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RESEARCH ARTICLE

REVISED Evaluation of the potential benefits of iron supplementation in organic pig farming

[version 2; peer review: 2 approved]

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v2 **First published:** 24 Jan 2022, **2**:11
https://doi.org/10.12688/openreseurope.14367.1
Latest published: 21 Mar 2022, **2**:11
https://doi.org/10.12688/openreseurope.14367.2

Abstract

Background: Iron from the stock acquired during foetal life and the ingestion of milk is not sufficient to cover the needs of the piglets during their first weeks of life. In organic farming, systematic supplementation with iron is problematic due to a strong limitation in pharmaceutic treatments.
Methods: Erythroid parameters around weaning were measured in piglets from organic outdoor and indoor farms, and related to indicators of the inflammatory status. Blood samples were collected from 28.9±2.6 piglets/herd at 42.0±3.2 days of age and 11.9±3.0 kg live weight (mean ± SD) in 21 farms from the west part of France. Among the 11 outdoor farms, only one had supplemented piglets with 200 mg iron while among the 10 indoor farms, only one had not supplemented piglets, one had supplemented them with 100 mg, 8 with 200 mg and one with 400 mg.
Results: Compared to outdoor piglets without supplementation, piglets kept indoors and receiving 200 mg iron had lower haemoglobin concentration (105 vs 118±2 g/l, mean ± SE) and red blood cell volume (56 vs 60±1 fl) (P<0.005). The reduction in haemoglobin concentration and red blood cell volume was more pronounced in indoor piglets supplemented with 100 mg of iron and even more when they had not received iron. The plasma concentration of haptoglobin was lower in outdoor than in indoor piglets (0.51±0.06 vs 0.78±0.09 g/l) whereas no effect of housing was observed for markers of oxidative stress (dROM, BAP). In the 14 farms where sow parity was known, the haemoglobin concentration was lower in piglets from primiparous than from multiparous sows (109 versus 114±2 g/l, P < 0.001).
Conclusion: With the exception of soils where the content of bioavailable iron is very low, piglets from outdoor farms do not require iron supplementation, unlike those raised indoors.

Open Peer Review

Approval Status ✓ ✓

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version 2 (revision) 21 Mar 2022	✓ view	
version 1 24 Jan 2022	? view	✓ view

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Keywords
Anaemia, haemoglobin, inflammation, outdoors, indoors, suckling piglet

This article is included in the Horizon 2020 gateway.

This article is included in the Sustainable Farming collection.

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Author roles: Prunier A: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Methodology, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Leblanc-Maridor M**: Resources, Supervision; **Pauwels M**: Formal Analysis, Investigation, Visualization; **Jaillardon L**: Resources; **Belloc C**: Conceptualization, Methodology, Resources, Supervision; **Merlot E**: Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This research was financially supported by the European Union's Horizon 2020 research and innovation programme under the grant agreement No 727495 (project CORE Organic Cofund).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Prunier A, Leblanc-Maridor M, Pauwels M *et al.* **Evaluation of the potential benefits of iron supplementation in organic pig farming [version 2; peer review: 2 approved]** Open Research Europe 2022, **2**:11
https://doi.org/10.12688/openreseurope.14367.2

First published: 24 Jan 2022, **2**:11 https://doi.org/10.12688/openreseurope.14367.1

REVISED **Amendments from Version 1**

The main differences between this new version and the previous one are:

- the enclosure of a new table to better describe the characteristics of the farms that were included in this experiment,
- the change of Figure 2 since we made a mistake (we copied the content of Figure 4 instead of the true content of Figure 2) in the initial version,
- we enclosed a discussion on the definition of the normal range of haemoglobin in piglets

Any further responses from the reviewers can be found at the end of the article

Plain language summary

Iron requirements for haemoglobin synthesis and muscle growth in piglets are very high during their first weeks of life. To meet these requirements, piglets use the iron stored during their foetal life and ingest iron from their mother's milk, but this is not sufficient. In a study supported by an Era-Net CORE Organic project (Power project), researchers from the French research institute INRAE (Saint-Gilles France) and the veterinary school of Nantes (Oniris, France) explored the iron status of organic piglets raised in free-range or indoor conditions and receiving or not an iron treatment during their first week of life. Such treatment is problematic in organic farming due to a strong limitation of chemically synthetized drugs. About 600 piglets from 21 organic farms in the west part of France were submitted to a blood sample at about six weeks of age, near weaning. The results show signed of anaemia, low haemoglobin content and low red blood cell size in piglets kept indoors receiving a low dose of iron (100 mg) or no iron supplementation. Moreover, the haematologic status was better in outdoor piglets not receiving iron supplementation than in those reared indoors injected with 200 mg dextran or gleptoferron iron. Therefore, except when soils are deficient in bioavailable iron, outdoor piglets do not require iron supplementation unlike those reared indoors. A solution with a continuous supply of iron in a natural form should be developed for organic piglets reared indoors.

Introduction

The piglet's iron requirement is high during its first weeks of life, about 21 mg per kg of gain, i.e. 5 to 7 mg per day, to accompany its rapid growth (Braude *et al.*, 1962; Mahan & Shields, 1998; Venn *et al.*, 1947). Iron is used, in particular, for the synthesis of haemoglobin in red blood cells and myoglobin in muscle. To meet the iron requirement, the new born piglet mobilizes its reserves stored during foetal life, but they only cover a few days of life (Mahan *et al.*, 2009; Venn *et al.*, 1947). A second source of iron is maternal milk, but the intake of about 1 mg per day (Szudzik *et al.*, 2018; Venn *et al.*, 1947) is very low compared to the need. Piglets would therefore become anaemic very quickly if they do not have access to other sources of iron. When lactating sows are kept outdoors, the absence of iron supplementation does not seem to be a problem, probably because piglets ingest iron from the soil (Brown *et al.*, 1996; Delbor *et al.*, 2000). When sows are kept indoors, this ingestion of soil does not occur and iron supplementation is necessary (Svoboda *et al.*, 2017; Svoboda & Pist'kova, 2018).

In conventional farms, farmers bring iron to piglets in the first few days of life by parenteral (intramuscular injection) or oral supplementation (Svoboda *et al.*, 2017; Svoboda & Pist'kova, 2018; Szudzik *et al.*, 2018). In organic farming this systematic supplementation is problematic since it breaks the principles of health (i.e. animal drugs should be avoided) and ecology (i.e. the production is to be based on ecological processes) (IFOAM, 2020). In accordance with these principles, the EU regulation for organic livestock farming limits strongly the use of chemically synthesised drugs (Regulation EC n°2018/848, 2018). For a pig to be certified as organic, only one drug treatment is allowed apart from parasite treatments, vaccines and compulsory treatments (e.g. mitigation of pain derived from male castration). Even if not systematic, iron preparations administered to piglets are considered, by some inspectors of certifying bodies in France, to be a drug treatment. In this case, the meat of a pig will be downgraded if it receives another treatment to treat a disease. This leads some farmers not to perform neonatal iron injections.

As little data are available on the risk of iron deficiency in organic pig farms, the main objective of this study was to measure haematological parameters as well as circulating concentrations of iron and ferritin in organic piglets raised outdoors or indoors and born from primiparous or multiparous mothers. Their inflammatory state was also assessed by measuring an inflammation protein (haptoglobin) and oxidative stress indicators because the inflammatory status can modulate the availability of iron for haemoglobin synthesis (Ganz & Nemeth, 2012).

Methods

Animals and sampling

All procedures were approved by the ethical committee for Clinical and Epidemiological Research from the Oniris Veterinarian School of Nantes in France (CERVO) and received the agreement # CERVO-2018–15-V. All efforts were made to minimise animal suffering. Farmers enrolled in the study were informed about the aims and content of the project and signed a letter of informed consent.

Twenty-one organic pig farms (11 outdoors, 10 indoors), located in the west part of France, were selected on a voluntary basis. The experiment was performed on domestic pigs (Sus scrofa domesticus) from various genetic lines (Table 1). It occurred in some farms that boars (one indoor and one outdoor farms) and sows (two indoor and one outdoor farms) were from two different genetic lines. Number of reproductive sows was also very variable between farms (Table 1). Weaning of piglets was performed around 6 weeks of age (Table 1). As indicated by the farmers, the average littersize at weaning in the farms was around 10, being slightly higher indoors than outdoors (Table 1). During lactation, piglets had access to a dry feed that could be the lactation

Table 1. Main characteristics of indoor and outdoor farms. Main characteristics of the farms where the experiment was performed. Means ± SD [minimal-maximal values] as well as number of farms between brackets are indicated.

	Indoor farms (n = 10)	Outdoor farms (n = 11)
Number of reproductive sows	78 ± 53 [28–180]	123 ± 130 [20–385]
Age at weaning, days	42.9 ± 2.9 [34–50]	43.2 ± 3.2 [41–57]
Littersize at weaning	10.7 ± 1.0 [9.5–12.5]	9.6 ± 1.2 [8.0–11.0]
Boar genetic lines	P (9), P + PxLW (1)	P (10), P + PxLW + Longué* (1)
Dam genetic lines	LWxLR (3), LW (2), Naima® (1), Danbred® (1), Danbred® + LWxLR (2), LWxBlanc de l'Ouest* (1)	LWxLR (7), LW (1), LRxSaddle Back (1), LWxLRxP (1) LWxLRx P + Longué* (1)
Type of feed during lactation[1]	Dam's lactation diet (2)[2] Creep feed,1st and/or 2nd age (9)[2]	Dam's lactation diet (3)[3], Creep feed, 1st and/or 2nd age (7)[3]
Age from which dam's diet available Age at starting creep feed	2 weeks of age (2) ~1 (2), 2 (1), 3 (6) weeks of age	birth (1), 3 (1), 4 (1) weeks of age 3 (4), 4 (2) weeks of age[4]

P: Pétrain, LW: Large White, LR: Landrace, *Local French breed

[1]The type of feed distributed to piglets was not recorded in one outdoor farm

[2]In one indoor farm, piglets from large litters received creep feed whereas only the dam's lactation diet was available to other litters

[3]In one outdoor farm, piglets received a creep feed between 20 and 30 days of age and the dam's lactation diet thereafter

[4]The age at starting the distribution of creep feed to suckling pigs was not known in one outdoor farm

diet of the dam or a diet for first- or second-age piglets (Table 1). According to farms, the dam's lactation diet started to be available to piglets from birth to 4 weeks of age and the distribution of creep feed started between 1 and 4 weeks of age (Table 1). As far as we know, all these diets were supplemented with ferrous or ferric iron (carbonate or sulfate or oxide form) and contained bentonite which is rich in ferrous or ferric iron. Piglets were selected within litters so that males and females, low, medium and large piglets were equally represented. In addition, only males with both testes descended were selected. In each farm, 28.9 ± 2.6 [18, 30] piglets (average ± SD, [minimum, maximum]) from four to seven litters (sows of different parities) were sampled at 42.0 ± 3.2 [34, 57] days of age, 11.9 ± 3.0 [5, 23] kg live weight and -1.1 ± 1.6 [-6, +1] days from weaning (606 pigs in total). Live weight was measured individually with a portable scale (HDB 30 K-2XL, 2336 Balingen, Germany). Blood was drawn from the jugular vein to collect 5 ml on a dry tube and 5 ml on an EDTA tube. Each piglet was kindly caught by the farmer or a technician. It was firmly held on its back in order to prevent any movement and to have the forelegs spread. The second operator, previously trained to blood sampling in piglets, performed the sampling. Immediately after, the piglet was weighed and returned to its home pen. The whole procedure lasted less than two minutes. The samples were stored, for a maximum of four hours, in a refrigerated cooler until arrival at the laboratory.

As far as possible, the identity of the mother, parity and date of birth were recorded. In order to link the piglets with their mother in outdoor farms, farmers were required to fix an identification tag on the ear of the piglets shortly after birth. When the exact date of birth was not known, a single date was recorded according to the farmer indication.

Laboratory analyses

On arrival at the laboratory, a complete haematological analysis was carried out on the blood collected on EDTA using a Procyte Dx Idexx automaton (Westbrook, Maine 04092, USA). Parameters measured included haemoglobin concentration (Hb), haematocrit, red blood cell and reticulocyte counts, mean Red Blood Cell Volume (RBCV), the mean Red Blood Cell Haemoglobin Concentration (RBCHb), the Reticulocyte Haemoglobin concentration (RetHb), the number of lymphocytes, monocytes, neutrophilic and eosinophilic granulocytes. These analyses were performed on the 606 piglets for all parameters except the leucocyte count for which three samples could not be analysed for technical reasons.

The remaining blood on EDTA was centrifuged before collecting the plasma. After clotting, the dry blood was centrifuged and the serum was collected. The aliquots were stored, for a maximum of four months, at -20°C until analysis. Haptoglobin was analysed on plasma, iron, ferritin, hydroperoxides (= oxidation products = dROM) and the antioxidant capacity of the blood (BAP) on serum. All analyses except for ferritin were performed in single assays with a laboratory robot (Konelab 20, Thermo Scientific, Waltham, MA USA) using commercial kits that are not species specific (total iron: Iron-981236, Thermo Scientific, Finland; haptoglobin: T801, Tridelta Ltd, Maynooth, Ireland; dROM and BAP: Diacron, Grosseto,

Italy). The intra-assay CV was 5.5%, 7%, 4% and 8%, respectively for iron, haptoglobin, dROM and BAP. Ferritin was measured in duplicates using an ELISA test specific for pigs (kit MBS741259, MyBioSource, San Diego, CA 92195–3308). The intra-assay CV was 3%.

These analyses were performed on 552, 566, 565, 563 piglets (18 to 30 piglets per farm), for iron, BAP, haptoglobin and dROM respectively, as they were not carried out in one outdoor farm (Farm U) and on a few pigs in eight other farms due to a lack of plasma or to a technical problem. Ferritin was measured in 200 piglets (10 piglets per farm selected to ensure a balanced sex ratio and diversity of weights within farms) from all farms except one outdoor farm (farm U).

Statistical analyses

All analyses were performed with the R software (version 4.0.2). Daily weight gain (DWG) was estimated by dividing the weight at the measure by the age at this measure since animals were not weighted at birth. This method overestimates slightly the real daily gain but overestimation occurs for all animals, even if it is slightly more marked in piglets that are heavier at birth. Mixed ANOVA (type 3) were carried using the lmerTest and Car packages. To analyse live weight and daily weight gain (DWG), sex (two modalities: Male vs Female) and housing (two modalities: Indoors vs Outdoors) were introduced as fixed effects and farm as a random effect. To analyse blood parameters, sex (two modalities: Male vs Female), housing (two modalities: Indoors vs Outdoors), live weight (quantitative variable) were introduced as fixed effects and farm as a random effect. In a subgroup of 13 farms where sow parity was known and piglets clearly linked to their dam, parity (two modalities: Primiparous vs Multiparous) was also included. A square root transformation was carried out before analysis for some parameters (number of lymphocytes, monocytes, neutrophils and eosinophils, iron, ferritin, haptoglobin and dROM concentrations, BAP) to normalise them and homogenise the variances. Pearson correlations between variables were calculated with the Hmisc package. Statistical levels were corrected for multiple comparison using the Holm method. Principal Component Analyses were performed with the FactoMineR package and missing values were imputed by the mean of each variable. Unless otherwise stated, the results presented in the text correspond to adjusted means ± SEM. P-values < 0.05 were considered statistically significant.

Results

In only one indoor farm (O), no iron supplementation was carried out. In the other indoor farms, piglets received: 100 (K) or 200 (A, B, C and R) mg dextran iron, 200 (E, F and J) or 400 (Q) mg gleptoferron iron (Figure 1). The injection was performed between one and four days of age except in the Q (on the day of birth) and C (four days of age) farms. Irrespective of the nature of the iron injected, descriptive statistics show that, indoors, the average haemoglobin concentration progressively increases between 0 (81.0 g/l), 100 (90.5 g/l) and 200 mg of iron (> 94.5 g/l), and between 200 (108.1 to 111.3 g/l) and 400 mg (116.7 g/l) of iron. In the outdoor farms, the piglets did not receive any iron supplementation except in one farm (S) where 200 mg gleptoferron iron was injected at seven days of age (Figure 1). In this farm, mean haemoglobin concentration was very close (124.8 g/l) to the mean measured in the four highest farms not injecting iron (121.2 to 124.6 g/l).

No statistical analysis was carried out to test the influence of the iron dose because only one farm injected 0, 100 or 400 mg of iron indoors and 200 mg of iron outdoors. Similarly, the number of farms was insufficient to statistically compare the two forms of iron when 200 mg iron were supplied.

Effects of housing, sex and live weight of piglets

Farms O, K, Q and S were excluded from the statistical analyses for the comparison of housing because of their particular practice of iron supplementation (no or 100 mg injection indoors, 200 mg outdoors, see above). Indoor farms supplementing with 200 mg iron using dextran or gleptoferron forms were grouped together in the same category. Finally, 7 indoor (206 piglets) and 10 outdoor (280 piglets) farms were compared.

Live weight (Indoors: 11.4 ± 0.6 kg, Outdoors: 12.7 ± 0.5 kg; P > 0.1), DWG (Indoors: 0.28 ± 0.01 kg/day, Outdoors: 0.29 ± 0.01 kg/day; P > 0.1) and age of the piglets (Indoors:

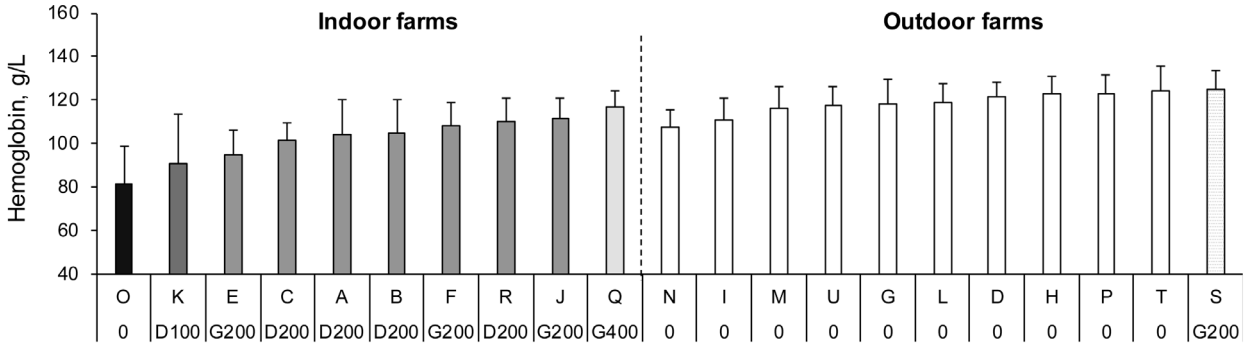


Figure 1. Mean blood haemoglobin concentration of piglets in 10 indoor and 11 outdoor organic farms. Blood haemoglobin concentration in the 21 farms enrolled in the study (mean ± SD). 0: no iron injection, D100: injection of 100 mg iron dextran, D200: injection of 200 mg iron dextran, G200: injection of 200 mg gleptoferron iron, G 400: injection of 400 mg gleptoferron iron.

40.8 ± 0.2 days, Outdoors: 43.3 ± 0.2 days; P < 0.001) at sampling were close in both housing systems even if a significant difference was observed for the age.

Parameters of the erythrocyte lineage, serum iron and ferritin

Haemoglobin concentration, haematocrit, red blood cell count, RBCV, RBCHb and RetHb were significantly higher in piglets kept outdoors compared to piglets kept indoors (P < 0.05, Figure 2). The reticulocyte count, the concentrations of iron and ferritin did not differ significantly between the two housing systems (P > 0.1).

There was a significant effect of sex only for RetHb and blood haemoglobin concentration, both parameters being significantly lower in males (haemoglobin: 110.0 ± 1.5 mg/ml; RetHb: 17.0 ± 0.2 µg/ml) than in females (haemoglobin: 113.0 ± 1.5 mg/ml; RetHb: 17.4 ± 0.2 10 µg/ml; P < 0.05). Live weight had a significant influence only on the reticulocyte count which increased with weight (P < 0.003).

Number of leukocytes and inflammatory status

The number of lymphocytes was significantly higher and the number of neutrophils lower indoors than outdoors (P < 0.05, Figure 3). The numbers of monocytes and eosinophils did not differ significantly between the two housing systems (P > 0.1). Serum haptoglobin concentration was higher in indoor than outdoor piglets (P < 0.05, Figure 3) whereas serum dROM and BAP were similar in both housing systems (P > 0.1, Figure 3).

A significant sex effect was only observed for neutrophils with a higher number in males than in females (8.21 ± 0.50 vs. 7.65 ± 0.48 106 cells/ml). Body weight had a significant effect only on the number of lymphocytes (P < 0.003), BAP (P <0.002), serum haptoglobin (P < 0.007) and dROM (P < 0.02). The number of lymphocytes and BAP increased with increasing body weight whereas serum haptoglobin and dROM decreased with increasing body weight.

Effects of parity

The comparison between primiparous and multiparous sows was only carried out in farms where parity was known and where at least two piglets of each sex and each type of sow had been measured. A total of five indoor and eight outdoor farms were included with 122 piglets from primiparous sows and 258 from multiparous sows (parities 2 to 9).

Parameters of the erythrocyte lineage, serum iron and ferritin

The influence of parity was significant for haemoglobin concentration, red blood cell count, RBCV (P < 0.05, Figure 4), haematocrit (Multiparous: 40.6 ± 0.7 %, Primiparous: 38.6 ± 0.7%, P < 0.05) and ferritin (Multiparous: 15.7 ± 1.1 pg/ml, Primiparous: 19.3 ± 1.1 ng/ml, P < 0.05) but not for RBCHb, reticulocyte count and RetHb (P > 0.1, data not shown).

Number of leukocytes and inflammatory status

Only the number of monocytes was significantly influenced by sow parity, piglets from primiparous sows (1.49 ± 0.11 10^6 cells/ml) having more monocytes than those from multiparous sows (1.31 ± 0.10 10^6 cells/ml; P > 0.004).

Relationships between parameters

Correlation analyses were carried out separately for the 7 indoor farms injecting 200 mg iron and for the 10 outdoor farms performing no supplementation. Only five correlations were

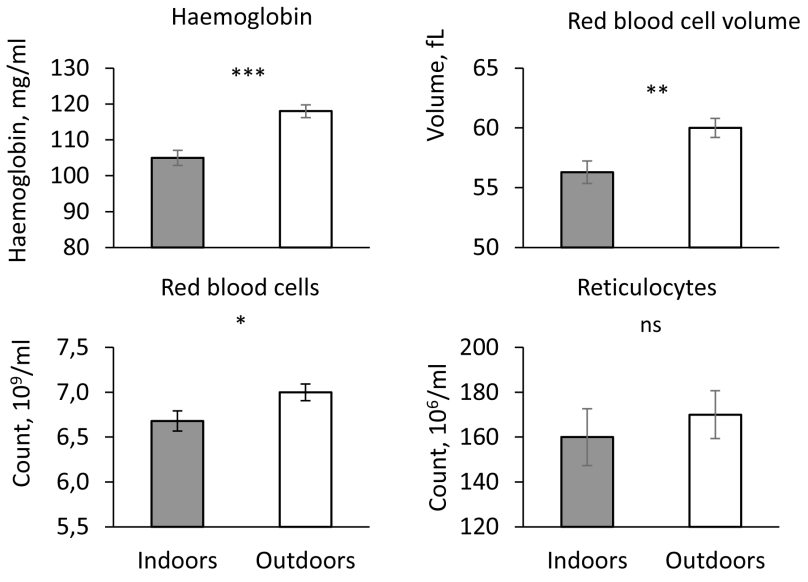


Figure 2. Haematologic indicators in indoor (200 mg iron supply) and outdoor piglets (no iron supply). Influence of housing (Indoors vs. Outdoors) on blood haemoglobin concentration, Reb Blood Cell Volume (RBCV), red blood cell and reticulocyte counts (adjusted mean ± SE). *** P < 0.001, ** P < 0.01, * P < 00.05, ns P > 0.1.

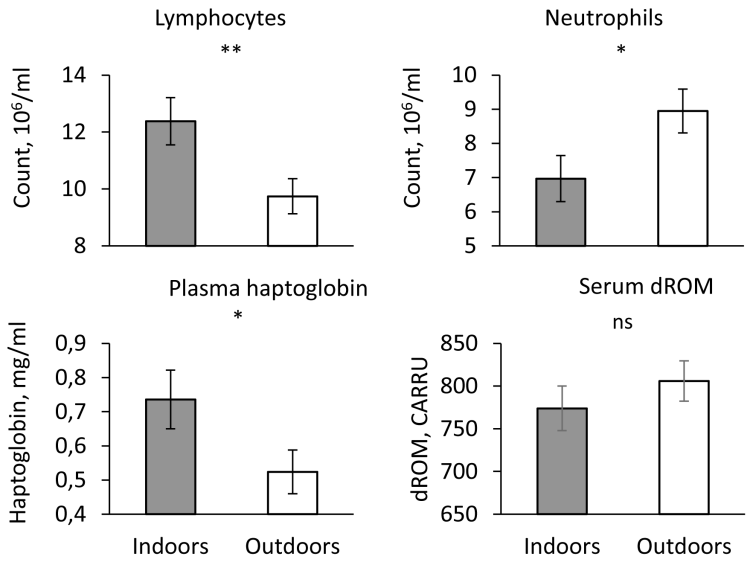


Figure 3. Health indicators in indoor (200 mg iron supply) and outdoor piglets (no iron supply). Influence of housing (Indoors vs. Outdoors) on lymphocyte and neutrophil counts, plasma haptoglobin concentration and serum dROM concentration (adjusted mean ± SE). ** P < 0.01, * P < 0.05, ns P > 0.1.

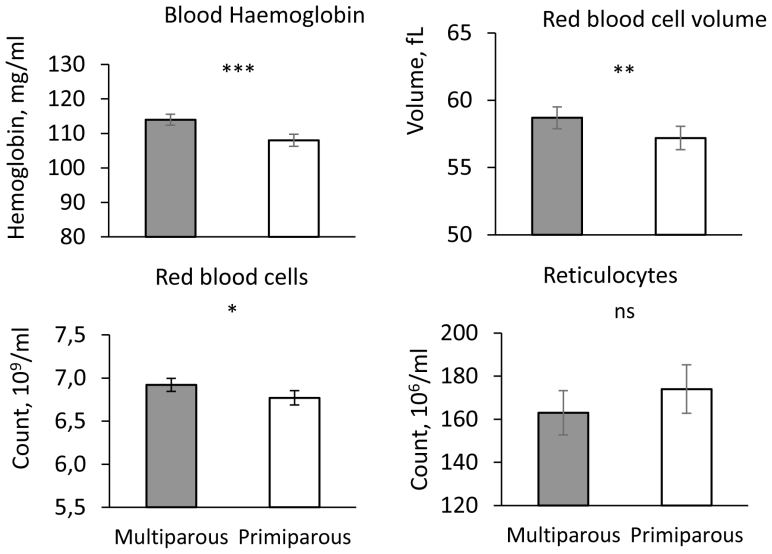


Figure 4. Haematologic parameters in piglets from primiparous and multiparous sows. Influence of sow parity (Primiparous vs. Multiparous) on blood haemoglobin concentration, Red Blood Cell Volume (RBCV), red blood cell and reticulocyte counts (adjusted mean ± SE). *** P < 0.001, ** P < 0.01, * P < 00.05, ns P > 0.1.

consistent in both housing systems: positive between blood Hb and RBCV as well as between blood Hb and the red blood cell count (P < 0.001 for both correlations and systems), and between plasma haptoglobin and serum dROM (P < 0.001 for both systems) but negative between serum haptoglobin and the reticulocyte count (Outdoors: P < 0.03, Indoors: P < 0.001) or between serum haptoglobin and serum iron (Outdoors: P < 0.07, Indoors: P < 0.001) (Table 2). One correlation was opposite in the two housing systems, that between plasma haemoglobin and average daily gain (Outdoors: positive and P < 0.07, Indoors: negative and P < 0.03, Table 2). Four correlations were significant only Outdoors and sixteen only Indoors (Table 2). The most interesting ones were positive correlations between serum iron on one side and plasma Hb or RBCV on the other side in Indoor pigs (P < 0.001 for both correlations, Table 2).

Table 2. Correlations between haematologic and health indicators in outdoor and indoor pigs. Pearson correlations between blood (haemoglobin concentration: Hb, red blood cell volume: RBCV, red blood cell count: nRBC, reticulocyte count: nRetic, square root of neutrophil count: rnNeutro, square root of lymphocyte: rnLympho), serum (square root of iron concentration: rIron, square root of hydroperoxide concentration: rdROM, antioxidant capacity: BAP) and plasma (square root of haptoglobin concentration (rHapto) characteristics, and daily weight gain (DWG). Values above the diagonal are calculated for Outdoor pigs (n = 242 to 280) and values below the diagonal are calculated for Indoor pigs (n = 188 to 203). Bold characters indicate that the correlation is significant (P < 0.05). Bold and Italic characters indicate that the correlation tends to be significant (P < 0.1). Levels of significance were corrected for multiple comparisons using the Holm method.

		Outdoors										
		Hb	**RBCV**	**nRBC**	**nRetic**	**rnNeutro**	**rnLympho**	**rIron**	**rHapto**	**rdROM**	**BAP**	**DWG**
Indoors	Hb	1.00	**0.53**	**0.75**	0.00	-0.09	0.07	0.10	-0.10	-0.05	0.15	***0.19***
	RBCV	**0.59**	1.00	-0.03	0.09	**-0.22**	0.18	-0.01	-0.15	-0.06	0.05	***0.18***
	nRBC	**0.48**	**-0.37**	1.00	-0.04	0.04	0.05	0.10	0.05	0.00	**0.22**	0.09
	nRetic	**0.27**	**0.48**	-0.11	1.00	0.03	0.11	0.15	**-0.22**	0.01	0.18	0.11
	rnNeutro	0.13	-0.09	**0.25**	-0.15	1.00	0.12	**-0.24**	0.06	0.02	0.04	0.09
	rnLympho	0.14	-0.14	**0.38**	0.02	0.17	1.00	0.01	-0.09	0.04	0.08	0.17
	rIron	**0.57**	**0.52**	0.05	**0.32**	-0.04	0.10	1.00	***-0.20***	-0.07	0.04	-0.06
	rHapto	**-0.29**	**-0.36**	0.04	**-0.31**	**0.29**	-0.18	**-0.41**	1.00	**0.35**	-0.10	***-0.20***
	rdROM	0.09	0.16	-0.05	-0.10	**0.26**	-0.12	0.00	**0.48**	1.00	**0.31**	-0.07
	BAP	-0.13	0.07	-0.13	0.10	**-0.23**	-0.03	-0.14	-0.07	0.10	1.00	0.12
	DWG	**-0.23**	-0.17	0.02	**0.27**	**-0.22**	0.02	-0.16	-0.13	**-0.30**	**0.25**	1.00

Multivariate analysis (PCA) was performed across housing systems using the 486 piglets from the same 7 indoor and 10 outdoor farms. Three traits related to the haematologic status (blood Hb, red blood cell and reticulocyte counts), five traits related to the health and oxidant status (neutrophil and lymphocyte counts, plasma haptoglobin, serum dROM and BAP), average daily gain and serum iron were included. Red blood cell volume was not included since it was highly correlated to the reticulocyte count. The first three components (a component is a direction that maximizes the variance of the projected data) of the PCA explained respectively 20.8, 16.1 and 12.7%, i.e. a total of 49.5% of the total variance. The highest contributors to the first component were blood haemoglobin, plasma haptoglobin, serum iron and red blood cell count in decreasing order. The highest contributors to the second component were red blood cell count, neutrophil count and dROM in decreasing order. The highest contributors to the third component were serum BAP and daily weight gain in decreasing order. The correlation structure between the 10 traits showed that (a) plasma haptoglobin was opposite to serum iron, blood haemoglobin and blood cell count, (b) serum iron, blood haemoglobin and blood cell count were positively linked, (c) BAP and daily weight gain were positively linked (Figure 5A and 5B). Projection of the piglets on to the first (Figure 5C) and second (Figure 5D) plots showed that indoor piglets were much more dispersed than outdoor ones. The difference between housing systems was essentially related to the projection on the first component with some indoor piglets characterized by high plasma haptoglobin, low serum iron, blood haemoglobin and blood cell count.

Discussion

This study assessed the possible consequences of different iron intakes on the haematologic status and the inflammatory state of piglets reared in organic pig farms either indoors or outdoors. They show that iron supplementation is not necessary in the open air, in line with most previous results (Brown *et al.*, 1996; Delbor *et al.*, 2000; Kleinbeck & McGlone, 1999). It is most likely the supply of iron through the consumption of soil that explains why piglets reared outdoors do not suffer from anaemia even if they do not receive iron supplementation (Venn *et al.*, 1947). In support of this, a three-week-old piglet reared outdoors was shown to have 513 mg of iron in its digestive contents (Venn *et al.*, 1947). However, the risk of anaemia exists when soils have a very low content of bioavailable iron. Indeed, Brown *et al.* (1996) showed an abnormally low average haemoglobin concentration at 3–4 weeks of age in one farm among the eight investigated in their study in Scotland (84 mg/ml compared with 103–124 mg/ml), and this farm was the one with the lowest iron content in its soil. More markedly, Szabo & Bilkei (2002) showed an extremely low mean haemoglobin concentration at five weeks of age (54.1 mg/ml) in a Hungarian outdoor farm where piglets did not receive iron supplementation.

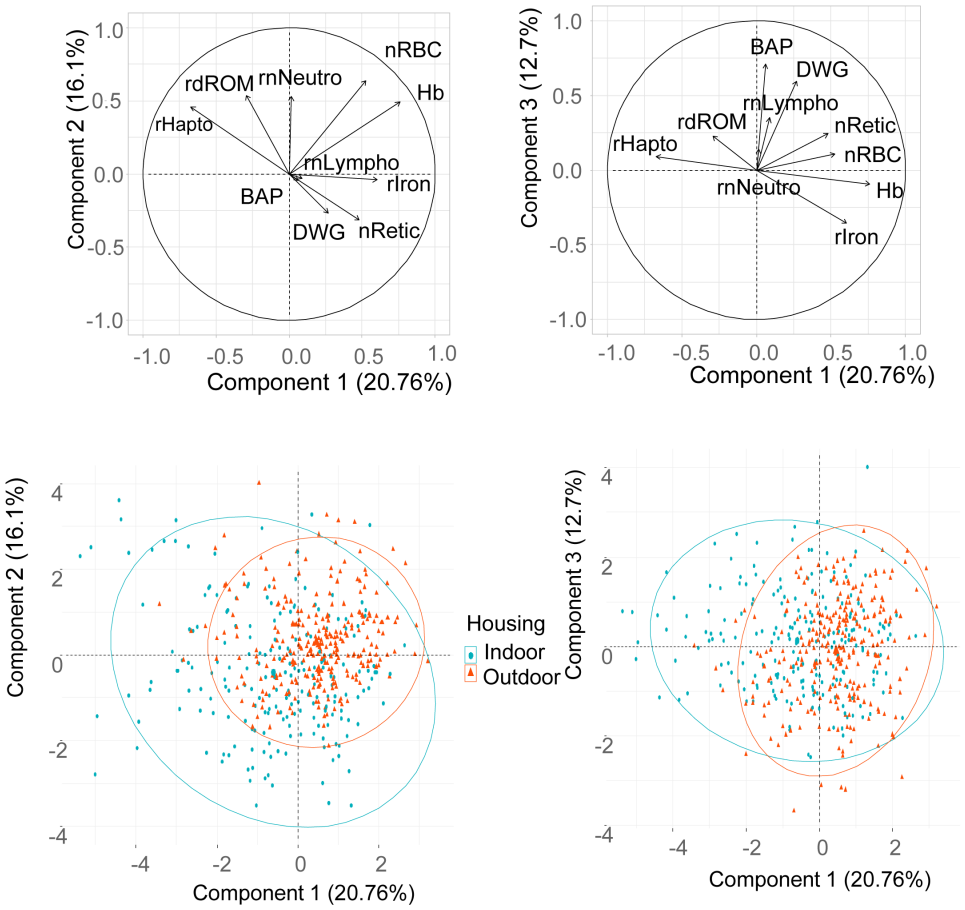


Figure 5. Representation of the links between haematologic and health indicators and projection of the piglets. Overall pattern of correlations between blood (haemoglobin concentration: Hb, reticulocyte count: nRetic, square root of neutrophil count: rnNeutro, square root of lymphocyte: rnLympho), serum (square root of iron concentration: rIron, square root of hydroperoxide concentration: rdROM, antioxidant capacity: BAP) and plasma (square root of haptoglobin concentration (rHapto) characteristics, and daily weight gain (DWG), presented on the first (first and second components, **A**) and second (first and third components, **B**) plots. Projection of the piglets on to the first (first and second components, **C**) and second (first and third components, **D**) plots. Ellipses include 95% of the individuals.

On the other hand, indoors, a dose of 200 mg of iron per piglet is a minimum to reach a sufficient level of haemoglobin. Without an iron injection or with a supplementation of 100 mg, the haemoglobin concentration measured in the indoor farms of the present study was respectively 81 and 90 mg/ml on average, which can be considered too low, even though the optimal haemoglobin concentration in piglets is still unclear as the literature is inconsistent on this issue (Svoboda *et al.*, 2017). However, in their review, Svoboda *et al.* (2017) referring to the National Research Council (1979) values for haemoglobin in pigs indicated that a haemoglobin concentration of 100 mg/ml or more can be considered normal, 80 mg/ml as a sign of borderline anaemia and 60 mg/ml of severe anaemia. Furthermore, beyond the question of defining a threshold, there may still be a positive effect of values above the normal ones. Indeed, Gentry *et al.* (1997) showed better growth rate and energy retention in the group of suckling piglets having 119 mg/ml haemoglobin in average compared to the group having 100.4 mg/ml, the two groups differing by the amount of iron-dextran injected at 3 days of age. Our results are in line with the bibliography which shows that 100 mg are clearly too low whereas 200 mg of iron would cover the iron requirement up to three weeks of age but would be insufficient beyond that, unless the piglets have access to an iron source complementary to milk (Svoboda *et al.*, 2017). Indeed, at three weeks of age, piglets would have consumed their prenatal reserve and the stock provided by the 200 mg neonatal supplementation, due to the very deficient balance between the high need for rapid growth and the relatively low intake from milk (Svoboda *et al.*, 2017).

Our study shows that the iron status of outdoor piglets is better than that of indoor piglets receiving an iron injection of 200 mg. All measured parameters were in the same direction.

Haemoglobin concentration, haematocrit, red blood cell count, mean corpuscular haemoglobin concentration, mean reticulocyte haemoglobin concentration, and red blood cell volume were lower in piglets raised indoors, suggesting an iron deficiency in those piglets compared to outdoor piglets. A priori, the iron intake from milk and solid feed did not differ between the two systems since piglets had comparable body weight and average daily gain. Thus, two hypotheses may explain this iron deficit in indoor pigs: iron sequestration due to a more pronounced inflammatory state or insufficient iron intake. Long lasting inflammation favours anaemia because it triggers the secretion of hepcidin, an hormone that reduces iron bioavailability by enhancing its sequestration in macrophages (Hentze *et al.*, 2010). The more pronounced inflammatory state indoors is suggested by a significantly higher plasma haptoglobin concentration even though the lack of effect of the environment on oxidative stress indicators suggests that the severity of this inflammation was limited. A significant correlation was also identified between plasma haptoglobin and serum iron, which was more marked indoors than outdoors. This supports that high inflammation may have contributed to an anaemic status of indoor piglets.

The more marked inflammatory state of piglets kept indoors reveals a higher activity of their immune system. We also observed more lymphocytes but fewer neutrophils indoors than outdoors in agreement with previous results (Kleinbeck & McGlone, 1999). The origin of the inflammatory response and change in leucocyte counts was not determined as we did not measure any clinical parameters and data from the literature can hardly be used. Indeed, to our knowledge, there is no study comparing the health of organic piglets raised outdoors and indoors. However, studies performed in older pigs suggest that there are more digestive and respiratory health problems indoors than outdoors (Delsart *et al.*, 2020; Leeb *et al.*, 2019).

The second hypothesis is that iron intake, through soil licking or consumption, allowed an adequate iron intake throughout lactation in outdoor pigs whereas in indoor pigs pre and postnatal stock were probably depleted by three weeks of age (see above) and intake by milk and feed were not sufficient to meet the need (see above). The iron intake from soil in outdoor piglets would have "compensated" or even be higher than that due to the neonatal injection of 200 mg of iron in indoor pigs. This superiority of iron intake via the soil can be understood in terms of quantity but also in terms of quality. Indeed, the neonatal injection of a massive dose of iron is likely to induce oxidative stress and to promote inflammatory states, such as arthritis (Svoboda *et al.*, 2017; Szudzik *et al.*, 2018) that may in turn favour the sequestration of iron. In addition, iron dextran injection was shown to induce a high expression of hepcidin and a low expression of ferroportin in the duodenum (Pu *et al.*, 2015) that may reduce iron absorption from milk and feed. The supply of iron from the earth is obviously very gradual and probably adapted to the needs of animals whose ancestral way of life, during which the species has evolved the longest, allowed the consumption of land.

A positive correlation between serum iron and blood haemoglobin was observed in indoor pigs (r = 0.57) in agreement with the literature in Human (Hinrichs *et al.*, 2010; Mahiou *et al.*, 1992) or cattle (Joerling & Doll, 2019). However, such a high correlation was not present in outdoor pigs (r =0.1) suggesting that the regulation of iron storage and the use of iron for haemoglobin synthesis were somewhat different in indoor and outdoor pigs. Interestingly, the results from the multivariate analysis indicated a greater heterogeneity regarding immune, inflammatory and red blood cell indicators among piglets raised indoors. This supports the hypothesis that piglets raised indoors are more at risk of developing anaemia and a health disorder whatever the cause.

Our results indicate a poorer haematologic status in piglets from primiparous sows than from multiparous sows, with a lower blood haemoglobin concentration and a lower average globular volume. This result cannot be compared with the bibliography as, to our best of knowledge, this is the first study to make this type of comparison. The difference could be explained by the fact that primiparous sows have not finished their growth so that the export of iron to the foetus or milk would compete with the sows' need for their own growth. Measurements in conventionally reared sows have shown a higher haemoglobin concentration in primiparous sows than in multiparous sows at different stages of gestation and lactation in France (Normand *et al.*, 2012) and the USA (Castevens *et al.*, 2020) and hence suggested iron deficiency in multiparous sows that may lead to iron deficiency in their progeny at the opposite of present results. However, they did not measure the iron stock at birth or the blood haemoglobin concentration in lactating piglets in the progeny to prove their hypothesis. In addition, it should be mentioned that these studies were carried out in conventional farms where lactation is significantly shorter (around four weeks in France and three weeks in the USA) than in our study (around six weeks) so that sows would have much less time to recover from iron transfer to their foetuses during gestation and blood loss at farrowing. This would result in a depletion of iron reserves over parities and, probably, lower transfer to their progeny in conventional multiparous sows which would not occur in organic farming.

Conclusion

This study shows that piglets reared outdoors find sufficient iron in their natural environment and do not need iron supplementation. Indoors, supplementation is necessary, but a single intramuscular injection of 200 mg of iron may be sub-optimal to prevent anaemia. Moreover, as this injection can be considered as a drug treatment in organic pig farming, alternative solutions need to be found to ensure a sufficient, natural and progressive iron supply to piglets kept indoors.

Data availability

Underlying data

Data INRAE: Iron supplementation in organic pig farming, https://doi.org/10.15454/QWRJHL.

This project contains the following underlying data:

- DataSetFinal_Power_21OrganicFarms.tab
- Variable_List_DataSetFinal_Power1.tab

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Acknowledgments

The authors would like to thank the farmers for their welcome as well as technicians from Biodirect for their help during the sampling, Raphaël Comte, Sophie Daré, Françoise Thomas (Inrae), Emmanuelle Blandin, Anne-Sophie Noël (Bioepar), Delphine Boucher, Léa Urffer, Maïlys Hilary (Laboniris) for their technical support.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 25 March 2022

https://doi.org/10.21956/openreseurope.15798.r28889

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✔ **Glen Almond**
Department of Population Health & Pathobiology, College of Veterinary Medicine, North Carolina State University (NCSU), Raleigh, NC, USA

The authors addressed this reviewer's concerns. It is challenging to control or measure all factors in an applied study over 21 farms. The information should prove useful. Field studies are challenging; however, the information is readily applied to commercial farms. Well done!

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: 40 years of basic and applied research in commercial pig production. Current research is directed at evaluating anemia in commercial pig farms. Previous research studies involved UTI's in sows and non-infectious reproductive failure in pigs.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 08 February 2022

https://doi.org/10.21956/openreseurope.15505.r28451

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✔ **Martin Svoboda**

Faculty of Veterinary Medicine, Ruminant and Swine Clinic, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

The article deals with a very important topic. Iron deficiency anaemia is a serious problem in swine production, it can cause significant economic losses. Therefore it is very important to find an optimal way of iron supplementation. The paper is very well written and also its technical quality is very good.

I recommend it pass peer review, but I have the following recommendations for any future versions:

- Please add information about the creep feet I mean iron content and from which day it was offered to the piglets.
- Please add information about the weaning age of piglets.
- Please give information about the breeds of pigs in different farms.
- I think that the limitation of the study is that the blood was taken only at the weaning and not for example in the first two weeks of life because with voluntary consumption I would expect differences in the first weeks of life of piglets. In my experience with voluntary consumption of iron, there is a usually high variability among piglets in the first weeks of life. So take this only as a recommendation for future studies.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 11 Mar 2022

armelle prunier

Thanks a lot for your valuable evaluation and comments.

- Please add information about the creep feet I mean iron content and from which day it was offered to the piglets.

It is an on-farm experiment and we do not have a precise information on that. We have added a table (Table 1) to describe this information we got during the farm visits together with some other characteristics of the farms as requested in other comments. We also added some comments in the text.

- Please add information about the weaning age of piglets.

Age at sampling and days from weaning were included in the text. Therefore, age at weaning could be deduced from that. However, we have added this information in Table 1 and added some comments in the text.

- Please give information about the breeds of pigs in different farms.

We have added this information in Table 1 and in added some comments in the text.

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We agree that it would have been very interesting to make measures at two stages, one around 2-3 weeks of age and one around weaning (6 weeks in organic pig production) but it was not possible in such a on-farm experiment where access to the piglets is difficult, especially in outdoor farms. However, we have performed a subsequent experiment in an experimental organic farm and performed measures at several time points during lactation as you recommend. Present data show that around 6 weeks of age, variations due to iron supply by neonatal injection, indoors, or due to the housing system are still noticeable. Therefore, it was a “sensitive” stage.

Competing Interests: No competing interests were disclosed.

Author Response 11 Mar 2022

armelle prunier

Thanks a lot for your valuable evaluation and comments.

- *Please add information about the creep feet I mean iron content and from which day it was offered to the piglets.*

It is an on-farm experiment and we do not have precise information on that. We have added a table (Table 1) to describe all the information we got during the farm visits together with some other characteristics of the farms as requested in other comments. We also added some comments in the text.

- *Please add information about the weaning age of piglets.*

Age at sampling and days from weaning were included in the text. Therefore, age at weaning could be deduced from that. However, we have added this information in Table 1 and added some comments in the text.

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- *I think that the limitation of the study is that the blood was taken only at the weaning and not for example in the first two weeks of life because with voluntary consumption I would expect differences in the first weeks of life of piglets. In my experience with voluntary consumption of iron, there is a usually high variability among piglets in the first weeks of life. So take this only as a recommendation for future studies.*

We agree that it would have been very interesting to make measures at two stages, one around 2-3 weeks of age and one around weaning (6 weeks in organic pig production) but it was not possible in such an on-farm experiment where access to the piglets is difficult, especially in outdoor farms. However, we have performed a subsequent experiment in an experimental organic farm and performed measures at several time points during lactation as you recommend. Present data show that around 6 weeks of age, variations due to iron supply by neonatal injection, indoors, or due to the housing system are still noticeable. Therefore, it was a "sensitive" stage.

Competing Interests: No competing interests were disclosed.

Reviewer Report 04 February 2022

https://doi.org/10.21956/openreseurope.15505.r28449

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?

Glen Almond
Department of Population Health & Pathobiology, College of Veterinary Medicine, North Carolina State University (NCSU), Raleigh, NC, USA

General Summary
The research evaluated the various blood parameters in piglets at approximately 42 days of age. Eleven outdoor farms and 10 indoor farms were included in the study. Iron supplementation varied among the indoor farms and only one outdoor farm injected piglets with iron. The indoor pigs had lower haemoglobin concentrations and red blood cell volume. Plasma haptoglobin concentrations were lower in the outdoor pigs than in the indoor pigs. The authors indicated that the piglets from primiparous sows had lower haemoglobin concentrations than pigs from multiparous sows. The authors concluded that iron supplementation is not required for piglets raised outdoors, provided that iron was bioavailable in the soils.

Specific Comments
Page 1:

- Background section – change the word "stock" to sow.

- Some basic demographics, such as the number of sows/farm, of the 21 farms would be

helpful.

- Blood samples were collected at 42 days of age and 11.9 kg live weight. Within the manuscript, daily weight gain is given and analyzed. At any time, were the pigs weighed prior to the end of the study? How was the DWG determined? In the results section, the DWG did not differ – this is confusing without starting body weights.

- What was the definition of anemia? Is 105 g/l indicative of anemia? This definition needs to be defined or a suitable reference included.

- What was the health status of the farms? And the preweaning mortality of the pigs on the 21 farms.

- It is concluded that the outdoor pigs do not require supplementation and that the outdoor pigs gain iron from the soil. At any time or for any farm, were soil samples collected to be analyzed for iron content? What are the bioavailabilities of the insoluble ferric oxides in soil? Would this be dependent on the soil pH? This comment also applies to the discussion on page 8.

Page 3:

- Provide the reference for the certifying bodies that consider iron administration as a drug treatment.

- Methods: the allocation of pigs by gender and size is appropriate. The age of pigs was 42 days, but the range was 34 to 57 days. At 57 days, would one expect that the iron injections in the first few days of life really maintain their influence? The influence of iron injections tends to decrease by 28 days of age. At any time, did the pigs receive solid feed, and if so, what was the iron content of the feed (including the sow feed).

- The SD is given; however in other parts of the manuscript, the SEM is given. Be consistent.

Page 4:

- Lab analyses: plasma or serum? Or both?

- The live weight and DWG (?) did not differ between housing systems but the age differed by 3 days. One could imply that the iron injection was required for the weight gains in the indoor pigs.

Page 5:

- Relationships between (or among parameters). What is meant by “side”? This is confusing.

Page 6:

- Figure 2: Are the titles on the X axis correct? Should they be indoor and outdoor rather than multiparous and primiparous?

- Figures 2 and 3: For these parameters, it appears that there are significant differences between the housing systems; however, are the values in the normal ranges for pigs at this age?

- … the first three components of the multivariate analysis. What are the components? Define

them. For Figure 5, which is interesting, it would be valuable to define the statistical components.

Page 7:

- Figure 4: Haematologic ..... parameters?

Page 8:

Table 1 Legend – check the P values. P<0.5 is not significant.

Page 9:

- 2nd paragraph. The authors suggest that the indoor pigs are affected with iron deficiency. Although there are differences between indoor and outdoor pigs, are the values for the indoor pigs within published normal ranges?

- Assuming that the outdoor pigs consume iron from the soil, how much is absorbed by the gut? In other words, is gut absorption sufficient? A key reference would be helpful.

- The influence of the 200 mg iron injections typically wanes after weaning at 21-28 days of age. The indoor pigs were much older than the pigs after a 3 or 4 week lactation. The transition to solid nursery diets is helpful for these indoor pigs. Did any of the indoor pig farms (or outdoor farms) provide creep feed or any type of solid feed prior to weaning at 40+ days?

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: 40 years of basic and applied research in commercial pig production. Current research is directed at evaluating anemia in commercial pig farms. Previous research studies involved UTI's in sows and non-infectious reproductive failure in pigs.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Mar 2022

armelle prunier

Thanks a lot for your valuable evaluation and comments. (Reviewer comments in italics)

Page 1

- *Background section – change the word "stock" to sow.*
  Thanks, we have made the change.

- *Some basic demographics, such as the number of sows/farm, of the 21 farms would be helpful.*
  We have added a table (Table 1) to describe this information together with some other characteristics of the farms as requested in all comments. We also added some comments in the text.

- *Blood samples were collected at 42 days of age and 11.9 kg live weight. Within the manuscript, daily weight gain is given and analyzed. At any time, were the pigs weighed prior to the end of the study? How was the DWG determined? In the results section, the DWG did not differ – this is confusing without starting body weights.*
  Sorry, we should have explained how DWG was calculated. Piglets were not weighted at birth. Therefore, we estimated the DWG by the dividing the weight at blood sampling by the age at blood sampling. Therefore, we have added an explanation in the MM part.

- *What was the definition of anemia? Is 105 g/l indicative of anemia? This definition needs to be defined or a suitable reference included.*
  We have modified the second paragraph of the discussion in order to define anaemia.
- *What was the health status of the farms? And the preweaning mortality of the pigs on the 21 farms.*
  This is an on-farm experiment and health status of the farm is a complex concept that was out of the scope of the present study. We did not record the mortality of the piglets during lactation (we know that it is very challenging in outdoor farms since it is very difficult to record the number of live piglets at birth). However, we recorded the average littersize at weaning as indicated by the farmers. This information is given in Table 1 and commented in the text.

- *It is concluded that the outdoor pigs do not require supplementation and that the outdoor pigs gain iron from the soil. At any time or for any farm, were soil samples collected to be analyzed for iron content? What are the bioavailabilities of the insoluble ferric oxides in soil? Would this be dependent on the soil pH? This comment also applies to the discussion on page 8.*
  That would have been very nice to take soil samples and do measures for bioavailability. We have thought to do it but we would have to solve problems of

representative samples of soil in the parks of the sows. Taking into account these problems and the time that was available to perform the samples and measures on animals, collect the information on the farms, we gave up.

Page 3

- *Provide the reference for the certifying bodies that consider iron administration as a drug treatment.*
  In fact, the EU regulation can be interpreted in different ways so that there are differences between countries (and hence certification bodies) and within a certification body between inspectors, at least in France. Therefore, we cannot give a list of certification bodies that consider iron administration as a drug treatment but we have modified the sentence.
- *Methods: the allocation of pigs by gender and size is appropriate. The age of pigs was 42 days, but the range was 34 to 57 days. At 57 days, would one expect that the iron injections in the first few days of life really maintain their influence? The influence of iron injections tends to decrease by 28 days of age.*
  We agree that it would have been very interesting to make measures at an earlier stage when the effects would have been more marked. However, as a first on-farm experiment on organic pigs, we make the choice of describing the situation at a critical moment in piglet life, that is around weaning. In addition, our data show that around 6 weeks of age, variations due to iron supply by neonatal injection, indoors, or due to the housing system are still noticeable.
- *At any time, did the pigs receive solid feed, and if so, what was the iron content of the feed (including the sow feed).*
  It is an on-farm experiment and we do not have precise information on that. We have added all the information we got in the farm visits in Table 1 and in the text.

- *The SD is given; however in other parts of the manuscript, the SEM is given. Be consistent.*
  Regarding the description of the Materials and Methods, we think that the SD is more obvious since the aim is to describe precisely the sample used for the experiment. Regarding the description of the results, we prefer to use the SEM since statistics are based on SEM. It is a common practice but, if really necessary, we can change to SEM all over the text.

Page 4

- *Lab analyses: plasma or serum? Or both?*
  Both were used as indicated in the text “Haptoglobin was analysed on plasma, iron, ferritin, hydroperoxides (= oxidation products = dROM) and the antioxidant capacity of the blood (BAP) on serum.”
- *The live weight and DWG (?) did not differ between housing systems but the age differed by 3 days. One could imply that the iron injection was required for the weight gains in the indoor pigs.*
  Yes, this is probably right. However, to prove it, we should have compared indoor pigs having or not received iron supplementation.

Page 5

- *Relationships between (or among parameters). What is meant by “side”? This is confusing.*

We have changed the text.

Page 6

- *Figure 2: Are the titles on the X axis correct? Should they be indoor and outdoor rather than multiparous and primiparous?*
  Very sorry for that, it is a mistake (Figure 2 and Figure 4 were the same). When I copied the figures from excel in the format sent to the journal, I made the mistake. Thanks for having pointed out this error. Now, I have sent the right figure comparing indoor and outdoor piglets.

- *Figures 2 and 3: For these parameters, it appears that there are significant differences between the housing systems; however, are the values in the normal ranges for pigs at this age?*
  The aim of the paper was not to determine what are the normal ranges but whether there are differences between housing systems and whether neonatal iron supply is needed. However, we agree that information on what are normal ranges can be very useful. Thanks for pointing that out. Therefore, we have modified the second paragraph of the discussion.
- *… the first three components of the multivariate analysis. What are the components? Define them. For Figure 5, which is interesting, it would be valuable to define the statistical components.*
  PCA is a descriptive analysis of the data. Here each piglet has 8 measures that were retained for the analysis. Therefore, the whole set of data (486 piglets) can be represented by an ellipsoid with 8 dimensions. In order to describe this ellipsoid, we make projections on 2 dimensional charts that are the best representations of the ellipsoid. In other words, these charts maximize the dispersion between individuals. They correspond to the first plot using the first and second first principal components, the second plot using the first and third components etc… These components can equivalently be defined as directions that maximize the variance of the projected data. These components are orthogonal. It is not possible to explain the principles of PCA in the text but we have added between brackets.

Page 7

- *Figure 4: Haematologic …… parameters?*
  Thanks for spotting that. We have added that in the legend.

Page 8

- *Table 1 Legend – check the P values. P<0.5 is not significant.TT*
  Thanks for spotting that. We have changed that in the legend.

Page 9

- *2nd paragraph. The authors suggest that the indoor pigs are affected with iron deficiency. Although there are differences between indoor and outdoor pigs, are the values for the indoor pigs within published normal ranges?*
  We have now changed the second paragraph of the discussion to indicate which values can be considered as normal.
- *Assuming that the outdoor pigs consume iron from the soil, how much is absorbed by the*

gut? In other words, is gut absorption sufficient? A key reference would be helpful.
We do not know how much soil and iron from soil can be absorbed by the gut of the piglets. We only know that high amount can be depicted in the gut and refer to the paper from Venn et al (1947) to illustrate that. However, our observations clearly show that Hb levels were relatively high in outdoor pigs without iron injection. Therefore, iron to synthetize Hb must come from somewhere and the best hypothesis is soil.

- *The influence of the 200 mg iron injections typically wanes after weaning at 21-28 days of age. The indoor pigs were much older than the pigs after a 3 or 4 week lactation. The transition to solid nursery diets is helpful for these indoor pigs. Did any of the indoor pig farms (or outdoor farms) provide creep feed or any type of solid feed prior to weaning at 40+ days?*
  We do not have precise information on the feed consumed by the piglets and from when. However, we have added all the information we got during the farm visits in Table 1 and some comments in the text.

Competing Interests: No competing interests were disclosed.