Soil ingestion, a key determinant of exposure to environmental contaminants. The case study of chlordecone exposure in free-range pigs in the French West Indies.

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Claire Collas [a,*], Jean-Luc Gourdine [b], David Beramice [c], Pierre-Marie Badot [d], Cyril Feidt [a], Stefan Jurjanz [a]

[a] *Université de Lorraine, INRAE, URAFPA, 54000 Nancy, France*

[b] *INRAE Centre Antilles-Guyane, UR-ASSET, 97170 Petit-Bourg, Guadeloupe, France*

[c] *INRAE Centre Antilles-Guyane, UE-PTEA, 97170 Petit-Bourg, Guadeloupe, France*

[d] *Université de Bourgogne Franche-Comté-CNRS, Laboratoire Chrono-environnement, 25030 Besançon Cedex, France*

* Corresponding author.

E-mail address: claire.collas@univ-lorraine.fr (C. Collas).

[1]Non-standard abbreviations sorted in alphabetical order

[1] BW: body weight, CLD: chlordecone, CR: Creole breed of pigs, DM: dry matter, FM: fresh matter, HP: high pasture paddock, LP: low pasture paddock, LW: Large White breed of pigs, MRL: maximum residue limit, MW: metabolic weight, SP: sweet potato paddock

ABSTRACT

Ingested soil may expose free-range animals to environmental pollutants. In pigs, soil ingestion is few described whereas their burrowing behaviour suggests that it could be high. Although highly productive pigs are generally reared indoor, free-range farming is increasing in view of ethical considerations for animal welfare and is a common practice for subsistence agriculture systems. The experiment lasted 8 weeks (2 for adaptation, 6 for measurements) with 24 growing pigs of Guadeloupean Creole (CR) or Large White (LW) breeds. Pigs were assigned to 3 outdoor treatments: high pasture HP (>60 days of regrowth), low pasture LP (35 days of regrowth), and sweet potato SP (sweet potato field). Titanium (soil marker) and chromium (faecal output marker) contents of faeces, vegetation and soil samples were used to estimate individual daily soil ingestions. The average, 10^{th} and 90^{th} percentiles were 440, 200 and 726 g of dry soil per 100 kg body weight, respectively, without significant differences between the 3 outdoor treatments or the 2 breeds but with a significant period (i.e. week of measurements) × treatment interaction ($P<0.001$). In the French West Indies, animals may be exposed to chlordecone (CLD), a very persistent organochlorine insecticide. Simulations of CLD tissue contamination due to ingestion of contaminated soil were carried out and compared to the maximum residue limit. These results show that grazing management needs to be adapted to effectively limit soil ingestion by pigs and the impact of a contaminated environment on the sustainability of pig systems.

Keywords*:* soil intake; feeding behaviour; plant cover; exposure risk; soil-bound pollutants; chlordecone

1. Introduction

Literature developed methodologies to show that free-range animals might ingest soil during exploration of outside runs or paddocks (Jurjanz et al., 2014; Mayland et al., 1975; Roberts and Longhurst, 2002) and to quantify it. Even if the ingestion of some invertebrates of the pedofauna can take place (Rose and Williams, 1983), the ingestion of soil is generally of little nutritional value, as the presence of soil in the digestive tract would decrease the digestibility of ingested diet (Jurjanz et al., 2014). In addition, the ingestion of soil has been shown to be a significant exposure pathway of free-range animals to environmental pollutants (Ayrault et al., 2016; Cooke et al., 1996; Johnsen and Aaneby, 2019; Rychen et al., 2013). Indeed, soil can keep over very long-time concentrations of heavy metals, organic pollutants or radionuclides (Comte et al., 2022). Therefore, the studies of soil ingestion are less motivated by nutritional or zootechnical questions but much more by environmental and food safety approaches. To the best of our knowledge, this question has been little studied in pigs where only one study gives quantifications for free-range lactating sows (Jurjanz and Roinsard, 2014).

Although some breeds of pigs are more often reared indoor, societal expectations in favour of animal welfare are encouraging farmers to provide outdoor areas for the animals, and this is a criterion in the specifications for organic farming if the control of African swine fever is met (Martínez Avilés et al., 2019). Moreover, pigs are reputed to their digging activity and to explore easily soil whenever they have an access to (Høøk Presto et al., 2008). Furthermore, pigs are frequently an element of self-catering agricultural systems as they are able to valorize different byproducts of the garden or the household and by consequence would efficiently participate in the food delivery for the family. These agricultural systems are of especial interest as they are more frequent in informal systems not covered by the governmental monitoring programs to control exposure to environmental pollutions (Kagira et al., 2010; Thutwa et al., 2020). Moreover, informal animal rearing would concern more often the less well-off parts of the population, which have generally a more vulnerable health status. The fact that soil may carry environmental pollutants in the food chain especially to these populations strengthen the need to evaluate the degree of soil ingestion of pigs in such informal rearing systems and how it can be limited.

In this frame, the Caribbean context has a higher importance as home reared pigs are part of the traditional food habits and were frequently raised outside in a tropical climate characterized by episodes of strong rainfall (Sousa Junior et al., 2014). Therefore, these free-range systems can cumulate all risk factors: species with an elevated digging activity raised on easily soiled vegetation due to humid climate. The presence of a sanitary crisis due to the pollution of the environment by the organochlorine pesticide chlordecone (CLD) would reinforce the need to quantify the soil ingestion by free-range pigs and to find out management tools to limit their soil ingestion. In Guadeloupe, as in many tropical and subtropical regions (Robinson et al., 2011), swine production is based on a variety of farming systems, including specialized industrial and landless farms with high pig density, or small family farms with Creole and/or crossbreeds reared in low input conditions (including free-range pigs or tethered to a tree) (Gourdine et al., 2021).

Therefore, the present work studies the ingestion of soil in free-range pigs raised on different types of tropical vegetation covers in Caribbean conditions. Besides this main objective, the study target to investigate potential differences in soil ingestion between the local Creole breed in comparison to the European Large White breed.

2. Materials and methods

2.1.Ethics statement

All animal care handling techniques and procedures as well as the procedures for sampling were approved by the Ethics Committee of French Guyana and West Indies n° 069 (Comité d'Ethique en Matière d'Expérimentation Animale des Antilles et de la Guyane, CEMEAAG) authorized by the French Ministry of Higher Education, Research and Innovation, under the project number APAFIS#6070-2016070721289156v3. The experiment was performed at the INRAE Experimental indoors facilities of PTEA (Plateforme Tropicale d'Expérimentation sur l'Animal) and outdoor facilities of PEYI (Plateforme Expérimentale sur le végétal et les agrosYstèmes innovants en milieu tropical) according to the certificate number A 971-18-02 of authorization to experiment on living animals issued by the French Ministry of Agriculture.

2.2.Experimental design and animal management

A total of 24 growing pigs of two different breeds (12 Creole CR and 12 Large White LW) were used on the experimental facilities of INRAE in Guadeloupe (GPS 16°12'13" N, 61°39'24"W). The experimental design consisted of three outdoors treatments: high pasture (HP, pasture with grass more than 60 days of regrowth age); low pasture (LP; pasture with grass at 35 days of regrowth age) and sweet potato (SP; sweet-potato field). The 24 pigs were randomly selected at 13 weeks of age (20.6 ± 3.6 kg and 28.9 ± 5.2 kg BW, for CR and LW pigs, respectively), from the same litters and were affected in each treatment to have half-siblings represented in each treatment (4 CR and 4 LW pigs). All the pigs fed the same before joining the different experimental treatments. The experiment began by 2 weeks of adaptation of the pigs to experimental conditions (wk1-wk2), followed by 6 weeks of measurements on animals, vegetation and soil (wk3-wk8) (Fig. SM1, supplementary material).

The total area for each treatment was 880 m² (10 x 88 m) of ferralitic alluvial soils, divided in one plot of 100 m² (10 x 10 m; 12.5 m² per pig) for adaptation (electric fences, pastures for HP and LP, and sweet-potato field for SP) and 6 plots of 130 m² (10 x 13 m²) for the measurement periods. The 8 pigs per treatment grazed a plot for one week, from Monday (day1) to Sunday (day7) (16.25 m² per pig per week). The plot was delimitated by a mobile electric fence powered by a solar battery. Every Monday morning (at about 07:00), the pigs were moved to another plot. For each treatment, the pigs had access to two shades (1 m² per pig) and to 2 barrels near the shades, containing 100 L of water each, which were filled twice per day so that the pigs never run out of water, for drinking and for mud wallow. The HP and LP pastures did not contain any cultivated crops, but only natural grasses. The SP field was the results of a planting of sweet-potato cuttings of more than three nodes every 20 cm on a row and 1.60 m between rows (corresponding to 33 000 cuttings per ha). The plots were clean weeded before planting and one month after planting. In LP treatment, the plots were previously mown to 3 cm 35 days before the introduction of the pigs, to ensure the same age of regrowth and the same vegetation stage. The LP and HP diets were based on grass available on the plot, cracked corn (containing <25 μg $Ti{\cdot}g^{-1}$ DM and <20 μg $Cr{\cdot}g^{-1}$ DM; 750 g $FM{\cdot}d^{-1}$ from wk1 to wk4, and 1000 g $FM{\cdot}d^{-1}$ from wk5 to wk8; DM = 87.8 % FM), and a supplementation of 250 g $FM{\cdot}d^{-1}$ of a protein feed (composed by 96% of soya bean meal and 0.6 % of chromium oxide; containing 100 μg $Ti{\cdot}g^{-1}$ DM and 4215 μg $Cr{\cdot}g^{-1}$ DM). The SP diets were based on sweet-potato leaves and tubers available on the plot (the pigs had to dig the soil to catch tubers), a limited amount of non-cultivated grasses available in the sweet-potato field, cracking corn (400 g $FM{\cdot}d^{-1}$) and the same supplementation (250 g $FM{\cdot}d^{-1}$) of protein feed than LP and HP treatments. The protein feed was given individually in feeding boxes for all treatments in two daily meals: 150 g fresh matter (FM) in the morning and 100 g FM in the afternoon.

2.3. Sampling, measurements and analysis

Pregrazing surface height of herbage sward of both HP and LP treatments were measured on Monday morning *via* a rising plate herbometer (Fig. SM1, supplementary material). Grass was sampled on the HP treatment plot (outside the experimental paddocks). Potato tubers and aerial parts (leaves and stems) of potatoes were harvested on the SP treatment plot (outside the experimental paddocks) and weighted to estimate the available biomass per surface unit. The potato allowance was estimated in kg dry matter (DM) per period, as well as in g DM per kg body weight (BW). Based on BW of each pig in each period, an average supply per CR pig and per LW pig was estimated (CR pigs being lighter than LW ones, the calculation was done separately for the two breeds). Samples of corn and protein feed were taken weekly. All feedstuffs samples were dried (65°C up to constant weight) and crushed in a ball mill.

The behaviour of the pigs was studied twice during the experimental period, at the beginning (the third day of wk3) and in the middle (the fourth day of wk7) of the experiment. During 24 continuous hours (from 06:00 to 06:00 of the next day), the physical (rest, feeding, rooting, …) and feeding (water, leaves, sweet potatoes, concentrate) activities were recorded every five minutes. Animals were weighed each Monday morning during the transfer to the new experimental barn on a scale (PM110, Maréchalle, Chauny). During the distribution of the protein feed, an individual sample of faeces was taken from each animal from Tuesday (day2) to Friday (day5), dried (65°C up to constant weight) and crushed in a ball mill.

Each morning (from Monday to Friday, at about 08:00), a standardized picture (from the south-east corner) of each plot (HP, LP and SP) was taken with a digital camera. Consequently, each plot within each treatment had a total of 6 pictures (from Monday to Friday, day1 to day5, and the Monday after pigs had changed plot, called day8) (Fig. SM1, supplementary material). These images were used to quantify the changes in monitor the disappearance of the vegetation cover (see section 2.5).

Soil samples were taken once the animals have been moved to the next barn (15 cm, 3 elementary samples grouped to one sample of each experimental barn, i.e. each week). After manual separation of gravel (>2 mm) and roots, soil samples were dried and crushed in a ball mill. Titanium (in the form of titanium dioxide TiO_2) and chromium (Cr) were analysed in all samples (vegetation, feedstuffs, faeces and soil) by SARM service of CNRS-CRPG laboratory (Vandœuvre-lès-Nancy, France). The samples were dissolved by melting with lithium metaborate and recovering the melt with diluted HCl. Melting was carried out in a muffle furnace progressively raised to 1000°C (200°C/h). TiO_2 was measured by UV-visible spectrophotometry (measurement at 470 nm of the titanium-chromotropic acid complex at pH 3.5) on an AGILENT CARY60 instrument. Cr was measured by flame atomic absorption spectrometry on an AGILENT SPECTRAA 240FS instrument. Regarding Quality Control, all measurements were validated by measuring certified reference materials included in the analysis series. The reference materials analysed are BE-N, UB-N, SO-1, SO-2, BCR-CRM060, GSS-7, CRM-055, BCR-CRM414, GXR-5 and GXR-2 (Jochum et al., 2005). For Quality Assurance, the limit of detection (LOD) was 25 ppm for TiO_2 and 20 ppm for Cr. It was calculated on the measurements of minimum 10 x experimental blank. Uncertainty values vary depending on the content determined in each sample and are based on statistical calculations extrapolated from measurements of reference materials and duplicates of the experimental samples. For Cr the relative uncertainty is <20% for contents below 100 ppm, and <5% for contents above 100 ppm. For TiO_2 the relative uncertainty varies from 10 to 20% for contents below 200 ppm, to <5% for contents above 1000 ppm.

2.4. Evaluation of soil ingestion

The ingestion of soil was estimated at each experimental week based on faecal samples taken between Tuesday (day2) and Friday (day5). The method consisted of estimating the amount of the internal soil marker TiO_2 excreted in faeces, by considering that all faecal TiO_2 was originated from soil according to the very low TiO_2 levels found in plants and crops, 200 times lower than those found in soil (Table 1). Therefore, the daily faecal output was estimated *via* the external marker Cr_2O_3. Indeed, the daily applied of chromium, obtained by multiplying the amount of protein feed daily ingested (refusals were considered) by the chromium content of the protein feed, was divided by the chromium concentration in the faeces:

Daily faecal output [kg $DM \cdot d^{-1}$] = protein feed ingestion [g DM] × Cr in protein feed [µg $Cr \cdot g^{-1}$ DM] / Cr in faeces [µg $Cr \cdot g^{-1}$ DM] × 1000

Then, the daily soil ingestion was estimated *via* the concentration of the soil-specific internal marker TiO_2 in faecal output and in soil:

Daily soil ingestion [$g \cdot d^{-1}$] = TiO_2 in faeces [µg $TiO_2 \cdot g^{-1}$ DM] × faecal output [g $DM \cdot d^{-1}$] / TiO_2 in soil [µg $TiO_2 \cdot g^{-1}$ dry soil].

2.5. Statistical analyses

Statistical analyses were performed using R software (version 4.1.1) (R Development Core Team, 2020). Pregrazing sward surface heights were analysed in a linear model to compare HP and LP treatments, period and treatment × period interaction. The latter was not significant and remove from final model. Tukey's *post-hoc* test was used for multiple comparisons between the 6 experimental periods.

For the behaviour data, the data collected every five minutes were converted in time spent in physical of feeding activities. Each behaviour trait (eating, rooting, …) was analyzed separately by robust analysis of variance (ANOVA) with the WRS2 package, using firstly a three-way robust ANOVA: the effects of treatment (HP, LP vs. SP), breed (CR vs. LW) and period (wk3 vs. wk7) and within-animal effects, due to repeated measurements (wk3 and wk7). Pairwise group comparisons were performed within each breed × treatment interactions and when interactions were found not significant (P>0.05), two-way ANOVA was performed and pairwise group comparisons were performed within each treatment (HP, LP and SP). Pearson correlations between estimated ingestion levels and the time spent for rooting activities were calculated to check the linear relationship between soil ingestion and rooting activities.

The daily pictures of plots were analyzed using the method proposed by Mahieu et al. (2019). The aim of this method is to discriminate defoliation patterns based on a pixel index, the visible atmospherically resistant index for green matter (VARIgreen). As reported by Mahieu et al. (2019), the pixel index values were distributed in three categories: i) category A contained pixels associated with bare soil, mud and/or soil litter; ii) category B contained pixels associated with stems and other senescent plant parts with low chlorophyll levels; and iii) category C contained pixels associated with green matter. The percentage of pixels were calculated for each category and were normalized with the arcsine of the square root of the proportions. The transformed data were analyzed using ANOVA of the R package agricolae, with the effects of treatment (HP, LP vs. SP), day (1 to 5 and 8) and the interaction between day and category (A, B and C).

Animal variables (BW, faecal production, soil ingestion) were tested using linear mixed models with type of paddock (HP, LP, SP), period, breed (CR, LW) and their two-by-two interactions as fixed effects; and individual as a random effect (R package nlme). Individual animals were used as the experimental unit. Type of paddock × breed interaction was never significant and remove from final models. Only the interactions of period with the type of paddock, or with the breed, were retained in the final models when they were significant (P<0.05) or tended to be (0.05<P<0.1). The 'lsmeans' function (R package lsmeans) was used for multiple comparisons (Tukey method for adjusting p.values).

3. Results

3.1.Paddock characteristics

Herbage and potato quality, as well as stocking rate, expressed in kg BW per m² per period, are detailed in Table 1. Pregrazing sward surface heights were significantly higher on HP than LP paddocks with an average gap of 12.9 cm (P<0.001; Table 1). For both LP and HP treatments, the sward heights in wk8 (27.0 cm) were significantly higher than the sward heights in weeks 3 to 6 (19.3 to 21.9 cm; P<0.001). Sward heights ranged from 14.2 to 21.2 cm and from 23.9 to 32.9 cm for LP and HP paddocks respectively. Treatment × period interaction was not significant.

The potato biomass on SP treatment was estimated to 38.4 and 18.7 kg DM per period for tubers and leaves/stems respectively. This corresponded to a daily potato allowance of approximately 20.1 and 10.1 g DM·kg^{-1} BW for tubers and leaves/stems respectively. In relation to the BW of the pigs this would be equivalent to a daily allowance of 615 and 756 g DM tubers per pig, and 300 and 368 g DM leaves/stems per pig, for CR and LW breeds respectively.

3.2.Pig behaviour and impacts on paddocks

3.2.1. Pig behaviour

The interaction effects of breed with treatment and period on rooting, drinking and resting activities were not significant (P>0.20). Rooting activities significantly increased from wk3 to wk7 in HP and LP treatment but remained the same in SP treatment (Fig. 1). Eating activities were found to be different between breeds (P <0.01). Irrespective of the treatment, CR pigs spent much more time eating than LW pigs. Eating (Fig. 2) and drinking (Fig. SM2, supplementary material) activities in outdoor conditions significantly increased or trended to increase from wk3 to wk7 (except for CR pigs in HP conditions and for pigs in SP conditions, for eating and drinking activities, respectively). Consequently, time dedicated for resting decreased from wk3 to wk7 (Fig. SM3, supplementary material).

3.2.2. Soiling and depletion of the vegetation

The ANOVA analysis of the percentage of pixels showed that the latest was not affected by either treatment (P>0.8) or week (P>0.6). At the opposite, the percentage of pixels was affected by the interaction between days of pasture and VARIgreen category (P<0.001). Figure 3 illustrates that the proportion of pixels corresponding to green matter (category C) but also to stems and senescent plant parts (category B) decreased with the day of pasture and it is closed to zero after pasture (day 8). At the opposite, the percentage of pixels corresponded to bare soil, mud and/or soil litter (category A) increased and the value was closed to 100 % after moving pigs to the next plot (day 8). The percentage of pixels of C category (green matter) dropped dramatically from the first to the second day of pasture and inversely the percentage of pixels of A category increased. These results of the picture analysis were in line with our observations (Fig. SM4, supplementary material).

3.3.Soil ingestion

3.3.1. Influence of the type of paddock and the period

There was a significant type of paddock × period interaction for the four variables: BW ($P<0.05$), daily faecal output ($P<0.05$), faecal TiO_2 content ($P<0.001$) and daily soil ingestions ($P<0.001$; Fig. 4; Table 2). Expressed in g DM per pig per day, the means per treatment and period ranged from 91.9 (wk4) to 130.6 (wk5), from 131.2 (wk7) to 262.1 (wk4), and from 80.3 (wk5) to 178.4 (wk8), for HP, LP and SP treatments respectively. Regarding the individual values, daily soil ingestions expressed in g DM per pig ranged from 38.5 to 258.6, from 58.4 to 514.5, and from 35.2 to 310.8, for HP, LP and SP treatments respectively. In LP treatment, two values were particularly high for one pig due to its high faecal output and high TiO_2 contents in faeces. The latest were kept in the dataset as they did not appear abnormal, but they pull the average of the LP treatment upwards (Fig. 4). Irrespective of the period (wk3 or wk7), no significant correlations were found between rooting behaviour and the level of soil ingestion (first behaviour data set during wk3: r = 0.18, $P=0.40$; second behaviour data set during wk7: r = -0.34, $P=0.10$).

3.3.2. Influence of BW and breed

Pig's BW was not different between the three treatments (i.e. the three types of paddock: HP, LP and SP) (33.0 ± 1.4 kg, mean ± SE) but was significantly higher for LW than CR pigs (*P*<.001; Table 1). Pigs' BW increased during the six periods from 26.9 to 39.3 kg (on average + 12.4 kg in 5 weeks). The effect of breed was only significant on daily soil ingestion when the latter was expressed per animal and not per unit BW. The breed effect was therefore confounded with the BW effect since CR pigs were lighter than LW ones. Indeed, BW was on average 29.3 kg for pigs of CR breed and 36.7 kg for pigs of LW breed. During the experiment, CR pigs have grown from 23.8 to 34.9 kg and LW pigs have grown from 30.0 to 43.6 kg. The BW difference between CR and LW pigs was on average 6.2 kg at the start and 8.6 kg at the end of the experiment. Daily soil ingestions were on average 116.2 and 162.9 g DM per pig, and 410.5 and 469.2 g DM per 100 kg BW, for CR and LW breeds respectively (Fig. 5). The 10^{th} and 90^{th} percentiles were 181.8 and 662.6 g DM per 100 kg BW for CR, and 218.1 and 789.8 g DM per 100 kg BW for LW. Period × breed and treatment × breed interactions were not significant for all variables tested (*P*>0.05; Fig. 5; Table 3).

4. Discussion

4.1.Soil ingestion in pigs and possible drivers

We hypothesized that soil ingestion would increase from HP, LP to SP due to i) the difference in pasture allowance between HP and LP; ii) the higher rooting activities in SP than LP and HP since pigs had to root to find sweet-potato tubers. In contrast to our expectations, soil ingestion levels did not differ between treatments, with a daily average of

440 g DM per 100 kg BW, while we observed higher rooting activities in SP than LP and HP and higher pregrazing sward heights in HP than LP. A high variability in soil ingestions between pigs and between periods was observed with average, 10th and 90th percentiles at 439.9, 200.2 and 726.3 g DM per 100 kg BW, respectively.

The BW of the animals remained similar between the three types of paddocks and the pigs grew by about 353 g per day (i.e. +12.4 kg between the 1st and 6th week of measurements). This growth is consistent with the references for these breeds fed with these types of diets in our conditions (Gourdine et al., 2018), so it appears that the three types of paddocks allowed the pigs to meet their nutritional requirements for maintenance and growth. Therefore, we consider that the animals on the LP treatment were not nutritionally limited.

Although the 24-hour behavioural observations in weeks 3 and 7 showed that pigs in the SP paddock spent more time for rooting activities, this did not result into higher soil ingestion. The higher rooting activities on the SP treatment may be explained by the expression of natural foraging behaviour reinforced here by the search for underground food in contrast to the aerial grass available on the LP and HP treatments. Several hypotheses can be put forward to try to explain the rooting and/or soil ingestion behaviour, such as the natural exploratory behaviour of pigs (Studnitz et al., 2007), the search for nutritional elements as pedofauna, minerals, trace elements or fibres. However, even if the nutrient intakes from the different diets of the three treatments have not been quantified, the possibility of a restriction in minerals or trace elements is unlikely.

According to the picture analysis of the dirty grass or bare soil evolution over a period, the condition of the paddocks remained fairly similar between the three treatments but with a clear degradation from one day to the next. From day 5 (i.e. the last day of faces collection), the paddocks had a fairly high proportion of bare soil (85.1 %). On the morning of the 8th day (i.e. the day the pigs were moved to a new paddock for a next period), the bare soil, mud and/or soil litter represented almost the entire surface. Faeces were collected from day 2 to day 5 of each period, so soil ingestion was assessed in the first half of each period when sufficient vegetation was still available to observe differences between treatments if there had been any.

The period effect on soil ingestion was not related to the increase in stocking rate over time, although the increase in grazing pressure from one period to another may increase the impact of trampling and soiling of the grass, but the highest soil ingestions not being concentrated in the last periods. The first experimental period was the rainiest with 49 mm the day the animals entered the first experimental paddock, and 45 mm the day before. These conditions could explain at least in part the higher soil uptake observed in wk3, particularly in the LP treatment. Such an effect of humidity on soil ingestion has been shown previously in sheep (Abrahams and Steigmajer, 2003) and cattle (Collas et al., 2019). The consequences of the heavy rainfall may be more impactful on the short grass of LP than on the HP and SP treatments where the tall grass, or the aerial parts of the potatoes, can more easily attenuate the "splash" effect linked to the soil projections on the grass (Dreicer et al., 1983; Hinton et al., 1995). The results of this study suggest that the amplitude of soil ingestion is more related to the natural rooting behaviour of pigs in comparison with other species, the variability in climatic conditions and the time spent in each plot.

4.2.Soil ingestion of pigs in comparison to other species

This very first quantification in growing pigs showed average daily ingestion of soil between 116 and 171 g per animal. That is quite similar to these reported for cattle, also in Caribbean systems (Collas et al., 2019), but the large different BW change their significations. Indeed, the comparison of ingestions are generally carried out for growing animals by putting the ingested amount in ratio to 100 kg of BW or for comparison between species relative to the metabolic weight (MW, correspond to $BW^{0.75}$). In such a frame, the measured soil ingestion of these free-range pigs of 368 to 548 g per 100 kg of BW are much higher than ingestions reported in growing cattle in Caribbean systems of less than 100 g per 100 kg BW (Collas et al., 2020, 2019; Jurjanz et al., 2017), or less than 200 g per 100 kg BW in growing broilers (Jurjanz et al., 2015). By expressing per kg MW, cattle and broilers ingested generally not more than 4 g DM per kg MW (Collas et al., 2019; Jurjanz et al., 2017, 2015), grazing horses and lactating sows around 5 g $DM \cdot kg^{-1}$ MW (Jurjanz et al., 2021; Jurjanz and Roinsard, 2014), whereas these free-range pigs in tropical conditions ingest much more with 9 to 13 g $DM \cdot kg^{-1}$ MW (Table 2). These comparisons confirm clearly that the natural digging behaviour of pigs would result in a significantly higher soil ingestion than in other species what would, by consequence, expose such rearing systems much more to pollutants in a context of contaminated areas.

4.3. Implications for animal exposure to soil-bound pollutants and human health – the case of chlordecone

During grazing, pigs ingest soil and this is enhanced by rooting activity. Although this natural behaviour may allow ingestion of pedofauna and roots or mineral supplementation (Edwards, 2003), it also presents risks as pigs are exposed to the full range of contaminants that may be present in the soil, which may have serious repercussions on their health and/or the safety of the products. In the French West Indies, nearly one fifth of the agricultural soil in Guadeloupe and two fifths of the agricultural soil in Martinique are at risk of CLD contamination (Comte et al., 2022; Ministère de la Transition Ecologique, 2017). Due to its strong persistence, this insecticide, used against the banana weevil (*Cosmopolites sordidus*) until its ban in the 1990s, caused soil pollution and the contamination of water and ecosystems. A major challenge for this territory, as for any territory affected by such a health crisis, is to maintain ecosystem services, in particular the food produced, and the economic activities that depend on them (Perrette et al., 2020).

The concentration of CLD in perirenal adipose tissue (reference at the slaughterhouse for comparison with the maximum residue limit MRL) was simulated as a function of the daily soil ingestion and the concentration of CLD in the soil (CLD_{soil}). The CLD concentration in serum (µg/mL) was first estimated by dividing the maintenance dose by the clearance. The maintenance dose is the amount of CLD daily ingested (µg $CLD \cdot kg^{-1}$ BW) obtained by multiplying daily soil ingestion by CLD_{soil}. Daily soil ingestions (CR: 164.2, 72.7 and 265.0 g DM per pig; LW: 246.3, 114.5 and 414.6 g DM per pig; average, 10th and 90th percentiles, respectively) were calculated for each breed using values expressed per 100 kg BW, obtained for CR and LW pigs in this study, and the average BW of free-range growing pigs (CR: 40 kg; LW: 52,5 kg; average between post-weaning and slaughtering BW considering linear relation, with 20 and 60 kg for CR pigs, 25 and 80 kg for LW pigs, respectively). Different CLD_{soil} from 0.01 to 0.1 $mg \cdot kg^{-1}$ were considered in these simulations. The clearance ($mg \cdot kg^{-1}$ per day) was obtained from Fourcot et al. (2020) for both breeds (average of the two breeds and separately). Finally, the CLD concentrations in perirenal adipose tissue (µg per g of fat) were obtained by dividing the serum concentration by the tissue partition coefficient (Kp). The Kp in tissue is obtained by the ratio of concentrations between serum and tissue (Fourcot, 2020). Simulated results show that when CLD_{soil} is 0.1 $mg \cdot kg^{-1}$, CLD concentrations in perirenal adipose tissue exceed the MRL set at 0.02 mg CLD per kg of fat regardless of breed and soil ingestion. When CLD_{soil} is 0.05 $mg \cdot kg^{-1}$, only the 10% of animals ingesting the least amount of soil complied for both breeds (with a concentration 2 times lower than the MRL). When CLD_{soil} is 0.03 mg·kg-1, only the 10% of animals that ingest the most soil do not comply for both breeds. For CLD_{soil} of 0.01 and 0.02 mg·kg^{-1}, all animals comply (Table SM5, supplementary material). These simulations on the compliance of animals at slaughter, in relation to their ingestion of soil and the level of soil contamination, may contribute to establishing thresholds for regulating soil use in agriculture (Li, 2020). Our results therefore raise the question of the values to be considered in the risk assessment. Should we consider the mean or median ingestion levels, the 90 or 95 percentiles or outliers? Since self-consumption is a widespread practice in the French West Indies, this point is of crucial importance for self-consumers, who can therefore be markedly overexposed. Free-range livestock farming is a relatively common practice in the tropics in self-sufficient animal husbandry and has many advantages in terms of economy, environment, animal welfare and legislation (European organic regulation), so it is important to be able to secure these farming systems against health risks. A precise determination of daily soil ingestion is therefore necessary for the assessment of the health risk.

Our study shows that a significant amount of soil can be ingested by pigs on a daily basis. In order to limit the exposure of pigs to environmental contaminants, especially persistent organic pollutants, it is necessary to adapt grazing practices (i.e. stocking rate, optimal time step for changing plots, to limit soil ingestion. Thus, soil quality (texture, structure and composition), particularly the amount of soil pollutants, and climatic parameters has to be considered in grazing management. Even if grass is initially available in the paddock, the foraging behaviour of the pig can quickly remove the grass, leaving bare or even turned over ground for the remainder of the time spent in the paddock (Edwards, 2003). Thus, maintaining sufficient grass availability and/or increasing the supply of concentrate feed may help to limit soil ingestion associated with foraging and rooting on pasture (Jakobsen et al., 2015; Stern and Andresen, 2003). Rivera Ferre et al. (2000) observed a high frequency of rooting in unrung sows compared to nose-rung sows, but animal welfare implications limit this practice as a recommendation to reduce soil ingestion (Horrell et al., 2001).

The present study focused on soil ingestion, which is an important route of exposure of animals to environmental contaminants. However, other matrices can also contribute to animal exposure such as food and water consumption. The biomass of sweet potato tubers on the SP treatment plot was assessed which provides information on the amount offered to the animals (i.e. 38.4 kg DM per paddock of 130 m² offered per week, which corresponds to 20.1 g DM/kg BW^{-1} per day). From the pictures of the paddocks (Fig SM4, supplementary material), it can be considered that the pigs consumed almost all the available potatoes, which allows to roughly estimate the daily intake of potato tubers (i.e. 615 and 756 g DM per pig for CR and LW breeds respectively) based on the average BW of the animals of both breeds. The CLD concentration in potato tubers was estimated according to Achard et al. (2007) using a proportional relationship with the CLD content of the soil and a coefficient adapted for ferralitic soils such as the study plots. These data make it possible to evaluate the intake of CLD via the ingestion of sweet potato tubers. Using the average soil ingestions obtained in this study for the CR and LW pigs in the SP treatment, together with the CLD contamination of the soil to be simulated, it is possible to estimate the intake of CLD via soil ingestion. For soil at 0.05 mg CLD.kg^{-1} for example, this would correspond to a CLD intake of 9.95 (CR) and 12.17 (LW) µg, with 5.92 (CR) and 7.21 (LW) µg via soil, and 4.03 (CR) and 4.96 (LW) µg via sweet potato tubers. The soil contribution to exposure would be 60% with this kind of diet.

Drinking water can also expose animals to pollutants as CLD, especially for unmonitored spring water or ponds. However, this route of exposure is easier to control if farmers can use potable water drinking supply as a limit has been set at 0.1 µg CLD.L^{-1}. Considering a water consumption of 10% BW (i.e. on average 0.29 and 0.37 L per day for CR and LW pigs respectively under the conditions of this study) (Chambre d'Agriculture de Bretagne, 2021), this would represent an intake of CLD up to 0.29 and 0.37 µg for CR and LW pigs respectively in the case of water reaching the potability threshold of 0.1 µg $CLD.L^{-1}$.

5. Conclusions

This study provided references on soil ingestion by free-range pigs by comparing two breeds (Creole and Large White) and three types of outdoor paddocks (pasture with two different grass availabilities and sweet potato field). The results show that daily soil ingestions are not influenced by breed or paddock type in this experiment, but this type of management exposes pigs to substantial soil ingestions about half a kg of dry soil per animal per day. When free-range farming is carried out in an area where the soil is contaminated, soil ingestion levels such as those observed in this study can result in contamination of pigs, and their tissues, at values sometimes exceeding the MRL. In this context, adapting farming practices to limit pig exposure to soil-bound pollutants, in particular persistent organic pollutants as some phytosanitary molecules, appears inevitable to ensure the safety of animal products.

Acknowledgements

This work was supported by The French National Research Agency (ANR, project INSSICCA; grant number: 16.CE21.0008.01) and the Region Guadeloupe (AGROECODIV project and CPER CRB-PSA project). The authors thank warmly Bruno Bocage, Mélain Bructer, Katia Benony and Laurent Dantec from PTEA Experimental Unit (INRAE), Harry Archimède, Matthieu Desfonds, Dalila Feuillet and Valérieuse Calif from UR-ASSET

(INRAE), Pamela Hartmeyer and Agnès Fournier from URAFPA (Université de Lorraine, INRAE), and Nadia Crini from Chrono-Environment Laboratory (Université de Franche-Comté-CNRS).

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Figures

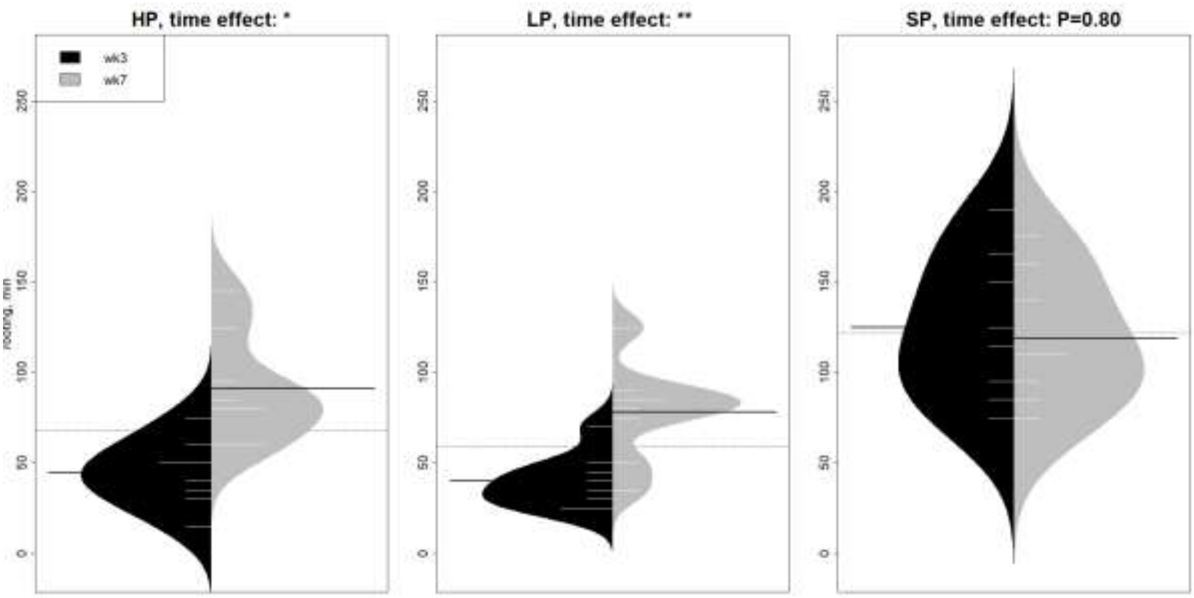


Fig. 1. Beanplot of rooting activities (time spent within 24-h, in min) of outdoor pigs reared in HP (pasture with grass more than 60 days of regrowth age), LP (pasture with grass at 35 days of regrowth age) or SP (sweet-potato field) conditions. The horizontal bars correspond to the means of the distributions (if they were normal): one mean for the black distribution, one mean for the grey and one mean for the joint distribution. Asteriks indicate significant difference (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$) between wk3 and wk7 within treatment.

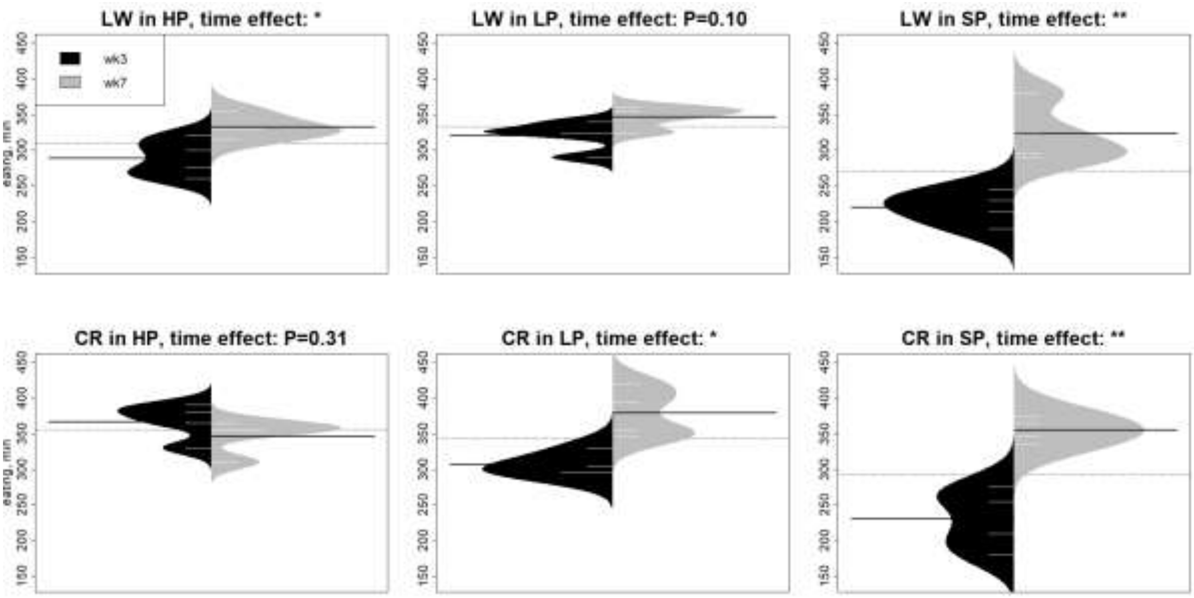


Fig. 2. Beanplot of eating activities (time spent within 24-h, in min) of outdoor pigs reared in HP (pasture with grass more than 60 days of regrowth age), LP (pasture with grass at 35 days of regrowth age) or SP (sweet-potato field) conditions. Asteriks indicate significant difference (*: *P*<0.05; **: *P*<0.01; ***: *P*<0.001) between wk3 (in black) and wk7 (in grey) within breed × treatment interactions (LW: Large White, CR: Creole).

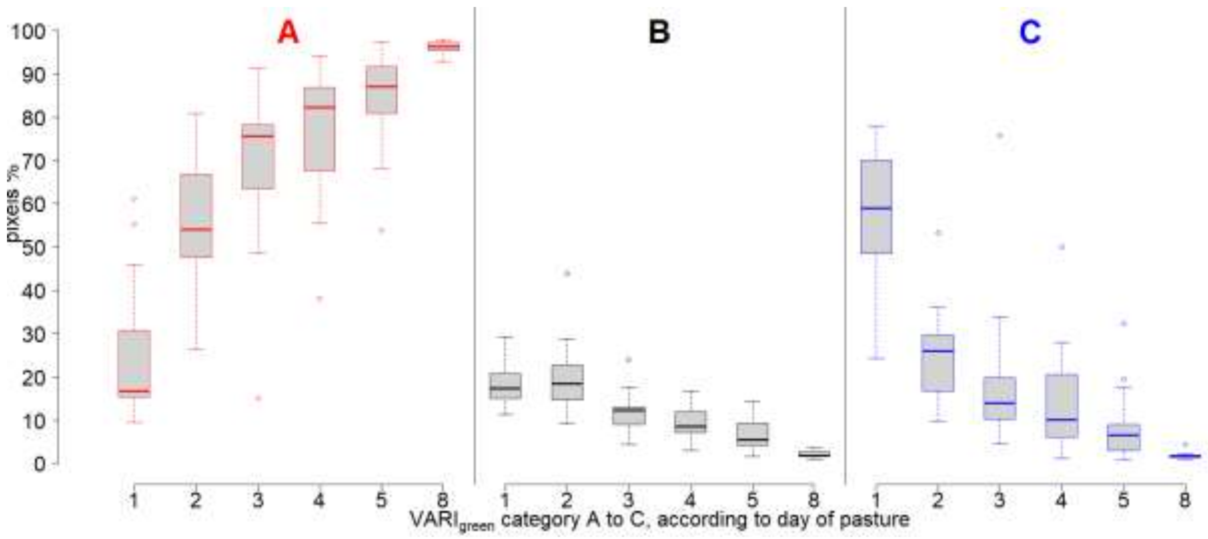


Fig. 3. Boxplot of the percentage of pixels in each VARIgreen category (A: bare soil, mud, and/or soil litter; B: stems and senescent plant parts; and C: green matter: leaves and sheaths) according to the day of pasture (1 to 5: first to fifth day of pasture; 8: the first day after pasture).

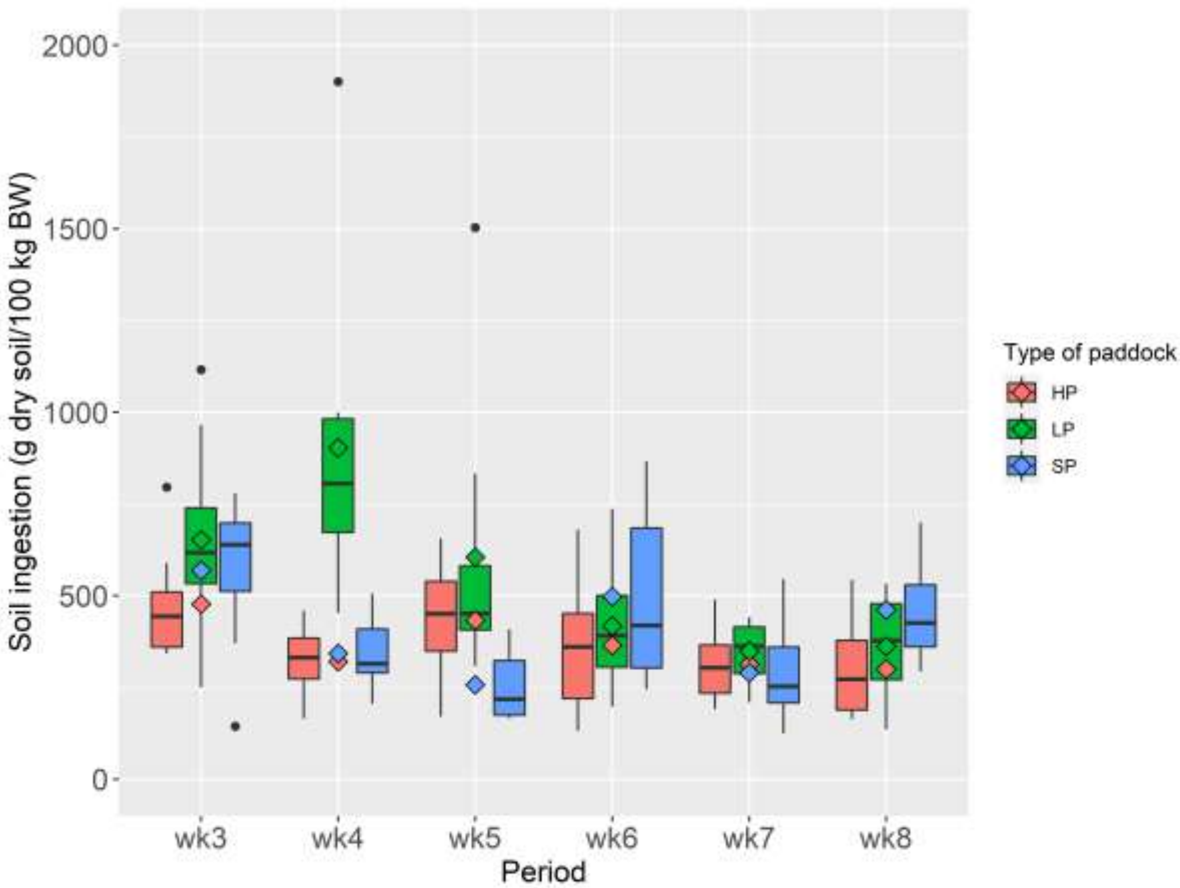
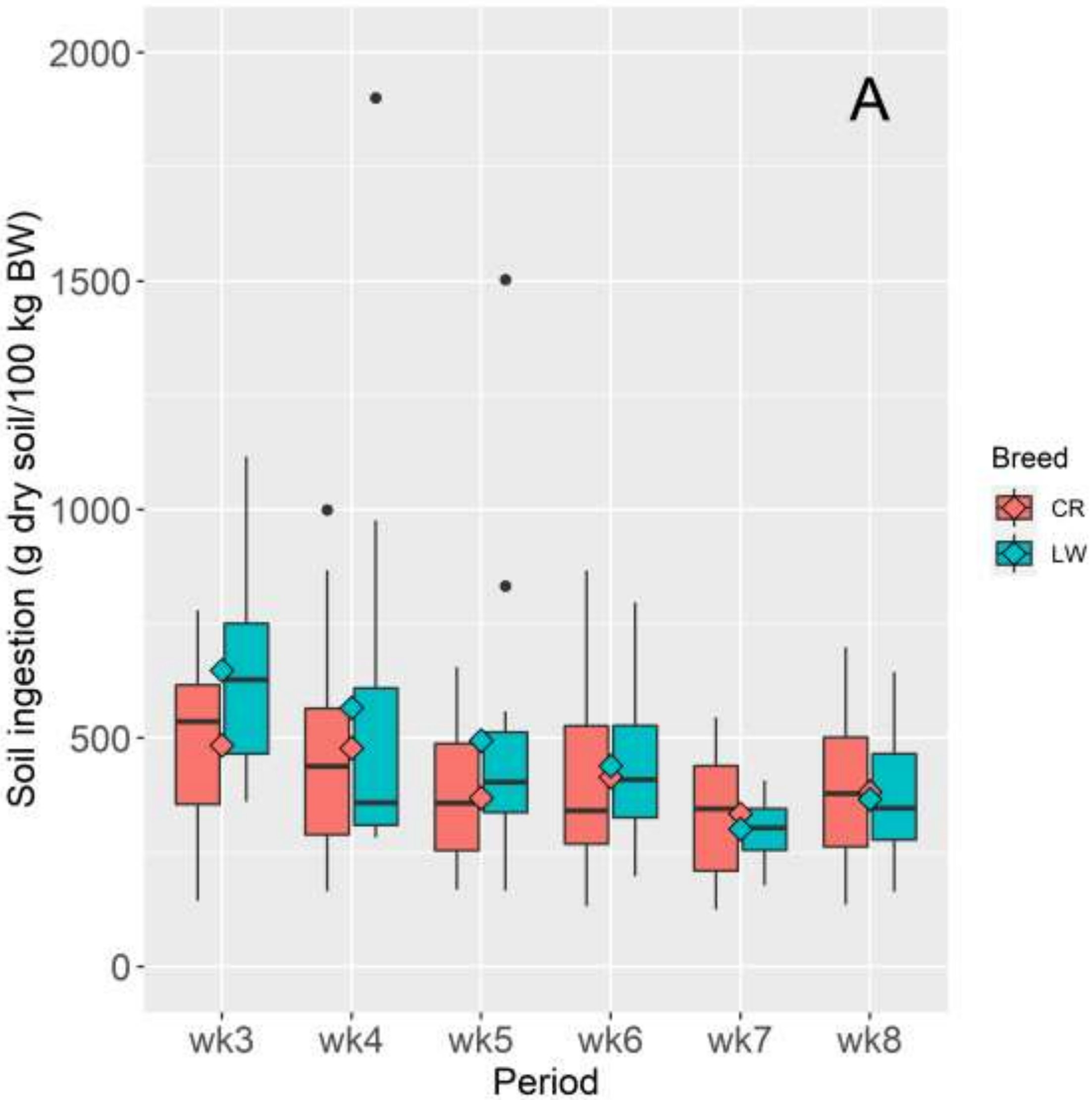


Fig 4. Effect of type of paddock (HP: pasture with grass more than 60 days of regrowth age; LP: pasture with grass at 35 days of regrowth age; SP: sweet-potato field) and experimental week (wk3 to wk8) on the ingestion of soil (the diamonds represent the averages of each treatment for each period).


A
Soil ingestion (g dry soil/100 kg BW)
2000
1500
1000
500
0
wk3
wk4
wk5
wk6
wk7
wk8
Period
Breed
CR
LW

698

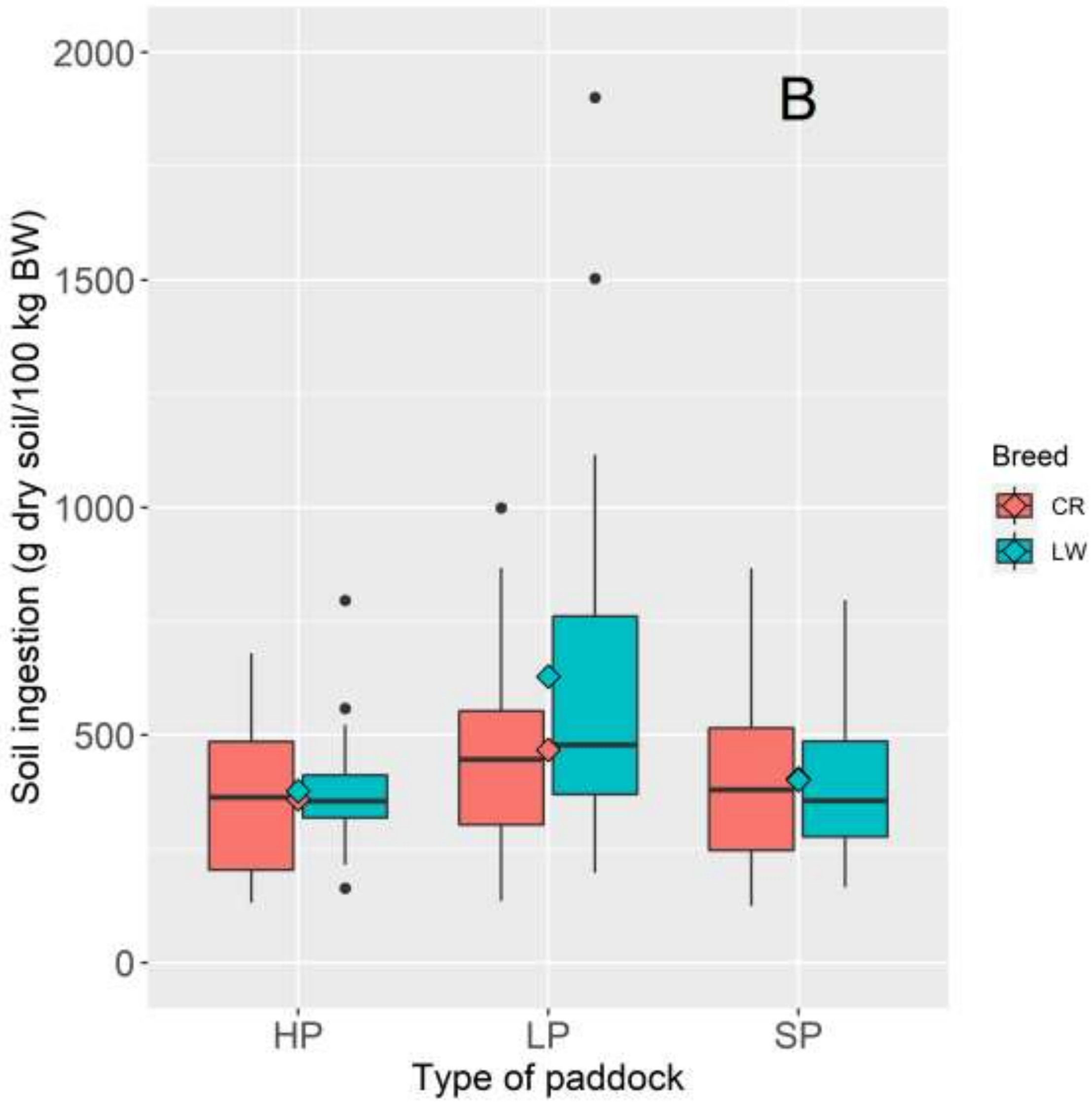


Fig. 5. Effect of breed (CR: Creole; LW: Large White) and experimental period (**A**) (wk3 to wk8) or type of paddock (**B**) (HP: pasture with grass more than 60 days of regrowth age; LP: pasture with grass at 35 days of regrowth age; SP: sweet-potato field) on the relative ingestion of dry soil, i.e. per 100 kg of BW.

Tables

Table 1. Grazing management, vegetation and soil characteristics according to the paddock type (HP: pasture with grass more than 60 days of regrowth age; LP: pasture with grass at 35 days of regrowth age; SP: sweet-potato field) (average value of the 6 weeks for each type of paddock).

Item	Type of paddock			
	HP	LP	SP	
			Tubers	Leaves/stems
Grazing management				
Daily surface area, m² per animal per period	16.25	16.25	16.25	
Stocking rate, kg BW per m²	2.00	2.05	2.04	
Stocking rate (week 3), kg BW per m²	1.64	1.66	1.67	
Stocking rate (week 8), kg BW per m²	2.39	2.44	2.42	
Sward height, cm	28.6	15.7	-	
Vegetation characteristics				
Dry matter content, $g \cdot kg^{-1}$	24.8	23.1	28.2	16.2
Organic matter content, $g \cdot kg^{-1}$ DM	779	-	881	801
Crude protein content, $g \cdot kg^{-1}$ DM	62	-	56	122
Neutral detergent fiber content, $g \cdot kg^{-1}$ DM	669	-	60	302
Cr[1] content, $\mu g \cdot g^{-1}$ DM	< 20	< 20	< 20	< 20
Ti[2] content, $\mu g \cdot g^{-1}$ DM	36	59	< 25	69
Ti and Cr levels in soils				
Cr content, $\mu g \cdot g^{-1}$ DM	<20	<20	<20	
Ti content, $\mu g \cdot g^{-1}$ DM	14223	14082	13282	

[1]Cr = chromium.

[2]Ti = titanium.

Table 2. Soil ingestion by pigs according to the type of paddock (HP: pasture with grass more than 60 days of regrowth age; LP: pasture with grass at 35 days of regrowth age; SP: sweet-potato field), period and their interaction.

Item	Type of paddock			RSD[1]	*P*-value		
	HP	LP	SP		Type of paddock	Period	Type of paddock × Period
Body weight (BW), kg	32.5	33.3	33.2	0.84	NS[2]	<.0001	0.043
Faecal production							
Faecal Cr[3] content, $\mu g \cdot g^{-1}$	3118	3050	3109	498.3	NS	0.004	0.068
Daily faecal output, g DM per pig	357	387	343	66.5	NS	0.058	0.015
Daily faecal output, g DM per 100 kg BW							
Faecal Ti[4] content, $\mu g \cdot g^{-1}$	4585[b]	6052[a]	4924[ab]	800.6	0.036	<.0001	<.0001
Soil ingestion							
Soil ingestion, g DM per pig	116[b]	171[a]	131[ab]	41.1	0.050	0.024	<.0001
Soil ingestion, g DM per 100 kg BW	368	548	403	145.0	0.081	<.0001	<.0001
Soil ingestion, g DM per kg MW[5]	8.7	12.9	9.6	3.26	0.069	NS	<.0001

[a–b]Means within a row with different superscripts differ (P <0.05).

[1]RSD = residual standard deviation.

[2]NS = non-significant (*P*>0.1).

[3]Cr = chromium.

[4]Ti = titanium.

[5]MW = metabolic weight.

Table 3. Soil ingestion by pigs according to the breed (CR: Creole; LW: Large White) and its interaction with the period (same model and continuation of the results presented in Table 2).

Item	Breed		RSD[1]	*P*-value	
	CR	LW		Breed	Breed × Period
Body weight (BW), kg	29.3	36.7	0.84	<.001	<.0001
Faecal production					
Faecal Cr[2] content, µg·g^{-1}	3676	2509	498.3	<.0001	0.009
Daily faecal output, g DM per pig	284	440	66.5	<.0001	0.019
Daily faecal output, g DM per 100 kg BW					
Faecal Ti[3] content, µg·g^{-1}	5442	4932	800.6	NS[4]	NS
Soil ingestion					
Soil ingestion, g DM per pig	116	163	41.1	0.015	0.089
Soil ingestion, g DM per 100 kg BW	411	469	145.0	NS	NS
Soil ingestion, g DM per kg MW[5]	9.4	11.4	3.26	NS	0.077

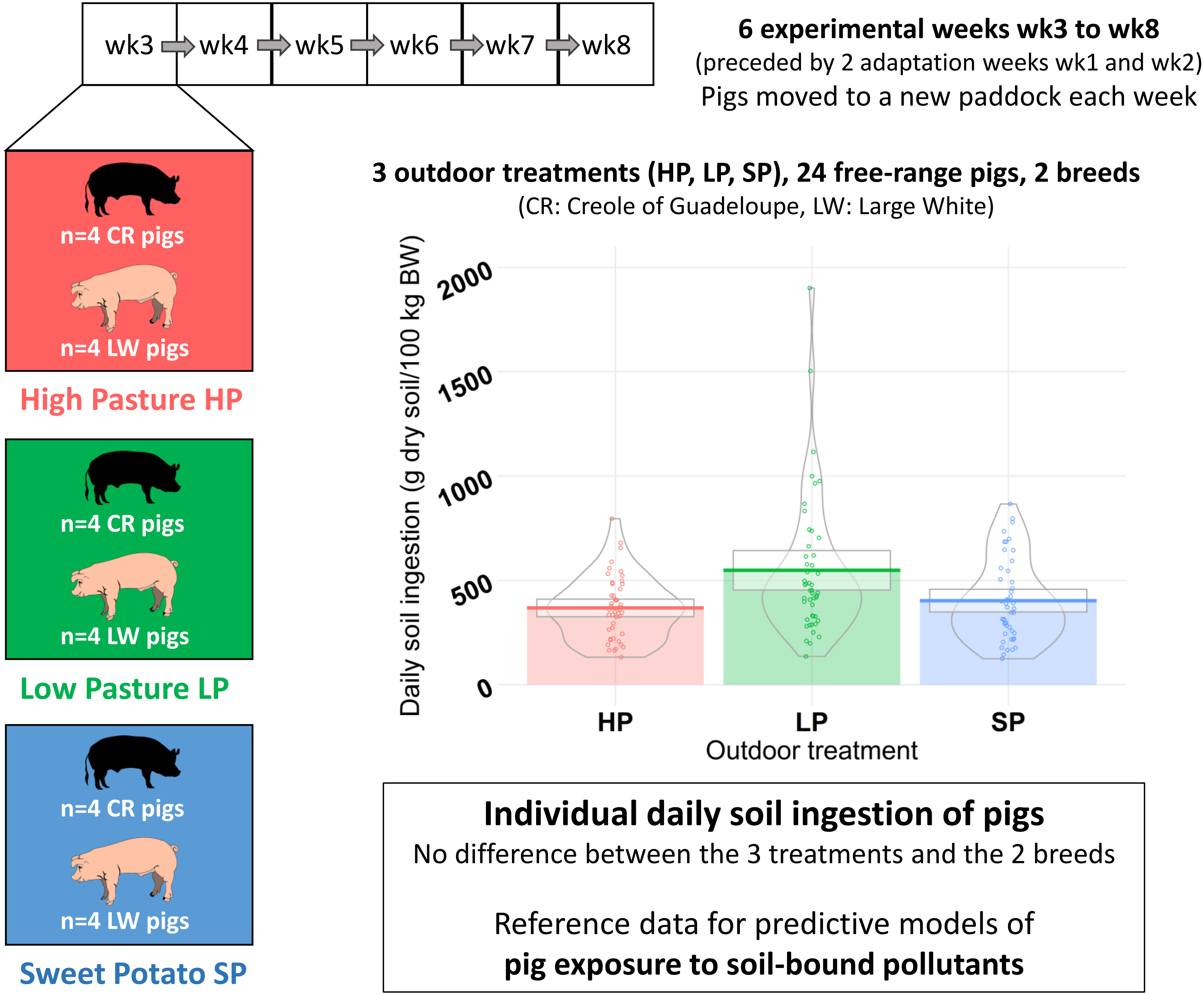
[1]RSD = residual standard deviation.

[2]Cr = chromium.

[3]Ti = titanium.

[4]NS = non-significant (*P*>0.1).

[5]MW = metabolic weight.


wk3
wk4
wk5
wk6
wk7
wk8
6 experimental weeks wk3 to wk8
(preceded by 2 adaptation weeks wk1 and wk2)
Pigs moved to a new paddock each week
n=4 CR pigs
n=4 LW pigs
High Pasture HP
n=4 CR pigs
n=4 LW pigs
Low Pasture LP
n=4 CR pigs
n=4 LW pigs
Sweet Potato SP
3 outdoor treatments (HP, LP, SP), 24 free-range pigs, 2 breeds
(CR: Creole of Guadeloupe, LW: Large White)
Daily soil ingestion (g dry soil/100 kg BW)
2000
1500
1000
500
0
HP
LP
SP
Outdoor treatment
Individual daily soil ingestion of pigs
No difference between the 3 treatments and the 2 breeds
Reference data for predictive models of
pig exposure to soil-bound pollutants