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Characterization of a novel encapsulated oral iron supplement to prevent iron deficiency anemia in neonatal piglets[1]

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ABSTRACT: The aim of this study was to characterize nonheme iron (NHI) and heme iron (HI) microparticles to elaborate a novel oral iron supplement, determining its effect on the iron nutritional status of neonatal piglets. Nonheme iron (ferrous sulfate) was dissolved at 20, 30, and 40% wt/vol, and HI (porcine blood cells) was dissolved at 30% wt/vol in a maltodextrin solution (40% wt/vol). Solutions were spray-dried, obtaining NHI microparticles (NHIM) A, B, and C, respectively, and HI microparticles (HIM). Microparticles were characterized by iron content and release profile at gastrointestinal in vitro pigs' conditions. NHIM-B was selected and blended with HIM (oral iron supplement). Neonatal pigs (n = 66) were supplemented with 200 mg of injectable dextran iron on d 2 of life (parenteral group; n = 22), with 126 mg oral iron supplement on d 2 and 8 (oral I; n = 22), or with 84 mg oral iron supplement on d 2, 8, and 14 (oral II; n = 22). Red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), free erythrocyte protoporphyrin (FEP), and serum ferritin (SF) were determined. The Hb content of the oral I group was lower (8.3 ± 1.3 g/dL) than the parenteral (10.1 ± 1.7 g/dL) and oral II (10.2 ± 1.4 g/dL) groups (P < 0.05). Moreover, RBC, Ht, MCV, MCHC, and SF were altered in the oral I group, indicating iron deficiency anemia. Iron deficiency anemia in piglets may be prevented by the administration of an oral encapsulated NHI/HI supplement (3 doses) presenting the same benefits as classical parenteral supplementation.

Key words: anemia, encapsulation, heme iron, nonheme iron, piglets

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INTRODUCTION

Iron deficiency anemia is a serious problem in neonatal piglets that is usually solved by a parenteral supplementation of 200 mg of dextran iron at d 1 to 3 postpartum. Several authors have investigated oral iron supplementation in piglets; however, results have not been as expected compared to a parenteral single-dose supplementation (Zimmerman et al., 1959; Svoboda and Drábek, 2002), which is explained by a high variation of nonheme iron (**NHI**) bioavailability (Lipiński et al., 2013) and by a low expression of NHI receptors and transporters (Lipiński et al., 2010). There are few examples of the use of heme iron (**HI**) supplement for pigs that have shown a greater iron bioavailability than NHI (Quintero-Gutiérrez et al., 2008; Lipiński et al., 2013). The emerging technology of encapsulation in animal nutrition could improve the bioavailability of NHI and HI, preventing iron deficiency in humans and rodents (Zimmermann, 2004; Xu et al., 2014). However, there is no information about the effects of NHI or HI encapsulation in pigs. Therefore, the aim of this study was to characterize NHI and HI microparticles to elaborate a novel oral iron supplement and to determine its effect on the iron nutritional status of neonatal piglets.

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MATERIALS AND METHODS

Encapsulation and Characterization of Iron Microparticles

Maltodextrin (Prinal S.A., Santiago, Chile) solution (40% wt/vol) in deionized water was prepared. Nonheme iron (iron sulfate heptahydrate; Merck S.A.) was suspended in maltodextrin solution at 20, 30, and 40% (wt/vol) and spray-dried (Buchi Mini Spray Dryer B-290; Buchi, Flawil, Switzerland), obtaining type A, B, and C NHI microparticles (**NHIM**), respectively. Porcine blood cells (Licán Alimentos S.A., Santiago, Chile) were suspended in maltodextrin solution at 30% wt/vol and were spray-dried obtaining HI microparticles (**HIM**). The total iron content of NHIM and HIM was determined using an atomic absorption spectrophotometer (905AA; GBC, Braeside, Australia) ($\lambda = 248.3$ nm). Heme iron content was determined according to Valenzuela et al. (2014). To simulate the pig digestive tract, 2 conditions were used: gastric fluid (**GF**; 2 g/L of NaCl containing 10 g/L of pepsin with pH adjusted to 2.0 with HCl 1 N) and intestinal fluid (**IF**; 50 g/L of pancreatin and 31.2 g/L of bile extract in an intestinal solution of 8.76 g/L NaCl and phosphate-buffered saline at 0.1 M, pH 7.4; the pH of IF was adjusted to 6.0 with HCl 1 N). The NHIM and HIM (0.5 g) were mixed in 100 mL of GF and IF and were incubated for 60 and 200 min, respectively, at 37°C with constant agitation at 150 rpm. The released pattern of total iron was measured from aliquots of 3 mL by atomic absorption spectroscopy at the end of each digestion.

Oral Iron Supplement

The NHIM-B were blended with HIM at 10:2 and 8:2 ratios wt/wt for 2 and 3 doses, respectively and were suspended into distilled water (2 mL). The supplement was prepared according pigs requirements (10 mg per day) (NRC, 2012).

Animal and Experimental Design

Experimental procedures were approved by the Bioethical Committee on Animal Experimentation of the Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile (certificate 05-2015). The experiment was conducted in a commercial pig farm (Región Metropolitana, Chile). A total of 66 male and female 1-d-old piglets, weighing 1.61 ± 0.09 kg were selected and allocated with 6 sows (11 piglets per litter) of the same parity number. Piglets were randomly assigned inside each litter to 3 experimental groups: 1) piglets supplemented with 200 mg of injectable dextran iron (Veterquímica, San Bernardo, Chile) into the thigh muscles on d 2 of life (parenteral group, $n = 22$), 2) piglets supplemented with 2 oral doses of the iron supplement on d 2 and 8 (oral I group, $n = 22$), or 3) piglets supplemented with 3 oral doses of the iron supplement on d 2, 8, and 14 (oral II group; $n = 22$). The oral supplement was given by using a blunt-tipped applicator within their snouts (2 mL liquid paste). Blood samples (2 mL) were taken from the piglets' jugular vein at d 1 and 21 by venipuncture to determine iron status. Red blood cell (**RBC**), hemoglobin (**Hb**), hematocrit (**Ht**), mean corpuscular volume (**MCV**), mean corpuscular hemoglobin concentration (**MCHC**) (Model ZBI; Coulter, Hialeah, FL and Cell-Dyn 1700; Abbott Diagnostic, Abbott Park, IL, USA), free erythrocyte protoporphyrin (**FEP**) (Hematofluorimeter model 206D; AVIV, Lakewood, NJ, USA), and serum ferritin (**SF**) (Pig ferritin, FE ELISA Kit; Cusabio) were determined at d 1 and 21. Animals were weighted at the beginning (d 1) and end (d 21) of the experiment.

Table 1. Microparticle characteristics of nonheme iron (NHIM) and heme iron (HIM).

Characteristic	NHIM-A	NHIM-B	NHIM-C	HIM
Total iron (mg/g)	74 ± 6^{a}	86 ± 8^{b}	94 ± 12^{c}	0.85 ± 0.12^{d}
HI (mg/g)	–	–	–	0.77 ± 0.15
Iron release GF (%)	26 ± 8^{a}	31 ± 10^{a}	38 ± 5^{b}	22 ± 4^{c}
Iron release IF (%)	72 ± 8^{a}	66 ± 13^{a}	61 ± 10^{a}	76 ± 5^{a}

[a–d]Means ± SD with different superscript letters are statistically different ($P < 0.05$).

Statistical Analysis

Statistix 8 software was used for all of the statistical analyses. The assays were processed by ANOVA, and means comparisons were adjusted by Tukey ($P < 0.05$).

RESULTS AND DISCUSSION

Encapsulated Oral Iron Supplement

Characteristics of NHIM and HIM are presented in Table 1. Nonheme iron microparticles showed a high total iron content that increased in relation with the NHI concentration. The iron released in GF was higher for NHIM-C, discarding this type of microparticle, since the maximum release was expected at the duodenum level where the iron is absorbed (Lipiński et al., 2013). A similar iron release was observed in all NHIM types in IF, where the maltodextrin matrix was disintegrated due to pancreatic amylase. Finally, NHIM-B was selected from the in vitro study due to its high iron content and adequate release of iron at the gastrointestinal tract. The total iron content for HIM was lower than that for NHIM despite the fact that HI presents a higher bioavailability than NHI (Quintero-

Table 2. Iron status biomarkers of 1 (T1) and 21-d-old (T21) piglets subjected to different iron supplementation protocols.

Biomarkers[1]	Parenteral		Oral I		Oral II		Cutoff value
	T1	T21	T1	T21	T1	T21	
RBC ($10^6 \times mm^3$)	6.0 ± 0.8^{aA}	6.0 ± 0.5^{aA}	5.9 ± 0.9^{aA}	5.6 ± 0.6^{bB}	6.0 ± 1.0^{aA}	6.3 ± 0.8^{aB}	<5.3[2]
Hb (g/dL)	11.5 ± 1.7^{aA}	10.2 ± 1.4^{aA}	11.3 ± 1.9^{aA}	8.3 ± 1.3^{bB}	11.7 ± 1.4^{aA}	10.1 ± 1.7^{aA}	<9.0[2]
Ht (%)	37.1 ± 5.4^{aA}	34.1 ± 3.5^{aA}	36.3 ± 5.8^{aA}	30.2 ± 3.0^{bB}	37.9 ± 4.9^{aA}	35.1 ± 4.8^{aA}	<32[2]
MCV (fL)	62.1 ± 3.4^{aA}	57.0 ± 4.6^{aA}	61.4 ± 3.0^{aA}	53.7 ± 2.9^{bB}	63.7 ± 3.1^{aA}	56.2 ± 3.3^{aB}	<50[2]
MCHC (%)	31.1 ± 0.7^{aA}	29.7 ± 1.6^{aA}	31.2 ± 0.8^{aA}	27.3 ± 2.3^{bB}	31.0 ± 0.8^{aA}	28.7 ± 1.5^{aA}	n.d.[3]
FEP (μg/dL RBC)	88 ± 19^{aA}	133 ± 37^{aB}	105 ± 30^{aA}	153 ± 29^{aB}	94 ± 18^{aA}	140 ± 31^{aB}	n.d.
SF (μg/L)	13.5 ± 4.4^{aA}	9.9 ± 4.4^{aB}	25.5 ± 4.8^{bA}	4.1 ± 1.3^{bB}	17.8 ± 4.8^{aA}	8.7 ± 3.9^{aB}	<12.1[4]

[a,b]Means ± SD with different superscript lowercase letters are statistically different ($P < 0.05$) to compare different treatments.

[A,B]Means ± SD with different superscript uppercase letters are statistically different ($P < 0.05$) within the same treatment for different days of sampling.

[1]RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume: MCHC, mean corpuscular hemoglobin concentration; FEP, free erythrocyte protoporphyrin; SF, serum ferritin.

[2]Zimmerman (1980).

[3]n.d., Not determined for nursery piglets in the literature.

[4]Adams et al. (1988).

Gutiérrez et al., 2008; Lipiński et al., 2013). The 91% of total iron of HIM corresponded to HI, which was expected by the origin of this product. The iron release pattern of HIM was lower in GF compared with NHIM, which is possibly due to the high gelling capacity of the iron source, retarding its release from the matrix (Valenzuela et al., 2014).

The oral iron encapsulated supplement contained 252 mg of the total iron, distributed in 2 or 3 doses of 126 or 84 mg of iron each, respectively.

Effects of Oral Iron Encapsulated Supplement on Iron Status of Neonatal Piglets

No differences were found on the body weight of piglets at d 21 between groups: 6.27 ± 0.91 kg (parenteral), 6.28 ± 1.01 kg (oral I), and 6.27 ± 0.93 kg (oral II).

Iron status biomarkers are presented in Table 2. At d 1, groups did not differ on RBC, Hb, Ht, MCV, MCHC, and FEP (baseline), presenting a similar iron nutritional status. However, the SF was higher in the oral I group, indicating better iron deposits in these animals. At d 21 parenteral and oral II animals increased their levels of RBC, Hb, Ht, MCV, MCHC, and SF due to supplementation compared with oral I piglets that presented Hb, Ht, MCV, MCHC, and SF below the cut-off points.

As iron deficiency anemia is defined as low Hb and 2 or more of any of the biomarkers altered (see cut-off in Table 2) (Zimmerman, 1980), both protocols (parenteral and oral II) prevented the development of iron deficiency anemia in the piglets of this study. In the oral I group, all of the biomarkers changed from baseline to d 21, indicating iron deficiency, from the depletion of the deposits of this metal (decreased SF) until exhaustion of functional iron (decreased Hb). For parenteral and oral II groups, the biomarkers most affected between 1 and 21 d were FEP and SF. FEP increase in both groups, which could indicate a lower availability of iron for the synthesis of Hb. Although there is no established cutoff for FEP in suckling pigs, Espinoza et al. (2014) determined a normal value of 106 ± 23 μg/dL RBC; thus, all of the groups presented this parameter altered at d 21. The SF was also lower at d 21, indicating a state of depletion of iron deposits in these animals. However, the SF values of these groups are higher than the ones from the oral I group, although these animals presented the highest values of SF at baseline.

Other authors also observed anemia with oral iron supplementation in neonatal piglets. However, to achieve a similar hematinic effect to parenteral supplementation, they used several doses (8 doses for 4 wk) (Zimmerman et al., 1959), making this management impracticable in the current conditions of pig farms. Svoboda and Drábek (2002) prevented piglet anemia at 21 d by delivering only 2 doses of 200 mg of ferrous fumarate; nevertheless, this value exceeds at least twice suckling piglets requirements of this mineral (NRC, 2012).

Iron deficiency anemia of neonatal piglets could be prevented with an encapsulated oral iron supplement divided into 3 doses of 84 mg of iron each, probably by the greater bioavailability of the NHI and HI sources due to the encapsulation process (Xu et al., 2014; Zimmermann, 2004) and/or a synergic effect of NHI/HI combinations on iron absorption.

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