociality, anxiety-related behaviours, and invasive exploratory activities are affected by the application of nose-clips—smaller and supposedly less invasive than nose-rings. To this purpose, we compared a physiological biomarker of stress (salivary cortisol), anxiety-related behaviours (frequency of displacement activities), excavation/exploratory activity, affinitive contacts, and aggression recorded in both clipped (experimental group) and non-clipped (control group) pigs over a 4-week period before and after nose-clip application onto the subjects belonging to the experimental group. Our predictions are detailed below.

Prediction 1

In pigs, the occurrence of anxiety-related behaviours rises in response to stressful events or disturbance (Norscia et al., 2021b). From a physiological standpoint, an increase in salivary cortisol levels is observed as a consequence of arousal, including arousal-related distress in the absence of other arousal-inducing factors (Koopmans et al., 2005). If the practice of nose-clipping - as it occurs for nose-ringing (Studnitz et al., 2003a, b)- impacts the physical and psychological well-being of pigs, we anticipate that cortisol levels would be higher after than before nose-clip application (*Prediction 1a*). Additionally, we anticipate that the variation of anxiety-related behaviours would follow the change in cortisol levels (*Prediction 1b*).

Prediction 2

One of the possible outcomes of anxiety increase – besides behavioural inhibition related to threat – is an increase in aggression (Rodgers et al., 1997; Ferrari et al., 1998; Porges, 2003; Sandi and Haller, 2015). Although the relationship between anxiety and aggression may not always be straightforward, anxiety can lead to aggression increase in several mammalian species, including rats (Neumann et al., 2010), monkeys (especially reactive aggression; Paschek et al., 2019) and domestic dogs (Kleszcz et al., 2022).

Given this perspective, we anticipate that the levels of aggression may be higher after than before the nose-clip application (*Prediction 2a*). Moreover, in their natural environment, domestic pigs frequently engage in social behaviours involving the nose, such as nose-to-body and nose-to-nose contacts and these interactions allow animals to recognise each other and maintain social hierarchies (Stookey and Gonyou, 1998; Kristensen et al., 2001; Mendl et al., 2002; McLeman et al., 2004; Horback, 2014). The use of nose-rings can reduce nose contacts, causing discomfort to pigs (Horrell et al., 2000, 2001). If the same applies to nose-clips, we expect that affiliative behaviours, especially those involving the nose, would decrease following the application of the nasal clips in the experimental group (*Prediction 2b*).

Prediction 3

Nose-ring application can lead to a reduction of excavation, via rooting and rasping, and promote less invasive exploratory behaviours such as sniffing and foraging (Horrell et al., 2000, 2001; Studnitz et al., 2003a, b). If the same applies to nose-clip application on free-ranging pigs, we expect that the frequencies of rooting and rasping would be lower after than before nose-clip application (*Prediction 3a*). Concurrently, we expect that the frequencies of exploratory behaviours, such as grazing/browsing and foraging, would be higher after than before nose-clip application (*Prediction 3b*).

Material and methods

Ethical statements

The present study was purely observational. The nose-clip application was carried out by the farmer as part of regular management activities and the moderate invasive procedures (i.e., size measurements and saliva samples collection) were carried out by the experimenters. The project was approved by the Bio-ethical Committee of the University of Torino (Approval nr.0615995 - 16/12/2022). The study species was not an endangered or a locally rare species. We never removed any individual from the group, and we did not interact with the pigs. No aberrant or stereotypic behaviour was recorded in the study group. We took all possible precautions to minimise the impact of our presence and we carried out a habituation process during the first days of the study, even though the animals were already habituated to human presence. In order to identify each subject, we simply marked pigs with non-toxic livestock painting spray. Marking was renewed every 4–7 days depending on weather conditions. This research is part of the broader projects "*So.Pig*" (*Socially Pig*) and "*Green Pig*".

The study group and site

Data collection was conducted in July-August 2021 on a group of semi-free ranging domestic pigs (*Sus scrofa*) at the Ethical Farm "Parva Domus" located at Cavagnolo (Turin, Italy). The animals could move freely and forage in a natural woodland habitat, within a fenced area of 3 ha. The study group was composed by 34 early adult pigs (Parma Black breed) including 16 males (9–10 months) and 18 females (8–9 months). The males had been castrated via the removal of testes within their first days of life, whereas females were potentially reproductive but the reproductive male was kept in a separate enclosure. The pigs were provided with feed (Ciclo Unico P, SILDAMIN®) once daily between 0830 and 1030 h and water was available *ad libitum*. The subjects could supplement their food intake with roots, leaves, fruits, seeds and bark naturally available in the environment.

Nose-clip application and study design

We applied a 2 × 3 design, considering two groups and three periods. To this purpose, we divided the study subjects into two groups of the same size (17 subjects, 8 males and 9 females) - Experimental Group (**EG**, in which subjects had nose-clips applied) and Control Group (**CG**). In CG, the individuals received a nose stitch – same nose handling as EG individuals – but the nose-clips were not actually applied. The individuals where randomly assigned to either group while ensuring a similar sex balance. Moreover, we considered three observational periods over a 4-week span: the week (7 days) before nose-clip application (hereafter, **PRE**) and the 1st (7 days) and 3rd (7 days) weeks after nose-clip application (hereafter, **POST1** and **POST2** for the 1st and 3rd weeks, respectively). (see Fig. 1).

Nose-clips (Veteur ©, four/pigs) were applied with pliers to the subjects of the EG after a week from the start of fieldwork (on the 22nd of July 2021). Nose-clips consisted of open steel wire rings (25 mm in diameter), clipped to the rim of the nose (each pig was fitted with 4 nose-clips to minimise the risk of the animals los-

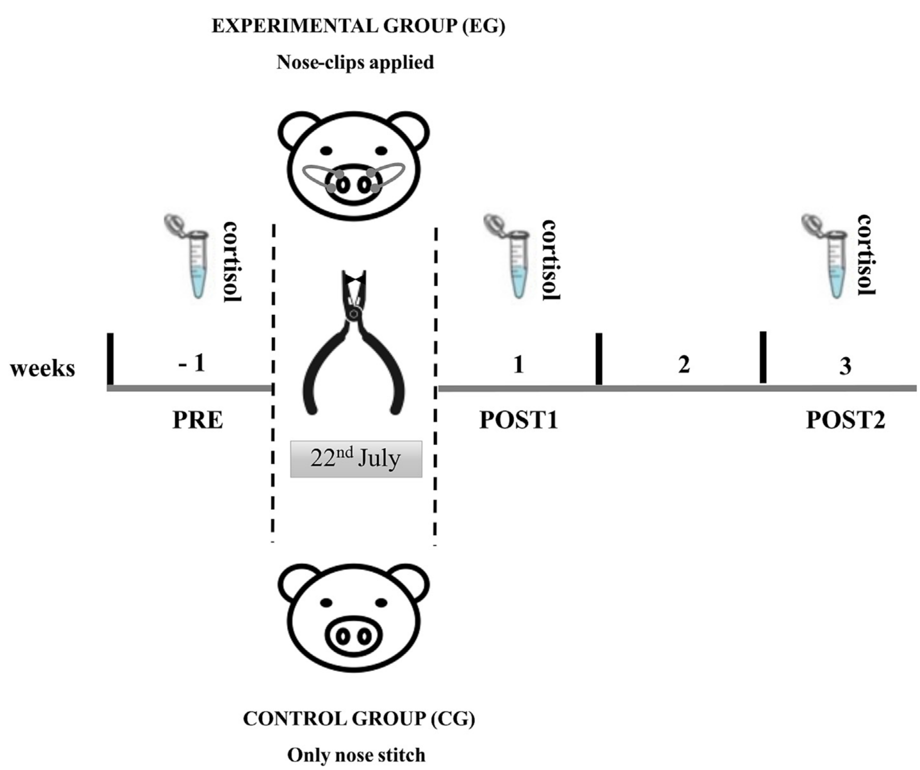

Fig. 1. Graphic abstract showing the protocol employed in the current study. A period of a week (−1) before the application of the nose-clips (PRE) and a period of 3 weeks (1, 2, 3) after the application of the nose-clips (POST) are represented with a dark grey line divided into equal parts (weeks) by black vertical lines. On the 22nd of July 2021, the nose-clips were applied with pliers to the individuals of the experimental group (EG; upper part of the figure) whereas in the control group (CG; lower part of the figure) the pigs' noses were pinched with the pliers, as if a clip were to be applied, but without actually applying the clip.

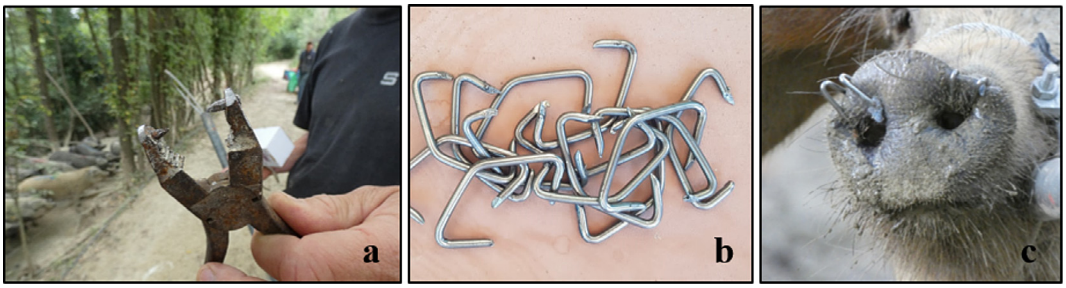

Fig. 2. Photos representing (a) the pliers used for applying nose-clips to pigs, (b) the model of nose-clips applied to animals (Veteur © model N. 5; INSVET, S.A., Huesca Spain), and (c) an individual of the experimental group with four nose-clips applied (2 × upper part of each nostril). (Photos by Giada Cordoni).

ing all the clips and to avoid a second application; see Fig. 2). The entire procedure (covering subjects of both EG and CG) lasted around 1.5 hr. Nose-clip application was not specifically carried out for this study: it was performed by the farmer as part of the management to allow pigs to roam around a natural setting without causing damage to the habitat, especially via rooting and digging. However, for the purpose of this study, part of the pigs were not clipped (CG) until the end of the data collection period.

Eco-ethological data collection

Environmental data and pig size estimation

Field data collection was carried out from 15th July to 13th August. Maximum and minimum values of temperature (°C) and humidity (%) were collected daily during the entire data collection period, via a portable Meteo Station (Elegiant; model EOX-9901). From the maximum and minimum temperature and humidity values, we calculated mean daily temperature and humidity. Two sets of size measurements - including body length and chest girth (cm) - were taken on each pig in the days before the start and in the days after the end of the data collection period. Body length and chest girth can explain around 90% of the weight variation and can therefore provide a reliable estimate of pig live weight (Mutua et al., 2011). The measurements were taken by the experimenters with the aid of a clothing tape while the subjects were feeding (with no necessity to constrain them). Body length was measured from the neck base to the tail base right above the shoulder; chest girth was determined by measuring the circumference of the chest area behind the forelegs (Birteeb et al., 2015). Considering pig age (8–10 months), weight (kg) was estimated by applying the following formula (Mutua et al., 2011): 0.39*length + 0.64*girth.

Behavioural data

Anxiety-related (vacuum-chewing, scratching/body-rubbing, head/body-shaking, and yawning), affiliative, aggressive, excavation (rooting and rasping), and exploratory (grasping/browsing and foraging) behaviours were collected via focal animal sampling (Altmann, 1974), which consists of recording all the behaviours performed by an individual in a given time window (in our case 15-min focal audio-videos in the time window from 0730 to 2030 h). One/two focal samplings were conducted daily for each individual throughout the data collection period, at different times of the day, to obtain 3 h of observation/individual/phase (a total of 9 h; PRE: 3 h; POST1: 3 h; POST2: 3 h), following a rotation of the time schedule, in order to obtain a balanced observation effort (PRE/POST1/POST2). The behaviours were categorised following the ethogram used by Norscia et al. (2021b) and described in Table 1. As regards affiliation, we considered (i) body contact interactions without the use of the nose (e.g., contact sitting/resting, head over), and (ii) body contact interactions with the use of the

Table 1
Affiliative, aggressive, anxiety-related, excavation and exploratory behaviours of domestic pigs considered in this study.

Behavioural patterns	Description
Affiliative behaviours NOT involving the nose	
Rest/Sit in contact	Two pigs lay down or sit touching one another with their bodies
Head-over	A pig puts its head above the neck of a companion
Affiliative behaviours involving the nose	
Nose-body contact	A pig touches/pushes with its nose a body part of a companion (excluding nose)
Nose-nose contact	A pig touches with its nose the nose of a companion
Aggressive behaviours	
Aggressive bite	A pig opens its mouth and closes its teeth tight on a companion's small piece of flesh, including tail
Aggressive head-knocking	A pig lurches or jerks its head hitting a companion
Aggressive lifting	A pig attempts to displace a companion by lifting or levering it with snout or head
Aggressive mount	A pig forces a companion to move away by rising upon its rear
Aggressive push	A pig presses its head, neck, shoulder or body against a companion thus causing it to move
Avoidance	A pig changes its route or moves away while a companion is approaching it
Chase	A pig pursues a companion
Flee	A pig runs away while it is chased by a companion
Anxiety-related behaviours	
Head/Body shaking	A pig vigorously shakes its head and/or body
Scratching/body-rubbing	A pig uses its legs or a substrate to rub part of its body
Vacuum-chewing	A pig chews with an empty mouth
Yawning	A pig performs deep, long inhalation with an open mouth
Excavation behaviours	
Rasping	A pig rasps with the snouts the ground on the surface, in search of food
Rooting	A pig digs the ground with its snout and eats from roots and other underground materials
Exploratory behaviours	
Foraging	A pig sniffs, touches, scrapes, nibbles, digs things and explores around the floor, or roots the floor or other things above the floor level
Grazing/ Browsing	A pig feeds from grass and plant leaves, bark

nose (e.g., nose-to-nose, nose-to-body). The videos were recorded by Maria Traversa with a Panasonic HC-V180 camera, and the camera was hand-held to follow the focal pig. The videos were then analysed via freeware VLC 3.0.6 and extension Jump-to-Time. Before starting the systematic video-analysis, Ivan Norscia and Giada Cordoni supervised Maria Traversa in a training period of 30 h to reach an interobserver reliability score (Cohen's k) never below 0.80.

To determine dominance relationships, each day during feeding all the aggressive events occurring over a time window of 15-min were noted (all-occurrences animal sampling; Altmann, 1974). Although distributed over a span of several meters to reduce aggression, the food was still provided within a limited area, which caused multiple agonistic events between pigs. For each aggression, the winner and loser of agonistic interactions were identified, with the loser being the opponent that retreated from the aggression or fled. We collected a total of 705 and 700 aggressive events in PRE and POST2 periods, respectively.

Saliva sample collection and hormonal analyses

Saliva samples were taken from a subgroup of individuals (17 subjects, 7 from EG, 10 from CG) because the individuals were roaming within a fenced area of 3 ha and it was not possible to collect all samples from all individuals. For each individual in this subgroup, three saliva samples were taken at different times: a first saliva sample was collected in the week prior to nose-clip application (PRE), a second saliva sample was collected within the 1st week after nose-clip application (POST1), a third saliva sample was collected within the 3rd week after nose-clip application (POST2). Each saliva collection round was carried out in the same morning slot, from 0730 to 0830 h, before the pigs were fed. Samples were collected by the experimenters wiping the surface of the pig's oral cavity with cotton swabs (Murase et al., 2019). Saliva samples were placed in a freezer at −20 °C within 1 h from collection and kept in dry ice when moved to the labs of the Department of Molecular Biotechnology and Health Sciences Department (University of Torino) where they were kept in a −20 °C freezer until the hormonal assays were carried out.

Chemicals

Analytical standards (purity > 98%) cortisol and internal standard (**IS**) cortisol-d3 were purchased from Sigma and LCG (Milan, Italy), respectively. Stock solutions for both analytes and IS were prepared at a concentration of 1 μg/mL for analytes and 25 μg/mL for IS using methanol and stored at −20 °C until use. Further dilutions were obtained in formic acid 0.1% in water/methanol 80:20. HPLC-MS grade ethyl ether, HPLC-MS hypergrade acetonitrile and methanol, and formic acid were purchased from VWR International (Milan, Italy). Ultrapure water was obtained from a VWR Water System.

Sample preparation

Swabs were fortified with cortisol-d3 IS (30 ng/mL final concentration), and 20 mL of ethyl ether was added to the samples. Organic solvent was recovered after shaking and ultrasonication for 5 min. The extracts were dried under a gentle stream of nitrogen, and the dried samples were reconstituted with 500 μL of 0.1% formic acid in water: MeOH 80:20 solution.

Liquid chromatography-mass spectrometry

The chromatographic separations were run on a Supelco Ascentis C18 column, 100 × 2.1 mm, 3 μm particle size (Merck-Sigma Aldrich, Milan, Italy) with an Isolute Env + 30 × 2.1 mm (Biotage, Milan, Italy) as a pre−analytical cartridge, both thermostated at 40 °C. Injection volume was 20 μL and flow rate 300 μL/min both for loading (C, formic acid 0.1%) and eluent (A, formic acid 0.1%; B, 0.1% formic acid in acetonitrile) solutions. Gradient mobile phase composition was adopted: 70:30 to 0:100 A/B in 12 min. A Shimadzu Nexera (Shimadzu, Kyoto, Japan) X2 UPLC SIL 30AC coupled with a Sciex 5500 Q-trap mass spectrometer (Sciex, Framingham, MA, USA) equipped with a Turbo Ion Spray atmospheric pressure interface (ESI ion source) was used. The LC column effluent was delivered into the ion source using nitrogen as sheath and auxiliary gas. The ion source temperature was set at 300 °C and the needle voltage at the 4.5 kV value. The acquisition method used was previously optimised for the analyte ions, and spectra were acquired in the positive ion MRM mode. Two transitions were selected for Cortisol, three for IS and all the MRM parameters for transition (Q1 → Q3), Declustering Potential, Entrance Potential, Collision energy and Collision Cell Exit Potential are reported in Table 2. The analytical protocol follows the protocol described and validated by Hauser et al. (2008).

Statistical analyses

Via the R 'steepness' package (https://CRAN.R-project.org/package=steepness; Leiva and de Vries, 2022), we calculated the dominance rank of the pigs, expressed as Normalized David's scores (**NDSs**). NDSs were assessed for each subject from decided aggressive encounters (i.e., it was possible to clearly distinguish a winner and a loser). The individual frequencies of decided encounters were entered in a sociomatrix. NDSs were determined from a dyadic dominance index (Dij) in which the observed proportion of wins (Pij) is corrected for the chance occurrence of the observed outcome. This chance occurrence is calculated based on a binomial distribution with each subject having an equal chance of winning or losing in every aggressive encounter (de Vries, Stevens and Vervaecke, 2006). We determined the NDS-based hierarchy in the two periods (PRE and POST2) by ordering the individuals according to their NDS−based ranks.

As a first step, we ran a series of preliminary analyses to check that periods (PRE/POST) and groups (EG/CG) were comparable. As a second step, we verified whether the nose-clip application modified the estimated weight gain and ranking positions in the study subject. Data were normally distributed, and so a *t*-test for two independent samples was applied to: (i) compare the mean temperature (Kolmogorov-Smirnov test: $N_{days\_PRE} = 7$, $N_{days\_POST} = 21$; $z \geq 0.113$, $P \geq 0.111$) and the mean humidity between PRE and POST the application of nose-clips (Kolmogorov-Smirnov test:

Table 2
Table shows the parameters set to carry out the chromatographic assays of domestic pig saliva samples, for declustering potential, input potential, collision energy and collision cell output potential.

Compound	Q1 Mass (*m*/*z*)	Q3 Mass (*m*/*z*)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
Cortisol	363.0	121.0	120	7	30	15
	363.0	309.0	120	7	21	25
Internal standard	366.0	121.0	59	11	29	18
	366.0	330.0	59	11	21	22
	366.0	272.0	59	11	27	16

Abbreviations: DP = declustering potential; EP = input potential; CE = collision energy; CXP = collision cell output potential.

N_{days_PRE} = 7, N_{days_POST} = 21; $z \geq 0.129$, $P \geq 0.200$); data were not paired as there were no specific association between a specific day in the period PRE and a specific day in the period post; (ii) compare the individual NDS difference ($NDS_{POST} - NDS_{PRE}$) between EG and CG (Kolmogorov-Smirnov test: $N_{subjects}$ = 17, $z \geq 0.358$, $P \geq 0.877$); (iii) compare the individual estimated weight gain (estimated $weight_{POST}$ − estimated $weight_{PRE}$) between the EG and CG (Kolmogorov-Smirnov test: $N_{subjects}$ = 17, $z \geq 0.713$, $P \geq 0.653$);

Then, we assessed whether the fluctuation of anxiety-related behaviours before and after nose-clip application followed a similar variation to the cortisol levels informing stress levels. To this purpose, we compared anxiety-related behaviour frequencies and salivary cortisol levels across three periods: the week before the nose-clip application (PRE), the 1st week after the nose-clip application (POST1) and the 3rd week after the nose-clip application (POST2). Due to the small sample size (N_{EG}=7; N_{CG} = 10), we applied the non-parametric Friedman test for k-dependent samples to compare the levels of salivary cortisol across periods. Furthermore, we compared the frequencies of anxiety-related behaviours displayed by individuals in both groups across the same three periods (PRE, POST1 and POST2). Owing to the prevalence of non-normal data distribution (Lilliefors-corrected Kolmogorov-Smirnov test: $z \geq 0.202$, $0.001 \leq P \leq 0.063$), we applied the Friedman test for k-dependent samples to compare the frequencies of anxiety-related behaviours across periods.

Finally, to evaluate the possible effects of nose-clips on excavation/exploratory activity and social behaviours, we compared the frequencies of anxiety-related, affiliative, aggressive, excavation (foraging + rasping) and exploratory (grasping/browsing + fora ging) behaviours (see Table 1) across three periods (PRE: during the week before the application of nose-clips; POST1: during the 1st week after the application of nose-clips; POST2: during the 3rd week after the application of nose-clips).

In the case of non-normal data distributions (Lilliefors-corrected Kolmogorov-Smirnov test: $0.001 \leq P \leq 0.200$), we applied non-parametric statistics for analysis (Siegel and Castellan, 1988). In particular, we applied the non-parametric Friedman test for k-dependent samples to compare the hourly frequencies of excavation behaviour (EG, CG), exploratory behaviour (EG, CG), affiliative behaviours involving the nose (EG, CG), proximity (EG, CG) and aggression (CG). Exact probabilities were selected for small sample sizes, following Mundry and Fischer (1998). Due to a normal distribution (Lilliefors-corrected Kolmogorov-Smirnov test: $P \geq 0.05$), we applied the parametric One-Way Anova test for k-dependent samples, to compare the hourly frequency of affiliative behaviours not involving the nose (EG, CG) and aggression (EG). The statistical significance threshold was set at α = 0.05.

Results

Preliminary analyses: cross-period and cross−group comparability

Temperature and humidity estimate. The periods preceding (PRE) and following (POST) the application of nose-clips (N_{days_PRE} = 7, N_{days_POST} = 21) had comparable mean temperature ($Mean_{PRE}$ ±-SE: 24.81 °C ± 0.65; $Mean_{POST}$ ± SE: 23.37 °C ± 0.51; *t*-test for two independent samples: t = 1.094, df = 26, P = 0.103) and humidity ($Mean_{PRE}$ ± SE: 63.00% ± 2.81; $Mean_{POST}$ ± SE: 68.88% ± 1.91; *t*-test for two independent samples: t = –0.442, df = 26, P = 0.109).

Estimated ranking position and weight difference. During PRE, the subjects of the experimental (EG) and control (CG) groups ($N_{subjects\_EG}$ = 17, $N_{subjects\_CG}$ = 17) had similar ranking positions expressed via NDS ($Mean_{EG}$ ± SE: 15.763 ± 0.953; $Mean_{CG}$ ± SE: 17.236 ± 0.588; *t*-test for two independent samples: t = 0.316, df = 32, P = 0.198) and size ($Mean_{EG}$ ± SE: 109.581 kg ± 2.551; $Mean_{CG}$ ± SE: 109.501 kg ± 3.651; *t*-test for two independent samples: t = −0.018, df = 32, P = 0.986). Hence, groups and periods were comparable. During the POST2, the subjects of EG and CG ($N_{subjects\_EG}$ = 17, $N_{subjects\_CG}$ = 17) showed similar NDS−based rank differences ($Mean_{EG}$ ± SE: –0.103 ± 0.353; $Mean_{CG}$ ± SE: 0.103 ± 0.180; *t*-test for two independent samples: t = 0.514, df = 32, P = 0.611) and size differences ($Mean_{EG}$ ± SE: 6.576 kg ± 21.353; $Mean_{CG}$ ± SE: 5.897 kg ± 1.754; *t*-test for two independent samples: t = −0.308, df = 32, P = 0.760). Therefore, the nose-clip influenced neither the overall weight gain nor the dominance rank of the subjects.

Prediction 1

Cortisol and anxiety behaviour levels

We found a significant difference in both frequencies of anxiety-related behaviours (Friedman test: N_{EG} = 17, χ^2 = 12.125, df = 2, P = 0.002; Fig. 3a) and salivary cortisol levels (Friedman test: N_{EG} = 7, χ^2 = 8.380, df = 2, P = 0.002, Fig. 3b) in EG across the three periods. Regarding anxiety-related behaviours, we detected an increase from PRE to POST1 (Dunn test, PRE vs. POST1: Q = −1.118, P = 0.003) and a decrease from POST1 to POST2 (Dunn test, POST2 vs. POST1: Q = 0.824, P = 0.049), whereas there was no difference between the PRE and POST2 periods (Dunn test, PRE vs. POST2: Q = –0.249, P = 1.000). Similarly, regarding salivary cortisol levels, we detected an increase from PRE to POST1 (Dunn test, PRE vs. POST1: Q = −1.286, P = 0.048) and a decrease from POST1 to POST2 (Dunn test, POST2 vs. POST1: Q = −1.286, P = 0.048), whereas there were no significant differences between PRE and POST2 (Dunn test, PRE vs. POST2: Q = −0.000, P = 1.000). In contrast, we found no significant differences in both frequencies of anxiety-related behaviour (Friedman test: N_{CG} = 17, χ^2 = 1.524, df = 2, P = 0.467) and salivary cortisol levels (Friedman test: N_{CG} = 10, χ^2 = 0.359, df = 2, P = 0.091) of CG across the three periods (PRE, POST1, POST2).

These results show that both anxiety and stress levels increased just after the nose-clip application (POST 1) but then decreased to pre-application levels (POST 2).

Prediction 2

Social behaviours

Body contacts with nose. In EG, the levels of affiliative behaviours involving the nose (i.e. nose-to-nose and nose-to-body contacts) were significantly lower in POST1 than in PRE and POST2, whereas they did not differ between PRE and POST2 (Friedman test: N_{EG} = 17, χ^2 = 10.698, df = 2, P = 0.005; Dunn test, PRE vs POST1: Q = 2.915, P = 0.011; PRE vs POST2: Q = 0.668, P = 1.000; POST1 vs POST2: Q = −2.487, P = 0.039, Fig. 4a). In CG, the frequencies of body contacts involving the nose did not significantly differ across the three periods (Friedman test: N_{CG} = 17, χ^2 = 2.375, df = 2, P = 0.305). Hence, the application of nose-clips temporarily reduced the levels of affiliative contacts involving the nose, which were restored to pre-application levels within the 3rd week.

Body contacts without nose. The frequencies of body contacts not involving the nose did not show any significant difference across the three periods (PRE, POST1, POST2) in both EG and CG (One-way ANOVA test: EG N = 17, χ^2 = 0.077, df = 2, P = 0.926; CG: N = 17, χ^2 = 2.636, df = 2, P = 0.082, Fig. 4b). Thus, nose-clips did not reduce affiliation not involving the nose.

Aggression. Frequencies of aggression did not significantly differ across the three periods in both EG and CG (EG: One-way ANOVA test, N_{EG} = 17, χ^2 = 2.922, df = 2, P = 0.063; CG: Friedman test, N_{CG} = 17, χ^2 = 4.388, df = 2, P = 0.111). Hence, the application of nose-clips did not cause an increase in aggressive events.

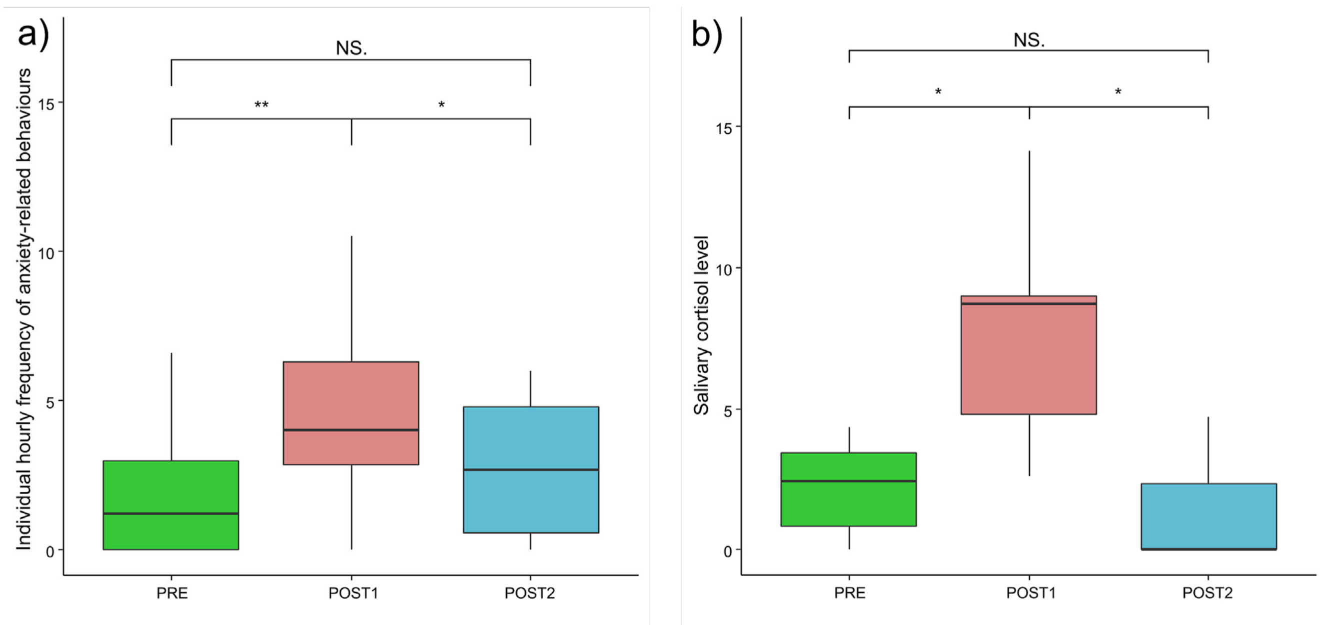

Fig. 3. Box plots showing that in the experimental group (EG) of free-ranging domestic pigs, the levels of anxiety-related behaviours (a) and salivary cortisol (b) were higher in the 1st week after nose-clipping (POST1) than in the week before (PRE) and the 3rd week after nose-clipping (POST2), there were no differences between the week before (PRE) and the 3rd week after nose-clipping (POST2). Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS = non-significant, **=P < 0.01, *=P < 0.05.

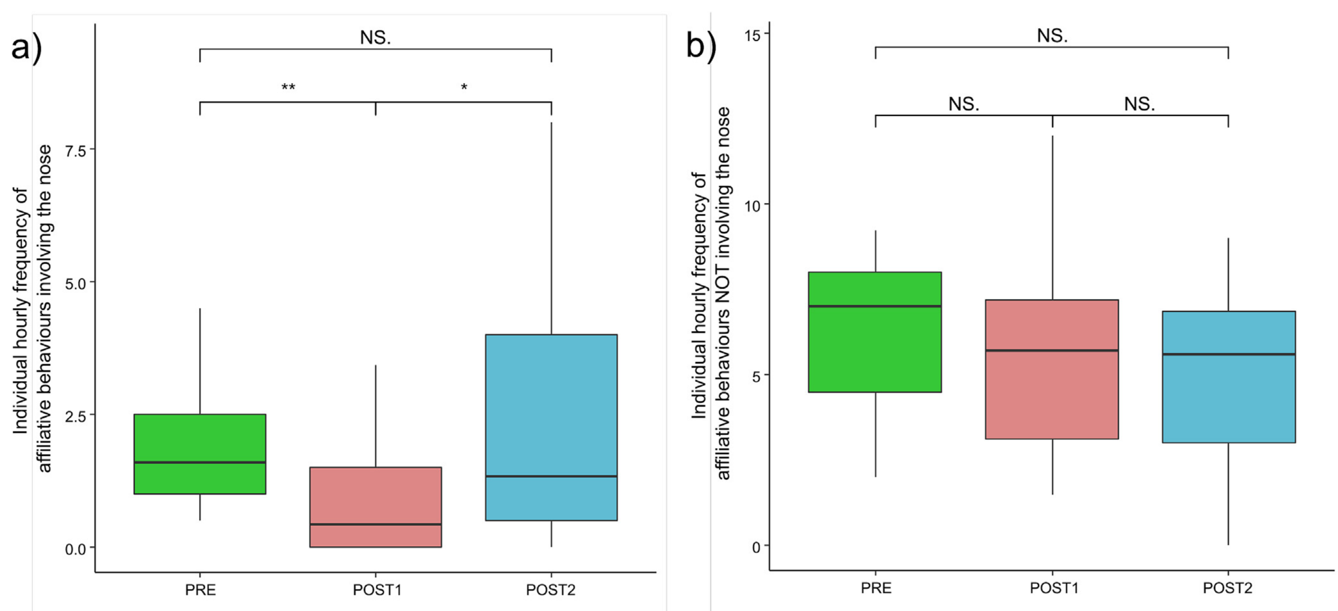

Fig. 4. Box plots showing that: (a) in the experimental group (EG) of free-ranging domestic pigs, the levels of affiliative behaviours involving the nose were lower in the 1st week after nose-clipping (POST1) than in the week before (PRE) and the 3rd week after nose-clipping (POST2), whereas there were no differences between the week before (PRE) and the 3rd week after nose-clipping (POST2); (b) in the experimental group (EG) of free-ranging domestic pigs, there were no differences in the levels of affiliative behaviours not involving the nose. Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS = non-significant, **=P < 0.01, *=P < 0.05.

Prediction 3

Excavation and exploratory behaviours

In EG, frequencies of excavation behaviour (rooting plus rasping) were significantly lower in both POST1 and POST2 compared to PRE, whereas there were no differences between POST1 and POST2 (Friedman test: N_{EG} = 17, χ^2 = 13.935, df = 2, P = 0.001; Dunn test, PRE vs POST1: Q = 3.087, P = 0.006; PRE vs POST2: Q = 3.087, P = 0.006; POST1 vs POST2: Q = 0.000, P = 1.000; Fig. 5a). Moreover, exploratory activity levels (taking/navigation plus foraging) did not differ across the three periods (Friedman test, N_{EG} = 17, χ^2 = 0.567, df = 2, P = 0.753, Fig. 5b).

In CG, we did not find any difference across the three periods in both the excavation (Friedman test, N_{CG} = 17, χ^2 = 3.169, df =

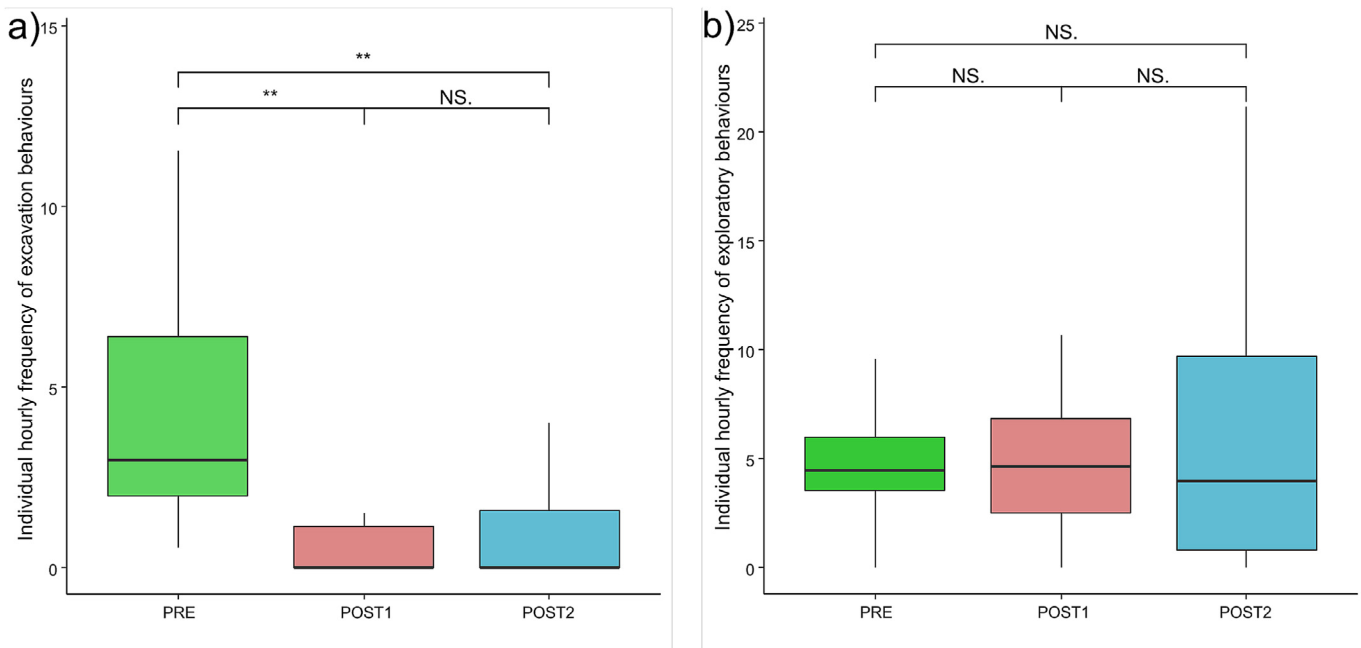

Fig. 5. Box plots showing that: (a) in the experimental group (EG) of free-ranging domestic pigs, the levels of rooting behaviours were lower in the 1st week (POST1) and in the 3rd week (POST2) after nose-clipping than in the week before nose-clipping (PRE), whereas there were no differences between the 1st week (POST1) and the 3rd week after nose-clipping (POST2); (b) in the experimental group (EG) of free-ranging domestic pigs, there were no differences in the levels of exploratory behaviours. Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS = non-significant, **=P < 0.01.

2, P = 0.205) and exploratory activities (Friedman test, N_{CG} = 17, χ^2 = 1.156, df = 2, P = 0.561). Therefore, in EG - but not in CG - rooting activities were lowered after nose-clip application while exploratory behaviours were not affected by nose-clips in both EG and CG.

Discussion

In the current study, we observed that the application of the nose-clips resulted in several outcomes: (i) a transient increase in anxiety, as indicated by self-directed behaviours and stress, measured by salivary cortisol levels. These effects peaked after clip application but returned to pre-application levels within the 3rd week, thus confirming *Predictions 1a* and *1b* (Fig. 3; Table 3); (ii) a temporary decrease in levels of affiliation involving the nose, specifically nose-to-nose and nose-to-body contacts. However, these levels were restored to their original pre-clip application state within the 3rd week, thus partially confirming *Prediction 2b* (Fig. 4a; Table 3). No significant variation was observed in other affiliation contacts or rates of aggression, contrary to *Prediction 2a.*; (iii) a stable reduction of rooting, thus confirming *Prediction 3a* (Fig. 5a; Table 3); (iv) no discernible variation in the exploratory activities, contrary to *Prediction 3b* (Fig. 5b; Table 3). In summary, the application of nose-clips appears to offer a viable compromise as it allows pigs to graze in natural areas while minimising negative impacts on both habitat integrity and animal welfare. This solution seems to preserve the animal freedoms (McCausland, 2014), including the freedom of pigs to express most of their

Table 3
Summary of the results obtained from the analysis of behaviours and saliva samples of domestic pigs.

Item	Summary of results
Anxiety and stress	
Anxiety-related behaviours	Experimental group (Friedman test: N_{EG} = 17, M_{PRE} = 0.020, $Median_{POST1}$ = 0.074, $Median_{POST2}$ = 0.044, χ^2 = 12.125, df = 2, P = 0.002)
	POST1 > PRE (Dunn test: Q = −1.118, P = 0.003)
	POST1 > POST2 (Dunn test: Q = 0.824, P = 0.049)
	PRE ≈ POST2 (Dunn test: Q = −0.249, P = 1.000)
	Control group (Friedman test: N_{CG} = 17, $Median_{PRE}$ = 0.013, $Median_{POST1}$ = 0.033, $Median_{POST2}$ = 0.022, χ^2 = 1.524, df = 2, P = 0.467)
	No statistical difference across the three periods
Cortisol levels	Experimental group (Friedman test: N_{EG} = 7, $Median_{PRE}$ = 2.420, $Median_{POST1}$ = 8.720, $Median_{POST2}$ = 0.000, χ^2 = 8.380, df = 2, P = 0.002)
	POST1 > PRE (Dunn test: Q = −1.286, P = 0.048)
	POST1 > POST2 (Dunn test: Q = −1.286, P = 0.048)
	PRE ≈ POST2 (Dunn test: Q = −0.000, P = 1.000)
	Control group (Friedman test: N_{CG} = 10, $Median_{PRE}$ = 6.465, $Median_{POST1}$ = 8.655, $Median_{POST2}$ = 8.670, χ^2 = 8.380, χ^2 = 0.359, df = 2, P = 0.091)
	No statistical difference across the three periods
Social domain: affiliation and aggression	
Body contact with nose	Experimental group (Friedman test: N_{EG} = 17, $Median_{PRE}$ = 1.600, $Median_{POST1}$ = 0.444, $Median_{POST2}$ = 1.333, χ^2 = 10.698, df = 2, P = 0.005)
	POST1 < PRE (Dunn test: Q = 2.915, P = 0.011)
	POST1 < POST2 (Dunn test: Q = −2.487, P = 0.039)

Table 3 (*continued*)

Item	Summary of results
	PRE ≈ POST2 (Dunn test: Q = 0.668, P = 1.000) Control group (Friedman test: N_{CG} = 17, $Median_{PRE}$ = 2.000, $Median_{POST1}$ = 2.000, $Median_{POST2}$ = 1.333, χ^2 = 2.375, df = 2, P = 0.305) No statistical difference across the three periods
Body contact without nose	Experimental group (One-way ANOVA test: N_{EG} = 17, $Mean_{PRE}$ ± SE = 2.885 ± 0.336, $Mean_{POST1}$ ± SE = 2.871 ± 0.506, $Mean_{POST2}$ ± SE = 2.677 ± 0.399, χ^2 = 0.077, df = 2, P = 0.926) No statistical difference across the three periods Control group (One-way ANOVA test: N_{CG} = 17, $Mean_{PRE}$ ± SE = 3.504 ± 0.423, $Mean_{POST1}$ ± SE = 3.524 ± 0.400, $Mean_{POST2}$ ± SE = 2.378 ± 0.389, χ^2 = 2.636, df = 2, P = 0.082) No statistical difference across the three periods
Aggression	Experimental group (One-way ANOVA test, N_{EG} = 17, $Mean_{PRE}$ ± SE = 5.099 ± 0.825, $Mean_{POST1}$ ± SE = 3.157 ± 0.575, $Mean_{POST2}$ ± SE = 3.123 ± 0.548, χ^2 = 2.922, df = 2, P = 0.063) No statistical difference across the three periods Control group (Friedman test, N_{CG} = 17, $Median_{PRE}$ = 3.500, $Median_{POST1}$ = 3.000, $Median_{POST2}$ = 2.857, χ^2 = 4.388, df = 2, P = 0.111) No statistical difference across the three periods
Impact on environment	
Excavation behaviours	Experimental group (Friedman test: N_{EG} = 17, $Median_{PRE}$ = 3.000, $Median_{POST1}$ = 0.000, $Median_{POST2}$ = 0.000, χ^2 = 13.935, df = 2, P = 0.001) POST1 < PRE (Dunn test: Q = 3.087, P = 0.006) POST2 < PRE (Dunn test: Q = 3.087, P = 0.006) POST1 ≈ POST2 (Dunn test: Q = 0.000, P = 1.000) Control group (Friedman test, N_{CG} = 17, $Median_{PRE}$ = 2.500, $Median_{POST1}$ = 1.333, $Median_{POST2}$ = 1.333, χ^2 = 3.169, df = 2, P = 0.205) No statistical difference across the three periods
Exploratory behaviours	Experimental group (Friedman test, N_{EG} = 17, $Median_{PRE}$ = 4.444, $Median_{POST1}$ = 4.667, $Median_{POST2}$ = 4.000, χ^2 = 0.567, df = 2, P = 0.753) No statistical difference across the three periods Control group (Friedman test, N_{CG} = 17, $Median_{PRE}$ = 4.000, $Median_{POST1}$ = 2.000, $Median_{POST2}$ = 2.000, χ^2 = 1.156, df = 2, P = 0.561) No statistical difference across the three periods

Abbreviations: EG = Experimental group; CG = Control group; PRE = 3rd week before the nose-clipping; POST1 = 1st week after the nose-clipping; POST2 = 3rd week after the nose-clipping.

natural behaviours and the freedom from distress, pain, and discomfort caused by certain artificial conditions, while simultaneously mitigating environmental damage.

During the 1st week after the application of the nose-clips (POST1), the experimental subjects showed an increase in both anxiety-related behaviours and salivary cortisol levels compared to the control group (Fig. 3a and b; Table 3). Our findings are in line with previous studies suggesting that cortisol levels increase in response to a stressor (Koopmans et al., 2005; Beerda et al., 1998; Riek et al., 2019) and that anxiety-related behaviours may be closely associated with a cortisol-mediated stress response (Beerda et al., 2000, Thompson, 2011; Kubota et al., 2014; Rinaldi, 2019). Pigs are not expected to exhibit stereotypical/abnormal behaviours under extensive farming conditions and are able to implement behavioural strategies (i.e., social buffering) which allow animals to manage the consequences of stressful events by restoring anxiety to pre-event levels (Norscia et al., 2021a, b). Thus, in such breeding conditions, even if the application of nose-clips causes a temporary increase in anxiety, this could be immediately buffered by the animals through their social mechanisms. We demonstrated a transient decrease in the frequencies of affiliative contacts involving the nose - but not in those not involving the nose - following nose-clipping (Fig. 4a and b; Table 3). In previous studies (Horrell et al., 2000, 2001), the application of the nose-ring led to a decrease in nose contact with surfaces or other individuals. On the contrary in our tested pigs, the reduction in affiliation involving the nose is temporary and levels of this kind of affinitive contacts were resumed within 3 weeks from the application of the nose-clips (Fig. 4a; Table 3). When pigs are free to interact under natural conditions, they use their snouts for recognition and engage in social contacts involving the nose that fosters social bonds (Camerlink and Turner, 2013). In this respect, the application of the nasal clips could allow free-ranging pigs to return to engage in social behaviours that involve use of the nose after a short time and furthermore without affecting the levels of affiliative interactions not involving the nose. Previous studies showed that stress can affect levels of affiliative behaviour (Biben and Champoux, 1999; Wilson, 2001; Klein et al., 2010; Norscia and Palagi, 2011). In our study, the decreased levels of affiliative behaviours involving the nose were temporarily associated with increased anxiety-related behaviour levels, with the original pre-clip application levels being restored within the 3rd week. In this regard, the increase in stress levels during the first post-clip application week (POST1) may affect negatively and specifically the levels of affiliative behaviours involving the nose.

In social mammals, including humans, anxiety can be buffered by affinitive interactions between subjects (i.e., social buffering; Kikusui et al., 2006; Thorsteinsson et al., 1998; Aureli and Yates, 2010; Kikusui et al., 2006). Moreover, in previous studies on pen and free-ranging (Reimert et al., 2014; Norscia et al., 2021b; Cordoni et al., 2023; Norscia et al., 2024), the level of anxiety-related behaviours was found to be significantly reduced after an affiliative interaction. Based on our findings, we can posit that affiliative behaviours (both involving and not involving the nose) may play a key role in restoring anxiety levels in the experimental group after nose-clipping.

As concerns aggressive behaviours, our results showed that in the experimental group, the frequencies of aggressive events did not differ before and after the nose-clip application (Table 3). Higher levels of anxiety can be associated with higher levels of aggression (Rodgers et al., 1997; Ferrari et al., 1998). However, the fact that the frequency of anxiety-related behaviours and salivary cortisol levels were restored already in the 3rd week post-clip application could explain the absence of a relevant variation in the frequencies of aggressive behaviours. Moreover, since the rank positions of the experimental subjects did not vary between PRE and POST (as indicated in the result section), the experimental group maintained their dominance relationships despite the application of nose-clips. Because aggression is the way to re-establish and change dominance hierarchy ranks (Drews, 1993), the use of aggression to restore one's rank was probably not necessary as hierarchy did not change before and after nose-clip application. According to previous studies indicating that positive social interactions can reduce tension within a group of pigs (Uvnäs-Moberg, 1998; Camerlink and Turner, 2013), our findings suggest that affiliative contacts could play an important role in reducing tension even after the transient increase in anxiety observed during the 1st week after the application of the nasal clip. Hence, all types of affiliative interactions may ensure the maintenance of social relationships and group cohesion, limiting the occurrence of aggressive behaviour, as observed in previous studies in other social mammals (Marler, 1976; Lehmann et al., 2007, De Dreu 2012).

Finally, we found that the invasive rooting activities decreased immediately after the application of the nose-clips (POST1) and remained at low levels even in the 3rd week after nose-clipping (Fig. 5a; Table 3). Therefore, our results are in line with previous studies that showed a decrease in excavation behaviour following the application of nose-rings (Horrell et al., 2000, 2001; Studnitz et al., 2003a, b). In contrast, less invasive exploratory behaviours (grazing/browsing and foraging) did not show significant differences after the application of nose-clips (Fig. 5b; Table 3). Rooting is a highly motivated exploratory behaviour in domestic pigs and it is even considered a behavioural need (Horrell et al., 2001; Studnitz et al., 2003a, b). In particular, the domestic pig needs to perform exploratory behaviour and uses its snout to explore the environment and objects (Studnitz et al., 2007). After the application of the nose-ring, pigs can replace rooting with other relevant exploratory behaviours and when ringed pigs were prevented from carrying out these exploratory activities a higher level of abnormal behaviour was observed (Studnitz et al., 2003b). However, our study showed that when pigs were unable to root, they were still able to engage in exploratory activities that did not involve the nose such as grazing/browsing and foraging. Contrary to previous studies (Studnitz et al., 2003a, b), our results do not show an increase in exploratory activities that do not involve the nose such as grazing/browsing and foraging. Therefore, we cannot assume that these behaviours can replace rooting behaviour after nose-clipping, as it did after the application of nose-rings. In our study, we recorded very few cases of behaviours such as digging with the legs or pawing that could replace the rooting behaviour after the application of the nose-rings, as suggested by previous studies (Studnitz et al., 2003a, b). In addition, previous studies have shown that nasal rings can negatively affect feeding efficiency in pigs by reducing their rooting behaviour (Horrell et al., 2000). However, the fact that - at the end of our study - we did not detect any difference between the experimental and control groups in relation to the individual body size (see Preliminary results) indicates that nose-clips did not affect the overall weight gain of the subjects. Hence, clipped-pigs were able to access food resources similar to non-clipped pigs. Probably, the extensive rearing conditions provided pigs with more variable and alternative food resources (other than the pellets provisioned by the farmer) that can be consumed without the use of the nose such as bark, leaves, fruits, and seeds. It has been hypothesised that the inability to perform highly motivated behaviours (such as rooting) may lead to a stress response in swine (Martínez-Miró et al., 2016). Therefore, the transient increase in both salivary cortisol levels and the frequency of anxiety-related behaviours may be affected by the inability to perform rooting behaviour. However, our results showed that anxiety levels returned to pre-application levels within the 3rd week unlike the frequency of rooting behaviour, which remained lower than pre-clip application levels. As described above, it is possible that other aspects including anxiety buffering mechanisms and extensive farming conditions are sufficient to restore anxiety and salivary cortisol levels. Previous studies have shown that if ringed pigs are allowed to explore the environment, individuals do not exhibit abnormal behaviour (Studnitz et al., 2003b; Studnitz et al., 2007). In line with these findings, nose-clipping in our study did not adversely affect non−harmful exploratory behaviours such as foraging, grazing, and navigation. This may also have helped to keep stress levels and anxiety-related behaviours under control.

Given the relevance of social interactions in alleviating anxiety among pigs (as observed, for example, in other groups from the same study site: Norscia et al., 2021a, b; Cordoni et al., 2023; Norscia et al., 2024), we cannot discount the possibility that the presence of unclipped individuals from the control group contributed to buffering anxiety more effectively than if only clipped pigs were present. Future studies may take this aspect into account and/or management measures may consider including unclipped individuals in the group. Moreover, we could not consider long follow-up periods and examine measures of more chronic stress, possibly related to limited rooting or other aspects (e.g. from hair cortisol; e.g. Wiechers et al., 2021). Further investigation may consider this issue. However, caution should be taken in setting the experimental design, as the more time elapses from clip application, the more variables can come into play and cause behavioural variation. These variables may include seasonal changes with temperature/humidity fluctuations, group composition changes due to culling, individual growth and development, and possible interference with other stressors coming along the way. The removal of confounding variables may not be totally possible in open, natural spaces and may require an indoor environment. Thus, attention should be paid to the study feasibility, which is necessary to draw solid and reliable conclusions. Finally, further investigation may delve into possible differences in how males and females respond to the nose clipping procedure. In principle, one sex could be more adversely affected than the other, and, if so, management plans should take this aspect into consideration.

In conclusion, the response of pigs to the application of nose-clips appears to involve a cascade of events aimed at coping with stressful conditions and restoring homeostasis. It is essential to recognise that attempting to eliminate all stressors may lead to a reduction in both environmental stimulation and the exchange of social support, which are crucial for promoting individual resilience to stressors and maintaining the physical and psychological health of animals (Špinka, 2006; Ozbay et al., 2007; Rault, 2012). Although ideally pigs should not be prevented from rooting, to maximise their welfare, nose-clipping emerges as a mild invasive technique, effective in preserving the natural environment without altering positive social dynamics. This approach is a good compromise that allows for a balanced consideration of both animal welfare and environmental concerns.

Ethics approval

All the procedures used in this study have been approved by the Bioethics Committee of the University of Turin (Protocol No. 0615995).

Data and model availability statement

The data that support the findings of this study were not deposited in an official repository. All data analysed during this study are available from the corresponding author upon reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare no conflicts of interest.

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