EFFECTS OF DIETARY PHOSPHORUS CONCENTRATIONS ON REPRODUCTIVE EFFICIENCY IN DAIRY HEIFERS

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INTRODUCTION

There have been conflicting reports concerning the effects of phosphorus on reproductive efficiency. Phosphorus concentrations in the diet ranging from .12 to .40% have been suggested as the cause of problems varying from anestrous cows to increased number of services per conception. On the other hand, regular cycling and normal fertility have been found in cows who have shown other phosphorus deficiency symptoms such as chewing on wood and rough hair coat.

Recommended phosphorus concentrations for growing heifers are .22 to .26% of the diet. Concentrations below .14% are generally considered to be conducive to reproductive problems. Today, economics is making it harder for the farmer to earn an adequate income. With the high cost of phosphorus in supplements, it is important to know the optimum dietary phosphorus requirements for maximum reproductive efficiency.

It is known that the ovarian hormones, estradiol and progesterone have an effect on the ability of an animal to reproduce. No studies to date have been done to examine the effects of dietary phosphorus on concentrations of estradiol and progesterone in the blood. It is thought that the amount of phosphorus could influence the synthesis of these hormones from the ovary. Phosphorus is required in adenosine 5'-triphosphate (ATP) and coenzymes such as nicotinamide adenine dinucleotide phosphate (NADP) for the synthesis of these steroid hormones.

The degree of estrus exhibited by a heifer is related to the amount of estradiol produced by the maturing ovarian follicles. If lowered dietary phosphorus would result in decreased synthesis of estrogens, the probability of a silent heat (ovulation without estrus) would be increased. This could be related to the poor reproductive performance in some herds.

The objective of this study was to investigate the relationship between dietary phosphorus concentrations and reproductive efficiency in dairy heifers. Specifically, the effects of a low level (.13 and .25%) were compared to the effects of supplementation to .40% phosphorus in the ration. Blood minerals, estradiol, progesterone and reproductive performance were evaluated.

CHAPTER II

REVIEW OF LITERATURE

Calcium and Phosphorus Requirements

National Research Council (1971) recommendations for dietary phosphorus range from .26% for 200 kg heifers to .22% for 500 kg dairy heifers. However, a supplemental report published by the NRC (1974) stated that these concentrations could be reduced by 10% with no adverse effect on the growth or reproduction of the animal. Huffman et al. (1933) reported the minimum phosphorus for dairy heifers for reproduction were .2% of the total dry matter intake. These animals showed some signs of phosphorus deficiency, such as loss of appetite, rough hair coat and chewing on wood, but still maintained proper growth rates and fertility.

The minimum calcium requirements for reproduction in dairy cattle range from .16 to .18% of the total ration on a dry matter basis (Fitch et al., 1932; Palmer et al., 1935). Studies with dairy heifers, demonstrated that heifers fed .16% calcium in the diet grew as well as heifers fed up to .50% calcium (Converse, 1954). NRC (1971) recommends .29 to .32% for growing heifers, although these concentrations are thought to be more than adequate for growing heifers.

Several factors which must be considered when determining these requirements are the physiological state of the animal, the presence of vitamin D and the ratio of calcium to phosphorus. Physiological status of the animal which affect the requirements includes growth, gestation and lactation (Maynard and Loosli, 1969; NRC, 1971; Cullison, 1975). During growth, additional calcium and phosphorus are needed for developing bones. Pregnancy requires the dam to supply nutrients to the fetus, while lactation exerts a heavy demand to supply the calcium secreted in milk.

Vitamin D has been shown to affect fertility, in relation to phosphorus, but only when the diet has been deficient in vitamin D. Cohen (1962) administered large doses of 5,000,000 to 10,000,000 units of vitamin D to anestrus cows at least ten weeks after calving and saw a significant increase in the percentage of cows coming into estrus as compared to controls fed no supplemental vitamin D. Hignett et al. (1953) demonstrated the effect vitamin D will have on fertility when comparing low and high concentrations of dietary phosphorus. When the vitamin D status was low, a rise in phosphorus intake resulted in improved fertility in both cows and dairy heifers. However, when the vitamin D status of the animal was high, conception rates were comparable for phosphorus concentrations ranging from 11 to 37 grams per day. Ward et al. (1971) compared the effects of 13,000 units of vitamin D to controls with 43,000 units of vitamin D per day on postpartum cows. Ample calcium and phosphorus were fed, 100 to 200 g of calcium and 80 g of phosphorus per day. The first postpartum estrus along with conception occurred earlier in the supplemented group although no difference in number of services per conception were observed. It was suggested that silent estrus could be attributed to a vitamin D effect on ovarian hormones. Massive doses of vitamin D increased the absorption of phosphorus although not net retention, since excretion of phosphorus also increased, particularly in the urine (Conrad et al., 1956).

The calcium to phosphorus ratio probably has little or no influence on fertility. Problems blaming extreme ratios have only occurred when other factors were involved such as a deficiency in vitamin D. Hansard and Barth (1962) reported that the level of calcium had no significant effect upon total blood phosphorus nor upon phosphorus excretion. Concentration of phosphorus in the blood was not affected by source or levels of calcium, although it was indicated that calcium absorption augmented metabolic excretion of phosphorus. Early work by Henderson et al. (1930) indicated ratios ranging from 1:1 to 7.2:1 had no effect on estrus or the estrous cycle. Littlejohn and Lewis (1960) studied Ca:P ratios ranging from 1:2 to 7.5:1 and found no difference in fertility whether the ratio was high or low. In one of their experiments, fertility for both controls and supplemented groups was considered to be low while the next year, with an almost identical approach, overall fertility turned out to be high. Yet these gross imbalances in the Ca:P ratio had no damaging effect on fertility or estrous behavior in either of the two years whether conception rate was high or low. Stevens et al. (1971) compared ratios of 3:1 and 1.5:1 demonstrating no effect of these ratios on reproduction in lactating dairy cows when levels of phosphorus were set at .4 and .6%.

Hignett et al. (1951) reported some breeding problems when low concentrations of phosphorus, 10 to 15 g per day, were fed. These problems decreased when dietary calcium was increased to 100 g per day. At high phosphorus concentrations, above 25 g per day, low calcium intake impaired fertility while increasing intake from 20 to approximately 100 g kept fertility at a normal level. The author, who concluded that calcium consumption should be in excess of phosphorus intake, obtained these results by observing 39 herds of dairy cattle. He did state however, that errors in sampling may have been made. These errors in conjunction with comparing bulls of varying fertility, leaves some question as to accuracy. Abrams (1952) pointed out that when phosphorus intake is low, the adverse effect of a wide Ca:P ratio can be eliminated if supplemental vitamin D is added to the ration.

Phosphorus and Calcium Concentrations in the Blood

Phosphorus concentrations in the blood are directly related to the amount of phosphorus in the diet. It has also been shown that as an animal matures, blood concentrations of phosphorus will decrease. NRC (1971) considers normal values for phosphorus to be 4 to 6 mg/100 ml of serum for cows and 6 to 8 mg/100 ml for calves under one year of age. Morrow (1969) recorded levels of 8 to 10 mg/100 ml in calves, 6 to 8 mg/100 ml in yearlings and 4 to 6 mg/100 ml in mature cows. Similarly, Lane et al. (1968) reported serum phosphorus decreased slightly from 6.3 mg/100 ml for cattle 18 to 30 months old to 5.7 mg/100 ml for dairy cattle at 8 to 9 years of age. One of the more reliable indicators of a phosphorus deficiency is serum inorganic phosphorus less than 4 mg/100 ml in adults and 6 mg/100 ml in yearlings (Church, 1974). Littlejohn and Lewis (1960) reported levels of blood phosphorus concentrations ranging from 4.0 to 7.6 mg/100 ml in dairy heifers prior to starting one of their experiments. Six months later concentrations had decreased to 1.4 to 4.1 mg/100 ml of serum, the majority being below normal, when feeding 9 to 10 g of phosphorus per day. However, this change in blood phosphorus is not a slow gradual process. Henderson et al. (1930) reported that as soon as the phosphorus in the feed is reduced, the inorganic blood phosphorus is decreased. Changes from 8 mg/100 ml to 4.5 mg/100 ml were recorded within a period of four days.

Average serum inorganic calcium concentrations measured by Church (1974) were 10 mg/100 ml with a normal range of 9 to 12 mg/100 ml of serum. Claypool (1977) discovered significant variations between seasons with calcium levels of 9.4 mg/100 ml in April compared to 10.3 mg/100 ml in October. Dietary levels of calcium ranging from .72 to 1.44% had no effect on blood calcium (Stevens et al., 1971). Studies with beef calves indicated calcium intake had no effect on blood calcium although calcium concentrations in the blood were decreased by raising dietary phosphorus concentrations (Wise et al., 1963).

Bone is the major storage site of both calcium and phosphorus (Guyton, 1971). When calcium ion concentration in the blood decreases, parathyroid hormone is secreted from the parathyroid gland causing bone absorption into the blood. This raises serum calcium levels back to normal, while at the same time, placing phosphorus from the bone into the blood. However, parathyroid hormone also increases the excretion of phosphate from the kidney, resulting in a net decrease of blood phosphorus. Therefore, a diet deficient in calcium could cause a diet with sufficient phosphorus to result in phosphorus deficient blood levels.

Effect of Phosphorus on Reproduction

There have been many conflicting reports as to the exact relationship between dietary phosphorus concentrations and fertility in cattle. Hignett et al. (1951) observed a rise in conception rate when he raised dietary phosphorus from 10 g to 26 g per day. Their conclusions, based on observations of 39 herds, is complicated by the use of bulls of varying fertility. Hignett and Hignett (1952) later reported decreased fertility in dairy heifers and recommended that 21 to 22 g of phosphorus should be fed daily. Other factors were also involved in the phosphorus deficiency. Rations were low in dry matter, initially deficient in vitamin A and cobalt, and since the animals were housed throughout the experiment, they probably lacked vitamin D. For these reasons additional phosphorus increased fertility even though overall conception rate was lower than normal.

Low conception rates in high producing dairy cows have been associated with low blood phosphorus concentrations ranging from 3.2 to 3.8 mg/100 ml (Snook, 1964). These animals had normal amounts of phosphorus in pasture grasses, however, when supplemented with 20 g of phosphorus per day, calving intervals was decreased. Serum phosphorus averaged 4.0 mg/100 ml in control cows for the three year study, while the supplemented animals had an average of 4.5 mg/100 ml. However, these data do not permit an evaluation of differences in feed intake following supplementation. Services per conception in low producing dairy cows were decreased when cows were fed .6% phosphorus versus .4% (Stevens et al., 1971). This decrease in fertility was mainly attributed to follicular cysts.

Morrow (1964) studied a herd of Guernsey heifers in which had a sudden infertility problem. The problem was attributed to intensified husbandry and cropping practices which depleted the soil of phosphorus. Dietary amounts of 9.7 to 11.2 g per day resulted in an average blood phosphorus of 3.9 mg/100 ml over two years, with signs of phosphorus deficiency. When dicalcium phosphate was made available free choice the deficiency symptoms disappeared. Services per conception decreased even though supplementation had no effect on the length of the estrous cycle or on the frequency of silent estrus. The insensitive criteria used to determine quality and quantity of intake does not allow an assessment of the possible effects of increased feed intake on fertility. In two New Zealand farms reduced fertility was observed when phosphorus concentrations in the blood fell from 4 to 5 mg/100 ml to 1.5 to 3 mg/100 ml (Clark, 1977).

Dietary phosphorus for beef cows nursing calves was recently examined by Taylor et al. (1976). Control cows were fed the NRC recommended levels of 23 g per day of phosphorus and 100% of the recommended levels for total digestible nutrients (TDN) while another group received 157% of the NRC recommendations for phosphorus, 36 g per day. The higher phosphorus group had 89% of the cows conceiving to first service and 11% conceiving to second service compared to 59% and 35% respectively in the control group.

Several workers have reported normal reproductive efficiency with cows on low phosphorus diets. Dairy cows fed a specific phosphorus deficiency continued to exhibit estrus normally (Eckles et al., 1935). Similarly, Theiler and coworkers (1937) observed normal estrus and normal ovulation in grossly deficient beef heifers fed 3 g of phosphorus per day for an extended period. In an experiment by Littlejohn and Lewis (1960) cows fed diets providing 51 g of phosphorus per day had no significant advantage in fertility over cows receiving 9 g of phosphorus. Blood concentrations in the low phosphorus group ranged from 1.4 to 4.1 mg/100 ml which is considered deficient, to levels of 7 to 8 mg/100 ml, the upper end of the normal range, in the supplemented group.

No differences in conception rates of dairy cows producing between 20 and 25 kg of milk per day were found when high concentrations of phosphorus, .4 and .6% were fed (Stevens et al., 1971). Noller et al. (1977) compared diets containing NRC recommendations for phosphorus, .30% as well as new lower recommended concentrations of .22%, with diets containing an additional .10% phosphorus to raise the levels to .40 and .32%. Results indicated no inhibition of estrus on the lower levels and no significant difference in fertility.

Role of Phosphorus in Hormone Synthesis

Estrogen synthesis occurs in the maturing follicles while progesterone synthesis takes place in the corpus luteum (Nalbandov, 1976). Both of these steroid hormones have cholesterol as their major precursor. Cholesterol is derived from acetate, a process that requires some 25 reaction steps, with key intermediates including mevalonic acid and squalene. In the synthesis of cholesterol, 3 ATP's (adenosine 5'-triphosphate) are required for energy while 2 NADPH's (nicotinamide adenine dinucleotide phosphate) are required as hydrogen donors (Lehninger, 1975; Popjak et al., 1960). With low blood phosphorus concentrations, less than 6 mg/100 ml in heifers, it could be possible that these enzymes may not be in sufficient quantities to synthesize normal amounts of cholesterol, and ultimately estrogen and progesterone, although there has not been any data to confirm this.

Cyclic AMP (adenosine 5'-monophosphate) is responsible for the mechanism of action of luteinizing hormone on target LH mediated progesterone synthesis cells (Niswender et al., 1972). The primary event in luteinizing hormone effect on steroid synthesis may be on the activation of membrane bound adenyl cyclase which causes an increase in intracellular concentration of cyclic AMP. It has been proposed by Goodwin and Margolis (1973) that cyclic AMP controls cholesterol biosynthesis. An enzyme catalysing melavonate synthesis could be regulated by phosphorylation due to cyclic AMP dependent protein kinase and dephosphorylation by a phosphoprotein phosphatase.

The Estrous Cycle

The length of the bovine estrous cycle is usually 21 days. In dairy cattle an average cycle length of 20.8 days with a range of 10 to 56 days was reported (Swanson et al., 1972). Long et al. (1969) and Hall et al. (1959) observed that the majority of cows, greater than 70%, will have estrous cycles ranging from 16 to 24 days. First estrus was observed at 43.3 weeks of age on the average in dairy heifers (Swanson et al., 1972).

The estrous cycle is usually divided into four periods. Proestrus, which immediately precedes estrus, will generally last from 2 to 3 days. Estrus, the period of sexual receptivity, will last approximately 18 to 19 hours. The estrus period, often referred to as the heat period, is followed by metestrus, lasting from 3 to 4 days (Hafez, 1974; McDonald, 1969). During this time, Kanchiv et al. (1976) observed declining estrogen levels with the development of the corpus luteum initiated. The longest period of the estrous cycle, diestrus, is characterized by the corpus luteum developing into a functional body, secreting large amounts of progesterone (Smith et al., 1975). Collectively, proestrus and estrus are called the follicular phase, meaning that they are under the control of the maturing follicles, while metestrus and diestrus are referred to as the luteal phase, being dominated by the corpus luteum (Hafez, 1974).

Plasma Hormone Concentrations During the Bovine Estrous Cycle

Lowest concentrations of estradiol occur during diestrus, with plasma concentrations as low as 2 pg/ml (Smith et al., 1975; Wettemann et al., 1972). Dobson and

Dean (1974) and Kanchiv et al. (1976) found minimum levels of estradiol of 5 to 6 pg/ml on days 6 and 7 of the estrous cycle. Maximum estradiol levels occur during late proestrus or estrus, due to the rapid growth of the maturing follicles. Chenault et al. (1975) and Wettemann et al. (1972) measured maximum levels of 6 to 9 pg/ml in contrast to Dobson et al. (1974), Kanchiv et al. (1976) and Smith et al. (1975) who found a maximum concentration of estradiol of 11 to 14 pg/ml. These maximums were observed on the day before estrus by Smith and Wettemann while Dobson, Kanchiv and Chenault found them on the day of estrus. Variations in maximum and minimum levels may be attributed to differences in type of assay, assay procedure or time of sampling.

Peripheral plasma progesterone concentrations have been correlated with corpus luteum function in the cow during the estrous cycle (Plotka et al., 1968). Lowest levels of progesterone occur at estrus, about 8 to 20 hours after the LH peak (Smith et al., 1975; Sprague et al., 1971; and Wettemann et al., 1972). Maximum plasma progesterone concentrations occur during midcycle (Sprague et al., 1971; Smith et al., 1975; Wettemann et al., 1972; Kanchiv et al., 1976).

Role of Estrogens and Progesterone in Ovulation and Fertilization

Increasing concentrations of estrogens during the follicular phase exert a positive feedback on the hypothalamus resulting in release of peak levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH)(Liefer et al., 1972). FSH is responsible for growth and maturation of the follicle prior to ovulation. It is possible that the peak LH levels are responsible for stimulating degradatory enzymes which digest the connective tissue in the wall of the follicle resulting in ovulation (Hafez, 1974).

Low fertility in cattle is due largely to defective ova or failure of the ova to fertilize (Nalbandov, 1976). Without sufficient estrogen to cause peak FSH and LH levels, ovulation might not occur. Since estrogens are responsible for estrus behavior, low concentrations would reduce or eliminate standing heat. Even if ovulation does occur, if circulating estrogen concentrations are not high enough, the cow might not stand, resulting in what is called a silent or missed heat.

Estrogens also play a vital role in sperm transport which is necessary for fertilization to occur in the oviduct. Proper transport of the sperm to the egg requires that the female reproductive tract be under control of estrogens. Cervical mucus strands found during the follicular phase may facilitate sperm transport to the oviduct. Under control of progesterone, sperm transport is inhibited by large numbers of leucocytes which will engulf the sperm (Jaszczak et al., 1973).

Progesterone functions in combination with estrogens to cause estrus behavior. It is also necessary for maintenance of pregnancy by inhibiting uterine contractions. High levels of progesterone will inhibit the LH surge, thus preventing ovulation (Hafez, 1974; Nalbandov, 1976).

Estrus Detection

Accurate estrus detection is a key to efficient reproduction in dairy cattle, and requires good management practices. Observing cows twice daily, in early morning and late afternoon, was found to be 81 to 90% effective in detecting estrus (Donaldson et al., 1968). Increasing observations to three times daily, resulted in no increase in percent detected. However, 98 to 100% of the cows were detected in estrus when the animals were observed continuously over the 24 hour period. This agrees with Williamson et al. (1972) who obtained similar results.

The best criterion, to determine if cows are in estrus is to see them stand when mounted by another animal (Foote, 1974). Estrus cows tended to stay in groups by themselves and move around more than non-estrus cows (Williamson et al., 1972). Mounting of estrus cows and ruffling and abrasion of the rump are both useful ancillary aids to diagnosis of standing heat. However, they stated that since none of the above signs are dependable on their own, they should be used in conjunction with some type of heat detection aid.

A commonly used device, a heat tape, is placed on the top of the rump. When the cow is mounted by another animal with sufficient force and duration, a colored dye is released in the marker. Baker (1965) observed 72 out of 80 animals positive, while the other 8 lost their markers. Four of the 72 were false positives or not really in heat. In his control group, 15 minute observations every 3 hours failed to detect 26 heifers. These false positives can almost be eliminated if the tapes are placed in the right spot. Wells and Adams (1972) had little difficulty with false positives and observed 96% in heat as compared to 87% when the animals were observed 2 to 3 times daily. Williamson et al. (1972) obtained significant help from the detectors by detecting 98% in heat as compared to twice daily and 24 hour continuous observations which only detected 56 and 89% in heat respectively. No problem with false positives was mentioned.

Another common and highly reliable aid to use is the chin ball marker bull (Foote, 1974). The device is a halter with a built in paint reservoir and marker. The marker rolls a band of paint on the back of the cow in heat when mounted by the wearer. Bulls can be prepared so as not to copulate by penectomy (Frazer, 1973), deflection of the penis (Jockle et al., 1973) or by blocking the penis from leaving the prepuce (Bieberly et al., 1973). Beerwinkle (1974) reported 95% of a herd of cows on pasture, with 30 cows per bull were marked well. Marker bulls detected 87% of cows in heat compared to 72% for cows observed twice daily for 30 minutes (Foote, 1974).

EFFECTS OF DIETARY PHOSPHORUS CONCENTRATIONS ON REPRODUCTIVE EFFICIENCY IN DAIRY HEIFERS

Summary

Seventy-six heifers were randomly assigned, within 2 age groups, to either a marginal phosphorus intake or supplemental intake. The younger group averaged 10.2 mo. in age. Young controls (I) were fed a ration containing .26% P while supplemented animals (II) received .4% by adding $NH_4H_2PO_4$. Older heifers averaged 19.2 mo. with controls (III) receiving .12% P and supplemented animals (IV) .4%. Energy and calcium levels were held constant within age group to meet requirements.

Blood samples were taken bi-weekly throughout the seven month study to determine Ca and P while weights were measured monthly. Heat observations were aided by deviated bulls with markers and visual observation twice daily. Plasma progesterone and estradiol were measured on day 12 and on the day of estrus of the same estrous cycle respectively by RIA.

Supplemented heifers had greater serum P concentrations, however, all groups were within the normal range for their age. Supplemented heifers did not differ from control heifers in number of heats per time period or in number of

conceptions per time period. Services required for conception averaged 1.35 and 1.50 for Groups III and IV vs. 2.0 for both younger groups. Neither plasma estradiol (pg/ml) or progesterone (ng/ml) were significantly affected ($P>.05$) by P supplementation. Mean plasma estradiol concentrations were 9.91, 8.01, 6.25 and 8.53 pg/ml, and mean progesterone concentrations were 6.72, 8.47, 8.63 and 8.76 ng/ml, respectively, for Groups I, II, III and IV. These results indicate that P requirements are likely lower than current recommendations and providing supplemented P has no effect on reproductive performance.

Introduction

Phosphorus is intimately associated with the normal function of all animal tissues playing an important role in energy exchange. The role of phosphorus in reproductive functions is not clear. Ovarian dysfunction, anestrus and reduced conception rates are often attributed to low or deficient phosphorus states. It is known that growth, reproduction and lactation will be retarded in a ruminant that is deficient in phosphorus (Little, 1970). However, there is little agreement as to the concentration of blood phosphorus associated with reproductive problems.

Regular estrous cycles and fertility were found in heifers exhibiting other symptoms of phosphorus deficiency (Eckles et al., 1935; Littlejohn et al., 1960). Huffman et al. (1933) observed normal reproductive efficiency in dairy heifers fed a diet with .2% phosphorus. In contrast, Hignett and Hignett (1952) observed that increasing amounts of phosphorus in the diet of dairy heifers resulted in increased ovarian activity and conception rates.

Phosphorus requirements proposed by NRC (1971) vary from .26% for 200 kg dairy heifers to .22% for 500 kg mature cows. These recommendations were found to be adequate for growth and reproduction (Nollar et al., 1977). There has been no research to date measuring the effects of phosphorus on ovarian hormones. The purpose of this study was to compare the effects of low or marginal dietary phosphorus and supplemental phosphorus on blood phosphorus, estradiol, progesterone and on reproductive performance.

Experimental Procedure

Seventy-six dairy heifers (38 Holsteins, 30 Ayrshires, 6 Guernseys and 2 Jerseys) were randomly allotted within age groups to either low phosphorus (control) or supplemental phosphorus intake. The younger heifers averaged 10.2 months of age and the older heifers 19.2 months at the start of the experiment. The study period was from June 30 to January 14. Young controls were fed free choice alfalfa hay, corn and pasture (Table I) resulting in .25 to .27% phosphorus in the diet. These levels are slightly higher than the .23 to .25% recommended by NRC (1971) for 250 kg heifers. A lower phosphorus concentration could not be achieved and still have maintained adequate protein, energy and calcium levels. The

TABLE I

ESTIMATED AVERAGE DAILY INTAKE OF HEIFERS FED VARYING AMOUNTS OF PHOSPHORUS

		Young		Old	
Item	Group	1[a]	2[b]	1	2
Dry matter intake, kg					
Prairie hay				9	9
Alfalfa hay		8	8		
Corn[c]		1.8	1.8		
Corn with urea[c]				1.8	1.8
Pasture		.8	.8	1	1
Nutrient intake					
Dig. energy, Mcal		65	65	58	58
Crude protein, kg		1.9	1.9	.8	.8
Phosphorus, %		.26	.40	.12	.40
Calcium, %		1.4	1.4	.3	.3

[a]1 = Controls.

[b]2 = Supplemented with phosphorus.

[c]Molasses was mixed in to insure total consumption.

older controls were fed prairie hay ad. libitum, corn with urea and pasture resulting in .10 to .13% phosphorus in their diet. Lower phosphorus concentrations could be achieved since these animals were older and did not have to grow as much as the younger heifers. Feed and hay samples were secured monthly for determination of composition. The supplemented heifers in both groups received a specific amount of monoammonium phosphate calculated monthly by measuring feed intake to achieve and maintain .4% phosphorus in the diet. Protein, calcium and energy levels (Table I) were according to NRC recommendations for each age group with the only difference being in the amount of phosphorus in the ration.

Ingredient and chemical composition of all feeds along with average daily intake are found in Table II. Orts of hay were measured periodically (every 4 weeks) to estimate average intake. Controlled phosphorus consumption was achieved by individually feeding 1.81 kg of corn, along with molasses and monoammonium phosphate as needed for the supplemented heifers. All animals were kept on pasture without housing.

The response criteria for this experiment were blood serum concentrations of calcium and phosphorus, rate of gain, regularity of estrus, conception rates and circulating plasma concentrations of estradiol and progesterone. Blood samples for calcium and phosphorus determinations were taken every 2 weeks via the tail vein. Analysis for phosphorus was done

TABLE II

CHEMICAL ANALYSIS OF FEEDS FED TO DAIRY HEIFERS THROUGHOUT THE STUDY

Ingredient (DM basis)	Phosphorus, %	Calcium, %
Prairie hay	.07	.49
Alfalfa hay	.32	1.54
Corn[a]	.33	.09
Corn with urea[a]	.32	.09
Pasture	.20	.50

[a]Molasses mixed in for analysis.

on a Gilford 240 atomic absorption spectrophotometer. An instrumentation laboratories 253 atomic absorption emission spectrophotometer was used in the calcium analysis. Serum inorganic phosphorus was determined by the procedure of Fiske and Subbarrow (1925) whereas serum calcium was determined by the procedure of Easley et al. (1965).

Blood samples obtained from the tail vein for progesterone analysis were taken on day 12 (from October to December) during the luteal phase of the estrous cycle as determined by the previous estrus. Quantification of plasma progesterone was done by radioimmunoassay similar to the method described by Kittock et al. (1973) as modified by Hallford (1975) and Fournier (1977). The antibody used was antiprogesterone #74, generously supplied by Hafs and coworkers at Michigan State University. Within each assay, samples of stripped steer plasma and steer plasma plus 4 ng of progesterone were quantified to determine the accuracy of the assay. When stripped steer plasma was used, its average value was 0.34±0.07 ng/ml (mean ± standard error) (n = 20). The average value for the steer plasma plus 4 ng progesterone was 4.24±0.80 ng/ml (n = 12). Coefficient of variation between assays was 7.9% and within assays it was 5.5%.

Blood samples for estradiol were taken on the day of estrus immediately following the progesterone sampling. Estradiol 17-B was quantified by use of a single antibody assay similar to that reported by Wettemann et al. (1972)

as modified by Hafs et al. (1974) and Hallford (1975). The antibody for this assay was generously supplied by G. D. Niswender, Colorado State University. Accuracy was determined by using stripped steer plasma and steer plasma plus 10 pg of estradiol 17-B. The average value for the stripped steer plasma was 0.93±0.69 pg/ml (mean ± standard error; n = 24) and the average value for the steer plus 10.17±0.97 pg/ml (n = 24). Coefficient of variation between assays was 11% and within assays it was 9%.

The heifers were observed for estrus twice daily, (8:00 AM and 5:00 PM), with the help of deviated bulls wearing chin-ball markers. All of the heifers had exhibited at least one estrus one month prior to the start of the breeding season. Animals observed in heat in the morning were bred by AI late that afternoon and animals found in heat in the afternoon were bred early the following morning. Pregnancy diagnosis by rectal palpation in conjunction with calving dates were used to establish exact service for conception.

Statistical analysis of the data were by procedures of Steel and Torrie (1960). Blood minerals, hormones and conception rates were analysed using analysis of variance for a randomized complete block design. Heat and AM versus PM data were tested by chi-square for significant differences.

Results and Discussion

Neither of the control groups exhibited any outward signs of phosphorus deficiency such as stiffness in the hind quarters, rough and dull hair coat, dull eyes, decreased appetite, chewing on wood and eating dirt (NRC, 1971). Huffman et al. (1933) reported seeing these signs of phosphorus deficiency when average blood phosphorus was 5.7 mg/100 ml in dairy heifers. Normal values of blood phosphorus in dairy heifers are considered to be in the range of 6 to 8 mg/100 ml (NRC, 1971).

Serum inorganic phosphorus for both groups of heifers were within the normal range. The younger heifers averaged 7.22 and 7.37 mg/100 ml of serum over the whole experiment for the control and supplemented animals, respectively. The older heifers averaged 6.47 and 7.57 mg/100 ml of serum, respectively, for control and supplemented animals (Table III). When phosphorus concentrations were averaged over the breeding season, similar values were obtained. Values for individual animals ranged from 4 to 10 mg/100 ml of serum. Supplemented animals fed .4% phosphorus in their ration had significantly higher serum phosphorus levels than controls ($P<.01$) in the older group from the fourth week of the study until its end (Figure 1). Supplementation in the younger heifers did not result in any significant difference in the blood levels of phosphorus ($P>.10$). However, the supplemented animals did maintain higher average phosphorus concentrations throughout the breeding season. These similar blood concentrations of phosphorus may be attributed to the higher levels of phosphorus fed to the younger

TABLE III

AVERAGE SERUM INORGANIC PHOSPHORUS AND CALCIUM CONCENTRATIONS AND RATE OF GAIN

	Young		Old	
Item Group[a]	1	2	1	2
No. of heifers	16	16	22	22
Phosphorus, mg/100 ml[d]				
For entire study	7.22±.2	7.37±.2	6.47[b]±.2	7.57[c]±.2
For breeding season	6.98±.2	7.26±.2	6.35[b]±.2	7.54[c]±.2
Calcium, mg/100 ml[d]	9.42±.2	9.29±.2	9.43±.2	9.24±.2
Initial weight, kg	236	234	395	389
Final weight, kg	351	348	447	435
Rate of gain, g/day	824	810	371	329

[a]1 = controls; 2 = phosphorus supplemented.

[b,c]Means within an age group not bearing common superscripts differ ($P<.01$).

[d]Blood concentrations ± standard error.

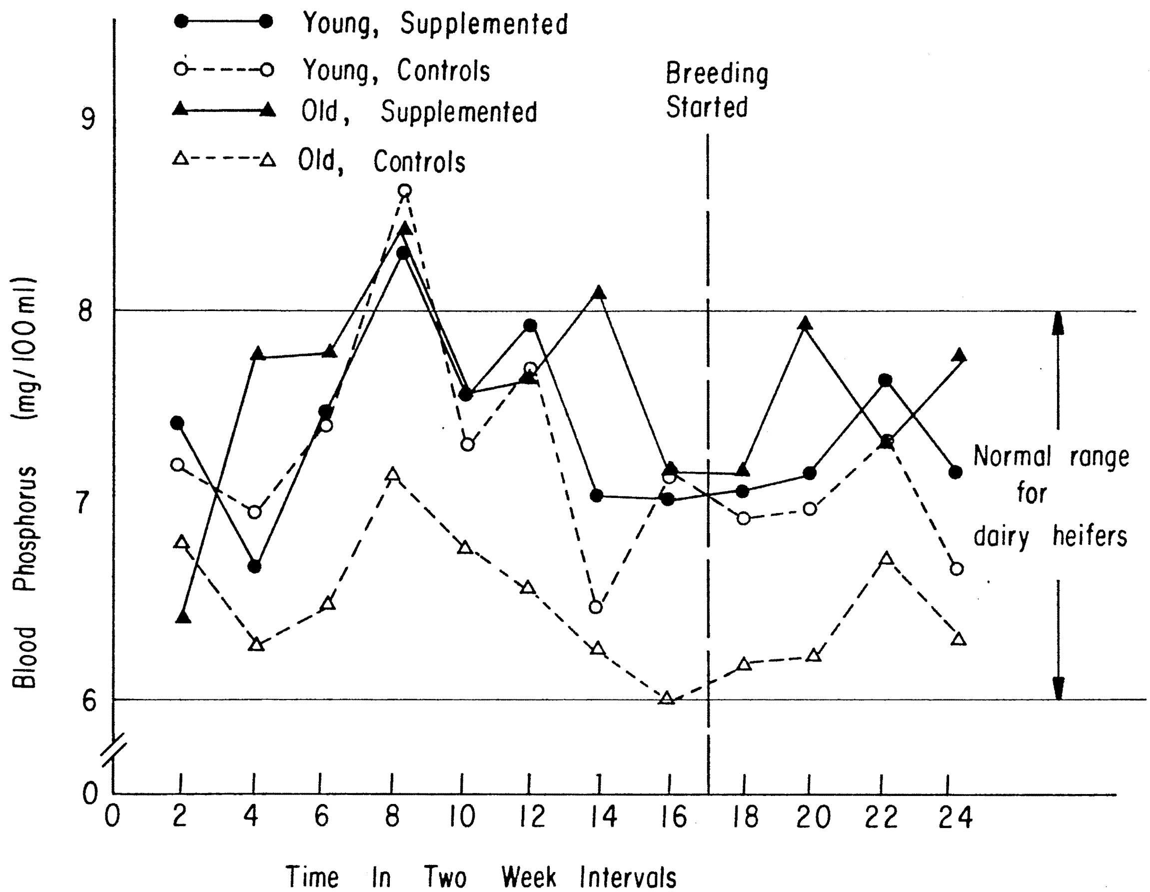

Figure 1. Serum Phosphorus Concentrations Before and During the Breeding Season

controls, .25%, versus the dietary intake of the older controls .12%.

Serum calcium concentrations did not differ significantly among all groups (P>.10) and fell within the normal range stated by NRC (1971). Although body weight gains were greater in control heifers than supplemented animals (824 to 810 g/day in the young group and 371 to 329 g/day in the older group, respectively, Table III), the increases were not significant (P>.10). As expected, the heifers averaging 10.2 months of age at the start of the trial had greater daily gain than the 19.2 month old heifers (817 vs. 350 g/day, respectively).

Phosphorus supplementation had no effect on reproductive efficiency in either the younger or the older animals (Table IV). Services per conception for both young groups averaged 2.0. The older supplemented heifers required 1.52 services per conception compared to 1.35 for the older heifers receiving no supplementation (P>.10). Conception to first service in the older heifers was 68% for both control and supplemented animals. Younger animals were somewhat less efficient with 44% of young controls and 50% of younger supplemented heifers conceiving to first service. Rate of conception, as determined by the number of animals conceiving in each succeeding 21 days of the breeding season, was similar for control and supplemented animals within each age group (P>.10). It was observed that the older heifers conceived earlier in the breeding period. The breeding

TABLE IV

EFFECT OF PHOSPHORUS SUPPLEMENTATION ON CONCEPTION RATES IN DAIRY HEIFERS

	Young		Old	
Item Group[a]	1	2	1	2
No. of heifers bred	16	16	22	22
Services per conception	2.0	2.0	1.35	1.52
Conception, no. of animals				
1st 21 days of breeding	6	8	13	13
2nd 21 days of breeding	4	3	5	4
3rd 21 days of breeding	1	2	1	2
4th 21 days of breeding	3	1	1	1
After 84 days	0	1	0	1
Open	2	1	2	1
Pregnant, %	87.5	93.8	90.9	95.5
Conc. to 1st service, %	44	50	68	68
Ave. day of year for conc.	312	311.7	298	303.8

[a]1 = controls; 2 = phosphorus supplemented.

season starting on October 7, (day 280 of the year). The older heifers conceived an average of 21 days into the breeding season with no significant differences (P>.10) due to treatment. The younger heifers required 31 days of the breeding season on the average to conceive, and no differences occurred between supplemented and control heifers (P>.10). Two animals failed to conceive in each control group compared to one in each supplemented group.

Phosphorus supplementation to increase dietary phosphorus to .4% appeared to have no effect on length or irregularity of the estrous cycle (Table V). Average length of the estrous cycle was comparable to that in previous studies (Swanson et al., 1972) averaging 21.7 and 21.6 days in the young animals and 21.9 and 20.8 days in the older group for control and supplemented heifers, respectively.

An irregular estrous cycle was defined as one lasting more than 24 days or less than 16 days as determined by twice daily heat observations aided by marker bulls. A majority of dairy cows, approximately 70%, will have estrous cycles that range from 16 to 24 days (Long et al., 1969; Hall et al., 1959). No significant differences existed between supplemented and control animals (P>.10). The younger heifers exhibited irregular estrous cycles 29.6% and 30.7% of the time as compared to the older heifers with 6% and 12% irregular cycles respectively for control and supplemented animals (Table V). The number of heifers exhibiting at least one irregular cycle was similar for both supple-

TABLE V

ESTROUS CYCLE LENGTH AND OCCURRENCE OF IRREGULAR CYCLES IN CONTROL AND SUPPLEMENTED DAIRY HEIFERS

	Young		Old	
Item Group[a]	1	2	1	2
Total no. of estrous cycles	75	81	100	106
No. of irregular cycles	23	24	12	7
Irregular cycles, %	29.6	30.7	6.6	12
No. of heifers	16	16	22	22
No. of heifers with at least one irregular cycle	10	11	6	6
Heifers, %	62.5	68.8	27.3	27.3
Ave. length of estrous cycle, days	21.7	21.6	21.9	20.8

[a]1 = controls; 2 = phosphorus supplemented.

mented and control groups (P>.10).

There were some differences found between age groups in conception rates and estrus regularity which may be attributed to the younger animals not reaching full sexual maturity at the start of the breeding season. Swanson et al. (1972) suggested that reproductive maturation continues beyond the first estrous cycle, although how far it continues is unknown.

Low dietary phosphorus, .4% fed to dairy cows averaging 23 kg of milk per day, resulted in ovarian dysfunctions which included follicular cysts, delayed ovulation and persistent corpus luteum (Stevens et al., 1971). These cows required hormone therapy to correct their problems and allow them to be bred. In the present study, the effect of circulating phosphorus levels on plasma estradiol and progesterone during the estrous cycle was measured. No significant differences (P>.10) were noted in circulating progesterone levels on day 12 of the estrous cycle or estradiol levels on the day of estrus due to supplementation with phosphorus (Table VI). The young heifers averaged 9.16±1.1 pg/ml and 7.63±1.0 pg/ml of plasma for control and supplemented animals, respectively. Older controls averaged 7.14±0.6 pg/ml while older supplemented animals averaged 7.95±0.6 pg/ml of plasma.

Phosphorus, calcium, progesterone and estradiol were not significantly correlated (P>.10) indicating that ovarian dysfunction may not be related to blood phosphorus concen-

TABLE VI

EFFECT OF SERUM INORGANIC CALCIUM AND PHOSPHORUS CONCENTRATIONS ON PLASMA CONCENTRATIONS OF PROGESTERONE AND ESTRADIOL

	Young		Old	
Item Group	1	2	1	2
No. of heifers	7	7	13	13
No. conc. to this estrus	2	3	9	9
Phosphorus, mg/100 ml[c]	7.16±.3	6.61±.3	6.01[a]±.2	7.40[b]±.2
Calcium, mg/100 ml	9.50±.3	10.11±.3	9.40±.2	9.18±.2
Progesterone, ng/ml	6.73±1.1	8.47±0.9	8.63±0.4	8.76±0.6
No. of heifers[d]	11	11	20	20
Estradiol, pg/ml	9.16±1.1	7.63±1.0	7.14±0.6	7.95±0.6

[a,b]Means within an age group not bearing common superscripts differ ($P<.05$).

[c]All concentrations ± standard error.

[d]Refers to animals used for estradiol determinations.

trations (Table VII). If blood phosphorus concentrations affected ovarian function, reproductive efficiency probably would have been altered.

No significant difference was found between the number of heats detected in the AM and the number expected when allowing 15 hours to elapse for heats to occur from the previous PM heat detection (Table VIII). One would expect 62.5% of all heats to occur in 15 hours on the average. In this study, 54.8% of the younger heifers and 64.9% of the older heifers were detected in heat in the AM. Conception rates were not significantly different in those heifers detected in heat in the morning and inseminated late that afternoon compared to those heifers detected in estrus in the PM and bred the following morning. AM cows required 1.34 services per conception on the average in the older animals, whereas PM cows required 1.78 services per conception. The younger animals required 1.76 services per conception to 2.16 services per conception for PM cows.

In the young age group no effect on appearance of animal, estrus behavior, weight gain, or conception rate was found when dietary levels of phosphorus were .25 and .40%. Since both these diets maintained normal blood phosphorus levels, which were not significantly different from each other, it would appear that .25% phosphorus is sufficient for proper growth, maintenance and reproduction of dairy heifers. This agrees with recent work by Nollar et al. (1977). Supplemental phosphorus did not improve reproductive efficiency in any

TABLE VII

CORRELATIONS BETWEEN SERUM INORGANIC CONCENTRATIONS OF PHOSPHORUS AND CALCIUM, PLASMA CONCENTRATIONS OF PROGESTERONE AND ESTRADIOL AND CONCEPTION RATE[a]

	Phosphorus	Calcium	Progesterone	Estradiol
Calcium	.10			
Progesterone	.09	.25		
Estradiol	-.27	.03	.02	
Conception	.03	.18	.08	.22

[a]No significant correlations, (P>.05). Correlation must be larger than .33 in order to be significant (P<.05).

TABLE VIII

REPRODUCTIVE PERFORMANCE OF HEIFERS DETECTED IN HEAT IN AM VERSUS HEIFERS DETECTED IN HEAT IN PM

	Young		Old	
Item Time[a]	AM	PM	AM	PM
Total no. of heats	91	75	152	82
Total no. of heats, %	54.8	45.2	64.9	35.1
Expected no. of heats[b]	103	63	146	87
Expected no. of heats, %	62.5	37.5	62.5	37.5
No. of heats serviced	30	26	43	16
No. conceiving	17	12	32	9
Services per conception	1.76	2.16	1.34	1.78

[a]AM refers to animals detected in heat in the morning and bred that afternoon.

[b]Number of heats expected to occur between 5:00 PM and 8:00 AM the next morning for AM heats and 8:00 AM to 5:00 PM for PM heat detection.

manner.

In the older group of animals where dietary phosphorus concentrations were .13% and .40%, significant differences in serum phosphorus were obtained. However, both of these groups still maintained normal values for their age group (NRC, 1971). Even when feeding as little as .13% phosphorus, which is quite uncommon unless one intentionally tries to feed very low concentrations, no deleterious effect on growth, maintenance or reproduction should occur. Neither age group showed any significant differences due to supplementation or any correlation between serum inorganic phosphorus concentrations and circulating concentrations of progesterone and estradiol.

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