



## The Effects of Dietary Phosphorus on the Growth Performance and Phosphorus Excretion of Dairy Heifers

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**ABSTRACT:** The objective of this study was to investigate the effects of reducing dietary phosphorus (P) on the frame size, udder traits, blood parameters and nutrient digestibility coefficient in 8- to 10-month-old Holstein heifers. Forty-five heifers were divided into 15 blocks according to the mo of age and were randomly assigned one of three dietary treatments: 0.26% (low P [LP]), 0.36% (medium P [MP]), or 0.42% (high P [HP]) (dry matter basis). Samples were collected at the wk 1, 4, 8. The results show that low dietary P had no effect on body measurement. The blood P concentration decreased with decreasing dietary P ( $p < 0.05$ ), while the blood calcium content of LP was higher than that of the MP and HP groups ( $p < 0.05$ ), though still in the normal range. The serum contents of alkalinephosphatase, potassium, and magnesium were similar among the treatments. No differences were found in all nutrients' apparent digestibility coefficients with varied dietary P. However, with P diet decreased from HP to LP, the total fecal P and urine P concentration declined significantly, as did fecal water soluble P ( $p < 0.05$ ). In conclusion, reducing the dietary P from 0.42% to 0.26% did not negatively affect the heifers' growth performance but did significantly lessen manure P excretion into the environment. (**Key Words:** Heifers, Growth Performance, Phosphorus, Phosphorus Excretion)

### INTRODUCTION

Phosphorus (P) is known to play an important role in various events of animal production and reproduction, including adenosine triphosphate (ATP), buffering systems, cell signaling, structure and strength of bones, and synthesis of cell walls, RNA and DNA (Hill et al., 2008; Geisert et al., 2010). Currently, the most critical environment problem in China is water pollution, and the excessive concentration of P has been recognized as a major cause of eutrophication in surface water (Correll, 1998; Imura, 2013). Previous studies demonstrated that the average amount of total P was 0.077 mg/L in Taihu Lake, of which animal and poultry manure P account for 46% (Li et al., 2000; Imura, 2013). Therefore, improving the efficiency of P utilization and lessening manure P excretion are the important ways to reduce the

potential P pollution of freshwater.

A recent survey demonstrated that livestock producers in eastern China fed heifers 0.46% to 0.72% dietary P on a dry matter (DM) basis (Guo, 2013), which was higher than the amount recommended by the NRC (2001) (0.25% to 0.29% diet P [DM basis], body weight [BW] from 250 to 350 kg, average daily gain [ADG] = 1,000 g). The high content of dietary P was enabling heifers to reach puberty and pregnancy faster (Hill et al., 2007). However, many studies indicated that overfeeding P is not necessary, because increase P in manure and augments the expense of the producer (Wu et al., 2000; Valk et al., 2002; Bjelland et al., 2011). Tallam et al. (2005) reported no effect on ovarian activity, reproduction, or lactation benefit when feeding multiparous Holstein cows 0.35% to 0.47% of dietary P. In addition, excess dietary P was simply excreted, suggesting that the supplied additional mineral P in grain-based diets is not necessary. There was a significant amount of information on P excretion reduction from lactating cows, but few data on heifers are available. Therefore, the objective of this study was to assess whether lessening dietary P affects the growth performance of dairy heifers in

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## MATERIAL AND METHODS

### Animals and experimental diets

The use of heifers was approved by the Animal Care Committee of Zhejiang University, Hangzhou, China. Forty-five Holstein heifers were divided into 15 blocks according to the mo of age ( $9.3 \pm 0.8$ ) and were randomly assigned one of the following treatments: 0.26% (low P [LP]), 0.36% (medium P [MP]), and 0.42% (high P [HP]) (Table 1). The LP diet contained no supplemental P, whereas the other 2 diets were obtained by adding different amounts of dicalcium phosphate. The amount of P in the LP diet was near the level recommended by the NRC (BW from 250 to 350 kg, ADG = 1,000 g), the MP dietary P level was close to the Chinese dairy cow feeding standard recommendations (NY/T 34-2004, BW from 250 to 350 kg,

ADG = 1,000 g), and the HP diet contained P commonly fed by livestock producers in the east region of China (Guo, 2013). In order to avoid excessive fattening, all heifers were limited feeding, 2.1% of BW, and average dry matter intake (DMI) of each group was measured weekly. Heifers were housed in a tie-stall barn with free access to water and fed 3 times daily at 06:30, 14:00, and 20:30 h. At each feeding time, mixed concentrates were offered first, and then corn silage and grass hay were provided. The experiment was conducted from October until December 2013.

### Measurements and analytical methods

A proportional amount of feed offered was collected weekly. Samples were dried in a forced oven at 55°C for 48 h and then milled through a Wiley mill with 1-mm screen, and analyzed for crude protein (CP), Ca, P, ash, ether extract (AOAC, 1990), fecal water soluble P (Dou et al., 2002), neutral detergent fiber (NDF; Van Soest et al., 1991), acid detergent fiber (ADF; Van Soest et al., 1991). Spot fecal and urinary samples collected at 07:00, 14:30, and 20:30 h on the d 3, 27, and 53 were mixed across hours with a day as described by Wang et al. (2014), and sampled for later analysis. The record of hip height, body high, body length, heart girth, and teat length were taken at the d 2, 26, and 52. Blood samples (5 mL) were collected from the coccygeal vein on the d 1, 25, and 51, and centrifuged at  $3,000 \times g$  for 10 min to collect serum, which were frozen at  $-20^\circ\text{C}$ . Alkaline phosphatase (ALP), Ca, P, magnesium (Mg) and potassium (K) serum concentrations were analyzed using a HITACHI (7020) Automatic Analyzer, and kits were provided by NINGBO MEDICALSYSTEM BIOTECHNOLOGY CO., LTD (Zhejiang, China).

### Calculations and statistical analysis

The BW of each cow was calculated based on the measurement of heart girth and body length using the following equation:  $\text{BW (kg)} = \text{heart girth}^2 \text{ (m)} \times \text{body length (m)} \times 96.475$  (Heinrichs et al., 1992; Shen et al., 2010; Yu et al., 2014). Apparent nutrient digestibility was calculated by using the following equation:  $\text{Apparent digestibility} = 100 - [(\text{N}_f/\text{N}_d) \times (\text{M}_d/\text{M}_f)] \times 100$ , where  $\text{N}_f$  = concentration of the nutrient in the fecal,  $\text{N}_d$  = concentration of the nutrient in the consumed diet,  $\text{M}_d$  = concentration of the acid insoluble ash (AIA) in the consumed diet and  $\text{M}_f$  = concentration of the AIA in the fecal (Stojanovic et al., 2014).

Data on DMI, P intake, body measurements, nutrient apparent digestibility coefficient, and fecal and urine P were analyzed using GLM of SAS (SAS Institute, 2000). Blood biochemical parameters were analyzed using PROC MIXED of SAS (SAS Institute, 2000); treatment, time, treatment  $\times$  time, and block were included as fixed effects in the model; Heifers were the random effect. Probability values of  $p < 0.05$  were used to define statistical significance

**Table 1.** Ingredients and nutrient composition of the diet

Items	Dietary treatment		
	HP	MP	LP
Ingredient, % DM basis			
Chinese wild rye	39.8	40.0	39.9
Corn silage	25.1	25.1	25.2
Corn	13.9	14.0	14.0
Barley	6.1	6.3	6.3
Rapeseed meal	4.6	4.7	4.7
Soybean meal	1.7	1.7	1.7
DDGS(corn)	4.8	4.9	4.9
Peptide protein	0.9	0.9	0.9
Mineral-Vitamin premix <sup>1</sup>	-	-	2.4
P mineral-Vitamin premix <sup>2</sup>	2.2	2.4	-
Di-calcium phosphate	0.99	-	-
Analyzed chemical composition			
CP	11.2	11.2	11.1
NDF	55.3	54.2	56.0
ADF	29.0	28.4	28.7
P	0.42	0.36	0.26
Ca	1.0	0.9	0.9
EE	2.5	2.3	2.4
Ash	6.9	6.9	6.8
NE <sub>G</sub> (Mcal/kg) <sup>3</sup>	0.95	0.96	0.96

LP, low phosphorus (P); MP, medium P; HP, high P; DM, dry matter; DDGS, distillers dried grains with solubles; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; NE<sub>G</sub>, net energy for gain.

<sup>1</sup> Mineral-vitamin premix per kg containing: Ca (g) 166; P (g) 0; Fe (mg) 1,800; Cu (mg) 630; Mn (mg) 630; Zn (mg) 2,940; Se (mg) 21; I (mg) 38; Co (mg) 8; Vitamin A (IU) 240,000; Vitamin D (IU) 60,000; Vitamin E (IU) 1,200.

<sup>2</sup> P Mineral-vitamin premix per kg containing: Ca (g) 166; P (g) 70; Fe (mg) 1,800; Cu (mg) 630; Mn (mg) 630; Zn (mg) 2,940; Se (mg) 21; I (mg) 38; Co (mg) 8; Vitamin A (IU) 240,000; Vitamin D (IU) 60,000; Vitamin E (IU) 1,200.

<sup>3</sup> As calculated by NRC (2001).

and values of  $p < 0.10$  and  $p \geq 0.05$  were accepted as statistical trends.

## RESULTS AND DISCUSSION

### Feed ingredients, experimental diets, dry matter intake and P intake

The experimental diets had a similar composition of ingredients but with different P concentrations of 0.26%, 0.36%, and 0.42% (DM basis, Table 1). The Ca:P is different, but it is still within the normal range. NRC (2001) recommended that Ca:P is not critical on absorption of P and Ca in ruminants, unless the ratio is  $>7:1$  or  $<1:1$ . DMI were similar among the treatments, average 2.1% of BW, which result from restricted feeding. Kertz (1987) believed that Holstein heifers must gain greater than 800 g/d and less than 1,000 g/d that not only could make heifers reach a BW of 570 kg at 24 mo of age, but also could avoid heifers' excessive fattening. The amount of P intake was increased with the increase of dietary P concentration (Table 2).

### Body measurements and serum parameters

The skeletal measurements and calculated BW of Holstein heifers fed diets containing 0.26%, 0.36%, and 0.42% P were presented in Table 3. Heifers fed 0.26% P were similar in every measure of frame growth compared to the other two groups, suggesting that low-P ration had no effect on skeletal development. These observations are supported by Bjelland et al. (2011) and Esser et al. (2009), who reported no differences in skeletal growth of heifers due to the dietary addition of supplemental P. Similar results were also obtained from Hill et al. (2007). Mammary development is one of the most important criteria used to estimate lactation performance, and high-producing heifers tended to have lengthier teats and larger distances between teats compared with poor-producing heifers (Lin et al., 1987). This study determined that front teat length, rear teat length and teat distance were not differ among the 3 treatments (Figure 1), which indicated that the mammary development of the heifer was not affected by LP. The serum ALP, K, Mg concentrations were similar among treatments (Table 4), although a numerical decrease in ALP was observed with increased dietary P ( $p = 0.09$ ). The serum P decreased with decreasing dietary P ( $p < 0.05$ ) and the serum P concentration of LP was consistent with results

**Table 2.** DMI and P intake of 8- to 10-month-old Holstein heifers

Item	Dietary treatment			SEM	p-value
	HP	MP	LP		
DMI (kg)	5.98	6.00	5.98	0.31	0.99
P intake (g/d)	25.13	21.60	15.57	1.12	<0.01

DMI, dry matter intake; HP, high phosphorus (P); MP, medium P; LP, low P; SEM, standard error of the mean.

**Table 3.** Calculated BW, and body measurements of 8- to 10-month-old Holstein heifers

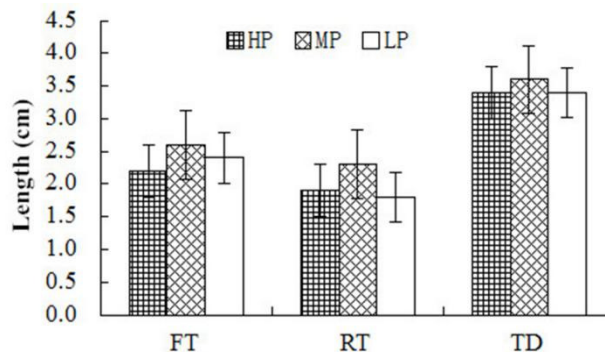
Item	Dietary treatment			SEM	p-value
	HP	MP	LP		
Calculated BW (kg)	289	291	297	4.86	0.52
Heart girth					
Initial (cm)	145.07	145.73	146.53	2.84	0.88
Final (cm)	153.00	152.80	152.33	2.70	0.97
Change <sup>1</sup> (cm/d)	0.14	0.13	0.10	0.02	0.20
Body length					
Initial (cm)	128.93	128.83	132.33	2.51	0.30
Final (cm)	134.87	135.13	136.60	2.67	0.78
Change (cm/d)	0.11	0.11	0.08	0.03	0.51
Body height					
Initial (cm)	107.80	108.60	110.80	2.37	0.43
Final (cm)	115.40	115.20	116.40	1.81	0.78
Change (cm/d)	0.13	0.12	0.10	0.02	0.14
Cannon bone <sup>2</sup>					
Initial (cm)	16.31	15.93	15.98	0.31	0.42
Final (cm)	16.48	16.12	16.11	0.35	0.49
Change (cm)	0.17	0.18	0.13	0.24	0.97

BW, body weight; LP, low phosphorus (P); MP, medium P; HP, high P; SEM, standard error of the mean.

<sup>1</sup> Change in body measurements from the beginning until the end of the trial.

<sup>2</sup> Value indicate cannon bone circumference.

reported by Bjelland (2011), who noting that blood P was 2.65 mmol/L with 0.30% dietary P in 8-month-old heifers. Wu et al. (2000) observed that serum P appeared higher for cows fed a high P diet compared with those receiving a low P diet; redundant dietary P was not utilized and was simply excreted in manure. The serum Ca content of heifers in LP was higher than that in MP and HP groups ( $p < 0.05$ ). Breves et al. (1985) reported that serum Ca increased when the amount of dietary P fed to sheep was decreased. Moreover, Kichura et al. (1982) believed that when dietary calcium is high, low dietary phosphorus seems helpful to enhance the activation of intestinal calcium absorption. No significant



**Figure 1.** Udder measures of 8- to 10-month-old Holstein heifers fed varied dietary phosphorus (P). LP, low P; MP, medium P; HP, high P; FT, front teat length; RT, rear teat length; TD, teat distance, distance around gland front to rear.

**Table 4.** Effect of different dietary phosphorus (P) on serum biochemical parameters of 8- to 10-month-old Holstein heifers

Item	Dietary treatment			SEM	p-value		
	HP	MP	LP		Diet	Time	Diet×time
ALP (U/L)	120.7	125.3	135.9	4.85	0.09	<0.01	0.95
Ca (mmol/L)	2.46 <sup>a</sup>	2.46 <sup>a</sup>	2.54 <sup>b</sup>	0.02	0.03	<0.01	0.49
P (mmol/L)	2.84 <sup>a</sup>	2.71 <sup>ab</sup>	2.64 <sup>b</sup>	0.05	0.02	0.03	0.50
K (mmol/L)	5.14	5.19	5.06	0.07	0.45	<0.01	0.36
Mg (mmol/L)	0.80	0.84	0.80	0.02	0.31	<0.01	0.06

LP, low phosphorus (P); MP, medium P; HP, high P; SEM, standard error of the mean; ALP, alkalinephosphatase.

Values with different superscripts (a, b) are significantly different ( $p<0.05$ ).

diet×time interaction was observed for serum P, Ca, K, Mg, and ALP.

#### Apparent digestibility of nutrients and manure P excretion

Even though the heifers in HP treatment consumed more P, the apparent P digestibility coefficients did not differ among the treatments (Table 5). Other research noted that the apparent digestibility of P in lactating cows less than 40% equates to an excessive of P intake (Wu et al., 2000), but it is still unknown for growing heifers. In regard to other nutrients, low dietary P had no effect on NDF, ADF, and the CP apparent digestibility coefficients, which is in agreement with previous results (Odongo et al., 2007; Xu et al., 2011). The excretion of P in manure was presented in Table 5. The total fecal P concentration decreased 35.62%, and the urine P concentration was reduced by 69.35% as dietary P decreased from 0.42% to 0.26% ( $p<0.05$ ).

**Table 5.** Fecal phosphorus (P) and urine P and apparent digestibility coefficients for CP, P, NDF, and ADF of heifers fed different dietary P concentrations

Item	Dietary treatment			SEM	p-value
	HP	MP	LP		
CP (%)	54.3	58.5	58.3	0.51	0.13
P (%)	32.7	32.2	33.1	0.98	0.98
NDF (%)	54.7	56.6	55.4	0.58	0.76
ADF (%)	52.1	52.7	50.3	0.62	0.79
Fecal total P (% of DM)	0.73 <sup>a</sup>	0.66 <sup>b</sup>	0.47 <sup>c</sup>	0.006	<0.01
Fecal water soluble P (% of DM)	0.38 <sup>a</sup>	0.41 <sup>a</sup>	0.26 <sup>b</sup>	0.005	<0.01
Urine P (g/kg)	0.62 <sup>a</sup>	0.22 <sup>b</sup>	0.19 <sup>b</sup>	0.025	<0.01

LP, low phosphorus (P); MP, medium P; HP, high P; SEM, standard error of the mean; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; DM, dry matter.

Values with different superscripts (a, b, and c) are significantly different ( $p<0.05$ ).

Nowadays, water soluble P has been seen as a viable index in environmental protection (Dou et al., 2002). This study determined that water soluble P account for more than 50% of total fecal P and similar with the results reported by Dou (2002) and Bernier (2014); and high dietary P not only led to higher total fecal P content, but it also increased the proportion of water soluble P ( $p<0.05$ ). Previous studies demonstrated that fecal P excretion decreased by 23% as dietary P lessened by 0.1 percentage points (Wu et al., 2000); similar reductions in fecal P were also reported by Tallam (2005), and our results are also consistent with their findings.

## CONCLUSIONS

In conclusion, this study revealed that reducing dietary P from 0.42% to 0.26% did not negatively affect heifers' growth performance, though it did significantly reduce manure P excretion into the environment. The dietary P content of 0.26% was sufficient for 8- to 10-month-old Holstein heifers in China. Depending on the feed ingredients used, this concentration of P can be obtained without the addition of inorganic P supplement to the feed. However, further studies are required to identify the long-term effects of low dietary P on heifers.

## CONFLICT OF INTEREST

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

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