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Utilization of milk minerals by Iberian suckling piglets

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Abstract

Little information is available concerning mineral metabolism in suckling piglets. The utilization of milk minerals and the mineral composition of Iberian (IB) suckling piglets were studied in two consecutive experiments at different ambient temperatures (trial 1, 27 ± 2°C; trial 2, 22 ± 2°C). Milk composition and the piglets' performance were determined weekly over a 34 days lactation period and, at the end, body mineral contents were analyzed and mineral retention and bioavailability were calculated. The ash content in IB sows' milk and in suckling IB piglets was found to be higher than that reported for lean genotypes. During lactation, mean ash content per unit of body weight gain in IB piglets (40.6 g kg^{-1}) was 42% higher than that observed for lean breeds, and weaned IB piglets contained 60% more Ca (11-12.7 g kg^{-1} empty body weight) and P (7-7.5 g kg^{-1} empty body weight), compared with conventional pigs. Minerals from milk were retained by the IB piglet with an overall efficiency of 78.3%, 66.3% and 48.7% for Ca, P and Mg, respectively. Significant differences between trials were observed in the mineral fraction composition of the milk, which were reflected in piglet body composition at weaning, presumably due to differences in ambient temperature. Present findings underline genotype differences in mineral composition of sow's milk and suckling piglets and may provide a useful starting point for formulating milk-replacer diets for IB piglets.

Additional key words: milk composition; mineral fraction; mineral retention; suckling Iberian piglets.

Introduction

Minerals have received little attention in swine nutrition, although they perform essential structural and metabolic functions (Underwood & Suttle, 2002). The piglet's reserves of minerals must be substantial to sustain the rapid growth and maturation of tissues in its early life; during pregnancy, many minerals are satisfactorily passed across the placenta to the fetuses but from birth, most of them are supplied in milk (Close & Cole, 2003). The main components of the mineral milk fraction are Ca, P and Mg; Ca and P are advantageous to bone growth, thereby contributing to the animal's overall strength, and Mg has many physiological functions and is involved in more than 300 enzymatic metabolic reactions (Underwood & Suttle, 2002).

The Iberian (IB) pig, a native breed from the southwest of the Iberian Peninsula, is much appreciated due to the special organoleptic characteristics and quality of its products, especially when the pigs are finished grazing acorns and grass. This is a low performing pig with a low genetic potential for lean tissue deposition (Nieto *et al*., 2002; Barea *et al*., 2007; Conde-Aguilera *et al*., 2011). The slower growth rate of the Iberian suckling piglet, compared with piglets from conventional or lean genotypes, seems to be related to a comparatively decreased efficiency of use of milk energy and protein in the former (Aguinaga *et al*., 2011a). Nowadays, no available data exist in the literature about the composition of the mineral fraction in IB sow milk. Moreover, as maternal milk provides the sole nutrient supply in the early life, evaluation of the utilization of minerals from milk —rather than gross mineral composition— would provide a suitable starting point for any formulation of a milk-replacer diet. However, there is no information about the body retention of minerals by the suckling IB piglet during lactation and, in general, there is very little information on

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Abbreviations used: ADG (average daily gain); BW (body weight); DM (dry matter); EBW (empty body weight); I (mineral intake); IB (Iberian); R (mineral retention); SEM (standard error of the mean).

this topic respect to suckling piglets of other pig genotypes.

The objectives of this experiment were to study the mineral composition of the Iberian sow's milk during a 34-day lactation period, and the body mineral composition and mineral balance in IB suckling piglets at weaning. The study was focussed to Ca, P and Mg, the major contributors to the mineral fraction of milk.

Material and methods

Husbandry and experimental design

Animals, management and general procedures of the experiment have been described elsewhere (Aguinaga *et al.*, 2011a). Briefly, 16 third-parity purebred IB sows (Silvela strain) were randomly selected from the Montecastilla farm (Granada de Rio Tinto, Huelva, Spain), and used in two consecutive trials ($n = 8$). In each one, four sows were selected to study milk production, litter performance and piglet's nutrient balance measurements, and the other four for milk sampling. During gestation, sows were fed a commercial diet that contained per kg, as fed, 12.13 MJ metabolizable energy, 140 g crude protein and 5.5 g lysine; the diet was offered at 1.8 kg day^{-1} for the first 70 days of pregnancy, when the feed was progressively increased to 2.5 kg day^{-1} until a week before farrowing. At this time, the sows were individually housed in farrowing crates (1.90 × 0.60 m) within pens (2.40 × 1.60 m) in a forced ventilated room. The environmental temperature of the farrowing room was 27 ± 2°C in the first trial and 22 ± 2°C in the second one (precision of ±0.2°C, thermometer E0207, Gesa Termómetros S.L., Vizcaya, Spain), during the subsequent 34 days of lactation period. The pens were equipped with a thermo-regulated piglet nest that maintained the temperature at 33-35°C during the first week of life by floor heating, declining progressively to 25-27°C at the end of the third week.

During the week prior to farrowing and the lactation period, the sows were fed a commercial lactation feed (PACSA-Sanders, Seville, Spain) containing per kg, as fed, 12.76 MJ metabolizable energy, 144 g crude protein and 6.8 g lysine. Analysis of the diets (n = 3 in each trial) revealed no significant differences ($p > 0.05$) in the mineral content between trials (g kg^{-1}, mean ± SEM: Ca, 10.32 ± 0.06; P, 7.70 ± 0.10; Mg, 1.86 ± 0.01). This diet was provided at a level of 1% of body weight (BW) until the day of parturition, when the sows were offered 1.5 kg of this diet. Thereafter, daily feed allowance was increased by 0.6 kg day^{-1}, reaching 4.5 kg day^{-1} by the fifth day of lactation, after which this level was maintained. No significant diet refusals were observed. The sows' live weight just after farrowing was 130-140 kg. The sows had permanent access to water, however, the only liquid intake of the litters was their mother's milk and they had no access to either creep feed or sow feed. The analyzed mineral composition of the water was as follows (mg L^{-1}, mean ± SE): Ca, 30 ± 0.07; P, non detectable; Mg, 21 ± 0.09. Due to the low mineral content, possible differences in water consumption between trials were considered non significant with respect to the sows' total mineral intake. Shortly after birth, the piglets were administered 200 mg Fe-dextran complex (Imposil Forte®; Alstoe Ltd, UK) via intramuscular injection. Litter size was homogenized to six piglets on the day after farrowing by cross-fostering, and four piglets in each litter were used to calculate milk intake, piglet performance and body composition. Procedures used in these experiments were approved by Bioethical Committee of Spanish National Research Council (CSIC).

Milk samples were collected from the four nursing sows on day 5, 12, 19, 26 and 34 *postpartum* as described in Aguinaga *et al.* (2011a) and stored in opaque plastic bottles at -20ºC until analyzed. This procedure allowed the collection of a great volume of milk (> 100 mL) without disrupting the suckling pattern of piglets involved in the nutrient balance measurements. An aliquot of milk was freeze-dried (FTS System, INC., TDS-3, NY, USA) for analysis of dry matter (DM), gross energy, ash and mineral composition, whereas total fat and protein (total N·6.38) were determined in aliquots of thawed milk. The weigh-suckle-weigh technique (Speer & Cox, 1984) was followed on days 5, 12, 19, 26 and 34 *postpartum* in order to calculate the piglets' individual milk intake during lactation. During each of these days, a total of eight determinations were obtained, at 75 min intervals, and the total amount of milk was extrapolated to a 24 hours period. A correction factor was applied to account for piglets' weight losses due to evaporative losses and physical activity during suckling (Klaver *et al.*, 1981). To calculate the total milk intake of each piglet during the 34 days of lactation, it was assumed that milk production changed linearly between the successive measurements (Aguinaga *et al.,* 2011a).

Piglets were individually weighed at birth and every seven days from day 5 of lactation. The average daily gain (ADG) was calculated for each period. Mineral retention was calculated from the difference between mineral body content at weaning and mineral body content at birth, following the comparative slaughter procedure. Within each litter, one piglet at birth and four of the six nursing piglets in the morning of day 35 of life, all with a BW close to the litter average, were anaesthetized by intra-peritoneal puncture of sodium pentobarbital (40 mg kg^{-1} BW) (Sodium Pentothal®; Abbott Lab.) and subsequently bled. After emptying the gut, collected blood, viscera, carcass, and head/feet/tail as a whole, were weighed and kept at –20°C until analysis. Carcass, viscera and head/feet/tail were ground and homogenized using a mincer and a cutter (Talleres Cato, Sabadell, Spain), and representative aliquots were lyophilized (FTS System, INC., TDS-3, NY, USA) for subsequent analysis. Samples were analyzed separately for DM, ash, protein (total N · 6.25), energy and mineral composition. Body fat was calculated assuming an energy content of 23.8 and 39.8 kJ g^{-1} for protein and fat, respectively (Wenk *et al*., 2001). Within each trial, body composition data from the initial slaughter group were used to estimate body composition of the other 16 piglets at birth.

Analytical techniques

All analyses, in milk and in piglet tissues, were performed by triplicate. The contents in DM, ash, N and fat were determined by standard procedures (AOAC, 1990). Gross energy was measured in an isoperibolic bomb calorimeter (PARR 1356, Biometa, IL, USA) and total fat in milk by the Gerber method (AOAC, 1990). Analyses of Ca and Mg were carried out by flame-atomic-absorption spectroscopy in a Perkin-Elmer Analyst 700 Spectrophotometer (Norwalk, CT USA). Total P was determined colorimetrically at 820 nm in a spectrophotometer (Shimadzu UV-1700, Model TCC-240A, Columbia, SC, USA) by the vanadomolibdate procedure (AOAC, 1990). Before analyzing the mineral composition, samples were completely digested by the addition of concentrated HNO_3:$HClO_4$ (1:4) and by heating to high temperatures (180-220°C) in a sand beaker (Block Digestor Selecta S-509; J.P. Selecta, Barcelona, Spain). Previous to Ca determination, 0.3% lanthanum chloride (Merck) was added to samples and standards. Pools of biological samples and milk were used as an internal control to assess analytical precision. The inter-assay coefficients of variation (CV %) in body tissues were: 2.07 for Ca, 5.16 for Mg and 0.73 for P, whereas in milk samples were: 1.99 for Ca, 3.74 for Mg and 1.87 for P. To test the accuracy of the mineral analysis, certified external standard of skim milk powder (BCR 063R) from the Community Bureau of Reference (Brussels, Belgium) was used. Certified values were: 13.49 ± 0.10 mg g^{-1} (Ca), 1.26 ± 0.02 mg g^{-1} (Mg) and 11.10 ± 0.13 mg g^{-1} (P); measured values (mean ± SEM of 10 determinations) were: 13.47 ± 0.04 mg g^{-1} (Ca), 1.27 ± 0.03 mg g^{-1} (Mg) and 11.04 ± 0.03 mg g^{-1} (P). All glassware and polyethylene sample bottles used for mineral analysis were washed with 10 mmol L^{-1} of nitric acid. Demineralised water (Milli-Q Ultrapure Water System, Millipore Corp., Bedford, MA, USA) was used throughout the study.

The efficiency of utilization or bioavailability (%) of each mineral was determined as $R \cdot I^{-1} \cdot 100$, where R is mineral retention (calculated from initial and final body content) and I is mineral intake (calculated from milk composition and intake).

Statistical analysis

Before analyzing the data, the normality of their distribution was tested for each variable included in the study using the Kolmogorov-Smirnov test. In all cases the *p*-values found were >0.05, indicating that all variables followed a normal distribution.

Data concerning milk nutrient composition and piglets' performance were analyzed using a repeated-measures two-way ANOVA randomized design to examine the effects of the trial and the period of lactation and the interaction between the two factors using the MIXED procedure of SAS (SAS, 2004). The lactation period was considered as the repeated effect. Data on piglets' body composition and mineral utilization were analyzed by one-way ANOVA with the trial as variation factor by the GLM procedure of SAS (SAS, 2004). Statistical significance was assessed using Fisher's LSD test. The level of significance was set at 5%.

Results

Milk composition

Data concerning energy, nutrient and mineral composition of milk samples obtained over the 34 days of

Table 1. Composition of Iberian sow's milk measured throughout 34 days of nursing period in two consecutive trials[1]

	Dry matter ($g\ 100\ g^{-1}$)	Energy ($kJ\ 100\ g^{-1}$)	Protein[2] ($g\ 100\ g^{-1}$)	Fat ($g\ 100\ g^{-1}$)	Ash ($g\ 100\ g^{-1}$)	Calcium ($mg\ 100\ g^{-1}$)	Phosphorus ($mg\ 100\ g^{-1}$)	Magnesium ($mg\ 100\ g^{-1}$)
Trial[3]								
1	18.16	469.9	5.45	6.31	1.079	286	215	13.3
2	17.73	459.3	5.22	5.59	0.952	243	180	11.6
Pooled SEM	0.369	13.68	0.087	0.344	0.0163	2.8	4.8	0.28
Day of lactation								
5	18.20	479.5	5.29[b]	6.28	0.890[d]	215[e]	181[c]	10.9[c]
12	18.38	489.7	4.94[b]	6.53	0.887[d]	235[d]	175[c]	10.9[c]
19	17.73	453.7	5.18[b]	6.16	0.990[c]	255[c]	188[c]	12.1[c]
26	17.41	441.9	5.28[b]	5.34	1.107[b]	293[b]	210[b]	13.5[b]
34	18.01	458.5	5.96[a]	5.46	1.203[a]	325[a]	235[a]	14.9[a]
Pooled SEM	0.582	21.59	0.142	0.531	0.0250	4.5	7.6	0.44
p-value[4]								
Trial			<0.100		<0.001	<0.001	<0.001	<0.001
Day			<0.001		<0.001	<0.001	<0.001	<0.001
Trial × Day	<0.10	<0.05		<0.10				

[1] Values are means of 4 sows in each trial [2] Total N × 6.38. [3] Trial 1, carried out at 27 ± 2°C; trial 2, carried out at 22 ± 2°C. [4] Repeated measures two-way ANOVA followed by LSD test. [a,b,c,d,e] Means with different superscripts within a column differ significantly ($p < 0.05$).

lactation period in the two trials are shown in Table 1. As lactation advanced, the DM, energy and fat content of the milk remained almost constant, whilst protein concentration increased at the end of the nursing period ($p < 0.001$). The analysis of the trial × day interaction for energy content ($p < 0.05$) revealed some minor differences between days for trial 1. Progressive and significant increases of the milk ash content were observed from day 12 until day 34 ($p < 0.001$), in parallel with rises of Ca, P and Mg ($p < 0.001$). No effect of the trial was observed for DM, energy and fat content of milk, whereas protein tended to increase ($p = 0.09$) and ash content was significantly higher ($p < 0.001$) in trial 1 compared with trial 2. In line with this change in ash concentration, increased contents of Ca, P and Mg in the milk obtained in trial 1 were noticed.

Milk intake and piglets' performance

During the 34 days of nursing period, significant increases of milk intake of piglets were observed ($p < 0.001$), whereas the ADG dropped ($p < 0.001$, Table 2). As a consequence, the efficiency of milk conversion (weight gain/milk intake) decreased over lactation ($p < 0.001$). In trial 1 (27°C) the milk intake was signi-

Table 2. Performance of Iberian suckling piglets measured throughout a 34 days of nursing period in two consecutive trials[1]

	Milk intake ($g\ day^{-1}$)	ADG[2] ($g\ day^{-1}$)	Gain/milk
Trial[3]			
1	811	167	0.215
2	899	176	0.203
Pooled SEM	15.7	3.6	0.0048
Day of lactation			
0-5	616[b]	189[a]	0.315[a]
6-12	885[a]	190[a]	0.215[b]
13-19	955[a]	180[ab]	0.189[c]
20-26	903[a]	163[b]	0.182[c]
27-34	915[a]	134[c]	0.144[d]
Pooled SEM	25.8	7.3	0.0082
p-value[4]			
Trial	<0.001		<0.10
Day	<0.001	<0.001	<0.001
Trial × Day			

[1] Values are means of 16 piglets (4 sows × 4 piglets per litter) in each trial. [2] ADG, average daily gain. [3] Trial 1, carried out at 27 ± 2°C; trial 2, carried out at 22 ± 2°C. [4] Repeated measures two-way ANOVA followed by LSD test. [a,b,c,d] Means with different superscripts within a column differ significantly ($p < 0.05$).

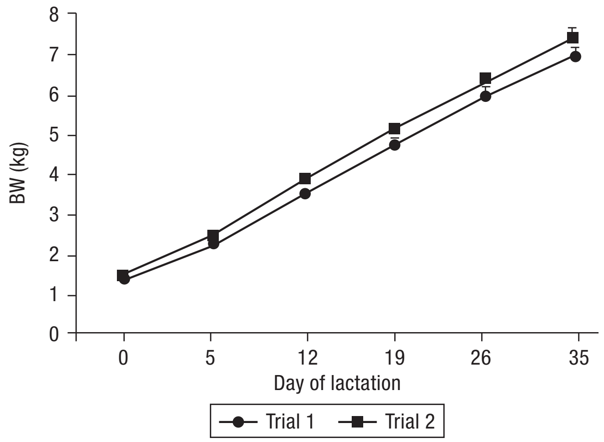

Figure 1. Body weight (BW) evolution of Iberian piglets throughout 34 days of nursing. Values are means ± SEM of 16 piglets (4 sows × 4 piglets per litter) in each trial. No significant differences were found between trials (LSD test). Trial 1, carried out at 27 ± 2°C; trial 2, carried out at 22 ± 2°C.

ficantly lower than in trial 2 (22°C) and, as there was no differences in ADG between trials, a tendency to a more efficient conversion of milk in weight gained was observed under the hot environment ($p = 0.07$). No significant interaction was detected for the variables studied. Similar BW evolution of piglets was observed in both trials (Fig. 1).

Body composition and mineral retention of the suckling piglet

Table 3 summarizes data on mineral composition of body and body components of piglets at birth and at weaning. Statistical analysis revealed no significant effect of the trial on the body composition of the IB piglets at birth and, thus, the data of newborn animals have been pooled (mean ± SEM). At birth, piglets contained an average of 40 g of ash per kg of empty body weight (EBW), 35% of which was Ca and 19.5% was P.

The ash content of the weaned IB piglets differed significantly between trials, whereas the other body components remained unmodified. Variations in the ash level were reflected in Ca, P and Mg body contents (g kg^{-1}), which were significantly higher in trial 1 than in trial 2. Expressed as a proportion of total ash, the main difference was found for Ca, which represented 31% *vs.* 28.5% of total minerals per kg EBW in trials 1 and 2, respectively.

The mineral balance (g retained day^{-1}) and the utilization efficiency of ash and minerals from milk are shown in Table 4. No significant differences were observed in the intake of total ash or the major milk minerals (Ca, P and Mg) by the piglets, as the lower mineral content found in milk in trial 2 was counteracted by the higher milk intake. Minerals from milk were retained by the IB piglet with an overall efficiency of 78.3%, 66.3% and 48.7% for Ca, P and Mg, respectively. Ca was found to be retained in higher quantity (g day^{-1}) and more efficiently in trial 1 than in trial 2 (both $p < 0.05$). The balance of the other minerals, as well as the utilization efficiency, remained unchanged between trials.

The mineral retention expressed as units of mineral per kg of piglet BW gain is depicted in Fig. 2. Each kg of piglet gain in trial 1 contained more ash (by 7.2%) than in trial 2, due to the increased content of Ca (17%, $p < 0.001$), P (6.7%, $p < 0.05$) and Mg (6.8%, $p < 0.05$).

Discussion

Milk composition

Data on the composition of IB sows' milk concerning organic nutrients, energy and amino acids have been published recently (Aguinaga *et al.*, 2011a,b). During the lactation period, the major changes were observed in the ash fraction which increased by 35%. Several studies have been reported on sows' milk

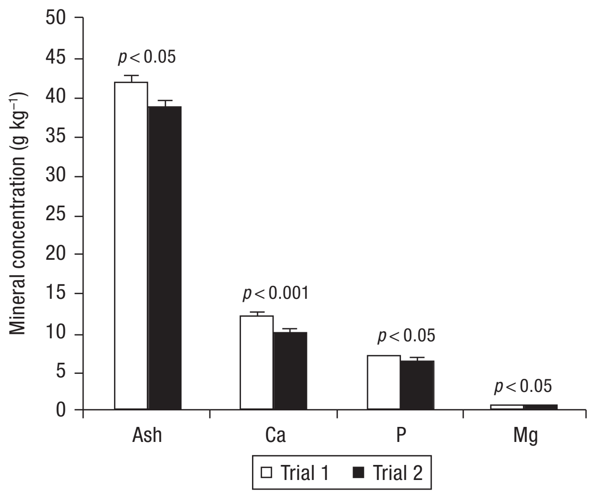

Figure 2. Ash and mineral content per kg of body weight gain in suckling Iberian piglets after 34 days of nursing period in two consecutive trials. Values are means ± SEM of 16 piglets (4 sows × 4 piglets per litter) in each trial. One-way ANOVA followed by LSD test. Trial 1, carried out at 27 ± 2°C; trial 2, carried out at 22 ± 2°C.

Table 3. Body composition and mineral composition of body components of Iberian piglets measured at birth and after 34 days of nursing period in two consecutive trials

	At birth[1]	At weaning[2]		*p*-value[4]
		Trial 1[3]	Trial 2	
Empty body weight (EBW)	1.26 ± 0.03	6.63 ± 0.13	6.93 ± 0.17	
Protein (g kg^{-1} EBW)	129 ± 3	161 ± 1	158 ± 1	
Fat (g kg^{-1} EBW)	26.4 ± 0.9	118 ± 3	125 ± 4	
Ash (g kg^{-1} EBW)	40.0 ± 1.3	40.8 ± 0.6	38.6 ± 0.6	< 0.05
Water (g kg^{-1} EBW)	805 ± 4	657 ± 4	655 ± 4	
Energy (MJ kg^{-1})	4.12 ± 0.06	8.53 ± 0.12	8.74 ± 0.15	
Calcium				
Total body (g)	17.7 ± 0.9	84.4 ± 2.5	76.1 ± 2.8	< 0.05
g kg^{-1} EBW	14.1 ± 0.7	12.7 ± 0.3	11.0 ± 0.4	< 0.001
Carcass (g kg^{-1})	5.20 ± 0.27	12.0 ± 0.3	10.0 ± 0.5	< 0.01
Blood (g kg^{-1})	0.03 ± 0.002	0.18 ± 0.01	0.22 ± 0.02	< 0.10
Viscera (g kg^{-1})	0.05 ± 0.003	0.16 ± 0.01	0.12 ± 0.01	< 0.01
Head/feet/tail (g kg^{-1})	8.77 ± 0.45	30.9 ± 0.7	28.6 ± 0.7	< 0.05
Phosphorus				
Total body (g)	9.80 ± 0.44	49.4 ± 1.2	48.7 ± 1.2	
g kg^{-1} EBW	7.80 ± 0.34	7.5 ± 0.1	7.0 ± 0.1	< 0.05
Carcass (g kg^{-1})	2.53 ± 0.11	7.6 ± 0.1	7.0 ± 0.2	< 0.05
Blood (g kg^{-1})	0.15 ± 0.006	0.06 ± 0.001	0.06 ± 0.004	
Viscera (g kg^{-1})	0.96 ± 0.042	0.69 ± 0.02	0.66 ± 0.01	
Head/feet/tail (g kg^{-1})	4.16 ± 0.18	15.1 ± 0.3	15.2 ± 0.3	
Ca:P	1.80 ± 0.04	1.71 ± 0.01	1.56 ± 0.05	< 0.05
Magnesium				
Total body (g)	0.46 ± 0.019	2.30 ± 0.07	2.25 ± 0.06	
g kg^{-1} EBW	0.36 ± 0.014	0.35 ± 0.001	0.33 ± 0.001	< 0.05
Carcass (g kg^{-1})	0.11 ± 0.004	0.35 ± 0.007	0.33 ± 0.008	< 0.10
Blood (g kg^{-1})	0.02 ± 0.001	0.11 ± 0.002	0.12 ± 0.004	
Viscera (g kg^{-1})	0.07 ± 0.002	0.163 ± 0.003	0.163 ± 0.002	
Head/feet/tail (g kg^{-1})	0.17 ± 0.006	0.59 ± 0.01	0.55 ± 0.01	< 0.05

[1] Values are means ± SEM of 8 piglets (4 × 2 trial). [2] Values are means ± SEM of 16 piglets (4 sows × 4 piglets per litter) in each trial. [3] Trial 1, carried out at 27 ± 2°C; trial 2, carried out at 22 ± 2°C. [4] Comparison of weaning values between trials (one-way ANOVA followed by LSD test).

composition (Klobasa *et al.*, 1987; Revell *et al.*, 1998; Alston-Mills *et al.*, 2000), most of which focused on evaluating the protein, fat and lactose milk content. However, there is little information on the mineral composition of the sow milk, and none related to IB sows' milk. Reported values of ash content in milk from conventional breeds range from 0.75-0.77% (Noblet & Etienne, 1986) to 0.80-0.84% (Csapó *et al.*, 1996; Renaudeau *et al.*, 2003), and are always below than that found for IB sows' milk (1.02% on average). Moreover, the milk ash content in conventional pig breeds does not increase during lactation at the high rate observed among the IB sows in the present assay. This increased ash milk content could be related to the higher bone-to-lean tissue ratios observed in IB pigs compared to leaner genotypes (Quiniou & Noblet, 1995; Mayoral *et al.,* 1999). In the present experiment, individual analysis of milk minerals shows that the sharp increase in ash content over lactation was due to significant rises in Ca, P and Mg. Our results agree with those of previous studies in which Ca concentration in milk increased from parturition to weaning (Miller *et al.,* 1994; Csapó *et al.*, 1996; Alston-Mills *et al.*, 2000). Nevertheless, while the Ca proportion reached 25.5-26.5% of the mineral fraction of the IB sow milk, the normal range in other genotypes is only

Table 4. Mineral balance of suckling Iberian piglets after 34 days of nursing period in two consecutive trials[1]

	Trial[2] 1	Trial[2] 2	*p*-value[3]
Ash			
Intake (I) (g day^{-1})	9.34 ± 0.26	8.73 ± 0.27	
Retention (R) (g day^{-1})	6.54 ± 0.18	6.39 ± 0.23	
% R · I^{-1}	70.1 ± 1.3	73.3 ± 1.8	
Calcium			
Intake (g day^{-1})	2.36 ± 0.07	2.23 ± 0.07	
Retention (g day^{-1})	1.91 ± 0.07	1.65 ± 0.08	< 0.05
% R · I^{-1}	81.8 ± 1.4	74.9 ± 2.6	< 0.05
Phosphorus			
Intake (g day^{-1})	1.78 ± 0.05	1.65 ± 0.05	< 0.10
Retention (g day^{-1})	1.13 ± 0.03	1.11 ± 0.04	
% R · I^{-1}	64.6 ± 1.0	68.1 ± 1.9	
Magnesium			
Intake (g day^{-1})	0.109 ± 0.003	0.106 ± 0.003	
Retention (g day^{-1})	0.052 ± 0.002	0.052 ± 0.002	
% R · I^{-1}	48.7 ± 0.90	48.7 ± 1.18	

[1] Values are means ± SEM of 16 piglets (4 sows × 4 piglets per litter) in each trial. [2] Trial 1, carried out at 27 ± 2°C; trial 2, carried out at 22 ± 2°C. [3] Comparison of weaning values between trials (one-way ANOVA followed by LSD test).

21-23% (Miller *et al.*, 1994; Csapó *et al.*, 1996). The milk from the IB sow presented also higher contents of P and Mg than those described by Csapó *et al.* (1996) in lean pigs.

Renaudeau & Noblet (2001) reported that the exposure to high environmental temperatures (29°C *vs.* 20°C) tended to increase DM, energy and ash contents in milk of conventional sows, without affecting protein, lactose or fat, suggesting that changes in milk composition could be related to the more intense mobilization of body reserves in hot conditions. On the contrary, subsequent observations have shown that sows' milk composition is not affected by ambient temperature, although mammary blood flow is higher at 28°C than under thermoneutral conditions, without modifying milk production (Renaudeau *et al.*, 2003). Mineral losses of sows affected by temperature have been scarcely investigated; Renaudeau *et al.* (2003) have found that the pattern of nutrient uptake from blood by the mammary gland, including Ca and P, is not affected by temperature. Without discarding other factors, variations in mineral concentrations of IB sows' milk observed in the current assay between trials could be related to the differences observed in ambient temperature; it is hypothesised that it may be an increased proportion of blood flow irrigating the mammary gland, or a higher mineral mammary extraction rate, under hot conditions. However, the mechanism involved in these selective changes remains unknown.

Milk intake and piglets' performance

In this study, the sows' feeding level in both trials was matched to 4.5 kg day^{-1} and all piglets suckled the same number of times, as described above, trying to avoid effects of feeding level of the sows and of suckling frequency in milk production (Quiniou & Noblet, 1999; Renaudeau & Noblet, 2001). As mentioned, milk intake of piglets increased during lactation, although after a drastic increase between days 5 and 12 from farrowing, values plateaued until the end of lactation. Other authors have also recorded this early breakpoint of milk yield in a warm environment (Noblet & Etienne, 1986), but under hot conditions, a more linear rise has been observed (Schoenherr *et al.*, 1989). A significant effect of the trial was detected ($p < 0.001$) but the lack of a significant trial × day interaction indicated that the milk intake variation during lactation was independent of the trial. The lower values of milk intake found in the piglets in trial 1 suggest a probably detrimental effect of the high ambient temperature on milk yield, in accordance with earlier findings. However, the differences observed in the present experiment cannot be attributed to the lower feed intake, as sows were fed at the same intake level in both trials and no refusal was observed; rather, they are likely due to an apparent inefficiency of the sow mammary gland at warmer conditions to dissipate body heat (Renaudeau *et al.*, 2003), or to endocrine adaptations mediated by declines of thyroid hormones and cortisol (Messias de Bragança *et al.*, 1998).

The ADG of the IB piglets was stable during the early lactation (until day 19), and decreased progressively in the two last weeks of the experimental period (Table 2), irrespectively of the trial. Neither there was any significant effect of the trial on the piglets' BW at birth (average 1.41 ± 0.02 kg) and at weaning (7.17 ± 0.13 kg), or during the entire nursing period (Fig. 1). Similar weaning BWs have been observed in lean pigs weaned at 21 days (Messias de Bragança *et al.*, 1998), and much higher ones when weaning is at 28 days

(Renaudeau & Noblet, 2001). This fact confirms the slower growth rate (corroborated by the lower ADG) of the IB piglets compared with conventional breeds, as pointed out in our previous paper (Aguinaga *et al.*, 2011a), mainly due to the limited ability of the IB piglet for protein accretion and the poor efficiency in the use of milk nutrients. Renaudeau *et al.* (2003) showed that, at a similar temperature range to ours (20-28°C), both milk yield and litter growth rate are unaffected. In the present experiment, the similar weight gains recorded at trials carried out at different temperature conditions, despite the lower milk intake in trial 1, might reflect a greater efficiency in converting milk to live weight gain under 27°C compared to 22°C ($p = 0.075$; Table 3). On the contrary, experiments on conventional pigs have reported a decreased weight gain per unit of feed as a result of exposure to hot ambient (Schoenherr *et al.*, 1989).

Body composition and mineral retention of the suckling piglet

Little information has been published concerning pig composition at birth (Noblet & Etienne, 1986 and 1987; Everts & Dekker, 1994). Moreover, there is scant information on the mineral distribution in body compartments. Thus, the data obtained in the present paper provide new information on the body composition of IB piglets at birth. Newborn IB piglets have higher contents (g kg^{-1}) of protein, ash, fat (more than double) and energy compared to conventional breeds (Elliot & Lodge, 1977). Values of Ca and P are also higher, while Mg levels are similar to those described by Everts & Dekker (1994) and others taken from previous literature.

After a lactation period of 34 days, IB piglets had similar body contents of protein, fat and energy to those described for conventional pigs weaned at 21 days but ash content was always higher in IB piglets (around 40 *vs.* 30 g kg^{-1} EBW) (Table 3) (Noblet & Etienne, 1987; Everts *et al.*, 1998; Renaudeau & Noblet, 2001). This discrepancy may be explained by variations due to genotype and by differences in the rate of weight gain of the litter, as ash body content at weaning is negatively correlated with ADG (Noblet & Etienne, 1987). Moreover, the higher ash content of the weaned IB piglet might be related to the composition of the IB sow milk, which is especially rich in minerals, as commented above. Compared with lean pigs (Large White × Dutch Landrace) weaned at 25 days, Iberian 34 days weaned piglets contained more Ca (11-12.7 *vs.* 7.4 g kg^{-1} EBW) and P (7-7.5 *vs.* 4.9 g kg^{-1} EBW) and similar values of Mg (0.33-035 *vs.* 0.3 g kg^{-1} EBW), with a lower mean BW (7.17 *vs.* 7.6 kg) (Everts *et al.*, 1998). Increasing Ca and P body levels at weaning could have important benefits since they play numerous roles; in addition to crucial functions in soft tissue metabolism, they are involved in maintaining bone integrity and preventing leg fractures (Mahan, 1990), and probably in improving the immune response (Galloway *et al.*, 1989; Altons-Mills *et al.*, 2000). Taking into account the weight of the different body fractions and their mineral content, it can be calculated that the carcass contains 60-65% of total body Ca, P and Mg, whereas in head/feet/tail are located nearly 40% of Ca, 33% of P and 27% of Mg. Results of the present assay offer, for the first time, information concerning mineral distribution in body tissues in weaned IB piglets. Unfortunately, to our knowledge, no data are available in the literature for comparison with those reported in the present paper.

Mean ash content per unit of BW gain in the weaned IB piglets (40.6 g kg^{-1} on average; Fig. 2) was considerably higher than that observed for lean breeds (28-29 g kg^{-1}) (Noblet & Etienne, 1986 and 1987; Everts *et al.*, 1998). Everts *et al.* (1998) performed a mineral balance in lean piglets weaned at 25 days, calculating overall data for the litter. According to these authors, 1-kg of litter gain contains about 6.5, 4.5 and 0.27 g of Ca, P and Mg, respectively, whereas in our IB piglets the average values were 11.6, 7.1 and 0.32 g, respectively. However, mineral balance expressed as units of mineral retained per day is not very different between IB and lean breeds being 1.7, 1.2 and 0.07 in the lean piglets (Galloway *et al.*, 1998) and 1.8, 1.13 and 0.05 in IB piglets for Ca, P and Mg, respectively (Table 4). Thus, differences in the mineral composition of piglets' gain between breeds may be due to the increased concentration of minerals in the IB sows' milk and to the lower ADG observed in the IB piglets.

As no differences were found between the trials with respect to final EBW, the tendency observed in trial 1 for a higher weight gain per unit of feed can be explained, at least in part, by the increased deposition of minerals, as the ash content per kg of gain was significantly higher in this trial (Fig. 2), mainly due to the increased Ca concentration (nearly 30% of total ash in trial 1 *vs.* 26% in trial 2). A higher ash content per unit of gain could indicate improved bone mineralization, and would be related to the increased Ca and P body contents and the higher Ca:P ratio found in the weaned piglets in trial 1 (Table 3). These differences could be mainly explained by the higher mineral content of milk in trial 1 and also by the significantly increased efficiency of utilization of milk Ca, as 82% and 75% of the Ca supplied in milk was retained in trials 1 and 2, respectively (Table 4). In mineral balance assays performed in 3-week-old piglets fed either a goat milk formula or a cow milk formula (Rutherfurd *et al*., 2006), Ca was retained with an efficiency of 89 and 92%, respectively, values that were inversely related to the Ca:P ratio in the milk. In our assay no differences between trials were observed in the Ca:P ratio of milk (1.33 and 1.35 in trials 1 and 2, respectively). A possible involvement of vitamin D in the increased Ca utilization found in the trial performed at the higher temperature cannot be discounted. Vitamin D (through its metabolite, the 1.25-dihydroxyvitamin D) is known to play an essential role in Ca absorption, and ambient temperature has been suggested as a contributing factor in the maintenance of adequate levels of vitamin D (Devgun, 1991). The efficiency of mineral retention in the study of Rutherfurd *et al*. (2006) were 96.2% and 55.3% for P and Mg, respectively, with no differences found between goat and cow milk. These values were higher than the mean percentages obtained in the present assay with piglets suckling IB sows' milk: 66% and 49% for P and Mg, respectively, although it should be noted that ash from IB sow's milk is retained with a higher efficiency than any other nutrient (Aguinaga *et al*., 2011a).

In conclusion, new data on the mineral composition of sow´ IB milk and of weaned piglets are shown in the present paper. The milk from IB sows supplies higher contents of Ca, P and Mg to the suckling piglet than those described in lean breeds. Body ash content in IB piglets at weaning is also higher than in conventional pigs, especially due to increased values of Ca and P. Significant differences were found between trials conducted under different thermal conditions, as sow milk and IB suckling piglets resulted significantly enriched in minerals at higher temperature. Also, an improved milk conversion efficiency (gain/food ratio) was found in the hottest ambient, which could be partly explained by an increased body deposition of minerals and a better utilization of dietary Ca. Variations in mineral metabolism found between trials could be presumably due to differences in ambient temperature, although interferences with other unknown factors cannot be discarded. Due to the importance of minerals in producing healthy animals at weaning, and the role of Ca, P and Mg in bone mineralization, the observed changes suggest the need for further studies to investigate the mechanisms involved in the utilization of minerals from the IB sows' milk at different environmental temperatures. Moreover, present findings provide a useful starting point for formulating milk-replacer diets for Iberian piglets.

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