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Abstract

This study investigated how co-mingling of non-littermates before weaning and feed familiarity at weaning in a 2 × 2 factorial design can minimize the adverse effects related to conventional weaning. Before weaning, piglets were either conventionally reared or co-mingled by grouping piglets from 3 litters during 10 days before weaning. At weaning, piglets from 3 litters were distributed over 3 pens in the nursery. Conventionally reared piglets from 3 litters were mixed, whereas the co-mingled piglets were housed with those they were co-mingled with before weaning. At weaning, piglets were either or not provided with 0.538 kg creep feed per piglet in creep feeders, next to the weaner diet. Creep feed was the same provided the last 4 days before weaning. Hence, the treatment groups were conventional with (n=9) or without creep feed (n=9), and co-mingling with (n=9) or without creep feed (n=9). Co-mingling non-littermate piglets did not affect weaning weight, daily weight gain and creep feed intake during d-

10-d0 before weaning. Co-mingling resulted in higher skin lesion scores of head and ears in piglets at d1 before weaning ($P < 0.05$), but in lower skin lesion scores at the snout, shoulders and flanks at d2 post-weaning compared to conventionally reared piglets ($P < 0.05$). Co-mingling did not affect salivary cortisol at d1 post-weaning . Hematocrit, hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin were higher in the co-mingled piglets at d2 post-weaning ($P < 0.05$). Co-mingling resulted in higher glucose concentrations in blood ($P < 0.05$) and in a tendency for lower NEFA concentration in serum at d2 post-weaning ($P < 0.10$). Feed familiarity at weaning increased average daily feed intake and weight gain during the first 5 days post-weaning ($P < 0.05$). During d5-14 post-weaning average daily feed intake tended to be lower ($P < 01.0$) and daily gain was lower ($P < 0.05$) in creep feed fed piglets compared to no creep feed fed piglets. Nevertheless, feed familiarity did not affect overall piglet performance during the first 2 weeks after weaning. Feed familiarity did not affect blood hematology and biochemical blood parameters, except the level of basophils at d2 post-weaning. Neither housing nor feeding strategy affected piglet performance between d14-42 post-weaning and ear and tail damage at d42 post-weaning . In conclusion, pre-weaning co-mingling of piglets and feed familiarity at weaning each contributed to a better adaptation of the weaning process but did not affect long-term piglet performance.

Keywords: Co-mingling, piglet, weaning, feed familiarity, creep feed

Introduction

Abrupt weaning of piglets, as is practised in current pig production, is a critical event for the piglet since multiple stressors culminate. The pig is removed from maternal care, switches from highly digestible milk to solid feed based on vegetable ingredients and devoid of protective antibodies, is regrouped with non-littermates and is transferred to a new environment. These events around weaning can be detrimental for animal performance and health (Campbell et al., 2013; Lallès et al., 2004). Continued feed intake around weaning is of paramount importance to maintain gut integrity (Bruininx et al., 2002). However, it remains a challenge to stimulate feed intake of piglets in the immediate post-weaning phase. Piglets consume little or nothing during the first days after weaning (Bruininx et al., 2001; Campbell et al., 2013). Energy needs for maintenance of weaned piglets are often not met until day 5 after weaning (Le Dividich and Herpin, 1994). It is therefore important that piglets consume feed immediately after weaning. It is hypothesized that by minimizing stressors at weaning the adverse effects on piglet feeding and social behaviour, performance and health can be reduced. A management strategy such as co-mingling of non-littermates before weaning focusses on the social development of piglets which may facilitate the social integration at weaning (Kanaan et al., 2012). This has been recently reviewed by Van Kerschaver et al. (2023). Indeed, after weaning piglets will not be regrouped with unfamiliar piglet, thus reducing social stress. Co-mingling unfamiliar piglets before weaning might also result in more robust, immunocompetent and mature piglets at weaning since contact with foreign antigens at a young age appears to be beneficial for the maturation of the immune system (Round and Mazmanian, 2009; van Nieuwamerongen et al., 2015). Moreover, pre-weaning co-mingling of non-littermates might also stimulate explorative and feeding behaviour of piglets (van Nieuwamerongen et al., 2015). By providing the same diet as given before weaning for the first days after weaning, feeding-related stress at weaning might be reduced and feed intake might be stimulated to overcome the acute weaning phase (Heo et al., 2018; Huting et al., 2021). Therefore, this research aimed to investigate the relative importance of a changing social environment and feeding scheme on the weaning transition. More specifically, the objective was to evaluate how these factors affect feed intake, animal behaviour and animal health during the immediate post-weaning phase, and if this impacts on animal performance up till 6 weeks post-weaning. It was hypothesized that additionally providing the same feed before and after weaning stimulates the immediate post-weaning feed intake, but moreover that social learning is responsible for promoting the transition for a creep feed diet to a weaner diet when the opportunity is presented (i.e. piglets that are co-mingled before weaning and hence are familiarized with each other, and have to opportunity to transiently shift from a creep feed diet to a weaner diet upon weaning).

Material and methods

The study was conducted in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes and by the Belgian royal decree (KB29.05.13) on the use of animals for experimental studies. The study was approved by the Ethics Committee for Animal Research of the Faculty of Veterinary Medicine and Bioscience engineering of Ghent University, Belgium (Ethical Approval Code: 2020-028).

Animals and housing

The study was conducted at a 220-head commercial sow farm (Bissegem, Belgium) from June until August 2020. The farm was operating in a 4-week batch system with alternating weaning schedule to accommodate an average weaning age of 23 days. The sows in this study had an average parity of 3.8 ± 2.3 (mean ± std), ranging from parity 1 to 9 and hence mirroring the parity distribution of the farm. A total of 36 primiparous and multiparous DanBred sows and their litters (461 piglets; Piétrain × Danbred) were studied in 2 batches. One week before expected farrowing sows were moved into conventional farrowing accommodation. Sows and their litters were housed in 2 separate farrowing rooms, each consisting of 14 individual farrowing pens, divided by a central feeding passage with 7 farrowing pens located on both sides. The farrowing pens (1.8 m x 2.8 m) contained a farrowing crate with partly solid and slatted floor underneath. Further, the farrowing pens had a heated piglet resting area and fully slatted floors in the rest of the area. At day 2 postpartum, equivalent to day 20 before weaning piglets received an iron injection (iron dextran, 200 mg/ml, Uniferon, Pharmacosmos A/S, Denmark) and were cross-fostered in order to equalize litters to 13-14 vital piglets. Cross-fostering exclusively entailed the removal of supernumerary piglets to foster sows outside the experiment. Piglets were tail docked, treated with toltrazuril (Cevazuril, 50 mg/ml oral suspension, CEVA Santé Animale N.V., Belgium) and vaccinated for Mycoplasma hyopneumoniae (Stellamune One, Elanco GmbH, Germany) and Shiga toxin-producing *Escherichia coli* (Ecoporc Shiga, IDT Biologika GmbH, Germany) at day 4 postpartum or day 18 before weaning. Sows and their litters were selected and allocated to the treatments on day 12 postpartum or day 10 before weaning based on litter piglet body weight (mean ± s.d., 3.42 ± 0.42 kg), litter size (mean ± s.d., 12.8 ± 0.7 piglets), age of the piglets (mean ± s.d., 11.8 ± 0.8 days) and parity (mean ± s.d., 3.75 ± 2.27). In the farrowing room, sows were fed a commercial transition diet from day 7 pre-partum until day 2 postpartum, and a commercial lactation diet from day 2 postpartum until weaning. Piglets were provided a milk replacer during day 2 – 5 postpartum or day 20 – 17 before weaning, and liquid feed during day 5 – 12 postpartum or day 17 – 10 before weaning. Furthermore, piglets were offered commercial solid creep feed in a round creep feeder located at the anterior side of the sow during d-10-d-4 before weaning (Table 1). Water was available *ad libitum*. At 22 days of age, a total of 432 piglets were weaned and assigned to 36 pens in the nursery unit. In each pen 12 piglets were housed. The pens (2.20 m x 1.32 m) were equipped with partly slatted and solid flooring, a three-space dry feeder, a nipple drinker and environmental enrichment (i.e. movable chain). Piglets were vaccinated for Porcine Circovirus Type 2 (CircoFlex, Boehringer Ingelheim Vetmedica GmbH, Germany) at d5 post-weaning. The piglets had *ad libitum* access to feed and water. Piglets were fed a commercial weaner diet during d0-14 after weaning and a commercial starter diet during d14-42 after weaning. Details about the composition of the diets can be found in Table 1.

Experimental design

The study included 2 factors, i.e. housing and feeding familiarity, arranged in a 2 × 2 full factorial design. With regard to housing, piglets were either kept under conventional conditions until weaning, i.e. littermates stayed together in the farrowing pen and had no contact with other litters, or piglets from 3 litters were co-mingled from day 10 before weaning (d-10) until weaning (d0) by removing the solid partitions between 3 farrowing pens. The treatments (conventional versus co-mingling) were allocated to the sows in order to stratify for sow parity and the age and mean litter body weight. At weaning, piglets from 3 litters were mixed and distributed over 3 pens in the nursery. Conventionally reared piglets were mixed with unfamiliar litters, whereas piglets from the co-mingling systems were mixed with those they were co-mingled with prior to weaning. Allocation of the piglets to the nursery pens within a group of 3 litters was done according to body weight, sex and sow, in such a way that 3 to 5 piglets from each sow were represented in one pen of the nursery. Each nursery pen contained 12 piglets in total.

Feed familiarity refers to the fact whether the same diet was provided before and after weaning or not. From weaning, piglets were either or not offered 0.538 kg creep feed per piglet (i.e. extra cost: € 0.30 per piglet based on the price difference between the weaner diet and creep feed diet and assuming that the total feed intake remains the same) in creep feeders (same creep feeders used in the farrowing pens), next to the weaner diet. The piglets were offered creep feed which was the same provided the last 4 days before weaning (Table 1). Important to mention is that piglets had free access to both the creep feed and weaner feed in these groups. Piglets that were not offered creep feed were fed the weaner diet only. The 4 treatments were thus as follows: (1) conventional housing – creep feed, where conventionally housed piglets were mixed with non-littermates at weaning and creep feed was provided, next to the weaner diet; (2) conventional housing – weaner diet, where conventional housed piglets were mixed with non-littermates at weaning and were fed the weaner diet only; (3) co-mingling – creep feed, where co-mingled piglets were mixed at weaning with those they were co-mingled with prior to weaning and creep feed was provided, next to the weaner diet; (4) co-mingling – weaner diet, where co-mingled piglets were mixed at weaning with those they were co-mingled with prior to weaning and were fed the weaner diet only. All four treatments were replicated in 9 pens with 12 piglets per pen.

Performance and health

Functional teats of the sows were recorded at d-10 and d-1 before weaning by scoring systems adapted from van der Peet-Schwering et al. (2015). This entailed counting the number of active mammary gland (i.e. pronounced visual develop of the gland) and excluding teats with a damaged milk duct. Piglets were individually weighed at d-10, d-1 before weaning and d5, 14 and 42 post-weaning. Creep feed intake of suckling piglets was recorded from d-10 before weaning until weaning and feed intake of nursery piglets was registered for d0-42 post-weaning. More specifically, feed intake post-weaning was registered at pen level by weighing the weaner feeders, creep feeders (i.e. in the treatments where creep feed was provided after weaning) and residual feed daily during d0-10 and on d12, 14 and 42 post-weaning. Differences in body weight, average creep feed intake, cumulative creep feed intake, average daily feed intake, cumulative feed intake and feed to gain ratio were calculated. Mortality and individual use of medical treatments of animals were registered. A faecal consistency score of piglets was assessed visually per pen daily during d-10 – d0 before weaning and d0-14 post weaning (score 0: no faeces visible; score 1: hard or slightly moist faeces; score 2: moist or soft faeces; score 3: watery or liquid faeces, indicative for diarrhoea). The highest faecal consistency score per pen was registered and used for calculations. When faecal consistency score 3 was found, individual pigs showing diarrhoea, i.e. wet, irritated backsides, were counted per pen for calculating diarrhoea incidence.

Saliva collection and cortisol analysis

Samples of saliva were collected at d1 post-weaning by allowing piglets to chew on synthetic sponges (Salivette®, Sarsted, Nümbrecht, Germany) fixed to the solid partitions of the pens by ropes until they were thoroughly moistened to absorb enough saliva. Samples were centrifuged immediately at 3000 × g for 10 min and then stored at -80 °C until further analysis of cortisol concentration. This was determined in triplicate using a commercially available cortisol saliva ELISA kit (no. RE52611, IBL-International, Hamburg, Germany) validated for pig saliva (Thomsson et al., 2014). Intra- and inter-assay coefficients of variation were < 10 %.

Blood sampling and analysis

Blood was collected by cranial caval vein puncture of 4 randomly selected piglets per pen at d2 post-weaning. NaF and EDTA whole blood samples were delivered to Animal Health Care Flanders (DGZ, Torhout, Belgium) for analysis of glucose concentration and hematology, respectively, following routine procedures. Serum was collected from clotted blood samples after centrifugation (3000 × g, 10 min) and was analysed for non-esterified fatty acids (NEFA), alkaline phosphatase activity and creatine kinase activity by Animal Health Care Flanders (DGZ, Torhout, Belgium) following routine procedures. The NEFA concentration was measured using a Gallery™ Discrete Analyzer (ThermoFisher Scientific, MA, USA) and Randox kits (no. FA115, Randox Laboratories Ltd, Ibach, Switzerland). Glucose, alkaline phosphatase activity and creatine kinase activity were determined with the same equipment using ThermoFisher

Scientific kits (no. 981779, no. 981832 and no. 981829, respectively, ThermoFisher Scientific, MA, USA). Hematological indices were determined using an IDEXX ProCyte Dx hematology analyzer (IDEXX Laboratories, Inc. Westbrook, ME, USA).

Skin lesions and damage

Skin lesions on the body of piglets were scored at d-1 before weaning and d2 post-weaning, based on protocols adapted from Kutzer et al. (2009), Parratt et al. (2006) and van der Peet-Schwering et al. (2015). Skin lesions at the snout were scored by a scoring system that ranged from 0 to 2 (0 = no scratches; 1 = only a few small scratches (≤ 5 mm); 2 = many small scratches or a number of larger scratches). Skin lesions at the head and ears and skin lesions at shoulders and flanks were scored by a scoring system from 0–3 (0 = no skin lesions; 1 = less than 5 superficial lesions (skin unbroken); 2 = 5 to 10 superficial lesions or less than 5 deep lesions (skin broken and evidence of haemorrhage); 3 = more than 10 superficial lesions or more than 5 deep lesions). Skin lesion scores were assessed on both the left and right side of the body and averaged separately for each body part of the piglet. Damage on ears and tail of the piglets were scored at d42 post-weaning, based on protocols from van Nieuwamerongen et al. (2015) and van Nieuwamerongen et al. (2017). Ear damage was scored by a scoring system that ranged from 0 to 4 (0 = no damage; 1 = small bite mark(s), ear is intact; 2 = small wound(s), ear is intact; 3 = medium-sized wound(s), ear is intact; 4 = severe wound(s), part of the ear is removed). Damage scores were assessed on both ears and averaged separately for each ear of the piglet. Tail damage was scored by a scoring system from 0-3 (0 = no tail damage; 1 = small bite mark(s), tail in intact; 2 = small wound(s), tail is intact; 3 = medium-sized wound(s), part of the tail is removed). Tail damage scores were averaged.

Statistical analysis

Data were analysed with IBM SPSS Statistics version 27.0 (SPSS Inc., Chicago, IL, USA). Data were tested for normality by Shapiro-Wilk test. Robust test of equality of means using Welch test was used for normally distributed heteroscedastic data, while and non-parametric tests using Kruskal-Wallis test was applied in case of non-normal data. When normality and homoscedasticity assumptions were satisfied, data were analysed as described below. For the statistical analysis of pre-weaning data, 3 sows and their litters in adjacent farrowing pens were considered the experimental unit for the co-mingling treatment (i.e. 6 replicates). Sow and respective litter in a single farrowing pen was the experimental unit for the conventional housing treatment (i.e. 18 replicates). During the experiment one sow from the co-mingling treatment died for unknown reasons and was replaced by another sow. Therefore, data related to this sow was excluded and data from the remaining 2 sows were considered the experimental unit. Analysis of variance (ANOVA) was performed using the General Linear Model module with housing as the fixed factor and batch as the random factor. For the statistical analysis of post-weaning data, pen was the experimental unit (i.e. 9 replicates per treatment). Analysis of variance (ANOVA) was performed using the General Linear Model module with housing, feeding familiarity and their interaction as fixed factors and batch as the random factor. Analysis of variance (ANOVA) was performed for average daily and cumulative creep feed intake post-weaning using the General Linear Model module with housing strategy as fixed factor and batch as the random factor. Robust test of equality of means using Welch test and non-parametric tests using Independent-Samples Kruskal-Wallis test were applied if appropriate. Data are presented as raw means with the standard error of the mean (SEM) in tables and standard error (SE) in figures. The data showed a normal and homoscedastic distribution unless stated otherwise in the tables or figures, and were consequently handled by the appropriate statistical tests as described above. Differences were considered significant at $P < 0.05$ and tendency was considered at $P \geq 0.05$ to $P < 0.10$.

Results

Pre-weaning

Animal performance and health

No significant differences in functional teats of sows from the conventional housing and co-mingling system were observed on day 10 before weaning (13.7 and 14.0 respectively) and day 1 before weaning (13.3 and 13.4 respectively). Medical treatments of individual sows during d-10-d0 were limited and did not differ between housing systems (conventional 1.11 vs. co-mingling 2.22 %). Body weight 1 day before weaning of the piglets did not differ from the conventional housing system (5.41 kg) and co-mingling system (5.44 kg). Average daily gain between day 10 and 1 before weaning of conventional and co-mingled housed piglets was similar (222 g/d). There was no significant effect of the housing system on creep feed intake of piglets during d-10-d0 before weaning (conventional 77 g vs. co-mingling 69 g). Average fecal consistency score during 10 days before weaning did not differ from conventional housing and co-mingling system (1.7 vs. 2.0) and no significant effects on diarrhoea incidence in the 10 days before weaning were found (3.30 and 1.48 % respectively). Individual medical treatments of piglets during d-10-d0 before weaning were not affected by the housing system (conventional 1.49 % vs. co-mingling 1.98 %). Only 1 piglet from the co-mingling system died for unknown reasons in the last 10 days before weaning.

Skin lesions

The skin lesion score of the snout of piglets 1 day before weaning was not affected by housing treatment (conventional 0.08 vs co-mingling 0.13). In contrast, 1 day before weaning the skin lesion score of head and ears of co-mingled piglets was significantly higher compared to conventionally housed piglets (0.32 vs. 0.12) and the skin lesion score of the shoulders and flanks of co-mingled piglets tended to be higher 1 day before weaning compared to conventionally housed piglets (0.52 vs. 0.28; $P < 0.10$).

Post-weaning

Animal performance and health

Body weight of co-mingled piglets tended to be higher at day 14 post-weaning ($P < 0.10$), but neither housing nor feeding strategy affected body weight of the piglets at day 5 and 42 post-weaning (Table 2). Between day 1 before weaning and day 5 post-weaning the average daily gain of piglets fed creep feed was 33 % higher than piglets fed no creep feed ($P < 0.05$). In contrast, piglets offered creep feed gained 18 % less than piglets not offered creep feed during d5-14 post-weaning ($P < 0.05$), but no effect of feeding strategy on average daily gain during d-1-14 post-weaning was observed (Table 2). There was a tendency for a higher weight gain during d-1-5 ($P < 0.10$) and d-5-14 ($P < 0.10$) post-weaning in co-mingled piglets compared to conventionally reared piglets. Likewise, average daily gain of co-mingled piglets was significantly higher during d-1-14 post-weaning (Table 2). Average daily feed intake in the first 5 days post-weaning was 43 % higher in creep feed fed piglets compared to non-creep feed fed piglets ($P < 0.001$) (Table 2; Figure 3; Figure 6). Feed intake of the weaner diet was higher during d2-5 post-weaning in the no creep feed fed piglets compared to the creep feed fed piglets ($P < 0.05$) (Figure 2; Figure 5). During d5-14 post-weaning, there was a tendency for creep feed fed piglets to have lower feed intake than piglets fed the weaner diet only ($P < 0.10$) and a tendency for co-mingled piglets to have higher feed intake than conventionally reared piglets ($P < 0.10$) (Table 2). Post-weaning creep feed intake was higher in co-mingled piglets than conventionally reared piglets but this was not significant (Figure 1; Figure 4). Average daily feed intake tended to be higher in co-mingled piglets than conventionally reared piglets during d0-14 post-weaning (Table 2). Regarding feed to gain ratio, no effects of housing or feeding familiarity was observed (Table 2). No treatment effects were found either for average fecal consistency score and diarrhoea incidence of the piglets during d0-14 post-weaning or for the individual use of antibiotics and piglet mortality during d0-42 post-weaning (data not shown).

Blood parameters

At day 2 post-weaning, hematocrit, hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin were higher in co-mingled piglets compared to conventionally housed piglets, concomitant with a trend for higher number of red blood cells (Table 3). In contrast, percentage and numbers of reticulocytes were lower in co-mingled piglets (Table 3). There was a tendency for more eosinophils in co-mingled piglets than conventionally housed piglets ($P < 0.10$) (Table 3). Numbers of basophils were higher for piglets fed only the weaner diet compared to piglets provided additionally with creep feed, but no effect of feeding strategy on other blood hematology parameters were found (Table 3). Glucose concentration was 10 % higher in co-mingled piglets compared to conventionally reared piglets (Table 4). There was a tendency for co-mingled piglets to have lower NEFA concentrations than conventionally reared piglets. Neither housing nor feeding strategy influenced activity of alkaline phosphatase and creatine kinase in serum at day 2 post-weaning (Table 4). Neither housing nor feeding strategy affected cortisol concentration in saliva of piglets at day 1 post-weaning (1.64 µg/dL).

Skin lesions

Skin lesion scores of the snout, shoulders and flanks were higher for conventionally reared piglets compared to co-mingled piglets at day 2 post-weaning. No effect of the housing system on the skin lesion score of the head and ears in piglets was found. Feeding strategy did not influence the occurrence of skin lesions in piglets (Table 5). Neither housing nor feeding strategy affected overall ear and tail damage of piglets at the end of the nursery phase.

Discussion

It was hypothesized that not mixing of piglets at weaning by applying co-mingling of non-littermates before weaning on the one hand and providing the same diet before and after weaning on the other hand would facilitate the weaning process in an beneficial manner resulting in improved piglet performance, immune competence and health in the post-weaning phase. However, no interactions between co-mingling and feed familiarity were found in the current study. Nonetheless, the interventions each contributed to an improved adaptation of the weaning process.

Although co-mingled piglets had more skin lesions at the head and ears and tended to have more skin lesions at the shoulders and flanks prior to weaning, a better weaning transition by co-mingling of non-littermates before weaning in the present study was clearly demonstrated by lower skin lesion scores at the snout, shoulders and flanks of the co-mingled piglets at day 2 post-weaning, suggesting a reduced incidence of fighting at weaning, which is also reported in several other studies in which piglets were socialized during lactation (Bohnenkamp et al., 2013; Hessel et al., 2006; Klein et al., 2016; Kutzer et al., 2009; Lange et al., 2020; Parratt et al., 2006; Pluske and Williams, 1996; Salazar et al., 2018; Schrey et al., 2019; van Nieuwamerongen et al., 2015; Van Kerschaver et al. 2021, Wattanakul et al., 1997a; Wattanakul et al., 1997b; Weary et al., 1999; Weary et al., 2002). In the long term, co-mingling did not affect the occurrence of skin lesions at the ears and tail of piglets at d42 post-weaning in the present study. Similarly, van Nieuwamerongen et al. (2015) found no differences in ear and tail damage scores at d33 post-weaning in piglets raised in a multi-suckling and conventional housing system. Also, Gentz et al. (2020) and Ko et al. (2021) observed no effect of pre-weaning socialization of piglets on tail lesions at any point during 6 weeks of rearing and ear biting lesions on d44 post-weaning, respectively. As suggested by Gentz et al. (2020), co-mingling of non-littermates before weaning might have more directly an impact on the level of aggressive behaviour in piglets related with establishing dominance hierarchy, rather than manipulative behaviour such as ear and tail biting. In addition, manipulation or non-aggressive biting behaviour seems to be a complex problem which might be influenced by many factors (Gentz et al., 2020; Prunier et al., 2020).

The current trial suggested that pre-weaning co-mingled piglets had less difficulties in making the weaning transition. During the first 2 weeks post-weaning co-mingled piglets showed better performances compared to conventionally reared piglets. Although creep feed intake before weaning did not differ, co-mingled piglets tended to consume 9 % more feed during 2 weeks post-weaning than conventionally reared piglets, regardless of the applied feeding strategy at weaning, which contributed to better weight gains of the co-mingled piglets in that period. Indeed, co-mingled piglets gained 15 % more weight during d0-14 post-weaning compared to the conventionally reared piglets. Also other studies in which piglets were socialized before weaning showed improved post-weaning piglet performance in terms of body weight and weight gain, although this was mainly in the first week after weaning (Schrey et al., 2019; van Nieuwamerongen et al., 2015; Weary et al., 2002). In the current study, only a tendency for higher weight gain in co-mingled piglets was observed during d-1-5, similar to the study of Hessel et al. (2006) where pre-weaning socialized piglets tended to gain more weight during the first week post-weaning. In studies of Bohnenkamp et al. (2013), Morgan et al. (2014), Parratt et al. (2006), Pluske and Williams (1996) and Weary et al. (1999) no effect of socializing unfamiliar piglets before weaning on the piglet performance during 2 weeks post-weaning was found. Nevertheless, the improved weight gain of the co-mingled piglets in the immediate post-weaning phase did not last until the end of the nursery period in current research. Neither average daily gain during d14-42 and d-1-42 post-weaning nor body weight at d42 post-weaning differed between the co-mingled and conventionally reared piglets. Also Bohnenkamp et al.

(2013) and Van Kerschaver et al. (2021) observed no effect of pre-weaning socialization on piglet performance after a rearing period of 6 weeks post-weaning. In contrast, Hessel et al. (2006) found a tendency for higher weight gain in co-mingled piglets during a rearing period of 5 weeks post-weaning. In studies of Kutzer et al. (2009) and van Nieuwamerongen et al. (2015) weight gain during 5 weeks of rearing in the nursery was even improved in piglets who were able to socialize during lactation compared to non-socialized piglets.

In the present study, hematocrit and hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin were significantly higher in the co-mingled piglets than in the conventionally reared piglets at day 2 post-weaning. Hematocrit represents the volume of red blood cells compared to the total blood volume, and hemoglobin is part of red blood cells which has an important role in the delivery of oxygen to the tissues (Billet, 1990). Mean corpuscular volume, which represents the size of red blood cells, and mean corpuscular hemoglobin, which defines the amount of hemoglobin per red blood cell, are calculated from hemoglobin, hematocrit and red blood cell values (Sarma, 1990). Perhaps the higher percentage of hematocrit and the higher amount of hemoglobin in the blood of the co-mingled piglets was caused by enhanced active behaviour of the co-mingled piglets during lactation. Pre-weaning socialization of non-littermates might result in an increase of overall activity during lactation (Salazar et al., 2018; Verdon et al., 2019) and it is known for example that hematocrit increases during exercise (Mairbaurl, 2013). The change in the hematological measures might be also caused by lower frequency of drinking behaviour of the co-mingled piglets since dehydration is evidenced by increased hematocrit levels (Steiger Burgos et al., 2001; Garcia et al., 2015). Rudine et al. (2007) explained differences in hematological measures between indoor and outdoor reared gilts by these two assumptions. However, it must be noted that information about activity or drinking behaviour in piglets is lacking since this was not measured in the current trial. The difference between co-mingled and conventionally reared piglets might also be explained by a more pronounced impact of weaning on hematological variables in conventionally reared piglets. For example, Chevalier et al. (2021) demonstrated similar variations in hematocrit and hemoglobin levels during the first few days post-weaning, although iron supplementation remains the main driver for the hematological status of the piglets. Bhattarai et al. (2015) showed that hematocrit and hemoglobin levels at weaning are positively related to the post-weaning average daily gain. Hence, the temporally improved post-weaning growth performance of co-mingled piglets in the current study could be attributed to an improved hematological status at weaning.

Blood levels of glucose and NEFA might not only be related to the nutritional status of pigs (Dunshea, 2003), the increase of glucose and NEFA concentrations in blood can be also suggested as mobilization of body energy sources in response to aggression (Tuchscherer et al., 1998). It was hypothesized that co-mingled piglets would fight less at weaning compared to the conventionally reared piglets which were mixed at weaning, and therefore would have lower levels of glucose and NEFA post-weaning. The concentration of NEFA at day 2 post-weaning tended to be lower in the co-mingled piglets in the present study. This might be a result of reduced fighting at weaning, evidenced by the lower skin lesion scores of the co-mingled piglets at day 2 post-weaning, rather than higher feed intake in the co-mingled piglets since feed intake in the first days post-weaning was not affected by the housing strategy. It is known that hyperglycemia can be stimulated by mixing unfamiliar pigs and aggressive behaviour between piglets might be related with increased plasma glucose levels (Fernandez et al., 1994). However, the glucose levels in the current study were in contrast higher in the pre-weaning co-mingled piglets at day 2 post-weaning compared to conventionally reared piglets which were grouped with unfamiliar piglets at weaning. Nevertheless, it should be emphasized that a good interpretation of the results of these metabolites is difficult since blood samples of the piglets were only taken after weaning and not prior to weaning and differences between sampling moments could therefore not be determined. Moreover, Fernandez et al. (1994) investigated the acute metabolic responses to aggressive interactions by taking blood samples during a short period, i.e. between 20 min before the encounter test and 1 hour after, whereas we examined the effect of the housing strategy at day 2 post-weaning, when most dominance relationships should already be established. Dominance hierarchy is determined within 48 to 72 h after mixing (Meese and Ewbank, 1973). Puppe et al. (1997) found an effect of changing the housing environment at weaning but no effect of mixing unfamiliar piglets at weaning on the plasma glucose levels between day 1 before weaning and day 4 after weaning. They also suggested that physiological effects are very transient. The higher blood levels of glucose at day 2 post-weaning in the co-mingled piglets in the present study might therefore be due to other factors rather than the reduced fighting behaviour associated with mixing.

Creatine kinase is suggested as a good indicator of the muscular activity or tissue damage since it is released from muscle fibers into the blood by exercise or tissue damage. Aggressive behavior between piglets due to mixing might affect levels of creatine kinase (Gade, 2008; Sutherland et al., 2009). It was therefore hypothesized that the conventionally reared piglets which were mixed at weaning would have higher levels of creatine kinase compared to the co-mingled piglets. However, no effect of the housing strategy at weaning on the levels of creatine kinase in piglets at day 2 post-weaning were found. In the study of Gade (2008) blood sampling was done at slaughtering in the immediate phase after mixing during transport and in the study of Sutherland et al. (2009) changes of creatine kinase were determined before and immediately after 60-min transport including mixing piglets, whereas in the current study blood sampling took place at day 2 post-weaning, which was perhaps already too late to find an effect. This might be also the case for the results of blood levels of alkaline phosphatase, a biomarker which can be related to stress such as mixing (Tuchscherer et al., 1998). The activity of alkaline phosphatase was also not affected at day 2 post-weaning by the housing strategy. Another relevant biomarker related to stress in piglets is cortisol. The activation of hypothalamic-pituitary-adrenal (HPA) axis by a stressful stimulus results in the production of glucocorticoids such as stress hormone cortisol (Martinez-Miro et al., 2016). Because the co-mingled piglets were not mixed at weaning compared to the conventionally reared piglets, it was anticipated that the amount of stressors in the co-mingled piglets would be reduced at weaning resulting in lower salivary cortisol concentrations. Salivary cortisol can be obtained without inducing stress by a non-invasive technique and reflects the free, circulating cortisol levels which might be adequate to evaluate HPA response (Escribano et al., 2012; Martinez-Miro et al., 2016). Several studies previously reported smaller salivary or blood cortisol increases after weaning in pre-weaning socialized piglets compared to the control piglets (Ko et al., 2021; Lange et al., 2020; Salazar et al., 2018). However, no effect of co-mingling non-littermates before weaning was found on the saliva cortisol concentrations in piglets after weaning in the current study, as also reported in studies of Pluske and Williams (1996), Turpin et al. (2017). Nevertheless, also Ko et al. (2021) also did not find an effect of the social environment of piglets before weaning on salivary cortisol levels at day 1 post-weaning. They observed an effect on day 2 post-weaning. Turpin et al. (2017) found an increase in plasma cortisol after weaning but without treatment effects. Pluske and Williams (1996) attributed the lack of finding an effect to the fact that they measured the concentration of plasma cortisol in piglets only at one time moment which was also the case in the current study. It was therefore unable to confirm an increase in cortisol after weaning since we only collected piglet saliva at day 1 post-weaning.

The feeding strategy at weaning clearly affected pig performance in the early post-weaning period in the present study. Piglets provided additionally with creep feed at weaning consumed 43 % more feed during d0-5 post-weaning than piglets not provided with creep feed at weaning, which reflected in a lower immediate post-weaning growth check. Indeed, creep fed piglets gained 33 % more weight during d-1-5 post-weaning compared to piglets not provided with creep feed at weaning. Feed familiarity at weaning thus might have reduced feeding-related stress in piglets, also evidenced by the higher feed intake of the creep feed fed piglets during d0-1 post-weaning, which might suggest that a high percentage of the piglets already started to consume feed while it is known that feed intake in piglets associated with commercial weaning at the first day post-weaning is very limited (Brooks and Tsourgiannis, 2003) or that only a limited percentage of weanling piglets had eaten at the first day after weaning (Bruininx et al., 2001). Also Heo et al. (2018) highlighted the importance of feed familiarity around weaning. In their study, piglets that consumed the same diet both 14 days before and after weaning performed better during the first 2 weeks post-weaning compared to piglets from which the diet changed at weaning. Remarkably, piglets provided with creep feed at weaning tended to consume less feed during d5-14 post-weaning and gained 22 % less weight during that period. The growth check during d5-14 post-weaning was perhaps caused by the transition of creep feed and weaner diet to the weaner diet only from approximately day 5-6 post-weaning onwards. In addition, 0.538 kg creep feed per piglet was provided in a round creep feeder at weaning and this amount was consumed by the piglets by approximately day 5-6 post-weaning. From then on piglets could only consume the weaner diet. Perhaps this transition in feed requested again an adaptation of the piglets which resulted in a lower weight gain during d5-14 post-weaning. Indeed, post-weaning dietary changes can result in decreased average daily gains (Carroll et al., 1998). Nevertheless, neither body weight at day 5 and 14 post-weaning nor overall average daily gain during d-1-14 post-weaning in piglets was affected by the feeding strategy in the current trial. Middelkoop et al. (2020) investigated the effect of pre-weaning creep feed provision and post-weaning creep feed supplementation through adding 80 g of creep feed per pen of 4 piglets twice a day on top of their weaner diet during 14 days post-weaning in a factorial way and did not find interaction effects on feed intake and growth during the first 2 weeks post-weaning. Similarly, van Oostrum et al. (2016) examined the impact of supplementation with a milk replacer 5 days before weaning and 5 days after weaning. After weaning piglets also had *ad libitum* access to a weaner diet. The authors also did not find interaction effects between the pre-weaning and post-weaning period during 14 days post-weaning. This might indicate that providing piglets the same diet as before weaning in a small amount during the post-weaning phase or for the first days in the acute weaning phase rather have a limited impact on piglet performance during first 2 weeks post-weaning. Moreover, the improved effect of feed familiarity in the very early post-weaning phase could not be maintained at the end of the nursery period in the present study, a finding which was also observed by Heo et al. (2018).

To evaluate if offering 0.538 kg additional creep feed per piglet was cost effective, an important observation is that the total feed intake during d0-14 (creep feed: 167 g/d, no creep feed:164 g/d) and d0-42 (395 g/d for both groups) post-weaning was not affected by this strategy. This signifies that, taking into account the price difference between the weaner diet and creep feed diet, this strategy indeed resulted in additional feed cost of approximately € 0.3 per piglet. Furthermore, since this strategy did not significantly improve the body weights at the end of the nursery period (creep feed: 17.2 kg, no creep feed:17.4 kg), we cannot conclude if this strategy resulted in a financial return. Likely some beneficial returns can be expected from transient effects in feed intake behaviour and social behaviour, which could affect animal health, but these financial benefits are hard to extrapolate from this experiment.

Conclusion

In summary, co-mingling of non-littermates before weaning and feed familiarity at weaning both facilitated the weaning process of piglets. Co-mingling of non-littermates might mainly have reduced the stress related to mixing in piglets at weaning, evidenced by lower skin lesion scores and improved piglet performances the first 2 weeks post-weaning. Providing the same feed as before weaning for the first days post-weaning might have minimized the nutritional stressor at weaning, indicated by higher feed intake and in consequence a lower growth check in the very early post-weaning phase.

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Declaration of interest Authors

The authors have no interest to declare. All authors read and approved the final manuscript

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Figure 1. Average daily creep feed intake during 5 days post-weaning of piglets in conventional or co-mingling housing system.

Figure 2. Average daily feed intake of the weaner diet during 14 days post-weaning of piglets in conventional or co-mingling housing system and either or not provided with creep feed at weaning. * Significant effect of feeding at P < 0.05. § Significant effect of housing at P < 0.05.

Figure 3. Average daily feed intake (total feed intake, i.e. weaner diet and creep feed) during 14 days post-weaning of piglets in conventional or co-mingling housing system and either or not provided with creep feed at weaning. * Significant effect of feeding at P < 0.05. § Significant effect of housing at P < 0.05.

Figure 4. Cumulative creep feed intake during 14 days post-weaning of piglets in conventional or co-mingling housing system.

Figure 5. Cumulative feed intake of the weaner diet during 14 days post-weaning of piglets in conventional or co-mingling housing system and either or not provided with creep feed at weaning. * Significant effect of feeding at P < 0.05.

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Table 1. Analysed nutrient composition of creep feed, weaner and starter diet

Table 2. Post-weaning animal performance of piglets in a conventional or co-mingling housing system and either or not provided creep feed at weaning (weaning referred as d0).

Table 3. Blood hematology of piglets at day 2 post-weaning in a conventional or co-mingling housing system and either or not provided creep feed at weaning.

Table 4. Biochemical blood parameters of piglets at day 2 post-weaning in a conventional or co-mingling housing system and either or not provided creep feed at weaning.

Table 5. Skin lesion scores of snout, head and ears, shoulders and flanks of piglets at day 2 post-weaning and ear and tail damage at day 42 post-weaning in a conventional or co-mingling housing system and either or not provided creep feed at weaning.

Supplementary Figure 1. Left picture: conventional housing of sows and their litter during the pre-weaning. Right picture: Co-mingling of 3 litters during 10 days before weaning.

Supplementary Figure 2. Left picture: piglets, either conventionally reared or co-mingled pre-weaning, housed in a nursery pen with access to the weaner diet in a three-space dry feeder and creep feed in a round creep feeder. Right picture:

piglets, either conventionally reared or co-mingled pre-weaning, housed in a nursery pen with only access to the weaner diet in a three-space dry feeder.

Declaration of interest Authors

The authors have no interest to declare. All authors read and approved the final manuscript

(Meese and Ewbank, 1973; Billett, 1990; Sarma, 1990; Fernandez et al., 1994; Le Dividich and Herpin, 1994; Pluske and Williams, 1996; Puppe et al., 1997; Wattanakul et al., 1997a; Wattanakul et al., 1997b; Carroll et al., 1998; Tuchscherer et al., 1998; Weary et al., 1999; Bruininx et al., 2001; Steiger Burgos et al., 2001; Bruininx et al., 2002; Weary et al., 2002; Brooks and Tsourgiannis, 2003; Dunshea, 2003; Lalles et al., 2004; Hessel et al., 2006; Parratt et al., 2006; Rudine et al., 2007; Barton Gade, 2008; Kutzer et al., 2009; Round and Mazmanian, 2009; Sutherland et al., 2009; Escribano et al., 2012; Kanaan et al., 2012; Bohnenkamp et al., 2013; Campbell et al., 2013; Mairbäurl, 2013; Morgan et al., 2014; Thomsson et al., 2014; Bhattarai and Nielsen, 2015; Garcia et al., 2015; van der Peet-Schwering et al., 2015; van Nieuwamerongen et al., 2015; Klein et al., 2016; Martínez-Miró et al., 2016; van Oostrum et al., 2016; Turpin et al., 2017; van Nieuwamerongen et al., 2017; Heo et al., 2018; Salazar et al., 2018; Schrey et al., 2019; Verdon et al., 2019; Gentz et al., 2020; Lange et al., 2020; Middelkoop et al., 2020; Prunier et al., 2020; Chevalier et al., 2021; Huting et al., 2021; Ko et al., 2021; Van Kerschaver et al., 2021; Van Kerschaver et al., 2023)

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Highlights (Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

- Co-mingling resulted in improved average daily gain the first 2 weeks post-weaning
- Feed familiarity temporary improved average daily gain and feed intake at weaning.
- Co-mingling combined with feed familiarity did not further improve the weaning response
- Co-mingling impacted on the hematological status at weaning.

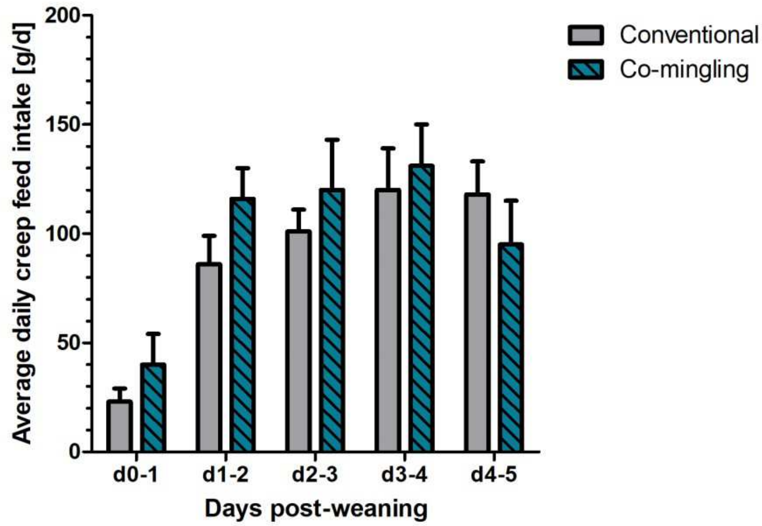
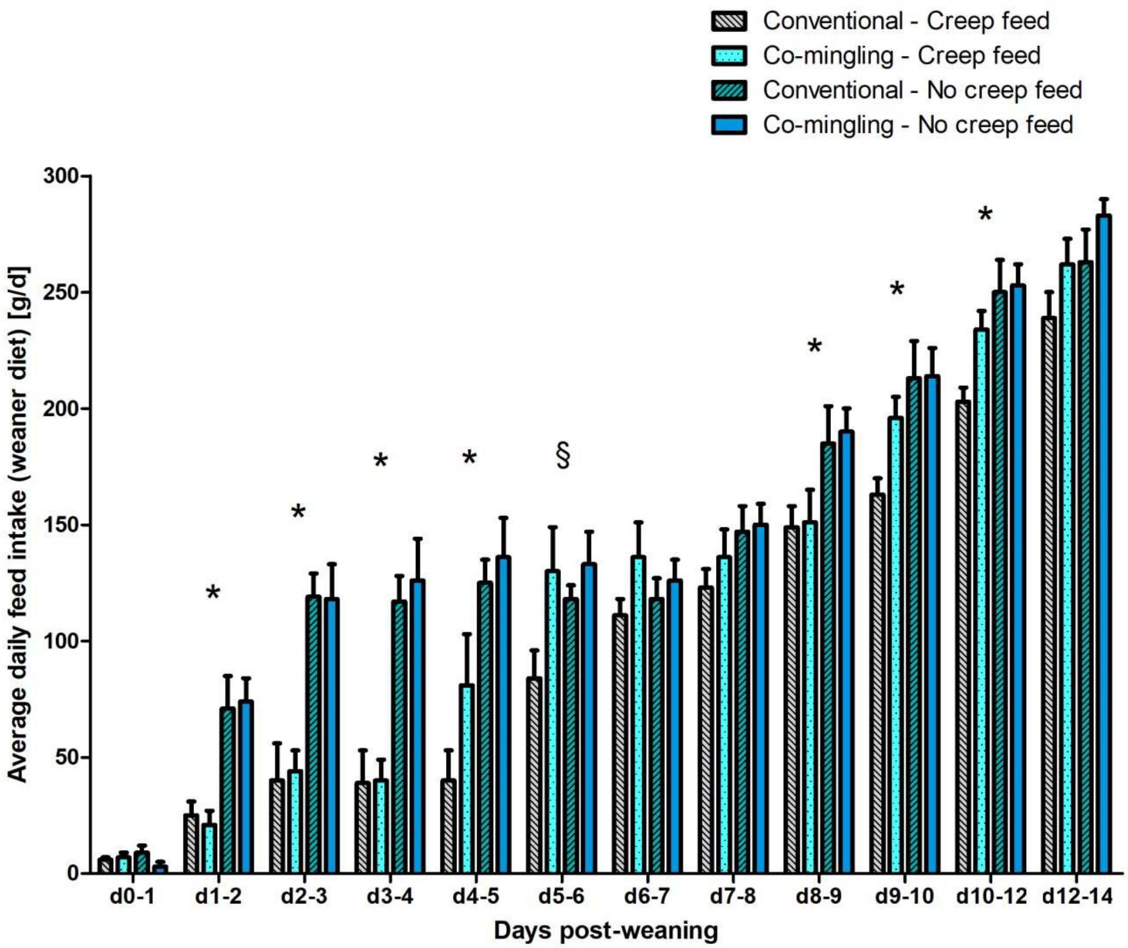


Figure 1. Average daily creep feed intake during 5 days post-weaning of piglets in conventional or co-mingling housing system.



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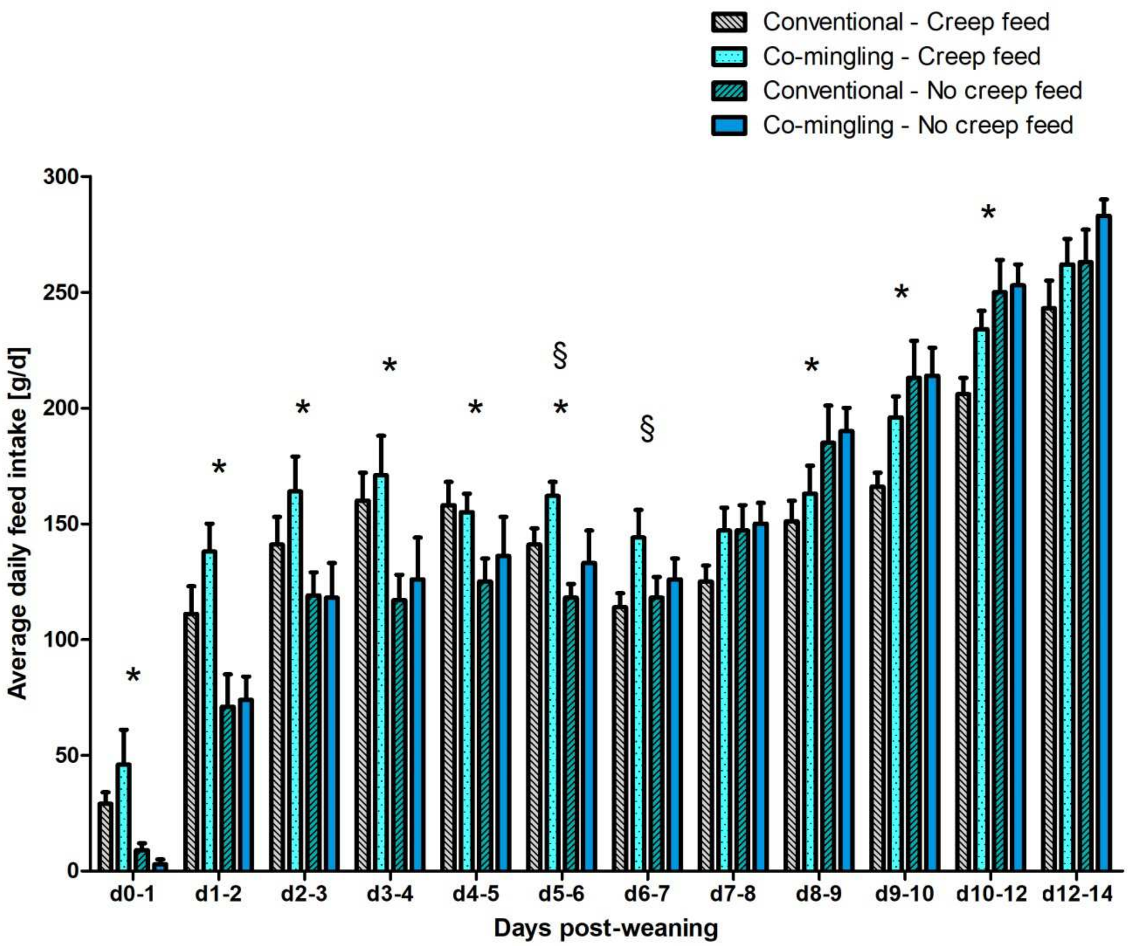

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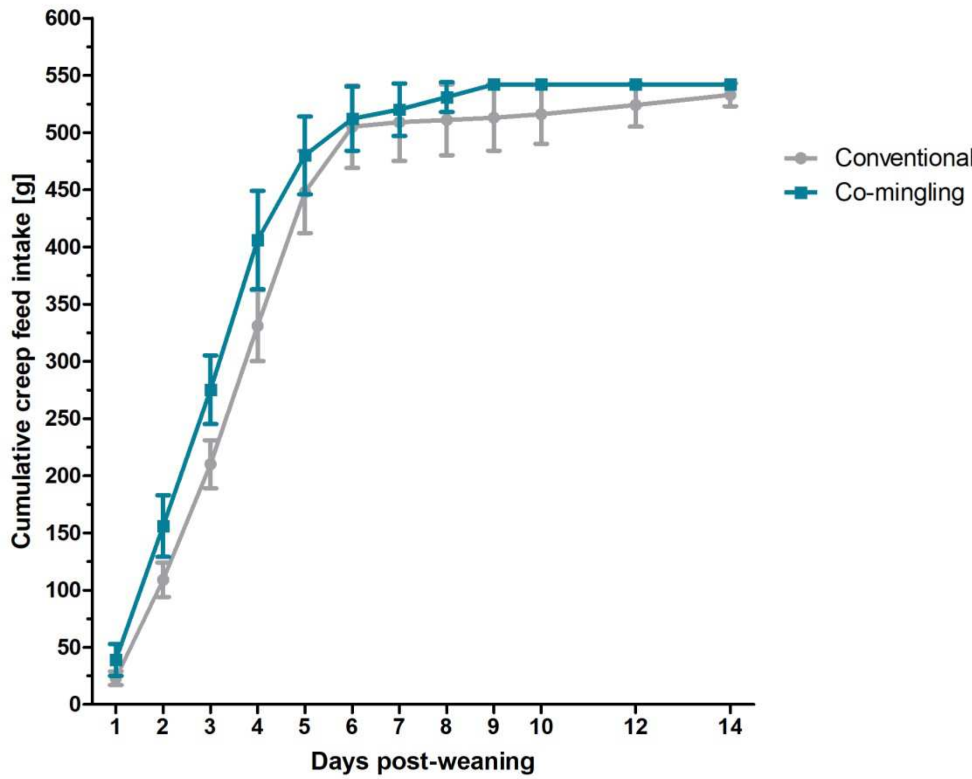
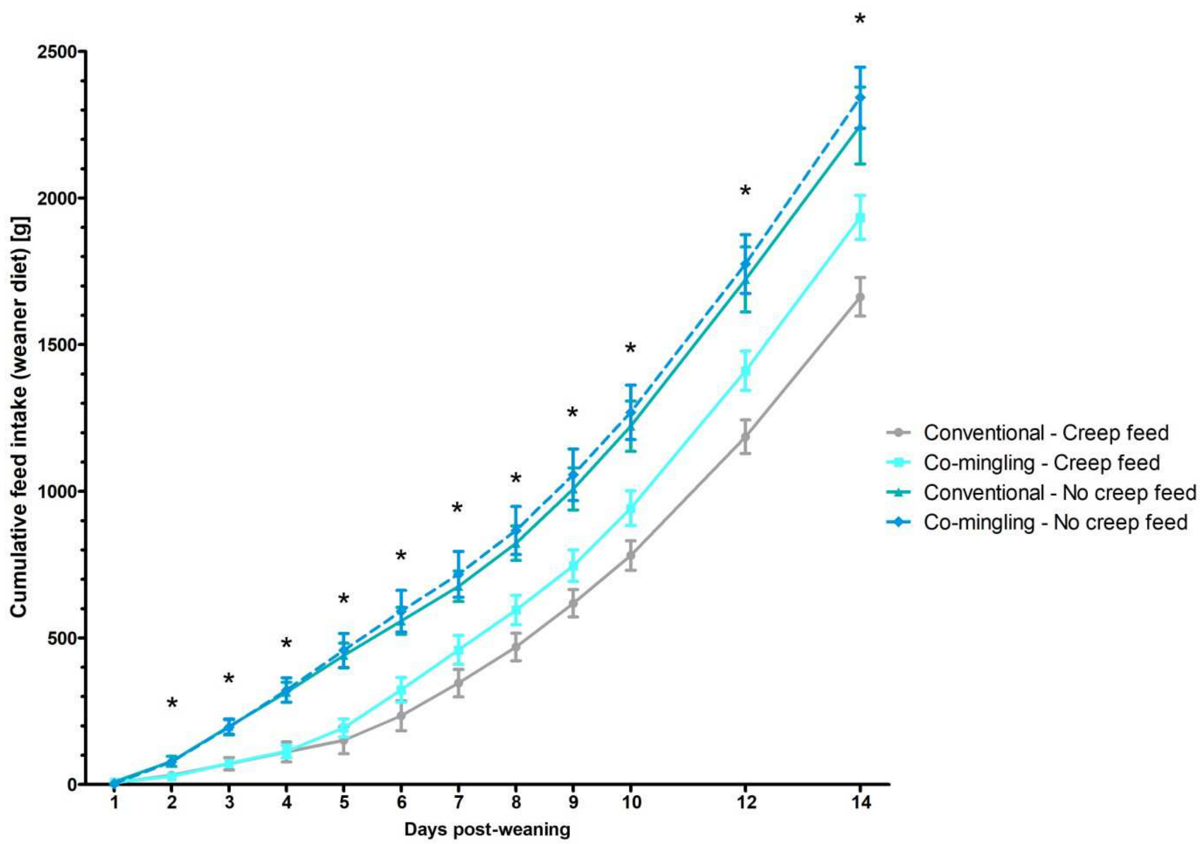


Figure 4. Cumulative creep feed intake during 14 days post-weaning of piglets in conventional or co-mingling housing system.



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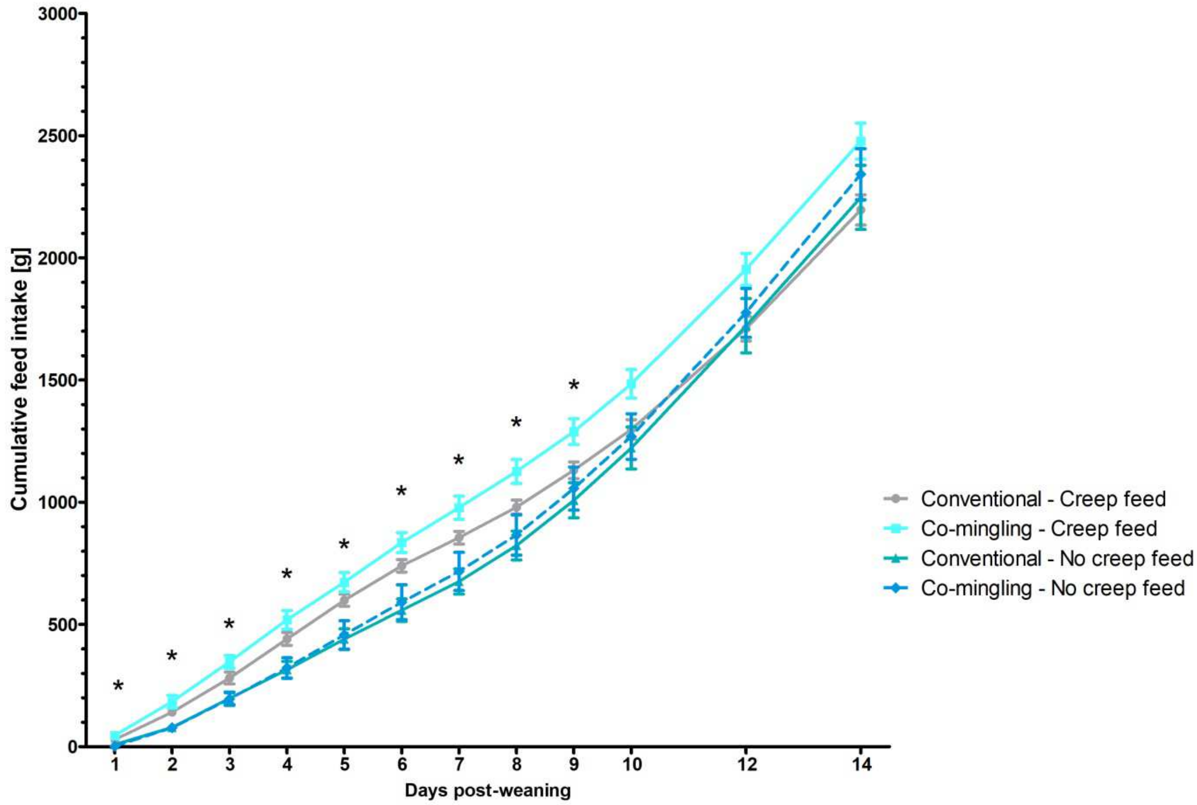

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Table 1. Analysed nutrient composition of creep feed, weaner and starter diet

	Creep feed (1)[1]	Creep feed (2)[2]	Weaner diet[3]	Starter diet[4]
Moisture, %	6.5	8.0	9.0	9.6
Crude protein, %	20.2	17.6	15.6	16.8
Crude fat (hydrolysis), %	10.8	6.2	5.3	6.4
Crude ash, %	5.6	4.9	4.8	5.0
Zinc, mg/kg	101	112	126	147
Copper, mg/kg	128	139	129	175

[1] Main ingredients: whey powder, wheat, extruded wheat, peeled and extruded oats, dehulled and extruded soybean meal, toasted soybeans, extruded maize, blood plasma (3.75 %), soybean oil, sugar, coconut and palm oil, dextrose, lactose, potato protein, wheat feed, wheat protein, monocalcium phosphate, salmon oil, sunflower oil, sodium chloride, sodium bicarbonate, calcium carbonate, calcium sodium phosphate, dried brewer's yeast, medium chain fatty acids esterified with glycerol, hydrolysed coconut flakes, fatty acid salts (butyric acid), by-products, coconut and palm kernel fatty acids

[2] Main ingredients: wheat, barley, whey powder, dehulled and extruded soybean meal, extruded wheat, peeled oats, peeled and extruded oats, wheat feed, sugar, blood plasma (2.5 %), extruded corn, refined coconut and palm oil, chicory pulp, potato protein, toasted soybeans, wheat protein, rice, sunflower oil, crude glycerine, monocalcium phosphate, salmon oil, sodium chloride, sodium bicarbonate, calcium carbonate, calcium sodium phosphate, dried brewer's yeast, medium chain fatty acids esterified with glycerol, hydrolysed coconut flakes, fatty acid salts (butyric acid), by-products, coconut and palm kernel fatty acids, dextrose

[3] Main ingredients: barley, wheat, toasted soybeans, whey powder, soybean meal, oat hulls, wheat feed, fish oil, wood cellulose, sodium salt of lactic acid, monocalcium phosphate, sodium sulfate

[4] Main ingredients: barley, wheat, soybean meal, toasted soybeans, maize, bakery by-products, wheat feed, wheat gluten feed, crude soya oil, dried sugar beet pulp, monocalcium phosphate, fish oil

Table 2. Post-weaning animal performance of piglets in a conventional or co-mingling housing system and either or not provided creep feed at weaning (weaning referred as d0).

	Conventional		Co-mingling		Housing		Feeding		SEM	P-value		
	Creep feed	No creep feed	Creep feed	No creep feed	Conventional	Co-mingling	Creep feed	No creep feed		Housing	Feeding	H × F
Body weight, kg												
d-1*	5.60	5.52	5.52	5.59	5.56	5.55	5.56	5.56	0.06	0.950	0.983	0.884
d5*	6.10	5.87	6.09	6.00	5.98	6.05	6.09	5.94	0.05	0.555	0.144	0.296
d14	7.00	7.03	7.15	7.25	7.01	7.20	7.08	7.14	0.07	0.093	0.955	0.742
d42	17.1	17.6	17.2	17.2	17.3	17.2	17.2	17.4	0.19	0.740	0.819	0.495
Average daily gain, g/d												
d-1-5	77	57	93	70	67	82	85[y]	64[x]	4	0.088	0.017	0.867
d5-14	99	128	118	139	114	128	109[x]	133[y]	5	0.097	0.009	0.631
d-1-14	90	100	108	111	95[a]	109[b]	99	106	3	0.027	0.328	0.627
d14-42*	362	376	359	356	369	358	360	366	5	0.278	0.596	0.601
d-1-42*	268	280	272	271	274	271	270	275	4	0.763	0.485	0.820
Average daily feed intake, g/d												
d0-5	120	88	135	91	104	113	127[y]	89[x]	5	0.285	<0.001	0.498
d5-14	177	201	200	209	189	205	189	205	4	0.054	0.051	0.369
d0-14	156	160	177	167	158	172	167	164	4	0.064	0.725	0.336
d14-42	513	526	509	497	520	503	511	512	8	0.239	0.736	0.356
d0-42	392	404	397	386	398	392	395	395	6	0.577	0.803	0.292
Feed to gain ratio, g/g												
d0-5	1.66	1.55	1.48	1.37	1.61	1.43	1.57	1.46	0.06	0.138	0.377	0.993
d5-14*	1.88	1.64	1.75	1.53	1.76	1.64	1.81	1.59	0.07	0.365	0.091	0.162
d0-14*	1.82	1.64	1.65	1.50	1.73	1.58	1.74	1.57	0.05	0.159	0.134	0.053
d14-42*	1.42	1.40	1.42	1.40	1.41	1.41	1.42	1.40	0.01	0.916	0.374	0.822
d0-42*	1.47	1.44	1.46	1.43	1.46	1.44	1.47	1.44	0.01	0.578	0.167	0.543

* Robust tests of equality of means (Welch test)
[a, b] Values with different superscripts within main effects housing are significantly different at $P < 0.05$.
[x, y] Values with different superscripts within main effects feeding are significantly different at $P < 0.05$.

Table 3. Blood hematology of piglets at day 2 post-weaning in a conventional or co-mingling housing system and either or not provided creep feed at weaning.

	Conventional		Co-mingling		Housing		Feeding		SEM	P-value		
	Creep feed	No creep feed	Creep feed	No creep feed	Conventional	Co-mingling	Creep feed	No creep feed		Housing	Feeding	H × F
Red blood cells, 10^{12}/L	5.56	5.51	5.80	5.68	5.54	5.74	5.68	5.60	0.06	0.065	0.569	0.759
Hematocrit, %	38.3	36.7	43.1	40.8	37.5[a]	41.9[b]	40.7	38.7	0.7	<0.001	0.144	0.758
Hemoglobin, g/dL	9.4	9.0	10.3	10.0	9.2[a]	10.2[b]	9.9	9.5	0.2	<0.001	0.255	0.758
Mean corpuscular volume, fL	68.8	65.9	74.7	72.2	67.3[a]	73.4[b]	71.8	69.0	1.1	<0.001	0.209	0.928
Mean corpuscular hemoglobin, pg	16.9	16.2	17.9	17.7	16.6[a]	17.8[b]	17.4	17.0	0.2	0.002	0.400	0.483
Mean corpuscular hemoglobin concentration, g/dL	24.7	24.6	24.0	24.6	24.6	24.3	24.3	24.6	0.1	0.178	0.242	0.153
Reticulocytes, %	2.0	2.0	1.5	1.5	2.0[b]	1.5[a]	1.8	1.7	0.1	0.019	0.919	0.898
Reticulocytes, K/μL	108.3	105.6	85.2	83.5	107.0[b]	84.3[a]	96.8	94.6	4.7	0.012	0.995	0.957
White blood cells, K/μL	11.9	12.6	12.5	13.2	12.2	12.8	12.2	12.9	0.4	0.466	0.347	0.926
Lymphocytes, %	52.0	46.2	47.5	47.9	49.1	47.7	49.8	47.1	1.0	0.407	0.258	0.066
Monocytes, %	8.1	7.3	7.2	7.0	7.7	7.1	7.6	7.2	0.3	0.327	0.519	0.692
Neutrophils, %	38.3	44.9	43.4	43.1	41.6	43.2	40.8	44.0	1.1	0.363	0.209	0.057
Eosinophils, %*	1.6	1.4	1.7	1.9	1.5	1.8	1.7	1.6	0.1	0.096	0.853	0.416
Basophils, %	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.424	0.051	0.909
Lymphocytes, K/μL	5.92	5.62	5.89	6.14	5.77	6.01	5.90	5.88	0.17	0.413	0.735	0.360
Monocytes, K/μL*	0.95	0.88	0.88	0.91	0.91	0.89	0.91	0.89	0.04	0.834	0.774	0.946
Neutrophils, K/μL	4.79	5.95	5.52	5.85	5.37	5.69	5.16	5.90	0.27	0.569	0.234	0.447
Eosinophils, K/μL	0.19	0.18	0.21	0.25	0.18	0.23	0.20	0.21	0.01	0.056	0.734	0.359
Basophils, K/μL	0.01	0.02	0.01	0.02	0.01	0.02	0.01[x]	0.02[y]	0.00	0.240	0.046	0.784
Thrombocyts, K/μL	452	512	430	445	482	438	441	479	20	0.265	0.273	0.571
Mean platelet volume, fL*	9.7	9.8	9.8	9.6	9.8	9.7	9.8	9.7	0.1	0.415	0.525	0.616

* Robust tests of equality of means (Welch test)

a, b Values with different superscripts within main effects housing are significantly different at P < 0.05.

x, y Values with different superscripts within main effects diet are significantly different at P < 0.05.

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Table 4. Biochemical blood parameters of piglets at day 2 post-weaning in a conventional or co-mingling housing system and either or not provided creep feed at weaning.

	Conventional		Co-mingling		Housing		Feeding		SEM	P-value		
	Creep feed	No creep feed	Creep feed	No creep feed	Conventional	Co-mingling	Creep feed	No creep feed		Housing	Feeding	H × F
Glucose, mmol/L*	6.53	6.27	7.13	6.92	6.40[a]	7.02[b]	6.83	6.59	0.14	0.019	0.398	0.059
NEFA, mmol/L	0.63	0.69	0.35	0.59	0.66	0.47	0.49	0.64	0.05	0.057	0.140	0.355
Alkaline phosphatase, IU/L	597	589	612	577	593	594	605	583	14	0.967	0.580	0.646
Creatine kinase, IU/L*	794	793	656	823	794	740	725	808	73	0.717	0.576	0.763

* Robust tests of equality of means (Welch test)

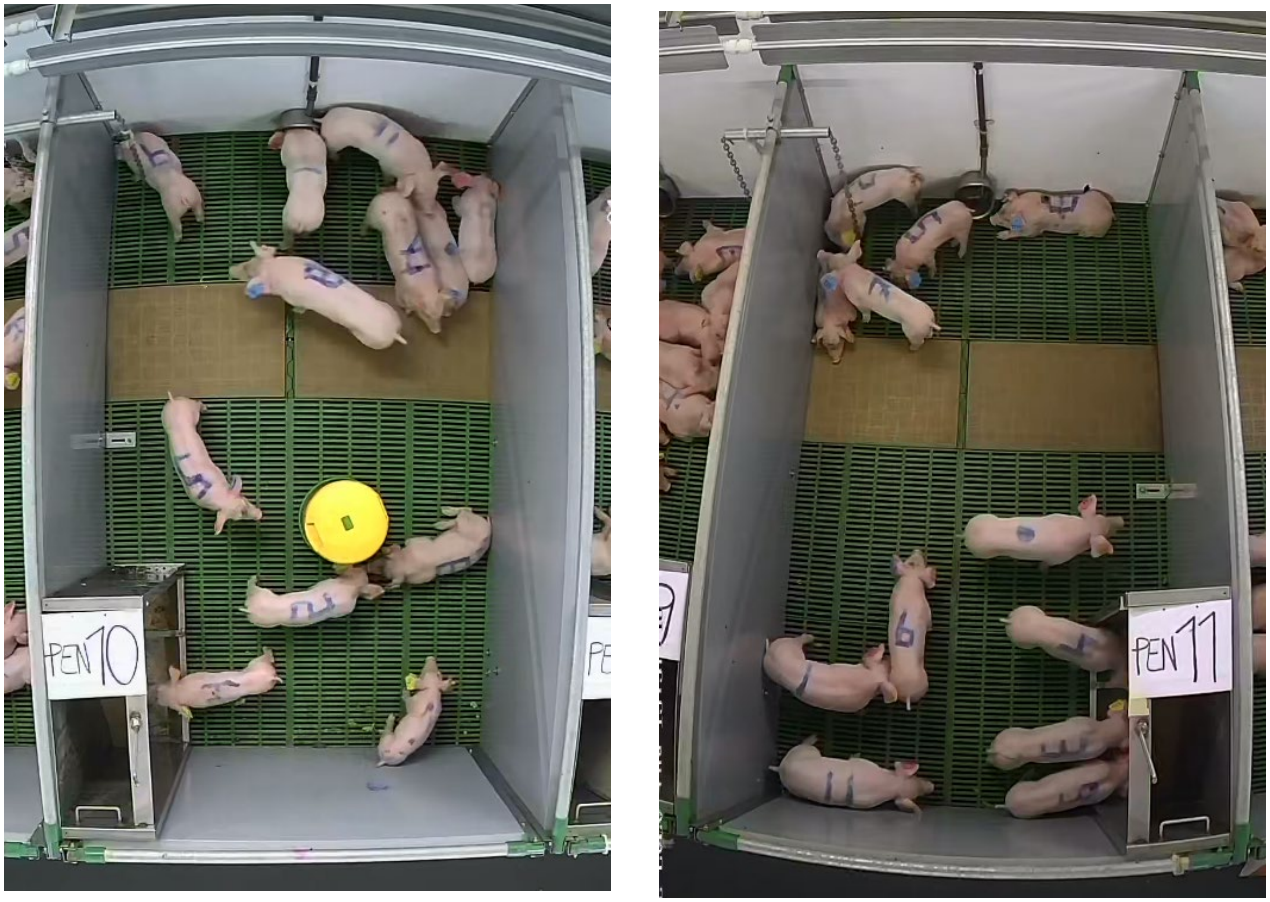
a, b Values with different superscripts within main effects housing are significantly different at $P < 0.05$.

Table 5. Skin lesion scores of snout, head and ears, shoulders and flanks of piglets at day 2 post-weaning and ear and tail damage at day 42 post-weaning in a conventional or co-mingling housing system and either or not provided creep feed at weaning.

	Conventional		Co-mingling		Housing		Feeding		SEM	P-value		
	Creep feed	No creep feed	Creep feed	No creep feed	Conventional	Co-mingling	Creep feed	No creep feed		Housing	Feeding	H × F
Skin lesions d2												
Snout*	0.44	0.27	0.18	0.18	0.35[b]	0.18[a]	0.31	0.22	0.04	0.048	0.355	0.291
Head and ears	0.79	0.82	0.58	0.69	0.81	0.64	0.69	0.76	0.05	0.101	0.431	0.710
Shoulders and flanks	1.22	1.22	0.57	0.61	1.22[b]	0.59[a]	0.90	0.92	0.08	<0.001	0.883	0.890
Damage d42												
Ear (left)	0.65	1.00	0.98	1.01	0.82	1.00	0.82	1.01	0.10	0.400	0.388	0.444
Ear (right)	1.32	1.21	1.67	1.14	1.26	1.41	1.49	1.18	0.11	0.536	0.207	0.371
Tail	0.31	0.19	0.27	0.14	0.25	0.20	0.29	0.16	0.03	0.494	0.077	0.911

* Robust tests of equality of means (Welch test)
a, b Values with different superscripts within main effects housing are significantly different at P < 0.05.

Supplementary Figure 1. Left picture: conventional housing of sows and their litter during the pre-weaning. Right picture: Co-mingling of 3 litters during 10 days before weaning.



Supplementary Figure 2. Left picture: piglets, either conventionally reared or co-mingled pre-weaning, housed in a nursery pen with access to the weaner diet in a three-space dry feeder and creep feed in a round creep feeder. Right picture: piglets, either conventionally reared or co-mingled pre-weaning, housed in a nursery pen with only access to the weaner diet in a three-space dry feeder.