NITROGEN AND CARBON BALANCE OF LACTATING HOLSTEIN COWS DURING EARLY AND MIDLACTATION

by

Jongsu Eun

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APPROVED:

Joseph H. Herbein, Chairman

C. E. Polan

M. L. McGilliard

W. E. Vinson

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Blacksburg, Virginia

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(ABSTRACT)

Thirty six Holstein cows in their first, second, third or fourth lactation were used in 2 x 2 factorial design to evaluate nitrogen (N) and carbon (C) partitioning to milk, urine, feces, and body tissue during early and midlactation. Diets containing 16% CP were formulated with 30 or 39% rumen undegradable protein (RUP) obtained by substituting blood meal (BM) for soybean meal (SBM). Each level of RUP was formulated with supplemental phosphorus from mono- and dicalcium phosphate or wheat bran. Dry matter intake was higher in midlactation compared with early lactation, and increased as parity increased. Addition of BM to the diets decreased milk protein percentage and yield compared with SBM. Fecal N excretion was higher for cows fed BM due to lower N digestibility (67 versus 63%). Cows fed SBM retained more N and partitioned more N into milk than cows fed BM. Cows partitioned approximately 49, 40, and 11% of absorbed N to urine, milk, and tissue, respectively. Concentration of plasma urea N was correlated with milk urea N ($r = .50$). Overall, data indicated that cows fed 16% dietary CP with SBM or BM met their requirements for milk and tissue protein synthesis. Carbon partitioning was very similar to N partitioning in response to parity. Using a fermentation balance equation, it was estimated

that .3 and 3.1 kg C were partitioned daily to methane and carbon dioxide, respectively. Estimated data indicated that 36% of intake C and 57% of absorbed C were lost to the atmosphere as carbon dioxide plus methane.

Key words: nitrogen, carbon, soybean meal, blood meal, carbon dioxide, methane.

Abbreviation key: **N** = nitrogen, **C** = carbon, **RUP** = rumen undegradable protein, **SBM** = soybean meal, **BM** = blood meal.

DEDICATION

This thesis is dedicated to my father, Kapcheul Eun, and my mother, Oksoon Choi, for all of their support and encouragement throughout my life and for teaching me what is truly important. Without their encouragement this degree would not have been possible. I thank them and I love them.

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Introduction

Optimizing dairy production with the aim to minimize the excretion of undesired end products to the environment requires a detailed knowledge of nutrient partitioning in the lactating cow. High producing dairy cows must consume and digest large amounts of feed to provide nutrients for milk production. Many factors affect digestive efficiency and animal productivity. Amount of feed consumed has a major influence on digestibility. Type of dietary ingredients, physical form, nutrient concentrations, and feeding strategy also may affect digestion or productivity. As a cow's milk production increases, supply of precursors for milk production becomes more critical. For example, flow of nitrogenous compounds to the small intestine is influenced by the extent of degradation of dietary protein and synthesis of microbial protein in the rumen. However, there is a considerable loss of nitrogen from feed protein in the waste produced by dairy cattle. Feeding excess rumen degradable protein inevitably leads to excretion of some additional nitrogen via the urine. With respect to environmental concerns, the excess nitrogen has the potential for pollution of surface and groundwater following land application of the waste produced by the dairy unit. In contrast, the dairy cow is expected to reduce loss of nitrogen when the balance between rumen degradable and undegradable protein in the diet is appropriate. In addition, minimizing nitrogen excretion by dairy cattle requires reduction of dietary CP intake to the level at which the supply of absorbed microbial and dietary amino acids meets requirements for maintenance, milk production, and tissue growth or replenishment.

From an environmental standpoint, dairy cattle present another area of concern. Associated with rumen fermentation is the emission of methane into the air, which is of concern with respect to global warming or the "greenhouse effect." Methane production in the rumen typically is proportional to the amount of feed ingested. To date, methanogenesis in dairy cattle has been the primary focus of discussions regarding methane production by domestic livestock. The following review summarizes the current understanding of nitrogen utilization by lactating cows and nitrogen and carbon losses to the environment.

Nitrogen Utilization in Dairy Cattle

The optimum feeding system for dairy cattle is one in which the cost of protein supplementation is kept low, while the complete requirement for essential amino acids is met by a combination of microbial and rumen undegradable feed proteins. Endogenous proteins, consisting primarily of sloughed ruminal epithelium cells, are an additional source of amino acids to the small intestine. Contribution by this source is difficult to quantify, and is considered to be low (Stern and Satter, 1980). Since delivery of essential amino acids to the small intestine depends upon factors such as feed source, intake, energy content of the diet, particle size, rumen microbial growth factors, and turnover rate, it is not possible to form a simple method for estimation of amino acid profile supplied to the intestine by a diet or feedstuff within a diet.

The crude protein (CP) intake to support production of up to 35 kg milk/d, as recommended by NRC (1989) for cows in early lactation has been gauged to be inadequate

by some (Forster et al.,1983; Kung and Huber, 1983; Polan et al.,1985), adequate by others (Maynard et al., 1979; Treacher et al., 1976), and unnecessarily high by Huber and Kung (1981). Because protein supplements are typically the most costly feed ingredients in a complete diet, factors influencing CP intake can have an economic, as well as physiological and environmental, impact.

Intake might be a limiting factor in meeting protein requirements of cows in early lactation. Kung and Huber (1983) found that intake was not a limiting factor in early lactation when dietary CP ranged from 14% to 17%. However, Polan et al. (1976) had previously shown that DMI decreased when dietary CP was increased from 12.8% to 16.2%. Intake is typically depressed by diets that fall below 11% CP, because fermentation by rumen microorganisms is uncoupled due to a deficiency in available nitrogen for microbial protein synthesis (Barney et al., 1981; Oldham et al., 1981). Cows in early lactation with inadequate CP intake can lose up to 25% of their total body protein mass as a result of homeorhetic control mechanisms that allow use of endogenous proteins in support of milk protein production (Bauman and Currie, 1980; Botts et al., 1979). After peak production, CP intake above the requirement will allow the cow to replenish her body protein mass. Dietary CP percentage is negatively correlated with the time required for full repletion (Barney et al., 1981).

Feedstuffs differ in their potential to serve as nitrogen sources in support of lactation. Therefore, isonitrogenous substitutions, as measured by CP, of one feedstuff for another will not necessarily provide the same milk production response. Soybean meal was superior to

isonitrogenous additions of urea to a complete feed (Satter and Roffler, 1975). Heat-treated soybean meal (Stern et al., 1985) and soybean meal treated with NaOH (Mir et al., 1984), compared with untreated soybean meal, increased nitrogen retention in lactating cows and growing calves. Blood urea N and rumen fluid ammonia (NH_3)-N concentrations indicated that treatment with NaOH or heat resulted in decreased proteolysis by microorganisms. Satter and Roffler (1975) suggested that reduced proteolysis of soybean meal resulted in increased flow of dietary amino acids to the small intestine. High NH_3-N concentration in the rumen is associated with N loss, because excess NH_3 absorbed from the rumen is converted to urea in the liver (Orskov, 1982). Blood urea not excreted via urine in the kidney can be recycled back to the rumen via the saliva. Bruckental et al. (1978) suggested that in the first month of lactation 10% of hepatic urea production was recovered in the milk and 22% in the urine, so that 68% of the urea was degraded or recycled, presumably to the digestive tract. Conrad (1972) reported that approximately 26% of total urea synthesis was excreted in urine of cows in midlactation.

Another mechanism that allows recycling of urea is transport from blood back through the rumen wall. Factors that influence such transfer include concentrations of ammonia in rumen contents, concentration of urea in blood, supply of fermentable organic matter to the rumen, and permeability of the rumen wall for urea (Kennedy and Milligan, 1980). Orskov (1982) cited evidence that this transfer was mediated by a carrier in the event that rumen NH_3 concentration was low. Armstrong and Weekes (1983) suggested this transfer only occurred when energy levels of rumen contents were high. Although urea recycling by saliva and

rumen wall transport allows conservation of N within the animal, recycling is not very efficient in terms of energy expenditure. Certainly, direct uptake of feed-derived NH_3 by the rumen microorganisms is going to be a more efficient process than is involved in the transport of NH_3 from the rumen to the liver, conversion to urea, recycling back to the rumen, and hydrolysis of urea by microorganisms to obtain NH_3.

Dietary Protein Degradation in the Rumen

Degradation of dietary protein is believed necessary to provide microbes with NH_3, alpha-keto acids, or intact amino acids for their own protein synthesis (Orskov, 1982). The extent to which a protein source is degraded by rumen microorganisms is a characteristic of the feedstuff. However, reported estimates of dietary protein degradation have a large variation due to the physical nature of diets, method of feeding, experimental animals, level of intake, and analytical errors (NRC, 1985). It is generally accepted that the mixture of proteins in a diet and their susceptibility to degradation in the rumen can have a substantial influence on the amount of N available for microbial protein synthesis. It is also important to assess degradation of dietary proteins with regards to the quality of undegraded protein leaving the reticulorumen (Ganev et al., 1979). The proportion of the protein source that resists degradation by the rumen microorganisms, and is delivered to the small intestine intact, is undegraded and is often called bypass protein. The proportion of bypass protein in feeds varies, but in most feeds it is between 20 and 60% of the CP (Chalupa, 1975; Mertens, 1977). Bypass protein is subject to enzymatic degradation in the abomasum and intestine, as is

microbial protein, and both serve as a source of essential and nonessential amino acids for the ruminant (Orskov, 1982; Van Soest, 1994). Many factors can influence the extent to which a protein source bypasses the rumen. Huber and Kung (1981) stated the extent of bypass may be affected by passage rate out of the rumen, by altering the microbial population balance or its growth, or by the particle size of the feedstuff.

Santos et al. (1984) reported values of 70, 45, 52, and 46% for degradability of CP in soybean meal (SBM), corn gluten meal (CGM), wet brewers grains (WBG), and dry distillers grains (DDG), respectively, when they were included in diets for lactating cow. Apparent absorption of amino acids from the small intestine was 70, 77, 71, and 66% of the amino acids entering the duodenum for the SBM, CGM, WBG, and DDG diets, respectively. Actual amounts absorbed (g/d) were lowest for the SBM diet. They concluded that diets containing CGM, WBG, or DDG generally supplied more total amino acids to the small intestine than a diet containing SBM. Net amounts of amino acids from bypass protein and rumen microorganisms flowing to the intestine also were higher for these diets, indicating adequate amounts of N were available to the microorganisms despite the lower degradability of the proteins. Loerch et al. (1983) reported that diet supplementation with blood meal (BM) or dehydrated alfalfa (DA) resulted in greater N flow to the small intestine of sheep when compared with a diet containing SBM. The degradable portion of SBM, BM, and DA was 71.3, 18.3, and 36.7%, respectively. Mathers et al. (1979) suggested that in general, animal protein sources such as BM had a greater undegradable fraction than plant proteins.

Zinn et al. (1981) estimated that rumen protein degradation of casein (CA), SBM,

cottonseed meal (CSM), or CGM in the rumen averaged 103, 85, 76, and 54 %, respectively. King et al. (1990) reported that the degradable fractions of BM, CGM, and CSM were 41.8, 53.4, and 56.2, respectively. Less rumen ammonia due to lower rumen degradability of BM or CGM protein may have resulted in greater recycling of urea to the rumen in cows fed BM or CGM compared with CSM (Chamberlain and Thomas, 1979).

Based on the above-mentioned reports, degradability ranged from 70 to 85% for SBM and from 18 to 42% for BM. The NRC (1989) estimated 65% and 28% degradability for SBM and BM, respectively.

Clark et al. (1987) reported that several factors affected a protein's ability to escape degradation in the rumen. Two of the more important factors were: 1) physical and chemical characteristics of the protein in the feed and 2) the method used in the processing of the feed. Stern et al. (1985) reported that extrusion of whole soybeans at 149 °C decreased protein degradation in the rumen from 80 to 60% and increased amino acid flow to the duodenum of lactating Holstein cows, when compared with raw soybeans. In a similar experiment with lactating cows, Kung and Huber (1983) showed that heat-treated SBM (140 °C for 2.5 h) was less degradable than SBM (55 versus 66%). Digestion of protein in the small intestine did not differ between treatments, indicating availability of amino acids for absorption from the two sources was similar. Results summarized by Chalupa (1975) showed that ruminal protein degradation was decreased by treatment with formaldehyde, and the amount of protein entering the small intestine increased significantly.

In summary, there is evidence that selection of undegradable protein sources or highly

degradable protein sources treated by chemical or physical means to reduce their degradability should increase protein flow to the duodenum of cattle. Also, a reduced loss of urea-N via urine could be expected due to reduced loss of NH_3-N from the rumen.

Protein Reserves

In contrast to energy, some vitamins, and certain minerals, dairy cattle do not store large amounts of protein in forms that can be mobilized to meet the needs of the animal when a protein deficient diet is fed (Swick and Benevenga, 1976). Thus, the protein needs should be supplied on a daily basis. Dairy cattle have reserves of labile protein which may supply key amino acids during early lactation (Botts et al., 1979). Labile protein reserves in the body are tissue proteins that can be depleted and contribute to the amino acid pool of the body (Allison and Wannemacher, 1965). Tissues associated with the mobilizable protein pool include liver, pancreas, blood, gut, skeletal muscle, and others.

Estimates of body protein reserves available for milk production ranged from 83 to 833 g/d (Belyea et al., 1978; Botts et al., 1979; Coppock et al, 1968). This range of protein would be sufficient for 60 to 600 kg of 3.5% FCM during early lactation. Coppock et al. (1968) calculated available body protein sufficient for 125 kg milk compared with 1000 kg of milk from energy stores. Belyea et al. (1978) reported losses of 50 kg of fat and 10 kg of protein during the first 2 mo following calving. Because much of the weight loss in early lactation is proportionally higher in energy than protein, increased dietary protein is needed for utilization of mobilized fat.

Paquay et al. (1972) reported that cattle had a potential reserve equal to 20% of total body protein. Using a N balance trial, Biddle et al. (1975) found labile N reserves to be about 6% of total body N in growing steers fed depletion followed by repletion diets. Tyrrell et al. (1970) noted that cattle in early stages of lactation mobilized up to 30% of the daily milk energy from fat depots but could obtain no more than 15% of their milk protein output from endogenous sources. Smith and Walsh (1984) found extensive mobilization of muscle lipids, even in well fed lactating ewes, along with losses of N. The ratios of RNA to DNA of tissue samples suggested decreased protein synthetic activity during early lactation. The authors cited other research showing a 25% reduction in muscle fibril diameter during early lactation in sheep. Trigg et al. (1980) measured energy and N balance in well-fed cows and their under-fed twins during early lactation. They found that both groups catabolized 85 to 95 g of tissue protein/d. Reid et al. (1979) reported that cows could mobilize up to 360 g of protein/d during lactation. Although the energetic value of this protein is small, its contribution of essential amino acids could be important during early lactation. In summary, cows apparently can mobilize body protein reserves at a rate of 80 to 360 g/d during early lactation.

Effects of Protein on Milk Production

Dietary protein can affect milk production by a) providing more amino acids, b) increasing available energy, and c) altering the efficiency or pattern of use of absorbed nutrients (Chalupa, 1984). Oldham (1984) suggested that 35 to 75% of the production

responses were due to the direct effects of protein, whereas 25 to 65% were the result of indirect energy effects. Effects via c) might be mediated, at least in part, by additional absorbed amino acids causing adjustments of endocrine status that affect lipid and glucose metabolism and increase nutrient supply to the mammary gland by increasing blood flow (Bines and Hart, 1982, Oldham, 1984).

Based on the report by Satter and Roffler (1975), peak milk production occurred at about 5 to 7 wk but dry matter consumption did not reach its maximum until 9 to 11 wk. Losses of body weight occurred during the first 6 wk, followed by a stabilization period of about 2 wk, after which there were gradual weight gains. Thus, the crucial period for depletion and repletion of nutrients was during the first 9 to 11 wk of lactation. Although a cow can mobilize protein and fat proportionate to need, the ration should be enriched with protein and energy to minimize depletion.

Requirements for protein increase more dramatically at the onset of lactation than energy requirements because milk solids contain about 27% protein (NRC, 1989). The demand for protein can be met by adjusting the protein content of the diet to correspond with expected feed intake in order to assure adequate daily intake of protein. Many researchers have reported that milk production increases as crude protein intake increases. McCarthy et al. (1989) reported that the source of dietary CP and energy fed to dairy cows significantly influenced the utilization of N and energy in the rumen and the flow of nutrients to the small intestine. Increases in milk production were large when dietary CP was increased from 9 to 14% of dietary DM (Clack et al.,1987). Norman et al. (1982) reported that differences in

daily milk production for cows fed diets that contained 15 or 11% CP were +3.5, +2.8, and - .1 kg/d during wk 1 to 8, 9 to 16, and 17 to 24 of lactation, respectively. However, further CP increases result in diminishing returns because bacterial destruction of dietary protein may be greater than the quantity of protein synthesized by microbes (Chalupa, 1985). Protein fed in excess of 14 to 16% of the dietary DM did not appear to increase either milk production or DMI significantly when typical diets were fed to dairy cows (Clack et al.,1987). NRC (1989) recommended, however, as much as 19% CP may be required in the diet for high producing dairy cows when DMI lags behind milk production during early lactation. Thus, microbial protein synthesis in the rumen may be insufficient to meet the protein needs of high producing dairy cows (NRC, 1989).

Digestibility and feed consumption were increased when concentration of dietary protein was increased (Chalupa, 1982; Oldham, 1984). Digestibility increases are usually considered to be the result of providing rumen microbes with optimum amounts and types of nitrogenous nutrients. Increased feed intake is partly related to improvement in digestibility, because rate of passage of digesta should increase as digestibility increases.

Other factors can affect production responses to increased intake of crude protein. Oldham (1984) discussed genotype, parity, and stage of the lactation cycle. Cows in first lactation usually did not respond to increased protein intake in the same way as mature cows. This was explained as a continuing drive in heifers to achieve mature size, which requires partition of a portion of absorbed nutrients towards growth. MacLoed et al. (1982), however, reported linear increases of feed intake and digestibility and a culvilinear increase

of milk yield when Holstein cows in first lactation were fed diets that contained 12, 15, or 18% CP.

Nitrogen Losses in Dairy Cattle

The main areas in which N losses occur are urinary excretion of urea synthesized from NH_3 derived from the rumen, fecal excretion resulting from indigestible or endogenous compounds, and urinary excretion due to an inefficient utilization of absorbed and endogenous protein for maintenance and for the synthesis of milk and body protein (Armstrong and Weekes, 1983). In addition to the economical and metabolic impacts of inefficient utilization of dietary N within the animal body, N loss in the feces and urine in regards to the environment is of growing concern. Urea and other organic forms of N are the primary forms of N in manure. Ammonia N (derived from degradation of urea) is rapidly volatilized into the atmosphere and can result in losses of up to 75% of total manure N (Van Horn et al., 1994). Much of the remaining N is likely converted to nitrate through nitrification while in storage or upon application onto soil.

Nitrous oxides association with global warming and nitrate contamination of ground water contribute to the pollution of the environment, causing many dairy farmers to face the challenges of proper manure disposal. Proper disposal will occur if land applications of excreted nutrients such as N are balanced with plant uptake. On average, a typical Holstein cow will excrete 296 g of N in her combined feces and urine/d (Van Horn et al., 1994). On a yearly basis, she will contribute 108 kg of N to our environment.

Nitrogen losses from the rumen

Important N losses may result from microbial degradation of dietary protein in the rumen not compensated for by microbial protein synthesis. Rumen losses are substantial for diets with N content above 24 g/kg of DM (i. e. 15% CP) (Tamminga, 1992). Reductions in rumen N loss are possible by reducing the dietary N level, by reducing the degradation of dietary protein, or by improving the efficiency of capture of ruminally degraded N through microbial protein synthesis. A disadvantage of a reduction in dietary N content to 24 g/kg of DM or even less is that this may reduce the digestion of other dietary ingredients. Oldham (1984) demonstrated that, in lactating cows, increasing N in the diet increased DM digestibility up to a dietary N level of almost 30 g/kg of DM (i. e. 18.8%). In nonlactating cows, the positive effect of increasing CP was minimal above 20 g of N/kg of DM. This difference probably results from differences in requirements, level of feed intake, and rate of digesta passage, all of which influence protein degradation and efficiency of microbial protein synthesis in the rumen (Tamminga et al., 1979). A shortage of ruminally degraded protein may limit the degradative activity of the microbes, resulting in a reduction in intake that further reduces supply of energy and protein for digestion. From the foregoing, it is apparent that for environmental reasons, N in diets for dairy cows should not exceed 30 g of N/kg of DM.

Nitrogen losses at maintenance

Maintenance requirements are difficult to measure and have a poor physiological basis.

Endogenous losses of N, considered unavoidable, can be separated into scurf, skin secretions, hair, and urinary and fecal losses (metabolic urinary and fecal N). Losses in scurf, skin, secretions, and hair are not well established but most likely are related to body surface area, which relates to BW in an exponential manner, varying between $BW^{.56}$ and $BW^{.62}$ (Swanson, 1980). Usually $BW^{.6}$ is accepted (NRC, 1989, Swanson, 1980), and losses are approximately .013 g of N/kg of $BW^{.6}$/d (Swanson, 1980). With the assumption that replacement of these losses is with an efficiency of .65, for a 600 kg dairy cow this would amount to a daily loss of 5.8 g of protein, the equivalent of that required for 200 mL of milk.

Unavoidable N losses in urine represent tissue maintenance requirements. They can be measured in experiments in which N-free diets are fed or by extrapolation to zero intake of N or of apparently digested N (ARC, 1984). Minimum losses determined in this way were calculated to be .44 g of N per $BW^{.5}$ (Swanson, 1980). Of these losses some 25 to 30% are in creatine and 5 to 10% in ammonia and urea. The remainder is in compounds like bilirubin, allantoin, hippuric acid, uric acid, and amino acids, including 3-methylhistidin (Swanson, 1980). For a 600 kg dairy cow, this means a daily loss of approximately 67 g of protein. Intragastric infusion studies revealed that urinary N losses may be much higher. Orskov et al. (ARC, 1984), using this technique, showed that losses were between 1 and 1.5 g of N/$BW^{.5}$. For a 600 kg cow, this means a protein loss of between 150 and 230 g/d, the equivalent of that required to produce 5 to 8 L of milk.

Nitrogen losses associated with in maintenance are relatively small (NRC, 1989; Owens, 1986), and no substantial benefit can be achieved by trying to reduce these

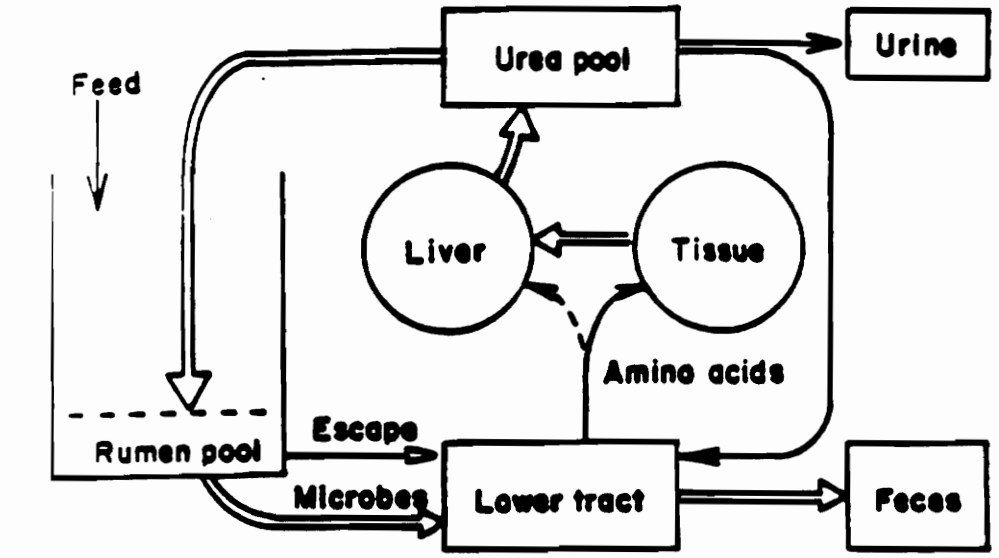
maintenance requirements. In addition, there is a continuous reduction in the proportion of N losses due to maintenance as milk production of cows increases.

Nitrogen losses in urine

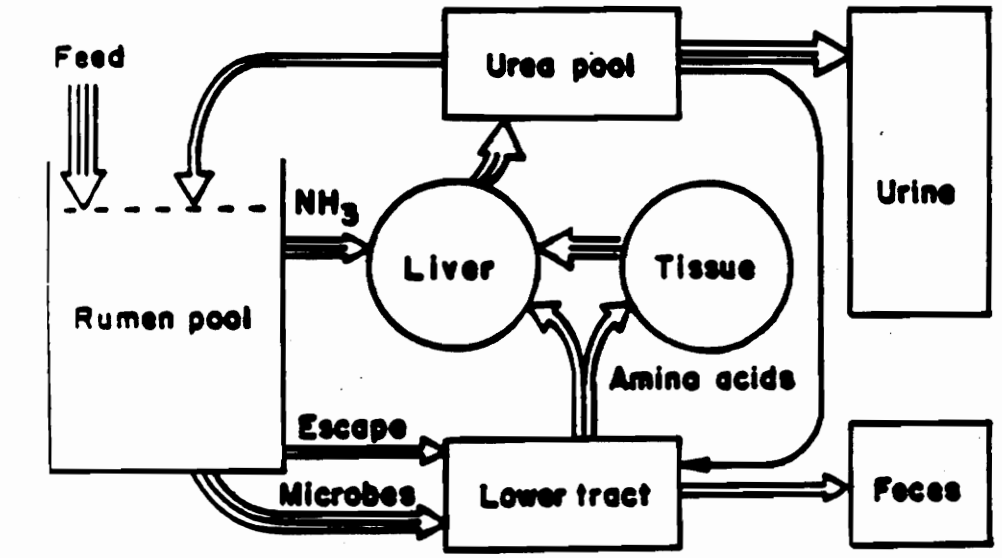
Urine is the primary excretory route for most waste products produced by metabolism of N-containing compounds in the body and many different nitrogenous compounds are found in urine. Urinary N includes excretions of maintenance, those incurred in utilization of absorbed amino acids and those arising from a positive flux from the rumen (Chalupa, 1984). Loss of N in urine originates from various sources, such as rumen loss, the replacement of metabolic losses in the gut, the incorporation of dietary protein N into microbial nucleic acids, loss in maintenance, and losses because of an inefficient conversion of absorbed amino acids into milk and body proteins. Proportions of these various compounds are indicative of the dietary N status of the animal.

Urea is the major end-product of N metabolism in nearly all mammals and amount of urea excreted by ruminants, as well as the proportion of total urinary N excreted as urea, have been shown to be positively correlated with dietary N intake (Van Soest, 1994). Figure I-1 gives an overall perspective of the shift in the N cycle that occurs when dietary N is increased. At low levels of N intake, a large portion of N metabolized within the animal is recycled, probably largely through the rumen, and very little appears in the urine. The net flow of urea shifts from the rumen toward the urine as dietary N is increased.

It has been reported that degradability of dietary protein could influence N excretion.



A. Low N intake



B. High N intake

Figure I-1. Comparison between nitrogen metabolisms at low nitrogen intake (A) and at high nitrogen intake (B), assuming equal dietary energy. Adapted from Van Soest (1994).

Christensen et al. (1993) noted that cows fed diets with the low ruminally undegradable protein (RUP) could excrete higher amounts of N to urine because concentrations of NH_3-N in ruminal fluid and of urea in plasma were higher than in cows high RUP diets. Buchanan-Smith (1995) reported that dietary protein level and degradability affected output of N in urine with greater amounts excreted when either protein content or degradability of protein in the diets increased. However, King et al. (1990) did not observe any differences in urinary N output when blood meal, corn gluten meal, and cottonseed meal were compared as supplemental proteins.

Nitrogen losses in feces

Feces contain undigested dietary protein plus protein originating in the gastrointestinal tract that is not reabsorbed. The latter is designated metabolic fecal protein (Chalupa, 1984). The largest single factor affecting quantity of fecal DM excreted is the amount of undigestible DM consumed by an animal. Fecal DM contains undigested cell walls of rumen bacteria, microbial cells from the cecum and large intestine, and residues of many endogenous substances including digestive enzyme, mucous and other secretions and epithelial cells sloughed from the walls of the alimentary tract. The proportion of materials of dietary origin relative to those of metabolic and endogenous origin would be greatest when diets containing substantial amounts of poorly digested feedstuffs (i. e., low quality forage) are fed. Conversely, animals consuming diets that are highly digestible (i. e., grains) excrete feces containing very little material of diet origin. True digestibility of feed protein is high and

often close to 90% (Van Straalen and Tamminga, 1990), so little improvement in digestibility seems possible. Reduction in the fecal excretion of undigested feed protein is not considered a promising way to realize a substantial reduction of N loss from the animal. The same is true for undigested microbial protein because it was shown that the intestinal digestion of microbial protein also is high and close to 85% (Storm et al., 1983). Tamminga (1992) noted that the most promising way to reduce fecal N excretion is the reduction of endogenous losses, which occurs because of the release of digestive enzymes and the sloughing of the epithelial cells of the gut.

Environmental Considerations in Nitrogen Excretion

Nitrogen intake and excretion by dairy cattle relative to environmental balance has been the topic of previous papers (Dewhurst and Thomas, 1992; Pell, 1992; Tamminga 1992; Van Horn et al.,1994). Van Horn et al. (1994) estimated total yearly N excretion to be 104 kg/cow/yr when a phase feeding system with an initial ration CP of 16% was used. However, this yearly excretion increased to 123 kg/cow/yr if the initial ration CP content was 17.5%.

The dairy cow excretes N via milk, urine, and feces. Milk N represented about 26 to 34% of total N intake in 3 recent reports (Crish et al., 1986; Wohlt et al., 1991; Andrew et al., 1991). Fecal N excretion ranged from 26 to 40% of total N intake. Urinary N excretion in those studies accounted for 20 to 43% of total N intake. In terms of quantities of N excreted daily, urinary N appears to increase more than fecal N with higher N intake (Pell, 1992). Excess N intake can also be monitored by measuring blood or milk urea N (Canfield

et al., 1990; Ciszuk and Gebregziabher, 1994; Roseler et al., 1993). Ciszuk and Gebregziabher (1994) proposed using milk urea N to estimate urinary N excretion in dairy cattle and goats.

To reduce N excretion to the environment, it is necessary to alter ration formulation to impact protein utilization and excretion. This does not infer that milk production will need to be reduced to attain this goal. Pell (1992) reported a very weak relationship between milk N and total excreted N. There have been a variety of approaches to control N excretion by changing ration formulation without compromising milk production. The overall goal has two basic components. One is to decrease feed N input. The other is to improve the efficiency of N utilization in the cow.

Improvement of forage quality

Higher quality forages contain more protein, are lower in fiber and have a greater energy content. Thus, they can provide a higher proportion of both protein and DM in the ration. Kaiser and Comb (1989) fed three qualities of first cutting alfalfa hay to early lactation cows. The CP contents of the alfalfa hays were 26.7 (A), 20.6 (B), and 18.7 (C) %. Total ration CP and NDF levels were similar for the 3 rations. Forage to concentrate levels were 68:32 (A), 53:47 (B), and 45:55 (C). Dry matter intake and milk yield were similar for all rations. Calculated concentrate CP contents were 9.5 (A), 18.5 (B), and 20 (C) %. Even though feed N inputs were higher for rations B and C, milk production was not altered compared to ration A.

Control of protein fractions in forages

The total CP content of a forage is only a part of the evaluation process. A second consideration is the proportion of the CP present in the soluble, degradable and undegradable fractions. The soluble protein in dry hays may represent 25 to 35% of the total CP. However, this fraction may increase to 50 to 80% of the total CP in silages. This conversion of true protein to soluble protein is the result of the silage fermentation process. As the solubility of the total ration increases, the risk of decreased N utilization increases. A recent trial concluded that protein was the first limiting nutrient when rations containing high levels of alfalfa silage were fed to dairy cows (Dhiman and Satter, 1993). The control ration in this study contained 19.2% CP on a DM basis. On a CP basis, this protein level would exceed NRC (1989) requirements. However, an 18% increase in milk production occurred when a high RUP supplement was provided. They concluded that the degradability of the alfalfa silage protein was the reason for this observation.

Consideration of the supplemental protein source

The NRC (1989) provided a basis for refining ration protein formulation by using the RUP/RDP system. Cozzi and Polan (1994) reported the results of a trial in which CGM or DBG were used as partial replacements for SBM. Corn silage and high moisture corn were the basal ration ingredients. The rations were formulated to be isonitrogenous. Even though ration RUP was increased in both the CGM and DBG rations, a response in milk production was obtained only for the DBG ration. The authors attributed this response to the DBG

ration to a better amino acid profile of the RUP fraction. This trial emphasized the need to consider amino acid profiles in addition to RUP content when selecting supplemental protein sources.

Consideration of amino acid balance

The amino acids available for absorption in the intestine come primarily from microbial protein and the RUP fraction of feeds. Schwab (1994) recently reviewed the current status of amino acid nutrition of the dairy cow. Microbial protein can be considered a high quality protein with an amino acid profile similar to milk protein. The amino acid content of the RUP is highly variable. As starting point, Schwab (1994) suggested that the percentages of the methionine and lysine in the duodenal ingesta should be 5 and 15% of the total essential amino acids. However, an imbalance of amino acids in the duodenal digesta may depress milk production and milk protein.

Carbon Losses to the Atmosphere

Digestion and metabolism causes unavoidable losses in fermentation and respiration. The major end product of metabolism is undoubtedly carbon dioxide (CO_2). In addition, CO_2 and methane (CH_4) are lost as end products of rumen fermentation. Large amounts of CO_2 and CH_4 accumulate in the rumen, with the average gas composition being about 65% CO_2 and 35% CH_4 (Brock and Madigan, 1991).

Based on estimates by Van Horn et al. (1994) (Figure I-2), approximately 35% of the

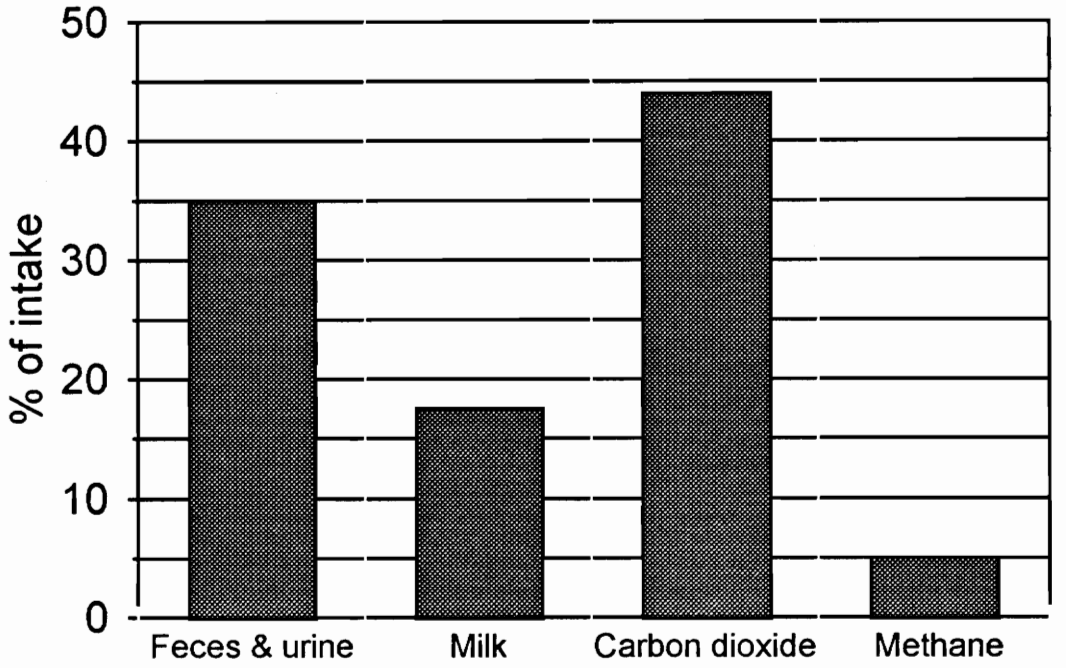


Figure I-2. Estimated excretion of carbon for a dairy cow producing 22.7 kg of milk. Adapted from Van Horn et al. (1994).

carbon (C) is contained in animal waste and provides the bulk of the solid's component. A major route for loss of intake C from the dairy cow is through metabolic heat production and fermentation in the rumen and hindgut, accounting for 40 to 44% of that consumed. This is dissipated to the atmosphere from the animal as CO_2 and should not be of environmental concern. At least 50% of the cow's diet can be corn silage or other forages, which by the photosynthetic cycle will effectively recycle the C back for animal feed. Methane, which also is of environmental concern with respect to global warming, is produced at a rate of 5%, with respect to C consumed, through fermentation in the rumen and hindgut. Milk captures some 18 to 20% of consumed C and the amount captured is directly related to production level.

Methane production in ruminants in terms of environmental concern

The concentration of CH_4 in the atmosphere has doubled over the past two centuries, which has been attributed to increasing emissions from human-related activities (Wilkerson et al., 1995). When the number of wild buffalo and other ruminants in America before 1800 and the current decline in the dairy cow population are taken into consideration, it is difficult to conclude that ruminants are responsible for the increase in atmospheric methane. However, CH_4 emissions from domesticated livestock in the US were estimated to range from 4.6 to 6.9 x 10^{12} g during 1990, and ruminants accounted for 95% of these emissions (US Environmental Protection Agency, 1993). Dairy cows, beef cows, and feedlot cattle were estimated to produce 4.4, 8.0, and 4.0% of total CH_4 emissions in the US, respectively. These classes of livestock have been targeted by the US Environmental Protection Agency

to estimate the contributions that ruminants make to CH_4 emissions. Reductions in CH_4 emissions depend on the accuracy of predicting CH_4 production by ruminants.

Ruminant digestive system

The ability of herbivorous mammals and particularly ruminants, to derive energy from a variety of fibrous feed sources is made possible to a large extent by their symbiotic relationship with the diverse microbial community inhabiting the digestive tract. Ruminal and hindgut microorganisms are capable of breaking down complex carbohydrates and proteins and utilizing them for their own growth. End products resulting from microbial degradation of carbohydrates are typically short chain volatile fatty acids (VFA), CH_4, CO_2, and microbial growth. From the standpoint of the host animal's metabolism, formation of VFA and microbes represents usable nutrients, while formation of CH_4 constitutes a net energetic loss to the animal. Therefore, understanding the factors influencing ruminal CH_4 production is important for developing nutritional strategies and livestock management practices to improve production efficiency of feed and fiber from ruminants in addition to concern over the potential global warming consequences.

Methanogenesis within the rumen

Rumen methanogenic bacteria are generally a very small fraction of the total population of microorganisms in the rumen. Although they can convert acetate (a fermentation product produced in the rumen) to CH_4 and CO_2, this pathway for CH_4

production in the rumen is believed to be of minor importance in animals fed adequate and balanced diets (Baldwin and Allison, 1983). Instead, the conversion of hydrogen (H_2) or formate and CO_2 (produced by fermentative bacteria) is believed to be the primary mechanism by which methanogenic bacteria produce CH_4 in ruminants.

The symbiosis between bacteria that ferment carbohydrates and the methanogens, such as *Methanobrevibacter ruminantium* and *Methanomicrobium mobilis* (Ogimoto and Imai, 1981), results in increased digestion and microbial production. The stoichiometry of VFA production during ruminal fermentation indicates that processes resulting in increased VFA and a shift in ratios toward acetate and butyrate production result in greater CH_4 production by ruminants.

The creation of CH_4 in the rumen represents energy which is subsequently not available to the host animal for maintenance or growth. Methods of reducing methanogenesis in ruminants have been investigated as part of the overall attempt to improve the efficiency of rumen metabolism. However, methanogenic bacteria play an important role in the complex ecology of the rumen, so that simply eliminating or suppressing the activity of methanogens in the rumen will not "free up" energy that can be used by the animal.

Measurement and prediction of methane yield

Commonly, CH_4 production is expressed in terms of CH_4 yield. This is defined on a caloric basis as the energy loss of methane per unit of gross energy intake (GEI). Methane yields from ruminants fed a variety of diets can range from 2 to 14% of GEI, although valves

of 6 to 10% are more commonly observed (Czerkawski, 1988). Van Horn et al. (1994) reported that dairy cows fed moderately high concentrate diets converted about 5% of their GEI into CH_4 and eructated this into the atmosphere. Blaxter and Czerkawski (1966) indicated for sheep, goats, and cattle fed mixed diets, CH_4 yield would be approximately 6 to 8% of GEI and for horses 2.5 to 3.0% of GEI. Quantities of CH_4 produced for each of these species when fed at a maintenance level of intake would be 40 L/d for a 50 kg sheep or goat, 300 L/d for a 500 kg steer and 250 L/d for a 1000 kg horse.

The measurement of CH_4 energy loss requires specialized equipment and considerable expenditures in time and labor, and must be subtracted to determine metabolizable energy (ME) valves for feedstuffs. To simplify the process of determining ME values, researchers have strived to develop empirical relationships for predicting CH_4 production in ruminants. Published equations for predicting CH_4 production (Table I-1) include one each from Axelsson (1949), Blaxter and Clapperton (1965), Blatzler and Forbes (1940), and Kriss (1930) and two from Moe and Tyrrell (1979). The approach used by Holter and Young (1992) to predict CH_4 energy involved the use of three equations, each used for a specific class of dairy cows: nonlactating cows, lactating cows not fed supplemental dietary fat, and lactating cows fed supplemental dietary fat. The equation of Blaxter and Clapperton and the equation of Holter and Young for nonlactating cows were incorrect as published, and the corrected equations are given in Table I-1.

The equation by Kriss (1930), which is based on DMI, was the simplest of the published equations for predicting CH_4 production that were evaluated. This equation was

Table I-1. Published equations used to predicted methane production by Holstein cows. Adapted from Wilkerson et al. (1995)

Kriss (1930)

Methane (g/d) = 18 + 22.5 x DMI (kg/d)

Bratzler and Forbes (1940)

Methane (g/d) = 17.68 + .04012 x digested carbohydrate (g/d)

Axelsson (1949)

Methane (Mcal/d) = -.494 + .629 x DMI (kg/d) - .025 x DMI^2 (kg/d)

Blaxter and Clapperton (1965)

Methane (kcal/100 kcal feed) = 1.30 + .112 x energy digestibility determined at maintenance intake (% of gross energy) + multiple of maintenance x (2.37 - .050 x energy digestibility at maintenance intake (% of gross energy))

Moe and Tyrrell (1979)

Intake of carbohydrate fractions

Methane (Mcal/d) = .814 + .122 x nonfiber carbohydrate (kg/d) + .415 x hemicellulose (kg/d) + .633 x cellulose (kg/d)

Intake of digested carbohydrate fractions

Methane (Mcal/d) = .439 + .273 x digested nonfiber carbohydrate (kg/d) + .512 x digested hemicellulose (kg/d) + 1.393 x digested cellulose (kg/d)

Holter and Young (1992)

Nonlactating cows

Methane (% gross energy) = 12.12 - .00542 x BW (kg) - .0900 x ADF (% DMI) + .1213 x ADF digestibility (%) - 2.472 x digestible energy (Mcal/kg of DM) + .0417 x neutral detergent solubles digestibility (%) - .0748 x cellulose digestibility (%) + .0339 x hemicellulose digestibility

Lactating cows fed supplemental dietary fat

Methane (% gross energy) = 2.898 - .0631 x milk (kg/d) + .297 x milk fat (%) - 1.587 x milk protein (%) + .0891 x CP (% DMI) + .1010 x forage ADF (% DMI) + .102 x DMI (kg/d) - .131 x fat (% DMI) + .116 x DM digestibility (%) - .0737 x CP digestibility

Lactating cows not fed supplemental dietary fat

Methane (% gross energy) = 2.927 - .0405 x milk (kg/d) + .335 x milk fat (%) - 1.225 x milk protein (%) + .248 x CP (% DMI) - .448 x ADF (%DMI) + .502 x forage ADF (% DMI) + .0352 x ADF digestibility

derived from 131 observations using 4 cows and 20 steers (54 observations from roughage and 77 observations from roughage plus grain diets).

The equation of Axelsson (1949) is also based on DMI but uses linear and quadratic terms of DMI for predicting CH_4 production. A total of 176 observations of adult cattle were used to develop this equation.

Blaxter and Clapperton (1965) developed an equation that varied the relationship between DMI and CH_4 production based on energy digestibility of the diet at maintenance and intake expressed as a multiple of maintenance. They defined maintenance as the metabolizable energy consumed at zero energy retention. They utilized 391 observations involving 48 diets fed to sheep to determine the effects of amount and type of diet on CH_4 production. The diets were classified as roughage, mixed diets, or pelleted diets, and regression analyses were performed on each class and on all diets. Blaxter and Clapperton (1965) found similar regression coefficients among the classes of diets and recommended that the overall equation be used to predict CH_4 production by ruminants. Their equation demonstrates that at low levels of feeding, CH_4 production increases with increasing digestibility but decreases with an increasing feeding level. The explanation is that at low feed intakes more substrate/unit feed ingested is degraded in the rumen without important changes in the VFA pattern.

Bratzler and Forbes (1940) related CH_4 production to the amount of digested total carbohydrate. Their equation was developed from 130 observations utilizing steers and cows of beef and dairy breeds. The equation of Bratzler and Forbes was developed using a

narrower range of digested total carbohydrates (.9 to 5.8 kg/d).

Fermentation biochemistry suggests that carbohydrates may differ in the amount of CH_4 produced in the rumen. Fibrous carbohydrates such as hemicellulose and cellulose typically result in higher proportions of acetate during fermentation than do nonfiber carbohydrates (NFC) and should result in greater CH_4 production. Moe and Tyrrell (1979) used the intake of NFC, hemicellulose, and cellulose to predict methane production. Their equations were developed from a data file containing 404 observations from Holstein cows used in studies to investigate the effects of dietary protein, proportions of concentrate, different feed ingredients, and physical form of corn on net energy. They concluded that the production of CH_4 was influenced by both the nature of the carbohydrate digested and the level of the diet intake, with relatively less effect being observed at low levels of intake. In contrast, Czerkawski and Breckenridge (1969), based on data from in vitro studies, concluded that CH_4 production was generally more contingent upon the amount of carbohydrate fermented and not necessarily the type of carbohydrate fermented.

Holter and Young (1992) used a more empirically statistical approach to identify cow and dietary factors that influence CH_4 production. They divided their data file into subsets to reduce cow and dietary effects. The subsets consisted of Holstein cows that included 60 observations for nonlactating cows, 107 observations for lactating cows fed supplemental dietary fat, and 90 observations for lactating cows not fed supplemental dietary fat.

Holter and Young (1992) reported that CH_4 production of nonlactating cows was positively related to the digestibility of ADF, neutral detergent solubles, cellulose,

hemicellulose, and forage ADF in the ration. They also observed a negative relationship between CH_4 production and dietary concentration of digestible energy. The equations of Holter and Young use variables that are not precursors of CH_4 production, such as milk production, milk fat, and milk protein (Table I-1). Additionally, the equations of Holter and Young have coefficients that are inconsistent with accepted relationships between diet and CH_4 production. Equations for nonlactating cows and lactating cows not fed supplemental dietary fat contain negative coefficients for dietary ADF and positive coefficients for ADF digestibility, suggesting that rations with high ADF content would have less CH_4 production than those with low ADF content. Similarly, the equation for lactating cows fed supplemental dietary fat has a positive regression coefficient for dietary CP and a negative regression coefficient for CP digestibility. Holter and Young (1992) concluded that increased percentages of fat in the ration reduced CH_4 production by lactating cows.

NITROGEN AND CARBON BALANCE IN LACTATING COWS

Introduction and Objectives

The research project described in this report had an overall objective to evaluate nitrogen and phosphorus partitioning to milk, feces, urine, and body tissue in lactating cows. Phosphorus partitioning data are not included in this report. Also not included are data concerning feed intake and milk production while cows were housed in the free stall area prior to determination of nutrient balances. Additional analyses and objectives, however, were added to consider carbon partitioning. Therefore, the revised objectives are as follows:

1. To evaluate nitrogen and carbon partitioning to milk, urine, feces, and body tissue when soybean meal or a combination of soybean meal and blood meal is used as the dietary protein supplement for lactating cows during early and midlactation.

2. To construct mathematical models to make estimates of total carbon and nitrogen flow from lactating cows to waste storage facilities on the basis of carbon and nitrogen balance determinations.

3. To estimate the amount of carbon lost as carbon dioxide and methane to the atmosphere during early lactation.

Materials and Methods

Cows, experimental design, treatments, and cow management

Thirty six Holstein cows in their first, second, third or fourth lactation were selected from the Virginia Tech dairy herd. Cows were assigned randomly at calving in a 2 x 2 factorial design. Dietary assignments were based on parity (Table II-1) and previous or estimated production. After calving, cows were housed in free stalls with a Calan® feeding system to determine milk yield and DMI for 4 wk. Between 25 and 45 DIM (period 1), groups of 4 to 8 cows were moved to tie stalls lined with rubber floor mats for 2 d of acclimation followed by 2 d of total collection of urine, feces, and milk. Water was always available. After completion of period 1, cows were returned to the herd for group feeding of a common diet until 112 to 126 DIM. At that time, cows were returned to free stalls with a Calan® feeding system, then again fed the diet assigned at calving to repeat the above sequence. At 143 to 164 DIM (period 2), cows were moved to tie stalls in groups of 4 to 8. Two days were allowed for acclimation followed by 2 d of total collection of feces, urine, and milk. Cows were weighed before and after periods 1 and 2.

Diets

Standard dietary ingredients (alfalfa silage, corn silage, ground corn, soybean meal, and trace mineral salt) were used to formulate a basal diet using Dair4 (Stallings et al., 1993). A diet was formulated with only soybean meal (S), then reformulated with a combination of S and blood meal (B) to obtain approximately 16% CP. Blood meal was substituted for

Table II-1. Number of cows per diet listed by parity.

	Diet[1]				
Parity	SPM	SWB	BPM	BWB	Total
1	2	3	2	2	9
2	3	3	4	4	14
3 or 4	3	4	3	3	13
Total	8	10	9	9	36[2]

[1]SPM = Soybean meal (S: 30% RUP) and mono- and dicalcium phosphate (PM)
SWB = Soybean meal (S) and wheat bran phytate (WB)
BPM = Blood meal (B: 39% RUP) and mono- and dicalcium phosphate (PM)
BWB = Blood meal (B) and wheat bran phytate (WB).

[2]Total observations: 36 x 2 periods = 72.

soybean meal to increase RUP content from 30% to 39% of total CP. The S and B diets were formulated to contain mono- and dicalcium phosphates (PM) as supplemental sources of inorganic phosphorus. The S and B diets then were reformulated using wheat bran (WB) to supply supplemental phosphorus in the organic form (phytate). Limestone and magnesium oxide were used to equalize calcium and magnesium across all diets. Thus, the four dietary treatments (Tables II-2 and II-3) were:

1) SPM = Soybean meal (S: 30% RUP) and mono- and dicalcium phosphate (PM)

2) SWB = Soybean meal (S) and wheat bran phytate (WB)

3) BPM = Blood meal (B: 39% RUP) and mono- and dicalcium phosphate (PM)

4) BWB = Blood meal (B) and wheat bran phytate (WB).

All concentrate ingredients were mixed in 2-ton batches and stored in bags. Alfalfa silage, corn silage, and concentrate were mixed daily and fed to individual cows in a quantity sufficient to allow 5 to 10% feed refusals. During each period, cows were fed equal portions of their daily allotment at 0700 and 1900 h with feed refusals weighed and sampled prior to feeding at 1900 h.

Measurements and sampling

Alfalfa silage, corn silage, and concentrate were sampled as each group of cows entered periods 1 and 2. Forages, concentrates, and feed refusals were dried in a forced-air

Table II-2. Ingredients in diet DM[1].

Item	Diet			
	SPM	SWB	BPM	BWB
	----------% of DM ----------			
Alfalfa silage	28.2	28.2	28.2	28.2
Corn silage	25.8	25.8	25.8	25.8
Corn, ground	32.69	27.60	35.54	30.22
Soybean meal, 48% CP	10.63	9.38	4.88	3.04
Megalac®[2]	1.01	1.01	1.01	1.01
Trace mineral salt[3]	.37	.37	.37	.37
Wheat bran	.28	7.41	.32	7.91
Limestone	.05	.23	.05	.18
Monocalcium phosphate	.23	-	.37	.09
Dicalcium phosphate	.09	-	.09	-
Peanut hull	.60	-	.60	-
Blood meal	-	-	2.58	3.13
Urea	-	-	.14	.05
Magnesium oxide	.05	-	.05	-

[1]Supplemented with 5060 IU vitamin A, 1520 IU vitamin D, and 2.5 IU vitamin E per kg diet DM.

[2]Calcium salts of fatty acids (Church & Dwight, Inc.) containing 8.0 to 9.6% Ca and a minimum of 80% fat.

[3]Contained 1.2 g Fe, 2.2 g Mn, 2.0 g Mg, .4 g S, .2 g Cu, .07 g Co, and .07 g I per kg.

Table II-3. Composition of diet DM.

	Diet			
Item	SPM	SWB	BPM	BWB
DM, %	63.8	63.8	63.7	63.3
CP, %	16.2	16.3	15.8	16.0
RUP, % of CP[1]	30.8	28.9	39.1	39.3
NDF, %[1]	31.6	34.0	31.6	34.2
ADF, %[2]	19.3	19.2	19.0	19.1
Fat, %[1]	4.1	4.1	4.1	4.1
NE_L, Mcal/kg[1]	1.6	1.6	1.6	1.6
Ca, %[2]	.65	.63	.65	.62
P, %[3]	.38	.34	.36	.34

[1]Calculated using Dair4 (Stallings et al., 1993).

[2]Analyzed using method from AOAC (1995).

[3]Analyzed using modified method from AOAC (1995). The use of a microtiter plate and a computer controlled plate reader substantially increased the speed and reduced the cost of analysis.

oven at 60 °C for 48 h, then ground in a Wiley mill (2-mm screen, Arthur H. Thomas Co., Philadelphia, PA). Samples of blood were taken by jugular venipuncture at the start of periods 1 and 2. Plasma obtained by centrifugation of blood at 7000 x *g* for 10 min was frozen until analyzed for plasma urea. Cows were milked in the tie stalls at 0630 and at 1830 h daily with a portable bucket-type system. Two milk samples were collected at each milking during both collection days of each period. One sample was sent to the Virginia Federation of DHIA laboratory for milk fat and protein analyses. The other sample was frozen until analyzed for total nitrogen (N) and urea.

Immediately after a cow defecated, the feces were placed in a sealed plastic container. Feces were weighed and thoroughly mixed daily. Fecal samples (400 g) were dried in a forced air oven at 60 °C, ground in a Wiley mill (2-mm screen), then stored in airtight containers.

For urine collection, a 22-Fr Foley catheter (75-cc, C. R. Bard, Covington, GA) was placed in the bladder by the method of Crutchfield (1968) approximately 6 h prior to the start of the 2-d collection. Urine was collected continuously in a polyethylene container and acidified after 4 h of collection with 22 mL of HCl/kg urine. After 24 h, acidified urine from the six 4-h intervals during each 24-h collection were combined, weighed, and thoroughly mixed. Two samples (150 mL) of urine were frozen until analyzed for total N and urea.

Chemical analyses

Kjeldahl and elemental analyses. Nitrogen analyses for feeds, feed refusals, feces, milk, and

urine were performed by the Kjeldahl method (AOAC, 1995) using a Kjeltec Auto 1030 Analyzer (Tecator AB, Hoganas, Sweden). To determine carbon (C) content of feeds, feed refusals, and feces from period 1, samples were ground again in a Retsch/Brinkmann-mill (.2 mm screen, Brinkmann Instruments Inc., Westbury, NY). Milk and urine samples from period 1 were freeze-dried at -25 °C for 4 d. All samples then were analyzed for C content using an elemental analyzer (CHNS/O Analyzer, Perkin-Elmer, Norwalk, CT). The elemental analyzer also determined hydrogen and N content (AOAC, 1995). Data for N content were compared to results from the Kjeldahl analysis. Data for hydrogen content are not included in this report.

Urea nitrogen analyses. Urea nitrogen in plasma, urine, and milk was determined using urease incubation and the indophenol reaction (Chaney and Marbach, 1962; Kaplan, 1965; Weatherburn, 1967). Five μL of each sample were incubated for 20 min with 100 μL of urease solution (Sigma Chemical CO., St. Louis, MO) containing .104 units urease per mL. One-half mL each of reagent one (50 g phenol and 250 mg sodium nitroferricyanide/L) and reagent two (25 g sodium hydroxide and 42 mL 8% sodium hypochlorite/L) were added. Samples were vortexed, diluted with 2.5 mL water, and incubated at room temperature overnight. Absorbance was read on a spectrophotometer (Microplate Autoreader, Bio-Tek Instruments Inc., Winooski, VT) at a wave-length of 630 nm. Results were expressed as mg urea N/dL.

Statistical analysis

All data dealing with N partitioning were analyzed using the general linear models (GLM) procedure of SAS (1990). The model was:

$$Y_{ijklm} = \mu + L_i + P_j + H_k + (PH)_{jk} + (LP)_{ij} + (LH)_{ik} + (LPH)_{ijk} + C_{(ijk)l} + R_m + (RL)_{mi} + (RP)_{mj} + (RH)_{mk} + (RPH)_{mjk} + (RLP)_{mij} + (RLH)_{mik} + (RLPH)_{mijk} + E_{(ijklm)},$$ where:

Y_{ijklm} = dependent variable;

μ = population mean;

L_i = fixed effect of lactation number i, i = 1, 2, or 3 (where 3 = third or fourth lactation);

P_j = fixed effect of supplemental protein source j, j = 1, 2;

H_k = fixed effect of supplemental phosphorus source k, k = 1, 2;

$(PH)_{jk}$ = fixed effect of interaction of protein j and phosphorus k;

$(LP)_{ij}$ = fixed effect of interaction of lactation i and protein j;

$(LH)_{ik}$ = fixed effect of interaction of lactation i and phosphorus k;

$(LPH)_{ijk}$ = fixed effect of interaction between lactation i, protein j, and phosphorus k;

$C_{(ijk)l}$ = random effect of cow l in lactation i, protein j, and phosphorus k;

R_m = fixed effect of period m, m = 1, 2;

$(RL)_{mi}$ = fixed effect of interaction of period m and lactation i;

$(RP)_{mj}$ = fixed effect of interaction of period m and protein j;

$(RH)_{mk}$ = fixed effect of interaction of period m and phosphorus k;

$(RPH)_{mjk}$ = fixed effect of interaction between period m, protein j, and phosphorus k;

$(RLP)_{mij}$ = fixed effect of interaction between period m, lactation i, and protein j;

$(RLH)_{mik}$ = fixed effect of interaction between period m, lactation i, and phosphorus k;

$(RLPH)_{mijk}$ = fixed effect of interaction between period m, lactation i, protein j, and phosphorus k;

$E_{(ijklm)}$ = random residual.

The following effects were tested using $C_{(ijk)l}$ as the error term: L_i , P_j , H_k , $(PH)_{jk}$, $(LP)_{ij}$, $(LH)_{ik}$, $(LPH)_{ijk}$. Tukey's multiple-comparison procedure was used to test differences between means for data grouped by parity. The effect due to stage of lactation (R_m) was tested using the error term ($E_{(ijklm)}$). Means were accepted as significantly different at $P <$. 05. An example of an ANOVA table is given in the Appendix (Table A-1).

Carbon balance data for period 1 were analyzed using the following model;

$Y_{ijkl} = \mu + L_i + P_j + H_k + (PH)_{jk} + (LP)_{ij} + (LH)_{ik} + (LPH)_{ijk} + E_{(ijkl)}$, where:

Y_{ijkl} = dependent variable;

μ = population mean;

L_i = fixed effect of lactation number i, i = 1, 2, or 3 (where 3 = third or fourth lactation);

P_j = fixed effect of supplemental protein source j, j = 1, 2;

H_k = fixed effect of supplemental phosphorus source k, k = 1, 2;

$(PH)_{jk}$ = fixed effect of interaction of protein j and phosphorus k;

$(LP)_{ij}$ = fixed effect of interaction of lactation i and protein j;

$(LH)_{ik}$ = fixed effect of interaction of lactation i and phosphorus k;

$(LPH)_{ijk}$ = fixed effect of interaction between lactation i, protein j, and phosphorus k;

$E_{(ijkl)}$ = random residual.

The overall gross and within-cow correlations between plasma urea N, urine urea N, and milk urea N were determined using the regression procedure of SAS (1990). Also, correlations between selected variables associated with N partitioning were determined. To make prediction equations for N excretion, the multiple regression procedure of SAS (1990) was used. Independent variables were removed from the models for multiple regressions by backward elimination.

Results and Discussion

Dry matter intake and body weight

The intake of DM differed in response to parity and stage of lactation (Table II-4). During early lactation cows did not consume as much feed as they did during midlactation. Journet and Remond (1976) estimated that DMI was depressed 15% on the average during the first 3 wk of lactation relative to later lactation. Despite greater body weight (BW) at midlactation compared with early lactation, DMI as a percentage of BW was greater at midlactation.

Overall, DMI did not differ for cows fed soybean meal (SBM) or blood meal (BM), but was lower for cows fed wheat bran compared with cows fed inorganic phosphorus sources (Table II-5). Clack et al. (1992) noted that DMI appeared to affect protein degradability in the rumen. At low levels of DMI, protein degradability in the rumen was high due to increased retention time. As intake of DM increased, rate of passage increased, retention time decreased, and degradability decreased. Owens (1986) calculated that protein degradability decreased from 83.1% to 72.8% as DMI increased from 1 to 2% of BW. He reported, however, at higher levels of DMI (> 2.5% of BW) increases in DMI probably would have a lesser effect on degradability. Based on the above information, protein degradability in this study probably was not affected by DMI because intake of DM was about 4.0% of BW. The amount of wheat bran (7 to 8% of DM) in diets fed in this study was necessary for comparisons of phosphorus balance, but is too high for practical diets fed to commercial dairy herds.

Table II-4. Least squares means for dry matter intake and body weight in response to parity and stage of lactation.

	Lactation				Stage of lactation[1]		
Item	1	2	3 & 4	SE	E	M	SE
Dry matter intake							
kg/d	20.0[a]	22.5[b]	25.0[c]	1.0	21.3[d]	23.6[e]	.3
% of body weight	3.79[a]	4.09[b]	3.92[ab]	.15	3.85[d]	4.01[e]	.05
Body weight, kg/d	527[a]	549[b]	638[c]	12	555[d]	588[e]	3

[1]E = Early lactation; M = Midlactation.

[a,b,c]Means in rows with different superscripts differ, $P < .05$, using Tukey's test.

[d,e]Means in rows with different superscripts differ, $P < .05$, using F-test.

Table II-5. Least squares means for dry matter intake and body weight in response to diet.

	Diet					Contrasts (*P*)		
Item	SPM	SWB	BPM	BWB	SE	Prot[1]	Phos[2]	INT[3]
Dry matter intake								
kg/d	23.9	21.4	23.4	21.2	1.11	.41	< .01	.75
% of body weight	4.15	3.82	4.07	3.69	.17	.15	< .01	.70
Body weight, kg	574	653	575	573	14	.27	.26	.22

[1]Prot = Supplemental protein sources (SPM and SWB compared with BPM and BWB).

[2]Phos = Supplemental phosphorus sources (SPM and BPM compared with SWB and BWB).

[3]INT = Prot x Phos interaction.

Milk production

Milk yield and milk component yields increased as parity increased (Table II-6). Milk and milk fat yields were greater in early lactation compared with midlactation, but milk protein yield was greater in midlactation due to a higher milk protein percentage. As mentioned in NRC (1989), DMI is a major factor regulating level of milk production.

Supplemental protein source did not affect milk production, but cows fed wheat bran had reduced milk yield (Table II-7). Clack et al. (1992) noted that as feed intake increased, passage of fluids and solids to the small intestine increased, amount of OM truly digested in the rumen increased, and ruminal pH decreased. Decreased ruminal pH and increased rate of passage from the rumen may increase ruminal escape of proteins with high ruminal degradability, such as SBM, more than bypass of proteins reported to have a low ruminal degradability, such as BM. However, as noted previously, such changes may have a greater effect when DMI is below 2% of BW as compared to the 4% of BW for cows in this study. Nocek and Russell (1988) postulated that the inability of RUP to increase milk production in some trials may have resulted because 1) dietary protein exceeded the cow's requirement for protein, 2) RUP was fed that was poorly digested in the small intestine, 3) unforeseen interactions occurred with mobilization or utilization of nutrients from body tissues, or 4) a depression in microbial protein synthesis in the rumen resulted from lowered concentrations of ruminal ammonia. In addition, they suggested that dietary incorporation of supplemental protein sources that are high in RUP stimulated milk production when CP intake is marginal (< 14% CP), but not when dietary CP exceeds 16%. In the current study, diets contained

Table II-6. Least squares means for milk production and composition in response to parity and stage of lactation.

	Lactation				Stage of lactation[1]		
Item	1	2	3&4	SE	E	M	SE
Yield, kg/d							
Milk	30.9[a]	34.9[b]	39.0[c]	1.8	36.3[e]	33.6[d]	.5
Fat	1.07[a]	1.23[b]	1.38[c]	.07	1.32[e]	1.13[d]	.02
Protein	.90[a]	1.00[b]	1.11[c]	.05	.99[d]	1.03[e]	.01
Content, %							
Fat	3.41	3.55	3.53	.15	3.62[e]	3.37[d]	.05
Protein	2.91	2.89	2.86	.06	2.72[d]	3.05[e]	.03

[1]E = Early lactation; M = Midlactation.

[a,b,c]Means in rows with different superscripts differ, $P < .05$, using Tukey's test.

[d,e]Means in rows with different superscripts differ, $P < .05$, using F-test.

Table II-7. Least squares means for milk production and composition in response to diet.

	Diet					Contrasts *(P)*		
Item	SPM	SWB	BPM	BWB	SE	Prot	Phos	INT
Yield, kg/d								
Milk	36.4	34.1	35.2	34.0	2.1	.36	.02	.38
Fat	1.28	1.19	1.33	1.10	.08	.60	< .01	.07
Protein	1.11	.97	.98	.96	.06	< .01	< .01	< .01
Content, %								
Fat	3.47	3.53	3.76	3.23	.17	.84	< .01	< .01
Protein	3.06	2.86	2.79	2.84	.08	< .01	.10	< .01

approximately 16.1% CP.

Milk fat percentage and yield were not affected by protein source in the diets, but wheat bran lowered fat yield due to lower milk production and milk fat percentage (Table II-7). Cows fed BM had lower milk protein percentage and yield compared to cows fed SBM. This result is in accordance with Wholt et al. (1991), who found milk protein yield was greater for cows fed SBM and fish meal compared to corn gluten meal. Tomlinson et al. (1994) reported that cows fed SBM had a higher milk protein percentage than cows fed BM in diets with 15 or 18% CP. In addition, an inadequate supply of amino acid (AA) or an imbalance could be the reason milk protein percentage was decreased when the diet contained BM. Schwab et al. (1976) suggested that methionine (Met) and lysine (Lys) were two of the most limiting AA for milk and milk protein synthesis. Therefore, failure to increase production of milk protein by feeding a high level of RUP suggests that inadequate amounts of total AA were absorbed from the small intestine for synthesis of milk protein by cows or that Met or Lys or both were deficient for cows fed a higher level of RUP. However, Christensen et al. (1993) observed that passage of Met to the small intestine was not altered significantly by inclusion of BM in the diet. Chandler (1989) reported an AA index for protein sources based on chemical score of AA. Within his index, SBM had the greatest biological valve. Polan (1992) noted "soybean meal is as balanced as any compared to AA in milk protein."

Nitrogen balance

Results concerning N balance are listed in Tables II-8 and II-9. Intake of N and means for most other parameters of N partitioning (g/d) differed due to parity and stage of lactation. Obviously, these results were caused by differences in DMI. Although cows in their first lactation consumed less N and DM, N digestibility was similar across parities. Also, in spite of higher milk production during early lactation, N intake, N digestibility, fecal N, urine N, and N balance were higher in midlactation.

Intake of N averaged 600 g/d and was less for cows fed BM (578 g/d) versus SBM (607 g/d) despite lack of a difference in DMI between them (Table II-9). This result may be explained by the fact that level of % CP was slightly higher (16.3 versus 15.9%) for cows fed SBM. However, fecal N excretion was higher for cows fed BM. Thus, N digestibility was lower for cows fed BM compared with cows fed SBM (63 versus 67%). Christensen et al. (1993) reported that N digestibility was 4.7 percentage units higher for the low RUP diets in which SBM was the main protein source than for high RUP diets in which BM was the main source of supplemental protein.

Five of 72 determinations of N balance were negative, three of the five were during early lactation, and four of the five were for cows fed BM. In addition to retaining more N than cows fed BM (64 versus 37 g/d), cows fed SBM also partitioned more N to milk than cows fed BM (157 versus 147 g/d). This was reflected as a greater milk protein percentage and yield for cows fed SBM.

Due to the fact that both milk N and retained N were higher for cows fed SBM versus BM, it can be concluded that N utilization by cows fed SBM was more efficient. Though BM

Table II-8. Least squares means for nitrogen partitioning, nitrogen digestibility, and absorbed nitrogen in response to parity and stage of lactation.

	Lactation				Stage of lactation[1]		
Item	1	2	3 & 4	SE	E	M	SE
N intake, g/d	516[a]	603[b]	658[c]	23	552[d]	633[e]	9
Fecal N, g/d	177[a]	207[b]	233[c]	8	198[d]	212[e]	3
Urinary N, g/d	148[a]	193[b]	212[c]	5	169[d]	200[e]	4
Milk N, g/d	136[a]	151[b]	169[c]	8	149	155	2
N balance, g/d[2]	54	53	45	12	36[d]	65[e]	7
N digestibility, %[3]	65.7	65.4	64.4	.7	64.1[d]	66.2[e]	.5
Absorbed N, g/d[4]	339[a]	397[b]	426[b]	17	354[d]	420[e]	8
% in urine	44.3[a]	49.6[b]	51.7[b]	2.7	48.8	48.3	1.1
% in milk	40.3	39.2	40.3	1.6	42.5[e]	37.4[d]	.8
% retained	15.5[b]	11.5[ab]	8.1[a]	3.3	9.1[d]	14.4[e]	1.6

[1]E = Early lactation; M = Midlactation.

[2]N balance (g/d) = N intake (g/d) - Fecal N (g/d) - Urinary N (g/d) - Milk N (g/d). N balance (g/d) = N retained (g/d).

[3]N digestibility (%) = ((N intakg (g/d) - Fecal N (g/d))/(N intake (g/d))) x 100.

[4]Absorbed N (g/d) = N intake (g/d) - Fecal N (g/d).

[a,b,c]Means in rows with different superscripts differ, $P < .05$, using Tukey's test.

[d,e]Means in rows with different superscripts differ, $P < .05$, using F-test.

Table II-9. Least squares means for nitrogen partitioning, nitrogen digestibility, and absorbed nitrogen in response to diet.

	Diet					Contrasts (*P*)		
Item	SPM	SWB	BPM	BWB	SE	Prot	Phos	INT
N intake, g/d	638	576	602	553	27	.03	< .01	.50
Fecal N, g/d	213	184	226	198	10	< .01	< .01	.90
Urinary N, g/d	189	185	184	179	6	.34	.45	.95
Milk N, g/d	166	148	150	145	9	< .01	< .01	.03
N balance, g/d[1]	70	59	42	33	14	.01	.32	.88
N digestibility, %[2]	66.5	67.8	62.5	64.0	.8	< .01	.05	.86
Absorbed N, g/d[3]	425	391	376	357	19	< .01	.02	.41
% in urine	44.8	47.9	49.4	52.1	3.2	< .01	.08	.87
% in milk	40.2	38.4	40.1	41.1	1.8	.25	.78	.24
% retained	15.1	13.9	10.6	7.2	3.9	.02	.33	.67

[1]N balance (g/d) = N intake (g/d) - Fecal N (g/d) - Urinary N (g/d) - Milk N (g/d). N balance (g/d) = N retained (g/d).

[2]N digestibility (%) = ((N intakg (g/d) - Fecal N (g/d))/(N intake (g/d))) x 100.

[3]Absorbed N (g/d) = N intake (g/d) - Fecal N (g/d).

largely escapes rumen fermentation, the imbalanced AA profile of BM may not allow a cow to achieve her potential for protein synthesis. Goedeken et al. (1990) reported that less metabolizable protein was required for maximal gain from SBM than from BM, which indicated that BM protein was used less efficiently than SBM. In their study, the protein efficiency for BW gain when SBM was fed was 2.4 times that of BM.

Cows partitioned approximately 49, 40, and 11% of absorbed N to urine, milk, and tissue, respectively. A greater proportion of absorbed N was excreted into urine by cows fed BM (51%) compared with SBM (46%). Retained N as a percentage of absorbed N was greater for cows fed SBM (14.5%) compared with BM (8.9%), but the percentage of absorbed N in milk did not differ due to dietary protein source.

Total excretion of urine and fecal nitrogen

Data for excretion of urine and feces are summarized by diet in Table II-10. Mean quantities of urine and feces excreted by cows consuming an average of 22.7 kg DM were 17.6 kg urine and 47.8 kg wet feces. Gross output of feces was higher for cows fed BM compared with cows fed SBM, but cows fed BM excreted less urine. Buchanan-Smith (1995) reported no effect of either protein level or degradability on gross output of feces, but urine output was higher when cows were fed diets with highly degradable protein compared with diets containing the level of degradable protein recommended by NRC (1989).

There were no differences in total waste production and total N in waste due to protein source. However, cows fed wheat bran produced less waste and excreted less N into

Table II-10. Least squares means for waste production in response to diet.

	Diet					Contrasts (*P*)		
Item	SPM	SWB	BPM	BWB	SE	Prot	Phos	INT
Feces								
Wet, kg/d	49.2	45.0	53.7	48.1	3.0	< .01	< .01	.67
N, g/d	213	185	227	198	10	< .01	< .01	.90
Urine								
Wet, kg/d	19.7	16.9	17.2	15.1	.9	< .01	< .01	.55
N, g/d	189	185	184	180	6	.34	.45	.95
Feces + Urine								
Wet, kg/d	68.8	61.9	70.9	63.2	3.5	.29	< .01	.95
N, g/d	402	369	410	377	13	.26	< .01	.90

waste compared with cows fed inorganic phosphorus sources. The differences were due to lower DMI when cows were fed wheat bran.

Table II-11 contains a comparison of N balance data from this trial with data from Fisher (1995). Both studies were conducted at the same location and time using the same source of forages. Fisher fed a diet with 17.5% CP supplied by multiple sources of RUP, including SBM, BM, whole cottonseed, corn gluten meal, fish meal, and feather meal. The four cows in his 4 x 4 Latin square study were in their third or fourth lactation. Intake of N was higher for cows fed 17.5% CP (700 g/d) than for cows fed 16.1% CP (659 g/d). However, there was no difference in productive N output (milk N plus retained N). Fecal N output was slightly higher when cows were fed 16.1% CP compared with 17.5% CP. In contrast, urinary N output was substantially higher for cows fed 17.5% CP (262 g/d) versus 16.1% CP (212 g/d). Based on the above comparison and other data from this study, the amount of N excreted in the urine of cows is influenced by level of dietary CP and source of supplemental CP. Absorption of N in quantities greater than required or absorption of an imbalanced supply of AA may lead to increased production of urinary urea as a result of deamination of AA by the liver. In addition, fecal N output is influenced by interactions between level of CP intake and CP digestibility (Table II-9).

Urea nitrogen in urine, plasma, and milk

Urinary urea N (UUN) excretion (g/d) was not influenced by diet (Table II-12), despite a higher concentration of UUN in cows fed BM compared with those fed SBM (822

Table II-11. Comparison of N partitioning by cows fed 17.5%[1] dietary CP with cows fed 16.1% CP[2].

Item	17.5% CP	16.1% CP	Difference[3]
Intake N, g/d	700	659	+41
Productive output			
Milk N, g/d	169	169	**0**
Tissue N, g/d	45	45	**0**
Waste output			
Feces N, g/d	224	233	-9
Urine N, g/d	262	212	+50

[1]Data from Fisher (1995), in which the same forages were fed with multiple sources of RUP (soybean meal, whole cottonseed, corn gluten meal, blood meal, fish meal, feather meal). DMI = 25.3 kg/d.

[2]Data from cows in this study in their third or fourth lactation. DMI = 25.0 kg/d.

[3]Difference = Mean for 17.5% CP - Mean for 16.1% CP.

versus 711 mg/dL). In addition, UUN output was a greater percentage of total urinary N (68.8 versus 65.2%), and percentage of absorbed N in cows fed BM compared with those fed SBM (34.7 versus 30.4%).

Concentration of plasma urea N (PUN) was not affected by diet (Table II-12). Roseler et al. (1993) noted that, overall, PUN increases as percentage of dietary CP increases from 12.2 to 17.6%. They reported that PUN concentrations for cows fed 30% or 38% RUP were 16.5 and 14.8 mg/dL when the diet contained 15 to 16% CP. Similarly, Baker et al. (1995) reported that PUN concentrations were 18.5 and 16.0 mg/dL when cows were fed 27% or 40% RUP in diets containing 15.1% CP. In both studies, a combination of BM and fish meal was used to obtain the higher level of RUP. Tomlinson et al. (1994) also reported a linear response of PUN to increasing dietary CP percentage from 12.1 to 18.6%. In their study, blood urea N were reported for cows fed diets containing 15% CP with SBM or SBM plus BM to obtain 32 or 39% RUP. Concentrations (11.2 and 10.4 mg/dL) were similar for cows fed 32 or 39% RUP.

Urea in milk arises primarily from passive transfer of urea from the blood. Concentration of milk urea N (MUN) was similar for dietary treatments (Table II-12). Roseler et al. (1993) noted that MUN concentration is sensitive to CP, RDP, and RUP, with elevations in MUN concentration indicating excess dietary N. They reported a significant difference in MUN concentration between cows fed 30% or 38% RUP at 15 to 16% CP (13.4 versus 11.6 mg/dL). However, MUN output did not differ (3.1 versus 3.0 g/d). Baker et al. (1995) reported that MUN concentrations were 18.6 and 15.1 mg/dL when cows were fed

Table II-12. Least squares means for concentration and output of urinary urea N (UUN), plasma urea N (PUN), and milk urea N (MUN).

	Diet					Contrasts (*P*)		
Item	SPM	SWB	BPM	BWB	SE	Prot	Phos	INT
UUN[1]								
mg/dL	633	789	779	866	45	< .01	< .01	.30
g/d	121	127	126	123	6	.87	.79	.40
% of total urine N	62.3	68.0	68.7	68.8	1.4	.04	.09	.11
% of absorbed N	28.0	32.7	33.8	35.6	2.3	.01	.03	.29
PUN[2], mg/dL	11.0	12.2	11.4	11.7	.4	.96	.21	.41
MUN[3]								
mg/dL	16.0	16.1	15.8	15.8	.7	.78	.97	.89
g/d	5.7	5.3	5.4	5.2	.5	.60	.39	.68
% of total milk N	3.4	3.6	3.6	3.6	.2	.41	.51	.52
% of absorbed N	1.3	1.4	1.4	1.5	.1	.15	.50	.91

[1]UUN = Urinary urea N.

[2]PUN = Plasma urea N.

[3]MUN = Milk urea N.

27 or 40% RUP at 15.1% dietary CP.

Correlations between urea nitrogen in urine, milk, and plasma

Efficiency of protein feeding is maximized when the N supplied in the diet matches the N required by rumen microbes and ruminant tissue. This balance is associated with a baseline concentration of urea in urine, plasma, and milk. Excess N supplied to the rumen and postruminal tissues or an imbalanced supply of AA increases the concentration of urea in plasma and milk above baseline values, thus increases the excretion of urea in urine.

In spite of accounting for only 1.4% of absorbed N, MUN output was correlated with UUN output (overall gross r = .52; within-cow r = .57) (Table II-13), and the overall regression describing the relationship between UUN and MUN output was

$$\text{UUN (g/d)} = 64.1 + 11.6 \times \text{MUN (g/d)};\ R^2 = .27.$$

However, the within-cow regression had higher coefficient of determination ($R^2 = .69$), and the regression is as follows:

$$\text{UUN (g/d)} = 48.9 + 14.4 \times \text{MUN (g/d)};\ R^2 = .69.$$

Ciszuk and Gebregziabher (1994) proposed that milk urea could be an estimator of urinary N excretion in dairy cattle. In their study, the overall regression describing the

Table II-13. Correlations between urea N outputs in urine and milk and between urea N concentrations in urine, milk, or plasma.

	Overall gross correlations		Within-cow correlations	
Item	r	*P*	r	*P*
UUN[1] vs. MUN[2]	.52	< .01	.57	< 01
CUUN[3] vs. CMUN[4]	- . 07	.55	- .38	.02
CUUN vs. CPUN[5]	- .12	.31	- .21	.21
CPUN vs. CMUN	.50	< .01	.61	< .01

[1]UUN = Urinary urea N output (g/d).

[2]MUN = Milk urea N output (g/d).

[3]CUUN = Concentration of urinary urea N (mg/dL).

[4]CMUN = Concentration of milk urea N (mg/dL).

[5]CPUN = Concentration of plasma urea N (mg/dL).

relationship between urinary N and MUN concentration was

Urinary N (g/d) = 34.1 + 10.6 x MUN (mg/dL); R^2 = .71.

In this study, urinary N excretion was correlated with MUN concentration (r = .52), and the overall regression describing the relationship between urinary N and MUN concentration was

Urinary N (g/d) = 88.9 + 6.2 x MUN (mg/dL); R^2 = .27.

However, the coefficient of determination (R^2 = .27) was lower in this study than in the previous study (Ciszuk and Gebregziabher, 1994; R^2 = .71), in which CP content of the diets varied between 11 and 19%. However, the within-cow regression describing the relationship between urinary N and MUN concentration elevated the coefficient of determination (R^2 = .83), and the regression is as follows:

Urinary N (g/d) = 72.4 + 7.2 x MUN (mg/dL); R^2 = .83.

The within-cow's effect on the relationship between concentrations of UUN and MUN established negative correlation (r = - .38) (Table II-13). Some factors could contribute to explain that relationship. Urine output was higher for cows fed SBM and inorganic

phosphorus, but urinary N output did not differ (Table II-10). Water intake could be involved in that process, and urea equilibrates in body water. As a result, UUN output did not differ in response to diet. But concentration of UUN was higher in cows fed BM and wheat bran. In contrast, MUN output and concentration did not differ.

Oltner et al. (1985) reported that the correlation between concentrations of MUN and PUN was .91. In this study, however, the correlation was lower (r = .50). The overall relationship between PUN and MUN (CPUN versus CMUN in Table II-13) is described by the following equation:

$$\text{MUN (mg/dL)} = 7.39 + .74 \times \text{PUN (mg/dL)}; R^2 = .25.$$

The within-cow correlation between MUN and PUN has been estimated in several reports (r = .88, Roseler et al., 1993; r = .96, Baker et al., 1995). In their studies, percentage of dietary CP ranged from 12 to 18%. The lower correlation coefficient in this study (r = .61) compared with previous studies may have resulted from feeding diets with a narrow range in dietary CP. The within-cow relationship between PUN and MUN in this study was:

$$\text{MUN (mg/dL)} = 5.06 + .94 \times \text{PUN (mg/dL)}; R^2 = .59.$$

Kinetic analysis has suggested that MUN concentration is a reasonable indicator of mean PUN concentration (Baker et al., 1995). Because milk samples are routinely collected

on dairy farms, MUN concentration could be measured to monitor protein utilization. Mean MUN concentration may be a good indicator of mean PUN concentration, but with respect to environmental concerns it also may be useful to use MUN output to predict UUN output.

Nitrogen excretion prediction equations

With respect to manure management, it would be very important to be able to predict N excretion from cow performance and dietary composition. Therefore, N excretion data for urine, feces, and a combination of urine and feces were subjected to regression analyses to determine importance of DMI, level of milk production, and parity to predict N excretion. A series of progressive steps were taken to determine the best prediction equation from the data set for this study.

For urine and fecal N excretion, the only significant regressions for all independent variables were:

$$TNURG = 71.29 + 22.82 \times Lact + 5.20 \times DMIKG - 1.39 \times MKKG$$

$$TNFECG = 3.05 + 6.25 \times Lact + 8.44 \times DMIKG$$

where:

TNURG = Total urine N excretion in g;

TNFECG = Total fecal N excretion in g;

Lact = Parity (Lactation number 1 = 1, 2 = 2, 3 and 4 = 3);

DMIKG = Dry matter intake in kg;

MKKG = Milk yield in kg/d.

Above models accounted for 54% and 79% of the variation (R^2) for TNURG and TNFECG. Due to the very high correlation between DMI and N intake (Table II-14), N intake was excluded in the models. Also, DMI was the major factor to influence N excretion.

To estimate N excretion in urine plus feces, the following equation was developed (Table II-15). This model accounted for 81% of the variation (R^2).

TNUFG = 73.35 + 28.92 x Lact + 13.58 x DMIKG - 1.32 x MKKG

where:

TNUFG = Total N excretion in urine and feces in g;

Lact = Parity (Lactation number 1 = 1, 2 = 2, 3 and 4 = 3);

DMIKG = Dry matter intake in kg;

MKKG = Milk yield in kg/d.

Comparison of nitrogen balance data between Kjeldahl and elemental analyses

Two methods were utilized to analyze N content of feeds, feed refusals, milk, and urine from period 1 only. Table II-16 contains comparisons between the two methods. The correlation coefficient for % N in urine was lower (r = .68) than coefficients for other samples (r = .74 to .81). It may be caused by some urinary N loss during freeze-drying for elemental analysis, because liquid urine and milk were used for Kjeldahl analysis. Similarly,

Table II-14. Correlations among lactation number, DM intake, milk yield, N intake, urinary N, fecal N, and combination of urinary and fecal N.

Item	LACT[1]	DMIKG[2]	MKKG[3]	TNIG[4]	TNURG[5]	TNFECG[6]
DMIKG	.47[8]	-	-	-	-	-
MKKG	.46	.58	-	-	-	-
TNIG	.46	.96	.50	-	-	-
TNURG	.60	.61	.28	.67	-	-
TNFECG	.51	.88	.54	.82	.54	-
TNUFG[7]	.63	.85	.47	.85	.87	.88

[1]LACT = Lactation number (1, 2, 3 & 4).

[2]DMIKG = Dry matter intake in kg/d.

[3]MKKG = Milk yield in kg/d.

[4]TNIG = Total N intake in g/d.

[5]TNURG = Total N in urine in g/d.

[6]TNFECG = Total N in feces in g/d.

[7]TNUFG = Total N in urine plus feces in g/d.

[8]r = Correlation coefficient. All correlation coefficients are significant at $P < .02$.

Table II-15. Variables used to predict N excretion in waste of Holstein cows (n = 72) during early and midlactation.

					Correlated with TNUFG	
Variable	LSM[1]	SD	Minimum	Maximum	r	*P*
TNUFG[2], g/d	396	70	243	586	-	-
Lactation number[3]	2.1	.78	1	3	.63	< .001
DMI, kg/d	22.7	4.0	14.3	31.0	.85	< .001
Milk yield, kg/d	35.4	6.6	18.3	48.7	.47	< .001

[1]LSM = Least square mean.

[2]TNUFG (Total N excretion in urine plus feces, g/d) = 73.35 + 28.92 x Lactation number + 13.58 x DMI (kg/d) - 1.32 x Milk yield (kg/d); R^2 = .81.

[3]Lactation number 1 = 1, 2 = 2, 3 & 4 = 3.

Table II-16. Comparison of N concentrations and N partitioning in early lactation from Kjeldahl versus elemental analyses.

Item	Kjeldahl analysis	Elemental analysis	r	*P*
Concentration	----------% N in DM----------			
Feeds	2.55	2.50	.81	< .01
Feed refusals	2.36	2.24	.74	< .01
Feces	2.67	2.61	.78	< .01
Urine	17.45	13.45	.68	< .01
Milk	3.50	3.34	.79	< .01
N partitioning	----------g/d----------			
N intake	560.2	545.1	.95	< .01
Fecal N	201.6	197.1	.96	< .01
Urinary N	171.2	132.4	.85	< .01
Milk N	150.8	144.1	.97	< .01
N balance[1]	36.6	72.2	.73	< .01
Absorbed N[2]	358.6	348.7	.82	< .01
% in urine	48.8	38.3	.83	< .01
% in milk	42.5	41.6	.82	< .01

[1]N balance (g/d) = N intake (g/d) - Fecal N (g/d) - Urinary N (g/d) - Milk N (g/d). N balance (g/d) = N retained (g/d).

[2]Absorbed N (g/d) = N intake (g/d) - Fecal N (g/d).

urinary N output was lower when estimated by elemental analysis (132 g/d) compared with Kjeldahl analysis (171 g/d). However, all correlation coefficients regarding parameters of N partitioning were still high, and suggest elemental analysis as an alternate method for determination of N content.

Carbon balance

Results of C balance, determined using elemental analysis, during early lactation are shown in Tables II-17 and II-18. Similar to N balance, C intake and means for most other parameters of C partitioning differed due to parity. Higher C digestibility by cows in their first lactation may be due to lower DMI. Tyrrell et al. (1988) reported slightly higher C digestibility (66.2%) than the average for this study (63.7%). Partitioning of C in response to supplemental protein source did not differ. Cows partitioned approximately 3.5 kg C to OTHER areas of C utilization, including carbon dioxide (CO_2), methane (CH_4), and tissue gain.

Other carbon partitioning

To estimate C partitioning to OTHER areas, a few assumptions from Wolin (1960) were used to permit calculation of the amounts of carbon dioxide (CO_2) and methane (CH_4) produced in the rumen and hindgut. For calculation of fermentation balance, the following assumptions were made:

Table II-17. Least squares means for carbon partitioning in response to parity during early lactation.

	Lactation			
Item	1	2	3 & 4	SE
C intake, kg/d	8.10[a]	9.43[b]	10.33[b]	.40
Fecal C, kg/d	2.77[a]	3.44[ab]	3.91[b]	.16
Urinary C, kg/d	.23[a]	.26[b]	.29[c]	.01
Milk C, kg/d	1.90[a]	2.14[a]	2.45[b]	.09
Other C, kg/d[1]	3.21	3.58	3.69	.25
C digestibility, %[2]	65.9[b]	63.3[ab]	62.0[a]	1.0

[1]Other C (kg/d) = C intake (kg/d) - Fecal C (kg/d) - Urinary C (kg/d) - Milk C (kg/d).

[2]C digestibility (%) = ((C intake (kg/d) - Fecal C (kg/d))/(C intake (kg/d))) x 100.

[a,b,c]Means in rows with different superscripts differ, $P < .05$, using Tukey's test.

Table II-18. Least squares means for carbon partitioning in response to diet during early lactation.

	Diet					Contrasts (*P*)		
Item	SPM	SWB	BPM	BWB	SE	Prot	Phos	INT
C intake, kg/d	9.51	8.72	9.88	9.04	.46	.45	.09	.96
Fecal C, kg/d	3.49	3.05	3.64	3.32	.18	.26	.05	.73
Urinary C, kg/d	.26	.26	.26	.25	.01	.47	.76	.45
Milk C, kg/d	2.23	2.03	2.27	2.11	.10	.58	.09	.87
Other C, kg/d[1]	3.52	3.37	3.72	3.37	.28	.74	.38	.73
C digestibility, %[2]	63.1	65.0	63.4	63.4	1.2	.55	.44	.42

[1]Other C (kg/d) = C intake (kg/d) - Fecal C (kg/d) - Urinary C (kg/d) - Milk C (kg/d).

[2]C digestibility (%) = ((C intake (kg/d) - Fecal C (kg/d))/(C intake (kg/d))) x 100.

a) The molar proportions of volatile fatty acids (VFA) found in rumen fluid are 70% acetic acid, 19% propionic acid, and 11% butyric acid. These proportions of VFA were similar to data for cannulated cows in a previous study (Rodriguez, 1994) fed similar diets.

b) The only fermentation products produced in addition to the above mentioned VFA are CO_2 and CH_4.

c) All fermentation products are formed from plant carbohydrates with the empirical formula $C_6H_{12}O_6$.

For the above molar distribution of VFA, the following balance equation with amounts expressed as moles was calculated:

$$55.5\ C_6H_{12}O_6 \text{ --------> } 70 \text{ acetate} + 19 \text{ propionate} + 11 \text{ butyrate} + 56\ CO_2 + 36\ CH_4 + 20\ H_2O$$

Organic matter intake was estimated to be 92.2% of DMI, based on a previous study (Fisher, 1995) with similar diets. To apply the above fermentation balance equation to OM fermentation, the following estimations, based on data from a previous study (Ellingson, 1993) with similar diets, were used.

a) 32% of OM intake was fermented in the rumen.

b) 68% of OM intake flowed to the duodenum (OMFD).

c) 4.8% of OMFD was fermented in the hindgut.

As a result of the above estimation, VFA, CO_2, and CH_4 proportions of OM fermented in the rumen and hindgut were calculated to be 65.9, 24.7, and 5.8%, respectively. The C content (kg) of CO_2 and CH_4 (27.3% and 74.9%) then was used to calculate C lost as CO_2 and CH_4.

For example:

If DMI = 21.3 kg/d,

Then OMI = 19.6 kg/d = (21.3 x .92 = 19.6)

OM fermented in rumen = 6.3 kg/d = (19.6 x .32 = 6.3)

OM fermented in hindgut = .6 kg/d = (13.3 x .048 = .6)

Total OM fermented = 7 kg/d = (6.3 + .6 = 6.9)

CO_2 production = 1.70 kg/d = (6.9 x .247 = 1.70)
CO_2 production = .46 kg C/d = (1.70 x .273 = .46)

CH_4 production = .40 kg/d = (6.9 x .058 = .40)
CH_4 production = .30 kg/d = (.40 x .749 = .30)

Total C lost in CO_2 plus CH_4 = .76 kg/d

To estimate the amount of C partitioned to tissue protein gain or loss, N retention (g/d) was multiplied by 6.25. Then, C retention in tissue (kg/d) was calculated as 47% of tissue protein, because animal proteins contain approximately 47% C. Finally, C partitioning to exhaled CO_2 was calculated by subtracting the sum of C lost during fermentation (CO_2 and CH_4) plus tissue C from OTHER C.

Table II-19 summarizes OTHER C partitioning in response to diet during early lactation. Supplemental protein sources did not affect OTHER C partitioning. Cows partitioned approximately .30 kg C to CH_4. Johnson et al. (1992) reported 5.6% of gross energy intake (GEI) was dissipated to the atmosphere as CH_4. They assumed GEI to be 4.45 Mcal/kg DMI, and methane energy to be 13.184 Mcal/kg. Based on these relationships, CH_4 produced by cows in early lactation consuming 21.3 kg DM/d in this study was calculated to be .40 kg/d, and C content of CH_4 was .30 kg (.40 x .75). Wilkerson et al. (1995) summarized data for seven studies using 382 lactating cows. They reported 4.6 Mcal GEI/kg DMI and energy in the methane equal to 5.5% of GEI as averages for all studies. Van Horn et al. (1994) reported that dairy cows fed moderately high concentrate diets converted about 5% of their GEI into CH_4.

It was estimated that cows partitioned approximately 2.6 kg C to respiration (aerobic) CO_2. At this point, it is necessary to verify whether this estimation is reasonable. In NRC (1989), a 550 kg lactating cow should consume 9.09 Mcal for maintenance heat production. For production, 41.75 Mcal of metabolizable energy (ME) are needed to produce 36.3 kg milk with 3.5% fat. Approximately 36% of the ME intake (15.03 Mcal) is dissipated as heat, and 64% is converted to milk energy. As a result, 24.12 Mcal is lost as heat for production plus maintenance. Assuming a respiration quotient (RQ) is equal to 1.0, 24.12 Mcal of heat production is equal to 9.385 kg CO_2 exhaled, which is equivalent to 2.56 kg C exhaled and similar to the 2.6 kg of C in respiration CO_2 listed in Table II-19.

Approximately, 3.08 kg of C was lost as CO_2 in the combination of fermentation and

Table II-19. Least squares means for estimated carbon partitioning to tissue, methane (CH_4), and carbon dioxide (CO_2) in response to diet during early lactation (n = 36).

	Diet					Contrasts (*P*)		
Item	SPM	SWB	BPM	BWB	SE	Prot	Phos	INT
Other C, kg/d	3.52	3.37	3.72	3.37	.28	.74	.38	.73
Tissue C, kg/d	.14	.11	.13	.05	.04	.51	.31	.69
Methane, kg/d	.31	.28	.32	.29	.002	.65	.07	.86
CO_2 (anaerobic), kg/d	.49	.44	.50	.45	.004	.65	.07	.86
CO_2 (aerobic), kg/d	2.47	2.55	2.78	2.53	.23	.55	.72	.50
CFER, kg/d[1]	.80	.72	.82	.75	.006	.65	.07	.86
$CLCO_2$, kg/d[2]	2.96	2.98	3.27	2.98	.22	.55	.61	.54
CLAT, kg/d[3]	3.27	3.26	3.59	3.27	.22	.54	.5	.57

[1]CFER = C to fermentation CH_4 and CO_2.

[2]$CLCO_2$ = C loss as CO_2 (anaerobic fermentation CO_2 plus respiration CO_2).

[3]CLAT = C loss to atmosphere(fermentation CH_4 + fermentation CO_2 + respiration CO_2).

aerobic respiration. Tyrrell et al. (1988) reported 5392 L CO_2 produced daily by lactating cows, and the total included CO_2 from respiration and fermentation. This 5392 L is equal to 10.6 kg CO_2, which contains 2.89 kg C. However, N balance in their study was negative (-21.1 g N). Consequently, 62 g C was mobilized as protein from tissue; thus, corrected C output as CO_2 from DMI was 2.82 kg. It is concluded that methods used to estimate CO_2 and CH_4 output in this study provided results similar to those obtained by other investigations using direct measurements or estimates based on direct measurements.

Overall carbon partitioning

Table II-20 summarizes overall C partitioning, and contains averages for all dietary treatments. Approximately, 36% of intake C and 57% of absorbed C were partitioned to the atmosphere as CO_2 and CH_4. Van Horn et al. (1994) reported estimations based on data from several sources. They estimated 49% of intake C and 71% of absorbed C were partitioned to the atmosphere. However, they did not consider C partitioning to tissue, and did not estimate fermentation CO_2 and respiration CO_2 separately. Furthermore, milk production was lower in their study (22.7 kg/d) compared with this study (36.3 kg/d).

Table II-20. Comparison of estimated carbon partitioning with another study.

	Elemental analysis			Van Horn et al. (1994)[1]		
Item	Carbon (kg/d)	% of intake C	% of absorbed C[2]	Carbon (kg/d)	% of intake C	% of absorbed C[2]
Intake	9.4	-	-	8.0	-	-
Feces	3.4	36	-	2.5	31	-
Urine	.3	3	4.4	.2	3	3.6
Milk	2.2	23	36.5	1.4	18	25.5
Tissue	.1	1	1.7	-	-	-
Methane	.3	3	5.1	.4	5	7.3
CO_2 (anaerobic)	.5	5	8.1	-	-	-
CO_2 (aerobic)	2.6	28	43.9	3.5	44	63.6

[1]Milk yield: 22.7 kg/d.

[2]Absorbed C = C intake (kg/d) - Fecal C (kg/d).

SUMMARY AND CONCLUSIONS

As body weight of cows increased from first through third or fourth lactation, milk and milk component yields increased. Overall, DMI in support of milk production increased from 20 to 25 kg/d due to increasing parity, with proportional increases in N intake (g/d) and mass of N partitioned to feces, urine, and milk. The percentage of absorbed N partitioned to milk, however, remained at 40% across parities. As parity and body weight increased, the percentage of absorbed N lost in urine increased from 44 to 52% and the percentage partitioned to body tissue (retention) decreased from 16 to 8%. Thus, as parity increases, greater amounts of waste N will be produced due to increasing N intake and a greater percentage of absorbed N will be lost via urine. Increasing amounts of C loss, primarily in feces, can also be expected due to increasing DMI and decreasing digestibility of C associated with increasing parity.

The lower milk production and higher DMI at midlactation compared with early lactation was expected due to the need of lactating cows to replenish body tissues lost during negative nutrient balances during early lactation. The higher DMI during midlactation was associated with higher N intake, higher amounts of N partitioned to feces, urine, and body tissue. Milk fat yield was lower at midlactation due to lower milk yield and milk fat percentage compared with early lactation, but milk protein yield was higher due to a higher milk protein percentage. The higher N intake in midlactation apparently supplied more than adequate amounts of AA for production, because the percentage of absorbed N partitioned

to milk was lower and the percentage partitioned to body tissue was higher than during early lactation. The amount of N absorbed at midlactation was higher partly due to higher DMI and partly due to higher N digestibility. The higher digestibility was not expected, but may have been due to the higher quality of corn silage fed during midlactation. The corn silage fed during early lactation was from the previous year, which had below average rainfall, and the amount remaining after completion of early lactation observations was not sufficient to complete the second half of the study. Thus, corn silage from the current year, with adequate rainfall, was used for midlactation observations.

After statistically accounting for effects due to parity and stage of lactation, significant effects due to BM substitution for a portion of the dietary SBM and use of wheat bran as an organic source of phosphorus were evident in N partitioning in lactating cows. The primary influence of wheat bran was reduction of DMI, which resulted in lower milk yield and milk component yields. Thus, N intake and absorbed N were lower for cows fed wheat bran, but percentages of absorbed N partitioned to urine, milk, and body tissues were similar to those for cows fed mineral sources of phosphorus. Unlike wheat bran, BM addition to the diet did not influence DMI, milk yield, or milk fat yield. Digestibility of N in diets containing BM was lower than N digestibility for diets containing only SBM; thus, reducing the amount of absorbed N. The lower amount of absorbed N resulted in lower milk protein percentage and yield. In addition, the percentage of absorbed N partitioned to urine was higher and percentage partitioned to body tissue was lower. These changes suggest an imbalanced supply of AA available for milk and body protein synthesis.

Concentration of UUN was higher in cows fed BM compared with those fed only SBM. In addition, UUN output was a greater percentage of total urinary N and percentage of absorbed N in cows fed BM compared with those fed SBM, suggesting an imbalanced supply of AA in BM. In contrast to the previous studies, concentrations of PUN and MUN did not differ due to dietary RUP content. Apparently concentrations of PUN and MUN are influenced primarily by CP intake, and CP intake in this study did not vary enough to cause major differences in PUN and MUN.

Based on comparison of N partitioning in mature cows fed 17.5% dietary CP with mature cows in this study fed 16.1% CP, cows fed 16.1% CP met their requirements for milk and tissue protein synthesis. Dietary CP above 16.1% was excreted as urinary N.

Carbon partitioning was very similar to N partitioning in response to parity, and differences were due primarily to differences in DMI. Based on the estimates made in this study, cows partitioned approximately 3.48 kg C to CO_2, CH_4, and tissue gain. The amount of C partitioned to CH_4 was .3 kg, which was equivalent to a daily loss of 5.36 Mcal of energy as CH_4. If GEI is assumed to be 4.3 Mcal/kg DMI for all diets, 5.9% of GEI was eructated as CH_4. It was estimated that cows partitioned approximately 2.6 kg C to respiration CO_2, and .48 kg C was lost as CO_2 from rumen and hindgut fermentation. Thus, cows dissipated 3.38 kg C to the atmosphere in the forms of CO_2 and CH_4. Carbon loss via gasses was 36% of intake C or 57% of absorbed C.

LITERATURE CITED

Agricultural Research Council. 1984. The nutrient requirements of ruminant livestock. Suppl. 1. Commonw. Agric. Bur., Slough, Engl.

Allison, J. B., and R. W. Wannemacher. 1965. The concept and significance of labile and overall protein reserves of the body. Am. J. Clin. Nutr. 16:445.

Andrew, S. M., H. F. Tyrrell, C. K. Reynolds, and R. A. Erdman. 1991. Net energy for lactation of calcium salts of long-chain fatty acids for cows fed silage-based diets. J. Dairy Sci. 74:2588.

Armstrong, O. G., and T. E. C. Weekes. 1983. Recent advances in ruminant biochemistry : Nitrogen digestion and metabolism. Int. J. Biochem. 15:261.

Association of Official Analytical Chemists International. 1995. Official Methods of Analysis. 16th ed. Method 984. 13. AOAC, Arlington.

Axelsson, J. 1949. The amount of produced methane energy in the European metabolic experiments with adult cattle. Ann. R. Agric. Coll. Sweden. 16:404.

Baker, L. D., J. D. Ferguson, and W. Chalupa. 1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. J. Dairy Sci. 78:2424.

Baldwin, R. L. and M. J. Allison, 1983. Rumen metabolism. J. Anim. Sci. 57:461.

Barney, D. J., D. G. Grieve, G. K. MacLeod, and L. G. Young. 1981. Response of cows to dietary crude protein during midlactation. J. Dairy Sci. 64:655.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation : A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514.

Belyea, R. L., G. R. Frost, F. A. Martz, J. L. Clark, and L. G. Forkner. 1978. Body composition dairy cattle by potassium-40 liquid scintillation detection. J. Dairy Sci. 61:206.

Biddle, C. N., J. L. Evans, and J. R. Trout. 1975. Labile nitrogen reserves and plasma nitrogen fractions in growing cattle. J. Nutr. 105:1584.

Bines, J. A., and A. C. Hart. 1982. Metabolic limits to milk production, especially roles of growth hormone and insulin. J. Dairy Sci. 65:1375.

Blaxter, K. L., and J. L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19:511.

Blaxter, K. L., and J. W. Czerkawski. 1966. Modifications of the methane production of the sheep by supplementation of its diet J. Sci. Fd. Agric. 17:417.

Botts, R. L., R. W. Hemken, and L. S. Bull. 1979. Protein reserves in the lactating dairy cow. J. Dairy Sci. 62:433.

Bratzler, J. W. and E. B. Forbes. 1940. The estimation of methane production by cattle. J. Nutr. 19:611.

Brock, T. D., and M. T. Madigan. 1991. Microbial ecology. p. 641 *in* Biology of Microorganisms. 6th ed. Prentice-Hall, Englewood Cliffs.

Bruckental, I., J. D. Oldham, and J. D. Sutton. 1978. Glucose and urea kinetics in cows in early lactation. Proc. Nutr. Soc. 37:107A.

Buchanan-Smith, J. D. 1995. Diet protein level and degradability on nitrogen in the manure of lactating cows. Dairy Res. Report 6.

Canfield, R. W., C. J. Sniffen, and W. R. Butler. 1990. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci. 73:2342.

Chalupa, W. 1984. Discussion of protein symposium. J. Dairy Sci.67. 1134.

Chalupa, W. 1982. Protein nutrition of dairy cattle. Page 101 in Proc. Dist. Feed Conf. Cincinnati, OH.

Chalupa, W. 1975. Rumen bypass and protection of proteins and amino acids. J. Dairy Sci. 58:1198.

Chamberlain, D. G., and P. C. Thomas. 1979. Ruminal nitrogen metabolism and the passage of amino acids to the duodenum in sheep receiving diets containing hay and concentrates in various proportions. J. Sci. Food Agric. 30:677.

Chandler, P. 1989. Achievement of optimum amino acid balance possible. Feedstuffs. 61(26):14.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130.

Christensen, R. A., M. R. Cameron, T. H. Klusmeyer, J. P. Elliott, J. H. Clack, D. R. Nelson, and Y. Yu. 1993. Influence of amount and degradability of dietary protein on nitrogen utilization by dairy cows. J. Dairy Sci. 76:3497.

Ciszuk, P and T. Gebregziabher. 1994. Milk urea as an estimate of urine nitrogen of dairy cows and goats. Acta. Agric. Scand. 44:87.

Clark, J. H., M. R. Murphty, and B. A. Crooker. 1987. Supplying the protein needs of dairy cattle from byproduct feeds. J. Dairy Sci. 70:1092.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304.

Conrad, H. R. 1972. Urea kinetics and amino acid entry rates in dairy cows. Page 69 *in* Tracer studies on non-protein nitrogen for ruminants. Vienna, Austria.

Coppock, C. E., H. F. Tyrrell, W. G. Merrill, and J. T. Reid. 1968. The significance of protein reserve to the lactating cow. Page 68 *in* Cornell Nutr. Conf. Proc., Ithaca, NY.

Cozzi, G. and C. E. Polan. 1994. Corn gluten meal or dried brewers grains as partial replacement for soybean meal in the diet of Holstein cows. J. Dairy Sci. 77:825.

Crish, E. M., J. E. Wohlt, and J. L. Evans. 1986. Insoluble nitrogen for milk production in Holstein cows via increases in voluntary intake and nitrogen utilization. J. Dairy Sci. 69:1576.

Crutchfield, W. O. 1968. A technique for placement of an indwelling catheter in the cow. Vet. Med. Small Anim. Clin. 63:1141.

Czerkawski, J. W. 1988. An introduction to rumen studies. Pergamon Press. Oxford.

Czerkawski, J. W. and G. Breckenridge. 1969. The effect of oxygen on fermentation and sucrose by rumen microorganisms in vitro. Br. J. Nutr. 23:67.

Dewhurst, R. T. and C. Thomas. 1992. Modelling of nitrogen transactions in the dairy cow and their environmental consequences. Livestock Prod. Sci. 31:1.

Dhiman, T. R. and L. D. Satter. 1993. Protein as the first-limiting nutrient for lactating dairy cows fed high proportions of good quality alfalfa silage. J. Dairy Sci. 76:1960.

Ellingson, T. A. 1993. Digestion and utilization of nutrients in diets containing feather meal and supplemental fat by lactating dairy cows. M.S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg.

Fisher, R. J. 1995. Partitioning of nitrogen by lactating cows fed diets varying in nonfibrous carbohydrate and rumen undegradable protein. Ph.D. Dissertation. Virginia Polytechnic Institute and State University, Blacksburg.

Forster, R. J., D. G. Grieve, J. G. Buchanan-Smith, and G. K. MacLeod. 1983. Effect of dietary protein degradability on cows in early lactation. J. Dairy Sci. 66:1653.

Ganev, G., E. R. Orskov, and R. Smart. 1979. The effect of roughage or concentrate feeding and rumen retention time on total degradation of protein in the rumen. J. Agric. Sci. Camb. 93:651.

Goedeken, F. K., T. J. Klopfenstein, R. A. Stock, R. A. Britton, and M. H. Snidt. 1990. Protein value of feather meal for ruminants as affected by blood additions. J. Anim. Sci. 68:2936.

Holter, J. B., and A. J. Young. 1992. Methane prediction in dry and lactating Holstein cows. J. Dairy Sci. 75:2165.

Huber, J. T., and L. Kung, Jr. 1981. Protein and non-protein nitrogen utilization in dairy cattle. J. Dairy Sci. 64:1170.

Johnson, D. E., G. M. Ward, and J. Torrent. 1992. The environmental impact of bovine somatotropin use in dairy cattle. J. Environ. Qual. 21:157.

Journet, M. and B. Remond. 1976. Physiological factors affecting the voluntary intake of feed by cows. A review. Livestock Prod. Sci. 3:129.

Kaplan, A. 1965. Urea nitrogen and urinary ammonia. Page 245-256 *in* Standard methods of clinical chemistry, Vol 5 ed. S. Meites. Academic Press, New York.

Kaiser, R. M. and D. K. Comb. 1989. Utilization of three maturities of alfalfa by dairy cows fed rations that contain similar concentrations of fiber. J. Dairy Sci. 72:2301.

Kennedy, P. M., and L. P. Milligan. 1980. The degradation and utilisation of endogenous

urea in the gastrointestinal tract of ruminants: A review. Can. J. Anim. Sci. 60:205.

King, K. J., J. T. Huber, M. Sadik, W. G. Bergen, A. L. Grant, and V. L. King. 1990. Influence of dietary protein sources on the amino acid profiles available for digestion and metabolism in lactating cows. J. Dairy Sci. 73:3208.

Kriss, M. 1930. Quantitative relations of the dry matter of the food consumed, the heat production, the gaseous outgo and the insensible loss in body weight of cattle. J. Agric. Res. 40:283.

Kung, L. Jr., and J. T. Huber. 1983. Performance of high producing cows in early lactation fed protein or varying amounts, sources, and degradability. J. Dairy Sci. 66:227.

Kung, L. Jr., J. T. Huber, and L. D. Satter. 1984. Influence of non-protein nitrogen and protein of low rumen degradability on nitrogen flow and utilization in lactating dairy cows. J. Dairy Sci. 66:1863.

Loerch, S. C., L. L. Berger, S. D. Plegge, and G. C. Fahey, Jr. 1983. Digestibility and rumen escape of soybean meal, Blood meal, meat and bone meal and dehydrated alfalfa nitrogen. J. Anim. Sci. 57:1037.

MacLeod, G. K., D. G. Grieve, and I. McMillan. 1982. Effect of varying protein and energy densities in diets of first lactation cows. J. Dairy Sci. 65 (Suppl. 1): 126. (Abstr.).

Mathers, J. C., R. J. Thomas, N. A. M. Gray, and I. L. Johnson. 1979. The nutritive value of feed proteins which escape degradation in the rumen. Proc. Nutr. Soc. 38:122A.

Maynard, L. A., J. K. Loosli, H. F. Hintz, and R. G. Warner. 1979. The proteins and their metabolism. Page 152 *in* Animal nutrition. McGraw-Hill, New York.

McCarthy, R. D., Jr., T. H. Klusmeyer, J. L. Vicini, J. H. Clark, and D. R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72:2002.

Mertens, D. R. 1977. Proc. Ga. Nutr. Conf. Feed Ind. p. 30-41.

Mir, Z., G. K. MacLeod, J. G. Buchanan-Smith, D. G. Grieve, and W. L. Grovum. 1984. Effect of feeding soybean meal protected with sodium hydroxide, fresh blood or fish hydrolysate to growing calves and lactating dairy cows. Can. J. Anim. Sci. 64:845.

Moe, P. W. and H. F. Tyrrell. 1979. Methane production in dairy cows. J. Dairy Sci. 62:1583.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, D. C.

National Research Council. 1985. Subcommittee on Nitrogen Usage in Ruminants. Ruminant Nitrogen Usage. National Academy Press. Washington, D. C.

Nocek, J. E., and J. B. Russell.1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070.

Norman, A. W., P. T. Chandler, and C. S. Park. 1982. The effect of ration protein on milk production responses at different stages of lactation. J. Dairy Sci. 65 (Suppl. 1): 126. (Abstr.)

Oldham, J. D. 1984. Protein-energy interrelationships in dairy cows. J. Dairy Sci. 67:1090.

Oldham, J. D., R. J. Fulford, and D. J. Napper. 1981. Source and level of supplemental N for cows in early lactation. Proc. Nutr. Soc. 40:30A.

Oltner, R., M. Emanuelson, and H. Wiktorsson. 1985. Urea concentration in milk in relation to milk yield, live weight, lactation number and amount and composition of feed given to dairy cows. Livest. Prod. Sci. 12:47.

Ogimoto, K. and S. Imai. 1981. Atlas of rumen microbiology. Jpn. Sci. Soc. Press. Tokyo, Japan.

Orskov, E. R. 1982. Protein Nutrition in Ruminants. Academic Press, New York.

Owens, F. N. 1986. Maintenance protein requirements. Page 187 *in* Agriculture: feed evaluation and protein requirement systems for ruminants. R. Jarrige and G. Alderman. 1982. Eur. Econ. Commun., Brussels, Belgium.

Paguay, R., R. De Baere, and A. Lousse.1972. The capacity of the mature cow to lose and recover nitrogen and the significance of protein reserves. Br. J. Nutr. 27:27.

PC-SAS® User's Guide: Statistics, Release 6.03 Edition. 1990. SAS Inst., Inc., Cary, NC.

Pell, A. N. 1992. Does ration balancing affect nutrient management? Proc. Cornell Nutr. Conf. p. 23. Ithaca, NY.

Polan, C. E. 1992. Protein and amino acids for lactating dairy cows. In: Large Dairy Herd

Management. H. H. Van Horn and C. J. Wilcox. Editors. American Dairy Science Association, Champaign, Illinois.

Polan, C. E., C. N. Miller, and M. L. McGilliard. 1976. Variable dietary protein and urea for intake and production in Holstein cows. J. Dairy Sci. 59:1910.

Polan, C. E., T. A. Herrington, W. A. Wark, and L. E. Armentano. 1985. Milk production responses to diets supplemented with dried brewers grains, wet brewers grains, or soybean meal. J. Dairy Sci. 68:2016.

Reid, I. M., R. A. Collins, G. D. Baird, C. J. Roberts, and H. W. Symonds. 1979. Lipid production rates and the pathogenesis of fatty liver in fasted cows. J. Agric. Sci. Camb. 93:253.

Robinson, P. H., R. E. McQueen, and P. L. Burgess. 1991. Influence of rumen undegradable protein levels on feed intake and milk production of dairy cows. J. Dairy Sci. 74:1623.

Rodriguez, L. A. 1994. Effect of dietary protein degradability and fat on rumen, blood, and milk components of Jersey and Holstein cows. M.S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg.

Roseler, D. K., J. D. Ferguson, C. J. Sniffen, and J Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in milk. J. Dairy Sci. 76:525.

Santos, K. A., M. D. Stern, and L. D. Satter. 1984. Protein degradation in the rumen and amino acid absorption in the small intestine of lactating dairy cattle fed various protein sources. J. Anim. Sci. 58:244.

Satter, L. D., and R. E. Roffler. 1975. Nitrogen requirement and utilization in dairy cattle. J. Dairy Sci. 58:1219.

Schwab, C. G. 1994. Optimizing amino acid nutrition for optimum yields of milk and milk protein. Proc. Southwest Nutr. and Management Conf. p. 114.

Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. J. Dairy Sci. 59:1254.

Smith, R. W., and A. Walsh. 1984. Effect of lactation on the metabolism of sheep adipose tissue. Res. Vet. Sci. 37:320.

Stallings, C. C., J. Quigley, D. Tomlinson, R. James, J. Kelley, G. Kroll, and C. Cassady. 1993. Dair4, ration evaluation software. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Stern, M. D., K. A. Santos, and L. D. Satter. 1985. Protein degradation in rumen and amino acid absorption in the small intestine of lactating dairy cattle fed heat-treated whole soybeans. J. Dairy Sci. 68:45.

Stern, M. D., and L. D. Satter. 1980. In vivo estimation of protein degradability in the rumen. Page 57 *in* Protein requirements for cattle: Symp. F. N. Owens, ed. Oklahoma State Univ., Stillwater.

Storm, E., D. S. Brown, and E. R. Orskov. 1983. The nutritive value of rumen microorganisms in ruminants. 3. The digestion of microbial amino acids and nucleic acids in, and losses of endogenous nitrogen from, the small intestine of sheep. Br. J. Nutr. 50:479.

Swanson, E. W. 1980. Estimation of metabolic protein requirements to cover unavoidable losses of endogenous nitrogen in maintenance of cattle. Page 183 *in* Protein requirements for cattle. F. N. Owens, ed. Oklahoma State Univ., Stillwater.

Swick, R. W., and B. J. Benevenga. 1976. Labile protein reserves and protein turnover. J. Dairy Sci. 60:505.

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75:345.

Tamminga, S., C. J. Van der Koelen, and A. M. Van Vuuren. 1979. Effect of the level of feed intake on nitrogen entering the small intestine of dairy cows. Livest. Prod. Sci. 6:255.

Tomlinson, A. P., H. H. Van Horn, C. J. Wilcox, and B. Harris, Jr. 1994. Effect of undegradable protein and supplemental fat on milk yield and composition and physiological responses of cows. J. Dairy Sci. 77:145.

Treacher, R. J., W. Little, K. A. Collis, and A. J. Stark. 1976. The influence of dietary protein intake on milk production and blood composition of high-yielding dairy cows. J. Dairy Res. 43:357.

Trigg, T. E., K. E. Jury, A. M. Bryant, and C. R. Parr. 1980. The energy metabolism of dairy cows underfed in early lactation. Energy Metabolism. Butterworths, London.

Tyrrell, H. F., A. C. G. Brown, P. J. Reynolds, G. L. Haaland, D. E. Bauman, C. J. Peel, and W. D. Steinhour. 1988. Effects of bovine somatotropin on metabolism of lactating dairy cows: Energy and nitrogen utilization as determined by respiration calorimetry. J. Nutr. 118: 1024.

Tyrrell, H. F., P. W. Moe, and W. P. Flatt. 1970. Influence of excess protein intake on energy metabolism of the dairy cow. Proc. 5th Symp. on Energy Metabolism, Vitznau, Switzerland. p. 69-72.

United States Environmental Protection Agency. 1993. Anthropogenic methane emissions in the United States, Rep. Congr., Global Change Div., Office Air Radiat., Environ. Protection Agency, Washington, DC.

Van Horn, H. H., A. C. Wilkie, W J. Powers, and R. A. Nordstedt. 1994. Components of dairy manure management systems. J. Dairy Sci. 77:2008.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. O & B Books, Inc., Corvallis, Oregon.

Van Straalen, W. M., and S. Tamminga. 1990. Protein degradation in ruminant diets. Page 55 *in* Feedstuff evaluation. J. Wiseman and D. J. A. Cole, ed. Butterworths, London, Engl.

Weatherburn, M. W. 1967. Phenol-hydrochlorite reaction for determination of ammonia. Anal. Chem. 39:971.

Wohlt, J. E., S. L. Chmiel, P. K. Zajak, L. Backer, D. B. Blethen, and J. L. Evans. 1991. Dry matter intake, milk yield and composition and nitrogen use in Holstein cows fed soybean, fish or corn gluten meals. J. Dairy Sci. 74:1609.

Wilkerson, V. A, D. P. Casper, and D. R. Mertens. 1995. The prediction of methane production of Holstein cows by several equations. J. Dairy Sci. 78:2402.

Wolin, M. J. 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43:825.

Zinn, R. A., L. S. Bull, and R. W. Hemken. 1981. Degradation of supplemental proteins in the rumen. J. Anim. Sci. 52:857.

APPENDIX

Table A-1. ANOVA for nitrogen intake (g/d).

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	47	816752	17378	6.15	.01
Error	24	67802	2825		
Corrected total	71	884554			

Source	DF	Type III SS	Mean square	F value	Pr > F
Lact[1]	2	206111	103056	8.20	.01
Prot[2]	1	15019	15019	1.20	.29
Phos[3]	1	52311	52311	4.16	.05
Prot*Phos	1	1302	1302	.10	.75
Prot*Lact	2	1623	812	.06	.94
Phos*Lact	2	28873	14436	1.15	.33
Prot*Phos*Lact	2	45560	22780	1.81	.18
Cow(Prot*Phos*Lact)	24	301452	12560	4.5	.01
Per[4]	1	111192	111192	39.5	.01
Per*Lact	2	215	108	.1	.96
Per*Prot	1	25120	25120	8.9	.01
Per*Phos	1	1367	1367	.5	.49
Per*Prot*Phos	1	2759	2759	1.0	.33
Per*Prot*Lact	2	1542	771	.3	.76
Per*Phos*Lact	2	4255	2127	.8	.48
Per*Prot*Phos*Lact	2	17224	8612	3.1	.07

(Continued on the next page)

Table A-1. ANOVA for nitrogen intake (g/d).

Tests of hypotheses using the type III MS for Per

Source	DF	Type III SS	Mean Square	F value	Pr > F
Per	1	111192	111192	39.5	.01

[1]Lact = Parity (lactation number).

[2]Prot = Supplemental protein sources.

[3]Phos = Supplemental phosphorus sources.

[4]Per = Stage of lactation (early or midlactation).

Comparison of Carbon Partitioning to Methane

To compare C partitioning (kg C) to fermentation CH_4 in response to diets, various CH_4 output prediction equations were used (Table I-1). Many independent variables in those equations were estimated using our previous studies (Ellingson, 1993; Fisher, 1995; Rodriguez, 1994), NRC (1989), and Dair4 (Stallings et al., 1993). The equations of Kriss (1930) and Bratzler and Forbes (1940) were converted from g of CH_4/d to Mcal of CH_4/d by multiplying by .013184 (.001 x 9.45 kcal/L of CH_4 divided by .7168 g/L of CH_4) (Wilkerson et al., 1995). The equations of Blaxter and Clapperton (1965) and Holter and Young (1992) were converted from the percentage of GEI lost as CH_4 to Mcal of CH_4/d. For purposes of converting kcal of CH_4 per 100 kcal of GE to volume or weight of CH_4, dietary DMI was considered to contain 4.3 kcal of GE/g (Holter and Young, 1992). In addition, in the equation of Bratzler and Forbes, the following formula was used to estimate carbohydrate (CHO).

Total CHO = 100 - Crude Protein - Ether Extract - Ash

All factors in this formula were estimated for each of the diets in this study using NRC (1989) and Dair4 (Stallings et al., 1993). In the equation of Moe and Tyrrell (1979), nonfiber carbohydrate (NFC) fraction was calculated using the following formula:

NFC = Total CHO - NDF

Amounts of hemicellulose and cellulose were estimated by NRC (1989). In the equation of Holter and Young (1992), forage ADF was estimated by Dair4 (Stallings et al., 1993), and ADF digestibility was accepted from data of Ellingson (1993).

Table A-2 summarizes C output as CH_4 as predicted by the above-mentioned equations. Supplemental protein source did not affect C partitioning to CH_4, but phosphorus source appeared to have an effect due to reduced DMI when wheat bran was in the diet. All prediction equations showed a same pattern of C partitioning to CH_4. In the equations of Moe and Tyrrell (1979) and Holter and Young (1992), the values for C lost as CH_4 were lower than those of the other equations. Wilkerson et al. (1995) reported that the equation of Moe and Tyrrell (1979) using intake of carbohydrate fractions ranked the best for predicting CH_4 production by lactating cows, based on its correlation coefficients and errors of prediction.

Table A-2. Least squares means for kg C to fermentation CH_4 in response to diet calculated by various CH_4 output prediction equations (n = 36).

	Diet					Contrasts (*P*)		
Equation	SPM	SWB	BPM	BWB	SE	Prot	Phos	INT
Kriss (1930)[1]	.39	.35	.39	.36	.003	.65	.07	.86
Blaxter & Clapperton (1965)[2]	.37	.33	.38	.35	.003	.64	.07	.86
Bratzler & Forbes (1940)[3]	.33	.29	.33	.31	.001	.63	.07	.79
Moe & Tyrrell (1979)[4]	.28	.25	.28	.26	.001	.65	.04	.85
Holter & Young (1992)[5]	.25	.23	.28	.24	.007	.11	< .01	.19

[1]Methane (Mcal/d) = (18 + 22.5 x DMI (kg/d)) x .013184 (Mcal/g of CH_4).

[2]Methane (Mcal/d) = (1.30 + .112 x energy digestibility determined at maintenance intake (% of gross energy) + multiple of maintenance x (2.37 - .050 x energy digestibility at maintenance intake (% of gross energy))) x (gross energy intake (Mcal/d)/100).

[3]Methane (Mcal/d) = (17.68 + .04012 x digested carbohydrate (g/d)) x .013184 (Mcal/g of CH_4).

[4]Intake of carbohydrate fractions
Methane (Mcal/d) = .814 + .122 x nonfiber carbohydrate (kg/d) + .415 x hemicellulose (kg/d) + .633 x cellulose (kg/d).

[5]Lactating cows not fed supplemental dietary fat
Methane (Mcal/d) = (2.927 - .0405 x milk (kg/d) + .335 x milk fat (%) - 1.225 x milk protein (%) + .248 x CP (% DM)- .448 x ADF (%DM) + .502 x forage ADF (% DM) + .0352 x ADF digestibility (%) x (gross energy intake (Mcal/d)/100).

VITA

Name: Jongsu Eun

Birthplace: Iri, Chonbuk, Korea

Date of Birth: May 5, 1965

Education

High School: Namsung High School

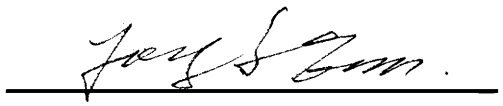
Iri, Chonbuk, Korea (1981-1984)

College: **B.S. Dairy Science**

Kon-Kuk University

Seoul, Korea (1986-1993)

Military Service: Korea Air Force (1986-1989)



Jongsu Eun