Effect of abomasal inorganic phosphorus infusion on phosphorus absorption in large intestine, milk production, and phosphorus excretion of dairy cattle

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***Short communication*: Effect of abomasal inorganic phosphorus infusion on phosphorus absorption in large intestine, milk production and phosphorus excretion of dairy cattle.** *By Feng et al.*, Page XX. Phosphorus (P) contamination of surface water can cause eutrophication, with impacts on aquatic life. Greater knowledge of the fate of the dietary P and its utilization in the digestive tract will improve our ability to optimize P feeding and reduce P runoff. In the current study, varying doses of a phosphate buffer solution were infused into the abomasum of ruminally and ileally cannulated cows. Increasing infused inorganic P linearly increased net absorption of total P and inorganic P in the large intestine.

***Short communication*: Effect of abomasal inorganic phosphorus infusion on phosphorus absorption in large intestine, milk production and phosphorus excretion of dairy cattle**

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ABSTRACT

The objective of the study was to evaluate the effect of inorganic phosphorus (**Pi**) infusion on P absorption in large intestine, milk production and phosphorus excretion. Four ruminally- and ileally-cannulated crossbred cows were used in a 4×4 Latin Square with 21 d periods. Cows were fed a total mixed ration containing 0.21% P, providing 50% of the cows' P requirement. Cobalt-EDTA (**Co-EDTA**) was used as marker to measure large intestine digesta flow. On d 13 to 21 of each period, each cow was infused daily with 0, 20.1, 40.2, or 60.3 g Pi into the abomasum and total collection was conducted on d 18 to 21. Ileal samples were collected every 9 h on d 18 to d 21. Feed, digesta, and fecal samples were analyzed for total P and Pi using the molybdovanadate yellow method and blue method, respectively. All data were analyzed using PROC GLIMMIX in SAS 9.3 using contrasts to evaluate linear, quadratic and cubic effects of Pi infusion dose. Dry matter (**DM**) intake, apparent DM digestibility, milk yield and milk total P were unaffected by Pi infusion. Ileal flow and fecal excretion of total P and Pi increased linearly with increasing infused Pi. In the large intestine, net absorption of TP and Pi were increased linearly with increasing infused Pi. The magnitude of absorption from the large intestine was greater than reflected in current models and raising questions that could be evaluated with longer infusion periods or dietary alteration.

Key Words: dairy cow, phosphorus absorption, phosphorus excretion

Short Communication

Manure phosphorus (**P**) contamination of surface water can impair growth and survival of aquatic species. The strong relationship between dietary P and manure P content in most species provides a useful approach to reduce environmental impact of livestock farms, but also makes important detailed knowledge of the fate of the dietary P and its utilization in the digestive tract. In dairy cattle, absorption of P mainly occurs in small intestine and is modulated by endocrine factors and nutritional factors (mineral content in the diet, P content of the diet and the forms of different P in diet). Absorption of P in the large intestine of dairy cattle is rarely reported. Net absorption of P from large intestine in sheep ranges from 2 to 30% of the P flow entering the large intestine (Breves and Schroder, 1991). Sklan and Hurwitz (1985) reported rapid absorption of many ions in the small intestine, but in the large intestine absorption of P, Ca, Mg and K were low. Smith et al. (1955) reported higher P concentration in the rectum compared to cecum in sheep, suggesting net secretion of P in the large intestine. Hoeller et al. (1988) reported net Pi secretion into the colon with a Pi-free infusate in the colon of sheep and net Pi uptake with infusate containing 2.5-6.5 mmol/L Pi. The objective of the current study was to determine the effect of infused Pi on phosphorus absorption in large intestine, milk production and phosphorus excretion of dairy cattle

All protocols and procedures were approved by Virginia Tech Institutional Animal Care and Use Committee. Four ruminally and ileally cannulated crossbred [Swedish Red or Brown Swiss X (Holstein X Jersey)] early lactation cows averaging 76 DIM (SD = 38) were fed a diet containing 0.21% P, providing ~50% of the cows' calculated P requirement (NRC, 2001). Treatments (abomasal infusion of 0, 20.1, 40.2, or 60.3 g/d inorganic phosphate solution) were imposed in a 4 × 4 Latin square design with 21 day periods. Cows were individually fed in

Calan doors (American Calan, Northwood, NH) once daily at 1200 h on d 1 to 7 and four times daily at 0600, 1200, 1800 and 2400 h on d 8 and d 9 of each period. Cows had constant access to feed except during milking. Feed was offered at 5-10% in excess of the previous day's intake (wet basis). From d 10 to 21 of each period, cows were housed in individual tie stalls with wood shavings as bedding (amount used recorded daily), milked twice daily at 0600 and 1800 h, fed four times daily as on d 8 and 9, with continuous access to diet and water. On d 13 to 21 of each period, each cow was infused daily with 0, 20.1, 40.2, or 60.3 g inorganic phosphate solution into the abomasum. The solution was made of monobasic potassium phosphate with potassium phosphate dibasic anhydrous in double distilled water and the P concentration of the solution was monitored daily. Cobalt-EDTA (**Co-EDTA**) was used (dosed 110 mg Co/day) as a marker to measure digesta flow. Marker was dosed into the rumen through the rumen cannula four times per day at each feeding on d 9 to 21 of each period. Feed and feed refusals were sampled daily and stored at -20°C. Samples were stored at -20°C. On d 19 to 21 ileal samples and urine samples were collected every 9 h. Total fecal collection was conducted on d 18 to 21 of each period. At 1800 h on each day feces were weighed, thoroughly mixed using an electric mixer, and a subsample was taken and stored at -20°C. Milk yield was recorded and milk samples collected at 8 consecutive milkings on d 18 to 21. Blood samples were obtained on d 20 and 21 of each period via venipuncture in coccygeal veins and saved in Vacuette tubes (Greiner bio-one, Monroe, NC). Serum was separated immediately by centrifugation at 3,000 rpm for 10 min at 4°C and then was stored at -20°C.

Feed, feed refusals, wood shavings and total collection fecal samples were thawed at room temperature then dried at 55°C forced air oven (Thermo Scientific Precision 645, Danville, IN) and ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA).

The ileal samples were thawed then pooled in equal wet basis over the 8 sampling times to yield a composite from each cow on each period. The pooled ileal samples were dried as described above. Ground feed and feed refusals were analyzed in duplicate for DM. Ground feed, feed refusals, wood shavings, total collection fecal samples and pooled ileal samples were ground further through a 0.2 mm screen (Z-grinder) and analyzed for total P (yellow molybdovanadate method) and Pi (molybdate blue method). Samples were digested by concentrated nitric acid and perchloric acid for total P analysis and extracted by 0.5% hydrochloric acid for Pi analysis. Milk samples were analyzed for fat, protein, solids non-fat, lactose, milk urea N, and somatic cell count (DHIA, Blacksburg, VA) and P. The composite ileal samples were also analyzed for Co using ICP-MS. Daily urine output was predicted using the equations presented in Holter and Urban (1992) and urinary P excretion was calculated by multiplying daily urine output by TP concentration in urine. The large intestine TP absorption in each treatment was calculated as the difference between ileal TP flow and fecal TP. Excretion of TP was calculated as fecal TP minus TP from wood shavings and urine TP. All data were analyzed using PROC GLIMMIX procedures of SAS (SAS Institute, 2011). In the model, treatment and period were fixed effects and cow was a random effect. Preplanned contrasts were used to evaluate linear, quadratic, and cubic treatment effects of treatment. Differences were declared significant at $P < 0.05$ and trends at $P < 0.1$.

Despite the very low P content of the basal diet (0.21%, ~50% of requirement), infused Pi dose did not affect digestibility of the DM or fecal DM excretion (Table 1). The P value for cubic contrast on DMI is significant, with cows supplied total 110% of their P requirement having DMI higher than in cows supplied 50, 80 or 140% of requirements. Valk et al. (2002) fed diets containing 67%, 80% and 100% of requirements and observed no effects of dietary P on

DMI or digestibility of DM in dairy cows. In contrast, Call et al. (1987) observed reduced DMI of the cows fed low P (0.24%) diet from 2 to 10 weeks after parturition as cows were allotted to the low P diet 2 months before expected parturition. Lack of effect of Pi dose on DM digestion in this study is likely due to the short term nature of the study.

Milk yield was not affected by treatment. The P content of milk ranged from 0.87 to 0.93 g/L, similar to values typically observed, with no effect of infused Pi observed. There was no effect of Pi infusion on milk composition except on milk. Call et al. (1987) reported insufficient P in diet decreased milk yield and reduced body weight in cows fed diets containing 0.24% in a 12 month study as compared to cows 0.32%P and 0.42% P diets, and suggested these measures as the initial signs of P deficiency. In the short term, P homeostasis is maintained by drawing from the skeletal reserves (Karn, 2001) likely explaining the minimal effects in the current study. While P intake must be very low for extended periods of time to impair milk yield, excess P intake has been reported to reduce milk yield. Carstairs et al. (1981) reported cows fed 35% more P than requirement produced 1.8 kg milk less per day during the experiment. In the present study, few detrimental effects of high and low P intake were observed but the greater DMI in cows supplied P near to the requirement is of interest.

In large intestine, the net absorption of TP and Pi increased linearly with increasing Pi infused ($P = 0.02$; $P = 0.004$) but absorption of these as % of ileal flow were not affected ($P = 0.32$; data not shown). Fecal TP and Pi increased linearly with increasing infused Pi ($P < 0.01$). Little quantitative information is available regarding P absorption from the large intestine of ruminants, especially dairy cattle. Feng et al. (2015) model predicted a small portion of P was absorbed in the large intestine of dairy cows (~6 g/d). In the present study as the infused Pi increased, net absorption of TP and Pi in large intestine increased linearly. Net absorption of TP in the large intestine was greater than absorption of Pi, suggesting microbial degradation of phytate or other organic P-containing compounds. In the current study P absorption from the large intestine and retention were higher than has been observed elsewhere, especially in the group of cows infused with Pi (12.3, 15.1, and 23.7 g/d). These values may represent the extreme high end of the normal range, but sources of bias must also be considered. The short infusion period and the reliance on single marker to estimate ileal flow may be sources of error; the former could be evaluated in future studies.

Blood Pi increased from 3.53 to 6.48 mg/dl with increasing infused Pi ($P = 0.003$). Excess absorbed P beyond animals' requirement is re-secreted from the blood into saliva (Ternouth, 1990) and then enters the digestive tract being reabsorbed or excreted into the feces. Decreased P intake lowered blood Pi concentration and also salivary Pi concentration (Valk et al., 2002) suggesting blood P as an indicator of P deficiency. Wu et al. (2000) and Knowlton and Herbein (2002) pointed out that with extended P deficiency resorption of bone P can occur making plasma P a less reliable indicator of body P status over time. In the current study, infused Pi linearly increased serum Pi concentration suggesting that blood P concentration might be used as an indicator of the P status in a short term.

It was concluded that increasing infused Pi into abomasum of cows fed diets supplying ~50% of total P requirement increased P flow entering the ileum, and increased fecal P excretion. In the large intestine, net Pi absorption linearly increased with increasing infused Pi. The magnitude of absorption from the large intestine was greater than reflected in current models and raising questions that could be evaluated with longer infusion periods or dietary alteration.

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Table 1. Effect of abomasal infusion of inorganic phosphorus (Pi) on DM intake and digestibility

	P supply, % of requirement[1]					*P*-value[2]		
Item	50	80	110	140	SE	Linear	Quad	Cubic
DMI, kg/d	19.8	19.6	23.2	20.7	1.03	0.16	0.22	0.04
P intake, g/d	42.7	41.5	45.4	43.9	2.85	0.50	0.96	0.36
Fecal dry matter, kg/d	6.22	7.19	6.96	7.92	0.71	0.11	0.99	0.40
DM digestibility, %	68.6	63.0	69.3	61.3	3.60	0.34	0.73	0.13

[1] Cow were abomasally infused with 0, 20.1, 40.2 and 60.3 g/d inorganic P (Pi) solution to supply 50, 80, 110 and 140% of their calculated P requirement.

[2] *P*-values for linear, quadratic, and cubic effects

Table 2. Effect of abomasal infusion of inorganic phosphorus (Pi) on milk yield, milk composition and milk total phosphorus concentration

	P supply, % of requirement[1]					*P*-value[2]		
Item	50	80	110	140	SE	Linear	Quad	Cubic
Milk yield, kg/d	27.4	26.6	29.1	26.4	1.88	0.91	0.38	0.10
Milk P, g/L	0.93	0.87	0.89	0.91	0.03	0.53	0.10	0.41
Fat, %	4.98	5.05	5.27	5.39	0.18	0.12	0.90	0.77
Protein, %	3.29	3.27	3.36	3.22	0.15	0.67	0.40	0.24
MUN, mg/dL	13.8	11.9	11.1	12.0	0.92	0.18	0.20	0.88
SCC, 1000/ml	29	77	40	75	20	0.27	0.76	0.11

[1]Cow were abomasally infused with 0, 20.1, 40.2 and 60.3 g/d inorganic phosphorus (Pi) solution to supply 50, 80, 110 and 140% of their calculated P requirement.

[2] *P*-values for linear, quadratic, and cubic effects

Table 3. Effect of abomasal infusion of inorganic phosphorus (Pi) on intake and digestibility of total phosphorus (TP) and Pi

	P supply, % of requirement[1]					*P*-value[2]		
Item	50	80	110	140	SE	Linear	Quad	Cubic
Total P								
Ileal TP flow, g/d	23.8	39.9	49.4	85.9	5.76	0.0001	0.09	0.18
Absorption of P from LI, g/d	6.25	12.3	15.1	23.7	3.75	0.02	0.74	0.61
Fecal TP, g/d	17.0	27.1	33.2	61.4	3.26	<0.0001	0.002	0.02
Milk TP, g/d	25.7	22.9	25.6	23.9	1.75	0.61	0.67	0.12
Urine TP, g/d	0.48	0.56	1.12	0.81	0.22	0.05	0.21	0.08
P Retention, g/d[3]	-0.98	10.1	22.9	12.5	3.33	0.005	0.008	0.09
Inorganic phosphorus (Pi)								
Ileal Pi flow, g/d	8.19	16.2	27.4	52.9	3.40	<0.0001	0.03	0.45
Absorption of Pi from LI, g/d	0.06	-1.64	6.36	15.4	2.64	0.004	0.09	0.49
Fecal Pi, g/d	8.13	17.8	21.0	37.6	4.03	0.0005	0.29	0.19
Serum Pi, mg/dL	3.53	5.87	6.64	6.48	0.38	0.003	0.03	0.75

[1]Cow were abomasally infused with 0, 20.1, 40.2 and 60.3 g/d inorganic P (Pi) solution to supply 50, 80, 110 and 140% of their calculated P requirement.

[2] *P*-values for linear, quadratic, and cubic effects

[3]P retention = dietary P + infused P – fecal P – urine P – milk P