POSTPARTUM REPRODUCTIVE FUNCTION OF DAIRY COWS AS INFLUENCED BY ENERGY AND PHOSPHORUS STATUS

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Summary

To determine if energy or phosphorus status would affect postpartum reproductive performance, we assigned primiparous heifers, six per group, to either high (135% NRC) or low (85% NRC) energy, and high (138% NRC) or low (98% NRC) phosphorus in a 2 x 2 factorial design. Treatments were continued from parturition through 84 days postpartum, after which standard diets were fed. The entire experiment was repeated. Reproductive function was almost identical among energy and phosphorus groups. However, no cow with peak serum progesterone below 2.7 ng/ml before insemination conceived to that insemination. Interval from parturition to first observation of serum progesterone (≥3 ng/ml) was correlated with serum phosphorus (r = -.34), glucose (r = -.33) and growth hormone (r = .51). In addition, body weight change, milk production and glycemia did not seem to affect reproduction markedly. These data suggest that neither energy nor phosphorus status, within the ranges studied, influences reproduction of dairy cows.

(Key Words: Reproduction, Energy Status, Phosphorus Status, Growth Hormone, Progesterone.)

Introduction

Effects of varying nutrient intake or nutrient imbalance on reproductive function have not been fully elucidated. Reports have suggested that diets with less than recommended energy impair fertility (Dunn et al., 1969; Wiltbank, 1974, 1977). Other studies have shown no significant effects on fertility (Gardner, 1969). Generally, when effects were observed, underfeeding was quite severe. Effects of less extreme deficiencies (10 to 20% below recommendations) have not been clearly defined.

Natural deficiencies of phosphorus were reported to cause infertility in cattle (Eckles et al., 1926; Theiler et al., 1928; Morrow, 1969). In contrast, reproduction was not affected in controlled deficiency experiments when blood phosphorus values were as much as one-half of normal (Eckles et al., 1935; Palmer et al., 1941). Supplementation of phosphorus has either improved reproductive performance (Theiler et al., 1928; Hart and Mitchell, 1965; Morrow, 1969; Taylor et al., 1976) or had no effect (Hecht et al., 1977; Noller et al., 1977).

The objective of the following study was to clarify the range of energy or phosphorus status that would allow postpartum cows to resume reproductive function and to conceive normally.

Experimental Procedure

Two levels of energy and phosphorus (>100 and 75% of NRC, 1971) were fed to 24 primiparous Holstein heifers from the Michigan State University here in an experiment using a 2 x 2 factorial design. This trial was repeated so that 48 heifers would complete the experiment. At parturition, heifers weighing between 405 and 633 kg were assigned alternately to one of four treatments (six heifers per treatment): (1) high energy, high phosphorus; (2) high energy, low phosphorus; (3) low energy, high phosphorus, and (4) low energy, low phosphorus. Heifers were assigned to represent equivalent genetic potential within each treatment. Treatment began on the day of parturition and was continued through 84 days postpartum. At that time, heifers were returned to standard diets and reproductive function was monitored for an additional 21 days, so the experiment ended at 105 days of lactation.
High energy diets were composed of (1) corn silage treated with NH$_4$OH, which was fed until feed refusals were about 10% of intake, and (2) a grain mixture (Carstairs et al., 1980) that was fed ad libitum. Grain was fed twice daily to these groups, but grain was restricted to 2.3 kg once daily for heifers on the low energy diets. All cows were fed 2.3 kg of alfalfa-brome hay daily. Phosphorus was restricted by replacement of dicalcium phosphate in the grain mix with corn and CaCO$_3$. In the second trial, a grain mixture containing dicalcium phosphate was mistakenly fed for 3 to 4 weeks to the high energy, low phosphorus group. Consequently, some heifers in this group were not in negative phosphorus status.

In both trials, daily records of disease, feed intake and milk production were kept, and these are reported elsewhere (Carstairs et al., 1980). Cows were weighed at parturition and at approximately the same time of the morning each week thereafter. Blood was collected via tail vein at approximately 1000 hr Monday and Thursday each week throughout the experiment. After collection, blood was maintained at room temperature for 1 hr and then at 5 C for 5 hr, after which it was centrifuged and the serum was removed. Serum was stored at -15 C until assayed for hormones and metabolites. Reproductive status was determined by concurrent examination of (1) serum concentration of progesterone and (2) data collected weekly from examination of reproductive organs by rectal palpation. These data, combined with herd records, were used for evaluation of the occurrence of ovulation, formation of corpora lutea and normal cycles. Number of days to first ovulation (it was not in all cases detected as an overt estrus) and number of days postpartum to reach a serum progesterone concentration of 3 ng/ml were determined. Time to reach 3 ng/ml serum progesterone was measured, because this is the level considered to be necessary before insemination in order for conception to occur (Folman et al., 1973; Corah et al., 1974; Rosenberg et al., 1977). It was anticipated that days to first ovulation and days to 3 ng/ml serum progesterone were regressed ately with means of biweekly values of energy and phosphorus status for each heifer before the time of ovulation or 3 ng/ml progesterone. The multiple regression equation used was:

$$y = a + \beta X_1 + \beta X_2 + \beta X_1 X_2,$$

where

- $y$ = days to first ovulation or days to 3 ng/ml progesterone,
- $X_1$ = energy status,
- $X_2$ = phosphorus status and
- $X_1 X_2$ = interaction of $X_1$ and $X_2$.

Throughout these data, when $P<.25$, the effect is designated nonsignificant.

Serum Analysis. Progesterone and growth hormone (GH) were measured by radioimmunoassay as described by Louis et al. (1973) and Purchas et al. (1970) in samples collected twice weekly from all heifers throughout the 15 weeks of lactation that were studied. Insulin was designated as detected or undetected on the basis of whether an estrus was recorded before the presence of a corpus luteum. Thus, undetected estrus included both absence of estrus (silent estrus) and unobserved estrus.

From composition measurements of feeds and NRC composition tables, a value for net energy and phosphorus was calculated for each feed used in the experiment. From these values and feed intake and analysis data, as well as milk production and milk composition measurements, a calculated energy status was determined for each heifer once every 2 weeks over the first 3 months of lactation (Carstairs et al., 1980). Thus, energy status was calculated from the difference between requirements and actual intake. Phosphorus status was calculated in the same way. Status, then, was an expression of intake of energy and phosphorus relative to requirements. All other nutrients in the diet were at or above requirements.
was radioimmunoassayed (Martin et al., 1979) by Dr. E. Veenhuizen of Eli Lilly and Co.*.

Glucose was assayed by reaction with 0-toluidine in hot acetic acid to form a Schiff base, in the first trial, and by glucose oxidase, in the second trial6. Phosphorus was measured colorimetrically as phosphomolybdate (Fiske and Subbarow, 1925).

**Results**

*Energy and Phosphorus Status.* High energy (HE) groups were in positive status throughout the experiment, while low energy (LE) groups were in negative status. Energy status (Mcal NE1/day) for HE and LE groups was 8.4 and -3.7 (P = .001), representing 135 and 85% of requirements.

In both trials, it was difficult to maintain negative phosphorus status when combined with a practical HE diet. Negative phosphorus status was achieved only in cows fed both LE and low phosphorus (LP) throughout the experiment. When data for the two trials were combined, the HE-LP group was not in negative status, but they were less positive than either of the high phosphorus (HP) groups. Phosphorus status for HP and LP groups was 20.6 and -1.9 g/day (P = .001), or 138 and 98% of requirements (phosphorus status for LP groups was the average of 110 and 86% of requirements).

*Days Postpartum to Reach 3 Ng/ml Serum Progesterone.* Groups fed LP diets tended to take longer (P = .10) to reach 3 ng/ml of progesterone than did HP groups (table 1). Given similar variation in data, approximately n = 100 would be needed to show a difference between phosphorus groups (at P = .05 level). Multiple regression analysis indicated no significant relationship between energy or phosphorus status and number of days for progesterone concentration to reach 3 ng/ml. As the average of weekly serum concentrations of phosphorus or glucose increased among heifers, 3 ng/ml progesterone was observed sooner, (r = -.34, -.33, P<.05, respectively). Days to 3 ng/ml progesterone was positively correlated with serum growth hormone concentrations (r = .51, P<.05).

Additionally, peak progesterone was tabulated for samples taken before inseminations that resulted in pregnancy or nonpregnancy. As a result, 41 and 16 progesterone peaks occurring before nonpregnancy and pregnancy, respectively, were recorded. Means for progesterone concentrations were similar (5.74 and 5.07 ng/ml, respectively). However, no heifer with a serum progesterone concentration before insemination below 2.7 ng/ml became pregnant to that insemination. Peak or maximal progesterone concentration before inseminations that did not result in conception ranged from .7 to 10.7 ng/ml.

*Days to First Ovulation.* Interval from parturition to the first ovulation did not differ between groups (table 2). HE groups, on the average, ovulated for the first time approximately 4 days before LE groups. HP and LP groups, on the average, ovulated at 27 days postpartum. Multiple regression analysis supports the hypothesis that increasing energy status decreases time to first ovulation. The standardized regression coefficient for energy status was −.38 (P = .07). Phosphorus status and interaction of the two main effects had no effect on the time when first ovulation occurred. Serum insulin was negatively correlated with days to first ovulation, (r = -.55, P<.01) in trial 2, but these parameters were not significantly correlated when data for both trials were combined.

Cows apparently ovulated, on the average, 18 days before progesterone concentration reached 3 ng/ml.

*Days Nonpregnant.* Cows were allowed to leave the herd only if one of the following conditions was met: (1) pregnancy was detected by rectal palpation; (2) they had been inseminated three times, or (3) they were more than 150 days in lactation. One-hundred and fifty days was selected as a realistic cutoff point

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**TABLE 1. NUMBER OF DAYS POSTPARTUM TO REACH 3 NG/ML SERUM PROGESTERONE**

<table>
<thead>
<tr>
<th>Energyb</th>
<th>Phosphorusb</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>High</td>
<td>44</td>
</tr>
<tr>
<td>Low</td>
<td>37</td>
</tr>
<tr>
<td>Mean</td>
<td>41</td>
</tr>
</tbody>
</table>

*Mean and standard error of mean for all heifers was 45 ? 3.5 days.

bEnergy = nonsignificant; phosphorus = P=.19; energy X phosphorus = nonsignificant.

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5Greenfield, IN 46140.
6Worthington Biochemical Corp., Freehold, NJ.
TABLE 2. NUMBER OF DAYS POSTPARTUM TO FIRST OVULATIONa

<table>
<thead>
<tr>
<th>Phosphorusb</th>
<th>Energyb</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>27</td>
<td>22</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>27</td>
<td>31</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>27</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aMean and standard error of mean for all heifers was 27 ± 2.3 days.

bEnergy, phosphorus and energy × phosphorus are nonsignificant.

TABLE 4. NUMBER OF INSEMINATIONS PER CONCEPTIONa

<table>
<thead>
<tr>
<th>Phosphorusb</th>
<th>Energy</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>2.56</td>
<td>1.86</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2.44</td>
<td>2.67</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.5</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aEnergy, phosphorous and energy × phosphorous are nonsignificant.

bNumber of cows pregnant in parentheses.

beyond which cows would normally be culled if apparently infertile.

Dates of pregnancy confirmed by rectal palpation were used for cows that became pregnant before 150 days. All other cows were arbitrarily classified as nonpregnant. When this was done, number of days nonpregnant was similar for all groups (table 3). Given the same variation in number of days nonpregnant, a sample size of at least 250 would be needed to show differences between phosphorus groups (8.6 days) and over 700 would be necessary to show differences between energy groups (5.0 days) in time to conception (at P = .05 level).

Inseminations per Conception. There were no significant differences between groups in inseminations per conception (table 4). Given similar variation, a sample size of about 280 would be needed to show differences between energy groups (at P = .05 level). In the HE and LE groups, either (33%) and three (12.5%) cows, respectively, did not conceive; six (25%) of the cows in the HP group and five (21%) in the LP group did not conceive. These differences, however, cannot be attributed to treatment.

Absolute Number and Percentage Undetected estrus. There were no differences between groups in either number or percentage of estrus undetected (table 5). Calculations indicated that, overall, 1.5 out of 3.3 ovulations, or 44%, were undetected during the first 15 weeks of lactation.

Although heifers fed excess phosphorus for the first 12 weeks of lactation had about 10% fewer undetected ovulations than those fed to requirements, an estimated sample size of 116 would be needed to detect differences at the P = .05 level.

Interval to Second and Third Ovulations. There were no significant differences in interval to second or third ovulation. Forty-four of 48 heifers had a second ovulation, and 38 of 48

TABLE 3. MEAN NUMBER OF DAYS NONPREGNANTa

<table>
<thead>
<tr>
<th>Phosphorusb</th>
<th>Energy</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>117.8</td>
<td>123.8</td>
<td>120.8</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>110.1</td>
<td>121.4</td>
<td>115.8</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>114.0</td>
<td>122.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aEnergy, phosphorous and energy × phosphorous are nonsignificant.

TABLE 5. NUMBER AND PERCENTAGE UNDETECTED ESTRUSa

<table>
<thead>
<tr>
<th>Energy</th>
<th>Phosphorus</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>1.3</td>
<td>1.6</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>(41.7)b</td>
<td>(48.6)</td>
<td>(45.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.2</td>
<td>1.7</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>(35.4)</td>
<td>(49.3)</td>
<td>(42.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.3</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(38.6)</td>
<td>(49.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aEnergy, phosphorous and energy × phosphorous are nonsignificant (P<.25).

bPercentage of undetected estruses in parentheses.
had a third ovulation during the first 13 weeks of lactation. Overall means were 20.6 and 21.2 days for second and third interovulatory intervals, respectively.

Mean number of ovulations (3.3 ovulations per heifer) did not differ between phosphorus groups during the 15-week experimental period. HE groups tended to have fewer ovulations \( (P<.25) \) than LE groups (3.1 vs 3.5); however, sample size was not large enough to detect statistical differences.

**Health.** Incidence of metritis was similar among groups (Carstairs et al., 1980). There was no indication that treatments significantly affected incidence of ovaries with palpable cysts, serum progesterone concentration coincident with these cysts or incidence of other reproductive problems. Increased serum progesterone concentration in the absence of pregnancy was used as an index to differentiate luteal from follicular cysts.

**Preovulatory Concentrations of Progesterone.** Increased concentrations of serum progesterone (up to 1.7 ng/ml) were seen from 1 to 8 days before first postpartum ovulation in six of the 48 cows (figure 1). The source of this progesterone is not clear at this time. This phenomenon has been observed previously (Donaldson et al., 1970) and has been compared with (or said to be analogous to) small progesterone peaks observed in prepubertal heifers.

**Discussion**

Evidence from these experiments suggests that energy and phosphorus status within the ranges studied did not affect postpartum reproductive function in any apparent way. Heifers fed HE diets ovulated for the first time only 4.5 days before heifers fed LE diets and only by regression analysis did this relationship approach significance \( (P = .07) \). There were no significant differences in time postpartum to reach 3 ng/ml progesterone; heifers usually ovulated once before reaching this concentration. In the study conducted by Rosenberg et al. (1977), heifers ovulated for the first time at 27 days postpartum and progesterone concentrations of 3 to 4 ng/ml were not observed until 40 to 50 days postpartum, findings comparable with those for heifers in the present studies. Rosenberg et al. (1977) noted that primiparous cows ovulated and reached 3 to 4 ng/ml progesterone later than multiparous cows; this has implications for the decision of when to breed first calf heifers if concentrations of progesterone are critical. Under the criteria of Folman et al. (1973), Corah et al. (1974) and Rosenberg et al. (1977), this would mean that the second ovum (not the first) in these heifers would be viable for conception. Although there appears to be no guarantee that progesterone concentrations of 3 ng/ml prior to insemination will insure pregnancy, there is a suggestion that at least that much progesterone is needed to allow conception.

No difference in days to conception could be attributed to energy or phosphorus status or differences in intake within the range studied. This observation agrees with one other study with dairy cows (Gardner, 1969) but contradicts several others in which diets deficient in energy increased the number of days nonpregnant (Dunn et al., 1969; Wiltbank et al., 1962, 1964). Different conclusions may have been reached in these studies because they were conducted with cows under more marginal nutritional conditions where energy status was at a more critical level. In addition, cows in the present study calved in good condition, and Wiltbank (1974, 1977) has suggested that, for beef cows, poor body condition precalving combined with poor feeding postcalving increases the incidence of postpartum fertility problems. Differences in management and physiology of beef cows may alter the animals' responses to energy and phosphorus status.

No evidence was found that lower intakes of phosphorus delayed conception; this supports previous work in which experimentally induced phosphorus deficiency did not impair ovulation or conception (Palmer et al., 1941). Severe phosphorus deficiency has been associated with fertility problems (Eckles et al., 1935; Morrow, 1969), but in the present experiment, heifers in the LP groups were, on the average, at NRC requirements for phosphorus. Serum phosphorus concentrations within a wide range have not been found to influence fertility (Parker and Blowey, 1976; Rowlands et al., 1977). Improved fertility in beef cows after the feeding of supplemental phosphorus (Ritson et al., 1971; Williams et al., 1971; Taylor et al., 1976) suggests control cows were extremely deficient in phosphorus. In a recent study, Call et al. (1978) reported no differences in growth, puberty, conception, gestation and lactation in beef heifers raised for 2 years on either 66 or 174% of NRC-recommended levels of phosphorus. There was no
Figure 1. Serum progesterone before and after first ovulation for each of the six heifers that showed preovulatory progesterone increases.
evidence in the present study that excessive phosphorus was associated with infertility, as had been suggested by Hewett (1974). There was some indication that increased serum P concentrations were associated with a decrease in the length of time to reach 3 ng/ml progesterone (progesterone concentration was highly correlated with interval from calving to ovulation). In both experiments, HP groups had higher serum phosphorus concentrations than LP groups. In trial 1, means were 7.1 and 6.3 ng/dl for HP and LP groups. In trial 2, means were 5.6 and 5.2 ng/dl for HP and LP groups. These concentrations would be considered within the normal range.

Differences in numbers of heifers conceiving may have been the result of management rather than nutritional problems, since some heifers became pregnant after treatment ended. There was no evidence that treatments affected occurrence of ovulations once cyclic behavior was established. Undernutrition has been quite severe (85% or less of maintenance) in cases in which estrous cycle length has been altered (Hill et al., 1970) or hormone concentrations changed (Apgar et al., 1975). HE groups had .4 fewer ovulations than did LE groups during the 15-week experimental period. This difference approached significance at the P = .1 level. Decreased numbers of ovulations in HE groups could not be attributed to pregnancies, since more HE heifers were nonpregnant at this time than were LE heifers (16 vs 13, respectively).

There are several hypotheses about how nutrition or other factors influence reproduction. Several investigators have suggested that increases (or minimal loss) in body weight pre- or postpartum are important for ovulation and conception (King, 1968; Folman et al., 1973; Wiltbank, 1974, 1977; Youdon and King, 1977; Holness et al., 1978). In the present study, on the average, heifers in the HE groups gained weight and those in the LE groups lost weight, but there was little difference between groups in time to first ovulation. This finding supports reports by several researchers (Gardner, 1969; Broster, 1973; Downie and Gelman, 1976) that weight change does not play an important role in postpartum reproduction under normal circumstances.

High milk production has also been reported to be associated with fertility problems (Morrow et al., 1966; Whitmore et al., 1974). In the present study, there was no evidence that level of milk production delayed time to ovulation or estrus, as had been observed by King (1968) and Simpson (1972). Heifers in the present trials, however, were not high producers (average 305-day yield was 5,908 kg), and this could account for the lack of correlation between these variables. Milk production data are discussed in more detail elsewhere (Carstairs et al., 1980).

The relationship between hypoglycemia and reproductive dysfunction has received much attention (Oxenreider and Wagner, 1971; McClure, 1972; McClure and Payne, 1978). Reports indicated that increasing blood glucose concentrations were associated with improved fertility. In this study, glucose concentrations of the different groups were similar (60 to 75 ng/dl) and would not be considered hypoglycemic. Serum glucose concentrations did not change significantly with days of lactation in any of the experimental groups. The only indication that glucose might be involved was a negative correlation with days to reach 3 ng/ml serum progesterone, which, as discussed, may be involved in conception. Blowey et al. (1973) and Hewett (1974) found no relationship between glucose concentrations and fertility after extensive study of many herds.

Serum insulin was negatively correlated with days to first ovulation in trial 2. Thus, higher glucose and insulin concentrations were associated with decreased number of days to reach 3 ng/ml progesterone and decreased number of days to ovulation. In trial 2, mean insulin concentrations for the HE and LE groups were 7.25 and 5.68 uU/ml, respectively. Serum GH, in contrast, was positively correlated with days to reach 3 ng/ml progesterone. During weeks 1 and 2, HE groups had higher GH concentrations than did LE groups (5.0 vs 3.8 ng/ml). From weeks 3 to 8, groups did not differ (mean = 4.4 ng/ml). From weeks 9 to 12, LE groups had higher GH concentrations than did HE groups (4.8 vs 3.6 ng/ml).

Reports that energy status might alter levels of reproductive hormones can neither be confirmed nor denied since only progesterone was measured in these experiments. Since there were no apparent differences in other reproductive function, it seems likely that progesterone secretion was not changed by either energy or phosphorus status. Finally, there was no evidence that heifers fed LE had a higher incidence of fertility disorders than did HE groups, as suggested by Francos (1974).
Literature Cited


