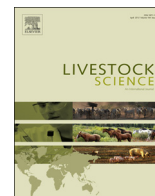




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Effects of straw provision, as environmental enrichment, on behavioural indicators of welfare and emotions in pigs reared in an experimental system

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ABSTRACT

The present study aimed to investigate whether straw provision in pigs increases positive emotions, indicated by tail movement, and reduces poor welfare indicators (agonistic and displacement behaviours), including indicators of negative emotions (ear movement). Comparisons between Straw and Control sessions were analysed from video recording for all parameters. 15 mini-pigs participated in a three-week study. Depending on the week, animals were included in Straw or Control sessions. During Straw sessions, pigs were placed in their own pens where straw was introduced and continuously provided for one week. During Control sessions, pigs remained in their pens with no additional stimulus. Pig behaviours were video-recorded four times during each session and scored. Results showed that ear movement frequency was significantly lower in the Straw than in Control session ($p = 0.005$); agonistic behaviour frequency and duration were significantly lower in the Straw than in Control session ($p = 0.013$ and $p = 0.0004$, respectively), and displacement behaviour frequency and duration were significantly lower in the Straw than in Control session ($p < 0.001$ and $p = 0.01$, respectively). Results suggest that straw provision reduces poor welfare but does not modify indicators of positive emotions selected for this study (tail movement frequency and duration). Our study also provides information about potential indicators of welfare, and more precisely, about emotions, which could also be useful to improve animal welfare assessment in pigs, obtaining more information about feasible behavioural indicators which could show the emotional state of the animals.

1. Introduction

The provision of straw in pig production systems is widely presumed to be beneficial to animal welfare (EFSA, 2014). There is weak evidence that the use of concrete flooring rather than straw is a risk factor for increased overall morbidity and mortality in pigs (Tuytens, 2005). Straw is the material that most reduces the occurrence of harmful redirected behaviours (Whittaker et al., 1999). It also reduces many other welfare problems (van de Weerd and Day, 2009), such as aggression, tail biting and stereotypies (Burbidge et al., 1994). Nevertheless, previous studies (Marcet-Rius et al., 2018a) suggest that providing straw in a pig experimental system does not modify putative physiological indicators of positive animal welfare, like oxytocin and serotonin: no

significant difference was observed between a group of pigs with a continuous provision of straw and a control group, over time; it thus appears that straw, and the possibility to perform exploratory and rooting behaviour, does not have an impact on plasma oxytocin and serotonin.

The concept of animal welfare includes not only physical welfare but also mental welfare, meaning that emotions are an important component of welfare (Broom, 1991; Mellor, 2012). It seems interesting to further analyse the real emotional state of the pigs when they have the opportunity to perform exploratory behaviour thanks to straw provision. To do so, a correct animal welfare assessment should not only include negative indicators, but should also analyse the production of positive emotions, in order to confirm that animals are in a positive

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welfare state (Boissy and Lee, 2014). The availability of behavioural postures analogous to facial expressions in humans could be extremely valuable for the evaluation of animal emotions (Boissy et al., 2011). In addition, it would be useful to have feasible indicators of emotions in pig production systems to avoid invasive and difficult practices such as blood sampling. Some authors have suggested that tail movement could serve as a behavioural indicator of positive emotions in pigs: both tail wagging and tail posture changes occurred more often during rewarding than aversive events (Reimert et al., 2013, 2015). Reimert et al. (2017) also showed that naive pigs tended to wag their tails more after the positive as compared to the negative treatment of the treated pigs. Recently, we (Marcet-Rius et al., 2018b,c) shed more light on the topic, supporting this hypothesis and results while adding new information and potential tools, such as precise measurements, as well as confirming that a high tail movement duration is linked to play behaviour. As it is generally assumed that play behaviour in mammals triggers positive emotions (Horback, 2014; Mellor et al., 2009), these results strongly suggested that long-duration of tail movement may be an indicator of positive emotions in pigs.

Regarding possible and feasible indicators of negative emotions, or a decrease of positive emotions, some authors have suggested that ear movements (i.e. changes between the ear postures 'front' and 'back') seem to be a potential behavioural indicator in pigs (Reimert et al., 2013; Marcet-Rius et al., 2018c). Flattened ears have also been associated with negative situations in pigs (Reimert et al., 2013, 2015; Goumon and Spinka, 2016) and in other species, namely sheep (Reefmann et al., 2009a,b), dogs (Kiley-Worthington, 1975) and horses (Freymond et al., 2014). It thus appears useful to analyse the appearance of both types of indicators to better understand the emotions of the pigs in a particular context.

Many indicators of poor welfare are currently used in animal welfare assessments. To identify the cause of a specific welfare outcome, several indicators need to be used (Welfare Quality, 2009). In our study, we decided to observe the presence of two of them, agonistic behaviour and displacement activities, to see if they would be emitted separately or together with tail movement and/or ear movement. A high incidence of agonistic behaviour or negative social behaviour could be considered as an indicator of poor welfare (Temple et al., 2011). Displacement behaviour is another interesting phenomenon to observe in this context. Displacement behaviours are thought to occur in conflict situations or in situations in which an animal is prevented from attaining its goal, so it is frustrated (Tinbergen, 1952). Its appearance is linked to a difficulty of the animal in coping with the environment (Landsberg et al., 2013), hence, it could indicate an inadequate environment, and a general poor welfare.

The aim of the present study was to investigate whether straw provision in pigs increases positive welfare, decreases negative welfare, or both. It is now widely recognised that the absence of negative welfare does not necessarily mean a high level of welfare, and that the expression of positive emotions is needed to achieve positive welfare (Boissy et al., 2007). Therefore, we used (i) certain potential indicators of positive emotions, such as tail movement duration, which could also be considered as an indicator of positive welfare, as welfare implicates mental and physical welfare (ii); certain potential indicators of negative emotions, or a decrease in positive emotions, in this case ear movements frequency, and (iii) some indicators of poor welfare, in this case a high incidence of agonistic and displacement behaviours.

2. Material and methods

The housing, husbandry and use of the animals involved in this experiment were performed according to French and European legislation and in respect of the principles of replacement, reduction and refinement. The project, including this experimental procedure, was approved by the IRSEA's (Research Institute in Semiochemistry and Applied Ethology) Ethics Committee (C2EA125) and the French

Ministry of Research (AFCE_201609_01).

2.1. Animals and housing

The mini-pigs (*Sus scrofa domestica*) ($n = 15$: Castrated males = 8; females = 7) involved in the study were a new strain resulting from cross-breeding of miniature breeds (Asian potbelly breeds: Vietnamese and Chinese) with conventional white hair breeds (Landrace and Large White), born and reared at the Specpig centre for breeding and biomedical research, in Barcelona, Spain. The pigs were entered into the study at sixteen months of age and had previously been involved in other non-invasive studies. Animals were previously socialised to humans and had a positive human-animal relationship. Pigs were housed in a controlled system in an experimental building designed for research, in two identical rooms (30 m²), with monitored environment parameters: mean ambient temperature of 22 °C, same ventilation by 2 artificial ventilators in each room in the same position and 60% humidity. In one room there were four castrated males and four females; in the other room there were four castrated males and three females. Two pigs of the same sex and age were housed in each pen (2.5 m²). Groups (pairs) were created after weaning to avoid fighting, so pigs were used to being together. Not all the pigs could be housed on pairs during the test, due to the death of some individuals before the beginning of the study. Apart from that, they were all in the same conditions. Pens were cleaned daily. Pigs were fed twice a day (at 8.00 a.m. and at 3.00 p.m.) with a special diet for mini-pigs maintained in restricted conditions for long-term trials (Special Diets Services, France) and had continuous access to drinking water. Lights were on from 8.00 a.m. until 6.00 p.m.

2.2. Procedure

The duration of the procedure was three weeks. Depending on the week, the pigs of one or both rooms participated in the study. In the first week, only the animals from room 1 were involved, participating in the Control session. In the second week, all the animals were involved: those from room 1 participated in the Straw session, while those from room 2 participated in the Control session. Finally, in the third week, only the animals from room 2 were involved, participating in the Straw session. This organisation was chosen for practical reasons so that each animal participated in the study for a total of two weeks into two different situations. All pigs first participated in a Control session followed by a Straw session, with each session lasting one week. Animals thereby served as their own controls. During the experimental procedure, pigs were housed in pairs, always with the same individuals, as in their normal life. As groups were created after weaning, fifteen months before the study, pigs were used to be together, being stressed when separated. Thus, for welfare reasons, as well as to not influence the normal behaviour of the pigs and so the results, it was decided to perform the study with the pigs housed in pairs. During the Straw session, pigs were placed in their own pens (2.5 m² in concrete floor), where 5 kg of straw were introduced in the floor of each pen (Straw from Coustenable, 1 bd DEWAVRIN- BP 60,044 – 62,260 - Auchel, France). Every morning, the animal-keepers removed the dirty straw and provided the same quantity to each pen, in order to always have the same amount of straw in each pen. Straw was continuously provided and renewed in that way throughout the week. During the Control session, animals were placed in their own pens, but no straw was provided; the pigs were in the normal situation in their pen, with no extra stimulus. Pigs were video-recorded four times during each session on day 1, 3, 5 and 7 of each week, for 30 min, always at the same time (from 10.30 a.m. to 11.00 a.m.). Behaviours were scored from videos using continuous recording with an ethogram (Table 1). The first 10 min were not analysed, as the presence of the operator entering the pen to switch the cameras on could influence the pigs' behaviour. The last 10 min were also not analysed, as when operators were putting the

Table 1
Ethogram used for video analysis of Straw and Control sessions.

Behaviour	Definition
Exploratory behaviour	It consists of rooting, chewing, sniffing and manipulating the available rooting material (Studnitz et al., 2007).
Tail movement	Tail swinging in any direction, but mostly from side to side, so lateral tail movements (Kiley-Worthington, 1975; Reimert et al., 2013).
Ear movements	Any ear movement or ear posture change, including one or two ears (i.e. changes between the ear postures 'front' and 'back') (Reefmann et al., 2009a; Reimert et al., 2013).
Agonistic behaviour for competition	Actively displacing another pig, ramming or pushing another pig with the head with or without biting, aggressively biting any part of another pig or actively pursuing another pig (Chaloupková et al., 2007).
Displacement behaviour	Behaviour patterns characterized by their apparent irrelevance to the situation in which they appear (Maestriperi et al., 1992), often as a result of frustration or conflict. It may also be observed in situations of arousal when there is no appropriate outlet for de-arousal (Landsberg et al., 2013). Displacement behaviour observed in pigs: for more than one-minute period, continuous biting or licking of one part of the wall and repetitive mastication with excessive salivation.

The frequencies and durations of each type of behaviour were analysed, except for ear movements, considered as “event” by Martin and Bateson (2007), where only frequencies were analysed.

Behaviours could be overlapped, not mutually exclusive.

Tail movement frequency means the number of times that a pig starts moving the tail from side to side on the two-minute period.

Tail movement duration is expressed in seconds and means that a pig is moving its tail, considering a new movement when it stops the movement for, at least, 2 s (Marcet-Rius et al., 2018b and 2018c).

Table 2
Tail movement frequency and duration and ear movement frequency: comparison between Straw and Control sessions, for a total of 2400 s (40 min).

	Session	N	Mean	Median	Std dev	Minimum	Maximum	p-value
TMF	Control	15	47	47	15	15	67	0.23
	Straw	15	40	38	16	17	66	
TMD (s)	Control	15	950	1000	446	123	1843	0.46
	Straw	15	850	785	514	83	1875	
EMF	Control	15	110	96	56	36	221	<0.01**
	Straw	15	70	66	23	42	109	

TMD: tail movement duration; TMF: tail movement frequency; EMF: ear movement frequency.

** high significance.

security overalls on and preparing the material in the airlock in order to switch the cameras off, animals could hear them, so it could influence their behaviour. Therefore, a total of 40 min per pig were analysed, for each session, meaning a total of 80 min per pig for the totality of the study.

2.3. Statistical analysis

Data analysis was carried out using SAS 9.4 software Copyright (c) 2002–2012 by SAS Institute Inc., Cary, NC, USA. The significance threshold was classically fixed at 5%. For all variables, the sum of the 4 videos was computed. Pen was included in the model as a random factor, to take into account the possible pen effect.

For tail movement duration, tail movement frequency and ear movement frequency, normality was verified, and comparisons between Control session and Straw session were carried out using the MIXED procedure. For agonistic behaviour frequency, agonistic behaviour duration, displacement behaviour frequency and displacement behaviour duration, normality was not verified and data was modelled with a general mixed Poisson model using the GLIMMIX procedure. As all the data consisted of discrete variables (times, for the frequencies, and seconds, for durations), the Poisson distribution was adapted.

Correlations between all parameters in each session were analysed using Spearman's or Pearson's correlation coefficient with the CORR procedure depending of the normality of data: Spearman's correlation coefficient “rho” was used when normality was not verified for at least one variable, and Pearson's correlation coefficient “r”, when normality was verified for both variables. According to Martin and Bateson (2007), $r = 0.4$ – 0.7 is considered to be a moderate correlation (substantial relationship), $r = 0.7$ – 0.9 is considered to be a high correlation (marked relationship) and $r = 0.9$ – 1.0 is considered to be a very high correlation (very dependable relationship).

3. Results

3.1. Comparisons between Straw and Control sessions

Comparisons between Straw and Control sessions were analysed for all parameters, except for exploratory behaviour with the rooting material, as the animals of the Control session did not have the material so the comparison between sessions was not possible. Even though, it was important to measure it as it confirmed that all the pigs performed exploratory behaviour when straw was provided (exploratory behaviour duration in Straw session - Mean = 2139 s; Median = 2340 s; Total of observation = 2400 s).

No significant difference was observed between sessions neither for tail movement frequency (df = 14; F value = 1.55; $p = 0.23$) nor for tail movement duration (df = 14; F value = 0.59; $p = 0.46$) (Table 2). Concerning ear movement frequency, a significant difference was observed between sessions (df = 14; F value = 11.31; $p < 0.01$): in the Straw session, ear movement frequency was significantly lower than in the Control session (Table 2).

Concerning the frequency of agonistic behaviour, a significant difference was observed between sessions (df = 11; F value = 8.70; $p = 0.01$): in the Straw session, agonistic behaviour frequency was significantly lower than in Control session (Table 3). Regarding the duration, a significant difference was observed between sessions (df = 11; F value = 25.07; $p < 0.001$): in the Straw session, agonistic behaviour duration was significantly lower than in the Control session (Table 3).

Regarding the frequency of displacement behaviour, a significant difference was observed between sessions (df = 14; F value = 34.24; $p < 0.001$): it was significantly lower in the Straw session than in the Control session (Table 3). About the duration, a significant difference was observed between sessions (df = 14; F value = 8.78; $p = 0.01$): it was significantly lower in the Straw session than in the Control session

Table 3
Agonistic and displacement behaviours frequency and duration: comparison between Straw and Control sessions, for a total of 2400 s (40 min).

	Session	N obs	Mean	Median	Std dev	Minimum	Maximum	p-value
ABF	Control	12	3	2	3	0	9	0.01**
	Straw	12	1	1	2	0	5	
ABD (s)	Control	12	7	4	8	0	20	<0.001***
	Straw	12	2	2	3	0	7	
DBF	Control	15	10	8	5	5	25	<0.001***
	Straw	15	1	0	2	0	8	
DBD (s)	Control	15	1773	1971	655	419	2378	0.01**
	Straw	15	59	0	176	0	686	

ABD: agonistic behaviour duration; ABF: agonistic behaviour frequency; DBD: displacement behaviour duration; DBF: displacement behaviour frequency.

** high significance.

*** very high significance.

(Table 3).

3.2. Correlations between all parameters in Straw and Control sessions

Concerning Straw session, some positive correlations were found between agonistic behaviour frequency and agonistic behaviour duration ($r = 0.94$; $p < 0.0001$), displacement behaviour frequency and displacement behaviour duration ($\rho = 0.99$; $p < 0.0001$); some negative correlations were found between exploratory behaviour duration and displacement behaviour frequency ($\rho = -0.73$; $p < 0.001$), exploratory behaviour duration and displacement behaviour duration ($\rho = -0.73$; $p < 0.001$), displacement behaviour frequency and tail movement duration ($\rho = -0.74$; $p < 0.01$) and displacement behaviour duration and tail movement duration ($\rho = -0.72$; $p < 0.01$). Regarding Control session, a positive correlation was found between agonistic behaviour frequency and agonistic behaviour duration ($\rho = 0.86$; $p < 0.001$).

4. Discussion

The aim of the present study was to investigate whether straw provision in pigs increases positive welfare, and precisely, positive emotions, or decreases negative welfare, including negative emotions, or both. The results of the video analysis confirmed that pigs performed exploratory behaviour after straw provision. We observed fewer indicators of poor welfare with the presence of straw, and we did not observe an increase of the indicator of positive emotions. More precisely, no significant difference was found between Control and Straw sessions regarding tail movement duration and frequency. Regarding ear movement frequency, a significant difference was found between sessions, being lower in the straw than in the Control session. Concerning agonistic behaviour frequency and duration, as well as displacement activities frequency and duration, all were significantly lower in the Straw session than in the Control session. Many interesting correlations were found regarding all these parameters in Straw session, providing more information about the relationship between these behaviours and intrinsic responses and their relationship with the expression of emotions.

The fact that ear movement frequency was significantly lower in the Straw than in the Control session suggests that straw provision reduces negative emotions in pigs. A previous study (Marcet-Rius et al., 2018c) showed that ear movement frequency was lower in a play situation compared to a control situation, in accordance with the present results for straw provision, as both situations are designed to enrich the pigs' environment. According to the literature (Reimert et al., 2013, 2015, 2017), it appears that ear movement (measured as ear posture changes) is more linked to negative situations, and is less present in positive situations, suggesting that it could be an indicator of negative emotions or a decrease in positive emotions. Besides, this study (Marcet-Rius et al., 2018c) adds further information about these relationships,

demonstrating that pigs showed less ear movement in a positive situation than in a control situation (with no stimulus or enrichment), and the results of the present study are in agreement with them.

Regarding the other indicators of poor welfare (agonistic and displacements behaviours) were significantly reduced in the straw provision situation compared to control (with no stimulus). These results suggest consistently that the straw reduces the appearance of poor welfare indicators, as the same result was obtained for agonistic behaviour frequency and duration as well as for displacement behaviour frequency and duration; both were significantly lower in the Straw session. Hence, it suggests that straw provision reduces poor welfare states, as indicated by the literature (Burbidge et al., 1994; Tuytens, 2005; van de Weerd and Day, 2009; Whittaker et al., 1999). Additionally, these results confirmed that the control situation produces poorer welfare or a decrease in welfare for the pigs compared to a straw provision environment, giving more light to ear movements as potential indicator of negative emotions (or a decrease of positive emotions), which was significantly higher in the Control session. Thus, this study also suggests very consistently that a high-frequency of ear movements in pigs could be considered as an indicator of negative emotions, and so, a poor welfare indicator, appearing at the same time and in the same context as these strongly validated indicators (Maestripieri et al., 1992; Protocol Welfare Quality, 2009; Temple et al., 2011).

The results for tail movement duration as an indicator of positive emotions showed no significant differences between Straw and Control sessions. No differences were found regarding tail movement frequency. Thus, these results suggested that, in our test conditions, straw provision did not induce positive emotions in pigs. Other studies have also shown that straw provision does not modify putative physiological indicators of positive welfare, like oxytocin and serotonin (Marcet-Rius et al., 2018a). Nevertheless, other studies have shown that tail movement duration increases in positive situations, as for instance, in a play situation (Marcet-Rius et al., 2018b,c), which is supposed to trigger positive emotions (Mellor et al., 2009). Besides, Bolhuis et al. (2005) suggested that the provision of straw induces positive emotions, as it induces play behaviour. It seems interesting to mention that, some authors (Fraser, 1975) showed that with the presence of straw, pigs tend to rest more than without the straw, a fact that was also observed, even if not scored, during the viewing of the video recordings. When pigs are resting, tail movements are absent (or imperceptible), so it could be that this intrinsic response of tail movement might be attenuated in the Straw session for this reason, with the result of no significant difference between sessions. Further research focalised on the activity budget of pigs in some specific contexts would be needed, taking the resting time into account and evaluating its putative influence on tail movement, as well as including other indicators of positive emotions.

These results suggest that straw provision reduces poor welfare, including negative emotions, but it does not increase the appearance of

the positive emotion indicators selected for this study. Hence, we may conclude that straw provision increases welfare, because poor welfare indicators are decreased, in accordance with the literature (Andersen and Bøe, 1999; Smith et al., 1998; Whittaker et al., 1999), but that it does not increase positive emotions, at least, not according to the indicator chosen for this study.

Much information was obtained concerning the correlations in the Straw session, in accordance with the results already mentioned. Most interestingly, a negative correlation was found between exploratory behaviour duration and displacement behaviour frequency, as well as between exploratory behaviour duration and displacement behaviour duration, confirming that when animals perform exploratory behaviour with the straw, they do not perform displacement activities. In addition, a negative correlation was found between displacement behaviour frequency and tail movement duration as well as between displacement behaviour duration and tail movement duration. These last correlations seem to indicate that when animals are in a poor welfare situation, represented by displacement behaviours, they move their tails less, which is coherent with the other results obtained and supports the use of long-duration tail movement as an indicator of positive emotions and positive welfare (Marcet-Rius et al., 2018b,c).

This research shows that enriching the environment with straw reduces the poor welfare situation induced by a poor environment, thereby reducing the suffering state of the animals, but it does not appear to produce positive emotions or pleasure in the pigs, at least, regarding our selected indicator. These results suggest that the straw provision compensates for the animals' poor environment, which would allow the pigs to "function" in the current intensive systems, or cope with this stressful environment. Nevertheless, this study suggests that it does not lead them to experience positive emotions, which seems essential to a positive welfare state. Further research would be necessary to explore more thoroughly this hypothesis such as investigating it in an intensive pig production system.

5. Conclusions

In conclusion, the straw provision reduces poor welfare in pigs, but it does not seem to produce positive emotions, at least, concerning the chosen indicator and in our test conditions. More precisely, it reduces the appearance of ear movements that are associated with negative emotions, and reduces agonistic and displacement behaviours, which seems important in the current production systems. Additionally, results show interesting correlations between different behaviours considered as positive or negative for the animals. Overall, these results may be useful to better understand the welfare state of the pigs in this context. It may also improve the animal welfare assessment of pigs, providing more information about simple and feasible behavioural indicators which reflect the emotional state of the animals. Our results also give rise to an important question about current pig intensive production systems: to ensure animal welfare, as well as the performance of the pigs, is it necessary to induce positive emotions in them or is it sufficient to simply eliminate the situations inducing poor welfare? Future studies including physiological and zootechnical measures as well as the incidence of positive emotions in a pig intensive system could be helpful to further explore this promising field.

Animal welfare implications

The main implication of this study was to understand the welfare state of the pigs when straw is provided, as a model of environmental enrichment, to determine whether it only reduces poor welfare or also increases positive emotions and so positive welfare. It also provides additional information about feasible behavioural indicators of animal welfare, which could be useful in improving pig welfare assessments.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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