scientific reports

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Proposing a short version of the Unesp-Botucatu pig acute pain scale using a novel application of machine learning technique

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Surgical castration of males is carried out on a large scale in the US swine industry and the pain resulting from this procedure can be assessed using the Unesp-Botucatu pig composite acute pain scale (UPAPS). We aim to propose a short version of UPAPS based on the behaviors best-ranked by a random forest algorithm. We used behavioral observations from databases of surgically castrated pre-weaned and weaned pigs. We trained a random forest algorithm using the pain-free (pre-castration) and painful (post-castration) conditions as target variable and the 17 UPAPS pain-altered behaviors as feature variables. We ranked the behaviors by their importance in diagnosing pain. The algorithm was refined using a backward step-up procedure, establishing the Short UPAPS. The predictive capacity of the original and short version of the UPAPS was estimated by the area under the curve (AUC). In refinement, the algorithm with the five best-ranked behaviors had the lowest complexity and predictive capacity equivalent to the algorithm with all behaviors. The AUC of Short UPAPS (89.62%) was statistically equivalent (*p* = 0.6828) to that of UPAPS (90.58%). In conclusion, the proposed Short UPAPS might facilitate the implementation of a standard operating procedure to monitor and diagnose acute pain post-castration in large-scale systems.

There is a forecast of an increase in the world's human population[1] which assumes an increase in demand for food. One-third of the protein for the human diet is provided by livestock, and pork is the second most consumed meat in the world[2,3]. In most of the swine industry, male piglets are castrated to avoid boar taint and tastes in the pork and to minimize undesirable aggressive and sexual behaviors[4]. Although alternative options exist to eliminate these issues without surgical procedures (e.g. immunological castration), these alternatives are not routinely performed yet in the first (China) and third (United States) largest swine producers in the world, and most of the piglets consistently are submitted to surgical castration without any anesthesia or analgesia[5–10]. In this context, meeting the demand for pork considering consumers' current concern about pigs' quality of life is a global animal welfare issue[3,5,11–13] and requires practical methods to identify animals experiencing pain on-field.

Pain is defined as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"[14] and directly impacts an animal's quality of life[15]. In addition to the ethical reason for pain relief, studies indicate that acute pain after surgical castration reduces weight gain in piglets, reinforcing the need to recognize and manage pain appropriately on farms[16–19]. Pain diagnosis in non-verbal mammals is considered a challenge, and in the case of piglet castration, the behavioral response has been used for this purpose[20]. There is no pain-altered behavior that is considered a pathognomonic sign of pain[21,22]. Therefore, pain scales usually combine behavioral categories related to pain or discomfort (e.g.: attention to the affected area, tail wagging, and lowering the head) and maintenance (e.g.: interaction with the environment and other animals, activity, and appetite) that can change when animals experience pain[23–26].

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In 2020, a species-specific behavioral scale was developed to assess acute pain in swine before and after surgical castration. The Unesp-Botucatu Pig Composite Acute Pain Scale (UPAPS) was validated for both weaned[23] and pre-weaned piglets[24] and underwent a psychometric validation process based on several statistical steps[27,28]. After completing the validation, 17 pain-altered behaviors categorized within five behavioral items were identified, defined, and used to assess pain via individual continuous observation for short intervals (4-min)[23,24]. UPAPS has been used not only as a diagnostic tool for acute pain but has also served to quantify the efficacy of analgesic intervention strategies[29,30]. However, pain assessment can often be time-consuming and require additional labor to assess pain during the surgical castration routines. Surgical castration is conducted on a large-scale system in commercial farms[15,31,32] and it can be challenging to implement pain monitoring and diagnosing with limited time and labor to assess acute pain across all animals. Therefore, identifying a more concise method to assess pain in pigs is needed intervention analgesia decision making for mitigating acute pain.

Recently, research from our lab utilized principal component analysis, canonical discriminant analysis, and logistic regression to rank each of the 17 UPAPS pain-altered behaviors and improve the pain diagnosis[33,34]. For sheep, we used a random forest algorithm to rank pain-altered behaviors[35]. It is a machine learning technique, in which the algorithm can capture a pattern in the data after training using several decision trees[36–38]. One of the advantages of this technique is it is less likely to promote overfitting and improve prediction accuracy[36–39]. Thus, the random forest can contribute to identifying important behaviors that drive pain assessment outcomes in castrated pigs. The refinement of pain scales to establish an concise version that still maintain accuracy, might facilitate a more realistic pain monitoring protocol for large-scale systems. This short pain scale version might enable on-farm delivery of pain mitigation and improve pig's welfare. However, to the best of our knowledge, pain-altered behaviors in pigs have not been ranked by random forest yet to propose a short version of the UPAPS. In addition, from a pain study perspective in general, the use of machine learning techniques is a novel approach to refine and to establish short pain scale versions across several species.

Therefore, we aimed to propose a short version of UPAPS based on the pain-altered behaviors best-ranked by the random forest algorithm using pigs before and after surgical castration.

Results

Reliability

Inter-observer reliability of the UPAPS total sum was considered "good" to "very good" according to the intraclass correlation coefficient (ICC) (Table 1). Specifically for the weaned pig database, the ICC ranged from 0.85 to 0.92, while the pre-weaned pig database was 0.98.

Training random forest

'Head down', 'Sits with difficulty', 'Wags tail', 'Interaction 2' (occasionally moves away from the other animals, but accepts approaches; shows little interest in the surroundings), and 'Activity 1' (moves with less frequency) respectively, were the five pain-altered behaviors best-ranked by the random forest algorithm to classify pigs in painful condition and those in pain-free condition (Fig. 1).

Testing random forest

The random forest algorithm had an AUC of 91.38%, which was statistically equivalent ($p = 0.8545$) to the AUC of UPAPS (90.58%) (Table 2).

Refining random forest

The algorithms with the four (AUC = 87.04%; $p = 0.0360$), three (AUC = 84.48%; $p = 0.0150$), and two (AUC = 79.00%; $p < 0.0001$) best-ranked pain-altered behaviors had lower AUCs than the algorithm containing all pain-altered behaviors (AUC = 91.38%) (Table S1 and Fig. 2). The other algorithms had AUC ranging from 89.12 to 91.44% and statistically equivalent ($p < 0.05$) to the algorithm with all pain-altered behaviors, and among them, the algorithm with the five best-ranked pain-altered behaviors had the lowest complexity (fewer pain-altered behaviors).

Short pain scale

The AUC of UPAPS (90.58%) was statistically equivalent ($p = 0.4940$) to its short version (89.62%) (Table 3). Short UPAPS total sum ranged from 0 to 5 and had an optimal cut-off point of 2 based on the upper 95% confidence interval.

Database	Observers	ICC	CI
Weaned pigs	Observer 1 vs. Observer 2	0.92	0.88–0.95
	Observer 1 vs. Observer 3	0.87	0.80–0.91
	Observer 2 vs. Observer 3	0.85	0.77–0.90
Pre-weaned pigs	Observer 4 vs. Observer 5	0.98	0.95–0.99

Table 1. Inter-observer reliability of the UPAPS total sum for each database[23,24]. UPAPS is Unesp-Botucatu pig composite acute pain scale; ICC is intraclass correlation coefficient; CI is 95% confidence interval; Interpretation: 0.81–1.0 = very good; 0.61–0.80 = moderate; 0.21–0.60 = reasonable; < 0.20 = poor[40].

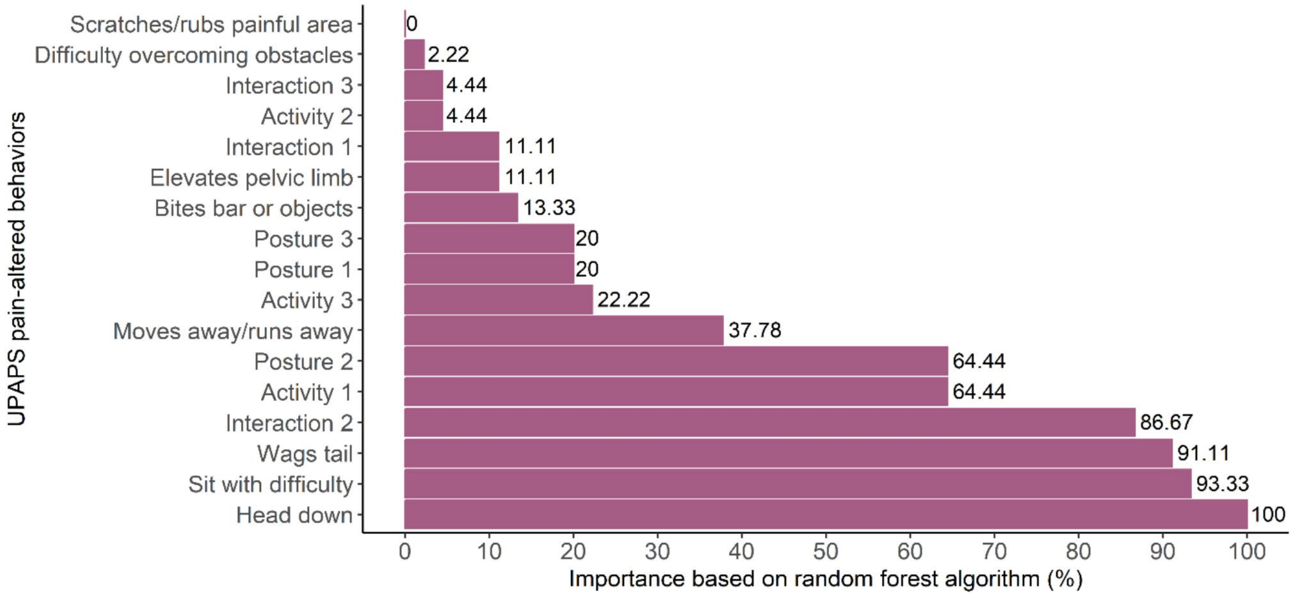

Fig. 1. Percentage of the importance of the 17 UPAPS pain-altered behavior based on the random forest algorithm using the pain condition (after castration) and pain-free (before castration) as target variable (UPAPS is Unesp-Botucatu pig composite acute pain scale).

Parameters	UPAPS#	Random forest&	*P*-value
Optimal cut-off point	02.50 (01.50–03.50)	62.08 (30.94–77.05)	
Specificity (%)	88.00 (78.00–96.00)	90.00 (78.00–98.00)	
Sensitivity (%)	90.00 (80.00–98.00)	88.00 (78.00–98.00)	
AUC (%)	90.58 (84.32–96.84)	91.38 (85.58–97.18)	0.8545

Table 2. Optimal cut-off point, specificity, sensitivity, and area under the curve of the UPAPS and random forest algorithm. UPAPS is Unesp-Botucatu Pig Composite Acute Pain Scale; # is total sum; & is probability of needing analgesia based on the algorithm; AUC is area under the curve; comparison between two AUCs was conducted with the DeLong test; the optimal cut-off point was determined by the Youden index (Specificity + Sensitivity – 1).

Discussion

Pain monitoring in the swine industry is a global animal welfare challenge[41] and the Unesp-Botucatu Pig Composite Acute Pain Scale (UPAPS) is the leading tool for assessing and diagnosing pain in pigs[23,24]. Our team previously ranked the importance of each UPAPS pain-altered behavior based on principal component analysis, canonical discriminant analysis, and logistic regression to optimize application[33,34], however, no work to date has ranked these pain-altered behaviors using machine learning techniques to develop a short version of this pain scale. Therefore, we aimed to propose a short version of UPAPS based on the pain-altered behaviors best-ranked by the random forest algorithm using pigs before and after surgical castration.

Acute pain in non-human mammals has been widely monitored by the behavioral response due to its practicality, non-invasiveness, non-intrusiveness, and low cost[32]. Despite the advantages, manual annotation of the behavior is considered an assessment with a certain degree of subjectivity, and it is essential to use more than one observer and estimate the level of reliability between them[42]. In the current study, we used five experienced observers in swine pain assessment, who had satisfactory inter-observer reliability for the UPAPS total sum. Based on this finding, we assumed behavioral assessments were reliable in the study.

When training the random forest algorithm, the importance of UPAPS pain-altered behaviors was heterogeneous, making it possible to rank this behavioral set. The five best-ranked pain-altered behaviors ('Head down', 'Sits with difficulty', 'Wags tail', 'Interaction 2: occasionally moves away from the other animals but accepts approaches; shows little interest in the surroundings', and 'Activity 1: moves with less frequency') are similar to behaviors that had already been well-documented. The literature suggests that each of these five behaviors is identified as consistent indicators of acute pain in swine. Following castration, piglets decreased their locomotion behaviors[16,17,43,44], interaction with the environment and conspecifics[5,43,45]. Furthermore, it has been reported that piglets post-castration have increased difficulty in getting up and or lying down[5,43,45,46], increased tail movements[17,43,44,46,47] and they remain with their head lowered (prostrate)[45–47].

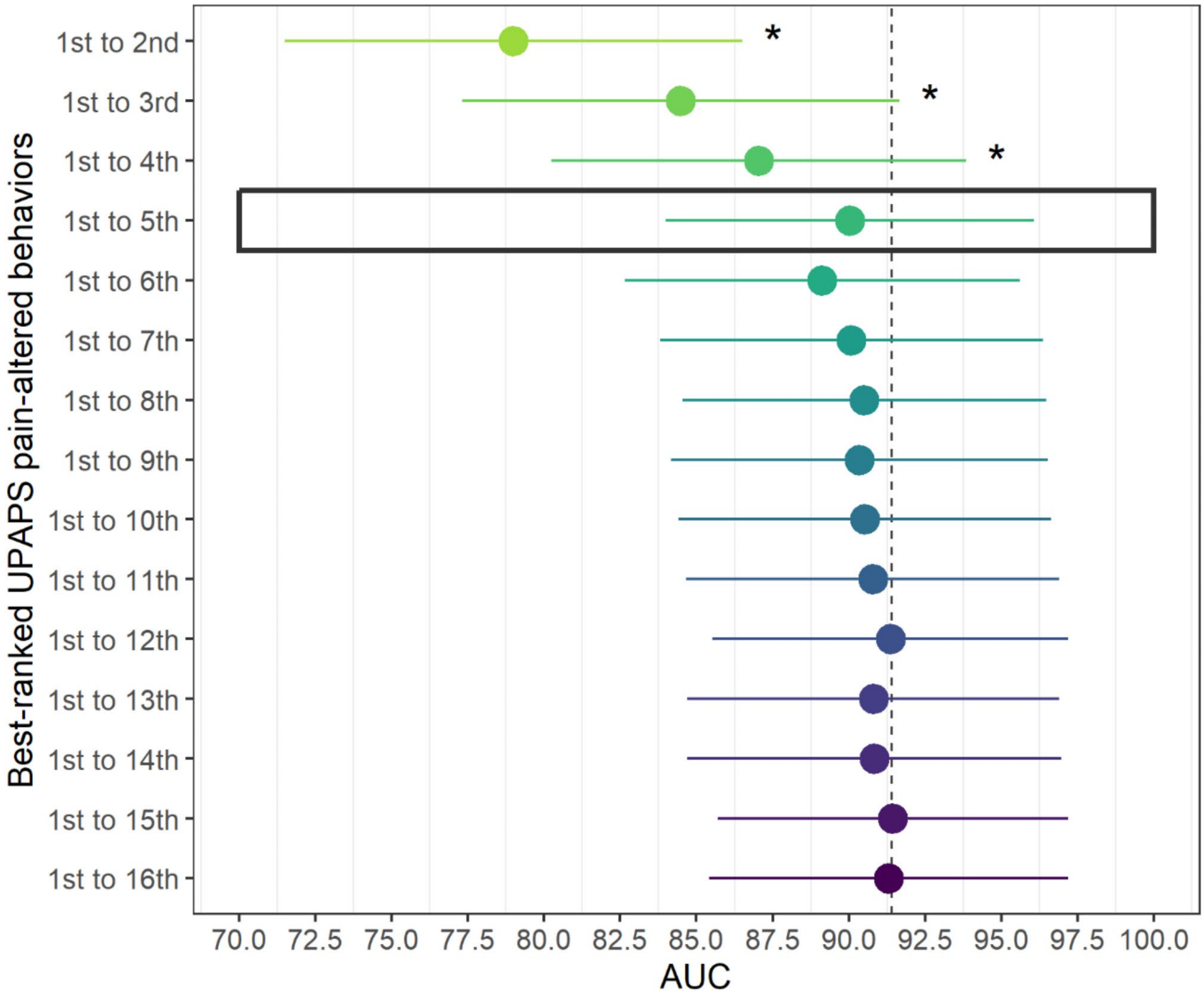

Fig. 2. Refinement of the random forest algorithm based on the importance ranking of UPAPS pain-altered behaviors (AUC indicates area under the curve; y-axis indicates the number of pain-altered behaviors according to the ranking by the algorithm; *indicates a significant difference in AUC in relation to the algorithm with all pain-altered behaviors; the dashed line indicates the AUC of the algorithm with all pain-altered behaviors; the black rectangle indicates the algorithm with the smallest number of pain-altered behaviors (lowest complexity) and having the AUC statistically equivalent to that of the algorithm with all pain-altered behaviors).

Parameters	UPAPS[#]	Short UPAPS	*P*-value
Optimal cut-off point	02.50 (01.50–03.50)	00.50 (00.50–01.50)	
Specificity (%)	88.00 (78.00–96.00)	80.00 (68.00–98.00)	
Sensitivity (%)	90.00 (80.00–98.00)	92.00 (68.00–98.00)	
AUC (%)	90.58 (84.32–96.84)	89.62 (83.50–95.74)	0.4940

Table 3. Optimal cut-off point, specificity, sensitivity, and area under the curve of the UPAPS and its short version. UPAPS is Unesp-Botucatu pig composite acute pain scale; Short UPAPS is total sum exclusively with the five better-ranked pain-altered behaviors; [#] is UPAPS total sum; AUC is area under the curve; comparison between two AUCs was conducted with the DeLong test; the optimal cut-off point was determined by the Youden index (Specificity + Sensitivity – 1).

The first ('Head down'), fourth ('Interaction 2: occasionally moves away from the other animals, but accepts approaches; shows little interest in the surroundings') and fifth ('Activity 1: moves with less frequency') best-ranked pain-altered behaviors can be understood as behaviors related to both a reduction of the animal's movement and its interaction with the environment and other individuals, while the second in the ranking ('Sits with difficulty') can be interpreted as a reduction in movement associated with the protection of the operated area. A plausible explanation for such behaviors is the evolutionary purpose of conserving energy and protecting the injured area to heal the inflamed tissue, avoiding activities that are not essential for survival[48–54]. Another justification may be the isolation that social mammals express in situations of imminent threat[55]. In addition to the reduction in movement and socialization, the increase in "Wags tail" was the third best-ranked pain-altered behavior and can be partially explained as a sign of discomfort due to an increase in neuromuscular responses in the surgical area. In humans[56,57], cats[58], and rats[58], pain can result in greater activation of the sympathetic nervous system, increasing muscle activity. In summary, the five best-ranked pain-altered behaviors indicate reduced movement and socialization, as well as increased protection of the affected area and discomfort in response to the change in homeostasis during acute pain[49,59].

The set of five best-ranked pain-altered behaviors in the present study are associated with pain, discomfort and or maintenance behaviors, as previously found in pigs[33,34], horses[60], and cattle[61]. In contrast, the five most important behaviors for pain diagnosis in sheep were exclusively from the maintenance category[35], reinforcing the relevance of ranking behaviors altered by pain on each species-specific scale. Specifically for pigs, the Head down, Wags tail and Interaction 2 ('occasionally moves away from the other animals, but accepts approaches; shows little interest in the surroundings') were among the five best-ranked pain-altered behaviors in our current and previous works[33,34]. However, the other two of five pain-altered behaviors were different across studies and might be explained by (i) the database used, (ii) the type of algorithms, and (iii) the type of target and feature variables used as input to the algorithm for each study. Further studies should explore new study population of pigs and observers to cover larger individual variability.

Testing the random forest algorithm, the predictive ability was 'good'[27] and statistically equivalent to the predictive ability of UPAPS, allowing the use of the ranking. Similar findings were found using other techniques to rank pain-altered behaviors in pigs[34] but different in previous studies on sheep[35] and horses[60] where the predictive capacity of the algorithms used was superior to the predictive ability of the original scales. For pigs, it is possible that the UPAPS in its original version already has an adequate balance between the behavioral items[23,24], which partially explains why the statistical weighting does not have a superior predictive capacity, even applying different techniques in the present and previous research[34].

The refinement of the random forest algorithm was conducted with a backward step-up procedure unprecedentedly designed to select a relevant set of pain-altered behaviors in non-human mammals. In our study, the algorithm with the five best-ranked pain-altered behaviors had the lowest complexity (smallest number of pain-altered behaviors) without losing the predictive capacity, considering the algorithm with all pain-altered behaviors, which follows the principle of parsimony[62]. Differently, another study applied principal component and canonical discriminant analyses but did not achieve a refinement of the piglet grimace scale[63].

One novelty of the present study was the Short UPAPS proposal based on the refinement by the random forest algorithm. The Short UPAPS and the original did not significantly differ in discriminatory ability. The optimal cut-off point above 2 on the Short UPAPS total sum determined that the occurrence of only two of the five best-ranked pain-altered behaviors indicates a painful condition. It is worth mentioning that Short UPAPS was effective in identifying acute pain, considering exclusively surgical castration of weaned and pre-weaned pigs.

One limitation of the study was the sample imbalance between weaned and pre-weaned pigs when we merged the databases. Despite this, the algorithm's performance metrics were satisfactory and future studies could expand the sample size. One of our databases[23] was composed of weaned pigs and it was collected during the first study developing the UPAPS because it is easier to recognize pain-altered behavior in older pigs than in young ones, but future studies should consider enlarging the sample size of pre-weaned pigs because this is the common age to castrate piglets. For both databases, although the observers were masked to the timepoints, in some cases surgical wounds could be recognizable, which makes the analysis partially blind. Another limitation was the step-up backward procedure used to refine the UPAPS because this method does not consider all the multiple combinations between the 17 pain-altered behaviors. However, the procedure adopted resulted in a short version of the pain scale with a satisfactory predictive capacity. Exhaustive methods to explore the all multiple combinations of feature variables in machine learning algorithms require a huge computational capacity that might be explored in the future. Lastly, future research should include a sham group to further refine the pain scale in being able to discriminate stress due to handling plus pain than only stress, avoiding false positive diagnoses[64]. However, the sham group in a previous study had maximum UPAPS total sum of 3, below the optimal cutoff point (UPAPS total sum ≥ 4) indicating positive pain diagnosis, and suggesting that stress effect did not induced false positive diagnoses[29].

One of the practical implications of our findings was the reduction from 17 to five pain-altered behaviors between the original scale and its short version. Although the original UPAPS can be used in real-time or video-recorded equivalently[65], farmers and veterinarians agree on the difficulty of recognizing pain in pigs in general[66–69]. Therefore, Short UPAPS has the potential to facilitate the training of caretakers on swine farms and implement a standard operating procedure to monitor acute pain in routine surgical castration for large-scale systems, promoting pigs' welfare. It is worth noting that a transition is taking place on pig farms towards the adoption of immunological castration instead of surgical castration[70,71], however, surgical castration is still commonly used worldwide and it is a welfare issue requiring attention[6–8]. Additionally, the methodological approach used to refine the algorithm and propose a short version of the UPAPS can be easily translated to pain scales in other species.

Our study represents the first step to optimize the pig pain assessment on farm, and subsequent steps are necessary before implement the proposed short version of the UPAPS as a pain monitoring system on farm. In the future, the short version of the UPAPS proposed in this research should be validated based on psychometric properties following the guidelines of the consensus-based standards for the selection of health measurement instruments (COSMIN) guidelines[28] as done for the original version[23,24]. The COSMIN guidelines aims to improve the selection of outcome measurement instruments both in research and in clinical practice by developing methodology and practical tools for selecting the most suitable outcome measurement instrument[28]. This psychometric validation should be performed using a new population of pigs. Furthermore, it could be investigated whether the use of Short UPAPS can be applied in life assessment by a presential observer with the same validation than video-record assessments, reduces observer's fatigue, assessment time-consumption, and enables to assess multiple pigs simultaneously. Lastly, the accuracy of the proposed short version of the UPAPS should be tested under different pain-mitigation strategies because the legislation across countries worldwide varies. For example, the guidelines of the second (Europe Union) and fourth (Brazil) largest swine producers in the world encourages farmers to conduct surgical castration under an anesthesia and analgesia protocol[72,73].

In conclusion, we identified a ranking of the importance of UPAPS pain-altered behaviors using a random forest algorithm and found that the predictive capacity of the algorithm was equivalent to UPAPS. Furthermore, we determined a short version of the UPAPS based on the five pain-altered behaviors best-ranked by the algorithm that had a discriminatory ability equivalent to the original version of the UPAPS. Finally, the proposed Short UPAPS might facilitate implementing acute pain monitoring in surgical castration routines in large-scale systems. Future studies are needed to validate the proposed Short UPAPS based on psychometric properties following the COSMIN guidelines.

Materials and methods

The current study was conducted with a database originating from two previous publications with weaned[23] and pre-weaned pigs[24], including new statistical analyses. The experiment procedures generating the weaned pig database were approved by the Ethics Committee for the Use of Animals in Research (protocol number 102/2014) of the School of Veterinary Medicine and Animal Science of the São Paulo State University (Unesp), Botucatu, Brazil, and followed federal legislation of the Brazilian National Council for the Control of Animal Experimentation (CONCEA)[23]. The study generating the pre-weaned pig database was approved under protocol number 19–796 by the North Carolina State University Animal Care and Use Committee[24]. Both previous studies followed the ARRIVE guidelines for reporting animal research[74]. All methods were performed in accordance with the relevant guidelines and regulations. We understand that database reuse contributes to two of the four R's of animal experimentation (reduction and responsibility)[75,76].

Database

Weaned pig database

The weaned pig database consisted of pain-altered behavioral recordings from 45 male pigs (*Sus scrofa domesticus*), of the Landrace, Large White, Duroc and Hampshire breeds, aged 38 ± 3 days and weighing 11.06 ± 2.28 kg and housed in iron pens (2.40 × 1.50 × 1.50 m of length x width x height) located side by side separated by bars in groups of five pigs. Before surgery, pigs underwent bilateral local anesthesia with 0.5 mL of 1% lidocaine without vasoconstrictor (Xylestesin®, Cristália, Itapira, São Paulo, Brazil) injected subcutaneously into each incision line, parallel to the diaphysis of the scrotum, followed by 1 mL intratesticularly injected into each testicle, and surgery was performed after five minutes. The pigs were filmed (DCR-SR68, SONY®, China) for 4 min at two perioperative timepoints: 24 to 16 h before surgery (pain-free condition) and 3.5 to 4 h after surgery (painful condition). Three observers conducted behavioral pain assessment on all videos. Two observers (Observer 1 and 2) were male professors with over 30 years of experience and one observer (Observer 3) was a female researcher with five years of experience in anesthesia and pain assessment in farm animals. In the original study, all observers assessed all videos (phase 1) and repeated all video assessments after an interval (phase 2) due to psychometric validation steps[23]. However, we used only the first phase of the assessment to merge the two databases, since in the pre-weaned pig database (described below) was performed only one assessment phase. Full details of management, procedures, protocols, and housing can be found in the previous publication[23].

Pre-weaned pig database

The pre-weaned pig database[24] consisted of pain-altered behavioral recordings from 14 Yorkshire-Landrace x Duroc piglets, five days old and weighing 1.62 ± 0.23 kg, housed with sows in individual farrowing crates (0.8 × 2.3 m of length x width) in fully slatted floors in a farrowing room with controlled environment conditions. No general or local anesthesia was administered due to standard management practices in the United States and the procedure followed the standard operating procedure approved by the treating veterinarian. The castration procedure occurred independently of research on all male animals on site. All animals enrolled in the pre-weaned pig dataset received intramuscular flunixin meglumine (2.2 mg/kg IM flunixin meglumine; Merck Animal Health, Millsboro, DE, USA) one hour after surgery. The pigs were filmed (AMCREST IP3M-943B; Houston, TX, US; one camera per crate) for 4 min at two perioperative time points: 24 h before surgery (pain-free condition) and 15 min after surgery (painful condition) only when pigs were awake. Two observers (Observer 4 and Observer 5) conducted behavioral pain assessment on all videos once. The observers were female researchers with more than two years of experience assessing pain in farm animals. Full details of management, procedures, protocols, and housing are described in the previous study[24].

Item	Score	Score/criterion	Links to videos
Posture	0	Normal (any position, apparent comfort, relaxed muscles) or sleeping	https://youtu.be/QSosCD2SD4E
	1	Changes posture, with discomfort	https://youtu.be/SpaWsFCrPxE
	2	Changes posture, with discomfort, and protects the affected area	https://youtu.be/VjSlsRrG8yA
	3	Quiet, tense, and back arched	https://youtu.be/pm4hJ5163ao
Interaction and interest in the surroundings	0	Interacts with other animals; interested in the surroundings or sleeping	https://youtu.be/-880STgYq2I
	1	Only interacts if stimulated by other animals; interested in the surroundings.	https://youtu.be/nXjOdwn3dyw
	2	Occasionally moves away from the other animals, but accepts approaches; shows little interest in the surroundings	https://youtu.be/2k2JDr5U6As
	3	Moves or runs away from other animals and does not allow approaches; disinterested in the surroundings	https://youtu.be/se70oYXcWFw
Activity	0	Moves normally or sleeping	https://youtu.be/cC75t7L5-YA
	1	Moves with less frequency	https://youtu.be/lQo9wq8LAn8
	2	Moves constantly, restless	https://youtu.be/YQRJjijLvpk
	3	Reluctant to move or does not move	https://youtu.be/Zyx0G3Wpt8o
Attention to the affected area		A. Elevates pelvic limb or alternates the support of the pelvic limb	https://youtu.be/UD99ftO7HE0
		B. Scratches or rubs the painful area	https://youtu.be/7idfFk1harE
		C. Moves and/or runs away and/or jumps after injury of the affected area	https://youtu.be/u-Pqubom278
		D. Sits with difficulty	https://youtu.be/ETNEOCVV4h0
	0	All the above behaviors are absent	
	1	Presence of one of the above behaviors	
	2	Presence of two of the above behaviors	
	3	Presence of three or all the above behaviors	
Miscellaneous behaviors		A. Wags tail continuously and intensely	https://youtu.be/pU5dGZFNRHc
		B. Bites the bars or objects	https://youtu.be/cF3dsq7gMtk
		C. The head is below the line of the spinal column.	https://youtu.be/ZcIgnglRpI
		D. Presents difficulty in overcoming obstacles (example: another animal)	https://youtu.be/HlvdOI3lGuY
	0	All the above behaviors are absent	
	1	Presence of one of the above behaviors	
	2	Presence of two of the above behaviors	
	3	Presence of three or all the above behaviors	
Total sum	0–15	Intensity of pain	

Table 4. Unesp-Botucatu pig composite pain scale without appetite or nursing item[23,24].

Behavioral pain scale

Observers were instructed to first watch each video and then score the behavioral indicators of the Unesp-Botucatu pig composite pain scale (UPAPS) described in the (Table 4)[23,24]. The observers were masked to the timepoint analyzed assessing the videos in a randomized order (blind analysis). The UPAPS was developed and published in an open access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source were credited.

In UPAPS, six behavioral items are assessed relating to posture, interaction and interest in the surroundings, activity, appetite (exclusive for weaned pigs), attention to the affected area, and miscellaneous behaviors[23,24]. These behavioral items are classified according to four descriptive levels ('0', '1', '2', and '3'). Level '0' indicates normal behaviors not associated with pain, while levels '1', '2' and '3' indicate pain-altered behaviors proportional to the intensity of pain[23,24]. Tutorial videos for each UPAPS behaviors can be seen on the Animal Pain website (https://animalpain.org/en/porcos-dor-en/) and in the Vetpain app (https://play.google.com/store/apps/details?id=com.vetpain.app for Android or https://apps.apple.com/ca/app/vetpain/id6462712970 for IOS).

Originally, the UPAPS for weaned pigs contained an item related to feeding (Appetite), however, in the validation of the UPAPS for pre-weaned pigs, an analogous item (Nursing) was considered sub-optimal and it was not included in the final version for pre-weaned pigs scale[24]. Therefore, in the current study, we disregarded both food-related items (Appetite and Nursing item) to combine the two databases. The total sum of the five behavioral items ranged from 0 to 15 and represented the intensity of pain according to UPAPS.

Statistical description

Statistical analysis was conducted in the R programming language, in the RStudio integrated development environment[77] (Version 4.3.0; RStudio, Inc., Boston, MA, USA). The functions and packages used were described as 'package::function' format corresponding to the programming language used. For all tests, a significance level of 5% ($p < 0.05$) was considered. Palette colors distinguishable by people with common forms of color blindness were used to construct the figures (ggplot2::scale_colour_viridis_d).

Sample size computation

The sample size was estimated using 80% power, with 0.05 as the significance level, and 0.90 of the area under the curve according to a receiver operating characteristic curve test (pROC::power.roc.test). Based on these premises, the minimum sample size estimated was six cases (painful conditions) and six controls (pain-free conditions).

Reliability

The inter-observer reliability of the UPAPS total sum was assessed by the degree of agreement between pairs of observers separately for each database. The Intraclass correlation coefficient (ICC) and its 95% confidence interval (CI) were calculated. The values obtained were interpreted using the Altman classification: 0.81–1.0 very good; 0.61–0.8 good; 0.41–0.6 moderate; 0.21–0.4 fair; and < 0.2 poor[40]. Reliability was conducted using 100% of all databases.

Train–test split

The database was separated into a division for training (training base) and another for testing (testing base). The training base contained 70% of the pigs (31 weaned and 10 pre-weaned) selected randomly, while the testing base had 30% of the remaining pigs (14 weaned and four pre-weaned). Both training and testing bases remained with five observers, two perioperative timepoints, and two age groups, exclusively changing the number of pigs and, consequently, the number of observations (226 and 100, respectively, for the training and testing base).

Random forest

Random Forest is considered an type of ensemble bootstrap aggregation machine learning technique for classification and regression[36,38,39]. In Random Forest, several (uncorrelated) decision trees are built with random samples from the training database, and the most common result among all trees in the forest is used to make classification predictions on the testing base[38,39]. One of the advantages of this technique is to reduce overfitting and improve prediction accuracy[36,38,39].

Training random forest

The random forest algorithm (caret::trainControl and caret::train) was built with a training base using the condition pain-free (before castration) or painful (after castration) as target variable. The pain-altered behavioral from the five UPAPS behavioral items (levels '1', '2', and '3') were converted into 17 dummy variables (0 = absence and 1 = presence of each level of each item) and used as feature variables in the algorithm (fastDummies::dummy_columns), as already proposed by our team[33–35,60]. In this way, each dummy variable represented one UPAPS pain-altered behavior. All trees were based on the Gini coefficient. The list of UPAPS items names andtheir respective dummy variables (pain-altered behaviors) used in the algorithm can be seen in the Table S2.

The optimization (tuning) of the algorithm was conducted adjusting the random forest hyperparameters and cross-validation parameters by using the grid search technique, testing (*i*) two to 17 feature variables in each decision tree, (*ii*) 501, 1001 and 2001 decision trees in the forest, (*iii*) two to 10 folds in k-fold technique and (*iv*) two to 10 repetitions. Based on the grid search technique, 3888 algorithms were evaluated with multiple combinations of random forest hyperparameters and cross-validation parameters, and their accuracy was on average 92.07 ± 0.66%. The random forest algorithm with the highest accuracy (94.26%) contained two feature variables in each decision tree, 501 trees in the forest, four-folds, and nine repetitions, which was assumed as the optimal complexity algorithm to be used in the study.

Finally, the ranking of the importance of each pain-altered behavior (caret::varImp) according to the classification of the selected algorithm was presented in a bar plot (ggplot2::ggplot).

Testing random forest

To evaluate the predictive capacity of the algorithm, the area under the curve (AUC) and its 95% confidence interval obtained with 1001 bootstrap repetitions were estimated (pROC::roc; pROC::ci.auc; and pROC::ci.coords). The AUC is obtained by the receiver operating characteristic curve (ROC) based on a predictor variable that represents a reference parameter for the phenomenon and a predictive variable that is the parameter to be tested[78]. AUC above 90 is considered 'good' and above 95 is 'excellent'. The ROC curve for each algorithm was constructed using the condition (painful and pain-free) as a predictive variable and the UPAPS total sum or the probability of each pig needing analgesia based each algorithm as a predictive variable (pROC::ROC). To obtain the AUC, it is necessary to establish an optimal cut-off point for each predictive variable, which was based on the Youden index (YI). The YI is estimated based on the sum of specificity and sensitivity subtracted from 1, calculated for each value of the predictive variable. The YI represents the maximum specificity and sensitivity concomitantly, attributing similar importance of specificity and sensitivity to the optimal cut-off point. The algorithms' AUC were compared with UPAPS's AUC by the DeLong test (pROC::roc.test).

Refining random forest

We use the ranking established by random forest training to reduce the number of pain-altered behaviors in the algorithm based on a step-up backward procedure. In this way, an algorithm was built using the training base, with exclusively the 16 best-ranked pain-altered behaviors, and this procedure was repeated sequentially until an algorithm was built with only the two best-ranked pain-altered behaviors. Using the testing base, the AUC of all algorithms with different numbers of pain-altered behaviors was compared with the AUC of the algorithm containing all behaviors using the DeLong test. The algorithm with the smallest number of pain-altered behaviors (lowest complexity) having an AUC statistically equivalent to or greater than the algorithm with all pain-altered behaviors was understood as the best refinement.

Short pain scale

The pain-altered behaviors contained in the algorithm with the best refinement were added together to make the Short UPAPS total sum, with each pain-altered behavior computed value '1' in the total sum regardless of its classification on the original scale. An optimal cut-off point was established for Short UPAPS based on the high 95% confidence interval of the YI, as described in the previous session. Finally, the AUC of the Short UPAPS was compared with the AUC of the UPAPS using the DeLong test. These steps were done with the testing base.

Data availability

The R script (Appendix file S1) and databases (Appendix file S2, S3, and S4) used for the current study are available in supplementary information files without restrictions. The R code was commented using # specifying each step following the heading sequence described at the statistical description section. In addition, the code is available in https://github.com/PeterTrindade/Proposing-a-short-version-of-the-Unesp-Botucatu-Pig-Acute-Pain-Scale.git.

Received: 15 February 2024; Accepted: 21 February 2025
Published online: 28 February 2025

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Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-91551-6.

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