Trop Anim Health Prod (2018) 50:477–484
https://doi.org/10.1007/s11250-017-1456-8

REGULAR ARTICLES

Supplementation of pig diets in the growth and termination phases with different calcium sources

Ana Lúcia Almeida Santana[1] · **Paulo Levi de Oliveira Carvalho**[2] · **Eliseu Carlos Cristofori**[2] · **Poliana Caroline da Silva Chambo**[2] · **Mariana Barbizan**[2] · **Ricardo Vianna Nunes**[2] · **Cristine Regina Gregory**[2] · **Jansller Luiz Genova**[2]

Received: 21 April 2017 / Accepted: 24 October 2017 / Published online: 7 November 2017

Abstract The aim of this study was to evaluate the effect of supplementation of pig diets in the growth and termination phases with different calcium sources. In experiment I, 36 whole males were distributed in randomized blocks in six groups, with six replications. A basal diet was formulated to meet the animals' nutritional requirements except for calcium (0.09%), and the sources evaluated (calcitic limestone, monodicalcium phosphate, calcinated bone flour, and oyster flour) replaced the basal diet to provide 0.59% of total calcium. To determine the endogenous calcium, a diet containing low calcium (0.019%) was given simultaneously to another group of animals. Feces and urine were collected for determination the coefficients of apparent and true digestibility. In experiment II, 160 piglets were distributed in randomized blocks in four treatments, with five replications and four animals per experimental unit. Carcass and performance parameters, calcium concentration in bone and serum, and bone parameters were evaluated. The data were submitted to analysis of variance and factorial. The calcium source did not influence the digestibility coefficients determined by total collection ($P > 0.05$). The digestibility of Ca from oyster flour estimated by collection with an indicator was higher than that from the other sources ($P < 0.05$). Calcium sources did not interfere in the evaluated parameters ($P > 0.05$). The sources studied in this work can be used to supplement growing pigs' diets.

✉ Ana Lúcia Almeida Santana
zootecana@gmail.com

[1] Universidade Federal da Bahia, Ondina Campus, Salvador, BA 40170-110, Brazil

[2] Universidade Estadual do Oeste do Paraná, Campus of Marechal Cândido Rondon, Marechal Cândido Rondon, PR 85960-000, Brazil

Keywords Calcium deposition · Digestibility · Oyster flour · Pigs

Introduction

In pigs, minerals are not decomposed or synthesized by chemical reactions in the body, and dietary supplementation is required to meet the nutritional requirements of these animals, since corn and soybean, the most commonly used ingredients in feed formulations, contain a relatively low concentration of calcium and moderate amounts of phosphorus. This is a result of the 5 to 25% of phytate content in these ingredients, which makes phosphorus and consequently calcium unavailable, as well as other nutrients, due to the formation of insoluble chelates (Cowieson et al. 2011).

Grain-based feeding without supplementation may result in rickets in young pigs (Suttle 2010). Among the sources that can be used in calcium supplementation are those from calcareous rocks (limestone and phosphates) and those of animal or mollusk origin (bone flour, meat and bones, and oysters).

Normally, bone and oyster flours present calcium concentrations above 30% (Rostagno et al. 2011) and greater solubility in relation to mineral sources, which can positively affect Ca availability. The solubility of the calcium sources is a factor indicative of quality and is strongly correlated with the bioavailability and intestinal absorption of calcium (Melo et al. 2006). However, inorganic sources are more often used in feed because they are found in greater abundance and are of low cost, a situation that may change in coming years, as these sources are not renewable.

Thus, the purpose of the present study was to examine the effect of supplementation of the pig diets in the growth and termination phases with different calcium sources.

Materials and methods

The project was undertaken in the Swine Sector of the Universidade Estadual do Oeste do Paraná—UNIOESTE, in two experiments.

Digestibility test

A total of 36 male pigs hybrid (Biribas'—BP 375) were used, with a mean initial weight of 29.83 ± 1.83 kg, distributed in a randomized block design in six groups, with six replicates and one animal per experimental unit, the initial weight and period being used as blocking factors. The animals were accommodated individually in metabolism cages (Pekas 1968), where they remained for 12 days, comprising 7 days of adaptation to the cages, diets, and regulation of consumption and 5 days for feces and urine collection.

A basal diet was formulated to meet the pigs' nutritional requirements (Rostagno et al. 2011) except for calcium (0.09%), and the evaluated sources replaced the basal diet in varying amounts to provide 0.59% of total Ca. The percentage of the basal diet replaced by the test source replacement was as follows: T1 = calcitic limestone (1.32%), T2 = monodicalcium phosphate (2.46%), T3 = calcinated bone flour (1.48%), and T4 = oyster flour (1.37%). To determine the endogenous calcium excreted in the feces, a diet containing low level of calcium (0.019%) was given simultaneously to another group of animals ($n = 6$) (Table 1).

At the same time, two feces collection methods were evaluated: total collection and fecal indicator. To each, experimental diet was added 1% of Ash Insoluble in Acid (AIA, Celite™), used as a fecal indicator. The amount of feed given to each animal in the collection period was determined according to its consumption in the adaptation phase, adjusted by the animal's metabolic weight ($LW^{0.75}$) (Sakomura and Rostagno 2007), and was given in two daily meals at 8 am and 4 pm. Water was provided freely.

The variables analyzed were as follows: dry matter intake; total calcium intake; calcium consumption of the test source, calcium content in the feed, feces, and urine; and serum calcium excretion and indigestible factor. Data were applied to the formulas to determine the apparent digestibility coefficients (CDACa) and true calcium (CDVCa) of the sources evaluated (Sakomura and Rostagno 2007).

Feces and urine collections were performed according to the procedures described by Sakomura and Rostagno (2007). At the end of the collection period, the animals were subjected to food fasting to collect the blood via the cranial vena cava and centrifuged at (Baby™ Centrifuge I—Model 206 B, FANEM, São Paulo, Brazil) at 1240×*g* for 15 min at 4 °C to obtain the serum, which was transferred to "eppendorf" polyethylene microtubes and frozen for analysis of the total calcium content. The calcium concentration was measured using the Automated Chemistry Analyzer using a commercial kit for analysis of calcium by direct colorimetry (ELITechGroup solutions. Record 80171840050, Vitória, Espírito Santo, Brazil).

The bromatological composition of the experimental diet was determined, as well as that of the initial feed samples and of the feces and urine samples. Protocols to obtain the mineral solution were carried out according to the methodologies described by AOAC (1990), and the analysis of Acid Insoluble Ash (AIA) was performed by digestion with hydrochloric acid (4N), following the procedures of Kavanagh et al. (2001). The mineral solutions obtained by nitroperchloric acid digestion

Table 1 Composition of the basal diet and low calcium

Ingredient	Proximate composition (kg/100 kg)	
	Basal diet	Low calcium diet
Corn	56.55	2.80
Pre-cooked corn1	–	69.92
Soybean meal (45%)	31.60	2.10
Starch	6.00	10.10
Celite	1.00	1.00
Sugar	3.00	9.00
Lysine sulfate (50.7% lys)	0.54	1.82
Soy oil	0.16	–
Salt	0.41	0.42
DL-Methionine	0.18	0.47
L-Threonine	0.15	0.61
Premix mineral-vitamin[a]	0.40	0.40
L-Tryptophan	0.004	0.18
L-Valine	–	0.50
L-Isoleucine	–	0.50
L-Arginine	–	0.19
Total	100	100
Calculated composition		
Calcium total (%)	0.092	0.019
Available phosphorus (%)	0.103	0.027
Metabolizable energy (Mcal/kg)	3.243	3.296
Analisated composition		
Calcium (% DM)	0.098	0.02
Total phosphorus (% DM)	0.321	0.159

Composition per kilogram of product: 99.90 mg folic acid; 4083.38 mg pantothenic acid; 12.50 mg biotin; 1995.66 mg copper; 20.81 mg ethoxyquin; 13.47 g iron; 75 mg iodine; 5500 mg licomycin; 3733.75 mg manganese; 5000 mg niacin; 75 mg selenium; 18.38 g triptophan; 1,499,999.90 UI vitamin A; 250 mg vitamin B_1; 3750 mg vitamin B_{12}; 1000 mg vitamin B_2; 499.99 mg vitamin B_6; 299,999.80 UI vitamin D_3; 5000 UI vitamin E; 29.99 mg vitamin K_3; 20 g zinc; *DM*, dry matter

[a] These are the corn meal previously treated with steam, with low calcium content (0.02%) (Rostagno et al. 2011)

(4:1) were read in an atomic absorption equipment (AAE) to obtain the total calcium concentration in the samples.

The minimum (25.84 ± 3.51 °C) and maximum (29.89 ± 3.26 °C) temperature of the metabolism room was recorded using a maximum and minimum analogue thermometer, which was installed in the center of the room at a height corresponding to that of the animals.

The normality of experimental error between treatments for the analyzed variables was previously evaluated using the Shapiro-Wilk test. The effects of the calcium sources and collection methodologies on the dependent variables were verified through analysis of variance, using the Statistical Analysis System (SAS 2002). The Tukey test and a 5% level of significance were adopted in all statistical analyses.

Performance testing

A total of 160 hybrid pigs (Biribas'—BP 375) (80 male immunocastrated and 80 female) with an initial weight average of 28.87 ± 3.23 kg were distributed in a randomized block design, in four treatments, with five replicates and four animals per experimental unit. The initial weight of the animals was used as blocking factors. The treatments consisted in four sources of calcium: T1 = calcitic limestone, T2 = monobloc calcium phosphate, T3 = calcined bone flour, and T4 = oyster flour. The animals were housed in a growth and finishing shed consisting of bays of 5.8 m^2, with semiautomatic feeders and pacifier type drinkers, where they remained for 113 days.

The diets were formulated to meet the nutritional requirements in the different growth phases (growth—30 to 70 kg; termination—70 to until slaughter) (Rostagno et al. 2011), except for calcium (Table 2). The feed and water were freely supplied.

The evaluated variables were as follows: final weight, daily feed intake, daily weight gain, and feed conversion; carcass quantitative and qualitative parameters; concentration of calcium in serum and bone.; weight, length, Seedor index,

Table 2 Composition of diets with different sources of calcium for pigs in the growing phase

Ingredient	Growth				Termination			
	LC	MP	BMC	OM	LC	MP	BMC	OM
Centesimal composition (%)								
Corn	63.84	63.53	65.53	64.27	80.89	80.27	81.41	80.75
Soybean meal (45%)	30.82	30.48	30.10	30.37	16.60	16.71	16.51	16.62
Soy oil	1.46	1.73	1.07	1.44	0.13	0.34	–	0.22
Salt	0.41	0.41	0.41	0.41	0.33	0.33	0.33	0.33
Lysine sulfate (50.7% lys)	0.58	0.57	0.58	0.57	0.37	0.37	0.38	0.37
DL-Methionine	0.17	0.17	0.17	0.17	0.02	0.02	0.02	0.02
L-Threonine	0.15	0.15	0.15	0.15	0.04	0.04	0.04	0.04
L-Tryptophan	0.01	0.01	0.01	0.01	–	–	–	–
Premix[a]	0.50	0.50	0.50	0.50	0.40	0.40	0.40	0.40
Limestone (calcitic)	1.32	–	–	–	0.82	–	–	–
Monodicalcium phosphate	–	2.46	–	–	–	1.53	–	–
Bone meal calcined	–	–	1.48	–	–	–	0.92	–
Oyster meal	–	–	–	1.37	–	–	–	0.85
Monoammonium phosphate	0.74	–	–	0.74	0.40	–	–	0.40
Total	100	100	100	100	100	100	100	100
Calculated composition								
Total calcium (%)	0.59	0.59	0.59	0.59	0.37	0.37	0.37	0.37
Calcium digestible (%)	0.41	0.42	0.39	0.42	0.26	0.27	0.24	0.27
Available phosphorus (%)	0.30	0.53	0.32	0.30	0.19	0.33	0.22	0.19
Metabolizable energy (Mcal/kg)	3.23	3.24	3.24	3.23	3.24	3.24	3.23	3.24

LC, limestone (calcitic); *MP*, monodicalcium phosphate; *BMC*, bone meal calcined; *OM*, oyster meal; *DM*, dry matter. Calcium digestible = obtained by the total collection method

[a] Composition per kilogram of product: 80.97 mg acid folic; 3269.04 mg pantothenic acid; 13.75 g zinc bacitracin; 8.75 mg biotin; 2492.29 mg copper; 222.86 mg ethoxyquin; 23.89 g iron; 299.90 mg iodine; 14.97 g manganese; 4024 mg niacin; 65.70 mg selenium; 35.52 g triptophano; 11,907,990.80 UI vitamin A; 196.98 mg vitamin B_1; 823.20 mg vitamin B_2; 376.30 mg vitamin B_6; 238,599.50 UI vitamin D_3; 4094 UI vitamin E; 254 mg vitamin K_3; 37.50 g zinc

maximum strength, and resistance to metatarsal breaking; and the carcass parameters.

Feed supply, leftovers, and wastes were recorded daily, and values are used to calculate daily feed intake (DFI), daily gain of weight (DWG), and feed conversion (G/F ratio) of the experimental unit. The animals were weighed weekly, and at the end of the finishing phase, the animals were weighed to obtain the final weight (FW), as well as the measurement of backfat thickness and loin depth at position P2 using the Sono-Grader device (Renco™). To evaluate the serum calcium concentration, the animals were subjected to a 12-h jejeum followed for blood collection via cranial vena cava. The blood was then centrifuged for serum extraction and used for calcium determination by direct colorimetry, using a commercial kit.

At the end of the experiment, the animals were sent to slaughter in a commercial slaughterhouse, and 80 animals were randomly selected, 20 per treatment, for tissue collection. The quantitative carcass characteristic evaluations (backfat thickness, loin depth, carcass weight, amount of meat) were carried out in a refrigerator using a Hennessy pistol. In order to evaluate qualitative parameters, samples of Longissimus dorsi were removed from the eighth and tenth vertebrae region in the left half of the carcass, on which analyses of marbling and firmness were performed using a pre-established numerical scale. Analyses of the L* (Brightness), a* (red-green component), and b* (yellow-blue component) using a Konica Minolta's portable CR-400 colorimeter, drip water loss, thawing and cooking, and shear force were performed according to Bridi and Silva (2009).

The paws were collected, cleaned, and removed from the third and fourth metatarsal bones. They were then weighed and measured, and these data were used to obtain the Seedor index (bone weight/length) (Seedor et al. 1991). The metatarsal bones were then pre-dried in a forced ventilation oven at 55 °C and sent for breakage or shear strength analysis using a Shimadzu equipment (AGX PLUS 100 KN) at a standard temperature of 23 °C. Dry matter content and mineral matter analyses, as well as determination of the calcium concentration in the bone, were carried out (AOAC 1990).

The minimum (23.67 ± 3.39 °C) and maximum (27.11 ± 3.65 °C) temperatures inside the shed were obtained using a maximum and minimum analogue thermometer, which was installed in the center of the shed at a height corresponding to that of the animals.

The normality of the experimental error between treatments for the analyzed variables was previously evaluated using the Shapiro-Wilk test. The effects of calcium sources on the dependent variables were verified through analysis of variance. Final weight (FW) lsmeans, daily feed intake (DFI), daily gain of weight, and feed conversion (F/G ratio) were estimated considering correction of the observed means for the covariate "initial weight." The Tukey test and the 5% level of significance were adopted in all statistical analyses (SAS 2002).

Results

Digestibility

The calcium balance (Table 3) shows that the sources evaluated did not interfere with the parameters related to this mineral ($P > 0.05$). The apparent and true digestibility determined by total fecal collection were not influenced by the calcium source ($P > 0.05$); however, the digestibility coefficients estimated by the collection with indicator varied according to the sources of calcium ($P < 0.05$). The digestible calcium calculated from the true digestibility is in Table 3.

Performance and carcass characteristics

The performance parameters were not influenced by the calcium sources ($P > 0.05$). In relation to the carcass quantitative-qualitative characteristics (Table 4), the sources of calcium did not have a significant effect on the pig carcass ($P > 0.05$). The bone parameters were not influenced by the calcium sources ($P > 0.05$). Analysis of variance indicated that there was no influence of the calcium source on the serum calcium and phosphorus concentration ($P > 0.05$) (Table 5).

Discussion

Digestibility

The results obtained for the calcium balance and the respective digestibility coefficients show that the different calcium sources presented similar behavior in relation to calcium digestibility in growing pigs. These results are relevant since calcium digestibility provides an estimate of its bioavailability (González-Vega and Stein 2014). Thus, the calcium contained in the sources used apparently presented similar absorption and was available for use by the animal with 59% of the calcium being retained.

Of the mean quantity of calcium consumed by the animals (4.49 g/d), 35 and 2% of the calcium was eliminated in feces and urine, respectively. The primary route of calcium excretion in pigs is the fecal route, which is an important mechanism for controlling mineral homeostasis. Thus, the fecal calcium comprises the non-absorbed dietary fraction as well as the endogenous fecal fraction (Sakomura and Rostagno 2007), eliminated from mucosal cells, saliva, gastric juice, pancreatic juice, and bile (Cozzolino 2009), justifying the 33% higher value for fecal excretion compared with urinary excretion.

Table 3 Calcium digestible and obtained by direct and indirect methods

Parameters	LC	MP	BMC	OM	*P* value
Total calcium (%)[a]	36.92	20.34	34.48	36.20	
Ca^{2+} consumed (g/d)	4.69 ± 3.05	4.63 ± 3.51	4.55 ± 2.29	4.59 ± 2.87	0.816
Ca^{2+} source feces (g/d)	1.63 ± 1.02	1.48 ± 2.57	1.73 ± 1.59	1.50 ± 1.66	0.540
Ca^{2+} endogenous (g/d)	0.197 ± 0.11	0.194 ± 1.23	0.191 ± 0.08	0.189 ± 0.10	0.802
Ca^{2+} urine (g/d)	0.10 ± 0.13	0.09 ± 0.11	0.09 ± 0.10	0.116 ± 0.15	0.249
Ca^{2+} retained (%)	62.97 ± 2.56	65.89 ± 3.74	59.42 ± 3.10	64.85 ± 2.70	0.514
Ca^{2+} in serum (mg/dL)	10.50 ± 0.67	10.16 ± 0.67	10.16 ± 0.54	10.20 ± 0.25	0.460
Method direct					
ADC (%)	65.16 ± 3.01	67.87 ± 2.40	61.47 ± 7.78	67.44 ± 5.66	0.470
TDC (%)	69.36 ± 3.02	72.06 ± 2.41	65.67 ± 7.79	71.64 ± 5.66	0.471
Digestible calcium (%)	25.62	14.66	22.64	25.92	–
Method indirect					
ADC (%)	59.28 ± 2.11[b]	60.42 ± 4.48[ab]	52.25 ± 2.80[c]	66.38 ± 6.43[a]	<0.01
TDC (%)	59.31 ± 2.12[b]	60.44 ± 4.47[ab]	52.28 ± 2.81[c]	66.41 ± 6.44[a]	<0.01
Digestible calcium (%)	21.99	12.29	18,03	24.04	–

LC, limestone (calcitic); *MP*, monodicalcium phosphate; *BMC*, bone meal calcined; *OM*, oyster meal; *ADC*, apparent digestibility coefficient; *TDC*, true digestibility coefficient; *P*, probability; mean ± standard deviation of the mean; *(g/d)*, grams per day. [a,b] Means followed by different letters in the lines differ from each other by the test of Tukey (5%)

[a] Analyzed value

The digestibility values estimated by the indicator collection showed a difference between the calcium sources, and oyster flour provided better calcium digestibility compared with calcareous limestone. According to Bünzen et al.

Table 4 Mean values of minimum squares (lsmeans) of the performance parameters and carcass characteristics of finished pigs

Parâmetros	Source of calcium				*P* value
	LC	MP	BMC	OM	
Final weight (kg)	127.92 ± 13.52	127.13 ± 11.51	127.91 ± 12.61	125.50 ± 10.12	0.636
Daily feed intake (kg)	2.50 ± 0.24	2.46 ± 0.20	2.45 ± 0.22	2.41 ± 0.22	0.164
Daily weight gain (kg)	0.99 ± 0.09	0.98 ± 0.08	0.98 ± 0.09	0.97 ± 0.07	0.598
Feed efficiency (kg:kg)	2.52 ± 0.14	2.49 ± 0.10	2.49 ± 0.11	2.49 ± 0.18	0.909
Quantitative characteristics of the carcass					
Carcass weight (kg)	94.35 ± 9.35	90.58 ± 8.94	92.54 ± 8.90	92.21 ± 6.65	0.271
Backfat thickness (mm)	15.76 ± 2.12	16.64 ± 3.60	14.15 ± 2.25	15.18 ± 2.87	0.067
Loin depth (mm)	59.69 ± 2.18	60.42 ± 5.61	60.38 ± 4.37	60.36 ± 2.35	0.959
Amount of meat (kg)	53.08 ± 4.28	50.69 ± 3.15	52.67 ± 4.17	51.75 ± 2.89	0.487
Qualitative characteristics of the carcass					
Loss of water by dripping (%)	5.31 ± 2.60	4.83 ± 2.33	5.75 ± 3.04	5.59 ± 2.48	0.746
Loss of water by thawing (%)	7.82 ± 2.58	9.36 ± 2.91	7.13 ± 2.05	8.60 ± 2.86	0.062
Loss of water by cooking (%)	22.82 ± 4.51	23.24 ± 5.85	23.18 ± 3.39	21.89 ± 4.32	0.765
Shear force (kgf)	3.74 ± 0.80	4.06 ± 1.25	3.73 ± 1.02	3.38 ± 0.82	0.237
Luminosidade—L*	49.26 ± 2.95	47.46 ± 2.90	49.51 ± 3.02	48.62 ± 5.33	0.362
Saturação—a*	4.50 ± 1.48	4.82 ± 1.70	4.60 ± 1.66	4.78 ± 1.65	0.926
Tonalidade—b*	3.24 ± 1.53	3.02 ± 1.51	3.30 ± 1.26	3.10 ± 1.34	0.935

LC, limestone (calcitic); *MP*, monodicalcium phosphate; *BMC*, bone meal calcined; *OM*, oyster meal; *P*, probability; *mm*, millimeters; *Kgf*, kilograms force; *Kg*, kilograms; *(g/d)*, grams per day; mean ± standard deviation of the mean. [a,b] Means followed by different letters in the column differ from each other by the *F* test (5%)

Table 5 Bone parameters assessed in finished pigs (126.94 ± 10.96 kg) fed with different sources of calcium

Parameter	Source of calcium				*P* value
	LC	MP	BMC	OM	
Metatarsal length (mm)	75.17 ± 1.54	75.78 ± 3.49	74.20 ± 3.47	74.23 ± 3.31	0.728
Fresh weight (g)	20.44 ± 1.47	20.97 ± 1.48	21.39 ± 2.05	20.27 ± 2,38	0.710
Pre-dry weight (g)	17.79 ± 1.35	18.17 ± 1.35	17.79 ± 2.03	18.03 ± 2.35	0.981
Seedor index (mg/mm)	271.53 ± 0.01	279.18 ± 0.01	286.03 ± 0.02	273.40 ± 0.02	0.618
Maximum force applied (N)	1528.27 ± 330	1539.97 ± 317	1458.80 ± 280	1353.36 ± 322	0.726
Maximum force applied (Kgf)	155.84 ± 33.67	157.03 ± 32.33	148.75 ± 28.61	138.00 ± 32.93	0.726
Bone resistance (MPa)	19.92 ± 3.07	21.47 ± 5.10	19.25 ± 3.81	17.77 ± 4.77	0.536
Bone resistance (Kgf/cm^2)	203.14 ± 31.27	219.00 ± 51.97	196.29 ± 38.88	181.24 ± 48.65	0.535
Calcium in bone (mg/dL)	119.12 ± 19.69	140.92 ± 37.75	131.59 ± 24.62	114.26 ± 7.20	0.07
Calcium in serum (mg/dL)	10.18 ± 0.82	10.01 ± 0.87	10.65 ± 0.74	9.92 ± 0.94	0.387
Phosphorus in serum (mg/dL)	8.74 ± 0.94	9.37 ± 0.95	9.42 ± 0.89	8.73 ± 0.98	0.149

LC, limestone (calcitic); *MP*, monodicalcium phosphate; *BMC*, bone meal calcined; *OM*, oyster meal; *P*, probability; mean ± standard deviation of the mean; *mm*, millimeters; *g*, grams; *mg*, milligrams; *cm*, centimeter; *dL*, deciliter; *N*, newton; *Kgf*, kilograms force; *MPa*, megapascal

(2009), the inclusion of alternative foods in the diets of pigs has been a constant practice and aims at reducing the costs of food using the raw material often considered as waste in some regions.

However, the indirect method may have underestimated the calcium digestibility, due to the low recovery of the indicator in the feces. As a result, it can overestimate fecal production and the indigestibility factor, underestimating digestibility, as observed in this study, where fecal production in all treatments was higher compared with total fecal collection. Furthermore, diets with animal ingredients may also contribute to overestimation of digestibility values in AIA assays, due to the higher concentrations of mineral residues in these sources (Zanatta et al. 2013).

According to Torres et al. (2009), the determination of the digestibility by direct method requires rigorous control of the intake and excretion, which makes the process more laborious. The indirect method, accomplished through the use of indicator, appeared in an attempt to reduce the work and time spent in this type of experiment, since it is possible to estimate consumption and fecal excretion, presenting advantages over total collection, for simplicity and convenience of use. However, the results obtained in this research, as well as the divergence in the data found in the literature, do not allow to affirm that the total collection can be replaced by the fecal indicator method with Celite™.

In relation to the calcium sources used in the present research, few studies regarding true digestibility are found in the literature, due to the difficulty in determining the endogenous calcium. The fact is that the calcium concentrations contained in these sources are expressed on the basis of total calcium (Rostagno et al. 2011), which results in higher values. Thus, mineral supplementation of pig diets would be more adequate if the feed formulations were based on the digestible calcium content, which would increase the use of dietary calcium by the animal organism, reducing the inclusion level of the sources and consequently the fecal excretion of the unabsorbed calcium fraction.

Performance and carcass characteristics

The performance parameters were similar among the treatments, suggesting that the evaluated sources can be used without impairing animal performance. Fialho et al. (1992), evaluating calcium sources for supplementation for pigs, reported that diets for growth and finishing can be supplemented with calcium from calcareous limestone, oyster flour, gypsum, or calcareous algae (*Lithothamnium calcareum*).

The similar daily intake among treatments may be an indication that the sources included did not modify the sensory feed characteristics nor influence the digestive processes in the gastrointestinal tract. In addition, the established calcium concentrations for each phase (Table 2) and the Ca:P ratio did not have a negative effect on the performance parameters. Excess calcium can reduce the feed palatability; it can also alter the Ca:P ratio, besides reducing the acidity of the stomach, impairing the digestibility of nutrients (Furlan and Pozza 2014).

In the present work, it was not possible to maintain the ratio 2.03:1 of total Ca:P availability established by Rostagno et al. (2011) between treatments, because the monodicalcium phosphate (T2) presents similar concentrations of calcium and phosphorus in its composition. However, it is worth mentioning that the 1.1:1 ratio obtained in the above treatment is within the NRC (1998) guidelines, which suggest ratios of 1:1 and 1.25:1 for corn and soybean feed and the results show

that this was not an interfering factor in the animals' performance, suggesting that the monodicalcium phosphate can be used as a mixed source.

The carcass parameters were not influenced by the different treatments (Table 4). It is worth noting that the literature is scarce in studies related to calcium sources and carcass characteristics of pigs, despite the relationship between calpain, an enzyme that regulates the meat tenderization process, and calcium. The factors and mechanisms responsible for the changes occurring during meat maturation are not fully understood (Koohmaraie and Geesink 2006). Some factors and enzymes are suggested, such as calpain, a calcium-dependent peptidase, which is directly related to the process of meat tenderization (Lage et al. 2009). The present results show that the different sources produce similar meat qualitative characteristics, such as the shear force, indicative of softness.

The results obtained for the carcass qualitative characteristics indicate that the sources evaluated do not compromise the final product quality. According to Argüello et al. (2005), the meat quality characteristics of major economic importance are the appearance (color, brightness, and cut presentation) and the softness, perceived in the tasting, since they determine the global acceptance of the cuts. Softness, as previously described, can be influenced by plasma calcium levels and, consequently, intracellular calcium, which is a mineral element necessary for the activation of the enzyme related to the process of meat softening.

The different calcium sources did not influence bone parameters (Table 5). The metatarsus in pigs is the fastest growing bone and is sensitive to calcium and phosphorus deficiencies softening. It is widely used in resistance studies, since bone is a dynamic tissue that constantly undergoes formation and reabsorption (Schmiel et al. 2016). Approximately 60 to 70% of the bone weight is composed of hydroxyapatite crystals formed by the association of calcium and phosphorus derived from the diet (Henn 2010), indicating that unbalanced or deficient feed would result in poor bone formation and even slow growth.

No signs of calcium deficiency were observed in the animals that consumed the diets supplemented with the different sources. The most severe signs of deficiency can be visually observed as they frequently result in locomotion problems. According to Teixeira et al. (2005), calcium and phosphorus deficiency can result in bone mineralization, reduced bone growth, and/or poor growth. Adult pigs consuming deficient diets mobilize calcium and phosphorus from their bones, resulting in brittle bones. These authors did not find differences in bone length, weight, and resistance when replacing dicalcium phosphate with monodicalcium and reported mean values of length (70.60 mm) and weight (19.42 g) similar to those obtained in the present study.

The Seedor index, obtained by the relation between weight and bone length, is used as an indicator of bone density (Murakami et al. 2009; Almeida Paz et al. 2010), along with the maximum force applied to the bone and the bone strength. Bone density is the result of mineralization, that is, of the amount of mineral deposited, and the higher the deposition, the greater the density and consequently the resistance. In this study, bone density was similar among the treatments, verifying that the sources were equally efficient for mineral deposition in bone.

The serum calcium concentration remained constant. In the case of calcium, this constancy is common, due to the control mechanisms that exist to ensure the ideal plasma level is maintained (Évora et al. 1999). Serum calcium concentration is one of the variables that is controlled with the greatest precision in animals. Among the calcium control mechanisms, bone mobilization and renal reabsorption, involving parathyroid hormone, calcitonin and vitamin D (Maiorka and Macari 2008) are most important. The mean concentration of total calcium in the pigs' serum was 10.09 mg/dL, which is within the range recommended in the literature (Henn 2010). According to González et al. (2000), the level of plasma calcium in animals is constant, but can vary between 8 and 12 mg/dL. In the termination phase, the phosphorus concentration remained constant, with a mean of 9.06 mg/dL (Table 5), being within the recommended (6.96 to 10.65 mg/dL) (Friendship and Henry 1992).

The different calcium sources promoted similar effects in terms of calcium digestibility and animal performance, as well as on calcium deposition in bone. This suggests that any of the sources evaluated could be used for calcium supplementation in pig feed within the established levels in the growth and termination stages, without causing injury and with acceptable performance.

Compliance with ethical standards

Statement of animal rights The project was undertaken in accordance with the regulations approved by the UNIOESTE Ethics Committee on Animal Use (Protocol No. 80/14). The manuscript does not contain clinical studies or patient data.

Conflict of interest The authors declare that they have no conflicts of interest.

References

Almeida Paz, I.C.L., Mendes, A.A., Balog, A., Martins, M.R.F.B., Almeida, I.C.L., Fernandes, B.C.S., Milbradt, E.L., Vulcano, L.C., Komiyama, C.M. and Cardoso, K.F.G., 2010. Níveis de cálcio e avaliação óssea e de ovos de avestruzes reprodutoras. Archivos de Zootecnia, 59, 459–462

AOAC - Association of Official Analytical Chemistry. Official methods of analysis, 1990. Washington, D.C., 15th, 684p

Argüello, A., Castro, N., Capote, J. and Solomon, M., 2005. Effects of diet and live weight at slaughter on kid meat quality. Meat Science, 70, 173–179

Bridi, A.M. and Silva, C.A., 2009. Avaliação da carne suína, (Publishing company Midiograf, Londrina)

Bünzen, S., Rostagno, H.S., Lopes, D.C., Gomes, P.C., Hashimoto, F.A.M., Apolônio, R.L. and Borsatto, C.G., 2009. Digestibilidade aparente e verdadeira do fósforo de alimentos de origem animal para suínos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 61, 903–909

Cowieson, A.J., Wilcock, P. and Bedford, M.R., 2011. Super-dosing effects of phytase in poultry and other monogastrics. World's Poultry Science Journal, 67, 225–235

Cozzolino, S.M.F., 2009. Cálcio. In.: Silva, A.G.H. and Cozzolino, S.M.F. (eds), Biodisponibilidade de Nutrientes, (Manole, Barueri, 3rd ed), 513–523

Évora, P.R.B., Reis, C.L., Ferez, M.A., Conte, D.A. and Garcia, L.V., 1999. Distúrbios do equilíbrio hidroelétrico e do equilíbrio acidobásico—uma revisão prática. Medicina, 32, 451–469

Fialho, E.T., Barbosa, H.P., Bellaver, C., Gomes, P.C. and Junior, W.B., 1992. Avaliação nutricional de algumas fontes de suplementação de cálcio para suínos. Biodisponibilidade e desempenho. Revista Brasileira de Zootecnia, 21, 891–905

Friendship, R.M. and Henry, S.C., 1992. Cardiovascular system, hematology, and clinical chemistry. In: Leman, A.D., Straw, B.E., Mengeling, W.L., D'allaire, S. and Taylor, D.J. (eds), Diseases of swine, (Ames: Iowa State University Press, 7th ed.), 3–11

Furlan, A.C. and Pozza, P.C., 2014. Exigências de Minerais para Suínos. In: Sakomura, N.K., Silva, J.H.V., Costa, F.G.P., Fernandes, J.B.K. and Hauschild, L. (eds), Nutrição de Não Ruminantes, (Funep, Jaboticabal, 1st ed.), 403–423

González F.H.D., Conceição T.R., Siqueira A.J.S., La Rosa V.L., 2000. Variações sangüíneas de uréia, creatinina, albumina e fósforo em bovinos de corte no Rio Grande do Sul. A Hora Veterinária, 20, 59–62.

González-Vega, J.C. and Stein, H.H., 2014. Calcium digestibility and metabolism in pigs (Invited review). Asian-Australian Journal Animal Science, 27, 1–9

Henn, J.D. Bioquímica do tecido ósseo. 2010. http://www6.ufrgs.br/favet/lacvet/restrito/pdf/osso_henn.pdf. Accessed in: 13 of November of 2015

Kavanagh, S., Lynch, P.B., Mara, O.F. and Cafrey, P.J. 2001. A comparison of total collection and marker technique for the measurement of apparent digestibility of diets for growing pigs. Animal Feed Science and Technology, 89, 49–58

Koohmaraie, M. and Geesink, G.H., 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Science, 74, 34–43

Lage, J.F., Oliveira, I.M. and Paulino, P.V.R., 2009. Papel do sistema calpaína-calpastatina sobre a proteólise muscular e sua relação com a maciez da carne em bovinos de corte. Revista electrónica de Veterinarias 10, 1–19

Maiorka, A. and Macari, M., 2008. Absorção de minerais. In: Macari, M., Furlan, R.L. and Gonzales, E. (eds), Fisiologia Aviária Aplicada a Frangos de Corte, (Funep, Jaboticabal, 2nd ed.), 167–173

Melo, T.V., Mendonça, P.P., Moura, A.M.A., Lombardi, C.T., Ferreira, R.A. and Nery, V.L.H., 2006. Solubilidad in vitro de algunas fuentes de cálcio utilizadas em alimentacion animal. Archivos de Zootecnia, 55, 297–300

Murakami, A.E., Garcia, E.R.M., Martins, E.N., Moreira, I., Scapinello, C. and Oliveira, A.F.G., 2009. Efeito da inclusão de óleo de linhaça nas rações sobre o desempenho e os parâmetros ósseos de frangos de corte. Revista Brasileira de Zootecnia, 38, 1256–1264

NRC. Nutrient requirements of swine., 1998. (National Academy Press, Washington, DC)

Pekas, J.C., 1968. Versatible swine labotarory apparatus for physiologic and metabolic studies. Journal of Animal Science, 2, 1303–1306

Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C., Oliveira, R.F., Lopes, D.C., Ferreira, A.S., Barreto, S.L.T. and Euclides, R.F., 2011. Tabelas brasileiras para aves e suínos, (UFV, Viçosa)

Sakomura, N.K. and Rostagno, H.S., 2007. Métodos de Pesquisa em Animais Monogástricos. (Funep, Jaboticabal)

SAS. System for Windows (Statistical Analysis System), 2002-2008. Version 9.2, (Cary: SAS Institute Inc)

Schmiel, S.E., Yang, J.A., Jenkins, M.K. and Mueller, D.L., 2016. Adenosine A2a receptor signals inhibit germinal center T follicular helper cell differentiation during the primary response to vaccination. The Journal of Immunology, 198, 623–628

Seedor, J.G., Quarruccio, H.A. and Thompson, D.D., 1991. The biophosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. Journal of Bone and Mineral Research, 6, 339–346

Suttle, N.F., 2010. Mineral nutrition of livestock, (Cambridge: CABI)

Teixeira, A.O., Lopes, D.C., Lopes, J.B., Vitti, D.M.S.S., Gomes, P.C., Lopes, J.B., Costa, L.F., Ferreira, V.P.A., Pena, S.M., Moreira, J.A. and Bünzen, S., 2005. Níveis de Substituição do Fosfato Bicálcico pelo Monobicálcico em Dietas para Suínos nas Fases de Crescimento e Terminação. Revista Brasileira de Zootecnia, 34, 142–150

Torres, L.C.L., Ferreira, M.A., Guim, A., Vilela, M.S., Guimarães, A.G. and Silva, E.C., 2009. Substituição da palma-gigante por palma-miúda em dietas para bovinos em crescimento e avaliação de indicadores internos. Revista Brasileira de Zootecnia, 38, 2264–2269

Zanatta, C.P., Gabeloni, L.R., Félix, A.P., Brito, C.B.M., Oliveira, S.G. and Maiorka, A., 2013. Metodologias para determinação da digestibilidade de dietas contendo fontes proteicas vegetal ou animal em cães. Ciência Rural, 43, 696–701