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POSTPARTUM REPRODUCTIVE AND LACTATIONAL PERFORMANCE OF COWS RELATIVE TO ENERGY, PHOSPHORUS AND HORMONAL STATUS

By

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ABSTRACT

POSTPARTUM REPRODUCTIVE AND LACTATIONAL PERFORMANCE OF COWS RELATIVE TO ENERGY, PHOSPHORUS AND HORMONAL STATUS

By

Jane Allison Carstairs

To determine if energy and phosphorus status would affect postpartum health, lactational and reproductive performance, dairy cows were assigned at parturition, six per group, to: (1) high energy, high phosphorus; (2) high energy, low phosphorus; (3) low energy, high phosphorus; (4) low energy, low phosphorus. Energy status (MCal/day) for high and low energy groups was 8.4 and -3.7, ($P = .001$), representing 135 and 85% of requirements. Phosphorus status (g/day) for high and low phosphorus groups was 20.6 and -1.9, ($P = .001$) or 138 and 98% of requirements. Treatment extended through 84 days postpartum after which standard rations were fed. Heifers were bled twice weekly. Reproductive status was monitored via serum progesterone palpation per rectum. The entire experiment was replicated. Milk yields were not statistically different until week 5 of lactation. From weeks 5-12, low phosphorus groups yielded 1.8 kg/day more milk than high phosphorus groups ($P \leq .03$). Energy did not affect milk yield until week 9 when persistency of low energy groups declined. Overall means for milk yield (kg/day) for high and low energy were 23.0 and 22.0, for high and low phosphorus 21.7 and 23.2. Low phosphorus groups produced 816 kg more milk ($P = .01$) in 305 days than high phosphorus

groups while energy groups did not differ in total yield. High energy groups had almost twice as much (P = .15) disease and higher (P = .006) rectal temperatures than low energy groups. Means were 38.9 and 38.7 C. All heifers had temperatures elevated above normal during the first month of lactation.

Body weight, serum glucose, insulin and propionate increased with energy status. Weight changes (kg/week) for high and low energy groups were 0.54 and -1.49, (P = .03). In trial 1 high energy groups had more (P = .06) serum glucose (mg/dl) than low energy groups, 75.5 versus 71.6. In trial 2, glucose values were approximately 14 mg/dl lower than in trial 1, 60.4 and 58.8 (P = .025) for high and low energy groups. Glucose for high and low phosphorus groups were also 60.4 and 58.8 (mg/dl) (P = .026). In trial 1, insulin concentrations were 3-4 fold more (P < .0005) in high energy than low energy groups, 16.5 versus 6.0 (μU/ml). In trial 2, values were lower but still different, 7.3 versus 5.7 (P = .001). Serum propionate increased (P = .001) with energy, 23.9 versus 15.3 μM for high and low energy groups. By 9 weeks of lactation serum growth hormone (ng/ml) was higher ($P \leq .02$) in low energy than high energy groups, 4.8 versus 3.5 for weeks 9-12. Growth hormone in high energy groups declined as lactation advanced, in low energy groups it did not change.

Serum acetate (over mean = 736.4 μM) was not different among groups. Serum butyrate and isobutyrate varied inconsistently with energy and phosphorus status.

Phosphatemia in low phosphorus groups indicated marginal phosphorus deficiency. In trial 1, high phosphorus groups had more (P = .02) serum phosphorus than low phosphorus groups, 7.1 versus 6.0 mg/dl. In trial 2, high phosphorus groups again had higher (P = .0006) serum phosphorus, 5.6

versus 5.2, but high energy groups exceeded low energy groups in serum phosphorus, 5.5 versus 5.3 ($P < .0005$). Serum calcium was inversely related to serum phosphorus, means for high and low phosphorus groups for serum calcium were 9.5 and 9.7 mg/dl ($P = .001$).

Regression analysis indicated that physiological concentrations of growth hormone, propionate, butyrate and isobutyrate varied with serum insulin ($P < .005$, $R^2 = 0.26$).

Reproductive function was almost identical among energy and phosphorus groups. No heifer with peak progesterone prior to insemination below 2.7 ng/ml conceived to that insemination. Days to reach 3 ng/ml progesterone were correlated with serum phosphorus, glucose ($r = -0.34, -0.33$, $P = .05$) and growth hormone ($r = 0.51$, $P = .05$).

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INTRODUCTION

This study was conducted to determine if energy or phosphorus status would affect postpartum performance of dairy cows. Performance was considered not only as milk production but health and reproduction also since all of these parameters are part of a successful lactation.

In order to accomplish this, performance was recorded for the first 3 months postpartum in Holstein heifers subjected to widely different intakes of energy and phosphorus. This early postpartum period is critical since lactational and reproductive performance during this time affects the total and subsequent lactations. Energy and phosphorus intake for each animal was calculated relative to published recommendations and performance was related to this calculated status. A number of metabolic parameters were measured to determine if they were correlated to energy or phosphorus status and attempts were also made to correlate these parameters with differences in performance of cows.

Efficiency of production is a primary goal of the livestock industry. If excess nutrients have detrimental or no beneficial effect on performance this has practical implications. Feed wastage is not economically or conservationally sound. Marginal deficiencies should also be evaluated for effects on dairy cow performance. The nutritional requirements of the postpartum cow are controversial. The aim of this research was to define requirements for early lactation more accurately and to relate these requirements to the physiology and endocrinology of the cow.

LITERATURE REVIEW

In order to assess the metabolic needs of the dairy cow in the postpartum period it is important to understand the changes that occur at this time. This literature review will first describe the changes in certain hormones that occur after calving and through lactation. Then, changes in certain metabolites will be discussed.

Next, the review will describe the changes in reproductive status which occur after calving and how energy or phosphorus intake might affect and be related to this.

Finally, the interactions between hormones and metabolites will be discussed within the context of how nutrient utilization might be controlled on a day to day basis by the lactating cow.

Hopefully, this review will form the basis for detailed discussion of the experimental data to follow.

Temporal and Non-Dietary Changes in Certain Hormones and Metabolites

This section will review the changes which occur in insulin, glucagon, growth hormone, glucocorticoids and prolactin in periparturient and postpartum cows. These hormones are closely involved with control of metabolism and utilization of nutrients. If we can understand how, and perhaps why, these hormones change in postpartum cows we can manipulate these hormones (perhaps by dietary means) so that nutrients might be more efficiently used at peak lactation. An understanding of these

hormonal signals might also enable us to control metabolic disorders where nutrient utilization is impaired.

Next, the changes in glucose, non-esterified fatty acids, calcium, phosphorus, magnesium and volatile fatty acids will be described. It is important to know whether serum values are indicative of dietary intake of a substance, what we can predict from examination of concentrations of serum metabolites and whether these have direct application to nutritional problems or lactational performance. Only when the normal is established can deviations from this be discussed and solutions arrived at.

Insulin

Plasma insulin in ruminants, as measured by radioimmunoassay, varies between 5 and 10 μU/ml, with basal concentrations of 10-59, 15-29 and 40 μU/ml for sheep, cattle and goats, respectively (Trenkle, 1972). Doubtless these ranges reflect no only inter-laboratory and time of sampling differences but also physiological and nutritional factors.

In normal cows there is no marked elevation in insulin concentrations at parturition (Schwalm and Schultz, 1976). Insulin concentrations do, however, increase with weeks after parturition (Koprowski and Tucker, 1973a; Smith et al., 1976; Gorewit, et al., 1977). Reported increases vary from 30% in the first 56 days of lactation (Smith et al., 1976), 30% increase in the first four months postpartum (Gorewit et al., 1977) and a more dramatic 2-3 fold increase between the fourth and twelfth weeks of lactation (Koprowski and Tucker, 1973a). Thereafter, insulin remains relatively stable. Differences in reported absolute values of insulin may, in part, be due to sampling time relative to feeding.

Eating causes release of insulin in sheep, cattle and other ruminants, consequently, time of sampling can influence values. Postprandial insulin response will be discussed later in this review.

Thus, insulin concentrations increase in early lactation, along with milk production and feed intake. Indeed, in early lactation, serum insulin was positively correlated with nutrient intake (either dry matter intake or estimated net energy intake) (Smith et al., 1976) and parallelled increasing serum glucose (Gorewit et al., 1977). In support of these observations that insulin reflects changing nutritional (energy) status, insulin concentrations were elevated almost 20% by an ad libitum grain-restricted roughage diet (Walker and Elliot, 1973), insulin concentrations doubled when straw was reduced from 47.5 to 17.5% of the diet and replaced by grain (Blair et al., 1974) and there was almost a three-fold difference in insulin concentrations between heifers fed more than 100% versus heifers fed 75% of their N.R.C. energy requirements (Carstairs, 1975). It should be noted that none of the above studies involved isocaloric diets. Not only does insulin seem to reflect energy level of the diet, however, it also may indicate the amount of roughage in isocaloric diets (Evans et al., 1975). Insulin concentrations were 20% higher in the low roughage group cows compared with the high roughage group. The insulin may have increased in response to increased propionate type fermentation or to more glucose reaching the lower gut.

It is not clear whether serum insulin concentrations are the same in lactating and non-lactating cows (Schwalm and Schultz, 1976; Chang et al., 1977) or higher in the latter (Hove, 1974). Multiparous cows have greater (33%) concentrations of insulin than primiparous cows (Koprowski and Tucker, 1973a). It is not clear at this time why this is

so. In addition, insulin decreases as pregnancy progresses but is not affected by sex of fetus or season of the year (Koprowski and Tucker, 1973a). Insulin is responsive to other metabolic stimuli, these will be discussed in the last section of this review.

Growth Hormone (GH)

It has been reported that serum GH concentrations gradually increased up to, and then doubled at, the time of parturition. This peak was of about 12 h duration, concentrations decreased to prepartum concentrations by about 4 days postpartum (Ingalls et al., 1973). Another study does not support these findings, reporting, on the average, no changes, although some cows did show peaks at parturition (Reynaert et al., 1976).

In contrast to insulin, GH decreases with time after parturition, and this is well documented (Koprowski and Tucker, 1973a; Smith et al., 1976; Halse et al., 1976). Smith et al. (1976) reported a 30% decrease by 41 days postpartum, Halse et al. (1976) reported a 70% decrease by 90 days. In longer term studies it has been shown that GH concentrations, if adjusted for stage of pregnancy and season of the year, linearly decrease by 50% from one to eleven months postpartum (Koprowski and Tucker, 1973a). As this suggests, GH decreases as lactation advances and increases as gestation progresses. GH is not affected by season or sex of fetus but in contrast to insulin it is not affected by cow parity either. The milking stimulus does not effect GH release (Koprowski and Tucker, 1973a).

Finally, it has been reported that GH concentrations in early lactation were negatively correlated with dry matter intake and estimated net energy intake (in contrast to insulin which was positively correlated)

(Smith et al., 1976). Further support for this idea that GH may reflect nutritional status (in the opposite way to which insulin might) is not available at the present time. Other regulators of GH release will be discussed in the last section of this review.

Glucocorticoids (GCD)

There is little doubt that GCD concentrations increase very rapidly at parturition. Concentrations may increase as much as 3, 5 or 8 times over prepartum values (Edgerton and Hafs, 1973; Convey et al., 1974; Arije et al., 1974; Paterson and Linzell, 1974).

During lactation, GCD concentrations are extremely variable. Values varied between 11 and 62 ng/ml from day 15 postpartum in a study with beef cows (Arije et al., 1974). One the average, however, it seems that GCD concentrations change little as lactation advances (Koprowski and Tucker, 1973a; Edgerton and Hafs, 1973; Convey, 1974). Not all studies support these findings, however, because Blom and Halse (1975) found the lowest GCD concentrations in cows within the first month of lactation and the highest at 2.5-3 months. Paterson and Linzell (1974) found GCD during lactation 3 times higher than in late pregnancy.

Neither gestation, season, fetal sex nor parity affect GCD concentrations but GCD is released by the milking stimulus and this release is not diminished as lactation proceeds (Koprowski and Tucker, 1973a).

At this time there is no evidence available to suggest that serum GCD concentration in any way reflects nutritional status. GCD may, however, be involved in the regulation of metabolism and this will be discussed later in this review.

Prolactin (PRL)

Serum PRL concentrations begin to increase a few days prepartum, increases 5-6 times at or just before parturition and remain elevated for some days or weeks after calving (Edgerton and Hafs, 1973; Arije et al., 1974; Convey, 1974). Beef cows perhaps have higher and more variable serum PRL concentrations in the peripaturient period (Arije et al., 1974). This may be due to the more freguent suckling stimulus applied by the calf.

PRL is dramatically affected by season (more specifically temperature and daylength) (Koprowski and Tucker, 1973b; Bourne and Tucker, 1975; Peters and Tucker, 1978). During April to September in Michigan (warmer temperatures and longer days) mean serum PRL concentrations are twice as high as from October to March (Koprowski and Tucker, 1973b). It is well documented that PRL is released in response to the milking (or suckling) stimulus. This response increases up to, and is highest at, about 8 weeks of lactation (corresponding to peak milk yield) and gradually declines as lactation advances. By 32 weeks of lactation (when milk yield was about 20 kg/day in this experiment) PRL was no longer released in response to milking (Koprowski and Tucker, 1973b).

Multiparous cows in the above experiment produced more milk and had greater PRL concentrations than primiparous cows. No pregnancy effect was noted but lactating non-pregnant cows did have greater PRL concentrations than lactating pregnant cows. There was no influence of fetal sex or stage of the estrous cycle on PRL concentrations (Koprowski and Tucker, 1973b).

Evidence for a direct effect of diet on PRL concentrations is not available at this time but its possible involvement with metabolic regulation will be discussed later.

Glucagon (GLG)

There is, unfortunately, not much information on serum GLG concentrations in cows because of the unavailability up to this time of a bovine GLG radioimmunoassay. The cow has both pancreatic and gut GLG as do the non-ruminants which have been studied (Manns, 1972). Separating these two GLG species from plasma, Manns (1972) showed that GLG like immunoreactivity (GLI) increases 3-5 fold in the initial days of lactation. This increase followed a similar course to milk production and was found to be primarily of gut rather than pancreatic origin. Concentrations remained elevated until 12 days postpartum when the experiment was terminated. It is possible, the author suggests, that the two-fold increase in grain intake with lactation might have resulted in glucose entering the small intestine thus elevating gut GLI secretion.

The role of gut GLG is not clear at this time but possibilities will be discussed in a later section of this review.

Serum GLG concentrations in monthly serum samples from dairy cows increased from 579 pg/ml at 1 month to a maximum of 693 pg/ml at 2 months, falling to 633 pg/ml at 4 months of lactation, (Gorewit et al., 1977). These data are limited and probably include both species of GLG but they are of the same order of magnitude as those reported by Manns (1972).

The role of GLG in metabolism will be discussed further. As yet there is not enough data to know whether GLG would reflect energy status.

Glucose

Serum glucose concentrations increase 56-66% at parturition and return to prepartum concentrations by 24 h postpartum (Schwalm and

Schultz, 1976; Bolenbaugh, 1977). For the first 3 or 4 months of lactation glucose concentrations increase gradually, after this time there is a decline (Blom and Halse, 1975; Smith et al., 1976; Gorewit et al., 1977). It should be noted that some studies have shown no variance of plasma glucose during pregnancy and lactation (Paterson and Linzell, 1974) and also wide variation (43-88 mg/dl) at different stages of lactation (Halse et al., 1976). No doubt these differences are due to factors such as experimental design, time and frequency of sampling and nutritional regimes.

Certainly, nutritional and metabolic status seems to influence glucose concentrations. Ketotic cows, immediately postpartum, have lower serum glucose concentrations than their non-ketotic contemporaries (Schwalm and Schultz, 1976). It has been reported that glucose concentrations were positively correlated with either dry matter intake or estimated net energy intake during the first 2 months of lactation (Smith et al., 1976), that glucose concentrations tended to increase as energy in the diet increased (Blair et al., 1974). In addition, mid-lactation cows, (150 days postpartum), showed an increase of 18 mg/dl glucose over initial values in response to grain feeding (Jenny et al., 1974) and cows fed 75 or 50% of their previous voluntary feed intake had significantly lower serum glucose concentrations than those fed 100% (Fisher et al., 1975).

Finally, there is some evidence that glucose concentrations may respond not only to energy intake in the diet but also to roughage content. It has been reported that glucose concentrations were significantly higher in cows fed a low, compared with a high, roughage diet even though these diets were isocaloric (Evans et al., 1975). Whether this

is due to increased propionate type fermentation or more carbohydrate reaching the lower gut in the low roughage fed cows is not clear.

Non-esterified Fatty Acids (NEFA)

Kronfeld (1965) reported mean jugular plasma NEFA concentrations of 286 μEq/l (14 mg/dl). The range was 46-864 μEq/l and distribution was skewed so that 88% of values were between 50 and 450 and another 11% between 450 and 659 μEq/l. This illustrates the variability of NEFA concentrations in blood.

NEFA concentrations either double (Reynaert et al., 1976) or increase as much as six times at parturition (Schwalm and Schultz, 1976). Concentrations remain elevated for 6 or 7 weeks postpartum and then begin to decline to prepartum values (Schwalm and Schultz, 1976; Chang et al., 1977). The largest NEFA concentrations usually occur at peak milk production (Bowden, 1971). The obvious conclusion from these data is that NEFA are a reflection of greater or lesser energy status (Belyea et al., 1975a) but there are limitations to this generalization. Indeed, some studies have shown that NEFA concentrations were closely associated with energy status (Erfle et al., 1974; Fisher et al., 1975; Carstairs, 1975), some have failed to show NEFA responding to dietary changes (Blair et al., 1974) possibly because the variability was too large to enable differences to be detected. In conclusion, it seems that using NEFA concentrations as a criterion of energy status during lactation is of limited usefulness.

The reader is referred to Bowden (1971) and Belyea et al. (1975a) for tables summarizing NEFA concentrations in cattle in various physiological and nutritional states from a number of additional literature sources.

Calcium (Ca)

Serum Ca concentrations in dairy cows are relatively stable. Analyses of serum from 4000 cows of various breeds showed a mean of 9.55 mg/dl, with a coefficient of variation of only 7.8% (Sansom, 1973). In another study utilizing samples from 236 Guernseys mean TCA soluble blood Ca was 7.4 mg/dl (range 3.7-12.0 mg/dl) (Lane et al., 1968).

Concentrations increase 1-2 mg/dl at parturition then begin to increase further postpartum (Belyea et al., Bolenbaugh, 1977). In weeks 1 to 10 of lactation serum Ca gradually increases (Belyea et al., 1975a; Carstairs, 1975). Month of lactation, however, seems negatively correlated with serum Ca (Lane et al., 1968).

Pregnancy does not influence Ca concentrations, season apparently does, although this may in part be due to nutritional programs which are seasonal (Lane et al., 1968). It has been shown by an extensive study (involving hundreds of cows) that there is a significant decrease in mean serum Ca concentrations with increasing age (Tumbleson et al., 1973). Mean serum Ca for Holsteins declined from 11.6 mg/dl at less than 0.5 hr of age to a mean of 10.5 mg/dl at 5 yr of age. From 3 through 10 yr of age, mean Ca was significantly lower in Holsteins than Guernseys. It should also be noted that plasma Ca concentrations are inversely correlated with plasma phosphorus concentrations (for plasma phosphorus between 3.7 and 26.0 mg/dl) (Symonds and Manston, 1974).

In addition, the reader is referred to a summary of Ca concentrations from various literature sources (Belyea et al., 1975a).

Phosphorus (P)

When 236 Guernseys were sampled a mean phosphorus concentration of 6.1 mg/dl (range 3.7-10.1 mg/dl) (Lane et al., 1968) was found. Neither season nor milk production affected serum concentrations. It is not clear at this time whether stage of pregnancy affects serum P levels. Serum P concentrations decrease at parturition and have increased almost to prepartum values 24 h after calving (Bolenbaugh, 1977).

Serum P significantly decreases as age of the animal increases (Lane et al., 1968; Tumbleson et al., 1973). At less than 0.5 yr of age mean serum P was 10 mg/dl and this decreased to 5.8 mg/dl by 4-6 yr of age (Tumbleson et al., 1973). The mean for 2-3 yr olds was approximately 6.6 mg/dl.

Except in cases of severe deficiency, it is not clear that serum P concentrations reflect intake variations. Certainly, when cows were fed differing amounts of P (although all diets provided all nutrients equal to or greater than N.R.C. requirements) serum P was not different among groups (Belyea et al., 1975a). Even when 75% of N.R.C. requirements were fed to heifers for the first 12 weeks of lactation there was only a one mg/dl serum P difference (significant nonetheless) between this group and heifers consuming greater than 100% of their requirements (Carstairs, 1975). In a practical situation a 15% difference such as this would be difficult to detect in individual animals. Only in extreme (rather than marginal) circumstances, in the field or in controlled experiments will P concentrations decrease to half of normal (2.5-3.0 mg/dl) (Eckles et al., 1935; Palmer et al., 1941; Morrow, 1969).

Magnesium (Mg)

Extensive studies with dairy cows have shown serum Mg concentrations to be about 2.4 mg/dl (with a coefficient of variation of about 17%) (Lane et al., 1968; Sansom, 1973). In ruminants Mg absorption rate varies considerably, low absorption is caused by high potassium content and/or low fibre content in feed and high pH in rumen fluid (Meyer, 1976).

Blood Mg concentrations are primarily controlled by renal excretion, if blood levels drop too low, excretion ceases. The following classification of Mg concentrations for sheep and cattle was described by Meyer (1976):

Normal	1.8-3.2 mg/dl
Slight hypomagnesemia	1.2-1.8 mg/dl
Severe hypomagnesemia	$\leq$ 1.2 mg/dl

Mg reserves of the body are quite low, mainly in the skeleton, this makes compensating for Mg deficiency quite difficult.

Pregnancy does not influence Mg concentrations but Mg is positively correlated with milk production (Lane et al., 1968). There is no change in serum Mg concentrations at parturition (Bolenbaugh, 1977). When intakes of Mg are adequate (relative to requirements) serum Mg does not seem to reflect intake variations (Belyea et al., 1975a). In addition, a summary of some typical Mg values are presented in the paper by Belyea et al., (1975a).

Volatile Fatty Acids (VFA)

There are not many data available where serum VFA have been examined in the periparturient cow. Schwalm and Schultz (1976) measured serum

acetate (C_2) from 20 days prior to calving to 48 days after. Prior to calving, C_2 remained fairly constant at about 1.3 mM, very little change was seen at calving, but after calving, in normal cows, C_2 increased to 1.67-2.0 mM. Cows that were sub-clinically ketotic however, had considerably higher serum C_2 concentrations of 2.5-3.33 mM and this difference persisted for at least 45 days. This two-fold difference between normal and ketotic cows was also noted in data summarized by Annison and Armstrong (1970), although in one experiment there was no difference between cows.

It is not clear whether lactating cows have different blood concentrations of VFA than non-lactating cows. If there are differences they are probably of dietary origin. Baird et al., (1975) reported that C_2 was higher in dry cows than in lactating cows (3.3 vs 2.5 mM), that serum propionate (C_3) was the same and that butyrate (C_4) in lactating cows was higher (0.042 vs 0.022 mM). In contrast, Annison and Armstrong reported data in which there was no difference in serum C_2 levels. Values of C_2 concentrations for lactating cows fed normal diets range from 1-2.5 mM (Huber et al., 1969; Yousef et al., 1969; Annison and Armstrong, 1970; Bickerstaffe et al., 1974; Baird et al., 1975). Only limited data is available for other serum VFA. Reported values for arterial concentrations, in lactating cows, for C_3 range from 0.04-0.13 mM (Bickerstaffe et al., 1974; Baird et al., 1975), for C_4, 0.042 mM was reported (Bickerstaffe et al., 1974).

In fasted goats or steers C_2 concentrations decrease about 50% (Annison and Armstrong, 1970). Serum VFA do seem to be sensitive to dietary changes, presumably this is due to the induction of different fermentation patterns with different feeds. It has shown that when

lactating cows were fed high grain, restricted roughage diets that plasma C_2 concentration decreased about 20% (Huber et al., 1969; Evans et al., 1975). C_4 levels did not change, both C_2 and isobutyrate ($isoC_4$) increased (45 and 56%, respectively) on the low roughage diet, reflecting the probable changes in fermentation (Evans et al., 1975). Neither the addition of whey or minerals ($NaHCO_3$ and MgO) had any further effect on plasma C_2 concentrations in cows fed high grain (Huber et al., 1969). In contrast, Walker and Elliot (1973) found no differences between cows fed high grain diets in either C_2 or C_3 concentrations, means were 2.5 and 0.115 mM for C_2 and C_3, respectively. The authors suggested that perhaps their assay was not sensitive enough to show differences.

In conclusion, data are limited, but there do seem to be dietary effects on serum VFA concentrations. Other environmental effects remain to be described. Involvement of VFA in regulation of metabolism will be discussed in the last section of the review.

Reproduction as Affected by Energy and Phosphorus Intake

Many theories have been postulated as to the cause of infertility, anestrus, cessation of ovulation and other reproductive failures. Nutrition has been one of the factors implicated in infertility. Rank deficiencies of certain nutrients are known to impair reproductive function. However, the effects of excesses, marginal levels of nutrients or nutritional imbalance have not been fully elucidated. Reproductive efficiency 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 part of the 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 also will be examined.

Energy and Reproduction

It has been suggested that 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 idea that low planes of nutrition can lead to anestrus and reduced conception rates is widely accepted. The evidence, however, is not so clear.

A ten percent decrease in liveweight from calving to the time of mating has been associated with infertility (McClure, 1970). 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 losing weight. Similarly, Schilling and England (1968) reported that with each kg of bodyweight lost from fall to spring there was a 0.123% reduction in calving rate and with each kg of bodyweight gained during the summer an increase of 0.203% in calving rate resulted. Youdan and King (1977) concluded that long term weight changes were associated with fertility and that managing cows so that they were gaining weight at the time of service resulted in improved fertility. No evidence was found to suggest that short term bodyweight gain was beneficial. Similarly, in Rhodesian beef cattle it was found that in cows fed either to gain 12-14% or lose 12-14% of their peak liveweight from early pregnancy to mid-breeding season (12-16 months), that estrus

occurred 9 days earlier in the high than the low plane cows but again, short term fluctuations in nutrient supply had no apparent effect on the incidence of estrus (Holness et al., 1978).

Using a condition scoring system where 0 represents 'emaciated' and 5, 'very fat' it has been shown in 15-20 suckler herds that optimum score for mating was 2-2.5 with extended calving intervals recorded for very lean and very fat cows (Lowman and Scott, 1976). Heifers with good/very good body condition score, fed at a high level of nutrition, showed evidence of ovarian abnormalities. The data suggest greater irregularities in estrus cycle length or that behavioral signs of estrus were manifested to a lesser extent (Leaver, 1977) than in those heifers in moderate condition. Similarly, in dairy cows, conception rate at first service was 52-60% when the condition score was 2 or less but 72-79% when the score was greater than two (Mulvany, 1977). After many studies with beef cows Wiltbank (1974, 1977) showed that when cows were fed low levels of feed following calving there was an increase in the number of cows that did not show heat and a decrease in the number that conceived to a single service. Wiltbank concluded that to successfully rebreed cows they should calve in moderate body condition and receive sufficient feed following calving to maintain their weight.

Not all studies, however, have found a significant relationship between liveweight change and fertility. Although Boyd (1972) found evidence for increased conception rates when cows were gaining weight, fertility differences were not statistically different between weight change groups and he concluded that weight loss had no adverse effect on fertility. Similarly, Broster (1973) quotes work by Munro (1970, unpublished) in which no conclusive relationship between fertility and

liveweight change was found. Downie and Gelman (1976) found that fertility seemed more related to serum glucose concentrations than to whether cows were gaining or losing weight. Finally, bodyweight changes were not found to be related to time of first ovulation in dairy cows (Stevenson, 1977). Liveweight change may indeed be related to fertility but it is probably only one of many factors involved.

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 not fully answered. King (1968), reviewing work in this area found no evidence to suggest that cows yielding more milk showed reduced fertility compared with lower yielding cows. Similarly, returns to service were equally distributed among cows of differing yield potential in New Zealand herds (Simpson, 1972). Dawson (1972) suggested that Simpson analyzed his data in a way that was biased towards finding no differences. 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 et al., 1966). Further reports, with some limitations, essentially support these data. Hewett (1968) showed that repeat breeders averaged 86.4 kg more milk during the first 120 days of lactation when compared to contemporary herd controls. 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. In another more extensive study of Swedish herds, Hewett (1974) failed to show that milk yield potential affected fertility.

Also in a recent study, 393 calving intervals were used to compare the effects of breeding at first postpartum estrus after 74 days as modified by two different levels of nutrition and two different genetic levels of production (Whitmore et al., 1974). 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. After an eleven year study of Czech dairy cattle it was concluded that when yields were above 3000-3500 kg conception rates significantly declined, the corresponding yield limit for older cows was 4000 kg (Brauner, 1975). Using Holstein dairy cows it was reported that interval to first ovulation was extended due to increased milk yield (Stevenson, 1977). Cows which ovulated after day 20 postpartum produced significantly more milk than those which ovulated earlier, cows which returned to estrus before day 15 produced significantly less milk.

Studies examining the effects of diets with below normal recommendations for energy have suggested that fertility was impaired. Dunn et al., (1969) studied these effects in 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 groups were further divided to assess the effects of low, moderate and high energy levels. 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 postpartum energy level. Pregnancy rates at 120 days were 87, 72, and 64% for cows fed high, moderate and low levels of energy postpartum, respectively. Estrus was delayed in the heifers receiving the low level of energy prepartum. Detrimental effects of feeding low levels of energy just prior

to parturition were partially overcome by feeding higher levels post-partum. Similarly, Brahman X Hereford heifers fed high energy pre and postpartum returned to estrus before those fed below recommendations (Randel and Welker, 1977). When heifers were fed high or low energy rations for 90 days prepartum and adequate energy postpartum, a higher percent of the high group were pregnant 60 days postbreeding compared with the low group (Bellows et al., 1972). Other studies have shown that low energy rations postpartum impair fertility (Macfarlane et al., 1977) and that energy supplementation for 6 weeks prior to, and for 6 weeks after mating improves conception (Drew et al., 1976). Nevertheless, not all work has established an effect of energy intake on fertility. In a study of 64 dairy cows throughout a lactation with high and low energy levels neither level of energy intake nor level of production before or after calving or both, had a significant effect on fertility (Gardner, 1969b).

Most evidence would suggest that adequate energy intake is more important or critical than protein intake for maintaining reproductive function (Wiltbank et al., 1962; Wiltbank et al., 1964; and McClure, 1968a). Protein intake, however, cannot be ignored or discounted. In a recent study of dairy herds in Israel involving 2725 total lactations it was shown that digestible protein intake, energy level and total dry matter fed were all significant factors influencing good conception rates. The nutrition of herds with good ($\geq$45%) and low (32%) conception rates was examined very closely and the authors concluded that proper amounts of these constituents in the ration during the entire lactation or inter-calving period was essential for providing appropriate fertility levels in high producing dairy herds (Francos et al., 1977).

It was also found in Israeli dairy herds, that incidence of many types of fertility disorders was much higher in herds fed a low plane of nutrition when compared to herds fed according to recommendations. Francos (1974) concluded that repeat breeder syndrome is associated with feeding a ration deficient in energy during all stages of lactation and the final stages of pregnancy.

The practice of overfeeding has also been implicated in fertility problems. Excessive feeding of energy and protein to medium, low yield and dry cows in the second half of pregnancy has been related to an increased incidence of mastitis after normal parturition. It was suggested that this practice predisposed the cow to faulty involution of the uterus and to metritis (Francos, 1970). Once the diet was corrected, the incidence of metritis declined. After an extensive survey of Swedish dairy herds Hewett (1974) also suggested that the incidence of infertility due to excess feeding was increasing.

Another aspect of feeding which has been associated with fertility problems is that of reduced roughage in the diet. It has been reported that there is a significant relationship between milk fat percent and conception rate (Francos, 1968, 1969). This Israeli data suggested that to maintain adequate fertility, the ration should be composed so as to ensure 3.3% milk fat, even at high milk yields. The observed decrease in fat percent was related to decreased acetate production in the rumen due to shortage of roughage, feeding pelleted concentrates, or both. Francos suggested that decreased acetate in the blood, as reflected by low milk fat %, somehow resulted in lower fertility. It could be that acetate insufficiency interfered with cholesterol and steroid synthesis, resulting perhaps in estrogen and progesterone deficiencies. This appears

to be a long term dietary effect since Francos noted that it took 4-5 months before low roughage diets induced a decline in fertility. A rise in conception rates were noted 1-2 months after fiber content of the ration was raised. Francos (1969) recommended that dry matter from roughage should make up at least 30% of the total dry matter in the ration.

Assuming that reproductive function is also impaired with decreased energy intake, several hypotheses have been suggested as to how this effect is mediated. Some years ago it was suggested that restricted energy intake caused hypoglycemia and that this in turn caused reproductive hypofunction. This observation was made after fertility was significantly improved in an Australian herd when randomly selected cows were fed an additional 5.6-6.4 kg of hay per day from calving to 3 weeks after mating (McClure, 1965). The blood concentrations of glucose of the fertile cows at the time of mating were increasing, and averaged 28 mg/dl, whereas concentrations in the infertile cows were decreasing, 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. Affected herds lost 5-10% of their body weight between parturition and mating and had low blood glucose concentrations of between 20 and 30 mg/dl. Supplementation of the rations of these herds with energy rich concentrations significantly increased both blood glucose and fertility (McClure, 1972).

Further support was given by a study in which cows were made hypoglycemic with insulin. Cows mated 0-2 days after 3-4 days of daily insulin treatment or those treated with insulin daily for the first 4 days after mating were significantly less fertile than cows treated with

insulin at other times, or control untreated cows (McClure, 1968b). Similar results were observed in mice (McClure, 1967). McClure (1967) suggested that the hypothalamus was failing to control the anterior pitultary as a direct failure of supply and utilization of glucose.

It should be noted that the nature of the malnutrition described by McClure (1968a) was apparently not a simple energy deficiency but was complicated by deficiencies of other nutrients such as protein, phosphorus, and carotene. Also, the nutritional and hypoglycemic conditions he describes are extreme when compared with a concentrate fed dairy cow in North America. McClures' hypothesis, however, was supported by Howland et al., (1966) who also suggested that hypoglycemic effects were mediated through the hypothalamus and resulted in a loss of ovarian activity. Additionally, a significant negative correlation was found between plasma glucose concentrations and postpartum interval to occurence 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. More recently, further support for this idea has been published. Downie and Gelman (1976) reported that increasing glucose concentrations during the 20 days preceding estrus was characteristic of fertile services in suckling beef cows regardless of body weight change. Parker and Blowey (1976) also reported that plasma glucose concentrations within + or - 3 days of first service of cows which conceived was higher than that of cows which did not conceive. This difference only approached significance at the 5% level but supports the above data. Additionally, in a large study of ovarian function and carbohydrate

status in dairy cows in Norway it was found that in periods before 24 days or after 40 days postpartum the cows without corpora lutea had significantly lower plasma glucose concentrations than cows with a corpus luteum (Benjaminsen, 1977).

On the negative side of this question neither Blowey et al. (1973) or Hewett (1974) were able to show any relationship between glucose concentrations and fertility in extensive surveys of herds in England and Sweden. The reasons for these discrepancies are not clear at this time. Throughout these studies, however, it is difficult to say that energy status or intake, lactation, and glucose are independent of each other. This leads to a problem of interpreting which is the main cause and which is effect.

Numerous endocrinologists have studied the effects of energy intake on postpartum ovarian activity and changes in serum hormone concentrations pre and postpartum have been studied. Unfortunately, metabolite concentrations 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 et al., 1974). Prepartum energy intake had no significant effect on either 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 the interval to first estrus was not influenced by the reduced energy intake. Of particular interest in this study was the marked elevation of progesterone in those cows which conceived, suggesting that a period of

elevated progesterone may be necessary for conception at first estrus. This effect has also been noted in diary cows maintained on high and low levels of nutrition (Folman et al., 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 progesterone required appeared to be around 3 ng/ml in this case. Also, during the luteal phase preceding insemination, cows that conceived after first insemination gained weight whereas cows that did not were losing weight, the difference approached significance. In a more recent study it was found that the timing of this peak may also be important. In fertile cows, peak progesterone occurred within 4-7 days prior to estrus rather than 8-11 days prior to estrus. This was found to be significant, however, only in the cows cycling in the summer and not in the winter (Rosenberg et al., 1977).

Other studies have shown that level of nutrition can affect peripheral reproductive hormone levels. Plasma luteinizing hormone (LH) and progesterone concentrations were examined for 3 estrous cycles in groups of Holstein heifers fed 100 or 62% of energy requirements (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 LH was also higher throughout the cycle. 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. Total progesterone and progesterone concentration in the corpora lutea of heifers fed 62% of their energy requirements was lower than in their normally fed counterparts.

Evidently, ovarian hypofunction in cases of energy deficiency is not due to reduced circulating levels of LH as was previously thought by such workers as Wiltbank et al., (1962). The first effect may be a reduced ability of the ovarian tissue to respond to LH. Decreasing progesterone has been noted before in heifers receiving 25% of the total feed consumed by the controls (Donaldson et al., 1970). Declines in plasma progesterone have occurred within even 5 days of reduction in feed intake (Hill et al., 1970). This 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. Corpora lutea weights 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 a lesser concentration of progesterone (Gombe and Hansel, 1973). The simplest suggestion is that restricted energy intake affects some step in steroidogenesis within the corpus luteum, resulting ultimately in reduced plasma progesterone concentrations. Several additional suggestions have been made as to how this effect is mediated (Gombe and Hansel, 1973).

Recently, using multiple regression analysis, factors affecting postpartum return to estrus were examined in dairy cows. The interaction between factors involved in calculated energy status and serum glucocorticoids accounted for more than 35% of the variation in days to first ovulation (Stevenson, 1977). Estradiol and the interaction between LH and estradiol also significantly contributed to the model. LH was negatively correlated to days to first ovulation. In a study with

Hereford Heifers fed either 115% or 60% of N.R.C. requirements pre and postpartum it was shown that level of energy did not affect estrogen secretion but, the pattern of LH release after GNRH was altered by feeding. The peak of LH after GNRH injection was 30 min later in the cows fed low energy (Lishman et al., 1977). Staigmiller et al., (1977) concluded that level of nutrition could influence follicular development in response to exogenous FSH but does not significantly affect or alter ovulation rate.

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

Phosphorus and Reproductive Function

A relationship between phosphorus and fertility was postulated many years ago and appears to be widely accepted. Evidence suggests that the phosphorus deficiency required to impair fertility is extremely severe. It has been more difficult to show effects of marginal deficiency on reproductive function. However, a sub-clinical or marginal deficiency may be of more importance since it may indeed cause production losses but not be overly detectable. Clinical signs of severe deficiencies may not be easily diagnosed but are easily noticeable.

The widespread occurrence of a natural deficiency of phosphorus

affecting cattle has been described by Tuff (1923), Theiler et al. (1924), Eckles et al. (1926), Hart and Guilbert (1928) and numerous others. As early as 1906, 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 (Jordan et al., 1966).

South African workers were among the first to report reproductive hypofunction in cattle maintained on veld deficient in phosphorus. By supplementing the naturally deficient animals with bonemeal, calf crops were increased from 51 to 80% (Theiler et al., 1928). Bonemeal supplementation also increased blood phosphorus concentrations from 2.2 mg/dl to normal levels in cows grazing phosphorus deficient pasture (Malan et al., 1928). Hypophosphatemia was reported in animals fed a hay-oats phosphorus deficient diet but effects on reproduction were not mentioned (Palmer and Eckles, 1927). Years later in Ireland both clinical and sub-clinical aphosphorosis of cattle grazing phosphorus deficient pasture was noted. The cattle developed all the symptoms of phosphorus deficiency plus 'temporary sterility' (Sheechy, 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 et al. (1935) followed the example of the South African workers such as du Toit et al. (1934), and tried to obtain experimental data regarding reproductive function in cattle on controlled uncomplicated phosphorus deficient rations. They recognized that much of the reported field data was confounded, knowingly or not, by definiencies of other nutrients. Eckles et al. (1935) fed phosphorus so as to maintain 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. The daily milk yields of these cows were low by today's standards, between 3.2 and 11.1 kg, and probably did not stress the animal excessively. After 3 years of experimentation no evidence was obtained to show that phosphorus deficiency influenced the estrous cycles of the cows. It did appear, however, that breeding efficiency was reduced. The authors concluded that the disturbances in estrus and reproductive function seen in naturally deficient areas were probably due to the complication of other nutrient deficiencies.

More recently, heifer infertility problems in a herd were attributed to phosphorus deficiency (resulting from depleted phosphorus in the soil and consequently in the crops), (Morrow, 1969). Calculations of intake and requirements showed that phosphorus intake was deficient. Clinical signs consisted of rough coat, depraved appetite and infertility. Low blood phosphorus was observed. Treatment of the condition with dicalcium phosphate supplements returned blood levels to normal and fertility was restored. The fertility problem was not due to length of estrous cycles or frequency of silent estrus but rather of conception failure.

In order to more accurately simulate a deficiency in the field the effects of a combined phosphorus and protein deficiency have been examined. Cows were observed for at least 24 months and up to 59 months on a protein and phosphorus deficient ration (Palmer et al., 1941). Cows showed delayed sexual maturity and silent heat but normal, regular ovulation and conception was not impaired. Breeding efficiency was not reduced. In the Northern territory of Australia, supplementation of range cows with 8 g P/head/day improved body weight and increased the pregnancy rate of lactating cows 20% over their controls (Hart and

Mitchell, 1965). The authors did comment, however, that the provision of protein in their opinion was of equal, if not more importance, than phosphorus.

In support of the latter comment, although bonemeal supplementation to native Rhodesian cattle grazing on veld pasture throughout the year, increased the weight of calves weaned by 21%, it did not appear to improve conception rates or have much effect on the duration of postpartum anestrus (Ward, 1968). Supplementation with groundnut cake in the winter, on the other hand, doubled conception rates over that of control cows indicating that protein was more limiting than phosphorus. Similarly, supplementation of Australian cows fed native hay with phosphorus had no significant effect on feed intake, liveweight change, milk production or reproductive activity. In cows which received adequate protein, however, estrus activity was markedly increased whether phosphorus was supplemented or not (Teleni et al., 1977).

Long term supplementation to dairy heifers of phosphorus above N.R.C. minimum daily requirements has also failed to show any reproductive advantages. No effects were noted in estrus exhibition, services per conception and pregnancies (Hecht et al., 1977) or on weight gains, feed conversion efficiency, animal health or conception rate (Noller et al., 1977). In contrast, studies with beef cows nursing calves under drylot conditions have shown that increasing P intake to 150% of N.R.C. requirements from 30 days postpartum to weaning improved rebreeding efficiency and apparently improved milk production. Eighty-nine percent of cows on high phosphorus treatment conceived to first insemination as compared to 59% of those fed the 100% N.R.C. diet (Taylor et al., 1976).

Another approach to the Australian problem of low fertility rates

in cattle grazing native pasture has been to supplement the pasture, rather than the cows, with superphosphate fertilizer. Application of a high level (377 kg/ha/yr) of this fertilizer significantly increased total pasture dry matter available and improved pregnancy rates and growth patterns of breeding cattle. Statistical analyses strongly suggest that improved conception was a direct effect of superphosphate and not attributable to liveweight differences (Williams et al., 1971; Siebert, 1973). At lower rates of fertilization, phosphorus content of the pasture was less than 0.15% for much of the year, apparently only at higher fertilization rates did the pasture approach an adequate phosphorus content to support pregnant and lactating cows (Ritson et al., 1971). It is not clear at this time whether further supplementation or protein additions would have improved fertility further.

Except in cases of severe deficiency it has been difficult to demonstrate a clear correlation between serum phosphorus levels and fertility in dairy cows. Attempts have been made to do this to aid in management of commercial dairy herds. Rowland et al. (1977) found no difference between these parameters in dairy cows between 40 and 100 days postpartum. Similarly, when serum phosphorus concentrations were examined in cows from 15 dairy herds there were no differences between those cows which conceived and those that did not (Parker and Blowey, 1976). It was suggested that when serum phosphorus concentrations were within the normal range it was unrealistic to expect to be able to detect differences.

Some years ago it was suggested that breeding efficiency of cattle might be related to the Ca:P ratio of the feed consumed, (assuming that the intake of each element alone was sufficient and adequate) (Hignett,

1950). It was suggested that a 1:1 ratio was ideal for fertility, whereas herd fertility could be impaired when the ratio was 2:1 or more, especially when the phosphorus intake was only slightly in excess of minimum requirements. After work was completed on another 802 cows, it was concluded that the generally accepted recommendations for phosphorus in England at that time were not high enough for high fertility in dairy cows (Hignett and Hignett, 1951). Decreased fertility was observed in rapidly growing heifers fed rations deficient in manganese and unbalanced in calcium and phosphorus (Hignett, 1959). It should be noted that even Hignett and Hignett (1951) regarded their results with caution. Most of their work was of the field survey type and only feed analyses and breeding data were used. No blood parameters were measured. Their caution appeared to have been justified since a large scale controlled experiment at Weybridge in 1958 and 1959 failed to demonstrate any significant relationship between the Ca:P ratio of the diet and fertility in dairy heifers (Littlejohn and Lewis, 1960). A high Ca:P ratio did, however, depress growth rate. In addition, in a study of 15 commercial dairy herds, where Ca:P ratios ranged from 1.19-3.55:1, no effect on fertility was noted (Parker and Blowey, 1976). Hignett (1950) originally, had also warned against the feeding of too much phosphorus, associating this with infertility. More recently this speculation was raised again when an extensive survey of Swedish dairy cattle showed that low herd fertility was associated with greater than normal serum concentrations of phosphorus, potassium, total protein and urea nitrogen (Hewett, 1974). Hewett interprets his results to indicate that excessive intake of certain nutrients occurs as a result of trying to provide the high yielding cow with sufficient energy, feeding proportionately more concentrates,

so that the ration is more likely to be in excess than that of low yielding cows. He suggests that sterility due to heavy feeding is steadily increasing and that 'starvation sterility' has little relevance today. In another study of dairy herds in British Columbia, however, it was found that the Ca and P content of the silage was important for fertility. As the intake of Ca, P and Cu/Mo was increased from silage as opposed to other sources, non-return rates decreased (Peterson and Waldern, 1977).

If phosphorus affects reproductive function, and evidence suggests that it may, the mechanism is not clear. It has been suggested that phosphorus deficiency acts at the anterior pituitary level causing '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; Kleiber et al., 1936). None of these reports are enlightening as to how deficiency causes these effects. Kleiber et al. (1936) reported that phosphorus deficiency lowers the total efficiency of energy utilization partly by depressing appetite and partly by lowering the utilization of the food eaten. Fasting catabolism did not appear to be altered. New evidence suggests that phosphorus intake could have its action directly in regulating cellular metabolism. It is postulated that cellular respiratory rate and NADH generation rate are regulated by the [ATP] / [ADP] • [PI] ratio. This means that cellular processes could be regulated by intracellular phosphorus rather than energy charge (Erecinska et al., 1977). It is possible then that phosphorus may directly affect metabolism of the reproductive tissues.

The Interrelationship Between Hormones and Metabolites

For many years attempts have been made to correlate peripheral concentrations of hormones and metabolites in ruminants. The idea that substrates and hormones might interact to regulate metabolism to the advantage of the animal is logical. The fact that the nutritional physiology of the ruminant is peculiar to that species suggested that the emphasis of regulation of metabolism should be different from that of the non-ruminant animal.

The most important features of ruminant metabolism and digestion which need to be considered in this context are: (1) the production from dietary carbohydrates by microbial fermentation in the rumen of large quantities of short chain fatty acids (principally C_2 (70%), C_3 (20%), and C_4 (10%)), and absorption of these acids from the rumen into portal blood either directly or after conversion of some C_3 to lactate and C_4 to βOHB in the rumen wall; (2) the virtual absence of hexose from the lower parts of the tract except on certain high grain starch containing diets; and (3) the degradation of much dietary protein and the synthesis of microbial protein in the rumen (Bassett, 1975). As a consequence of this, ruminants are almost entirely dependent on gluconeogenesis for provision of glucose in the fed and fasted state and utilize acetate (C_2) instead of glucose as a major substrate for energy storage and oxidation in the fed state.

As a consequence, the rate of gluconeogenesis is highest and the availability of gluconeogenic precursors (C_3, amino acids) are greatest in the immediate postprandial period. Bassett (1975) suggested that the hormonal responses to nutrient intake in ruminants, therefore, should favour C_0 oxidation or incorporation into fat and the maintenance of

high rates of glucose synthesis to preserve metabolic homeostasis. Bassett suggests that the responses seen may differ little from other species consuming a low carbohydrate or predominantly protein diet. However, ruminants differ from other species eating low carbohydrate diets, in that C_3 is a major precursor of glucose and may account for about 50% of the glucose produced.

Although the processes of digestion and absorption in adult ruminants are prolonged when compared with those in monogastrics, some diurnal variations of hormone and metabolite patterns have been observed that are associated with feed ingestion. The examination of postprandial changes has been useful. The alternative approach has been to observe changes after administration of hormones or metabolites to the animal.

In both sheep and cows, plasma insulin concentrations increase significantly after feeding (Trenkle, 1971a; McAtee and Trenkle, 1971; Ross and Kitts, 1973; Bassett, 1974a, 1974b, 1975; Jenny and Polan, 1975; Evans et al., 1975). The increase which occurs postprandially may peak anywhere from 2-6 h postfeeding (McAtee and Trenkle, 1971a; Bassett, 1974a; Jenny and Polan, 1975; Evans et al., 1975). The act of ingestion itself causes vagal reflexes which cause insulin release. This can be seen in suckling lambs at 5 min after the initiation of suckling, there is a second peak at 45-90 min after feeding which is not a neural effect but due to absorbed glucose (Bassett, 1974b). In adult sheep, an extra feeding, given 12 h after the first or usual feeding, resulted in a repetition of the changes in plasma insulin concentrations seen after the normal feed (Bassett, 1974a). With sheep fed once daily on 3 different quantities of a mixed hay/grain diet Bassett (1974a) showed that the time that insulin was high increased as the amount of food increased. Bassett

(1975) concluded that the amount of food ingested by sheep clearly influenced the magnitude of the insulin response. It has been reported that insulin levels increase less after feeding hay than concentrates (Bassett, 1975). Also, lactating cows fed a high grain ration had significantly higher insulin concentrations at all hours postfeeding when compared to control cows (Jenny and Polan, 1975). Bassett suggests that these differences are probably due to slower digestion of forage type diets and a smaller amount of digestible organic matter. The flow of protein digesta into the abomasum and small intestine may also be important in determining insulin levels in sheep (Bassett et al., 1971; Bassett, 1975). This was suggested after it was found that insulin levels were higher in sheep fed a diet of protected protein.

As would be expected from the above data, fasting decreases insulin concentrations (Trenkle, 1970a; McAtee and Trenkle, 1971a; Trenkle, 1976). It seems that dietary effects are greater than those of age or sex. No differences in plasma insulin could be related to age (18-371 days old) or breed (Angus, Hereford, Shorthorn and crossbred cattle) in a study conducted by Irvin and Trenkle (1971). Steers had higher insulin concentrations than heifers at 371 days of age, but, the steers were fed a finishing ration, the heifers a lower energy ration and thus, the difference was judged to be dietary. Certainly, in another study yearling steers fed finishing rations increased insulin concentrations during the feeding period (Trenkle, 1970b).

There is also evidence that serum GH concentration in both young and adult ruminants are influenced by feeding and dietary factors. The most striking features of plasma GH in adult ruminants is their great variability, suggestive or episodic secretion of the hormone (Bassett,

1975). Fasting does not cause any consistent increase in plasma GH (Bassett, 1975; Trenkle, 1971, 1976), however, it appears that fasting increased the halflife of injected GH from 9.2 to 13.4 min in sheep and from 20.3 to 31.9 min in calves (Trenkle, 1976).

Oscillations in GH concentrations seem greatest in animals not fed and it appears that one affect of feeding may be to decrease the size and frequency of these secretory bursts (Bassett, 1975). It was recently suggested that this suppression may in part be due to somatostatin release from the intestine and/or the pancreas (Bassett, 1975; Trenkle, 1978). In sheep fed maintenance diets once daily, feeding causes a marked decrease in plasma GH and suppresses the oscillations for 2-4 h (Bassett, 1974a, 1975). After this time concentrations return, with marked oscillations, towards prefeeding values. In sheep fed larger quantities of food GH levels tend to be generally lower and an effect of feeding is less clearly seen (Bassett, 1974a). In sheep fed every 3 h mean plasma GH concentrations were negatively correlated with digestible organic matter intake and with plasma insulin concentrations (Bassett et al., 1971). The response of plasma GH levels in lambs to milk feeding differs substantially from the changes following feeding in adult sheep (Bassett, 1974b). Suckling initially causes a rapid decrease in plasma GH but this period is quickly followed by increases in plasma GH levels to high values 30-90 min after milk ingestion. The functional significance of these differences is not clear at this time, but is possibly partly due to rumen bypass and difference in composition of the diet. Since GH appears to be episodically secreted, unless samples are taken fairly frequently, interpretation becomes difficult.

The logical question is, by what mechanisms are hormone concentration

influenced by diet and how might this control metabolism? It cannot be assumed, for example, that insulin and glucagon are regulated by the same mechanisms as in a non-ruminant fed a low carbohydrate diet because of the special features of ruminant digestion.

In view of the extensive metabolism of dietary hexose to VFA in the rumen, attention has naturally been directed towards the VFA as possible regulators of insulin (and glucagon) release in ruminants. It is now well established that intravascularly administered acids C_3-C_8, but not C_2, stimulate insulin secretion in sheep and cattle (Manns and Boda, 1967; Manns et al., 1967; Horino et al., 1968; Hertelendy et al., 1969; McAtee and Trenkle, 1971a; Trenkle, 1971a). Usually, C_4 is more insulinogenic than C_3. In some experiments where glucose was tested at the same time, glucose was much less effective than C_3 and C_4 in stimulating insulin secretion (Manns and Boda, 1967; Trenkle, 1971a; McAtee and Trenkle, 1971a). In addition to C_2, neither acetocetate nor β-hydrobutyrate (βOHB) is insulinogenic, either in vivo (Manns and Boda, 1967; Horino et al., 1968) or in vitro (Jordan and Phillips, 1978). While C_3 has no effect on insulin secretion in rats, rabbits or pigs (Horino et al., 1968) it is interesting to note that VFA do stimulate insulin secretion in newborn lambs (Hertelendy et al., 1969), suggesting that the capacity of the ruminant to respond to short chain fatty acids is a constitutional rather than an acquired characteristic. Due to these reports it is tempting to conclude that the VFA were regulators of insulin secretion in response to food ingestion in ruminants.

Despite these studies there is still considerably controversy about the physiological importance of VFA in insulin release. Bassett (1975) summarizes these problems: (1) C_3 and C_4 are largely removed from the

blood by a single passage, yet C_2, the only VFA occurring in peripheral plasma in substantial quantities, has no significant effect on insulin secretion and probably none on glucagon secretion either since it does not cause hyperglycemia; (2) the hyperglycemic and insulinogenic actions of VFA have been observed following injections and infusions of probably unphysiologically high concentrations, hence, perhaps reflecting a pharmacological effect; and (3) when VFA were administered via the gastrointestinal tract in physiological quantities they were ineffective (Stern et al., 1970). On the other hand, as Bassett (1975) points out, in their experiments, increases in insulin and glucagon in sheep fed once daily occur at the same time as increases in plasma VFA levels. Ross and Kitts (1973) found that plasma C_4 and isoC_4 were most related to postprandial insulin concentrations in sheep and Trenkle (1978) reports work in which intraruminal infusion of physiological concentrations of C_2, C_3 and C_4 in sheep fasted for 36 h increased insulin concentrations. It is suggested that Stern et al. (1970) failed to find a response because the animals in that study had been allowed free access to feed during the experiments and that the higher concentrations of insulin in ad libitum fed goats may have masked any additional response to VFA (Trenkle, 1978).

Certainly, VFA do increase in the circulation after feeding, despite the high extraction of C_3 and C_4 by liver and metabolism of C_4 to βOHB in the rumen wall (Ross and Kitts, 1973; Bassett, 1975; Evans et al., 1975). The timing of these increases relative to changes in insulin is variable. At any rate it appears that maximum concentrations occurring do not approach 200 μM and may be 10-fold lower (Bassett, 1975). Bassett (1975) suggests that it would be surprising if such low

concentrations were to have any marked influence on insulin secretion although this question remains to be definitively answered. Certainly, it is not likely that VFA are the only way in which insulin and glucagon secretion are regulated in ruminants.

Due to the absence of hexose from the digesta in ruminants, changes in plasma glucose have not been generally considered important in regulating insulin release. Bassett (1975) suggests that plasma glucose concentrations are the end result of hormonal regulation. Generally, evidence is weak that increases in insulin after feeding are the result of increases in glucose. Correlations between postprandial glucose and insulin concentrations are usually low (Bassett et al., 1971; McAtee and Trenkle, 1971a, Ross and Kitts, 1973; Evans et al., 1975; Bassett, 1975; Trenkle, 1978). Insulin concentrations may be more related to glucose entry rates rather than to concentrations per se (Bassett et al., 1971).

The next obvious candidate for a regulator of insulin secretion would be protein or amino acids. In fact, it has been shown that intravascular administration of leucine, phenylalanine, arginine or hydrolysed casein will significantly increase insulin concentrations in ruminants (Hertelendy et al., 1969; McAtee and Trenkle, 1971a; Davis, 1972; Trenkle, 1978). This increase was apparently 'much more than could be accounted for by the glucogenic effects of amino acids' (McAtee and Trenkle, 1971a). The amounts of amino acids infused, however, are generally considered to be unphysiological and in fact, after once daily feeding, plasma amino nitrogen levels decrease rather than increase and so do not suggest a significant role for regulating plasma insulin (Bassett, 1975; Trenkle, 1978). One study has shown that plasma

of tyrosine, valine, isoleucine, and phenylalanine were correlated with plasma insulin concentrations in sheep, the highest correlation, however, was with the amount of protein digested in the intestine (Bassett et al., 1971).

The effects of protein on insulin and glucagon release may be mediated by the secretion of gastrointestinal hormones (Bassett, 1975). In non-ruminants gastrin, secretin and pancreozymin can stimulate insulin secretion and gastrin and pancreozymin can also stimulate glucagon release (Bassett, 1975). These hormones also seem able to potentiate the insulin secretory responses to glucose and amino acids although there is, as yet, no evidence that any of them are effective at physiological concentrations. The GI hormones secretin, and cholecystokinin-pancreozymin stimulate insulin secretion in ruminants (Baile et al., 1969; Trenkle, 1972), and it has been proposed that these hormones may be involved in the initial increase in plasma insulin after feeding (Bassett, 1975; Trenkle, 1978). Perhaps gut glucagon as well as other GI hormones are involved in this mechanism too, although the function and regulation of these hormones in ruminants is poorly defined. In view of the long time-course of digestion in ruminants and the apparently well utilized by-pass protein and fat in some ruminant diets however, one might speculate that these hormones may well be involved in the potentiation of responses to other stimuli of insulin and glucagon secretion.

Several mechanisms seem to be involved in regulation of serum insulin concentrations in response to ingestion and digestion of feed. The first response is probably a release of GI hormones resulting from passage of feed into the intestine which causes the first release of insulin. The second increase in plasma insulin is probably caused by a combination

of shortchain fatty acids from the rumen as well as absorption of glucogenic precursors from the digestive tract, (Trenkle, 1978).

Relationships between GH and metabolites at physiological concentrations are not well established. A number of studies have shown that GH is released in a number of situations. Decreasing plasma glucose (from induced hyperglycemic levels) seem to stimulate GH release, while hypoglycemia per se does not (Hertelendy et al., 1969; Trenkle, 1971b; McAtee and Trenkle, 1971b). Short chain fatty acids (C_2, C_3 and C_4) do not seem to affect GH levels (McAtee and Trenkle, 1971b). Although one study reported that very high doses of C_4 released GH (Reynaert et al., 1975). Glucose and VFA do not seem to be important in the regulation of GH concentrations in normal animals. Amino acids have been shown to stimulate GH secretion many times. Arginine and also leucine significantly increase plasma GH concentrations when administered intra-vascularly into cows, heifers, steers, lambs and sheep (Hertelendy et al., 1969; McAtee, 1970; Hertelendy et al., 1970; McAtee and Trenkle, 1971b, Davis, 1972).

There is some evidence that fasting alters the sensitivity of the pituitary to factors which cause secretion of GH since there are greater responses to arginine in fasted than fed heifers (McAtee and Trenkle, 1971b).

Trenkle (1978) also reports that GH can be released by injections of unphysiological amounts of glucose, insulin and glucagon. These increases are not due apparently to altering blood glucose concentrations since increases in GH occurred both during a decrease (during administration of insulin and C_4) and an increase (during administration of glucose and amino acids) of blood glucose. Furthermore, inhibition of glucose

utilization with 2-deoxy-glucose did not change plasma GH (McAtee and Trenkle, 1971b). Even though intra-vascular infusion of amino acids increases plasma GH, plasma concentrations of amino acids do not seem to play a significant role in secretion of GH, because concentrations of GH in sheep plasma are correlated negatively with free amino acids in plasma and the amount of protein digested in the intestine after feeding (Bassett et al., 1971). A decrease in free fatty acids in plasma, which is common with the injection of hormones and metabolites that result in increases in GH, has been suggested to induce the rise in plasma GH (Reynaert et al., 1975).

In general, it is suggested that GH tends to increase when availability of nutrients becomes limiting. Especially when there are increased metabolic demands during pregnancy and lactation. The increase in GH would mobilize energy from adipose tissue to satisfy the needs for metabolism (Bassett, 1975; Trenkle, 1978). This hypothesis is supported by the observation that GH concentrations were lower in ketotic than non-ketotic cows (Reynaert et al., 1977).

GH and insulin are involved in homeostasis of nutrient metabolism. The pattern of postprandial secretion is such that higher insulin with adequate GH favors uptake of glucose and amino acids by non-hepatic tissues for tissue synthesis or storage. Increasing glucagon favors hepatic removal of glucogenic precursors and favors synthesis of glucose. These hormonal changes after feeding favor efficient use of dietary nutrients, which usually are consumed periodically rather than continuously (Trenkle, 1978).

In times of greater metabolic demand GH and GCD usually increase, insulin and glucagon decrease, insulin relatively more than glucagon.

Higher GH alone with low insulin favors transfer of calories from adipose tissue to other tissues. Decreased uptake of amino acids by non-hepatic tissues combined with GCD action on muscle to result in loss of amino acids eventually results in increased gluconeogensis. This is facilitated by higher GCD and a higher glucagon: insulin ratio (Trenkle, 1978).

Although data are limited at this time, PRL and GCD are perhaps also involved in metabolism and utilization of nutrients. PRL appears to follow the pattern of insulin, increasing at feeding, decreasing with fasting and increased metabolic demands (Trenkle, 1978). GCD although not apparently changing with feeding may increase with fasting and greater metabolic demand (Trenkle, 1978). The role of these hormones in the integration of metabolism remains to be clearly defined.

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 2 x 2 factorial design. This trial was then replicated so that in total 48 heifers completed the experiment. At parturition these heifers weighed between 437 and 625 kg in the first trial and between 405 and 633 kg in the second trial. At parturition heifers were assigned to one of four treatment groups, in groups of six to: (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 some measurements were taken for a further 21 days (the experiment ending at 105 days of lactation).

In the first trial the high energy rations were corn silage treated with NH_4OH fed until feed refusals were about 10% of intake and grain mixture fed to the maximum which the heifers would consume. Grain was fed daily at 0800 and 1300 h. Grain intake was restricted to 2.3 kg at 0800 h for the low energy rations. All cows were fed 2.3 kg of alfalfa-brome hay once daily. The corn silage was fed once daily in the morning. Phosphorus was restricted by replacing the dicalcium phosphate

in the grain mixture with corn and $CaCO_3$. Composition of the concentrate mixtures may be seen in Appendix Table 1.

In the second trial the dietary regime was essentially the same, the only important difference was the time of feeding. In this case, the grain was fed daily at 1800 and 0800 h. For the low energy rations, grain intake was again restricted to 2.3 kg at 1800 h. Corn silage was fed once in the evening and hay was fed once during the day. It is also important to note that in the second trial a mistake was made in mixing the high energy, low phosphorus grain at the feed mill. This grain was not low in phosphorus and was fed for approximately 3 or 4 weeks before it was noticed and corrected. Consequently, some of the heifers in this group were not in negative phosphorus balance for some time. The levels of intake will be clarified further in the results.

For both trials 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. Milk fat in both trials was analyzed by standard Dairy Herd Improvement Association (DHIA) procedures by DHIA personnel (E. Lansing, MI). In the first trial, protein content was measured by routine microKjeldahl analysis. In the second trial, the first few samples were analyzed by the microKjeldahl method and the rest were measured using a dye method, (Udy Analyser Co., Boulder, CO). The dye binding method used acid orange 12 dye, a measured amount of this was added to 2.240 ml of thoroughly mixed milk sample. After shaking vigorously and filtering, the percent transmission of the filtrate was measured with a spectrophotometer (Udy Color Analyser) set to a reference point. Unknowns were calculated from a conversion table supplied by the manufacturer. For several weeks both Kjeldahls and dye

binding methods were performed. Since values were in close agreement, following this, only the dye binding method was used.

Body weights were measured at parturition and once a week thereafter. This was done at approximately the same time each week. Once weekly, throughout the second trial only, rectal temperatures were recorded. Blood was collected at approximately 1000 h twice weekly throughout the experiment via the tail vein using a 20 guage 25 mm vacutainer needle and 20 ml vacutainer tubes (Becton Dickenson Inc., Rutherford, NJ). The blood was allowed to stand at room temperature for 1 h after collection and was then placed in a 5C cooler for 5 h. It was then centrifuged for 20 min at 1000 x g and the serum was removed. Serum was stored at -15C until assayed for hormones and metabolites. Reproductive status was determined by the concurrent examination of serum progesterone concentrations 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, OH).

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. (Feed assay data and NE and other values used are shown in Appendix Tables 2 and 3.) From these values and the feed intake data, milk production and composition measurements, a calculated energy status was determined for every heifer once every two weeks over the first 12 weeks of lactation.

First, energy requirements (MCal NE lactation/day) were calculated for maintenance and lactation from N.R.C. (1971) tables using the two

week average for body weight, milk production and milk fat percent. N.R.C. recommendations to add 20 percent to the maintenance allowance for growth during first lactation and an increase of 3 percent feed for each 10 kg of milk produced over 20 kg/day were not included in these calculations. Then energy intake was calculated (MCal NE lactation/day) using feed intake data and the energy values of the feeds. Thus, energy status was calculated from the difference between the requirements and actual intake. Phosphorus status was calculated in the same way. Energy status or balance indicates the difference between input and output of energy. 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 in 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.). Thus, status is an expression of intake of energy and phosphorus relative to requirements.

Statistical Analysis

Because of the variation in the energy and phosphorus status of the cows in both trials, data were analyzed not only by standard analysis of variance procedures but also be regression analysis. In this way, since absolute status values were used in the analysis, inaccurate group designations did not affect subsequent conclusions. Absolute values of energy and phosphorus 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 status

X_2 = phosphorus status

X_1X_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 in each trial. In addition to regression analysis all data were analyzed by two way analysis of variance to determine if means were different. Also, because the experiments were repeat measure designs the possibility of interactions between the main effects and time period could not be ignored. Consequently, all data were analyzed as shown below to test for time confounding factors:

Source of Variance	D.F.	M.S.	F.
Energy status (E)	1		
Phosphorus status (P)	1		
E x P	1		
Error A (Anim./EP)	20 (44)*		
Period	5		
E x Period	5		
P x Period	5		
E x P x Period	5		
Error B (Anim. x time)	100 (220)*		
Total	143 (287)*		

*Degrees of Freedom (D.F.) for when both experiments were included in the analysis.

Error B was used to test for significance of the period terms in the analysis. If E x Period, P x Period or E x P x Period were significant the data were shown to be confounded with time and reanalyzed by considering each period separately. In this way, means from each period were compared and tested and the repeat measure confounding factor was removed. If these terms were not significant then no further analysis of variance was necessary. Results in all cases were prepared by analyzing each trial independently as well as together where possible (i.e. where parameters were measured in both trials and were comparable).

All variables were analyzed to see if they were correlated with time, whether the parameter increased, decreased or did not change with time (or stage of lactation).

Other specific analyses were carried out to test specific hypotheses and these will be discussed further in the results.

Analysis of Serum Hormones

Progesterone

Progesterone was measured by radiommunoassay as described by Louis et al. (1975). Progesterone was measured twice weekly for all heifers throughout 15 weeks of lactation in both trials.

Insulin

Insulin was measured by radiommunoassay courtesy of Dr. E. Beehuizen of Eli Lilly and Co., Indianapolis, IN. Insulin was measured once every two weeks in the first trial and once weekly in the second trial.

Growth Hormone

Growth hormone was measured weekly, in the second trial only,

by radioimmunoassay, as described by Purchas et al., (1970).

Analysis of Serum Metabolites

In the first trial serum glucose, calcium and phosphorus were measured using a Hycel Mark X clinical autoanalyzer, (Hycel Inc., Houston, TX). The test principles used by this analyzer are described under individual metabolite headings. In the second trial, since the autoanalyzer was no longer available, alternative methods were used. These are described below also.

Glucose

In the first trial, using the autoanalyzer, the test principle used was of O-toluidine reacting with glucose in hot acetic acid to form glucosylamine and a Schiff base. The end product was blue-green and was quantified spectrophotometrically.

In the second trial, glucose was measured by a coupled reaction enzyme system, using glucose oxidase and peroxidase, of a Glucostat Reagent set (Worthington Biochemical Corporation, Freehold, NJ). Again, the glucose is quantified spectrophotometrically.

In both trials glucose was measured weekly in all heifer throughout the experimental period.

Calcium and Phosphorus

In the autoanalyzer method serum albumin bound calcium was first released by acid. The total serum calcium was then complexed by cresopthalein complexone. This was measured colorimetrically after the complex was turned purple with base. Serum phosphorus was measured by reacting serum inorganic phosphate with ammonium molybdate, this forms ammonium-phosphomolybdate which was then measured colorimetrically.

In the second trial serum calcium and phosphorus were measured by alternative methods using glassware washed with a 2:1, water:hydrochloric acid solution. All solutions used in the assays were made with deionized distilled water. Both assays required deproteinization of serum. Serum was deproteinized in duplicate with 12.5% trichloracetic acid (TCA). The serum was diluted 1:5 (0.5 ml serum plus 2 ml TCA). The solution was vortexed, allowed to stand for 5-10 min and centrifuged at 1000xg for 15 min. The supernatant was decanted into another set of acid washed tubes for calcium and phosphorus analysis. Composite standards were used in all assays and were run through all procedures beginning with deproteinization. In addition, a blank deionized distilled water sample was run with all assays to use in the phosphorus analysis.

Calcium

0.5 ml of supernatant was diluted with 3 ml of 10000 ppm strontium solution, (strontium as strontium chloride, $SrCl_2 \cdot 6H_2O$). The solution was vortexed and read on an atomic absorption spectrophotometer (Model IL 453, Instrumentation Lab. Inc., Lexington, MA).

Phosphorus

The Gomorri modification of the Fiske-SubbaRow inorganic phosphorus determination was used (Fiske and SubbaRow, 1925). To 0.5 ml of supernatant, 2.5 ml of molybdate-sulphuric acid solution and 0.25 ml of p-methyl-amino-phenolsulphate solution were added. The samples were vortexed and incubated at room temperature for 45 min at which time they were read at 700 mμ on a Gilford 2400S spectrophotometer, (Gilford Instrument Laboratories Inc., Oberlin, OH).

Reagents

10000 ppm Sr solution: 60.86 g $SrCl_2 \cdot 6H_2O$ and 10.0 g NaCl were diluted to 1 liter with deionized distilled water. This was diluted 1:1 with deionized distilled water to give 10000 ppm Sr solution.

Molybdate-sulphuric acid solution: 5 g of sodium molybdate ($Na_2MO_4 \cdot 2H_2O$) in 500 ml deionized distilled water, 14 ml H_2SO_4 (conc.) diluted in approximately 200 ml of deionized distilled water was added to the molybdate solution. This solution was then made up to 1 liter with deionized water.

P-methyl-amino-phenolsulphate (elon) solution: 1 g of elon was dissolved in 100 ml of 3% sodium bisulphite ($NaHSO_3$). It was protected from the light while mixing. The solution was stored in a brown bottle.

Serum Volatile Fatty Acids

Serum VFA determinations were done using the ethanolic extraction method described by Remesy and Demigne (1974). The concentrated extract was quantified with a Model No. 5730A gas liquid chromatograph (Hewlett Packard, Avondale, PA).

Two hundred µl of serum were placed in a 16 x 100 ml glass tube and 1 ml of pure ethanol was added. The solution was then vortexed and centrifuged for 15 min at 1000 X g. The supernatant was then transferred into a 12 x 75 mm glass culture tube and made alkaline with 20 µl of 0.1 M NaOH. It was then evaporated in a slight air current at 20 C. The dry residue was redissolved in 15 µl of water a few minutes before analysis. Just before injection on the column, 5 µl of 25% (v/v) orthophosphoric acid was added. Acidification at the last moment avoided the risk of volatilization of the VFA and of micellar separation of isobutyric acid, which is poorly soluble in acid conditions. Thus, a

solution with a concentration 10 times that of the original sample was obtained. Using a microsyringe (Hamilton Co., Reno, Nev.) 3 µl of the 20 µl was injected on the column.

Conditions

Column: glass, 2.7m long, 2mm i.d.

Packing: 3% carbowax 20M, 0.5% H_3PO_4 on 60/80 mesh carbopack B (Supelco Inc., Bellefonte, PA).

Oven temperature: 160 C

Port and Detector temperature: 200 C

Detector: Hydrogen flame ionization.

Carrier gas: Nitrogen, flow rate 25 ml/min.

Peak area: Measured by an electronic integrator. Unknowns were calculated by comparing peak areas of a standard mixture of VFA with peak areas of unknowns. Standards were about the same concentration as VFA in serum. They were subjected to the same treatment as the unknowns. The standard solution contained acetate, propionate, butyrate and insobutyrate.

RESULTS

Feed Intake

Dry matter intakes of corn silage, hay and grain for trial 1 and 2 are shown in Table 1. In the first trial high energy (HE) groups consumed 7.1 kg more grain but 2.8 kg less corn silage daily than low energy (LE) groups. Hay intake was approximately constant, each row was offered 2.3 kg/day and consumed on average 1.7 kg/day.

Table 1.--Mean dry matter intake (kg/day) of corn silage, hay and grain for the first 12 weeks of lactation in trials 1 and 2.

Feed	Trial	High Energy		Low Energy	
		High Phos.	Low Phos.	High Phos.	Low Phos.
Corn silage (with NPN) (kg/day)	1	7.83	7.50	10.74	10.19
	2	6.46	6.58	7.28	7.22
Hay (kg/day)	1	1.65	1.64	1.78	1.77
	2	2.08	2.08	2.08	2.09
Grain* (kg/day)	1	8.92	9.20	2.00	1.98
	2	8.71	8.91	2.02	2.03

*Dicalcium phosphate replaced by $CaCO_3$ and corn in low P grain.

In the second trial, heifers consumed less corn silage, more hay and approximately the same amount of grain as in the first trial. Depressed silage intake was partly due to the higher moisture content of the silage and also due to deliberate restriction of silage fed, to control energy intake. Treatment means are shown in Table 1. The HE

groups consumed 6.8 kg more grain but 0.7 kg less corn silage. Hay intakes were not different among groups. There was no difference in silage intake between high and low phosphorus groups.

Consequently, in trials 1 and 2 the HE groups consumed 49% of their dry matter intake as grain mix, 51% of their intake as corn silage and hay. In trial 1, the LE group consumed 14% and 86% of their dry matter intake as grain and corn silage/hay respectively. In trial 2 the corresponding figures were 18 and 82% for the LE group.

Summary of Data Analysis

Data for both trials have been combined throughout this presentation except where trial affected results.

Because trials were repeat measure experiments all data were tested for period-nutrient status interaction. Where data were confounded by period, data were analyzed within periods, otherwise data are presented analyzed over all periods.

Energy and Phosphorus Status

Where status is an expression of intake of energy on phosphorus relative to requirements.

Energy Status

HE groups were in positive energy status throughout the experiment, and thus were receiving more than N.R.C. (1971) requirements, while LE groups were receiving less than requirements for maintenance and milk production. Energy status data from both trials are presented in Table 2.

Energy status for treatment groups is represented as a function of stage of lactation versus energy expressed as a percent of N.R.C.

requirements in Figure 1. This enables a slightly different perspective to be taken of the data than absolute values. In all groups energy status increases with time, particularly in the HE groups, due to increasing dry matter intake.

Table 2.--Mean energy status (MCal/day) of high and low energy groups as a function of weeks of lactation for trials 1 and 2.

	Weeks of Lactation					
	1-2	3-4	5-6	7-8	9-10	11-12
High energy*	3.72	5.94	8.44	9.71	11.01	11.78
Low energy	-5.03	-4.27	-3.76	-3.09	- 3.37	- 2.46

*HE > LE (P = .001) in all weeks. Differences between HP and LP were N.S. There was no significant interaction of E and P status.

Phosphorus Status

Phosphorus status was not as easily controlled as energy status. In both trials it was difficult to maintain negative phosphorus status when combined with a high energy ration. Further complications were added when in trial 2 the high energy, low phosphorus grain was incorrectly mixed and the resulting grain was not restricted in phosphorus content. Before this was discovered and corrected, some of the heifers were not in negative phosphorus status.

Phosphorus status data for both trials are presented in Table 3. It can be seen from this study only the low energy, low phosphorus group was in negative phosphorus status throughout the experiment. When the two trials were combined, the high energy, low phosphorus group was not in negative status (for the previously mentioned reasons) but generally, they were much less positive than either of the high phosphorus groups.

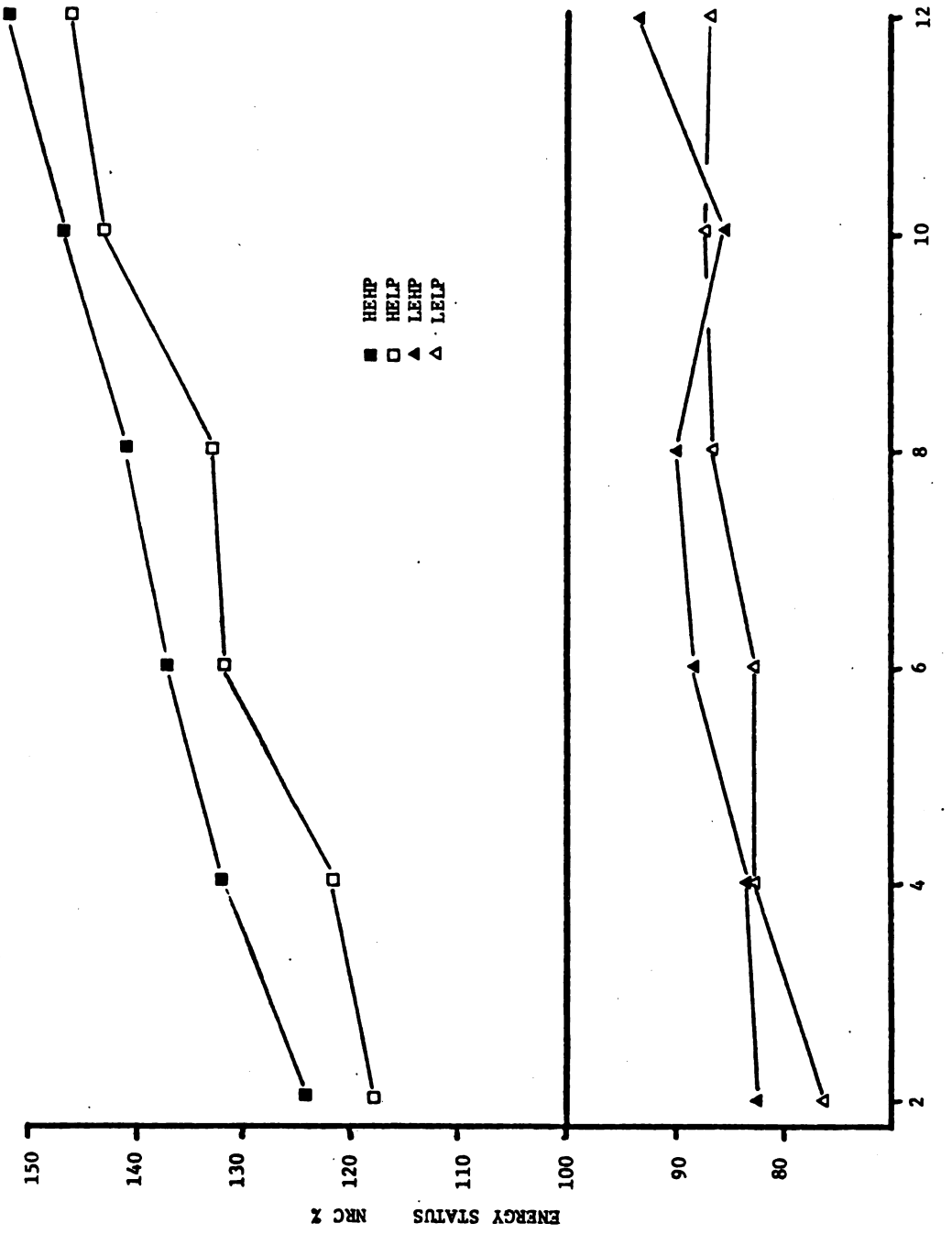


Figure 1. Mean energy status (NRC %) for the first 12 weeks of lactation

Table 3.--Mean phosphorus status (g/day) as a function of weeks of lactation for both trials.

Weeks of lactation	High Energy*		Low Energy	
	High P**	Low P	High P	Low P
1-2	16.90	4.69	11.64	- 6.55
3-4	22.93	3.55	8.72	- 9.14
5-6	27.62	6.02	9.84	- 9.76
7-8	35.19	4.50	12.18	-10.35
9-10	38.42	5.77	10.17	- 9.00
11-12	40.66	5.82	13.40	- 8.70

* In weeks 1-12, HE > LE groups in P status ($P \leq .002$).
**In weeks 1-12, HP > LP groups in P status ($P = .001$).
In weeks 1-6, interaction EXP, NS.
In weeks 5-12, interaction EXP, ($P \leq .04$)

Thus, only the low energy, low phosphorus were marginally deficient in phosphorus status. Only the high energy, high phosphorus group tended to increase in phosphorus status as lactation advanced, whereas the other groups were quite stable. Phosphorus status expressed as a percent of N.R.C. requirements and as a function of stage of lactation is shown in Figure 2. When high phosphorus groups were averaged they represented 138% of requirements. When low phosphorus groups were averaged they represented 98% of requirements (average of 110% and 86%).

Milk Production

(a) Average daily milk yield

Milk yield for both trials combined is presented in Tables 4 and 5.

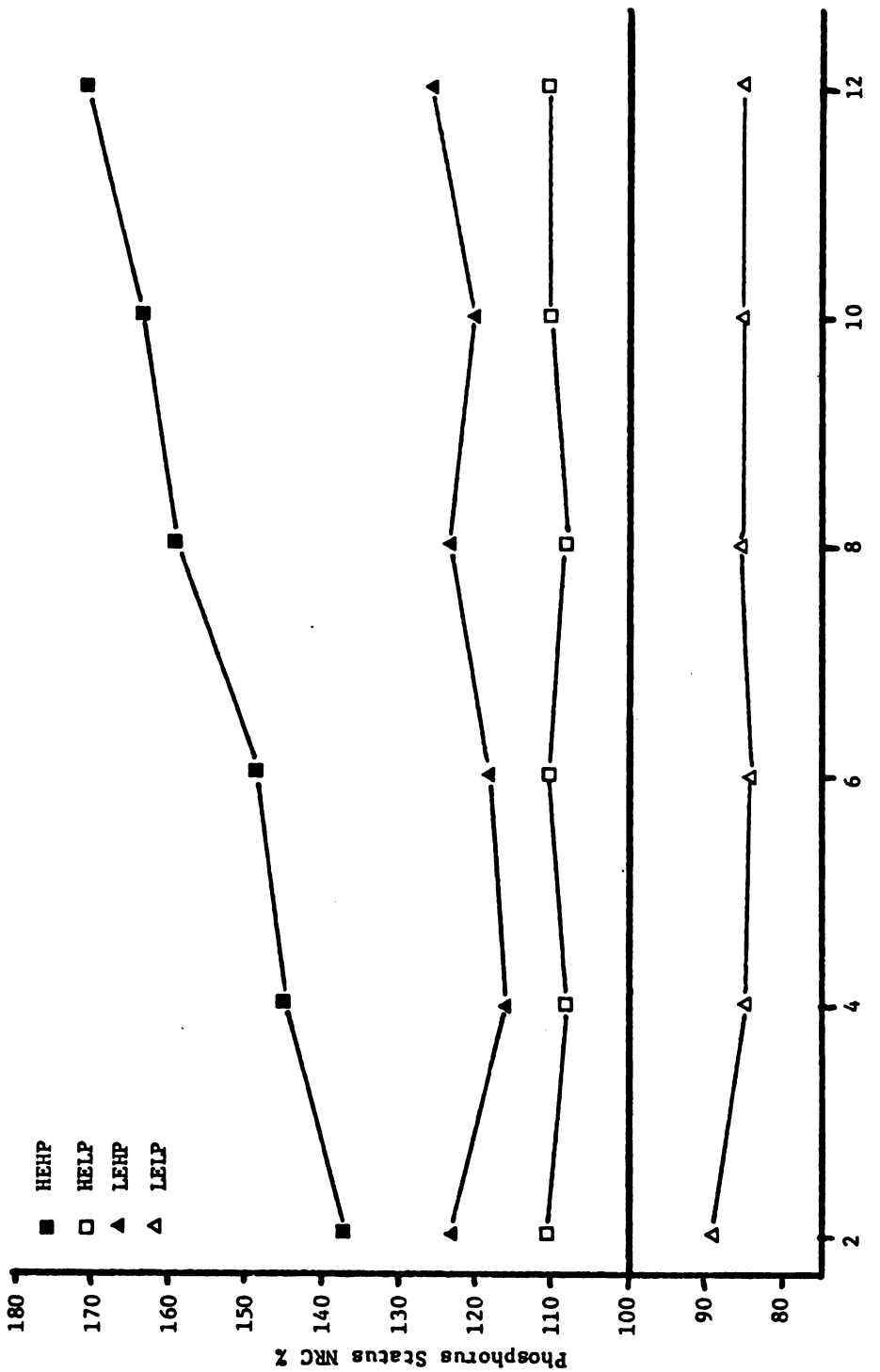


Figure 2. Mean phosphorus status (NRC %) for the first 12 weeks of lactation.

Table 4.--Mean daily milk yield (kg/day) for both trials as a function of weeks of lactation.

Weeks of lactation	High Energy		Low Energy	
	High P	Low P	High P	Low P
1-2	17.02	18.71	19.21	19.19
3-4	21.12	23.33	22.74	23.00
5-6	22.74	26.21	23.43	23.81
7-8	22.83	26.31	22.33	23.76
9-10	23.17	25.86	22.27	22.87
11-12	22.33	26.26	21.13	22.00
Mean	21.54	24.45	21.85	22.44

Table 5.--Mean daily milk yield (kg/day) for both trials for high and low energy and high and low phosphorus groups.

Weeks of lactation	High Energy*	Low Energy	High P**	Low P***
1-2	17.60	19.20	18.12	18.95
3-4	22.27	22.87	21.97	23.16
5-6	24.48	23.62	23.09	23.81
7-8	24.57	23.05	22.58	25.04
9-10	24.52	22.57	22.72	24.37
11-12	24.30	21.56	21.73	24.13
$\bar{X}$	22.96	22.15	21.70	23.24

* Weeks 1-8, HE and LE NS.
Weeks 9-12, HE > LE ($P < .05$).
** Weeks 1-4, HP and LP NS.
Weeks 5-8,11-12, LP > HP ($P < .03$).
Weeks 9-10, LP > HP ($P = .10$).
***Low P = 98% of NRC P requirements.
(Mean of 110 and 86%).

These data show there was no significant difference in mean daily milk yield among groups for the first 4 weeks of lactation. From the third through the twelfth week the daily yield of the low phosphorus (LP) group exceeded that of the high phosphorus (HP) group. This difference ranged from 0.72 kg/day in weeks 5-6 to 2.46 kg/day in the weeks 7-8. Only in weeks 8-12 of lactation did the HE group yield significantly more milk ($P \leq .05$) than the LE group. This difference was 1.95 kg/day in weeks 9-10 and 2.75 kg/day in the weeks 11-12. There were no significant differences in average daily milk yield between heifers designated best, worst and control genetic groups, (means were: 23.4, 22.0 and 21.9 kg/day, respectively).

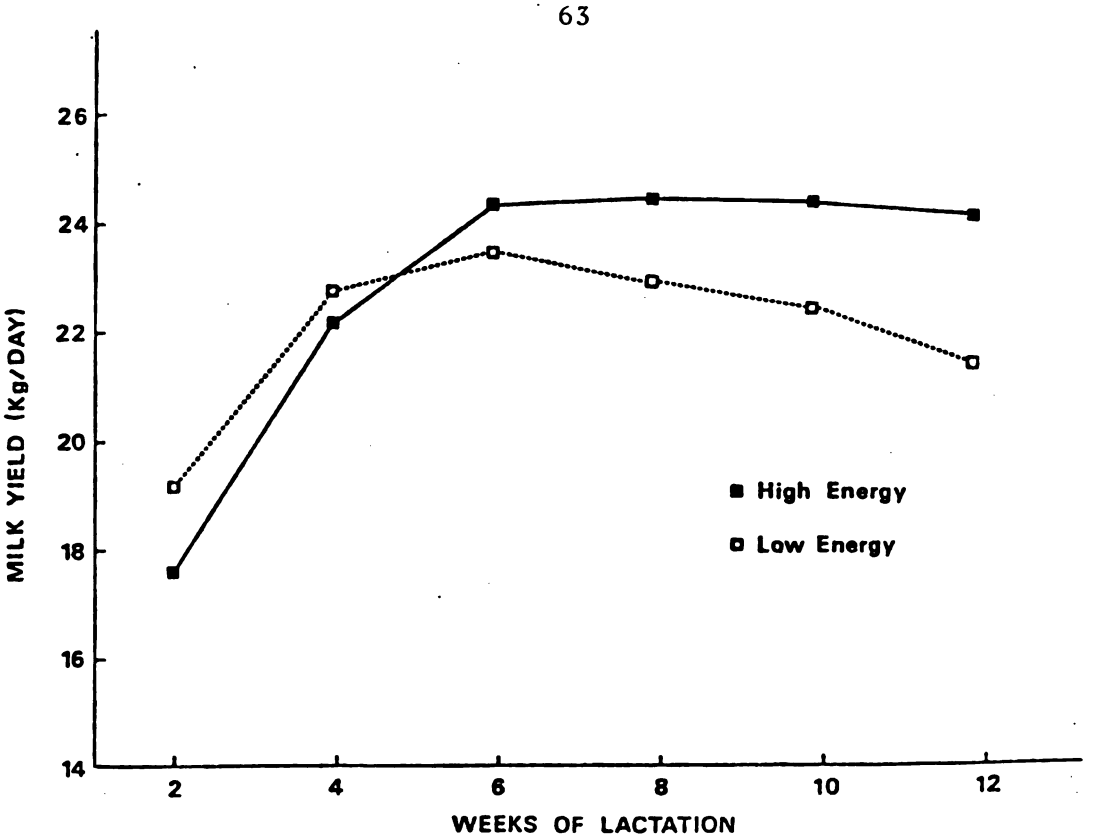
Milk yields for HE and LE and HP and LP groups are graphically represented in Figure 3. This graph shows that HE groups did not begin to yield more milk until approximately 5 weeks of lactation, and after this were more persistent than the LE groups. The second graph shows that LP groups (which averaged 98% of N.R.C. requirements) consistently produced more milk than HP groups throughout the experiment. When groups are ranked by average milk yield the order is as follows:

1. HELP
2. LELP
3. LEHP
4. HEHP

When ranked by energy and phosphorus groups, the order is:

1. LP
2. HE
3. LE
4. HP

Regression analysis support the above data by showing that milk yield increased as energy status increased and declined as phosphorus status increased. Regression coefficients show that, relatively,



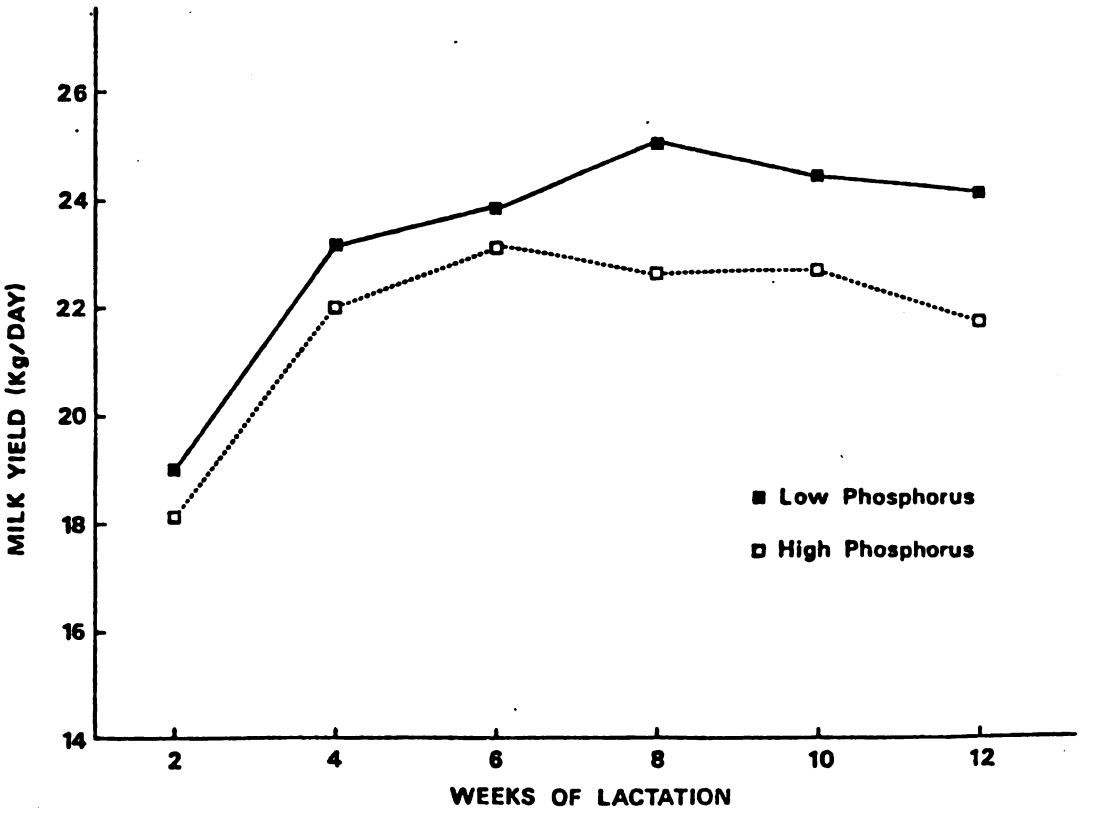


Figure 3. Mean milk yield (kg/day) for the first 12 weeks of lactation.

phosphorus status was a more important component of the regression equation than energy status (Table 6).

Table 6.--Regression of energy and phosphorus status with milk yield, milk fat % and milk protein % for both trials.

Dependent Variable	Standard Regression Coefficient* Energy Status	Phos. Status	EXP
Milk yield	0.30	-0.52	NS**
Milk fat %	-0.57	NS**	0.31
Milk Prot. %	0.17	0.35	-0.19

* Standardized coefficients so that comparison may be made within variables without regard to units.
**NS = P > .05.

(b) Milk fat percent

Milk fat percent combined data can be seen in Table 7. LE groups had more (P = .02) fat percent than the HE groups. This was probably due to the LE group yielding slightly less milk than the HE groups. Phosphorus groups did not significantly differ. This was a significant

Table 7.--Mean milk fat percent for the first 12 weeks of lactation for both trials.

Energy	Phosphorus High	Low	Mean
High	3.32	3.13	3.22
Low	3.34	3.52	3.43
Mean	3.33	3.32	
	E P = .02		
	P NS		
	EXP P = .04		

interaction between energy and phosphorus status. This was due mostly to the low energy, low phosphorus group which had the highest milk fat percent of 3.52%. As expected milk yield and milk fat % were negatively correlated, r = -0.15, ($P < .05$). These data are supported by regression analysis which indicated that fat percent decreases as energy status increases ($P < .0005$). The interaction between energy and phosphorus status was also significant (Table 6).

(c) Milk protein percent

HE groups had greater ($P < .0005$) milk protein percent than LE groups and HP groups had greater ($P = .01$) protein percent than LP groups (Table 8).

Table 8.--Mean milk protein percent for the first 12 weeks of lactation for both trials.

Energy	Phosphorus High	Phosphorus Low	Mean
High	3.31	3.27	3.29
Low	3.18	3.02	3.10
Mean	3.24	3.14	
	E P < .0005		
	P P = .01		
	EXP NS		

As expected milk yield and protein percent were negatively correlated, r = -0.44, ($P < .05$). Regression analysis supports the conclusions of the analysis of variance. As energy and phosphorus status increased, protein percent increased. Coefficients indicate that phosphorus status had more effect on protein percent than energy status. The negative

interaction coefficient shows that energy and phosphorus effects were less than additive (Table 6).

(d) 305 day actual milk yields

Since the first part of the lactation curve, including peak milk yield, determines to a large extent the total of the lactation, it was considered important to examine the total first lactation yields of heifers involved in both trials. It seemed important to determine if the treatments imposed in the first 3 months of lactation had any adverse or beneficial effects on the whole lactation relative to their performance in the first 3 months. Of course this assumes that even though heifers were assigned to various other experiments after 3 months that it is probable that treatment differences in subsequent experiments were balanced and cancelled out. Table 9 shows the 305 day milk yields for heifers from both trials.

Table 9.--Cumulative 305 day milk yield (kg) for heifers from both trails.

Energy	Phosphorus High	Low	Mean
High	5357	6515	5936
Low	5642	6117	5880
Mean	5500	6316	
	E NS P P = .01 EXP NS		

There were no significant differences between energy groups in yield. Consistent with the results in the first 3 months however, heifers fed at 98% requirements in early lactation yielded 816 kg more (P = .01) milk

in 305 days than heifers fed excessive phosphorus in early lactation. Ranking in milk production was HELP, LELP, LEHP and HEHP and LP, HE, LE, HP. These are the same order as they were after 3 months of lactation. Thus, it could be that differences which occurred in the first 3 months of lactation may have affected the total lactation. It is not clear whether these differences were due to nutritional treatment or whether the 305 day yields, or 3 month yields, reflect differences in genetic ability among cows.

When these differences are expressed over the total 305 day lactation heifers which were fed excessive phosphorus for 84 days postpartum gave 2.68 kg of milk less per day throughout lactation than those fed at about N.R.C. requirements (i.e., the LP groups). HE and LE groups were approximately the same, means were 19.46 and 19.28 kg/day, respectively.

Further examination of the data showed that in the first 84 days of lactation heifers had produced on the average just over 30% of their total 305 day yield. This is shown below by group:

HEHP	HELP	LEHP	LELP
33.8%	31.5%	32.5%	30.8%

If the 3 month treatment period is excluded and yield for the last 221 days of lactation was examined the groups ranked in yield as before: HELP, LELP, LEHP and HEHP. When the average daily yield of the first 84 days is compared with the average daily yield of the last 221 days it was found that for the HEHP group yield per day in the last 221 days was 74.5% of the first 84 days, the corresponding value for the HELP group was 82.6%. For the LEHP and LELP groups values were 78.8 and 85.3%, respectively.

Health

(a) Health scores

Records were kept of the incidence of postpartum health problems such as retained placenta, metritis, mastitis, displaced abomasum, respiratory and foot and leg problems. 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 3 days from the last record of mastitis. Thus, a score was established for each group. Prior to the second trial it was decided to add another more objective indicator of health, and rectal temperatures were taken weekly on all heifers throughout the second experiment. Also, culling data were collected from all heifers which were culled after the first lactation, reasons for leaving the herd were noted.

Mean health scores for each group with combined trials are shown in Table 10.

Table 10.--Mean incidence of disease in both trials for the first 12 weeks of lactation.

Energy	Phosphorus High	Low	Mean
High	2.0	2.6	2.3
Low	1.1	1.6	1.3
Mean	1.5	2.1	
	E P = .15 P NS EXP NS		

Although means were not highly statistically different, the trend was the same in both trials, HE groups tended to have more health problems than LE groups, means were 2.3 and 1.3, respectively. There was little difference in disease incidence for HP and LP groups. Generally, it is difficult to show disease differences in small experiments but the biological significance of these data should not be ignored. Twice as many cows would be needed to show significance at P = .05 level given similar variance.

Health problems involved in these scores are shown for HE and LE groups in Table 11. These data show that incidence of mastitis and

Table 11.--Types of health problems in high and low energy groups for the first 12 weeks of lactation.

Health Problems	High Energy		Low Energy	
	Number of Incidences	Percent	Number of Incidences	Percent
Mastitis	17	32	16	49
Metritis	12	22	10	30
Respiratory/pneumonia	8	15	3	9
Foot and leg problems*	5	9	0	0
Displaced abomasum and digestive upsets*	6	11	0	0
General infections*	4	7	0	0
Edema	2	4	1	3
Retained placenta**	0	0	3	9
Total	54	100	33	100

* HE > LE (P = .05).
**LE > HE (P = .05).

metritis are similar in HE and LE groups. HE groups, however, had more respiratory, foot and leg, digestive, general infection and edema problems. Total number of disease incidences for HE groups was 54, almost twice as high as for the LE groups with a total score of 33.

(b) Rectal temperatures

First of all, intra-animal correlations with time showed that in each group rectal temperatures significant ($P < .05$) declined over the first 12 weeks of lactation, $r = -0.44$ to -0.55. During this time the HE groups had higher ($P = .006$) temperatures than LE groups. Means were 38.87 and 38.74 C, respectively (38.61 ≡ 101.5F ≡ normal temperature). There were no differences between phosphorus groups and no interaction between the main effects. Average temperatures for high and low phosphorus groups were 38.78 and 38.83 C (Table 12).

Table 12.--Mean rectal temperature (C) for the first 12 weeks of lactation for the second trial.

Energy	Phosphorus High	Phosphorus Low
High	38.88	38.85
Low	38.69	38.80
E	P = .006	
P	NS	
EXP	NS	

Regression analysis did not show that rectal temperature was dependent upon energy or phosphorus status. The reason for this contradiction in results is not clear.

When temperature was examined as a function of time it was clear it declined as lactation advanced (Figure 4). Temperatures for HE and LE

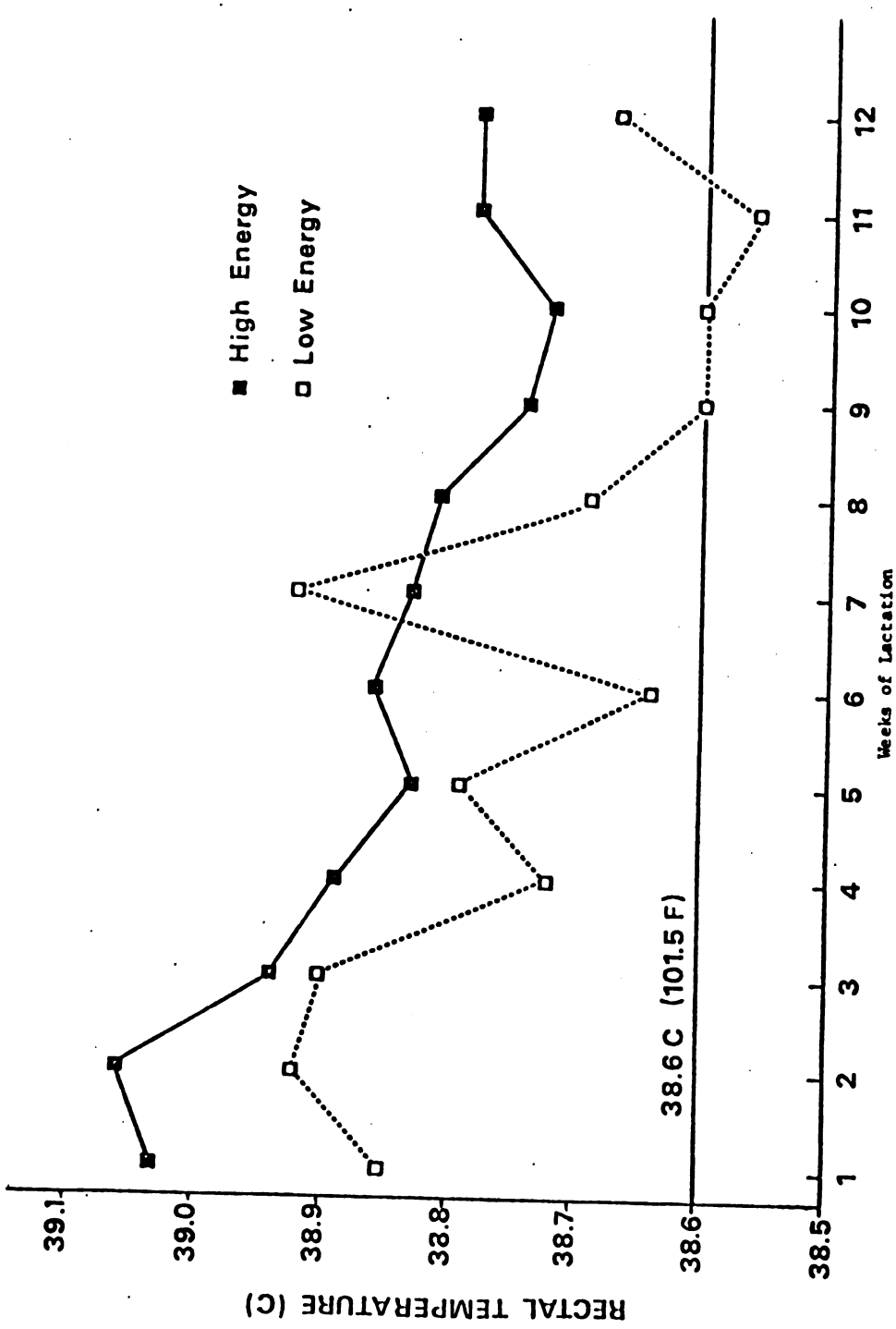


Figure 4. Mean rectal temperature (C) for the first 12 weeks of lactation.

groups in the first week of lactation were 39.0 and 38.9 C. Temperatures did not decline to normal levels (38.6 C) until approximately 8 or 9 weeks of lactation in LE groups, whereas by week 12, HE group temperatures were still elevated. It was interesting that both milk yield and corn silage intake were negatively correlated to rectal temperature r = -0.30 and -0.43, respectively, ($P < .05$).

(c) Culling after first lactation

Examination of culling records showed that 42% (or 10/24) of heifers fed excessive energy in the first 3 months of lactation were culled compared with only 12.5% (or 3/24) of those fed low energy rations, ($P = .05$). Culling percentages were 3.33 (8/24) and 20.8% (5/24) for HP and LP groups, respectively. It should also be noted that no heifer was culled after first lactation in the low energy, low phosphorus group in either trial. Of 10 heifers culled in the HE group, 5 were because of fertility problems, three were for poor condition and health and 2 were because of udder problems. All three of the heifers culled in LE groups were because of fertility problems. These culling data are somewhat biased, however, since no cows were culled for production due to an on-going genetics experiment.

Energy Status Relationship

(a) Body weight change

Change in body weight expressed as kg/week of the treatment period generally followed energy status of cows in both trials. When data from both trials were combined HE groups gained on average 0.54 kg/week whereas the LE groups lost 1.49 kg/week ($P = .034$). There was no difference in weight loss between phosphorus groups and no interaction

of energy and phosphorus status (Table 13).

Table 13.--Change in body weight (kg/week) for the first 12 weeks of lactation for both trials.

Energy		Phosphorus High	Phosphorus Low
High		0.475	0.575
Low		-1.18	-1.75
	E	P = .034	
	P	NS	
	EXP	NS	

Variation within each group was considerable. For example, the range of weight change in the HEHP groups was -5.15 to +5.73 kg/week, and range for the LELP group was -6.33 to +1.85 kg/week. This shows why, when averaged, body weight changes seem small. Of the four groups: HEHP, HELP, LEHP and LELP: 6, 7, 3 and 4 out of 12 heifers were gaining weight over the 12 week period, whereas the rest, on average, lost weight. Regression analysis shows that body weight change in dependent upon energy status but not in a highly predictive manner ($r = 0.51$, $P < .01$) (Table 14). The groups that were gaining and losing the most weight, HELP and LELP, were ranked first and second in milk production. Body weight change and milk production were negatively correlated, $r = -0.54$, ($P < .01$).

Table 14.--Regression of energy and phosphorus status with body weight change.

Variable		Energy Status	Phos. Status	EXP
Body weight change	Trial 1	0.54*	NS	NS
	Trial 2	0.44**	NS	NS
	Combined trials	0.51***	NS	NS

* P = .07.
** P = .15.
***P = .007.

(b) Serum glucose

Response of serum glucose to energy and phosphorus status differed slightly in each trial. Since, also, serum glucose should be examined by trial when considering insulin responses later in these results, glucose data will be presented separately.

In the first trial serum glucose concentrations of HE groups were greater (P = .06) than those of LE groups by approximately 4 mg/dl (Table 15). Neither phosphorus status nor the interation of the main effects had any influence on glucose concentrations.

In the second trial glucose concentrations were, on average, 13-14 mg/dl less than in the first trial. It is not clear if this is due to assay differences, real differences in cows due perhaps to feeding schedule differences (since cows in trial 1 were sampled nearer to feeding) or due to some factor the author is not aware of. However, serum glucose concentrations were higher (P = .025) in cows fed excess energy than in those fed low energy. Glucose also increased, however, (P = .026), in those cows fed HP when compared to those fed LP (Table 16). There was no significant interaction between energy and phosphorus status and

Table 15.--Means serum glucose concentrations (mg/dl) for the first 12 weeks of lactation for the first trial.

Energy	Phosphorus High	Low	Mean
High	75.9	75.0	75.5
Low	70.4	72.8	71.6
	E P = .06		
	P NS		
	EXP NS		

Table 16.--Mean serum glucose concentrations (mg/dl) for the first 12 weeks of lactation for the second trial.

Energy	Phosphorus High	Low	Mean
High	61.1	59.8	60.4
Low	59.8	57.8	58.8
Mean	60.4	58.8	
	E P = .025		
	P P = .026		
	EXP NS		

glucose levels. Serum glucose increased 1.6 mg/dl when energy or phosphorus was increased. Thus, these glucose data do not reflect energy status quite as well as data from the first trial.

Regression analyses support analysis of variance results. Coefficients from the first trial show that as energy status increases, glucose concentrations increase (Table 17). However, energy and phosphorus were not entirely independent of each other since the interaction term was significant. In the second trial only the phosphorus status term was

Table 17.--Regression of energy and phosphorus status with serum glucose concentrations.

Dependent Variable		Standard Regression Coefficients Energy Status	Phos. Status	EXP
Serum glucose	Trial 1	0.42	NS	0.20
	Trial 2	NS	0.45	NS

significant (Table 17). This appears to be due to a wider range of phosphorus status values compared to the range of energy status values and this, coupled with glucose increasing equally when energy or phosphorus was increased meant phosphorus status expressed glucose concentrations better than energy status in the regression equation.

Finally, analysis showed that in no group did serum glucose increase or decrease significantly as lactation advanced. Serum glucose was positively correlated with energy status, $r = 0.30$, ($P < .01$). Serum glucose was negatively correlated to serum acetate, $r = -.19$, ($P < .05$) but was not significantly correlated to other serum VFA's measured.

(c) Serum insulin

Since insulin data from each trial were different, each will be discussed separately. In the first trial serum insulin concentrations responded markedly and positively to energy status increases (Table 18). The difference between energy groups, except, in weeks 9-10, was quite dramatic. On the average HE groups had four-fold higher insulin than LE groups. It is important to remember that these differences were seen in samples taken approximately 3 h postfeeding of the main feed of the day. In no week was there any difference between phosphorus groups or a significant interaction between energy and phosphorus status.

Regression analysis strongly support the analysis of variance results, showing that as energy status increased, insulin concentrations increased ($P < .0005$), (Table 19). Figure 5 shows the relationship between HE and LE groups graphically as a function of stage of lactation.

Table 18.--Mean serum insulin concentrations (μU/ml) for the first trial as a function of weeks of lactation.

Weeks of lactation	High Energy*		Low Energy	
	High P	Low P	High P	Low P
1-2	15.10	12.62	4.03	3.23
3-4	15.68	10.83	5.03	3.98
5-6	27.00	18.57	3.50	4.42
7-8	23.73	13.48	5.77	6.32
9-10	14.83	13.48	11.23	15.70
11-12	18.70	14.37	2.02	5.38

*In weeks 1-8, and 11-12, HE > LE ($P \leq .05$).
In weeks 9-10, HE and LE NS.

Table 19.--Regression of energy and phosphorus status with serum insulin concentrations.

Dependent Variable		Energy Status	Phos. Status	EXP
Serum insulin	Trial 1	0.50	NS	NS
	Trial 2	0.51	NS	NS

In the second trial the response in serum insulin concentrations to changing energy status was not as marked. This may be in part due to difference in sampling times relative to feeding. In the second trial the LE group were sampled approximately 15 h postfeeding, the HE group

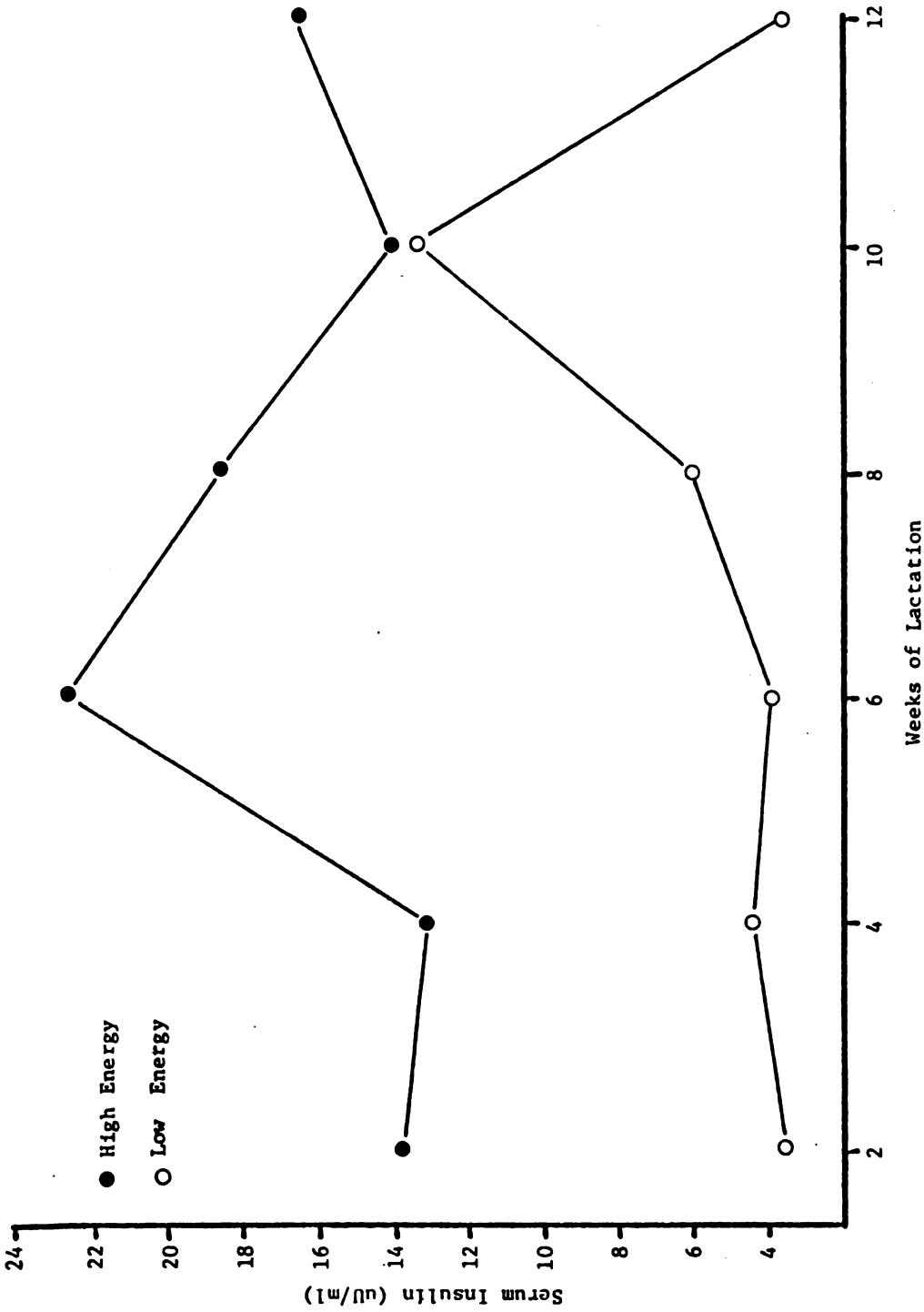


Figure 5. Mean serum insulin (uU/ml) for the first 12 weeks of lactation in trial 2.

was sampled approximately 3 h after half of the grain ration was fed.

Insulin concentrations in the HE group were 28% higher (P = .001) than in the LE group (Table 20). There were no differences between phosphorus groups in insulin concentrations. There was a significant energy and phosphorus status interaction due to the HELP group which had the highest mean insulin concentration. These results were supported by the regression analysis which again showed that insulin concentrations were highly dependent (P < .0005) on energy status (Table 19). The interaction term in the regression equation was near significance (P = .063) showing that, as in the analysis of variance, energy and phosphorus

Table 20.--Mean serum insulin concentrations (μU/ml) for the first 12 weeks of lactation in the second trial.

Energy	Phosphorus High	Low	Mean
High	6.46	8.03	7.25
Low	5.96	5.41	5.68
	E P = .001		
	P NS		
	EXP P = .02		

status were not entirely independent in their influence on serum insulin. Figure 6 shows serum insulin in the second trial for HE nad LE groups as a function of stage of lactation. From this figure it can be seen that after only 2 1/2 weeks of lactation the separation between HE and LE groups is clear.

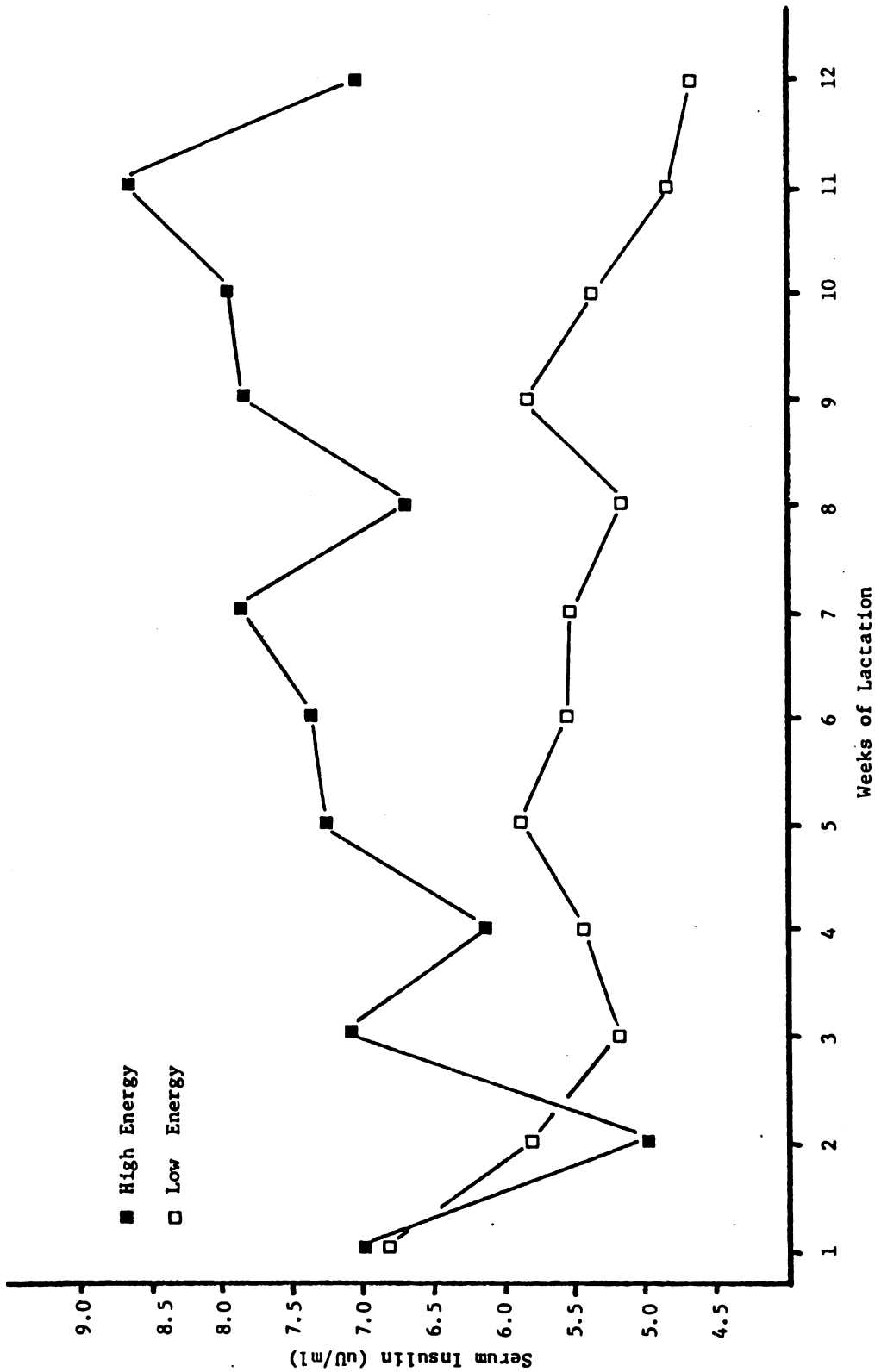


Figure 6. Mean serum insulin (uU/ml) for the first 12 weeks of lactation in trial 2.

(d) Serum volatile fatty acids

(1) Serum acetate

Acetate (C_2) was measured in the second trial only. There were no significant differences between groups in serum acetate concentrations overall mean was 736.4 μM (Table 21).

Table 21.--Mean serum acetate concentrations (μM) for the first 12 weeks of lactation in the second trial.

Energy	Phosphorus High	Phosphorus Low
High	713.5	823.7
Low	761.5	737.0
E	NS	
P	NS	
EXP	NS	

Regression analysis supports these conclusions showing that energy and phosphorus status had no significant influence on serum C_2 concentrations (Table 22).

Table 22.--Regression of energy and phosphorus status with serum volatile fatty acids.

Dependent Variable	Standard Partial Regression Coefficients Energy Status	Phos. Status	EXP
Serum acetate	NS	NS	NS
Serum propionate	0.37	NS	NS
Serum butyrate	0.57	NS	NS
Serum isobutyrate	NS	NS	NS

Serum C_2 was negatively correlated with serum glucose, r = -0.19, (P < .05), but was positively correlated with milk yield and corn silage intake, r = 0.24 and 0.22, respectively (P < .05).

(2) Serum propionate

Serum propionate (C_3) was greater (P = .001) in the HE than in the LE groups, means were 23.9 and 15.3 μM, respectively. C_3 was also different between phosphorus groups, the LP groups had greater (P = .022) C_3 concentrations than the HP groups, means were 22.5 and 16.6 μM (Table 23). The significant interaction between energy and phosphorus status

Table 23.--Mean serum propionate concentrations (μM) for the first 12 weeks of lactation in the second trial.

Energy	Phosphorus High	Low
High	17.8	29.9
Low	15.4	15.2
E	P = .001	
P	P = .022	
EXP	P = .02	

(P = .02) was due mainly to the high energy low phosphorus group which had the highest C_3 level. Serum C_3 and grain intake were positively correlated, r = 0.29, (P < .05) but C_3 was not significantly correlated to serum glucose. Regression analysis showed that serum C_3 increased as energy status increased (P = .013) but not that phosphorus status or the interaction of energy and phosphorus status had any effect (Table 22).

(3) Serum butyrate

In general, where means were different (Table 24), HE groups had higher ($P \leq .044$) butyrate (C_4) concentrations than LE groups and LP groups had higher ($P \leq .09$) serum C_4 than HP groups. Only in weeks 5-6 was this relationship not consistent. It is not clear why this is so. Regression analysis showed that as energy status increased, serum C_4 increased. Regression analysis did not, however, show that phosphorus status or the interaction between energy and phosphorus status had any influence on serum (C_4) (Table 22). Serum C_4 was not correlated to serum glucose but was positively correlated to grain intake, $r = 0.37$ ($P < .05$).

Table 24.--Mean serum butyrate concentration (μM) for the first 12 weeks of lactation in the second trial.

Weeks of lactation	HE*	LE	HP**	LP
1-2	12.52	5.15	4.55	13.13
3-4	17.32	7.45	8.42	16.35
5-6	7.43	20.14	19.94	7.63
7-8	14.52	5.00	8.23	11.29
9-10	11.23	3.01	4.62	9.62
11-12	10.88	6.34	7.74	9.47

* Weeks 1-2,5-6, NS.
Weeks 3-4,7-12, HE > LE ($P \leq .044$).
**Weeks 5-8,11-12, NS.
Weeks 1-4,9-10, LP > HP ($P \leq .09$).
Weeks 3-4 only, EXP ($P = .003$).

(4) Serum isobutyrate

Analysis of variance showed no differences between energy groups in serum isobutyrate. The LP group had higher (P = .005) serum isobutyrate ($isoC_4$) than the HP group, means were 2.50 and 1.73 μM. The significant interaction of energy and phosphorus status was due to the high energy low phosphorus group and the high energy high phosphorus group which had the highest and lowest means, respectively (Table 25).

Table 25.--Mean serum isobutyrate concentration (μM) for the first 12 weeks of lactation in the second trial.

Energy	Phosphorus	
	High	Low
High	1.40	2.84
Low	1.92	2.17
E	NS	
P	P = .005	
EXP	P = .05	

Regression analysis showed that only energy status was near significance (P = .1) in influencing $isoC_4$ concentrations (Table 22). It is not clear why this is contradictory to analysis of variance results.

Finally, all serum VFA were positively correlated with one another, (P < .05), values are shown in (Table 26).

Table 26.--Simple correlations between serum VFA.

VFA	Simple Correlation
C_2 x C_3	0.63
C_2 x C_4	0.65
C_2 x $isoC_4$	0.46
C_3 x C_4	0.77
C_3 x $isoC_4$	0.46
C_4 x $isoC_4$	0.46

(e) Growth hormone

Serum growth hormone results are presented by weeks in Table 27. In weeks 1-2 HE groups had greater (P = .08) serum GH than LE groups. In weeks 3-8 there was no significant difference between HE and LE groups but by week 9 it was apparent that HE groups had decreased whereas LE groups had not changed in GH concentrations. In weeks 9-12 LE groups had greater ($P \leq .02$) GH concentrations than HE groups. GH as a function of stage of lactation for HE and LE groups is presented in Figure 7. Regression analysis indicated that GH was not significantly influenced by energy or phosphorus status.

Analysis showed that GH in the HEHP and HELP groups declined as lactation advanced, r = -0.39 and -0.43, respectively ($P < .05$). There was no significant increase or decline in the two low energy groups. GH was negatively correlated with milk yield, r = -0.23, ($P < .05$), and corn silage intake, r = -0.22, ($P < .05$). It was positively correlated with serum C_4 concentrations, r = 0.23 ($P < .05$).

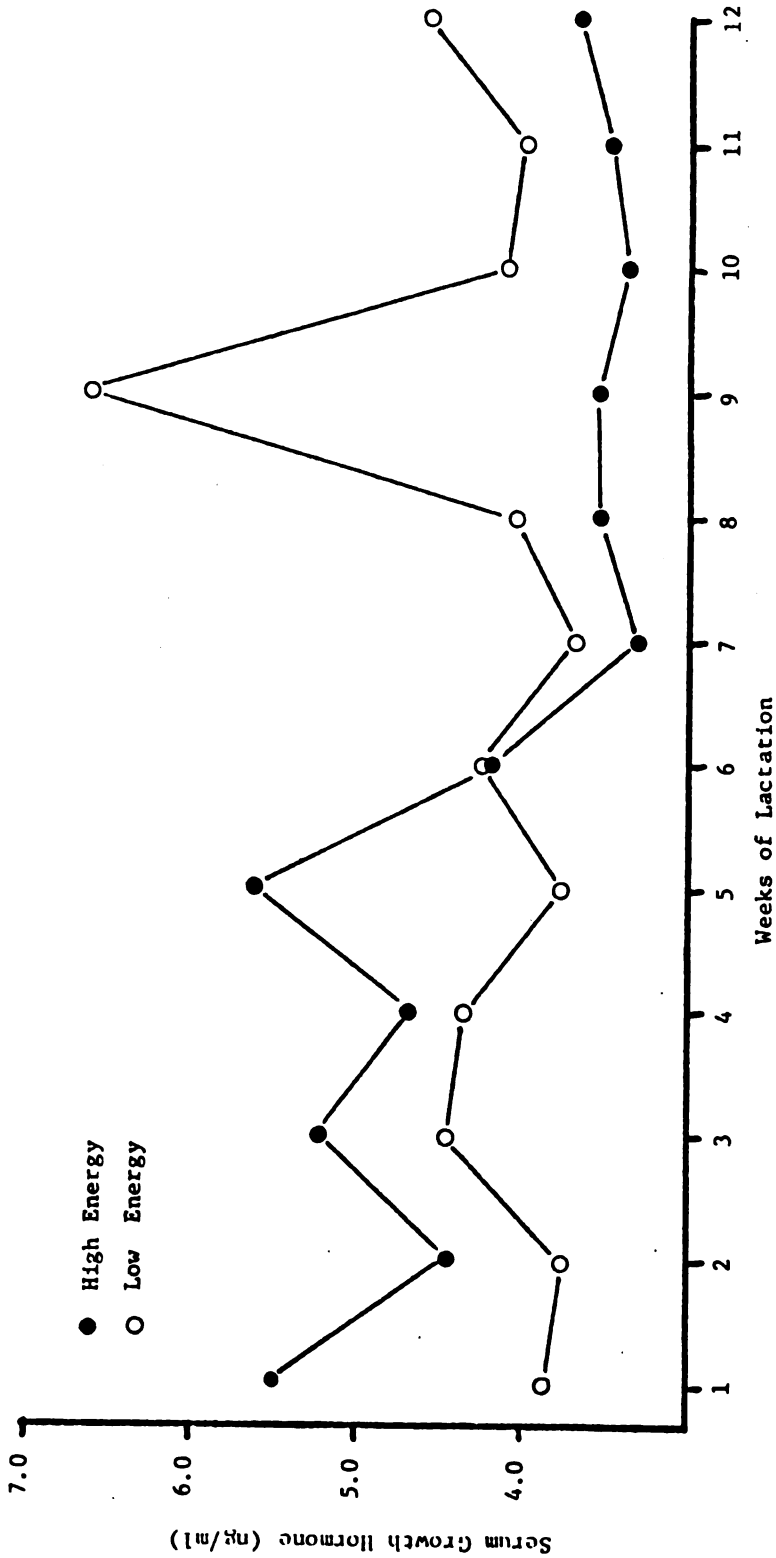


Figure 7. Serum growth hormone (ng/ml) for the first 12 weeks of lactation in trial 2.

Table 27.--Mean serum growth hormone concentrations (ng/ml) as a function of weeks of lactation.

Weeks of lactation	HE*	LE	HP**	LP
1-2	5.00	3.82	4.13	4.69
3-4	5.75	4.59	4.57	5.77
5-6	4.93	4.01	4.43	4.51
7-8	3.48	3.88	3.59	3.78
9-10	3.50	5.39	3.95	4.94
11-12	3.51	4..	3.89	3.84

* Weeks 1-2, HE > LE, P = .08.
Weeks 3-8, HE and LE, NS.
Weeks 9-12, LE > HE, $P \leq .02$.
**Weeks 1-12, HP, LP, NS.

Indicators of Phosphorus Status

(a) Serum phosphorus

Because analysis of variance results were not the same in each trial results are presented separately. In the first trial serum P reflected P status since the HP groups had greater (P = .02) serum P concentrations than the LP group. Neither energy status nor the interaction of energy and phosphorus status affected serum P levels (Table 28). Regression analysis showed that serum P increased as phosphorus status increased, decreased as energy status increased and that these two effects were not additive (Table 30).

In the second trial HP groups had greater serum P than LP groups ($P < .005$) but the HE groups also had greater (P = .0006) serum P than the LE groups (Table 29).

Table 28.--Mean serum phosphorus concentrations (mg/dl) for the first 12 weeks of lactation in the first trial.

Energy	Phosphorus High	Low
High	6.67	6.22
Low	7.52	5.68
Mean	7.1	6.0
	E NS	
	P P = .02	
	EXP NS	

Table 29.--Mean serum phosphorus concentrations (mg/dl) for the first 12 weeks of lactation in the second trial.

Energy	Phosphorus High	Low	Mean
High	5.72	5.33	5.53
Low	5.54	4.97	5.25
Mean	5.63	5.15	
	E P = .0006		
	P P < .0005		
	EXP NS		

Regression analysis confirmed that as phosphorus status increased, serum P increased but did not show that energy status influenced serum P concentrations (Table 30).

Table 30.--Regression of energy and phosphorus status with serum phosphorus.

Dependent Variable		Standard Regression Coefficients Energy Status	Phos. Status	EXP
Serum Phos.	Trial 1	-0.33	0.59	-0.25
	Trial 2	NS	0.44	NS

(b) Serum calcium

Since there were no important differences between trials data are presented for both experiments combined. Serum Ca was greater (P = .001) in the LP groups than the HP groups, means were 9.69 and 9.45 mg/dl for LP and HP. Thus, there is an inverse relationship between serum Ca and P. There were no differences in serum Ca between energy groups but the interaction of energy and phosphorus status was significant (P = .04) (Table 31).

Table 31.--Mean serum calcium concentrations (mg/dl) for the first 12 weeks of lactation for both trials.

Energy	Phosphorus High	Low
High	9.56	9.65
Low	9.35	9.73
E	NS	
P	P = .001	
EXP	P = .04	

Regression analysis supports the conclusion that serum Ca decreases as phosphorus status increases and the interaction of energy and phosphorus

status increases and the interaction of energy and phosphorus status (Table 32). This interaction is due to increasing energy blocking the effect of phosphorus. This may be the result of confounding of energy and phosphorus particularly in trial 2. In all but the HEHP group serum Ca increased as lactation advanced, r = 0.41 to 0.51, ($P < .05$).

Table 32.--Regression of energy and phosphorus status with serum calcium.

Dependent Variable	Standard Regression Coefficients		
	Energy Status	Phos. Status	EXP
Serum Ca combined trials	NS	-0.31	0.32

Regulation of Serum Insulin

These experiments presented an opportunity to examine serum insulin as it responded to differences in energy intake and to attempt to relate these changes to other serum hormones or metabolites.

Because more data were available in trial 2 a number of variables were selected from the second trial for a regression analysis involving serum insulin as the dependent variable. Variables for analysis were chosen because they were significantly correlated with serum insulin. Grain intake was significantly correlated with insulin but since it was so highly related to energy status (r = 0.95) it was not included.

By analysis of variance and regression analysis insulin was shown to be related to energy and phosphorus status. Table 33 shows variables that insulin was correlated with in both trials 1 and 2. Serum glucose and insulin concentrations were significantly correlated, as were energy status and insulin. However, in trial 2, serum C_2, C_3 and C_4 were more

correlated with insulin concentrations than glucose. Serum C_2, C_3, C_4 and $isoC_4$ were not measured in trial 1 and so comparable information is not available. Although milk yield was not well related to insulin in either trial it was chosen as one of the variables for analysis.

Table 33.--Simple correlations with insulin in trials 1 and 2.

	Simple Correlations	
	Trial 1	Trial 2
glucose x insulin	0.35*	0.22*
C_2 x insulin	--	0.26**
C_3 x insulin	--	0.42**
$isoC_4$ x insulin	--	-0.048
C_4 x insulin	--	0.40**
GH x insulin	--	-0.27*
E status x insulin	0.54*	0.32*
P status x insulin	0.33*	0.10
milk yield x insulin	-0.17*	0.13

* $P < .05$, $r = 0.159$, $n = 144$.
**$P < .05$, $r = 0.205$, $n = 92$.

Figure 8 shows the stepwise deletion regression analysis used. In dependent variables that significantly contributed to the equation ($P < .0005$, $R^2 = 0.26$) were serum GH, C_3, C_4 and $isoC_4$. Variables with $P > .1$ were deleted (that is, energy status was deleted first, glucose last). Coefficients in Figure 8 are absolute values and are not standardized. Table 34 shows standard regression coefficients of the previous equation (Figure 8) so that independent variables may be compared with each other.

$$y = 6.4 + 177.6x_1 - 1092.9x_2 - 0.23x_3 + 143.6x_4$$

where y = insulin

x_1 = butyrate

x_2 = isobutyrate

x_3 = growth hormone

x_4 = propionate

($P < .0005$, $R^2 = 0.26$)

Deleted: (1) E status (2) P status (3) milk yield (4) acetate (5) glucose

Figure 8. Deletion regression analysis with serum insulin as dependent variable.

Table 34.--Deletion regression analysis with serum insulin as dependent variable: standard coefficients.

Variable	Stand. Regression Coeff.	R^2 Deletes*	Sig.
Growth hormone	-0.338	0.16	.001
Propionate	0.310	0.22	.040
Butyrate	0.265	0.24	.088
Isobutyrate	-0.261	0.21	.016

*Original $R^2 = 0.26$.

Examination of these coefficients show GH and C_3 concentrations to be the most important variables since they have the highest coefficients. GH and insulin were negatively related and C_3 and insulin were positively related. C_4 and $isoC_4$ follow with approximately equal coefficients. The R^2 deletes column indicates the R^2 or proportion of total variance that would result if that single variable were dropped from the equation. R^2

decreases the most when GH is eliminated from the equation, (from R^2 = 0.26 to 0.16). If either C_3, C_4 or $isoC_4$ are excluded the R^2 also declines (Table 34). C_4 was positively related to insulin, $isoC_4$ was negatively related. This analysis suggests that serum GH and serum VFA (excluding C_2) are more important in regulation of serum insulin concentrations than serum glucose concentrations or other parameters tested.

Reproduction Data

Serum progesterone concentrations were used in conjunction with rectal palpation data to establish occurrence of estrus, ovulation and normal cycles. Number of days to first ovulation (it was not in all cases detected as an overt estrus), and number of days postpartum to reach a progesterone concentration of 3 ng/ml were calculated. Time taken to reach 3 ng/ml was measured because this level is considered to be the peak needed in the estrous cycle preceding insemination in order to permit conception (Folman et al., 1973; Corah et al., 1974; Rosenberg et al., 1977). It was hoped that these two parameters would provide an independent measure of reproduction, however, days to each 3 ng/ml progesterone and days to first ovulation were well correlated, r = 0.74, ($P < .01$).

Days to reach 3 ng/ml progesterone and days to first ovulation were regressed with only energy and phosphorus status data from periods preceding these events for each heifer. Of course, energy or phosphorus status after an event does not affect the event. Throughout these reproductive data, where $P > .25$ the effect is designated non-significant.

(a) Days postpartum to reach 3 ng/ml progesterone

Although there were no significant differences between groups, LP groups took longer (P = .19) to reach 3 ng/ml than HP groups (by approximately 9 days) (Table 35). Calculation shows that given the same variation in data approximately n = 100 would be needed to show a difference between P groups (at P = .05 level).

Table 35.--Number of days postpartum for serum progesterone to reach 3 ng/ml in both trials.

Energy	Phosphorus High	Low	Mean
High	43.8	52.8	48.3
Low	36.7	46.5	41.6
Mean	40.3	49.7	
	E NS P NS (P = .19) EXP NS		

When these data were analyzed by regression with energy and phosphorus status there was little indication that energy or phosphorus status influenced progesterone concentrations reaching 3 ng/ml. Correlation data showed that as serum phosphorus or glucose increased, days to reach 3 ng/ml progesterone decreased (occurred sooner), r = -0.34, -0.33 (P < .05), respectively. Days to 3 ng/ml progesterone was positively correlated with serum GH concentrations (r = 0.51, P < .05).

(b) Days to first ovulation

HE groups, on average, ovulated for the first time approximately 4 days prior to LE groups. HP and LP groups both ovulated at 27 days

postpartum. There were no significant differences between groups by analysis of variance (Table 36).

Table 36.--Number of days postpartum to first ovulation.

Energy	Phosphorus High	Low	Mean
High	27.0	22.0	24.5
Low	26.8	31.2	29.0
Mean	26.9	26.6	
	E, P, EXP, NS		

Regression analysis supports the hypothesis of increasing energy status decreasing time to first ovulation. The regression coefficient for energy status was -0.38 ($P = .07$), phosphorus status and the interaction of the two main effects had no effect on first ovulation. Serum insulin was negatively correlated to days to first ovulation, $r = -0.55$, ($P < .01$) in trial 2, but these parameters were not significantly correlated when both trials were combined. Heifers apparently ovulated before progesterone concentrations reached 3 ng/ml, on average this difference was 18 days.

(c) Days non-pregnant

Heifers could leave the herd only if one of the following conditions was met: (1) they were diagnosed pregnant; (2) they had been inseminated 3 times; (3) they were more than 150 days in lactation. One hundred and fifty days was selected as a realistic cut off point beyond which heifers would normally be culled if apparently infertile.

Actual pregnancy dates were used for heifers which became pregnant prior to 150 days postpartum. All other heifers were arbitrarily assigned 150 days non-pregnancy. When this was done all groups had similar days non-pregnant (Table 37).

Table 37.--Mean number of days non-pregnant for both trials.

Energy	Phosphorus High	Low	Mean
High	117.8	123.8	120.8
Low	110.1	121.4	115.8
Mean	114.0	122.6	
	E, P, EXP, NS		

Given the same variation calculations indicate a sample size of at least 250 would be needed to show differences between phosphorus groups and over 700 to show differences in energy groups in pregnancy (at P = .05 level).

(d) Inseminations per conception

There are no significant differences between groups in insemination per conception (Table 38).

Given the same variation a sample size of about 280 would be needed to show differences between energy groups (at P = .05 level).

There were some differences however, in number of cows pregnant. For HE and LE groups, 8 (or 33%) and 3 (or 12.5%) heifers, respectively, did not conceive six (25%) of HP and 5 (21%) of LP groups did not conceive. These differences cannot be attributed to treatments, however.

Table 38.--Mean number of inseminations per conception for both trials.

Energy	Phosphorus High	Low	Mean
High	2.56 (9)*	1.86 (7)	2.2 (16)
Low	2.44 (9)	2.67 (12)	2.6 (21)
Mean	2.5 (18)	2.3 (19)	
	E, P, EXP, NS		

*Number cows pregnant.

(e) Absolute number and percent undetected estrus

There were no differences in either number or percent of undetected estrus (Table 39). Overall means were 1.45 (out of 3.3 ovulations) or 43.8% undetected estrus during the first 15 weeks of lactation.

Table 39.--Mean number and percent undetected estrus for both trials for the first 15 weeks of lactation.

Energy	Phosphorus High	Low	Mean
High	1.3 (41.7)*	1.6 (48.6)	1.5 (45.2)
Low	1.2 (35.4)	1.7 (49.3)	1.4 (42.4)
Mean	1.3 (38.6)	1.6 (49.0)	
	E NS P NS ($P < .25$) EXP NS		

*Percent undetected estrus.

An estimated sample size of 116 would be needed to detect differences between phosphorus groups at the P = .05 level.

(f) Interval to second and third ovulations

There were no significant differences in interval to second or third ovulation. Forty-four of 48 heifers had a second ovulation, 38 of 48 had a third. Overall means were 20.6 days and 21.2 days for second and third interestrual intervals, respectively. All first, second and third ovulations used in these analyses occurred within 13 weeks postpartum, during and just after the dietary treatment period.

There were no differences between phosphorus groups in mean number of ovulations that occurred within the 15 week experimental period, mean was 3.3 ovulations per heifer. HE groups had less ovulations ($P < .25$) than LE groups, means were 3.1 and 3.5, however, it was estimated that a sample size of 116 would be needed to detect differences at P = .05 level.

Simple Correlations

Other correlations which were statistically significant but are not reported in the text and which may be interest are listed in Appendix Table 4. Non-significant correlations are not presented.

DISCUSSION

Results of these experiments show postpartum energy and phosphorus status affects milk production and possibly health. Certain serum metabolites and hormones responded to changes in energy or phosphorus status. Relationships between energy and phosphorus status and reproductive function were not definitive. Status, is an expression of intake of energy or phosphorus relative to requirements for maintenance and milk production. This must be distinguished from intake per se. Analysis of variance really tested differences in intake rather than status. Whereas multiple regression analysis tested for influence of status on variables measured, not of intake. This explains why results of these two analyses are not always the same.

Milk production responses of heifers to energy and phosphorus status were of practical significance. Heifers fed excessive phosphorus (138% N.R.C.) in the first 3 months of their lactation yielded 6.5% less milk per day for 84 days postpartum than those heifers which were fed almost to requirements (98% N.R.C.). When 305 day yields were compared, heifers fed excessive phosphorus early in lactation yielded 13% less milk (816 kg) than heifers fed to recommendations. If excess phosphorus suppressed milk yield it is not immediately clear how this occurred. The excess phosphorus may have affected metabolism at the tissue level. The possibility that differences between phosphorus groups were due to differences in genetic potential, post-treatment or unknown factors cannot be

excluded. There was no apparent difference in health of phosphorus groups so this at least was probably not the cause of differences in yield.

Because of fertilization and natural soil content, phosphorus levels in feeds may well meet requirements of cows without additional phosphorus supplementation. It cannot be assumed that phosphorus 30-50% in excess of requirements is without effect. In fact, data from these experiments suggest it is better to feed just below requirements rather than over them. Energy status also affected milk yield. HE groups did not begin to yield more milk than LE groups until week 5 of lactation and this difference did not become significant until week 8 when LE groups were less persistent.

These responses could in part be due to postpartum disease since heifers fed in excess of requirements had nearly twice as many incidences of disease when compared with heifers fed below requirements. Also, whereas the majority of disease incidence in LE and HE groups occurred within the first 4 weeks of lactation, HE groups also had more disease problems from week 5 onwards, (17 versus 5 incidences for weeks 5-12 for HE and LE groups). Prolonged reoccurrences of health problems may have suppressed milk yield potential in HE groups. By 9 weeks incidence of problems was low in all groups. Incidences of mastitis and metritis were approximately equal between HE and LE groups. HE groups, however, had more respiratory, pneumonia and edema problems. LE heifers had no displaced abomasums or digestive problems, no general infections or foot and leg problems. Previous studies have linked liberal pre- and post-partum grain feeding to increased disease but not always with statistical significance. The type of disease varies from trial to trial suggesting

a nonspecific mechanism, (Armstrong et al., 1966); Emery et al., 1969; Trimberger et al., 1972; Belyea et al., 1975a). It is possible that feeding excess energy lowers resistance to disease in some way.

Rectal temperatures in all groups declined as lactation advanced and reached 'normal' levels at approximately 9 weeks. It is tempting to suggest that HE groups had higher temperatures because of higher incidence of disease, especially since after 9 weeks, disease incidence was practically zero and temperatures were 'normal'. Both HE and LE groups had elevated temperatures at the beginning of lactation which is consistent because both groups had a fairly high incidence of disease at this time. However, if one examines heifers with a zero disease score, (no clinical disease problems), which also have rectal temperature measurements then HE group heifers still had higher temperatures than LE heifers for the first 4 weeks of lactation and in both groups temperatures were elevated above normal. These limited data suggest that energy status rather than disease per se affected temperature. Also, temperatures at beginning of lactation seemed to be above normal regardless of nutritional regime or health status. This elevation is probably not due to increased feed since feed intake was increasing as temperatures were declining. It could be that temperature effects observed were due in part to health and in part to energy status and attempts of cows to dissipate extra heat from feed. As far as the author is aware, temperature elevation at the beginning of lactation has not been previously examined.

Subsequent culling data support excess energy status-health problem associations. Three times more heifers were culled from the original HE groups than LE groups and most of these heifers culled in HE

groups were for reasons of poor condition and health or udder problems. Heifers culled in LE groups were all for fertility problems.

In addition to health problems, heifers fed energy in excess of requirements were apparently not as efficient in utilization of energy in feeds offered as heifers deficient in energy. This is probably another reason why heifers fed excess energy did not yield more milk. Some of the extra energy was partitioned into body tissue since HE groups on average gained weight. Failure, however, to find a perfect correlation between weight change and energy status must be due to changing body composition and to variation in efficiencies of nutrient utilization. Calculations indicated that the average HE group heifer partitioned less than 10% of the excess energy fed to body tissue. The rest was presumably wasted since it was obviously not partitioned to the mammary gland. Weight losses in this study were quite similar to those reported by Gordon (1977) for cows fed low and high amounts of concentrates and to those reported by Belyea et al. (1975b) for cows in early lactation fed to meet requirements.

The beneficial effects of feeding limited rations early in lactation have previously been reported. 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 (Thomas et al., 1974). These data however, disagree radically with work by Broster et al. (1975) who found a marked response over the full lactation to extra food given in weeks 1 to 4 of lactation in first calf heifers. Broster et al. (1975) concluded that level of intake in the first few weeks of lactation appeared more critical in increasing yield than supplementary

feeding for the first several weeks of lactation. Heifers in the above study were fed three times daily and this may have helped utilization of food offered in early lactation. In addition, Broster had no group that was restricted in feed and then switched to high energy feeding to compare with data in the present study.

These milk production and health data strongly suggest that heifers should be fed rations moderate in energy immediately after calving, gradually building up their energy intake to a high level by 4-5 weeks of lactation. Phosphorus should be fed according to recommendations during this time. These data suggest there can be no justification for feeding in excess of energy or phosphorus requirements in early lactation. If rations are fed to these recommendations milk yield should be optimized and health problems minimized. Management systems are presently available that would enable these recommendations to be followed.

Now, responses of various serum metabolites to energy or phosphorus status will be discussed. Within the range of phosphorus intake or status in this study serum P did not reflect either very well. In extreme conditions serum P may decrease by 50%, but in the present study serum P only decreased by 11% in LP groups. No symptoms of phosphorus deficiency were noticed. LELP groups in both trials (which were approximately 85% N.R.C. for P) had the lowest mean serum P concentrations. According to data of Tumbleson et al. (1973) LELP group values are rather low for 3 year old Holsteins. These authors quoted mean serum P of around 7.0 mg/dl for heifers for this age. Previous studies which involved experimental or accidental severe deficiencies reported much lower values. Eckles et al. (1935) reported 2.5 mg/dl in phosphorus deficient cows 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 fed phosphorus deficient rations (Morrow, 1969). In the present study cows fed phosphorus deficient rations were consuming 45-65 g/day, considerably more than in the previous studies cited. They were, however, producing 2 or 4 times as much milk as cows studied by Eckles et al., (1935) or Palmer et al., (1941). In a recent study it was concluded that cows fed at or greater than N.R.C. for phosphorus did not reflect differences in intake very well (Belyea et al., 1975a). Cows in the present study were at most 15% below N.R.C. requirements and serum P concentrations did not indicate that there was a deficiency problem of any proportions. This means that serum P will not indicate cases of anything but severely inadequate P intake in a practical situation. Feed analysis and actual calculation of intake and requirements would be needed.

Serum Ca was inversely related to serum P. Serum Ca concentrations generally decrease as phosphorus status and serum P increase. The negative correlation between Ca and P has been previously reported (Symonds and Manston, 1974). Serum Ca concentrations were quite stable and differences between groups were small. In all but the HEHP group in trial 2, serum Ca concentrations gradually increased as lactation progressed as previously shown by Ward, Blosser and Adams (1952), Belyea et al.,

(1975c) and Belyea et al., (1975a). Concentrations in the present study were comparable to those in other reports cited. In the short term, anyway, both serum P and Ca did not appear to be detrimentally affected by the range of P intake in this study. Body weight change, serum glucose, certain VFA's, insulin and GH all responded to changes in energy status. If these parameters were measured in a herd with apparent nutritional problems however, it would be difficult to accurately predict that energy was limiting or in excess in the rations.

Serum glucose concentrations were a better indicator of energy status in the first trial than the second. In general, cows fed excess energy had significantly higher serum glucose levels than those deficient in energy. In the second trial, however, serum glucose increased also with excess phosphorus. Glucose concentrations have previously been associated with dry matter intake and estimated net energy intake in the first 2 months of lactation (Smith et al., 1976), increasing amounts of concentrates (and therefore energy) in diets (Blair et al., 1974; Jenny et al., 1974). In the present studies glucose was correlated with grain intake ($r = 0.21$, $P < .05$) but was not correlated with hay or corn silage intake. Glucose concentrations, however, did not significantly increase or decrease during the first 3 months of lactation confirming reports of Paterson and Linzell (1974) and Halse et al., (1976) but contradicting Blom and Halse (1975), Smith et al., (1967) and Gorewit et al. (1977) who reported increased glucose concentrations in the first 3-4 months of lactation. These differences are probably due to experimental design, time and frequency of sampling and nutrient regimes. LE groups were not hypoglycemic and the slightly lower serum glucose concentrations of the LE groups did not appear to limit milk production.

Not all serum VFA responded to changes in energy status. Serum C_2 was not different among groups, neither did regression analysis indicate a significant relation between C_2 and energy status. This agrees with a previous report that cows fed high or normal levels of grain did not differ in serum C_2 concentrations (Walker and Elliot, 1973). In contrast, however, other reports (Huber et al., 1969; Evans et al., 1975) found a 20% decrease in C_2 concentrations in cows fed high grain, restricted roughage diets. It is possible that since heifers in the present study were not roughage restricted that sufficient C_2 fermentation occurred in the rumen in both groups so that C_2 levels did not change. C_2 values for cows in this study are similar to those previously reported for lactating cows fed normal diets (Huber et al., 1969; Emery et al., 1969; Annison and Armstrong, 1970; Annison and Linzell, 1974; Baird et al., 1975; Evans et al., 1975; Schwalm and Schultz, 1976).

Serum C_3 increased as energy status increased, however, by analysis of variance phosphorus groups were also significantly different. Serum C_3 was also positively correlated with grain intake ($r = 0.29$, $P = .05$). This confirms previous work where serum C_3 increased 45% with a low roughage diet (Evans et al., 1975) but does not agree with another study (Walker and Elliot, 1973). Means in the present study were lower than in the studies just cited (also Bickerstaffe et al., 1974 and Baird et al., 1975). Differences between heifers fed high and lower amounts of concentrates were presumably due to greater C_3 production ruminally and subsequent greater absorption into the blood. Also, since more glucose would probably be absorbed from the intestines with concentrate by-pass, gluconeogenesis may have decreased somewhat and C_3 may have been spared, hence increasing in serum.

Serum $isoC_4$ did not reflect energy status but was increased in LP groups compared with HP groups. Serum $isoC_4$ has previously been shown to increase 56% when roughage was restricted and replaced with grain (Evans et al., 1975). This increase was possibly due to increased valine degradation in the rumen. Values for $isoC_4$ in the present study were about one tenth as high as those reported by Evans et al. (1975) and its relationship to energy or phosphorus status is not clear. It appears that when HE was combined with excess phosphorus, $isoC_4$ levels were depressed, but when HE was combined with phosphorus below or around requirements $isoC_4$ was increased (over either of LE groups). This accounts for the significant interaction term in the analysis of variance.

It is not apparent that serum VFA in these studies affected performance of different treatment groups in early lactation. However, their relationship to serum insulin was of interest and will be discussed later in this section.

In the first trial insulin was a very sensitive indicator of energy status. Differences in energy groups were easily noticeable. Cows fed excess energy had serum insulin concentrations three times as high as cows fed deficient energy. These differences were in part due to excess energy but were also accentuated by sampling close to feeding time. Response to high energy or grain feeding and increases in serum insulin have been previously reported (Walker and Elliot, 1973; Blair et al., 1974; Jenny et al., 1974 and Evans et al., 1975). Increases in insulin postprandially are also well documented (Trenkle, 1971a; McAtee and Trenkle, 1971a; Ross and Kitts, 1973; Bassett, 1974a, 1974b, 1975; Jenny and Polan, 1975; Evans et al., 1975).

In the second trial, although serum insulin increased with energy status and differences between HE and LE groups were significant, they were not as great as in the first trial (increases of 28% compared to 300-400%). This may partly be due to feeding schedule changes between first and second trials resulting in insulin samples being taken many hours postfeeding. This may also account for why values in the second trial are low compared with other reports, even for primiparous cows (Koprowski and Tucker, 1973a; Trenkle, 1972; Jenny and Polan, 1975; Walker and Elliot, 1973; Evans et al., 1975). Values were comparable, however, to those reported by others (Smith et al., 1976; Schwalm and Schultz, 1976; Blair et al., 1974; Gorewit et al., 1977).

Insulin has been reported to increase with time after parturition (Koprowski and Tucker, 1973a; Smith et al., 1976; Gorewit et al., 1977), in the present studies only the LELP group in the first trial and the HEHP group in the second trial significantly increased with time, the rest did not significantly increase or decrease. This would suggest that insulin may not follow intake or milk yield as well as previously reported.

In the first trial insulin was negatively correlated with milk yield ($r = -0.17$, $P = .05$), in the second trial the correlation was not significantly different from zero ($r = 0.13$, NS). Other studies which have shown or implied a negative relationship between insulin and milk yield are numerous (Kronfeld et al., 1963; Schmidt, 1966; Koprowski and Tucker, 1973a; Jenny et al., 1974). In contrast, a positive relationship is implied by data from Baldwin et al. (1973) in which cows treated with insulin did not experience a fall in production whereas milk yields of untreated controls fell 18% in the same period. Apparent contradictions

among these trials may be due to variation in energy status and glucose concentrations of the animals and other unknown factors. At any rate, in the present study, insulin was not well correlated with milk yield in either experiment. This suggests that insulin concentrations do not directly influence milk yield in lactating cows.

In the first trial insulin was negatively correlated with milk fat percent, ($r = -0.33$, $P = .05$) confirming reports by others (Walker and Elliot, 1973; Blair et al., 1974; Jenny et al., 1974). In the second trial, insulin and milk fat were not significantly correlated. Insulin, as expected, was related to other variables, which will be discussed further later in this section.

Serum GH, although taking some weeks, did seem to respond to increasing energy status by decreasing in concentration in an opposite manner to the response of insulin. These data suggest that in response to excess energy supply metabolic demands of HE cows declined and GH decreased in response to this. In LE heifers on the other hand, metabolic demands of lactation in the face of deficient energy supplies were considerable and GH remained at normal postpartum levels possibly to facilitate endogenous energy mobilization.

GH has previously been reported to decline as lactation advanced, presumably due to decreasing metabolic demands as feed intake increased and milk yield declined (Koprowski and Tucker, 1973a; Halse et al., 1976; Smith et al., 1976). None of the cows in the above studies were fed restricted diets. LE groups in the present study did not significantly increase or decrease, suggesting that GH only declines in response to increasing and sufficient energy intake. GH was negatively correlated with dry matter, estimated net energy intake and digestible organic

matter intake (Bassett et al., 1971; Smith et al., 1976). Increasing GH has been previously associated with increasing metabolic demands (Bassett, 1975; Trenkle, 1978). Data from the present studies support the hypothesis that GH increases and insulin decreases in time of greater metabolic demand. It is possible that GH and insulin responded as they did to maintain endogenous energy supplies. In HE groups, higher insulin and lower GH would favor substrates moving into tissue for synthesis and storage, whereas in LE groups, low insulin and higher GH would favor endogenous mobilization of substrates necessary to maintain milk production. The fact that insulin responds so quickly and GH much more slowly to dietary changes suggests that insulin responds to energy intake whereas GH responds to changes in energy status. Undoubtedly, glucagon, corticoids and other hormones are involved in this regulation of nutrient supply. Knowledge of optimum hormonal status for milk production presents opportunities to improve milk yield in future work.

GH was not correlated with serum glucose concentrations ($r = -0.075$), Halse et al. (1976) did not find a high correlation between these variables either ($r = -0.15$, $P = .05$). GH was negatively correlated with milk yield ($r = -0.23$, $P = .05$), this does not agree with results from Koprowski and Tucker (1973a) who reported no significant correlation between GH and milk yield early in lactation, (up to 12 weeks) although correlations were largely negatively, especially after 24 weeks postpartum. GH concentrations in this study were comparable to those reported by other workers for early lactation cows, (Koprowski and Tucker, 1973a; Convey, 1974; Reynaert et al., 1976; Halse et al., 1976) but much lower than those reported by Smith et al. (1976).

Results from this investigation help explain regulation of serum insulin. Physiological concentrations of serum C_3, C_4 and $isoC_4$ (but not C_2), may be involved in insulin regulation. These data are supported by the studies of Ross and Kitts (1973) who concluded that circulating C_4 and $isoC_4$ were the most important VFA with respect to postprandial insulin concentrations in sheep. VFA concentrations from that study were physiological and very similar to levels in the present study.

Why GH was retained and energy status, phosphorus status, milk yield, acetate and glucose were deleted from the equation is not clear. There is a possibility that GH and insulin may directly influence each other, perhaps at the receptor level. The fact that GH responds so slowly, however, and insulin responds so quickly to energy or metabolic status changes weakens the suggestion of a direct effect. If GH and insulin indirectly affect each other, the mediator in this indirect effect is not obvious. It cannot be glucose since GH and glucose were not correlated and glucose was deleted from the equation anyway. Neither is it likely to be glucose entry rate since this is highly related to milk yield and GH was not strongly correlated with milk yield and milk yield was also deleted from the equation. Alternative suggestions are that GH is reflecting the amount of protein digested. Bassett et al. (1971) found a very high correlation between insulin concentrations and protein digested in the small intestine, additionally, insulin and GH were highly negatively correlated. It is however, not likely that in the present study that there was enough difference in protein reaching the lower gut to account for these changes in insulin and GH concentrations. Finally, it may be that GH varies with insulin merely because GH is decreasing and insulin increasing with advancing lactation.

Evidence from these experiments suggest that energy and phosphorus status did not affect postpartum reproductive function in any dramatic way. Heifers fed HE rations ovulated for the first time only 4.5 days prior to heifers fed LE rations and only by regression analysis did this relationship approach significance (P = .07). There were no significant differences in time postpartum to reach 3 ng/ml progesterone, LP groups, however, took 9 days longer than HP groups. Heifers usually ovulated once before reaching this level of progesterone, average time between these events were 18 days. Using the criteria of Folmann et al. (1973), Corah et al. (1974) and Rosenberg et al. (1977) this would mean that only the second ovulation in these heifers would be viable for conception. In order to test the hypothesis that serum progesterone concentrations should be at least 3 ng/ml for conception to occur, data from this study were examined. Progesterone peaks preceding inseminations resulting in pregnancy or non-pregnancy were tabulated. As a result 41 and 16 peaks occurring prior to non-pregnancy and pregnancy were recorded. Means were similar, 5.74 and 5.07 ng/ml, respectively. However, no heifer with a serum progesterone peak below 2.7 ng/ml became pregnant to the subsequent insemination. Peak progesterone before nonconceiving inseminations ranged from 0.7-10.71 ng/ml progesterone. Thus, although there appeared to be no guarantee that 3 ng/ml would result in pregnancy, there was a suggestion that at least that much was needed to permit conception, confirming reports by previously cited authors that a critical amount of progesterone is needed for conception. Heifers in the study of Rosenberg et al. (1977) ovulated for the first time at 27 days postpartum (some time after multiparous cows) and this compares well with data from the present study. Also, heifers in Rosenber'g study reach 3.4 ng/ml of

progesterone at a time comparable to those in the present study (40-50 days). When best, worst and control genetic groups were examined, days to first ovulation were 27.0, 27.5 and 25.9 days, respectively. Thus, as far as designated genetic potential there was very little difference.

There were no differences in days to conception which could be attributed to energy or phosphorus status or intake differences. This confirms one other study with dairy cows (Gardner, 1969b) but contradicts several other reports where deficient energy rations did lengthen days nonpregnant (Wiltbank et al., 1962, 1964; McClure, 1968a; Dunn et al., 1969; Bellows et al., 1972; Drew et al., 1976; Randel and Welker, 1977; Macfarlane et al., 1977). These studies, however, were either conducted with beef cows or dairy cows under marginal nutritional conditions where energy status was at a more critical level.

No evidence was found that lower phosphorus intake delayed pregnancy which confirms previous work in which experimental phosphorus deficiency, (where blood values of P were half normal), did not impair ovulation or conception (Palmer et al., 1941). Severe phosphorus deficiency has been implicated with fertility problems (Eckles et al., 1935; Morrow, 1969) but heifers in the present experiment were on average, at N.R.C. recommendations for P. Serum P concentrations within a wide range have not been shown to influence fertility (Parker and Blowey, 1976; Rowlands et al., 1977). Experiments with beef cows where phosphorus supplementation improved fertility (Williams et al., 1971; Taylor et al., 1976; Ritson et al., 1971) suggest that published P requirements for beef cows are too low and also that cows were supplied with greatly insufficient phosphorus to start with. Other studies with similarly poor nutritional conditions have not shown improvements in fertility

after phosphorus supplementation (Ward, 1968; Teleni et al., 1977). There was no evidence in the present study that excessive phosphorus was associated with infertility as suggested by Hewett (1974). In all cases, if anything, phosphorus at recommended levels caused problems. In this study serum P concentrations were negatively correlated with days to 3 ng/ml serum progesterone ($r = -0.34$, $P = .05$). Although serum P was not correlated to days to ovulation, ovulation and time to reach 3 ng/ml progesterone were highly related.

There were no significant differences among treatment groups in inseminations per conception, although, three times as many HE heifers did not become pregnant compared with LE heifers. These differences in pregnancies may have been the result of management rather than nutritional problems, since some heifers became pregnant after treatment ended.

There were no dramatic differences in number or percent undetected estrus among treatment groups. The trend was that groups fed excess phosphorus had slightly less undetected estrus than heifers fed to requirements. Phosphorus deficiency has previously been associated with silent estrus (Palmer et al., 1941), temporary sterility (Sheehy, 1946) and 'anestrus or estrus with repeated failures to conceive after service', (O'Moore, 1950). It must be remembered, however, that these were apparently cases of severe deficiency where serum P concentrations were depressed as much as half. Morrow (1969) did not observe silent estrus in heifers in his study.

There was no evidence that treatments affected subsequent ovulations once cyclic behavior was established. Intervals to second and third ovulations were normal (20.6 and 21.2 days). Undernutrition has been

quite severe (85% of maintenance) in cases where estrous cycle length was altered (Hill et al., 1970). HE groups had 0.4 fewer ovulations that LE groups in the 15 week experimental period. This difference approached significance at the P = 0.1 level. At any rate decreases in ovulations in HE groups could not be attributed to pregnancies since more HE group heifers were open at this time than LE groups (16 and 13 open, respectively).

As previously summarized in the literature review several hypotheses exist as to how nutrition or other factors influence reproduction. Several workers have suggested that increases in body weight were not important for ovulation and conception (King, 1968; Schilling and England, 1968; McClure, 1970; Folman et al., 1973; Wiltbank, 1974; Youdan and King, 1977; Holness et al., 1978). In the present study there was little evidence for differences between groups in time to first ovulation. Thus, although on average HE groups gained weight and ovulated a few days sooner than LE groups, which on average lost weight, there was much variability and evidence is certainly not conclusive. This confirms reports by several workers (Gardner, 1969b; Boyd, 1972; Broster, 1973; Downie and Gelman, 1976 and Stevenson, 1977) that body weight change does not play an important role in postpartum reproduction under normal circumstances.

High milk production has often been cited as a cause of delayed first estrus and ovulation in postpartum cows (Morrow et al., 1966; Hewett, 1968; Whitmore et al., 1974; Brauner, 1975; Stevenson, 1977). Since there was no significant difference in milk production between energy groups for the first 8 weeks of lactation this cannot be used as a reason for HE and LE groups differing in ovulation times (which they did not, significantly). There were no differences among phosphorus groups in

time to first ovulation even though LP groups gave significantly more milk from weeks 5-12 than HP groups. This confirms conclusions of King (1968) and Simpson (1972). Heifers in the present trials, however, were not excessively high producers (average 305 day yield was 5908 kg) and this may be one reason for lack of a correlation between these variables.

Francos (1968, 1969) suggested that 3.3% milk fat was required for optimum fertility. Since all groups, on average, in this study met this criterion, low milk fat and not enough roughage in the ration cannot be cited as a reason for fertility problems.

Hypoglycemia has received much attention in association with reproductive hypofunction (McClure, 1965, 1967, 1968a, 1968b, 1972; Howland et al., 1966). In neither trial in this study was there very much difference in glucose concentration between groups and neither would they be considered hypoglycemic. In this study the only data suggesting that glucose might be involved was that serum glucose was negatively correlated ($r = -0.33$, $P = .05$) with days to reach 3 ng/ml progesterone which, as already discussed, may be involved in conception. Similarly, Oxenreider and Wagner (1971) found a negative correlation between glucose and postpartum interval to occurrence of a 10 mm follicle and ovulation in primiparous dairy cows. McClure and Payne (1978) found that non-return rates increased as blood glucose concentrations increased. In contrast, Blowey and Davis (1973) and Hewett (1974) found no relationship between glucose concentrations and fertility after extensive study of many herds.

Most reports that energy status might alter levels of reproductive hormones can neither be confirmed nor denied since only progesterone was measured in these experiments. Since there were no dramatic differences

in other reproductive functions measured it seems unlikely that progesterone was altered significantly by either energy or phosphorus status. Previous reports would suggest that undernutrition or energy deprivation had to be quite severe to effect changes in progesterone concentrations or corpus luteum size and function (Hill et al., 1970; Donaldson et al., 1970; Gombe and Hansel, 1973). In fact recent evidence, where beef heifers were fed one-third of recommended energy, showed no differences in blood levels of progesterone or LH, number of follicles nor follicular or luteal volume. However, the ovary containing the corpus luteum was 57% larger in heifers fed adequate energy than in those restricted in energy intake (Spitzer et al., 1978). Reduced pregnancies in heifers restricted in energy appeared not to be a fertilization failure, but due to some later causative factor.

Finally, there was no evidence that heifers fed LE in this present study had a higher incidence of fertility disorders compared with HE groups as suggested by Francos (1974).

CONCLUSIONS

Cows supplied with excess phosphorus yielded less milk throughout the first 3 months of lactation than those fed just below recommendations. Phosphorus in excess of recommendations may have had negative effects on milk production. Cows fed excess energy did not begin to yield more milk than cows fed deficient energy until the fifth week of lactation. By eight weeks cows fed excess energy had greater persistency than those deficient in energy. It is not clear why excess energy did not improve milk production further. Health may have contributed to this since cows fed high energy rations had almost twice the incidence of disease of those fed deficient energy. Incidences of mastitis and metritis were similar among energy groups. Heifers fed excess energy, however, had more other types of disease and also higher rectal temperatures than those fed deficient energy rations. All heifers, however, had elevated temperatures at the beginning of lactation. In addition, three times more heifers were culled after first lactation from groups fed excess energy for three months postpartum compared with those fed deficient energy. Differences between phosphorus groups in milk production persisted throughout the 305 day lactation. Whether these differences in culling and total production were a result of treatments imposed in early lactation is not know.

It is suggested that heifers should be fed rations moderate in energy immediately after calving, gradually building up their energy

intake to a high level by 4-5 weeks of lactation. During this time phosphorus should be fed at recommended levels. These observations concerning negative effects of excess phosphorus and energy on production and health warrent further investigation.

Body weight, serum glucose, insulin and propionate increased with increases in energy status. None of these alone, except postprandial insulin concentrations, would be suitable for diagnostic purposes, how ever, since differences would be too small to detect by sampling just a few animals. Serum growth hormone, after some weeks, seemed to respond to increasing status by significantly decreasing. Growth hormone decreased in cows fed excess energy, possibly due to declining metabolic demands. By eight weeks postpartum growth hormone was significantly lower in cows fed excess energy. In cows fed deficient energy growth hormone did not significantly change. Serum acetate was not different among groups. Serum butyrate and isobutyrate were not consistently related with energy or phosphorus status.

Serum phosphorus was the only indicator of phosphorus status and it showed that a marginal phosphorus deficiency was accomplished at least in the low energy, low phosphorus groups. Serum calcium was inversely related to serum phosphorus.

Results from this investigation help explain regulation of serum insulin. Physiological concentrations of serum propionate, butyrate, isobutyrate and growth hormone may be involved in insulin regulation. Whether growth hormone has a direct or indirect effect on insulin is not known. These parameters only accounted for 26% of variance in serum insulin concentrations and thus were not the only variables involved but these data add to evidence implicating serum VFA in insulin regulation.

Energy and phosphorus status did not affect postpartum reproductive function significantly. Progesterone data indicated that no heifer with peak progesterone prior to insemination below 2.7 ng/ml conceived to that insemination. Days to reach 3 ng/ml serum progesterone was negatively correlated with serum phosphorus and glucose. Examination of estrous cycles and pregnancy data indicated that milk yield, body weight change and hypoglycemia did not affect postpartum reproduction.

APPENDIX

Table A1.--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

Table A2.--Dry matter, net energy and phosphorus values used to calculate NE intake and phosphorus intake for trial 1.

Feed	Dates Value Used	Dry Matter (%)	Net Energy (Mcal/kg)	Crude Protein (%)	Phosphorus (%)
HEHP Grain	Throughout	87.00	1.96	16.4	0.636
HELP Grain	Throughout	86.00	1.96	18.4	0.397
LEHP Grain	Throughout	88.00	1.63	47.3	1.773
LELP Grain	Throughout	87.00	1.54	49.7	0.685
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	0.271
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	0.258
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

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

Table A3.--Dry matter, net energy and phosphorus values used to calculate NE intake and phosphorus intake for trial 2.

Feed	Dates Value Used	Dry Matter (%)	Net Energy (Mcal/kg)	Crude Protein (%)	Phosphorus (%)
HEHP Grain	11/8/75-1/17/76	88.8	2.16	12.6	0.48
HEHP Grain	1/18/76-end exp.	89.8	2.33	15.3	0.68
HELP Grain	11/8/75-1/17/76	88.5	2.20	16.9	0.42
HELP Grain	1/18/76-end exp.	89.8	2.33	17.0	0.32
LEHP Grain	Throughout	90.2	2.02	51.6	1.32
LELP Grain	Throughout	90.3	2.07	50.9	0.63
Hay	11/8/75-4/11/76	91.4	1.19	14.9	0.36
Hay	4/12/76-end exp.	95.9	1.19	15.4	0.28
Corn Silage	11/8/75-2/9/76	24.3	1.69	12.4	0.49
Corn Silage	2/10/76-end exp.	29.9	1.69	10.6	0.34

ALL NE, P and CP % values expressed on a dry basis.

Table 4.--Simple correlations from both trials.

Variables	r
E Status x P Status	0.64
E Status x Glucose	0.30
E Status x Insulin	0.38
E Status x Fat %	-0.36
E Status x Serum P	-0.27
P Status x Serum P	0.17
P Status x Insulin	0.20
P Status x Milk	-0.23
P Status x Protein %	0.33
Serum Ca x Serum P	-0.43
Serum Ca x Glucose	-0.41
Serum Ca x Fat %	0.17
Serum P x Glucose	0.46
Serum P x Fat %	0.40
Glucose x Fat %	-0.35
Glucose x Wt. change	0.33
Glucose x Protein %	0.18
Insulin x Fat %	-0.32
Insulin x Protein %	0.26
Milk x Wt. change	-0.42
Fat % x Wt. Change	-0.41
Temperature x GH*	0.22
Temperature x Fat %*	0.28
Temperature x C_2*	-0.28
GH x Serum Ca*	-0.18
E Status x C_3*	0.28
E Status x C_4*	0.35
Serum P x Temperature*	-0.25
Fat % x isoC_4*	-0.20

*These variables from trial 2 only, ($P \leq .05$), ($r = 90\text{-}144$). All other variables reported from both trials combined ($P \leq .05$), ($r = 288$, except where wt. change used, $r = 48$).

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