Animal 18 (2024) 101194

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Effects of natural oral alternatives to parental iron supplementation on haematological and health-related blood parameters of organic piglets

E. Merlot[a,*], C. Clouard[a], R. Resmond[b], C. Robert[a], S. Ferchaud[c], A. Prunier[a]

[a] PEGASE, INRAE, Institut Agro Rennes-Angers, 35590 Saint Gilles, France
[b] IGEPP, INRAE, Institut Agro Rennes-Angers, Université de Rennes 1, 35653 Le Rheu, France
[c] GENESI, INRAE, Venours, 86480 Rouillé, France

ARTICLE INFO

Article history:
Received 4 February 2024
Revised 7 May 2024
Accepted 13 May 2024
Available online 21 May 2024

Keywords:
Anaemia
Health
Lactation
Peat
Soil

ABSTRACT

The most common and efficient iron supply to prevent neonatal anaemia in piglets is the injection of iron dextran or gleptoferron. This treatment is problematic in organic farms because organic specifications strictly limit the use of chemically synthesised allopathic drugs. Based on the observation that piglets raised outdoors rarely develop anaemia, we hypothesised that piglets satisfy their iron needs by ingesting soil from their environment. Therefore, we compared the efficacy of a 100-mg intramuscular iron dextran injection (Iron, 8 litters, n = 98 piglets) at 4 days (**d**) of age (d4), to a daily *ad libitum* supply of dried soil (Soil, 8 litters, n = 101) or dried peat-like river silt (Peat, 8 litters, n = 102) from d4 to weaning (at 49 days of age, d49). Pigs were raised according to organic farming rules. Blood was collected on three males and three females per litter on d4, 20, 41, 50 and 69. BW was similar in the three groups on d4, 20, 41, 50 and 69 ($P > 0.1$). During the experiment, piglets were affected by a severe digestive *E. coli* episode but litter mortality rate between d4 and d69 did not differ between groups ($P > 0.1$). Blood haemoglobin concentration (**Hb**) was similar in all groups on d4, 50 and 69. However, on d20, Hb was higher in Peat and Iron groups than in the Soil group ($P < 0.001$), and on d41 and d50, Hb was higher in the Peat group than in Iron and Soil groups ($P < 0.001$). Mean red blood cell volume (**RBCV**) remained stable over time in the Peat group. In comparison, RBCV dropped in the Soil group on d20 and d41 ($P < 0.001$), and in the Iron group on d41 ($P < 0.001$). Soil and Iron group RBCV returned values similar to the Peat group by d69 ($P > 0.1$). In conclusion, soil supply in the pen was not sufficient to ensure a satisfactory iron intake in piglets, unlike peat-like river silt, which enable to reach haemoglobin concentrations above 80 mg/mL for over 90% of the piglets from d20 and, over 100% of piglets at weaning. The daily supply of the silt proved more efficient than the 100-mg iron injection beyond 20 days.

Implications

When raised indoors, iron supplementation of suckling piglets is essential for their good health. Injecting iron to the neonate will adequately prevent anaemia, provided the dose is appropriate for the weight and growth rate of the piglet. However, injectable formulations contain synthetic products, which contradicts the principles of organic production. The present study shows that very small quantities of a peat-like silt substrate, provided in a trough on a daily basis from the 1st week of life until weaning, provide a sufficient iron supply for the whole litter.

Introduction

In the 1st weeks after birth, domestic piglets face a risk of anaemia because they are born with very low iron reserves and receive insufficient iron from sows' milk relative to their needs for haemoglobin (**Hb**) synthesis and muscle growth (Csapo et al., 1996). Without other iron sources, piglets would become anaemic within days of birth. This risk disappears after weaning, when piglets receive adequate amounts of iron from cereal-based feed. When lactating sows and piglets live outdoors, in most cases, the soil appears to be a sufficient source of iron (Brown et al., 1996; Prunier et al., 2022). Indoors, without soil contact, farmers need to supplement piglets with iron in the 1st days after birth. They generally administer supplements by injection, more rarely by gavage (Svoboda et al., 2017; Svoboda and Pist'kova, 2018). The usual dose ranges from 100 to 200 mg per piglet, in the form of

* Corresponding author.
E-mail address: elodie.merlot@inrae.fr (E. Merlot).

https://doi.org/10.1016/j.animal.2024.101194
1751-7311/

iron-dextran or gleptoferron, administered in one or two injections (Egeli and Framstad, 1999; Kleinbeck and McGlone, 1999; Svoboda et al., 2017). This relatively simple therapy efficiently prevents anaemia.

In organic farms, suckling piglets and their mothers can be raised either indoors or outdoors. The European regulation on organic farming strongly limits the use of chemically synthesised drugs (Commission Regulation EC n° 889/2008). Some certifying bodies consider injectable iron a drug rather than a simple nutritional supplement, because the preparations contain iron in combination with other chemical compounds. This interpretation leads some organic farmers not to perform neonatal iron injection. In those farms, because efficient oral alternatives approved for organic farming are lacking, piglets are at risk to develop anaemia (Prunier et al., 2022). Besides, administering a single high dose of iron may be toxic for piglets. It induces massive hepatic iron accumulation, generating oxidative stress (Lipinski et al., 2010). It is also suspected of favouring arthritis in piglets (Svoboda et al., 2017). Furthermore, it activates the regulatory hepcidin-based system, inhibiting the piglet's gut ability to absorb iron from food for an extended period (Starzynski et al., 2013).

Therefore, the main objective of this study was to test oral, progressive, and natural solutions for iron supplementation of piglets. Based on the observation that piglets raised outdoors satisfy their iron needs by ingesting soil, we tested the daily provision of small amounts of soil from the experimental farm. We also intended to test peat, of which iron content is of similar order of magnitude to that of soil (Koch et al., 2021). Brière River silt was preferred over classic peat because peat is a scarce resource, whereas silt is renewable. The efficacy of these two products on haematological parameters and basic blood indicators of piglet's health was compared to that of an intramuscular iron injection. The piglets were followed from the start of the treatment until 3 weeks after weaning (from 4 to 69 days of age). The preliminary results of this study have been published in an abstract form (Merlot et al., 2021).

Material and methods

Animals

The experiment was conducted at the INRAE's organic pig farm (Rouillé, France, https://doi.org/10.15454/1.5572415481185847E12) from May to October 2020. We used Large White × Piétrain piglets from 24 litters across two consecutive cohorts, with 6-week intervals between cohorts and twelve litters per cohort (12.6 ± 2.3, mean ± SD, piglets born alive per litter [range: 9–17]; male to female ratio = 1.3). All sows were primiparous since the farm was newly established. Piglets of the first cohort suffered an unexpected episode of severe diarrhoea when they were in their 5th week after birth. Some pigs were still sick when the study ended. Although the animal keepers administered individual treatment as soon as symptoms appeared, following the farm veterinarian's recommendations, 34 of 158 (21.5%) and 39 of 144 (27%) piglets died before weaning in the two cohorts, respectively.

From 105 days of gestation, sows were kept in 10 m^2 individual farrowing pens, where they were confined in crates until 4 days after farrowing, after while they were released. Fresh straw bedding was added daily on the floor. Pens were equipped with a heated nest for piglets. Animal keepers did not crop piglets' teeth or tails, nor did they castrate males. They performed cross-fostering within the first 3 days of life if needed. From 11 days after farrowing (d11), sows and their litters had free access to a 6.25 m^2 outdoor area adjacent to the pen, with a concrete floor. Piglets had permanent access to solid feed from 20 days of age (d20) onwards.

On d48, piglets were separated from their mothers and transferred to postweaning pens together with piglets from other litters (33 ± 2 pigs per pen). Pens consisted of a covered 39 m^2 area with a concrete floor and deep straw bedding, and a 30 m^2 outdoor area with a concrete floor. Fresh straw was added weekly. Pens were equipped with two feeders and two drinking nipples. From weaning onwards, pigs had *ad libitum* access to the same solid pelleted diet distributed during lactation (ingredients: barley, peas, wheat, soya meal, oats, rapeseed meal, maize, salt, agglomerated alfalfa, monocalcium phosphate, sodium carbonate, bentonite – montmorillonite, sunflower vegetable oil, nutritional composition in Table 1). After the experiment, pigs remained in the farm's production cycle and were marketed for meat once reaching standard weight.

Experimental design

On d4 (mean piglet age: 4.5 ± 1.3 days), litters were randomly allocated to one of the three treatments (Iron, Soil or Peat) resulting in four litters per treatment per cohort. However, one litter allocated to the Peat group had to be weaned and excluded from the protocol at d30 for a reason independent of the experimental protocol. Piglets in the Iron group received a single intramuscular injection of iron on d4, according to the manufacturer's recommendations (1 mL containing 100 mg iron as dextran and 50 mg phenol as excipient, Cofafer, Dopharma France, Vair-sur-Loire, France). Soil and Peat litters had free access to small amounts of soil or peat from d4 afternoon to d48. Total daily rations were 150 g per pen from d4 to d12, 200 g from d13 to d26, and 250 g from d27 to d47, to allow access close to *ad libitum* without excessive waste.

The soil was collected from molehills in the field adjacent to the farm. The peat was actually silt from the banks of the Brière River (a marsh located north of the Loire estuary), kindly provided free of charge by the Florentaise company (Saint-Mars-du-Désert, France), which uses it as a fertiliser in horticulture. The mineral composition of soil and river peat (Table 2) was analysed by the INRAE soil analysis laboratory (Arras, France). Substrates were sterilised in 1 L glass jars immersed in boiling water for 1.5 h, without prior drying, to preserve the texture. Rations were distributed daily in a small circular feeder located in the nest, inaccessible to the sow. Every morning, the animal caretaker removed the previous day's substrate before adding fresh substrate.

Table 1
Chemical composition (as fed-basis) of the piglet diet.

Item	Composition
CP, %	16.5
Ash, %	6.3
Crude cellulose, %	5.5
Crude fat, %	3.1
Lysine, g/kg	8.7
Methionine, g/kg	2.4
Vitamin A, UI/kg	12 000
Vitamin D3, UI/kg	2 000
Vitamin E, UI/kg	60
Calcium, %	0.65
Phosphorus, %	0.60
Sodium, %	0.42
Copper ($CuSO_4 \cdot 5\ H_2O$), mg/kg	150
Iodine ($Ca(IO_3)_2$), mg/kg	1.0
Iron ($FeSO_4 \cdot H_2O$), mg/kg	159
Manganese (MnO), mg/kg	60
Selenium (Na_2SeO_3), mg/kg	0.40
Zinc (ZnO), mg/kg	110

Table 2
Total trace element composition of the soil and the peat distributed to the piglets.

Element	Soil	Peat
Total elements, mg/kg [1]		
Aluminium (Al^{3+})	39.8×10^3	36.7×10^3
Cadmium (Cd^{2+})	0.194	0.221
Calcium (Ca^{2+})	2.17×10^3	12.7×10^3
Chrome (Cr^{3+})	85.4	39.2
Cobalt (Co^{2+} and Co^{3+})	21.2	9.30
Copper (Cu^{+} and Cu^{2+})	13.9	15.2
Iron (Fe^{2+} and Fe^{3+})	49.7×10^3	26.1×10^3
Lead (Pb^{2+})	48.1	62.5
Magnesium (Mg^{2+})	1.52×10^3	7.08×10^3
Manganese (Mn^{2+} and Mn^{4+})	2.03×10^3	743
Molybdene (Mo)	0.853	0.950
Natrium (Na^{+})	3.15×10^3	4.81×10^3
Nickel (Ni^{2+})	23.7	21.9
Phosphorus (P)	677	830
Potassium (K^{+})	12.7×10^3	9.91×10^3
Thallium (Tl)	0.648	0.482
Zinc (Zn^{2+})	69.6	113
EDTA-extractible elements, mg/kg [1]		
Copper (Cu^{+} and Cu^{2+})	2.92	1.51
Iron (Fe^{2+} and Fe^{3+})	91	1.8×10^3
Manganese (Mn^{2+} and Mn^{4+})	353	523
Zinc (Zn^{2+})	4.25	54.8

[1] Peat and soil samples were first dried and crushed (diameter < 250 μm), and total trace elements were assayed by MS.

Measures and behavioural observations

At distribution of soil or peat and at 1330 h, the animal caretaker noted on a grid the presence of wasted substrate on the ground next to the trough and the filling level of the feeder (0: empty, 1: less than 50%; 2 > more than 50% of the quantity distributed left in the trough). On d4, 11, 20, 41, 50 and 69, experimenters caught all piglets individually for weighing and visual inspection of their health status (coughing, sneezing or other respiratory issues, diarrhoea (soft or liquid faeces), body cleanliness, bursitis, hernia, lesions, lameness, skin condition). Dead piglets were weighed and the cause of death was recorded.

Immediately after d20 and d41 blood samplings, the 6 experimental piglets per litter were individually marked with a symbol sprayed on their back. Video footage was continuously recorded. On d21 and 42, behavioural activities were scored using 5-min scan sampling during four sessions of 2 h per day (0800–1000 h, 1100–1300 h, 1400–1600 h, 1700–1900 h), resulting in a total of 96 scans per pig per day, where the activity of eating soil or peat (having the head over the feeder and exploring/eating the soil or peat) was recorded. It was expressed as proportions of total scans, as a proxy of proportions of total time.

Blood collection and laboratory analyses

Within each litter, three males and three females of light, medium and heavy weight relative to their litter were selected and ear tagged. Sick piglets or those weighing less than 1.1 kg at birth were not selected. These piglets (n = 48 per treatment group) were blood sampled on d4, d20, d41, d50 and d69. When a piglet died from diarrhoea, it was replaced by a littermate whenever possible (29 replacements in total, 47 Iron, 42 Peat and 41 Soil piglets sampled on d69).

During the sampling procedure, a trained experimenter restrained the pigs while the sampler took the blood from the jugular vein. The animals were caught and handled calmly and as gently as possible, in a room adjacent to their living quarters. Until d20, pigs were held on their back on a table. Thereafter, pigs were restrained with a wire metal nose snare. The blood sampling was timed and stopped after 2 min in order to minimise the piglets' stress. Blood was collected into 5-mL BD Vacutainer tubes (one ethylenediaminetetraacetic acid (**EDTA**), one heparinised) and kept on ice until processing.

Within 1 h, a complete haematological analysis was carried out on EDTA blood using a cell counter calibrated for pigs (MS9; Melet Schloesing Laboratories, Osny, France). Parameters measured included haemoglobin concentration (Hb), haematocrit, red blood cell count (**RBCC**), mean red blood cell volume (**RBCV**), the mean cell haemoglobin concentration, the number of lymphocytes, monocytes, neutrophils and eosinophils.

Thereafter, samples were centrifuged at 1 800 g for 10 min at 4 °C and plasma was stored at −20 °C. Ferritin was assessed in EDTA plasma using a commercial pig ELISA kit (Cusabio, Houston, TX, USA). The intra-assay CV was 3%. The concentration of hydrogen peroxides was assessed in heparinised plasma using a diacron Reactive Oxygen Metabolites (**dROM**) kit (H&D, srl, Parma, Italy). Concentrations of dROM were expressed in Carratelli Unit (**CARRU**, 1 CARRU = 0.08 mg H_2O_2/100 mL), and intra- and inter-assay CVs were 6 and 8%, respectively. The ferric−reducing ability of plasma (**FRAP**) was assayed in heparinised plasma as previously described (Sierzant et al., 2019) and was expressed as molar Trolox equivalents/L. The acute phase protein haptoglobin was assayed in the heparinised plasma using commercial kits (Tridelta Development Ltd, Maynooth, Ireland). The minimum concentration detectable was 0.033 mg/mL, and the intra- and inter-assay CV were 7 and 24%, respectively. All these assays were performed on a multianalyzer apparatus (Konelab 20i, ThermoFisher Scientific, Courtaboeuf, France).

Statistical analyses

Analyses were performed with the R software (version 4.0.2). Mixed ANOVA (type 3) were carried using linear mixed models with the lmerTest package, and lsmeans were calculated using the emmeans package. For weight and blood variables, treatment (three modalities: Iron, Peat, Soil) in interaction with the sampling day (five modalities: d4, 20, 41, 50 and 69) and sex (two modalities: Male vs Female) were introduced as fixed effects. For the proportion of time spent eating or exploring the substrate, fixed effects included treatment (Peat, Soil) in interaction with the sampling day (d2, d42) and sex (Male vs Female). The experimental piglet and the sow nested within the batch were introduced as random effects in all these models. If model residuals did not meet the assumptions for normal distribution and equality of variances, variables were square root or log−transformed (physiological variables) or arcsin square root transformed (behavioural variables), to fit these assumptions. For mortality (percentage of dead piglets per litter between d4 and d69), diarrhoea expression at each time point (percentage of piglets expressing or not diarrhoea), and anaemia occurrence at each time point (percentage of piglets having a haemoglobin concentration lower than 80 mg/mL), logistic regression were used, with sex and treatment introduced as fixed effects, and the sow nested within the batch as a random effect. Data are presented as adjusted least-squares means ± SE of the mean. The P-values (corrected with Tukey method) were considered statistically significant if < 0.05.

Results

Peat and soil usage

Time spent eating or exploring the feeder was influenced by the day × treatment interaction ($P < 0.05$), with piglets spending significantly more time eating or exploring the peat on d21 than d42 ($P < 0.01$). On the contrary, time spent eating or exploring the soil remained stable over time (Fig. 1A, $P > 0.10$). With regard to the emptying level of the troughs, the frequency of litters receiving a score of 2, indicating low substrate utilisation, fell from 64 to 17% between the first and 2nd weeks of substrate distribution ($P < 0.001$) and then decreased to 4% on the week before weaning ($P < 0.05$). The frequency of litters that received a score of 0 (trough empty) was 1% on the 1st week, then continuously increased until reaching 33% in the 4th week of distribution (d32-38, $P < 0.001$), and remained stable until the weaning week (between 18 and 29%, Fig. 1B). No substrate wastage was observed on the ground for the first batch but there was from the 4th week onward in the second batch.

Growth, morbidity and mortality

The mortality rate during the whole experimental period was not statistically different between the three treatment groups: 15 of the 98 Iron pigs (15.3%), 25 of the 102 (14.7%) Peat pigs and 33 of the 102 Soil pigs (32.4%) died. The percentage of pigs experiencing diarrhoea was also the same in the three groups on every control day (Fig. 2A). BW and average daily gain (**ADG**) were influenced by the day ($P < 0.001$) and its interaction with treatment ($P < 0.001$). ADG between d41 and d50 was greater in the Peat group (224 ± 20 g/day) than in the Soil and Iron groups (151 and 153 ± 18 g/day, respectively, $P < 0.05$). Nevertheless, BW never differed between the three treatment groups at any time point (Fig. 2B).

Blood assays

Concentrations of haptoglobin (Fig. 2C), hydroperoxides (dROM, Fig. 2D) and FRAP in the plasma of piglets were influenced by the treatment × day interaction ($P < 0.001$). For haptoglobin, the three groups had similar concentrations on all time points, except on d20, when the concentration was greater in Soil compared to Peat piglets ($P < 0.05$). Hydroperoxides were higher in Iron piglets than in Peat piglets on d4, before treatment start ($P < 0.05$) and, than Soil piglets on d20 ($P < 0.05$). There were no differences at other time points. Regarding FRAP, on d4, the Iron group had lower levels than the Soil group ($P < 0.001$) and tended to have lower levels than the Peat group ($P = 0.05$, Iron: 65, Peat: 70 and Soil: 78 ± 2 molar Trolox equivalents/L). Ferritin concentrations were assessed at 3 time points. They increased from d4 to d20 and then from d20 to d50 (d4: 17 ± 0.7, d20: 20 ± 0.8, d50: 24 ± 1.0 ng/mL, $P < 0.01$), but were not influenced by treatment ($P > 0.1$).

Red blood cell lineage

Haemoglobin, RBCV, RBCC, cell Hb concentration (Fig. 3) and haematocrit (not shown) were influenced by the treatment × day interaction ($P < 0.001$). All groups started with similar values on d4. On d20, Iron and Peat animals had greater Hb ($P < 0.001$), haematocrit ($P < 0.01$), RBCV and RBCC ($P < 0.001$) and cell Hb concentration ($P < 0.001$) than Soil pigs. On d41 and d50, Peat animals had greater Hb (d41: $P < 0.001$ and d50: $P < 0.05$), haematocrit ($P < 0.01$), RBCV ($P < 0.001$ and < 0.05, respectively) and cell Hb concentration ($P < 0.001$ and < 0.05, respectively) than Soil and Iron pigs. However, regarding RBCC, Peat and Iron pigs had similar counts ($P > 0.1$) on d41, but higher than in the Soil group ($P < 0.01$ and $P = 0.09$, respectively). On d50, the three groups had similar RBCC. On d69, the three treatment groups had similar Hb, RBCC, RBCV, cell Hb concentration and haematocrit. On d20, the percentage of piglets with Hb levels lower than 80 mg/mL was greater in the Soil group than in the Peat ($P < 0.05$) and Iron groups ($P < 0.01$, Fig. 4). From d41, this percentage was not different anymore between treatment groups.

Regarding the variation with age, Soil and Iron groups reached their maximal Hb concentration with a delay compared to the Peat group (d50 compared to d41). Furthermore, in the Iron group, a decrease in Hb was observed between d21 and d41 ($P < 0.05$). In the Soil and Iron groups, RBCV and cell Hb concentration decreased from d20 to d41 compared to d4 ($P < 0.05$) and did not get back to their initial values on d69 ($P < 0.05$). In contrast, in the Peat group, RBCV never decreased and the decrease of cell Hb concentration compared to d4 was considerably smoothed, reaching a transiently significant decrease on d50 only.

White blood cell lineage

Total white blood cell numbers and among them, the numbers of lymphocyte, monocyte and neutrophil subsets increased with time ($P < 0.001$, Fig. 5). Neutrophil and monocyte numbers were influenced by the treatment × day interaction ($P < 0.05$). On d4, the Peat group displayed lower neutrophil numbers than the Iron group ($P < 0.01$, Fig. 5B). More interestingly, the age-related

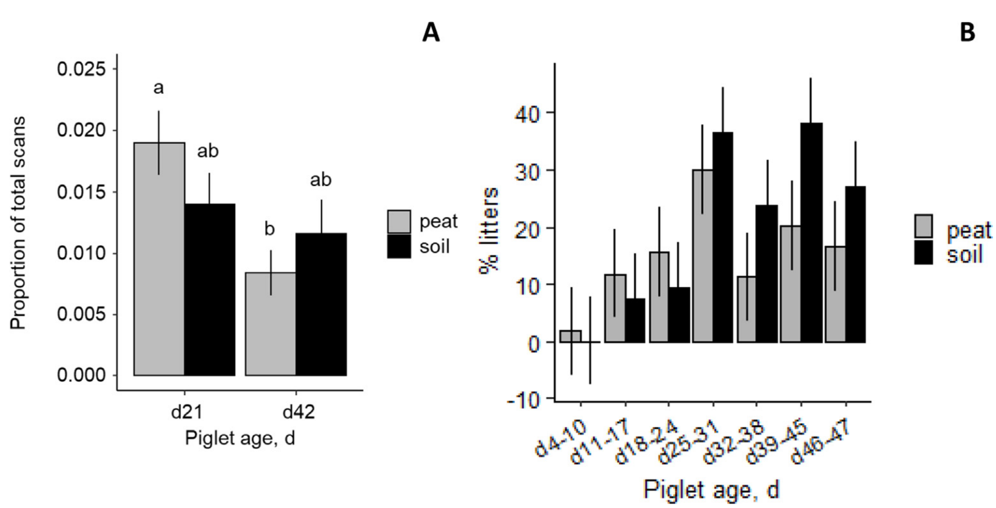

Fig. 1. Proportion of total scans when piglets of the Soil and Peat treatment groups were eating or exploring substrate in the feeder at 21 (d21) and 42 (d42) days of age (A) and percentage of litters with an empty trough at control times from the first (d4-10) to the last (d46-47) weeks of lactation (B). Adjusted means ± SEM, bars with different letters are significantly different at $P < 0.05$.

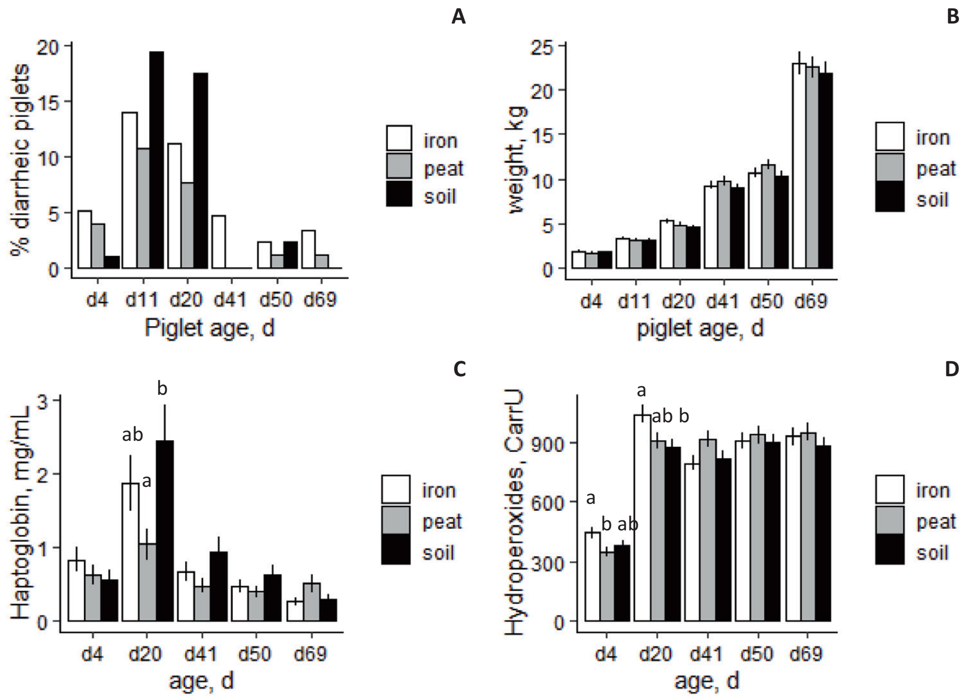

Fig. 2. Percentage of piglets displaying diarrhoea (A), BW (B), plasma haptoglobin (C), and plasma hydroperoxide concentrations (D) in Iron, Peat and Soil treatment groups (adjusted means ± SEM). For each time point, bars with different letters are significantly different at $P < 0.05$. CarrU = Carratelli Unit.

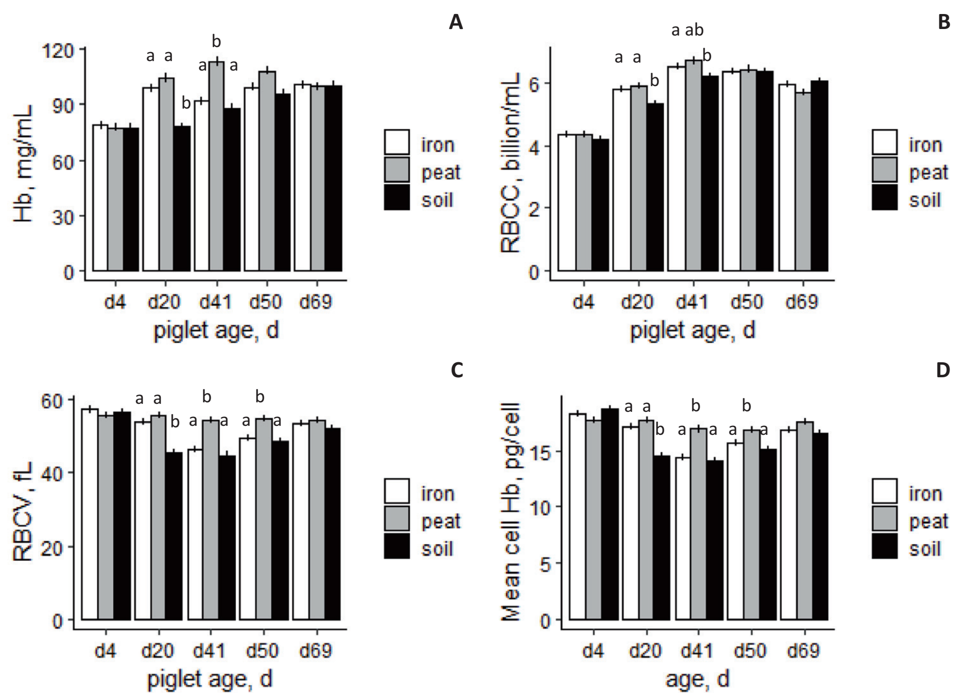

Fig. 3. Haemoglobin (Hb) concentration (A), red blood cell count (RBCC, B), red blood cell volume (RBCV, C) and mean cell Hb concentration (D) in piglets of the Iron, Peat and Soil treatment groups (adjusted means ± SEM). For each time point, bars with different letters are significantly different at $P < 0.05$.

increase in neutrophils and monocytes was delayed in the Soil group, leading to lower numbers in this group on d20 compared to Peat and Iron pigs.

Finally, platelet numbers were influenced by the treatment × day interaction ($P < 0.001$, Fig. 5D). They reached a peak on d20 in the Soil and Peat groups, after which they decreased continuously until d69, but platelet numbers in the Soil group remained higher than in the Peat group from d20 to d50 ($P < 0.01$). In the Iron group, the platelet numbers were stable between d20 and d41. They were greater than in the Peat group ($P < 0.001$) and similar to the Soil group ($P > 0.1$) on d41 and d50. On d69, only Peat piglets were back to values similar to d4 ($P > 0.1$).

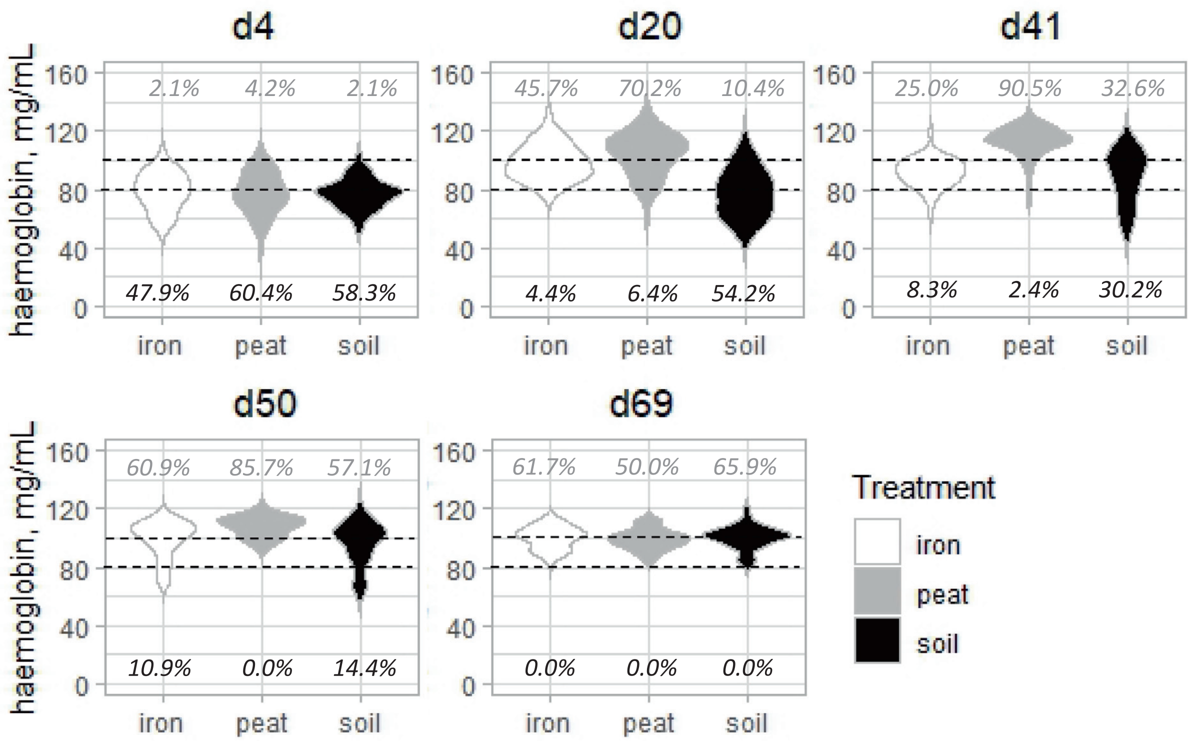

Fig. 4. Violin plots of the distribution of haemoglobin from d4 to d69, and percentages of piglets with a haemoglobin concentration lower than 80 mg/mL (black numbers) or higher than 100 mg/mL (grey numbers) in Iron, Peat and Soil treatment groups.

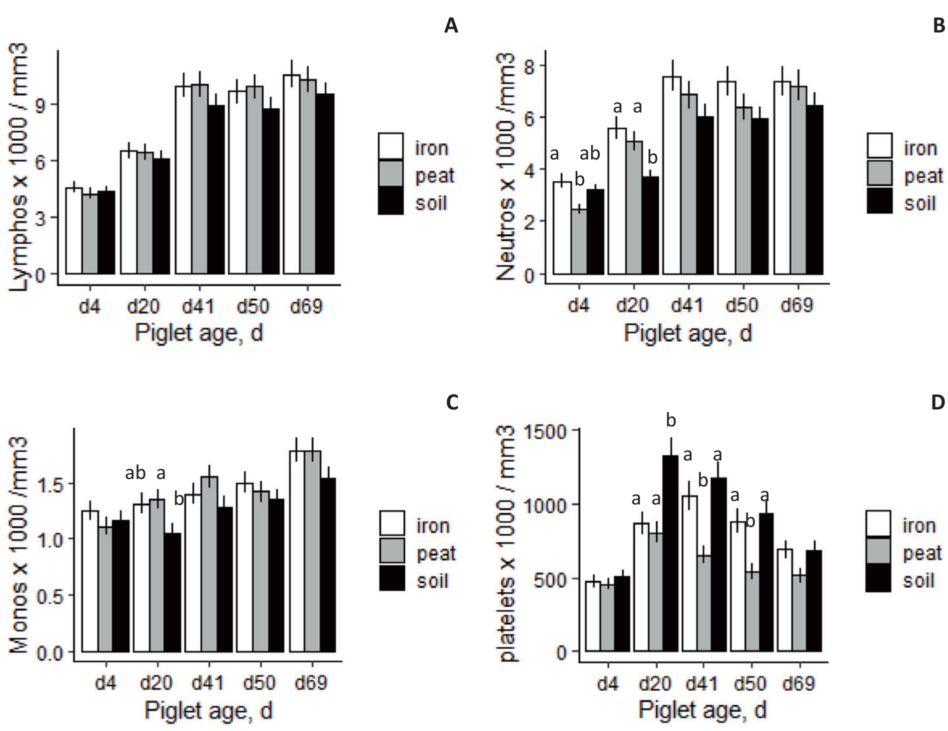

Fig. 5. Blood numbers of circulating lymphocytes (A), monocytes (B), neutrophils (C) and platelets (D) in piglets of the Iron, Peat and Soil treatment groups (adjusted means ± SEM). For each time point, bars with different letters are significantly different at $P < 0.05$.

Discussion

In this study, two oral solutions, based on soil or peat-like river silt, were tested as alternatives to iron injection for the prevention of anaemia in piglets raised indoors. Their efficacy was compared to that of an intramuscular iron injection. The results showed that the peat provision allowed maintaining the majority of piglets at a good level of Hb throughout lactation, with similar or even better results than the injection of 100 mg iron. By contrast, piglets administered soil presented lower measures from d20 to weaning (Hb, RBCC) and even still 1 week after weaning (RBCV and cell Hb concentration) compared to the other two groups. Soil piglets presented a high haptoglobin concentration, low neutrophil and monocyte counts, and high platelet counts on d20, suggesting that iron deficiency altered their haematopoietic process.

Rational for using peat or soil

The piglet gut can efficiently absorb iron from 4 days after birth, when the expression of transporters responsible for iron absorption increases in enterocytes (Lipinski et al., 2010). In farms, oral iron supplementation is most often administered as a paste directly into the mouth for one or a few consecutive days (Egeli and Framstad, 1999; Svoboda and Pist'kova, 2018). Various chemical forms of iron have been tested (salts, chelated, encapsulated), but to our knowledge, the provision of soil or peat without artificial iron addition had never been tried (Svoboda and Pist'kova, 2018). We tested soil provision based on the observation that outdoor piglets satisfy their iron needs without supplementation, probably by ingesting the soil of their enclosure (Prunier et al., 2022). Since pigs do not actually eat soil, but rather forage in it, it is likely that piglets have a high efficiency for absorbing iron from small amounts of soil, as previously demonstrated in rats (Guja and Baye, 2018). In humans also, sufficient iron can be obtained from very small amounts of non-food sources, such as cooking in iron pots or eating plant-based food contaminated with soil during traditional threshing on the floor (Guja and Baye, 2018). The peat treatment was included in the study to test a different substrate in terms of composition, and to provide a solution for farmers who are unable or unwilling to harvest soil from their immediate environment. We knew that pigs would use peat because it is a popular substrate for pigs to forage in, and it is sometimes used as a matrix for nutritional elements, such as iron. In a context of peat scarcity, we tested an equivalent substrate still unexplored to date in pig farming, but available in large quantities: the organic-rich silt of estuaries from marshy areas, which is already used as a fertiliser for plants. Here, both substrates were offered in troughs and the animals ingested it freely. From a practical perspective, this procedure avoided catching and force-feeding the piglets.

Indicator interpretation

Several indicators were used in this study. Iron deficiency anaemia in suckling piglets is typically a hypochromic microcytic anaemia characterised by a decrease in red blood cell parameters such as hematocrit, blood Hb, RBCV and mean cell Hb concentration (Szudzik et al., 2018). RBCV and mean cell Hb concentration are earlier indicators of iron-deficient erythropoiesis than Hb and RBCC (Svoboda et al., 2008). Ferritin is used as an indicator of the level of iron stores (Daru et al., 2017). There is no consensus in the literature on the threshold haemoglobin values that indicate anaemia in piglets (Svoboda et al., 2017). In their review, Svoboda et al. (2017) indicated that a Hb concentration of 100 g/L or more can be considered normal, 80 g/L as a borderline anaemia, and 60 g/L as severe anaemia. These thresholds are used to discuss the present data.

Efficiency of 100 mg dextran iron

The dose recommended by the supplier of the injectable iron preparation was between 100 and 200 mg of iron per piglet. Using a dose of 100 mg, we observed that mean Hb concentrations were greater than 80 g/L from d21 and until the end of the experiment. However, at weaning, it was in the low range of what is commonly observed in indoor farms (Prunier et al., 2022). Furthermore, less than half of the piglets presented a satisfactory Hb concentration above 100 g/L on d21, and this proportion decreased until the weaning time (d41). Meanwhile, the percentage of piglets with less than 80 g/L increased from 4.4 to 8.3%.

As piglets grow, their blood volume, and thus their haemoglobin and iron need, expand consequently (Svoboda et al., 2017). If the iron storage from the injection is insufficient, Hb concentration drops during lactation (Miller et al., 1982; Kegley et al., 2002). Our data suggest that the dose of 100 mg iron could meet the needs of piglets until approximately 20 days of age, when piglets weighed 4.95 ± 0.15 kg. Indeed, on that day, the Hb concentration had increased and RBCV and mean cell Hb concentration were stable compared to d4. On d41, when piglets had reached a mean BW of 9.32 ± 0.28 kg, Hb, RBCV and mean cell Hb concentration were decreased compared to d20. Thus, 100 mg of iron is not enough in an indoor organic system, where pigs are weaned after 42 days of age. This is in agreement with several studies indicating that even 200 mg iron might not be sufficient until the age of 28 days for heavier piglets (Murphy et al., 1997; Heidbüchel et al., 2019). However, growth, health indicators, and blood cell numeration followed normal time courses, which indicated that this dose nevertheless prevented the occurrence of severe anaemia. Hb, RBCV and mean cell Hb concentration started to rise again as early as 1 week after weaning. This confirms that solid cereal-based feed is a satisfactory source of iron as soon as the animals consume it as their main feed.

This study could not verify that iron injection causes oxidative stress, for two reasons. First, the differences in hydroperoxides observed on d20 were not very clear-cut: hydroperoxides were greater in the Iron group compared to the Soil group but not the Peat group. Second, inexplicably, hydroperoxide concentration was already higher and the anti-oxidant FRAP capacity lower in the Iron group at the start of the study compared to the other groups. It is therefore impossible to determine whether differences observed on d20 were due to the iron injection or to a pre-existing difference in oxidative status.

Efficiency of peat supply

The river silt treatment, used as a renewable alternative to peat, performed very well. Our raw observations at the pen level suggested that the piglets learned during their 1st week to use the substrate. In this group, Hb increased continuously in piglet blood until weaning age, reaching higher concentrations than in the other groups the day before weaning. Furthermore, the stability of RBCV, whose fall is a reliable indicator of anaemia (Egeli and Framstad, 1999), suggested that peat prevented anaemia. However, the very limited decline in mean cell Hb concentration on d50 indicates that iron supply by peat was below what piglets are able to use to synthesise haemoglobin. It was not possible to evaluate the amount of peat that individual piglets consumed. However, a large majority of piglets in the Peat group had reached an Hb concentrations greater than 100 g/L as early as d20, which suggests little heterogeneity in their behaviour regarding peat consumption and, and indicates that the small daily rations were enough for the entire litter. Behavioural observations showed a reduction in trough use on d42 compared with the Soil group. This suggests that the attractiveness of the substrate to piglets is directly linked to their iron status. Indeed, piglets in the peat group had reached their optimal haemoglobin status by d41, which was not yet the case for animals in the soil group. The better inflammatory status in Peat piglets on d20 indicates also a better global health, either due to their better iron status, either to the presence in the peat of unknown compounds with a positive influence on gut health.

Efficiency of farm soil supply

In the Soil group, many piglets had haemoglobin concentrations below 80 g/L until d50, with a drop in RBCV and mean cell Hb concentration as early as d20, revealing an anaemic status throughout lactation. This status was confirmed by a delay, compared to the two other groups, in the developmental increase in neutrophil and monocyte numbers, which should rather occur during the 1st weeks after birth (Pomorska-Mol and Markowska-Daniel, 2011). The increase in platelet numbers observed from d20 to d50 in this group probably also arised from anaemia, perhaps in combination with an inflammatory state (as indicated by the high haptoglobin concentration in Soil group on d20). Indeed, in human infants and children, infection, chronic inflammation and iron deficiency are known to generate moderate thrombocytosis (Brissot et al., 2021).

The frequency of deaths and diarrheic piglets was not statistically different between experimental groups. However, numerically, Soil piglets seemed to have a higher occurrence of diarrhoea and mortality, and they presented a higher concentration of haptoglobin on d20, indicating a greater inflammation. It can be assumed that the anaemic status of the piglets in the Soil group had increased their susceptibility to pathogens and hence to diarrhoea. During inflammation, inflammatory cytokines induce hepcidin secretion and iron is sequestered in the liver, enterocytes and macrophages (Lee et al., 2005; Muñoz et al., 2011). The depletion of iron from the blood and extracellular liquid is a part of the non-specific defence mechanism against infection, since all organisms, including pathogenic bacteria, require iron for their basic cellular processes (Gerner et al., 2020). Therefore, it could also be hypothesised that the diarrhoeic episode and its associated inflammatory response contributed to anaemia. However, the difference between the Peat group and the two other treatment groups concerning red blood cell characteristics appeared at d20, that is before the first cases of diarrhoea.

The low efficiency of soil supply in the present experiment was puzzling, especially since the iron concentration in the soil was twice as high as in the peat. In most outdoor farms, piglets present an excellent iron status (Brown et al., 1996; Prunier et al., 2022), although very low haemoglobin levels can sometimes be observed (Szabo and Bilkei, 2002). These different results are probably due to different soil compositions in these areas. In experimental conditions, increasing doses of ferrous sulphate monohydrate added directly into the feed improved linearly the haemoglobin status of piglets (Lee et al., 2008), but in field conditions, there is a lack of correlation between soil iron content and blood haemoglobin concentrations (Brown et al., 1996). Therefore, the bioavailability, rather than the total iron quantity, is likely the main limiting factor. The free-iron fraction, which is not embedded in silicates and is accessible to plants, microbes and animals, includes iron in the form of salts or associated with organic matter (Baize, 2018). At the neutral or basic pH of the small intestine, most ferric iron precipitates if not chelated, and becomes unavailable for absorption. Thus, notwithstanding heme–iron from animal products, the iron chelated to organic matter is believed to be the most bioavailable (Benito and Miller, 1998). In a soil sample, this organic fraction is estimated by assessing the EDTA-extractible iron fraction (Baize, 2018). In our experiment, the amount of EDTA-extractible iron was twenty times higher in the peat than in the soil. This high content in organic iron reflects the almost exclusively plant-based origin of the silt and may explain the better results obtained in the Peat piglets compared to the Soil piglets.

Another possible limiting factor is the competition in the gut between minerals due to similarities in absorption and transport mechanisms through the gut barrier (Bjorklund et al., 2017). In our experiment, the ratios of the organic form of these minerals to organic iron were clearly more advantageous for iron bioavailability in the silt substrate compared to the soil (Zn/Fe: 0.03 vs 0.05, Mn/Fe: 0.3 vs 3.9, and Cu/Fe: 0.001 vs 0.032, in silt and soil, respectively). The access to iron contained in vegetal matrixes can also be limited by the poor digestibility of the vegetal matrix or other molecules that sequester organic iron (Rousseau et al., 2020). Lastly, in the large intestine, colonic microbiota can produce siderophores that modulate colonic iron availability (Yilmaz and Li, 2018). We do not know in what proportion these two phenomena were occurring in the soil and silt groups.

Conclusion

This study demonstrates that the daily provision of small amounts of river silt, used as a renewable alternative to peat, directly distributed in the pen, allowed piglets to reach a satisfactory status in iron. The substrate was palatable enough to be consumed by the vast majority of piglets throughout the lactation period. This solution is in line with the principles of organic farming and would also enable a more gradual iron intake than neonatal iron injection. However, this solution has two major limitations: the silt is not readily available, as it is mainly marketed today in the form of horticultural mixes, and sterilisation and daily distribution are time-consuming. In addition, further studies are needed to better specify the doses and distribution protocol.

Ethics approval

The protocol was approved by the local Ethics Committee in Animal Experiment of Poitou-Charentes, France, and the French Ministry of Higher Education and Research, according to the EU Directive 2010/63/EU for animal experiments (agreement APAFIS#21892-2019071611422718v2).

Data and model availability statement

The dataset that supports the study findings is publicly available on the Data INRAE data repository, under the project name "Biological responses to 3 different iron supplementation solutions for organic piglets" (https://doi.org/10.57745/MQR1IO).

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to improve English wording and grammar. After using this service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Author ORCIDs

Merlot E: https://orcid.org/0000-0003-2300-0970.
Robert C: no ORCID.

Clouard C: https://orcid.org/0000-0002-5562-6057.
R Resmond: https://orcid.org/0000-0003-4805-9688.
Ferchaud S: https://orcid.org/0009-0004-9364-8785.
Prunier A: https://orcid.org/0000-0003-3070-6613.

CRediT authorship contribution statement

E. Merlot: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **C. Clouard:** Writing – review & editing, Methodology, Investigation, Formal analysis. **R. Resmond:** Formal analysis. **C. Robert:** Validation, Investigation, Formal analysis. **S. Ferchaud:** Validation, Resources, Investigation. **A. Prunier:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of interest

None.

Acknowledgements

The authors would like to thank Olivier Loréal for his kind and critical reading of the manuscript; Doryan Grivault, Stéphane Moreau and Tony Terrasson for taking care of the animals and helping at sampling; Raphaël Comte, Sophie Daré, and Françoise Thomas for their technical support in the lab. We are also grateful to the piglets that were included in the study.

Financial support statement

This research was supported by INRAE and by the European Union's Horizon 2020 program (grant agreement No 727495, project CORE Organic Cofund).

References

Baize, D. (Ed.), 2018. Guide Des Analyses En Pédologie. Éditions Quae, Versailles, France, pp. 155–172.
Benito, P., Miller, D., 1998. Iron absorption and bioavailability: an updated review. Nutrition Research 18, 581–603.
Bjorklund, G., Aaseth, J., Skalny, A.V., Suliburska, J., Skalnaya, M.G., Nikonorov, A.A., Tinkov, A.A., 2017. Interactions of iron with manganese, zinc, chromium, and selenium as related to prophylaxis and treatment of iron deficiency. Journal of Trace Elements in Medicine and Biology 41, 41–53.
Brissot, E., Troadec, M.B., Loréal, O., Brissot, P., 2021. Iron and platelets: a subtle, under-recognized relationship. American Journal of Hematology 96, 1008–1016.
Brown, J.M.E., Edwards, S.A., Smith, W.J., Thompson, E., Duncan, J., 1996. Welfare and production implications of teeth clipping and iron injection of piglets in outdoor systems in Scotland. Preventive Veterinary Medicine 27, 95–105.
Csapo, J., Martin, T.G., Csapo-Kiss, Z.S., Hazas, Z., 1996. Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days. International Dairy Journal 6, 881–902.
Daru, J., Colman, K., Stanworth, S.J., De La Salle, B., Wood, E.M., Pasricha, S.-R., 2017. Serum ferritin as an indicator of iron status: what do we need to know? The American Journal of Clinical Nutrition 106, 1634S–1639S.
Egeli, A.K., Framstad, T., 1999. An evaluation of iron-dextran supplementation in piglets administered by injection on the first, third or fourth day after birth. Research in Veterinary Science 66, 179–184.
Gerner, R.R., Nuccio, S.-P., Raffatellu, M., 2020. Iron at the host-microbe interface. Molecular Aspects of Medicine 75, e100895.
Guja, H., Baye, K., 2018. Extrinsic iron from soil contributes to Hb regeneration of anaemic rats: implications for foods contaminated with soil iron. British Journal of Nutrition 119, 880–886.
Heidbüchel, K., Raabe, J., Baldinger, L., Hagmüller, W., Bussemas, R., 2019. One iron injection is not enough—iron status and growth of suckling piglets on an organic farm. Animals 9, e651.
Kegley, E.B., Spears, J.W., Flowers, W.L., Schoenherr, W.D., 2002. Iron methionine as a source of iron for the neonatal pig. Nutrition Research 22, 1209–1217.
Kleinbeck, S.N., McGlone, J.J., 1999. Intensive indoor versus outdoor swine production systems: genotype and supplemental iron effects on blood hemoglobin and selected immune measures in young pigs. Journal of Animal Science 77, 2384–2390.
Koch, F., Kowalczyk, J., Wagner, B., Klevenhusen, F., Schenkel, H., Lahrssen-Wiederholt, M., Pieper, R., 2021. Chemical analysis of materials used in pig housing with respect to the safety of products of animal origin. Animal 15, 100319.
Lee, P., Peng, H., Gelbart, T., Wang, L., Beutle, E., 2005. Regulation of hepcidin transcription by interleukin-1 and interleukin-6. Proceedings of the National Academy of Sciences 102, 1906–1910.
Lee, S.H., Shinde, P., Choi, J., Park, M., Ohh, S., Kwon, I.K., Pak, S.I., Chae, B.J., 2008. Effects of dietary iron levels on growth performance, hematological status, liver mineral concentration, fecal microflora, and diarrhea incidence in weanling pigs. Biological Trace Element Research 126, S57–S68.
Lipinski, P., Starzynski, R.R., Canonne-Hergaux, F., Tudek, B., Olinski, R., Kowalczyk, P., Dziaman, T., Thibaudeau, O., Gralak, M.A., Smuda, E., Wolinski, J., Usinska, A., Zabielski, R., 2010. Benefits and risks of iron supplementation in anemic neonatal pigs. American Journal of Pathology 177, 1233–1243.
Merlot,E., Robert, C., Clouard, C., Resmond, R., Frechaud, S., Prunier, A. 2021. Testing natural alternatives to iron for organic piglets. In: Proceedings of the 72nd Annual Meeting of the European Federation of Animal Science (EAAP), 30 August - 3 September 2021, Davos, Switzerland, p. 361.
Miller, E.R., Waxler, G.L., Ku, P.K., Ullrey, D.E., Whitehair, C.K., 1982. Iron requirements of baby pigs reared in germ-free or conventional environments on a condensed milk diet. Journal of Animal Science 54, 106–115.
Muñoz, M., García-Erce, J.A., Remacha, Á.F., 2011. Disorders of iron metabolism. part 1: molecular basis of iron homoeostasis. Journal of Clinical Pathology 64, 281–286.
Murphy, K.A., Friendship, R.M., Dewey, C.E., 1997. Effects of weaning age and dosage of supplemented iron on the hemoglobin concentrations and growth rate of piglets. Swine Health and Production 5, 135–138.
Pomorska-Mol, M., Markowska-Daniel, I., 2011. Age-dependent changes in relative and absolute size of lymphocyte subsets in the blood of pigs from birth to slaughter. Bulletin of the Veterinary Institute in Pulawy 55, 305–310.
Prunier, A., Leblanc-Maridor, M., Pauwels, M., Jaillardon, L., Belloc, C., Merlot, E., 2022. Evaluation of the potential benefits of iron supplementation in organic pig farming [version 2; peer review: 2 approved]. Open Research Europe 2, 11.
Rousseau, S., Kyomugasho, C., Celus, M., Hendrickx, M.E.G., Grauwet, T., 2020. Barriers impairing mineral bioaccessibility and bioavailability in plant-based foods and the perspectives for food processing. Critical Reviews in Food Science and Nutrition 60, 826–843.
Sierzant, K., Perruchot, M.-H., Merlot, E., Le Floc'h, N., Gondret, F., 2019. Tissue-specific responses of antioxidant pathways to poor hygiene conditions in growing pigs divergently selected for feed efficiency. BMC Veterinary Research 15, e341.
Starzynski, R.R., Laarakkers, C.M.M., Tjalsma, H., Swinkels, D.W., Pieszka, M., Stys, A., Mickiewicz, M., Lipinski, P., 2013. Iron supplementation in suckling piglets: how to correct iron deficiency anemia without affecting plasma hepcidin levels. Plos One 8, e64022.
Svoboda, M., Pist'kova, K., 2018. Oral iron administration in suckling piglets - a review. Acta Veterinaria Brno 87, 77–83.
Svoboda, M., Ficek, R., Drabek, J., 2008. Reticulocyte indices in the diagnosis of iron deficiency in suckling piglets. Bulletin of the Veterinary Institute in Pulawy 52, 125–130.
Svoboda, M., Vanhara, J., Berlinska, J., 2017. Parenteral iron administration in suckling piglets - a review. Acta Veterinaria Brno 86, 249–261.
Szabo, P., Bilkei, G., 2002. Short communication - iron deficiency in outdoor pig production. Journal of Veterinary Medicine Series a-Physiology Pathology Clinical Medicine 49, 390–391.
Szudzik, M., Starzyński, R.R., Jończy, A., Mazgaj, R., Lenartowicz, M., Lipiński, P., 2018. iron supplementation in suckling piglets: an ostensibly easy therapy of neonatal iron deficiency anemia. Pharmaceuticals (basel) 11, e128.
Yilmaz, B., Li, H., 2018. Gut microbiota and iron: the crucial actors in health and disease. Pharmaceuticals (basel) 11, e98.