# aJava

Asian Journal of Animal and Veterinary Advances



# Effect of Dietary Phosphorus on Phosphorus Utilization and Excretion in Thai-Indigenous Heifers

Parita Suppaso, Songsak Chumpavadee and Anut Chantiratikul Faculty of Veterinary Medicine and Animal Science, Mahasarakham University, Muang, Maha Sarakham, 44000, Thailand

Abstract: The objective of this study was to determine effect of dietary phosphorus (P) on P utilization and excretion in Thai-indigenous heifers. The experimental design was a 4×4 Latin square design with 21 day period. Four Thai-indigenous heifers, average body weight of 107±4.53 kg, were randomly received 1 of 4 diets containing 0.10, 0.20, 0.30 and 0.40% P at 2% of BW. The results found that feed intake and nutrient digestibility of heifers were not significantly different (p>0.05) among treatments. Intake of P, P excretion, P absorption, P retention and plasma P concentration significantly increased (p<0.05) with increasing dietary P. Additionally, P absorption and P retention of heifers fed 0.30 % dietary P were not significantly different (p>0.05) with those of heifers fed 0.40% dietary P. Based on the results, it could be concluded that dietary P levels affected significantly (p<0.05) P utilization and excretion in Thai-indigenous heifers. The P requirement of heifers was lower than 0.4% of diet.

Key words: Phosphorus utilization, phosphorus excretion, Thai-indigenous heifers

# INTRODUCTION

Phosphorus is an essential element for body structure and plays a vital role in energy metabolism (Pfeffer *et al.*, 2005). Therefore, osteomalacia, rickets, reduce feed intake and low fertility can occur when animal received inadequate P (Underwood and Suttle, 1999). Extensive areas of P-deficient soils occur throughout the world, especially in tropical area and a deficiency of this element can be regarded as the most widespread and economically important of all the mineral disabilities affecting grazing livestock (McDonald *et al.*, 1995). On the other hand, excess P intake is liked to increase P loss in manure. The excretion of P is associated with environmental concern or eutrophication of water lakes (Valk *et al.*, 2000; Ekelund *et al.*, 2006). Reducing P intake by cattle and thus also that excreted in the manure will contribute to reducing environmental pollution (Valk and Beynen, 2003). Consequently, the optimal way to decrease P excretion must be to develop diets that closely match the requirement of the animal (Ekelund *et al.*, 2006).

The tropical crossbred beef heifers of 100 to 300 kg of BW required 0.17 to 0.35% of dietary P for maintenance and growth (Kearl, 1982). National Research Council (1996) estimated the P requirement for finishing cattle as 0.20% of diet DM. However, Erickson *et al.* (1999) found that P requirement for finishing yearlings was 0.14% of diet DM or less. Erickson *et al.* (2002) consistently observed P requirement for finishing feedlot steers was lower than 0.16% of diet DM. The P requirement is dependent on breed, body weight, weigh gain, pregnant stage, milk production and age of the animal (McDonald *et al.*, 1995). Thai-indigenous cattle is a small frame (<250 kg of BW) and lower growth rate than the temperate beef cattle (Ubunratchatani Livestock Breeding Station, 1999).

They probably have the different P requirement from other cattle. Furthermore, their P requirement has not been investigated. Therefore, the objective of this study was to determine effect of dietary P on P utilization and excretion in Thai-indigenous heifers.

# MATERIALS AND METHODS

Four-Thai-indigenous yearling heifers an initial body weight of  $107\pm4.53$  kg were used. The animals were dewormed by Ivomectin and injected with AD<sub>3</sub>E vitamin-complex prior to the beginning of the experiment. They were housed in individual stall and daily fed at 2.0% of BW at 0800 and 16:00 h. Drinking water was available at all time.

Animals were randomly allocated to one of four dietary treatments in a  $4\times4$  Latin Square Design with 21 day periods. Each period consisted of 14 day of adaptation and 7 day test. Dietary treatments (Table 1) contained 0.1, 0.2, 0.3 and 0.4% of dietary P. Monosodium phosphate (Sigma-Aldrich, Germany) was used as P source in the diets. The diets were prepared in total mixed ration with rice straw as a roughage source and formulated to be isonitrogenous and isocaloric diets according to National Research Council (1996).

Feed intake was monitored daily throughout the experiment, however only the intake during the 7 day test period was used. During each collection period, feed and orts were collected daily and composite by animal for chemical analysis. Simultaneously, feces of each animal was weighed, mixed and a 10% subsample was taken, stored at -4°C and later pool individually the end of each period. Fecal samples were dried at 65°C for 72 h and ground through 1 mm screen for chemical analysis. The urinary sample was collected via modified urinary cap and kept into plastic container pre-added with 250 mL of 10%  $\rm H_2SO_4$  (v/v). Acidified urine was kept at -20°C for P determination. Rumen fluid and blood samples were taken at the end of collection period. Rumen fluid was sampled using esophageal tube. The sample was filtered through a muslin cloth and rumen fluid was immediately acidified  $10\% \rm H_2SO_4$  (v/v) and frozen at -20°C for rumiual P determination. Blood was drawn from jugular vein. Plasma was harvested by centrifugation of the whole blood for 15 min at 3000 g and kept at -20 °C for P analysis.

Feed offered, orts and fecal samples were analyzed for DM, OM, CP (AOAC, 1996), ADF and NDF according to Robertson and Van Soest (1981). Nitrogen in feed, orts and urine was determined by macro-Kjeldahl method (Association of Official Analysis Chemists, 1996). Nutrient digestibility and N utilization were estimated using the procedure of Schnieder and Flalt (1975) and McDonald *et al.* (1995), respectively.

#### Statistical Analysis

The data were analyzed using the general linear models procedure of Statistical Analysis System Institute SAS (1996) according to the following statistical model:  $Y_{ijk} = \mu + A_i + P_j + D_k + e_{ijk}$ , where A, P and D are animal, period and diet effects, respectively. The differences among means were compared by Least Significant Different (Steel and Torries, 1989). Significance was declared at p<0.05.

#### RESULTS AND DISCUSSION

The analyzed P concentrations in the dietary treatments were 0.15, 0.22, 0.32 and 0.4% (Table 1). The obtained values were similar to the calculated value of 0.1, 0.2, 0.3 and 0.4% of dietary P, respectively. Dietary P did not significantly alter (p<0.05) feed intake and digestibilities of DM, OM, CP, NDF and ADF of the indigenous heifers (Table 2). The outcomes are consistent with the foregoing reports by Erickson *et al.* (1999, 2002) and Hurley *et al.* (2003). Those reports found that feed intake, daily gain and feed efficiency of feedlot and finishing steers were not affected by

dietary P. Furthermore, Valk *et al.* (2002) and Wu *et al.* (2003) were similarly reported that no effect of dietary P on nutrient digestibility was observed. The ruminal P concentration of heifers fed diets containing 0.1 to 0.4% of P ranged from 137.33 to 431.50 mg L<sup>-1</sup> (Table 3). Generally, rumen fluid contained 150 to 600 mg L<sup>-1</sup> of P (Cohen, 1980). Ruminal microorganisms require P for their activities especially in fiber digestion. The microbial digestion of cellulose and hemicelluloses, therefore, decreased significantly when P content in the rumen was lower than 50 mg L<sup>-1</sup> (Komisarczuk *et al.*, 1987). Additionally, it has been reported that dietary P level of less than 0.25% can reduce rumen microbial growth resulting in less microbial protein and lower ration digestibility (Durand and Kawashima, 1980). The present findings demonstrated that ruminal P content of heifers was adequate for fiber digestion of microorganism. Therefore, the levels of dietary P (0.1 to 0.4 %) did not negatively influent nutrient digestibility in the indigenous heifers.

P absorption (g/day), P retention (g/day) and P excretion (g/day) of heifers increased significantly (p<0.05) with increasing dietary P concentration. However, P digestibility (%) decreased dramatically (p<0.05) in heifers fed diet containing 0.4% of P (Table 3). The other works (Wu *et al.*, 2001, 2003)

Table 1: Ingredients and chemical composition of dietary treatments

	Dietary P (%DM)						
Ingredients	0.10	0.20	0.30	0.40			
Rice straw	30.00	30.00	30.00	30.00			
Cassava chip	55.00	54.60	54.20	53.80			
Soybean meal (44% CP)	6.00	6.00	6.00	6.00			
Urea	2.00	2.00	2.00	2.00			
Molasses	5.00	5.00	5.00	5.00			
Sulfur	0.25	0.25	0.25	0.25			
Limestone	0.50	0.50	0.50	0.50			
Salt	0.75	0.75	0.75	0.75			
Premix <sup>1</sup>	0.50	0.50	0.50	0.50			
Monosodium phosphate	-	0.40	0.80	1.20			
Total	100.00	100.00	100.00	100.00			
Chemical composition (%DM)							
Dry matter	90.03	88.52	88.66	91.12			
Organic matter	90.79	89.73	89.08	89.43			
Crude protein	9.35	9.85	9.70	9.91			
Neutral detergent fiber	36.17	36.10	36.33	36.45			
Acid detergent fiber	21.16	21.33	21.17	21.33			
Acid detergent lignin	4.33	4.35	4.64	4.47			
Ca	0.32	0.34	0.39	0.48			
P	0.15	0.22	0.32	0.40			
ME2,3 (Mcal kg <sup>-1</sup> M)	1.99	1.98	1.97	1.96			

 $^1\text{The}$  premix provided per kilogram of diet: 10,000 IU vitamin A; 2,000 IU vitamin D<sub>3</sub>; 20 IU vitamin E; 0.01 g Cu; 0.08 g Mn; 0.04 g Zn; 0.05 g Fe; 0.0008 g I; 0.0003 g Co; 0.0003 g Se; 0.005 g Ethoxiquin and 0.05 g SiO<sub>2</sub>.  $^2\text{ME}$ : Metabolisable Energy.  $^3\text{Calculated values}$ 

Table 2: Effect of dietary P on feed intake and nutrient digestibility in Thai-indigenous heifers

Items	Dietary P (%	(DM)			SEM <sup>1</sup>
	0.10	0.20	0.30	0.40	
Total feed intake					
(kg day <sup>-1</sup> )	2.14	2.11	2.10	2.12	0.03
BW (%)	1.87	1.88	1.85	1.87	0.01
$g kg^{-1} BW^{0.75}$	61.02	61.22	60.37	60.99	0.29
Digestibility (% of intak	e)				
Dry matter	70.09	71.88	70.90	71.56	0.10
Organic matter	73.38	75.16	74.10	74.97	0.89
Crude protein	75.09	75.80	73.39	73.90	1.02
NDF <sup>2</sup>	45.99	49.79	48.13	49.66	1.79
ADF <sup>2</sup>	36.03	37.48	31.88	32.88	2.31

<sup>1</sup>SEM: Standard Error of the Means. <sup>2</sup>NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber

Table 3: Effect of dietary P on P utilization, plasma P and ruminal P in Thai-indigenous heifers

Items	Dietary P (%DM)					
					SEM <sup>1</sup>	
	0.10	0.20	0.30	0.40		
P balance (g day <sup>-1</sup> )						
P intake	$2.73^{d}$	4.03°	$6.03^{b}$	7.70°	0.50	
P feces	$0.69^{d}$	$1.09^{\circ}$	$1.86^{\rm b}$	3.15a	0.25	
P urine	$0.04^{\circ}$	0.11°	$0.30^{b}$	0.49 <sup>a</sup>	0.05	
P absorption	2.04°	2.94 <sup>b</sup>	4.18ª	4.55ª	0.27	
P retention	$2.00^{\circ}$	2.83 <sup>b</sup>	3.88⁴	4.07ª	0.23	
P digestibility (%)	75.33ª	74.93ª	68.87ª	56.90 <sup>b</sup>	2.33	
Plasma P (mg L <sup>-1</sup> )	32.66°	37.39°	45.74 <sup>b</sup>	51.37ª	1.98	
Ruminal P (mg L <sup>-1</sup> )	137.33	279.41	375.25	430.50	38.69	

<sup>&</sup>lt;sup>a-c</sup>: Means on the same row with different superscripts are significantly different (p<0.05). <sup>1</sup>SEM: Standard error of the means. <sup>3</sup> NS: Not Significant

previously indicated that fecal P excretion increased (p<0.05), however, P digestibility declined significantly (p<0.05) when dietary P was increased. Normally, cattle have the ability to absorb the amount of P needed, when P is provided in sufficient amount or in excess (Morse et al., 1992). Passive absorption prevails when sufficient or excessive amount of P are consumed (Wasserman and Taylor, 1976). The decreased P digestibility was also due to depressing 1, 25 Dihydroxy vitamin D synthesis in the kidney that inhibit intestinal mucosa to absorb excessive dietary P (Reinhardt et al., 1988). Subsequently, the surplus or indigested P was mainly excreted in the feces. (Valk and Beynen, 2003). The highest levels of apparent digestibility were obtained when P is not supplied in excess (Ekelund et al., 2006). It is probably that the animal adapted to the low dietary P by increasing the transport capacity in the small intestine (Huber et al., 2002). Furthermore, P absorption and retention of heifers fed diet containing 0.3 and 0.4% of P were not significantly different (p>0.05). The results in the present study obviously indicated that 0.4% of dietary P was an excessive level of P requirement for the indigenous heifers. The P digestibility of heifers varied between 56.90 to 75.33% (Table 3). The obtained values were higher than those previously reported (24 to 52.5%) in lactating dairy cows (Wu et al., 2001; Ekelund et al., 2003, 2006). However, Bravo et al. (2003) mentioned that the efficiency of dietary P digestibility in lactating cows (0.69) was generally lower than that of growing cattle (0.76). Unfortunately, there was no comparable information of P digestibility of Thai-indigenous cattle to clarify the current results. Grazing livestock in the tropics, including in Thailand (Vijchulata et al., 1983; Ngampongsai et al., 1999) were usually deficient in P due to insufficient of P in forage and soil (McDowell, 1992). Thai-indigenous cattle are familiar with this harsh condition. They have maybe adjusted themselves by enhancing P digestibility as mentioned above (Huber et al., 2002). The further research is needed to verify this issue.

Plasma P concentrations of the indigenous heifers increased dramatically (p<0.05) with increasing dietary P (Table 3). Wu *et al.* (2001), Lopez *et al.* (2004) and Peterson *et al.* (2005) consistently reported the concentration of P in blood serum was higher (p<0.01) for cows fed the highest P diet than for those fed the lower P diets. The normal level of plasma P in cattle was 31 to 46.5 mg L<sup>-1</sup> (Underwood and Suttle, 1999). Hence, plasma P (32.66 to 51.37 mg L<sup>-1</sup>) of indigenous heifers was in the normal range. Plasma P levels is directly affected by P intake. It is therefore may have more value as an indicator of dietary P, than as a P status indicator. Because it is evident that the physiological stage of production and the length of time on a P-deficient all affect an animal's P status and thus have a modulating effect on blood P levels (Karn, 2001).

# CONCLUSION

Dietary P levels influenced significantly (p<0.05) P utilization and excretion in Thai-indigenous heifers by increasing P absorption, P excretion and P retention. The 0.4% of dietary P was excessive level of requirement for Thai-indigenous heifers.

# ACKNOWLEDGMENT

This research project No. 5003026 was funded by budget fiscal year 2007 of Mahasarakham University.

# REFERENCES

- Association of Official Analysis Chemists, 1996. Official Methods of Analysis. AOAC. Washington, DC.
- Bravo, D., D. Sauvant, C. Bogaert and F. Meschy, 2003. I. A bibliographic database for quantitative analysis of phosphorus flow in ruminants. Reprod. Nutr. Dev., 43: 251-269.
- Cohen, R.D.H., 1980. Phosphorus nutrition in rangeland ruminant nutrition: A review. Livest. Prod. Sci., 7: 25-37.
- Durand, M. and R. Kawashima, 1980. Influence of Minerals in Rumen Microbial Digestion. In: Digestive Physiology and Metabolism in Ruminant, Ruckebusch, Y. and P. Thivend (Eds.). Lancaster, England, pp: 375-408.
- Ekelund, A., R. Sporndly, H. Valk and M. Murphy, 2003. Influence of feeding various phosphorus sources on apparent digestibility of phosphorus in dairy cows. Anim. Feed Sci. Technol., 109: 95-104.
- Ekelund, A., R. Sporndly and K. Holtenius, 2006. Influence of low phosphorus intake during early lactating on apparent digestibility of phosphorus and bone metabolism in dairy cows. Livest. Sci., 99: 227-236.
- Erickson, G.E., C.J. Klopfenstein, C.T. Milton, D. Hanson and C. Calkins, 1999. Effect of dietary phosphorus on finishing steer performance, bone status and carcass maturity. J. Anim. Sci., 77: 2832-2836.
- Erickson, G.E., C.J. Klopfenstein, C.T. Milton, D. Brink, M.W. Orth and K.M Whittet, 2002. Phosphorus requirement of finishing feedlot calves. J. Anim. Sci., 80: 1690-1695.
- Huber, K., C. Walter, B. Schroder and G. Breves, 2002. Phosphate transport in the duodenum and jejunum of goats and its adaptation by dietary phosphate and calcium. Am. J. Physiol., 283: 296-302.
- Hurley, L.A., T.L. Stanton and D. Schutz, 2003. Phosphorus and vitamin D in beef finishing diets. J. Anim. Sci., 65: 251-254.
- Lopez, H., Z. Wu, L.D. Satter and M.C. Wiltbank, 2004. Effect of dietary phosphorus concentration on estrous behavior of lactating dairy cows. Theriogenology, 61: 437-445.
- Karn, J.F., 2001. Phosphorus nutrition of grazing cattle: A review. Anim. Feed Sci. Technol., 89: 133-153.
- Kearl, L.C., 1982. Nutrient Requirements of Rumiuants in Developing Countries. 1st Edn. Utah Agricultural Experiment Station. Utah State University, Logan, Utah, USA.
- Komisarczuk, S., R.J. Merry and A.B. McAllan, 1987. Effect of different levels of phosphorus on rumen microbial fermentation and synthesis determined using a continuous culture technique. Br. J. Nutr., 57: 279-290.
- McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan, 1995. Animal Nutrition. 5th Edn. Longman Singapore Publishers (Pte) Ltd., Singapore.
- McDowell, L.R., 1992. Mineral in Ammal and Human Nutrition. 1st Edn. Academic Press, Inc., San Diego.
- Morse, D.H., H.H. Head and C.J. Wilcox, 1992. Disappearance of phosphorus in phytate from concentrates *in vitro* from rations fed to lactating dairy cows. J. Dairy. Sci., 75: 1979-1086.

- National Research Council, 1996. Nutrient Requirements of Beef Cattle. 6th Edn. National Academy of Science, Washington, DC.
- Ngampongsai, W., P. Chitprasan and W. Pralomkarn, 1999. Evaluating the mineral status of beef cattle in Songkhla province, southern Thailand. I. marco-minerals, hemoglobin and hematocrit. Songklanakarin. J. Sci. Technol., 21: 285-292.
- Peterson, A.B., M.W. Orth, J.P. Goff and D.K. Beede, 2005. Periparturient responses of multiparous Holstein cows fed different dietary phosphorus concentrations prepartum. J. Dairy Sci., 88: 3582-3594.
- Pfeffer, E., D.K. Beede and H. Valk, 2005. Phosphorus Metabolism in Ruminants and Requirements of Cattle. In: Nitrogen and Phosphorus Nutrition of Cattle, Pfeffer, E. and A. Hristov (Eds.). Biddles Ltd, King's Lynn, UK., pp. 195.
- Reinhardt, T.A., R.L. Horst and L.P. Goff, 1988. Calcium, phosphorus and magnesium homeostasis in ruminants. Vet. Clin: North America. Food Amm. Prac., 4: 331-350.
- Robertson, J.B. and P.J. Van Soest, 1981. The Detergent System of Analysis and its Application to Human Foods. In: The Analysis of Dietary Fiber, James, W.P.T. and O. Theander (Eds.). Marcel Dekker, New York, pp: 123.
- SAS, 1996. User's Guide: Statistics, Version 6. 12th Edn. SAS Inst., Inc., Cary, NC.
- Schneider, B.H. and W.P. Flatt, 1975. The Evaluation of Feeds though Digestibility Experiments. 1st Edn. The University of Georgia Press, Athen, US.
- Steel, R.G.D. and J.H. Torries, 1989. Principle and Procedure of Statistic: A Biomaterial Approach. 2nd Edn. McGraw-Hill, New York, USA.
- Ubunratchatani Livestock Breeding Station, 1999. Progress Report of Genetic Improvement on Characteristic of Thai Native Cattle Project during 1992-1998. Animal Husbandry Division, Department of Livestock Development, Ministry of Agriculture and Cooperation, 42 p. (in Thai)
- Underwood, E.J. and N.F. Suttle, 1999. The Mineral Nutrition of Livestock. 3rd Edn. CAB Int., Wallingford, Oxon, UK.
- Valk, H., J.A. Metcalf and P.J.W. Withers, 2000. Prospects for minimizing phosphorus excretion in ruminants by dietary manipulation. J. Environ. Qual., 29: 28-36.
- Valk, H., L.B.J. Sebek and A.C. Beynen, 2002. Influence of phosphorus intake on excretion and blood plasma and saliva concentrations of phosphorus in dairy cows. J. Dairy Sci., 85: 2642-2649.
- Valk, H. and A.C. Beynen, 2003. Proposal for the assessment of phosphorus requirements of dairy cows. Livest. Prod. Sci., 79: 267-272.
- Vijchulata, P., S. Shipadpanich and L.R. McDowell, 1983. Mineral status of cattle raised in villages in central Thailand. Trop. Amm. Prod., 8: 131-137.
- Wasserman, R.H. and A.N. Taylor, 1976. Gastrointestinal Absorption of Calcium and Phosphorus. In: Handbook of Physiology: Proceeding 7th International Endocrinology, Parathyroid Gland, Vol. VII, Aubach, G.D. (Ed). American Physiology Society, Washington DC., pp. 137-155.
- Wu, Z., L.D. Satter, A.J. Blohowiak, R.H. Stauffacher and J.H. Wilson, 2001. Milk production, estimated phosphorus excretion and bone characteristics of dairy cows fed different amounts of phosphorus for two or three years. J. Dairy Sci., 84: 1738-1748.
- Wu, Z., S.K. Tallam, V.A. Ishler and D.D. Archibald, 2003. Utilization of phosphorus in lactating cows fed varying amounts of phosphorus and forage. J. Dairy Sci., 86: 3300-3308.