RESEARCH ARTICLE

J Anim Sci Technol 2022;64(5):871-884
https://doi.org/10.5187/jast.2022.e53

JAST
Journal of Animal Science and Technology
pISSN 2672-0191 eISSN 2055-0391

Calcium chloride is a better calcium source rather than calcium carbonate for weanling pigs

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Received: Apr 29, 2022
Revised: Jun 2, 2022
Accepted: Jun 16, 2022

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Competing interests
No potential conflict of interest relevant to this article was reported.

Funding sources
Not applicable.

Abstract

Two experiments were conducted to evaluate the effects of calcium (Ca) levels in weanling pigs (Landrace × Yorkshire × Duroc). In experiment 1, one hundred and eighty weanling pigs were randomly allotted to one of the three treatments. The treatments were low (Ca 0.60% in phase 1 and 0.50% in phase 2), standard (Ca 0.72% in phase 1 and 0.66% in phase 2), and high (Ca 0.84% in phase 1 and 0.72% in phase 2). In experiment 2, hundred and forty weanling pigs were randomly assigned to one of four treatments differing in Ca levels (high and low) and sources ($CaCl_2$ and $CaCO_3$) in a 2 × 2 factorial arrangement. There were 10 pigs per replicate in both experiments, with 6 replicates in each treatment, and they were conducted in two phases (phase 1, days 0–14; phase 2, days 15–28). In experiment 1, body weight (BW), average daily gain (ADG), and growth to feed ratio (G/F) increased as the Ca level decreased ($p < 0.05$). P digestibility was higher in the low-Ca diet group than in the high-Ca diet group ($p < 0.05$). In experiment 2, the final BW, ADG, and G/F increased in the $CaCl_2$ diet group compared with the case in the $CaCO_3$ diet group ($p < 0.05$). The digestibility of crude protein (CP), Ca, and P was higher in the $CaCl_2$ diet group than in the $CaCO_3$ diet group ($p < 0.05$). Cl^- levels were higher in the $CaCl_2$ diet group than in the $CaCO_3$ diet group ($p < 0.05$). The bicarbonate (HCO_3^-), base excess (BE), and electrolyte balance (EB) levels were lower in the $CaCl_2$ diet group than in the $CaCO_3$ diet group ($p < 0.05$). Hematocrit increased as the Ca level decreased ($p < 0.05$). The HCO_3^- interacted with the Ca sources and thus, affected the Ca levels ($p < 0.05$). Bone ash, Ca, and P were downregulated in the low-Ca diet group compared with the case in the high-Ca diet group. Overall, the low dietary Ca supplementation led to greater growth performance. Furthermore, $CaCl_2$ appeared to be a better Ca source than $CaCO_3$ because of the greater digestibility of CP, Ca, and P, and improved EB.

Keywords: Electrolyte, Digestibility, Growth performance, Absorption, Pig

INTRODUCTION

In recent years, feed cost has been a critical factor in the swine industry, accounting for 50%–85% of the total production expenses [1]. Improving feed efficiency can be a sound solution for reducing feed costs. Recently, the effects of dietary concentration and the relative ratio of calcium (Ca) to phosphorus (P) on growth performance, feed efficiency, and bone mineralization have been mainly discussed with

Acknowledgements
Not applicable.

Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Mun JY, Kim JS.
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Ethics approval and consent to participate
The animal care and experimental protocols used in the present study were approved by the Institution of Animal Care and Use Committee, Kangwon National University (Ethical code: KW-210707-1).

controversial [2,3]. Ca and P are widely acknowledged as essential microminerals in pig nutrition and the deposition of lean tissue, maintenance and synthesis of skeletal structure, and many other functions [4–6]. The safety of P in diets of swine is frequently calculated using small margins. Typically, P excretion will increase when it is overestimated [7,8]. In contrast, the danger of an abundant Ca supply in swine diets occurs because of its low cost and the absence of environmental issues [9].

The digestibility and absorption of both minerals are affected by the Ca and P ratios [10]. Therefore, swine diets should supply appropriate Ca and P ratios as well as the individual requirements for both minerals. An imbalance in the Ca and P ratio leads to a reduction in growth performance and bone mineralization, particularly when insufficient P is formulated in pig diets [11–14]. Furthermore, the indigestible Ca-phytate-P structure in the small intestine is created by a high Ca or Ca and P ratio, which exacerbates the function of exogenous phytases [15,16].

Limestone (calcium carbonate [$CaCO_3$]) is mainly used as the Ca source. However, $CaCO_3$ frequently binds to acids in the gastrointestinal tract, reducing nitrogen and P digestibility by lowering protein and P solubility [17,18]. Therefore, dietary $CaCO_3$ levels are negatively correlated with P consumption. Moreover, high dietary $CaCO_3$ and P supplementation in swine diets leads to excessive pig manure excretion during intensive production, resulting in environmental issues. One of the most widely employed nutritional strategies in recent years to overcome environmental P pollution and promote P utilization in farm animals is the incorporation of phytase in animal diets. Calcium chloride ($CaCl_2$) is an alternative to $CaCO_3$ because of its high solubility in water. This may cause differences in bioavailability and digestibility. Consequently, the main aim of our current study was to determine how changes in dietary Ca (levels and sources) affect the growth performance, nutrient digestibility, blood profile, and bone mineralization of growing pigs.

MATERIALS AND METHODS

The protocol for this study was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea (ethical code: KW-210707-1).

Animals and experimental design

Experiment 1

A total of hundred and eighty growing pigs (Landrace × Yorkshire × Duroc [LYD]) with an average body weight (BW) of 10.37 ± 0.01 kg were randomly allotted to one of the three treatments on the basis of initial BW. The experiment was conducted in a randomized complete block design at the Research Center of Animal Life Sciences at Kangwon National University. There were 10 pigs per replicate, with 6 replicates in each treatment. The treatments included low (Ca 0.60% in Phase 1 and 0.50% in Phase 2), standard (Ca 0.72% in Phase 1 and 0.66% in Phase 2), and high (Ca 0.84% in Phase 1 and 0.72% in Phase 2). The experimental diets were supplemented for 28 d in two phases: phase 1 (days 0–14) and phase 2 (days 15–28). The pigs were group-housed in partially slatted concrete floor pens with a 2.80 m × 5.00 m pen size. All pens contained a self-feeder and nipple drinker to allow access to feed and water ad libitum. The diets were formulated to provide all nutrients to meet or exceed the nutrient requirements listed in the NRC [19], except Ca (Table 1).

Experiment 2

Two hundred and forty growing pigs (LYD) with an average BW of 10.32 ± 0.01 kg were randomly assigned to one of the four treatments based on initial BW. A randomized complete block design was conducted at the Research Center of Animal Life Sciences of Kangwon National University.

https://doi.org/10.5187/jast.2022.e53

Table 1. Ingredient composition of diets in experiment 1 (as-fed diets), phase 1 and 2[1)]

Ca level	Phase 1			Phase 2		
	Low	Standard	High	Low	Standard	High
Ingredients (%)						
Corn	44.123	43.808	43.492	53.727	53.305	52.884
Soybean meal	20.223	20.223	20.223	24.305	24.305	24.305
Cookie powder	9.000	9.000	9.000	7.000	7.000.	7.000
Wheat bran	7.500	7.500	7.500	7.000	7.000	7.000
Whey powder_sweet	7.500	7.500.	7.500	-	-	-
Soy oil	2.399	2.399	2.399	3.468	3.468	3.468
Sugar	3.000	3.000	3.000	1.000	1.000	1.000
Fish meal	2.500	2.500	2.500	-	-	-
DCP	0.700	0.700	0.700	0.906	0.906	0.906
Limestone (fine)	0.374	0.689	1.005	0.402	0.824	1.245
L-Lysine_HCl 98% (lysine 78%)	0.588	0.588	0.588	0.474	0.474	0.474
Salt	0.300	0.300	0.300	0.450	0.450	0.450
DL-Methionine_99%	0.319	0.319	0.319	0.238	0.238	0.238
Mineral premix[2)]	0.275	0.275	0.275	0.275	0.275	0.275
L-Threonine_99%	0.306	0.306	0.306	0.192	0.192	0.192
Prohacid advance	0.200	0.200	0.200	0.200	0.200	0.200
ZnO	0.250	0.250	0.250	-	-	-
Choline_50%	0.056	0.056	0.056	0.150	0.150	0.150
Vitamin premix[3)]	0.100	0.100	0.100	0.100	0.100	0.100
L-Tryptophan_100%	0.107	0.107	0.107	0.063	0.063	0.063
Valine (99%)	0.130	0.130	0.130	-	-	-
Phytase	0.050	0.050	0.050	0.050	0.050	0.050
Chemical composition						
ME (kcal/kg)	3,560	3,548	3,536	3.611	3,595	3,580
DM (%)	91.29	91.32	91.34	90.53	90.57	90.60
CP (%)	18.52	18.50	18.48	18.03	18.00	17.97
CF (%)	6.51	6.50	6.49	7.31	7.30	7.29
Ash (%)	5.07	5.38	5.69	4.73	5.14	5.55
Ca (%)	0.60	0.72	0.84	0.50	0.66	0.82
P (%)	0.69	0.69	0.69	0.66	0.66	0.66
Total Ca : total P	0.87 : 1	1.04 : 1	1.22 : 1	0.76 : 1	1.00 : 1	1.24 : 1

[1)]Phase 1: Low, 0.60%; Standard, 0.72%; High, 0.84%; Phase 2: Low, 0.50%; Standard, 0.66%; High, 0.82%.

[2)]Supplied per kg of diet: 100 mg Fe, 6 mg Cu, 4 mg Mn, 0.3 mg Se, 0.14 mg I, 0.25 mg Co.

[3)]Supplied per kg of diet: 16,000 IU vitamin A (palmitate), 2.00 mg vitamin B_1 (thiamin), 5.00 mg vitamin B_2 (riboflavin), 2.00 mg vitamin B_6 (pyridoxine), 0.03 mg vitamin B_{12} (cyanocobalamin), 25.00 mg niacin, 0.40 mg folic acid, 0.05 mg biotin, 5.00 mg ethoxyquin, 2,000 IU vitamin D_3 (cholecalciferol), 75.00 mg vitamin E (dl-α-tocopheryl acetate), 2.00 mg vitamin K_3 (menadione).

DCP, dicalcium phosphate; ME, metabolizable energy; DM, dry matter; CP, crude protein; CF, crude fat.

There were 10 pigs per replicate, with 6 replicates in each treatment. Four diets differing in Ca levels (high and low) and sources ($CaCl_2$ and $CaCO_3$) were formulated and fed to the pigs from day 14 post-weaning (Table 2) in a 2 × 2 factorial arrangement. The pigs were fed a basal diet of mash feed *ad libitum*. The experimental diets were fed for 28 days in two phases: phase 1 (d 0–14), and phase 2 (d 15–28). The pigs were housed in partially slatted concrete floor pens with a pen size of 2.80 m × 5.00 m. All the pens contain a self-feeder and nipple drinker to allow *ad libitum* access

Table 2. Ingredient's composition of diets in experiment 2 (as-fed diets), phase 1 and 2[1)]

Ca level/Ca source	Phase 1				Phase 2			
	Low		Standard		Low		Standard	
	$CaCO_3$	$CaCl_2$	$CaCO_3$	$CaCl_2$	$CaCO_3$	$CaCl_2$	$CaCO_3$	$CaCl_2$
Ingredients (%)								
Corn	44.385	44.156	44.123	43.971	54.151	53.941	53.927	53.764
Soybean meal	20.223	20.223	20.223	20.223	24.305	24.305	24.305	24.305
Cookie powder	9.000	9.000	9.000	9.000	7.000	7.000	7.000	7.000
Wheat bran	7.500	7.500	7.500	7.500	7.000	7.000	7.000	7.000
Whey powder_sweet	7.500	7.500	7.500	7.500	-	-	-	-
Soy oil	2.399	2.399	2.399	2.399	3.468	3.468	3.468	3.468
Sugar	3.000	3.000	3.000	3.000	1.000	1.000	1.000	1.000
Fish meal	2.50	2.500	2.500	2.500	-	-	-	
DCP	0.700	0.700	0.700	0.700	0.906	0.906	0.906	0.906
$CaCl_2$	-	0.341	-	0.526	-	0.388	-	0.565
Limestone (fine)	0.112	-	0.374	-	0.178	-	0.402	-
L-Lysine_HCl 98% (lysine 78%)	0.588	0.588	0.588	0.588	0.474	0.474	0.474	0.474
Salt	0.300	0.300	0.300	0.300	0.450	0.450	0.450	0.450
DL-Methionine_99%	0.319	0.319	0.319	0.319	0.238	0.238	0.238	0.238
Mineral premix[2)]	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275
L-Threonine_99%	0.306	0.306	0.306	0.306	0.192	0.192	0.192	0.192
Prohacid advance	0.200	0.200	0.200	0.200	-	-	-	-
ZnO	0.250	0.250	0.250	0.250	-	-	-	-
Choline_50%	0.056	0.056	0.056	0.056	0.150	0.150	0.150	0.150
Vitamin premix[3)]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
L-Tryptophan_100%	0.107	0.107	0.107	0.107	0.063	0.063	0.063	0.063
Valine (99%)	0.130	0.130	0.130	0.130	-	-	-	-
Phytase	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Chemical composition					-	-	-	-
ME (kcal/kg)	3,560	3,560	3,560	3,560	3,611	3,611	3,611	3,611
DM (%)	91.29	91.29	91.29	91.29	90.53	90.53	90.53	90.53
CP (%)	18.52	18.52	18.52	18.52	18.03	18.03	18.03	18.03
CF (%)	6.51	6.51	6.51	6.51	7.31	7.31	7.31	7.31
Ash (%)	5.07	5.07	5.07	5.07	4.73	4.73	4.73	4.73
Ca (%)	0.50	0.50	0.60	0.6	0.40	0.40	0.50	0.50
P (%)	0.69	0.69	0.69	0.69	0.66	0.66	0.66	0.66
Total Ca : total P	0.72 : 1	0.72 : 1	0.87 : 1	0.87 : 1	0.61 : 1	0.61 : 1	0.76 : 1	0.76 : 1

[1)]Phase 1: Low, 0.60%; Standard, 0.72%; High, 0.84%; Phase 2: Low, 0.50%; Standard, 0.66%; High, 0.82%.

[2)]Supplied per kg of diet: 100 mg Fe, 6 mg Cu, 4 mg Mn, 0.3 mg Se, 0.14 mg I, 0.25 mg Co.

[3)]Supplied per kg of diet: 16,000 IU vitamin A (palmitate), 2.00 mg vitamin B_1 (thiamin), 5.00 mg vitamin B_2 (riboflavin), 2.00 mg vitamin B_6 (pyridoxine), 0.03 mg vitamin B_{12} (cyanocobalamin), 25.00 mg niacin, 0.40 mg folic acid, 0.05 mg biotin, 5.00 mg ethoxyquin, 2,000 IU vitamin D_3 (cholecalciferol), 75.00 mg vitamin E (dl-α-tocopheryl acetate), 2.00 mg vitamin K_3 (menadione).

$CaCO_3$, calcium carbonate; $CaCl_2$, calcium chloride; DCP, dicalcium phosphate; ME, metabolizable energy; DM, dry matter; CP, crude protein; CF, crude fat.

https://doi.org/10.5187/jast.2022.e53

to feed and water. The diets were formulated to provide all nutrients to meet or exceed the nutrient requirements listed in the NRC [19], except Ca.

Growth performance

All experimental pigs were weighed individually on day one of the experiment and the last day of the experiment, and feed consumption was recorded during the entire duration of the experiment. This was used to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G/F) at the end of each experiment.

Nutrient digestibility

To determine the effects of each treatment on nutrient digestibility, Cr_2O_3 was formulated at 0.25% in the treatments. The pigs were fed for five days with diets before collection. Fecal samples were collected four days before the end of each phase to evaluate the digestibility of dry matter (DM), crude protein (CP), crude fat (CF), ash, Ca, and P. To start sample collection, previous fecal samples were eliminated, and the fecal samples were pooled within the pen. Samples were collected and then placed in a freezer at -20℃ until analysis. Fecal samples were thawed, dried at a temperature of 60℃ for 72 h in a forced-air oven, ground in a 1-mm screen Wiley mill (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA), and analyzed for calculating digestibility. Each sample was analyzed in triplicate for measuring the levels of DM (Method 930.15), CP (Method 990.03), CF (Method 960.39), ash (Method 942.05), Ca, and P (method 985.01; 16) according to the methods described by the AOAC [20].

Bone measurements

On the last day of the experiment, one pig per replicate with a BW that was the closest to the average of the replicate was slaughtered via captive bolt stunning. The left front leg was removed, stored at −20℃, and later autoclaved for 55 min at 125℃. The tibia was then extracted from the leg. The bone marrow was removed, and the tibia was dried and soaked in petroleum ether under a chemical hood for 72 h to remove the remaining marrow and fat. The bones were dried overnight at 130℃ and ashed at 600℃ for 16 h.

Blood electrolyte balance

The acid-base status of the animals was evaluated. Venous blood samples from three pigs per treatment were collected via jugular venipuncture into 3-mL non-heparinized vacuum tubes and were then analyzed within approximately 10 min after sampling for pH: sodium (Na^+), potassium (K^+), ionized Ca (iCa^{++}), chloride (Cl^-), bicarbonate (HCO_3^-), base excess (BE), hematocrit (HTC), and electrolyte balance (EB) using an i-STAT Portable Clinical Analyzer with EC8+ cartridges (i-STAT, Princeton, NJ, USA).

Statistical analysis

Data from this trial were analyzed by ANOVA using the GLM procedure in SAS (9.2, SAS Institute, Cary, NC, USA). Initial BW was used as a covariate for growth performance but was eliminated from the model when it was not significant. Each pig was an experimental unit for growth performance, feed intake, nutrient digestibility, blood EB, and bone measurement. The Tukey means comparison test was applied for treatment mean separation at $p < 0.05$. A probability level of less than 0.1 was considered a tendency.

RESULTS

Experiment 1

Growth performance

Mortality was not observed during the experiment. The growth performance results are listed in Table 3. BW increased as the Ca level decreased ($p < 0.05$). ADG was also greater in the low-Ca diet group. Similarly, G/F was improved in the low-Ca diet compared to the high-Ca diet. However, there was no difference in ADFI across the treatments.

Nutrient digestibility

Nutrient digestibility is shown in Table 4. Although there was no difference in the DM, CP, CF, ash, and Ca digestibility as Ca level changed, P digestibility was higher in the low-Ca diet group than in the high-Ca diet group ($p < 0.05$).

Bone mineralization

Bone mineralization is shown in Table 5. No difference was noted in the levels of bone ash, Ca, P, and Mg as the Ca levels changed.

Experiment 2

Growth performance

The results of the growth performance on Ca level and source are shown in Table 6. Final BW increased in the diets with $CaCl_2$ compared with the case for the $CaCO_3$ diets ($p < 0.05$). In the $CaCl_2$ diet group, the ADG was higher and G/F was improved, compared with the case for the $CaCO_3$ diet group. However, there was no difference in the ADFI of the pigs.

Table 3. Effects of dietary Ca concentration on growth performance in pig (experiment 1)[1)]

Ca level	Low	Standard	High	SEM	*p*-value
BW (kg)					
Initial	10.37	10.36	10.39	0.01	0.999
Final	29.88[a]	27.94[ab]	26.17[b]	0.86	0.015
Phase 1 (1–14 d)					
ADG (g)	519[a]	506[ab]	485[b]	8.79	0.006
ADFI (g)	806	799	803	8.96	0.708
G/F	0.64[a]	0.63[ab]	0.60[b]	0.01	0.047
Phase 2 (15–28 d)					
ADG (g)	803[a]	750[ab]	684[b]	28.99	0.004
ADFI (g)	1,322	1,298	1,317	39.68	0.815
G/F	0.61[a]	0.57[ab]	0.53[b]	0.02	0.002
Overall (1–28 d)					
ADG (g)	661[a]	628[a]	585[b]	15.69	0.001
ADFI (g)	1,050	1,057	1,064	20.67	0.804
G/F	0.62[a]	0.59[a]	0.58[b]	0.01	0.001

[1)]Phase 1: Low, 0.60%; Standard, 0.72%; High, 0.84%; Phase 2: Low, 0.50%; Standard, 0.66%; High, 0.82%.

[a,b]Means values within a row with unlike superscript letters were significantly different ($p < 0.05$).

BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G/F, gain feed ratio.

https://doi.org/10.5187/jast.2022.e53

Table 4. Effects of dietary Ca concentration on nutrient digestibility in pig (experiment 1)[1)]

Ca level	Low	Standard	High	SEM	*p*-value
Phase 1, 14 d (%)					
DM	75.96	75.50	74.90	0.86	0.487
CP	73.29	72.84	73.07	0.35	0.481
CF	72.82	72.16	71.47	0.83	0.621
Ash	63.43	64.16	64.37	0.52	0.212
Ca	65.70	65.47	64.50	0.62	0.170
P	52.11[a]	50.54[ab]	48.24[b]	0.89	0.003
Phase 2, 28 d (%)					
DM	72.88	72.52	72.70	0.44	0.721
CP	71.69	70.84	71.07	0.71	0.335
CF	70.62	70.56	70.07	0.47	0.480
Ash	61.81	61.16	60.97	0.63	0.409
Ca	63.60	64.15	63.26	0.84	0.588
P	52.25[a]	50.19[ab]	46.93[b]	1.08	0.005

[1)]Phase 1: Low, 0.60%; Standard, 0.72%; High, 0.84%; Phase 2: Low, 0.50%; Standard, 0.66%; High, 0.82%.
[a,b]Means values within a row with unlike superscript letters were significantly different ($p < 0.05$).
DM, dry matter; CP, crude protein; CF, crude fat.

Table 5. Effects of dietary Ca concentration on bone mineralization in pig (experiment 1)[1)]

Ca level	Low	Standard	High	SEM	*p*-value
Bone ash (%)	62.96	62.57	61.83	3.95	0.959
Bone Ca (%)	35.13	36.18	36.29	1.02	0.498
Bone P (%)	16.89	17.84	18.35	1.97	0.763
Bone Mg (%)	4.91	5.02	5.60	0.68	0.586
Bone ash (g)	6.68	6.78	6.70	0.42	0.971
Bone Ca (g)	2.34	2.45	2.43	0.16	0.794
Bone P (g)	1.13	1.20	1.23	0.14	0.797
Bone Mg (g)	0.32	0.33	0.37	0.03	0.451

[1)]Phase 1: Low, 0.60%; Standard, 0.72%; High, 0.84%; Phase 2: Low, 0.50%; Standard, 0.66%; High, 0.82%.

Nutrient digestibility

The effects of Ca level and source on nutrient digestibility are shown in Table 7. There were no significant differences in DM, CF, and ash digestibility due to Ca level and source variation. CP, Ca, and P digestibility was higher in the $CaCl_2$ diet group than in the $CaCO_3$ diet group ($p < 0.05$).

Blood electrolyte balance

The effects of Ca levels and sources on blood electrolytes are shown in Table 8. Cl^- was upregulated in the $CaCl_2$ diet group compared to the $CaCO_3$ diet group ($p < 0.05$). The HCO_3^-, BE, and EB levels were lower in the $CaCl_2$ diet group than in the $CaCO_3$ diet group ($p < 0.05$). The HTC increased as Ca levels decreased ($p < 0.05$). HCO_3^- interacted with the Ca sources, and thus, influenced the Ca levels ($p < 0.05$).

Bone mineralization

The effects of Ca levels and sources on bone mineralization are presented in Table 9. Bone ash, Ca, and P were downregulated in the low-Ca diet group compared with the high-Ca diet group. There was no difference in bone ash, Ca, P, and Mg as the Ca sources changed.

Table 6. Effects of dietary Ca concentration and source on growth performance in pig (experiment 2)[1)]

Ca level/Ca source	Low		Standard		SEM	Ca level	Ca source	Ca level × source
	$CaCO_3$	$CaCl_2$	$CaCO_3$	$CaCl_2$				
BW (kg)								
Initial	10.32	10.24	10.24	10.39	0.01	0.812	0.812	0.999
Final	26.05[c]	27.37[b]	27.72[b]	29.12[a]	0.26	0.016	0.020	0.861
Phase 1 (1–14 d)								
ADG (g)	435[c]	468[b]	476[b]	502[a]	5.19	0.064	0.080	0.222
ADFI (g)	696	695	702	698	2.28	0.182	0.258	0.795
G/F	0.62	0.67	0.68	0.72	0.01	0.056	0.062	0.219
Phase 2 (15–28 d)								
ADG (g)	689[c]	755[b]	773[b]	837[a]	13.65	0.010	0.013	0.938
ADFI (g)	1,330	1,336	1,354	1,331	9.56	0.619	0.673	0.488
G/F	0.52	0.57	0.57	0.63	0.01	0.055	0.061	0.753
Overall (1–28 d)								
ADG (g)	562[c]	612[b]	624[b]	669[a]	8.97	0.027	0.034	0.774
ADFI (g)	1,013	1,015	1,028	1,014	4.75	0.533	0.608	0.443
G/F	0.55[c]	0.60[b]	0.61[b]	0.66[a]	0.01	0.023	0.025	0.858

[1)]Phase 1: Low, 0.50%; Standard, 0.60%, phase 2: Low, 0.40%; Standard, 0.50%.

[a–c]Means with different superscripts within a row differ ($p < 0.05$).

BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G/F, gain feed ratio.

Table 7. Effects of dietary Ca concentration and source on nutrient digestibility in pig (experiment 2)[1)]

Ca level/Ca source	Low		Standard		SEM	Ca level	Ca source	Ca level × source
	$CaCO_3$	$CaCl_2$	$CaCO_3$	$CaCl_2$				
Phase 1 (1–14 d)								
DM	79.88	79.57	80.37	80.09	0.43	0.118	0.354	0.965
CP	78.01[b]	78.86[a]	77.28[c]	79.10[a]	0.34	0.326	0.001	0.058
CF	78.44	78.82	78.79	79.02	0.37	0.310	0.260	0.784
Ash	64.19	63.58	64.57	63.69	0.52	0.518	0.059	0.717
Ca	61.84[b]	63.29[a]	62.08[b]	63.46[a]	0.54	0.605	0.001	0.926
P	50.85[b]	51.76[a]	50.54[b]	51.21[a]	0.48	0.225	0.033	0.723
Phase 2 (15–28 d)								
DM	77.13	77.19	77.71	77.03	0.42	0.487	0.322	0.238
CP	74.85[b]	76.02[a]	74.09[c]	75.75[a]	0.35	0.053	0.001	0.336
CF	75.74	76.24	75.55	75.94	0.70	0.622	0.381	0.912
Ash	62.28	61.68	61.71	60.58	0.69	0.105	0.093	0.599
Ca	60.35[b]	62.25[a]	61.32[b]	62.78[a]	0.74	0.172	0.005	0.686
P	50.45[b]	51.65[a]	50.61[b]	51.47[a]	0.55	0.973	0.017	0.678

[1)]Phase 1 : Low, 0.50%; Standard, 0.60%; Phase 2 : Low, 0.40%; Standard, 0.50%.

[a–c]Means with different superscripts within a row differ ($p < 0.05$).

DM, dry matter; CP, crude protein; CF, crude fat.

https://doi.org/10.5187/jast.2022.e53

Table 8. Effects of dietary Ca concentration and source on blood electrolyte in pig (experiment 2)[1)]

Ca level/Ca source	Low		Standard		SEM	Ca level	Ca source	Ca level × source
	$CaCO_3$	$CaCl_2$	$CaCO_3$	$CaCl_2$				
Phase 1 (14 d)								
pH	7.32	7.27	7.24	7.30	0.07	0.668	0.916	0.243
Na^+ (mmol/L)	142.5	141.4	141.9	140.8	0.92	0.397	0.119	0.940
K^+ (mmol/L)	6.23	6.31	6.76	6.20	0.36	0.430	0.361	0.221
iCa^{++} (mmol/L)	1.46	1.48	1.50	1.51	0.04	0.321	0.645	0.892
Cl^- (mmol/L)	99.8^c	101.0^b	99.3^c	103.4^a	0.28	0.001	0.001	0.001
HCO_3^- (mmol/L)	31.41^a	29.98^b	32.45^a	28.66^c	0.28	0.483	0.001	0.001
BE (mmol/L)	7.61^a	6.16^b	7.80^a	6.05^b	0.12	0.701	0.001	0.095
HTC (%)	46.16^a	46.33^a	42.66^b	42.16^b	0.52	0.001	0.656	0.377
EB (meq/L)	48.91^a	46.78^a	49.38^a	43.61^b	1.01	0.076	0.001	0.020
Phase 2 (28 d)								
pH	7.37	7.32	7.24	7.29	0.06	0.107	0.976	0.282
Na^+ (mmol/L)	141.9	142.7	142.0	141.1	0.74	0.169	0.975	0.115
K^+ (mmol/L)	6.16	5.85	6.18	5.73	0.34	0.841	0.134	0.789
iCa^{++} (mmol/L)	1.41	1.43	1.45	1.47	0.03	0.143	0.372	0.940
Cl^- (mmol/L)	98.8^c	101.7^b	99.6^c	103.8^a	0.54	0.003	0.001	0.091
HCO_3^- (mmol/L)	30.21^a	28.33^b	31.20^a	27.60^b	0.53	0.743	0.001	0.033
BE (mmol/L)	7.50^a	5.71^b	7.45^a	5.35^b	0.23	0.231	0.001	0.359
HTC (%)	44.83^a	45.50^a	41.50^b	41.00^b	1.13	0.001	0.919	0.477
EB (meq/L)	49.23^a	46.73^b	47.75^{ab}	42.88^c	0.84	0.001	0.001	0.045

[1)]Phase 1 : Low, 0.50%; Standard, 0.60%; Phase 2 : Low, 0.40%; Standard, 0.50%.

[a–c]Means with different superscripts within a row differ ($p < 0.05$).

BE, base excess; HTC, hematocrit; EB, electrolyte balance.

Table 9. Effects of dietary Ca concentration and source on bone characteristics in pig (experiment 2)[1)]

Ca level/Ca source	Low		Standard		SEM	Ca level	Ca source	Ca level× source
	$CaCO_3$	$CaCl_2$	$CaCO_3$	$CaCl_2$				
BBS (N)	247^b	239^b	297^a	307^a	9.35	0.001	0.991	0.193
Bone ash (%)	59.31^b	57.56^b	66.65^a	66.82^a	1.59	0.001	0.494	0.406
Bone Ca (%)	32.61^b	34.15^b	40.37^a	42.56^a	1.48	0.001	0.090	0.758
Bone P (%)	14.44	16.41	19.75	20.62	3.37	0.060	0.560	0.821
Bone ash (g)	6.30^b	6.11^b	7.08^a	7.10^a	0.17	0.001	0.494	0.406
Bone Ca (g)	2.05^b	2.09^b	2.86^a	3.02^a	0.12	0.001	0.269	0.469
Bone P (g)	0.91^b	0.99^b	1.39^a	1.45^a	0.21	0.005	0.616	0.960

[1)]Phase 1: Low, 0.50%; Standard, 0.60%; phase 2: Low, 0.40%; Standard, 0.50%.

[a,b]means with different superscripts within a row differ ($p < 0.05$).

BBS, bone breaking strength.

DISCUSSION

Experiment 1

Ca and P are interdependent minerals that should be used in moderate quantities. Inadequate inclusion of these two diets may have a deleterious effect on the digestibility of other minerals or nutrients [21]. For instance, excess Ca has been shown to be detrimental to obtaining optimal pig growth performance, and more marginal or lower than P requirements in swine diets exacerbate the situation [11,12,14]. Reduction in P digestibility is manifested by this adverse effect when the dietary Ca level is higher than the requirements [10]. In the gastrointestinal tract, Ca-P-insoluble compounds reduce P digestion and absorption [22,23]. Dietary P addition may help to mitigate the negative effects of excessive dietary Ca on the growth performance of pigs. To improve pig growth performance and mineral digestibility, an appropriate Ca:P ratio should be established during the feed formulation.

In Experiment 1, high concentrations of Ca lowered ADG, G/F, and P digestibility, corresponding to an estimated Ca:P ratio greater than 1.22:1. The decrease in P digestibility could be attributable to the deleterious effect of higher dietary Ca levels on ADG and G:F. [24] reported that an increase in Ca levels reduced G:F. Similarly, Wu et al. [14] observed that the ADG, ADFI, and G: F of nursery piglets fed P-deficient diets decreased with increasing Ca levels. Additional supplementation with P in the diet alleviated these negative effects. Furthermore, González-Vega et al. [11,12] and Merriman et al. [13] similarly reported the negative effects of high Ca or Ca:P ratios in 11- to 25-kg pigs, 25- to 50-kg pigs, and 100- 130-kg pigs that were supplemented without additional phytase in their diets.

In our study, we did not find any variation in bone mineralization, including bone ash, Ca, P, and Mg per tibia. Numerous studies have demonstrated that the Ca requirement for maximizing growth performance is different from maximizing bone mineralization, as numerous studies have demonstrated [16,25]. To increase bone mineralization, a higher Ca and P ratio should be supplied more than pig`s requirement [11,13,25]. Bone ash concentration did not change when the dietary Ca and P ratios were increased. This result suggests that bone mineralization changes dramatically when a low-Ca diet is formulated to maximize growth performance.

Experiment 2

Two Ca sources and levels were used for the diets. Therefore, $CaCO_3$ (provided as limestone) was replaced in the diets of growing pigs at varying Ca levels. In our study, different Ca sources affected the growth performance of pigs. Although dietary Ca levels and sources had no effects on ADFI, $CaCl_2$ in pig diets showed a higher growth rate among pigs than $CaCO_3$ in pig diets throughout the trial. This may be because $CaCl_2$ alters the dietary electrolyte balance (dEB) values. In a previous study, Austic et al. [26] observed that dEB values for optimum growth performance of pigs should be in the range of 100–300 mEq/kg.

Similarly, Patience et al. [27] found that diets with dEB values of approximately 175 mEq/kg might provide ideal optimal growth performance. Dersjant-Li et al. [28] suggested that pigs fed diets containing 200 and 500 mEq/kg showed higher growth rates than those fed diets containing 100 mEq/kg. Similarly, Haydon [29] indicated that increasing the dEB value from 25 to 400 mEq/kg can linearly increase daily feed intake. According to Budde and Crenshaw [30], however, dietary dEB values (ranging from –35 to 212 mEq/kg) did not affect piglet growth or feed intake, and Patience and Chaplin [31] suggested that a low dEB value (–20 mEq/kg) in pig diet is a better choice than a high dEB value (104 or 163 mEq/kg) with a tendency for growth rate improvement. Guzmán-Pino et al. [32] observed that pigs fed 16 and 133 mEq/kg diets had greater ADG than

https://doi.org/10.5187/jast.2022.e53

pigs fed 269 mEq/kg diet.

In contrast to the findings of Experiment 1, we found that lower levels of dietary Ca or lower Ca and P ratios were harmful to growth performance. This is because lower levels of dietary Ca are insufficient to satisfy the Ca requirement for growth. In both experiments, dietary Ca levels that were either low or too high showed an aberrant growth rate, which was consistent with the narrow-calculated Ca and P ratios. Excessive dietary P and low dietary Ca compared with greater dietary Ca levels may bind with abundant dietary P and deficient dietary Ca, which reduce ADG [25]. Contrary to the findings of the present study, González-Vega et al. [12] and Merriman et al. [13] reported that low levels of dietary Ca had no effect on pig growth performance. Previous studies used heavier experimental animals, whereas our study examined different calculated Ca and P ratios. A well-balanced dietary nutrient composition is required for younger pigs than for older pigs. As a result, younger pigs in our study may require more dietary Ca for their growth and maintenance in comparison to those in the previous study.

The diet supplemented with $CaCO_3$ reduced the apparent digestibility of CP, Ca, and P compared to diets supplemented with $CaCl_2$. For optimal growth of pigs, both amino acids and zinc should be included in the diet as key nutrients [33,34]. Piglets have low acid activity in their stomach at weaning, which, along with other factors such as low lactic acid concentration and/or irregular large meal intake, can lead to an increased gastric pH, even more than 5.0 [35].

In this regard, the existence of several carbonate sources in the pig diet, such as sodium bicarbonate or $CaCO_3$ has a high acid-binding capacity, which may increase the pH of the stomach of weaned pigs [35]. After weaning, increased gastric pH decreases protein digestibility [36]. Pepsinogen protein digestive enzyme is transformed into pepsin at pH 5.0 in the stomach. However, sources of carbonate increase gastric pH. Moreover, the solubility of other minerals, such as Ca, P, and Zn, could also be affected by elevated gastric pH by encouraging the formation of Zn–Ca–phytate precipitates [37]. The negative effects on performance observed in these growing pigs may be due to the decreased CP, Ca, and P digestibility of the $CaCO_3$ diet. The discrepancy in the results observed in the literature regarding the effects of using carbonate sources on pig performance was explained by the digestibility of intrinsic effects in minerals. However, to evaluate the relationship in this argument, further research with additional CP, Ca, and P supplements is required.

In contrast to pigs fed with $CaCO_3$ diets, blood chloride, HCO_{3^-}, BE, and EB concentrations were shown to be lower in pigs fed with $CaCl_2$ diets for 14 and 28 days. The acidogenic behavior of the $CaCl_2$ diet and the influence of the acid-base balance in pigs were demonstrated in this experiment. For instance, Patience et al. [27] reported that supplementation of pig diets with chloride sources can decrease BE and HCO_{3^-} concentrations. Similarly, Patience and Chaplin [31] and Dersjant-Li et al. [38] found that blood BE, HCO_{3^-}, and pH were decreased when pigs fed on $CaCl_2$ sources in their diet were compared with pigs fed on $CaCO_3$ sources in their diet, respectively.

Different from Exp. 1, we observed less deposition of bone ash, Ca, and P in Exps. 2. Greater bone mineralization in Exp. 2 compared to Exp. 1, caused by the different dietary Ca levels. The existence of Ca and P can influence the deposition of bone minerals, and they accumulate at a constant ratio (2.2:1) during the formation of hydroxyapatite [4]. The fact that pigs can accumulate considerably more bone Ca and P than is required for optimal pig growth performance has been suggested in several studies [11–13,25]. This means that the Ca and P concentrations required to achieve great muscle growth are lower than those required to achieve maximum skeletal tissue synthesis. As a result, our observations indicate that additional Ca supplementation in the diet is not required for bone mineralization if P meets the requirements.

CONCLUSION

If dietary P is at or above this requirement, dietary Ca levels could have detrimental effects on growth performance. Although the lowest dietary Ca level decreased bone Ca and P levels, bone parameters were less sensitive to dietary Ca levels than growth performance. A Ca and P ratio of 1.04–1.22: 1 is required for maximizing growth performance in growing pigs. Dietary Ca sources also changed the acid–base balance and apparent digestibility of pigs, as changes in blood chloride, HCO_3^-, BE, and EB concentrations reduced apparent CP, Ca, and P digestibility. This was observed when the piglets were fed a Ca-carbonated diet. In this study, $CaCl_2$ was a better choice for Ca source in the diet rather than $CaCO_3$. However, further studies are required to elucidate the mechanisms underlying these responses.

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