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GROUP MANAGEMENT OF PREGNANT SOWS AT MIXING: IMPLICATIONS FOR ANIMAL WELFARE AND REPRODUCTIVE PERFORMANCE

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Contents

ABSTRACT

In conventional intensive farming, sows experience several highly relevant welfare consequences. As regards pregnant sows, the welfare consequences are primarily associated with confinement in individual gestation stall, group stress related with aggression required to establish the social hierarchy at mixing and in general inability to perform exploratory/foraging behaviour.

The first step of this thesis was to conduct a systematic review aimed at assessing how the scientific community addressed the subject of pregnant sow welfare over the past 30 years, which were the publishing country of the studies, the interventions studied and the welfare outcomes considered. Although the number of publications on group management and environmental enrichments increased over the last 10 years, their limited number suggests the need for further investigations, especially regarding the stage of reproductive cycle at the time of mixing after service (6 papers); group pen management strategy (4 papers) and provision of environmental enrichments (8 papers). In light of the paucity of publications on this topic and of the forthcoming revision of the European animal welfare legislation regarding the use of individual stalls, a second step was to evaluate the effects of reducing the time pregnant sows spend in gestation stalls on animal welfare measures and reproductive performance. The results of the first field study suggest that sows can be mixed into static groups with floor feeding early after insemination (within 4 days post-service) rather than 28 days after, without negatively affecting welfare indicators (skin lesion and salivary cortisol), reproductive performance (pregnancy and farrowing rate), litter size or back fat thickness. The results of the second field study indicate that mixing unacquainted groups of sows into individual free access feeding stalls pens early after insemination (within 2 days post-service), rather than 28 days after, increase the number of fighting recorded the day after mixing, but neither skin lesion nor locomotion were affected. However, the negative trend reported in sows mixed early after

insemination for reproductive performance suggested the need for further investigation, with larger sample size.

Given the importance of finding methods that reduce the stress and the aggressions resulting from mixing unacquainted groups of sows and the paucity of papers that investigated the effect of environmental enrichments on sow welfare, a further field study evaluated the effect of providing access to straw in racks and manila ropes on aggressive behaviour after mixing. The results indicate an overall decrease in aggression, although the effect was reduced on the day of mixing. Moreover, the role played by back fat thickness and parity order on the level of aggressive behaviour was investigated, revealing an effect only in the initiated aggressive behaviour, which was most frequently performed by sow with low back fat depth.

In conclusion, the implications of these studies are that they indicate the possibility to reduce the time pregnant sows spend in gestation stalls and they could provide further useful data on how best to respond to the citizens' request to put an end to the "Cage Age" and to direct farmers in the best management of this important change.

RIASSUNTO

Negli allevamenti intensivi convenzionali le scrofe sono esposte a numerose e rilevanti conseguenze in termini di benessere. Per quanto riguarda le scrofe gravide, le conseguenze sul benessere sono principalmente associate al confinamento in gabbia individuale, allo stress di gruppo legato all'aggressività necessaria per stabilire la gerarchia sociale al momento dell'imbrancamento e all'incapacità di eseguire comportamenti esplorativi e di foraggiamento.

Il primo passo di questa tesi è stato quello di condurre una revisione sistematica volta a valutare come la comunità scientifica ha affrontato il tema del benessere delle scrofe gravide negli ultimi 30 anni, quali sono stati i Paesi che hanno pubblicato gli articoli, quali gli interventi studiati e quali i risultati in termini di benessere utilizzati. Sebbene il numero di pubblicazioni sulla gestione delle scrofe in gruppo e sugli arricchimenti ambientali sia aumentato negli ultimi 10 anni, il loro limitato numero suggerisce la necessità di ulteriori indagini, in particolare per quanto riguarda lo stadio del ciclo riproduttivo al momento dell'imbrancamento (6 articoli); le strategie da utilizzare per la formazione del gruppo (4 articoli) e l'utilizzo di arricchimenti ambientali (8 articoli).

Alla luce della scarsità di pubblicazioni su questo argomento e dell'imminente revisione della legislazione europea per quanto riguarda l'uso delle gabbie individuali, un secondo passo è stato quello di valutare gli effetti della riduzione del tempo trascorso dalle scrofe nelle gabbie gestazione sulle misure di benessere e sulle performance riproduttive. I risultati del primo studio di campo suggeriscono che le scrofe possono essere imbrancate in gruppi statici con sistema di alimentazione a terra subito dopo l'inseminazione (entro 4 giorni dalla copertura) anziché 28 giorni dopo, senza influenzare negativamente gli indicatori di benessere (lesioni cutanee e cortisolo salivare), le prestazioni riproduttive (fertilità ecografica e portata al parto), le dimensioni della nidiata o lo

spessore del grasso dorsale. I risultati del secondo studio di campo hanno indicato che l’imbrancamento subito dopo l'inseminazione (entro 2 giorni dal servizio), piuttosto che 28 giorni dopo, in box con poste singole di alimentazione ad accesso libero, aumenta il numero di combattimenti registrati il giorno successivo all’imbrancamento, ma non vengono influenzate né le lesioni cutanee né la locomozione. Tuttavia, la tendenza negativa registrata nelle scrofe imbrancate precocemente dopo l'inseminazione per quanto riguarda le prestazioni riproduttive ha suggerito la necessità di ulteriori indagini, con una maggiore dimensione del campione.

Data l'importanza di trovare dei metodi che possano ridurre lo stress e le aggressioni derivanti dalla formazione dei nuovi gruppi di scrofe e la scarsità di articoli che hanno studiato l'effetto degli arricchimenti ambientali sul benessere delle scrofe, un ulteriore studio di campo ha valutato l'effetto della fornitura di paglia in rastrelliere e di corde di fibra naturale sul comportamento aggressivo dopo l’imbrancamento. I risultati hanno indicato una diminuzione complessiva dell'aggressività, sebbene l'effetto sia stato ridotto nel giorno dell’imbrancamento. Inoltre è stato analizzato il ruolo svolto dallo spessore del grasso dorsale e dall'ordine di parto sulla manifestazione dei comportamenti aggressivi, rivelando un effetto significativo solo sui comportamenti aggressivi proattivi, che sono stati messi in atto più frequentemente dalle scrofe con uno spessore di grasso dorsale inferiore rispetto alla media.

In conclusione, le implicazioni di questi studi riguardano la possibilità di ridurre il tempo che le scrofe gravide trascorrono nelle gabbie gestazione e potrebbero fornire ulteriori dati utili per capire come rispondere al meglio alla richiesta dei cittadini di porre fine all' “Era delle Gabbie” e per indirizzare gli allevatori nella miglior gestione di questo importante cambiamento.

CHAPTER 1

General introduction

1.1 EUROPEAN LEGISLATION ON THE WELFARE OF DRY AND PREGNANT SOWS

European legislation regarding dry and gestating sows has changed little over the past few decades. Indeed, although 91/630/EEC and 2001/88/EC established important improvements for the welfare of pregnant sows, no further directives have been introduced that would change the way gestating sows are managed or housed. Council Directive 91/630/EC prohibited the use of tethers for sows and gilts, Council Directive 2001/88/EC banned the use of stall housing from 4 weeks after the service to 1 week before the farrowing, established that *sows and gilts shall have permanent access to manipulable material* and *must be given a sufficient quantity of bulky or high-fibre and high-energy food* , sanctioned the minimum floor area available to each gilt and sow when they are kept in groups and stipulated the requirements for flooring surfaces. Finally, the Directive now in force (2008/120/EC) made no changes to what was previously regulated in the 2001/88/EC with regard to the housing and management of sows. However, a new breakthrough in this legislative deadlock seems very imminent. In fact, in June 2021, the European Commission (European Commission, 2021) announced that a new legislative proposal will be presented by the end of 2023, with the aim to phase out and finally prohibit the use of cages from 2027 for all the animals species and categories referred to the initiative, including sow. This Commission Communication came in response to the European Citizens 'Initiative (ECI) "End the Cage Age" (2018), which called on the European Commission to end cage housing system for farm animals. Within a year, the organisers of the petition, with the support of more than 170 non-governmental organisations across Europe, collected 1.4 million signatures, making it the first ECI calling for action on the welfare of farmed animals to have reached the required numerical threshold of 1 million signatures. The huge success of this initiative demonstrates the strong and growing attention of consumers towards animal welfare and the desire for a transition towards more ethical and sustainable farming systems.

Some EU Member States have already introduced a total or partial crates ban. For example, since 1988 in Sweden sow stalls have been completely banned, since 2013 the Netherlands restricted the use of individual stalls to 4 days after insemination and in Austria to 10 days after insemination. In 2020 Germany passed a regulation introducing a ban on sow stalls, but it will become mandatory from 2030. In Denmark, sow stalls have been banned in new buildings since 2015, with the possibility of stalling sows for a maximum of 3 days during oestrus, and will be completely banned from the time of weaning with full effect from 2035 (European Commission, 2021).

Concerns about the welfare of sows in individual stalls and crates had already been raised in the 2007 EFSA Report ([EFSA] European Food Safety Authority, 2007), in which it was reported that crating sows for four weeks from the time of weaning and during farrowing/lactation severely restricts sow freedom of movement, their opportunity to interact socially and therefore causes stress and frustration.

Against this background, changes in the EU animal welfare legislation are likely to lead to increased use of group-housing systems for sows after weaning and in early pregnancy and of loose farrowing systems. In view of the above, it is important that the scientific community provides indications on how best apply the future provisions and on how reduce the risks associated with group-housing and loose farrowing systems. The present thesis has addressed the issue of group-housing systems for gestating sows.

1.2 BEHAVIOURAL BACKGROUND

1.2.1 SOWS IN NATURAL AND SEMI-NATURAL CONDITIONS. Considering that the behaviour pattern of the "modern commercial sow" is still very similar to that of the wild swine (Jensen et al., 1987;

Jensen, 1986), studying the behaviour of feral sows in a natural setting can provide useful information about their housing and behavioural needs (D'Eath and Turner, 2009).

1.2.2 SOCIAL BHEAVIOUR AND SOCIAL STRUCTURE. Pigs are gregarious animals, that in nature form stable group of between two and four sows with their piglets (Graves, 1984; Mauget, 1981). These animals live in a close association, their use of time and space is synchronised and appear to have strong inter-individual bonds (Mauget, 1981). Their home range can be large 6000 hectares (Janeau and Spitz, 1984), and although it may be overlapped with different herds, the groups tend to actively avoid open confrontation with each other (Gabor et al., 1999). Family group do not disperse until the litters are mature (7-8 month old) or the sows are ready to farrow again (Mauget, 1981). The sub-adult females may form sub-groups within the herd or separate off to form a new one. Sub-adult boars also form sub-groups, eventually becoming solitary by 1 or 2 year of age (Marchant-forde, 2009). Female become solitary when they are ready to give birth, and afterwards reform into small groups at about 12 days post-partum (Jensen and Redbo, 1987).

1.2.3 AGONISTIC BHEAVIOUR**.** Between herd members aggressive behaviour is absent or rare (Marchant-forde, 2009), thanks to a stable simple linear social hierarchy, mostly determined by age and size (Mauget, 1981). In established groups, aggression is regulated by an “avoidance order”, whereby the display of certain behaviours can limit the attacks made by dominant individuals (Jensen, 1982). However, agonistic interactions occur between pigs which are unfamiliar to each other (Arey and Edwards, 1998). Aggression is based on offensive moves with head movements aimed at pushing up or down the head or body of the opponent, trying to bite or lift its opponent (Mcglone, 1985). Pigs try to minimise the number of bites they receive by adopting parallel or inverted parallel postures (Jensen, 1980). The submissive sow attempts to escape, initially moving her head away from the body of the dominant, and then quickly backing away (Houpt et al., 2000).

In addition to escape attempts, submissive sows display a series of surrender signals, leading to the end of the fight: head tilt and 180° body rotation (Jensen, 1982; Mcglone, 1985).

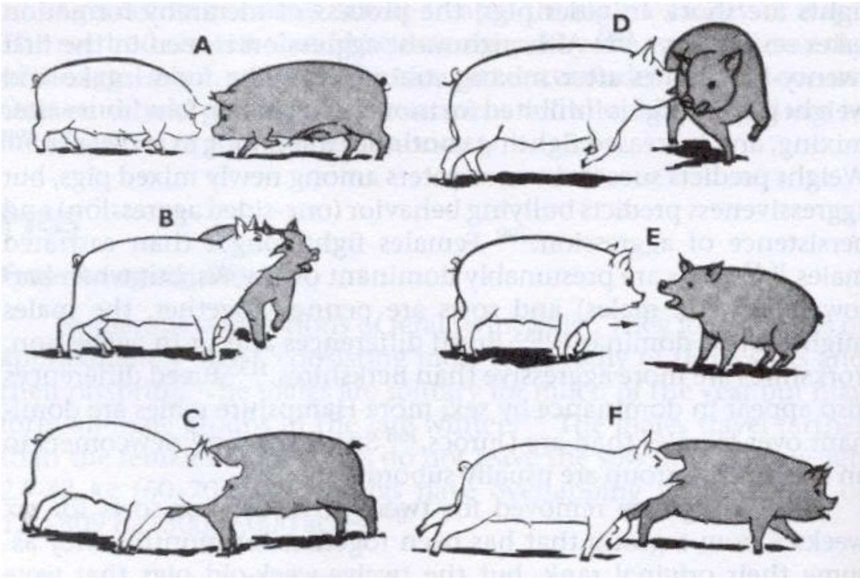

Figure 1. Fighting behaviours (Houpt et al., 2000): A. Scratch the ground at the beginning of the fight. B. They strut their stuff. C. Shoulder-shoulder contact and attempts to injure the opponent. D. Attack with perpendicular biting. E. Submission of the pig to the right. F. Pursuit of the defeated subject.

1.2.4 ACTIVITY PATTERN. Sows have a diurnal activity pattern. In semi-natural environments, sows are engaged in foraging-related activities, including rooting, grazing and exploring substrates with their snout for 75% of their daytime activity (Stolba and Wood-Gush, 1984). Members of a group forage together for the most part of the time, with an average distance of nearly 4m from their nearest neighbour (Marchant-forde, 2009; Stolba and Wood-Gush, 1984). During the resting period, they spend most of their inactive daylights in characteristic day beds, formed with grass, twigs and other bedding material (Graves, 1984).

1.2.5 BREEDING BEHAVIOUR. There is very limited information available in respect of sexual behaviour in female pigs in natural or semi-natural conditions. However a marked increase in social activities around oestrus seem part of the natural oestrus behaviour in feral sows (EFSA, 2007; Babu et al., 2004). These activities include sniffing, flank nosing and snout contacts (Pedersen, 2007). When females exhibit sexual receptivity, adult males associate with them and their herd (Graves, 1984).

1.3 WELFARE IMPLICATION OF PREGNANT SOWS IN INDOOR SYSTEM

Welfare problems are generally the result of a mismatch between the behavioural and housing needs of pigs and the farming environment and management (D'Eath and Turner, 2009).

1.3.1 CONFINEMENT IN INDIVIDUAL STALLS. Implications for sows' welfare and health due to individual housing in stalls mainly relate to the restricted ability to move and socialise (EFSA, 2007; EFSA AHAW Panel, 2022). Indeed, in individual gestation stalls, the sow is not able to turn around, to lie comfortably or to perform unhindered getting up and down movements (Marchant and Broom, 1996; Taylor and Friend, 1988). This may lead to variation from the normal standing up and lying down sequence (Mumm et al., 2020), prolonged ventral lying (Marchant, 1994), standing or dog-sitting. Such restriction may also disrupt the quantity and quality of sleep (EFSA AHAW Panel, 2022). Space confinement while lying in gestation stalls are exacerbated by the progressive increase in body size due to age (O'Connell et al., 2007; Mcglone et al., 2004; Nielsen et al., 2018) and genetic selection (Moustsen et al., 2011). Besides, the lack of exercise reduces cardiovascular fitness (Marchant et al., 1997) and bone and muscle strength (Marchant and Broom, 1996b). Inactivity due to restriction, combined with difficulties in manoeuvring in stalls, also increases the incidence of callosities and limb injuries (Calderòn Diaz et al., 2014; Leeb et al., 2001). Moreover, sows housed in this kind of system have limited possibility to social interact with conspecifics, causing stress and frustration, especially during the pre-oestrus and oestrus periods when they are highly motivated to do so (Pedersen, 2007; Pedersen et al., 1993).

1.3.2 GROUP STRESS. In natural condition aggressive behaviour is absent or rare thanks to a stable simple linear social hierarchy that is maintained among herd members. However, in indoor systems sows are mixed into groups with unfamiliar conspecifics at least once for each gestation, resulting in high level of aggression associated with the formation of a dominance hierarchy (Verdon and Rault, 2018). Rank order fights can be severe, but generally do not last more than 1 – 2 days following the mixing of unfamiliar animals (e.g. Arey and Edwards, 1998; Spoolder et al., 1997). Aggression once a hierarchy has been established typically occurs over competition for access to limited resources, as food (D'Eath et al., 2018), drinker (Chapinal et al., 2010), foraging material (Bench et al., 2013) or preferred lying area (Strawford et al., 2008). The nature of this aggression is generally short in duration, but can occur frequently (Spoolder et al., 2009). In comparison to aggression at mixing, this aggression is more likely to include single aggressive bouts (e.g., knock, bites) and little to no fighting (Karlen et al., 2007), with a greater use of non-physical aggression, such as displacement and threats (Randolph et al., 1981). Scratches or cuts on the skin are the most common lesions caused by aggression (Spoolder et al., 2009). Aggression can also lead to leg problems, especially if the design of the pen is such that animals cannot easily avoid an incident (due to lack of space) or if the floor does not offer sufficient support to the claws during an interaction (too slippery or poor quality slats) (Spoolder et al., 2009). Finally, aggression inevitably causes physiological and psychological stress in the animal (Spoolder et al., 2009). Besides the welfare consequences related to the establishment of the dominance hierarchy at mixing and to competition for resources, the risks for welfare consequences in group systems are greater after weaning, as it is increased by the mounting behaviour exhibited during oestrus, which can cause inability to avoid unwanted sexual behaviour particularly for low-ranking animals (Pedersen, 2007; Pedersen et al., 1993), bone lesions (Hartnett et al., 2019; Rydhmer et al., 2006) and locomotory disorders (Di Muzio et al., 2023; Rydhmer et al., 2004), especially for those weaned sows that are physically compromised by lactation (Giesemann et al., 1998; van Riet et al., 2016).

1.3.3 ABSENCE OF EXPLORATORY OR FORAGING OPPORTUNITIES. Exploratory behaviour is an intrinsic need of pigs which evolved in their wild counterparts (Studnitz et al., 2007). The inability to perform this natural behaviour within a commercial setting due to the absence of suitable materials at which it could be directed results in frustration and/or boredom, and is linked to the development of abnormal behaviour, as aggression, stereotypies and restlessness (Fraser, 1975; Rushen, 1985; Spoolder et al., 1995; Terlouw et al., 1991; Whittaker et al., 1998). The consequence on pregnant sow welfare of lacking access to a suitable substrate for foraging and exploration are considered to be an high relevance due to the fact that they are restrictively fed with concentrate diet, which is low in bulk and consumed in short time. Thus feeding motivation remain high and sows do not feel behaviourally satisfied (D'Eath et al., 2009; Lawrence and Terlouw, 1993). Therefore pregnant sows motivation to explore are predominantly appetitive foraging, and materials that contain edible parts are probably the most appropriate to satisfy their motivation to explore (EFSA, 2007).

1.4 FACTORS AFFECTING SOW WELFARE IN GROUP HOUSING SYSTEMS

Elucidating the factors affecting sow welfare in group housing system is complicated as many aspects are often inextricably linked to each other (Edwards, 2000). However the main factors that have been shown to impact the welfare of group housed sow are explored in this chapter.

1.4.1 SPACE ALLOWANCE. Limited space allowance in group housed sows can increases the adverse consequences of aggressive behaviours (EFSA, 2007). For instance, in pens with insufficient space sows have reduced opportunities to avoid aggression, e.g. by showing submissive behaviour or fleeing from the attacker (Spoolder et al., 2009). Thus, time and level of agonistic interactions increase and the establishment of the social hierarchy is prolonged (Barnett et al., 1992; Marchant-

Forde et al., 2011; Rault, 2017). Moreover, not being able to perform a full behavioural repertoire is a source of frustration and stress for animals (Martínez-miró et al., 2016), which can leads to elevated cortisol levels (Grimberg-henrici et al., 2018). The need for a large flight distance can be reduced by including solid visual barriers that facilitate active avoidance by subordinate sows from more dominant animals (Marchant-Forde and R. M., 2005). Insufficient space allowance in group housed sows can also increase skin lesions, caused by contact with pen fittings or flooring, or non-agonistic interactions between individuals stepping on one another (Harris et al., 2006).

1.4.2 FLOOR TYPE. The flooring should allow sows to move easily without causing leg injuries and to rest comfortably (EFSA, 2022). To prevent claw and leg injuries floor should provide good foothold, should not be slippery, abrasive or hard (Webb and Nilsson, 1983). Concrete slatted floors have been identified as a high-risk factor for leg disorders (Cador et al., 2014; Calderón Díaz et al., 2013), especially where the slat width is too narrow, exerting high pressure on sole points, the gap width is too wide, causing trapping and twisting of the claw or tearing of the dew claw, or the slats have sharp edges (EFSA, 2022). In addition, fully slatted concrete floors are associated with discomfort lying (Calderón Díaz et al., 2013) and increasing risk for shoulder lesions (Schubbert et al., 2020). Use systems with well-maintained solid or partially slatted floor could therefore be advantageous regarding leg injuries and comfort resting, especially when it is covered with sufficient bedding material like straw (Spoolder et al., 2009). In housing system which are not suitable for the use of straw bedding, the provision of rubber mats can be considered as an alternative to offer a comfortable lying area for gestating sows (Calderón Díaz et al., 2013; Elmore et al., 2010; van Riet et al., 2016). However there is disagreement on the question of whether mats reduce the risk of leg lesions, as pens covered with rubber mats can become slippery, due to insufficient slurry drainage (Calderón Díaz et al., 2013; Huneau-salaün et al., 2021).

1.4.3 GROUP SIZE AND TYPE. The typical social unit for feral pigs is a basic and relatively stable group of 2 to 4 related sows and their offspring (Gonyou, 2001). Therefore, keeping sows in small stable groups means to guaranteeing them a condition closer to their natural state. Stable groups prevent that the addition of new individuals continuously disrupts the dominance hierarchy, resulting in an increased intensity of fighting to establish the rank order (Chapinal et al., 2008; Simmins, 1993). The effects of group size in commercial setting on sow welfare are instead controversial. Indeed, there are benefits associated with large groups and consequently large amount of shared pace. In such systems there is more opportunities for subordinate sows to avoid the aggressive encounters by hiding behind others or escaping into the group (Spoolder et al., 2009) and more space to exercise (Durrell et al., 2002).

1.4.4 FEEDING SYSTEM. Competition for food or access to food is particularly important for gestating sows, which are usually on a restrictive diet (D'Eath et al., 2018). The type of feeding system, and in particular the level of protection provided to the feeding animal, influences the level of aggression related to competition for food (Spoolder et al., 2009). Feeding systems that do not provide protection during feeding, such as floor or troughs feeding, are obviously the most competitive, and can thus exacerbate and prolong aggression within a group (Andersen et al., 1999). Feeding systems with protection ensure the access of all individuals to their allocated amount of feed, but they can also have certain negative aspects. Electronic sow feeders (ESF) provide for the greatest control over individual sow intake (Verdon and Rault, 2018), but forces sows to feed in sequence, potentially increasing the level of aggression that occurs as sows queue at the entrance of the ESF (Bench et al., 2013; Verdon et al., 2015). Individual feeding stalls allow sows to eat simultaneously, avoiding aggression associated with queuing, but if the length of the barriers do not protect the full body and feeding stalls are not lockable, getting access to the feeding stalls can lead to competition and aggression (Bench et al., 2013) and sows that finish their ration first may try to displace other sows out of their stalls, increasing the number of vulva injuries (Andersen et al., 1999).

1.4.5 ENVIROMENTAL ENRICHMENT. Council Directive 2008/120/EC establishes that "*pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities"*. Enrichment material should enable pigs to fulfil their essential needs and for this reason they should be edible, chewable, investigable and manipulable (EU/2016/336). In addition they should be regularly replaced and replenished, accessible for oral manipulation, given in sufficient quantity, clean and hygienic (EU/2016/336). Providing pigs with appropriate enrichment allows for cognitive stimulation and, depending on the type of substrate, has the potential to reduce stereotypic (Hoorweg et al., 2022) and aggressive behaviour (Beattie et al., 2001; Durrell et al., 1997; Vanheukelom et al., 2011).

1.5 REPRODUCTIVE PHYSIOLOGY

Directive 2008/120/EC currently in force finds in the reproductive physiology of the sow the reasons for allowing individual cage housing up to 28 days after insemination. The sensitivity to stress in the early gestational phases is in fact the critical point that has long called into question the group-housing of sows from the early stages of pregnancy. In particular, maternal recognition of pregnancy and implantation are commonly recognised as the moments of greatest risk of embryo loss due to the complexity of factors involved in these phases.

Maternal recognition of pregnancy (between days 10 and 13 post-estrus) and implantation (days 14-19) are key events in the establishment and development of early pregnancy (Waclawik et al., 2017). Pregnancy recognition is the process that includes the set of signals that are expressed by the conceptus to support corpus luteum function. In pigs these signals are modulated by a biphasic production and release of estrogen into the uterine lumen by porcine conceptus. The first increase in conceptus estrogen occurs during the period of elongation on days 11 to 13 of pregnancy, while the second occurs during the period of uterine attachment and placental development from day 17 through 25 (Meyer et al., 2019).

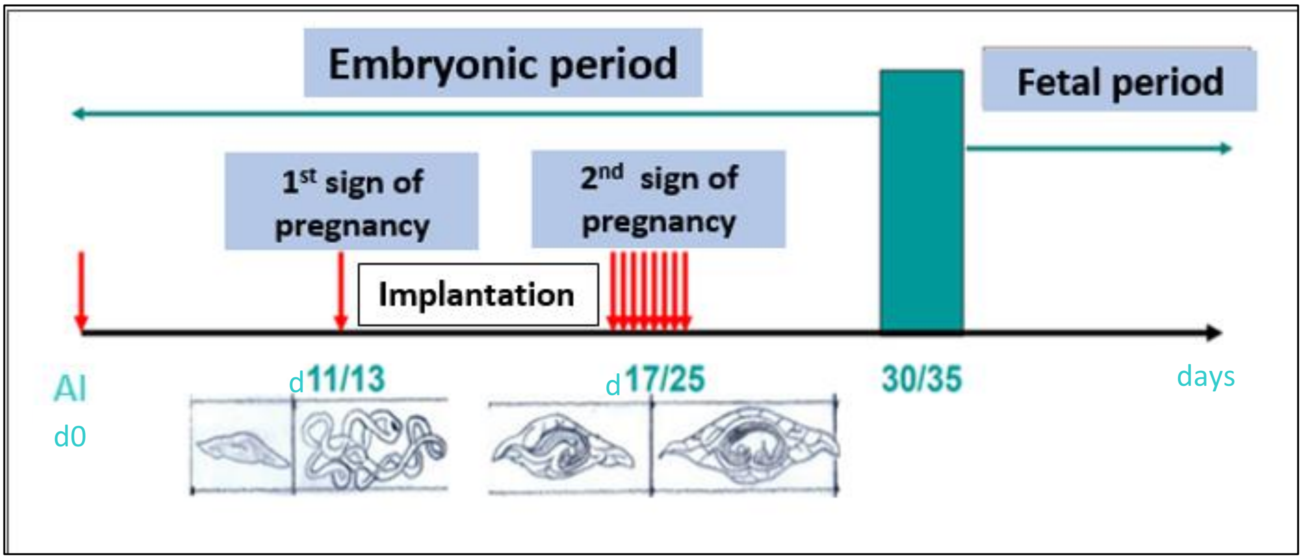

Figure 2. Important events during early pregnancy in pigs (adapted from Martineau, 2003)

Conceptus estrogens increase stimulation of luteal progesterone secretion, increase luteal Luteinizing hormone (LH) receptor concentration and redirect luteolytic Prostaglandin F2α (PGF2α) secretion from the uterus venous drainage to the uterine lumen, thus inhibiting the luteolytic effect of PGF2α (Bazer and Thatcher, 1977). Maternal recognition of pregnancy is thus the mechanism that allows the maintenance of the corpus luteum, the steroidogenic structure that sustains progesterone synthesis throughout pregnancy.

During the peri-implantation period, the action of progesterone is required for developing uterine receptivity to embryo implantation (Waclawik et al., 2017). Progesterone stimulates the uterine luminal and glandular epithelium to produce and secrete pro-inflammatory factors (citokines and growth factors), nutrients, transport proteins, enzymes and other substances that together support conceptus development, implantation, and placentation. Progesterone also downregulates the expression of progesterone receptors (PGR) in the uterine luminal epithelium (LE), with a consequent downregulation in the expression of Mucin-1 (MUC1), a component of the apical surface of the uterine LE that physically inhibits attachment of the conceptus trophectoderm to the uterine LE. Reduction of MUC1 allows a formation of a stable adhesion between integrins present on the coceptus trophectoderm and uterine epithelium. The development of interditigal microvilli between conceptus' trophectoderm and uterine epithelium ensure nutritional exchange for fetal-placental development throughout pregnancy. Between days 15 and 20 of pregnancy, the interface between the conceptus trophectoderm and uterine LE cells becomes increasingly complex as it undergoes a functional from histotrophic to hemotrophic nutrient transport. Finally, adhesion progresses into placentation by progressively developing of interdigitating microvilli between the conceptus trophectoderm and uterine LE cells that extends into the peripheral zone by day 26 of gestation (reviewed by Johnson et al., 2021). The pig has a epitheliochorial placenta in which both the uterine LE and the chorionic epithelium remain intact throughout gestation (reviewed by Geisert et al., 2015).

Implantation, with the associated maternal recognition of pregnancy, is a period that relies on a balance of hormones and factors that make it particularly sensitive to alterations (Spoolder et al., 2009). During these weeks it is therefore essential to avoid stress that may compromise embryo implantation and, consequently, sows reproductive efficiency.

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CHAPTER 2

The changing face and associated drivers

of research on welfare of the gestating sow

The changing face and associated drivers of research on welfare of the gestating sow

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ABSTRACT

The housing and management of commercial breeding sows is of crucial importance for their productivity and welfare. The aim of the present study is to evaluate how the scientific community addressed the subject of pregnant sow welfare, how it has changed over the past 30 years and what were the drivers of this change. A search of the literature in Scopus identified 318 articles, which were screened for inclusion criteria. Over one hundred of these publications (n=102) were deemed relevant for the systematic review. Globally, the number of papers on sow welfare during pregnancy increased, but in the last 5 years the trend changed both in terms of publishing country, the interventions studied and the welfare outcomes employed. Up to 2014, published papers about gestating sow welfare came from Europe and North America, with housing system as the most studied topic, followed by nutrition and feeding, and behaviour as the most common welfare outcome. In the last five years, publications from Europe and North America decreased, while publications from Oceania, America Latina and Asia started to appear. Papers on the management of sows in groups and to a lesser extent, environmental enrichment, increased in number. In addition, while prior to 2009 behaviour was the most common welfare outcome, a more diverse range of welfare outcomes were employed in papers in the last 10 years. In order to support new legislation and to respond to consumer requests, future studies should focus on improving sow comfort during gestation.

INTRODUCTION

Clearly, optimal sow reproductive performance is crucial to the efficiency and financial profitability of the pig industry. Good welfare during gestation is an important driver of optimal sow performance as stress may impair reproduction by interfering with the endocrine events which in turn induce oestrus, ovulation, and early pregnancy (Turner et al. 2005; Einarsson et al. 2008; Lagoda et al. 2021a, 2021b). It is also a crucial aspect from an ethical point of view, considering that gestation is the longest phase in the sow's productive life, and for a marketing aspect, given consumers' growing interest towards animal welfare (EC 2016).

However, ensuring an adequate state of welfare is a complex issue. Sow welfare may be influenced by many different factors, such as housing systems, group management, feeding plan, but also by animal characteristics (genetics, parity as well as by human–animal interactions) (Verdon et al. 2015). Moreover, not only is there no established method to assess animal welfare, but it may differ according to both the different concepts of animal welfare and the purpose of the research (Sejian et al. 2011). Indeed, animal welfare is a multidimensional concept involving three main conceptual frameworks, (biological function, affective state and natural living) (Fraser 2003). In measuring animal welfare then, methodologies draw on indicators from multiples disciplines that include animal behaviour science, stress physiology, animal science, veterinary science, psychology, immunology and neurophysiology (Hemsworth et al. 2015).

In the light of the above-mentioned considerations, it is important that research aims to ensure and improve welfare of gestating sow, in order to support new legislation, to respond to consumers' request and to be in step with the progress and changes achieved by genetic breeding programs. For example, modern hyper-prolific crossbred sows have become heavier and longer than their ancestors in 1994 (Moustsen et al. 2011) and this aspect must be taken into consideration in the design of stalls and pens.

European pig welfare legislation covers all phases of production but several aspects require updating, especially regarding management of the sow. The latest Scientific Opinion of European Food Safety Authority concerning the welfare of sow (EFSA 2007) was last updated in 2007. As regards pregnant sows, the report underlines the need for further research on the welfare and health of group-housed sows, especially from weaning to 4 weeks after mating. Indeed, housing sows in individual stalls during this vulnerable period ensures that stressors associated with mixing do not adversely affect embryo implantation and survival (Spoolder et al. 2009), but severely restricts their freedom to move and socialise. The report also stresses that in order to make more precise recommendations, there is a need for more knowledge on how sow claw health is affected by different flooring conditions, on bulky content of pregnant sows feed, on access to rooting materials and also on slurry systems that can handle straw or other organic materials. Despite these recommendations, Europe has not made any legislative intervention on sow housing and husbandry since 2001. This is likely to change in coming years in line with the requirements of the European Union's new Farm to Fork (F2F) strategy where animal welfare has an integral role (EC 2020). Indeed, the European Food Safety Authority (EFSA) are currently undertaking a comprehensive evaluation of the EU's animal welfare legislation (EFSA 2020). This is further driven by the successful outcome of the European Citizens' Initiative (End the Cage Age 2018) calling for an end to the use of cages for farmed animals.

Therefore, the first aim of the present study is to evaluate if the scientific community has addressed the subject of pregnant sow welfare and how it has changed since the past 30 years. We will then interpret such changes in the light of associated socio- logical, legislative, scientific and practical drivers. Informed by these findings, we will conclude with suggestions for an appropriate direction that future research should take on the welfare of gestating sows.

METHODS

Protocol

Guidelines for conducting a systematic review were obtained from McMullen et al. (2020). This protocol, reported in accordance with PRISMA-ScR guidelines (Tricco et al. 2018), was published at the University of Guelph's institutional repository (https://atrium.lib.uoguelph.ca/xmlui/handle/10214/17788), and regis- tered online with Systematic Reviews for Animals and Food (SYREAF) available at: http://www.syreaf.org.

Search strategy

The literature search was conducted on 4th of September 2020 in Scopus database, using a string designed to obtain any articles published in the past 30 years that provided data on gestating sow (or gilt) welfare. The search strategy was developed using key concept terms and words, connected using Boolean operators 'AND', 'OR'. In the study, the search string used was:

TITLE-ABS-KEY (((sow OR gilt) AND (gestati* OR pregnan*) AND (welfare OR well-being))) AND DOCTYPE (ar) AND PUBYEAR > 1989 AND PUBYEAR < 2020

Study Selection

All the articles collected from the online search were exported to Excel (2013) and were scanned to remove those that were included because of keywords that were different to those provided by the author. Indeed, KEY code is a combined field that searches in Author Keywords, but also in Index Terms, Trade Name and Chemical Name. We then manual screened all the publications to remove articles that did not include the chosen search terms in the Title, Abstract or Author Keywords. Thereafter, we removed duplicates from the reference list.

The remaining titles and abstracts were assessed for relevance using the following primary screening questions by two independent reviewers:

1. 'Is the title and/or abstract available in English?'
2. 'Does the title and/or abstract assess a gestation intervention in sows and/or gilts?'
3. 'Does the title and/or abstract describe a relevant welfare outcome?'
4. 'Does the title and/or abstract describe an analytic primary research study?'

All questions included a response for YES, NO, and UNCLEAR. References were only excluded if both reviewers responded 'NO' to any of the questions. Disagreements were resolved by consensus with mediation by a third member of the review team if an agreement could not be reached.

The full-text articles of the citations deemed eligible by the previous stage of screening were assessed using the same four questions and the same eligibility criteria.

Study characteristics

Study characteristics were extracted for all studies included after full-text screening. They included year of publication, country of the first author, journal title, number of citations, study population (sow, gilt or both) and inclusion criteria (interventions and out- comes, see below).

Eligible interventions

The eligible interventions employed in the gestation period were classified as follows:

- **Housing** interventions: included **housing system** (individual stall, group housing or tether, indoor or outdoor); **stall size**; **floor space allowance**; **floor characteristics**.
- **Group management** interventions: group **size**; **static or dynamic** group; group **strategy** (parity composition, familiarisation, use of tranquilizers); **stage of reproductive cycle at mixing**.

- **Nutrition** and **feeding** interventions: feeding system and frequency; level of dietary fibre; feed supplements and additives
- Provision of **environmental enrichment**/foraging material

Eligible outcomes

Outcomes eligible for inclusion in the review were the indicators for the assessment of animal welfare, categorised as:

- **Behavioural** indicators: observation of sow behaviour, including stereotypies, posture, aggressive behaviour, locomotory/activity and others
- **Physical** indicators: body lesions and locomotory problems
- **Physiological** indicators: cortisol level, immune traits, heart rate, antioxidant status
- **Reproductive** indicators: wean-to-oestrus interval, conception rate, farrowing rate, litter size, piglet birth weight and others
- **Growth/productive** indicators: body condition score, body weight, backfat thickness and others

RESULTS AND DISCUSSION

Study selection

Results of the search and flow of studies through the screening process are presented in Figure 1. The data- base search identified 318 articles. After the first scan through the 'Key' process, there were 260 articles. Of these publications, seven were removed as duplicates. The remaining 253 articles were screened by title and abstract, and 113 were deemed eligible. Of these publications, 102 were deemed as relevant when the inclusion and exclusion criteria were applied to the full-text.

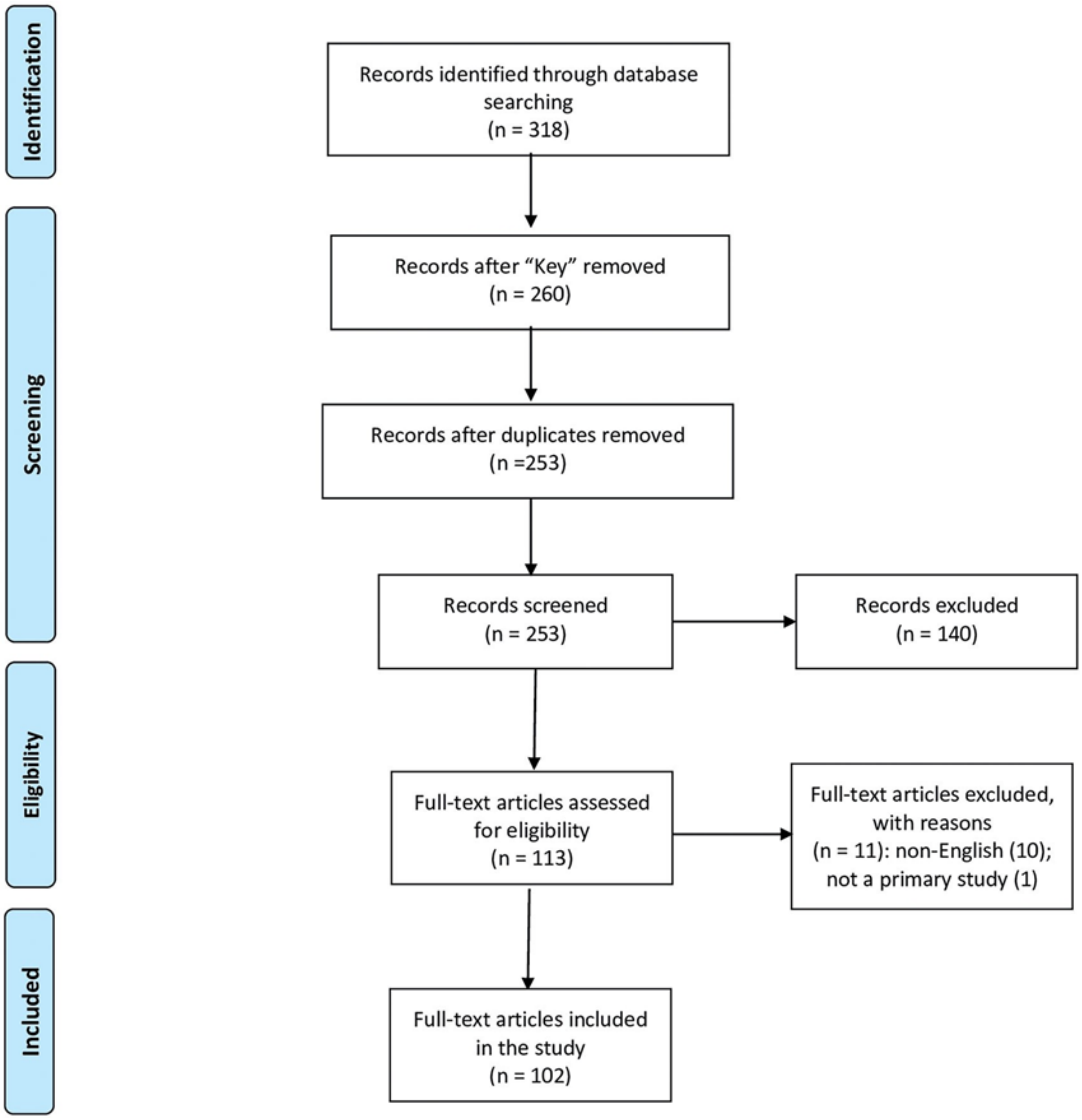

Figure 1. PRISMA study flow diagram (Moher et al. 2009) documenting the process of identification, screening, eligibility and inclusion for the systematic review of welfare of gestating sow.

Study characteristics

Studies on the welfare of gestating sows were assigned to 21 countries according to affiliation of the first author, most frequently in Europe (42%) and North America (40%). In Europe, the top four publishing countries are United Kingdom, Belgium, France and Ireland (n=8; n=7; n=7; n=7, respectively).

Figure 2 shows the distribution of the 102 papers according to the publication year (divided into 6 5- year intervals) and subdivided based on the first author's country.

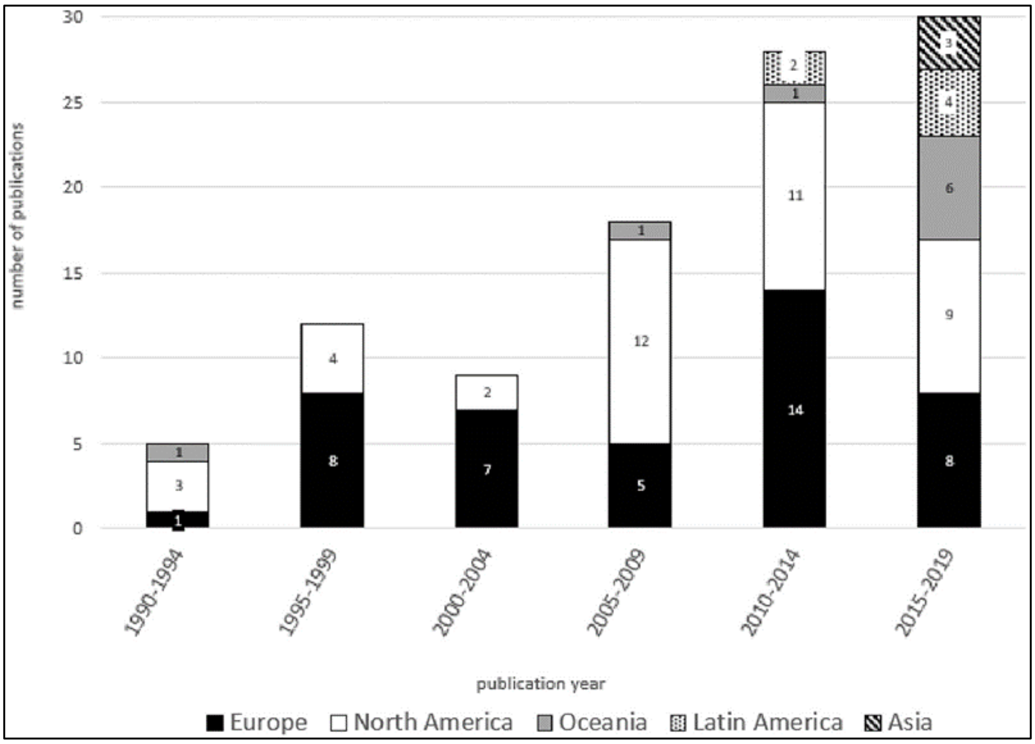

Figure 2. Number of papers of welfare of gestating sow distributed according to the publication year in the different continents.

Looking globally, in the last 30 years the number of papers about the welfare of gestating sows increased, except during the period 2000–2004 in which there was a slight reduction. From 1990 to 2014, Europe and North America were substantially the two continents that published papers about the welfare of the gestating sow. In the last five years, Oceania, America Latina and Asia also started publishing articles on this topic and even though they have major pig industries they have minimal or no legislation protecting pig welfare.

China is the world's largest pork producer (USDA 2020), but it has not yet enacted animal welfare legislation, and the reason may be in part due to the lack of animal welfare literature coming from the country (Sinclair et al. 2020). In this review, the lack of scientific research is evident: of the three articles from Asia, only one is Chinese. However, a recent review that searched for literature on animal welfare in a Chinese database (Sinclair et al. 2020) identified 164 articles on pregnant sow welfare. This could be an opportunity to increase knowledge transfer by making key Chinese animal welfare papers available in English and vice versa, and so establishing closer collaboration with Chinese partners and ultimately improving animal welfare globally. Brazil is the only Latin American country to produce scientific literature on the welfare of gestating sows and it is also the largest pork producer in Latin America. It ranked number 3 in the world pork producers and, since 2014, grew 1.2% year on year (OECD 2019). Its growth in production appears to be accompanied by a growth in publishing, but not in law-making. Indeed, there are not any regulations with regards to the rearing of farm animals (API 2020a). However, even if sow stalls are not prohibited by legislation, Brazil's largest pork producers and processors recently announced that they are phasing out gestation crates (Cardoso et al. 2017). Australia represents publishing on sow welfare during gestation in Oceania. Australian pork production increased by 2.2% year on year since 2014 (OECD 2019) and this was matched by substantial growth in publishing in the last five years. Nevertheless, there are no national laws relating to farm animal welfare. Commonwealth Government developed a series of National Model Codes of Practice for the Welfare of Livestock, but it serves as a voluntary guide and is not legally binding (API 2020b).

Europe and North America addressed the topic of dry sow welfare at different times. Europe began to publish a greater number of papers from the second half of the 1990s, probably influenced by the enactment of European directives in those years. However, though 91/630/EC and 2001/88/EC established import- ant improvements for the welfare of pregnant sows, namely the prohibition of stall housing from day 28 of pregnancy, no further directives were produced that would change the way in which gestating sows were managed or housed. However, this deadlock at the legislative level was not accompanied by a deadlock in scientific research. Rather there was a peak in publications in the 2010–2014 interval. This could be due to the prolonged time that directives allowed for the application of the changes introduced. The most important provisions relating to group housing only became mandatory in 2013 and in the preceding years, scientific research sought the best solution for this change. Some European countries approved more ambitious legislation, exceeding the baseline set at EU level: Sweden (1994), United Kingdom (1999), banned sow stalls entirely, since 2013 the Netherlands restricted individual stall to four days after insemination and in 2020 Germany passed a regulation introducing a ban on sow stalls, but it will become mandatory from 2030. However, except for United Kingdom, these countries are not the same top publishing countries, suggesting that the drivers for change probably had another origin.

North America, on the other hand, began publishing a greater number of papers starting from the 2005-2009 interval. It was precisely in those years that some American states enacted individual state provisions to regulate the living conditions of farm animals, including the ban of gestation crates (Centner 2010). However, recommendations for the minimal floor space allowance, for requirements of flooring surfaces, or as regards manipulable material do not exist in the United States.

The 102 papers selected were published in 33 Journals with the Journal of Animal Science (n=26) and Applied Animal Behaviour Science (n=20) being the most popular. These publications generated 1931 citations. Three articles collected more than 100 citations (Robert et al. 1993 with 115 citations; Broom et al. 1995 with 102 citations, Barnett et al. 1992 with 101 citations). These articles, although old, certainly represent points of reference for the literature on the welfare of the gestating sow. Sows represented the study population in 61 articles, gilts in 20 articles and both were represented in 21 articles.

Interventions

Table 1 shows the distribution of the selected publications according to the interventions studied. Housing was the subject of the majority of the selected publications (63%), followed by nutrition and feeding (34%), group management (24%) and environmental enrichment (8%). In a few papers, more than one topic was considered. A number of specific characteristics of each intervention were studied. Housing system was the most commonly studied aspect of housing (59%), and in particular, the comparison between individual stalls and groups (87%). This was followed by floor type (27%) and space allowance (23%). The two main aspects related to nutrition and feeding were feeding system and frequency of feed distribution (43%) and the level of dietary fibre (40%). Group size (42%) and whether static or dynamic (42%) comprised most of the group management studies. Eight publications investigated different objects and material available as environmental enrichment, such as straw, wood, rope, rubber sticks and mats, plastic discs and music.

Table 1. Number and percentage of publications of welfare of gestating sow per topic during the 30-year period.

TOPIC	NUMBER OF PAPERS	%
Housing	**64**	**63**
Housing system	*38*	*59*
Floor characteristics	*17*	*27*
Space allowance	*15*	*23*
Stall size	*6*	*9*
Nutrition and feeding	**35**	**34**
Feeding system and frequency	*15*	*43*
Level of dietary fiber	*14*	*40*
Feed supplement and additive	*3*	*9*
Others	*6*	*17*
Group management	**24**	**24**
Group size	*10*	*42*
Static/dynamic	*10*	*42*
Stage of reproductive cycle at mixing	*6*	*25*
Group strategy	*4*	*17*
Environmental enrichments	**8**	**8**

The total number of the selected papers is 102. Several papers include more than one topic, and the same topic can include more than one specific interventions. The percentages of the main topics have been calculated on the 102 papers, while the percentages of the specific interventions have been calculated on the number of papers of the main topic.

Figure 3 shows the distribution of the selected papers during the 30-year interval according to the research topic. Until 2014, sow housing was the most studied topic, followed by nutrition and feeding, but in the last five years (6th interval) the trend changed such that publications on group management and to a lesser extent, environmental enrichment, increased in number. Looking at these data it might seem that, as regards European legislation, scientific research is supportive not so much for the issuance of regulations, as for their best application. In fact, the European Union introduced the requirement to keep sows in groups and to provide them with manipulable materials since 2001/88/EC.

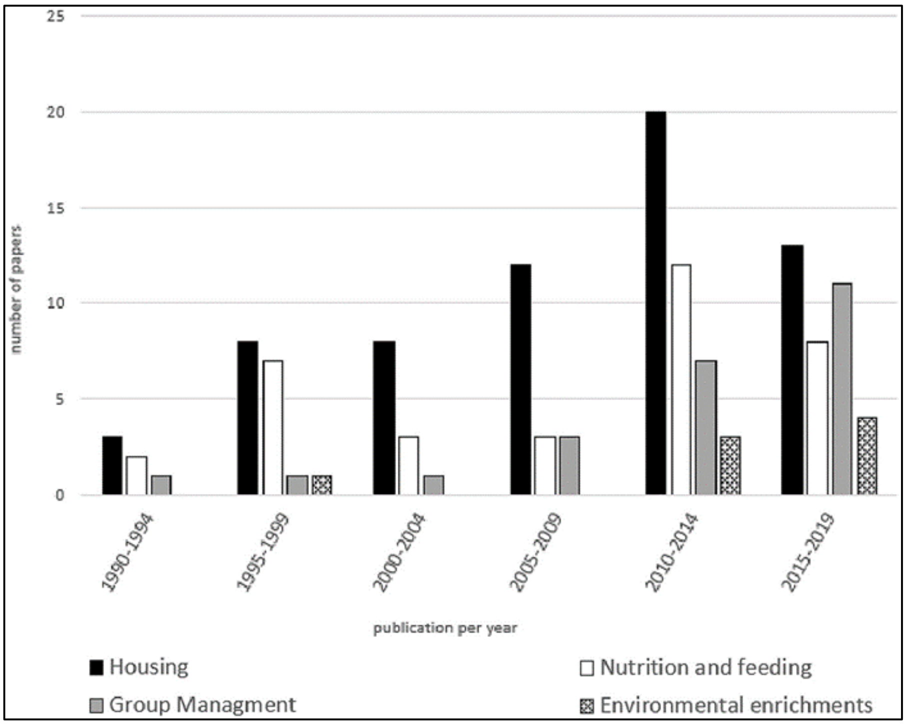

Figure 3. Number of papers of welfare of gestating sow distributed according to the publication year and classified according to the four topics considered.

However, scientists only became interested in these research topics at a later stage, starting from the 2010–2014 interval (Figure 4), when the provisions became mandatory to all holdings. This could be due to the fact that EU legislation gives only a few instructions and obligations on how apply it. As regards group management, legislation made precise provisions only regarding the period in which sows are in groups. Scientific research may have focussed on other management aspects, like group size and type (static or dynamic), in order to give indications on how best to implement the provisions. Also, as regards environmental enrichments, EU legislation provides some examples of manipulable material but it does not give precision indications on quantity, on how it must be provided nor does it distinguish between the different ethological needs for the different categories of pigs. This lack of information, combined with the need to find environmental enrichment suited to both the needs of pregnant sows and of farmers, could be what has prompted more research in this area in the last 10 years. Publications from North America concerning group management started to emerge from 2005 to 2009 (Figure 4(a)). This period coincided with the ban on gestation stalls in some US states, and therefore this seems consistent with the increase in scientific interest in managing sows in groups. However, most USA States do not place any limits on the use of individual stalls and private food companies seem to be the main driver. Since 2007, over 40 companies and several grocery store giants, including Smithfield Foods, United States' largest pig producer, McDonalds, Cargill, Hormel Foods, and the two largest foodservice companies in the world, announced plans to phase out the use of gestation crates (HSUS 2013). As regards the companies mentioned above, all seem (except for the Compass group, which does not disclose this information) to have actually undertaken to reduce or eliminate the use of gestation crates, albeit with different results. For example, Smithfield Foods fulfilled its commitment to provide group-housing systems for pregnant sows on all company-owned farms in the United States. McDonald's, rather, in the latest statement reports that 50% of the U.S. pork supply chain has phased out the use of gestation stalls for pregnant sows. Regarding instead Sodexo, the second largest foodservice company in the world, in the end of 2020, it states that only 24% of their pork came from trace- able reduced gestation stalls supply chains, and a small amount from completely crate-free operations. As regards environmental enrichment, North American researchers began to publish papers starting from the 2010 to 2014 interval, but this appears to be due to public concerns about farm animals and market forces and not to legislative pressure. Indeed, US swine farmers are not required by federal law to pro- vide rooting materials, but they may provide these materials for market driven reasons if they voluntarily adhere to certain animal welfare certification programs (Horback et al. 2016). Looking globally, it is also clear that the increase in publications on these two topics in the last 5 years is mainly due to the addition of papers from the other continents which were absent previously. In the last five years, both Brazil and Australia published two papers about group management and one paper about environmental enrichment. This recent scientific interest gives hope for a new sow welfare reform. This appears to be imminent in Australia compared to Latin America. Indeed, a recent nationally representative survey (Futureye Pty Ltd. Department of Agriculture and Water Resources 2018) shows that the vast majority (95%) of respondents consider farm animal welfare in Australia to be an issue to some degree and 91% require at least some reform to address it. This indicates that the Australian Government are out of step with public expectations, and it is being strongly encouraged to ban the extreme confinement of farm animals (API 2020b). In contrast, little is known about the view of citizens from developing countries (von Keyserlingk and Ho€tzel 2015). Brazilian citizens have a low level of awareness regarding livestock production system (Yunes et al. 2017). However, different studies show that increasing information tends to result in increased opposition to intensive livestock production practice and system (Bonamigo et al. 2012; Souza et al. 2013). It appears that citizens prefer systems that are not associated with behavioural restrictions (Yunes et al. 2017; Ho€tzel et al. 2017). Despite the different pressures from public opinions, several important Brazilian (Aurora, Brazil Foods and JBS S.A.) and Australian (Australian Pork) industries committed to voluntary phasing out sow stalls. In the absence of a national law banning gestation stalls, these corporate polices appear very important to meet consumers' expectations or to avoid the risk that certain housing and management practices may undermine the socially sustainability of the current farming system once the public becomes aware of them. As regards Asia's papers about these two topics, only Israel published one paper on sow group management in 2018. Although pig production in Israel is limited to nearly 38.000 sows (FAO 2019), since 2014, according to the Israeli Swine Legislation, the use of individual confinement stalls during gestation is not allowed. The lack of scientific research from Asia is evident, not only for these two topics but for the overall welfare of gestating sows. As mentioned above, the paucity of Chinese publications is probably due to the unavailability to the English-speaking global scientific community (Sinclair et al. 2020). Little is known about the other Asian countries, some of which, such as Vietnam and South Korea have large pig industries.

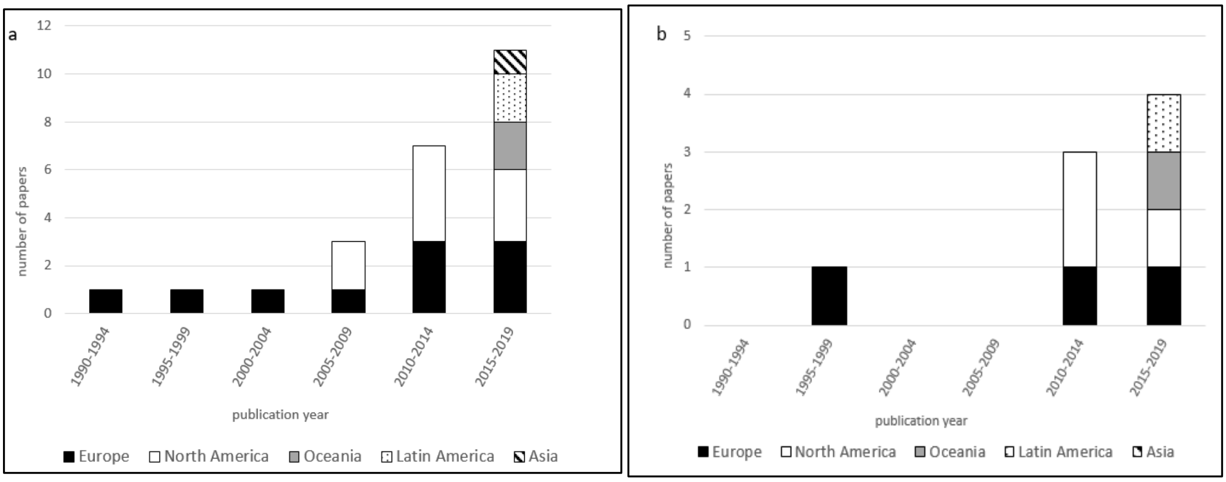

Figure 4. Number of group management (a) and environmental enrichments (b) papers distributed according to the publication year in the different continents.

Housing, nutrition and feeding papers decreased sharply in the last 5 years (Fig.3). Concerning housing interventions (Fig.5), it is mainly due to the limited number of articles comparing individual stalls and group housing (Fig.5a). From 2015 to 2019 there were no European publications on this topic, and only limited publications from North America. Floor space allowance papers followed the same trend, but in recent years publications from Australia contributed to an increase (Fig.5b). Additionally the number of papers on floor quality reduced sharply in the last 5 years, due to fewer European publications (Fig.5c).

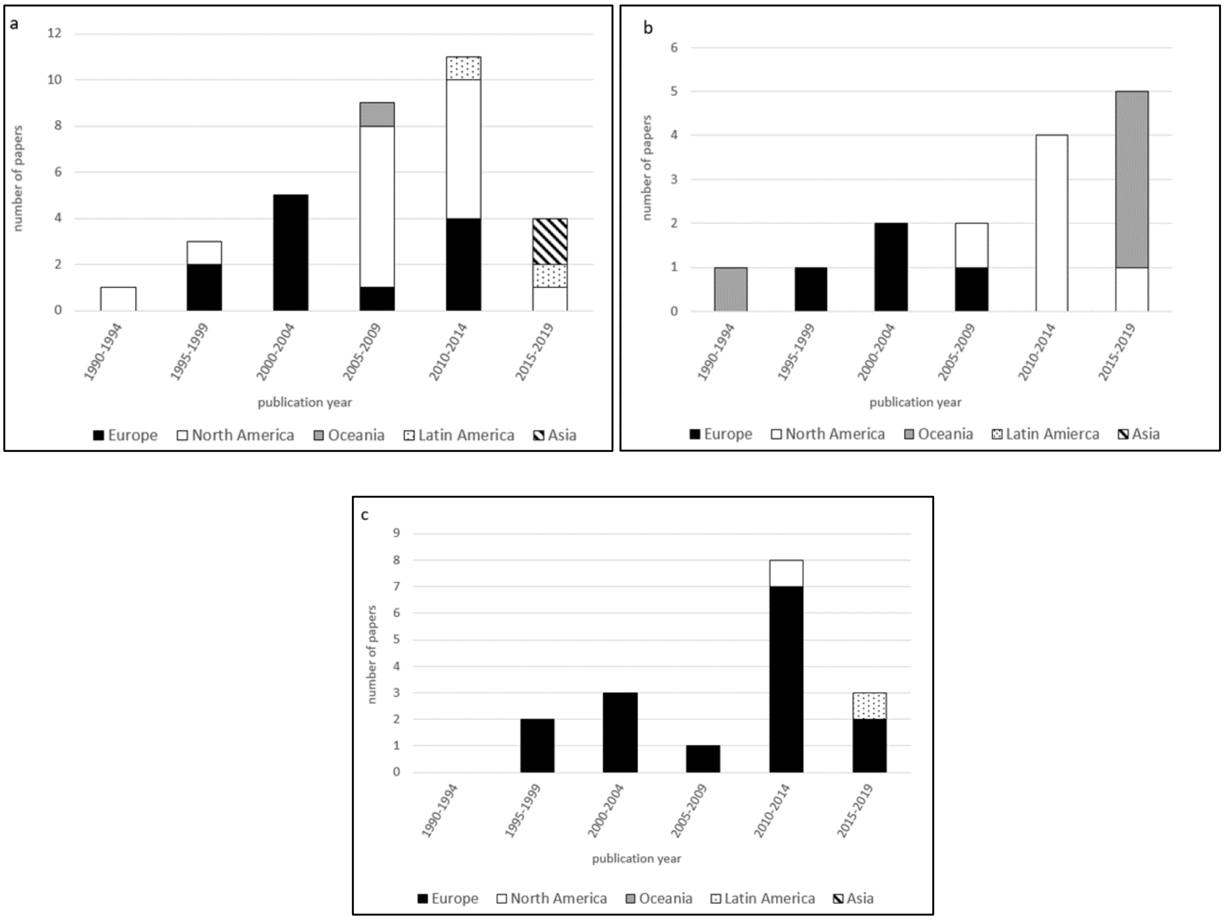

Figure 5. Number of papers of the three main housing inter- ventions (individual stalls vs. group housing (a); space allow- ance (b); floor characteristics (c)) distributed according to the publication year in the different continents.

The decrease seen in publications relating to nutrition and feeding (Figure 3) is mainly due to the limited number of articles comparing different feeding systems and frequency (Figure 6a) (main topic for nutrition and feeding until then). In the last five years, only one paper was published on this topic from the United States, while the number of papers on fibre remained roughly unchanged (Figure 6(b)). On the other hand, we found two articles on 'feed supplement and additive', topic cited in one article only, until 2014. From these data, it seems that research interest in topics that are already tightly regulated by the EU is decreasing. This includes the use of individual stalls/ cages, floor space allowance and quality as well as sow feeding systems. All of these topics are very important to the welfare of the gestating sow, especially when housed in groups (Verdon et al. 2015) and deserve research attention to better refine group- housing systems for gestating sows. Moreover, taking into account the successful recent European Citizens' Initiative End the Cage Age which calls on European Union to end cage confinement of farm animals, it is very important that the European scientific community takes account of the new consumers requirements, and therefore implements research on topics already regulated by legislation, but which may need to be modified. The European Commission in response to the initiative 'intends to propose to phase out and finally prohibit the use of such cage systems. This will be included as one of the key objectives of the revision of animal welfare legislation that the Commission has committed to propose by the last quarter of 2023.' This is a displayed quotation (EC C(2021) 4747 2021, p. 9).

The decreasing number of publications in the last five years in these topics, like the use of individual stalls, floor allowance and quality is also evident in North America, even if in the vast majority of the American countries there is still no regulation of these practices.

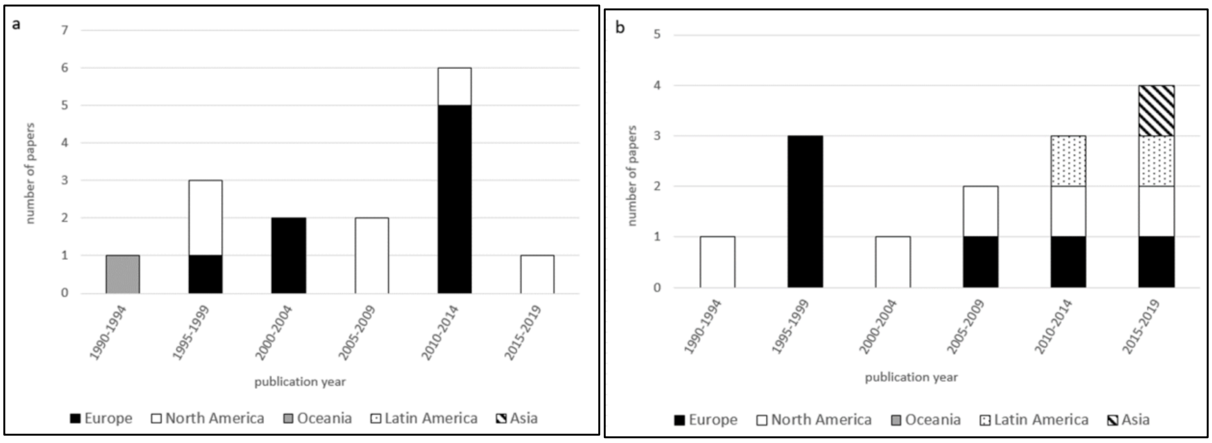

Figure 6. Number of papers of the two main Nutrition and Feeding interventions (feeding system and frequency (a); level of dietary fibre (b)) distributed according to the publication year in the different continent.

Outcomes of the selected studies

Of the 120 publications included in the study, behaviour was the most commonly reported welfare outcome (74%), followed by physical indicators (57%). All the other outcomes (reproductive performance 50%, productive performance 48% and physiological indicators 47%) were similar (Table 2). In Table 2, the specific aspects of each welfare outcome are also detailed. As regards behavioural observation, the main aspects of sow ethogram were utilised equally, and the different approaches developed during this 30- year period are not evident from this review. Only a few studies introduced motivation and positive social contact in their behavioural research and none used a relatively new scientific method to evaluate the expression quality of animal behaviour and emotions: Qualitative Behaviour Assessment. This behaviour assessment is included in the Welfare Quality® (2009) to identify sow emotional state, but the scientific community did not embrace this measure in studies of gestating sow welfare.

Table 2. Number and percentage of publications of welfare of gestating sow per welfare outcomes during the 30- year period.

TOPIC		NUMBER OF PAPERS	%
Behavioral observation		**75**	**74**
	Postural	*44*	*59*
	Locomotory	*39*	*52*
	Aggressive	*38*	*51*
	Steroetypies	*33*	*44*
	Others	*33*	*44*
Physical indicators		**58**	**57**
	Body lesion	*51*	*88*
	Locmotory problems	*33*	*57*
Reproductive performance		**51**	**50**
	Litter size	*51*	*100*
	Birth weight	*32*	*63*
	Farrowing rate	*23*	*45*
	Wean-to-estrus-interval	*10*	*20*
	Conception rate	*7*	*14*
	Gestation length	*6*	*12*
	Others	*3*	*6*
Productive performance		**49**	**48**
	Body weight	*38*	*78*
	Backfat	*35*	*71*
	BCS	*16*	*33*
	Others	*10*	*20*
Phisiological indicator		**48**	**47**
	Cortisol	*38*	*79*
	Immune traits	*19*	*40*
	Heart rate	*8*	*17*
	Antioxidant status	*1*	*2*
Others		**13**	**13**

The total number of the selected papers is 102. Several papers include more than one welfare outcomes, and the same outcome can include more than one specific indicators. The percentages of the main topics have been calcu- lated on the 102 papers, while the percentages of the specific indicators have been calculated on the number of papers of the main outcome.

This can be correlated with the recent growing interest in the other indicators. Indeed, if up until 2009 behaviour was the most common welfare outcome, in the last 10 years each of the other welfare outcomes were employed at similar frequencies, and in the last five years reproductive performance and physical indicators were utilised more often than all other welfare assessment (Figure 7). This reflects the increasing multidisciplinarity and importance of welfare assessment (Sejian et al. 2011) but it is also probably due to the increased number of group management papers.

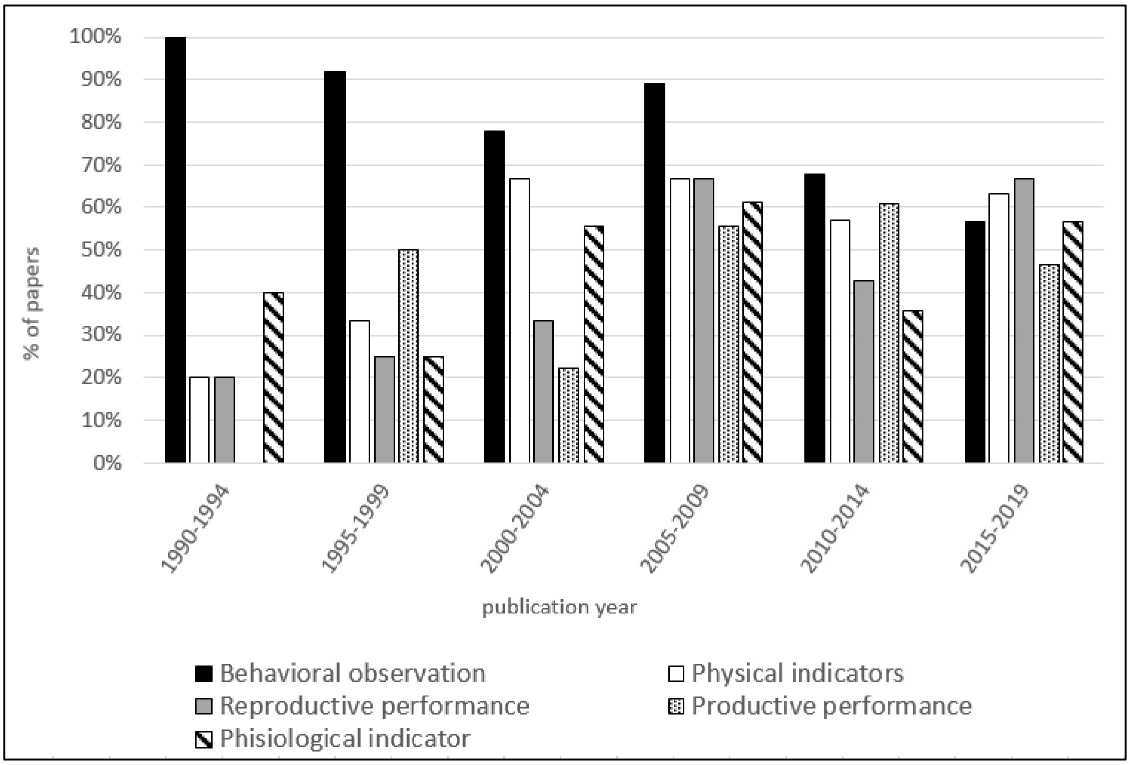

Figure 7. Percentage of papers of welfare of gestating sow distributed according to the publication year and classified according to the welfare outcomes considered.

This is in fact the only intervention in which sow welfare was most frequently evaluated through physical indicators and reproductive performance (Figure 8). Group management papers turn out to have these different percentages probably because aggression is the most obvious welfare implication in group housing (Bench et al. 2013), and its consequences on injuries (e.g. Turner et al.

2006; Schneider et al. 2007) and reproductive performance (Salak-Johnson 2017) are some of the most important indicators for both sow welfare and herd productivity. As regards physical indicators, body lesions were the most frequently evaluated indicator (88%) followed by locomotory problems (57%), while reproductive performance was evaluated through litter size for all publications, 63% used birth weight, 20% far- rowing rate, 14% conception rate and 12% gestation length (Table 2). Concerning the other two welfare outcomes, body weight and backfat thickness were the most employed indicators of productive performance (78% and 71%, respectively), while cortisol (79%; salivary 44% and serum 33%) was by far the most frequently employed physiological indicator followed by measures of immune function (40%). Although there is no dispute over the assumption that stressful experiences cause the synthesis and release of cortisol from the adrenal gland (Seyle 1935), its use in assessing animal welfare has limitations related to the fact that its levels can be affected by many other factors (Lane 2006). Furthermore, the process of sampling can in itself be a stressful event, interfering with the stress marker. In particular, this could occur with invasive sampling methods, such as blood samples (Sheriff et al. 2011), and therefore, there is a growing interest in less invasive sampling techniques. Our findings indicate that despite this evidence, serum cortisol is still often used and the only non-invasive assessment of cortisol used is saliva sampling. This technique is quite practicable in sow herds and minimally invasive, but its level may rise if sampling is contaminated with food (Heimbu€rge et al. 2019). Only one paper used an alternative measurement, urinary cortisol, and none of them considered others alternatives like hair cortisol. This measurement is minimally invasive and has proven to be a useful biomarker of long-term stress in sows and growing pigs (Bacci et al. 2014; Casal et al. 2017a, 2017b; Lagodaet al. 2021b).

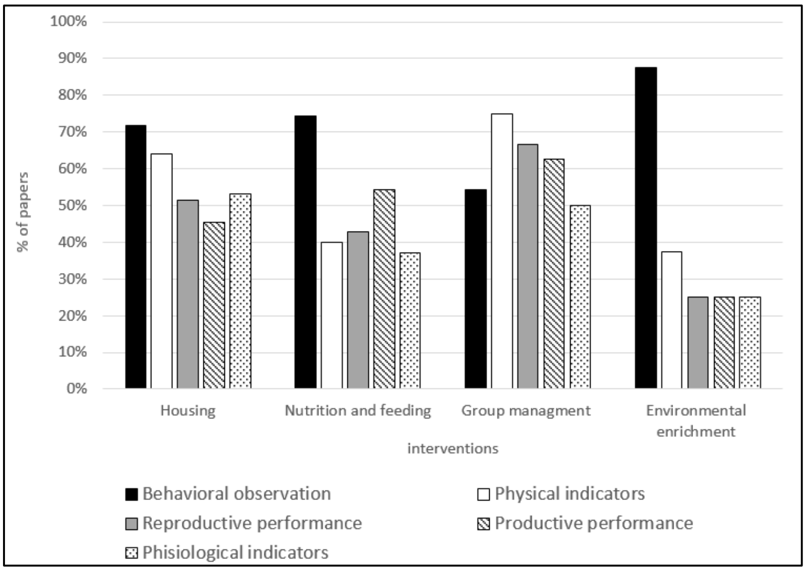

Figure 8. Percentage of papers of welfare of gestating sow classified according to the welfare outcomes considered for each of the four main topics (housing, nutrition and feeding, group management and environmental enrichment).

CONCLUSION

Clearly, it appears that scientific research on the welfare of gestating sows is increasing globally but some topics, such as provide high fibre diets and environ- mental enrichment, need more attention. Importantly, further studies on the effects of management of sows in groups are required as the industry moves away from stall housing internationally. Research is particularly needed on the time of introducing sows to groups which is pertinent giving the current review of EU legislation which permits housing sows in gestation stalls for the first 28 days post-service. In order to ensure the sustainability of pig production, it is clear that research needs to be in step with societally driven legislative and industry changes in pig production. This requires proactive research policies underpinned by targeted funding, international research groups that can find the best solutions to be adapted in different farming systems and structures, better communication between legislation, the scientific world, farmers and consumers and finally, better sharing of research in non-English publications. Until recently, the limitations that research may have encountered could lie in the difficulty of undertaking research projects, both due to the challenges in finding farms capable of hosting a research project, and due to the lack of funded projects. Countries further behind in legislation and research on the welfare of pregnant sows could interface with the most advanced countries, thus catching up more easily and quickly. Even the most advanced countries, such as those in Europe, should continue research in this area in order to give greater support to further legislative changes required to meet societal demands, as stated in the Farm to Fork strategy (European Commission 2020). The increasing demands for more welfare-friendly farming methods and the importance that citizen has in directing legislation (End the Cage Age Initiative) and consequentially in research, has given (European Commission 2021), and most likely will give, the tools to change the legislation. In this scenario, the aim of scientific world should be to make easier to align legislation with social expectations and to make it easier to enforce these changes.

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CHAPTER 3

Can we further reduce the time

pregnant sows spend in gestation stalls?

*Pen with floor feeding system

Can we further reduce the time pregnant sows spend in gestation stalls?

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ABSTRACT

There is growing societal pressure to eliminate close confinement of farm animals. However, under current EU legislation, sows can be kept in gestation stalls for up to 28 days post-service. Pregnant sows fight to establish a dominance hierarchy at mixing. This poses welfare and productivity concerns particularly if sows are mixed in early pregnancy when the pregnancy is less well established and they are still recovering from the demands of lactation. The aim of this study was to compare the effects of day of mixing (PEN4D:4 days or PEN28D:28 days post-insemination) on measures of animal welfare (skin lesion and cortisol concentrations) collected at mixing, 3 and 7 days after and on body condition (back fat thickness), reproductive performance (pregnancy and farrowing rate) and litter size. Sows included in the study (n=144) were of mixed parity (parities 2-7) and they were all weaned 4 weeks after farrowing and moved from farrowing to the breeding area where they were placed into individual stalls. Once inseminated, sows were assigned according to parity order and genetics to one of the two experimental treatments (PEN4D and PEN28D) and they were maintained as a static group until 1 week before farrowing. The group size was 21, and sows were housed on solid concrete floor, with a slope to convey manure in a drain, and with a floor space allowance of 2.25 m2/head. One block of wood on chain and one chain were provided as enrichments. Feed was distributed manually, spreading it in a wide clean area of the pen floor and in the troughs in the individual stall. There were no treatment effects on salivary cortisol concentrations or on the number of fresh skin injuries. For the old scratches score, a significant effect of treatment was observed only at three days after mixing, where PEN28D sows had a higher anterior lesion score than PEN4D sows (P=0.005). Furthermore, there were no treatment effects on backfat thickness, reproductive performance or litter size. Results of this experiment indicate that mixing sows into groups early after insemination (within 4 days post AI) rather than 28 d after has no effect on reproductive and welfare indicators measured in the short term after mixing.

INTRODUCTION

Currently, under the EU Directive (Council of the European Union, 2008/120), sows and gilts can be kept in gestation stalls for four weeks after service. The main reason behind the decision to keep sows in individual stalls during this period was related to the fact that new social groupings can lead to extreme stress and, if experienced at the critical time of embryo implantation, could negatively influence the reproductive process, particularly ovulation and embryo survival (European Commission, 1997). To date, the effect of reproductive cycle at mixing on sow welfare and performance has not been widely investigated (Galli et al., 2021), especially during the first month post-service. In addition, as regards reproductive performance, there is good consensus in the literature on the possibility of avoiding the detrimental effects of mixing during the four week period post-service. This can be achieved by grouping sows outside the stress-sensitive stage of the implantation interval, and therefore immediately after service ([EFSA] European Food Safety Authority, 2007), or at least prior to embryo implantation in the uterine wall, which starts 11-12 days after insemination (Dantzer and Winther, 2001; Spoolder et al., 2009). In fact, if grouping takes place immediately after service, reproductive performance can be as good as that at grouping four weeks after service (Bampi et al., 2020; Cunha et al., 2018; Kirkwood and Zanella, 2005; Mheen et al., 2003; Stevens et al., 2015). However, other studies reported lower farrowing rates for sows mixed very soon after insemination compared to sows mixed four weeks later (Knox et al., 2014; Li and Gonyou, 2013). In addition, with regard to the welfare effects, few studies evaluated the effects of mixing sows at different times during the first month post-service on welfare parameters, especially in the short-term period. Indeed, despite there are substantial data in the scientific literature that confirm that the effects of mixing pigs on injuries, aggression and stress are most pronounced within the first 24-48h (Dolf, 1986; Meese and Ewbank, 1973; Mount and Seabrook, 1993), only two studies assessed skin injuries and cortisol concentrations in the short term in relation to the stage of gestation at mixing in the first month post-service (Knox et al., 2014; Stevens et al., 2015). They report that sows mixed early in gestation had more skin injuries than those mixed later. Where skin injuries were evaluated in the long term, some studies found no effects of stage of gestation (Knox et al., 2014; Stevens et al., 2015; Strawford et al., 2008) while others (Cunha et al., 2018; Li and Gonyou, 2013) found that sows mixed early in gestation had fewer skin lesions than sows mixed post-implantation. Stevens et al. (2015) found that sows grouped early after insemination had higher salivary cortisol concentrations at mixing than sows grouped at day 35 of gestation. Meanwhile Knox et al. (2014) found that sows grouped early after insemination had a lower increase in serum cortisol concentrations from baseline during day 3 and 9 after mixing. In the long term assessment Stevens et al (2015) found no effects of stage at gestation, while Strawford et al. (2008) found greater cortisol concentrations in sows mixed later after insemination than those mixed earlier. Currently a deeper understanding of the effects of mixing sows during earlier stages of gestation on reproductive performance and welfare, and an identification of the variables (e.g. feeding system, floor quality, group size, genetics, season) which may be responsible for some of the contradictory findings and inconsistencies in the literature are urgently needed. This comes in light of the evidence that crating sows for four weeks from the time of weaning severely restricts sow freedom of movement, their opportunity to interact socially and therefore causes stress and frustration ([EFSA] European Food Safety Authority, 2007) especially during the pre-oestrus and oestrus periods when they are highly motivated to do so (Pedersen, 2007; Pedersen et al., 1993).

Moreover, the success of the European Citizens Initiative (ECI) 'End the Cage Age' (End the Cage Age, 2018) clearly reflects the negative attitude of European citizens towards the practice of sow crating, and their agreement with the need for a ban on the continued use of gestation stalls for pregnant sows. According to the EFSA Report and the "End the Cage Age" petition, a total ban of individual stalls housing system would be required. However, the risk of leg injures due to mounting behaviour during oestrus is high ([EFSA] European Food Safety Authority, 2007; Elmore et al., 2010) and the lack of a consolidated oestrus detection protocol in group-weaned sows system (Kemp et al., 2005; Rault et al., 2014) pose serious concerns for sow welfare and reproductive performance.

In order to address some of the inconsistencies and knowledge gaps outlined above, we tested the effects of mixing sows into groups where they were floor fed immediately after mating or four weeks later on reproductive performance, litter size and sows welfare measures (in- juries and cortisol concentrations) on the day of mixing, 3 and 7 days later.

MATERIAL AND METHODS

Animals and treatments

The study was carried out on a 600-sow farrow-to-finish commercial farm in Northern Italy. The sows were of 4 genetic backgrounds: TN60 and TN70 (Topigs Norsvin, Italy); ANAS (Associazione Nazionale Allevatori Suini, Italy) and Goland (Gorzagri, Italy). The sows were weaned 4 weeks after farrowing and were moved from the farrowing accommodation to the breeding room where they were placed in individual stalls. From the first day after weaning, the sows were checked for oestrus once daily in the morning using fence-line exposure to a mature boar with application of the back-pressure test. Sows were artificially inseminated (AI) with 3.0 109 sperm/dose at the onset of oestrus and at 24-h intervals until no longer standing in oestrus. Once inseminated, all sows within replicate were allocated according to parity order and genetics to one of the two following experimental treatments:

PEN4D (n 209): sows were mixed into static groups within 4 days after service.

PEN28D (n 171): sows remained in individual stalls until 28 d after service and were then mixed into static groups.

The study was performed in 8 replicates using batches of weaned sows bred from December 2020 through May 2021. All sows were maintained in their treatment group until approximately 110 d of gestation when they were moved into the farrowing crates. Genetics were represented across PEN4D-PEN28D, with a range of 39-34% for Anas, 28-37% for TN60, 23-23% for Goland, 10-6% for TN70. Only multiparous sows were included in the study (from parity 2 to parity 7). Sows of all parities were represented in each treatment with a range within PEN4D and PEN28D respectively of: 3 and 3% (parity 2), 49 and 47% (parity 3 and 4), 22 and 25% (parity 5), 8 and 9% (parity 6), 18 and 16% (parity 7).

Housing and feeding

Sows were housed in stalls in the breeding barn which had mechanical ventilation and a light cycle of 9-15h according to the season each day was maintained with artificial lights turn on at 06:00 a.m. The breeding barn had 162 individual stalls, the anterior half of the floor was solid concrete while the posterior part was of concrete slats. Stalls were 1.9 x 0.65 m (l X w). Each stall was equipped with a nipple drinker. The barn with group housing had 15 pens with a solid concrete floor, with a slope to convey manure in a drain. Sows were housed in groups of 21 sows/pen, with a floor space allowance of at least 2.25 m2/sow. One block of wood on chain and one chain were provided as enrichment. Drinking water was available ad libitum from two nipple drinkers per pen. During gestation, sows were fed once daily with an average of 3 kg of a standard barley-corn-roughage-soybean diet (9.7 MJ net energy/kg DM, 6.6% crude fibre, 2.7% crude fat and 0.7% lysine), which was slightly adjusted through the gestation period. Feed was distributed manually, spreading it in a wide clean area of the pen floor allowing sows to explore and root, and on the trough in the individual stall.

Data collection

Reproductive performance were assessed for all sows in all replicates, while backfat thickness, skin injuries and salivary cortisol measurements were conducted only in 4 replicates (PEN4D= 84 sows, PEN28D=62 sows).

Backfat thickness and reproductive performance

As a proxy indicator of body condition and good feeding, the backfat thickness (BFT) was measured at the last rib, 6 to 7 cm off the midline on the left and right side (P2 position) using an ultrasonic scanner (Lean- meter, Renco, Minneapolis, MN). BFT was recorded at weaning and at the end of gestation before sows entered the farrowing crate (c. 110 days of gestation). Changes in BFT during pregnancy were calculated as the difference between these two measurements. As indicators of reproductive efficiency, pregnancy rate (proportion of sows inseminated that resulted in pregnancy at 30 d after a real-time ultrasound examination), farrowing rate (proportion of sows inseminated that farrowed) and litter size (total born, born alive, stillborn and mummified piglets) were recorded.

Skin injuries

The number and severity of skin injuries were assessed by visual inspection of both sides of the sow body. The body was divided into 9 areas: head, ears, neck and shoulders (anterior region), flanks and back (middle region), rump, thighs and vulva (posterior region). Each area was scored according to a three-scale injury score, based on the classification used by Strawford et al. (2008): 0 = no scratches; 1 = 1-3 scratches; 2 = 4-6 scratches; 3 ≥ 7 scratches. The same scale was used to score fresh and old injuries, therefore each body region had two scores, one referring to fresh injuries and the other referring to old injuries. The score for each region was calculated as a sum of the scores of each area. In addition, the number and severity of cuts, swelling and abscesses on each area of the body was also recorded. The scale used to score each of the injuries was also based on the classification used by Strawford et al. (2008) (Table 1). The injuries were assessed on days 0 (approximately 5 h after introduction in the group pen, T0), 3 and 7 days after mixing (T3 and T7).

Table 1. The scoring system used to record cuts, swellings and abscesses.

Score	**Cut**	**Swelling**	**Abscess**
0	No cuts	No inflammation	No evidence of an abscess
1	Minor cut: no flesh exposed	Inflammation, slight swelling	Previous wound healed (scar)
2	Major cut: can see flesh	Marked swelling	Presence of a scab
3	-	-	Open wound

Salivary cortisol

Saliva samples for cortisol measurements were collected on the day of mixing (approximately 6 h after introduction, T0) and 3 days after mixing (T3). Saliva was collected by allowing each sow to chew on cotton swabs (Salivettes®, Sarstedt, Nümbrecht, Germany) for approximately 30 s. All the samples were collected between 13:00 and 14:00 h to minimise the effect of cortisol diurnal variation. The samples were centrifuged at 3000 X g for 20 min, the saliva was then collected into a 1.5 ml Eppendorf tube and stored at 20◦C until analysed. Salivary cortisol concentration was measured by using the electrochemiluminescence immunoassay Elecsys Cortisol II kit, and the automated Cobas e601 analyser (Roche Diagnostics GmbH, Mannheim), following manufacturer instruction. Intra and inter-assay coefficients of variation of the saliva assay were lower than 10%; the limit of detection was 1.5 nmol/L.

Statistical analysis

The statistical analysis were carried out using SAS (SAS Institute, Inc., Cary, NC). Data were analysed based on their distribution tested by Shapiro-Wilk test for normality. Changes in BFT were processed using ANCOVA, with treatment and replicate included as fixed factors and backfat at weaning as covariate. The effects of treatment (PEN4D vs PEN28D) on pregnancy rate and farrowing rate (counting data) were examined using two proportion Chi- square test. The analysis of the number of total born piglets and born alive piglets were carried out using ANOVA model with the treatment and replicates as fixed factors, while the percentage of stillborn and mummified piglets were analysed with a non-parametric test (Mann- Witney test). Skin injuries were analysed by considering the sum of the scores for each body region. The effect of treatment (PEN4D vs PEN28D) on both fresh and old skin injuries was examined using Mann-Witney test, while the time effect (T0 vs T3 vs T7) was examined using Kruskal-Wallis test. Cortisol was analysed using repeated ANOVA mixed model, with treatment and replicate as fixed factors and animal as repeated random effect.

RESULTS

Body condition and reproductive measures

At the time of weaning, there was no differences for BFT between treatments (PEN4D 15.1 mm vs PEN28D 16.4 mm; P 0.11). As reported in Table 2, no differences were also detected in BFT changes during pregnancy (P 0.42). Indicators of reproductive efficiency (pregnancy rate and farrowing rate) as well as total number of piglets born, numbers born alive, stillborn or mummified (Table 2) were not significantly affected by the experimental treatment.

Table 2. Effect of stage of gestation at mixing (PEN4D=4 days after service and PEN28D=28 days after service) on measurements of backfat, reproductive performance and litter size.

Measurement:	**PEN4D**	**PEN28D**	**P Value**
Change in BFT [1] (mm)	4.7 ± 0.49	4.1 ± 0.59	0.42
Pregnancy rate [2] (%)	88 (183/209)	85 (146/171)	0.64
Farrowing rate [2] (%)	84 (175/209)	81 (138/171)	0.52
Total piglets born[1] (n.)	14.4 ± 0.29	14.3 ± 0.31	0.81
Piglets born alive[1] (n.)	13.0 ± 0.26	12.8 ± 0.27	0.80
Stillbirth [3] (%)	8 (0-13)	7 (0-14)	0.76
Mummified [3] (%)	0 (0-0)	0 (0-6)	0.17

[1]data normally distributed were reported as ls-means ± standard error

3 count data were analysed using two proportions z-test

[3] data not normally distributed were reported as median (interquartile range)

Skin injuries

There was no significant effect of treatment on the number of fresh injuries in the different regions, although there were a tendency for sows of PEND28D to have higher injury scores in the anterior region at T0 and T7 than sows of PEN4D (P 0.05; Table 3). Regarding old scratches, significant effect of treatment was observed only at 3 days after mixing, in which sows of the PEN28D treatment had a higher anterior lesion score than PEN4D sows (P 0.005; Table 3).

Regardless of the experimental treatment, significant effects of time (P<0.0001) were found for fresh and old injuries in all of the body regions (Table 3). As regards fresh injuries, on the day of mixing (T0) these were always greater than at T3 and T7 (P<0.0001). Fresh injury scores decreased between T3 and T7 but they were not statistically different, except for PEN4D in the anterior region (T3 0.6 vs T7 0.1; P 0.010) and for PEN28D in the middle region (T3 0.3 vs T7 0.0; P 0.011), in which the decreases were statistically significant. As regards old injuries, at T3 and T7 these were always greater than at T0 (P<0.0001). Old injury scores decreased between T3 and T7 but these were not statistically different, except for PEN28 in the anterior region (T3 7.2 vs T7 4.3, P<0.0001), in which the decrease was statistically significant. Cuts, swelling and abscess were rarely recorded (Table 4).

Table 3. Effect of stage of gestation at mixing (PEN4D=4 days after service and PEN28D=28 days after service) and of time (days after mixing) on skin injuries. Values presented as median (minimum-maximum).

Body region		**Time**	**PEN4D**	**PEN28D**	**P Value**
Anterior	fresh	T0	5.1*a* (0-17)	5.9*A* (0-15)	0.05
		T3	0.6*b* (0-5)	0.3*B* (0-4)	0.19
		T7	0.1*c* (0-3)	0.3*B* (0-3)	0.05
		P Value	**< 0.0001**	**< 0.0001**	
	old	T0	0.0*b* (0-1)	0.0*C* (0-0)	0.12
		T3	5.6*a* (0-14)	7.2*A* (2-15)	**0.005**
		T7	5.2*a* (0-14)	4.3*B* (0-12)	0.13
		P Value	**< 0.0001**	**< 0.0001**	
Middle	fresh	T0	2.2*a* (0-6)	1.7*A* (0-6)	0.10
		T3	0.2*b* (0-2)	0.3*B* (0-3)	0.15
		T7	0.1*b* (0-1)	0.0*C* (0-1)	0.47
		P Value	**< 0.0001**	**< 0.0001**	
	old	T0	0.0*b* (0-1)	0.0*B* (0-0)	0.37
		T3	2.4*a* (0-7)	2.3*A* (0-6)	0.75
		T7	2.2*a* (0-6)	1.8*A* (0-5)	0.19
		P Value	**< 0.0001**	**< 0.0001**	
Posterior	fresh	T0	1.8*a* (0-6)	1.4*A* (0-5)	0.44
		T3	0.1*b* (0-2)	0.3*B* (0-5)	0.30
		T7	0.0*b* (0-2)	0.1*B* (0-2)	0.81
		P Value	**< 0.0001**	**< 0.0001**	
	old	T0	0.0*b* (0-0)	0.1*B* (0-2)	0.05
		T3	1.9*a* (0-6)	1.8*A* (0-5)	0.81
		T7	1.6*a* (0-7)	1.5*A* (0-4)	0.72
		P Value	**< 0.0001**	**< 0.0001**	

Different letters mean significant different values along columns

Table 4. Effect of stage of gestation at mixing (PEN4D=4 days after service and PEN28D=28 days after service) and of time (days after mixing) on the percentage of animals with at least one cuts, swelling or abscess.

Time	**PEN4D**	**PEN28D**	**P Value**
T0	10 %	6 %	0.59
T3	18 %	8 %	0.14
T7	11 %	3 %	0.12
P Value	0.31	0.65	

Salivary cortisol

There was no significant effect of treatment on the salivary cortisol concentrations, whereas there was a significant time effect (P<0.001). Cortisol concentrations decreased between T0 and T3 for both PEN4D (T0=9.8 nmol/l vs T3=5.4 nmol/l, P<0.001) and PEN28D (T0=7.8 nmol/l vs T3=4.0 nmol/l, P<0.001) (Table 5).

Table 5. Effect of stage of gestation at mixing (PEN4D=4 days after service and PEN28D=28 days after service) and of time (days after mixing) on salivary cortisol concentrations (nmol/l).

Time	PEN4D	PEN28D	P Value
T0	9.8	7.8	0.82
T3	5.4	4.0	0.31
P Value	**<0.001**	**<0.001**	

DISCUSSION

This study suggests that mixing sows into groups early after insemination (within 4 days post AI) rather than 28 d after did not have a negative impact on reproduction and measures of sow welfare. It is important to highlight that these results were obtained on a commercial large-scale farm, in which all the housing, environmental and management conditions were less standardized compared to that of a small-scale experimental settings.

The similar BFT variation recorded during pregnancy in both mixing treatments shows that it is possible to group sows in early pregnancy without adverse effects on body condition. This finding is especially important given that the sows in this study were floor fed which is a highly competitive feeding system (Edwards, 1992), often resulting in considerable variation between sows in body condition score/BF (Brouns and Edwards, 1994). Moreover, backfat depth is a good indicator of good feeding both considering the amount of feed delivered and sows ability to access it. During pregnancy, sows have a need for nutrients for maintenance and to support the growing foetuses, they also need to restore the low energy balance resulting from the previous lactation period (Spoolder et al., 2009). Therefore, low backfat gain may negatively affect farrowing rate and litter size (Kongsted, 2006). Two previous studies (Cunha et al., 2018; Stevens et al., 2015) obtained similar results with non-competitive feeding systems (Electronic Sow Feeding and external feeding stalls).

Mixing sows into groups early or later after insemination had no effect on reproductive performance (pregnancy rate and farrowing rate) or litter size. Indeed, based on the physiology of pregnancy, it is commonly accepted that the most suitable time to group sows to avoid reproductive failure is prior to embryo implantation to the uterine wall which starts 2 weeks after insemination, or to wait until attachment is complete 2 weeks after (Morrison et al., 2011; Spoolder et al., 2009). Results from the present study and from those of Bampi et al. (2020), Cunha et al. (2018), Stevens et al. (2015), Kirwood and Zanella (2005) and Van der Mheen et al. (2003) confirm this physiological indication. In contrast, there are some studies that reported lower farrowing rates for sows mixed early after insemination compared to those mixed later (Knox et al., 2014; Li and Gonyou, 2013). The discrepancy in results for reproductive performance among studies as a result of early time of mixing could be influenced by several variables, including different group management (static or dynamic), size and composition, floor quality and space, feeding system, pen design and genetics (Spoolder et al., 2009).

The absence of an effect on litter size is supported by other studies (Bampi et al., 2020; Cunha et al., 2018; Kirkwood and Zanella, 2005; Knox et al., 2014; Li and Gonyou, 2013; Stevens et al., 2015) and demonstrates that, in spite of the competitive feeding arrangement in the current study, the stress associated with mixing were not severe enough to affect embryo survival. Based on a review of Turner et al. (2002) it appears that only stressors that can lead to severe and pro- longed elevation of cortisol, like high stocking density or chronic fear of the stockperson, may negatively affect embryo survival.

In the present study, the results obtained from salivary cortisol and skin lesions confirm the acute nature of stress related to mixing sows, as they both sharply decreased 3 days after mixing, regardless of the day that mixing occurred. There are substantial data in the scientific literature that confirm that mixing pigs increases injuries, aggression and stress within the first 24-48h (Dolf, 1986; Meese and Ewbank, 1973; Mount and Seabrook, 1993), but less literature comparing the short-term effect of mixing related to grouping sows at different stages of gestation. In the present experiment, there were no significant short-term effects of stage of gestation at the time of mixing on skin lesions and salivary cortisol. In agreement with Strawford et al (2008) the average skin lesion score did not differ between sows mixed in groups early or late in respect of the day of insemination. However, it is important to note that Strawford et al. evaluated skin lesions in both the short and long term and presented the results as the average value of measurements carried out at 0, 3, 28 and 63 days after mixing. The studies of Li and Gonyou (2013) and Cunha et al. (2018) found that sows mixed early in gestation had fewer skin lesions than sows mixed post-implantation, but the assessments took place at the end of gestation, so they concern the long-term effects. Moreover, Li and Gonyou (2013) only recorded swellings, wounds and cuts, which could be caused by trauma from the physical environment, they did not include scratches which are caused by fighting. Cunha et al. (2018) also evaluated the short-term effects as they assessed lesions at 3, 12 and 23 d after mixing, nevertheless the lack of a statistical comparison between treatments in relation to the day after mixing (and not to the day of gestation) makes it difficult to draw conclusions. In contrast, other studies (Knox et al., 2014; Stevens et al., 2015) report that sows mixed early in gestation had more skin injuries than those mixed later, but only in the short-term. Indeed, this effect was not seen at 91 d of gestation

(Stevens et al., 2015) nor in the period between 12 d after mixing to 110 d of gestation (Knox et al., 2014).

In contrast to our finding of a lack of an effect of stage of gestation at mixing on cortisol concentrations on both the day of mixing and 3 days after, Stevens et al. (2015) found that sows grouped early after insemination had higher salivary cortisol concentrations at mixing than sows grouped at day 35 of gestation. This difference was not seen 7 or 91 days after mixing. Even if in the study of Stevens et al. (2015) the effect of time was not analysed, the data presented in the paper shows that 7 days after mixing salivary cortisol concentrations were lower than on the day of mixing. These values are in accordance with our results, in which cortisol concentrations decreased between T0 and T3 for both PEN4D and PEN28D. The only other two studies that evaluated cortisol concentrations related to time of mixing found higher cortisol concentrations in sows mixed later after insemination than those mixed early (Knox et al., 2014; Strawford et al., 2008). Both papers, however, did not measure cortisol concentrations on the day of mixing and they did not evaluate the data from different days separately. Indeed, Knox et al. (2014) analysed serum cortisol concentrations only as a change in the average of 3 and 9 days after mixing from the pre-treatment baseline mean. Furthermore, Strawford et al. (2008) evaluated salivary cortisol concentrations only as an average of 1, 2, 3, 28 and 63 days after mixing. Strawford et al. (2008) also analysed the mean salivary cortisol concentrations on the different days after mixing, but they did not evaluate effect of time on the different stage of gestation at grouping. They found that salivary cortisol levels increased throughout gestation, therefore the higher salivary cortisol levels in the sows mixed later in gestation may be due to their physiological state and not due to stress. However, in contrast with ours and Stevens et al. (2015) findings, this increase was also found in the short-term (between 1 and 3 days after mixing).

CONCLUSION

Results of this experiment suggest that sows can be mixed into static groups with floor feeding early after insemination (within 4 days post-AI) rather than 28 d after, with little impact on sows' reproduction performance and welfare indicators (skin lesion and salivary cortisol) collected in the short-term after mixing. The present findings supports the proposal to reduce the use of the gestation stalls since this would not harm farm productivity or animal welfare. However, further research is necessary to confirm these results in other types of group housing systems, in other genetics and also in the other seasons. It is indeed possible that these favourable results were positively affected by the favourable season for reproduction (winter/spring), the solid concrete flooring or the floor feeding system. The latter allows sows to eat simultaneously and if spread over a wide area of the pen floor as in this study, fulfils some elements of natural behaviour by allowing sows to explore and root.

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CHAPTER 4

Does reducing time in gestatio stalls in early pregnancy

affect sow welfare and performance?

*Pen with individual feeding stalls system

Does reducing time in gestation stalls in early pregnancy affect sow welfare and performance?

*Pen with individual feeding stalls system

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SUBMITTED TO LIVESTOCK SCIENCE

ABSTRACT

EU legislation (2008/120/EC) allows sows to be kept in gestation stalls up to 4 weeks after service, but there are increased societal calls to reduce and/or ban their use. However, high levels of aggression are commonly observed in newly formed groups after mixing, pose a concern with regards the impact that mixing sows in early gestation may have on their welfare and performances. The aim of this study was to determine the effects of mixing sows (6 groups/treatment, 12 sows/group) in pens with individual free access feeding stalls 2 days (PEN2D) or four weeks (PEN28D) after service on aggression, skin lesions locomotory ability, reproductive performance and litter size. Direct observation of aggressions (all-occurrence sampling, 2hrs/day) were conducted immediately after mixing (T0), 24hr (T1), 8 days (T8) and 3 week post-mixing (T21). Skin lesions were counted at T1, T8 and T21. Sows were locomotion scored using a visual analogue scale 8 weeks post-mixing and in late pregnancy. An effect of treatment was observed only on the number of fights recorded at T1, which was higher in PEN2D, but neither skin lesion counts nor locomotion were affected. The negative trend reported in PEN2D for reproductive performance suggested the need for further investigation, with larger sample size.

INTRODUCTION

At the present time, the EU Directive allows sows and gilts to be kept in gestation stalls until four weeks after service (Council of the European Union, 2008). The main reason behind the decision to keep sows in individual stalls for the first month post-service was to protect them from stressors associated with grouping and social competition during the early phase of pregnancy, in particular during the phase of embryo-implantation(European Commission, 1997). In pigs this phase occurs from approximately day 11-13 to day 17-25 after service when the embryos implant in the uterine wall (Dantzer and Winther, 2001; Spoolder et al., 2009). Stress during this particularly cortisol-sensitive period could potentially cause implantation failures leading to loss in litter size or a complete loss of the pregnancy (Salak-Johnson, 2017; Spoolder et al., 2009). However, the physical confinement in gestation stalls inevitably results in compromise sow's welfare, as it severely restricts sow movement and socialization, causing stress and frustration ([EFSA] European Food Safety Authority, 2007; EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare) et al., 2022). In view of the above, the European citizens' initiative End the Cage Age("End the Cage Age," 2018) calls on the European commission to propose legislation banning the use of stalls for pregnant sows. Against this background, it appears important to define the stage of reproduction at which it would be beneficial for the welfare and not detrimental to reproductive performance to mix unacquainted sows into the group housing system.

As regards the welfare consequences, the latest EFSA scientific opinion (EFSA AHAW Panel, 2022) reports that the few papers that have studied welfare animal-based measures (ABMs) associated with different grouping times show little or no consistency in the direction of the findings. In any case it seems clear that, besides the welfare consequences related to the establishment of the dominance hierarchy at mixing, the risk for welfare consequences are greater after weaning, as it is increased by the mounting behaviour exhibited during oestrus, which can cause inability to avoid unwanted sexual behaviour particularly for low-ranking animals (Pedersen, 2007; Pedersen et al., 1993), bone lesions (Hartnett et al., 2019; Rydhmer et al., 2006) and locomotory disorders (Di Muzio et al., 2023; Rydhmer et al., 2004), especially for those weaned sows that are physically compromised by lactation (Giesemann et al., 1998; van Riet et al., 2016).

Also in term of the effects of timing of grouping on reproductive performances, the limited number of studies yielded conflicting and therefore inconsistent results (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare) et al., 2022; Schubbert et al., 2022). Taking as an example the farrowing rate, different studies show no effect of grouping 3 compared to 28 days post-insemination (Bampi et al., 2020; Cunha et al., 2018; Galli et al., 2022; Kirkwood and Zanella, 2005; Mheen et al., 2003; Stevens et al., 2015), however the two studies with bigger sample sizes reported lower farrowing rate for sows grouped very soon after insemination compared to sows grouped 4 weeks later (Knox et al., 2014; Li and Gonyou, 2013). The results of the analysis conducted in the EFSA Scientific Opinion (EFSA AHAW Panel, 2022) to understand the effects of grouping sows at different stages on farrowing rate are inconclusive, however the outcome supports that grouping sows in the period between 8 and 21 days post-service should be avoided due to possible detrimental effects on farrowing rate. The period between 1 and 7 days post-service could also revealed to be a period sensitive to stress, but requires further investigation (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare) et al., 2022; Verdon et al., 2015).

The limited number of studies (Galli et al., 2021) as well as the wide variation between researches in the feeding systems used, group size and composition, space allowance and pen design, group management (static or dynamic) and floor as well as the considerable variation in the way the welfare consequences were measured, likely explains the lack of conclusive evidence on the effect of grouping time on welfare measures and reproduction performances.

In order to fill with new informations some of the inconsistencies and knowledge gaps outlined above, we examined the effects of mixing sows into individual free access feeding stalls groups of 12 sows/pen early after mating or four weeks later on aggression, skin lesions and locomotory ability.

MATERIALS AND METHODS

Animals and treatments

The study was carried out on a commercial 2000-sow farrow-to-finish farm in Co. Cork, Ireland and it was performed in 6 replicates using batches of sows weaned between October 2021 and June 2022 (see Table 1 for experimental schedule dates).

Table 1. Experimental schedule dates.

	WEANED	A.I.	PEN2D MIXING	PEN28D MIXING
REPLICATE 1	30/09/2021	04/10/2021	06/10/2021	02/11/2021
REPLICATE 2	07/10/2021	11/10/2021	13/10/2021	09/11/2021
REPLICATE 3	27/01/2022	31/01/2022	01/03/2022	02/02/2022
REPLICATE 4	03/02/2022	07/02/2022	09/02/2022	08/03/2022
REPLICATE 5	02/06/2022	06/06/2022	08/06/2022	05/07/2022
REPLICATE 6	09/06/2022	13/06/2022	15/06/2022	12/07/2022

The sows (Large White X Landrace) were weaned 4 weeks after farrowing and were moved from the farrowing accommodation to the breeding room where they were placed in individual stalls. From the first day after weaning the sows were checked for oestrus once daily in the morning using fence-line exposure to a mature boar with application of the back-pressure test. Sows were artificially inseminated (AI) at the onset of oestrus and at 24-h intervals until no longer standing in oestrus. Once inseminated, 24 sows per replicate were selected for the experiment out of approximately 60 to 80 sows per batch and were allocated in a way that the both treatments were balanced according to parity order and back-fat thickness to one of the two following experimental treatments:

PEN2D (n=72): 12 sows per replicate were mixed into static groups within 2 days after service.

PEN28D (n= 72): 12 sows per replicate remained in individual stalls until 28 days after service and where then mixed into static groups.

Only multiparous sows were included in the study (from parity 2 to parity 6). Sows of all parities were represented in each treatment with a range within PEN2D and PEN28D respectively of: 14 and 17% (parity 2), 29 and 29% (parity 3), 36 and 32 % (parity 4), 20 and 21 % (parity 5), 1 and 1 % (parity 6). In order to balance the treatments according to body condition, back fat thickness was measured the day after the A.I. at the last rib, 6 to 7 cm off the midline on the left and right side (P2 position) using an ultrasonic scanner (Lean-meter, Renco, Minneapolis, MN), and the two values averaged. The mean ± standard deviation for back fat (mm) was 10.1 ± 1.8 in PEN2D and 10.6 ± 2.6 in PEND28D. All sows were maintained in their treatment group until approximately 110 d of gestation when they were moved into the farrowing crates.

Housing and Feeding

Sow were housed in the breeding barn in fully-slatted individual stalls (2.3 m length x 0.55 m width), each one equipped with a nipple drinker. The fully slatted gestation pens (5m width x 6.6m length) had 12 individual free access feeding/lying stalls (0.55m width x 2.3 length) with a gate at the back end of the stall. All rear gates of the stalls in a pen can be opened and closed simultaneously by using a valve lever. Sows were free to move around the remainder of the pen (roaming area behind feeding stalls: 2.7m width x 6.6m length). Sows were therefore housed in groups of 12 sows/pen, with a floor space allowance of 2.75m2/sow. One block of wood on chain and one chain suspended within the group area were provided as enrichment. Drinking water was available ad libitum from 3 nipple drinkers per pen. Sows were fed a liquid gestation diet (Table 2) twice per day.

Table 2. Ingredient and chemical composition of the gestation diet.

INGREDIENT	**%**
Barley	41.1
Maize	7
Wheat	23
Suguar beet pulp	4
Hi pro soya	15
Soya hulls	4
Soya oil	2.5
Min	2.4
COMPOSITION	
C Protein	15.6
C Oil	4.9
C Fibre	5.3
C Ash	5.7
De	13.3
Lysine	0.9
M+C	0.53
Threonine	0.61
Calcium	0.95
Av Phos	0.36
Salt	0.58

Data collection

Aggressions

Aggressions were monitored immediately after mixing (T0), 24hr (T1), 8 days (T8) and 3 week post-mixing (T21). All sows were identified by individual marks on their backs. During the observation days, all occurrences of various aggressive behaviours and the identity of the sows involved in the encounter were recorded for 2 hours continuously. The ethogram of the aggressive behaviours which were recorded was adapted from Stewart et al. (Stewart et al., 2008) and is listed in Table 3. The behaviours biting, head knock and chase were merged for the analysis and defined as "aggressive behaviours".

Table 3. Description of the aggressions recorded in the study (adapted from Stewart et al., 2008).

<table>
<tr><td colspan="2">Behaviour</td><td>Description</td></tr>
<tr><td colspan="2">Fighting</td><td>Mutual pushing parallel or perpendicular, ramming or pushing of the opponent with the head, with or without biting in rapid succession. Lifting the opponent by pushing the snout under its body</td></tr>
<tr><td rowspan="3">Aggressive behaviour</td><td>Biting</td><td>Biting any part of another sow, but not as a part of head thrust</td></tr>
<tr><td>Head knock</td><td>Ramming or pushing another sow with the head (with or without biting)</td></tr>
<tr><td>Chase</td><td>Moving rapidly in pursuit of another sow</td></tr>
</table>

Skin lesions

Skin lesions were counted 24hr (T1), 8 days (T8) and 3 weeks post-mixing (T21), following a method validated by Turner et al. (2006). Skin lesions were counted on the anterior (head, neck, shoulders, front legs), middle (flanks, back), and posterior (rump, hind legs), on the left and right sides of the body. Counts included both fresh and old lesions, identified by colour and the estimated age of scabbing, therefore each body region had two counts, one referring to fresh injuries and the other referring to old injuries. All counts were summed to calculate one total fresh and one total old skin lesion count for each sow per inspection.

Locomotion

Locomotion was assessed before mixing (T0, used as a baseline value), 8 weeks post-mixing (T56) and in late pregnancy (T108) using a visual analogue scale (VAS) developed by Lagoda et al. (2021). Animals were encouraged to take at least 6 strides on the fully-slatted, concrete floor of the group pen and an observer scored the locomotory ability of the animals.

Reproductive performance

As indicators of reproductive efficiency, the following data were collected: pregnancy rate (proportion of sows inseminated that resulted in pregnancy at 30 d after a real-time ultrasound examination), farrowing rate (proportion of sows inseminated that farrowed) and litter size (total born, born alive, stillborn and mummified piglets).

Statistical analysis

The statistical analysis were carried out using SAS (SAS Institute, Inc., Cary, NC). Data were analyzed based on their distribution tested by Shapiro-Wilk test for normality. The effects of treatment (PEN2D vs PEN28D) on pregnancy rate and farrowing rate (counting data) were examined using two proportion Chi-square test. The analysis of the number of total born piglets and born alive piglets were carried out using ANOVA model with the treatment and replicates as fixed factors, while the percentage of stillborn and mummified piglets were analysed with a non-parametric test (Mann-Witney test). The aggression (fighting and aggressive behaviour), skin lesions and locomotion were analysed using generalized linear mixed model (PROC GLIMMIX), with days of measure, treatment (PEN2D vs PEN28D) and their interaction as fixed effects and sow as a random and repeated effect. For locomotion scores, the model included the measures at d0 as covariates.

RESULTS

We found that anticipating mixing from day 28 to day 2 had a significant difference on aggression only considering the number of fights recorded at 24 hours post mixing (T1), with PEN2D sows showing a higher number of fights per sow (P=0.04; Table 4). The effect of time followed the same trend in both treatments, with the average number of fights sharply decreased 24 hours after mixing (T1, P<0.0001), while the average number of aggressive behaviour decreased 8 days later (T8, P<0.0001).

There was no effect of treatment on total fresh and old skin lesion counts at any point (P>0.05; Table 4). Fresh lesion counts sharply decreased between T1 and T8 in both treatments (P<0.0001). Sows were considered lame if the VAS score was scored 60 mm or higher (≥ 60) . Locomotion scores were low throughout pregnancy (PEN2D= 9.9 ± 11.8; PEN28D=10.3 ± 14.3), with low occurrence of lameness (PEN2D n = 2; PEN28D n = 3 lame sows). There was no effect of treatment and time on locomotory ability at any point throughout pregnancy (P > 0.05; Table 4).

Table 4. Effect of stage of gestation at mixing (PEN2D=2 days after service and PEN28D=28 days after service) on measurements of aggression (fighting and aggressive behaviour), skin lesion count (fresh and old) and locomotory ability.

Variables		PEN2D	PEN28D	P Value
No. of fighting, per sow/ 2h				
	T0	1.59 ± 0.36	0.88 ± 0.22	0.185
	T1	0.29 ± 0.09	0.05 ± 0.03	**0.036**
	T8	0.14 ± 0.05	0.02 ± 0.02	0.202
No. of aggressive behaviour, per sow/ 2h				
	T0	1.39 ± 0.33	0.76 ± 0.20	0.434
	T1	0.71 ± 0.19	0.26 ± 0.09	0.169
	T8	0.25 ± 0.09	0.17 ± 0.07	0.991
	T21	0.20 ± 0.08	0.11 ± 0.06	0.984
Fresh skin lesion count				
	T1	24.97 ± 2.64	29.49 ± 3.11	0.875
	T8	12.13 ± 1.50	11.36 ± 1.44	0.999
	T21	9.44 ± 1.26	7.84 ± 1.18	0.940
Old skin lesions count				
	T8	26.98 ± 2.12	29.26 ± 2.30	0.884
	T21	23.61 ± 1.90	21.60 ± 1.87	0.876
Locomotion[1], mm				
	T56	9.98 ± 5.53	11.24 ± 6.29	0.992
	T108	11.03 ± 6.10	10.86 ± 6.12	1.000

[1]Locomotion scored using a VAS ranged from 0 mm (perfect) to 150 mm (the most severe impairment possible)

Pregnancy rate and farrowing rate, as well as litter size measurements (total born, born alive, stillbirth and mummified piglets) were not affected by the time of mixing (Table 5).

Table 5. Effect of stage of gestation at mixing (PEN2D=2 days after service and PEN28D=28 days after service) on reproductive performance and litter size.

Measurements:	PEN2D	PEN28D	P Value
Pregnancy rate[1] (%)	83 (60/72)	94 (68/72)	0.06
Farrowing rate[1] (%)	80 (58/72)	88 (64/72)	0.25
Total piglets born[2] (n.)	15.3 ± 0.51	15.6 ± 0.48	0.67
Piglets born alive[2] (n.)	14.2 ± 0.42	14.4 ± 0.40	0.75
Stillbirth[3] (%)	5 (0-11)	7 (0-12)	0.07
Mummified[3] (%)	0 (0-0)	0 (0-0)	0.37

[1] count data were analysed using two proportions z-test
[2] data normally distributed were reported as ls-means ± standard error
[3] data not normally distributed were reported as median (interquartile range)

DISCUSSION

In agreement with Strawford et al (Strawford et al., 2008) and Knox et al. (Knox et al., 2014), we found that the frequency of fights on the first day of mixing was similar for sows mixed at either 2 days or 28 days post-insemination. In contrast Stevens et al. (Stevens et al., 2015) found a reduction in the number of bouts of aggressive behaviour during the day of mixing for sows mixed at day 35 of gestation compared to sows mixed early after insemination, although the duration of bouts was similar. In the present study we also recorded the number of fights 24 hours after mixing and we found that, even if the frequency was lower than the day of mixing for both treatments, it was higher in PEN2D sows. However, in light of results obtained from the skin lesion counts which showed no difference in the number of fresh skin lesions assessed 24 hours post-mixing (and collected after the T1 aggression observations), it could be suggested that the intensity and/or the duration of the encounters were similar between the two treatments, despite the difference in the frequency. Although in the present study the average number of fresh and old skin lesions did not differ at any point between sows mixed in groups early or late in respect of the day of insemination and that fresh lesion counts sharply decreased between T1 and T8 in both treatments, it might be interesting to note that the number of lesions at T8 appears quite high in both treatments. Even if other studies measured skin lesions associated with different grouping times, make a comparison between the numbers in the present study and those of the others appears difficult, as they assessed skin lesions by using score systems (Cunha et al., 2018; Galli et al., 2022; Knox et al., 2014; Li and Gonyou, 2013; Strawford et al., 2008) , and also some papers presented the results as the average of different days(Knox et al., 2014; Strawford et al., 2008). However, comparing fresh and old lesions counts at T8 with the only comparable study (Stevens et al., 2015), it emerged that in the present study the number of lesions was almost three times for fresh lesions and almost twice for old lesions.

The high number of lesions recorded at T8 could explain the lower pregnancy and farrowing rate of PEN2D compared with PEN28D. Indeed, although this difference was not statically significant, it is important to note that the sample size could not be big enough to reach a power suitable to pick up real meaningful differences for these parameters and that these strong trends could have an important impact on the reproductive efficiency of a sow herd. The high number of lesions recorded at T8 in both treatments could be an evidence that the present housing system, the free access feeding stalls pen, could lead to prolonged aggression between sows for the establishment of the social hierarchy. In the free access feeding stall pens, sows are often observed occupying feeding stalls for almost the entire time (Schubbert et al., 2022)(Lagoda under review) due to the absence of a resting area and especially when the loose area is not provide with a concrete floor or comfortable rubber mats (Schubbert et al., 2022), and this presumably leads to a prolongation of the encounters over time. Although in the PEN28D this prolongation did not lead to pregnancy problems as the implantation attachment is completed, in the PEN2D treatment it would mean that the aggressions continued until the critical period of embryo implantation, thus probably causing problems of implantation and maintenance of pregnancy. To support this hypothesis, we found that 6 of the 12 sows of PEN2D which resulted not in pregnancy at 30 d after a real-time ultrasound examination had a higher number of fresh skin lesions at T8 compare to the average, while the 3 sows of the PEN28D who lost the pregnancy after the ultrasound examination at T8 had a lower number of skin lesions compared to the average number. The limited number of studies that investigate the effects of the timing of grouping 3 compared to 28 days post-insemination on farrowing rate reported discrepant results. Some research studies show no effect of grouping 3 compared to 28 days post-insemination(Bampi et al., 2020; Cunha et al., 2018; Galli et al., 2022; Kirkwood and Zanella, 2005; Mheen et al., 2003; Stevens et al., 2015), however the two studies with bigger sample sizes reported lower farrowing rate for sows grouped very soon after insemination compared to sows grouped 4 weeks later (Knox et al., 2014; Li and Gonyou, 2013). Differences among studies in feeding system, pen design, floor space, group size and composition may be responsible for the contradictory findings, as all these factors may affect the duration and the intensity of aggression and thereby influence the level of stress during early pregnancy. The absence of an effect on litter size between the two times of mixing reported in the present experiment is instead shared among all the studies (Cunha et al., 2018; Galli et al., 2022; Kirkwood and Zanella, 2005; Knox et al., 2014; Li and Gonyou, 2013; Stevens et al., 2015).

In the present study locomotory ability of sows did not differ between sows mixed in groups early or late in respect of the day of insemination. Making rigorous comparison between the few studies which measured welfare ABMs related to lameness and associated with grouping early or later after insemination appears difficult, as there was considerable variation in the way in which it was measured, in addition to the differences in floor type and pen design. Li and Gonyou (Li and Gonyou, 2013) measured the percentage of lame sows, and reported no differences in the prevalence of lameness in sows mixed at 2-9 days after insemination or 35 d later. Knox et al. (Knox et al., 2014) evaluated the incidence of lameness and leg inflammation and found that sows mixed 35d post-insemination had a higher percentage of lameness during the first 12 d after mixing, but this incidence and the leg inflammation scores decreased in these sows late after mixing. In sows mixed 3d post-insemination, whereas, the incidence of lameness was lower but, together with leg inflammation incidence, it increased late after mixing. Lastly, Cunha et al. (Cunha et al., 2018) evaluated the frequency of claw lesions and lameness and the culling rate by locomotor problems. The lack of a statistical comparison between treatments in relation to the day after mixing (and not to the day of gestation) makes it difficult to draw conclusions regarding claw lesions and lameness, but the paper reported a greater culling rate by locomotor problems in sows mixed 5-10d after mating compared to those mixed 28-33 after mating.

CONCLUSION

The current study indicates that it is possible to mix unacquainted groups of sows into individual free access feeding stalls pens early after insemination (within 2 days post AI), rather than 28d after, without negative effect on measures of sows welfare (aggressive behaviour, skin lesions and locomotory ability). Considering the negative trend reported in PEN2D for pregnancy and farrowing rate, the effect that this grouping time could have on reproductive performance requires further investigation, with bigger sample size. The free access feeding stalls pens certainly protect sows from aggression while feeding, but more research may provide further clarity on the effects of feeding stalls on the duration of hierarchy formation, and its possible effects on reproductive performances in early mixed sows.

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CHAPTER 5

The role of environmental enrichment and back fat depth in the intensity of aggressive behaviour performed by sows during the establishment of the dominance hierarchy

The role of environmental enrichment and back fat depth in the intensity of aggressive behaviour performed by sows during the establishment of the dominance hierarchy

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ABSTRACT

For sows introduced into new groups, the aggressive behaviour associated with establishing a social hierarchy represents a period of severe stress. The aim of this study was to investigate the effect of providing sows with an improved pen environment (straw in racks and ropes) on aggressive behaviour after mixing and to understand the role played by sow back fat thickness and parity order. At 29 d post-service, sows were mixed into IMPROVED or CONTROL pens with individual feeding stalls (6 groups/treatment, 20 sows/group). Aggressive behaviour was recorded for 2h at mixing (T0) and 24 h (T1) and 3 weeks post-mixing (T21). Overall, the sows in the CONTROL pens performed more fighting behaviour compared to the IMPROVED sows ($p < 0.001$). This difference was significant only at T21 ($p < 0.001$). Additionally, the sows in the CONTROL pens generally initiated more aggressive behaviours than the sows in the IMPROVED pens ($p = 0.02$). The sows with a low back fat thickness initiated more aggressive behaviours, but parity had no significant effect on any of the aggressive behaviours. These results indicate a beneficial effect of improvements to the pen environment on the aggression performed by group-housed sows between the time of mixing and three weeks later. The effect was reduced on the day of mixing, which is in accordance with the necessity for sows to employ aggression to establish the dominance hierarchy.

INTRODUCTION

In current indoor gestation housing systems, sows experience several highly relevant stressors with welfare consequences. The major stressor for pregnant sows is being mixed into new groups (Varley and Stedman, 1994; Verdon et al., 2015). The associated aggression required for sows to establish the social hierarchy often has detrimental implications for sow welfare and production. In light of this and of the in-creased use of group housing systems for sows in response to the forthcoming European ban on stalls, as called for by the European Citizens' Initiative "End the cage age" ("End the Cage Age," 2018), finding methods that reduce this stress would be necessary nowadays.

Most agonistic behaviours resulting from mixing unacquainted groups of pigs occur within the first hours after mixing, and the dominance order tends to be stable within 48 h (Dolf, 1986; Meese and Ewbank, 1973). Agonistic behaviour includes offensive and defensive contact and non-contact events, such as fighting, biting, pushing, and pursuing, as well as communicative elements such as threats expressed through body movements and vocalizations (Lanthony et al., 2022). There are various measures that are effective in mitigating this aggression, such as the use of a specialized mixing pen, increasing the space allowance, or the provision of enrichment materials (EFSA AHAW Panel, 2022). Environmental enrichment effectively reduced post-mixing aggression in weaner and finisher pigs (Beattie et al., 1996; Blackshaw et al., 1997; Olsen et al., 2002; Schaefer et al., 1990). However, the impact of environmental enrichment on sow aggression is less investigated (Galli et al., 2021; Weerd and Ison, 2019), especially around mixing, and the limited literature works that are available report contradictory findings. Previous studies found that the provision of straw in individual racks for sows housed in small static groups with individual feeding cubicles (Stewart et al., 2011) or of point-source materials in floor-fed pens of 12 sows (Greenwood et al., 2019) had no effect on aggression at mixing. Meanwhile, Durrell et al. (Durrell et al., 1997) reported that sows housed in small static groups with individual feeding stalls with spent mushroom compost in suspended racks showed a lower frequency of overall agonistic behaviour on the day of mixing than sows in barren pens. In contrast, Stewart et al. (Stewart et al., 2008) found higher levels of post-mixing aggressive behaviour when sows in a large dynamic group had access to straw provided in a single rack.

Indeed, Commission Recommendation (EU) 2016/336 (European Commission, 2016) recommends that all pigs in a group should be able to access materials that are edible or feed-like, chewable, investigable, and manipulable. Straw is a valued material, as it has all the necessary characteristics to be effective and should satisfy the need to express foraging motivation (Fraser, 1975; Spoolder et al., 1995; Whittaker et al., 1998). However, if it is only provided in limited amounts/circumstances to a large group of sows, it could be perceived as a limited resource and increase aggression be-tween sows (Stewart et al., 2008). In the present study, straw was provided at three different locations in the loose area of a pen where 20 sows each had access to their own feeding stall. In addition, we suspended a length of natural fiber rope in each stall such that all sows in the group had individual access to a pliable and easily destructible material.

Animal factors such as body weight/size and age (e.g., sow parity) influence a pig's position in the dominance hierarchy. Some studies found a positive correlation between social rank and weight and parity (Arey, 1999; Brouns and Edwards, 1994; Martin and Edwards, 1994; Norring et al., 2019), while others found no correlation (Meese and Ewbank, 1973) or a negative correlation (Lanthony et al., 2022). However, only a few studies (Andersen et al., 2000; D'Eath, 2002; Mount and Seabrook, 1993; Strawford et al., 2008) investigated the impact of these parameters on aggressive be-havior, and these report contradictory results. Considering that the individual level of aggressiveness is not necessarily correlated with the pig's dominance rank (Erhard et al., 1997; Meese and Ewbank, 1973), there is a need to better determine the role of animal factors in sow aggressive behaviour.

We hypothesized that sows housed in pens and provided with straw in racks and manila ropes, in addition to two blocks of wood and two chains, would engage with these materials, which would function as distractions to reduce aggressive behaviour during hierarchy establishment. To test the above hypothesis, aggressive interactions were recorded immediately after mixing and 24 h and 3 weeks post-mixing. Moreover, we also investigated the role played by back fat thickness and parity order on the level of aggressive behaviour and potential interactions with environmental complexity.

MATERIAL AND METHODS

Animals and Treatments

The study was carried out on a commercial 2000-sow farrow-to-finish farm in Co. Cork, Ireland, as part of a larger study described by Lagoda et al. (under review). The study was performed in six replicates using batches of sows weaned between July 2021 and November 2021. In total, 240 Large White X Landrace sows were used in the study. The sows were artificially inseminated in stalls in the service house within 24 h of dis-playing signs of oestrus post-weaning and remained in the stalls without environ-mental enrichment until day 28 post-insemination. On day 25 post-insemination, 40 sows within the replicate were selected for the experiment in such a way that both treatments were balanced according to the parity order (parity 1–5, mean ± standard deviation; 2.4 ± 1.03) and back fat thickness. The experiment started on the day that the sows were moved to gestation pens and mixed (day 28.9 ± 0.37 post-insemination) into one of the two following experimental treatments:

CONTROL: A total of 20 sows per replicate were moved to a fully slatted gestation pen with two rows of ten individual free-access feeding stalls, in which two blocks of wood on chains and two simple chains were provided as enrichment in the middle of the loose slatted area. IMPROVED: A total of 20 sows per replicate were moved to similar pens but in which the floors of the feeding stalls were covered with rubber mats (EasyFix Rubber Products, Ballinasloe, Galway, Ireland). In the middle of the loose area, there were two blocks of wood on chains and two simple chains, and a portion of the floor was covered with rubber mats, in the middle of which a rooting tower holding straw was mounted. A straw rack was also mounted on the gates of the pen at either end of the loose area and suspended above a steel collection plate on the floor. Manila ropes (1 m manila rope; Marine Suppliers & Co., Ltd., Howth, Dublin, Ireland) were suspended at a height of one meter within each feeding stall. The racks were filled with straw each day throughout the trial, while manila ropes were replaced as often as necessary to provide continuous access.

In each pen, the space allowance per sow was 2.62 m^2/sow, and 20 individual free-access feeding/lying stalls (0.55 m width × 2.3 m length) were available. The sows were free to move around the remainder of the pen (7.2 m width × 7.3 m length; loose roaming area between two rows of feeding stalls: 2.7 m width × 7.3 m length), and they had free access in and out of the feeding stalls through a rear closing gate which could also be locked into position (open or closed) using a valve lever.

The sows were fed a standard, restricted gestation diet twice daily (Table 1), while drinking water was available ad libitum with a ratio of 3 drinkers/20 sows per pen.

All sows were maintained in their treatment group until approximately 110 d of gestation, when they were moved into the farrowing crates.

Table 1. Ingredient and chemical composition of the gestation diet.

INGREDIENT	%
Barley	41.1
Maize	7
Wheat	23
Suguar beet pulp	4
Hi pro soya	15
Soya hulls	4
Soya oil	2.5
Min	2.4
COMPOSITION	
C Protein	15.6
C Oil	4.9
C Fibre	5.3
C Ash	5.7
De	13.3
Lysine	0.9
M+C	0.53
Threonine	0.61
Calcium	0.95
Av Phos	0.36
Salt	0.58

Aggressive Behaviours

Aggressive behaviours were monitored by two trained observers, who alternated between the two treatments in each replication, at three times: immediately after mix-ing (T0), 24 h post-mixing (T1) and 3 weeks post-mixing (T21). All sows were identified by individual marks on their backs. Before the observations commenced, the animals were allowed 10 min to get used to the presence of the observer, who was positioned in the corridor outside the pen. During the observation days, all occurrences of various aggressive behaviours and the identities of the sows involved in the encounters were recorded for 2 h continuously from 8:00 to 10:00. The list of aggressive behaviours recorded was adapted from Stewart et al. (2008) [17] and is summarized in Table 2. For each behaviour, except for fighting, the sows receiving the aggressive behaviour were al-so identified. The aggressive behaviours of biting, head knock, and chase were merged for the analysis and defined as “initiated” behaviours, while bitten, head knocked, and chased were considered as “received” behaviours.

Table 2. Description of aggressive behaviours recorded in the study (adapted from Stewart et al., 2008).

Behavior		**Description**
Fighting		Mutual pushing parallel or perpendicular; ramming or pushing of the opponent with the head; with or without biting in rapid succession. Lifting the opponent by pushing the snout under its body
Initiated	Biting	Biting any part of another sow, but not as part of a head knock
	Head knock	Ramming or pushing another sow with the head (with or without biting)
	Chase	Moving rapidly in pursuit of another sow
Received	Bitten	Being bitten by another sow, but not as part of a head knock
	Head knocked	Being rammed or pushed by another sow with the head (with or without biting)
	Chased	Moving rapidly/running away from another sow
Threat		Interaction expressed through body movements without physical contact, with a sow actively withdrawing (avoid)
Avoid		Active withdrawal of a sow being threated without physical contact

Back Fat Thickness

The back fat thickness (BFT) was recorded at 25 days post-service. BFT was measured at the last rib, 6 to 7 cm off the midline on the left and right side (P2 position), using an ultrasonic scanner (Lean-meter, Renco, Minneapolis, MN, USA).

Statistical Analysis

All the behaviours recorded (fight, initiated and received aggressive behaviour, threat, and avoidance) were calculated as the number of events per hour. For the purposes of the analysis, the sows were categorized on the basis of the BFT of the sows within each pen in “LOW”, “MEDIUM”, and “HIGH” sows. “MEDIUM” sows were the animals that belonged to the interquartile range of their pen (8.5–14.5 mm), while “LOW” and “HIGH” sows were the animals that belonged to the lower (5.5–10 mm) and upper (12.5–21 mm) quartiles of their pen, respectively. Regarding parity order, the sows were divided into young (parity 1–2) and old (parity 3–5).

The statistical analyses were carried out using the software package SAS (SAS Institute, Inc., Cary, NC, USA). Normality tests of data distribution and residuals were performed for every variable evaluated with the PROC UNIVARIATE using the Shapiro–Wilk test. None of the variables recorded in the study were normally distributed; therefore, the GENMOD procedure with Poissson distribution was used for the data processing. The model considered the pen as the experimental unit, while the replicate, day of measure, treatment (control vs. improved), back fat thickness (low vs. medium vs. high), and parity order (young vs. old) and their interaction were considered as fixed effects. The pen by day was considered as a repeated effect. For all statistical tests, the significance level was established at $p < 0.05$.

RESULTS

Overall, there was an effect of treatment on the average number of fights, with the sows in the control pens performing more fighting behaviour compared to the improved sows ($p < 0.001$). Within time, this difference was significant only at T21 ($p < 0.001$, Figure 1). Moreover, there was an overall effect of time, with more fights on T0 compared to T1 and T21 ($p < 0.001$). However, in the control pens, a similar number of fights were recorded on T1 and T21, whereas the number of fights decreased significantly between T1 and T21 in the improved pens ($p < 0.001$; Figure 1).

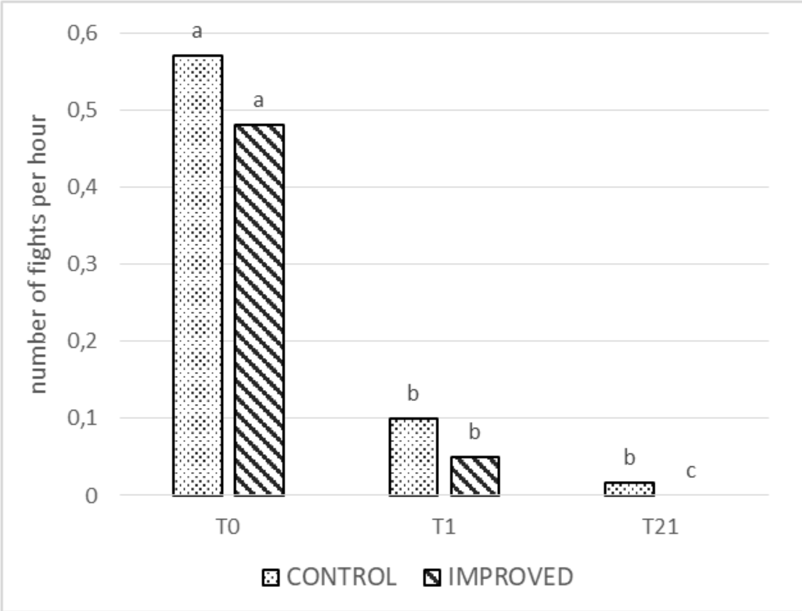

Figure 1. Number of fights per hour in the CONTROL and IMPROVED pens on the day of mixing (T0), the day after mixing (T1), and three weeks later (T21). a, b, c: Different letters indicate significant differences ($p < 0.05$) between the treatment and time.

There was an effect of treatment on the average number of initiated aggressive behaviours, being higher in the control pens compared to the improved pens ($p = 0.02$; Figure 2). There was no treatment effect on threats and no interactive effects for either variable ($p > 0.05$).

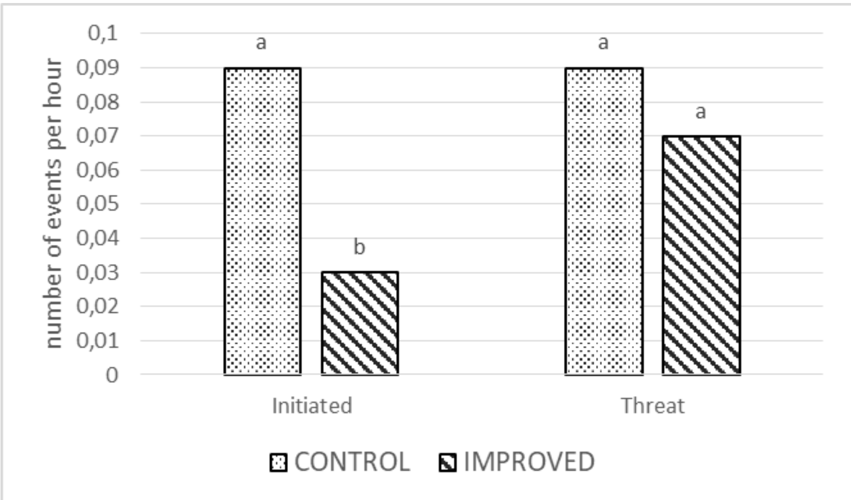

Figure 2. Number of initiated aggressive behaviours and threats per hour in the CONTROL and IMPROVED pens. a, b: Different letters indicates significant differences ($p < 0.05$) between treatments.

Moreover, there was an effect of time on both initiated aggressive and threat behaviours. There were more initiated aggressive behaviours on T0 than on any other day (p < 0.001; Figure 3). There were more threat behaviours on d0 and d21 (p = 0.002; Figure 3).

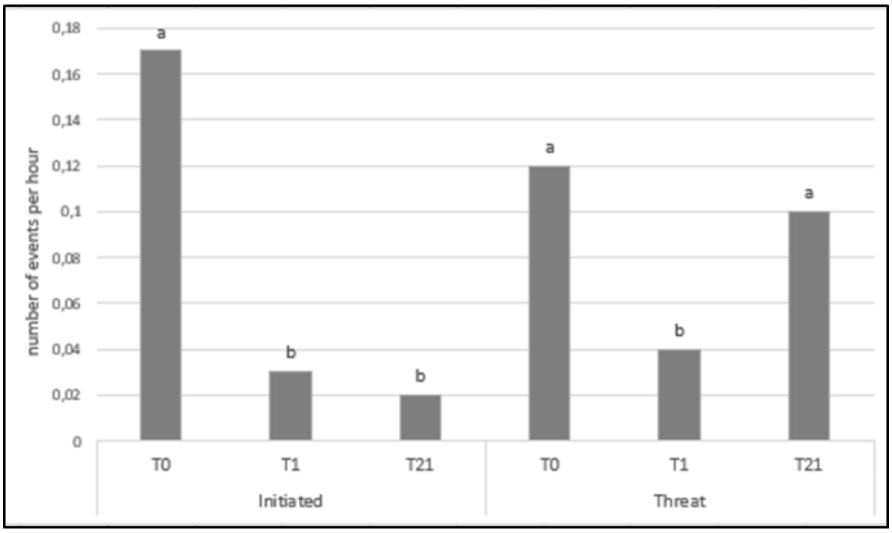

Figure 3. Average number of initiated aggressive behaviours and threats per hour according to the time after mixing: the day of mixing (T0), the day after mixing (T1), and three weeks later (T21). a, b, c: Different letters indicate significant differences (p < 0.05) over time.

There was no interaction between treatment and back fat thickness regarding the number of any of the different aggressive behaviours (p > 0.05). However, regardless of the environmental complexity, there was an effect of back fat thickness on the expression of initiated aggressive behaviours (Table 3), which were performed more by low-BFT sows (p < 0.0001).

No interaction was found between treatment and parity order. Regardless of the environmental complexity, the parity order did not have a significant effect on the dis-play of any of the aggressive behaviours (p > 0.05).

Table 3. Number of aggressive behaviours per hour according to back fat thickness (low, medium, and high).

Behavior	Back Fat Thickness			*p*
	Low	Medium	High	
Fight	0.003 ± 0.001	0.002 ± 0.000	0.002 ± 0.000	NS
Initiated	0.13 [a] ± 0.03	0.06 [b] ± 0.01	0.02 [b] ± 0.01	<0.001
Received	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.02	NS
Threat	0.10 ± 0.02	0.05 ± 0.02	0.09 ± 0.02	NS
Avoid	0.05 ± 0.02	0.09 ± 0.01	0.06 ± 0.02	NS

a, b: Different letters within a row indicate significant differences between means.

DISCUSSION

The present data support our hypothesis that improvements to the environment of a group housing system with free-access stalls reduces aggressive behaviour including fighting, likely mediated by the distraction provided to the sows by good environ-mental enrichment. Given the need for sows to employ aggression to establish the dominance hierarchy, the reduced effect on aggression on the day of mixing is perhaps unsurprising.

Regarding fighting behaviour, the comparison between treatments revealed significant differences overall and specifically on d21, confirming the hypothesis that the sows in the improved pens would fight less frequently than the sows in the control pens. However, on d0 and on d1, despite the sows performing numerically fewer fights, the difference was not significant. This is likely because the dominance hierarchy was still in formation and is in line with previous research with sows (Durrell et al., 1997; Greenwood et al., 2019; Stewart et al., 2011). These studies and the current study confirm that, irrespective of the housing system, fighting at mixing is mostly unavoidable, and it is an essential component of the establishment of the dominance hierarchy (Stewart et al., 2011).

Indeed, given the evolutionary importance of the dominance hierarchy to group stability (Meese and Ewbank, 1973), it is not surprising that it takes precedence over other less valuable activities, such as, in this case, interacting with the enrichment materials or even eating the straw. This is also in spite of the sows used in the study not having prior experience with such materials, which would likely have heightened their interest in them (Day et al., 2002). Our findings suggest that it is not possible to influence sows fighting at mixing by providing these materials as a distraction (Greenwood et al., 2019).

However, it is important to note that, 21 days after mixing, there were significantly more fights in the control pens than there were in the improved pens, and the reduction in the frequency of fights between day 1 and day 21 was faster in the latter pens. This indicates that environmental enrichment has an important role later, when the social hierarchy was established. This is in contrast to Durrell et al. (1997) and Greenwood et al. (2019), who found no differences between barren and enriched pens in terms of the number of fights observed between day 2 and day 14/20 after mixing. The type and the number of materials provided may be responsible. In the study of Greenwood et al. (2019), no foraging substrate, such as straw, was provided, and the number of ropes was lower than the number of sows in the pen, probably decreasing the attractiveness of this enrichment material. Additionally, Durrell et al. (1997) only used one form of enrichment, whereas both straw and natural fiber ropes were used in the current study.

As already shown by previous studies (Durrell et al., 1997; Greenwood et al., 2019), it is clear that, regardless of the material provided, fights are frequent on the day of mixing and decrease considerably thereafter. This result is consistent with other findings, namely, the finding that, in domestic sows, the dominance order tends to stabilize within 24–48 h of mixing (Meese and Ewbank, 1973; Mount and Seabrook, 1993).

Within time, no significant differences were found between treatments in terms of initiated aggressive behaviours. However, the overall number of these behaviours was significantly lower for sows in improved pens. In contrast, Greenwood et al. (2019) found that the material provided (ropes, plastic disk swings, and rubber mats) had no effect on bites and head knocks performed by sows. Durrell et al. (1997) found a significant difference on day 1 after mixing in the frequency of agonistic behaviour, particularly the behaviour “head-thrusting”, with the sows in the barren pens showing a higher frequency than the sows in the enriched pens. Although fighting and aggressive behaviours were not distinguished separately by Stewart et al. (2008, 2011), they showed that the provision of straw to sows in large dynamic groups increased the average proportion of aggressive behaviour, while access to straw had no effect on the occurrence of aggressive behaviour in the post-mixing period in small static groups. Regard-less of the experimental treatments, initiated aggressive and threat behaviours showed a different trend over time. Initiated aggressive behaviour decreased soon after the day of mixing, while threat behaviours showed the same frequency on d1 and on d21. These findings suggest that once the social hierarchy is established, sows replace aggressive behaviour with threat displays.

Regardless of the environmental complexity, there was an effect of back fat thick-ness on the expression of aggressive behaviour only for initiated aggressive behaviours, which were performed more by low-BFT sows. This finding is in contrast with the few studies that investigated the role of body condition in determining the outcomes of aggression in pigs. Indeed, Andersen et al. (2000) and D’Eath et al. (2002) reported that heavier pigs initiated more aggressive acts and were more involved in fighting. Conversely Mount et al. (1993) found no correlation between the number of aggressive interactions initiated and received and body weight. So far, the scientific literature has mainly focused on the correlation between weight and the position of animals within the dominance hierarchy based on the success in winning agonistic interactions (Arey, 1999; Brouns and Edwards, 1994; Lanthony et al., 2022; Martin and Edwards, 1994; Norring et al., 2019). The limited number of studies that have investigated the correlation between body condition and frequency of aggressive behaviour makes it difficult to find an explanation as to why low-BFT sows initiated more aggressive behaviour. However, a possible explanation could lie in the fact that fatter sows may engage in less aggressive behaviour because their size alone scares the other sows away.

Regardless of the environmental complexity, the parity order did not have a significant effect on the display of any of the aggressive behaviours. This is in line with the findings of Mount et al. (1993). By contrast, Strawford et al. (2008) found that old sows were involved in a greater number of aggressive encounters than young and intermediate sows. In light of the small differences found in the present study in terms of the frequency of aggressive behaviour depending on back fat thickness and parity order, it seems that, in pens with free-access, full-length individual feeding/lying stalls, these intrinsic factors have a marginal impact on aggressive behaviour.

CONCLUSION

This study confirms the benefit to sow welfare, through reduced aggression, of improving the housing environment. The benefit was likely largely driven by the sows' interest in the substrates provided. The findings indicate that aggression at mixing is unavoidable, as it is essential for the establishment of the dominance hierarchy and group stability, but enrichment materials could at least have an effect on reducing its frequency. The current study indicates that the body condition (measured as back fat thickness) and parity do not have a great impact on sow social behaviour, even though the thinnest sows were those that most frequently performed initiated aggressive behaviours.

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CHAPTER 6

General conclusion

GENERAL CONCLUSION

Outcomes of the systematic review revealed that the scientific research on the welfare of gestating sows requires more attention, especially on topics such as the time of mixing sows into groups after service, group pen management strategy and the provision of environmental enrichment, which are less investigated but particularly important giving the current review of EU animal welfare legislation which will most likely further increase group-housing for pregnant sows.

Results of the study suggest that it is possible to reduce the time in which sows are motor and social deprived in gestation stalls without negatively affecting their welfare and litter size by grouping them immediately after mixing (2-4 days after service) either into floor feeding or individual feeding stalls group systems. As regards the effect that early grouping could have on reproductive performance, results show that, in groups with floor feeding systems, pregnancy and farrowing rate were not affected by stage of gestation at mixing, while in groups with individual feeding stalls a lower trend of these parameters were found in sows mixed early after insemination. A possible explanation could lie in the fact that the free access feeding stalls could lead to a prolongation over time of the aggression between sows, as they often occupied the feeding stalls, thus postponing until the critical period of embryo implantation the encounters necessary to establish the dominance hierarchy.

In order to reduce the detrimental effects of aggressive behaviour, attractive environmental enrichments, like straw and natural ropes, seems to have a reduced effect on the day of mixing when sows have to establish the dominance hierarchy, but seems to be beneficial in reducing the overall frequency of aggressions the days after.

In conclusion, aggression at mixing is unavoidable as it its essential to ensuring the establishment of the dominance hierarchy and thereby achieving group stability, but it is possible to find the optimal mixing time, group system and housing environment such that the reduction of the time sows are confined in gestation stalls does not negatively affect sows' welfare and performance.