Effect of moderate forced physical activity on behaviour, lameness and osteochondrosis in growing pigs from two divergent lines selected for feed efficiency

Anne Boudon, M. Karhapää, H. Siljander-Rasi, E. Cantaloube, Ludovic Brossard, Nathalie Le Floc'H, Marie-Christine Meunier-Salaün

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A. Boudon [a,b,*], M. Karhapää [b], H. Siljander-Rasi [b], E. Cantaloube [a], L. Brossard [a], N. Le Floc'h [a], M.C. Meunier-Salaün [a]

[a] PEGASE, INRAE, Institut Agro, 35590 Saint Gilles, France
[b] Luke, FI-01370 Vantaa, Finland

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ABSTRACT

In pig farming, physical constraints and genetic selection for high production are risk factors for the development of leg disorders, such as degraded locomotor activity. Interactions between both factors need to be explored. The study was carried out on two replicates of 80 pure-bred Large White growing-finishing pigs from the 8th generation of two divergent lines selected for low and high residual feed intake (**LRFI**, **HRFI**). Each replicate included 40 LRFI pigs and 40 HRFI pigs, housed on partly slatted flooring in a room equipped with a sorter allowing access to electronic self-feeders during two replicates. Ear tags determined the side of the room to which the pigs were oriented after the sorter exit and the distance back to the sorter (short: spontaneous activity, long: forced activity (**FA**)). Lameness was assessed individually weekly using visual gait scoring. At slaughter (weight of 100 kg), *postmortem* quantification of osteochondrosis (**OC**) lesions was performed on both the proximal and distal extremities of the humerus and femur. Low RFI pigs showed a lower feed conversion ratio ($P < 0.001$). They also showed lower individual numbers of sorter crossings per day and a lower proportion of standing pigs, which confirmed their lower physical activity. Forced activity clearly increased the number of sorter crossings/d/pig ($P < 0.001$), and the magnitude of the effect of FA was clearly lower in LRFI pigs than in HRFI pigs. The occurrence of gait was low (less than 9% of recorded scores). The proportion of scores classified as stiffness was higher for LRFI pigs than in HRFI pigs ($P < 0.0001$). The average lameness score was also higher for LRFI pigs and lower with FA ($P < 0.05$). The pigs of the LRFI line showed higher OC scores on both the proximal humerus and femur ($P < 0.001$) and lower OC scores on the distal humerus with surface evaluation ($P < 0.05$). The carcasses of LRFI pigs were heavier with a higher lean meat percentage ($P < 0.001$). Most OC scores were unaffected by FA. Only the OC scores of the distal femur (slice method) were higher with increased activity in LRFI pigs, whereas they were lower in HRFI pigs ($P < 0.05$). Seric biomarkers of cartilage synthesis and degradation were higher for pigs from the LRFI line, but no correlation could be observed between individual OC scores and cartilage biomarker contents.

Implications

Pigs selected for high feed efficiency seemed less likely to increase physical activity when constraints for reaching food were applied, which puts into question their adaptability to farming conditions with increased surfaces. These pigs could also be more prone to locomotor disorders. This suggests that a high level of selection should be accompanied by specific attention to leg health by early detection of osteochondrosis and automatic detection of lameness, for instance, or adaptation of housing.

* Corresponding author at: PEGASE, INRAE, Institut Agro, 35590 Saint Gilles, France.
E-mail address: anne.boudon@inrae.fr (A. Boudon).

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Specification table

Subject	Behaviour and Health Management
Type of data	Table, Figure
How data were acquired	Electronic self-feeders, ESFs (IPF-Intelligent Precision Feeder(s), AgroICT – University of Lleida, Lleida, Spain & Exafan, San Mateo de Gállego, Spain) and weighing sorter (Selfilab, ASSERVA, Lamballe-Armor, France) ELISA tests (Varioscan ThermoFisher)
Data format	pre-treated data
Parameters for data collection	Pigs were housed on a partly slatted floor (0.76 m^2/pig) in a room equipped with eight electronic self-feeders.
Description of data collection	Data were collected from automatic recordings of electronic self-feeders and sorter for data related to feed consumption and growth; after slaughter thanks to a visual observation of the surface or slices of bone extremities for OC scores; by weekly visual observations for gait scores; from video recordings for posture screening; from ELISA analyses for seric concentration of OC biomarkers.
Data source location	Institution: INRAE City/Town/Region: St-Gilles/Brittany Country: France Latitude and longitude: 48°8′37.332″N, 1°49′56.595″; 48.14370346069336, -1.8323876857757568
Data accessibility	Repository name: Data INRAE Data identification number: (https://doi.org/10.15454/RMZX3M) DUA: CC0 Public Domain Dedication (https://dataverse.org/best-practices/dataverse-community-norms).

Introduction

In pig farming, management practices, such as space restrictions associated with the hard and abrasive floors used in indoor housing, can place strong physical constraints on animals and are commonly associated with a high risk of leg disorders. In growing pigs and sows, leg disorders, which can be defined as a poor walking ability, are recognised as a major part of production diseases (Etterlin et al., 2014; Stavrakakis et al., 2014). Clinically, leg disorders can be diagnosed by the prevalence and severity of lameness *in vivo*, and/or joint lesions at slaughter. Societal concerns about animal welfare have promoted increased surfaces and enriched housing in pig farming, which increases the possibility of physical activity, and more specifically mobility (EFSA, 2014). Several studies have shown a beneficial effect of exercise in preventing visual abnormalities of posture, leg weakness or low bone density (Perrin and Bowland, 1977; Petersen et al., 1998), while physical activity that is too severe may have deleterious effects (Schenck et al., 2008). The consequences of increased surface in pig farming on physical activities and leg disorders remain to be evaluated in various conditions to increase result inference.

Selection to improve feed efficiency in livestock remains a major objective in most species. The development of electronic self-feeders (**ESFs**) has allowed individual recording of feed intake in pig farming, so individual measures of feed efficiency can be used for selection. For instance, residual feed intake (**RFI**), i.e. the difference between observed and predicted feed intake (**FI**) for production and maintenance needs, is a criterion for feed efficiency that can be measured in numerous pigs and used for selection (Gilbert et al., 2017). Studies in pigs have demonstrated an association between a decreased RFI and an increased behavioural activity (De Haer et al., 1993; Gilbert et al., 2017; Sadler et al., 2011; Meunier-Salaün et al., 2014), which was already known in laying hens (Luiting et al., 1991) and beef cattle (Herd et al., 2004). After ten generations of divergent selection based on RFI in pigs, Gilbert et al. (2017) reported reduced maintenance energy requirements for more efficient growing pigs (low RFI), which was mainly explained by lower physical activity in individually and group-housed pigs. The lower prevalence of leg disorders, i.e. lameness, as well as leg lesions or bursitis, in the low RFI line (Meunier-Salaün et al 2014) is contradictory with the idea that a moderate physical activity could help prevent leg disorders.

Osteochondrosis (**OC**), which is a non-infectious and degenerative disease corresponding to ischaemic necrosis of cartilage growth (van Grevenhof et al., 2011; Etterlin et al., 2014), can be a cause of leg disorders (Jensen and Toft, 2009). Because a main identified predisposing factor of OC is the breed or the genetic line (Petersen et al., 1998; Etterlin et al., 2015), it could be interfered that the susceptibility of the lines to OC could differ. More generally, a question is also to determine whether the expected beneficial effect of increased physical activity differs in lines selected for improved feed efficiency. Thus, the purpose of the present study was to assess the interaction between selection for increased feed efficiency and forced physical activity on behaviour and prevalence and severity of OC lesions and lameness, in growing pigs housed indoor on concrete floor.

Materials and methods

Animals and experimental design

The study was carried out at INRAE experimental unit UE3P (Saint-Gilles, France, https://doi.org/10.15454/1.5573932732039927E12) on two replicates of 80 pure-bred Large White growing-finishing pigs from the 8th generation of two divergent lines selected for low and high RFIs (**LRFI**, **HRFI**) (Gilbert et al., 2017). All pigs in a batch were housed as one group. Each replicate included 40 LRFI pigs and 40 HRFI pigs, with a 1:1 ratio of females and castrated males. Pigs were housed from 11 weeks of age until slaughter (weight of 101.0 ± 9.26 kg on average, with an objective of 100 kg, between 25 and 26 weeks of age) on a partly slatted floor (0.76 m^2/pig) in a room equipped with an electronic weighing device associated with a sorter allowing the pigs access to eight ESFs (University of Lleida, Lleida, Spain) (Supplementary Fig. S1). The experimental room had two symmetrical sides with four areas: testing, feeding, sorting, and resting, and a path between the two resting areas in the back of the room. Animals were equipped with RFID (Radio Frequency Identification) ear tags for electronic identification. In both lines, half of the pigs were assigned to one of two activity treatments, "spontaneous activity" (**SA**) or "forced activity" (**FA**). Within genetic lines, pigs were allocated to experimental treatments according to sex, body weight and origin (siblings or half-siblings). Upon exiting the sorter, pigs assigned to the SA group were directed to the right side of the feeding area where they could come directly back to the sorter. Pigs submitted to FA were automatically directed to the left side where they had to go

to the back of the room to enter the sorter again and, once in the sorter, could access the feeding area through only one of the two passages. The distance the pigs had to walk to go back to the sorter once they exited the feeding area was 3 m for SA and 2 × 30 m for FA. The treatments were applied from two weeks after the entrance into the experimental room until slaughter. Before the application of the treatments, the sorter remained opened to the animals to become familiarised with the room, and no data were collected. The electronic feeders provided *ad libitum* access to a pelleted diet composed of cereals and soybean meal containing 10 MJ NE/kg and 160 g CP/kg, with a minimum of 0.80 g digestible Lys/MJ NE. Water was supplied *ad libitum* through 10 drinkers in the resting area. Six fixed chains were hung 1 m above the ground to enrich the environment.

Measurements

Growth performance and feeding activity

Pigs were weighed just before the entrance into the experimental room, each time they crossed the sorter associated to feeders and at 21 weeks of age to determine slaughtering dates. The ESF automatically recorded the time, duration and FI of each visit. The daily number of meals and amount of FI per meal were calculated for each animal with a meal criterion set at two minutes. The individual average daily gain (**ADG**) and the feed conversion ratio (**FCR**) were calculated weekly.

Behavioural and lameness recordings

Physical activity was assessed by counting the number of times individual pigs crossed the sorter and by video recordings (24 h at each time) at weeks 2, 7 and 13 after entrance into the experimental room, i.e. just before activity treatment start, 6 weeks and 11 weeks after treatment start. Videos were recorded for 24 h, after the last pigs were marked, spread over two days (Day 1: 1230–1930, Day 2: 0730–1230). They were analysed for posture screening (standing, sitting, lying, Supplementary Table S1) on 11-h daylight period (Day 1: 1400–1900, Day 2: 0830–1430). The results were expressed at the group level in terms of the number of pigs according to the treatment (line and activity treatment) for each item (standing, sitting, lying). The identification of the line and treatment applied to animals for the video recording analysis was performed by drawing marks on their backs using a combination of strokes and dots. Details about the video recording procedure are given as supplementary materials.

Lameness was assessed weekly in the home pen, by grouping all the pigs in the right rest area and then passing each pig through in an isolated space 5 m long thanks to barriers towards the left rest area (solid and slatted floor). The scoring was performed by two experienced observers and was based on the degree of reduction in weight bearing for a leg during walking and using a visual gait scale from 0 to 5 (Supplementary Table S2) adapted from Stavrakakis et al. (2014). A pig was defined as lame with a score equal to or higher than two, as having stiffness with a score of one and as non-lame with a score of zero.

Measurements of carcass and bones

At slaughter, pigs were stunned by electronarcosis and exsanguinated. Cold carcasses were dissected 24 hours after slaughtering for weighing of the ham, loin, shoulder, belly, and back fat. After dissection, the EC reference lean meat percentage (European-Community, 2008, Annex IV, partial dissection) was calculated from the weights of the above-mentioned cuts. Joints from both the humerus and femur on the right carcass were extracted during the dissection and frozen (−20 °C) before examination for OC scores a few weeks later. The same two trained observers examined all joints and bones simultaneously. Osteochondrotic lesions of both the distal and proximal extremities of femurs and humeri were first graded after examination of the surfaces of the joints according to the scale proposed by van Grevenhof et al. (2011) from zero to five (Supplementary Table S3). A score of zero corresponded to the absence of lesions, scores of two to four corresponded to irregular joint surfaces with increasing severity of irregularity/invagination of the cartilage, and a score of five corresponded to *osteochondrosis dissecans*. A score of one was added to the scale of van Grevenhof et al. (2011) after the detection of numerous bone extremities with slight irregularity of cartilage associated with strong colour change. The distal extremities of femurs and humeri were then cross-sectioned into two 10-mm-thick slices (Supplementary Fig. S2), and the severity of OC was macroscopically scored from the four slice surfaces for each bone, as described by Etterlin et al. (2014) (Supplementary Table S4). The maximal score of the four slice surfaces analysed for each animal and bone was considered for statistical analyses. A score of zero corresponded to evenly thick cartilage, a score of one to four corresponded to uneven cartilage thickness with eventual clefts and separation in the osteochondral junction with eventual subcondral hyperhaemia and/or necrosis, and a score of five corresponded to *osteochondrosis dissecans*.

Blood sampling and cartilage biomarker analyses

Blood was sampled in the morning after a night of fasting between two and four days before slaughtering depending on the slaughtering batch of the pigs, using a nose snare. Samples were taken from the jugular vein with a dry Vacutainer® and left to coagulate at room temperature before centrifugation (3 000 rpm, 10 min, 4 °C) and subsequent storage at −80 °C. Serum contents of collagen type II cleavage (**C2C,** biomarker of cartilage degradation) and procollagen II C-propeptide (**CPII**, biomarker of cartilage formation) were analysed during replicate 1 using ELISA (ITEM #60-1001-001 for C2C and ITEM #60-1003-001 for CPII, from IBEX Pharmaceuticals Inc., Montréal, Canada). Each sample was analysed in duplicate. A common control was used for each assay. The intra- and interassay coefficients of variation were 8.39 and 20.4%, respectively, for C2C and 9.74 and 14.56% for CPII. Due to high interassay coefficients of variation, seric C2C and CPII were corrected in proportion to the value of the control used for all assays.

Statistical analyses

Quantitative continuous variables obtained once per animal were analysed by ANOVA with a linear mixed model using the MIXED procedure of SAS (9.2, SAS Institute, Cary, North Carolina). The model included the fixed effects of genetic line, activity treatment, replicate, sex, their interactions and a random effect of the individual. Variables resulting from repeated quantitative measures were analysed with the same model as described before, and the fixed effect of time and its interactions with all other factors were also included as fixed effects. Time was recorded in weeks for variables resulting from sorters or feeders and in a 30-min interval (within recording days) for variables resulting from video analyses. The covariance matrix structure retained was an autoregressive moving average model (ARMA(1,1)) for most variables except the daily number of meals, which was analysed with an autoregressive model of order 1 (AR(1)) after selection of the best fitting model by comparisons of the AIRs. Data related to the percentage of standing pigs were transformed using an arcsin root transformation to achieve a normal distribution. Discrete variables such as lameness and OC scores were analysed using the GENMOD procedure with an ordinal model for multinomial data. Because shapes of score distribution remain closed between lines and treatments, gross averages of scores were given to synthetise the score

distribution. For continuous data obtained once per animal, the model included the fixed effects of the line, the treatment, the replicate, the sex, and their interactions. The statistical units for analysis of the occurrence of lameness scores were groups of pigs (by line, sex and activity treatment) × week of measurement (N = 72). All SAS scripts are given as Supplementary Materials.

Results

The replicate affected some parameters, but its interactions with other factors were very barely significant. Therefore, its effect was only described when a significant effect of its interactions with line, activity or line × activity was observed. Because of technical sorter failure at week 2 of replicate 1, a daily number of crossings were not included in the analyses for both first weeks of replicate 1. Due to adaptation difficulties or health problems not related to leg disorders, pigs had to be discarded during the experiment; 2 LRFI pigs submitted to SA treatment, 5 LRFI pigs from FA treatment, 5 HRFI pigs from SA treatment and 6 HRFI pigs from FA treatment.

Growth performance and feeding activity

Pigs of the LRFI line showed a higher ADG (811.5 vs 762.0 g/d between 3 and 14 weeks, Fig. 1, $P < 0.001$) and a lower FCR (2.85 vs 3.19 g/g, $P < 0.001$) than pigs of the HRFI lines. Pigs from the LRFI also showed a lower daily FI ($P < 0.05$) and a higher live weight ($P < 0.001$) throughout the growing period. Forced activity did not affect the FCR ($P > 0.10$) and tended to decrease the ADG, daily FI and live weight ($P < 0.10$). The dynamics of ADG, FCR, and daily FI did not differ according to the lines (interaction 'week × line', $P > 0.10$). The live weight of the pigs assigned to FA was increasingly lower than that of the pigs assigned to SA between week 2 and week 13 (interaction 'week × line', $P < 0.05$). Average daily gain, FCR, FI and live weight were unaffected by the interaction line × activity ($P > 0.10$). The carcass of LRFI pigs was heavier ($P < 0.001$) with a higher lean meat percentage ($P < 0.001$) due to the higher loin percentage and lower belly and back fat percentage. Forced activity decreased back fat thickness (18.9 vs. 20.1 mm, $P < 0.05$) and belly percentage ($P < 0.01$) and increased carcass lean meat percentage ($P < 0.05$). Feed intake per meal was higher for the

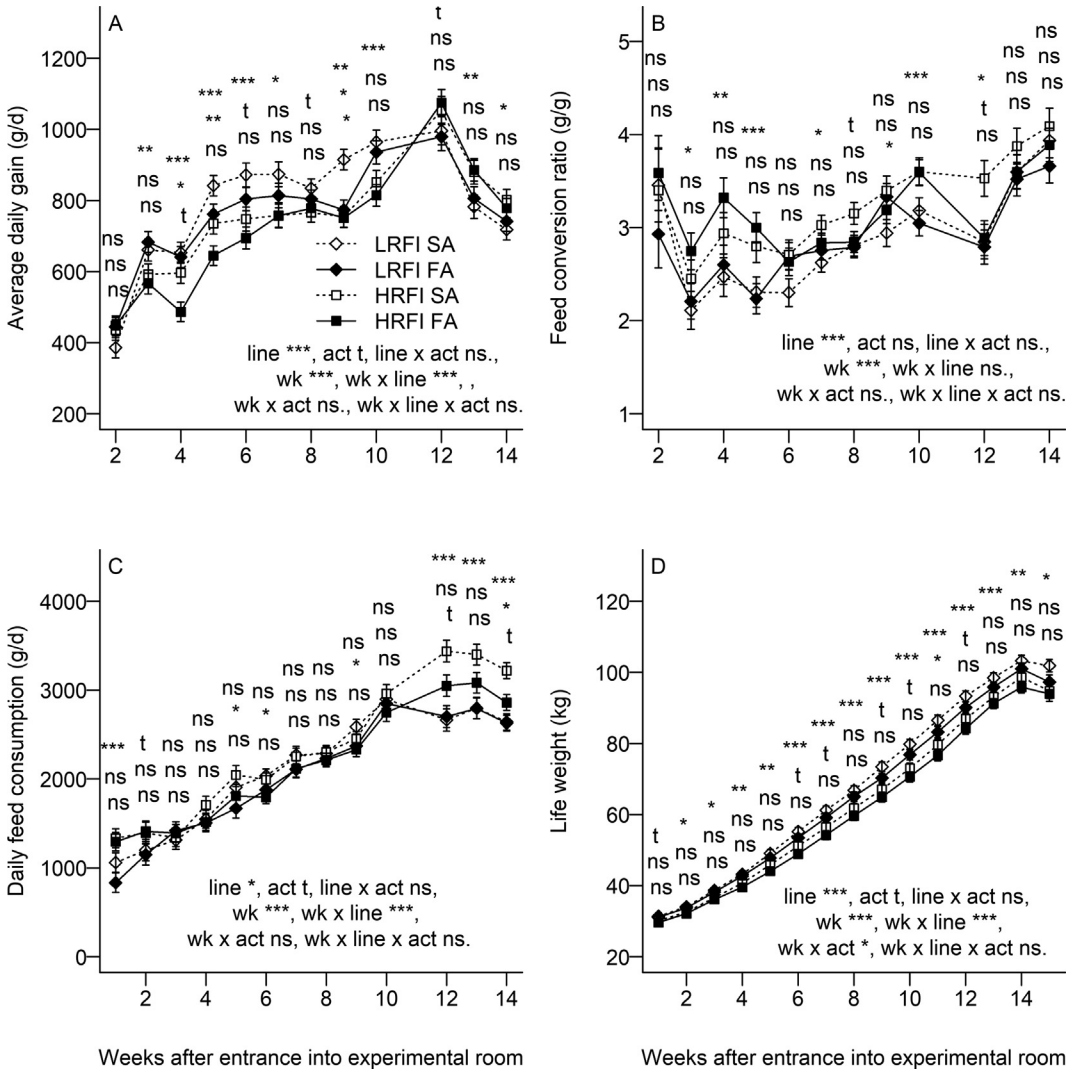

Fig. 1. Effect of line (HRFI high, LRFI low residual feed intake) and activity (SA: spontaneous activity or FA: forced activity) on average daily gain (ADG), feed conversion ratio (FCR), feed intake and live weight of the pigs between weeks 1 and 14 after their entrance into the experimental room (activity treatments were differentiated after week 2, pigs were slaughtered between weeks 15 and 16). Symbols represent, from top to bottom, the significance of the effect of the line, the treatment and their interaction (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, t $P < 0.10$, ns, $P \geq 0.10$). Bars represent SEM of the adjusted means per modality of line × treatment.

LRFI pigs than for the HRFI pigs ($P < 0.001$, Fig. 2), and their daily number of meals was lower ($P < 0.001$). Forced activity increased the FI per meal and decreased the daily number of meals ($P < 0.001$).

Physical activity

Low RFI pigs showed a lower individual number of sorter crossings/d than HRFI pigs (3.85 ± 0.33 vs 4.89 ± 0.34 crossings/d between weeks 3 and 15, $P < 0.001$, Fig. 3). Forced activity clearly increased the number of sorter crossings/d/pig ($P < 0.001$), but the magnitude of the effect of FA was lower in LRFI pigs (4.26 vs. 3.44 crossings per day between FA and SA) than in HRFI pigs (5.97 vs. 3.82 crossings per day, interaction $P < 0.05$).

The proportion of standing pigs was always lower for the LRFI lines than for the HRFI lines throughout the experimental period (Fig. 4, $P < 0.001$). Forced activity increased the proportion of standing pigs at week 13, at the beginning of the recording period, and in the afternoon ($P < 0.05$). However, the proportion of pigs standing was also affected by FA on week 3 before the activity treatment started, but the difference was numerically small ($P < 0.05$).

Lameness scoring

Throughout the growing period, the prevalence of lameness was very low. Less than 9% of the recorded scores characterised lame pigs, and the number of days a pig was recorded lame throughout the nine recording days was 0.55 ± 0.07 d (Table 1). On average, the lameness score was higher for LRFI pigs ($P < 0.05$) and was lower with FA ($P < 0.05$), but this effect was mainly due to low scores for HRFI pigs with FA, even though the effect of the interaction was not significant. The proportion of scores classified as stiffness was higher for LRFI pigs than for HRFI pigs (38.3 vs 29.6%; $P < 0.0001$). This line effect was numerically higher with FA, but the effect of the interaction was not significant. The distribution of lameness scores according to the recording week is given in Supplementary Table S5.

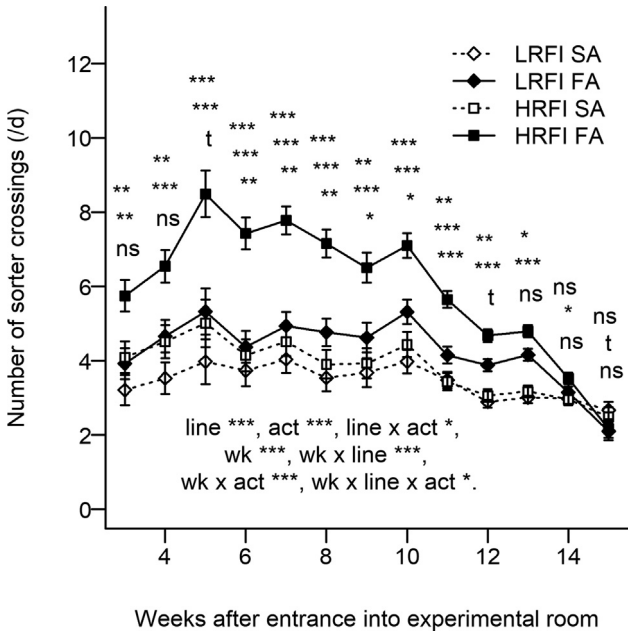

Fig. 3. Physical activity within groups of pigs, expressed as the average number of times an individual pig crossed the sorter per day during the whole experimental period according to the line (HRFI high, LRFI low residual feed intake) and the activity (SA spontaneous activity or FA forced activity) applied 2 weeks after the entrance into the experimental room. Symbols represent, from top to bottom, the significance of the effect of the line, the treatment and their interaction (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, t $P < 0.10$, ns, $P \geq 0.10$), bars represent SEM of the adjusted means per modality of line × treatment. Because of sorter failure in replicate 1 of week 2, data of weeks 1 and 2 were not included in the analyses.

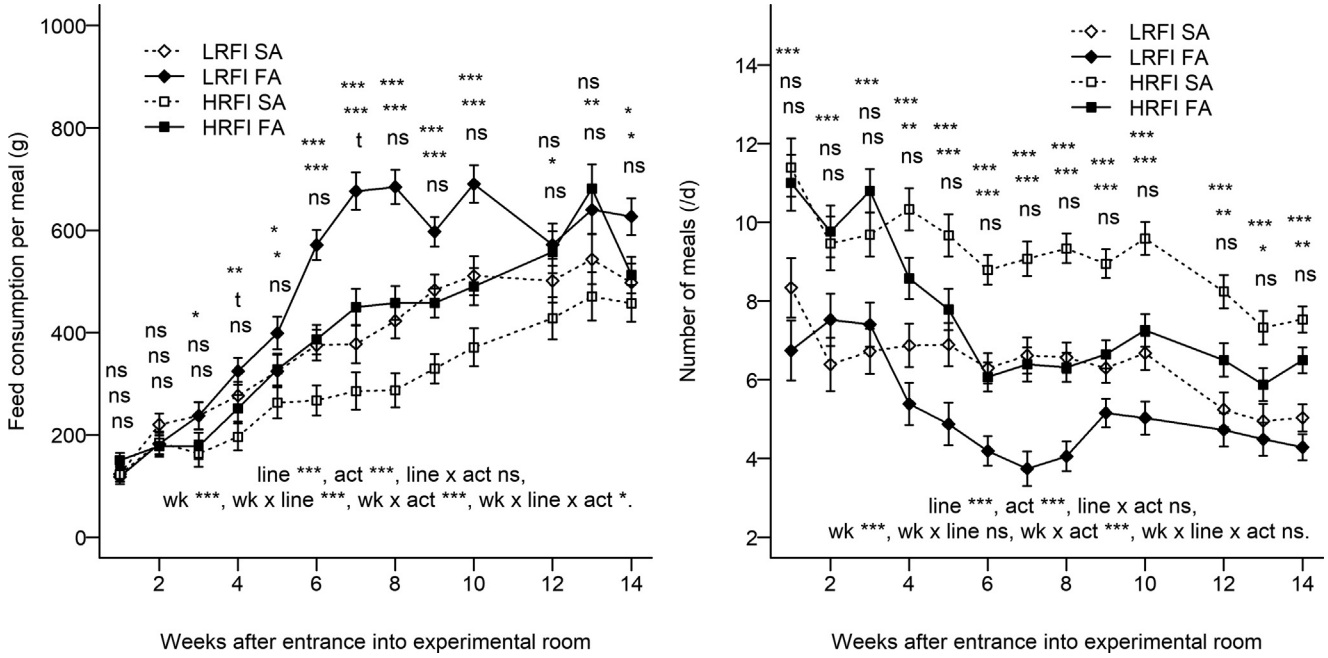

Fig. 2. Effect of line (HRFI high, LRFI low residual feed intake) and activity (SA: spontaneous activity or FA: forced activity) on feed consumption per meal and daily number of meals of the growing pigs between weeks 1 and 14 after their entrance into the experimental room (activity treatments were differentiated after week 2, and pigs were slaughtered between weeks 15 and 16). Symbols represent, from top to bottom, the significance of the effect of the line, the treatment and their interaction (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, t $P < 0.10$, ns, $P \geq 0.10$), bars represent SEM of the adjusted means per modality of line × treatment.

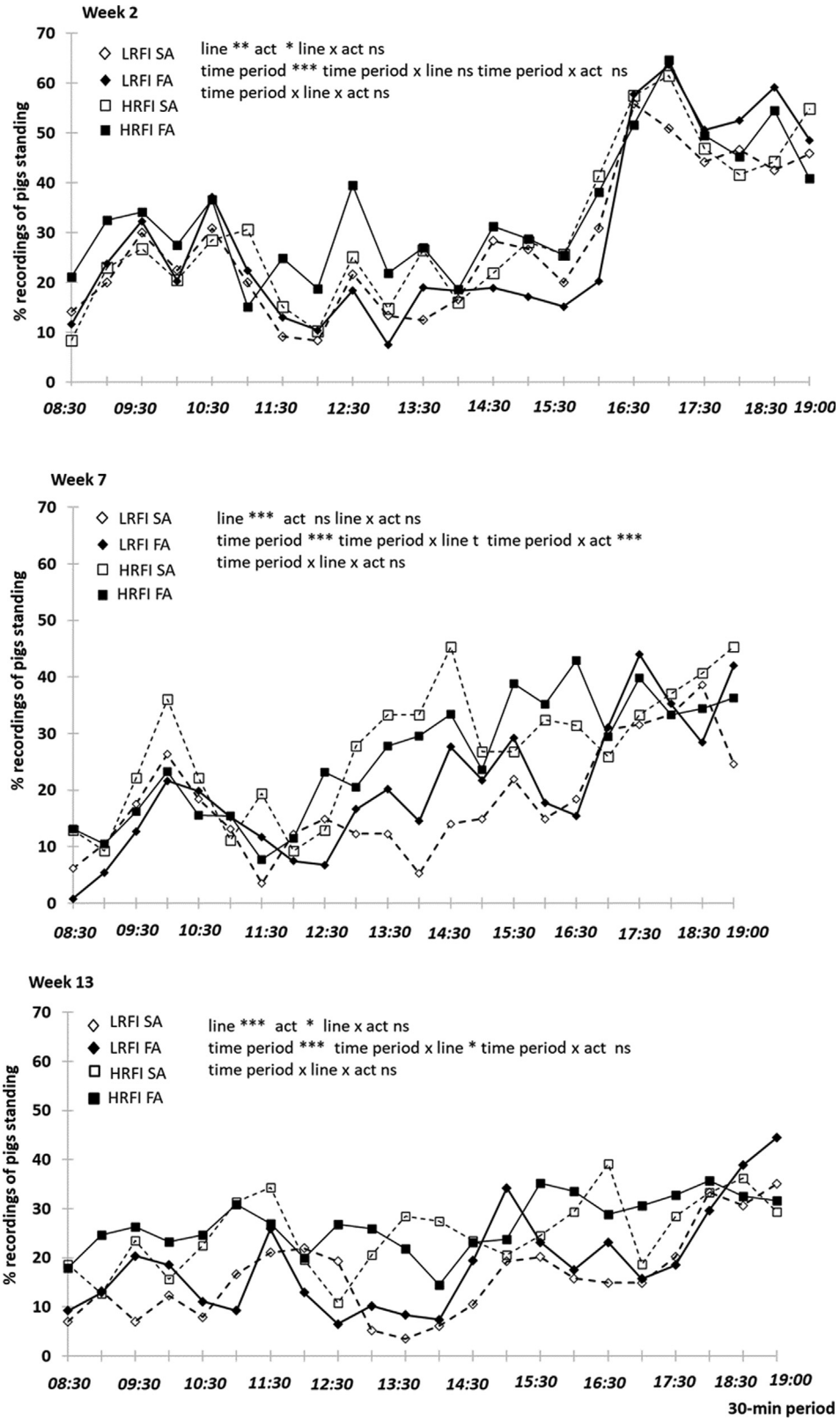

Fig. 4. Physical activity within groups of pigs, expressed as the number of pigs standing during the 11-h daily period (30-min period time) at weeks 3, 8 and 13 after entrance into the experimental room, according to the line (HRFI high, LRFI low residual feed intake) and the activity (SA spontaneous activity or FA forced activity) applied 3 weeks after entrance into the experimental room. Significant effects of the 30-min period, line, treatment and their interaction: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, t $P < 0.10$, ns $P \geq 0.10$). Analysis was performed on arcsin root transformed data.

Table 1
Effect of selected lines on RFI and activity of growing pigs on lameness, osteochondrosis scores, carcass composition and serum analyses.

					P-values[1,2]			
	LRFI SA	LRFI FA	HRFI SA	HRFI FA	Line	Treat	Line × treat	Sex
Average number of days with lameness per pig[1]	0.60 ± 1.05	0.69 ± 0.98	0.47 ± 0.78	0.41 ± 0.60	0.19	0.82	0.47	0.32
Gait, score[2]	0.52	0.57	0.51	0.33	*	*	0.51	0.92
Occurrence, % of total recordings[2]								
Lameness score ≥ 2	7.08	8.75	6.17	4.95	0.32	0.51	0.81	0.51
Stiffness score = 1	39.08	37.5	34.09	25.08	***	0.28	0.35	0.11
Osteochondrosis scores[2]								
Proximal Humerus (Surface[3])	2.16	2.2	1.53	1.36	***	0.71	0.28	0.06
Distal Humerus (Surface[3])	2.44	2.16	2.75	2.58	*	0.25	0.83	0.23
Proximal Femur (Surface[3])	1.75	1.67	1.18	1.13	***	0.78	0.76	0.79
Distal Femur (Surface[3])	1	1.15	1.27	1.27	0.07	0.53	0.63	0.81
Distal Humerus (Slices[4])	1.57	1.24	1.66	1.94	0.14	0.88	0.37	**
Distal Femur (Slices[4])	0.91	1.11	1.52	0.99	0.10	0.19	0.03	***
Carcass weight (kg)[1]	83.8 ± 1.05	82.2 ± 1.09	78.4 ± 1.11	76.6 ± 1.09	***	0.12	0.94	*
Back fat thickness (mm)[1]	20.3 ± 0.52	19.1 ± 0.54	19.8 ± 0.55	18.7 ± 0.55	0.42	*	0.88	***
Carcass lean meat percentage[4] (%)[1]	74.6	75.1	73.9	74.1	***	*	0.41	***
Ham percentage (%)[1]	23.2	23.5	23.0	23.3	0.76	0.94	0.46	***
Loin percentage (%)[1]	26.3	26.8	25.2	25.2	**	0.85	0.87	**
Shoulder percentage (%)[1]	24.1	24.1	23.8	23.9	0.91	0.45	0.33	1.00
Belly percentage (%)[1]	12.7	12.4	13.5	13.4	***	**	0.33	***
Back fat percentage (%)[1]	7.2	6.6	7.9	7.5	***	0.14	0.22	0.89
Serum biomarkers of cartilage degradation and formation[1,5]								
CPII (ng/ml)	2720 ± 187.3	2501 ± 191.2	2068 ± 198.7	2380 ± 191.0	*	0.81	0.17	0.10
C2C (ng/ml)	533.7 ± 25.2	578.8 ± 25.7	464.3 ± 26.8	493.9 ± 25.7	***	0.15	0.77	0.08

Abbreviations: HRFI = high residual feed intake; LRFI = low RFI; SA = spontaneous activity; FA = forced activity; C2C = collagen type II cleavage; CPII = procollagen II C-propeptide.
[1] *P*-values of serum biomarker contents, number of days with lameness per pig, carcass weight and back fat thickness were obtained from the mixed procedure of SAS, and LSmeans ± standard errors of the mean are given. P-values of anatomical proportions of the carcass were obtained from the mixed procedure of SAS after transformation of the variable by the arcsin function, and back transformation of the LSmeans are given. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[2] *P*-values of gait and osteochondrosis scores were obtained from the Genmod procedure of SAS, and gross averages are given.
[3] Scale from van Grevenhof et al. (2011).
[4] Scale from Etterlin et al. (2014).
[5] Data obtained from replicate 1. The effects of the replicate and its interaction were discarded from the model. P-values were obtained from the mixed procedure of SAS after transformation by the log function. Reciprocal transformation of the LSmeans is given.

Osteochondrosis evaluation and carcass parameters

The distribution of OC scores is given in Supplementary Table S6. It differed according to the joint and the evaluation method. With surface evaluation, the proportions of pigs with OC lesions were between 31.7% for the proximal femur humerus and 82.4% for the distal humerus. These proportions were calculated considering scores between two and five because score one may not have been specific to OC. For slice evaluation, the proportions of pigs with OC reached 57.3% for the distal humerus and 61.3% for the distal femur (scores different from zero). No pigs with *osteochondrosis dissecans* (score five) of the femur were observed regardless of the extremity and method of evaluation or of the proximal femur with surface evaluation. However, on the distal humerus, *osteochondrosis dissecans* was observed in 12% of animals with surface evaluation (17 animals) and 14% of animals with slice evaluation (20 animals).

The LRFI pigs showed higher OC scores on the proximal humerus (2.18 vs 1.45, $P < 0.001$, Table 1) and femur (1.71 vs 1.16, $P < 0.001$) with surface evaluation than the HRFI pigs. They also showed lower OC scores on the distal humerus with surface evaluation (2.30 vs 2.67, $P < 0.05$) and a tendency of lower scores on the distal femur with surface (1.08 vs. 1.27, $P = 0.07$) and slice evaluation (1.01 vs. 1.26, $P = 0.10$). Most OC scores were unaffected by either the activity treatment or its interaction with the line. Only OC scores of the distal femur (evaluated by the slice method) were higher with FA for LRFI pigs, whereas they were lower for HRFI pigs ($P < 0.05$).

The OC scores obtained by slice evaluation were also higher for castrated males than for females ($P < 0.01$ for distal humerus, $P < 0.001$ for distal femur). However, surface evaluation of the proximal humerus showed that scores tended to be slightly lower for males than for females (1.72 vs. 1.91, $P = 0.06$).

Cartilage biomarkers

Seric concentrations of CPII and C2C were higher in LRFI pigs than in HRFI pigs (CPII $P < 0.05$, C2C $P < 0.001$, Table 1). These concentrations were unaffected by the activity treatments. The seric content of CPII and C2C also tended to be higher for males than for females (CPII $P = 0.08$, C2C $P = 0.10$). No correlation was observed between individual scores of OC and cartilage biomarker concentrations both in the general population and within the lines (maximal correlation coefficient of 0.14 except for the correlation coefficient between the C2C concentration and surface OC scores of the proximal femur that reached 0.17).

Author's point of views

Experimental conditions

In agreement with previous studies (Gilbert et al., 2017), pigs from the LRFI line had a lower FCR and carcasses with more muscle and less fat. Our results also confirmed that LRFI pigs are less active than HRFI pigs (Gilbert et al., 2017), which was illustrated by the lower daily number of sorter crossings, the lower daily number of meals and the lower proportions of pigs standing. The feeding patterns were also consistent with what has been widely described, with LRFI pigs exhibiting a lower number of visits, shorter daily eating time, and increased feeding rate (Meunier-

Salaün et al., 2014; Gilbert et al., 2017). Observations of group-housed pigs usually show that the majority of movements occur around feeding and suggest that the distance walked by pigs for feed is essentially a representation of the mobility intensity and the distance covered by animals according to access to the feeder (Hansen et al., 1982). Thus, we assumed that the daily number of sorter crossings can be a good predictor of physical activity.

Forced activity had little effect on performance irrespective of the line. The distance to be covered by pigs may have been too low for depressing the performances considering that the pigs had *ad libitum* access to feed. Moreover, pigs increased the FI per meal and decreased the daily number of meals to counteract the constraint for feed access.

The line with the lower feed efficiency was more prone to increased physical activity with forced activity treatment

Forced activity induced a higher daily number of sorter crossings, and this effect was greater for the HRFI line before week 10. This shows that HRFI pigs, contrary to LRFI pigs, had a higher propensity to increase their physical activity when submitted to a constraint to feed access. This may indicate that LRFI pigs could be less prone to increased physical activity when constraints are applied to reach food, which may put into question their adaptability to farming conditions with increased surfaces.

The daily number of sorter crossings decreased throughout the growing period for the HRFI line. This could be related to the general decrease in activity over the growing-finishing period in growing pigs, as reported in groups of growing pigs submitted to reduced space allowance over time or to crowded conditions (Vermeer et al., 2014). While growing, the HRFI pigs may have experienced more constraints travelling through the group, to reach the sorter and thus may have reduced the daily number of sorter crossings and meals. However, FA had a limited effect on the percentage of pigs standing, which must be related to the important time spent resting in groups during the nycthemeral cycle in many rearing systems (Averós et al., 2010).

Higher susceptibility to locomotor disorders is suspected in the line with higher feed efficiency

Higher OC scores of the proximal humerus and femur and higher proportions of stiff gait and lameness scores were observed in LRFI pigs than in HRFI pigs, even though inverse but less significant results could be observed for OC scores of the distal humerus. Higher average OC scores were associated with higher ADG, live weight and lean meat contents, as already reported (Lundeheim, 1987; Jørgensen and Andersen, 2000), even though the existence of a relationship between those traits is under debate (Ytrehus et al., 2007). The causality of the relationship between OC scores and stiff gait cannot be proven, but our results illustrate that LRFI pigs may be more affected by locomotion disorders. This may explain their likely lower motivation to move when submitted to FA treatment. Because of the low number of lameness occurrence in our study and because lower OC scores on the distal humerus could also be observed in LRFI pigs, the link between the prevalence of OC lesions and lameness should be discussed with caution. We hypothesise that the time window for the appearance of lameness and OC lesion development is not the same. The development of OC lesions is due to focal failure of vessels in cartilage canals at certain predilection sites that mainly occur between 10 and 16 weeks of age (Ytrehus et al., 2007). Osteochondrosis lesions can then disappear until a point of non-return, anterior to 23 weeks of age (Bertholle et al., 2016). Lameness has been identified as an important reason for early culling in sows (Engblom et al., 2007), whereas it is less frequent in fattening pigs.

Our results did not demonstrate that FA can reduce the appearance of leg disorders. The occurrence of lameness was low, which could be explained by proper housing conditions with individual space allowances larger than the European guidelines (Directive EU 2008/120/UE), a partially slatted floor and optimal management, avoiding a slippery floor (EFSA, 2014). We observed that FA reduced the OC scores of the distal femur (slice evaluation) of HRFI pigs, which was consistent with the observation that FA mainly increased the activity of HRFI pigs. However, no effect of FA could be observed on any other OC scores.

Methodological issues for osteochondrosis evaluation

Compared with the literature, the proportions of pigs with OC in this experiment were high, but OC prevalence strongly depended on the joint and the considered studies (Lundeheim, 1987; Jørgensen et al., 1995; Storskrubb et al., 2010; van Grevenhof et al., 2011; Etterlin et al., 2015). Large differences in OC prevalence between countries and breeds have been reported (Storskrubb et al., 2010). Even though these discrepancies may be explained by differences in specific population-related factors, the most likely reason may be the scoring systems. The effects of the lines on OC scores vary with the bone and the considered extremity. Accordingly, other studies have demonstrated that variations in occurrence and severity of OC lesions between individuals are not consistent for all joints (Jørgensen and Andersen, 2000).

However, our results also showed differences between lines depending on whether the OC lesions were evaluated with surface or slice observations, which may be due to differences in the sensitivity and specificity of the two methods. An OC lesion is an area of ischaemic necrosis of chondrocytes within the epiphyseal growth cartilage centred on one or several necrotic cartilage canals (Olstad et al., 2014). Its differential diagnosis relies on histopathological examination that was not possible in a large number of animals. Visual observations are quicker and do not require heavy equipment (Olstad et al., 2014), but their sensitivity and specificity for the detection of OC lesions are questionable. Visual observation of OC is based on indirect evaluation of the consequences of necrosis: focal thickening of cartilage for the lowest scores and subchondral lesions for the highest scores (Jørgensen et al., 1995) that can be directly observed on slices (Etterlin et al., 2014). However, the cut area of the bone represents a limited part of the surface that may not include the lesion, which may explain the low sensitivity of this method for low scores and perhaps the lack of significance of the line effect. In contrast, the consequences of cartilage necrosis can only be indirectly detected by the presence of defects on the surface (van Grevenhof et al., 2011), and some defects may also have been caused by the slaughter process or handling of the carcass. For all these reasons, surface measurements even if less specific are more sensitive than slice measurements.

The different time windows for OC lesion and lameness development and the established effect of heredity on OC susceptibility highlight the need for early detection of OC lesions for selection purposes, which is difficult with visual observations. Analyses of the seric contents of biomarkers of cartilage formation and degradation were performed (Frantz et al., 2010) because they do not require an expensive imagery apparatus. Seric concentrations of CPII were higher in our LRFI pigs, consistent with higher OC scores on both the proximal femur and humerus. This positive relationship was consistent with previous studies in pigs (Frantz et al., 2010) and horses (Billinghurst et al., 2004). It reflects an increased synthesis of type II collagen, which may be an attempt to promote a repair process through the synthesis of new cartilage, consistent with the thickening of cartilage that can be observed with OC lesions (Etterlin et al., 2014). The increase in seric concentrations of C2C in LRFI lines could also be related to an increase in proximal

humerus and femur OC scores and may be related to cartilage necrosis that can be observed with higher OC scores (Etterlin et al., 2014). The link between OC and C2C is weaker than that between CPII and OC in the literature (Frantz et al., 2010). These seric analyses did not seem to be an informative test for the early detection of OC in our study because we did not observe any significant correlation between these parameters and OC scores. A major limitation of those biomarkers is the low repeatability and reproducibility of their analyses. It cannot be determined whether the systemic variation in the concentrations of those biomarkers due to OC development may be limited if we considered OC to be due to small focal failures of endochondral ossification. Ultimately, imagery tools may be the solution for the objective of early detection of OC (Olstad et al., 2014).

Conclusion

In agreement with previous data, the physical activity of LRFI pigs was lower than that of HRFI pigs, but this experiment also showed that LRFI pigs were less likely to increase their physical activity when faced with a constrained access to feeders, which may put into question their adaptability to farming conditions with increased surfaces. The LRFI trait may be associated with higher susceptibility to locomotive problems, such as stiff gait, and more OC lesions. Due to the low prevalence of lameness, it was difficult to make a link between OC and lameness in this study. Animals with severe OC lesions were not systematically lame, but the observed severity of some OC lesions raises the question of induced pain. The present study did not allow us to estimate whether pigs with OC lesions felt pain and how severe the pain was, which is an important issue from an animal welfare point of view. The links between the severity of OC, lameness and animal welfare remain challenging and need further investigation. This study did not confirm that biomarkers of cartilage synthesis and degradation can be used as indicators of the presence of OC lesions because of the low repeatability and reproducibility of the related ELISA tests. Imagery tools remain the best way for the early detection of OC.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.anopes.2022.100010.

Ethics approval

The experimental protocol (APAFIS#495-2015040710599641v 3) was approved by French Ministry of Higher Education and Research.

Author ORCIDs

AB: https://orcid.org/0000-0001-8734-3310.
MK: https://orcid.org/0000-0003-4007-7259.
LB https://orcid.org/0000-0001-6727-5066.
NLF: https://orcid.org/0000-0001-9858-1584.
MCM: https://orcid.org/0000-0001-8667-9453.

Author contributions

Anne Boudon: Formal analysis, Writing - Original Draft.
Maïja Karhapää & Hilkka Siljander-Rasi: Conceptualization, Methodology.
Emma Cantaloube: Investigation.
Ludovic Brossard: Methodology, Validation, Formal analysis.
Nathalie Le Floc'h: Supervision, Funding acquisition.
Marie-Christine Meunier-Salaün: Conceptualization, Project Administration.

Declaration of interest

None.

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