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Effects of Calcium Sources and Levels on Growth Performance and Calcium Bioavailability in Weaning Piglets

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ABSTRACT

The objective of this study was to investigate the effects of different calcium sources and levels on performance and bioavailability of calcium and phosphorus in weaning piglets. A total of 90 LY (Landrace×Yorkshire) weaning piglets were randomly assigned to 2×8 factorial completely randomized arrangement. The two calcium sources were calcium carbonate or calcium citrate; Eight dietary calcium levels were 0.37, 0.50, 0.60, 0.70, 0.80, 0.90, 1.00 and 1.10%. Each treatment consisted of 3 replicate pens of 2 piglets. Digestibility trial was conducted from 29 to 32 day of experiment using the total collection method. No significant difference was observed on average daily gain, average daily feed intake and feed conversion rate in piglets ($p>0.05$) from calcium sources or interactions between calcium sources and calcium levels. There was a quadratic response of average daily gain to increasing calcium levels ($p<0.05$), with the optimum dietary calcium level of 0.6%. Piglets receiving dietary ranging from 0.37 to 0.80 had a higher calcium, phosphorus apparent digestibility than piglets receiving dietary ranging from 0.90 to 1.10%. Calcium citrate had significantly higher calcium apparent digestibility than calcium carbonate in piglets ($p<0.05$). It is concluded that calcium citrate is a good calcium source with comparable in bioavailability as calcium carbonate for weaning piglets. The piglets fed 0.6% calcium grew better, regardless of dietary calcium source.

Key words: Weaning piglets, performance, calcium carbonate, calcium citrate, bioavailability

INTRODUCTION

Calcium (Ca) is an essential mineral element for the development and maintenance of skeletal system and performs many other physiologic functions. Young piglets have a particularly high growth rate which needs adequate Ca supply to promote the bone development and support whole body metabolism. A considerable amount of studies had been conducted to determine the Ca requirements of piglets, which suggested 0.8% of diet for 5-10 kg piglets (Combs *et al.*, 1991; Miller *et al.*, 1962; Reinhart and Mahan, 1986; Zimmerman *et al.*, 1963). It should be noted that maximum bone ash as an indicator had been utilized by many researchers to determine the pig's Ca requirements. However, the question remains whether maximum calcification is needed by piglets for optimum growth.

Generally, Ca content and availability in raw materials is low and piglets need additional Ca mainly as form of calcium carbonate and calcium phosphate. Notably, calcium carbonate and calcium phosphate had higher acid-binding capacity (Lawlor *et al.*, 2005) and their dissociations need a great amount of gastric acid for the subsequent absorption. Adult piglets have sufficient gastric acid secretion for dissociation of Ca before absorption whereas weaning piglets have a difficulty in performing this function since weaning piglets had insufficient gastric acid secretion due to immature digestive system (Kidder and Manners, 1978; Manners, 1976). Consequently elevated gastric pH lead to a poor nutrient utilization (Kidder and Manners, 1978) and intestinal dysfunction (Bolduan *et al.*, 1988). Alternatively, Ca source from a low acid biding capacity seems to be an appropriate choice to improve the growth performance of weaning piglets. Early studies revealed that calcium citrate had superior dissolvability than calcium carbonate (Heaney *et al.*, 1999), Additionally, calcium citrate could be dissolved independent of gastric acid (Dolinska *et al.*, 2011), which was believed to be have a higher bioavailability for weaning piglets. Therefore, the current study was to investigate the effects of different Ca sources and Ca levels on growth performance and bioavailability of Ca in weaning piglets.

MATERIALS AND METHODS

Animal, experimental design and diets: The experiment was carried out from May 12 to June 12, 2011. The experiment protocol was approved by the Animal Care and Use Committee of Sichuan Agricultural University. A total of 90 LY (Landrace×Yorkshire) piglets with initial average weaning (at 21 days of age) body weight of 7.13 kg±0.19 were purchased from Mianyang branch of Chia Tai Group (Mianyang, Sichuan, China). A complete randomized design involving a 2×8 factorial design was used in this experiment. The two supplemental Ca sources were calcium carbonate and calcium citrate. The calcium carbonate was feed grade (Tongda Company, Chengdu, China) and its purity was about 95% containing 38.42% Ca on analytical basis. The calcium citrate was also feed grade (Zeweier Feed Company, Guangxi, China) and its purity was about 98% containing 20.11% Ca on analytical basis. Basal diets (Table 1, containing Ca 0.37% by calculation)

Table 1: Composition of experimental basal diet

Ingredients (kg 100 kg^{-1})	Basal diet	Calculated analysis	Basal diet
Corn	29.11	Crude protein (%)	19.83
Extruded corn	22.39	Digestible energy (MJ kg^{-1})	14.09
Extruded soybean	10.00	Calcium (%)	0.37
Soybean meal	10.00	Total phosphorus (%)	0.60
Soy protein concentrate	5.00	Available phosphorus (%)	0.40
Fish meal	5.00	Lysine (%)	1.34
Dry whey	10.00	Methionine+ Cysteine (%)	0.68
Sucrose	1.50	Threonine (%)	0.74
Glucose	0.33	Tryptophane (%)	0.22
Sodium dihydrogen phosphate	0.33		
Salt	0.35		
Bentonite	3.61		
DL-Methionine	0.07		
L-Lysine HCl	0.34		
L-Threonine	0.11		
L-tryptophan	0.04		
Choline chloride	0.10		
Multi-nutrient premix[1]	0.55		
Colistin sulfate	0.002		
Total	100		

[1]Provided per kilogram of diet: Fe 100 mg, Cu 150 mg, Mn 40 mg, Zn 100 mg, I 0.5 mg, Se 0.3 mg, vitamin A, 18,000 IU; vitamin D_3, 4,000 IU, vitamin E, 40IU, vitamin K3, 4 mg; vitamin B_1, 6 mg, vitamin B_2, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg, biotin, 0.2 mg, folic acid, 2 mg, biacin, 50 mg, pantothenic acid, 24 mg

Table 2: Ca concentrations in diets (as-fed basis)

Ca source	Predicted Ca level (%)	Calculated $CaCO_3$ or Ca citrate supplements (%[1])	Analyzed Ca level (%[2])
Basal diet	0.37	0.00	0.31
$CaCO_3$	0.50	0.33	0.49
	0.60	0.59	0.58
	0.70	0.85	0.66
	0.80	1.11	0.77
	0.90	1.37	0.92
	1.00	1.63	0.97
	1.10	0.89	1.07
Ca citrate	0.50	1.13	0.60
	0.60	1.62	0.69
	0.70	2.12	0.76
	0.80	2.62	0.76
	0.90	3.11	0.91
	1.00	3.61	0.99
	1.10	3.61	1.06

[1]Treatment calcium levels were added at the expense of bentonite, [2]Date were means of triplicates of dietary chemical analysis, SE: Standard errors, Ca: Calcium

were corn-soybean which provided 19.83% CP, 14.09 MJ kg^{-1} DE and 0.60% total phosphorus recommended by NRC (1998). Dietary Ca levels were designed by adding different ratio of calcium carbonate or calcium citrate to basal diet at the expense of bentonite to get eight total Ca levels: 0.37, 0.50, 0.60, 0.70, 0.80, 0.90, 1.00, or 1.10%. Because the two Ca sources shared one Ca-unsupplemented basal diet, there were a total fifteen dietary treatment groups, with 3 replicate pens of 2 piglets each. Sodium dihydrogen phosphate was the supplemental Phosphorus (P) source in the diet. The dietary Ca concentrations are listed in Table 2. Digestibility trial was conducted from 29 to 32 d of experiment using the total collection method. During the digestibility experiment piglets from each treatment were housed in metabolism cage (one pig/cage) for faeces collection. During the feeding experiment, all piglets were housed in slotted and concrete floor pens. The pen size was 240× 165× 60 cm, equipped with a nipple waterer and a stainless feeder. During the entire experiment the temperature and humidity were managed at 22-25°C and 45-60%, respectively. Piglets had free choice of feed and water. The whole trial lasted for 32d.

Sample collection: Ca and P contents of corn, soybean meal, fish meal, soy protein concentrate and dry whey were determined before the experiment to ensure Ca and P contents in the diets. The calcium carbonate and calcium citrate were also sampled for analysis of the actual contents of Ca and P. Feed consumption was recorded by pen weekly and body weight was recorded by pig weekly, which were then used to calculate Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Rate (FCR). To study the effect of dietary Ca sources and Ca levels on diarrhea rate, fecal consistency was measured daily at 9:00-11:00 using a macroscopic score (Ball and Aherne, 1982). The diarrhea index was calculated as a percentage of summation of the scores of affected piglets in each pen during the experimental period divided by the total number of piglets in each pen (Miller *et al.*, 1984).

At the end of feeding experiment, blood samples were collected via jugular venipuncture from each pig. Blood samples were collected in 10 mL tubes. These samples were centrifuged at 3,000×g at 4°C for 10 min for serum separation and then stored at -20°C for further analysis of Ca and P contents and Alkaline Phosphatase Activity (AKP).

All faeces collected from each pig for 4 day were pooled together in equal ratio and oven-dried at 60°C until constant weight. Dry samples were weighed and frozen at -20°C for Ca and P contents analysis.

Sample analysis: Concentrations of Ca and P in feed ingredient or diet samples, or faeces were determined by the method described by AOAC (1984).

Serum Ca and P concentrations and AKP activity were determined using Kit (Nanjing Jiancheng BioSciences Corporation, China) according to the manufacture's recommendations. The spectrophotometer (721, Implen, Germany) was used to measure their absorbance.

Statistical analysis: Data were analyzed by two-way analysis of variance using the General Linear Models (GLM) procedure of SAS (SAS, 1985). The model includes the main effects of Ca sources, Ca levels and their interactions between Ca sources and Ca levels. Average daily gain based on calcium levels were analyzed by polynomial regression analysis using the GLM procedure of SAS. Differences among means were tested by the LSD method. Data were presented as Mean±SEM. Statistical significance was based on $p<0.05$.

RESULTS

The Ca sources and interactions between Ca sources and Ca levels did not affect ($p>0.05$) ADG of weaning piglets (Table 3). There was a quadratic response of ADG as increasing dietary Ca levels, with the optimum Ca level of 0.60% ($p<0.05$). No significant differences could be observed from any dietary treatment on ADFI and FCR ($p>0.05$). These results indicated that calcium citrate did not improve growth performance of weaning piglets.

Table 3: Effects of Ca sources and levels on growth performance of pigs

Item	Ca level (%)	Initial weight (kg)	Final weight (kg)	ADG (g day^{-1})[1]	ADFI (g day^{-1})[2]	FCR (g g^{-1})[2]
Level effect	0.37	7.06	17.52	373.57^{bc}	552.98	1.49^{ab}
	0.5	7.08	18.25	398.9?^{abc}	569.91	1.43^{b}
	0.6	7.07	19.7	428.57^{a}	593.71	1.38^{b}
	0.7	7.07	18.53	409.29^{ab}	594.24	1.45^{b}
	0.8	7.33	18.50	398.92^{abc}	586.12	1.43^{b}
	0.9	7.15	17.96	386.07^{abc}	585.89	1.52^{ab}
	1.0	7.11	17.61	375.17^{bc}	576.62	1.54^{ab}
	1.1	7.10	17.24	361.97^{c}	579.81	1.62^{a}
Pooled SE		0.28	0.56	13.27	21.42	0.05
Source effect	$CaCO_3$	7.09	18.01	390.09	584.19	1.50
	Ca citrate	7.16	18.16	392.84	575.63	1.48
Pooled SE		0.14	0.30	6.63	1.071	0.03
p value						
Source				0.772	0.576	0.669
Level				0.029	0.891	0.046
Source×level				0.259	0.245	0.350

Treatment means within columns followed by different superscripts are significantly different ($p<0.05$), [1]Means and standard errors are based on six pigs of 3 pens per treatment taken during 28 d period, [2]Means and standard errors are based on 3 pens each treatment taken during 28 day period., SE: Standard errors, Ca: Calcium, ADG: Average daily gain, ADFI: Average daily feed intake, FCR: Feed conversion rate

Table 4: Effects of Ca sources and levels on diarrhea of pigs

Item	Diet Ca level (%)	Diarrhea index[1]
Level effect	0.37	1.21[b]
	0.5	1.21[b]
	0.6	1.91[b]
	0.7	1.23[b]
	0.8	1.27[ab]
	0.9	1.50[a]
	1.0	1.52[a]
	1.1	1.48[a]
Pooled SE		0.11
Source effect	$CaCO_3$	1.36
	Ca citrate	1.29
Pooled SE		0.04
p value		
Source		0.189
Level		0.012
Source×level		0.786

Treatments means within columns followed by different superscripts are significantly different ($p<0.05$), [1]Mean and standard errors based on 3 pens each treatment taken during 28 d period, SE: Standard errors; Ca: Calcium

The dietary Ca sources and interactions between Ca sources and Ca levels had no effect on the diarrhea index of piglets ($p>0.05$) (Table 4) . However, dietary Ca levels affected diarrhea index of piglets. The diarrhea index was lowest in dietary Ca of 0.60% for piglets ($p<0.05$). Increasing the dietary Ca content from 0.9 to 1.1% rised diarrhea index of piglets ($p<0.05$).

As data shown in Table 5, the Ca sources, Ca levels and their interactions did not affect serum Ca concentrations or AKP activity ($p>0.05$). Meanwhile, serum P concentrations were not affected by Ca sources or the interactions between Ca sources and Ca levels ($p>0.05$). However, as dietary Ca levels increased, serum P concentrations were reduced ($p<0.05$).

The effects of dietary treatments on Ca and P intake and excretion and digestibility were shown in Table 6. The dietary Ca levels linearly increased Ca intake and excretion ($p<0.001$). Increasing the Ca levels of the diets from 0.37 to 1.10% had significant effects on P excretion and digestibility ($p<0.05$). High dietary Ca levels increased P excretion and reduced P digestibility ($p<0.05$). The piglets fed the diets containing range 0.37 to 0.80% dietary Ca had higher Ca, P apparent digestibility than the piglets fed the diets containing high dietary calcium (0.90-1.10%). As expected, the piglets supplemented calcium citrate had a higher Ca digestibility than those supplemented calcium carbonate ($p<0.05$).

DISCUSSION

Results in the present experiment indicated that diet containing calcium citrate would not improve growth performances of weaning piglets compared with diet containing calcium carbonate, which agreed with the previous results (Gottlob *et al*., 2006) in which calcium formate was proposed to be more effective in simple corn-soybean meal diets than complex diet. In present study, protein sources in diets were not only based on soybean meal, but also soy protein concentrate and fish meal. Additionally, the lactose in diets would generate lactic acid, which may facilitate the absorption of calcium carbonate.

Table 5: Effects of Ca sources and levels on Ca, P concentrations and AKP activity in the serum of pigs

Item	Ca Level (%)	Ca (mmol L^{-1})[1]	P (mmol L^{-1})[1]	AKP (Kin g unit/100 mL)[1]
Level effect	0.37	2.26	3.11[a]	54.98
	0.50	2.29	3.14[a]	51.99
	0.60	2.44	3.00[ab]	50.23
	0.70	2.35	2.75[bc]	5.49
	0.80	2.34	2.87[ab]	47.70
	0.90	2.29	2.45[d]	52.32
	1.00	2.32	2.50[cd]	50.39
	1.10	2.41	2.46[d]	52.45
Pooled SE		0.03	0.04	1.62
Source effect	$CaCO_3$	2.33	2.75	52.03
	Ca citrate	2.35	2.83	50.63
Pooled SE		0.06	0.09	0.81
p value				
Source		0.620	0.209	0.225
Level		0.256	<0.001	0.114
Source×level		0.117	0.319	0.931

Treatments means within columns followed by different superscripts are significantly different ($p<0.05$), [1]Mean and standard errors based on six pigs of 3 pens each treatment taken during 28d period, SE: Standard errors, Ca: Calcium, P: Phosphorus, AKP: Alkaline phosphatase

Table 6: Effects of Ca sources and levels on Ca and P utilization of pigs

Item	Ca Level(%)	Intake Ca (g day^{-1})[1]	Faecal Ca (g day^{-1})[1]	Intake P (g day^{-1})[1]	Faecal P (g day^{-1})[1]	Ca apparent digestibility (%)[1]	P apparent digestibility (%)[1]
Level effect	0.37	2.13[e]	0.63[e]	4.27	2.24[c]	72.54[abc]	47.48[a]
	0.5	4.37[d]	1.24[d]	5.31	2.71[b]	72.42[abc]	41.95[ab]
	0.6	4.48[d]	1.31[cd]	5.15	5.76[b]	75.82[a]	46.34[a]
	0.7	5.05[d]	1.37[cd]	5.07	2.87[ab]	73.32[ab]	44.20[ab]
	0.8	6.38[c]	1.48[c]	5.33	2.72[b]	76.26[a]	48.09[a]
	0.9	7.53[b]	2.08[b]	5.35	3.31[a]	71.41[bc]	38.09[bc]
	1.0	7.67[b]	2.24[b]	4.88	3.30[a]	70.67[bc]	31.98[cd]
	1.1	8.77[a]	2.70[a]	4.76	3.28[a]	68.92[c]	31.42[d]
Pooled SE		0.25	0.07	0.21	0.15	1.38	2.23
Source effect	$CaCO_3$	5.60[b]	1.67[a]	5.02	2.91	71.25[b]	42.14
	Ca citrate	6.00[a]	1.58[b]	5.00	2.89	74.08[a]	40.24
Pooled SE		0.12	0.03	0.11	0.08	0.69	1.12
p value							
Source		0.030	0.041	0.905	0.879	0.005	0.231
Level		<0.0001	<0.0001	0.007	<0.0001	<0.0001	<0.0001
Source×level		0.158	0.798	0.323	0.317	0.267	0.141

Treatments means within columns followed by different superscripts are significantly different ($p<0.05$), [1]Mean and standard errors based on six pigs of 3 pens each treatment taken during 4 day period, SE: Standard errors, Ca: Calcium, P: Phosphorus

The ADG of weaning piglets showed a quadratic response with increasing supplemental Ca level. And maximal body weight gain was achieved at Ca level of 0.60%, which was consistent with the results of Zimmerman *et al.*(1963) and (Miller *et al.*, 1962). One possible explanation for the optimum growth performance of piglets containing 0.60% calcium is that diarrhea index is lower than piglets fed high dietary calcium level (0.9-1.1%) in this experiment. (Pallauf and Hüter, 1993) reported that high dietary Ca resulted in lower daily gain, which was consistent with our results.

Dietary Ca level increasing from 0.65 to 0.95% reduced growth rate of piglets and they found that the high dietary Ca level could inhibit P absorption through the mineral balance experiment. (Bayley *et al*., 1975). In fact, our results had indicated high dietary Ca level increased P excretion and reduced P digestibility, which may be related to the unbalanced ratio of Ca to P range from 1.5:1 to 1.83:1 compared with the optimum total Ca: P ratio (between 1:1 and 1.25:1) as suggested by NRC(1998).

Serum P concentrations were reduced as dietary Ca level increased, which was consistent with earlier studies (Bayley *et al*., 1975; Coalson *et al*., 1972; Cromwell *et al*., 1972; Qian *et al*., 1996). However, dietary treatment had no effect on serum Ca concentrations. It is important for maintaining Ca homeostasis that isoionic Ca deposit and release on bone surfaces, which is a process regulated by AKP derived bone (Bronner, 2001). Serum AKP activity is negatively related to skeletal deposits in fasting and healthy animal (Withold *et al*., 1996). Nevertheless, no difference was observed in the serum AKP among treatments in present study. Therefore, our results indicated dietary Ca level might have no adverse effect on skeletal development, as indicated by Bayley *et al*. (1975) and Crenshaw (1986) and Stockland and Blaylock (1973).

Increased dietary Ca level resulted in increased Ca and P excretion and the piglets fed the diets containing range 0.37 to 0.80% dietary Ca level had higher Ca, P apparent digestibility than the piglets fed the diets containing high (0.90-1.10%) dietary calcium level. This was consistent with the results of Qian *et al*.(1996) who found the digestibility of Ca and P were linearly reduced as the ratio of the Ca: P increased in the diets.

The data collected from Ca, P apparent digestibility in our study showed that calcium citrate resulted in a higher Ca bioavailability in weaning piglets, which was supported by the results in humans (Hanzlik *et al*., 2005; Harvey *et al*., 1990; Nicar and Pak, 1985). Calcium is absorbed across the gut wall in ionized form and solubility of Ca source plays a important role in its absorption (Pak *et al*., 1989). Heaney *et al*. (1990) reported that solubility of calcium citrate is higher than calcium carbonate and calcium citrate can be effectively soluble independently of archenteric environment pH (Dolinska *et al*., 2011). This might be the reason for the improvement in Ca apparent digestibility of calcium citrate.

CONCLUSION

In conclusion, our results indicated that calcium citrate had a superior Ca bioavailability for weaning piglets. A appropriate dietary Ca level was needed by piglets to maintain optimum growth which was obtained at 0.60% dietary Ca level regardless of Ca source.

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