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Dietary Requirement of True Digestible Phosphorus and Total Calcium for Growing Pigs

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ABSTRACT : Sixty healthy growing pigs (Duroc×Landrace×Yorkshire with an average BW of 21.4 kg) were used to determine the true digestible phosphorus (TDP) requirement of growing pigs on the basis of growth performance and serum biochemical indices. Pigs were assigned randomly to one of five dietary treatments (12 pigs/diet), representing five levels of TDP (0.16%, 0.20%, 0.23%, 0.26% and 0.39%). There were three replications per treatment, with four pigs (2 barrows and 2 gilts) in each replication (2 pigs/pen) A randomized-block design was used, with pen as the experimental unit. Experimental diets were formulated to provide the 5 TDP levels with a total calcium (Ca) to TDP ratio of 2:1, and offered to pigs at 5% BW for 28 d. The total Ca contents of the five diets were 0.33, 0.38, 0.45, 0.51 and 0.79%, respectively. During the 28-d experimental period, the ADG of pigs was affected by dietary TDP levels as described by Equation 1: $y = -809,532x^4+788,079x^3-276,250x^2+42,114x-1,759$; ($R^2 = 0.99$; $p<0.01$; y = ADG, g/d; x = dietary TDP, %). The feed:gain ratio for pigs was affected by dietary TDP levels as described by Equation 2: $y = 3,651.1x^4-3,480.4x^3+1,183.8x^2-172.5x+10.9$ ($R^2 = 0.99$; $p<0.01$; y = feed:gain ratio; x = dietary TDP, %). Total P concentrations in serum were affected by dietary TDP levels as described by Equation 3: $y = -3,311.7x^4+3,342.7x^3-1,224.6x^2+195.6x-8.7$ ($R^2 = 0.99$; $p<0.01$; y = total serum P concentration and x = dietary TDP, %). The highest ADG (782 g/d), the lowest feed:gain ratio (1.07), and the highest total serum P concentration (3.1 mmol/L) were obtained when dietary TDP level was 0.34%. Collectively, these results indicate that the optimal TDP requirement of growing pigs is 0.34% of the diet (e.g., 5.1 g/day for a 30-kg pig that consumed 1.5 kg feed daily) at a total Ca to TDP ratio of 2:1. (**Key Words :** Calcium, Pigs, Growth, Biochemical Indices, True Digestible Phosphorus)

INTRODUCTION

Phosphorus (P) is an essential element, being the second most abundant mineral in the animal's body after calcium (Ca). Phosphorus plays a key role in metabolism, including 1) the development and maintenance of skeletal tissue, 2) maintenance of osmotic pressure and acid-base balance, 3) energy utilization and transfer, 4) protein synthesis, 5) cell differentiation and growth, 6) appetite control, 7) intestinal integrity and function, 8) cell signaling and nutrient metabolism, 9) the efficiency of feed utilization, and 10) reproductive function (Anderson, 1991; Wu, 1998; Jobgen et al., 2006). These metabolic and physiological functions are essential for the survival, growth, and development of organisms. Symptoms of P deficiency vary but, in all cases, they are associated with reduced feed intake, impaired growth, and compromised health in animals. P deficiency will ultimately result in a reduction in plasma phosphate concentrations, followed by resorption of Ca and P from bones, thereby minimizing weight gain and growth performance of livestock (Fan et al., 2005; Yang et al., 2007).

A large proportion of P in plant feed ingredients exists as phytates, whose phosphate is not available for pigs due to a lack of mammalian digestive enzyme, phytase. Thus, swine diets should contain inorganic phosphate sources. However, an excessive use of inorganic phosphates in diets has emerged as an increasingly important problem due to the environmental pollution from phosphate-enriched swine

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Table 1. Fecal apparent and true digestibility of phosphorus (%)

Feedstuff	Fecal apparent digestibility	Fecal true digestibility
Corn (Yellow)	24.31±5.16	49.15±4.16
Corn (White)	17.16±5.78	40.33±5.24
Paddy meal	32.20±4.16	43.65±3.88
Barley meal	35.61±4.09	56.68±3.56
Wheat meal	32.70±4.10	49.74±4.92
Oat meal	35.98±590	27.45±3.83
Buckwheat meal	30.36±6.23	49.72±5.43
Sorghum meal	31.02±5.89	42.34±5.35
Rough rice meal	28.61±5.67	41.89±5.84
Broken rice meal	31.91±4.88	44.62±3.22
Rough rice bran	11.82±409	23.41±3.44
Defated rice bran	14.54±4.09	26.08±4.06
Wheat middlings meal	40.19±4.89	63.93±4.42
Wheat bran	41.25±3.99	56.82±1.59
Soybean meal	27.59±309	51.30±2.61
Cottonseed meal	15.02±5.90	30.22±4.61
Rapeseed meal	27.67±400	39.30±2.50
Field bean meal	31.60±5.89	50.76±5.86
Peas meal	26.33±744	36.56±6.50

Data are means±SEM, n = 5.

manure (Jongbloed et al., 1997; Correll et al., 1999; Mallin, 2000; Naqvi et al., 2000; Pouslen, 2000; Yang et al., 2007). Thus, research on accurately determining P requirement and improving the efficiency of its utilization by pigs has become an important issue in swine nutrition (Cromwell and Coffey, 1991; Jongbloed et al., 1991). Considerable research has been conducted to assess the requirements of total P by swine (Chapman et al., 1962; Combs et al., 1962; Miller et al., 1964; Bayley et al., 1969; Libal et al., 1969; Cromwell et al., 1970; Harmon et al., 1970; Cromwell et al., 1972). Most recently, Lin et al. (2002a, b) evaluated the requirement of available P in swine diets. Because P bioavailabilities vary greatly among feed ingredients, P requirements should be based on true digestible P (TDP) rather than total P or available P in feeds. However, despite considerable progresses in many areas of swine nutrition, TDP has not previously been established for pigs at any stage of the life cycle. The objective of this study was to determine the TDP requirement of growing pigs.

MATERIALS AND METHODS

Digestibility trial

Knowledge about the true digestibility of P in feed ingredients is necessary for designing a sound experiment to determine the TDP requirements for swine growth. Thus, we decided to determine the fecal apparent and true digestibility of P in 19 different feedstuffs from different parts of China over a 2-year period. This digestion study was conducted using a 5×5 Latin square design, according to the methods of Yin et al. (2004), Yang et al. (2007), and Fang et al. (2007a, b). The results are summarized in Table 1 as a basis for preparing diets used in the growth-performance trial.

Growth performance trial

Animals, diets, and experimental design : Sixty healthy cross-bred pigs (Duroc×Landrace×Large White) with an average BW of 21.4 kg were assigned randomly to one of the five dietary treatments. Each treatment consisted of 3 replications, with 4 pigs (2 barrows and 2 gilts) in each replication. Each pen housed 2 pigs. Five corn- and soybean meal-based diets were formulated on the basis of the TDP values of the ingredients (Table 1) to provide five levels of TDP: 0.16%, 0.20%, 0.23%, 0.26% and 0.39% and total Ca: 0.33%, 0.38%, 0.45%, 0.51% and 0.79% (Table 2), but the total Ca to TDP ratio was kept at 2:1 for all diets. Except for Ca and P, other nutrients were adequate for growing pigs (NRC, 1998) and were similar among the experimental diets. Feeds were offered to pigs at 5% BW for 28 d. Pigs had free access to drinking water via low-pressure drinking nipple (Chen et al., 2005; Fan et al., 2005; Yang et al., 2007; Kong et al., 2007a,b).

Measurement of body weight and sample collection : The BW of pigs was measured after a 12-h fast in the morning of the first and last days of the growth trial to calculate average daily gain (ADG). Feed intake was recorded daily during the 28-d period to determine average daily feed intake (ADFI) and the feed:gain ratio. On the first and last days of the experiment, after the fasted pigs were weighed, jugular venous blood samples (10 ml) were obtained from 1 pig of each pen into heparin-free tubes, as previously described (Tang et al., 2005). Blood samples were centrifuged at 4,000×g for 15 min. The supernatant fluid (serum) was collected and immediately frozen at -20°C for biochemical analyses (Yang et al., 2005; Chen et al., 2006; Huang et al., 2005; 2007; Deng et al., 2007a,b).

Serum analyses

An Automated Biochemistry Analyzer (Synchron CX Pro, Beckman Coulter, Fullerton, CA, USA) was used to determine the concentrations of total P, total Ca, and alkaline phosphatase (ALP) in serum. All the kits were purchased from Beijing Chemlin Biotech Co., Ltd (Beijing, China).

Statistical analyses

The ANOVA and the orthogonal analyses were performed according to a randomized-block design, using the General Linear Model procedures (Proc GLM) of SAS (SAS, 2000). Pen was the experimental unit in the statistical analysis (Linear and curvilinear regression analyses were conducted, using SAS (2000). For the orthogonal contrast

Table 2. Composition of experimental diets and nutritive values for growing pigs

Ingredients (%)	Diet				
	1	2	3	4	5
Corn	58.00	58.00	58.00	58.00	58.00
Soybean meal	22.00	22.00	22.00	22.00	22.00
Cornstarch	1.94	1.70	1.45	1.20	0.07
Rice bran meal	5.00	5.00	5.00	5.00	5.00
Cottenseed meal	3.00	3.00	3.00	3.00	3.00
Wheat middlings meal	5.00	5.00	5.00	5.00	5.00
Soybean oil	3.00	3.00	3.00	3.00	3.00
Lys	0.13	0.13	0.13	0.13	0.13
NaCl	0.30	0.30	0.30	0.30	0.30
$CaCO_3$	0.63	0.67	0.72	0.77	1.00
$CaHPO_4$	0.00	0.20	0.40	0.60	1.50
Premix[1]	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutritive values[2]					
CP (%)	17.34	17.34	17.34	17.34	17.34
DE (Mcal/kg)	3.44	3.43	3.42	3.41	3.36
Calcium (%)	0.33	0.38	0.45	0.51	0.79
Total phosphorus (%)	0.44	0.47	0.51	0.54	0.69
True digestible phosphorus (%)	0.16	0.19	0.23	0.26	0.39
Ca:true digestible phosphorus	2:1	2:1	2:1	2:1	2:1
True digestible lysine (%)	0.83	0.83	0.83	0.83	0.83
TMet+TCys (%)[3]	0.51	0.51	0.51	0.51	0.51
True digestible threonine (%)	0.58	0.58	0.58	0.58	0.58
True digestible tryptophan (%)	0.19	0.19	0.19	0.19	0.19

[1] Supplied the following minerals and vitamins (mg/kg diet): $CuSO_4·5H_2O$, 11.8; KI, 0.6; $FeSO_4·H_2O$, 152; $NaSeO_3$, 0.3; $ZnCO_3·7H_2O$, 95.9; $MnSO4·H_2O$, 6.2; retinyl palmitate, 5.2; cholecalciferol, 0.38; all-rac-α-tocopherol acetate, 44.0; menadione, 3.0; riboflavin, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B-12, 0.012; thiamine, 1.1; choline chloride, 550.0; pyridoxine, 1.1; d-biotin, 0.1; and folic acid, 0.6.

[2] Calculated values on an as-fed basis.

[3] True digestible methionine-true digestible cysteine.

among the treatment groups, the linear and quadratic effects were tested using SAS (2000). Probability values <0.05 were taken to indicate statistical significance.

RESULTS

Serum biochemical indices

Neither ALP activities nor total Ca concentrations in serum differed (p>0.05) when dietary TDP levels increased from 0.16% to 0.39% (Table 3). However, feeding different levels of TDP had a quadratic effect (p<0.05) on total P concentrations in serum (Table 3). A regression equation relating total serum P concentrations to dietary TDP levels was established: $y = -3,312x^4+3,343x^3-1,225x^2+196x-9$; $R^2 = 0.99$, $p<0.05$; where x = dietary TDP level (%) and y = total serum P concentration (mmol/L). Total P concentration in serum was the highest (3.1 mmol/L; p<0.05) when dietary TDP was 0.34% (or 5.1 g/day for a 30-kg pig that consumed 1.5 kg feed/day). Total P concentration in serum (0.22 mmol/L or 1.36 mg/100 ml) was lower (p<0.05) in pigs fed a diet containing 0.16% TDP than in pigs fed diets providing 0.26% and 0.39% TDP.

Growth performance

Feeding different levels of TDP had no linear effects (p>0.05) on ADG, ADFI, or the feed:gain ratio in growing pigs during a 28-d trial (Table 4). However, dietary TDP levels exhibited a quadratic effect (p<0.05) on ADG and the feed:gain ratio (Table 4). An equation was developed to relate the ADG of pigs to a dietary TDP level: $y = -809,532x^4+788,079x^3-276,250x^2642,114x-1,759$; $R^2 = 0.99$; $p<0.01$; where x = dietary TDP level (%) and y = ADG of pigs (g/d). The ADG of pigs was the greatest (750 g/d) when the dietary TDP level was 0.34%. Notably, the ADG of pigs decreased (p<0.05) when dietary TDP levels exceeded 0.34%.

The feed:gain ratio of growing pigs decreased (p<0.05) from 1.71 to 1.56 when dietary TDP levels increased from 0.16% to 0.26%, but increased (p<0.05) from 1.56 to 1.67 when dietary TDP level was elevated from 0.26% to 0.39%. The relationship between the feed:gain ratio for pigs and dietary TDP levels could be described by the following equation: $y = 3,651.1x^4-3,480.4x^3+1,183.8x^2-172.5x+10.9$;

Table 3. The Effect of dietary true digestible phosphorus (TDP) levels on serum biochemical indices in growing pigs

Diet	1	2	3	4	5	SEM	P (linear)	P (quadratic)
Total P (%)	0.44	0.47	0.51	0.54	0.69			-
Apparent P (%)	0.14	0.17	0.20	0.23	0.37			-
TDP (%)	0.16	0.20	0.23	0.26	0.39			-
Total calcium (%)	0.33	0.38	0.45	0.51	0.79			
Serum alkaline phosphatase activity (U/L)	186	183	168	166	159	18	0.808	0.782
Total serum P concentration (mmol/L)	2.72^{b}	2.83^{b}	2.86^{b}	2.94^{a}	2.94^{a}	0.11	0.565	0.049
Total serum Ca concentration (mmol/L)	2.57	2.63	2.66	2.75	2.67	0.05	0.147	0.521

$^{a, b}$ Values in the same row with different superscript letters differ (p<0.05).
Data are means with pooled SEM for 6 pigs per treatment group.

Table 4. The effects of dietary true digestible phosphorus (TDP) levels on growth performance in growing pigs

Diet	1	2	3	4	5	SEM	P (linear)	P (quadratic)
Total P (%)	0.44	0.47	0.51	0.54	0.69			-
Apparent P (%)	0.14	0.17	0.20	0.23	0.37			-
TDP (%)	0.16	0.20	0.23	0.26	0.39			-
Total calcium (%)	0.33	0.38	0.45	0.51	0.79			
Initial weight (kg)	21.38	21.58	21.34	21.36	21.29	0.35	0.973	0.995
Finial weight (kg)	38.31	39.04	39.18	40.08	40.00	1.06	0.753	0.458
Average daily feed intake (g/d)	1,029	1,059	1,069	1,060	1,062	88	0.998	0.700
Total P intake (g/d)	4.5^{a}	4.9^{a}	5.5^{ab}	5.7^{ab}	7.3^{b}	0.49	0.019	0.219
Apparent P intake (g/d)	1.4^{a}	1.8^{ab}	2.1^{ab}	2.4^{b}	3.9^{c}	0.22	0.001	0.308
TDP intake (g/d)	1.6^{a}	2.1^{ab}	2.4^{ab}	2.7^{b}	4.1^{c}	0.24	0.001	0.209
Average daily gain (g/d)	604^{b}	623^{b}	637^{ab}	668^{a}	668^{a}	34	0.640	0.045
Feed:gain ratio	1.71^{a}	1.72^{a}	1.69^{a}	1.56^{b}	1.67^{a}	0.14	0.984	0.0344

$^{a, b}$ Values in the same row with different superscript letters differ (p<0.05).
Data are means with pooled SEM for 12 pigs per treatment group.

R^2 = 0.99; p<0.01; where x = dietary TDP level (%) and y = the feed:gain ratio for growing pigs. The feed:gain ratio was the lowest (1.07) when the dietary TDP level was 0.34%.

DISCUSSION

Only the true P digestibility value can reflect the actual digestion and absorption of P in the diet. There were several disadvantages for using apparent P digestibility to estimate the efficiency of P utilization by pigs. For example, when the contribution of endogenous P to intestinal or fecal P is not taken into consideration, the fecal apparent P digestibility was 20% to 25% lower than the fecal true P digestibility for feed ingredients (Table 1), resulting in a substantial underestimation of digestible P in swine diets. Additionally, apparent P availabilities of the same feed ingredient vary considerably (as much as 15% to 35%) with swine diets (Fan et al., 2001 and 2002; Shen et al., 2002; Fang et al., 2007b). Further, 3) the apparent P digestibility values are not always additive in single feed ingredients for growing pigs (Fan et al., 2002; Fang et al., 2007b). In contrast, available evidence shows that true P digestibility values are additive in ingredients containing low levels of phytate phosphorus and antinutritional factors (Fang et al., 2007b). Thus, compared with the trditional total dietary P content and apparent P digestibility systems, the use of TDP in formulating swine diets offers a distinct advantage of accuracy in meeting P requirements. In support of this view, results from the present study indicate that changes in the ADG of growing pigs and total serum P concentrations were positively correlated with dietary TDP levels, but not with total dietary P or fecal digestible P (Table 3 and 4).

A major factor that affects the determination of P requirements by animals is the Ca to P ratio (Combs et al., 1991). This ratio greatly influences the availability of dietary Ca and P that can enter the portal circulation (Anderson, 1991). The NRC (1998) recommended that the ratio of total Ca to total P in a typical corn- and soybean meal-based diet be between 1:1 and 1:1.2 and that the ratio of total Ca to available P be between 2:1 and 3:1. The ARC (1981) suggested that the ratio of total Ca to total P should not exceed 2:1 for pigs in the growing phase and that a ratio of total Ca to total P ratio between 1:1 and 1.2:1 for diets containing phytic acid is beneficial for growth performance and bone function in the pigs. The phytate and phosphatase concentrations differ among dietary ingredients from different sources. As a result, there were marked disparities in P availability in the swine alimentary tracts. Therefore, formulating diets on the basis of a total Ca to total P ratio will not accurately reflect the actual requirements of these two minerals by growing pigs. This shortcoming can be best corrected using a ratio of total Ca to TDP to precisely meet the metabolic requirements of both Ca and P by swine. The results from our extensive research have established that a total Ca to TDP ratio of 2:1 is optimal for growth performance and the efficiency of utilization of dietary P in growing pigs (Yin, 2005).

The results of present study indicate that serum ALP activity was not affected by dietary Ca contents when the ratio of total Ca to TDP was kept at 2:1 among all the treatment groups. Also, varying dietary Ca intakes at a constant ratio (2:1) of total Ca to TDP had no effect on total Ca concentrations in the serum of growing pigs (Table 3). Similar findings were reported by Lin et al. (2002a) and Wang et al. (2002). However, total P concentrations in serum increased with increasing dietary TDP levels (Table 3). These results are comparable to those reported by Lin et al. (2002a, b) for weanling pigs as well as growing-finishing pigs. Taken together, the findings from the present study and the work of Lin et al. (2002a) demonstrate that total P concentrations in serum rise as dietary P levels increase to an optimal level. Moreover, our findings shows that an optimal TDP level in the diet is 0.34% (on an as-fed basis) for 20- to 40-kg pigs. Thus, a further increase in dietary TDP levels beyond 0.34% did not increase total P concentrations in serum (Table 3). Interestingly, Wang et al. (2002) reported that serum P concentrations increased progressively with increasing dietary P levels in Xiangzhu Chinese pigs. It is possible that there are significant differences in Ca or P digestion and metabolism between different breeds of pigs. Future studies using molecular biology and proteomics technologies (Wang et al., 2006) are warranted to test this important hypothesis.

Besides the data on serum P concentrations (Table 3), our findings also show that growing pigs exhibited the highest ADG and highest feed efficiency when they were fed a diet containing 0.34% TDP and 0.68% Ca (Table 4). This diet provided the 20- to 40-kg pigs with daily TDP, available P and total P intakes of 3.76, 3.65, and 6.81g/day, respectively (Table 4). Note that the recommended requirements (NRC, 1998) of the available P in the diet was 0.23% with daily intakes of available P and total P intake being 4.27 and 9.28 g/day, respectively, for pigs with BW of 20-50 kg. Thus, the NRC-recommended dietary intake of total P by growing pigs (NRC, 1998) is 36% greater than the optimal value (6.81 g/day) obtained from the present study. Clearly, NRC (1998) overestimated substantially P requirement by growing pigs fed corn- and soybean meal-based diets. We suggest that this overestimation be corrected in its next version of swine nutrient requirements.

In summary, results of the present study indicate that, on the basis of growth performance and serum biochemical indices, the optimal TDP requirement of 20- to 40-kg pigs is 0.34% of the diet (on an as-fed basis) at a total Ca to TDP ratio of 2:1. This corresponds to 5.1 g TDP/day for a 30-kg pig that consumes 1.5 kg feed daily. Our findings suggest that dietary P requirement by growing pigs is substantially overestimated in the current version (10^{th} edition) of NRC-recommended nutrient requirements of swine.

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