POSTPARTUM REPRODUCTION AND METABOLITES IN THE COW AS AFFECTED BY ENERGY AND PHOSPHORUS INTAKE

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ABSTRACT

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By

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A study was designed to examine the influence of energy and phosphorus intake on postpartum reproductive function and metabolite levels of heifers. High and low energy and phosphorus refer to 100 and 75% of requirements. Primiparous Holsteins were assigned at parturition in a 2x2 factorial design in groups of six to: (1) high energy, high phosphorus (HEHP); (2) high energy, low phosphorus (HELP); (3) low energy, high phosphorus (LEHP); (4) low energy, low phosphorus (LELP). Treatment extended through 84 days postpartum. At this time, heifers were returned to a standard herd ration and observed for a further 21 days, the experiment ending at 105 days postpartum. Heifers were bled twice weekly and serum was assayed for several hormones and metabolites. Reproductive status was monitored via serum progesterone and rectal palpation.

Mean energy statuses (mcal/day) for high and low energy groups were 5.4 and -3.4, ($P < .0005$). Mean phosphorus statuses (g/day) for high and low phosphorus groups were 16.9 and -5.0 ($P < .0005$). Mean milk yields (kg/day) for high and low energy groups were 17.4 and 20.1

(P = .12). High energy groups had higher (P = .19) incidence of ill health compared to low energy groups.

Body weight change, serum urea nitrogen, glucose, non-esterified fatty acids (NEFA) and insulin were highly related to energy status. Mean weight changes (kg/week) for high and low energy groups were 1.75 and -0.14 (P = .12). Urea nitrogen levels (mg/dl) were higher (P < .0005) in low energy groups than high energy groups, means were 15.8 and 10.4. Mean glucose levels (mg/dl) for high and low energy groups were 75.5 and 71.6 (P = .06). Mean NEFA levels (nmoles/ml) for high and low energy groups were 221.3 and 258.9 (P = .3). Mean NEFA levels (nmoles/ml) for high and low phosphorus groups were 221.8 and 258.5 and were also different (P = .3). Mean insulin levels (μU/ml) were higher (P < .0005) in high energy groups than in low energy groups, means were 16.5 and 6.0. Both insulin and glucose were negatively related to milk yield.

Serum phosphorus was a good indicator of phosphorus status. Mean serum phosphorus levels (mg/dl) for high and low phosphorus groups were 7.1 and 5.9 (P = .02). Serum levels for the low phosphorus groups reflected marginal phosphorus deficiency.

Serum calcium, cholesterol, creatine phosphokinase, aspartate aminotransferase, and alkaline phosphatase were influenced by energy and phosphorus intake but were not useful as indicators of energy or phosphorus status. Cholesterol levels increased (P < .001) with time postpartum (r = 0.9), as did alanine aminotransferase (r = 0.88, P < .001). Regression analysis on these data on energy and phosphorus status tended to support the analysis of variance. After the end of the treatment period, values returned to normal by 105 days.

Groups imbalanced for energy and phosphorus took longer ($P = .12$), postpartum to reach 3 ng/ml progesterone than balanced groups. Means were 41 and 31 days. Imbalanced groups also took longer ($P = .27$), to experience first ovulation than balanced groups. Means were 27 and 21 days. For second and subsequent estrus', the tendency was for high energy to lengthen and high phosphorus to shorten estrous cycle length.

Mean incidence (%) of undetected heats for imbalanced and balanced groups was 40.6 and 56.4 ($P = .14$). Mean number of days non-pregnant for balanced and imbalanced groups were 145 and 117 ($P = .14$). High phosphorus groups needed more ($P = .08$) services/conception than low phosphorus groups, 3.3 and 2.2, respectively. Cystic follicles were equally distributed among groups.

This study demonstrates that postpartum energy and phosphorus intake influence metabolites and reproductive function in primiparous Holstein cattle.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table		Page

LIST OF FIGURES

INTRODUCTION

The relationship between nutrition and reproductive efficiency has been studied for many years. This efficiency of production is of even more importance today in view of the growing need for food production in a world with an expanding population. Infertility may be the result of a single, or a combination of nutrient deficiencies. Combined deficiencies have caused difficulties in assessing the effect of dietary intake on reproduction.

Deficiencies in energy and phosphorus have been implicated in delayed onset of puberty, postpartum estrus and other reproductive failures. These effects of energy and phosphorus are discussed in the Review of Literature. Previous studies have tended to be oriented towards a specific aspect of the deficiency. Some workers have emphasized the hormonal changes resulting from various deficiencies. Others have examined mainly metabolite changes and only general reproductive differences, such as conception rate. This study was designed so that both hormonal and metabolic responses could be integrated and related to the nutrient deficiency.

In order to do this, parameters were measured which would indicate the general status of the cow over the postpartum period. Primiparous Holstein heifers were used since these animals were still growing and consequently the nutrient deficiency should have caused more of a stress on reproduction and lactation. Energy and phosphorus balances were calculated so that a precise description of the status of each animal would be available. In addition several serum metabolites and enzymes were measured so that responses to the diets could be monitored. Serum progesterone and rectal palpation were used to monitor reproductive status.

Hopefully, this investigation will describe, more fully than before, the relationship between energy, phosphorus, metabolites and reproduction. Hopefully, the parameters measured can be related to differences in performance of the heifers. Perhaps also, certain of these parameters can be used in the future as indicators of energy and phosphorus status and enable marginal deficiencies to be detected.

LITERATURE REVIEW

Many theories have been postulated as to the cause of sterility, anestrus, cessation of ovulation and other reproductive failures. Nutrition has been one of the factors implicated in infertility and much work has been devoted to this area. Rank deficiencies of certain nutrients are known to impair reproductive function. However, the effects of excesses, marginal levels of nutrients or nutritional imbalances have not been fully elucidated. Reproductive efficiency at this point in time is of prime importance to the producer. The characterization and application of the relationship between nutrition and infertility will greatly aid this efficiency.

In this review the influence of energy and phosphorus on postpartum estrus and fertility will be discussed. Possible mechanisms for the action of these nutrients on reproductive function will also be examined.

Level of Energy

The cow divides her food, excluding maintenance needs, between milk production and liveweight gain (Broster, 1972). This division of nutrients is related to the milk yield potential of the cow, the stage of lactation and the level of food intake. It has been suggested the liveweight change is a measure of plane of nutrition and evidence has been reported that there is a positive relationship between plane of nutrition and fertility (Broster, 1973). The hypothesis that low planes of nutrition can lead to anestrus and reduced conception rates is widely accepted. The evidence in the literature, however, is not quite as clear cut and varying reasons as to how this effect is mediated have been suggested.

A ten percent fall in liveweight from calving to the time of mating has been associated with infertility (McClure, 1970). Similarly, a one percent change in conception to first service per one percent change in liveweight has been noted by King (1968). In that study the cows that were gaining weight had a much higher conception rate at first service than those that were losing weight. Attempts to repeat and confirm such statements have not been as successful. Although Boyd (1972) did find evidence for increased conception when cows were gaining weight, fertility differences between weight change groups were not statistically different and he concluded that weight loss had no adverse effect on fertility. Similarly, Broster (1973) quotes work by Munro (1970, unpublished) in which he could find no conclusive relationship between fertility and liveweight change. There appears to be a limitation to the relationship of liveweight and fertility and the factors that may influence it.

The question of the greater vulnerability of the cow with a higher yield potential to the adverse effects of undernutrition compared with the lower yielding cow is controversial. King (1968), reviewing work in this area found no evidence to suggest that cows yielding more milk showed reduced fertility compared to lower yielding cows. Similarly, returns to service were reported to be equally distributed amongst cows of differing milk yield potential in New Zealand herds by Simpson (1972). From here, however, the evidence is not as clear. Simpson's work was strongly criticized by Dawson (1972) who suggested that Simpson analyzed his data in such a way that it was biased towards finding no differences. Dawson (1972) quoted recent European evidence disagreeing with Simpson's (1972) results. In the United States a positive correlation between milk production and the interval from calving to conception as well as services per conception, especially for animals producing more than 7272 kg of milk per lactation, has been reported (Morrow, Roberts, McEntee and Gray, 1966). Hewett (1968) showed in Swedish herds that repeat breeders averaged 86.4 kg more milk during the first 120 days of lactation when compared with contemporary controls. He classified a repeat breeder as a cow which was declared non-pregnant after at least three inseminations or a cow which became pregnant after at least four inseminations. Each repeat breeder cow had a strictly selected control cow. This control had to belong to the same herd, be born within the same four months, calve within 35 days of the repeat breeder cow and have conceived within 100 days of calving after not more than three inseminations. This study may have some limitations due to the fact that two-thirds of the herds examined had less than 15 cows and in some cases a control could not be found and thus some repeat breeders must have been excluded from the analysis. More recently 393 calving intervals were used to compare the effects of breeding at the first postpartum estrus after 74 days as modified by two different levels of nutrition and two different genetic levels for production (Whitmore, Tyler and Casida, 1974). The incidence of silent heats for first postpartum ovulation was greater in the high nutrition groups. The interval to first postpartum estrus was longer in cows with superior genetic potential for milk production than for those which were genetically inferior producers and longer for cows on high compared to average nutrition. Finally, Simpson (1972), who did not report any infertility associated with increased milk yield did add a cautionary note in his report which although it does not explain the results of the scientific research in this area does suggest why the relationship between milk yield and infertility is commonly accepted. He notes that this relationship is observed, particularly by veterinarians, because poor producers are less likely to be presented for treatment, thus, the proportion of infertile cows could be biased toward the cow producing more milk. The situation is obviously in need of clarification.

Turning now to the effects of energy intake alone, Dunn, Ingalls, Zimmerman and Wiltbank (1969) studied the effects of energy intake on reproductive performance of two year old heifers from 140 days prepartum to 120 days postpartum. Two levels of digestible energy, low and high, were fed prepartum. At parturition the low group was divided further in half: one half were fed a moderate diet and one half were fed a high diet. The high group was divided into thirds, one third were fed a low diet, one third moderate and one third high. It should be noted that these designations were for convenience only, all were below the levels recommended by the National Research Council. Pregnancy rate 120 days after calving was directly related to the postpartum energy level. Eighty seven percent of the cows fed the high level after calving were pregnant 120 days postpartum compared with 72% of those fed the moderate level and 64% of those fed the low energy level. Estrus was delayed in the heifers receiving the low level of energy prepartum. The detrimental effects of feeding low levels of energy just prior to parturition were partially overcome by feeding higher levels postpartum.

Evidence to date would suggest that adequate energy intake is more critical than protein intake for maintaining reproductive function (Wiltbank et al., 1962; Wiltbank, Rowden, Ingalls and Zimmerman, 1964; and McClure, 1968a). Assuming that reproductive function is impaired with decreased energy intake, several hypotheses as to how this effect is mediated have been suggested. Some years ago it was hypothesized that restricted energy intake caused a hypoglycemia and that this in turn caused reproductive hypofunction. This suggestion was made after fertility was significantly improved in an Australian herd when randomly selected cows were fed an additional 5.5-6.4 kg of hay per cow per day from calving to 3 weeks after mating (McClure, 1965). The involvement of hypoglycemia was noted because the blood levels of glucose of the fertile cows at the time of mating were rising and averaged 28 mg/dl whereas the levels in the infertile cows were falling and averaged 22 mg/dl. Body weight followed the same pattern. This hypothesis was reiterated a few years later (McClure, 1968a) when low fertility syndrome herds in Australia and New Zealand were characterized as returning to estrus after irregular but often lengthened intervals. The affected cows lost 5-10% of their body weight between parturition and mating and also had low blood glucose levels of between 20 and 30 mg/dl. In addition, supplementation with energy rich concentrates to the rations of these infertile hypoglycemic herds resulted in significant increases in both blood glucose levels and fertility (McClure, 1972). Further support was given by a study in which cows were made hypoglycemic with insulin. The cows mated 0-2 days after daily insulin treatment for 3 or 4 days and those treated with insulin daily during the first four days after mating were significantly less fertile than either cows treated at other times with insulin or control untreated cows (McClure, 1968b). These results are similar to those in mice in which hypoglycemia was induced by fasting or insulin or glucose metabolism was inhibited by 2-deoxy-D-glucose (McClure, 1967). The hypothesis was that the hypothalamus was failing to control the adenohypophysis as a direct consequence of failure of the hypothalamus to be supplied with, or to utilize glucose (McClure, 1967).

It should be noted that the nature of the malnutrition described by McClure (1968a) was not well characterized. He observed infertility under two different conditions: (a) when the pasture was dry, of low digestibility and deficient in protein, phosphorus and carotene, or (b) when the pasture was green, lush and young (McClure, 1968a). Thus, his underfeeding is not just a simple case of energy deficiency but appears to be complicated by other nutrients. It should also be noted that the nutritional and hypoglycemic conditions he describes are extreme when compared with a concentrate fed dairy cow in North America. Support, however, to McClure's hypothesis is lent by Howland, Kirkpatrick, Pope and Casida (1966) who also suggest that the hypoglycemia exerted its influence through depression of hypothalamic function thus resulting in loss of ovarian activity. Additionally, a significant negative correlation was found between plasma glucose level and postpartum interval to occurrence of a 10 mm follicle and ovulation in a study of primiparous cows (Oxenreider and Wagner, 1971). Both energy and lactation had a significant effect on plasma glucose levels during the first 8 weeks postpartum. Lactation and low energy significantly delayed postpartum follicular growth and ovulation. Throughout these studies the independence of energy level, lactation and glycemia can be questioned. It is difficult to say that one or the other is the main cause or effect. One of the limitations in interpreting these data is that hormones involved in reproduction were not examined, thus, conclusions must to some degree be questionable.

At a more physiological level, the effects of energy intake on postpartum ovarian activity and changes in serum hormone levels pre and postpartum have been studied. Unfortunately, metabolite levels were not concurrently measured with alterations in reproductive function. Normal ovarian and hormonal changes have been reported by numerous workers such as Echternkamp and Hansel (1973).

The effects of different energy intakes prepartum on postpartum progesterone and estradiol concentrations in beef heifers have been examined (Corah, Quealy, Dunn and Kaltenbach, 1974). The rations were approximately equal in crude protein content and postpartum rations were adequate in energy. Blood was collected from 14 days prepartum to 20 days after the first postpartum estrus. There was no significant effect of nutrition on peripheral concentrations of progesterone or estradiol either prior to, or following, parturition. Reducing energy intake for 100 days prior to calving markedly reduced body weight and fat cover but in contrast to the work of Dunn et al. (1969) and Bellows, Varner, Short and Pahnish (1972), the interval from parturition to first estrus was not influenced by the reduced energy intake. Of particular interest in this study was the marked elevation of progesterone only in those cows conceiving, suggesting that a period of elevated progesterone may be necessary for conception at first estrus. This effect has also been noted in dairy cows maintained on high and low levels of nutrition (Folman, Rosenberg, Herz and Davidson, 1973). Cows that conceived after one insemination had significantly higher progesterone concentrations during the estrous cycle preceding insemination than did cows that did not conceive. The concentration of serum progesterone required appeared to be around 3 ng/ml. Level of nutrition had a profound effect in cows that needed more inseminations for conception. During the luteal phase preceding insemination, cows that conceived after the first insemination gained weight whereas cows that did not conceive lost weight, the difference approached significance.

In contrast to the work in which level of nutrition did not appear to affect peripheral progesterone levels per se, other studies have shown an effect. Plasma luteinizing hormone (LH) and progesterone concentrations in groups of six Holstein heifers fed 100 or 62% of energy requirements have been examined during three estrous cycles

(Gombe and Hansel, 1973). Plasma LH increased progressively from the first to the third estrous cycle in heifers fed the low energy ration. This increase was first seen in the maximum LH of the cycle but by the third cycle this increase was seen throughout the cycle and was also higher than that of the control heifers. During the first cycle progesterone was slightly higher in the cows fed 62% compared to 100% of their energy requirements but became progressively lower in the subsequent cycles. When the corpora lutea were examined on the tenth day of the third cycle it was found that total progesterone and progesterone concentration in the corpora lutea were lower in the heifers fed 62% of their energy requirements than in their normal counterparts. Evidently ovarian hypofunction in cases of energy deficiency is not due to reduced circulating LH as was previously thought by such workers as Wiltbank, Rowden, Ingalls, Gregory and Koch (1962). The first effect may be a reduced ability of the ovarian tissue to respond to LH. This effect of decreasing progesterone has been noted before in heifers receiving 25% of the total feed consumed by the controls (Donaldson, Basset and Thorburn, 1970). Declines in plasma progesterone have occurred within even 5 days of the reduction in feed intake (Hill, Lamond, Dickey and Niswender, 1970). The undernutrition which was about 85% of maintenance, also temporarily reduced the number of medium sized follicles, altered the length of the estrous cycle and reduced the proportion of heifers with normal fertilized ova. Weights of corpora lutea formed in undernourished heifers were about 70% of control values. It should be noted that Donaldson *et al*. (1970) and Hill *et al*. (1970) varied both energy and protein content of the ration whereas Gombe and Hansel (1973) reduced only energy intake. Evidence suggests that the observed reduction in plasma progesterone is a reflection of a smaller corpus luteum, containing less total and concentration of progesterone (Gombe and Hansel, 1973). Further, the simplest suggestion is that the first effect of this restricted energy intake is at some step in steroidogenesis within the corpus luteum, causing the observed reduction in plasma progesterone levels. Several additional suggestions have been made as to how this effect is mediated (Gombe and Hansel, 1973).

Accumulating evidence suggests there is an interrelationship of energy intake and reproductive function in the cow. The characteristics of this relationship are still not clearly defined and the mechanism of action is still not fully elucidated. The evidence does suggest that restricted energy intake can cause reproductive failures and that this relationship is not simple but must be considered concurrently with body weight, stage of lactation and other physiological factors.

Phosphorus and Reproductive Function

Variations in blood phosphorus levels in the bovine have been partially explained by pregnancy, parturition, lactation and age (Lane, Campbell and Krause, 1968). These changes in blood phosphorus levels with physiological state are not surprising in view of the fact that phosphorus functions in energy metabolism, skeletal growth and milk production.

The National Research Council (1971) quotes normal values for bovine plasma phosphorus as 4-6 mg/dl for cows and 6-8 mg/dl for calves under one year of age and states that unlike some other nutrients, blood concentrations will decline before any clinical signs develop. A relationship between phosphorus and fertility was postulated many years ago and appears to be widely accepted. The evidence for this relationship is often questionable since the phosphorus deficiency required to impair fertility is usually extremely severe. This does not tell how marginal the condition has to be in order to show reproductive effects. Clinical signs of phosphorus deficiency are easily noticeable and easily treated. A sub-clinical or marginal deficiency may be even more important as it may indeed cause production losses and yet not be overtly detectable.

Research in the severely deficient animal is quite well documented while research in the marginally deficient animal is indeed lacking. The situation is complicated when it is realized that most reports are confounded, knowingly or not, by deficiency of another nutrient. The widespread occurrence of a natural deficiency of phosphorus affecting cattle has been described by Tuff (1923), Theiler, Green and du Toit (1924), Eckles, Becker and Palmer (1926), Hart and Guilbert (1928) and numerous others. As early as 1906 however, in a study of the effect of phosphorus compounds in the diet of milking cows, it was noted that phosphorus deficiency was accompanied at times by the cessation of estrus (Jordon, Hart and Patten, 1906).

South African workers were among the first to report reproductive hypofunction in cattle maintained on veld deficient in phosphorus. In one study, it was shown that reproductive efficiency was increased from a 51% calf crop in the control group to an 80%

calf crop in the experimental group, simply by supplementing the naturally deficient animals with bone meal (Theiler, Green and du Toit, 1928). It was also noted that blood phosphorus was around 2.3 mg/dl in phosphorus deficient pastures whereas with bone meal supplementation the concentrations doubled (Malan, Green and du Toit, 1928). Hypophosphatemia was noted in animals being fed on a hay-oats phosphorus deficient diet also but the effects on reproduction were not mentioned (Palmer and Eckles, 1927). Years later in Ireland both clinical and sub-clinical aphosphorosis of cattle was noted in phosphorus deficient pasture. The cattle developed all the symptoms of phosphorus deficiency plus "temporary sterility" (Sheehy, 1946) or "anestrus or estrus with repeated failures to conceive after service" (O'Moore, 1950). Hypophosphatemia was noted in all these cases and phosphorus supplementation seemed to solve the problem.

In the United States, Eckles, Palmer, Gullickson, Fitch, Boyd, Bishop and Nelson (1935) were some of the first to try and obtain experimental data regarding reproductive function in cattle on controlled uncomplicated phosphorus deficient rations. They were following the example of workers such as du Toit, Malan and Groenewald (1934). Eckles et al. (1935) did not have a definite feeding level of phosphorus. They tried to supply each cow so as to maintain the plasma phosphorus at approximately 2.5 mg/dl, about one-half normal. This concentration was selected as being representative of cattle in a severely deficient area. These concentrations were accomplished and indicated severe phosphorus deficiency. The daily milk yields in these cows were between 3.2 and 11.1 kg, somewhat low by today's standards, and probably did not cause a large strain on the metabolic system. Even though this experiment was run for about three years no evidence was gained to show that the phosphorus deficiency influenced the estrous cycles of the cows. All of the cows came into heat quite regularly, it did appear, however, that breeding efficiency was reduced. It was suggested from this study that the disturbances in estrus and reproductive function in naturally deficient areas was probably due to the complication of other nutrient deficiencies.

Concurrently with the study on cows, rats were fed a diet containing 0.1% P and the following types of reproductive failures were noted: complete cessation of estrus sometimes following a successful pregnancy or successful lactation; regular estrus cycles but no normal breeding; fetal resorptions *in utero*; irregular cycles; undersized weak litters (Eckles *et al.*, 1935).

More recently a herd with heifer infertility problems was described (Morrow, 1969). These problems were attributed to phosphorus deficiency presumably resulting from depleted phosphorus in the soil and consequently in the crops. Calculations of intake and requirements for protein, energy, calcium and phosphorus showed that the phosphorus intake was deficient. Clinical signs consisted of rough coat, depraved appetite and infertility. Low blood phosphorus was also observed. The condition was treated with the feeding of dicalcium phosphate. Blood levels returned to normal and fertility was restored. The phosphorus deficiency did not appear to affect the length of the estrous cycles or the frequency of silent estrus, but the problem appeared to be in conception.

The effects of a combined phosphorus and protein deficiency have also been examined. Cows were observed for at least 24 months and up to 59 months on a protein and phosphorus deficient ration (Palmer, Gullickson, Boyd, Fitch and Nelson, 1941). It was felt that this was more analogous to a real deficiency in the field. The cows showed delayed sexual maturity, silent heat but normal, regular ovulation and conception was not interfered with. Breeding efficiency was not reduced. Feeding trials with cows on phosphorus deficient pastures in the Northern territory of Australia showed positive effects regarding fertility (Hart and Mitchell, 1965). Supplementation of range cows with 8 g P/head/day improved body weight and fertility in the lactating cows. A pregnancy rate of 60% in the treated group compared with 41% in the controls was observed. The authors did comment, however, that the provision of protein in their opinion was of equal, if not more importance, than phosphorus.

Phosphorus and calcium have also been linked in causing infertility. Hignett (1950) suggested from the results of a herd survey in England that breeding efficiency of cattle might be related to the Ca:P ratio of the feed consumed, always assuming that the amount of each element was sufficient in itself. It was suggested that a 1:1 ratio was ideal for fertility. The risk of herd fertility being impaired was great when the ratio was 2:1 or more, especially when the phosphorus intake was only slightly in excess of minimum requirements. The feeding of too much phosphorus was also warned against since Hignett (1950) associated infertility with excess levels of phosphorus. After work was completed on another 802 cows, it was concluded that the generally accepted recommendations for phosphorus (23 g/day for maintenance plus 19 g for each 4.5 kg of milk) were not enough for high fertility in dairy cattle (Hignett and Hignett, 1951). It was observed later that fertility was decreased in rapidly growing heifers which were fed rations deficient in manganese and unbalanced in calcium and phosphorus (Hignett, 1959).

Hignett and Hignett (1951) warned against the exaggeration of the importance of the influence of calcium and phosphorus intakes on fertility and their caution is justified. Most of their work was of the field survey type and only feed analyses and breeding data were used. No blood parameters were measured. As support to this caution, a large scale controlled experiment was carried out at Weybridge in 1958 and 1959 and this failed to demonstrate any significant relationship between the Ca:P ratio of the diet and fertility in dairy heifers, whether the herd fertility was high or low (Littlejohn and Lewis, 1960). A high Ca:P ratio depressed growth rate. These data are probably more reliable than the survey work reported by Hignett (1950) and Hignett and Hignett (1951).

In contrast, it has been suggested that phosphorus deficiency acts at the anterior pituitary level and causes "cessation of estrus, lack of sexual libido, testicular and accessory organ atrophy" (Guilbert, 1942). Also the "ovary in phosphorus deficiency becomes quiescent and infantile" (Guilbert and Hart, 1930) and (Kleiber, Goss and Guilbert, 1936).

Finally, it has been reported that phosphorus deficiency lowers the total efficiency of energy utilization mainly by lowering the appetite and secondly by lowering the partial efficiency of energy utilization. It does not seem to influence fasting catabolism (Kleiber *et al.*, 1936).

From the evidence to date, it does appear that there is some link between severe phosphorus deficiency and infertility. The extent to which this effect is related to deficiencies in other nutrients such as protein, other minerals, or energy is not yet entirely clear. The mechanism for its action has not been fully described. More work needs to be done, not only in the more marginally deficient animal, but also on how phosphorus interacts with other nutrients and what influence this may have. It is to be hoped that the following study will answer some of these questions and help further the understanding of nutrient deficiencies and their relationship to fertility.

MATERIALS AND METHODS

Experimental Design

Two levels of energy and phosphorus (100 and 75% of N.R.C., 1971), requirements were fed to 24 primiparous Holstein heifers from the Michigan State University herd using a 2x2 factorial design. These heifers weighed between 437 and 625 kg at parturition. Heifers were assigned at parturition in groups of six to one of four treatment groups: (1) high energy, high phosphorus; (2) high energy, low phosphorus; (3) low energy, high phosphorus; (4) low energy, low phosphorus. Heifers were assigned so as to represent equivalent genetic potential within each treatment group. Treatment began on the day of parturition and extended to 84 days postpartum. At that time, heifers were returned to the herd ration and measurements were taken for a further 21 days (the experiment ending at 105 days postpartum).

The high energy rations were corn silage treated with non protein nitrogen fed until feed refusals were about 10% of intake and grain fed to the maximum which the heifers would consume. Grain was fed daily at 0800 and 1300 hours. Grain intake was restricted to 2.3 kg at 0800 hours for the low energy rations. All cows were fed 2.3 kg of the alfalfa brome hay and the corn silage once daily. Phosphorus was restricted by replacing the dicalcium phosphate in the grain mix with corn and $CaCO_3$ (Table 1).

Table 1.--Composition of grain mixes (%).

Component	High Energy		Low Energy	
	High P	Low P	High P	Low P
Shelled Corn	73.9	74.3	--	1.8
Soybean Meal	18.8	18.8	86.1	86.1
Molasses	5.0	5.0	5.0	5.0
Dicalcium Phosphate	1.2	--	6.0	--
Limestone	0.5	1.3	0.2	4.4
Trace Mineral Salt	0.6	0.6	2.7	2.7
Total	100.0	100.0	100.0	100.0

Daily records of disease, feed intake and milk production were kept. Composite morning and evening milk samples were taken every two weeks and analyzed for fat and protein. Body weights were measured at parturition and once a week thereafter. This was done as far as possible at the same time each week.

Blood was collected at approximately 1000 hr twice weekly throughout the experiment via the tail vein using a 20 gauge 25 mm vacutainer needle and 20 ml vacutainer tubes (Becton Dickinson Inc., Rutherford, N.J.). The blood was allowed to stand at room temperature for one hour after collection and was then placed at 5 C for 5 hours. It was then centrifuged at 1000 x g for 20 minutes and the serum was removed. Serum was stored at -15 C until assayed. Reproductive status was determined by the concurrent examination of serum progesterone and weekly rectal palpation data.

Feed samples were taken and analyzed for dry matter, protein and phosphorus (analysis was done in the analytical laboratory of the Department of Biochemistry, M.S.U. or in the analytical laboratory at Wooster, Ohio). From these composition measurements of the feed and N.R.C. feed composition tables a value for Net Energy (NE) and phosphorus was calculated for each feed used in the experiment (Table 2). From these values and the feed intake data, milk production and composition measurements, a calculated energy balance was determined for each heifer once every two weeks over the first 14 weeks of lactation.

First, energy requirements (mcals NE lactation/day) were calculated from N.R.C. (1971) tables using the two week average for body weight, milk production and milk fat percent. Then energy intake was calculated (mcal NE lactation/day) using feed intake data and the energy values of the feeds. Thus, energy balance was calculated from the difference between requirements and actual intake. Phosphorus balance was calculated in the same way. Energy balance indicates the difference between input and output of energy. A requirement is estimated according to N.R.C. standards to account for the energy needed for maintenance and milk production. If the energy consumed is not enough to meet these requirements then the heifer would have to supply this energy difference from endogenous sources or decrease production. The heifer would under these circumstances be in negative energy balance or status. If the amount consumed is equivalent to the amount required then the animal is at zero balance (or 100% N.R.C.).

Table 2.--Dry matter, net energy and phosphorus values used to calculate NE intake and phosphorus intake.

Feed	Dates Value Used	Dry Matter (%)	Net Energy (Mcal/kg)	Crude Protein (%)	Phosphorus (%)
HEHP Grain	Throughout	87.00	1.96	16.4	0.553
HELP Grain	Throughout	86.00	1.96	18.4	0.341
LEHP Grain	Throughout	88.00	1.63	47.3	1.560
LELP Grain	Throughout	87.00	1.54	49.7	0.596
Herd Grain	Throughout	88.00	1.85	18.1	1.420*
Haylage	Throughout	66.00	1.14	13.3	0.300*
Hay	10/8/73-2/26/74	86.30	1.19	17.6	0.234
Hay	2/27/74-end exp.	88.70	1.19	15.9	0.300*
Corn Silage	10/8/73-1/31/74	40.00	1.69	13.6	0.103
Corn Silage	1/31/74-2/26/74	44.00	1.69	12.0	0.360*
Corn Silage	2/27/74-3/15/74	43.00	1.69	12.1	0.330*
Corn Silage	3/16/74-3/21/74	39.00	1.69	--	0.330*
Corn Silage	3/22/74-end exp.	30.00	1.69	--	0.345*

*Phosphorus percent expressed on a dry matter basis, analyzed at the analytical laboratories at Wooster, Ohio. All other values for phosphorus on a wet basis.

All NE and CP % values expressed on a dry basis.

Energy and phosphorus balance or status and their interaction were used as the independent variables in a multiple regression analysis. The regression equation is described below:

$$Y = \alpha + \beta X_1 + \beta X_2 + \beta X_1 X_2$$

where:

Y = dependent variable

α = constant

X_1 = energy balance

X_2 = phosphorus balance

$X_1 X_2$ = interaction of X_1 and X_2

Each heifer had a calculated X_1 and X_2 for each two week period of the experiment. Thus, there were six values for each heifer and 144 observations were used in the regression analysis.

Blood Serum Hormones

Progesterone.

Progesterone was measured by radioimmunoassay as described by Louis, Hafs and Seguin (1973). Progesterone was measured twice weekly for all heifers throughout the experiment.

Insulin.

Serum insulin was measured by radioimmunoassay courtesy of Dr. E. Veenhuizen of Eli Lilly and Co., Indianapolis, IN. Serum insulin was measured in all heifers once every two weeks throughout the experiment.

Metabolites and Enzymes in Blood Serum

Nonesterified Fatty Acids (NEFA).

NEFA were measured using the methods of Ho (1970) as modified by Bieber (1974). NEFA were extracted from 0.2 ml serum with 1.0 ml of Dole extraction mixture (isopropanol:heptane:1NH_2SO_4, 40:10:1) (Dole, 1956). Samples were vortexed and placed in ice. After 10 minutes of standing, 0.2 ml of heptane and 0.2 ml of water were added, vortexed, and placed on ice to allow the separation of organic and aqueous phases. An aliquot of the heptane layer (0.2 ml) was removed and placed in a 1.5 ml polypropylene centrifuge tube with an attached cap (Brinkman Instruments Inc., Westbury, N.Y.). Chloroform (0.8 ml) was added to each tube, the tube capped and placed on ice.

Nickel reagent was made by dissolving 0.4050 g of $NiCl_2 \cdot 6H_2O$ in 100 ml of 1 M triethanolamine giving a final Ni concentration of 1 mg/ml of reagent. Radioactive ^{63}Ni (1.17 mCi/100 μgNi; Amersham Searle Corp., Arlington Heights, IL.) was added to give approximately 10^6 cpm per 0.1 ml of Ni reagent.

Nickel reagent (0.1 ml) was added to each tube, vortexed vigorously for 45 seconds and placed in an ice bath. The organic and aqueous phases were separated by centrifugation at 500 x g for 5 minutes. The aqueous phase was removed by aspiration and a 0.5 ml aliquot of the organic phase transferred to a scintillation vial and evaporated to dryness. Ten ml of scintillation fluid (Appendix Table 1) were added and ^{63}Ni counted in a Nuclear Chicago liquid scintillation counter.

Palmitic acid was used as standard for calculation of unknown NEFA concentration. The concentration of NEFA in serum was obtained from an equation derived from regression of palmitic acid (Y) in concentrations from 0 to 100 nmoles on counts for blank activity (X). Standards were carried through the same manipulations as serum. Serum NEFA's were measured once weekly in all heifers throughout the experiment.

Serum Parameters Measured by Autoanalytical Techniques

Serum glucose, calcium, phosphorus, urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase and alkaline phosphatase were measured on a Hycel Mark $\underline{\bar{X}}$ clinical autoanalyzer (Hycel Inc., Houston, TX.). These parameters were measured once a week in all heifers throughout the experiment. The methodology is described on the following pages (Table 3).

Table 3.--Methodology of assays used by the Hycel Mark X clinical autoanalyzer.

Variable	Location	Test Principle	Range and Normal Value for Cattle	Reference
Phosphorus (mg/dl)	80% in bones. In blood, mainly in red cells. In serum as phospholipids and as inorganic P.	iP + ammonium molbydate forms ammonium phosphomolybdate. Measure colorimetrically.	6.4 5.1-7.9	Taussky and Shorr (1953)
Calcium (mg/dl)	In bones. In blood, ionized, protein bound and complexed with organic ions.	Serum albumin bound Ca released by acid. Total serum Ca complexed by cresolpthalein complexone. Measure colorimetrically after base turns complex purple.	9.9 8.3-11.5	Kessler and Wolfman (1964) Gitelman (1967)
Total Cholesterol (mg/dl)	In serum as cholesterol and cholesterol esters.	Extracted cholesterol + H_2SO_4 form 3,5-cholestadiene. Measure colorimetrically as monosulphonic acid.	196.0 109.0-257.0	Kaser (1953)
Urea Nitrogen (mg/dl)	Synthesized in liver.	Measured colorimetrically when 2,3-butane-dione condenses with urea and thiosemicarbide to form pink complex.	11.7 6.8-16.4	Crocker (1967)

Table 3.--Continued.

Variable	Location	Test Principle	Range and Normal Value for Cattle	Reference
Glucose (mg/dl)	Blood.	0-toluidine reacts with glucose in hot acetic acid to form glucosylamine and Schiff base. End product blue-green.	65.0 57.0-77.0	Dubowski (1962) Hyvarinen and Nikkila (1962)
Creatinine (mg/dl)	From creatinine produced during muscle contraction.	Creatinine reacts with picrate ion in alkali to give red complex.	1.1 0.8-1.3	London, Freiberger and Marymount (1967)
Aspartate Aminotransferase (U/l)*	Heart, liver > skeletal muscle, kidney > pancreas, spleen, lung	L-aspartate + α-ketoglutarate → oxaloacetate. Measure colorimetrically after coupling with azoene dye.	113.0 71.0-190.0	Babson, Shapiro, Williams and Phillips (1962)
Alkaline phosphatase (U/l)	Intestinal mucosa ≃ placenta > kidney ≃ bone > liver ≃ lung ≃ spleen.	Hydrolysis of magnesium thymolphthalein monophosphate at pH 9.85. Determined colorimetrically.	13.0 6.0-33.0	Coleman (1966)
Creatine phosphokinase (U/l)	Striated muscle, brain and heart.	CPK catalyses creatine + ATP creatine phosphate + ADP + iP at pH 8.94. iP measured colorimetrically. Corrected for endogenous phosphate.	45.0 31.0-74.0	Okinaka *et al.* (1961)

Table 3.--Continued.

Table 3.--Continued.

Variable	Location	Test Principle	Range and Normal Value for Cattle	Reference
Alanine aminotransferase (U/l)	Liver > kidney > heart ≃ muscle.	L-alanine + α-ketoglutarate → pyruvate + L-glutamate. Pyruvate + 2,4-dinitrophenylhydrazine → 2,4-dinitrohydrazone. Measured colorimetrically.	58.0 36.0-87.0	Reitman and Frankel (1957)

*U is an international unit, where 1U is the amount of enzyme which will catalyse the reaction of 1 μmole of substrate per minute. Normal values from: H. Kitchen (1974) unpublished data. Further detail on methodology can also be found in Hycel Chemistry Methods, Hycel Inc., 1974.

RESULTS

Feed Intake

The treatment means for intake of corn silage, hay and grain are shown in Table 4. The high energy groups consumed 8.2 kg more grain but 7 kg less corn silage daily than the low energy groups. Hay intake was approximately constant, the cows were all offered 2.3 kg/day.

Table 4.--Mean intakes (kg/day) of corn silage, hay and grain for the first 12 weeks of lactation.

	High Energy		Low Energy	
	High P	Low P	High P	Low P
Corn silage (with NPN) (kg/day)	18.64	17.83	25.57	24.25
Hay (kg/day)	1.90	1.89	2.05	2.03
Grain* (kg/day)	10.25	10.70	2.27	2.27

*Dicalcium phosphate replaced by $CaCO_3$ and corn.
(Intakes expressed on an as fed basis).

Energy and Phosphorus Status

The high energy groups were in positive energy status and thus, were receiving more than NRC (1971) requirements, while the low energy cows were receiving less than requirements. Mean energy and phosphorus status expressed as a function of time are shown for each group (Figures 1 and 2). It can be seen from Figure 1 that the low energy groups were not as negative as the high energy groups were positive. The low energy high phosphorus group became marginally positive at eight weeks of lactation. Phosphorus status (Figure 2) was more difficult to maintain at the desired level. The high energy, high phosphorus group was in positive status but on the average was over twice as high as the other high phosphorus group. Negative phosphorus status was achieved in the low energy, low phosphorus group but the other low phosphorus group was on the average marginally positive after about five weeks of lactation. It was difficult to restrict phosphorus intake by this group when coupled with high energy.

There was considerable variation in energy and phosphorus status among cows within each group, mainly caused by the difficulty of adjusting intake along with rapidly changing milk production. When energy and phosphorus status were analyzed by a two way analysis of variance it was found that differences did exist among the four groups. The mean energy statuses of the high and low energy groups were 5.4 and -3.4 mcal/day respectively ($P < .0005$, Table 5). Phosphorus did not significantly reduce energy status and there was no interaction of dietary energy and phosphorus on energy status.

A different phosphorus status was achieved between the high and low phosphorus groups. The mean phosphorus status for the high phosphorus group was 16.9 g/day as opposed to a mean of -5.0 g/day

Fig. 1.--Mean energy status (mcal/day) for the first 12 weeks of lactation.

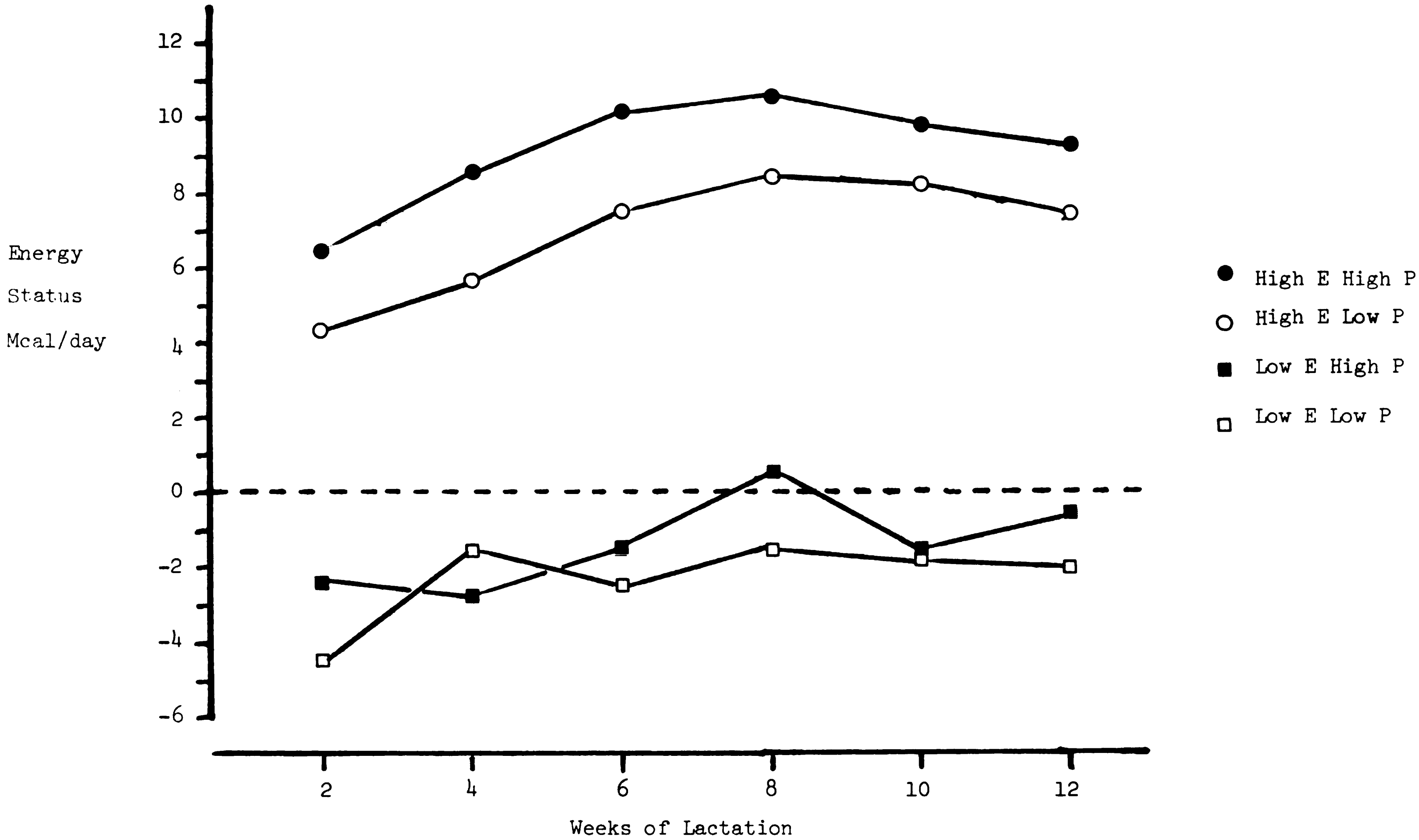
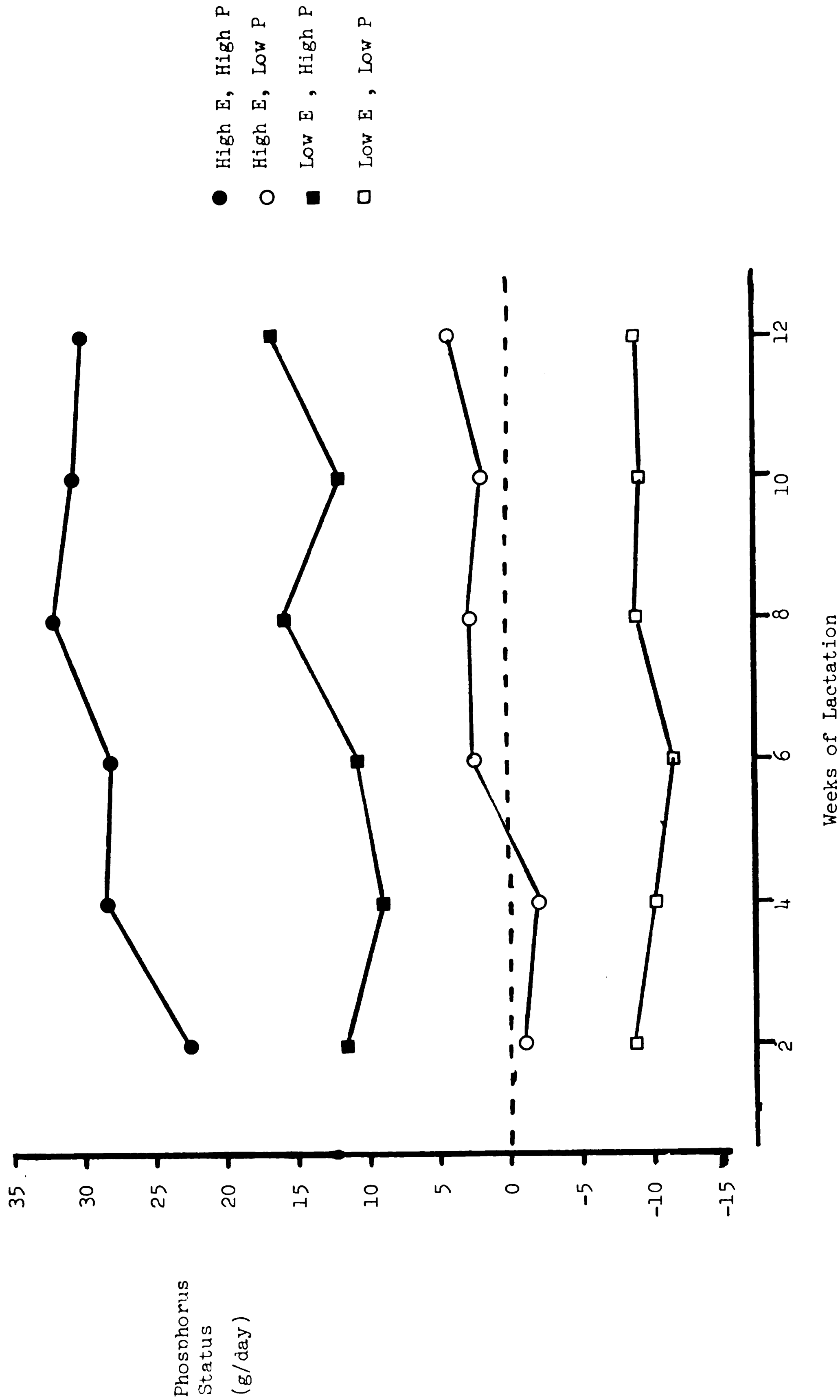

Energy
Status
Mcal/day
12
10
8
6
4
2
0
-2
-4
-6
2
4
6
8
10
12
Weeks of Lactation
High E High P
High E Low P
Low E High P
Low E Low P

Fig. 2.--Mean phosphorus status (g/day) for the first 12 weeks of lactation.



High E, High P
High E, Low P
Low E , High P
Low E , Low P
Phosphorus Status (g/day)
35
30
25
20
15
10
5
0
-5
-10
-15
2
4
6
8
10
12
Weeks of Lactation

Table 5.--Mean energy status (mcal/day) for the first 12 weeks of lactation.

Energy		Phosphorus High	Phosphorus Low
High		6.54	4.18
Low		-2.67	-4.19
	Energy	$P < .0005$	

for the low phosphorus groups ($P < .0005$, Table 6). The high energy, high phosphorus group and the low energy, low phosphorus group accounted for the most positive and the most negative phosphorus statuses.

In addition, the mean phosphorus status for the high energy group was 10.5 g/day and for the low energy group 1.4 g/day ($P = .03$). This difference was not desired but does reflect the difficulty of maintaining or restricting intakes at the level needed. However, means of the balanced and imbalanced groups were not different.

Body Weight

Body weight change generally followed the energy status, however, there was variation among cows in the same group. The high energy cows tended to gain or maintain body weight, whereas the low energy groups tended to either lose or maintain weight. The difference in weight loss between the high and low energy groups approached significance ($p = .12$). The high energy groups gained an average of 1.75 kg/week against the low energy groups who lost 0.14 kg/week. The high energy, high phosphorus group gained more weight/week than

Table 6.--Mean phosphorus status (g/day) for the first 12 weeks of lactation.

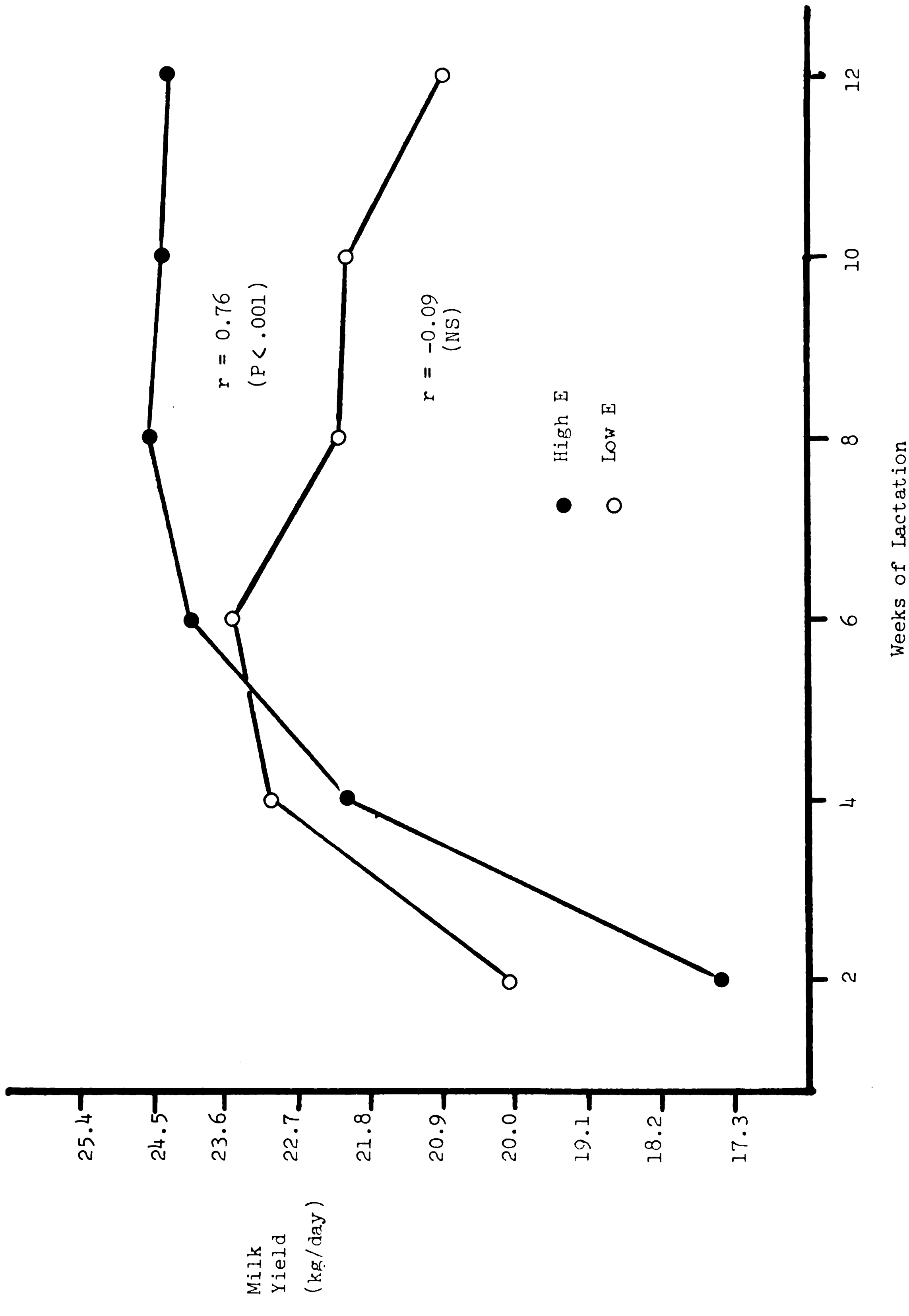
Energy		Phosphorus High	Phosphorus Low
High		22.34	-1.32
Low		11.53	-8.73
	Energy	$P = .03$	
	Phosphorus	$P < .0005$	

any other group (2.14 kg/week), the low energy, high phosphorus group lost the most/week (-0.32 kg/week). Neither phosphorus nor the interaction term had any significant effect on weight change.

Milk Yield

The high energy, high phosphorus group had the lowest average milk yield. When considered together, the high energy groups started at 17.4 kg/day at 2 weeks of lactation as opposed to a mean of 20.1 kg/day for the low energy groups. By the sixth week of lactation the high energy group had reached an average of 24.2 kg/day, surpassing the low energy group who were giving an average of 23.6 kg/day (Figure 3). From this point the high energy group continued to produce more milk than the low energy groups, who were starting to decline in milk production. The high energy group peaked at 24.6 kg/day about the eighth week of lactation. Milk yields increased with time ($r = 0.76$, $P < .001$) in the high energy groups, whereas low energy groups showed a nonsignificant negative correlation with time ($R = -.09$, NS).

Fig. 3.--Mean milk yield (kg/day) for the first 12 weeks of lactation.


Milk Yield (kg/day)
25.4
24.5
23.6
22.7
21.8
20.9
20.0
19.1
18.2
17.3
2
4
6
8
10
12
Weeks of Lactation
r = 0.76
(P < .001)
r = -0.09
(NS)
High E
Low E

The high energy groups had a lower mean milk yield than the low energy groups--a mean of 17.4 kg/day as opposed to 20.1 kg/day ($P = .12$). This was primarily due to the high energy, high phosphorus cows whose mean milk yield was only 16.4 kg/day and the lowest of the four groups.

Milk Fat Percent

There were no significant differences in milk fat percent among the treatment groups. The mean milk fat percent for the four groups was 3.59%.

Milk Protein Percent

Milk protein percent was significantly and positively related to energy and phosphorus status from the regression of milk protein on energy and phosphorus status. The analysis of variance showed that the cows in the high energy groups had higher ($P < .0005$) milk protein percent levels than the low energy groups, the means were 3.33 and 3.08%, respectively. The high phosphorus groups also had higher ($P = .02$) milk protein percent levels than the low phosphorus groups, the means were 3.27 and 3.14% respectively. These percentage differences were, however, due to total daily milk production differences. Milk protein percent fell with time ($r = -.18$, $P = .05$), when all cows were considered, obviously a total milk yield effect.

Serum Calcium

The main effects of energy and phosphorus status were not related to serum calcium levels but the interaction of energy and phosphorus was significantly related to calcium levels. The imbalanced groups had lower ($P = .07$) calcium levels than the balanced groups (Table 7), and this was primarily due to the low energy, high phosphorus group in which serum calcium seemed to be depressed relative to the other groups throughout the 12 weeks examined (Figure 4).

Table 7.--Mean serum calcium levels (mg/dl) for the first 12 weeks of lactation.

Energy		Phosphorus	
		High	Low
High		9.13	8.85
Low		8.57	9.08
	Interaction	$P = .07$	

Calcium showed a significant correlation with time, rising gradually as lactation progressed ($r = 0.48$, $P < .001$). The low energy, high phosphorus group at week 2 of lactation had a mean serum calcium level of 8.6 mg/dl, rising to 9.1 mg/dl at week 10 ($r = 0.48$, ($P < .05$). The other three groups had a mean calcium level of 9.0 mg/dl at week 2, rising to a peak value of 9.5 at week 8 ($r = 0.48$, ($P < .01$).

Serum Phosphorus

The low phosphorus groups had lower ($P = .02$) serum phosphorus than the high phosphorus groups, the means were 5.9 mg/dl and 7.1 mg/dl, respectively (Table 8).

There was no difference in serum phosphorus between the high and low energy groups, or the balanced and imbalanced groups, the

Fig. 4.--Mean serum calcium (mg/dl) for the first 12 weeks of lactation.

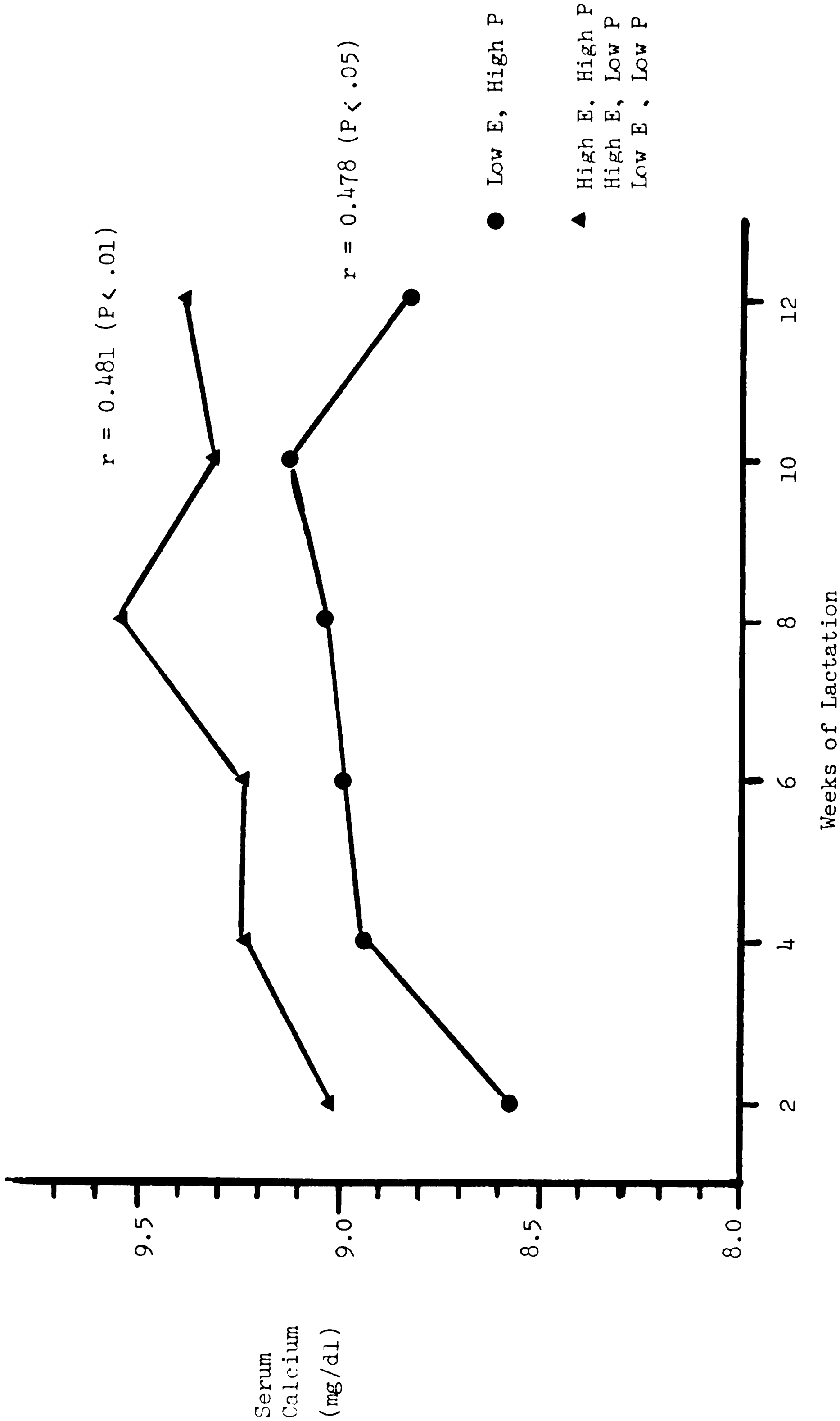

r = 0.481 (P < .01)
r = 0.478 (P < .05)
Low E, High P
High E, High P
High E, Low P
Low E, Low P
Serum Calcium (mg/dl)
9.5
9.0
8.5
8.0
2
4
6
8
10
12
Weeks of Lactation

Table 8.--Mean serum phosphorus (mg/dl) for the first 12 weeks of lactation.

Energy		Phosphorus High	Phosphorus Low
High		6.67	6.22
Low		7.52	5.68
	Phosphorus	P = .02	
	Interaction	P = .15	

means were 6.4, 6.6 mg/dl and 6.2, 6.9 mg/dl, respectively. Although the difference between the balanced and imbalanced groups did approach significance (P = .15) with the imbalanced groups having the higher serum phosphorus levels, this difference was primarily due to the low energy, high phosphorus group. It should be noted that this group had the lowest serum calcium level.

Creatinine

There were no significant differences in creatinine levels between treatment groups. The mean creatinine level for the four groups was 0.98 mg/dl.

Urea Nitrogen

Serum urea nitrogen was significantly ($P < .0005$) higher in the low energy groups than in the high energy groups. The means for high and low energy groups being 10.4 and 15.8 mg/dl, respectively. The other main effect, phosphorus status and the interaction term did not have any significant effect on urea nitrogen levels.

Total Cholesterol

There was no difference in cholesterol between energy levels, the means for the high and low energy groups being 104.5 and 96.3 mg/dl, respectively. There was however a difference (P = .10) between phosphorus groups, the high phosphorus groups having a higher mean cholesterol level (110.1 mg/dl) than the low phosphorus groups (90.8 mg/dl).

The difference between the balanced and imbalanced groups approached significance (P = .11), the balanced groups having a mean cholesterol level of 109.6 mg/dl against the level of the imbalanced groups, 91.3 mg/dl. The case was generally one of more phosphorus, more cholesterol but was particularly evident when high energy was coupled with high phosphorus. The high energy, high phosphorus group mean was 123.4 mg/dl as opposed to 96.8 mg/dl when low energy was coupled with high phosphorus (Table 9).

Table 9.--Mean serum total cholesterol (mg/dl) for the first 12 weeks of lactation.

Energy		Phosphorus	
		High	Low
High		123.4	85.7
Low		96.8	95.8
	Phosphorus	P = 0.10	
	Interaction	P = 0.11	

Total cholesterol also showed a significant and positive correlation with time, rising markedly as lactation progressed. The high phosphorus groups achieved a cholesterol level of 180.0 mg/dl by the eighth week of lactation and then plateaued while the low phosphorus groups required 12 weeks to reach the same level (Figure 5).

Glucose

The low energy groups had lower (P = .06) mean serum glucose concentrations when compared with high energy groups. Mean levels were 71.6 and 75.5 mg/dl for low and high energy groups. There were no significant differences in glucose between either phosphorus or imbalanced and balanced groups (Table 10).

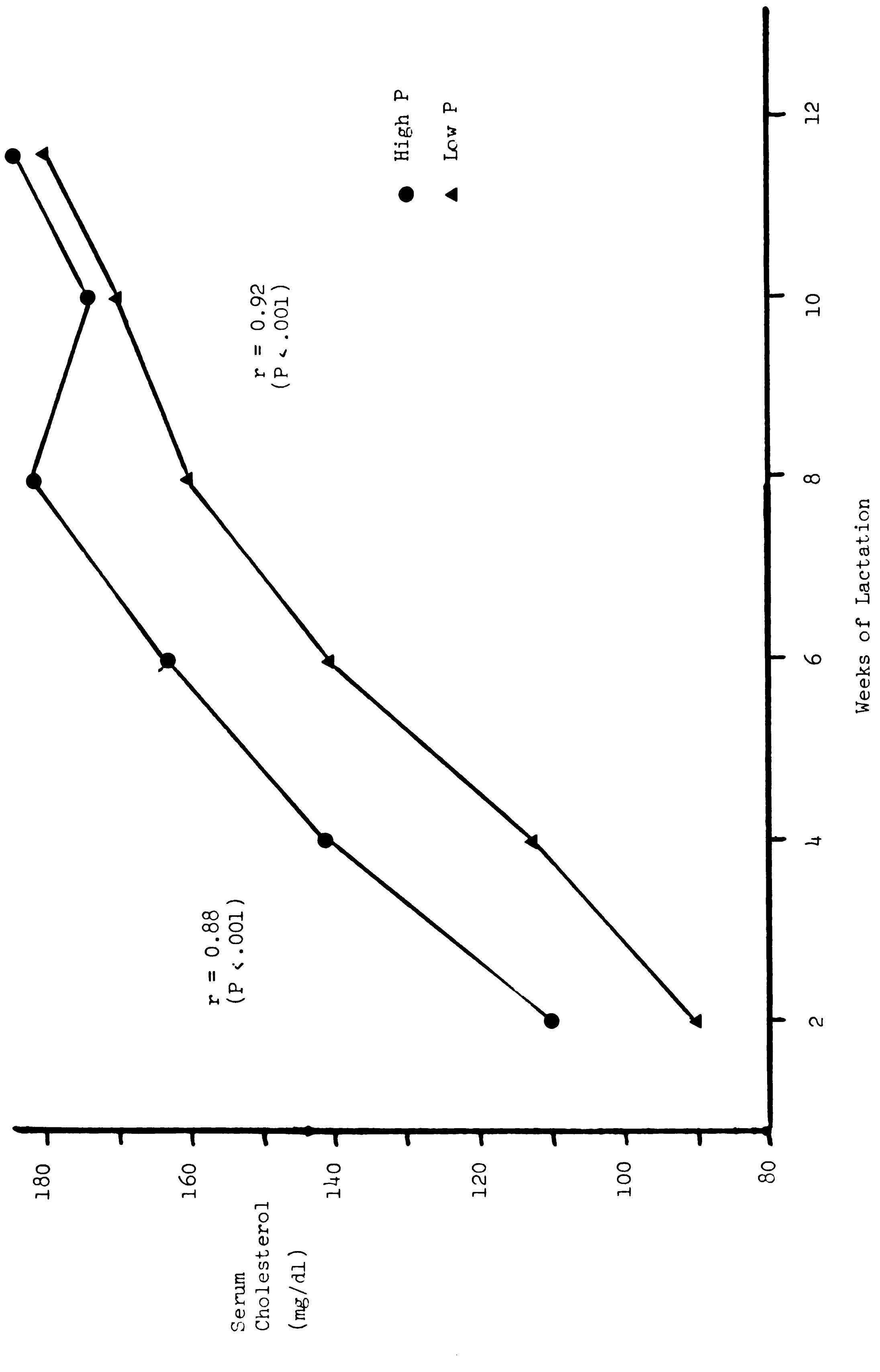
Table 10.--Mean serum glucose levels (mg/dl) for the first 12 weeks of lactation.

Energy		Phosphorus	
		High	Low
High		75.9	75.0
Low		70.4	72.8
	Energy	P = .06	

Alkaline Phosphatase

There were no significant differences among treatment groups in serum alkaline phosphatase. The mean value for all groups was 14.5 U/l.

Fig. 5.--Mean serum cholesterol (mg/dl) for the first 12 weeks of lactation.


Serum Cholesterol
(mg/dl)
180
160
140
120
100
80
2
4
6
8
10
12
Weeks of Lactation
r = 0.88
(P <.001)
r = 0.92
(P <.001)
High P
Low P

Alanine Aminotransferase

There were no significant differences among treatment groups in serum alanine aminotransferase. The mean level for the four groups was 29.9 U/l.

Alanine aminotransferase did significantly increase with time in all groups, irrespective of treatment ($r = 0.88$, $P < .001$). At the second week of lactation the mean concentration was 29.9 U/l, by the twelfth week the concentration was 42.6 U/l (Figure 6).

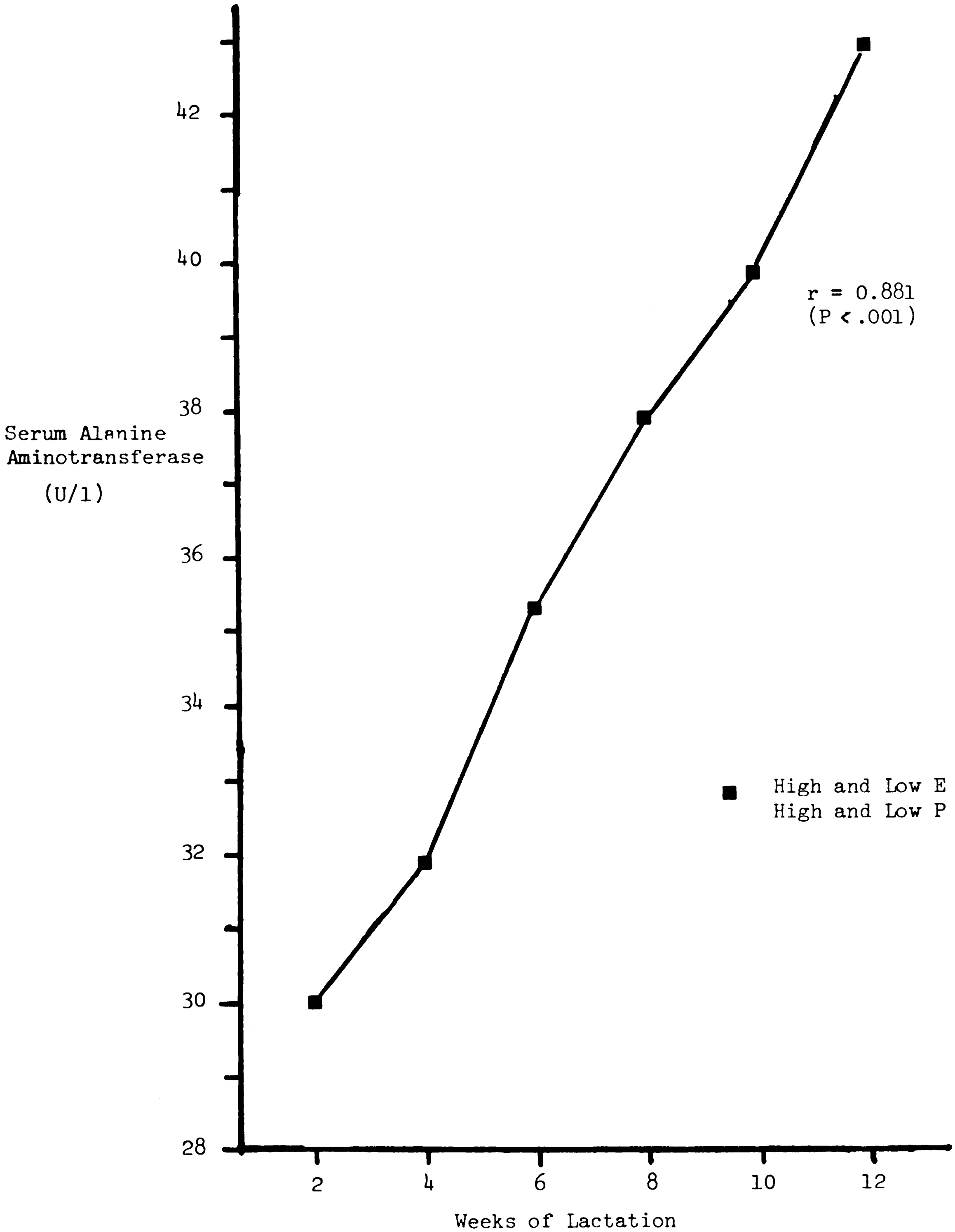
Aspartate Aminotransferase

The interaction of energy and phosphorus status was the outstanding effect regarding aspartate aminotransferase. The balanced groups had significantly ($P = .008$) higher aspartate aminotransferase concentrations than the imbalanced groups, the means were 105.2 and 91.5 U/l respectively (Table 11). The difference between the energy groups did however approach significance ($P = .14$), the high energy

Table 11.--Mean serum aspartate aminotransferase levels (U/l) for the first 12 weeks of lactation.

Energy		Phosphorus	
		High	Low
High		104.0	85.6
Low		97.3	106.4
	Energy	P = .14	
	Interaction	P = .008	

Fig. 6.--Mean serum alanine aminotransferase (U/l) for the first 12 weeks of lactation.


Serum Alanine
Aminotransferase
(U/l)
28
30
32
34
36
38
40
42
2
4
6
8
10
12
Weeks of Lactation
r = 0.881
(P < .001)
High and Low E
High and Low P

groups had a lower mean aspartate aminotransferase level (94.8 U/l) than the low energy groups (101.9 U/l).

Creatine Phosphokinase

There were no differences in creatine phosphokinase either between energy groups or between balanced and imbalanced groups. The differences between phosphorus groups did approach significance however ($P = .15$), the high phosphorus groups having higher mean creatine phosphokinase concentrations (65.5 U/l) than the low phosphorus groups (47.0 U/l). There was considerable variation in creatine phosphokinase values but generally the case holds with more phosphorus, more creatine phosphokinase (Table 12).

Table 12.--Mean serum creatine phosphokinase levels (U/l) for the first 12 weeks of lactation.

Energy	Phosphorus High	Phosphorus Low
High	65.8	51.0
Low	65.3	43.0
Phosphorus	P = .15	

Insulin

The low energy low phosphorus group was the only group to show a significant ($r = 0.44$, $P < .01$) increase in insulin with time. At two weeks the mean serum insulin was 3.2 μU/ml, peaking at 15.7 μU/ml at 10 weeks. The insulin concentration in the three other groups were not significantly related to time and were also on the average more than the low energy low phosphorus group (Figure 7).

The high energy groups had significantly higher ($P < .0005$) mean insulin concentrations than the low energy groups. The means for the high and low energy groups were 16.5 and 6.0 μU/ml, respectively. The insulin concentrations of the phosphorus groups were not different. The balanced groups had higher ($P = .08$) levels than the imbalanced groups, the means were 12.9 and 9.6 μU/ml, respectively. This was due primarily to high energy increasing insulin but not as much when it was coupled with inadequate phosphorus (Table 13).

Table 13.--Mean insulin levels (μU/ml) for the first 12 weeks of lactation.

Energy		Phosphorus	
		High	Low
High		19.17	13.89
Low		5.35	6.59
	Energy	$P < .0005$	
	Phosphorus	$P = .27$	
	Interaction	$P = .08$	

Glucose:insulin ratios and insulin:glucose ratios were also calculated and analyzed. It was found that energy significantly affected both ratios and in addition the interaction term was significant for the mean insulin:glucose ratio (Tables 14 and 15).

Fig. 7.--Mean serum insulin (μU/ml) for the first 12 weeks of lactation.

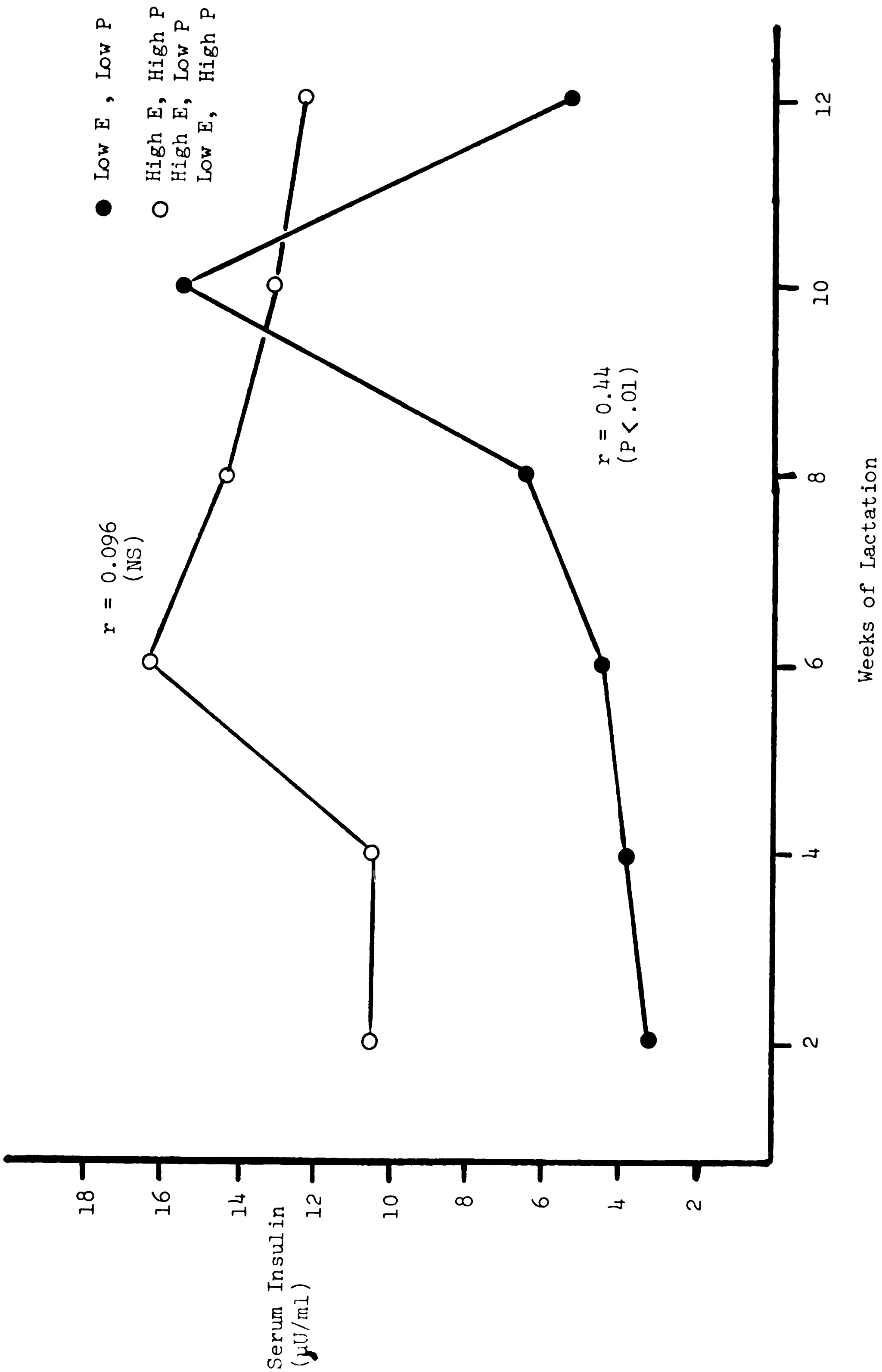

Low E , Low P
High E, High P
High E, Low P
Low E, High P
r = 0.096
(NS)
r = 0.44
(P < .01)
Serum Insulin
(μU/ml)
18
16
14
12
10
8
6
4
2
2
4
6
8
10
12
Weeks of Lactation

Table 14.--Means of glucose:insulin ratio for the first 12 weeks of lactation.

Energy		Phosphorus High	Phosphorus Low
High		14.4	16.4
Low		25.5	23.6
	Energy	P = .001	

Table 15.--Means of insulin:glucose ratio for the first 12 weeks of lactation.

Energy		Phosphorus High	Phosphorus Low
High		0.24	0.18
Low		0.08	0.10
	Energy	P < .0005	
	Interaction	P = .09	

Finally, a multiple regression analysis was done using milk as the dependent variable and glucose and insulin as the independent variables. It was found that milk yield varied more directly with glucose than with insulin (Table 16).

Table 16.--Standard partial regression coefficients for milk yield, glucose and insulin.

Dependent Variable	Glucose	Insulin
Milk	-0.15	-0.11
	(P = .08)	(P = .20)

Non Esterified Fatty Acids (NEFA)

It was found from the analysis of variance and the regression analysis that NEFA's were significantly and negatively related to energy and phosphorus status of the cows. In addition, the regression analysis showed a significant and positive relationship between NEFA's and the interaction of the two statuses. The high energy groups had significantly lower (P = .03) NEFA levels than the low energy groups (Table 17). The high phosphorus groups also had significantly lower (P = .03) NEFA levels than the low phosphorus groups.

It appears that the significance of the interaction term in the regression analysis is due to the fact that when going from low to high energy and phosphorus that the depression in NEFA levels is not quite as much as would be expected from examining the effects of going from high to low energy or phosphorus singly. That is, the effects of energy or phosphorus status are not absolutely additive.

Table 17.--Mean NEFA levels (nmoles/ml) for the first 12 weeks of lactation.

Energy		Phosphorus	
		High	Low
High		208.3	234.4
Low		235.3	282.5
	Energy	P = .03	
	Phosphorus	P = .03	

NEFA's decreased significantly (r = -0.21, P = .01) as lactation progressed. This effect was mainly due to the low phosphorus groups. All groups, except the high energy, high phosphorus group, displayed a sharp drop in NEFA concentrations from the second to the fourth week. There was an unexplained rise in all groups between the fourth and sixth week.

Reproduction Data

The reproduction data was examined in a number of ways. Basically, serum progesterone levels were used in conjunction with rectal palpation data to establish occurrence of estrus, ovulation and normal cycles. The number of days to first ovulation (it was not in all cases detected as an overt estrus), and the number of days postpartum to reach a level of 3 ng/ml progesterone were calculated. The time it took to reach 3 ng/ml was measured because this level is considered by some physiologists to be the amount of progesterone needed in the peak of the previous cycle in order that the next estrus will be one that is ovulatory, capable of conception and capable of maintenance of pregnancy (Folman et al., 1973 and Corah et al., 1974). This measurement will give us, hopefully, an independent measure of the reproductive capabilities of the cow.

The imbalanced groups of heifers took on the average 10 days longer (P = .12) to reach a progesterone level of 3 ng/ml than did the balanced groups (Table 18). The main effects of energy and phosphorus status did not significantly affect time to achieve this level of progesterone. The times to first ovulation are shown in Table 19. These times show essentially the same pattern as the progesterone levels. The imbalanced heifers took approximately a week longer to ovulate for the first time postpartum (P = .27). It should be noted that the heifers generally ovulated once before their progesterone levels rose to or surpassed 3 ng/ml. The main effects of energy and phosphorus status had no significant effect on time to first ovulation.

In considering the analysis of interval to second, third and fourth estrous cycles it was decided to analyze the data with the cycles in which cystic ovaries occurred included and excluded. This decision was made because the abnormal length of cycles with ovarian cysts can change the length or interval to estrus in the group or groups in which they occur. All cystic ovaries recovered spontaneously, no treatment was given to the cows that were diagnosed cystic. There was no significant difference in the occurrence of cystic ovaries among groups.

The estrous cycles which occurred within the first 13 weeks were examined. This included all cycles within the 12 week experimental period and those that were already two-thirds over by the end

Table 18.--Number of days postpartum for serum progesterone to reach 3 ng/ml.

Energy		Phosphorus	
		High	Low
High		28.7	44.5
Low		36.8	33.0
	Interaction	P = .12	

Table 19.--Number of days postpartum to first ovulation.

Energy		Phosphorus	
		High	Low
High		19.2	26.7
Low		27.7	22.3
	Interaction	P = .27	

of that period. Next, the estrous cycles occurring in the total 15 weeks were examined. This included the period when all the heifers were consuming the standard herd ration. This was to determine if there was any carry over effect of the diet. The mean values for interval to second, third and fourth estrus are shown in Appendix Table 2. Interval to second estrus averaged 18.5 days in the high phosphorus groups as opposed to 20.2 for the low phosphorus groups (P = .25). High energy lengthened the interval to third estrus (P = .22) when cystic cycles were included, means were 22.6 and 20.4 days for high and low energy groups. When the cystic cycles were excluded the interaction of energy and phosphorus status was more apparent. The balanced ration groups showed a trend of having shorter (P = .25) cycles, the means were 20.7 and 22.0 days for balanced and imbalanced groups. Energy or phosphorus balance did not affect interval to fourth estrus.

On examining the cycles occurring in 15 weeks (Appendix Table 3) the results for interval to second and third estrus were the same as for the 13 week analysis. Interval to fourth estrus showed significant effects of both energy and phosphorus status. High energy lengthened (P = .06) cycles. The high energy group had a mean interval of 23.9 days compared to 21.3 days for the low energy group. High phosphorus, on the other hand, shortened (P = .10) cycle length. The means were 21.5 and 23.7 days for high and low phosphorus groups respectively. There did appear to be some carry over effects of the diets on reproductive efficiency.

Basically, the same results were found when the mean overall interestrual interval was considered. These values are shown in Table 20. Overall interestrual interval in the 15 week period including cycles with cystic ovaries showed again excess energy lengthened ($P = .08$) cycle length. The means were 21.8 and 20.2 days for high and low energy groups, respectively. This tendency was also present when the cycles with cysts were included ($P = .26$). When cycles with cysts were taken out, the shortening effects of phosphorus on estrous cycle length were noted ($P = .18$). The means were 20.4 and 21.5 days for high and low phosphorus.

Overall interestrual interval in 13 weeks including cycles with cysts showed energy lengthened cycles ($P = .15$) with means of 21.3 and 19.9 days for high and low energy groups, respectively. When the cycles with cysts were excluded from the analysis, the significant effects were not present but the tendency was for high energy to lengthen and for high phosphorus to shorten estrous cycle length.

The overall tendency is for excess energy and excess phosphorus to lengthen and shorten estrous cycle length, respectively.

The number of undetected heats, cystic follicles, number of days open and number of services per conception were also recorded (Table 21). The low energy, high phosphorus group had the least undetected heats (9 out of 22, or 40.9%) while the low energy, low phosphorus group had the most (14 out of 23, or 60.8%). The imbalanced groups had fewer ($P = .14$) undetected heats than the balanced groups, a mean of 40.6%, as opposed to 56.4% for the

Table 20.--Mean overall interestrual interval.

	HEHP	HELP	LEHP	LELP
15 weeks including cystics[a]	21.4 (17)*	22.1 (15)	19.9 (16)	20.4 (17)
15 weeks excluding cystics[b]	20.6 (16)	22.1 (15)	20.1 (15)	20.8 (16)
13 weeks including cystics[c]	21.2 (16)	21.3 (13)	19.7 (15)	20.0 (15)
13 weeks including cystics[d]	20.3 (15)	21.3 (13)	19.9 (14)	20.4 (14)

*Figures in parentheses represent n.

[a]Energy P = 0.08 (21.8, 20.2, HE lengthens).
Phosphorus P = 0.26.

[b]Energy P = 0.26.
Phosphorus P = 0.18 (20.4, 21.5, HP shortens).

[c]Energy P = 0.15 (21.3, 19.9, HE lengthens).

[d]Energy P = 0.46.
Phosphorus P = 0.35.

Cows were excluded from groups on the basis of cystic follicles, no estrus or no estrus in 13 weeks but final one in 15 weeks.

Table 21.--Treatment means for number of undetected heats, cystic follicles, days non-pregnant and services per conception.

	Percent Undetected Heats	Percent Cystic Follicles	Days Non-Pregnant	Services per Conception
High E High P	52	4.3	160.4*	3.6
High E Low P	40.3	4.5	113.2*	2.0
Low E High P	40.9	4.5	120.6**	3.0
Low E Low P	60.8	4.3	130.0	2.3

*One cow sold open after 3 unsuccessful breedings, n = 5.

**One cow sold open after at least 7 breedings and 1 abortion, n = 5.

balanced groups. Thus, the imbalanced groups took longer to reach 3 ng/ml of progesterone, took longer to ovulate but had a lower incidence of undetected heats.

There was no difference in incidence of cystic ovaries among groups. The high energy, high phosphorus group was non-pregnant longer than any of the other three groups (160.4 days). The high energy, low phosphorus group was non-pregnant the shortest time (113.3 days). The balanced groups were non-pregnant longer (P = .14) than the balanced groups, the means were 145.2 and 117.0 days, respectively. So even though the imbalanced groups took longer to ovulate for the first time after parturition, on the average they were pregnant 28 days before the balanced groups.

Services per conception appeared to be influenced by dietary phosphorus. The high phosphorus groups needed more services per conception (P = .08) than the low phosphorus groups. The means were 3.3 and 2.2 for high and low phosphorus, respectively.

Multiple Regression and Simple Correlation Analysis

Because of the difficulty of maintaining the energy and phosphorus status at the desired level with rapidly changing feed intakes and milk production, it was decided that the data should be additionally analyzed by multiple regression. This regression analysis generated standard partial regression coefficients, shown in Table 22. Generally, the regression analysis is supportive to the basic analysis of variance and perhaps provided a fuller description of the relationship than the analysis of variance. These effects and how they link together will be examined in the discussion of results. In addition to the regression analysis, a simple correlation matrix was generated, some of these are presented in Table 23. Other correlations were found to be significant but are explained or represented through the analysis of variance and the regression analysis and are not shown here.

Health

Records were kept of the incidence of postpartum health problems such as retained placenta, metritis, mastitis and foot rot. A scoring system of one point was used for each recorded incidence of disease. In the case of mastitis another point was scored for each incidence more than three days from the last record of mastitis. Thus, a score for each group was established. The mean score for the cows is shown in Table 24.

Table 22.--Standard partial regression coefficients (P < .07).

Dependent Variable	Energy Status	Phosphorus Status	E X P
Milk Yield	0.27	0.35	NS
Milk Fat (%)	-0.56	NS	NS
Milk Protein (%)	0.40	0.25	NS
Body Weight Change	0.54	NS	NS
Serum:			
Ca	0.25	-0.48	0.28
P	-0.33	0.59	-0.25
Creatine phosphokinase	NS	0.43	NS
Alkaline phosphatase	NS	NS	0.41
Aspartate aminotransferase	-0.53	NS	0.33
Urea nitrogen	-0.78	-0.24	NS
Glucose	0.42	NS	0.20
Insulin	0.50	NS	NS
NEFA	-0.38	-0.29	0.33

Table 23.--Simple correlations ($P < .05 = 0.159$, $n = 144$).

	r
Energy status x phosphorus status	0.63
Milk yield x alkaline phosphatase	-0.35
Milk fat (%) x alkaline phosphatase	0.22
Milk yield x insulin	-0.17
Glucose x insulin	0.35
Glucose x alkaline phosphatase	0.20
NEFA x Insulin	-0.25

Table 24.--Mean incidence of disease for the first 12 weeks postpartum.

Energy	Phosphorus	
	High	Low
High	2.2	3.2
Low	1.5	1.2
Energy	$P = .19$	

The high energy groups had a higher (P = .19) incidence of ill health, (2.7 against 1.4) than the low energy groups. There was no difference between the phosphorus groups or the balanced and imbalanced groups. Mastitis and metritis accounted for most of the ill health in all groups. Metritis accounted for 25% of the incidence of ill health in the high energy groups. Mastitis accounted for 50% of the total score in the high energy groups and 44% in the low energy groups. Other problems such as retained placenta, elevated temperatures and foot rot accounted for 25% of the ill health in the high energy groups and only 6% in the low energy groups.

The high energy groups then, in terms of actual numbers, had a higher incidence of mastitis and other health problems than the low energy groups. The occurrence of metritis was approximately the same as in the low energy groups.

Post Experimental Responses in Energy and Phosphorus Status, Milk Yield and Serum Parameters

Energy and Phosphorus Status

All heifers were in positive energy status following the return to a normal herd ration (Table 25). The overall mean was 7.7 mcal/day. There was little difference between groups. All heifers were in positive phosphorus status, the mean being 86.2 g/day.

Milk Production

The overall mean for milk production during this post experimental period was 20.6 kg/day. The group which had previously been

Table 25.--Mean energy and phosphorus status and milk production for the two week period immediately following the end of the experimental treatments.

	High Energy		Low Energy		Overall Mean
	High P	Low P	High P	Low P	
Energy status (Mcal/day)	6.60	8.39	7.83	8.12	7.74
Phosphorus status (g/day)	83.91	103.25	75.39	82.30	86.21
Milk production (kg/day)	20.46	24.72	20.84	20.40	21.60

the high energy low phosphorus group had the highest yield (24.7 kg/day).

Feed Intake

Mean intakes are shown in Table 26. The heifers were fed a ration consisting of grain, hay, haylage and corn silage. Some of the heifers assigned later in the experiment received no corn silage because none was available. One heifer received only grain and corn silage in this period.

Body Weight Change

In the 3 weeks following the treatment period in which all heifers were fed a standard herd ration, 14 of 24 heifers gained weight, five maintained weight and five lost weight. Breaking this down by the groups to which they had previously been assigned, in the high energy high phosphorus group, four of six gained weight and two lost weight. In the high energy low phosphorus group, two lost,

Table 26.--Mean intakes (kg/day) of grain, hay, haylage and corn silage for the two week period immediately following the end of the experimental treatments.

Number of Cows	Grain*	Hay	Haylage	Corn Silage
15	8.21	3.71	6.47	16.13
8	8.32	4.50	12.06	---
1	8.59	--	--	23.30

*Grain fed was the normal herd grain described in Table 2.
(Intakes expressed on an as fed basis).

two gained and two maintained weight. Three gained and three maintained weight on the low energy high phosphorus group. It was found that five gained weight and one lost weight in the low energy low phosphorus group.

The designation of energy and phosphorus groups is used only to show how these groups responded to being changed from the experimental diets, all cows in this period were fed the same normal herd ration.

Serum Calcium

In the three week post-experimental period, serum calcium either stayed the same or declined. The high energy, high phosphorus group at week 12, the last week of the treatment period, had a mean calcium of 9.2 mg/dl, at week 15 the level was 9.3 mg/dl. The other three groups showed a decline in calcium concentration, the high energy low phosphorus group falling from 9.6 mg/dl at week 12 to 9.2 mg/dl at week 15. The low energy, high phosphorus group fell from 8.8 to 8.5 mg/dl and the low energy low phosphorus group from 9.4 to 9.1 mg/dl.

Serum Phosphorus

In the low phosphorus group, serum phosphorus markedly increased within two weeks of the end of the treatment period. The high energy, high phosphorus group had a mean serum phosphorus of 6.3 mg/dl at week 12, rising to 7.2 mg/dl at week 14 and falling again to 6.5 mg/dl at week 15. The high energy, low phosphorus group rose from 7.0 mg/dl to 8.3 mg/dl one week after the end of treatment, falling to 7.3 mg/dl by week 15. The low energy low phosphorus group went from 7.2 to 8.4 mg/dl within a week, falling to 7.1 mg/dl by week 15. The low energy high phosphorus group did not follow this pattern, starting at 7.6 mg/dl then rose to 8.0 mg/dl by week 15 after a slight decline in levels after being placed on a standard herd ration.

Creatinine

There was very little change in creatinine levels after the treatment period ended, except that the creatinine concentration in high phosphorus groups increased at week 14, falling again by week 15.

Urea Nitrogen

The high energy groups had an increase in urea nitrogen levels in the post experimental period. The low energy groups declined considerably, the low energy high phosphorus group going from 16.7 mg/dl at week 12 to 12.7 mg/dl at week 13 and the low energy low phosphorus group from 17.1 to 14.7 mg/dl. By week 15 the high energy groups had a mean level of 12.5 mg/dl and the low energy groups had a mean of 12.7 mg/dl.

Total Serum Cholesterol

Cholesterol levels in all groups continued to rise through week 15. At week 12 all groups were below 200 mg/dl cholesterol, by week 14 all groups were over 200 mg/dl, reaching an average concentration, for all groups, of 229.9 mg/dl at week 15.

Glucose

Glucose decreased in all groups following the end of the treatment period. By week 15 concentrations in all groups were back to approximately the same levels as week 12. At week 12 the high energy groups had a mean of 77.0 mg/dl, the low energy groups, 70.7 mg/dl. By week 15 this had changed to 73.3 mg/dl for the high energy groups and 71.0 mg/dl for the low energy groups.

Alkaline Phosphatase

The activities in the high energy low phosphorus group dropped from 14.0 U/l at week 12 to 9.0 U/l at week 15. In the high energy, low phosphorus and low energy, low phosphorus groups activities rose somewhat and by week 15 were declining again, below the original week 12 value. The levels in the low energy, high phosphorus group were still rising at week 15.

Alanine Aminotransferase

Activity of alanine aminotransferase in all groups continued to rise through week 15. At week 12 the mean for all four groups was 42.5 U/l rising to a mean of 48.6 U/l by week 15.

Aspartate Aminotransferase

Levels of aspartate aminotransferase in all groups continued to rise through week 15. As in the treatment period the groups which had been imbalanced had lower levels than the balanced groups. The means at week 12 for the balanced and imbalanced groups were 98.3 U/l and 85.7 U/l respectively. By week 15 the means were 101.4 U/l and 94.5 U/l.

Creatine Phosphokinase

By week 13 activity of creatine phosphokinase in all groups had risen. Subsequently, activity had begun to decline in all groups except the low energy, high phosphorus group by week 15. Activities in the low energy, high phosphorus group were very high at week 15.

Insulin

Both of the high energy groups showed a decline in serum insulin after being removed from the experimental diet. The low energy groups both increased in insulin levels. These values are shown in Table 27.

NEFA

After the end of the treatment period concentrations of NEFA's in all groups decreased slightly and the groups tended to come together

Table 27.--Mean insulin levels (μU/ml) for the two week period following the end of treatments.

Group	Mean Insulin (μU/ml) Levels Week 12	Week 14
HEHP	18.7	11.7
HELP	14.4	9.0
LEHP	2.0	13.5
LELP	5.4	8.9

in NEFA concentration by week 14. The animals apparently adapted back to a normal diet and their levels were stabilizing.

DISCUSSION

The results of this experiment show postpartum energy and phosphorus intake affects certain serum metabolites and hormones. There does appear to be a relationship between energy and phosphorus status and postpartum reproductive function also. At this point it might be advantageous to remind the reader that when discussing the effects of positive status what we are examining is the result of excess nutrient over the requirement for maintenance and milk production. Thus, when the terms high energy or high phosphorus are used in the context of this study this means an excess supply of energy or phosphorus over the requirements of the animals concerned. Conversely, low energy or low phosphorus indicates a deficiency in basic requirements. This is to be distinguished from the amount of nutrient offered to a group of animals.

Production of milk is obviously of prime importance and the response in milk yield to energy and phosphorus intake was surprising. The heifers supplied with excess energy in this study had a lower average milk yield than heifers deficient in energy. This was surprising in view of the logical, highly positive relationship between milk yield and energy status shown by the regression analysis which would indicate that the more positive the energy or phosphorus status the more milk will be produced. The low average was primarily due to the high energy, high phosphorus group whose mean milk yield was the lowest of the four groups. On examining the milk yield data as a function of time, the high energy group started their lactation with a lower milk yield than the low energy group and indeed took about six weeks to reach the same level of production. After this time the high energy cows did produce more milk, accounting for the positive relationship observed between energy and milk yield. The possibility exists that this observation may be a random one, the cows in the high energy group may have initially produced less purely by chance. However, high energy rations in the early postpartum period might actually depress or suppress milk yield potential. Certainly it would seem advantageous to examine the milk yield curves of the heifers in this experiment. Perhaps heifers should be fed a ration low in energy immediately after calving, gradually building up their energy intake to a high level in the first few weeks of lactation. Support is added to this when the trial conducted by Thomas, Emery and Brown (1974) is examined. It was found that cows fed limited grain for the first 45 days postpartum and fed grain *ad libitum* from day 46 to 180 gave the greatest amount of milk, fat-corrected milk, fat and solids-not-fat when compared with cows fed *ad libitum* from parturition. Alternatively, the response in milk yield could conceivably be due to postpartum disease causing the observed lower production since in addition to lower milk yield, animals fed in excess of their energy requirements had a higher incidence of ill health (mainly mastitis and metritis), when compared with cows deficient in energy.

These responses are not conclusive at this time but warrant further investigation.

Previous studies have linked high grain feeding prepartum to an increased incidence of milk fever and mastitis postpartum. No effect, however, was shown on metritis, retained placenta or indigestion (Emery, Hafs, Armstrong and Snyder, 1969). In contrast, another study found no relationship between liberal postpartum grain feeding and the incidence of mastitis, milk fever and ketosis but digestive disturbance and abomasal displacements were significantly increased (Trimberger, Tyrrell, Morrow, Reid, Wright, Shipe, Merrill Loosli, Coppock, Moore and Gordon, 1972). High levels of grain feeding had no significant relation to the incidence of diseases such as mastitis, metritis, ketosis and indigestion (Armstrong, Brown, Thomas and Getty, 1966). There is evidence to support the hypothesis that excess energy intake under certain conditions can cause health problems, which health problems and why is not clear.

Several parameters were reasonably good indicators of energy status. Certain of these could be used as estimators of energy status in postpartum cows. Body weight change, serum urea nitrogen, glucose, NEFA's and insulin were all good estimators of energy status. Some of these parameters, however, have inherent problems in interpretation under certain conditions.

Body weight change followed energy status. By the regression analysis, energy status was the only significant influence on weight change. It is logical that the more energy there is in the system the more is left after maintenance and milk production needs have been removed for synthesis of body tissue. As expected, cows offered excess energy gained more weight than cows deficient in energy. Cows offered both energy and phosphorus in excess gained the most weight. This group gave the least milk (Figure 3, Table 25), indicating a partitioning of energy to body tissue. This is not an ideal situation since heifers do not need to be gaining much weight in this period, certainly not at the expense of expressing their potential for milk production.

Weight change seems to be a reasonable measure of energy status. Increasing weight would generally indicate positive energy status. The failure, however, to find a perfect correlation must be due to changing body composition and to variation in efficiencies of nutrient utilization. Certainly, at this time use of this single measurement cannot be relied upon as an estimator of energy status.

Serum urea nitrogen was found to be a good estimator of energy status. It was negatively related to energy and phosphorus status but more strongly to energy. The more positive the energy status, the less serum urea nitrogen was present. Cows fed excess energy had significantly lower urea nitrogen levels than those fed deficient energy rations. This is logical since urea nitrogen is produced from ammonia which is derived from deamination of amino acids and cows in negative energy status would be mobilizing body tissue, including muscle.

This relationship only holds however when cows consuming rations equivalent in crude protein content are considered, hence use of urea nitrogen as an indicator of energy status is limited if diets are not equal in protein content. Increases in plasma urea nitrogen levels have been shown to be related to urea and crude protein level in the diet (Van Horn, Jacobson and Graden, 1969). Van Horn and Jacobson (1971) showed that plasma urea nitrogen levels were 6.8, 11.4 and 14.6 mg/dl on a basal, 11.4% CP, diet, a 13.3% CP soybean based diet and a 15.1% CP soybean based diet, respectively. A similar pattern was observed with an 11.4% CP basal diet, and 13.2 and 15.0% CP urea based diets. More recently, increasing serum urea nitrogen concentrations were attributed to increasing protein intake in post-partum cows fed 3 different forage diets by Belyea, Coppock and Lake (1975).

In this experiment crude protein content of the diets were approximately equivalent, thus, urea nitrogen levels expressed energy status well, but obviously its use as an indicator is limited to situations where crude protein is equivalent so that protein intake does not confound urea levels.

Serum glucose was found to be a good estimator of energy status. Cows fed excess energy had significantly higher serum glucose levels than those deficient in energy. Increasing levels of glucose have been related previously to increasing amounts of concentrates (and therefore energy) in the diet (Blair, Christensen and Manns, 1974; Jenny, Polan and Thye, 1974).

NEFA's were found to be related to energy and phosphorus status. Results indicate that a deficiency in energy intake causes NEFA levels to increase. It was also found, however, that deficient phosphorus also causes increasing NEFA levels. This effect of phosphorus status on NEFA's appears to be a new and unique observation. Thus, NEFA levels appear to be a fairly good indicator of energy status but are also confounded somewhat by phosphorus intake. This, coupled with its variability during early lactation may limit its usefulness.

NEFA's are often suggested to be a reflection of greater or lesser energy status (Belyea et al., 1975). In a study on the evaluation of certain metabolites as criteria of energy status of cows in early lactation, NEFA levels were more closely associated with energy status than were other metabolites (Erfle, Fisher and Sauer, 1974). The correlation they found between NEFA's and energy status was higher than the one observed in this experiment but their conclusions about its usefulness were similar. It was reported that NEFA's did not respond to dietary changes and did not reflect energy intake differences in a study examining low, medium and high straw diets (Blair et al., 1974). NEFA levels per se appear to have limited usefulness as an indicator of energy status. It is clear that even if glucose and NEFA's were equally sensitive as estimators, it would be more logical to measure glucose because of the precision and ease of the glucose assay.

Insulin was a very sensitive indicator of energy status. Cows fed excess energy had serum insulin concentrations three times as high as cows fed deficient energy. As the glucose:insulin ratio shows, insulin changes more per unit of energy status change than glucose does. Support is added to this result of serum insulin increasing more than serum glucose by a study showing a doubling in serum insulin when glucose levels only increased about 10 mg/dl (Blair _et al._, 1974). A similar, though not so dramatic effect was noted by Jenny _et al._ (1974).

At this point it would be advantageous to discuss the relationship between serum glucose, insulin, NEFA's and milk yield in this experiment. Excess energy did not immediately produce higher milk production, cows fed in excess of their energy requirements lagged behind cows fed below their requirements for milk production by some six weeks. Insulin and glucose, in contrast, changed more dramatically in response to excess energy, increasing in concentration almost immediately, NEFA's decreased in response to excess energy.

Glucose was positively correlated with insulin but no significant relationship was found between glucose and NEFA's. It has been reported that blood glucose is more related to acetoacetate and β-hydroxybutyrate than with NEFA's (Erfle _et al._, 1974). NEFA levels were negatively correlated to insulin levels and positively correlated with milk fat percent, no relationship, however, was indicated between NEFA's and milk yield. Insulin was negatively correlated with milk fat percent. This has previously been reported (Jenny _et al._, 1974; Walker and Elliot, 1973; Blair _et al._, 1974).

Serum insulin, in the present study, was negatively correlated to milk yield. This relationship between milk yield and endogenous insulin and glucose has previously been reported by Koprowski and Tucker (1973) and Jenny _et al._ (1974).

Other studies have been conducted which would lead one to conclude that there is a negative relationship between insulin and milk yield. Kronfeld, Mayer, Robertson and Raggi (1963) examined the short term effects of long-acting insulin injections. Blood glucose concentration and milk yield both declined. When the plasma glucose was restored to normal by administration of glucose milk yield also returned to normal. Hypoglycemia seems to be more of a direct cause of hypogalactia than is insulin. Results of a study by Schmidt (1966) tend to confirm the results of Baldwin et al. (1963). Short acting insulin injections resulted in a decrease in milk production and in blood glucose levels. Infusion of glucose with the insulin injections restored the milk yield to near normal. These data suggest that insulin does not have an effect on milk yield independent of glucose.

In contrast, long term insulin treatment did not depress milk yield in a study conducted by Baldwin, Reichl, Louis, Smith, Yang and Osborn (1973). Cows injected with insulin daily for four weeks did not experience a fall in production whereas the milk yield of the untreated controls fell 18% in the same period. This would lead one to conclude that the exogenous insulin in this study was positively related to milk yield. Unfortunately, Baldwin et al. (1973) did not measure blood glucose concentrations.

The apparent contradiction among these trials may be due to variation in energy status and serum glucose of the animals which in turn affects serum insulin concentration. Exogenous insulin may influence serum glucose quite differently than endogenous insulin. If high doses of exogenous insulin are administered then insulin levels in this case are independent of glucose control.

Glucose and insulin from the present study were negatively related, by regression analysis, to milk yield, with milk yield and glucose having a stronger relationship than milk yield and insulin (Table 16). It appears, however, that if glucose levels are held constant the effects of insulin on milk yield do not entirely disappear. This suggests that the effects of insulin on milk yield cannot be accounted for just by glucose concentration. Insulin may be influencing milk production, directly or indirectly, through other metabolites which regulate milk production as well as glucose.

In order to examine these relationships further we must make some assumptions: if we assume that milk production controls glucose levels and to some extent insulin, either directly or indirectly, it appears that in the cows supplied with excess energy, who had a lower milk yield, there is less demand for glucose at the mammary level. This, coupled with an excess in the supply of energy substrates could be the cause of rising glucose levels and this in turn could cause a response whereby insulin levels are increased also. The difference in glucose levels between groups is small compared with the difference in insulin response but of course absolute levels of serum glucose do not necessarily reflect glucose turnover. In the higher yielding animal (the low energy group heifers), glucose turnover may be greater. Certainly this, coupled with more demand for glucose at the mammary gland and less input of energy substrates would suggest that serum glucose and extraction into the mammary gland would be greater in the higher yielding animals, resulting in lower serum glucose. These conditions could explain the observed differences in the experiment and indeed it is not surprising that the milk production of the cow should have such a strong influence on glucose and insulin in view of the mammary gland's overriding metabolic demand in the early part of lactation. If the above relationships are true, it is logical that insulin and milk yield are inversely related even though it seems to be contradictory at first sight. Although NEFA's were correlated with insulin, they appeared to be independent of glucose and milk yield. Thus, where NEFA's fit into this scheme is not clear. Interpretation of the results as regards NEFA's is difficult due to the apparent lack of correlation with milk yield, which is surprising in view of the relationship of NEFA's and energy status.

In this experiment, the low energy, low phosphorus group was the only one where insulin levels significantly correlated with time. Koprowski and Tucker (1973) found a 2-3 fold increase in insulin levels between the fourth and twelfth week of lactation, thereafter the levels remained relatively stable. The insulin levels they reported were higher than were found in this trial, however, it was noted by Koprowski and Tucker that the primiparous cows in their experiment had lower mean insulin levels than the multiparous cows they examined and this could account for some of the difference.

Serum phosphorus appeared to be the only useful indicator of phosphorus status. Cows fed rations deficient in phosphorus had significantly lower serum phosphorus levels than those fed excess phosphorus. Belyea et al. (1975) also noted a significant positive correlation for plasma phosphorus with intake of phosphorus. An extreme decrease in serum phosphorus concentration is observed with severe phosphorus deficiency but it appears that marginally deficient animals show a small but significant decrease without any overt symptoms of phosphorus deficiency resulting from this.

The low energy, low phosphorus group had the lowest mean serum phosphorus concentration, 5.68 mg/dl, which at first sight would not be considered low. I submit, however, that for a three year old Holstein female this is indeed rather low according to the data of Tumbleson, Wingfield, Johnson, Campbell and Middleton (1973). These authors quoted a mean serum phosphorus level of around 7.0 mg/dl for 3 year old Holsteins. This would indicate that cows fed below their requirements for phosphorus in the present study did have low serum phosphorus levels compared to those in positive phosphorus status. Serum phosphorus levels quoted in previous experiments, however, were much lower. Eckles et al. (1935) reported levels of 2.5 mg/dl in phosphorus deficient cows. This is half of normal and "no difficulty was experienced in producing a state of phosphorus deficiency in any of the animals." This was accomplished by using a ration of prairie hay (grown on phosphorus deficient soil), and a grain mix low in phosphorus. It was calculated that cows producing 9.1 kg of milk per day were consuming about 19 g or less of phosphorus per day. Palmer et al. (1941) reported serum phosphorus values of 2.5-3.0 mg/dl with an average intake of phosphorus of about 6 g per day, these animals, however, had very low milk yields. In the field, serum values of 4.0 mg/dl were reported for yearling heifers on phosphorus deficient rations (Morrow, 1969). In the present study cows on deficient phosphorus rations were consuming 45-65 g/day, considerably more than in the previously cited studies. They were, however, producing two or four times as much milk as the cows studied by Eckles _et al_. (1935) or Palmer _et al_. (1941).

It does appear that the cows in this study were marginally rather than severely deficient in phosphorus. This provides us with information on the effects of marginal phosphorus deficiency on reproduction and blood metabolites.

Other serum metabolites and enzymes were related to energy and phosphorus intake. These relationships were interesting but were of no value at this time as indicators of energy or phosphorus status.

Serum calcium was related to energy and phosphorus in an exactly opposite manner to that of serum phosphorus. The imbalanced groups had lower serum calcium levels than the balanced groups. High phosphorus in the presence of low energy depressed serum calcium levels throughout the first 12 weeks of lactation. Low phosphorus and high energy coupled together elevated levels slightly. Serum calcium levels were actually very stable and the differences between groups were small. The physiological significance of this variation is not known. As previously shown by the data of Ward, Blosser and Adams (1952) and more recently by Belyea, Martz and Ricketts (1975) serum calcium levels rose gradually as lactation progressed. Serum calcium and phosphorus were found to be negatively correlated in the present study lending support to the results of Symonds and Manston (1974) who reported an inverse correlation between calcium and phosphorus in the range of 3.7 to 26.0 mg/dl serum phosphorus.

Cows fed excess phosphorus had higher serum cholesterol levels than those deficient in phosphorus, particularly when excess phosphorus was coupled with excess energy. This apparently is a new observation with implications far beyond the objectives of this dissertation. The most striking observation on cholesterol, however, was the correlation with time. Stage of lactation accounted for the majority of change in levels over the first 12 weeks postpartum. This phenomena has been noted by several workers (Arave, Miller and Lamb, 1975; Belyea et al., 1975; Bolenbaugh, 1975). It has been suggested that the observed increasing levels of cholesterol reflect increased lipid intake during early lactation (Belyea et al., 1975). Data from the present study, however, indicates cholesterol is independent of energy intake. Thus, it seems that cholesterol levels have no use as an indicator of energy or phosphorus status. Cholesterol is confounded with stage of lactation in the early postpartum period and this may overwhelm any effects of energy or phosphorus status. The physiological significance of this increase with time is not clear.

Several serum enzymes were measured and there was a relationship between some of these and energy and phosphorus balance. At this time, however, their measurement is of limited value in describing the effects of energy and phosphorus intake.

Cows fed excess phosphorus had higher levels of creatine phosphokinase than those fed deficient phosphorus. How CPK levels are related to phosphorus status is not clear but it does appear that excess intake of phosphorus does elevate CPK levels slightly. Whether this effect is mediated through ATP is not obvious at this time. Extremely high levels of CPK are indicative of skeletal muscle diseases, heart or brain damage (Okinaka et al., 1961). This is obviously not the case in this study. CPK levels are limited in their use, they are too variable, the normal range is wide and small differences are of doubtful physiological significance.

Aspartate aminotransferase is of little use in indicating nutrient status. Since it is so ubiquitous in animal tissues it is very difficult to interpret the physiological significance of a change in concentration. Cows fed excess energy had lower aspartate aminotransferase levels compared to those fed deficient energy. Cows fed unbalanced rations had lower levels than those fed balanced rations. It could be reasoned that animals under a metabolic strain to provide energy substrates from an endogenous source would have elevated levels of aspartate aminotransferase due to increased muscle and tissue mobilization. This could account for the difference in the high energy and low energy groups but does not explain the strong interaction effect. It would seem logical that the unbalanced cows would be under more of a strain than the balanced cows but in fact the levels in the unbalanced groups were lower.

Alkaline phosphatase was related to the interaction of energy and phosphorus status. This is not easily explained. Alkaline phosphatase as it is measured here is in fact the measurement of several alkaline phosphatases; this makes interpretation difficult. Alkaline phosphatase was negatively correlated with milk yield and glucose. Several possibilities were explored to explain this relationship but at this time it is still not clear. Normal total serum alkaline phosphatase activity varies considerably in ruminants (Allcroft and Folley, 1941). In a trial of this kind it may be of little help in understanding nutrient status.

The majority of variation in alanine aminotransferase levels were accounted for by stage of lactation. Levels increased in all cows, as lactation progressed, irrespective of treatment. This has been previously reported (Bolenbaugh, 1975). It is important when reporting alanine aminotransferase levels to note the stage of lactation. Elevated levels are indicative of liver damage, there is no reason to think liver damage occurred in this experiment. It is of little value as an indicator of nutrient status.

From observations on the cows after the experimental period had ended, values of all parameters were returning to normal in all cows by the end of the third week on a standard herd ration. The effects of excess or deficient energy and phosphorus did not seem to have long lasting results.

Evidence from this experiment suggests that energy and phosphorus status affects postpartum reproductive function. The effects of imbalanced energy and phosphorus intake were noted almost immediately after calving. Heifers fed imbalanced diets tend to experience their first postpartum estrus or ovulation later than the balanced group. This trend was also apparent when time to reach a serum progesterone level of 3 ng/ml was examined. Using the criteria of Folman _et al_. (1973) and Corah _et al_. (1974) this would suggest that it would be the second estrus in most cases which would be viable and capable of conception. Interval to first postpartum ovulation was longer in the imbalanced heifers. This would suggest that the imbalanced heifers were reproductively disadvantaged. The data, however, do not bear this out.

After experiencing first estrus, the subsequent estrus' appeared normal in most cases. There was, however, variation in cycle length and some of this was accounted for by excess energy and phosphorus intake. Excess energy tended to lengthen while excess phosphorus tended to shorten estrous cycle length. It did not appear that subsequent estrus' were as affected by energy and phosphorus imbalance as was the interval to first estrus. Nutrient status appears to affect cycle length in normally cycling heifers. Which part of the estrous cycle is lengthened or shortened is difficult to pinpoint at this time. It is also hard to establish what importance this variation in length may have but the observation is of great interest and to our knowledge has not been reported before. More animals are needed to confirm this finding.

Cows fed imbalanced rations had a lower incidence of undetected heats even though the interval to first postpartum ovulation was longer in these cows. This may partially account for the cows fed imbalanced rations becoming pregnant about 28 days before the balanced groups. If the cows fed imbalanced rations had more overt estrus' they had a better chance of being observed in estrus, bred and thus becoming pregnant earlier than the balanced groups.

Reproductive status was not detrimentally affected by imbalanced energy and phosphorus intake delaying interval to first estrus. This result could be partially attributed to management factors since perhaps not all cows were given an equal opportunity to conceive if their estrus was not detected. It should also be noted that breeding efficiency was not affected by deficient phosphorus. Cows fed excess phosphorus needed an average of one more insemination per conception compared to those deficient in phosphorus. Energy and phosphorus status did not affect the incidence of cystic follicles, these being equally distributed among all four groups.

As previously discussed in the review of literature, many theories have been postulated as to how nutrient deficiency causes infertility. Body weight loss is often cited as a cause of infertility (McClure, 1970; King, 1968). Munro (1970) as quoted by Broster (1973) and Boyd (1972) concluded that weight loss had no detrimental effects on fertility. The data from this experiment confirm the conclusions of Munro and Boyd. Cows who lost the most weight in this study did not have the lowest fertility. Weight change cannot be cited as a cause of infertility according to our data.

It has been hypothesized that high yielding cows are more susceptible to infertility (Morrow et al., 1966; Hewett, 1968). Examination of the data from the present study does not support this theory. This confirms the conclusions previously made by King (1968) and Simpson (1972). Heifers in this experiment who had the lowest average milk yield were non-pregnant longer than any other group. This result may be partially explained by the relatively small range in production observed in this experiment. The effects of extremely high production cannot be ruled out entirely.

Hypoglycemia has been linked to infertility (McClure, 1965) but the results of this trial indicate no relationship between glucose levels and fertility. Certainly, glucose levels were reduced in cows in negative energy status but this could not be linked to fertility differences. There is no support for the theory of Oxenreider and Wagner (1971) who related glucose levels to follicular development nor for the hypothesis of Howland et al. (1966) who suggested that hypoglycemia suppressed hypothalamic function thus depressing ovarian activity. It seems unlikely that the glucose levels observed in the present study could cause depression of function of any organ.

Postcalving energy intake was not directly related to fertility, in contrast to the results of Whitmore et al. (1974) and Dunn et al. (1969). In the present study imbalance of energy and phosphorus intake appeared to have a more important effect of fertility than an excess or deficiency per se. Unfortunately, the effects of imbalanced nutrient intake have not been as intensively studied as the effects of nutrient deficiency along, thus, it is difficult to compare these results with the literature. It does appear, however, that simple energy deficiency may not always cause fertility problems and that imbalance may cuase problems not yet fully examined.

Phosphorus deficiency in this experiment was not severe, no deficiency symptoms were observed. The marginality of the deficiency may account for the lack of effects of fertility and estrus caused by low phosphorus per se. Marginal phosphorus deficiency alone did not appear to directly cause fertility problems. Excess phosphorus or phosphorus imbalanced with energy intake, however, does appear to have reproductive effects. Obviously the effects of balance and imbalance should be studied further to clarify the relationship it has to reproductive function.

CONCLUSIONS

Cows supplied with excess energy started lactation with a lower average milk yield than those fed deficient energy. This effect may have been due to a sample biased toward genetically inferior cows because of the small sample size. The lower milk production of cows supplied with excess energy may also have resulted from a higher incidence of postpartum disease. Finally, excess energy, through some unknown mechanism, may have depressed milk production. Cows in positive energy status had a higher incidence of ill health than those fed deficient energy. The feeding of high energy rations in the early postpartum period may cause problems which warrant further investigation.

Body weight change, serum urea nitrogen, glucose, NEFA's and insulin were highly related to energy status. Glucose, insulin, urea nitrogen and perhaps body weight change were the best indicators and could be used in the future as estimators of energy status. Both insulin and glucose were negatively correlated to milk yield. Milk yield varied more directly with glucose than with insulin.

Serum phosphorus was found to be the only good indicator of phosphorus status. A marginal phosphorus deficiency was accomplished and this was apparently sufficient to influence metabolites and reproductive function. Certain other metabolites and enzymes, calcium, cholesterol, creatine phosphokinase, aspartate aminotransferase, alkaline phosphatase and alanine aminotransferase, were affected by energy and phosphorus intake. They were, however, not useful as indicators of energy or phosphorus status. All parameters returned to normal after cows had returned to a standard herd ration.

Energy and phosphorus intake affected reproductive function. Imbalance of energy and phosphorus intake delayed the first postpartum estrus but imbalanced heifers experienced less undetected heats and became pregnant sooner than those balanced in energy and phosphorus intake. High energy tended to lengthen, while excess phosphorus tended to shorten second and subsequent estrous cycle length. There was no treatment effect on incidence of cystic follicles. Cows in positive phosphorus status needed on average one more service per conception than those deficient in phosphorus.

These data suggest that postpartum energy and phosphorus intake affects certain serum metabolites and hormones. There is apparently a relationship between energy and phosphorus status and postpartum reproductive function. It is hoped that further study will clarify this relationship more fully.

APPENDIX

APPENDIX

Table A-1.--Composition of scintillation fluid.

Xylenes	770 ml
p-Dioxane	770 ml
Absolute ethanol	460 ml
Napthalene	160 g
2,5-Diphenyloxazole	10 g
1,4-bis(2-(4-methyl-5-phenyloxazolyl))-benzene	0.1 g

Table A-2.--Mean interval to second, third and fourth estrus in the first 13 weeks of lactation.

	Int. 2nd Estrus		Int. 3rd Estrus		Int. 4th Estrus	
	Incl. cystics[a]	Excl. cystics[a]	Incl. cystics[b]	Excl. cystics[d]	Incl. cystics[c]	Excl. cystics[c]
HEHP	18.8 (6)*	18.8 (6)	23.2 (6)	20.8 (5)	21.8 (4)	21.8 (4)
HELP	20.5 (6)	20.5 (6)	22.0 (6)	22.0 (6)	22.0 (1)	22.0 (1)
LEHP	18.2 (6)	18.2 (6)	21.2 (5)	22.0 (4)	20.9 (4)	20.9 (4)
LELP	19.8 (6)	19.8 (6)	19.5 (6)	20.6 (5)	20.7 (3)	20.7 (3)

*Figures in parentheses represent n.

[a]Phosphorus P = 0.25.

[b]Energy P = 0.22.
Phosphorus P = 0.43.

[c]NS

[d]E. X P P = 0.25.

Cows were excluded from groups on the basis of cystic follicles, no estrus or no 3 or 4th estrus within 13 weeks.

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