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Novel edible toys as iron carrier to prevent iron deficiency of postweaned pigs

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ABSTRACT

The current preventive treatment for iron deficiency in pigs is inefficient, resulting in a high prevalence of iron-deficient or anemic postweaned pigs. The aim of this study was to develop and characterize edible toys (**ETs**) to be used as oral iron supplements, and to assess their effect on feeding behavior and iron status of postweaned pigs. Three types of ETs, varying in sweetness, were produced by ionic gelation, using whey, sodium alginate, ferrous sulfate and atomized bovine erythrocytes. ET control (**ETC**) was developed without sweetener, ET1 contained 15% w/v sucrose and ET2 contained 0.03% w/v of Sucram (98% sodium saccharin, 1% neosperidine dihydrocalcone and 1% maltol). ETs were mainly composed of carbohydrates and protein, with a similar concentration of iron (2.2–2.7 mg/g). The ETs were offered to 24 postweaned pigs to measure acceptability and preference. The animals preferred ETC and ET2 over ET1. To assess the nutritional benefit of the ETs, 24 postweaned pigs were distributed into three groups: ETC (without iron), ETC-Fe (ETC with iron) and ET2-Fe (with iron and Sucram). Iron-loaded ET (ETC-Fe and ET2-Fe) significantly increased the concentration of red blood cells (from 6.1 to $7.5 \cdot 10^6$ x mm^3 for ETC-Fe and from 6.2 to 7.8 for ET2-Fe), hematocrit (from 32.8 to 37.9% for ETC-Fe and from 32.3 to 35.1 for ET2-Fe), serum iron (from 28.6 to 120.6 μmol/L for ETC-Fe and from 34.9 to 145.4 for ET2-Fe) and serum ferritin (from 7.8 to 18.5 μg/L for ETC-Fe and from 8.1 to 20.2 for ET2-Fe). In conclusion, the ETs developed in this study were accepted by the pigs and provided adequate iron to improve the iron status of postweaned pigs.

Implications

Introducing iron-enriched edible toys to weaned pigs will improve current methods of iron supplementation by reducing the cost and stress associated with handling the animals raised under intensive conditions, all while achieving optimal nutritional status of iron in the postweaning stage. The expected impact of the development of these innovative edible toys is the ability to easily provide needed nutrients to postweaned pigs without having to physically manipulate the animals.

Introduction

Iron deficiency is a major issue in suckling pigs raised in intensive production systems. To prevent iron deficiency, an intramuscular dose of dextran iron (100–200 mg) is commonly provided during the first days of life (Lipiński et al., 2010; Szudzik et al., 2018). However, this parenteral administration is not totally effective. Previous studies described that at weaning pigs can be anemic (6% prevalence), iron-deficient (28%) (Perri et al., 2016) or have their iron deposits depleted (Antileo et al., 2016; Churio et al., 2019). This deficiency becomes more acute 3 weeks after weaning, where the prevalence of anemia (6–32%) and iron deficiency (29–74%) increases which could affect the performance of the animals (Perri et al., 2016).

Iron deficiency is commonly observed in intensive pig production systems (Payne et al., 2005; Peters and Mahan, 2008; Bhattarai and Nielsen, 2015). Possible causes of iron deficiency include the higher potential growth of commercial lines and their larger number of piglets produced by hyperprolific sows (Kim et al., 2018; Szudzik et al., 2018). Moreover, dextran iron delivered by intramuscular injection can increase the mRNA expression of hepcidin, which blocks iron transportation into circulation by ferroportin decreasing its bioavailability (Lipiński et al., 2010; Starzyński et al., 2013) and increasing piglets' oxidative stress

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(Egeli and Framstad, 1998; Lipiński et al., 2010). Bhattarai and Nielsen (2015) reported that an increase in pigs' hemoglobin improved daily weight gain of weaned pigs, suggesting that it is necessary to implement strategies to improve the hematological values at weaning.

To reduce iron deficiency and assure iron homeostasis, a strategy that combines using lower and repeated doses of dextran iron during the nursing stage, and increasing iron bioavailability through oral iron supplementation may be effective (Kim et al., 2018). Oral supplementation of iron has been extensively researched but has yielded little success partially because the iron used has low bioavailability as most come from inorganic sources (Yu et al., 2000; Quintero-Gutiérrez et al., 2008). Previous studies proposed that by mixing inorganic and heme-iron, iron bioavailability in pigs increase (South et al., 2000; Quintero-Gutiérrez et al., 2008; Lipiński et al., 2013). Nonetheless, pigs and other mammals reject iron supplements orally, such as oral supplements delivered into the mouths of suckling pigs or iron used as sow's nipple paint, due to its aversive organoleptic characteristics (Valenzuela et al., 2016a). Several studies have described that encapsulation technology improves the adverse organoleptic characteristics of iron and enhances its bioavailability (Zimmermann, 2004; Xu et al., 2014; Churio et al., 2019).

Various three-dimensional forms can be used to create an edible toy (**ET**) in order to attract the attention of animals and stimulate consumption. Sodium alginate has been used as an encapsulating material to form an ET matrix because it carries iron (Durán et al., 2020) and can release it in the small-bowel, where iron is absorbed (Valenzuela et al., 2016b). Another material used to develop ETs is milk whey, which is accepted by pigs, and is already included in most transition diets (Zijlstra et al., 1996). In a previous study, our group demonstrated that these ingredients attract the attention of pigs (Durán et al., 2019). Pigs prefer sweet tasting compounds, so to improve consumption by the animals, different sweeteners can also be included in ETs (Bolhuis et al., 2009).

To our knowledge, there are no studies demonstrating the ability of ETs to act as vehicles of micronutrients in pigs. Thus, we hypothesized that the combination of encapsulation technology, a mix of ferrous sulfate and heme-iron, and sweeteners to decrease animals' taste aversion, would allow the development of ETs as iron vehicles that could improve the iron status of postweaned pigs. The goal of this study was to develop and characterize ETs as oral iron supplements, and to assess their effect on feeding behavior and iron status of postweaned pigs.

Material and methods

Material

Sodium alginate (viscosity of 25.7 centipoise at 25 °C, 2 g/100 mL solution; Sigma-Aldrich, USA) and dried sweet whey (Prinal S. A, Chile) were used as the ET matrix forming materials. Ferrous sulfate heptahydrate (Merk S.A, USA) and bovine spray-dried blood cells (LICAN Alimentos S.A, Chile) were used as inorganic iron and heme-iron sources, respectively. Sucrose was used in the formulation as a natural caloric sweetener and Sucram as a non-caloric artificial sweetener (98% sodium saccharin, 1% neosperidine dihydrocalcone and 1% maltol; Pancosma Jiangsu Feed Additives Co., Ltd., China). Reagents were all of analytical grade and purchased from Merck S.A.

Preparation of edible toys

ETs were prepared according to Durán et al. (2020), using ionic gelling encapsulation technology, based on a solution of sodium alginate at 2% w/v in distilled water and dried sweet whey at 40% w/v (blend base) plus 1% w/v of ferrous sulfate and 1% w/v of bovine spray-dried blood cells (proportion 1:1, 2.4 mg of heme-iron and 21.6 mg of non-heme-iron). Three ETs were developed: (1) control (**ETC**): without sweetener, (2) ET1: with 15% w/v of sucrose added to the formulation, and (3) ET2: with 0.03% w/v of Sucram. The sweetening power of both compounds differs dramatically, as sodium saccharin is 500 times sweeter than sucrose (Carloni et al., 2003). These blends were homogenized with a mechanical agitator and poured into silicone molds (20 mL capacity), which were refrigerated at 4 °C ± 0.5 °C for 48 h. Matrices were then demolded and immersed in aqueous gelling solution of 5% w/v $CaCl_2$ for 30 min. Next, the matrices were drained, deposited in aluminum trays, and dried in an oven at 50 °C for 48 h.

Characterization of edible toys

Digital photography (Sony DSC-HX1, Sony Corporation, Japan) was used to record the appearance of ET. Height and width dimensions were measured with a digital Vernier caliper. The weight was determined with an analytical balance. Color was measured using a colorimeter (Konica-Minolta CR-300, Japan). The proximate chemical analysis was performed to determine moisture (method 945.15), CP (Kjeldahl method 945.18), ether extract (method 945.16), crude fiber (method 962.09), and ash (method 920.153) according to Association of Official Analytical Chemists (AOAC) (1996), and nitrogen-free extract was calculated by difference. The total iron content was determined by acid digestion (method 999.11; AOAC, 1996) and atomic absorption spectroscopy (GBC, 905AA, Australia). All of these measurements were determined in a pool made up of five replicates in triplicate ET.

Animal studies

Studies were performed in the Center for Research, Technological Innovation and Training for the National Pig Industry (CICAP) at the Pontifical Catholic University of Chile, Pirque, Metropolitan Region, Chile.

Study 1: Acceptability and preference for edible toys in postweaned pigs

Animals and housing. A total of 24 postweaned piglets (21 days of age), castrated males and females ([Large White × Landrace] × Pietrain), of similar BW (range: 6.6–7.2 kg), coming from 6 different litters (cross-fostering) were randomly allocated into 12 pens (two pigs per pen) inside a room equipped with automatic forced ventilation and slatted floors. Each pen (2.3 m^2) had a feeder and an independent water supply by nipple. All pigs were supplemented with 200 mg of dextran iron on the third day of life as is commonly used in the pig industry. Pigs did not receive creep feeding before weaning. Animals were allowed 24 h to adapt to their new environment before beginning the experiment; this adaptation period was immediately after weaning. Pigs were fed with a diet formulated according to their nutritional requirements (Table 1) containing 200 ppm of ferrous sulfate as recommended by the National Research Council (NRC) (2012) during the weaning period. Water and feed were provided *ad libitum*. Two hours before experimental tests, feeders were removed from each pen. Immediately after the preference and acceptability tests the pigs had access to their diets.

Procedures. A summary of study 1 is presented in Fig. 1. The acceptability of ETs was analyzed on days 2, 3 and 4 after weaning. Animals had access to one option of ET per day (ETC, ET1 or ET2) during 10 min (Fig. 2A). Two ETs from the same treatment were placed in a pan feeder at the front of each pen (Fig. 2B). Feeders

Table 1
Pig diet composition (as-fed basis).

Item	g/kg
Ingredients	
Corn	500
Dried whey	164
Fish meal	110
Mixomeal55®[a]	77
Wheat bran	30
Soy meal 48%	43
Nupro®[b]	25
Canola oil	15
Dicalcium phosphate	8.0
Oyster shell	2.0
L-Lysine	3.37
DL-Methionine	2.40
L-Threonine	1.72
DL-Valine	1.10
L-Tryptophan	0.95
Salt	1.0
MIXzyme PPRO®[c]	1.75
Premix[d]	2.0
Zinc oxide	3.0
Choline chloride	1.2
Citric acid	7.5
Composition	
Metabolizable energy (kcal/kg)	3 450
CP (%)	21.4

[a] Micronized soybean (Nutrimel S.A., Chile).
[b] Yeast-based protein source (Alltech, USA).
[c] Feed enzyme with xylanase (250 unit per kg of diet) and phytase (400 unit per kg of diet) (EURO-NUTEC PREMIX S.A., Mexico).
[d] Vitamins and minerals (per kg of diet): A (9 900 UI), D (1 650 UI), E (77 UI), K (4.4 mg), choline (330 mg), niacin (44 mg), riboflavin (9.9 mg), B12 (44 mcg), folic acid (770 mcg), biotin (154 mcg), thiamin (3.3 mg), pyridoxine (4.4 mg), iron (200 mg as ferrous sulfate), manganese (50 mg as manganese oxide), copper (18 mg as copper sulfate), iodine (1 mg as potassium iodine), cobalt (1 mg as cobalt chloride) and selenium (0.3 mg as sodium selenite).

were weighed at the beginning and at the end of each test in order to calculate the pigs' intake. The order in which ETs were presented through the days (ETC, ET1 or ET2) were balanced across pens to avoid possible order bias.

A preference test between ETs was then performed on day 5 after weaning. Two pieces of each ET were placed in a pan feeder and simultaneously offered for 10 min to pigs (ETC *vs* ET1 *vs* ET2) (Fig. 2C). The position of ETs (left, center or right) was balanced across pens to avoid side bias. Similar to acceptability tests, feeders were weighed at the beginning and at the end of each test to calculate the pigs' intake. The relative preference for each ET was calculated using the following formula:

$$P(\%) = \frac{C1}{C1 + C2 + C3} \times 100$$

Where:

P (%): Preference, expressed as percentage.
C1: consumption of one type of ET.
C1 + C1 + C2: consumption of all ETs.

Study 2: Effect of edible toys on iron status of postweaned pigs

Animals and housing. A total of 24 postweaned piglets were used. The pigs (21 days of age), castrated males and females ([Large White × Landrace] × Pietrain), of similar live weight (range: 6.8–7.3 kg) were randomly allocated to 12 pens (two pigs per pen). General housing and feeding conditions were the same as for Study 1. These animals also received iron injections at day 3 of life.

Procedures. The objective of this study was to determine the change in iron status of postweaned pigs before and after iron supplementation with ET. The higher preferred ETs, determined in Study 1 were selected (ETC and ET2). As a control treatment, an ET without iron was prepared. The iron content of the ETC without iron used in this study was determined by acid digestion (method 999.11; AOAC, 1996) and atomic absorption spectroscopy (GBC, 905AA, Australia).

Pens were randomly assigned to one of three experimental treatments 1) provision of ETC without iron, 2) provision of ETC-Fe (with iron), 3) provision of ET2-Fe (with iron and Sucram). For this study, two ETs placed in a pan feeder were delivered every 2 days for 14 days (Fig. 1) at the front of each pen. Feeders were weighed at the beginning and at the end of each test to calculate ET intake. The pigs were weighed at the beginning and end of the study.

Iron status. To assess the iron status, blood samples of 5 mL were drawn from the piglets by jugular veniepuncture at days 2 and 16 (Fig. 1). Total blood (1 mL) was used to determine biomarkers of iron status red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht) and mean corpuscular volume (MCV) through a Cell Dyn 3200 counter (Abbott Laboratories, Abbott Park, IL). Serum was separated by centrifugation at 1 400*g* for 3 min, and then immediately stored at

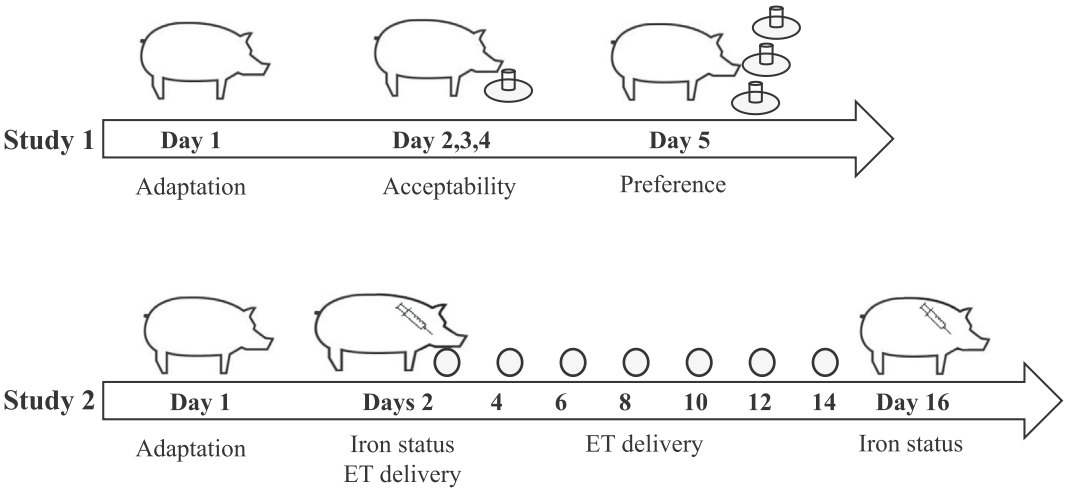

Fig. 1. Experimental design of studies. Study 1 was designed to determine the acceptability and preference of edible toys (ET) for pigs. Study 2 was to determine the effect of ET consumption on iron status of pigs.

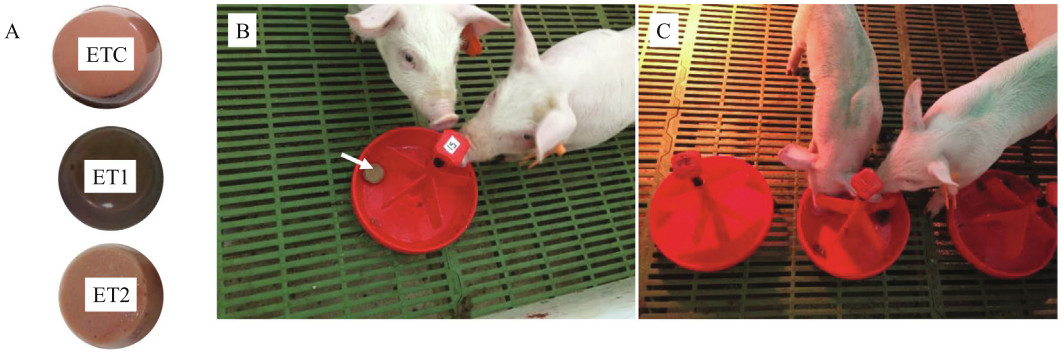

Fig. 2. Aspects of edible toys (ETs) with iron: control (ETC), plus sucrose (ET1) and plus Sucram (ET2) (A). Images of the acceptability (B) and preference (C) tests in pigs. The white arrow indicates the ET in feeders.

−20 °C. Total iron binding capacity (TIBC) was determined in serum by a colorimetric method (Fischer and Price, 1964). Serum iron (SFe) was determined by atomic absorption spectrophotometry (Simaa 6100, PerkinElmer, Waltham, Massachusetts, USA), and serum ferritin (SF) with a commercial kit (Pig ferritin, ELISA Kit; Cusabio, Hubei, China).

Statistical analysis

For Study 1, ANOVA and Tukey tests ($P < 0.05$) were used to analyze ET characteristics (nutritional properties based on proximal chemical analysis, iron content and color). The results were presented as mean ± SD. Data of acceptability and preference tests were analyzed with Kruskal–Wallis test and comparison of multiple ranges ($P < 0.05$). The results were presented as mean ± SE of the mean. These analyses were performed using Statdistix (version 8, Analytical Software 2003, Tallahassee, FL, USA).

For Study 2, the supplementation effect on iron biomarkers were analyzed with a Two-Way ANOVA for repeated measures using the GLM procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Mean values are presented as least-square means adjusted by Tukey. The α level used for the determination of significance was 0.05. The following statistical model was used:

$$Yijk = \mu + \alpha i + \beta j + \alpha i * \beta j + \varepsilon ij$$

where Y is the iron status biomarker, μ is the general mean of all observations, α is the effect of treatment (ETC, ETC-Fe or ET2-Fe), β is the effect of the period (2 and 16 days), $\alpha i * \beta j$ is the interaction between variables (treatment and period) and ε is the random error.

Results

Characterization of edible toys

The appearance of ETs is shown in Fig. 2A and the size and weight of the three ETs are shown in Table 2. ETs have similar physical characteristics; oval in shape and of similar size and weight. ETC had a light brown color, similar to ET2, unlike ET1 which presented a dark brown color (Fig. 2A and Table 2).

The proximal chemical analysis and iron content of ETs are presented in Table 2. The moisture content was low for all ETs, with ET1 having a higher percentage of moisture. ETs were mainly composed of nitrogen-free extract (carbohydrate-equivalent) and protein. The protein content was lower in ET1, compared to the other ET, and the nitrogen-free extract content was higher in ET1 than the other ET. Crude fiber and ether extract content were similar and low for all the ETs. The ash content of ET1 was lower compared to ETC and ET2 and the iron content was similar in all ETs, with the exception of ETC that was formulated without iron in study 2 (0.08 ± 0.02 mg/g).

Table 2
Nutritional characteristics, dimensions and weight of edible toys with iron and without sweetener (ETC), with 15% w/v of sucrose (ET1) and with 0.03% w/v of Sucram (ET2).

Parameters	ETC	ET1	ET2
Nutritional characteristics			
Moisture (%)	4.8 ± 0.0[a]	5.5 ± 0.1[b]	4.6 ± 0.0[a]
CP (%)	11.8 ± 0.1[a]	9.0 ± 0.3[b]	11.9 ± 0.2[a]
Crude fiber (%)	0.9 ± 0.01[a]	1.0 ± 0.1[a]	1.0 ± 0.1[a]
Ether extract (%)	0.03 ± 0.01[a]	0.05 ± 0.02[a]	0.04 ± 0.01[a]
nitrogen-free extract (%)	73.1 ± 0.1[a]	78.0 ± 0.1[b]	71.3 ± 0.3[a]
Ash (%)	9.2 ± 0.0[a]	4.9 ± 0.1[b]	9.9 ± 0.1[a]
Iron (mg/g)	2.5 ± 0.8[a]	2.2 ± 0.6[a]	2.7 ± 0.5[a]
Color			
L^*	31.8 ± 5.2[a]	15.3 ± 2.3[b]	33.1 ± 4.5[a]
a^*	12.6 ± 2.1[a]	4.9 ± 1.2[b]	12.0 ± 2.9[a]
b^*	31.1 ± 2.7[a]	49.5 ± 4.4[b]	34.4 ± 3.6[a]
Dimensions			
Height (cm)	1.1 ± 0.1[a]	1.1 ± 0.2[b]	1.1 ± 0.2[a]
Width (cm)	3.8 ± 0.2[a]	3.9 ± 0.3[b]	3.8 ± 0.2[a]
Weight (g)	9.6 ± 0.3[a]	9.8 ± 0.2[b]	9.7 ± 0.4[a]

ET = edible toy.
Different letters indicate significant differences in the same row (Tukey's test, $P < 0.05$).
Color parameters: L^* (lightness value, 0 to black and 100 to white), a^* (green–red component, green with negative values and red with positive values), b^* (blue–yellow component, blue with negative values and yellow with positive values).

In vivo studies

Study 1: Acceptability and preference for edible toys

Results of the acceptability and preference analysis are shown in Fig. 3. No differences were observed in the acceptability of pigs for ETs ($P > 0.05$, Fig. 3A). ET1 was preferred less than ET2 ($P = 0.004$) during the choice test, while no difference in preference was observed between ETC and ET1 ($P = 0.097$; Fig. 3B).

Study 2: Iron nutritional status

One unit of an ET (with iron) contained on average 24 mg of iron, where 2.4 mg correspond to the heme-iron provided by bovine spray-dried blood cells and the difference is non-heme-iron from ferrous sulfate. The pigs consumed 100% of the ET on all days of the supplementation trial.

The effect of ET supplementation on iron status in pigs is presented in Table 3. Significant differences were observed in all biomarkers after supplementation. At the end of supplementation, the ETC (without iron) showed lower values of red blood cells, hematocrit, serum iron and serum ferritin than did the ETC-Fe

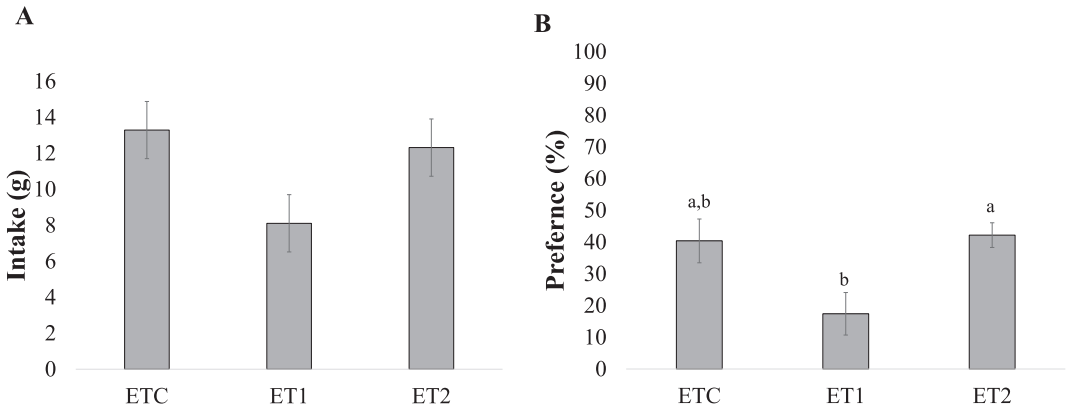

Fig. 3. Acceptability determined as grams of edible toys (ETs) consumed per pair of pigs (A) and preference calculated as proportion of each ET on the total intake (B). ET control (ETC), ET plus sucrose (ET1) and ET plus Sucram (ET2). Different letters indicate significant differences ($P < 0.05$).

and ET2-Fe groups. No effect of ETs on the live weight of pigs was observed (Table 3).

Discussion

The excessive handling of animals that occurs with the current oral iron supplementation methods for pigs, produces stress in animals (Valenzuela et al., 2016a). Also, multiple doses are required to achieve optimal iron nutritional status (Antileo et al., 2016), which is not feasible in intensive production farms. Therefore, in this study we developed ETs that are attractive to pigs and can be consumed voluntarily, avoiding excessive handling.

The ETs in this study were similar in shape and color, because equal molds were used to prepare them. The brown coloration common to all ETs is explained by the incorporation of atomized bovine erythrocytes into the base mixture as a source of heme-iron (Churio et al., 2018). The darker brown color of ET1 can be attributed to a non-enzymatic browning reaction that occurred when ETs were subjected to drying at elevated temperatures (Martins et al., 2000).

The moisture content in ETs were low (<12%), being an advantage for storage. The higher moisture content of ET1 could be a consequence of the higher hygroscopic capacity of sucrose (Sardar and Singhal, 2013). Whey provided the protein and carbohydrate content of ETs (12% of CP and 75% of nitrogen-free extract) (Batal et al., 2016). Nitrogen-free extract content was higher in ET1 due to the addition of 15% sucrose. The ash content of ET1 was lower than ETC and ET2 due to a dilution effect by sucrose.

When determining ET preference, we did not observe higher consumption of ET formulated with sweeteners. In fact, ET1 was the least preferred probably due to the generation of bitter-tasting compounds by non-enzymatic browning (Martins et al., 2000). Several studies have described that pigs reject bitter compounds, in an innate protective response to potentially toxic compounds (Tinti et al., 2000).

ETs were consumed on a voluntary basis, which is interesting because it is common for postweaned pigs to exhibit neophobia and decreased feed consumption due to the acute stress of this stage (Oostindjer et al., 2011). Studies have shown that environmental enrichment elements can reduce neophobia and play behaviors stimulate food consumption, which may explain the high acceptability of ETs by the pigs (Beattie et al., 2000; Oostindjer et al., 2011; Durán et al., 2019). The large size of the ET may have also helped, as pigs prefer whole and large-sized feeds over ground feeds (Maxwell and Carter, 2001). Pigs in a transition stage have a high preference for whey due to its odor and taste (Zijlstra et al., 1996) and because its flavor generates a link with consumption of maternal milk. Finally, it has been observed that

Table 3
Effect of supplementation with edible toys, control (without iron and sweetener), ETC-Fe (with iron and without sweetener) and ET2-Fe (with iron plus Sucram) on iron status and live weight of pigs.

Biomarkers	ETC		ETC-Fe		ET2-Fe		SEM	*P-value*		
	Day 2	Day 16	Day 2	Day 16	Day 2	Day 16		Treatment	Period	Interaction
Iron status										
RBC ($10^6 \times mm^3$)	6.1^{A}	6.3^{aB}	6.1^{A}	7.5^{bB}	6.2^{A}	7.8^{bB}	0.239	0.004	<0.001	0.008
Hemoglobin (g/dL)	8.5^{A}	9.8^{aB}	8.8^{A}	10.2^{aB}	8.4^{A}	11.0^{aB}	0.263	0.156	<0.001	0.031
MCV (fL)	51.8^{A}	53.3^{aB}	50.5^{A}	53.9^{aB}	52.3^{A}	53.2^{aB}	0.907	0.825	0.015	0.338
Hematocrit (%)	32.4^{A}	34.2^{aB}	32.8^{A}	37.9^{bB}	32.3^{A}	35.1^{bB}	0.781	<0.001	<0.001	<0.001
Serum iron (μmol/L)	26.4^{A}	65.8^{aB}	28.6^{A}	120.6^{bB}	34.9^{A}	145.4^{bB}	8.867	<0.001	<0.001	<0.001
TIBC (μg/dL)	595.4^{A}	389.0^{aB}	564.5^{A}	316.4^{aB}	557.3^{A}	304.6^{aB}	32.21	0.135	<0.001	0.733
Serum ferritin (μg/L)	7.7^{A}	11.2^{aB}	7.8^{A}	18.5^{bB}	8.1^{A}	20.2^{bB}	1.330	<0.001	0.002	0.005
Weight performance										
Live weight (kg)	7.0^{A}	9.5^{aB}	7.1^{A}	9.2^{aB}	7.1^{A}	9.8^{aB}	0.012	0.856	0.029	0.765

ET = edible toy; RBC = red blood cell; MCV = mean corpuscular volume; TIBC = total iron-bonding capacity. Treatment groups: ETC = Control group (without iron and sweetener); ETC-Fe = pigs exposed to ETC with iron and without sweetener and ET2-Fe = pigs exposed to ET2 with iron plus Sucram.
Period: refers to the effect of the time period between day 2 (start of supplementation) and day 16 (end of supplementation).
Interaction: refers to the interaction of treatment by period factors.
Mean values are presented as least-square means showing the SEM of the treatment × period interaction.
Different lowercase letters indicate significant differences between treatments at the end of the study (Tukey's test, $P < 0.05$).
Different capital letters indicate significant differences for the time period (day 1 *vs.* day 16) within each treatment (Tukey's test, $P < 0.05$).

pigs show a preference for soft, easy-to-chew feeds, which are also attributes of the ET (Durán et al., 2019).

Iron deficiency anemia has become a problem in the weaned stage of commercial pigs, and common prophylactic practices in the nursing stage are not efficient to improve their iron status (Bhattarai and Nielsen, 2015; Antileo et al., 2016; Perri et al., 2016; Kim et al., 2018). Moreover, intramuscular injections of 200 mg dextran iron are associated with high oxidizing capacity producing toxicity and disturbing the iron homeostasis in newborn pigs (Egeli and Framstad, 1998; Lipiński et al., 2010; Starzyński et al., 2013). Therefore, oral iron supplementation strategies at weaning are necessary.

The ETs delivered to pigs as iron supplements were effective in improving their iron status. The iron biomarker levels of all pigs after supplementation were expected, because all animals received sources of iron. Thus, ETC pigs ingested iron in their commercial feed, and ETC-Fe and ET2-Fe groups received iron in feed and through ET. However, at day 16 the pigs in the ETC-Fe and ET2-Fe groups showed higher values in several biomarkers such as: RBC, hematocrit, serum iron and serum ferritin, compared to the ETC group. Weaned pigs present a low intake of solid feed or even anorexia during the first 48 hours postweaning (Bolhuis et al., 2009). For ETC the only iron source was their commercial diet, therefore if they do not consume or consume a small amount of feed they will not ingest enough iron to maintain iron levels. Moreover, pigs provided with iron supplemented ETs consumed in addition heme-iron, which is more bioavailable than ferrous sulfate for pigs (Quintero-Gutiérrez et al., 2008; Lipiński et al., 2013). It has been described in iron-deficient suckling pigs that hemoglobin, a source of heme-iron, was efficiently absorbed and increased the expression of genes responsible for heme-iron transport in the duodenum (Staroń et al., 2017). It has also been described that some intestinal receptors and non-heminic iron transporters are not adequately expressed in pigs from birth to 3 weeks of age (Lipiński et al., 2010). Therefore, the absorption of non-heminic iron at this stage may be insufficient and explains why pigs that consumed ETC-Fe or ET2-Fe showed higher values of the mentioned biomarkers.

Another explanation is based on the effect of iron encapsulation in ET. A previous study showed that suckling pigs orally supplemented with encapsulated iron had a higher concentration of serum ferritin than pigs not given encapsulated iron (Churio et al., 2019),because encapsulation technology protects iron as it passes through the gastrointestinal tract, reducing the precipitation of non-heme-iron in the stomach. This generates a better absorption of iron and an increase in its bioavailability, which has been described in several studies in humans and other mammals (Zimmermann, 2004; Xu et al., 2014). Also, the encapsulating material used, sodium alginate, has the property of releasing the iron into the small intestine, where the iron is absorbed (Valenzuela et al., 2016b; Churio et al., 2018).

It is known that in humans more than 80% of the absorbed iron is used for the formation of hemoglobin. This absorption increases in anemic or iron-deficient pigs (Lipiński et al., 2013; Valenzuela et al., 2013). A pig's iron reserves represented by serum ferritin only begin to increase as the iron requirements for erythropoiesis are satisfied. Serum iron is a more immediate indicator of iron consumption, which rapidly increases after iron intake. This explains why after supplementation an improvement in hemoglobin was observed for all groups. However, large differences in serum ferritin and serum iron were found between ETC *versus* ETC-Fe and ET2-Fe. The iron consumed by ETC pigs was insufficient to allocate iron surpluses to reserves; resulting in an increase of 3.5 μg/L. In contrast, ETC-Fe and ET2-Fe groups increased by 10.7 and 12.1 μg/L, before and after supplementation, respectively.

Finally, regarding ET safety, one ET unit contains 24 mg of iron, which represents about 12% of the daily iron requirement of weaned pigs according NRC (200 mg of iron/kg of diet) (NRC, 2012). Based on this calculation, we assumed that the possibility of toxicity due to the consumption of ETs every 2 days is very low. Other studies have described that high doses of oral iron are required to cause toxicity. Doses greater than 1 000 mg/kg of iron in the pigs' diet are considered high and increases the destruction of tocopherols (Dove and Ewan, 1990). With a dose of 4 000 mg/kg, growth rate and inorganic phosphorus in serum were reduced and with 5 000 mg/kg, ash content of the femur also was diminished (O'Donovan et al., 1963; Furugouri, 1972).

Conclusion

In conclusion, whey/alginate-based ETs were developed with heme and non-heme-iron sources with encapsulation technology, to generate novel iron vehicles that were selected and consumed by weaned pigs without excessive handling. The iron-loaded ET improved the iron status of pigs; proving ET to be an effective and safe method for improving the health of postweaned pigs.

Ethics approval

The experiment was conducted in accordance with the Chile Guidelines for Animal Welfare and the experimental protocol was approved by the Institutional Committee on Animal Care and Use from the University of Chile (17064-VET-UCH).

Data and model availability statement

None of the data were deposited in an official repository.

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Declaration of interest

The authors declare no competing financial interest.

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