

Dietary maifanite supplementation did not affect the apparent total tract digestibility of calcium and phosphorus in growing pigs

Li Li Bai¹, Dong Xu Ming¹, Shu Ren Dong², Zhong Yue Yang¹, Wen Hui Wang¹, Shuai Zhang¹,
Xiang Shu Piao¹, Ling Liu^{1,*}, and Feng Lai Wang^{1,*}

* Corresponding Authors:

Ling Liu

Tel: +86-10-62731210, Fax: +86-10-62733688,

E-mail: liul@mafic.ac.cn

Feng Lai Wang

Tel: +86-10-62733586, Fax: +86-10-62733586,

E-mail: wangfl@cau.edu.cn

¹ State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

² Tianjin National Breeding Pig Farm, Tianjin 301500, China

ORCID

Ling Liu

<https://orcid.org/0000-0002-8832-9366>

Feng Lai Wang

<https://orcid.org/0000-0002-7538-0082>

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Objective: This study was conducted to determine the effects of dietary maifanite supplementation and fecal collection method on the apparent total tract digestibility (ATTD) of calcium (Ca) and phosphorus (P) and blood parameters in growing pigs.

Methods: Thirty-six growing barrows (Duroc×Landrace×Yorkshire; 27.0±2.6 kg) were allotted to six dietary treatments with 6 pigs per treatment according to body weight in a completely randomized design. The experimental treatments were: i) Low Ca+cornstarch (2.25%), ii) Low Ca+maifanite (2.25%), iii) Medium Ca+cornstarch (1.42%), iv) Medium Ca+maifanite (1.42%), v) High Ca+cornstarch (0.64%), and vi) High Ca+maifanite (0.64%). Feces were collected by the total collection (TC) and indicator method (IM). At the beginning and the end of the experiment, blood samples were collected from each pig.

Results: For the TC method, there were no difference in Ca intake, fecal Ca output, Ca retention and the ATTD of Ca between cornstarch and maifanite diets at the same dietary Ca level. However, urinary Ca excretion was lower ($p = 0.01$) in pigs fed low Ca diets without maifanite supplementation compared with other dietary treatments. Dietary maifanite supplementation had no effect on the P metabolism in growing pigs. For the IM method, there was no difference in Ca digestibility between cornstarch and maifanite diets at the same dietary Ca level. The ATTD of P was greater ($p < 0.01$) in pigs fed the high Ca diet with maifanite supplementation compared with the high Ca diet with cornstarch treatment. Dietary inclusion of maifanite had no effect on blood parameters in growing pigs.

Conclusion: Dietary maifanite supplementation had no effect on the ATTD of Ca and P and serum parameters in growing pigs. The IM resulted in lower digestibility values than the TC method.

Keywords: Apparent Total Tract Digestibility; Calcium; Fecal Collection Method; Growing Pigs; Maifanite; Phosphorus

INTRODUCTION

Maifanite is a kind of granitoid silicate and displays a high porosity and surface area [1]. The total percent of silica (SiO₂) and aluminum oxide (Al₂O₃) in maifanite is more than 70% [2]. Maifanite is widely used in the fields of feed additive, purifying water, medicine, fertilizer and so on [3-5]. Recent studies showed that the addition of maifanite to pig diets could reduce the detrimental effects of cadmium [6], aflatoxin B1 [7] and zearalenone [1]. Liao et al [8] indicated that maifanite has the ability to regulate the balance and metabolism of trace elements in rats suffering cadmium poisoning. Chen et al [9] reported significant improvements in the digestibility of Ca and P as a result of either 1% or 2% Biotite V (aluminosilicate clay) supplementation. Thacker [10] found no improvement in P digestibility as a result of including Biotite V in diet in growing-finishing pigs. Maifanite supplementation could decrease the pH value during pig manure composting [11].

To our knowledge, there is relatively limited data on the effect of maifanite supplementation on calcium (Ca) and phosphorus (P) metabolism of growing pigs. Thus, we hypothesized that maifanite may have effects on the digestibility of Ca and P in growing pigs.

The total collection (TC) and indicator method (IM) are commonly used to estimate apparent total tract digestibility (ATTD) of pig diets [12]. Several studies demonstrated that the IM results in lower ATTD values of nutrients than the TC method [13-16]. However, Kemme et al [17] reported that the ATTD of Ca and P were greater in the IM compared with the TC method in pigs fed corn-soybean meal-based diet. Hence, it is necessary to compare TC and IM methods to determine the digestibility of nutrients in growing pigs housed in stainless steel metabolism crates.

Therefore, the objectives of the present study were i) to evaluate the effect of maifanite on apparent digestibility of Ca and P and serum parameters in pigs fed diets with different Ca concentrations, and ii) to compare the TC with IM methods on measuring the apparent digestibility of diets with or without maifanite in growing pigs.

MATERIALS AND METHODS

The China Agricultural University Laboratory Animal Welfare and Animal Experimental Ethical Inspection Committee (Beijing, China) reviewed and approved the protocol used in the study. This study was conducted in the Metabolism Laboratory in the Swine Nutrition Research Center of the Ministry of Agriculture Feed Industry Center (Chengde, Hebei Province, China). The maifanite used in this study was provided by Tonglixingke Agricultural Science and Technology Company Limited (Beijing, China). The quality standard of the maifanite is as follows: SiO₂ ≥65%, Al₂O₃ ≥16%.

Animals, diets and experimental design

Pigs were placed individually in stainless steel metabolism crates (1.4×0.45×0.6 m) for adaptation 1 week before the experiment. During this period, pigs were allowed *ad libitum* access to a corn-soybean diet containing 0.60% Ca and 0.50% P.

Thirty-six crossbred barrows (Duroc×Landrace×Yorkshire; 27.0±2.6 kg) were assigned to six dietary treatments with 6 pigs per treatment according to body weight in a completely randomized design. The individual pig was the experimental unit.

Diets were corn-soybean meal based. Treatments were comprised of 3 levels of Ca: 0.39% (low Ca), 0.70% (medium Ca), and 0.99% (high Ca), respectively. Limestone was added at the expense of cornstarch or maifanite to adjust dietary Ca levels. The experimental treatments were as follows: i) Low Ca+cornstarch (2.25%), ii) Low Ca+maifanite (2.25%), iii) Medium Ca+cornstarch (1.42%), iv) Medium Ca+maifanite (1.42%), v) High Ca+cornstarch (0.64%), and vi) High Ca+maifanite (0.64%). The dietary P level was formulated at 0.62% by supplying of monosodium phosphate and dicalcium phosphate. Vitamins, minerals, and

amino acids were supplemented in all diets to meet or exceed the estimated nutrient requirements for growing pigs recommended by NRC [18]. The composition and chemical analysis of the experimental diets are shown in Table 1.

The daily feed allowance was equivalent to 4% of BW at the beginning of the experiment [12]. The allotments of feed were divided into two equal meals and provided at 08:30 and 15:30 h daily. The feeding level was progressively increased for the first two days in order to reach fixed intakes. Feed refusals and feed spillage were collected, dried and weighed accurately to calculate feed intake. Throughout the experiment, pigs had free access to water from a low-pressure drinking nipple. The room temperature was maintained at 22°C±2°C to meet the environmental needs of the pig.

Adaptation and collection procedures

The TC and IM methods were used for each pig at the same time to obtain separate fecal collections. Pigs were fed experimental diets for 12 days. From d 6 to 12, pigs were fed diets mixed with 0.3% chromic oxide (Cr₂O₃) as an indigestible marker for 7 days. Following 5-day adaptation (d 6 to d 10) to the Cr₂O₃ containing diets, fecal samples were collected during the last 2 days (d 11 and d 12). Feces collection during these 2 days from each pig were pooled, and 300 g of feces was obtained and kept separated in plastic bags to be analyzed by the IM method. Feces were also collected from the same pig from d 8 to d 10, which were kept separated in plastic bags and labeled. Those fecal samples were pooled with the remaining feces collected on d 11 and d 12 (equivalent to collection in 5 continuous days), then analyzed and treated as samples for the TC method.

At the end of the collection period, all fecal samples were dried for 72 h in a 65°C drying oven, allowed to equilibrate for 24 h at room temperature and then ground through a 1-mm screen for chemical analyses. Urine samples were collected from d 8 to 12. Urine was collected into buckets containing 50 mL of 6 N HCl and emptied each afternoon. Urine volume was recorded daily and 10% of the daily urinary excretion from each pig was collected then stored at -20°C. At the end of the experiment, all the urine was thawed, pooled by pig, homogenized and sub-sampled.

At the beginning and the end of the experiment, blood samples were collected from each pig. After an overnight fasting, barrows were bled via the anterior vena cava into 10-mL heparin-free vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Blood samples were centrifuged (Biofuge22R; Heraeus, Hanau, Germany) at 3,000×g for 10 min, and serum samples were stored at -20°C until analysis.

Chemical analysis

All samples were analyzed in duplicate. Diets and fecal samples were analyzed for dry mater (DM; method 930.15), Ca (method 935.13), and total P (method 965.17) [19]. Crude protein (CP) of diets was analyzed according to the method 988.05 of the AOAC

Table 1. Ingredient and nutrient composition of the experimental diets (as-fed basis)

Items	Treatment						
	Ca (%):	Low (0.39)		Medium (0.70)		High (0.99)	
	Maifanite (%):	0	2.25	0	1.42	0	0.64
Ingredients (%)							
Corn		66.00	66.00	66.00	66.00	66.00	66.00
Soybean meal		22.00	22.00	22.00	22.00	22.00	22.00
Wheat bran		6.00	6.00	6.00	6.00	6.00	6.00
Dicalcium phosphate		0.98	0.98	0.98	0.98	0.98	0.98
Monosodium phosphate		0.50	0.50	0.50	0.50	0.50	0.50
Limestone		0.27	0.27	1.10	1.10	1.88	1.88
Salt		0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride		0.20	0.20	0.20	0.20	0.20	0.20
L-lysine HCl		0.40	0.40	0.40	0.40	0.40	0.40
DL-methionine		0.20	0.20	0.20	0.20	0.20	0.20
L-threonine		0.10	0.10	0.10	0.10	0.10	0.10
Maifanite		-	2.25	-	1.42	-	0.64
Cornstarch		2.25	-	1.42	-	0.64	-
Vitamin and mineral premix ¹⁾		0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide		0.30	0.30	0.30	0.30	0.30	0.30
Analyzed composition ²⁾							
GE (MJ/kg)		16.03	15.76	15.88	15.88	15.68	15.63
DM (%)		87.26	87.72	87.05	88.24	86.85	86.91
CP (%)		16.08	16.57	16.84	16.87	16.50	16.42
Ca (%)		0.44	0.44	0.71	0.71	0.95	0.94
Total P (%)		0.63	0.65	0.63	0.64	0.61	0.64
Calculated composition							
DE (MJ/kg)		13.60	13.60	13.60	13.60	13.60	13.60

GE, gross energy; DM, dry matter; CP, crude protein; Ca, calcium; P, phosphorus; DE, digestible energy.

¹⁾Vitamin and mineral premix provided the following per kg of complete diet for growing pigs: vitamin A, 6,000 IU; vitamin D₃, 1,500 IU; vitamin E, 15 IU; vitamin K₃, 1.5 mg; thiamine, 0.8 mg; riboflavin, 3 mg; niacin, 18 mg; pantothenic acid, 9.5 mg; pyridoxine, 1.5 mg; vitamin B₁₂, 10 µg; biotin, 25 µg; iron, 80 mg; copper, 60 mg; zinc, 65 mg; manganese, 20 mg; iodine, 0.3 mg; selenium, 0.2 mg.

²⁾Analyzed values are the result of the chemical analysis conducted in duplicate.

[19]. Gross energy (GE) of diets was measured by an automatic adiabatic oxygen bomb calorimeter (Parr 6300 Calorimeter, Moline, IL, USA). Urine samples were microwave digested in nitric acid and perchloric acid and then analyzed for Ca by Atomic Absorption Spectrometer (Z-2000, Hitachi, Tokyo, Japan), and P was analyzed according to the vanadate colorimetric method [19]. Chromium concentration in the diets and fecal samples were analyzed using an Atomic Absorption Spectrometer (Z-2000, Hitachi, Japan) according to the procedure of Williams et al [20].

Calculation of apparent total tract digestibility

The ATTD of nutrients was calculated for both TC and IM methods using the following formulas according to Adeola et al [12]: digestibility (%) by TC = $(\{[\text{nutrient consumed, g} - \text{nutrient in feces, g}]/\text{nutrient consumed, g}\} \times 100)$, and digestibility (%) by IM = $(100 - [100 \times \{\% \text{ Cr in feed}/\% \text{ Cr in feces}\} \times \{\% \text{ nutrient in feces}/\% \text{ nutrient in feed}\}])$.

Blood analysis

Serum Ca and P levels and alkaline phosphatase (ALP) activities were measured using an Automatic Biochemistry Analyzer (Hi-

tachi 7020, Japan). All commercial kits were purchased from the Biosino Biotechnology and Science Company (Beijing, China) and used following the standard procedures described by the manufacturer.

Calculations and statistical analysis

Data were checked for normality and outliers were detected using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC, USA). To determine the dietary treatment effect, data were analyzed using the general linear model procedure of SAS, with individual pig as the experimental unit. The model included dietary treatment as the fixed effect. Means were separated using the LSMEANS statement, and the multiple comparison was adjusted using the SNK test. To determine the method effect, data were compared within each dietary treatment group using the TTEST procedure of SAS. A p-value less than 0.05 indicated a significant difference, whereas a p-value larger than 0.05 but less than 0.10 indicated a statistical trend.

RESULTS AND DISCUSSION

All pigs consumed their diets and remained healthy throughout the experiment. Values of analyzed Ca concentration in diets were 0.01% and 0.05% greater than formulated values in medium and low Ca groups, respectively, and ~0.05% lower in high Ca group. Values for analyzed P in diets were 0.01% to 0.03% different than formulated value (0.62%) in each group (Table 1). However, these differences are assumed not large enough to affect the experiment results.

Digestibility measurement by TC method

The daily intake of Ca increased ($p < 0.01$) as the concentration of Ca in the diets increased (Table 2). Fecal Ca output also increased ($p < 0.01$) as the dietary level of Ca increased. Pigs fed high Ca diets had lower ($p = 0.01$) Ca digestibility compared with pigs fed low and medium Ca diets regardless of maifanite or cornstarch supplementation. Jolliff and Mahan [21] found that increasing dietary Ca levels decreased the digestibility of Ca. Other studies also reported that Ca digestibility was significantly reduced by a high dietary Ca level [22,23], which were all in accordance with our results. There were no significant difference in Ca intake, fecal Ca output, Ca retention and the ATTD of Ca between cornstarch and maifanite diets with the same dietary Ca level. However, urinary Ca excretion was lower ($p = 0.01$) in pigs fed low Ca diets without maifanite supplementation than other dietary treatments. Urinary Ca excretion was slightly increased by zeolite (hydrated crystalline aluminosilicate) supplementation in growing goats [24]. In the present study, dietary supplementation levels of maifanite had no effect on the Ca digestibility for growing pigs. However, Chen et al [9] reported significant improvements in the digestibility of Ca and P as a result of supplementation with either 1% or 2% Biotite V (aluminosilicate clay). It has been proposed that clays reduce the speed of passage of feed along the digestive tract in chickens which would improve the nutrient digestibility [25].

The different results with our findings may be related to the type of clays and supplemental content.

In regard to P, the daily intake and retention of P were not significantly affected by the dietary treatments. The fecal P excretion was lower ($p < 0.01$) in pigs fed low Ca diets without maifanite supplementation compared to pigs fed high Ca diets. Urinary P excretion were greater ($p < 0.01$) in pigs fed low Ca diets compared to pigs fed the medium and high Ca diets. Pigs fed high Ca diets without maifanite had lower ($p < 0.01$) P digestibility than other dietary treatments. Excess Ca is known to interfere with P absorption and usage [26]. Studies also showed that the utilization of absorbed P is dependent on the dietary Ca levels and Ca:P ratio [27-29]. This may be due to the formation of Ca-P complexes in the small intestine, which reduces the availability of P for absorption [30,31]. Observations for P metabolism were similar to those for Ca as there was no difference between cornstarch and maifanite diets treatments with the same dietary Ca level. Previous study has indicated that the P digestibility was not improved as a result of including aluminosilicate clay in the diet in growing-finishing pigs [10]. These findings also suggested that dietary supplementation with 2.25% maifanite had no effect on the P metabolism in growing pigs.

Digestibility measurement by IM method

The ATTD of Ca were lowest ($p < 0.01$) in pigs fed high Ca diets regardless of maifanite or cornstarch supplementation (Table 3). There were no significant differences in Ca digestibility between cornstarch and maifanite diets with the same dietary Ca level. These results are similar to those gained using the TC method. However, the ATTD of P were lowest ($p < 0.01$) in pigs fed high Ca diet without maifanite supplementation compared to the other dietary treatments. For the high Ca diets treatments, the ATTD of P was increased as the supplementation of maifanite. The

Table 2. Daily balance and apparent total tract digestibility (ATTD) of Ca and P in pigs fed experimental diets with the total collection (TC) method (as-fed basis)

Items	Treatment							SEM	p-value
	Ca, %:	Low (0.39)		Medium (0.70)		High (0.99)			
		Maifanite, %:	0	2.25	0	1.42	0		
Number of pigs		6	6	6	6	6	6	-	-
Ca (g/d)									
Intake		4.69 ^c	4.71 ^c	7.56 ^b	7.48 ^b	10.12 ^a	9.91 ^a	0.34	<0.01
Feces		2.08 ^c	2.24 ^c	3.37 ^b	3.60 ^b	5.72 ^a	5.93 ^a	0.26	<0.01
Urine		0.04 ^c	0.10 ^{ab}	0.07 ^{ab}	0.16 ^{ab}	0.29 ^a	0.26 ^{ab}	0.06	0.01
Retention		2.58	2.44	3.77	3.69	3.86	3.72	0.42	0.08
ATTD (%)		55.31 ^a	52.80 ^a	54.92 ^a	54.96 ^a	40.64 ^b	39.75 ^b	3.45	0.01
P (g/d)									
Intake		6.71	6.96	6.71	6.74	6.50	6.75	0.29	0.93
Feces		3.14 ^b	3.43 ^{ab}	3.27 ^{ab}	3.59 ^{ab}	3.84 ^a	3.85 ^a	0.15	0.01
Urine		0.89 ^a	0.79 ^a	0.28 ^b	0.33 ^b	0.02 ^b	0.08 ^b	0.10	<0.01
Retention		2.87	2.74	2.85	2.82	2.59	2.98	0.26	0.93
ATTD (%)		54.58 ^a	52.30 ^{ab}	48.69 ^{ab}	49.69 ^{ab}	40.72 ^c	45.27 ^{bc}	1.94	<0.01

SEM, standard error of the mean; Ca, calcium; P, phosphorus.

^{a-c} Within a row, means without a common superscript differ ($p < 0.05$).

Table 3. Apparent total tract digestibility (ATTD) of Ca and P in pigs fed experimental diets with indicator marker (IM) method (as-fed basis)

Items	Treatment						SEM	p-value		
	Ca, %:		Low (0.39)		Medium (0.70)				High (0.99)	
	Maifanite, %:	0	2.25	0	1.42	0			0.64	
Number of pigs		6	6	6	6	6	6			
Ca (%)		45.70 ^{ab}	46.52 ^{ab}	50.61 ^a	53.26 ^a	27.92 ^c	36.40 ^{bc}	3.13	<0.01	
P (%)		43.87 ^a	47.09 ^a	45.36 ^a	45.49 ^a	30.19 ^b	43.31 ^a	1.92	<0.01	

SEM, standard error of the mean; Ca, calcium; P, phosphorus.

^{a-c} Within a row, means without a common superscript differ (p < 0.05).

different outcomes may be due to the differences in the methodologies used for nutrient digestibility estimates in the experimental diets. According to these results, we suggest extending the days for feces collection when using the IM method to guarantee more precise results.

Comparison of TC and IM for estimation of the apparent total tract digestibility

The ATTD of Ca were significantly different only in pigs fed high Ca diets without maifanite supplementation when compared between TC and IM methods (Figure 1). The ATTD of P did not differ significantly between IM and TC methods in pigs fed medium Ca diet without maifanite or high Ca diet with maifanite treatments. Several comparative studies have shown that the two methods can yield different estimations of digestibility [14-17,32]. The overall mean digestibility of Ca and P calculated with the TC method was higher than that with the IM method, regardless of the dietary treatments. Similar to the present findings, Agudelo et al [32] reported that the values of digestibility for Ca and P were lower by IM vs TC. A possible reason may be that the index (Cr₂O₃) recovery was below 100% [14].

Blood serum parameters

Serum Ca and P levels and ALP activities were not significantly affected by dietary treatments when P level was constant among all the treatment groups (Table 4). The results of the present study indicate that pigs fed increasing concentrations of Ca in corn-soybean meal diets were able to maintain blood Ca within the normal range. Similar findings were reported by Nicodemo et al [33], Larsen et al [34], Li and Stahl [35], and Metzler-Zebeli et al [36]. The reported results further confirmed that serum Ca homeostasis was resistant across a range of increasing dietary Ca intake [37]. The present result indicated that total P concentrations in serum were not affected by dietary Ca levels without the supplementation of maifanite. However, Metzler-Zebeli et al [36] found that serum P concentrations was raised in pigs fed the high Ca diets as compared to those fed adequate Ca diets. The different results may be because the dietary Ca:P ratio used by Metzler-Zebeli et al [36] was constant for all diets. Measurement of ALP enzymatic activity is the most commonly used serum marker to assess bone formation [38]. As we all know, Ca is the critical nutritional factor for bone health. Hens fed high-Ca diet had higher bone strength and lower serum ALP activity than those fed the low Ca diet [39]. Eklou-Kalonji et al [40] also found that growing

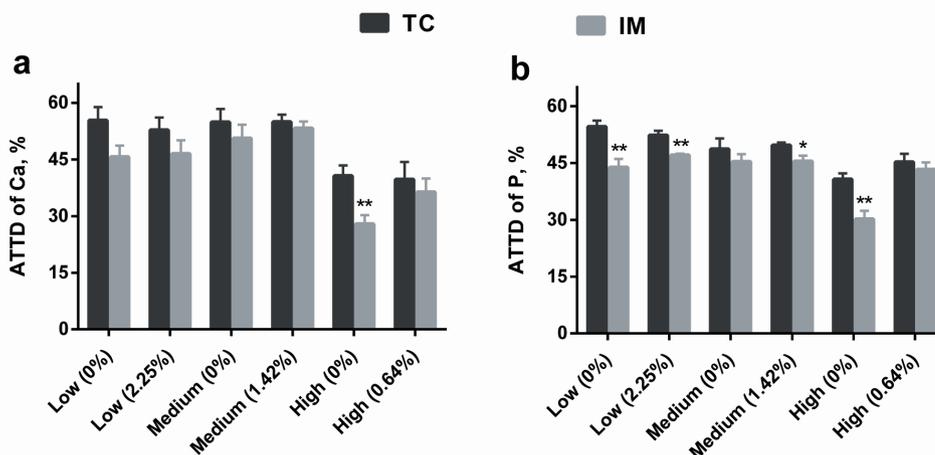


Figure 1. Comparison of apparent total tract digestibility (ATTD) of Ca and P in diets between total collection (TC) method and indicator method (IM). (a) The ATTD of Ca; (b) The ATTD of P. Values are means with their standard errors (** Significant difference between collection methods, p < 0.01; * Significant difference between collection methods, p < 0.05; n = 6). Low (0%), low Ca diet without maifanite supplementation; Low (2.25%), low Ca diet with maifanite supplementation; Medium (0%), medium Ca diet without maifanite supplementation; Medium (1.42%), medium Ca diet with maifanite supplementation; High (0%), high Ca diet without maifanite supplementation; High (0.64%), high Ca diet with maifanite supplementation.

Table 4. Effect of dietary Ca levels and maifanite on serum Ca, P concentrations and alkaline phosphatase (ALP) activity in growing pigs

Items	Treatment						SEM	p-value		
	Ca, %:		Low (0.39)		Medium (0.70)				High (0.99)	
	Maifanite, %:	0	2.25	0	1.42	0			0.64	
Number of pigs		6	6	6	6	6	6			
Day 0										
Ca (mmol/L)		2.22	2.24	2.22	2.13	2.20	2.24	0.05	0.59	
P (mmol/L)		3.02	3.11	2.88	2.96	2.96	3.11	0.11	0.59	
ALP (U/L)		283	284	259	268	290	306	18.98	0.56	
Day 12										
Ca (mmol/L)		2.09	2.12	2.14	2.15	2.17	2.20	0.03	0.15	
P (mmol/L)		2.72	2.62	2.75	2.56	2.58	2.69	0.07	0.32	
ALP (U/L)		241	264	233	250	302	262	21.12	0.22	

SEM, standard error of the mean; Ca, calcium; P, phosphorus.

pigs fed very low Ca (0.1%) diet had higher plasma ALP than pigs fed low Ca (0.4%) and control Ca (0.9%) diets. The unaffected serum ALP activity by dietary Ca levels in the present study may be due to the degree of dietary Ca restriction. Therefore, serum ALP is not sensitive enough to detect moderate stimulation of bone metabolism in growing pigs fed the low Ca diet.

In the current study, maifanite supplementation had no effect on blood parameters compared with cornstarch treatment regardless of dietary Ca levels. Several studies have demonstrated a similar effect concerning serum Ca, P, and ALP activity in growing pigs as a result of feeding aluminosilicate clays [7,41]. In agreement with these results, our study indicated that dietary maifanite supplementation (levels up to 2.25%) is unlikely to promote adverse effects on serum mineral concentrations in pigs.

In conclusion, the results of this experiment indicate that dietary supplementation with maifanite had no influence on the ATTD of Ca and P and the serum parameters in growing pigs. In addition, using TC method can lead to greater ATTD of Ca and P compared to the IM method in growing pigs.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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