AN ABSTRACT OF THE THESIS OF

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Title: RELATIONSHIP OF DIETARY CRUDE PROTEIN TO FACTORS IN UTERINE SECRETIONS AND PLASMA IN HIGH PRODUCING POSTPARTUM DAIRY COWS

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Eighteen multiparous, high producing (7,836 kg 4% FCM) Holstein dairy cows were assigned randomly to isocaloric rations containing either 12 or 23% crude protein (CP) on day 40 postpartum. After a 10-day adaptation period, uterine secretion and blood samples were collected on the day of estrus (day 0-1), day 5 and day 15 of the first estrous cycle after day 50 postpartum and on the day of estrus (day 0-2) of the subsequent estrous cycle. Days to first postpartum estrus, days to first experimental estrus, days from first to second experimental estrus, bodyweights, parity and feed intake did not differ between treatments. Cows fed 23% CP required a 1.9-fold increase ($P<.06$) in services per conception (SPC) the year of the experiment as compared to the preceding years, but cows fed 12% CP required the same number of SPC. The ammonia concentration in plasma of cows fed 23% CP was elevated (8.0 ± .5 µg/ml) over cows fed 12% CP (6.3±.3 µg/ml; $P<.01$). Total protein concentration in the uterine secretions

increased 88% from day 0-1 (8.9±1.5 mg/ml) to a peak at day 15 (16.7±1.7 mg/ml; P<.05) and then declined again by day 0-2 (4.6±.7 mg/ml), although it did not change with treatment. Plasma urea concentration was 3.5-fold lower in cows fed 12% CP than 23% CP rations (4.8±.3 and 16.8±.5 mg/100 ml, respectively; P<.01). Uterine secretion urea concentration was 2.7-fold higher in cows fed 23% CP (17.2±1.1 vs. 6.4±.7 mg/100 ml). Plasma glucose concentration was lower on days 0-1 and 5 than on days 15 and 0-2 (60.9±.7 and 65.9±1.4 mg/100 ml, respectively; P<.01), but did not differ with treatment. Protein level did not affect actual milk production, % milk fat or 4% FCM. Uterine secretion Ca was higher during the luteal phase (159.0±11.1 ppm), days 5 and 15, than during estrus (81.5±4.0 ppm; P<.01), but did not vary with treatment. Plasma Ca (105.1±1.1 ppm) and Mg (23±.3 ppm) did not change with time or treatment. Cows fed 12% CP had higher Mg in the uterine secretions on day 5 and 15 of the estrous cycle than cows fed 23% CP (66.7±11.1 and 38.6±4.3 ppm, respectively; P<.01). Plasma P was higher (P<.01) in cows fed 23% CP (6.1±.2 mg/100 ml) than in cows fed 12% CP (5.2±.2 mg/100 ml). However, uterine secretion P was higher in the 23% CP group (6.9±1.1 vs. 5.3±.8 mg/100 ml; P<.01), but only on days 5 and 15 of the estrous cycle. Plasma Zn concentration declined from 1.4±.2 to 1.1±.1 ppm

from the first to second experimental estrus in cows fed 12% CP, but rose from 1.2±.1 to 1.4 ±.1 ppm during the same interval in cows fed 23% CP. Uterine secretion Zn declined from .92±.13 to .41±.04 ppm in cows fed 12% CP and from .72±.12 to .52±.10 ppm in cows fed 23% CP from the first to second experimental estrus. Cows fed 23% CP had higher plasma K concentrations than cows fed 12% CP (224±3 and 216±2 ppm, respectively; P=.06). Potassium was higher in the uterine secretions of cows fed 12% CP on days 5 and 15 (1059±124 µg/ml) as compared to cows fed 23% CP (799±61 µg/ml; P<.05). Plasma progesterone was elevated during the luteal phase, but no treatment differences were observed in the peak concentration, although the progesterone level was 19% lower in the cows fed 23% CP on day 15 of the estrous cycle. The interval from estrus to peak progesterone and the number of days the plasma progesterone concentration was <1 ng/ml did not differ with treatment. The decreased fertility without lengthened estrous cycles suggests excess dietary CP affects events preceding implantation, such as ovum or sperm viability, fertilization or early embryonic cleavage and survival. Further, these results indicate protein and its metabolites alter the uterine secretions by decreasing P, Mg, and K the luteal phase of the estrous cycle in cows fed 23% CP relative to cows fed 12% CP.

Relationship of Dietary Crude Protein to Factors in Uterine Secretions and Plasma in High Producing Postpartum Dairy Cows

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CHAPTER 1

INTRODUCTION

Milk production from cows on Dairy Herd Improvement Association test has increased 37% in the past 20 years, from 4,907 kg/cow/yr in 1960-61 to 6,721 kg/cow/yr in 1979-80. During the same time interval the number of cows/herd has increased 78%, from 45 to 80 (King et al., 1980). The combination of increased milk production, larger herds and total confinement housing has created a stress contributing to the decreased reproductive efficiency noted by dairy producers.

As milk production has increased, dairy producers have also increased crude protein (CP) and energy levels to their dairy cow rations by feeding higher proportions of concentrates. Studies have been conducted in beef and dairy cattle to evaluate the effects of varying levels of protein and energy supplementation pre- and post-partum on reproductive parameters such as days open, services per conception (SPC) and days to first postpartum estrus. Most nutrition research has evaluated the effects of protein and energy, or energy alone. The effect of protein on reproduction was evaluated at Oregon State University recently by feeding isocaloric rations with three

different levels of CP (Jordan and Swanson, 1979a). Days open and SPC were increased in animals fed 19.3% CP compared to animals fed 12.7% CP.

This study was designed to evaluate changes in the reproductive tract secretions and plasma between cows fed low and high levels of dietary CP, which would influence the aforementioned reproductive parameters. Since previous workers have shown uterine secretions vary due to hormonal influences, a second objective was to define changes in uterine secretion composition during the estrous cycle, as affected by protein level.

This study should provide a foundation for future studies to elucidate the cause and determine the mechanism by which high levels of dietary CP reduce reproductive efficiency in dairy cows. For example, urea can prevent the initiation of epididymal sperm motility (Turner and Howards, 1978). If urea levels in the uterine secretions are elevated by high CP, its effect on sperm motility would provide information at the mechanistic level. Research investigating the effect of nutrition on reproduction, with consequent improvements in fertility, can be of economic benefit to the dairy industry. An additional economic benefit can be gained from reduction in feed costs as dairy producers reduce the CP level of their

rations from the 19% CP rations currently fed by some to the 16% CP rations recommended by the National Research Council (1978).

CHAPTER 2

REVIEW OF LITERATURE

Protein and Energy Effects on Reproduction

In past years, many researchers have examined the effect of pre- and post-partum nutrition on reproductive efficiency. Dunn et al. (1969) reported the detrimental effect of feeding low levels of energy (8.7 megcal. digestible energy) to beef cows prepartum was manifested postpartum as a delay in the onset of first estrus. However, the 120-day pregnancy rate in beef cows was related directly to the postpartum energy level, with 87% of the animals on a high energy ration (48.2 megcal. digestible energy) conceiving, but only 72% on a moderate energy level (27.3 megcal. digestible energy) and 64% on a low energy level (14.2 megcal. digestible energy) conceiving by 120 days postpartum. Failure to exhibit estrus was a major cause for the low pregnancy rate in the cows fed low levels of energy postpartum (Dunn et al., 1969).

Folman et al. (1973) reported that lactating dairy cows on a high plane of nutrition (6 kg hay and ad libitum concentrates) required fewer inseminations and conceived 19 days earlier than cows maintained on a standard plane of nutrition (6 kg hay and concentrates for production). The cows on the high plane of nutrition

started cycling 23 days earlier than cows maintained on a standard plane of nutrition. In cows which conceived to the first insemination, nutritional level did not affect plasma progesterone concentration during the estrous cycle preceding insemination. However, in cows which did not conceive, progesterone concentrations from day 8 to 15 of the estrous cycle preceding the first ineffective insemination were significantly higher in animals on the high plane of nutrition.

Effect of plane of nutrition has been studied in other ruminants such as the ewe. In 1966, Howland et al. reported ewes fed hay and 0.9 kg grain/head/day had more corpora lutea (1.7) than ewes fed only hay (1.4). In addition, ewes fed hay and grain had more follicles with a diameter >4 mm, larger volumes of follicular fluid and larger anterior pituitaries.

The effects of protein alone have not been examined thoroughly in cattle. Jordan and Swanson (1979a) reported high producing dairy cows fed 19.3% CP had more SPC and a greater number of days open than did cows fed 12.7% CP. In animals fed 19.3% CP, serum progesterone was lower on day 14 during the first observed cycle postpartum (3.5 vs. 4.9 ng/ml) and during the cycle of conception (3.4 vs. 4.5 ng/ml) in comparison to animals fed 12.7% CP. Results from Drori and Folman (1971) were similar in that cows fed

11.5% CP were open 88 days compared to 98 days when cows were fed 16% CP. Only 10% of the cows fed 11.5% CP were culled for sterility while 29% of the cows fed 16% CP were culled for sterility.

When Julien et al. (1977) fed dairy cows high levels of CP (15%) prepartum, a higher incidence of general health problems (i.e. abortion, parturient paresis and displaced abomassum) and metabolic disorders (10-fold increase) was observed. Hibbitt et al. (1969) observed that cows fed a high level of dietary protein had an increase in the incidence of ketosis, a syndrome occurring in negative energy balance, and had lower blood glucose levels. Declining blood glucose has been associated with reduced fertility by some workers (Downie and Gelman, 1976).

Sonderegger and Schurch (1977) analyzed herd fertility records from six dairy farms to determine the influence of energy and protein supply on fertility. Digestible protein surpluses (>250-300 g digestible protein/cow/day) lengthened the interval between parturition and first service. In contrast, high levels of energy, particularly the first 60 days postpartum, decreased the interval from

first service to conception and from parturition to conception.

In the ewe, Shelton and Huston (1968) reported high protein (.13 kg digestible protein daily) tended to have an adverse effect on reproduction in a high temperature environment when compared to low protein (.08 kg digestible protein daily). The ewes fed low protein had heavier lambs and a lower lamb mortality. They hypothesized that the higher heat increment required for protein degradation was a cause of the adverse effects of high protein feeding.

The effects of high levels of dietary protein have been examined more extensively in monogastrics. The embryonic loss was greatest in rats when dams were fed very high levels of protein (39.9%) and very low levels of starch (2.5%) (Saitoh and Takahashi, 1977). These authors hypothesized the embryonic loss may have been caused by inhibition of progesterone synthesis by the ovary. In chickens, egg production and fertility are not influenced by raising protein levels from 16 to 32%, however hatchability is decreased (Patel and McGinnis, 1977).

Examination of the effects of energy on hormone levels has yielded equivocal results. Apgar et al. (1975) reported luteinizing hormone (LH) levels decreased in

Holstein heifers fed 60% of Morrison's recommended total digestible nutrients (TDN) compared to heifers fed 100% of Morrison's recommendation. In contrast, Gombe and Hansel (1973) reported higher basal and peak levels of plasma LH in Holstein heifers fed 62% of Morrison's recommended TDN than in control heifers fed 100% of Morrison's recommendation. Heifers with restricted energy consumption had lower plasma progesterone levels during the second and third estrous cycles after initiation of the experiment than control heifers. However, Dunn et al. (1974) reported that mature Hereford cows fed a restricted ration (3.6 kg cubed alfalfa) had higher progesterone and peak LH concentrations in plasma relative to cows fed a normal ration (3.6 kg cubed alfalfa and 2.3 kg ground barley).

Nitrogen Metabolism in the Ruminant

Ruminants are unique animals in that the microflora of the rumen permits efficient utilization of dietary non-protein nitrogen. The rumen microbes can convert low quality dietary protein into high quality microbial protein (Satter and Roffler, 1975). As early as 1891, Zuntz hypothesized that rumen bacteria preferentially use amides, amino acids and ammonium salts instead of protein (cited by Maynard and Loosli, 1969). In 1937, Fingerling and coworkers, using calves in nitrogen-balance

studies, determined urea can be utilized by the rumen microbes to provide a portion of the protein required for growth (cited by Maynard and Loosli, 1969). Besides the ability to utilize low quality protein and nonprotein nitrogen, ruminants can survive on low levels of nitrogen by utilizing nitrogen recycled into the reticulo-rumen from saliva and from blood across the rumen wall. However, the rumen microflora can be disadvantageous, since under some conditions more dietary protein is destroyed than synthesized by the microbes (Satter and Roffler, 1975).

The net requirement of an animal for nitrogen is the amount required for maintenance, production (milk, growth and wool) and reproduction. Rumen nitrogen is obtained from dietary sources, from saliva and from across the rumen wall (Hogan, 1975). When natural protein is consumed, approximately 40% escapes rumen degradation while the remaining 60% is digested almost entirely into ammonia (Satter and Roffler, 1977). Some of the end products of protein degradation in the rumen are: ammonia (major), amino acids, amines, guanosine, adenosine, pyrimidine nucleosides and pyrimidine bases; however, no purine bases are produced (Smith, 1969).

Frequency of feeding, type of dietary carbohydrates, rumen pH and extent of dietary protein degradation have been implicated in influencing ruminal ammonia concentration

(Satter and Roffler, 1977). Pearson and Smith (1943a) reported that pH affects rumen liquor urease activity in vitro, with the optimum pH being between 7 and 9. Urease activity was minimal above pH 9.5 or below pH 3. In vitro the optimal temperature for conversion of urea-N to ammonia in rumen liquor was 49 C. During ingesta degradation approximately 62% of the gas formed was carbon dioxide, which accelerated the degradation of urea in the presence of starch. When the concentration of urea was increased in vitro, a slight increase in conversion of urea to ammonia resulted. Thus, alterations of the rumen environment influenced protein degradation and ammonia production.

An increase in solubility of dietary protein increased the ability of rumen microorganisms to degrade amino acids to ammonia and other products (El-Shazly, 1952b). Solubility was affected by pH, salt, ionic strength, temperature, degree of agitation, extraction time and chemical treatment (Wohlt et al., 1973). Wohlt et al. (1973) examined the solubility of purified casein and isolated soy protein in vitro in rumen fluid and in mineral mixture at pH 5.5, 6.5 and 7.5. The protein solubility nearly doubled as the pH was increased from 5.5 to 7.5. Nitrogen solubility of some common feedstuffs, as measured in vitro using a mineral mixture, varied widely.

i.e. dried milk, 93%; rye, 41%; rapeseed meal, 39%; wheat, 30%; barley, 17%; soybean meal, 13%; corn meal, 12% and cottonseed meal, 7%. Majdoub et al. (1978) reported cows fed a ration with low (22%) nitrogen solubility had higher milk production than cows fed a ration with higher (42%) nitrogen solubility. Average daily intake of dry matter, CP or net energy for lactation were not affected by nitrogen solubility. However, daily intake was increased when dietary CP was changed from 13 to 15%.

Natural feeds utilized in ruminant rations contain nitrogenous components which consist of a combination of protein and non-protein nitrogen. The non-protein nitrogen fraction varies with the type of plant, maturity, weather and method of harvesting and storing. Drying forage for hay or haylage increases non-protein nitrogen from 25 to 50%, and non-protein nitrogen in unwilted silage increased to 60-75% during storage (Waldo, 1968). In immature soybeans, >30% of the nitrogen is non-protein nitrogen, while only 4-5% of the nitrogen is non-protein nitrogen in mature seeds (Krober and Gibbons, 1962). Drought and cool, wet weather also increase the percentage of non-protein nitrogen.

Utilization of non-protein nitrogen and proteins is dependent upon the quantity of carbohydrates available. An inadequate energy source or one of diminished

availability reduces nitrogen utilization (Waldo, 1968). Conversely, low dietary nitrogen content limits voluntary dry matter intake due to reduced microbial activity in the rumen and a reduced rate of digesta degradation (Church, 1979).

In 1973, Broster examined the relationship between energy and protein and developed a quadratic equation from experimental data, concluding that excessive protein intake adversely affected production (milk, growth and wool). The adverse effect of feeding high levels of protein may have resulted from recycling excess urea through the digestive tract or from lower efficiency of carbon utilization from amino acids relative to fats and carbohydrates. The latter effect is associated with the increased metabolic cost required for excretion of nitrogen from metabolism of excess amino acids (Tyrrell et al., 1970).

Although the ultimate function of dietary nitrogen is the maintenance of tissue or synthesis of tissue and milk, dietary nitrogen is used as a nutrient by rumen microorganisms also. Microbial enzymes metabolize non-protein nitrogen and a majority of the dietary protein into amino acids and ammonia (Hogan, 1975). Of the 89 strains of rumen bacteria isolated by Bryant and Robinson (1962), 56% utilize ammonium or casein hydrosylate as

the nitrogen source. And ammonium is essential for 25% of the rumen bacteria strains which can not utilize casein hydrosylate. Rumen protozoa usually require amino acids and preformed purine and pyrimidine bases rather than ammonia. Engulfment and digestion of bacteria or small ingesta particles by the protozoa probably provides most nutrients, although they may ingest some free amino acids and pyrimidines (Smith, 1969).

Rumen utilization of ammonia by bacteria was determined by the population and growth rate of bacteria, as well as the availability of other nutrients. Pearson and Smith (1943b) measured the concentration of non-protein nitrogen, ammonia and protein in rumen liquor incubated in vitro. As non-protein nitrogen decreased, a parallel decline in ammonia-N occurred, suggesting that synthesized microbial protein was produced from ammonia and not from other non-protein nitrogen sources. The quantity of microbial protein derived was equivalent to the quantity of cell organic matter synthesized, less the quantity destroyed within the rumen (Hogan, 1975). The cell destruction in the rumen occurred due to autolysis, attack by bacteriophages, and engulfment by protozoa, all of which increased with time in the rumen. The amount of organic matter synthesized was dependent upon the physical environment, the efficiency of nutrient utilization

by the different organisms, and the rate of organism removal (Hogan, 1975). Maximal microbial cell yield occurred in vitro when microorganisms were in a fermentation system for 2 to 5 hours and yield was depressed when the retention time was of longer or shorter duration (Hobson and Summers, 1967).

Virtanen (1966) reported a change in the rumen microflora as cows were adapted to urea over a six month period. The number of protozoa declined sharply and in some cases virtually disappeared. Concurrently, the number of bacteria rose from 25×10^8 bacteria/ml in cows on a natural protein ration to 12×10^{10} bacteria/ml in cows on a purified diet with urea as a nitrogen source. Milk production of cows on the purified ration was comparable to their previous lactations; however, maximum production was only 4,217 kg.

Loosli et al. (1949) fed lambs a purified ration with urea as a nitrogen source. The lambs remained in a positive nitrogen balance gaining 104 g/day compared to 136 g/day by lambs fed a ration with casein as the nitrogen source. Rumen contents of the lambs on the purified diet were analyzed for arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The rumen contents had 9 to 20 times the amount of amino acids present in the diet,

further indicating that rumen microorganisms can synthesize amino acids.

In 1965, Cook et al. demonstrated that some amino acids (glycine, serine, threonine, methionine sulfoxide, aspartic acid, asparagine, glutamine, isoleucine and leucine) could be absorbed intact from the rumen. However, Hogan (1975) found the major site of amino acid absorption was the small intestine. Ammonia was absorbed through the rumen wall and entered the portal blood system. Rumen ammonia and portal ammonia concentrations were correlated over a wide range of dietary protein intakes, but peripheral blood levels of ammonia were only correlated with rumen and portal levels at higher nitrogen intakes (Lewis et al., 1957).

Differences in pH across membranes influenced diffusion of ammonia (Visek, 1968). As pH rose above 7.3, urea toxicity was observed in sheep fed high levels of nitrogen, whereas when pH remained below 7, urea toxicity was not apparent, although rumen ammonia concentrations were similar (Coombe et al., 1960). Fatty acids also increased the absorption of ammonia near neutral pH (Hogan, 1975). El-Shazly (1952a) reported a parallel evolution of ammonia and volatile fatty acids in sheep as protein was degraded in the rumen. In addition to pH and fatty acids, oils have been implicated in ammonia transport

from the rumen. If nitrogen is < 3% of the ration, added oils depress rumen ammonia; however, if nitrogen is > 3% of the ration, added oils increase rumen ammonia (Waldo, 1968).

The excess ammonia absorbed from the reticulo-rumen or from the lower gut is termed "ammonia overflow" (Satter and Roffler, 1977). Excess ammonia is converted by the liver into urea which may be disposed of either through urinary excretion or by recycling to the rumen via saliva or across the rumen wall (Smith, 1969). Regulation of urinary urea excretion is influenced by the concentration of plasma urea as well as the renal processes of glomerular filtration rate, concentrating ability of the kidney and urine flow rate (Thornton and Wilson, 1972). When plasma urea concentrations are high, approximately 65% of the filtered urea is excreted in the urine, whereas, as little as 10% is excreted under conditions of low plasma urea.

The time required to attain peak plasma urea nitrogen levels increases as dietary CP increases. Fenderson and Bergen (1976) observed that changing steers abruptly from a diet containing 11% CP to 20%, 32% or 40% required 2, 3 and 5 days, respectively, before maximal blood urea nitrogen levels were attained. The steers had rumen ammonia levels over 100 mg/100 ml of rumen liquor after

10 days on the ration, but no signs of urea toxicity were evident. Probably, the simultaneous synthesis of volatile fatty acids from the digestion of carbohydrates and preformed proteins maintained an acidic rumen pH, thereby slowing ammonia absorption.

Theurer et al. (1966) reported that lambs fed a semi-purified ration utilizing soybean meal as a protein source had the highest portal and jugular plasma urea levels 24 hours postprandially. Urea cycle enzyme activities (Fig. 1) increased as the nitrogen content of rations fed to sheep increased from .75% to 5.7% (Payne and Morris, 1969). Dosage of sheep on the high protein diet with 0.5 kg urea/kg bodyweight failed to alter ammonia concentration in the blood. However, the same dose given to sheep on the low protein ration resulted in a rapid and fatal rise in blood ammonia concentration. Tolerance to ammonia or urea toxicity may have been a function of urea cycle enzyme activity in the liver.

Acid-Base Balance in Ammonia Toxicity

With the utilization of urea in ruminant rations, acid-base problems and ammonia or urea toxicity have arisen and been investigated in both sheep (Singer and McCarty, 1971; Coombe et al., 1960) and cattle (Bartley et al., 1976; Davidovich et al., 1977; Word et al., 1969).

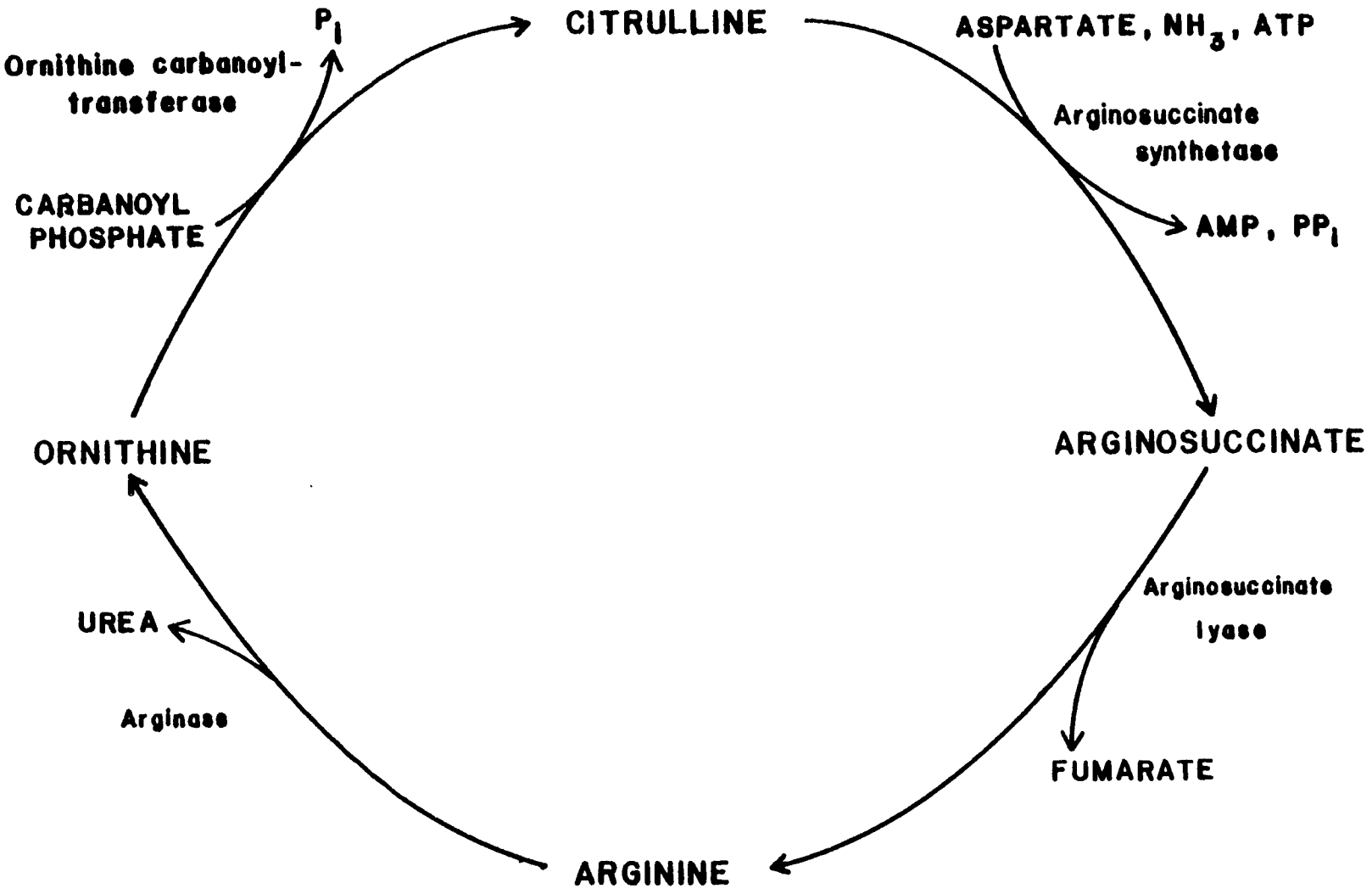

FIG. 1. The urea cycle (Lehninger, 1975).

Toxicity was not related to rumen ammonia and blood urea concentrations although it was correlated positively with rumen pH and blood ammonia (Bartley et al., 1976). As rumen pH rose to 7.0, rumination time diminished and complete rumen stasis occurred at pH 7.3. High rumen ammonia levels were not the cause of rumen stasis, since similar ammonia concentrations at lower pH did not inhibit rumen motility (Coombe et al., 1960).

As proteins are hydrolyzed in the rumen to produce volatile fatty acids and ammonia (El-Shazly, 1952a) and as urea is converted by urease to ammonia and water, hydrogen ions are utilized in converting ammonia to ammonium. This results in increased pH unless adequate carbohydrates and proteins are available to produce acids. By the time toxic effects are noted, hydrogen ions from the body are being utilized by the rumen for ammonium production, body pH has arisen, and metabolic alkalosis occurs (Davidovich et al., 1977).

The body has three mechanisms by which to combat disturbances in acid-base balance: chemical buffering, respiratory adjustment of carbonic acid concentration and excretion of ions by the kidneys (Houpt, 1977). The ruminant apparently uses a respiratory adjustment to compensate for the metabolic alkalosis occurring with urea toxicity, since Singer and McCarty (1971) reported labored

breathing and death due to respiratory failure in sheep. Examination of the blood at death indicated terminal acidosis secondary to the metabolic alkalosis, indicating the respiratory system had overcompensated. According to Houpt (1977), respiratory acidosis results primarily from an increase in the blood partial pressure of carbon dioxide. A depression of the central nervous system respiratory center, abnormal chest wall or respiratory muscles, and an obstruction to gas movement or diffusion in the lungs can all cause increased carbon dioxide concentration.

Davidovich et al. (1977) demonstrated the brain probably absorbs ammonia-N since the concentration in carotid blood averaged 1.47 mg/100 ml at toxicity, while jugular concentration at toxicity was only 0.95 mg/100 ml. In patients with hepatic coma (Phillips et al., 1952; Sherlock, 1958) the administration of ammonia or other nitrogenous substances led to neurological complications. Juhász (1972) reported a decrease in production of cerebrospinal fluid and its pressure in animals in a state of ammonium intoxication. This leads to the conclusion that the respiratory center of the central nervous system has been depressed. Other neurological complications may be confused with clinical signs of ammonia toxicity, such as tetany, mydriasis and tachycardia.

A decrease in salivary secretion and a gradual increase in diuresis have been observed by Juhász (1972) when urea or casein was fed. During the first hour after feeding, blood Na decreased but returned to normal within seven hours, while K decreased similarly but failed to return to normal within seven hours. In 1975, Juhász et al. also reported that although the K concentration in urine did not increase, increased urine output resulted in increased K excretion. Utilization of K isotopes indicated a decreased activity of K within the erythrocytes and in whole blood. Juhász hypothesized ammonia inhibited the active transport of K into erythrocytes; however, in vitro studies indicated that although ammonia enhanced passive transport from the erythrocyte, it did not interfere with active transport into the cell (Juhász et al., 1976). From these observations it was apparent that changes in the acid-base equilibrium could alter cell function by changing the concentration of cations and anions. The pH change coupled with hyperglycemia, increased concentrations of amino acids and urea, hematuria and albuminuria which were observed (Singer and McCarty, 1971) may have resulted from urea toxicity.

Reproductive Tract Environment

The reproductive tract environment is critical for

sperm, ova and embryo survival and maturation. In vivo insemination and ovulation must be synchronized for syngamy. Cows bred 0 to 24 hours prior to ovulation have a 65.1% conception rate as compared to 42.1% for those bred greater than 24 hours before ovulation and 40.3% for those bred after ovulation (Shannon, 1978).

Cross and Brinster (1970) reported 3.2% of two-cell ova obtained by collecting immature oocytes from mouse ovaries, incubating them under oil until matured and then fertilizing them in vitro, developed into 15-day-old fetuses when transferred to foster mothers. However, when tubal oocytes (matured in vivo) were collected and fertilized in vitro 23.6% of the two-cell ova transferred to foster mothers developed into 15-day-old fetuses. Optimal in vitro fertilization of oviductal matured oocytes requires adherence of the cumulus cells and media containing 30 mg/ml albumin.

Sperm viability and motility were influenced by pH, ions present and osmolality. Extremes in pH (4.2 and 10.2) reduced sperm motility (Turner and Howards, 1978), although motility was adequate in a wide range of monovalent and divalent ionic solutions. Ammonia levels of 4, 8, 12 and 16 mM resulted in oxygen uptake inhibition of 11, 16, 30 and 33%, respectively (Dietz and Flipse, 1969). In addition, the citric acid cycle was inhibited by 49%

at an ammonia concentration of 8 mM. Epididymal spermatozoa remained immotile when transferred into a 300 mOsm solution of urea. However, treatment with other nonionic compounds such as sucrose and mannitol resulted in transient motility (Turner and Howards, 1978). Caudal epididymal fluids in the rat had an osmotic concentration of 375 mOsm, but the sperm became motile as the concentration was decreased to 300 mOsm. Further reduction of osmolality to 285 mOsm caused loss of motility (Turner and Howards, 1978). Harrison et al. (1978) found that ionic strength had little or no effect on motility in bull, boar, rabbit, ram and stallion sperm, while the addition of albumin maintained sperm motility and diminished agglutination which normally occurs with time.

Sperm capacitation is affected by the uterine environment as well. In porcine uterine secretions a glycosaminoglycan stimulates the conversion of proacrosin to acrosin, aiding in the penetration of the zona pellucida of the oocyte (Wincek et al., 1979). Sperm incubated in Brackett's medium plus the uteroglobin fraction of rabbit oviductal fluids have a greater acrosome loss than those incubated with media alone or media plus total estrous oviductal or uterine fluids (Feigelson et al., 1977). Thus, the uterine and oviductal environment can influence fertilization by the effects on sperm capacitation and penetration.

Timing is critical for survival of the conceptus. In the bovine, the 16-cell stage embryo enters the uterus between 72 and 96 hr following ovulation, when the uterus is under the influence of progesterone produced by the developing corpus luteum (Robinson, 1977). The embryo transfer experiments of Rowson and Moor (1966) demonstrate the importance of an adequately prepared uterine environment for normal development of the blastocyst. During this time the embryo is dependent on uterine secretions, which are regulated by maternal hormones, for nourishment (Sauer, 1979).

Pond et al. (1969) reported dietary protein intake was especially important on days 16 to 20 of pregnancy, the time of implantation in swine. Gilts fed 16% protein on days 16 to 20 of pregnancy and .5% protein prior to and after this period had litters with increased birth weights and growth rates compared to litters from gilts fed .5% protein throughout gestation. Interestingly, feeding rations with no protein or 2% protein did not affect the ability of the uterus to secrete protein (Murray et al., 1979).

Uterine secretion protein composition varied with stage of the estrous cycle and differed from serum. Heap (1962) reported protein N in cow uterine flushings increased from 64 to 155 µg/ml from estrus to the luteal

phase of the estrous cycle. Schultz et al. (1971) observed an increase in uterine secretion total protein from a low of 4.6 mg/100 ml at estrus to 9.6 mg/100 ml on day 13 and 15 of the estrous cycle. Total N and protein N also increased in the uterine secretions during the luteal phase in rabbits and sheep (Heap, 1962), but decreased in rats (Heap and Lamming, 1962; Heap, 1962). Fahning et al. (1967) reported the concentration of the amino acids glycine, leucine, alanine, phenylalanine, proline, valine, aspartic acid, tyrosine, serine, isoleucine and taurine in bovine uterine secretions was greater during the luteal phase than in the follicular phase of the bovine estrous cycle, with peak concentrations occurring from day 8 to 10. β-N-acetylgalactosaminidase and β-N-acetylglucosaminidase were elevated at diestrus in bovine oviductal fluids (Roberts et al., 1975). The amino acids, ethanolamine, β-alanine, and cystine, were found in bovine uterine secretions, but not in serum (Fahning et al., 1967). In addition, a protein identical to milk lactoferrin and an acid phosphatase have been isolated (Dixon and Gibbons, 1979). Fractionation of bovine oviductal fluid by gel filtration or affinity chromatography detected non-serum proteins at estrus and diestrus (Roberts et al., 1975). Laster (1977) isolated a bovine pregnancy specific protein (50,000-60,000 MW) present by day 15

postmating in the uterine flushings as well as in the uterine endometrium. The pregnancy specific protein was 1% or less of the uterine endometrial protein.

The day 14 and 15 sheep conceptus contains a heat-labile luteotropic substance which maintains the corpus luteum when it is repeatedly infused into the uterus starting on day 12 of the estrous cycle (Rowson and Moor, 1967). However, Roberts et al. (1976) could not isolate this substance although they did observe increased quantities of glycoprotein at estrus. In addition, β-N-acetyl-galactosaminidase, β-N-acetylglucosaminidase, β-L-fucosidase, β-D-galactosidase and α-D-glucosidase are elevated between day 11 and 15 of pregnancy in the uterine fluids of ewes. On the basis of studies with radioactively labeled amylase, albumin, IgG and catalase, Oliphant et al. (1978) hypothesized that absorption of serum components into the oviduct fluid is based on molecular weight in the rabbit.

Protein was not the only component in uterine secretions to change with stage of the estrous cycle. Potassium increased from 11.5 mEq/l at estrus to 20.6 mEq/l at day 16 of the estrous cycle in bovine uterine secretions (Schultz et al., 1971). Heap (1962) reported higher K concentrations during the luteal phase of the estrous cycle in the rabbit, sheep and cow, but the reverse in

the rat. However, Lamothe and Guay (1970) and Lamothe et al. (1976) failed to detect a change in bovine uterine secretion K concentration associated with stage of the estrous cycle. Magnesium concentration in the uterine secretions of normal cows decreased from 4.95 mg/100 ml at estrus to 2.89 mg/100 ml at proestrus (Lamothe and Guay, 1970), but did not change with stage of the estrous cycle in a subsequent experiment (Lamothe et al., 1976). In the ewe, Mg rose slightly from 1.33 µg/uterine horn at estrus to 1.49 µg/uterine horn during the luteal phase, but declined from estrus to diestrus in the rat and rabbit (Heap, 1962). In bovine uterine secretions, Ca concentration decreased from 4.3 mg/100 ml at estrus to 1.1 mg/100 ml on day 12 of the estrous cycle, but rose to 4.2 mg/100 ml 2 days later (Schultz et al., 1971). Lamothe et al. (1976) reported bovine uterine secretion Ca concentrations rose from 4.39 mg/100 ml at estrus to 6.12 mg/100 ml at diestrus. However, Olds and VanDemark (1957) reported the Ca concentration in uterine fluids dropped from 18.6 mg/100 ml around estrus to 8.5 mg/100 ml between day 11 and 17 postestrus. Phosphorus concentrations decreased from 22.8 mg/100 ml at estrus to 10.5 mg/100 ml at proestrus in the bovine according to Lamothe and Guay (1970). Heap (1962) reported P concentrations were higher in the rabbit, sheep and cow, but were lower in the rat during the luteal

phase of the estrous cycle. Cook and Hunter (1978) found Zn concentrations in the uterus did not change substantially between anestrus and the luteal phase of the estrous cycle in sheep, but increased during pregnancy. Hagenfeldt et al. (1977) reported cyclic variations in human endometrium levels of Zn with peak concentrations during the secretory phase of the estrous cycle.

In addition to analyzing changes in the uterine secretions which occurred during the estrous cycle, Ayalon (1978) and Lamothe and Guay (1970) have examined differences in uterine secretion composition between cows with and without reproductive problems. Ayalon (1978) reported uterine flushing Ca concentration was 178.5 ± 17.4 mg/100 ml on day 7 post-estrus in cows with abnormal embryos, while only 14.3 ± 2.2 and 12.2 ± 4.8 mg/100 ml in cows with normal embryos and cows which were not bred, respectively. Zinc and K concentrations also were elevated in cows carrying abnormal embryos. Total protein in bovine uterine secretions on days 6, 7 and 8 postestrus of cows which were repeat breeders averaged 115 µg/ml in contrast to cows with normal fertility which averaged 176 µg/ml (Ayalon, 1978). In cows with normal fertility, Na was greater at estrus and lower at proestrus; K was lower throughout the estrous cycle; and P was greater at estrus and postestrus than in repeat-breeder cows (Lamothe and Guay, 1970).

Progesterone is essential for the maintenance of

early pregnancy and in most species is a prerequisite for implantation. The rat and mouse require post-ovulatory estrogen if the endometrial environment is to be conducive to implantation and normal blastocyst development (Sauer, 1979). The uterus has the capability to concentrate both estrogen and progesterone from plasma, with estradiol being concentrated 100- to 200-fold in non-pregnant sheep caruncles, while progesterone is concentrated to a lesser degree (Challis et al., 1976; Henricks and Harris, Jr., 1978). In the rabbit progesterone and, to a lesser extent, estrogen stimulate the synthesis of uteroglobin, which functions to enhance blastocyst development in vitro by promoting blastocyst expansion and RNA synthesis (Feigelson et al., 1977). Thus, the increased potency of progesterone may compensate for its decreased concentration in stimulating uteroglobin synthesis.

Heap et al. (1979) reviewed estrogen synthesis in the pig blastocyst. On day 11 of gestation, progesterone, estrone and estradiol-17β have been detected in pig blastocysts and by day 22 progesterone can be metabolized to estrogens. The concentration of estrone and estradiol-17β increased in luminal fluid of the uterus due to steroid synthesis in the preimplantation blastocyst. The estrogens then passed into the endometrium where they were converted to estrogen sulfate (Heap et al., 1979). Sauer

(1979) proposed that estrone, synthesized and secreted by the pig blastocyst and sulphated in the endometrium, was transported to the ovary where it was hydrolyzed back to estrone, providing a luteotrophic stimulus.

Shemesh et al. (1979) reported measurable amounts of progesterone in bovine blastocysts, which had or had not been cultured, at 13, 15 and 16 days after insemination, with the cultured blastocysts producing more progesterone. Estradiol-17β was detected in some of the day 15 and 16 blastocysts which had been cultured, but not in blastocysts which had not been cultured. Testosterone was detectable by day 15 and 16, with the cultured embryos containing more testosterone. A steady and significant increase in prostaglandins F and E was noted as the blastocysts increased in age from day 13 to 16. The cultured blastocysts had a 4- to 17-fold increase in prostaglandin concentration relative to the blastocysts which were not cultured. Heap et al. (1979) hypothesized steroid synthesis by the blastocyst may function to assist in maternal recognition, to facilitate implantation, or to suppress the uterine release of prostaglandin $F_{2\alpha}$.

Running Head: Relationship of crude protein and reproduction

CHAPTER 3

Relationship of Dietary Crude Protein to Reproduction in High Producing Postpartum Dairy Cows. I. Nitrogenous, Glucose, Production and Fertility Parameters. [1]

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ABSTRACT

Eighteen multiparous, high producing (7,836 kg 4% FCM) Holstein dairy cows were assigned randomly to iso-caloric rations containing either 12 or 23% crude protein (CP) on day 40 postpartum. After a 10-day adaptation period, uterine secretion and blood samples were collected on the day of estrus (day 0-1), day 5 and day 15 of the first estrous cycle after day 50 postpartum and on the day of estrus (day 0-2) of the subsequent estrous cycle. Days to first postpartum estrus, days to first experimental estrus, days from first to second experimental estrus, bodyweights, parity and feed intake did not differ between treatments. Cows fed 23% CP required a 1.9-fold increase ($P=.06$) in services per conception (SPC) the year of the experiment as compared to the preceding years, but cows fed 12% CP required the same number of SPC. The ammonia concentration in plasma of cows fed 23% CP was elevated ($8.0 \pm .5$ µg/ml) over cows fed 12% CP ($6.3 \pm .3$ µg/ml; $P<.01$). Total protein concentration in the uterine secretions increased 88% from day 0-1 (8.9 ± 1.5 mg/ml) to a peak at day 15 (16.7 ± 1.7 mg/ml; $P<.05$) and then declined again by day 0-2 ($4.6 \pm .7$ mg/ml), although it did not change with treatment. Plasma urea concentration was 3.5-fold higher in cows fed 23% CP than 12% CP rations ($16.8 \pm .5$ and $4.8 \pm .3$ mg/100 ml, respectively; $P<.01$). Uterine

secretion urea concentration was 2.7-fold higher in cows fed 23% CP (17.2 ± 1.1 vs. 6.4 ± .7 mg/100 ml, P<.01). Plasma glucose concentration was lower on days 0-1 and 5 than on days 15 and 0-2 (60.9 ± .7 mg/100 ml and 65.9 ± 1.4 mg/100 ml, respectively; P<.01), but did not differ with treatment. Protein level did not affect actual milk production, % milk fat or 4% FCM. The decreased fertility without lengthened estrous cycles and increased plasma ammonia, uterine secretion urea-N and plasma urea-N, suggests excess dietary CP affects events preceding implantation, such as ovum or sperm viability, fertilization or early embryonic cleavage and survival.

INTRODUCTION

Cattle in Dairy Herd Improvement Association (DHIA) tested herds averaged 4,907 kg of milk/cow/yr in 1960-61 compared to 6,721 kg of milk/cow/yr in 1979-80. During the same time interval, the number of cows per herd increased from 45 to 80 (King et al., 1980). Increased milk production, larger herds and total confinement housing have created stress contributing to decreased reproductive efficiency. As milk production has increased, dairy producers have also increased crude protein (CP) and energy levels in dairy cow rations by feeding higher proportions of concentrates.

Dunn et al. (1969) reported that beef heifers fed high energy prepartum showed earlier postpartum estrus than those on low energy prepartum rations. In 1973, Folman et al. reported lactating dairy cows maintained on a high level of nutrition required fewer inseminations than those on a standard level. These cows were individually fed either a high-energy ration ad libitum or a ration to meet production requirements. Most nutrition research has involved changes in both protein and energy or only energy; consequently, conclusive evidence has not been reported on the reproductive effects of protein alone. Jordan and Swanson (1979a) reported that feeding high levels of protein (19.3% CP dry matter) to dairy cows impaired fertility by increasing the days open and services per conception (SPC), and consequently increasing the calving interval, as compared to lower levels of dietary protein (12.7% CP, dry matter). Drori and Folman (1971) and Edwards et al. (1980) found similar declines in dairy cow fertility as protein was increased from 11.5 to 16.0% CP and from 13 to 17% CP, respectively.

The reduced fertility observed with increased dietary CP intake may be indicative of a clinical syndrome in which the products of protein metabolism alter the uterine environment or function of the reproductive organs in ruminants. The objective of this experiment was to

elucidate possible mechanisms by which high levels of dietary CP reduce fertility by determining if: 1) nitrogenous components of the uterine secretions and plasma differ in cows fed 12 or 23% CP rations, and 2) the nitrogenous components vary during the estrous cycle. In addition, milk production, fertility, plasma glucose and bodyweight changes were examined.

MATERIALS AND METHODS

Eighteen multiparous, high producing (7,836 kg of 4% FCM) Holstein dairy cows were assigned randomly on day 40 postpartum to isocaloric rations (Table 1) containing either 12 or 23% CP. The rations, consisting of 56% corn silage, 11% chopped alfalfa hay and 33% concentrate (as fed), were mixed and fed once daily as complete rations. The concentrate was formulated to meet the protein requirements of each ration by altering the ratio of barley to soybean meal, but the percent concentrate within each ration remained the same throughout the experiment. The rations were balanced for minerals and in addition each group had ad libitum access to trace mineralized salt and a mineral mix containing Ca, P and Mg. The cows remained on the rations an average of 41 days (range 31-58 days). Feed intakes were determined for each group.

Twice daily visual observations for mounting activity,

Table 1. Composition of the experimental rations.

Ingredient	11.9% CP	22.6% CP
	% DM	
Alfalfa hay	18.0	18.0
Corn silage	28.6	28.6
Pellet	53.4	53.4
Barley, Pacific Coast	87.78	51.91
Soybean Meal, 47.5% CP	2.01	38.94
Cane molasses	7.70	7.56
Limestone	.92	.91
Dicalcium phosphate	.88	--
TM salt	.46	.45
Vitamin premix	.23	.23

restlessness and vulval mucus discharge were used in conjunction with tailhead chalk for the detection of estrus. Any cow not exhibiting estrus by 75 days postpartum was removed from the experiment. Fenderson and Bergen (1976) reported rumen NH_3-N and plasma urea-N levels plateaued by day 7 and 3, respectively, after switching Holstein steers from a 10.7% to 32.5% CP ration. Therefore, the cows were maintained on the respective rations a minimum of 10 days prior to sample collection. Uterine secretions and blood samples were obtained 2 hr after feeding on the day of estrus (day 0-1), day 5 and day 15 of the first estrous cycle after day 50 postpartum and on the day of estrus (day 0-2) of the subsequent estrous cycle. (Preliminary observations on non-lactating dairy heifers indicated 2 hr post-prandial was optimum for whole blood ammonia and plasma urea-N analysis.) After uterine secretion collection at the second estrus each cow was artificially inseminated. Cows were removed from the feeding trial 24 hr after insemination which averaged 81 days postpartum.

Uterine secretions were collected through an infusion pipette using a 30 cc syringe for aspiration. Blood samples were collected from the coccygeal vessel into heparinized sealed tubes. Samples were placed immediately on ice and transported to the laboratory. Uterine secretions were transferred to conical tubes and centrifuged at 1100 g

for 20 min at 4 C. The supernatant was removed and the volume determined. Secretions were diluted with an equal volume of distilled water, mixed and stored at -20 C.

Triplicate one ml aliquots of whole blood were analyzed for ammonia within 30 min for collection using the colorimetric method developed by Ternberg and Hershey (1960) and modified by Chaney and Marboch (1962). The remainder of the blood sample was centrifuged 30 min post-collection; plasma was removed and stored at -20 C. Subsequently, uterine secretions and plasma were analyzed by the method of Lowry et al. (1951) for total protein, a colorimetric dye-binding method for albumin (Spectru (TM) AB2 Albumin Reagent, Pierce Chemical Co.), a diacetyl monoxime colorimetric method for urea (Urea Nitrogen (BUN) Rapid Stat (TM) Kit, Pierce Chemical Co.) and an o-toluidine colorimetric method for glucose (Glucose Rapid Stat (TM) kit, Pierce Chemical Co.).

The number of days to first estrus postpartum; the number of days to first experimental estrus; the length of time from the first to the second experimental estrus; the number of SPC the year of the trial; the mean number of SPC during the preceding years; initial and final body-weights; and parity were determined. Daily milk weights were recorded starting 10 days prior to initiation and continuing for 10 days after termination of the feeding

trial. Milk samples were collected once weekly and analyzed for milk fat concentration (Modified Banco Procedure, Anderson Laboratories, Inc.). Four percent fat corrected milk (4% FCM) was computed using the equation: .4 (kg milk) + 15 (kg fat) = 4% FCM. The rate of increase or decrease (persistency) of milk production, fat production and 4% FCM was calculated for two time periods to assess treatment effects: 1) the first two weeks of the feeding trial as compared to the week preceding the trial, and 2) the week after termination of the feeding trial as compared to the last two weeks of the trial. The previous 305-day, 2X daily milking, mature equivalent 4% FCM and fat production records were obtained from DHIA records.

Data were analyzed by analysis of variance, orthogonal contrasts, regression and correlations to determine the effects of dietary CP intake on uterine secretion and plasma composition, reproduction and milk production. Feed intake was analyzed using analysis of covariance with the number of cow days at a particular intake level as a covariate.

RESULTS

The days to first postpartum estrus, days to first experimental estrus, days from first to second experimental estrus, bodyweights, parity and feed intake did not

differ between treatments (Table 2). Mean number of SPC the preceding years did not differ significantly between treatments, although cows on the 23% CP ration had averaged 21% fewer services. During the experimental year, this trend was reversed with cows fed 23% CP requiring an increased (1.9-fold; p=.06) number of SPC, whereas cows fed 12% CP did not. One cow in each group was not included in the fertility information since she was not rebred at the second experimental estrus for reasons unrelated to the experiment.

Only two cows in each group conceived at first service, indicating the collection technique may have reduced fertility. However, since the number of services per conception required by cows fed the 12% CP ration did not increase the year of the experiment, the dietary protein rather than the collection technique probably accounted for the decreased fertility observed.

Ammonia concentration in whole blood averaged 1.7 μg/ml higher in cows fed 23% CP than in cows fed 12% CP (8.0 ± .5 and 6.3 ±.3 μg/ml, respectively; $P<.01$), but did not change during the estrous cycle.

Plasma concentration of total protein did not change due to treatment or stage of the estrous cycle, averaging 46 ± 1 mg/ml. Total protein concentration in the uterine secretions did not differ with treatment, but increased

Table 2. Reproductive parameters, bodyweight, parity and feed intake of cows fed 12 or 23% CP rations.

Criteria	% CP	
	12	23
Days to first postpartum estrus	30.9 ± 6.5[a]	29.1 ± 5.2
Days to first experimental estrus	59.1 ± 1.9	61.7 ± 3.1
Days from first to second experimental estrus	20.6 ± .6	21.8 ± .5
Services per conception, preceding years	2.0 ± .5	1.5 ± .2[b]
Services per conception, year of trial	2.0 ± .3	2.8 ± .6[b]
Initial bodyweight, kg	580 ± 13[c]	630 ± 20
Final bodyweight, kg	585 ± 14[c]	646 ± 18
Parity	3.7 ± .7	3.3 ± .5
Feed intake, kg/cow/day (DM)	21.7 ± .5	24.2 ± .5

[a]Mean ± SE

[b]P = .06

[c]Observations from one cow which lost greater than 2 S.D. of weight were omitted from the analysis.

88% from day 0-1 (8.9 ± 1.5 mg/ml) to a peak at day 15 (16.7 ± 1.7 mg/ml; $P < .05$) and then declined by day 0-2 (4.6 ± .7 mg/ml; Fig. 1). The concentration of albumin in the uterine secretions and plasma was not affected by stage of the estrous cycle or by protein treatment, averaging 5.20 ±.45 mg/ml and 26.4 ±.5 mg/ml, respectively.

Urea concentration in the uterine secretions (Fig. 2) was affected by treatment, but not stage of the estrous cycle, with the cows fed 23% CP having a 2.7-fold higher concentration of urea than cows fed 12% CP (17.2 ±1.1 and 6.4 ±.7 mg/100 ml, respectively; $P<.01$). Concentration of urea in the plasma was 3.5-fold higher in cows fed 23% CP rations than in animals fed the 12% CP rations (16.8 ± .5 and 4.8 ± .3 mg/100 ml, respectively; $P<.01$; Fig. 2).

Plasma glucose concentrations were not affected by treatment. Across treatments, plasma glucose concentrations were lower the first experimental estrus and day 5 of the estrous cycle (60.9 ± .7 mg/100 ml) than at day 15 of the estrous cycle and the second experimental estrus (65.9 ± 1.4 mg/100 ml; $P < .01$; Table 3).

Daily milk production from cows fed 12 or 23% CP did not differ with treatment, but declined linearly (11%) during the experiment ($\hat{Y} = 42.14 - 2.14X_1 - 1.26X_2$, where $\hat{Y}$ = daily milk production (kg), X_1 = treatment and X_2 = week of experiment; $R^2 = .16$; $P<.05$). Daily milk production

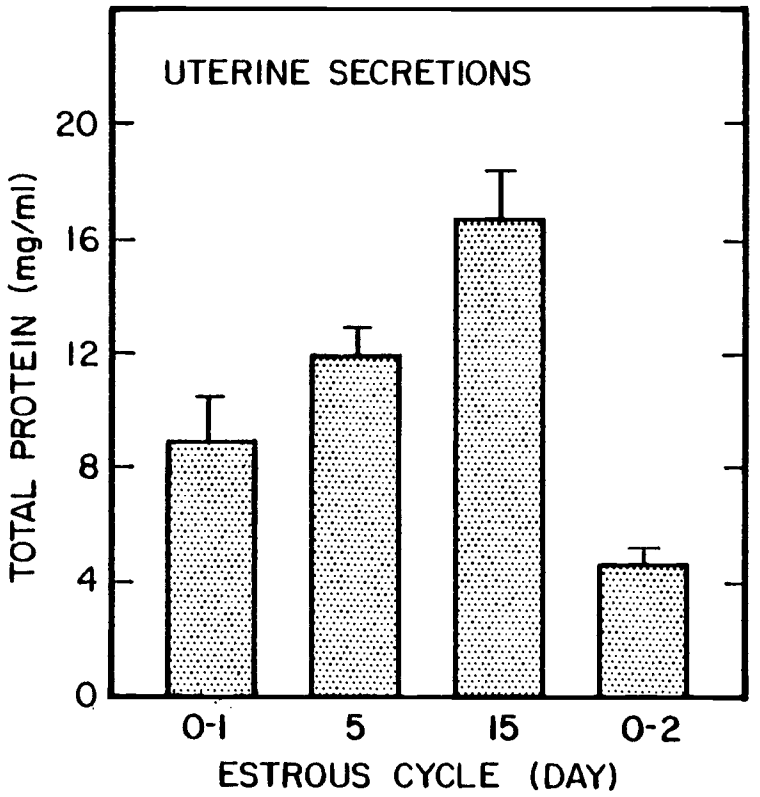

FIG. 1. Total protein in uterine secretions (Mean ± SE) during the estrous cycle pooled from cows fed two levels of crude protein (12 and 23% CP). Day 0-1 is the day of the first experimental estrus and Day 0-2 is the day of the second experimental estrus.

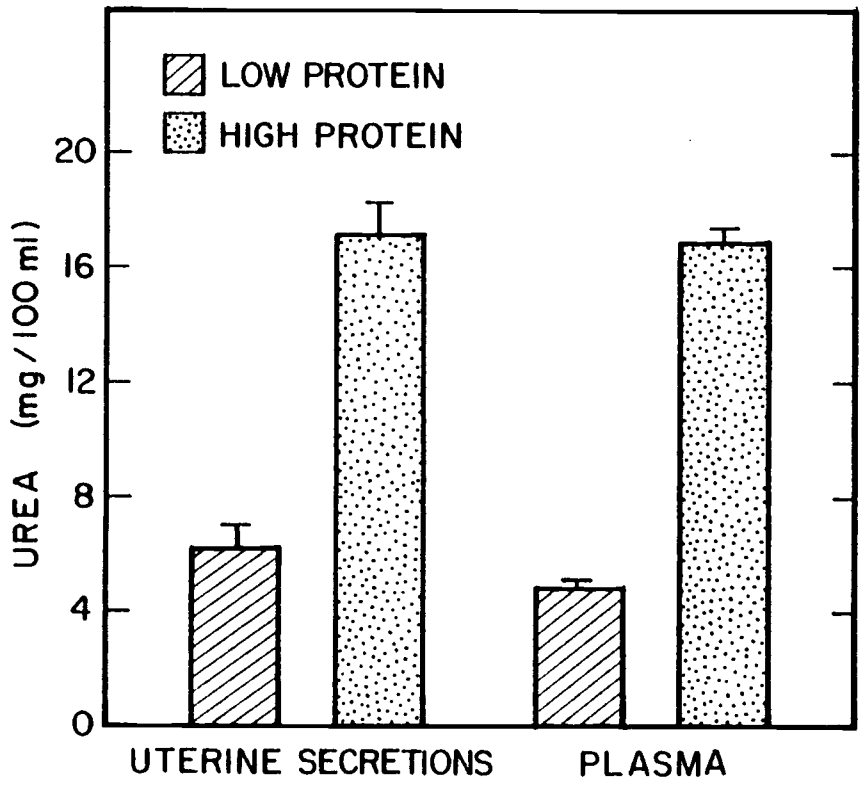

FIG. 2. Uterine secretion and plasma urea-N (Mean ± SE) concentration in cows fed low (12% CP) or high (23% CP) protein rations, pooled over time.

was not altered by the change from the herd to either experimental ration (see Period 1, Table 4), although % milk fat and 4% FCM decreased when the experiment was initiated and then rebounded when the cows were returned to the herd ration (Period 2, Table 4). The 4% FCM and % milk fat had significant time-treatment interaction. In the high protein group (23% CP), % milk fat (thus 4% FCM) decreased after the second week whereas in the low protein group (12% CP), % milk fat decreased after the first week (Fig. 3).

DISCUSSION

Mean number of SPC during the preceding years and number of days to first postpartum estrus during the experimental lactation (67% of the cows exhibited estrus by day 40 postpartum) were similar for both groups, indicating the cows in this experiment were probably of equivalent reproductive status prior to the initiation of the feeding trial (Table 2). The interval from first to second estrus during the experiment was 21 ± .4 days in both groups, indicating the rations did not affect estrous cycle length. The same number of cows conceived at first service in both groups, but the cows fed 23% CP ration required 1.9-fold more SPC the year of the experiment than during the preceding years, suggesting the detrimental effects of protein on fertility continued even though 88% of the cows were

Table 3. Pooled plasma glucose concentrations during the estrous cycle of dairy cows fed 12 and 23% CP rations.

Day of estrous cycle [a]	mg/100 ml
0-1	62.1 ± .9[b]
5	59.7 ± 1.0[b]
15	65.5 ± 2.3[c]
0-2	66.1 ± 1.6[c]

[a] 0-1 and 0-2 represent the first and second estrus after day 50 postpartum.

[b,c] Mean ± SE with different superscripts are significantly different ($P<.01$).

Table 4. Increase or decrease in milk production (persistency) when changing to and from the experimental ration.

Criteria	Dietary protein level	Persistency[a] Period 1	Period 2
Daily Milk Production	12	94 ± 2%[b]	100 ± 2%
	23	100 ± 2%	98 ± 4%
% Fat	12	93 ± 5%	106 ± 6%
	23	91 ± 6%	116 ± 5%
4% FCM	12	91 ± 3%	108 ± 5%
	23	95 ± 3%	108 ± 5%

[a] PERIOD 1 is the first two weeks of the feeding trial as compared to the week preceding the trial; PERIOD 2 is the week after termination of the feeding trial as compared to the last two weeks of the trial.

[b] Mean ± SE.

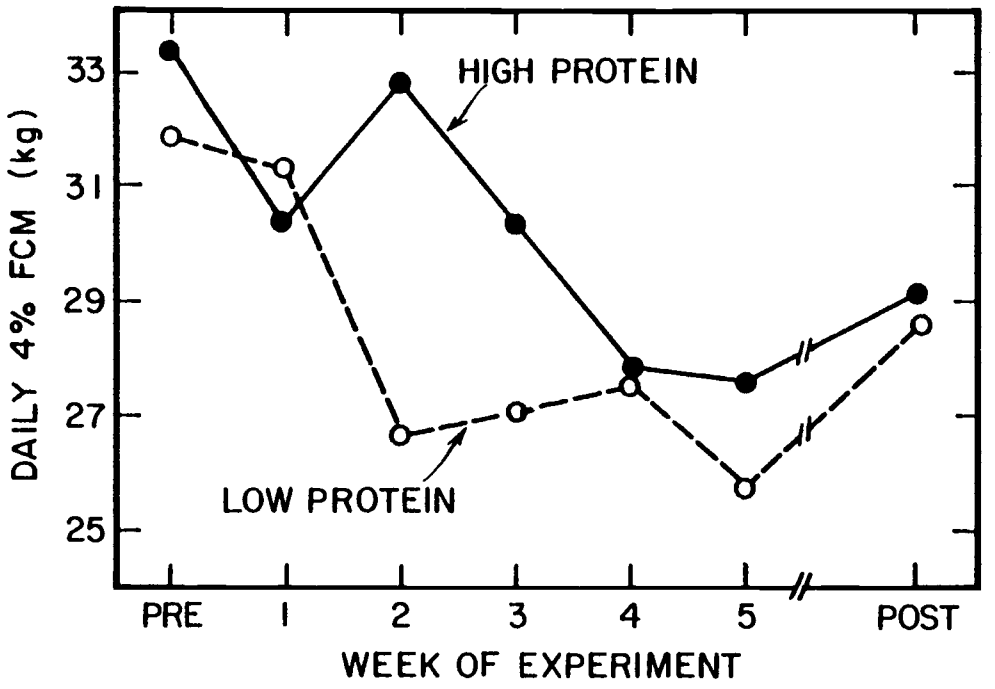

FIG. 3. Daily 4% FCM produced by cows fed low (12% CP) or high (23% CP) protein rations, illustrating the time-treatment interaction ($P<.05$) occurring at week 2 of the experiment. The week preceding the experiment is indicated as pre and the week after is post.

returned to their normal ration prior to conception. The increase in SPC in our study is similar to that reported by Jordan and Swanson (1979a) when cows were fed 19.3% CP until conception occurred. Excess dietary CP may affect the events preceding implantation, such as ovum or sperm viability, fertilization or early embryonic cleavage and development.

Satter and Roffler (1975) reported ruminal NH_3-N concentrations rose from .8 to 56.1 mg/100 ml as dietary CP increased from 8 to 24%. Excess ammonia produced in the reticulo-rumen is absorbed either there or in the lower gastrointestinal tract, transported to the liver and converted to urea. The hepatic concentrations of the urea cycle enzymes, arginine synthetase, ornithine transcarbamoylase and arginase, increase when animals are fed high protein rations (Payne and Morris, 1969). The increased plasma urea-N and ammonia concentrations we observed in cows fed 23% CP (Fig. 2) suggest the urea cycle enzymes did not convert all of the excess ruminal ammonia into urea-N, possibly because uptake into the hepatic cells is insufficient. Ammonia inhibits the citric acid cycle in sperm (Dietz and Flipse, 1969). If the ammonia concentration in the uterine secretions increased in cows fed high protein rations, as in plasma, fertility could be

reduced. (We could not measure ammonia in uterine secretions due to inadequate sample volume.) Ammonia and urea-N concentrations in plasma and urea-N concentrations in uterine secretions did not vary with time in our study, indicating the ten-day adjustment period was adequate for adaptation to the different CP rations. Uterine secretion urea and plasma urea were correlated with blood ammonia ($r=.24$; $P<.05$ and $r=.46$; $P<.01$, respectively).

In vitro, urea-N inhibits the binding of ^{125}I-HCG to the LH receptors on the corpus luteum (Haour and Saxena, 1974). Inhibition of LH binding to its receptors could decrease progesterone concentration as we reported previously (Jordan and Swanson 1979b). The urea-N concentration was elevated in cows fed 23% CP in the uterine secretions and plasma in our study (Fig. 2). A significant relationship ($r=.80$) between the concentration of urea in the plasma and uterine secretions indicates urea readily diffuses into the uterine lumen where it may act as an antagonist to fertility. For example, epididymal spermatozoa remained immotile when diluted with isotonic urea (Turner and Howards, 1978).

Plasma total protein and albumin did not change as ration CP increased, similar to findings of Jordan and Swanson (1979a) and Treacher et al. (1976). However, the concentration of total protein in the uterine secretions increased 88% from estrus to day 15 of the estrous cycle and declined again by the following estrus (Fig. 1).

Schultz et al. (1971) have reported a similar increase in total protein concentration in uterine secretions. Since the volume of uterine secretions decreased after estrus (Schultz et al., 1971), the total quantity of protein may have been the same. The collection technique used did not permit determination of uterine secretion volume in this study, so total quantity of protein could not be determined, only the concentration. Uterine secretion albumin did not change with treatment or stage of the cycle, indicating the changes in uterine secretion total protein were a result of alterations in proteins other than albumin.

Downie and Gelman (1976) have reported beef cows were infertile when plasma glucose and bodyweight were both declining, but were fertile when plasma glucose was increasing and bodyweight was decreasing. Since the glucose concentration did not vary with treatment, alterations in this component were not contributing to the decreased fertility we observed in the high protein group. The increases in plasma glucose from days 0-1 and 5 to days 15 and 0-2 (Table 3) may be related to the stage of lactation. In general, peak milk production is reached 5-7 weeks postpartum, but maximum dry matter consumption is not reached until 9-11 weeks postpartum (Satter and Roffler, 1975). Thus, during early lactation, high producing cows are frequently in a negative energy balance due to the cow's limited capacity or appetite (Clark and Davis, 1980). The

cows in this trial averaged 8½-9 weeks postpartum for the day 0-1 and 5 samples and 10½-11½ weeks postpartum for the day 15 and 0-2 samples. Consequently, the cows may have been changing from a negative to positive energy balance during this time interval. Fifteen of the eighteen cows had gained weight by the termination of the trial (Table 2), supporting this conclusion.

Differences in uncorrected milk, % milk fat and average 4% FCM were not significant between treatments, similar to reports by Claypool et al. (1980). A recent review of the literature (Palmquist and Jenkins, 1980) indicates fat added to dairy cattle rations is effective in maintaining milk production and percent fat. The experimental rations did not contain added fat, although before and after the experiment the cows consumed whole cottonseed (2-2½ kg/head/day), a source of fat. This may account for the decrease in fat production at the initiation of the trial and the increase after termination of the trial (Table 4).

Results reported herein indicate 1) urea-N and ammonia concentrations are increased in plasma and uterine secretions as CP intake increases, but do not vary with stage of the estrous cycle; 2) total protein concentration of the uterine secretions increases during the luteal phase of the estrous cycle, but is not affected by CP intake; and 3) fertility is impaired when CP is increased to 23%

CP. Although the exact mechanism for reduction of fertility in cows fed high protein rations has not been elucidated in our study, several possibilities have arisen. Alterations in the uterine secretions may affect fertility by reducing ovum or sperm viability, decreasing fertilization or impairing early embryonic cleavage and development. The uterine environment also may be altered indirectly due to changes in steroid secretion.

Running Head: Relationship of crude protein and reproduction

CHAPTER 4

Relationship of Dietary Crude Protein to Reproduction in High Producing Postpartum Dairy Cows. II. Minerals and Progesterone.[1]

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ABSTRACT

This experiment was designed to examine the effect of crude protein (CP) intake on progesterone and minerals in plasma and uterine secretions at various stages of the estrous cycle. Eighteen multiparous, high producing (7,836 kg) Holstein dairy cows were assigned randomly to isocaloric rations (74% TDN) containing either 12 or 23% CP on day 40 postpartum. Uterine secretions and blood samples were collected at estrus, day 5 and day 15 of the first estrous cycle after day 50 postpartum and at the subsequent estrus. Uterine secretion Ca was higher during the luteal phase (159.0 ± 11.1 ppm), days 5 and 15, then during estrus (81.5 ± 4.0 ppm; P <.01), but did not vary with treatment. Plasma Ca (105.1 ± 1.1 ppm) and Mg (23 ±.3 ppm) did not change with time or treatment. Cows fed 12% CP had higher Mg in the uterine secretions on day 5 and 15 of the estrous cycle than cows fed 23% CP (66.7 ± 11.1 and 38.6 ± 4.3 ppm, respectively; P<.01). Plasma P was higher (P<.01) in cows fed 23% CP (6.1 ± .2 mg/100 ml) than in cows fed 12% CP (5.2 ± .2 mg/100 ml). However, uterine secretion P was higher in the 12% CP group (6.9 ±1.1 vs. 5.3 ±.8 mg/100 ml; P<.01) but only on days 5 and 15 of the estrous cycle. Plasma Zn concentration declined from 1.4 ±.2 to 1.1 ±.1 ppm from the first to second experimental estrus in cows fed 12% CP, but rose from 1.2 ±.1 to 1.4 ±.1 ppm during the same interval in cows fed 23% CP. Uterine secretion Zn

declined from .92 ±.13 to .41 ± .04 ppm in cows fed 12% CP and from .72 ± .12 to .52 ± .10 ppm in cows fed 23% CP from the first to second experimental estrus. Cows fed 23% CP had higher plasma K concentrations than cows fed 12% CP (224 ± 3 and 216 ± 2 ppm, respectively; P=.06). Potassium was higher in the uterine secretions of cows fed 12% CP on days 5 and 15 (1059 ± 124 µg/ml) as compared to cows fed 23% CP (799 ±61 µg/ml; P<.05). Plasma progesterone was elevated during the luteal phase, but no treatment differences were observed in the peak concentration, the interval from estrus to peak progesterone or the number of days the plasma progesterone concentration was <1 ng/ml. These results indicate dietary CP intake can alter uterine secretion mineral content, although the mechanism is not known.

INTRODUCTION

Approximately 25% of the nation's dairy cows culled annually are removed due to reproductive failure. In 1979, Jordan and Swanson reported services per conception (SPC) in high producing Holstein cows increased from 1.47 to 2.47 as crude protein (CP) in the isocaloric rations increased from 12.7 to 19.3% CP. A concomitant increase in days open and calving interval also occurred. Similar trends have been reported by Drori and Folman (1971) and Edwards et al. (1980).

The decline in fertility as protein intake increases may be caused by an alteration in steroid production or in the composition of uterine secretions. Lower plasma progesterone levels on day 14 of the first postpartum estrous cycle and the cycle of conception in cows fed 19.3% CP were observed by Jordan and Swanson (1979b). Increased concentration of plasma urea-N in cows fed 23% CP rations (Jordan et al., 1981) may reduce LH binding to its receptor (Haour and Saxena, 1974), decreasing progesterone synthesis.

Ion composition (Ca, Mg, P, K, Zn) of the reproductive tract secretions, which may be altered by protein or nitrogen content of the ration, affects viability of sperm, ova and zygotes via their effect on cell metabolism. When ruminants graze pastures which have been fertilized with high levels of nitrogen, lowered blood Mg results, probably due to reduced absorption (Bartlett et al., 1954; Head and Rook, 1955; Chicco et al., 1972). Zinc absorption in rats and ruminants declines when low levels of dietary protein are consumed (Van Campen and House, 1974; Stake et al., 1973). Juhász et al. (1975) reported decreased ^{42}K activity in plasma and whole blood from sheep after intra-ruminal administration of urea (0.6 to 0.8 g/kg body-weight).

Steroids and minerals play a vital role in establishing a reproductive tract environment conducive to ova and sperm

viability, fertilization and embryo cleavage and survival prior to implantation. This experiment was conducted to examine the effect of CP intake on levels of progesterone and minerals in plasma and uterine secretions at various stages of the estrous cycle.

MATERIALS AND METHODS

On day 40 postpartum, eighteen multiparous, high producing (7,836 kg) Holstein dairy cows were assigned randomly to isocaloric rations (74% TDN) containing either 12 or 23% CP. Uterine secretions and blood samples were collected 2 hr after feeding, at estrus (day 0-1), day 5 and day 15 of the first estrous cycle after day 50 postpartum and at estrus (day 0-2) of the subsequent estrous cycle. Collection and storage techniques were described previously (Jordan et al., 1981).

Subsequently, uterine secretions and plasma samples were analyzed for Ca, Mg, K and Zn by atomic absorption spectrophotometry. Zinc concentration in uterine secretions was not determined on day 5 and 15 of the estrous cycle due to insufficient sample volume. Plasma and uterine secretions were analyzed for P by a colorimetric method using a molybdenum trioxide polymer (Phosphorus Rapid Stat® Kit, Pierce Chemical Co.). Daily blood samples were collected during the estrous cycle for radioimmunoassay analysis of progesterone, using dextran-charcoal to separate bound from free steroid (Koligian

and Stormshak, 1977). The progesterone data were standardized to a 21-day estrous cycle. From the daily progesterone data, peak progesterone concentration, days to peak progesterone concentration and percent of days in the cycle with <1 ng progesterone/ml were determined. Concentrations of plasma ammonia, urea, albumin, total protein and glucose and uterine secretion urea, total protein and albumin; reproductive parameters; and milk production records were reported in the previous paper (Jordan et al., 1981).

Data were analyzed by analysis of variance, orthogonal contrasts, regression and correlations to determine the effect of dietary CP intake on mineral composition of uterine secretions and plasma, as well as on progesterone concentrations in the plasma.

RESULTS

Concentrations of Ca in plasma (105 ± 1.1 ppm) did not differ with time or treatment. Although uterine secretion Ca did not differ with treatment, it was 2-fold higher during the luteal phase, days 5 and 15 (159.0 ± 11.1 ppm), than during estrus (81.5 ± 4.0 ppm; $P<.01$; Fig. 1).

Plasma P was higher in cows fed the 23% CP ration as compared to cows fed the 12% CP ration (6.1 ± .2 and 5.2 ± .2 mg/100 ml, respectively; $P<.01$), but did not change significantly with time. However, uterine secretion P

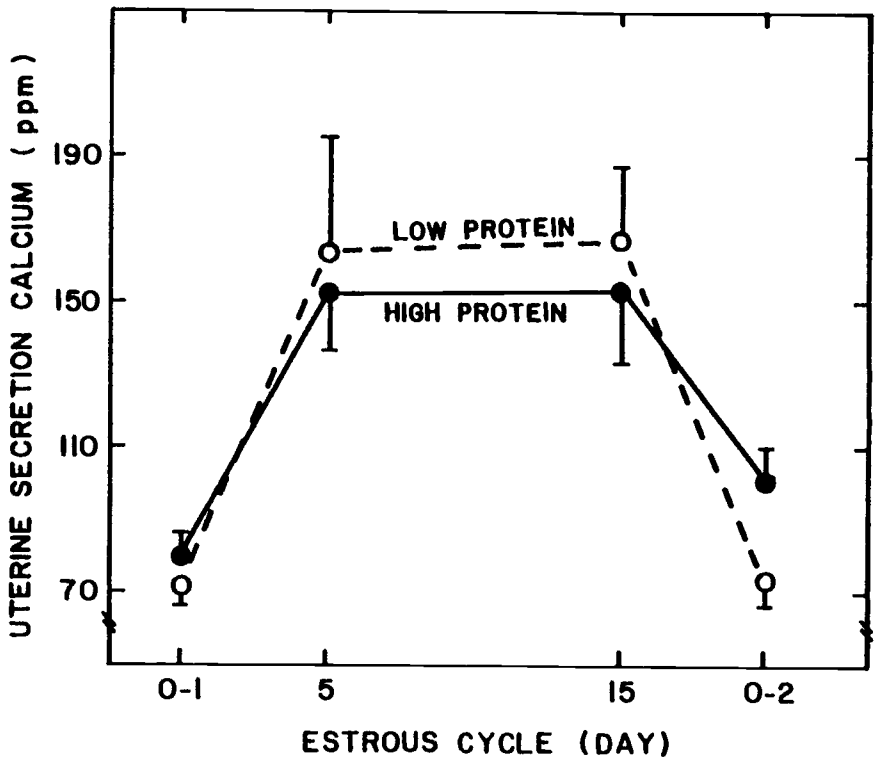

FIG. 1. Calcium concentrations (Mean ± SE) in the uterine secretions of cows fed 12 or 23% CP rations at estrus (day 0-1), day 5 and day 15 of the first experimental estrous cycle and the subsequent estrus (day 0-2).

concentration was 30% higher on days 5 and 15 of the estrous cycle (Fig. 2) in the 12% CP group than in the 23% CP group (6.9 ± 1.1 and 5.3 ± .8 mg/100 ml, respectively; $P<.01$). Phosphorus concentration increased 67% from day 0-1 to day 5, declined slightly on day 15, then dropped precipitously at the next estrus ($P<.01$).

No significant differences in plasma Mg (23 ± .3 ppm) were observed with treatment or time. The cows fed 23% CP had higher (66.7 ± 11.1 ppm) Mg in the uterine secretions on days 5 and 15 of the estrous cycle than the cows fed 23% CP (38.6 ± 4.3 ppm; $P<.01$). The concentration of Mg, similar to Ca and P, rose from day 0-1 to days 5 and 15 and declined by day 0-2 in both groups of animals (Fig. 3; $P<.01$).

Significant time-treatment interactions existed for plasma ($P<.05$; Fig. 4) and uterine secretion ($P<.01$; Table 1) Zn concentrations. In plasma, Zn concentration declined from 1.4 ±.2 ppm at the first experimental estrus to 1.1 ± .1 ppm at the second experimental estrus in cows fed 12% CP, whereas in cows fed 23% CP, plasma Zn concentrations rose from 1.2 ±.1 to 1.4 ± .1 ppm from the first to the second experimental estrus. Uterine secretion Zn concentration declined 55% from the first to the second experimental estrus in cows fed 12% CP but only 28% in cows fed 23% CP (Table 1).

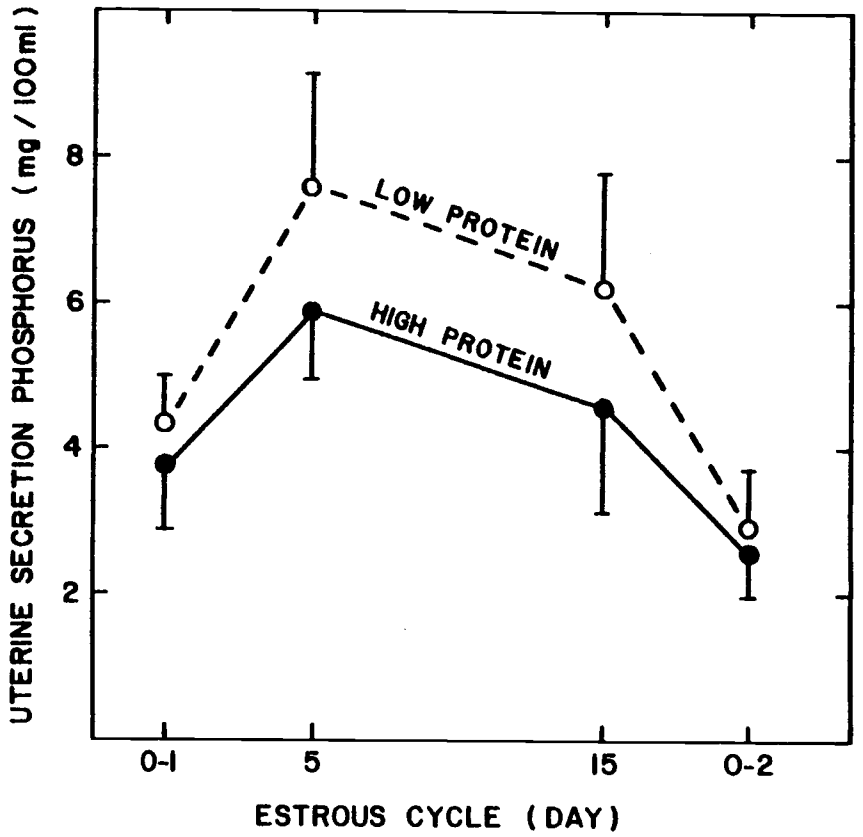

FIG. 2. Uterine secretion phosphorous concentration (Mean ± SE) of cows fed 12 or 23% CP rations at estrus (day 0-1), day 5 and day 15 of the first experimental estrous cycle and the subsequent estrus (day 0-2).

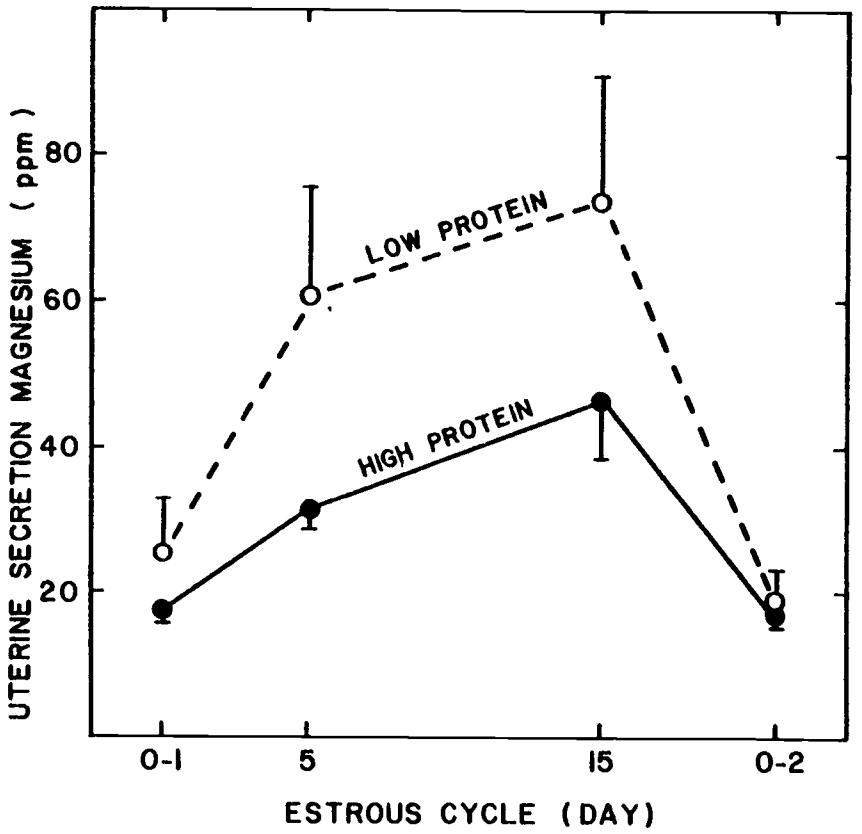

FIG. 3. Magnesium concentration (Mean ± SE) in the uterine secretions at estrus (day 0-1), day 5 and day 15 of the first experimental estrous cycle and the subsequent estrus (day 0-2) of cows fed 12 or 23% CP rations.

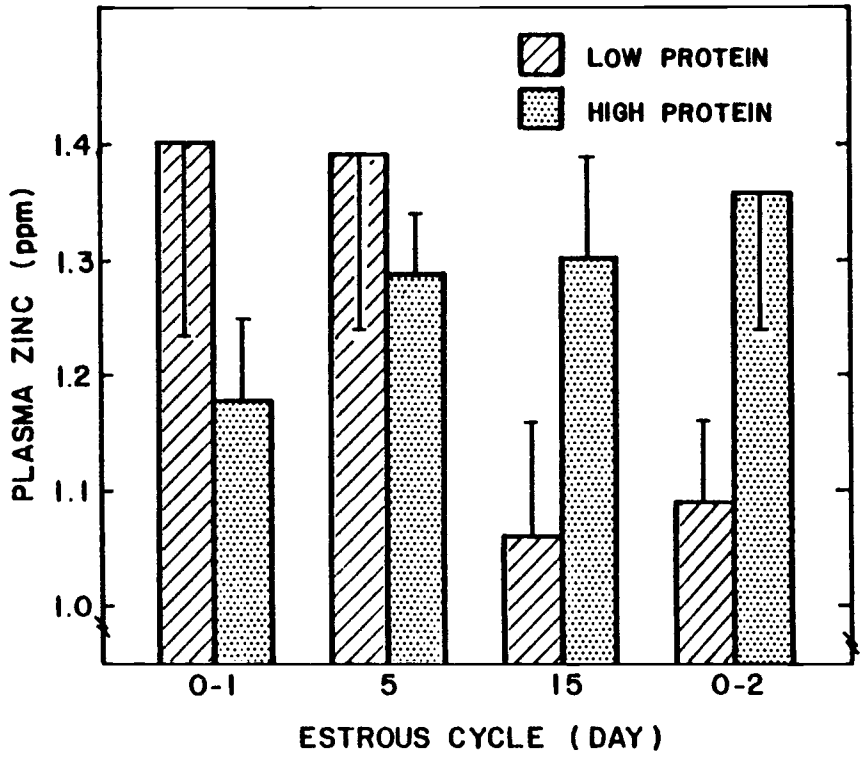

FIG. 4. Plasma zinc concentrations (Mean ± SE) of cows fed 12 or 23% CP rations at estrus (day 0-1), day 5 and day 15 of the first experimental estrous cycle and the subsequent estrus (day 0-2).

Table 1. Uterine secretion zinc concentrations at estrus.

Ration	Sample Time	
	0-1[a]	0-2[b]
	ppm	
12% CP Ration	.92 ± .13[c]	.41 ± .04
23 CP Ration	.72 ± .12	.52 ± .10

[a]0-1 indicates the day of the first experimental estrus.

[b]0-2 indicates the day of the second experimental estrus.

[c]Mean ±SE.

Higher plasma K concentrations were observed (P=.06) in cows fed a 23% CP ration than in cows fed a 12% CP ration (224 ± 3 and 216 ± 2 ppm). Uterine secretion of K concentration increased from day 0-1 (536 ± 36 ppm) to day 5 (873 ± 68 ppm) and 15 (992 ± 133 ppm) and returned to day 0-1 levels by the next estrus (535 ± 38 ppm; P<.01; Fig. 5). Potassium was higher in the uterine secretions of cows fed 12% CP on days 5 and 15 (1059 ± 124 µg/ml) as compared to cows fed 23% CP (799 ± 61 µg/ml; P<.05).

No treatment differences were observed in plasma progesterone levels (Fig. 6), but as expected, plasma progesterone was elevated during the luteal phase, fitting the regression equation: $\hat{Y} = -.19 + .29X - (.26 \times 10^{-4})X^4$ where $\hat{Y}$ equals the predicted progesterone concentration and X equals time in days; $R^2 = .32$; P<.01). The peak progesterone concentration did not differ between the high and low protein ration groups (4.4 ± 2.0 and 3.7 ± .9 ng/ml, respectively), nor did the interval from estrus to peak progesterone concentration. The plasma progesterone concentration was <1 ng/ml 38 ± 4% of the days of the estrous cycle in the 23% CP group and 34 ± 3% in the 12% CP group.

DISCUSSION

Phosphorus, Ca, Mg, Zn and K have been implicated in many vital physiological functions. Garbers and Kopf

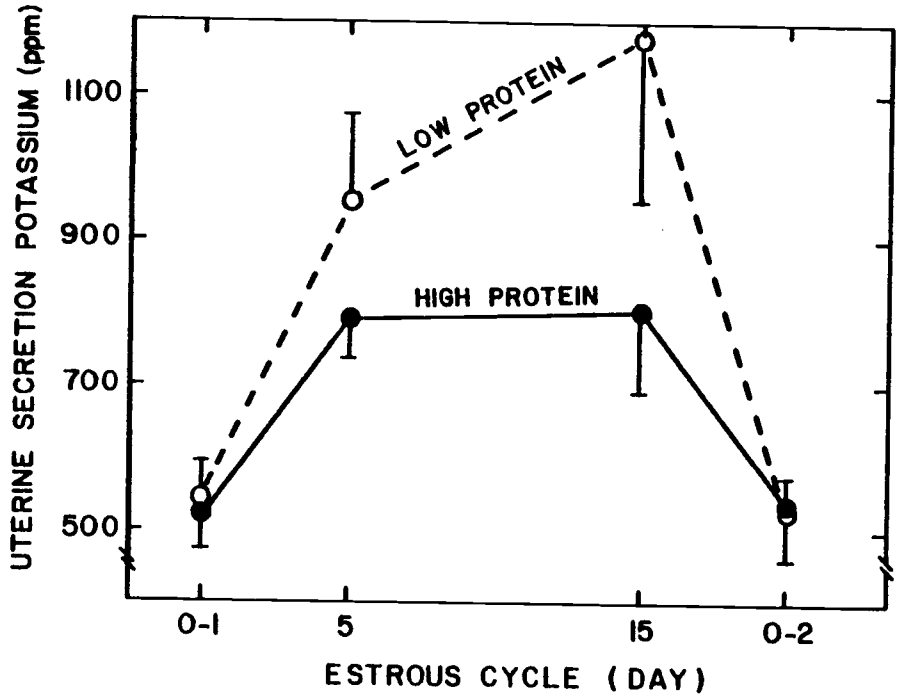

FIG. 5. Uterine secretion potassium concentration (Mean ± SE) of cows fed 12 or 23% CP rations at estrus (day 0-1), day 5 and day 15 of the first experimental estrous cycle and the subsequent estrus (day 0-2).

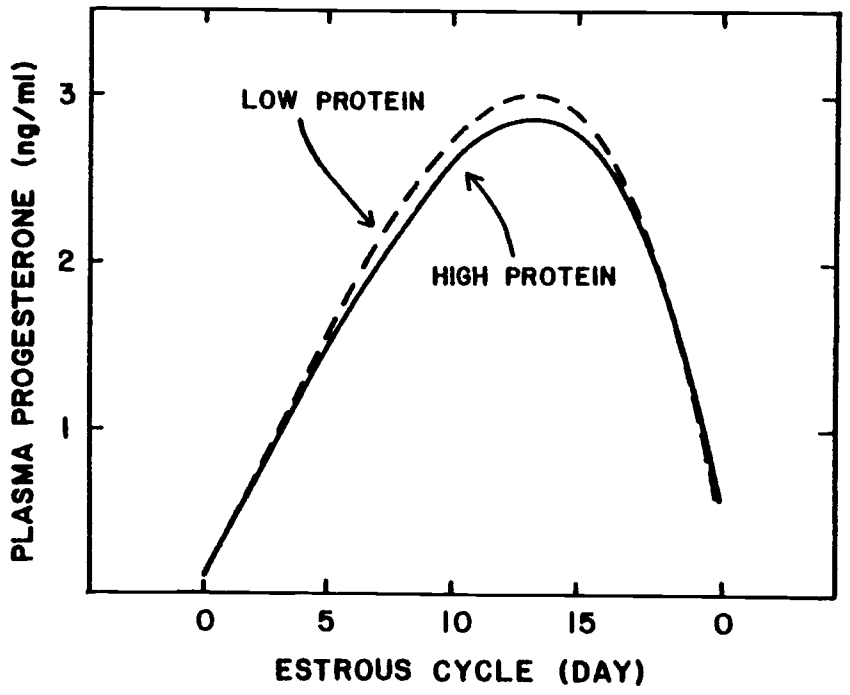

FIG. 6. Regression of the plasma progesterone concentrations of cows fed 12 or 23% CP rations standardized to a 21-day estrous cycle.

(1980) have hypothesized that Ca may induce the acrosomal reaction in spermatozoa. Ca is required for in vitro fertilization (Miyamoto and Ishibashi, 1975) and cellular adhesion in sea urchin larva (Herbst, 1900, cited by Aikawa, 1971). More recently, the role of Ca in hormone action and the assembly and disassembly of microtubules, as mediated by calmodulin, has been elucidated (Klee et al., 1980).

Ayalon (1978) observed a 13.5-fold increase ($P<.01$) in uterine secretion Ca concentration on day 7, but not on day 6 or 8, of the estrous cycle in cows with abnormal embryos compared to cows which were not bred or cows carrying normal embryos. This elevation may result in or arise from an impairment in normal hormone action and cellular division. Miyamoto and Ishibashi (1975) reported decreased in vitro sperm penetration and fertilization of mouse ova at Ca concentrations above or below an optimum of 1.71 mM (68.5 ppm). The 50% reduction in Ca concentration to 81.5 ppm observed in our study at estrus may be required to permit sperm penetration and fertilization assuming the reduction is maintained into metestrus. Lamothe and Guay (1970) reported Ca concentrations from 5.61 to 6.64 mg/100 ml (56.1 to 66.4 ppm) from proestrus to postestrus, but did not report the increase during the luteal phase we observed (Fig. 1). Uterine secretion Ca and total protein

were positively correlated ($r=.32$; $P<.01$; Table 2), possibly due to the increase in each observed on days 5 and 15 of the estrous cycle or the involvement of Ca in uterine protein synthesis or transport.

The mode of P and Ca transport into the gravid uterus prior to implantation has not been elucidated, although transport across the placenta is a Vitamin D-independent process (DeLuca, 1980). Our results indicate the transport mechanism for P into the non-gravid uterus may be influenced by a product of protein metabolism, since cows fed 12% CP had greater uterine secretion concentration of P (Fig. 2) whereas cows fed 23% CP had greater plasma P concentration. Phosphorus is essential to gametes and zygotes through its involvement in energy transfer in cellular metabolism. In addition, Batra (1978) reported myometrial microsome uptake of Ca is potentiated by inorganic P, indicating P may affect sperm, ova or zygote transport.

Our uterine secretion P concentrations agree with those reported by Olds and VanDemark (1957), but are 1.5- to 5-fold less than those reported by Schultz et al. (1971) and Lamothe et al. (1976). Undiluted uterine secretions were collected by simple aspiration in our study, while Schultz et al. (1971) utilized a vacuum pump to aspirate the secretions under a negative pressure and Lamothe et al. (1976) collected uterine flushings. Thus, collection

Table 2. Significant plasma and uterine secretion correlations for cows fed 12 and 23% CP rations.[c]

Uterine Secretion	Uterine secretion					Plasma			
	Albumin	Ca	P	Mg	K	Total Protein	Albumin	Urea-N	Progesterone
Total Protein	.67**	.32**	.67**	.38**	.33**	--	--	--	.50**
Albumin		--	.46**	--	--	--	--	--	.28**
Ca			--	.36**	.40**	--	--	--	.37**
P				.22[a]	.25*	--	.23*	--	.37**
Mg					.79**	-.31**	--	-.25*	.37**
K						-.29*	--	-.20[b]	.46**

* $P<.05$

** $P<.01$

[a] $P=.06$

[b] $P=.10$

[c] Total protein, albumin and urea-N data are from Jordan et al., 1981.

technique may have influenced P content. Although the P concentrations reported by Schultz et al. (1971) differed from those we reported, they observed an increased level of uterine secretion P during the luteal phase of the estrous cycle as reported in the bovine by us and in the rabbit and sheep by Heap (1962). The positive correlation of uterine secretion P with uterine secretion total protein (r=.67) and plasma albumin (r=.23, Table 2), suggests albumin may act as a carrier for P.

Magnesium concentration may also affect Ca and P levels due to its requirement by the kidney mitochondrial mixed function monooxygenase, 1-hydroxylase, which converts 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. Although plasma Mg levels did not differ between groups, the higher uterine secretion concentrations in the low protein group (Fig. 3) may also be indicative of higher concentrations in the kidney. Uterine secretion Mg is correlated (Table 2) with uterine secretion Ca (r=.36) and P (r=.22), while plasma progesterone is correlated positively to uterine secretion Mg (r=.37) and Ca (r=.37). Since all three minerals, Ca, P and Mg, are elevated during the luteal phase as compared to estrus, the cyclic nature of progesterone, estrogen or the ratio of progesterone to estrogen may influence mineral transport into the uterus.

Rayssiguier et al. (1979) reported marginal Mg deficiencies impaired uterine involution and collagen metabolism

in rats. We rectally palpated all cows prior to initiation of the experiment, verifying that uterine involution was not a cause of the decreased fertility observed in the high protein group. The observation of Herbst (1900, cited by Aikawa, 1971) that Mg was important in cellular adhesion and other reports of fetal malformations with resorptions and abortions associated with severe Mg deficiency (Hurley et al., 1976), suggest that low Mg levels may be associated with infertility.

Fetal deformities also have been associated with Zn deficiency (Hurley and Mutch, 1973), possibly due to the Zn requirement for DNA and RNA polymerase. Conversely, excess Zn reduces progesterone binding to its receptor (Habib et al., 1980). In our study, uterine secretion Zn concentrations were higher at day 0-1 than 0-2 (Table 1), although the time-treatment interaction indicated the rate of decline from first to second experimental estrus differed with treatment. Uterine secretion Zn concentrations were similar to those reported by Ayalon (1978). Although we did not determine luteal phase Zn concentrations, Hagenfeldt et al. (1977) reported the Zn content of human endometrium is cyclic with peak levels during the luteal phase.

Potassium ions are important in maintaining homeostasis, the active transport of glucose via Na^+-K^+ ATPase, nerve

impulses and muscle contraction. In general, our uterine secretion K concentrations were similar to those reported in the bovine by Ayalon (1978) and Lamothe and Guay (1970), with K levels being higher in the mid-luteal phase of the estrous cycle when glucose transport into the embryo would be necessary. McKenna et al. (1978) reported K modulates in vitro aldosterone biosynthesis. At 6 mEq/l (234.6 ppm) the biosynthetic pathways from cholesterol to pregnenolone and pregnenolone to aldosterone were stimulated positively as compared to 0 mEq/l. In our experiment uterine secretion K was correlated positively (r=.46; Table 2) with plasma progesterone. Uterine secretion K concentrations were higher in cows fed low protein rations (Fig. 5) but whether progesterone mediates the K concentration in the reproductive tract or vice versa is yet to be determined. Plasma urea-N and total protein were correlated negatively with uterine secretion K (r=-.20 and r=-.29, respectively; Table 2), indicating the ion transport mechanism may be affected by protein or protein metabolites.

Plasma progesterone concentrations did not vary significantly between treatment groups; however, on day 15 of the standardized estrous cycle, plasma progesterone was 19% higher in cows fed a 12% CP as compared to 23% CP ration. The difference was not as great as the 40% increase on day 14 reported previously by Jordan and Swanson (1979b). Folman et al. (1973) hypothesized plasma

progesterone concentrations > 4 ng/ml at the peak of the luteal phase, at least one estrous cycle preceding insemination, were positively associated with conception. Thus lower progesterone in the cows fed 23% CP also may be a factor in the decreased fertility we observed. Although plasma concentrations of progesterone were not altered significantly, the uterus may be exposed to different local levels of steroids when cows are fed high and low CP rations, since progesterone and estradiol are concentrated in the endometrium (Challis et al., 1976; Henricks and Harris, 1978).

In summary, Ca, P, Mg and K concentrations were elevated during the luteal phase of the estrous cycle as compared to estrus. Plasma progesterone and uterine secretion total protein were positively correlated with each mineral in the uterine secretions. In addition, cows fed 23% CP exhibited lower concentrations of P (30%), Mg (73%) and K (32%) in uterine secretions on day 5 and 15 of the estrous cycle than cows fed 12% CP while Ca and Zn were not altered. Conversely, plasma concentrations of P (17%) and K (4%) were higher in cows fed 23% CP while Ca, Mg and Zn were unchanged and progesterone was decreased (19%). These changes may contribute to the decreased fertility observed in this and previous experiments (Jordan and Swanson, 1979a). However, we did not observe any obvious differences in the plasma and uterine secretions of the 4 cows

which conceived to first service compared to the 14 which did not.

Our results indicate dietary CP intake can alter uterine secretion mineral content, although the mechanism is not known. Small changes in protein metabolites or progesterone production may alter ion transport mechanisms. The mode of action by which the ion changes affect sperm or egg viability, fertilization, embryo transport or embryo cleavage remain to be elucidated.

APPENDIX

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Plasma Estradiol. Blood and Uterine Secretion pH, pCO_2 and pO_2.

The objectives of this experiment were to determine if plasma estradiol concentration or uterine secretion and whole blood pH, pCO_2 and pO_2 differed between cows fed 12 or 23% CP rations.

On day 40 postpartum eighteen multiparous, high producing (7,836 kg, 4% FCM) Holstein dairy cows were assigned to isocaloric rations of 12 or 23% CP. Uterine secretion and blood samples were collected and plasma obtained as described previously (Chapter 3, Materials and Methods). Uterine secretions and whole blood used for pH, pCO_2 and pO_2 were kept on ice in sealed syringes until analysis on the BMS 3 MK 2 Blood Micro System with the PHM 73 pH/Blood Gas Monitor (Radiometer, Copenhagen). The pH, pCO_2 and pO_2 were determined only at the first and second experimental estrus, due to inadequate uterine secretion volume on day 5 and 15 of the estrous cycle. Plasma estradiol concentration was determined by radioimmunoassay utilizing dextran-charcoal to remove unbound steroid.

Results and Discussion

The estradiol concentration in the plasma was not affected by treatment, but was lower during the luteal phase ($P<.01$), averaging 12.0 ± 1.0 pg/ml at estrus and

8.7 ± .7 pg/ml on day 5 and 15 of the estrous cycle (Appendix Table 1). Henricks and Mayer (1977) and Echternkamp and Hansel (1973) reported peak estrogen levels occurred at estrus, ranging from 7.8 to 20 pg/ml, and fluctuated from 1 to 5 pg/ml during the luteal phase of the estrous cycle. Thus, our luteal phase estradiol values of 9.6 ± .9 and 7.8 ± 1.0 pg/ml were high compared to those reported by others. (Subsequent analysis has indicated we have had low level contamination in our extraction procedure.) The decrease in estradiol concentration during the luteal phase of the estrous cycle and the lack of effect of treatment may be valid results, but the absolute values are suspect. The sample volumes remaining are inadequate to reanalyze most samples. Opposed to the positive correlations of progesterone, estradiol was correlated negatively with uterine secretion Mg ($r=-.33$, $P<.01$) and K ($r=-.34$, $P<.01$) concentrations reported in Chapter 4.

Whole blood pH did not differ with time or treatment in this experiment (Appendix Table 2). The pCO_2 and pO_2 were not analyzed statistically since some samples consisted of venous blood and others arterial (Appendix Table 2). The pO_2 changed the most from arterial to venous blood, although pCO_2 and pH were affected to a lesser degree (Tenney, 1977; Swenson, 1977). Atmospheric pO_2 was approximately 158 mm Hg (Tenney, 1977), so values above this were

APPENDIX TABLE 1. Estradiol concentration in the plasma during the estrous cycle pooled from cows fed 12 and 23% CP rations.

Day of the estrous cycle	Estradiol
	---pg/ml----
0-1[a]	11.8 ± 1.4[b]
5	9.6 ± .9
15	7.8 ± 1.0
0-2[a]	12.3 ± 1.5

[a]0-1 is the first experimental estrus and 0-2 is the second experimental estrus.

[b]Mean ± SE

APPENDIX TABLE 2. Whole blood pH, pCO_2 and pO_2 for cows at the first and second experimental estrus when fed 12 or 23% CP rations.

Cow No.	First Experimental Estrus			Second Experimental Estrus		
	pH	pCO_2	pO_2	pH	pCO_2	pO_2
		mm Hg			mm Hg	
12% CP Ration						
1287	7.434	38.9	54.5[b]	7.385	57.0	41.3[b]
20	7.517	27.4	(176.1)	7.415	----	118.3
66	7.376	----[a]	50.7[b]	7.295	51.5	31.4[b]
39	7.346	----	50.5[b]	7.294	57.8	(192.9)
1374	7.277	----	44.2[b]	7.415	39.8	(131.0)
P-21	7.430	----	(194.7)	7.394	40.6	(168.5)
1171	7.354	36.9	114.6	7.365	40.3	75.3
56	7.322	54.3	42.6[b]	7.395	43.8	102.8
41	7.276	31.8	42.6[b]	7.359	42.8	43.5[b]
Mean	7.367[c]	NA	NA	7.366[c]	NA	NA
23% CP Ration						
1210	7.510	28.0	110.7	7.301	39.2	90.4
8	7.467	38.4	(188.4)	7.370	46.0	78.3
40	7.394	47.3	46.8[b]	7.423	----	(120.3)
36	7.431	42.5	102.3	7.328	----	42.8[b]
1299	7.406	----	(122.5)	7.399	----	113.7
61	7.345	----	31.3[b]	7.345	46.8	38.8[b]
1293	7.432	----	115.9	7.374	29.5	94.8
1443	7.398	37.2	(246.4)	7.413	40.6	42.7[b]
1373	7.350	51.7	33.8[b]	7.370	49.0	36.3[b]
Mean	7.412[c]	NA	NA	7.368[c]	NA	NA

( ) indicates values which were outside the acceptable range for pO_2.

[a]Indicates missing values.

[b]Venous samples.

[c]Means for pH are computed by converting to eq/l. H^+ = antilog (-pH).

not possible. Davidovich et al. (1977) reported arterial pO_2 values from 72.1 to 104.4 mm Hg and pCO_2 values from 32.3 to 42.6 mm Hg in cows before and after dosing with urea. Since some sample pO_2 values were above 110 mm Hg, the mean concentration in inspired air (Tenney, 1977), an upper limit of 120 mm Hg for pO_2 was defined and since the cows were not hyperventilating a lower limit of 25 mm Hg for pCO_2 was established. Values outside these limits were suspected of being invalid. Uterine secretion pH tended to be higher in cows fed 12% CP rations, particularly at the first experimental estrus, than in cows fed 23% CP (Appendix Table 3). The uterine secretion pO_2 and pCO_2 (Appendix Table 3) were not analyzed statistically due to missing values and values outside the acceptable range for pCO_2 and pO_2 discussed previously. The missing values for whole blood and uterine secretions resulted from leaking seals on the BMS 3 MK 2 Blood Micro System, which surrounded the pCO_2 and pO_2 electrodes, causing wide fluctuations in readings. The values outside the acceptable range for pO_2 in uterine secretions and whole blood may have resulted from air bubbles being in contact with the electrode, although repeated observations were the same. Drying of the electrode membranes may also have caused some of the high pO_2 values, although the membranes were changed frequently and stored with moisture.

APPENDIX TABLE 3. Uterine secretion pH, pCO_2 and pO_2 for cows at the first and second experimental estrus when fed 12 or 23% CP rations.

Cow No.	First Experimental Estrus			Second Experimental Estrus		
	pH	pCO_2	pO_2	pH	pCO_2	pO_2
		mm Hg			mm Hg	
12% CP Ration						
1287	6.922	39.0	(124.5)	6.890	40.0	57.3
20	7.005	28.3	55.4	7.143	----[a]	67.1
66	7.045	----	(124.9)	6.668	42.8	110.1
39	6.973	----	(150.8)	6.540	26.4	(138.4)
1374	7.200	----	70.2	6.749	28.8	(145.0)
P-21	6.744	----	111.4	6.933	29.3	(167.7)
1171	6.774	31.3	(149.2)	6.470	27.8	75.3
56	6.881	47.4	41.7	6.915	43.2	84.7
41	7.165	42.7	(127.8)	7.347	(9.4)	(211.7)
Mean	6.936[b]	37.7[c]	69.7	6.739[b]	34.0	78.9
SE	NA	3.5	15.1	NA	2.9	9.0
23% CP Ration						
1210	6.863	40.1	(144.3)	6.889	34.3	(156.5)
8	6.468	42.3	(152.9)	6.848	46.7	(120.3)
40	6.439	34.3	98.6	6.745	----	78.2
36	6.957	51.8	(123.2)	7.144	----	(146.3)
1299	6.495	----	108.6	6.888	----	74.7
61	6.790	----	92.6	6.020	40.5	(127.7)
1293	6.762	----	101.8	7.550	44.9	100.7
1443	6.539	38.4	(141.9)	6.915	36.7	(149.4)
1373	7.287	(9.3)	83.8	6.814	38.2	(191.3)
Mean	6.665[b]	41.4	97.1	6.673[b]	40.2	84.5
SE	NA	2.9	4.2	NA	2.0	8.1

( ) indicates values which were outside the acceptable range for pCO_2 or pO_2.
[a]Indicates missing values.
[b]Means for pH are computed for converting to eq/l. H^+ = antilog (-pH).
[c]Means for pCO_2 and pO_2 only include values within the acceptable range.

In the future an artery which can be punctured repeatedly and with accuracy should be selected instead of the coccygeal vessel if pH, pCO_2 and pO_2 measurements are to be made. In aspirating uterine secretions as we did, it is impossible to prevent some contact with air, which alters the sample pH, pO_2 and pCO_2. In addition, small air bubbles are present in aspirated secretions and the secretions can not be mixed well due to viscosity. Consequently, a new technique should be devised if accurate measurement of pH, pCO_2 and pO_2 are going to be made on uterine secretions.