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Behavior of suckling pigs supplemented with an encapsulated iron oral formula

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ABSTRACT

Parental iron supplementation in neonatal piglets is a routine management to solve anemia but may cause welfare problems. The aim of this study was to assess behavior responses generated after oral or parenteral iron supplementation. Parenteral supplementation consisted of 200 mg of iron dextran intramuscular. The novel oral iron supplement was a combination of encapsulated nonheme/heme iron (252 mg of total iron) delivered orally. Two litters formed by 11, 2-day-old piglets were assigned to each treatment. The litters were video recorded for 6 hours, 3 before and 3 after iron supplementation. Scan sampling every 3 minutes was used to register the occurrence of 7 behaviors. The highest overall percentage of time was allocated to resting, 46.5% (167.4 minutes) for the parenteral group and 42.4% (152.6 minutes) for the oral group; and suckling 24.6% (88.6 minutes) for the parenteral group and 27.8% (100.1 minutes) for the oral group. Resting time was higher after 1 hour of iron supplementation in the parenteral group 51.9% (31.1 minutes) vs. 33.8% (19.7 minutes). In conclusion, the oral iron supplementation resulted in a higher behavioral disruption in neonatal pigs, probably associated to increased handling time and aversive flavor of the supplement.

Introduction

Iron-deficiency anemia is the most common mineral deficiency in swine, with a multicausal origin, and high morbidity in intensive pig farming systems (Lipiński et al., 2010). Within the first days of life, neonatal piglets are routinely subjected to parenteral iron supplementation (injection intramuscular of 100 to 200 mg of dextran iron), practice that can be stressful for piglets and sows (Brown et al., 1996). Alternative methods such as oral iron supplementation have also been proved to decrease iron deficiency (Quintero-Gutiérrez et al., 2008; Svoboda and Drábek, 2002). However, generally inorganic sources of nonheme iron, which have low bioavailability, are used as oral supplement. In contrast, Quintero-Gutiérrez et al. (2008) used sources of heme iron in pig supplementation, which showed a higher bioavailability than nonheme iron. The encapsulation method for iron has been widely used in human nutrition to prevent iron-deficiency anemia with good results (Zimmermann, 2004), but to our knowledge, this technology has not been applied for pig iron supplementation.

Current information on iron supplementation methods has focused on measuring the iron biomarkers in blood and serum, and the productive parameters of piglets (Lipiński et al., 2010; Quintero-Gutiérrez et al., 2008; Svoboda and Drábek, 2002). Any effects of parenteral and/or oral iron supplementation on behavior and welfare of pigs have been little studied compared with other stressful husbandry practices such as castration, tail docking, teeth resection, ear notch, and identification. The few studies in this area have focused on measures of vocalization, stress-related hormones, and escape attempts (Brown et al., 1996; Marchant-Forde et al., 2009, 2014). To date, there are no assessments of the effects of supplementation on the behavioral time budget of piglets.

One disadvantage of obtaining blood or saliva samples for measuring stress hormones is that the amount of restraint required might be a stressor itself (Blackshaw and Blackshaw, 1989). Saliva can be considered less invasive, but sampling time can take as long as 5 minutes in piglets (Blackshaw and Blackshaw, 1989), time that could have an effect on results. Kobelt et al. (2003) reported that up

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to 4 minutes can be taken to collect a saliva sample from dogs without producing an effect on the cortisol measured (Kobelt et al., 2003).

Assessment of behaviors such as vocalization may require special equipment for acoustic analysis and to discriminate vocalizations associated with pain, such as screams (Marx et al., 2003).

The aim of this study was to evaluate the general behavioral effects on the time budget of piglets associated with 2 iron supplementation methods: parenteral versus oral.

Materials and methods

Animals and housing

The experiment was conducted in a commercial pig farm (Región Metropolitana, Santiago, Chile). A total of 22 male and 22 female 2-day-old piglets, weighing 1.61 $\pm$ 0.09 kg were used. The 4 sows used were hybrid commercial pigs that had the same parity (third) and litter size. All experimental procedures were approved by the Bioethics Committee of the Faculty of Veterinary and Animal Sciences, University of Chile, certificate N° 05-2015.

Iron supplements

Oral iron supplement

A novel oral iron supplement was developed by encapsulating in a maltodextrin matrix heme and nonheme iron. Nonheme iron (iron sulfate heptahydrate, Merck S.A) at 30% w/v was suspended in maltodextrin solution (40% w/v in deionized water), and spray-dried (Buchi Mini Spray Dryer B-290, Switzerland), producing a nonheme iron encapsulated. Porcine blood cells (Lican Alimentos S.A, Chile) at 30% w/v were suspended in maltodextrin solution (40% w/v in deionized water), and spray-dried, producing heme iron encapsulated. Both iron encapsulated forms were blended at 10:2 (nonheme iron encapsulated:heme iron encapsulated) ratio, and suspended into distilled water (2 mL), obtaining a total iron content of 252 $\pm$ 12 mg per dose.

Experimental design

At day 2 after birth, 2 litters of 11 piglets each were assigned to each group as follows:

Parenteral group

Piglets received an intramuscular injection of 200 mg of dextran iron (2 mL) (Veterquímica, Chile) into the thigh muscles. For this, the operator stretched back one of the hind legs of the piglets while they were standing or nursing and applied the injection. The time between picking up the hind leg and final release was recorded as handling time in seconds.

Oral group

Piglets were supplemented orally with 2 mL of the novel iron supplement with a blunt-tipped applicator. For administration, piglets were held up by an operator with both hands by the belly and taken from the farrowing cage to allow a second operator to open the mouth, introduce the applicator, and deliver the supplement. Once the piglet had swallowed the supplement, it was returned into the cage with the sow. Handling time (seconds) was calculated from the moment the piglet was held up by the operator until it was replaced in the farrowing cage.

A control group without iron supplementation was not included because according to the commercial farm managers, no piglets could be left without iron supplementation.

Behavioral observations

Two days before the piglets' probable date of birth, 4 double infrared video cameras (IM-CIR50600NS IR Outdoor Cameras 700tvl 1/3 cmos Sony, SENKO SA, Santiago, Chile) were installed, one in each farrowing cage. The video information was captured and stored using a digital video recording system and an external memory drive. The cameras began with the recording mode 3 hours before (H-3, H-2, and H-1) to iron supplementation and up to 3 hours after (H1, H2, and H3) as described in Leslie et al. (2010). All video images were analyzed by 1 observer with the Observer XT 2011 (Noldus software, version 11, Noldus Information Technology, The Netherlands). Scan sampling every 3 minutes was used, which allowed us to capture the briefest behavioral state of interest. The number of piglets performing each of the 7 behaviors described in Table 1 was registered at each sampling point (144 sampling points). The number of piglets and time spent out of sight was also registered.

Statistical analysis

For the time budget, minutes and percentages of time allocated to each behavior before and after supplementation were calculated. For differences between treatments, for each behavior, and differences between handling times, the Wilcoxon test was applied. Kruskal-Wallis and the post hoc multiple pairwise comparison tests were applied for the analysis between hours within treatments. A significance level of $P < 0.05$ was applied.

Results and discussion

Time budgets

Rest and suckling behaviors occupied the major part of the overall time budget of neonatal pigs (Table 2), with an overall average allocation of time to resting of 167.4 minutes (46.5%) and 152.6 minutes (42.4%) for parenteral and oral groups, respectively; and 25% and 28% in parenteral and oral groups for suckling. These values are in accordance with the literature, where it has been reported that, together, resting and suckling can occupy over 70% of newborn pigs' daily time budget (Fraser and Broom, 1997, Leslie et al., 2010).

The others studied behaviors showed a low expression for both groups (Table 2) with an overall average of 3.4 and 6.8 minutes (1.7 and 1.9%) for normal locomotion, 4 minutes (1%) for exploration, and 5.8 and 1.4 minutes (1.6% and 0.4%) for positive interaction in both parenteral and oral groups, respectively. This information is also in accordance to the literature because newborn piglets present the highest resting rates among farm animals during their

Table 1
Description of behaviors of 2-day-old piglets according to Fraser and Broom (1997) and Leslie et al. (2010)

Behaviors	Description
Suckling	Teat in the mouth. Vigorous rhythmic movements.
Positive interactions	Includes allo-grooming behavior between piglets or between piglet and sow, play behavior such as locomotor play (run, jump, spin) between piglets or directed toward the sow or parts of the crate by a piglet.
Rest	Recumbent position, resting or sleeping with head up or legs and head outstretched.
Sitting	Body weight supported by hind-quarters and front legs.
Standing	Body weight supported by all 4 legs.
Normal locomotion	Forward movement in a 4-time gait from point A to point B, all 4 limbs are involved.
Exploration	Piglet extends neck toward part of the environment and looks at or sniffs at an object.

Table 2
Estimated minutes and percentage of time (%) dedicated hourly and by periods to each of the behaviors observed in the parental and oral groups

Behaviors	H-3	H-2	H-1	Total pre	H1	H2	H3	Total post	Total
Parenteral									
Resting	28.9 (48.1)	27.9 (44.9)	24.2 (40.3)	79.9 (44.4)	31.1 (51.9)[d]	30.9 (51.5)	24.4 (42.4)	87.5 (48.6)	167.4 (46.5)
Suckling	19 (31.6)	10.6 (17.6)	15.5 (25.9)[d]	44.3 (24.6)	11.3 (18.9)	12.6 (21)	20.3 (33.9)	44.3 (24.6)	88.6 (24.6)
Positive interaction	0.5 (0.8)	0.2 (0.4)	1.0 (1.7)	1.8 (1.0)	1.8 (3.0)	0.8 (1.3)	1.3 (2.1)	3.8 (2.1)	5.8 (1.6)
Sitting	0 (0)	0.1 (0.2)	0 (0)	0.2 (0.1)	0 (0)	0 (0)	0.1 (0.2)	0.2 (0.1)	0.4 (0.1)
Standing	0.1 (0.2)	0.7 (1.1)[d]	0.5 (0.8)	1.3 (0.7)	0.4 (0.6)	0.2 (0.4)	0.7 (1.1)	1.4 (0.7)	2.5 (0.7)
Normal locomotion	0.1 (0.2)	0.1 (0.2)	0.5 (0.9)	0.7 (0.4)	2.6 (4.4)	1.9 (3.2)	0.8 (1.3)	5.4 (3)	3.4 (1.7)
Exploration	0.5 (0.9)	0.6 (1)	1.0 (1.7)	2.2 (1.2)	0.7 (1.1)	0.5 (0.8)	0.6 (1)	1.8 (1)	4 (1.1)
Out of sight	11.3 (18.9)	20.8 (34.7)[d]	17.2 (28.6)[d]	49.3 (27.4)	12.1 (20.1)[d]	13.1 (21.8)[d]	10.7 (17.9)	35.8 (19.9)	85.3 (23.7)
Oral									
Resting	23.4 (39)[abc]	27.7 (46.2)[abc]	18.1 (30.1)[c]	69.1 (38.4)	19.7 (32.8)[bc]	30.7 (51.1)[ab]	32.9 (54.9)[a]	27.8 (46.3)	152.6 (42.4)
Suckling	20.2 (33.7)[ab]	16.7 (27.8)[abc]	23.9 (39.8)[a]	60.8 (33.8)	15.4 (25.6)[abc]	9.3 (15.5)[c]	14.8 (24.6)[bc]	5.2 (21.9)	100.1 (27.8)
Positive interaction	0.2 (0.4)	0.1 (0.2)	0.2 (0.4)	0.5 (0.3)	0.2 (0.4)	0.5 (0.8)	0 (0)	0.7 (0.4)	1.4 (0.4)
Sitting	0.1 (0.2)	0 (0)	0.2 (0.4)	0.4 (0.2)	0.2 (0.4)	0.1 (0.2)	0 (0)	0.4 (0.2)	0.7 (0.2)
Standing	1 (1.7)	0 (0)	0.9 (1.5)	2 (1.1)	1 (1.7)	1.3 (2.1)	0 (0)	0.8 (1.3)	4.3 (1.2)
Normal locomotion	0.9 (1.5)	0.2 (0.4)	2.5 (4.2)	3.6 (2.0)	2.2 (3.6)	0.5 (1.5)	0.1 (0.2)	1.1 (1.8)	6.8 (1.9)
Exploration	0.4 (0.6)	0.1 (0.2)	0.5 (0.8)	0.9 (0.5)	1.8 (3)	1.3 (2.1)	0 (0)	3.1 (1.7)	4 (1.1)
Out of sight	13.7 (22.9)[a]	15.1 (25.2)[ab]	13.7 (22.9)[ab]	42.7 (23.7)	19.6 (32.6)[ab]	16 (26.7)[b]	12.2 (20.3)[b]	15.9 (26.5)	90.4 (25.1)

[abc]Indicates differences within groups among hours for a specific behavior ($P < 0.05$).
[d] Indicates differences between groups for a specific behavior ($P < 0.05$).

neonatal stage, whereas other behaviors are poorly observed until the first week of life (Fraser and Broom, 1997).

Out-of-sight values were high in both groups with an overall average of 85.3 min (23.7%) and 90.4 min (25.1%) for parenteral and oral groups, respectively. This high percentage of time could have several reasons: (1) while suckling newborn pigs tended to fall asleep on each other, hindering their filming; (2) the same situation occurs when they are resting; and (3) although not measured in a quantifiable way, it was observed in both types of iron supplementation, that when sows were disturbed they stand up, decreasing cameras visibility.

Effects of iron supplementation on behavior of neonatal pigs

Iron supplementation had an effect on resting behavior. At H1, pigs given oral supplements spent significantly lower time resting (19.7 min) than did those given parenteral supplements (31 minutes) (Table 2). This difference could be the result of an increase of standing, restless, gasping and tongue shaking, and biting and exploring bedding behaviors, behaviors which all may be associated with the unpleasant metallic taste of iron, especially nonheme iron (Stevens et al., 2006). A high rejection rate for iron-fortified foods has been reported in humans, who describe color changes and unpleasant metallic taste (Hurrell, 2002).

Although both iron forms present in the supplement are encapsulated, which reduces undesirable organoleptic characteristics generated by the nonheme iron (Li et al., 2014), the doses used for iron requirement of pigs are high, due to the high requirements of this metal (7-10 mg of iron per day) in lactation stage of these animals (NRC, 2012), and the metallic taste could still be present (Table 2).

Treatments had no effect on suckling behavior, as in Leslie et al. (2010). Increased time spent in exploration after treatment in the oral group was probably compensated by a decrease in resting and positive behaviors, without alteration in suckling behavior. Leslie et al. (2010) also only reported a significant increase for the time spent awake inactive, probably also associated to the numerical decrease reported by the authors in sleeping behavior.

With respect to the amount of time piglets could not be seen, significant differences at H-2, H-1, H1, and H2 were found between treatments (Table 2). After iron supplementation at H1 and H2, the piglets in the oral supplementation group were out of sight significantly more often. Piglets in the oral supplementation group had to be taken by the abdomen and removed from the cage for delivery of the supplement. These maneuvers generated behavioral changes in pigs, which did not occur in the parenteral group, making the sow stand up for longer time and consequently obstructing the view of some piglets, as directly observed but not quantified by the observer.

Both methods of supplementation differed significantly ($P < 0.001$) in the handling time required to deliver the iron supplement. The parenteral method required and average handling time per pig of 5.06 ± 1.6 seconds while the oral supplement 33.7 ± 10.2 seconds per pig. This difference in handling time could explain why there was more behavioral disruption with the oral supplement. Both movement restriction, and increases in handling time have been reported as sources of stress for animals (Noonan et al., 1994). Other authors have also classified oral iron supplementation as more stressful than parenteral supplementation (Marchant-Forde et al., 2014) and noted that parental iron supplementation is more effective and easier to carry out (Polner et al., 2002). However, Marchant-Forde et al. (2009) described found no significant behavioral differences, despite the longer amount of time it took to deliver the oral supplement.

Conclusions

This study showed that oral iron supplementation required significantly more handling and more time to deliver than the parenteral compound and resulted in more negative behavioral changes than did the parenteral form commonly used form. When developing new husbandry alternatives, training personnel to reduce handling time should be considered as a way to reduce negative welfare effects on piglets.

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Valenzuela. The article was written by Carolina Valenzuela, Tamara Tadich, and Jaime Figueroa.

Ethical considerations

The experiments associated to the article entitled: "Behavior of suckling pigs supplemented with a novel encapsulated iron oral formula" have all been approved by the Bioethics Committee of the Veterinary Faculty of the Universidad de Chile. The experiments are contained and approved in the certificate N° 05-2015.

Conflict of interest

The authors declare no conflict of interest.

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