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Effect of dietary phosphorus concentration on estrous behavior of lactating dairy cows☆

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Abstract

The objective of this study was to determine the effect of dietary phosphorus (P) concentrations of 0.38 (adequate) or 0.48% (excess) of the total mixed ration (TMR) (dry matter basis) on estrous behavior of lactating cows as measured by a radiotelemetric system (HeatWatch; De Forest, WI, USA). At calving, 42 Holstein cows ($n = 21$ per treatment) were randomly assigned to one of two dietary P treatments. Cows were milked twice daily and milk weights were recorded. Cows were housed in a free-stall barn and were fitted with a radiotelemetric transmitter 40 days postpartum to record estrous mounting activity. The total number of estruses recorded for the 42 cows were 72 (37 and 35 for cows in the adequate and excess P groups, respectively). The mean duration of estrous cycles was 22 ± 0.6 days and 21 ± 0.4 days for cows fed the adequate and excess P diets, respectively ($P = 0.14$). The mean duration of estrus was 8.9 ± 1.1 h and 8.6 ± 1.2 h ($P = 0.86$), the average number of mounts during estrus was 7.0 ± 1.2 and 8.2 ± 1.7 ($P = 0.57$), and the total mounting time was 27.1 ± 4.3 s and 30.8 ± 6.5 s ($P = 0.64$) for cows fed the adequate and excess P diets, respectively. Phosphorus treatment had no significant effect on intensity or duration of estrus.

Keywords: Dairy cow; Estrus; Phosphorus requirement

1. Introduction

There is a widespread notion among producers and consultants that reproductive performance can be improved by feeding phosphorus (P) above recommended levels [1]. This idea appears to be based on the results of early research on range cattle maintained

☆ Trade names and the names of commercial companies are used in this report to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the USDA or an endorsement over products not mentioned.

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on P-deficient pastures [2–8]. In these early reports, poor reproductive performance was often accompanied by clinical signs of P deficiency (i.e. osteoporosis, osteomalacia, osteophagia, cachexia, anorexia), probably reflecting the extremely low levels of dietary P. One common characteristic for most of these reports was the interruption and even suppression of estrus [2,3,8]. Although these reports are the compilation of field observations rather than experimental data obtained under properly controlled conditions, they initially suggested irregular expression of estrus as a characteristic of cattle under conditions of natural phosphorus deficiency.

Later studies evaluated the effect of dietary P concentration on milk production and reproductive performance of dairy cattle [9–16]. Most of these studies compared diets varying in P content in relation to the National Research Council (NRC) recommendations [17–19]. The current NRC recommendations for early lactation (90 DIM) diets are 0.36% P (dry matter basis) for cows milking 45 kg per day and 0.35% P for cows milking 35 kg per day. The NRC (2001) recommends up to 0.42% for the highest producing cows during the first few weeks of lactation. However, this higher amount of P may not be necessary since P from mobilized bone contributes a significant amount of P during this period of lactation [20]. De Boer et al. [14] reported no effect of dietary P levels of 0.34, 0.51, and 0.69% in days to first ovulation, days to first estrus, days to first service, services per conception, and days open. Similarly, Call et al. [11] reported no effect of dietary P of 0.24, 0.32, and 0.42% on the same reproductive parameters. Brodison et al. [9] reported no differences in days to first progesterone rise, days open, and conception rates of lactating cows fed low P diets (0.40–0.45%) compared to cows fed high P diets (0.60–0.64%). Wu and Satter [21] summarized eight studies relating dietary P to reproduction of dairy cows. They found that reproductive performance of cows fed low P diets (0.31–0.40%) was similar to cows fed high P diets (0.39–0.55%).

Although evidence does not support the idea that excessive supplementation of P improves reproductive performance, it is still common for dairy producers to increase dietary P above the NRC recommendations in an attempt to increase expression of estrus and to improve reproductive performance of the herd. Ten years ago it was common to formulate dairy diets to contain 0.5–0.6% P (dry matter basis) [22]. Phosphorus feeding levels have been reduced somewhat in recent years, but they are still well in excess of NRC recommendations. This overfeeding of P has important environmental implications. Phosphorus excretion increases linearly as P intake is increased above the requirements [16,23,24]. Once P requirements are met, all of the excess dietary P is excreted in the feces [16,24]. This excess P accumulates in the environment, primarily by the recycling of manure to land as fertilizer for crop production. The surface runoff of this excess P promotes eutrophication of surface waters [25,26]. Therefore, close monitoring of P inputs in the livestock industry is important to reduce the risk of eutrophication of lakes and streams [27,28].

In order to accurately characterize the length and intensity of behavioral estrus, it is essential that cows be continuously monitored. Although increasing expression of estrus is one of the most common reasons for feeding excessive P, previous studies have only used visual heat detection to evaluate length or intensity of estrus. However, detecting cows in estrus and characterizing estrous behavior by visual observation is limited by incomplete information on the precise number and timing of mounts. The introduction of a radiotelemetric system for continuous monitoring of estrus has alleviated the need for estrous detection by visual observation [29–31]. The objective of this study was to determine the effect of dietary phosphorus (P) concentrations of 0.38 (adequate) or 0.48% (excess) of the total mixed ration (TMR) (dry matter basis) on estrous behavior of lactating cows using a radiotelemetry system that allows continuous monitoring of mounting activity.

2. Materials and methods

Observations on estrous behavior presented in this report were collected during the first year of a 2-year trial that analyzed milk production and reproductive performance of dairy cows fed two concentrations of dietary P [21]. Results for the first year of that study are presented in Table 1.

At calving, 42 Holstein cows ($n = 21$ per treatment) were randomly assigned to one of two dietary P treatments. Cows were fed either a diet that was close to the NRC recommendations (0.38% P: adequate), or a diet that was in excess of the NRC recommendations (0.48% P: excess). Formulation of the diet was the same for both groups with the only difference being P level. The adequate P diet contained no supplemental P, while the excess P diet was obtained by adding monosodium phosphate (NaH_2PO_4) and dicalcium phosphate ($CaHPO_4$) to the TMR. All animals were housed in a free-stall barn with concrete flooring and fed the TMR during the first 34 weeks of

Table 1
Lactation performance and reproductive measures of lactating dairy cows fed diets containing adequate or excess P[a]

Measure	Adequate P (0.38%) $n = 21$	Excess P (0.48%) $n = 21$	S.E.M.	P-value
Dry matter intake (kg per day)	20.7	20.4	–	–
Milk (kg/308 days)	9131	8860	283	0.50
3.5% fat-corrected milk (kg per day)	29.6	29.3	0.9	0.85
Milk fat (%)	3.46	3.58	0.09	0.37
Milk fat (kg per day)	1.02	1.03	0.03	0.85
Milk protein (%)	3.05	3.16	0.04	0.06
Milk protein (kg per day)	0.91	0.93	0.02	0.73
Milk lactose (%)	4.84	4.86	0.04	0.76
Milk solids not fat (%)	8.62	8.73	0.06	0.17
Milk somatic cell count (10^3/ml)	301	347	74	0.66
Days to first estrus[b]	52.2	43.4	3.8	0.11
Days to first AI[c]	76.4	76.8	4.1	0.93
Days open[d]	115	120	11	0.76
Services/conception[d]	2.5	2.6	0.4	0.80

[a] Adapted from [21].
[b] First estrus detected by visual observation.
[c] First service applied after visual detection of estrus after 52 days postpartum.
[d] Includes only the cows that ultimately became pregnant.

lactation. The diets were offered once daily ad libitum (5–10% refusal). Weekly samples of the ration were collected and dry matter (DM) content was determined by oven-drying at 60 °C for 48 h. Diets were adjusted for changes in DM content of ingredients if required. Composition of the diets has been previously reported [21]. For analysis of P, ground samples of alfalfa silage and corn silage were composited approximately every 4 weeks, and grain samples approximately every 12 weeks. These composites were processed as described by Nelson and Satter [32] and analyzed for P content by direct current plasma emission spectroscopy, by adapting the procedure described by Combs and Satter [33].

Blood was sampled periodically for later determination of serum inorganic P concentrations. Sampling times occurred within the following weeks of lactation: 1–5, 6–15, 16–25, and 26–34. Approximately 10 ml of blood were obtained from each cow via coccygeal venipuncture 3 h after feeding. Samples were centrifuged at $2200 \times g$ for 15 min, and serum was analyzed for inorganic P at the Marshfield Laboratories (Marshfield, WI, USA) by the molybdovanadate colorimetric procedure, according to AOAC [34].

Visual detection of estrus was performed by the farm crew during the day and while cows were in the holding area prior to milking. Cows were inseminated following the AM-PM rule at the first estrus detected by visual observation after 52 days postpartum. Information on estrus collected by visual observation was only used to breed cows and not to characterize estrous behavior. Pregnancy was confirmed by rectal palpation approximately 30 days after insemination. Cows were milked at 05:00 and 17:00 h and milk yields were recorded daily. Milk samples were collected biweekly from two consecutive milkings and analyzed at the Ag-Source Milk Analysis Laboratory (Menomonie, WI, USA) for fat, protein, lactose, total solids, and somatic cell count. Cows were weighed after milking at the beginning of lactation and approximately every 4 weeks during lactation.

In order to characterize estrous behavior, all cows ($n = 21$ per dietary treatment) were fitted with a radiotelemetric patch and a transmitter 40 days postpartum. Transmitters remained active until pregnancy was confirmed by rectal palpation ~30 days after AI. This system was used to collect information on mounting activity related to estrus. The radiotelemetric system used in this trial included battery-powered, pressure-sensitive transmitters with a 0.4-km range, a signal receiver that was situated approximately 100 m from the free-stall barn where the cows were housed, a buffer for receiving and storing mounting activity data, and PC-compatible software for interpreting the information. Activation of the pressure-sensitive transmitter by the weight of a mounting cow for a minimum of 2 s results in a radio-wave transmission generating real time data. The transmitted data were analyzed by the software using a mount data log that recorded information for each mount (date, time, duration, cow number, and transmitter number) [35]. An estrous event was defined as a period containing a minimum of two mounts within a 4-h period and duration of estrus was defined as the time interval from the first to last mount. Onset of estrus was identified by the first activation of the transmitter. Single mounts were not included in the analysis of estrous behavior.

In general, short estruses were more intense (as determined by the number of mounts/h) than long estruses. Therefore, in order to evaluate the relationship between dietary treatment and duration and intensity of estrus, the mean duration of estrus was calculated and this measure was used to classified estruses by duration as short or long and the intensity of estrus was analyzed separately for short or long periods of estrus.

Categorical data were analyzed for treatment effects using the FREQ procedure of SAS with chi-square and Fisher's exact tests [36]. Continuous data were analyzed by the GLM procedure of SAS [37]. Serum P concentrations were analyzed by using the MIXED procedure of SAS. Autoregression correlation coefficient (AR) for unequally spaced time data was calculated to determine the level of correlation between estrous events expressed by the same cow by using the MIXED procedure of SAS with a repeat measures statement [36,38]. Characteristics of estrous behavior were analyzed by Student's *t*-test.

3. Results

The total number of estruses recorded for the 42 cows was 72 (37 and 35 for cows in the adequate and excess P groups, respectively). Estrous events expressed by the same cow were not correlated ($AR = 0.62$; $P = 0.15$) and therefore were analyzed as independent events. The average days in milk (DIM) during the period when estruses were recorded was 86.8 ± 2.4 days with a range of 46–125 days. The mean duration of estrous cycles was 22 ± 0.6 days ($n = 36$) with a range of 16–29 days and 21 ± 0.4 days ($n = 34$) with a range of 18–28 days for cows fed the adequate and excess P diets, respectively ($P = 0.14$). The length of estrus was 8.9 ± 1.1 h and 8.6 ± 1.2 h for cows fed the adequate and excess P diets, respectively ($P = 0.86$). The number of mounts per estrus was 7.0 ± 1.2 and 8.2 ± 1.7 ($P = 0.57$), for a total mounting time during estrus of 27.1 ± 4.3 s and 30.8 ± 6.5 s ($P = 0.64$) for cows fed the adequate and excess P diets, respectively. Dietary P had no detectable effect on the length of estrus, the number of mounts per estrus, and the mounting time during estrus (Table 2).

The effect of dietary treatment on duration and intensity of estrus was analyzed. The mean duration of estrus was calculated (8.8 h) and estruses were classified by duration as short (<8.8 h) or long (≥8.8 h). In general, cows with shorter duration of estrus had a higher intensity of estrus (as determined by the number of mounts/h) than cows with longer duration of estrus ($P = 0.002$). Therefore, the intensity of estrus was classified separately for cows with short or long periods of estrus. The mean intensity for short estruses was calculated (1.6 mounts/h) and short estruses were classified by intensity as low

Table 2
Characteristics of estrous events (mean ± S.E.M. (range)) for cows fed diets containing adequate or excess P

Characteristic	Adequate P (0.38%) $n = 37$[a]	Excess P (0.48%) $n = 35$	*P*-value
Duration of estrus[b]	8.9 ± 1.1 (1.5–25.1)	8.6 ± 1.2 (1.5–24.8)	0.86
Total mounts (*n*)	7.0 ± 1.2 (2–45)	8.2 ± 1.7 (2–60)	0.57
Total mounting time (s)	27.1 ± 4.3 (4–151)	30.8 ± 6.5 (4–155)	0.64
Average days in milk[c]	87.0 ± 3.3 (48–125)	86.5 ± 3.6 (46–121)	0.69
Average milk production (kg)[d]	35.1 ± 1.2 (20.3–51.5)	34.4 ± 1.3 (15.5–47.6)	0.91

[a] Number of estrous events.
[b] Number of hours between the first and the last recorded mount of an estrous period.
[c] Days postpartum when information on estrous behavior was collected by radiotelemetry.
[d] Average milk production for 5 days preceding the day of estrus.

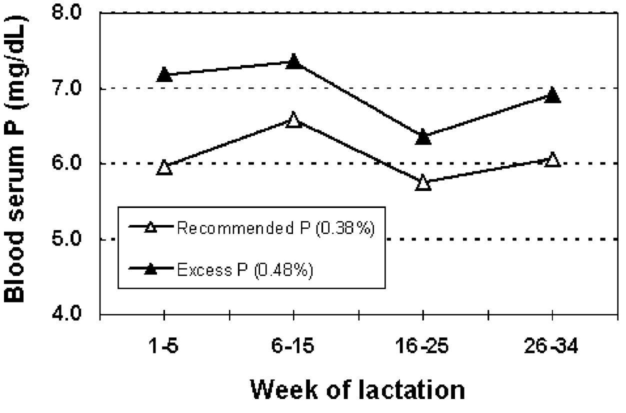

Fig. 1. Concentration of inorganic phosphorus (P) in blood serum of cows fed diets containing adequate (0.38%) or excess (0.48%). Effect of diet ($P < 0.0001$) and time ($P < 0.0001$), and diet by time interaction ($P = 0.27$) during the experimental period.

(<1.6 mounts/h) or high (≥1.6 mounts/h). Similarly, the mean intensity for long estruses was calculated (0.8 mounts/h) and long estruses were classified by intensity as low (<0.8 mounts/h) or high (≥0.8 mounts/h). Dietary P had no detectable ($P = 0.66$) effect on the distribution of estrous periods by duration and/or intensity. There were 22 (59.5%) and 21 (60.0%) short estruses and 15 (40.5%) and 14 (40.0%) long estruses for cows fed the adequate and excess P diets, respectively ($P = 0.96$). Within the short estruses, there were 13 (59.1%) and 15 (71.4%) low-intensity estruses and 9 (40.9%) and 6 (28.6%) high-intensity estruses for cows fed the adequate and excess P diets, respectively ($P = 0.39$). Within the long estruses, there were 12 (80.0%) and 9 (64.3%) low-intensity estruses and 3 (20.0%) and 5 (35.7%) high-intensity estruses for cows fed the adequate and excess P diets, respectively ($P = 0.42$).

The concentration of inorganic P in blood serum was higher ($P < 0.0001$) for cows fed the excess P diet as compared to the concentration in cows fed the adequate P diet (Fig. 1). All concentrations (range 4.5–7.9 mg/dl) were within the normal range (4–8 mg/dl) typically seen in lactating dairy cows [39].

4. Discussion

This is the first study that has analyzed the characteristics of estrus for lactating cows fed two different concentrations of dietary P (0.38% P: adequate or 0.48% P: excess) using a device designed to continuously monitor estrous mounting activity. Dietary treatment did not have significant effect on duration or intensity of estrus.

The duration of estrus, the number of mounts, and the total mounting time during estrus for the present study were similar to those reported in some studies [31,40,41], but greater than those reported in other studies [42,43] using a similar system for estrous detection. Dransfield et al. [31] summarized the characteristics of 2055 estruses for dairy cows from 17 herds (rolling herd average and size of herds ranged from 7330 to 10,862 kg per year and from 56 to 556 cows, respectively) under diverse management conditions (from total confinement feeding to primarily grazing systems). In that study, the duration of estrus was 7.1 ± 5.4 h (range ± S.D. 5.1 ± 3.8 h to 10.6 ± 6.8 h) and the number of mounts during estrus was 8.5 ± 6.6 (range ± S.D. 6.2 ± 5.1 to 12.8 ± 9.9). Walker et al. [40] reported duration of estrus of 9.5 ± 0.8 h, number of mounts of 10.1 ± 0.6, and mounting duration of 24.1 ± 1.5 s for 77 estruses from lactating cows. Xu et al. [41] reported similar results for duration of estrus (8.6 ± 0.4 h), number of mounts (11.2 ± 0.9), and mounting duration (29.0 ± 2.7 s) for 89 estruses for dairy cows on pasture.

Other studies have reported shorter duration of estrus and less mounting activity during estrus than those found in the present study. At-Taras and Spahr [42] reported duration of estrus of 5.8 ± 0.8 h and 5.6 ± 1.0 h and number of mounts of 6.7 ± 0.7 and 5.4 ± 0.8 for 68 natural and 52 synchronized estruses for dairy cows housed in free-stall barns. Timms et al. [43] analyzed the characteristics of estrus in relation to seasonal housing differences. In that study the duration of estrus was 8 and 4 h, the number of mounts during estrus was 4.1 and 3.4, and the mounting duration was 14 and 10 s for estruses recorded during the months of May to October and November to April, respectively. Lopez et al. [44] analyzed the characteristics of estrus for high-producing (11, 336 ± 1053 kg/305 days) dairy cows. In that study, the average length of estrus was 3.6 ± 0.8 h and the number of mounts per estrus was 4.3 ± 0.3 for a total mounting duration of 14.6 ± 1.4 s.

In general, reports on duration of estrus and mounting activity reveal extensive variation due to multiple factors, including footing surface [45–47], lactation number [47], environmental temperature [42,47], number of herdmates simultaneously in estrus [48], and social factors among the herd [49].

In the present study, the length of estrus, the number of mounts, and the mounting time during estrus did not differ between cows fed the adequate P or the excess P diet. This contradicts a widely held notion that feeding high P diets can improve the ‘‘strength of heat’’ [1]. This widespread concept may have originated from findings between 1900 and 1950 in which low dietary P was related to long periods of anestrus and/or irregular expression of estrus in range cattle [2,3,8]. In these early studies, dietary P was extremely low, and other dietary deficiencies associated with the low quality diets fed may have contributed to the irregularities in the expression of estrus. It has been reported that dietary P levels of less than 0.25% can reduce rumen microbial growth [50] resulting in less microbial protein, lowered ration digestibility, and decreased energy supply. Additionally, low levels of dietary P can reduce feed intake, causing coincidental deficiencies of energy, protein, and other nutrients. A decrease in DMI, milk production, and body weight was reported for dairy cows fed a diet containing 0.24% P when compared to cows fed diets ranging from 0.28 to 0.42% [11,15]. It is probably through these mechanisms that low levels of dietary P may have an indirect effect on the expression of estrus in the range cattle utilized in early reports. However, modern dairy diets usually do not approach the low P levels required to impair function of rumen microbes or depress DMI. The P content in current dairy diets usually ranges from 0.33 to 0.40% before P supplementation [16]. Therefore, supplementation of P above the NRC recommendations is not an appropriate practice to improve the expression and intensity of estrus in modern dairies.

In summary, supplementation of dietary P above levels recommended by the NRC (2001) did not improve duration or intensity of estrus.

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