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Consistency of Convenience Sampling Order and its Association With Response to Handling and Weaning in Domestic Pigs (*Sus scrofa*)

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ABSTRACT

Convenience sampling in animal experiments may affect findings due to individual difference in temperament. The aim in this study was to assess the consistency of convenience sampling order (ORDER), and to study the association between potential factors (i.e., behaviors related to handling, and coping ability to weaning) and ORDER in pigs (n = 325). ORDER was recorded by catching the closest pig in the pen on Day (D) 1, 14, 23, 27, 31, 38, 69 and 79, while weighing pigs. Response to handling was assessed on all weighing days except D69. For D1 to 38, vocalization and attempt of escaping were recorded on a Yes/No basis; for D79, a scoring system from 'very difficult' to 'easy to handle' was used for 3 handling phases: 'Moving into the crate,' 'In the crate,' and 'Leaving the crate'. ORDER within each pen was categorized to 4 quartiles. To study the association between coping ability to weaning and ORDER, salivary cortisol and chromogranin A (CgA) were determined on pre- and post-weaning. The response variables for statistical analysis were ORDER: either 1) the log-transformed percentage of ORDER; or 2) first quartile (1QT, i.e., first-caught) vs. the rest; or 3) fourth quartile (4QT, i.e., last-caught) vs. the rest. An individual effect on ORDER was found ($P < 0.001$), suggesting that a pig that had been caught either first, middle, or last was very likely to be caught in a similar order in the following handling sessions. On the other hand, neither responses to handling nor coping ability to weaning were associated with ORDER ($P > 0.05$). To conclude, although ORDER is not associated with responses to human handling and coping ability to weaning, convenience sampling in pigs should be cautious as ORDER is highly consistent and long-lasting.

Introduction

A recent report conducted in 2017 indicated that only 11.2% of the analyzed publications clearly described which method of randomization was used to allocate animals to different treatment groups in the experiments. On the other hand, 64.2% of the analyzed publications stated using randomization (Macleod, The Nature Publication Quality Improvement Project (NPQIP) Collaborative Group, 2017). In animal studies, randomization is a common practice to reduce bias (Bespalov, Wicke and Castagné, 2020). Randomization should be taken into account, especially during sample selection, for several reasons. Animal temperament is among the reasons of concern, because the way an animal reacts when being sampled (i.e., handled) would likely affect the study results.

Temperament, often known as *personality* or *behavioral syndrome* synonymously in literature (MacKay and Haskell, 2015), refers to the individual behavioral differences within a population, with the expectation that the differences remain consistent over time and across situations (Réale et al., 2007). It has been observed in a number of species, from insects, reptiles, to fish, birds and mammals (Brehm and Mortelliti, 2018), including pigs (O'Malley et al., 2019). Temperament might be a source of bias and potentially affect the validity of results especially in behavioral and physiological studies, and their interpretation, due to

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unrepresentative group profile (Biro and Dingemanse, 2009; Carter et al., 2012; Michelangeli et al., 2016). For instance, Montiglio et al. (2012) found that chipmunks (*Tamias striatus*) that were faster explorers, were trapped more frequently, showed an increased sympathetic activity during restraint, and had a relatively stable fecal cortisol level over 5 months, compared to slower explorers.

As animal temperament is associated with the mechanism of how the individuals respond to potential risks or fearful stimuli (e.g., human approach or handling) (D'Eath et al., 2009), and how they adapt to novelty or an environment (hereafter we refer to this mechanism as 'coping strategy' or 'coping style') (O'Malley et al., 2019), several studies have suggested an effect of temperament on capture (i.e., trapping) in wild animals during sampling (Michelangeli et al., 2016). For example, Carter et al. (2012) confirmed that bold wild lizards (*Agama planiceps*) are likely to be trapped sooner than the shy ones. Wilson et al. (2011) also discovered that angling technique captures more timid bluegill sunfish (*Lepomis macrochirus*). Recent evidence also discovered that animal temperament plays an important role on fitness (e.g., stress response) when the individual interacts with the social and physical environment (Michelangeli et al., 2016; O'Malley et al., 2019).

In most of the publications, it is considered to be acceptable to report the randomization procedure by giving statements like "X animals were randomly assigned to the Y treatment group" (Bespalov et al., 2020), which may lead to room for doubt about the methodology of randomization. We wondered whether the order of the pigs caught would be consistent over repeated handling sessions, if a handler remain at the same location of the pen, and randomly catch the closest pig at his/her convenience (i.e., convenience sampling), one-by-one. We also asked if convenience sampling can affect validity of results in studies due to the individual difference in temperament. Scarce literature is available on the consistency of convenience sampling order (i.e., handling order, ORDER) in group-housed domestic pigs (*Sus scrofa*), and whether ORDER is associated to other behavioral traits such as temperament and coping strategy. The objectives of the study were to study the consistency of ORDER in pigs, and to study the association between responses to handling, coping styles and ORDER. We hypothesized that ORDER in pigs would remain consistent in different handling sessions (i.e., first-caught pigs would often be caught first; last-caught pigs would often be caught last). We also hypothesized that responses to handling and copying styles would be associated with ORDER.

Materials and methods

The study took place in a commercial farm in summer 2017 in Lleida (Spain). Pigs (n = 325) were followed from birth to fattening. Pigs were managed as per routine practices (feeding, cleaning, and caring) by experienced farm staff.

Animals and housings

Piglets were first raised in farrowing pens with their sows and littermates for 25 days, then to nursery pens for 46 days, and then to fattening pens until they were transported to a slaughterhouse. Raising pigs from birth to slaughter took about 6 months.

Twenty-three Danbred sows (10 primiparous and 13 multiparous) were housed in a farrowing unit of 6 rooms 4 days before parturition until weaning. Each sow was confined in a farrowing crate (190 × 62.5 cm) of a pen (253 × 168 cm) with complete slatted flooring. There were 325 piglets at birth (male: 154; female: 171). Within 24 h post-farrowing, new-born piglets were cross fostered to standardize the litter size, making the litter size 14.1 ± 0.1 piglets per sow on average. During the suckling period, 64 piglets were dead or lost to follow-up (e.g., transferred to other housings). At 25 days of age, 261 piglets (approximately 5 kg on average) were weaned and regrouped (based on similar body size) to a nursery with 8 pens (220 × 200 cm slatted flooring and 100 × 200 cm solid heated flooring) in the same room. The initial stocking density of the nursery pen was ~0.20 m^2/animal. During the nursery period, 53 pigs were dead or lost to follow-up (e.g., transferred to other housings). At 71 days of age, 208 pigs (approximately 17 kg on average) were regrouped (based on similar body size) to a fattening unit with 19 pens (314 × 206 cm concrete slatted flooring and 314 × 60 cm concrete solid flooring) in the same room. The initial stocking density of the fattening pen was ~0.62 m^2/animal. In nursery and fattening pens, pigs had unrestricted access to water from nipple drinkers and were fed *ad libitum* with commercial diets corresponding to their production stages: a three-phase feeding program during nursery (2,480, 2,470, and 2,460 Kcal/kg) and a two-phase feeding program during fattening (2,488 and 2,477 Kcal/kg).

Experimental design

Sampling order and weighing

Piglets were individually identified with numbered ear-tags (Importvet, Centelles, Spain) after birth (Day [D] 1). The ORDER of each pig was recorded (ranked from 1 to *n* animals in each pen) while weighing them individually. Weighing occurred on D1, 14, 23, 27, 31, 38, 69 and 79. All the handlers were from our university and had the previous experience in pig handling. Only 1 handler could enter the pens and randomly catch/handle the nearest pig. Ear-tag number could only be recognized from a close distance but not possible to read it without restraining the pig. Thus, for the weighing tasks from D1 to D69, piglets were gently lifted from the ground by holding 1 of its back legs with 1 hand and supporting its chest with another hand. Piglets were then placed into a plastic crate (55 × 40 × 40 cm), which sat on a weighing scale (PB-4040-60, Balanzas Cobos, L'Hospitalet de Llobregat, Spain), one by one. The bottom of the crate was attached with anti-slip tape to minimize slipping while pigs were in the crate. After the body weight was recorded, the upper back of the piglet was marked with a crayon (RAIDEX, Dettingen an der Erms, Germany) to be easily distinguished from the remaining piglets, and were released to their home pens with the same lifting technique from the crate.

For the weighing task of D79, pigs were unable to be lifted due to their weight but were instead guided to a mobile weighing crate (Meier Brakenberg, Germany) suitable for the body size. The mobile weighing crate was placed in front of the entrance of each home pen. The handler entered the pen with a sorting panel and a paddle stick to facilitate the weighing process. After the body weight was recorded, pigs were spray-marked (RAIDEX, Dettingen an der Erms, Germany) and guided to a corridor until all their pen mates were weighed, and then they returned to their home pen altogether.

Assessment of the responses to handling during weighing

Response to handling was assessed on all weighing days, except for D69 due to the absence of the observers. For the weighing tasks from D1 to D38, vocalization and attempt of escaping were recorded by 2 observers on a Yes (1) / No (0) basis, while a piglet was in the plastic crate. An attempt of escaping was considered when the piglet showed a high frequency of locomotion in the plastic crate and/or lifted and leaned its front legs on the plastic crate. For the weighing task of D79, a scoring system from D'Eath et al. (2009) was applied to assess the response to handling during weighing by 1 observer (Table 1). The scoring system includes 3 phases of the handling: 'Moving into the crate' (MIC) (from 1 to

Table 1
Scoring system extracted from D'Eath et al. (2009) to assess the response to handling during weighing.

Score	Description
Moving into the crate (MIC)	
1	Pig is very difficult to move and is trying to escape.
2	Pig is difficult to move into the crate.
3	Pig moves into the crate with some assistance from the handler.
4	Pig walks into the crate with little or no encouragement.
5	Pig runs forward into the crate.
In the crate (ITC)	
1	Pig moves around a lot during weighing, jumping and crashing around.
2	Pig moves around during weighing.
3	Pig stands still during weighing.
Leaving the crate (LTC)	
1	Pig resists and is very difficult to push out of the weigh crate.
2	Pig moves out of the weigh crate after some pushing.
3	Pig leaves of its own accord once the door is opened.

5), 'In the crate' (ITC) (from 1 to 3), and 'Leaving the crate' (LTC) (from 1 to 3), with 1: very difficult to handle, to 3 or 5: easy to handle.

Saliva collection around weaning and salivary analysis

Weaning is a common practice in commercial farms which poses multiple social and environmental challenges to piglets, from diet and housing transitions, mixing with unfamiliar conspecifics, to abrupt separation from the sows (Campbell et al., 2013). It has therefore been considered as an ideal model to study the coping ability (i.e., adaptive stress response) of piglets towards stress (Royer et al., 2016). Seventeen out of the 23 litters were selected randomly for saliva collection. From these litters, 6 piglets per litter were pre-selected for saliva samples: a male and a female piglet of the heaviest, the middle, and the lightest birth weight within each litter. On the weighing task of D23, before releasing the piglets back to their home pens, those piglets that were selected for saliva sampling were spray-marked in advance. Saliva samples were collected on 1D pre-weaning (D24) and 1D post-weaning (D26). Saliva samples were obtained by first gently lifting the marked piglets 1 by 1 and introducing the cotton swabs into piglets' mouths for 1 minute. The cotton swab was available in the Salivette tube (Sarstedt AG & Co., Nümbrecht, Germany). Those piglets were spray-marked again on their upper back on D24, so that the mark remained until D26. Right after the collection, each piglet was released, and samples were centrifuged (Avanti J-20 XP, Beckman Coulter, California, USA) for 10 minutes at 3000 rpm and were stored at -20°C until analysis. Salivary cortisol (CORT) (μg/dL) and chromogranin A (CgA) (μg/mL) were determined from the saliva samples. An automated chemiluminescence immunoassay (Immulite 1000 Cortisol, Siemens Medical Solutions USA, Malvern, USA) was used to detect CORT (Escribano et al., 2012) and time-resolved immunofluorometry assays (TR-IFMA) were used to detect CgA (Escribano et al., 2013). The intra- and inter-assay coefficients of variations (CV) were lower than 16% and 10% for CORT and CgA, respectively. The detection limit was 0.016 μg/dL and 4.27 ng/mL for CORT and CgA, respectively.

Statistical analysis

Results were analyzed and figures were generated in RStudio version 2022.02.3 (R Foundation, Austria). Statistical significance was accepted when $P < 0.05$ and a tendency was considered when $0.05 < P \leq 0.10$. Results were reported with least square means ± standard errors.

Consistency of the sampling order

To analyze the consistency of ORDER, a Spearman correlation test was performed, in which the ORDERs of an individual pig recorded throughout the study period were correlated altogether. Additionally, a linear model was applied: the response variable was the log-transformed percentage of ORDER (i.e., ORDER/number of animals per pen), and the fixed effects were day and individual.

To analyze the degree of consistency of ORDER, several chi-square goodness of fit tests were applied, by comparing the observed and the expected values of ORDER consistency. ORDER within each pen was first categorized into 4 quartiles in each handling session. The response variables were either the first quartile (1QT, i.e., first-caught pigs) vs. the rest, or the fourth quartile (4QT, i.e., last-caught pigs) vs. the rest. ORDER in different production periods was analyzed separately. To have similar probability of being caught in 1QT and in 4QT within a pen, the data on D79 were removed because it was the only handling session in the fattening period. In the suckling period, only the probability of 0.29 in each pen and each handling session was kept for the analysis, meaning only when the pen size was 14 piglets, making it around 295 piglets. In the nursery period, the probability between 0.24 and 0.26 in each pen and each handling session was kept for the analysis, making it around 318 pigs. The response variable of the observed values was created, based on the number of times a pig was in 1QT or 4QT in the total handling sessions (values between 0 and 3 for the suckling, and between 0 and 4 for the nursery). The response variable of the expected values was determined by the goodness of fit test with the theoretical binomial distribution (size = 3 and probability = 0.29 for the suckling; and size = 4 and probability = 0.25 for the nursery period). Bonferroni correction was conducted after the multiple analyses.

Association between the responses to handling and the sampling order

For the responses to handling in a crate from D1 to D38, 2 linear mixed effect models (LMM) were applied: the log-transformed percentage of ORDER as the response variable, the individual nested in the production period as the random effect, with the following fixed effects in each model: 1) day, sex, relative body weight, and vocalization (0/1); 2) day, sex, relative body weight, and attempt of escaping (0/1). Relative body weight of an individual was calculated by [actual weight of the individual – mean of the weight on the day].

For the responses to handling on D79, the response variable of the following LMMs was the log-transformed percentage of ORDER, and the random effect was the fattening pen, with the following fixed effects in each model: 1) sex, relative body weight, and MIC score; 2) sex, relative body weight, and ITC score; 3) sex, relative body weight, and LTC score.

Association between the coping ability to weaning stress and the sampling order

Salivary stress biomarkers (CORT and CgA) were used as proxies of weaning stress (Yang et al., 2018; Escribano et al., 2019). Two LMMs were applied, the log-transformed percentage of ORDER as the response variable, the farrowing pen as the random effect, with the following fixed effects in each model: 1) sex and the difference of CORT between D24 and D26; 2) sex and the difference of CgA between D24 and D26.

Results

Consistency of the sampling order

ORDER was highly consistent throughout the handling sessions ($P < 0.001$). It reflects the great likelihood that the pigs that were caught first would be caught first again in the following sessions, and the same was true for those that were caught in the middle or the last. The result of the correlation test showed that ORDERs were more positively correlated with each other during the same production period. During the suckling period, $r_{D1\text{-}14} = 0.17$ and $r_{D14\text{-}23} = 0.18$ (both $P < 0.01$), and during the nursery period, $r_{D27\text{-}31} = 0.40$, $r_{D27\text{-}38} = 0.29$, $r_{D31\text{-}38} = 0.47$, $r_{D31\text{-}69} = 0.25$, and $r_{D38\text{-}69} = 0.40$ (all $P < 0.001$), the correlation coefficients suggest that ORDER was repeatable while pigs were in the same pen. The degree of ORDER consistency in 1QT and 4QT during the suckling and the nursery periods is shown in Figure 1(a) - (d), as they represent the number of handling sessions a pig was caught in the same quartile. The expected values (purple bars) represent ORDER if that would be randomized, which was significantly different from the observed values (green bars) in both quartiles (**[a]** 1QT during suckling: $X^2{}_3 = 83.93$; **[b]** 4QT during suckling: $X^2{}_3 = 89.12$; **[c]** 1QT during nursery: $X^2{}_4 = 75.43$; **[d]** 4QT during nursery: $X^2{}_4 = 49.48$; *all* $P < 0.001$). In Figure 1 **(c)** and **(d)**, it appeared that more pigs than expected were repeatedly caught in the same quartile during the nursery period (i.e., the observed values were greater than the expected ones) (1QT: when $x = 1$, 3, and 4; 4QT: when $x = 1$, 2, 3, and 4). On the other hand, compared to the nursery period, there were less pigs than expected that were repeatedly caught in the same quartile during the suckling period (1QT: when $x = 1$ and 2; 4QT: when $x = 0$), as shown in Figure 1 **(a)** and **(b)**. This highly repeatable ORDER pattern in the nursery period than the suckling period is also reflected on the correlation test as mentioned above.

Association between the responses to handling and the sampling order

For the handling sessions in the plastic crate from D1 to 38, sex, relative body weight, vocalization ($P = 0.72$), and attempt of escaping ($P = 0.25$) were not associated with ORDER. For the handling session of D79, the MIC score ($P < 0.001$) was negatively associated with ORDER, but the ITC ($P = 0.95$) and LTC scores ($P = 0.45$) were not associated with ORDER, indicating that early-caught pigs were easier to handle when entering the weight crate (i.e., higher MIC score), and last-caught pigs were more difficult to handle (i.e., lower MIC score). In addition, sex was associated with ORDER during the handling session on D79 ($P < 0.05$), in which male pigs appeared to be easier to handle than the female pigs.

Association between the coping ability to weaning stress and the sampling order

CORT ($n = 65$, $P = 0.36$) and CgA ($n = 59$, $P = 0.79$) were not associated with ORDER. Our results suggested that the coping ability to weaning stress may not be associated with the sampling order in pigs.

Discussion

In this study, we explored whether ORDER was consistent in repeated handling of group-housed pigs from birth to fattening. We also studied possible factors that could be associated with ORDER, combining some behavioral and physiological traits related to response to handling and weaning stress, which are known to be associated with temperament.

We found that ORDER was highly consistent in pigs during eight handling sessions, which lasted almost 3 months. This statement is based on the fact that, when comparing the observed values (i.e., our results, when catching was at our convenience) with the expected values (i.e., if catching was randomized), we discovered that more pigs than expected were either in the first or the last quartile more times than it would be in repeated handling sessions, especially during the nursery period. In the studies of Réale et al., (2000) and Le Cœur et al. (2015), they also found a repeated individual trappability in bighorn ewes (*Ovis canadensis*, multi-year study) and Siberian chipmunk (*Tamias sibiricus*, 1-year study), respectively. The phenomenon of this repeatable ORDER in pigs may reflect a systematic bias if convenience sampling (e.g., for blood, saliva, feces, or behavior) is carried out in the captive environment, which could affect the validity of results and statistical assumptions (Carter et al., 2012). A relatively higher consistency of ORDER during the nursery period, compared to the suckling period, could be due to the absence of the sow and the farrowing crate in the environment, which may complicate the catching procedure.

The practice of weighing consists of several challenges for pigs, which includes novel environment, isolation, confinement and human handling (D'Eath et al., 2009). Previous studies based on 3 classical temperament tests of coping style (including human approach test, novel object test and open door test), described that rapid approach to human or novel object, or rapid exit the home pen was considered as proactive coping style (similar to first-caught pigs), whereas slow approach or reluctant to exit was considered as reactive copying style (similar to the last-caught pigs) (Brown et al., 2009). The association between response to handling and coping style (represented by ORDER) was confirmed by our results. At the weighing task of D79, we found that the MIC score was negatively correlated with ORDER, which means that pigs showing an easier handling (high MIC score) were caught first, and difficult handling (low MIC score) were caught last. However, attempt of escaping and ITC score did not seem to be associated with ORDER while weighing. Although immobility or fight/flight represents the passive or active responses towards fearful situations (Erhard and Mendl, 1999), we did not find the association between attempt of escaping/ITC score and ORDER. It could be that the duration of the isolation in the crate was relatively short (few seconds) to observe different fear responses among individuals in our study, compared to other studies (e.g., 1 minute in Bolhuis, 2004; 10 minutes in Adcock et al., 2015).

In the present study, pigs hardly vocalized when being isolated in the crate, and we did not find a strong association between vocalization and ORDER either. Current knowledge regarding the association between vocalization and temperament in pigs is rather inconsistent. In Hessing et al. (1993) and (1994), passive pigs (i.e., shy, or reactive coping style) vocalized more. However,

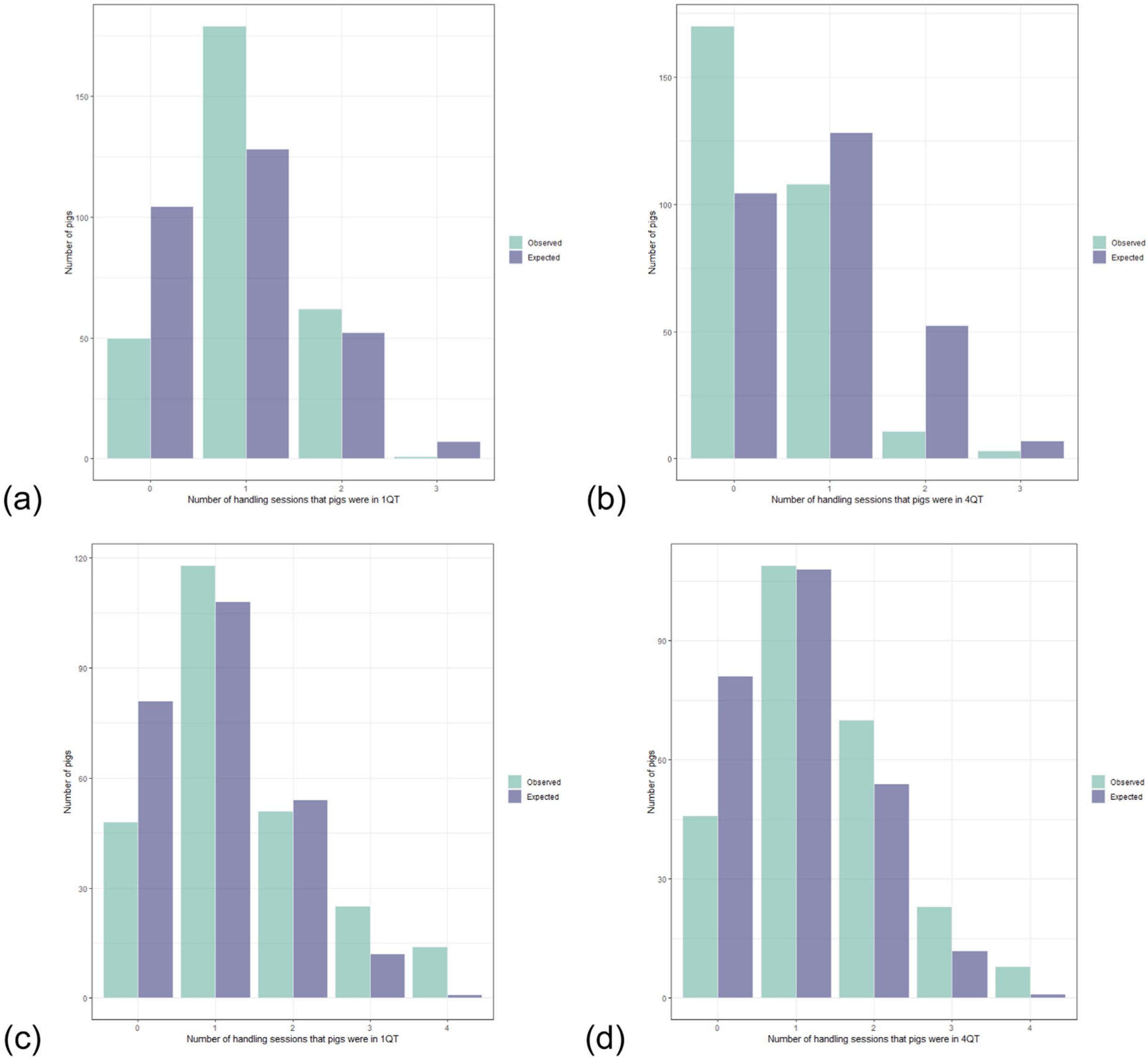

Figure 1. Bar charts presenting the observed (green) and expected (purple) values of the degree of sampling order consistency in **(a)** the first quartile (1QT) during suckling, **(b)** the fourth quartile (4QT) during suckling, **(c)** the 1QT during nursery, and **(d)** the 4QT during nursery. The degree of sampling order consistency was calculated as follows: if a pig was caught in the same quartile (either 1QT or 4QT) of the sampling order for three times out of three handling sessions, it got the value of three; if a pig was caught for one time out of three handling sessions, it got the value of one. The distribution of the expected values was determined by using the chi-square goodness of fit tests with the binomial distribution (size = 3, i.e., D1-23 and probability = 0.29 for the suckling period; size = 4, i.e., D27-69 and probability = 0.25 for the nursery period). The observed and the expected values in all figures were significantly different ($P < 0.001$).

in Geverink et al. (2002), high-resisting pigs (i.e., bold, or proactive coping style) vocalized more than low-resisting ones (i.e., reactive). Although the frequency of vocalization could differ due to different handling process (backtest restraint in Hessing et al. (1993), and nose sling restraint in Geverink et al. (2002)), more research is needed to confirm the association between vocalization and temperament in pigs (O'Malley et al., 2019).

In terms of coping ability to weaning stress, both ADG around weaning and salivary stress biomarkers were not associated with ORDER. The association between stress axis activation and temperament has not been consistent in the literature (Bolhuis, 2004). For instance, Koolhaas et al. (1999), Ruis et al. (2000) and Adcock et al. (2015) explained that proactive pigs represent a higher general activity, and show low reaction of hypothalamic-pituitary-adrenal (HPA) axis and high reaction of sympathetic-adrenomedullary (SAM) axis (i.e., fight/flight), whereas reactive pigs, who respond more with immobility and avoidance, show high reaction of HPA axis and low reaction of SAM axis (i.e., conservation/withdrawal). Nevertheless, Ruis et al. (2000) reported that low-resisting pigs (i.e., proactive) showed a higher activity of HPA axis than high-resisting ones (i.e., reactive). More studies are therefore necessary to confirm the association between stress axis activation and temperament traits.

Additionally, as proposed in Koolhaas et al. (2010), animal temperament is a two-tier model which is divided by 2 independent dimensions, including the quality of the response towards a challenging circumstance (i.e., coping style) and the quantity of that response (i.e., stress reactivity). This model suggests the possibility for the proactive individual having a strong tendency to act with low stress reactivity; and the reactive individual initiating little behavior with high stress reactivity, which could be the case for last-caught pigs (i.e., reactive) in our study. With the data from the present study, the response to handling cannot assign the first- or last-caught pigs to the two-absolute temperaments (i.e., proactive/reactive; active/passive; bold/shy; low-resisting/high-resisting), and thus the association between response to handling and coping style needs further investigation before any conclusion could be drawn.

Conclusions

In the present study, we found that pigs handled in repeated sessions showed a high consistency in ORDER when they were caught at convenience in a group. This ORDER consistency reflects an individual's long-lasting effect towards human handling in pigs (i.e., from suckling to fattening). ORDER was not determined by sex or relative body weight within a group. However, behavior used as temperament indicators such as reluctance to enter the weight crate, occurred more frequent in the last-caught pigs. In conclusion, sampling pigs at the handler's convenience within a group may lead to a systematic bias.

Authorship statement

The idea for the paper was conceived by Heng-Lun Ko and Pol Llonch. The experiments were designed by Heng-Lun Ko, Xavier Manteca, and Pol Llonch. The experiments were performed by Heng-Lun Ko, Pol Llonch, and Qiai Chong. The data were analyzed by Heng-Lun Ko, and were interpreted by Heng-Lun Ko, Xavier Manteca, and Pol Llonch. The paper was written by Heng-Lun Ko. The paper was reviewed and edited by Heng-Lun Ko, Xavier Manteca, and Pol Llonch. The final version to be submitted was approved by Heng-Lun Ko, Xavier Manteca, and Pol Llonch.

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Ethical Considerations Statement

All the experimental procedures were approved by the ethical committee of Universitat Autònoma de Barcelona (FUE-2016-00441221).

Conflict of Interest

The authors declare no conflict of interest.

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