

Incidence of anemia in piglets and the use of supplemental iron on the quality of meat from muscles with different myoglobin content

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Abstract:

Two continuous experiments were established to evaluate the productive response, blood parameters, meat quality, and myoglobin (Mb) content in the *Longissimus dorsi* (LD) and *Psoas major* (PM) muscles of pigs after the use of iron (Fe): Exp-1, Fe supplementation in the diet of sows, and the application of iron dextran (FeD) in their offspring, and Exp-2, Fe supplementation during the growth-fattening of pigs. Exp-1, supplemental Fe in sows did not alter their blood parameters (≈ 10 % of anemic sows, Hb= 11.55 ± 1.281 g/dL) and productive parameters. Piglets were born deficient in Fe (Hb= 10.04 ± 1.418 g/dL), and there was an increase in the presence of anemic pigs on the third day to wean ≈ 40 % of the population with Hb<10 g/dL after applying FeD. In Exp-2, the lack of Fe supplementation did not alter the productive performance and the Hb level ($P > 0.30$). Nonetheless, continuous Fe supplementation to pigs affected ($P < 0.02$) the

lightness (L^*), lipid oxidation (TBARS), and protein oxidation (met-myoglobin) of PM meat and drip water loss in LD. Apparently, the Fe contribution of the ingredients was sufficient to correct postweaning anemia ($Hb = 13.63 \pm 1.059$ g/dL, <5 % Fe-deficient animals). The Mb content (LD = 1.23 ± 0.384 and PM = 1.40 ± 0.301 mg/g) was the same for any state of anemia or use of dietary Fe. Therefore, Fe supplementation must be adjusted to the contribution of the ingredients and to the prevention of oxidative processes in meat.

Keywords: Pigs, Iron, Anemia, Myoglobin, Meat quality.

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Introduction

Iron (Fe) is a mineral that is abundant and highly reactive in the free state and is present in two forms: hemic and non-hemic⁽¹⁾. Multiple factors, both intrinsic and extrinsic, affect its solubility and absorption in pigs^(2,3). In piglets, the total concentration of Fe at birth is ≈ 47 mg, distributed mainly between the blood, spleen, liver, and skeletal muscle⁽⁴⁾, requiring 7 mg/d only to satisfy erythropoiesis^(5,6). In addition, poor maternal-filial transfer of Fe (≈ 2 μ g/ml) during gestation and lactation and rapid growth of piglets can lead to Fe deficiency and the presence of microcytic-hypochromic anemia^(3,7-9). Despite the above, there is a lack of knowledge about the incidence of anemia in piglets expressed in hemoglobin (Hb) values and its consequences on meat production and quality⁽¹⁰⁾, which has led to the recommendation of a single application of iron dextran (200 mg/piglet IM) in the first days of life of the newborn^(9,11,12) and in the Fe supplementation in postweaning diets based on mineral premixes that mostly exceed nutritional requirements⁽¹³⁾. Although some studies report stunting in anemic pigs^(8,14,15), they do not investigate how dietary iron management influences myoglobin (Mb) content, and therefore, they do not investigate the consequences on color stability in muscles with different levels of Mb⁽³⁾, being one of the main consumer purchasing arguments⁽¹⁶⁾.

Therefore, the work aimed to evaluate the productive response and meat quality in two muscles (*Longissimus dorsi* and *Psoas major*) in pigs through inferences in the concentration of Mb and Hb in piglets after applying a dose of iron dextran (FeD) and supplemental Fe management (via diet) from weaning onwards, satisfying 100 % of the nutritional requirements during the growth-

fattening stage. The work consisted of two independent but continuous experiments: the first (Exp-1) describes the prevalence of anemia in pigs up to day 63 of life in response to the use of dietary Fe in sows and the application of FeD to the offspring on the third day of life. The second experiment (Exp-2) monitored blood parameters, productive behavior, Mb content, and meat quality in two muscles with different Mb content (*Longissimus dorsi* and *Psoas major*) resulting from the management of dietary Fe from 70 d of life until the finishing stage.

Materials and methods

Facilities

The experiments were conducted at the Pig Livestock Unit of the CENID Physiology of INIFAP, located in Ajuchitlán, Colón, Querétaro at 20°41'44" N, 100° 00'59" W, and 1,964 m asl. The facilities used were: 1) Gestation, open building with individual stainless-steel cages, concrete floor with slope at the back, cement canoe-type feeder, and a nipple drinker. 2) Maternity, a closed building with natural ventilation through windows, with steel cages, a rigid slotted plastic floor, an individual feeder, and two nipple drinkers at different heights. Each cage had a fiberglass piglet crate and a heat source, with two access routes for piglets at the front of the cage. 3) Weaning, a building with a controlled environment by means of a gas heater and natural ventilation, with raised pens with a slotted plastic floor, a stainless-steel feeder with seven slots, and a nipple drinker on the opposite side of the feeder. 4) Growth, open building with concrete-floor pens, a Dutch-type plastic feeder for wet feeding, and an extra nipple drinker. 5) Fattening, open building with 1.95 m² individual cages delimited by a stainless-steel structure with a concrete front floor (60 %) and woven steel mesh (40 %) in the back, with a feeder and a nipple drinker on the opposite side of the feeder. The experimental protocol was approved by the internal ethics committee of CENID with SIGI number 15142034838, considering the Official Mexican Regulations to ensure animal welfare^(17,18).

Experiment 1 (Exp-1)

To generate the individuals in the experiment, 30 sows (Landrace × Large White) inseminated with semen from a terminal line male (PIC 337) were used. The work was based on a design of randomized complete blocks with a 2×2 factorial arrangement, where the first factor was the Fe

supplementation in the diets of sows (gestation-lactation) according to the productive stage [first 80 days of gestation (Gestation 1), with 0 or 75 ppm; from day 81 of gestation to farrowing (Gestation 2), with 0 and 66 ppm, and during the entire lactation with 0 and 100 ppm], and the second factor was the application of FeD (0 and 200 mg/piglet) intramuscularly on the third day of life. The blocking criterion was the farrowing group (2 consecutive groups). Before insemination, considering reproductive age (number of farrowings) and body weight, sows were randomized to the treatments, which were: CC) control diets without Fe supplementation, and CF) diets equal to CC plus inclusion of Fe from iron sulfate heptahydrate ($\text{FeSO}_4 \bullet 7\text{H}_2\text{O}$). The diets were calculated with a formulation program at a minimum cost based on the nutritional requirements for each production stage⁽⁵⁾. During gestation, fibrous ingredients, such as dehydrated alfalfa and oat hay, soybean meal, rapeseed meal, and soybean oil, were included, the resulting diets were: Gestation 1 (from day 1 to 80 of gestation) with 2.94 Mcal/kg of ME, 12.02 % of CP, 0.35 kg/t of L-Lysine-HCl; and Gestation 2 (from day 81 to farrowing) with 3.03 Mcal/kg of ME, 13.02 % of CP, 0.64 kg/t of L-Lysine-HCl. In lactation, the diet had 3.32 Mcal/kg of ME, 17.40 % of CP, 2.25 kg/t of L-Lysine-HCl, and 0.45 kg/t of L-Threonine. Vitamin and mineral premixes were included to meet or exceed the requirement⁽⁵⁾, and conventional additives (enzymes and probiotics) for sows were also used. In the CF diets, iron supplementation was 75, 66, and 100 ppm of $\text{FeSO}_4 \bullet 5\text{H}_2\text{O}$ at the expense of sorghum for Gestation 1, Gestation 2, and Lactation, resulting in a final content of 84.27, 136.54, and 150.28 ppm of iron, respectively.

Proximate chemical analysis⁽¹⁹⁾ and determination of Fe content⁽¹⁹⁾ were performed in all diets using inductively plasma-coupled equipment ICP-AES (iCAP 6000, Thermo ScientificTM, USA). On the third day of life, piglets were randomized within their litter according to weight and sex to the second factor (the application or not of FeD, Lectron 20[®], Virbac, Mexico), resulting in four treatments: 1) CC-NoD, piglets without FeD application from CC-fed sows; 2) CC+FeD, piglets with the application of FeD from CC-fed sows; 3) CF-NoD, piglets without the application of FeD from CF-fed sows; and 4) CF+FeD, piglets with the application of FeD from CF-fed sows.

Sow management

Sows were weighed individually at insemination, at 30, 80, 109 days of gestation, and at the end of lactation (≈ 18 d). Feeding during gestation was restricted so as not to exceed an intake of 8.0 Mcal of ME/d, divided into two moments (0700 and 1600 h), and at freedom during lactation on three occasions (0800, 1400 and 2000 h). Two diets were used during gestation, one for the first 80 d and the second from d 81 until farrowing. During lactation, feed increased at a rate of 0.5

kg/d starting from 2.0 kg offered on the day of farrowing⁽²⁰⁾. The sows had free access to water at all times. Feed intake (DFI) was measured weekly by the difference between the food offered and the food rejected, as were the individual weights to estimate daily weight gain (DWG) and feed efficiency as the G×I ratio.

Piglet management

Immediately after farrowing, the piglets were weighed and individually identified by notching their ears. During the first 48 h postpartum, the litters were homogenized within the same treatments of the sows according to the weight and sex of the piglets, to randomize the piglets in their litter to the FeD factor. During lactation, piglets only had access to breast milk and special attention was paid to animal health and welfare, so possible symptoms associated with the presence of anemia were monitored. If animals with Hb < 9.0 mg/dL were identified, they were removed from the study and treated pharmacologically to deal with signs of anemia. At the end of the lactation period, the individual weight of the piglet was recorded to estimate the average daily gain (DWG). At weaning, piglets were randomized to the new experimental units (EUs) considering litter of origin, weight, and sex. The EUs were made up of 6 pigs: 3 castrated males and 3 females. The experimental period lasted 42 d postweaning, at which time the pigs reached ≈63 d of age.

Sample collection and analysis

Blood samples were collected from fasting sows and piglets (0700 h) in 6 mL vacuum tubes without additives and 0.5 mL tubes with EDTA-K2. In sows, blood sampling was performed at 30, 80, 109 d of gestation, and at 18 d of lactation; and in piglets, at 3, 11, 15, 18, and 63 d of age. Blood parameters were analyzed with an automated ADVIA[®] 60 equipment (Hematology Systems, Bayer HealthCare, Siemens, Germany): erythrocyte count (RBC), reticulocytes (Rt), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and mean corpuscular Hb concentration (MCHC).

Experiment 2 (Exp-2)

The complete progeny of two production groups (280 pigs, castrated males and females) of the same cross as in Exp-1 were used. All piglets received intramuscular application of FeD (200 mg/piglet, Lectron 20[®], Virbac) at d 3 of life, as well as dietary Fe supplementation at 100 % of the nutritional requirement⁽⁵⁾, based on FeSO₄•7H₂O, for 42 d postweaning. Based on a CRD, pigs were randomized to two treatments at 64 d of age, considering litter of origin, sex, and weight: NOF) diets without Fe supplementation in the diet; and FES) diets with Fe supplementation in the diet, satisfying 100 % of the nutritional requirement⁽⁵⁾. The feeding program from weaning consisted of eight phases with different durations each (Table 1).

Table 1: Experimental diets by feeding phase (kg/t) for pigs, Experiments 1 and 2

Phase	1	2	3	4	5	6	7	8
Length, days	7	14	21	21	21	21	21	21
Yellow corn, grain	257.42	250.26	250.37	241.38	64.86	--	--	--
Dehydrated whey	216.00	120.00	90.00	--	--	--	--	--
Sorghum, grain	200.00	310.00	310.00	354.00	600.00	663.72	725.54	791.84
Soybean, meal	100.00	130.00	180.00	272.00	221.00	233.00	168.11	81.55
Plasma protein	60.00	--	--	--	--	--	--	--
Fish, meal	50.00	50.00	50.00	--	--	--	--	--
Soybean, protein concentrate	35.31	54.00	30.00	6.10	--	--	--	--
Rapeseed, meal	30.00	30.00	40.00	60.00	65.00	70.00	80.00	100.00
Soybean, oil	26.00	28.80	25.00	33.00	20.00	11.00	6.00	6.00
Calcium carbonate	7.50	5.65	4.60	8.70	8.30	8.03	7.58	7.36
L-Lysine·HCl	4.20	6.20	5.65	5.55	4.75	2.66	2.52	3.36
Iodized salt	4.00	4.00	4.00	4.00	3.60	3.60	3.60	3.60
Mono-dicalcium phosphate	3.90	3.40	3.41	9.00	7.63	5.45	4.18	2.97
DL-Methionine	1.70	2.40	2.24	1.43	1.39	0.27	0.09	--
Choline-HCl	1.45	1.25	1.25	1.12	0.67	0.67	0.67	0.67
Trace minerals. pmx [‡]	1.00	1.00	0.90	0.90	0.80	0.80	0.70	0.70
Vitamins. pmx [¥]	0.50	0.50	0.50	0.50	0.40	0.40	0.40	0.40
L-Threonine	0.51	1.75	1.45	1.79	1.15	--	0.21	1.15

L-Tryptophan	0.11	0.39	0.23	0.13	0.05	--	--	--
Others [§]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
ME, Mcal/kg	3.45	3.42	3.40	3.35	3.30	3.27	3.24	3.20
CP, % [‡]	22.88	20.75	21.43	20.90	18.75	19.14	16.94	14.25
Fe from ingredients, ppm [‡]	81.47	98.36	111.66	108.27	89.72	86.37	86.89	81.47
Total Fe, ppm	191.47	208.36	201.66	188.27	169.72	166.37	156.89	191.47

[‡] The premix of Fe-free trace minerals contributed per kg of premix: Mg, 30,000 ppm; Zn, 120,000 ppm; Cu, 12,000 ppm; I (EDDI), 800 ppm; Co, 600 ppm. Iron was supplemented based on FeSO₄•5H₂O. [§] The vitamin premix provided per kilogram of finished feed (Phases 1 to 4): retinol acetate, 10,000 IU; cholecalciferol, 2,000 IU; α-tocopherol acetate, 120.0 mg; menadione, 8.04 mg; biotin, 0.30 mg; cyanocobalamin, 0.05 mg; folic acid, 1.50 mg; niacin, 60.00 mg; pantothenic acid, 35.00 mg; pyridoxine, 6.00 mg; riboflavin, 10.00 mg; thiamine, 3.50 mg. The vitamin premix provided per kg of finished feed (Phases 5 to 8): retinol acetate, 8,000 IU; cholecalciferol, 1,600 IU; α-tocopherol acetate, 96.0 mg; menadione, 6.43 mg; biotin, 0.24 mg; cyanocobalamin, 0.04 mg; folic acid, 1.20 mg; niacin, 48.00 mg; pantothenic acid, 28.00 mg; pyridoxine, 4.80 mg; riboflavin, 8.00 mg; thiamine, 2.80 mg. [§] Ronozyme-VP[®] (enzyme complex with action on non-starch polysaccharides), 0.30 kg/t; Ronozyme HiPhos[®] (Phytase), 0.10 kg/t. [‡] Determined value.

Pig management

The management and feeding of the pigs until d 63 of life was carried out following the Exp-1 procedures, with a period of six more days of adaptation to the individual housing in order to reduce the stress of the new housing. Water and food consumption were *ad libitum*, the feed was offered on two occasions (0700 and 1600 h). The assessment began at 70 d of age and productive behavior was measured: daily feed intake (DFI), daily weight gain (DWG), and feed efficiency (G×I). At the end of the experiment (≈168 d of age), the animals began a total fast of 24 h before slaughter, from the cessation of feeding to the start of slaughter, including a previous rest of 4 h in pens of a Federal Inspection Type slaughterhouse (TIF-117).

Sample collection and analysis

Longissimus dorsi (LD) and *Psoas major* (PM) muscles were collected from two pigs from random litters at weaning (≈21 d) and at day 105 of age, resulting in 14 pigs/age/treatment, to determine the Mb content⁽²¹⁾. Additionally, random blood samples were taken from 80 % of the population of a production group to monitor changes in Hb and blood parameters. On d 168 of life, all pigs in a production group were shipped to and processed at the slaughterhouse. During

the process of obtaining the meat, at 45 min and 24 h postmortem, the pH and temperature in the dorsal region were measured at the height of the last rib with a potentiometer and a puncture electrode (HI 99163, Hanna[®], Romania), which were previously calibrated. The weight of the hot carcass (45 min postmortem) and the cold carcass (24 h postmortem) was recorded, both records included head and legs. During carcass butchering, LD and PM muscles were obtained, which were measured for pH, subjective color and marbling⁽²²⁾, and the instrumental color CIE L*a*b* (MiniScan[®] EZ, D65/10°, HunterLab, USA) with spectral scanning to estimate the ratio of myoglobin Redox species^(23,24): deoxy-myoglobin (Deo-Mb), oxy-myoglobin (Oxy-Mb) and met-myoglobin (Met-Mb). In addition, drip water loss⁽²⁵⁾ was determined, as were the oxidative status by lipid oxidation techniques by the methodologies for 2-thiobarbituric acid reactive substances (TBARS)⁽²⁶⁾ and the antioxidant capacity by the ferric reducing ability of plasma (FRAP)⁽²⁷⁾.

Statistical analysis

The results are shown as least squares means and the standard error of the mean (SEM). All analyses were performed using the SAS[®] 9.4 statistical package (SAS Institute Inc., Cary, NC, USA). The first experiment (Exp-1) employed a completely randomized block design (CRBD) in a 2x2 factorial arrangement. The data were analyzed using the GLM procedure, with repeated measures over time evaluated using the MIXED procedure with a previous test of covariances (AR1, UN, and CS) considering the best BIC value. To compare means, the PDIFF option was applied with the adjustment of type III mean squares (SS3), considering a significance level of $P < 0.05$ to determine statistical differences. In addition, the UNIVARIATED and FREQ processes of the same software were applied to explore the normal distribution of response variables. The second experiment (Exp-2) used a completely randomized design (CRD) with two treatments. The data were analyzed through the GLM and MIXED procedures. As in the first experiment, PDIFF/SS3 was applied for the comparison of means, establishing a significance criterion of $P < 0.05$.

Results and discussion

Experiment 1

The supplemental dietary Fe factor in sows did not interact with the application of Fe dextran ($P > 0.09$) in the neonate, so the productive parameters and blood parameters of the progeny were

not affected, so the discussion of major effects is presented. As expected, the productive behavior (Table 2) and blood parameters (Table 3) of the sows were similar between treatments ($P>0.15$). Sow productivity was 14.34 ± 1.788 piglets born and 13.13 ± 1.786 piglets born alive, with a total litter weight of 18.86 ± 2.056 kg. At weaning, sows lost 2.21 % of their weight during lactation, with a DFI= 5.19 ± 1.032 kg/d, weaning 10.88 ± 1.659 piglets at 18 ± 3.54 d of lactation. Some studies mention that the pregnant sow has the ability to put the supply of nutrients to the fetus before any other factor that could demand a lack of nutrients, such as Fe^(28,29). During gestation and lactation, the sow is in a physiological state that is very demanding of nutrients, with the Fe requirement in pregnant sows being approximately 168 mg/d and 477 mg/d during lactation⁽⁵⁾; although the Fe content of the ingredients could contribute to the total contribution, its availability is uncertain. During pregnancy, with supplementation of 100 % of the requirement and restricted intakes of 2.2 - 2.5 kg of food, the CF treatment had a total Fe intake 76 % higher than the requirement (296 mg Fe/d); for its part, in CC (without iron supplementation), it was 148 mg/d on average, equivalent to 88.5 % of the requirement. Regarding the Hb value, throughout gestation, at least 10 % of the sows had Hb values <9 g/dL (frankly anemic), and 33 % had iron deficiency ($9 < \text{Hb} < 11$ g/dL). Apparently, at the beginning of the last third of gestation (d 80), the Hb value increased slightly to 12.19 ± 1.249 g/dL, reducing the population of anemic sows to 3 %. The other blood parameters are within the previously reported response⁽⁷⁾.

Table 2: Effect of iron supplementation during gestation and lactation on the productive parameters of sows [‡]

	CC	CF	SEM	P-value
Postpartum sow weight, kg [‡]	208.13	213.35	20.619	0.50
Litter weight, kg	19.23	18.48	4.254	0.64
Total number of piglets born, n	15.14	13.53	3.205	0.18
Total number of piglets born alive, n	14.00	12.26	3.237	0.16
Lactating sow DFI, kg	5.02	5.35	1.073	0.42
Sow weight at weaning, kg	203.54	208.63	18.350	0.24
Litter weight at weaning, kg	50.33	51.27	14.678	0.87
Weaned piglets, n	11.00	10.76	2.784	0.83

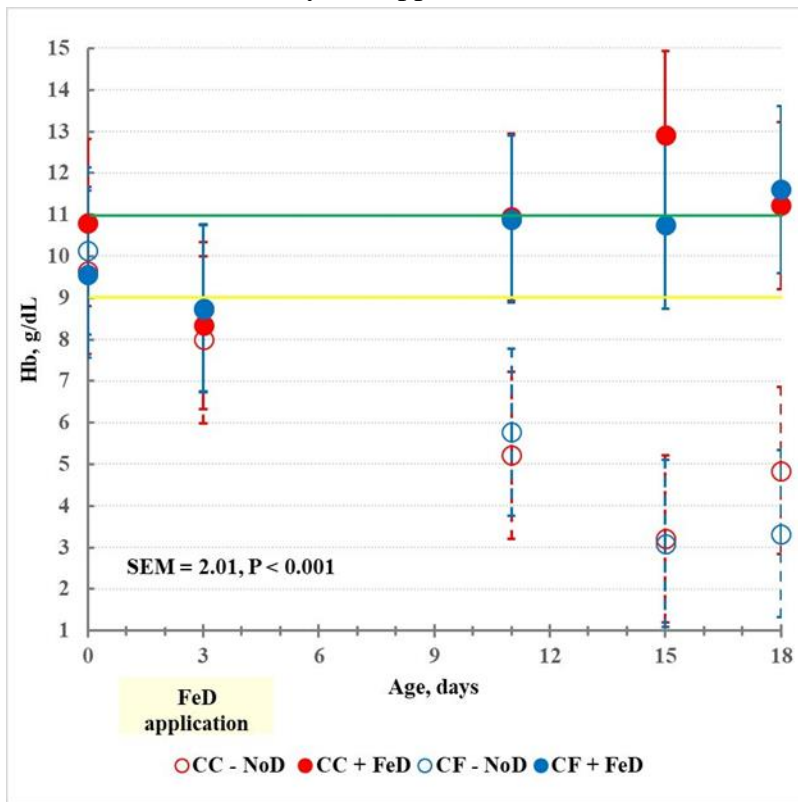
[‡] Least squares means and standard error of the mean (SEM).

CC= without Fe supplementation; CF= supplemented with Fe; n=15 sows/treatment. Age= 2.6 ± 0.63 farrowings ($P=0.63$). Lactation length= 18 ± 3.54 days. [‡] Estimated postpartum weight, kg= $-5.39 + (0.875 \times \text{pre-farrowing sow weight in kg}) - (1.281 \times \text{total litter weight at farrowing in kg}) + (0.962 \times \text{total number of piglets})$; $R^2= 0.97$ ⁽²⁰⁾.

Clinically, in pigs, Fe deficiency is defined as a microcytic-hypochromic anemia, and the clinical signs are observed as: loss of appetite, pallor in mucous membranes and, therefore, weight loss; in severe stages, dyspnea, tachycardia and tachypnea are identified⁽¹⁾. In piglets at birth, the Hb

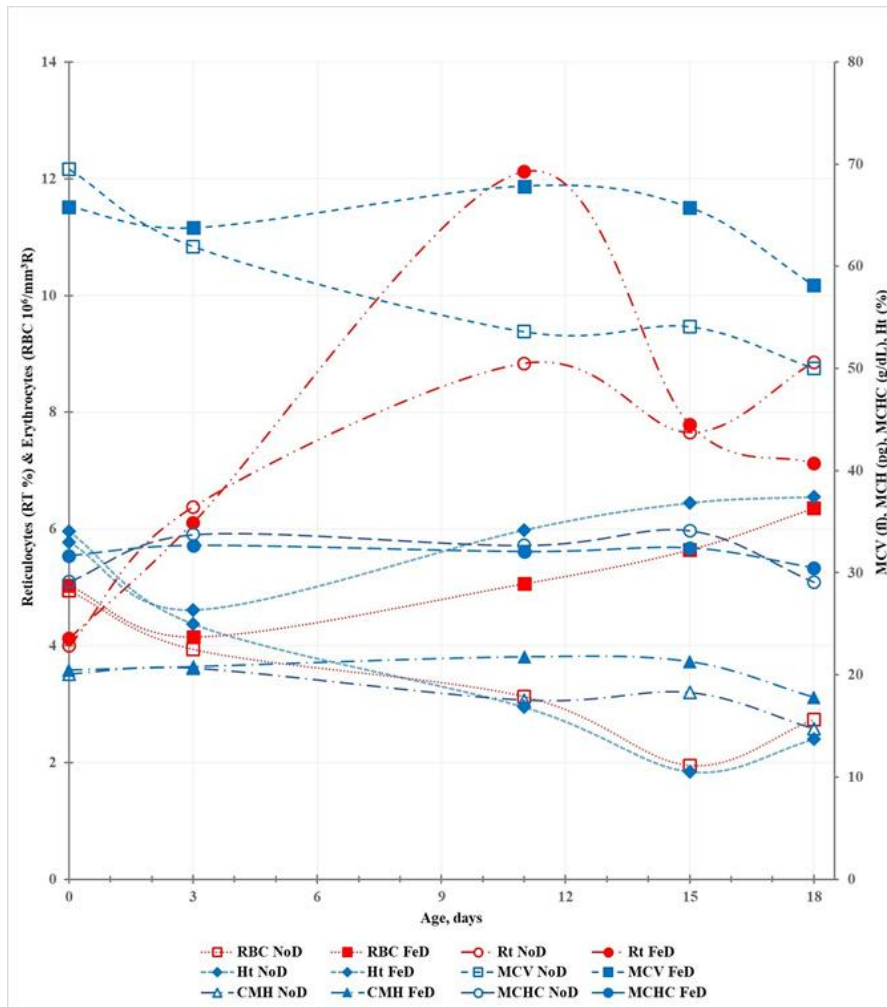
level was similar ($P>0.40$) regardless of iron supplementation in sows. Figure 1 shows that, on average, 27 % of piglets are apparently anemic ($Hb < 9$ g/dL), and up to 75 % could be deficient in Fe ($11 > Hb > 9$). On the third day of life, the average Hb decreased to 8.45 g/dL in treatments, which was considered transient anemia in about 75 % of the population ($Hb < 9$ g/dL). At 11 d of age, in piglets that receive FeD, Hb increased to 10.91 g/dL, whereas in pigs without FeD application (NoD), it decreased to 5.50 g/dL (severe anemia in >90 % of the population), which was associated with a low RBC (6.35 vs $2.74 \times 10^6/\text{mm}^3$) and a low Ht count (37.45 vs 13.70 %), a behavior similar to other reports, but which could underestimate the requirement of Fe in the blood^(6,12,14). At weaning (18 d), pigs with FeD application had ≈ 11.4 g Hb/dL, where about 60 % of the population was found at values >11 g/dL, data also reported by other researchers^(6,8,9,30). The rest of the blood parameters (Figure 2) maintained the differences associated with the use of FeD within the normal range^(4,7,11,31).

Figure 1: Changes in the hemoglobin of piglets (n=28) during lactation, an effect of iron dextran (FeD) and dietary Fe supplementation in the sow



Dots (solid and hollow) represent the least squares means and bars represent the standard error of the mean (EEM). The green line corresponds to the Hb value accepted as normal and the yellow line corresponds to the limit of a Fe deficiency, and the onset of a state of severe anemia. The treatments were CC–NoD) piglets without FeD application from sows without Fe supplementation; CC+FeD) piglets with FeD application on the third day of life from sows without Fe supplementation; CF–NoD), piglets without FeD application from sows supplemented with Fe; and CF+FeD), piglets with the application of FeD on the third day of life from sows supplemented with Fe.

Figure 2: Changes in the blood parameters of piglets (n= 55) during lactation ($P<0.04$), an effect of iron dextran (FeD) application at third day of life



Markers (solid and hollow) represent the least squares means. The treatments were: NoD, piglets without the application of FeD; and FeD, piglets with the IM application of 200 mg/piglet of FeD. The blood parameters were RBC, erythrocytes; Ht, hematocrit; MCV, mean corpuscular volume; HCM, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration, and Rt, reticulocytes.

Table 4 only shows the effect of FeD application on piglet production parameters up to day 14 after weaning (time when the EU were complete) and from weaning to 63 d of age with the remaining population. Pigs with Hb < 9 g/dL were removed from the experiment and underwent parenteral recovery therapy for anemia.

Stunting of NoD pigs ($P<0.01$) continued after weaning, even with Hb levels within normal (12.72 g/dL). The initial weight at weaning (5.02 vs 4.05 kg) and at 14 d postweaning (5.58 vs 3.09 kg) of piglets was higher in treatment with FeD application, which was reflected in an expected daily weight gain of 40 g/d, and a weight loss of 60 g/d in NoD. In addition, the DFI

tended ($P=0.09$) to decrease in NoD animals by $\approx 50\%$, leading to a negative feed efficiency of -1.51 ± 1.462 ($P=0.04$). These results are consistent with reports on productive detriment, even with different plans for the application of Fe^(11,12).

Table 4: Effect of the application of Fe dextran (FeD) on the postweaning productive behavior of pigs[‡]

	NoD	FeD	SEM	P-value
Experimental units, n [‡]	20	20		
Weaning weight, kg	4.05	5.02	0.106	0.01
Cumulative response to day14 postweaning				
DFI, kg	0.04	0.08	0.015	0.09
DWG, kg	-0.06	0.04	0.010	0.01
G×I, kg	-1.51	0.50	0.039	0.04
Weight at 14 days postweaning, kg	3.09	5.58	0.155	0.01
Pigs, n [§]	27	118		
DWG, kg	0.39	0.57	0.118	0.01
Weight at 42 days postweaning, kg	18.50	29.83	6.561	0.01

[‡] Least squares means and standard error of the mean (SEM). Weaning was at 18 ± 3.54 days. NoD= piglets without the application of FeD; FeD= piglets with the IM application of 200 mg/piglet of Fe dextran on the third day of life.

[‡] Pens with 6 pigs (3 castrated males and 3 females).

[§] Viable pigs at 63 days of age, with Hb values ≥ 9 g/dL. The value of n is different because the animals below the Hb value were treated parenterally to counteract the signs of anemia and removed from the experiment.

Experiment 2

The productive behavior (DWG, DFI, and G×I) of the pigs was not different due to the lack of Fe supplementation in the diets after 70 d of life; ending with an average weight of 122.23 ± 17.16 kg ($P>0.30$, Table 5). In this regard, other studies using Fe in the diet as the only source of variation^(8,9,14) or in combination with other minerals⁽²⁾ also found no differences in the productive behavior at a higher age of the pig. This could be associated with the Fe provided by the ingredients (87.7 ± 1.80 ppm in the diets of this work), even with a low bioavailability value that can be assigned, the levels in complex diets can be very close to the contribution of the nutritional requirement. Although the treatment x age interaction showed a trend ($P>0.07$) for Mb, the concentrations of Mb and Hb were equal between treatments at the end of the study ($P \geq 0.65$), only the age of the individual ($P<0.01$) influenced their concentrations. The rest of the blood parameters showed the same behavior, with no differences due to the use of Fe, in agreement with other studies^(2,8), which indicate that once Hb levels ≥ 11 mg/dL are reached, the

immune status can be improved, which leads to fully expressing productive performance. Regarding the quantification of Mb, in this work, measurements were made at critical moments of the productive life of the pig for slaughter, which are associated with high stress factors (birth and weaning) or large physiological changes (105 d of age), without observing a direct effect on the content of Mb in both muscles, which suggests that even with a lower contribution of Fe, without reaching a deficiency or total deficiency, the Mb is preserved and only the changes are attributed to age in the animal.

Table 5: Effect of Fe supplementation in pig diets on their productive potential, muscle myoglobin content, and blood parameters [‡]

	FES	CON	SEM	P-value
Productive behavior				
Final weight, kg	122.13	120.64	2.230	0.63
DFI, kg	2.56	2.49	0.049	0.33
DWG, kg	0.94	0.94	0.018	0.95
G×I, kg	0.37	0.38	0.005	0.30
Myoglobin in muscles [¥]				
<i>L. dorsi</i> , mg/g	1.22	1.24	0.054	0.72
<i>P. major</i> , mg/g	1.41	1.38	0.066	0.65
Blood parameters ^{¥, §}				
Hb, g/dL	13.20	13.27	0.133	0.72
RBC, 10 ⁶ /mm ³	7.42	7.49	0.066	0.47
Ht, %	42.92	43.57	0.479	0.33
MCH, pg	17.71	17.95	0.138	0.21
MCHC, g/dL	30.66	30.73	0.154	0.76
Rt, %	2.03	2.02	0.084	0.93

[‡] Least squares means and standard error of the mean (SEM). n= 58 pigs/treatment, except for Mb (n= 12 pigs/treatment). Initial weight at 70 d= 35.85±7.783 kg ($P=0.26$). The treatments were applied for 98 d:

FES= supplemented at 100 % of the requirement based on FeSO₄•7H₂O; CON= without Fe.

[¥] Age effect, $P<0.01$.

[§] Hb= hemoglobin; RBC= erythrocytes; Ht= hematocrit; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; Rt= reticulocytes.

The meat quality parameters for the LD and PM muscles are shown in Table 6. The temperature (T) and pH of the meat of each muscle was equal between treatments ($P>0.09$). For LD, the final T was 6.1°C (SEM= 0.38) with a pH of 5.51 (SEM= 0.015); on the other hand, in the PM, the T was 6.7°C (SEM= 0.32) with a pH of 5.72 (SEM= 0.023) at ≈22.5 h postmortem. In the LD, the assessment of color ($P>0.13$) and oxidative status ($P>0.30$) by TBARS and FRAP were the same.

These results differ from some studies that report a higher oxidation in the LD⁽¹⁶⁾, which could be due to the fact that, in their design, they evaluated a high concentration of Fe one month before slaughtering the animals. In addition, the redox species of myoglobin were not affected, and acceptable percentages were found, which are representative of fresh meat. This indicates that with supplementary levels of 100 % of the nutritional requirement or with ingredients that provide about 70 % of Fe (without considering their bioavailability), the antioxidant systems are not altered in LD meat. Nevertheless, the water retention capacity, which was assessed by drip weight loss, was higher ($P<0.02$), 2.5 % for FES compared to non-supplementation of Fe, 2.10 % (CON). These results, although at acceptable values, differ from what was reported⁽⁸⁾. In this case, the process of slaughter and fasting plays a very important role in the decrease of pH, a regulatory factor of the stability of post-mortem proteins on their water retention capacity.

Table 6: Drip weight loss, color, oxidative status, and myoglobin in the meat of two pig muscles[‡]

	<i>Longissimus dorsi</i>			<i>Psoas major</i>		
	FES	CON	SEM	FES	CON	SEM
Drip weight loss, %	2.50 ^a	2.00 ^b	0.108	0.61	0.60	0.051
Color, NPPC scale	2.97	3.10	0.449	-	-	-
Lightness, L*	55.17	54.61	0.284	48.96 ^a	47.66 ^b	0.292
Red tones, a*	17.56	17.63	0.141	22.81	22.64	0.161
Yellow tones, b*	9.03	9.10	0.133	7.01	6.64	0.171
FRAP, mg eq. Trolox/kg meat	53.81	57.83	2.604	58.86	54.03	3.440
TBARS, mg MDA/kg meat	0.14	0.13	0.010	0.13 ^a	0.10 ^b	0.006
Myoglobin, mg/g	0.96	0.95	0.063	1.35	1.39	0.063
Deo-Myoglobin, g/100 g	21.58	21.00	0.790	18.80	19.87	0.873
Met-Myoglobin, g/100 g	23.20	23.28	0.495	29.63 ^b	28.71 ^a	0.265
Oxy-Myoglobin, g/100 g	55.26	55.75	0.796	51.57	51.57	0.753

[‡] Least squares means and standard error of the mean (SEM). n= 42/treatment. The treatments were applied for 98 days: FES) pigs supplemented at 100 % of the requirement based on FeSO₄•7H₂O; and CON) pigs without supplemental Fe in the diet.

^{a,b} Different letters within the same muscle indicate differences ($P<0.02$).

Regarding PM meat, there were differences ($P<0.02$) in lightness (L*), where the value is higher with FES (48.96) vs CON (47.66), which could be associated with its higher lipid oxidation (0.13 vs. 0.10 mg MDA/kg). Apparently, oxidation also produced changes in Mb Redox species in the PM, increasing the met-myoglobin content ($P<0.01$) in FES= 29.63 vs CON= 28.71 g/100 g of

meat. The increase in met-myoglobin was previously observed in LD⁽¹⁶⁾, and associated with the pro-oxidative potential of iron, which can be reduced by supplementing vitamin E (≈ 200 mg of alpha-tocopherol acetate/kg food); it has also been recommended to include vitamin C; however, its excess can lead to greater absorption of Fe and enhance oxidation⁽³²⁾. Together, both the level of Hb and Mb can induce oxidation (auto-oxidation, mainly Hb), changing their oxidation state from 2+ to 3+. The oxidation of Met-Mb will release ferriprotoporphyrin IX, which can oxidize the phospholipids of the muscle membranes⁽¹⁰⁾, and consequently affect the water retention capacity. The use of supplemental Fe throughout the growth-fattening of the animal apparently induced an oxidative imbalance at the lipid level of the PM muscle membranes (FES= 0.13 vs CON= 0.10 mg MDA/kg meat, $P < 0.02$) which, having an oxidative metabolism, is prone to more oxidation reactions⁽³³⁾, with an exceeded FRAP antioxidant activity ($P = 0.06$), possibly associated with the conventional level of vitamin E used in the feed in this study (30 mg alpha-tocopherol acetate/kg).

Conclusions and implications

The blood parameters in the breeding sows are strongly regulated and given the lack of Fe supplementation in their diet, the contribution of the ingredients seemed to be sufficient not to affect the blood parameters and the myoglobin content in the offspring. In piglets, the application of Fe dextran decreased the proportion of piglets with Hb < 9 g/dL (anemic) at weaning by 80 %. In contrast, in the growth-finishing stage, pigs with Hb values between 9 and 11 g/dL, classified as iron deficient, had normal growth; nevertheless, the intake of Fe beyond the requirement (ingredient contribution + supplemental Fe) apparently exacerbated a greater oxidative imbalance in the *P. major* muscle than in *L. dorsi* muscle, making the changes more noticeable in the color of myoglobin than in the water retention capacity of the meat. Therefore, it could be feasible to adjust Fe in diets also considering the contribution of ingredients, as well as the inclusion of antioxidants to reduce lipid and hemoprotein oxidation processes.

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Table 3: Effect of Fe supplementation during gestation and lactation on blood parameters in sows (n= 15) [‡]

Days	RBC, 10 ⁶ /mm ³		Hb, g/dL		Ht, %		MCV, fl		MCH, pg		MCHC, g/dL		Rt, %	
	CC	CF	CC	CF	CC	CF	CC	CF	CC	CF	CC	CF	CC	CF
<i>Gestation</i>														
30	5.85	6.21	11.48	11.89	35.55	37.46	60.78	60.46	19.67	19.21	32.27	31.81	1.69	1.19
80	6.09	6.18	12.24	12.14	37.87	38.02	62.19	61.49	20.11	19.59	32.40	31.91	1.33	1.23
109	5.77	5.98	11.63	11.80	34.85	36.07	60.50	60.42	20.21	19.74	33.38	33.69	1.17	1.34
<i>Lactation</i>														
18	5.62	5.55	11.65	11.10	34.48	33.53	61.14	60.41	20.75	20.01	33.95	33.11	1.50	1.23
SEM	0.818		1.642		4.936		2.575		1.315		1.778		0.757	
P-value	0.81		0.44		0.64		0.54		0.87		0.76		0.81	

[‡] Least squares means and standard error of the mean (SEM).

CC= without the supplementation of Fe; CF= supplemented with Fe.

RBC= erythrocytes; Hb= hemoglobin; Ht= hematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular Hb; MCHC= mean corpuscular Hb concentration; Rt= reticulocytes.

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