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Painful Standard Management Practices with Piglets: Does Bundling and/ or Anesthesia Improve Animal Welfare?

S. Van Beirendonck¹, B. Driessen¹ and R. Geers²

¹K. H. Kempen University College, Kleinhoefstraat 4, B-2440 Geel, Belgium ²Quality Care in Animal Production, K.U.Leuven, Bijzondere weg 12, B-3360 Lovenjoel, Belgium

Abstract

Newborn piglets are subjected to several painful management practices during their first week of life. The objective of this study was to investigate whether the bundling of these painful management practices as such and in combination with anesthesia influenced the behavior and/or production results of piglets positively compared to a random application. There were 515 piglets included in this study, which consisted of two experiments. In the first experiment, management practices were carried out spread over the first week ("separate" group, n = 168) or bundled at one week of age ("together1", n = 144). In the second experiment management practices were all bundled at one week of age without anesthesia ("together2" group, n = 97), or bundled at one week of age while the piglets were anesthetized with 100% CO₂ ("anesthesia" group, n = 106). Behavior of the piglets in both experiments was observed from the day of birth until weaning. Behavioral categories were lying down, udder activity, walking, social cohesion, interactive behavior, pain related behavior and postures (sitting, standing and kneeling). Results demonstrate that piglets seem to cope better with pain if painful interventions are not combined. Moreover, the applied CO, anesthesia has facilitated the pain experience after treatment, since lying, interactive and walking behavior indicated more discomfort for the anesthetized piglets. Anesthetized piglets had only an advantage when considering nursing behavior. The question remains how aversion against CO, might have impaired the healing process after castration when combined with other painful interventions. Hence, both farmers and veterinarians, who have to back up farmers for application of painful procedures, should take into account these results in their decision making.

Keywords: Anesthesia; Animal welfare; Pain; Pig

Introduction

Commentary

Piglets have to endure several painful management procedures during their first week of life [1]. Processing of pain is considered as being analogous to humans and animals, according to the principle of analogy based on anatomical and physiological similarities between humans and higher animals [2]. Molony V et al. [3] described pain in animals as an aversive sensory and emotional experience that represents the animal's notion of damage or threat to the integrity of the tissues. It influences the physiology of the animal and the behavior to reduce or avoid damage, to minimise the chance on reoccurrence and to improve recovery. Anand KJS et al. [4] suggested adjusting the traditional vision, which emphasizes mostly self assessment as a measure for pain. These authors stated that behavioral changes caused by pain are a form of self assessment for non-verbal individuals, and that behavior cannot be perceived as a 'surrogate measurement' of pain. They accentuated this in the first place for human neonates, but also for other individuals not capable of expressing their pain in a direct way, and by extension for animals. Pain experience depends on several factors, e.g. the nature of the pain, location, duration and intensity. This experience can vary from a negligible discomfort to a completely debilitating condition [5]. The response of an animal to a painful stimulus can vary according to species, age, sex, previous experience, and the response can be altered by individual experience [6].

The management procedures that are carried out in the piglets' first week of life are ear tagging, tooth shortening (although routinely prohibited, except when recommended by a veterinarian), tail docking (although routinely prohibited, except when recommended by a veterinarian), injections (vaccination and iron injection) and castration (only male piglets). Ear tagging of newborn piglets causes pain and stress [7-9], as being expressed vocally and by behavior [9]. Tooth

J Anesthe Clinic Res ISSN:2155-6148 JACR an open access journal shortening is not routinely allowed, but it is still frequently applied to minimise wounds and skin lesions in other piglets and the sow's udder. Two techniques are used: clipping or grinding. In comparison to grinding, clipping had a more negative impact, namely teeth fractures, gum haemorrhages, infection of gums and tooth cavity... [10-11]. However, piglets with clipped canines appeared to induce less skin lesions than piglets with ground canines. This was probably because of the fact that piglets with clipped canines showed more pronounced pain suffering [12]. Shortening canines, whichever technique was used, led to teeth lesions and intense pain [11].

Tail docking occurs directly or some days after birth with a heated knife or other instruments, and mostly without anesthesia or analgesia. Tails are snipped off and the wound is sealed at the same time. Sometimes an ordinary cutter or bistouri knife is used, and antiseptic products might be applied on the tail wound. The length of the removed tail varies from a little top to almost the complete tail where the tail remainder is reduced up to approximately 1.5 cm. The piglets' tail was cut shorter in case of biting problems [13]. It is known that tail docking is painful since the peripheral nerves in the tip of the tail are already fully developed in newborn piglets [14]. Iron is administered to prevent anemia [15], and is correlated with a reduced mortality of piglets during

*Corresponding author: S. Van Beirendonck, K. H. Kempen University College, Kleinhoefstraat 4, B-2440 Geel, Belgium, Tel: +3216468139; Fax: +3216468159; E-mail: sanne.van.beirendonck@khk.be

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the nursery period [16]. Although it is recognised that needle injections can be painful, hardly any investigation has been conducted for further evaluation in animals [8]. Research in human medicine made it clear that needle injections can be painful. These pains were influenced by numerous factors, particularly needle length, needle sharpness; needle diameter (gauge), needle temperature and patient's age [17-18]. Every year approximately a 100 million piglets in the European Union are castrated, which is about 83% of the male pig population in the EU [19]. From behavioral observations [20] and vocalisation registration where the frequency and the intensity of screaming were analysed during castration [21], it appears that castration without anesthesia is a painful intervention.

The objective of this study was to investigate in a first experiment whether the bundling of painful management practices influences the behavior and/or production results of piglets. The bundling of these painful events on a later age also has the advantage that the piglets only have to be picked up once (time saving for the farmer) and that the piglets are left alone the first days of their life which gives weaker piglets the chance to make a good start also. In a second experiment, the objective was to examine whether the bundling of painful management practices with anesthesia influences the behavior and/or production results of piglets positively compared to a random application without anesthesia. The hypothesis that bundling of the procedures might improve welfare was based on the phenomenon of 'pain memory'. Studies on rats [22] and humans [23-24] have indicated that individuals who were repeatedly exposed to neonatal pain develop a lowered pain threshold. Wounding and tissue damage at a young age can cause changes in the central nervous system that persist after the wound is healed, and influence the behavioral response on painful procedures months later in the sense that they display a much stronger pain response [24].

The absent pain experience during the procedures for piglets of the anesthetized group is undoubtedly advantageous to piglet welfare, but the objective of the study was to look at the effects afterwards. The present study attempts to contribute to the debate on farm animal welfare and the discussion on painful management practices. Both veterinarians and farmers have to be aware, since veterinarians are legally responsible for advising farmers on the application of painful procedures.

Material and Methods

Animals and housing

Hybrid piglets (Piétrain x Hypor), being heterozygous for the halothane gene, were used. All piglets (515 out of 41 litters) were raised in the same housing conditions at the Zootechnical Centre (ZTC) – K.U.Leuven R&D (Belgium, Europe), and were individually marked with an ear tag number at day of birth. The number of gilts and boars within a litter and the total number of piglets per pen was more or less equal, i.e. 12 to 13, but the total number was not standardized. Cross fostering was applied during the week of birth in order to balance litters for body weight and number of piglets. All piglets from a single litter (male and female piglets) received the same experimental treatment, except for castration. Hence, a treatment was allocated to a litter.

The farrowing house was temperature controlled using floor and air heating so that piglets were kept within their thermal neutral zone. The housing environment was automatically controlled with a computerized heating and ventilation system, so that the required temperature was managed independently of the outside temperature. Piglets were weaned at week 4. From weaning till about 22 kg, the piglets were kept in weaning pens with slatted plastic floor and a warming floor element. The fattening period started at about 22 kg, and pigs were kept within the same group on concrete slatted floors during fattening. Pigs had ad libitum access to water and a commercial diet. Management was based on the "all in – all out" principle for each room.

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Experimental design

Piglets were handled according to the Belgian law on the protection of animals, and the experimental protocol was agreed by the Ethical Committee on the use of experimental animals. Two experiments were conducted in 2 different trials.

Experiment 1.

In the first experiment, the following 2 treatment groups were compared:

- Management practices carried out as normal, spread in the first week of life (SEPARATE, n = 168 out of 12 litters):
 - o On day 1: iron injection, ear tagging, tooth resection, tail docking
 - o On day 7: Vaccination for Mycoplasma hyopneumoniae (Stellamune Mycoplasma, Intervet), 0.5 ml Amoxycilline (Duphamox, Fort Dodge) injection, castration (male piglets, about half of the litter)
- Management practices all carried out together on day 7 (TOGETHER 1, n = 144 out of 12 litters).

All management practices were executed before the piglets reached the age of 1 week (EU Directive 91/630/EEG). Piglets were weighed regularly: on the day of birth, on the day of castration, at about 20 kg, 45 kg, 75 kg and before transport to the slaughterhouse (\pm 110 kg).

Experiment 2.

In a subsequent experiment, bundling of management practices was examined deeper. Two treatment groups were compared:

- Management practices all carried out together on day 7, (TOGETHER 2, n = 97 out of 9 litters).
- Management practices all carried out together on day 7, piglets were anesthetized with 100% CO₂ before the procedures (ANESTHETIZED, n = 106 out of 8 litters).

Anesthesia with CO₂ has generated different opinions. The noxious effect was too large according to [25], while [26] concluded that CO₂ -anesthesia can be induced safely and rapidly. A concentration of 70% CO₂ was considered admissible to avoid pain during castration by [27]. CO₂ is already being used to stun animals before slaughter. Only high concentrations above 80% are used for this to keep the aversion period as short as possible, i.e. about 70 sec, and reducing the risk of inadequate anesthesia [28]. Therefore, in this experiment 100% CO₂ was used during 25s as being found to be the optimal duration in a preliminary experiment. Anesthesia was administered through a mouth mask.

All management practices were executed before the piglets reached the age of 1 week (EU Directive 91/630/EEG). Piglets were weighed regularly: on the day of birth, on the day of castration, at about 20 kg, 45 kg, 75 kg and before transport to the slaughterhouse (\pm 110 kg).

Behavioral observations

Behavioral observations were carried out in the same way for the 2 experiments, according to a scan sampling procedure. The time interval between 2 successive scan samplings was about 2 minutes. The behavior of each piglet within a pen was scored, and then the observer moved on to the next pen and repeated the action up to the last pen. This sequence was repeated until every pen was observed 4 times. This procedure was followed in the morning as well as in the afternoon. All observations were performed by a single observer (de visu) standing in the central corridor of the farrowing or weaning room. Piglets were observed the first time at the day of birth and behavior was followed up regularly until weaning (every Monday, Wednesday and Friday).

All behavioral categories were mutually exclusive and are described in Table 1. Teeth grinding and tail wagging might be related to respectively tooth resection and tail docking, but were included in the

"Non-specific" behaviors			
Suckling	Teat in the mouth. Vigorous rhythmic suckling move- ments.		
Udder massage	Nose in contact with the udder, leaning against it. Ample and rhythmic up and down head movements.		
Teat seeking	Attempts to find a teat by walking and pushing other piglets, while most of the others are suckling.		
Nosing	The snout is close to or in contact with a substrate or a pen-mate. Snout movements may be observed.		
Chewing	Nibbling at littermates (ears, tail or foot, etc.) or substrates.		
Licking	Rubbing the tongue over littermates, floor or pen walls.		
Playing	Head shaking, springing (sudden jumping or leap- ing), running with vertical and horizontal bouncy movements. Can involve partners (gentle nudging or pushing, mounting, chasing).		
Aggression	Forceful fighting, pushing with the head or biting lit- termates in a violent manner.		
Walking	Slowly moving forward with one leg at a time.		
Running	Trot or gallop without sudden change in direction or speed.		
Sleeping	Lying down, eyes closed.		
Pain-castration related b	ehaviours		
Huddled up	Lying with at least 3 legs tucked under the body.		
Trembling	Shivering as with cold. The animal may be lying, sit- ting or standing.		
Spasms	Quick and involuntary contractions of the muscles under the skin, of a leg.		
Scratching	Scratching the rump by rubbing it against the floor or the pen walls.		
Tail wagging	Tail's movements from side to side or up and down.		
Postures			
Lateral lying	Motionless; body weight supported by side. Shoulder in contact with floor.		
Ventral lying	Motionless; body weight supported by belly. Sternum in contact with floor.		
Sitting	Motionless; body weight supported by hind-quarters and front legs.		
Standing	Motionless; body weight supported by the 4 legs.		
Kneeling	Motionless; body weight supported by front carpal joints and hind legs.		
Social cohesion			
Isolated	Apart from other piglets, alone or with one pen-mate at the most. A distance of at least 40 cm (about the width of two piglets) separates the animal from the closest group of littermates.		

 Table 1: Description of behavioral categories.

category 'pain related behavior'.

In addition to the piglets, sows' behavior was also scored. A difference was made between lying down, sitting and standing (Table 1).

Statistical analysis

All data were analyzed with SAS 9.2 (SAS Inst, Inc, Cary, NC, USA 2008) software. A significance level of 0.05 was used. Categories of behavior were grouped for analysis in order to have a sufficient number of observations within each grouped category, so that the empirical estimation could be carried out on a sufficient number of observations. A condition of the applied statistical model is that the performance of the empirical estimation passes the convergence test [29], being dependent on the number of observations within each category. Hence, lateral lying, ventral lying and sleeping were grouped under 'lying'; teat seeking, suckling and udder massage were grouped under 'udder activity'; huddled up, trembling, spasms, scratching and tail wagging were grouped under 'pain related behavior'; nosing, chewing, licking, playing and aggression were grouped under 'walking'; and sitting, standing and kneeling were grouped under 'postures'.

Behavioral data were not normally distributed and were dichotomized using the median as cut-off value. The binary data were analyzed using the logistic mixed model, with the fixed effects being treatment and observation period (time related variations are therefore taken into account), and the random effect being piglet. Random effects accounted for the variability between the piglets within and between litters, hence also for a litter effect. The applied procedure made it possible to allocate a random effect to a variable [29], so that piglets could be regarded as the experimental units. There were 2 factors considered in defining this random effect: variability between litters and variability between individual piglets. When taking into account the lowest level in the model, i.e. individual piglets, the variability between piglets partially represented the variability between litters. The behavior of the sow was also included in the model to take into account the variability between litters and the possible influence of sow's behavior on piglets' behavior. Sows' behavior was divided into three classes, the lowest class representing resting behavior and the highest class representing more restless behavior; and was added in the model as a covariate. Values are presented as means ± S.E.M.

Data concerning body weight were analyzed using a linear mixed model (SAS, Inst, Inc, Cary, NC, USA 2008), with the litter effect taken into account through the random effect, and were covariated for starting weight.

Data analysis was the same for experiment 1 and 2. However, both data sets were not merged for a global analysis, because experimental conditions were not exactly the same. However, it can be assumed that all potential influential factors, but out of an intended treatment effect, were at random distributed in the same way between treatments within each experiment. Hence, a difference between treatments can be compared between experiments, especially in reference to the common treatment.

Results

For both experiments, results are reported for all data taken together over time, and separately for every week before weaning, in order to infer the effect of the bundling of the painful management procedures, whether or not in combination with anesthesia, on the behavioral categories studied. Attention will be focused on the complementarities of the behavioral events that are considered to be indicative of pain and discomfort, and also on the so-called positive behavior (interactive behavior, social cohesion) that is important for animal welfare [30-31].

Experiment 1

Behavior, all observation periods together: Piglets of the "together1" group were lying down more but showed less activity at the udder less interactive behavior, less walking and less postures than piglets of the "separate" group (Table 2).

Behavioral observations, per week: In week 3 and 4, piglets of the "separate" group were more active than piglets of the "together1" group by displaying more udder activity, interactive behavior, walking and postures, and less lying down and isolated behavior. More interactive behavior was also shown by the "separate" group in week 1. Piglets of the "together1" were more active at the udder in week 2 (Table 3). No differences were found in pain related behavior. (Table 3).

Body weight, from week 1 until slaughtering: Mean body weights at castration were 2.37 kg for the "separate" group and 2.16 kg for the "together1" group. There were no statistically significant differences on body weight between treatment groups from day of castration until slaughtering.

Experiment 2

Behavior, all observation periods together: The "together2" group showed more activity at the udder, but less sitting, standing and kneeling compared to the "anesthetized" group (Table 4).

	SEPARATE	TOGETHER1	P-value
Lying down	0.592 ± 0.00515ª	0.641 ± 0.00526 ^b	< 0.0001
Isolated behavior	0.00584 ± 0.000673	0.00696 ± 0.000819	
Postures	0.0374 ± 0.00177^{a}	0.0301 ± 0.00169b	0.0259
Walking	0.113 ± 0.00298ª	0.0979 ± 0.00315 ^b	0.0009
Interactive behavior	0.0419 ± 0.00182^{a}	0.0289 ± 0.00169b	< 0.0001
Pain related behavior	0.00734 ± 0.000946	0.00876 ± 0.00115	
Activity at the udder	0.202 ± 0.00406 ^a	0.189 ± 0.00453 ^b	0.0068

Table 2: Behavioral data of all observation periods of experiment 1 taken together, presented as means \pm S.E.M. a,bWithin a row and variable, means without a common superscript differ (P < 0.05). Only significant P-values are shown. Means and S.E.M. are from the non-transformed data. Significant differences were obtained after analysis of the dichotomized data.

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Week	Behavior	SEPARATE	TOGETHER1	P-value
1	Interactive	0.0120 ± 0.00244	0.00413 ± 0.00178	0.0230
2	Udder activity	0.172 ± 0.0065	0.210 ± 0.00818	0.0020
3	Lying down	0.528 ± 0.0110	0.653 ± 0.0107	< 0.0001
	Udder activity	0.262 ± 0.00914	0.166 ± 0.00792	< 0.0001
	Interactive	0.0414 ± 0.00392	0.0249 ± 0.00330	0.0052
4	Lying down	0.457 ± 0.00824	0.588 ± 0.00914	< 0.0001
	Udder activity	0.200 ± 0.00644	0.166 ± 0.00678	0.0005
	Interactive	0.0861 ± 0.00438	0.0568 ± 0.00413	< 0.0001
	Walking	0.179 ± 0.00620	0.125 ± 0.00612	< 0.0001
	Isolated	0.00505 ± 0.00114	0.00936 ± 0.00167	0.0427
	Postures	0.0735 ± 0.00427	0.0477 ± 0.00380	0.0004

Table 3: Behavioral data per week in experiment 1, presented as means \pm S.E.M. Only behavioral categories which differ significantly (P < 0.05) are shown. Means and S.E.M. are from the non-transformed data. Significant differences were obtained after analysis of the dichotomized data.

Behavioral observations, per week: The piglets of the "together 2" group were more active in week 2 and 3, by showing more udder activity, walking and interactive behaviour than the "anesthetized" group (Table 5). The latter group was walking most during week 1, lying most during week 2, sitting, standing and kneeling most in week 3, and showed most udder activity in week 4 (Table 5). No differences were found in relation to pain related behaviour and isolated behaviour. (Table 5).

Body weight, from week 1 until slaughtering: There are no significant differences on body weight for the two treatment groups, from the day of the treatment until slaughtering.

Discussion

Experiment 1

Lying: Lying down of the piglets was considered by [3] as a way to protect themselves from their littermates when they are in pain, by shielding their painful body parts away from the other piglets. Following this reasoning, the differences in lying behavior in the present study turned out in the benefit of the "separate" group. This group was lying down the least during the complete observation period and in week 3 and 4.

Udder activity: Concerning activity at the udder, piglets of the "separate" group were more active than piglets of the "together1" group for the overall period of observation. This was also the case in week 3 and 4, while in week 2 piglets of the "together1" group were more active at the udder than piglets of the "separate" group. Noonan GJ et al. [32] correlated increased udder activity with pain because of the release of endorphins during suckling, which have an analgesic effect. Hay Met al., McGlone JJ et al. [20,33] on the other hand, found that piglets spent less time at the udder during the first hours after castration, when they

	TOGETHER2	ANESTHETIZED	P-value
Lying down	0.544 ± 0.00711	0.539 ± 0.00752	
Isolated behavior	0.00521 ± 0.00122	0.00823 ± 0.00158	
Postures	0.0598 ± 0.00307ª	0.0659 ± 0.00332 ^b	0.0013
Walking	0.141 ± 0.00452	0.152 ± 0.00518	
Interactive behavior	0.0250 ± 0.00184	0.0294 ± 0.00223	
Pain related behavior	0.00285 ± 0.000652	0.00518 ± 0.00120	
Activity at the udder	0.216 ± 0.00531ª	0.200 ± 0.00567 ^b	0.0296

Table 4: Behavioral data of all observation periods of experiment 2 taken together, presented as means \pm S.E.M. a,bWithin a row and variable, means without a common superscript differ (P < 0.05). Only significant P-values are shown. Means and S.E.M. are from the non-transformed data. Significant differences were obtained after analysis of the dichotomized data.

Week	Behavior	TOGETHER2	ANESTHETIZED	P-value
1	Udder activity	0.253 ± 0.0140	0.158 ± 0.0111	0.0002
	Walking	0.0798 ± 0.00841	0.131 ± 0.00924	0.0464
2	Lying down	0.623 ± 0.0109	0.543 ± 0.0133	0.0168
	Udder activity	0.183 ± 0.00813	0.241 ± 0.0105	0.0003
	Walking	0.102 ± 0.00649	0.102 ± 0.00787	< 0.0001
3	Lying down	0.452 ± 0.0130	0.518 ± 0.0136	< 0.0001
	Udder activity	0.232 ± 0.0107	0.204 ± 0.0122	0.0022
	Interactive	0.0469 ± 0.00491	0.0376 ± 0.00507	0.0064
	Postures	0.0827 ± 0.00733	0.0828 ± 0.00740	0.0471
4	Udder activity	0.214 ± 0.0102	0.177 ± 0.0107	0.0155

Table 5: Behavioral data per week in experiment 2, presented as means ± S.E.M.Only behavioral categories which differ significantly (P < 0.05) are shown. Means</td>and S.E.M. are from the non-transformed data. Significant differences were obtained after analysis of the dichotomized data.

were in pain. In week 2, the "together1" group has just been put through all the painful procedures, which could suggest that that result agrees with [22]. In week 3 and 4, it was already a few weeks since painful procedures were carried out, so that it is difficult to relate the activity at the udder to possible pain experience in this case. It is also possible that the effect of the painful procedures on activity at the udder is limited in time.

Interactive behavior: Piglets of the "separate" group displayed more interactive behavior than those of the "together1" group for the overall observation period and for the first and third week. In the fourth week however, piglets of the "together1" group showed more interactions. Interactive behavior in this study included nosing, chewing, licking, aggression and playing. Llamas Moya S et al. [34] found that castrated piglets were being less playful in comparison with sham-castrated piglets, which might indicate poor welfare. Blackshaw JK et al. [31] specifically label playing behavior as a positive indication of animal welfare. Hay M et al. [20] suggested that reduced oral exploration, such as licking and chewing, may be associated with pain.

In the present study, this would suggest that the "separate" group might have a better state of welfare for the overall observation period and in the first and third week, but in week 4 the "together1" group might have a better state of welfare.

Walking: Piglets of the "separate" group tended to walk more than the piglets of the "together1" group in the overall period of observation and in week 4. Llamas Moya S et al. [34] already stated that piglets after castration, in pain, may avoid certain activities such as walking and postures like dog-sitting, to minimize pain. The reduced running around of piglets of the "together1" group, having to cope with a lot of painful procedures at the same time, may possibly be interpreted in this way.

Isolated behavior: Concerning social cohesion, there was only a difference in the last week before weaning. Piglets of the "together1" group isolated themselves more from their littermates than piglets of the "separate" group. Other studies [20,34] observed that piglets after castration, when they were in pain, tended to be more isolated than their pen mates. The most plausible explanation for this is that they tried to avoid contacts with pen-mates that might create more pain. In the present study there was only a difference between the treatment groups in week 4, where this could suggest a better welfare for the "separate" group. However, since all painful procedures took place in week 1, it is not certain that this behavioral difference is due to the painful procedures performed some weeks earlier.

Postures: Looking at postures, piglets of the "separate" group sat, stood and kneeled more than the other piglets in the overall observation period and in week 4. Taylor AA et al. [35] observed increased sitting and standing after castration, which could be a result of the painful experience. Other studies [33,36,37] however, showed reduced standing after castration. Concerning postures, studies do not agree. Hay M et al. [20] therefore suggested that changes in posture are not fully reliable to assess pain in piglets.

Pain related behavior: There are no differences observed between treatment groups, for the overall observation period and every week separately. It would be expected that piglets of the "separate" group, because of the prolonged (repeated) pain experience, would be more

subjected to pain. However, apart from udder activity the other results (interactive behavior, walking), do not point in that direction.

Body weight: There were no differences in body weight between the two treatment groups, so that the treatment applied in the farrowing room had no effect on body growth until slaughter.

Experiment 2

Lying: In week 2 and 3, the "anesthetized" group lay down more than the "together2" group. Applying the conclusions of [3] again, who considered lying down as a strategy of piglets to shield their painful body parts from other piglets; this could be interpreted as a better state of welfare for the "together2" group. In experiment 1 lying behavior was more beneficial for the "separate" group, which might indicate that in experiment 2 the anesthesia procedure might have increased the impact of the treatment compared to the "together2" group. Hence, the statement of [38], that aversion before losing consciousness is compensated by the fact that piglets experience complete anesthesia and analgesia during castration, might not be completely true when considering the period after treatment.

Udder activity: For the overall observation period as well as in week 1, 2 and 3, piglets of the "together2" group displayed more udder activity, confirming the results in experiment 1. In discussing experiment 1, it is already mentioned that piglets in pain increased in udder activity because of the release of endorphins during suckling [32]. Extrapolating this to the results of experiment 2, there is an indication that piglets of the "together2" group perceived more pain than piglets of the "anesthetized" group.

Interactive behavior: Piglets of the "together2" group performed more interactive behaviors than piglets of the other group in week 3. Several studies [20,31,34] linked interactive behaviors with an improved welfare. Results of the present study therefore speculate a better state of welfare for piglets of the "together2" group in week 3, based on interactive behavior. In experiment 1, interactive behavior was more beneficial for the "separate" group, which might indicate that the anesthesia procedure might have increased the impact of the treatment within the "together2" group.

Walking: Walking behavior shifted after 1 week. In the first week, piglets of the "anesthetized" group walked around more than piglets of the "together2" group, while in week 2 it was the contrary. Because treatments were only carried out at the end of the first week, the difference in week 2, right after the treatment, can be considered the most important. Llamas Moya S et al. [34] interpreted avoiding walking in piglets as a way to minimize pain after a painful procedure like castration. For the present study, that would imply that piglets of the "anesthetized" group experience more pain in week 2, after the treatments are carried out. This emphasizes again the additional impact of anesthesia on the pain experience afterwards.

Postures: For the overall observation period as well as week 3, piglets of the "anesthetized" group sat, stood and kneeled more than piglets of the "together2" group. As already mentioned for experiment 1, a number of studies [20,33,35-37] disagree on sitting and standing in piglets, which makes these behavioral parameters not fully reliable for assessing pain in piglets [20].

Body weight: There were no differences in body weight between

the two treatment groups, so that the treatment applied in the farrowing room had no effect on body growth until slaughter.

Conclusions

The painful effect of castration is demonstrated again, but the interaction with other painful interventions is new information. Piglets seem to cope better with pain if painful interventions are not combined. Moreover, the applied CO2 anesthesia has facilitated the pain experience after treatment, since lying, interactive and walking behavior indicated more discomfort for the anesthetized piglets. Anesthetized piglets had only an advantage when considering nursing behavior. The question remains how aversion against CO₂ might have impaired the healing process after castration when combined with other painful interventions. Although the beneficial effect of anesthesia during painful procedures is not really confirmed by the results, these results should be interpreted as a 'delayed' pain experience for anesthetized piglets rather than an additional pain experience. The post-operative pain may be present in both treatments, but the absent pain experience during the procedures for piglets of the anesthetized group is still advantageous to piglet welfare. The present study attempts to contribute to the debate on farm animal welfare and the discussion on painful management practices. Both veterinarians and farmers have to be aware, since veterinarians are legally responsible for advising farmers on the application of painful procedures.

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