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True Digestibility of Phosphorus in Different Resources of Feed Ingredients in Growing Pigs

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ABSTRACT : To determine the true digestible phosphorus (TDP) requirement of growing pigs, two experiments were designed with the experimental diets containing five true digestible P levels (0.16%, 0.20%, 0.23%, 0.26% and 0.39%) and the ratio of total calcium to true digestible P (TDP) kept at 2:1. In Experiment 1, five barrows (Duroc×Landrace×Yorkshire) with an average initial body weight of 27.9 kg were used in a 5×5 Latin-square design to evaluate the effect of different dietary P levels on the digestibility and output of P and nitrogen. In Experiment 2, sixty healthy growing pigs (Duroc×Landrace×Yorkshire) with an average body weight (BW) of 21.4 kg were assigned randomly to one of the five dietary treatments (12 pigs/diet), and were used to determine the true digestible phosphorus (TDP) requirement of growing pigs on the basis of growth performance and serum biochemical indices. The results indicated that the true digestibility of P increased ($p<0.05$) linearly with increasing dietary TDP level below 0.26%. The true P digestibility was highest (56.6%) when dietary TDP was 0.34%. Expressed as g/kg dry matter intake (DMI), fecal P output increased ($p<0.05$) linearly with increasing P input. On the basis of g/kg fecal dry matter (DM), fecal P output was lowest for Diet 4 and highest ($p<0.05$) for Diet 5. The apparent digestibility of crude protein (CP) did not differ ($p>0.05$) among the five diets, with the average nitrogen output of 12.14 g/d and nitrogen retention of 66% to 74% ($p>0.05$), which suggested that there was no interaction between dietary P and CP protein levels. During the 28-d experimental period of Experiment 2, the average daily gain (ADG) of pigs was affected by dietary TDP levels as described by Eq. (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, %), F/G for pigs by Eq. (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 = F/G; x = dietary TDP, %), and Total P concentrations in serum by Eq. (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 F/G (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 at a total Ca to TDP ratio of 2:1. (**Key Words :** True Digestible Phosphorus, Growing Pigs, Growth Performance, Biochemical Indices, Calcium)

INTRODUCTION

As the second most abundant mineral in the animal body, phosphorus (P) has many metabolic and physiological functions, including energy utilization and transfer, cell differentiation and growth, cell signaling and nutrient metabolism, development and maintenance of skeletal tissue, intestinal integrity and function, protein synthesis and maintenance of osmotic pressure and acid-base balance (Anderson, 1991; Wu, 1998). 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.

Phytate P is the major form, however, it is poorly

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

Feedstuffs	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
Defatted 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.

digested by pigs due to a lack of phytase (Jongbloed and Lenis, 1998). So producers always add excessive phosphate in feed in order to fill the requirement of pigs, which can result in the following problems: (1) excessive P output in swine manure enters our waterways where it can stimulate algal blooms and eutrophication. (Mallin, 2000; Pouslen, 2000; Sacakli et al., 2006; Fang et al., 2007a); (2) excessive P in feed adds to the cost of swine production; (3) as an anti-nutrient factor, inorganic P combines with Ca and fat in diets into an insoluble compound -saponin- which decreases the digestibility of other nutrients; and (4) as a crucial component of all forms of life on earth, inorganic phosphate is a limited and non-renewable natural resource and the conservation of P has become a global issue (Abelson, 1999). Thus, research on determining P requirement accurately and improving the efficiency of its utilization by pigs has become an important issue in swine nutrition (Bhanja et al., 2005; Shen et al., 2005; Ruan et al., 2007).

Considerable research has been conducted to assess the requirements of total P by swine (Miller et al., 1964; Cromwell et al., 1972; Lin et al. 2002; Ruan et al., 2007). Because P bioavailabilities vary greatly among feed ingredients and endogenous P and nitrogen (N) were included in the feces, so the apparent digestibility underestimated the biological values of amino acid and P, only true digestibility reflects the process of digestion and absorption. So P requirements should be based on true digestible P (TDP) rather than total P or available P in feeds. Despite considerable progress in many areas of swine nutrition, TDP has not been previously established for pigs

Table 2. Composition of experimental diets and nutrient indexes for growing pigs

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Corn	57.80	57.80	57.80	57.80	57.80
Soybean meal	22.10	22.10	22.10	22.10	22.10
Cornstarch	2.04	1.80	1.55	1.30	0.17
Rice bran meal	5.00	5.00	5.00	5.00	5.00
Cottonseed meal	3.00	3.00	3.00	3.00	3.00
Wheat middling 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:					
Crude protein (%)	17.34	17.34	17.34	17.34	17.34
Digestible energy (MCal/kg)	3.43	3.42	3.41	3.40	3.35
Total Ca (%)	0.32	0.40	0.46	0.52	0.78
Total phosphorus (%)	0.44	0.47	0.51	0.54	0.69
True digestible phosphorus (%)	0.16	0.20	0.23	0.26	0.39
Total Ca:true digestible P	2:1	2:1	2:1	2:1	2:1
True digestible Lys (%)	0.83	0.83	0.83	0.83	0.83
True digestible (Met+cys) (%)	0.51	0.51	0.51	0.51	0.51
True digestible Thr (%)	0.58	0.58	0.58	0.58	0.58
True digestible Trp[12] (%)	0.19	0.19	0.19	0.19	0.19

[1] Supplied the following vitamins and minerals (mg/kg diet): 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; $FeSO_4 \cdot H_2O$, 152; $ZnCO_3$, 95.9; $MnSO_4 \cdot H_2O$, 6.2; $CuSO_4 \cdot 5H_2O$, 11.8; KI, 0.6; Na_2SeO_3, 0.3.

at any stage of the life cycle. Thus the objective of these studies was to determine the TDP requirement of growing pigs and to study the effect of dietary P levels on the digestibility and output of P and N. The studies offer a scientific basis for making diets with low pollution characteristics.

MATERIALS AND METHODS

Digestibility trial and experimental diets

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, the fecal apparent and true digestibility of P in 19 major feedstuffs of China were determined over a 2-year period. in our laboratory. These studies were conducted using a 5×5 Latin square design, according to the methods of Yin et al. (Yin et al., 1995; Yin and McCracken, 1996; Yin et al., 2000a, b, c; 2002a, b) Fan et al. (2005) and Fang et al. (2007a, b). The results are summarized in Table 1 as a basis for preparing diets used in the trials.

Five corn- and soybean meal-based diets were formulated on the basis of the TDP values of the ingredients mentioned above to provide five levels of TDP: 0.16%, 0.20%, 0.23%, 0.26% and 0.39% and the ratio of total calcium to TDP was 2:1 (Table 2). Except for Ca and P, other nutrients were adequate for growing pigs (NRC, 1998) and were similar among the experimental diets. Chromic oxide (0.4%) was added as an inert marker for estimation of the coefficient of total tract apparent digestibility (Huang et al., 2005; Yang et al., 2005).

Animals, experimental design and management

In Experiment 1, five Duroc×Landrace×Yorkshire barrows, with an average initial body weight (BW) of 27.86 kg, were fitted with a simple T-cannula at the distal ileum. The pigs were housed individually in stainless steel metabolism crates in a temperature-controlled room (20-25°C) and fed the five experimental diets (Table 2) according to a 5×5 Latin square design. The experiment had 5 periods and during a 7-d recovery period, the pigs were fed a regular grower diet. During the experimental period the pigs were fed twice daily, equal amounts for each meal at 08:00 and 20:00 h (Yin et al., 1993). The dietary allowance was 4% of body weight (Yin et al., 2001a, b, c, d; Yin et al., 2004).

In Experiment 2, 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 (Table 2). Each treatment consisted of 3 replications, with 4 pigs (2 barrows and 2 gilts) in each replication. Each pen housed 2 pigs. Feeds were offered to pigs at 5% BW for 28 d (Yin et al., 1991a, b;Yin et al., 1993a, b; Yin et al., 1994; Yin 1994; Yang et al., 2007).

Drinking water was freely available to all the pigs via low-pressure drinking nipples.

Sample collection and treatment

In Experiment 1, each experimental period comprised 8 d. After a 4-d adaptation, all possible fecal samples were collected on d 5, 6, 7 and 8. The ileal digesta samples were collected in soft plastic boxes which were attached to the barrel of the cannula with Velcro tape. The box contained 10ml of a solution of formic acid (2.86 ml/L) to minimize further bacterial activity and was replaced as soon as it was partially filled with sample. Samples were immediately frozen at -20°C. Then samples were freeze-dried, pooled within the same barrow and period of the same diet, ground through a 1-mm mesh screen and mixed before analysis. Samples of the diets were ground similarly. Analyses were performed in duplicate (Huang et al., 2003).

In Experiment 2, 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(F/G). On the 1st and 28th day of the experiment, the fasted pigs were weighed, and 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 (Deng et al., 2007a, b; Kong et al., 2007a, b, c).

Chemical analysis

Analysis for dry matter (DM) was carried out according to AOAC methods. Chromic oxide was determined (Saha and Gilbreath, 1991) by using an atomic absorption spectrometer (SpectrAA-10/20, Varian, Mulgrave, Australia). Diet and fecal samples were weighed into 60-ml Pyrex beakers and ashed overnight at 550°C. Cr_2O_3, as part of the ash, was then oxidized to dichromate by digestion in 6 ml of phosphoric acid (16.7 mol/L)-manganese sulfate (13.5 m mol/L) solution mixed with 8 ml of potassium bromate (0.27 mol/L) solution on a hot plate. Potassium dichromate was used as standard (Deng et al., 2007a, b; Huang et al., 2007).

Analyses of total inorganic P in samples were carried out by spectrophotometric analysis at 355 nm (Heinoen and Lahti, 1981). Potassium monobasic phosphate was used as standard.

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 in serum (Kong et al., 2007a, b; Li et al., 2007). All the kits were purchased from Beijing Chemlin Biotech

Co., Ltd (Beijing, China).

Estimation principles

Determination of the gastrointestinal endogenous P outputs by the regression analysis technique relies on establishing linear relationships between P outputs in feces and their dietary inputs (Fan and Sauer, 1999).

The total outputs of P in feces, expressed as g/kg dry matter diet intake (DMI), are calculated from Eq. (1) according to the previous studies.

$$P_O = P_I \times (I_D / I_I) \quad (1)$$

where P_O represents the outputs of P in feces (g/kg DMI), P_I is the content of P in feces (g/kg DM feces), I_D is the Cr_2O_3 concentration in the diets (g/kg DMI) and I_I is the Cr_2O_3 concentration in feces (g/kg DM feces).

The outputs of P in feces have both dietary and endogenous origins. If there are linear relationships between P outputs in feces and the graded levels of P inputs from diets, when expressed as g/kg DMI, their relationships can be expressed according to Eq. (2).

$$P_{Oi} = P_E + D_I \times P_{Di} \quad (2)$$

where P_{Oi} represents the output of P in feces collected from animals fed the ith assay diet, determined using Eq. (1) (g/kg DMI), P_E represents the level of the gastrointestinal endogenous P in feces (g/kg DMI), D_I is the percentage of dietary P that is truly indigestible going through the gastrointestinal tract (%) and P_{Di} is the P content in the ith assay diet (g/kg DMI). D_T is the true fecal P digestibility values (%) in the P-containing assay ingredient and can be calculated according to Eq. (3), once D_I is estimated from regression analysis according to Eq. (2).

$$D_T = 100\% - D_I \quad (3)$$

Eq. (2) represents a simple linear regression model in which P_{Oi} and P_{Di} are the dependent and independent variables, respectively. P_E and D_I are the regression coefficients and are estimated by fitting the simple linear regression model. If there are linear relationships between P outputs in feces and graded levels of P inputs from the diets with significant intercepts, then the endogenous P level in feces can be determined directly by extrapolating the dietary inputs of P to zero by obtaining the intercepts of the linear regression Eq. (P_E).

To determine true fecal P digestibility values in a P-containing ingredient, a series of assay diets are formulated to contain graded dietary levels of P, but only from the assay ingredient. The contents of other dietary factors, such as anti-nutritive factors that likely affect P digestion and endogenous P outputs, should be controlled between the assay diets.

The apparent fecal digestibility values of DM and P in the experimental diets were calculated according to Eq. (4).

$$D_{Ai} = 100\% - ((I_D \times P_I)/(I_I \times P_D)) \times 100\% \quad (4)$$

where D_{Ai} represents the apparent fecal P digestibility values in the assay diets (%, on as-fed basis), I_D is the digestibility marker concentration in the ith assay diet (%, on as-fed basis), P_I is the P concentration in feces (%, on as-fed basis), P_D is the P concentration in the ith assay diet (%, on as-fed basis) and I_I is the digestibility marker concentration in feces (%, on as-fed basis).

On the basis of the apparent ileal and fecal P digestibility values and the levels of endogenous P extrapolated with regression analysis, the true P digestibility values in the assay diets and also in the test ingredient, SBM, can be determined according to Eq. (5).

$$D_{Ti} = D_{Ai} + (P_E / P_{Di}) \times 100\% \quad (5)$$

Alternatively, the endogenous P outputs corresponding to individual diets can also be calculated according to Eq. (6), if corresponding true ileal and fecal P digestibility values are determined.

$$P_E = ((D_{Ti} - D_{Ai}) \times P_{Di})/100\% \quad (6)$$

where D_{Ti} represents the true fecal P digestibility values in the assay diets (%), D_{Ai} represents the apparent fecal P digestibility values in the assay diets (%), P_E represents the levels of endogenous P in feces (g/kg DM diet intake) and P_{Di} is the P concentration in the assay diets (g/kg DM diet).

Statistical analyses

The digestibility values were first subjected to ANOVA for a 5×5 Latin square design. The treatment effect was therefore partitioned and tested according to equally spaced orthogonal polynomial analyses (Steel and Torrie, 1980). The ANOVA and the orthogonal polynomial analyses were carried out using the General Linear Procedures of SAS (SAS, 2000). Related linear and curvilinear regression analyses were conducted using the Fig.P program (FigP Sofware Corporation, 1992). The comparison of true P digestibility values and the endogenous P outputs between feces was conducted according to the pooled t test (Byrkit, 1987). Pen was the experimental unit in the statistical analysis. For the orthogonal contrast among the treatment groups, the linear and quadratic effects were tested using

Table 3. Dry matter, crude protein, apparent and true phosphorus digestibility values in experimental diets (%)

Items	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P
Dietary true digestible phosphorus concentration	0.16	0.20	0.23	0.26	0.39	-	-
Dry matter digestibility values	91.74	91.03	90.88	90.04	91.00	0.75	0.6315
Apparent crude protein digestibility values	80.86	81.55	82.60	81.76	81.23	0.85	0.6794
Apparent phosphorus digestibility values	25.60	31.05	32.57	34.49	34.59	2.16	0.0676
True phosphorus digestibility values	48.40	51.03	51.68	52.69	49.56	2.16	0.6567

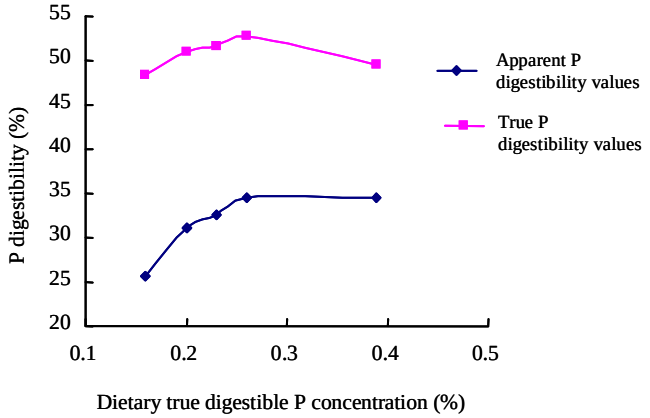

Figure 1. The effects of dietary true digestible phosphorus concentration on the apparent and true phosphorus digestibility.

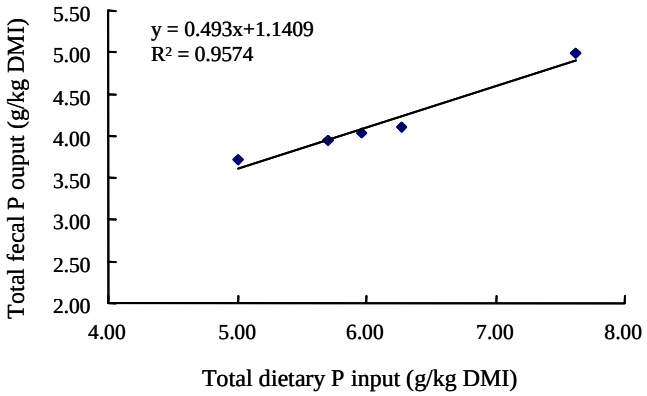

Figure 2. Linear relationship between phosphorus output in feces and dietary phosphorus input.

SAS (2000). Probability values of <0.05 were taken to indicate statistical significance.

RESULTS

All the pigs remained healthy and symptoms of Ca and P deficiency were not found. There was no effect of individual animal and experimental period on the result according to the statistical anaysis.

The digestibility of P and endogenous output

The apparent P digestibility could be calculated according to Eq. (4) and increases with increasing dietary TDP concentration in growing pigs. The apparent P digestibility values increased from 25.60% to 34.59% (Table 3 and Figure 1).

When expressed as g/kg DMI, the fecal P output could be calculated according to Eq. (1). Fecal P output increased significantly (p = 0.0002) linearly with increasing dietary TDP input (Table 4). So it can be concluded that fecal P output was influenced by the dietary TDP concentration. Endogenous P output was calculated according to Eq. (5). Endogenous P output of growing pigs was calculated as 1.14 g/kg DMI in Experiment 1.

True P digestibility was calculated according to Eq. (5) or Eq. (6). From Table 3 and Figure 1, true P digestibility increased with increasing dieatry TDP concentration when dietary TDP concentration was below 0.26%. When dietary TDP concentration increased from 0.26% to 0.39%, the true P digestibility decreased from 52.69% to 49.56% and the true P digestibility was highest when dietary TDP

Table 4. Dietary phosphorus input and the partitioning of phosphorus flow in feces

Items	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P
Total dietary phosphorus intake (g/kg DMI)	5.00	5.71	5.97	6.27	7.26	-	-
Apparent digestible phosphorus in feces (g/kg DMI)	1.28^{a}	1.77^{b}	1.94^{b}	2.16^{b}	2.64^{c}	0.13	0.0001
True digestible phosphorus in feces (g/kg DMI)	2.42^{a}	2.91^{b}	3.01^{b}	3.30^{b}	3.78^{c}	0.13	0.0001
Total fecal phosphorus output							
g/kg feces DM	21.86^{ab}	23.77^{c}	23.21^{bc}	21.28^{a}	25.82^{d}	0.49	0.0002
g/kg DMI	3.72^{a}	3.94^{a}	4.02^{a}	4.11^{a}	4.99^{b}	0.13	0.0002
Endogenous fecal phosphorus output							
g/kg feces DM	7.35	6.70	6.20	5.15	6.27	0.58	0.1772
g/kg DMI	1.25	1.12	1.08	1.01	1.22	0.13	0.6710
Fecal phosphorus output of dietary origin							
g/kg feces DM	15.15^{a}	16.86^{a}	16.62^{a}	15.33^{a}	19.67^{b}	0.44	0.0001
g/kg DMI	2.58^{a}	2.80^{a}	2.88^{a}	2.96^{a}	3.84^{b}	0.13	0.0002

Values in the same row with different superscript letters differ significantly (p<0.05).

Table 5. The balance of phosphorus and nitrogen for growing pigs

Items	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P
Dietary true digestible phosphorus concentration (%)	0.16	0.20	0.23	0.26	0.39	-	-
Dietary phosphorus input (g/d)	6.45[a]	7.36[b]	7.84[b]	8.02[b]	9.62[c]	0.18	0.0001
Fecal phosphorus output (g/d)	3.38[a]	3.55[a]	3.86[a]	3.98[a]	5.39[b]	0.23	0.0004
Urinary phosphorus output (g/d)	0.025[a]	0.034[b]	0.035[b]	0.042[c]	0.072[d]	0.0020	0.0001
Total phosphorus output (g/d)	3.40[a]	3.58[a]	3.90[a]	4.02[a]	5.47[b]	0.23	0.0003
Detention phosphorus (g/d)	3.05[a]	3.18[b]	3.94[b]	4.00[b]	4.26[b]	0.17	0.0043
Phosphorus retention (%)	47.67	51.26	50.29	49.90	43.30	2.77	0.3715
Dietary nitrogen input (g/d)	40.85	40.53	41.73	40.41	39.85	0.91	0.6829
Fecal nitrogen output (g/d)	6.17	6.92	6.47	6.01	4.78	0.79	0.4402
Urinary nitrogen output (g/d)	6.07	6.57	6.28	5.76	5.69	0.48	0.6857
Total nitrogen output (g/d)	12.24	13.49	12.74	11.77	10.47	0.92	0.2596
Retentive nitrogen (g/d)	28.61	27.04	28.99	28.64	29.38	1.03	0.5790
Nitrogen retention (%)	70.25	66.60	69.46	70.92	73.64	2.13	0.2834

Values in the same row with different superscript letters differ significantly ($p<0.05$).

concentration was 0.34% (Figure 1). There were no significant differences among the 5 diets.

Fecal P flow

As shown in Table 4, apparent fecal digestible P output increased with increasing dietary TDP concentration. Apparent fecal digestible P output increased from 1.28 g/kg DMI to 2.64 g/kg DMI. The apparent fecal digestible P output of diet 1 was significantly lower than diet 2 ($p = 0.022$) and highly significantly lower than diet 3 ($p = 0.007$), diet 4 ($p = 0.003$) and diet 5 ($p = 0.001$). The apparent fecal digestible P output of diets 2, 3 and 4 were highly significantly lower than diet 5 ($p<0.001$).

True fecal digestible P output increased from 2.42 g/kg DMI to 3.78 g/kg DMI with increasing dietary TDP concentration. The true fecal digestible P output of diet 1 was significantly lower than diet 2 ($p = 0.022$) and highly significantly lower than diets 3 ($p = 0.007$), 4 ($p = 0.003$) and 5 ($p = 0.001$). The true fecal digestible P output of diets 2, 3 and 4 were highly significantly lower than diet 5 ($p<0.001$).

Expressed as g/kg DMI, fecal P output increased linearly with increasing dietary P input. The linear equation was $y = 0.493x+0.1409$, $R^2 = 0.9574$, where x represents dietary P input (g/kg DMI), y represents fecal P output (g/kg DMI). When x was extrapolated to zero, the y value obtained from the intercept of the linear regression equation was endogenous P output (Figure 2). The average endogenous P output of growing pigs in the experiment was 1.14 g/kg DMI. The fecal P output of diet 5 was very significantly higher than diets 1, 2, 3 and 4 ($p<0.001$). The differences among other diets were not significant (Table 4). The ratio of fecal P output to dietary P input decreased from 74.4% to 65.5%, then increased to 68.7% with increasing dietary TDP concentration.

Expressed as g/kg feces, there was no clear relationship in fecal P output with increasing dietary TDP concentration and diet 5 was higher than diets 1 ($p<0.001$), 2 ($p = 0.021$), 3 ($p = 0.005$) and 4 ($p<0.001$). The fecal P output of diet 4 was very significantly ($p<0.001$) lower than diets 2 and 5 and significantly ($p = 0.029$) lower than diet 3. The fecal P output of diet 1 was significantly ($p = 0.03$) lower than diet 2.

After calculating the true P digestibility, endogenous P output could be calculated according to Eq. (5) or (6). Endogenous P output decreased with increasing dietary P input when dietary P input was lower than 6.27 g/kg DMI. When dietary P input increased from 6.27 g/kg DMI to 7.26 g/kg DMI, endogenous P output increased from 1.01 g/kg DMI to 1.22 g/kg DMI. There were no significant differences among experimental diets in endogenous P output and fecal P output of dietary origin of diet 5 was very significantly ($p<0.001$) higher than the other 4 diets. Expressed as g/kg DM feces, fecal P output of dietary origin of diet 5 was very significantly ($p<0.001$) higher than the other 4 diets, while expressed as g/kg DMI, faecal P output of dietary origin increased with increasing dietary P input.

P balance

Fecal P output of pigs fed diet 5 was very signifiantly ($p<0.01$) higher than with the other 4 diets. Fecal and urinary P output increased with increasing daily dietary P input. Urinary P output of diet 5 was very significantly ($p<0.001$) higher than diet 4. Urinary P output of diet 4 was significantly higher than diets 2 ($p = 0.034$) and 3 ($p = 0.046$) and very significantly higher than diet 1 ($p<0.001$). There were differences of 2.02 g/d between the highest (diet 5) and lowest diets (diet 4) in fecal P output and 0.047 g/d in urinary P output (Table 5). Total P output was influenced by fecal P output and urinary P output, so it increased with increasing daily dietary P input, and the highest value was 2.07 g/d higher than the lowest ($p<0.01$).

Retention of P in the body increased with increasing daily dietary P input. Retained P of diet 1 was very significantly ($p<0.01$) lower than the other 4 diets. There

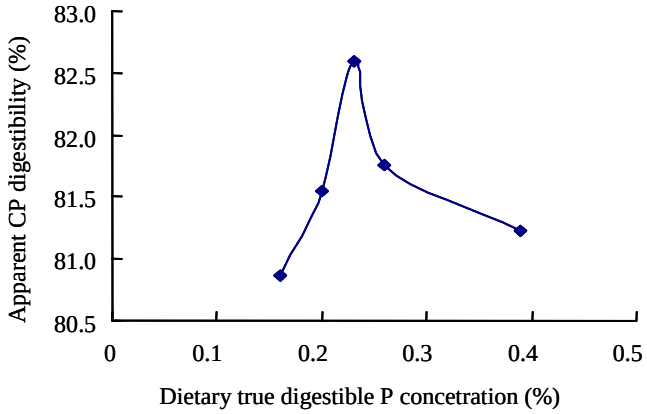

Figure 3. The effects of dietary true digestible phosphorus concentration on the apparent crude protein digestibility values.

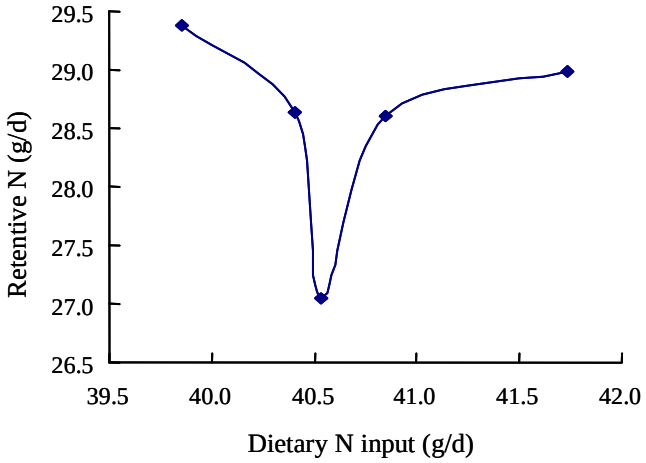

Figure 4. The relationship between dietary nitrogen input and nitrogen retention.

was no clear relationship for P retention in the body with values from high to low being diet 2, 3, 4, 1 and 5. The highest value was 7.96% higher than the lowest, but there were no significant differences among the diets.

Apparent N digestibility and N balance

The average apparent N digestibility was 81.6%. There was no significant effect of dietary TDP concentration on apparent N digestibilty among diets (Table 3). The effect of dietary TDP concentration on apparent N digestibility showed a parabolic trend (Figure 3). The highest point of apparent N digestibility was evident when dietary TDP concentration was 0.23%. The apparent N digestibility ranged from high to low on diets 3, 4, 2, 5 and 1 with respective values of 82.60%, 81.76%, 81.55%, 81.23% and 80.86%.

There was no relationship in fecal N, urine N and total N output with increasing dietary N input and there were no significant differences among the diets (Table 5). The amount of retained N decreased initially and then increased with increasing dietary N input (Figure 4). The lowest point of retained N in the body was evident when dietary TDP concentration was 0.20%, and its value was 27.04 g/d. There were no significant differences in retained N among the 5 diets. The highest point was evident at 29.38 g/d when dietary TDP concentration was 0.39%.

The retention of N in the body decreased with increasing dietary P input from 7.36 to 9.62 g/d, but the differences among diets were not significant. The lowest value was observed at a dietary TDP concentration of 0.20% and the highest value was present at a dietary TDP concentration of 0.39% (Table 5).

Growth performance

Feeding different levels of TDP had no linear effects ($p>0.05$) on ADG, ADFI, or the F/G in growing pigs during the 28-d trial (Table 6). However, dietary TDP levels exhibited a quadratic effect ($p<0.05$) on ADG and the F/G (Table 6). An equation was developed to relate the ADG of pigs to dietary TDP level: $y = -809{,}532x^4+788{,}079x^3-276{,}250x^2+42{,}114x-1{,}759$; $R^2 = 0.99$; $p<0.01$; 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

Table 6. The effects of dietary true digestible phosphorus levels on growth performance in growing pigs

Items	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P (linear)	P (quadratic)
Total phosphorus (%)	0.44	0.47	0.51	0.54	0.69	-	-	-
Apparent phosphorus (%)	0.14	0.17	0.20	0.23	0.37	-	-	-
True digestible phosphorus (%)	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
Final 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 phosphorus 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 phosphorus intake (g/d)	1.4^{a}	1.8^{ab}	2.1^{ab}	2.4^{b}	3.9^{c}	0.22	0.001	0.308
True digestible phosphorus 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

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

Table 7. The effects of dietary true digestible phosphorus levels on serum biochemical indices

Items	Diet1	Diet 2	Diet3	Diet4	Diet5	SEM	P (linear)	P (quadratic)
Total phosphorus (%)	0.44	0.47	0.51	0.54	0.69	-	-	-
Apparent phosphorus (%)	0.14	0.17	0.20	0.23	0.37	-	-	-
True digestible phosphorus (%)	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 phosphorus 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.

level was 0.34%. Notably, the ADG of pigs decreased ($p<0.05$) when dietary TDP levels exceeded 0.34%.

The F/G 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 F/G for pigs and dietary TDP levels could be described by the following equation: $y = 3{,}651.1x^4-3{,}480.4x^3+1{,}183.8x^2-172.5x+10.9$, $R^2 = 0.99$, $p<0.01$; where x = dietary TDP level (%) and y = the F/G for growing pigs. It was calculated that the F/G was the lowest (1.07) when the dietary TDP level was 0.34%.

Serum biochemical indices

Neither alkaline phosphatase activities nor total Ca concentrations in serum differed ($p>0.05$) when dietary TDP levels increased from 0.16% to 0.39% (Table 7). 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/d for a 30-kg pig that consumed 1.5 kg feed/d). 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 on diets providing 0.26% and 0.39% TDP.

DISCUSSION

Determination of true P digestibility and endogenous P output

There are several disadvantages in using apparent P digestibility to estimate P efficiency, and only true P digestibility value can reflect the actual digestion and absorption of P in the diet. Apparent P digestibilty underestimates the biological values of P because endogenous P contribution to intestinal or fecal P is not taken into consideration, so apparent fecal P digestibilities were 20% to 25% lower than true P digestibility for feed ingredients (Table 1). Apparent P availability of the same feed ingredient may vary considerably (as much as 15% to 35%) with swine diets (Weremko et al., 1997; Fan et al., 2001 and 2002; Shen et al., 2002; Fang et al., 2007). Further, apparent P digestibility values are not always additive in single feed ingredients for growing pigs (Fan et al., 2002; Fang et al., 2007). 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., 2007). Compared with the traditional 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. 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 (Tables 6 and 7).

Exact determination of endogenous P output in animals is the key to determining the true P digestibility. However, it is difficult to determinine true P digestibility because P recirculated in the body. The traditional method of determining endogenous P output is to feed animals a diet with no P, then the fecal P output is only endogenous P, but animals fed diets without P will suffer perturbations of digestion and metabolism caused by the shortage of P. The endogenous P output determined under these conditions is far different from that under nomal conditions. Fan et al. (2001) found that piglets suffered diarrhea and shiver after feeding a no-P diet for 5 to 7 days. ^{32}P tracer has been used to determine endogenous P output, but fast recycling of P in the gastrointestinal tract creates difficulty in handling radioactive material in routine whole animal studies, so this area of research has been relatively unexplored (Fan et al., 1999). Fan et al. (1999) have successfully determined endogenous P output and true amino acid digestibility by using a Regression Analysis Technique. Fan et al. (2001) and Shen et al. (2002) used this method to determine endogenous P output and true P digestibility. Zhang (2004) used this method to determine true P and Ca digestibility.

These experiments proved the feasibility of this method which is cheaper, safer and more convenient.

Endogenous P determined in the present study was 1.14 g/kg DMI which is similar to the experimental result (1.08 g/kg DMI) of Zhang (2004) and higher than that of Shen (2002). The endogenous percentage in total P and TDP requirement was 17% and 30.54%, respectively, in this experiment, compared to were 18% and 39.13% recorded by Zhang (2004). Fan et al. (2001) determined that the endogenous P output of pigs (body weights were 5 to 20 kg) was 0.31 g/kg DMI, which was 5.8% to 12.8% of total P requirement and 9.5% to 24.1% of TDP requirement. Later, Shen et al. (2002) determined the value in growing pigs (BW 20-45 kg) was 0.67 g/kg DMI which was 12.3% of total P requirement and 26.6% of TDP requirement. There are gender differences and at different periods even in the same gender and period, because endogenous P output can be influenced by factors such as the ratio of Ca to P, P levels and feed management.

According to the present results, it can be deduced that true P digestibility reaches the highest point when dietary TDP concentration is 0.34%. When dietary TDP concentration exceeds the requirement of pigs, the excess would be excreted from the body so that the percentage of absorbed P in total dietary P input is reduced. Thus, true P digestibility decreased when dietary TDP concentration exceeded the pig's need. Except for diet 1 the experimental diets contained dicalcium phosphate. The biological availability of P in dicalcium phosphate is higher than in plant ingredients so the higher true P digestibility of diets 2, 3, 4 and 5 compared to diet 1 was attributable to dicalcium phosphate. True P digestibility increased progressively to the highest point, and then decreased when dietary true digestible TDP concentration increased from 0.26% to 0.39%. However, the range of dietary TDP concentration between diets 4 and 5 was too wide to determine accurately the change of true P digestibility and the deduced result needs confirmation.

The effect of dietary TDP concentration on P output and P balance

Fecal and urinary P output increased with increasing dietary P input which indicated that there was a positve correlation between fecal and urinary P output and dietary P input. The result was in accord with the results of Vipperman et al. (1974) and Miller et al. (1964). Vipperman et al. (1974) found urinary P output increased with increased dietary P input when dietary Ca concentration was higher (7.5%) and increased linearly with increasing dietary P input when dietary Ca concentration was lower (2.5%). They also found that urine P output decreased with increasing dietary Ca input when dietary P level was higher and urinary P output was nearly zero when dietary Ca level exceeded dietary P level. Miller et al. (1962) found that the retention of P increased with an increasing dietary P level and the amount of retentained P was influenced primarily by urinary P output.

In this study, when expressed as g/kg DM feces, P output was lowest when dietary TDP concentration was 0.26% which indicated that diet 5 may be better than the other experimental diets in terms of P availability and P output. When analysed further, there was a lower point of P output between dietary TDP concentration of 0.26% and 0.39%. The lower point was apparent when dietary TDP concentration was approximately 0.34% according to the results. When expressed as g/kg DMI, fecal P output increased with increasing dietary TDP concentration, but the ratio of fecal P output to dietary P input decreased from 74.4% to 65.6%, then increased to 68.7%. When the ratio of fecal P output to dietary P input was analysed, the lower point was evident when dietary TDP concentration was near to 0.34% according to the results. The above two conclusions were in accord with the result of another part of the study, which proved that the performance of growing pigs was best when dietary TDP concentration was 0.34%. It is concluded that the optimum level of addition of TDP was 0.34%.

Ca to P ratio

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). NRC (1998) recommended a ratio of total Ca to total P in a typical corn- and soybean meal-based diet of between 1:1 and 1:1.2 and a ratio of total Ca to available P of between 2:1 and 3:1. ARC (1981) suggested that the ratio of total Ca to total P should not exceed 2:1 for growing pigs 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 pigs. The phytate and phosphatase concentrations differ among dietary ingredients, and thus there were marked disparities in P availability in the swine alimentary tract. Therefore, formulating diets on the basis of a total Ca to total P ratio cannot accurately reflect the actual requirements for these two minerals by pigs. To meet the metabolic requirements of both Ca and P by swine precisely, using a ratio of total Ca to TDP is a better choice. 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).

Mudd et al. (1969) found retentive Ca and P increased with increasing dietary Ca and P level while the retention

decreased gradually, which was proved by this study, but the retention was lower when dietary TDP concentration was lower(0.16%). The retentive Ca in this study differed to that of Mudd et al. (1969), and the reason may be that the ratio of Ca to P were not equal in the two experiments. Vipperman et al. (1974) found that the retentive P decreased when dietary Ca level was added without additional dietary P level, and retentive P increased with increasing dietary Ca level when dietary P level was higher. The results of the present study indicated that serum alkaline phosphatase activity was not affected by dietary Ca content when the ratio of total Ca to TDP was kept at 2:1. Also, varying dietary Ca intakes at a constant ratio (2:1) of total Ca to TDP had no effect on total Ca concentration in the serum of growing pigs (Table 7). Similar findings were reported by Lin et al. (2002) and Wang et al. (2002). However, total P concentration in serum increased with increasing dietary TDP levels (Table 7). These results are comparable to those reported by Lin et al. (2002) for weanling pigs as well as growing-finishing pigs. Taken together, the findings from the present study and the work of Lin et al. (2002) demonstrate that total P concentrations in serum rise as dietary P levels increase to an optimal level. Moreover, the research 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 concentration in serum (Table 7). Interestingly, Wang et al. (2002) reported that serum P concentration increased progressively with increasing dietary P level in Chinese Xiangzhu pigs. It is possible that there are significant differences in Ca or P digestion and metabolism between different breeds. Further study is needed to test this important hypothesis.

Besides the data on serum P concentrations, growing pigs exhibited the highest ADG and highest feed efficiency when fed a diet containing 0.34% TDP and 0.68% Ca (Table 6). This diet provided the 20- to 40-kg pigs with daily TDP, available P and total P intakes of 3.76, 3.65, and 6.81 g/d, respectively (Table 6). 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/d 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% more than the optimal value (6.81 g/d) obtained in the present study. Clearly, NRC (1998) overestimated substantial P requirement by growing pigs fed corn- and soybean meal-based diets.

The effect of dietary TDP concentration on apparent N digestibility and N balance

The influence of dietary TDP concentration on apparent N digestibility might be attributable to saponin formed by the connecting of P with Ca and fat which restrained the digestibility of amino acids. Vipperman et al. (1974) found that retentive N decreased when dietary Ca level was added without increasing dietary P level. Mudd et al. (1969) found that the effect of dietary P level on the amount of retentive N was greater than the effect of dietary Ca level. In this study, dietary CP level was 17.34% and the average total fecal N output was 12.14 g/d which was lower than the value of 16.6 g/d obtained by Cromwell (1996) when dietary CP level was 16%. The retention of N was over 66% in this study which is higher than the result of Fan et al. (1999). The reason for N output being higher in the latter experiment may be that the phytate phosphorus level was higher and available phosphorus was lower than in this experiment and, as a result, more saponin was formed.

Fecal and urinary N output decreased all along in Experiment 1 and may have been related to the decrease of dietary N input. The results indicated that dietary TDP concentration could influence the apparent N digestibility, but not significantly. These studies also validated the experimental result of Cromwell (1996) which concluded that there was no interaction between dietary P and CP levels.

CONCLUSIONS AND IMPLACATIONS

The results showed that true P digestibility and total P output increased with increasing dietary TDP concentration. When dietary TDP concentration was 0.34%, the ratio of total P output to dietary P input was the lowest, fecal P output was not the lowest but the most appropriate. Apparent N digestibilities were similar at different levels of dietary TDP, which indicated that dietary TDP concentration may not influence dietary apparent N digestibility and N output. N output was influenced mainly by dietary CP level.

In summary, results of the present studies indicate that the appropriate dietary TDP level 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/d for a 30-kg pig that consumes 1.5 kg feed daily. The 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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