Whole-animal nitrogen balance in cattle

June 2004

Chapter 5

Table of Contents

1 Importance of Nitrogen Balance

1.1 Environmental Issues.

Disposal of animal manure has dramatically escalated to be one of the foremost research problems for dairy nutrition and management in the US (Meyer and Mullinax, 1999; Nelson, 1999; Van Horn and Hall, 1997) and internationally (Kohn *et al*., 1997; Kuipers *et al.*, 1999; Castillo *et al.*, 2000; Castillo *et al.*, 2001b). Major concerns include environmental consequences of nitrogen (N) and phosphorus (P) loading. The greatest environmental effect of N loss in manure is a result of rapid conversion of urea to ammonia and its subsequent volatilization (Nelson, 1999; James *et al.*, 1999), which affects the acidity of precipitation (Van Horn and Hall, 1997), formation of long-lasting aerosols (James *et al.*, 1999), and reduction of the N:P ratio of the residual manure below plant requirements (Van Horn and Hall, 1997; Van Horn *et al.*, 1996). We note that reduction of excreted P (see other chapters) influences this ratio favorably. Mechanical costs of reducing ammonia volatilization are up to $20/kg of N surplus reduced

(Kuipers *et al.*, 1999). Therefore, the most effective solution to reduce this problem entails more efficient nitrogen capture in the form of body components, milk, and wool. This review will focus on improvement of whole body N balance (i.e., the retention of dietary N in body tissue or milk) but also specific components of N excretion with respect to dietary N inputs for beef and dairy cattle.

1.2 Beef and dairy cattle production systems

Beef Cattle. Much of the beef production in the US is concentrated in large feedlots. The majority (80 to 90%) of N fed to feedlot cattle is excreted, with 50 to 75% of that excretion in the form of urinary N (Satter *et al.*, 2002). Therefore, relatively modest improvements in N efficiency can be magnified considerably, particularly if the amount of N lost as urinary urea can be reduced. Reduction of N loss in manure has important ramifications with regard to manure distribution as fertilizer relative to the cropland used to support grain production (Klopfenstein and Erickson, 2002), but this topic is beyond the scope of this review.

In recent reviews, Klopfenstein and Erickson (2002) and Satter *et al.* (2002) discussed implementation of metabolizable protein systems to feedlot cattle according to their changing growth phases in order to improve the efficiency of dietary N utilization. 'Metabolizable protein' is defined as the protein reaching the small intestine and digested therein. Clearly, improvements in gain:feed ratio should reduce N excreted per animal because of fewer days on feed. Even if feed efficiency is not increased, however, feeding protein according to requirements should allow less N excretion because cattle protein requirements decrease proportionately with increasing maturity. In both scenarios, 'N balance' (N intake – N lost in feces and urine; also termed 'tissue N retention') was similar between treatments when calculated as g/d , but decreasing N inputs decreased N lost into the environment by 12 to 21%. However, few studies have continued during the growth phase all the way to finishing. Compensatory growth could potentially make up for any short-term limitations in daily gain (i.e., metabolizable protein slightly below requirements) for cattle retained in the feedlot until finishing (Firkins and Fluharty, 2000), so the potential to reduce N loss might be even greater in such systems.

In order for phase feeding to be adopted more widely, beef producers need to be confident in the supply and requirements of metabolizable protein for cattle under various circumstances (Klopfenstein and Erickson, 2002). Aside from various factors affecting protein requirements (NRC, 2000), the supply is influenced by the ruminal degradability and intestinal digestibility of protein sources. Concomitantly, feed libraries and analyses are improving in both precision and accuracy (Stern *et al.*, 1997). In addition, systems are developing that will improve prediction of requirements of metabolizable amino acids for beef cattle (see Chapter 2). However, Klopfenstein and Erickson (2002) cited studies in which the ruminal degraded protein (RDP) requirements were estimated to be 6.3, 8.3, and 10.0% of dry matter for feedlot cattle fed dry rolled, steam-flaked, and high-moisture corn, respectively, due to increased ruminal availability of carbohydrate to support microbial protein production. If RDP limited growth of amylolytic microbes, digestion of starch in the small and large intestines could compensate (Firkins *et al.*, 2001) and theoretically could increase efficiency of energy usage (Harmon and McLeod, 2001). Transfers of N among gut and blood urea pools could have a strong influence on N efficiency in beef feedlot cattle (see later section).

Grazing Beef Cattle. Many beef cow/calf operations in the US rely heavily on grazing of poor quality grass. Nitrogen recycling between the blood and gut helps compensate for low protein intake (see later section), but protein supplementation to increase RDP has improved productivity in some studies (Firkins and Fluharty, 2000). In this regard, studies with lactating beef cows have found that the benefit of RDP supplementation is not reduced when the frequency of RDP feeding is reduced to as low as once every 4 d (Coleman and Wyatt, 1982; Krehbiel *et al.*, 1998). This may reflect recycling of non-protein N (NPN) between the gut and body pools (Krehbiel *et al.*, 1998) or the short-term deposition of amino acids in labile protein pools (Waterlow, 1999). In addition, because of the low energy availability for microbial protein production, some researchers have reported responses to supplementation of rumen undegraded protein (RUP). Moreover, the requirement by the cow for metabolizable protein might be lower than the rumen microbes' requirements for RDP. The low cost/low intensity management of these operations compared with the large area of land usage probably lowers the potential benefit of improved protein usage from an environmental standpoint and will not receive further attention in this review.

Dairy Heifers. Dairy calves are primarily raised intensively and must be evaluated differently from beef calves. Mammary development can have residual effects on lifetime milk production, promoting the concept of target weight gains (NRC, 2001). Because heifers need to be grown for about two years before milk production, needing more replacements to meet the demands for milk production would have a profoundly negative impact on N usage. With economic pressure to increase growth rate, having an adequate supply of metabolizable protein for heifers might be very important in reducing the negative effects of energy for rapid growth (Whitlock *et al.*, 2002). Despite a lack of differences in average daily gain, feed efficiency improved with increasing concentration of crude protein (CP) in the diet, and structural growth measurements tended to improve (Gabler and Heinrichs, 2003). Even if CP requirements are targeted for weight gain, it seems likely that CP concentration in the field will not decrease, particularly with those producers adopting accelerated growth programs. Increasing CP intake above requirements for growth primarily increased the loss of N in urine (James *et al.*, 1999). Future research is needed to better document the requirements of metabolizable protein and amino acids for dairy heifers so that N input can be decreased reliably without potentially impacting lifetime milk production.

Dairy Cows. For lactating cows, the secretion of high amounts of protein into milk prioritizes the importance of the amount and profile of amino acids reaching the duodenum. Nitrogen balance can be influenced positively by improved ration balancing to capture more dietary N as milk protein. Considerable research has been done to increase milk protein concentration, particularly when dietary fat is fed (Wu and Huber, 1994). In fact, the NRC (2001) elaborated on the complexities involved with synchronizing ruminal fermentation with microbial protein synthesis for incorporation into a system to meet requirements for metabolizable amino acids (see later section). With gaining emphasis on formulating rations to meet requirements for specific amino acids and with improved advances in technology (e.g., reproductive aids and bovine somatotropin) and housing systems, there is a strong potential to multiply a modest gain in efficiency of N usage per cow to reduce environmental impact. Satter *et al.* (2002) noted that increased milk production will dilute maintenance N costs, so restricting protein supply below requirements to reduce manure N excretion should be avoided; yet still they emphasized that feeding more than about 17.5% CP (18.5% in certain circumstances) on a

dry matter (DM) basis to high producing cows would only divert more dietary N into urine if the diets have corn silage to dilute the high RDP in legume silages and have protein balanced for degradability. In Europe, excess protein is often fed to lactating dairy cows because it is relatively inexpensive, provides a safety margin against drops in forage CP concentration, and generally increases milk yield through effects on intake of grass silage and other forages. Generally, changes in milk and milk protein yield with differences in dietary CP below 14 to 15% of DM have been attributed to metabolic effects of metabolizable protein supply, whereas, above this threshold, changes in milk yield are typically accompanied by changes in dry matter intake (DMI) attributed to effects on rumen or total tract digestion (Clark and Davis, 1980; Oldham and Smith, 1980; Reynolds, 2000).

Currently, various amino acid supplements are available on the market, but further research is needed to determine when to use them economically, with impending environmental regulations ostensibly increasing their economic feasibility. Balancing to meet metabolizable lysine requirements using conventional protein sources and then supplementing rumen-protected methionine could reduce the total CP fed, potentially reducing N excretion by 13 to 20% compared with current practice (Satter *et al.*, 2002). Sloan (1997) further discussed this 'ideal protein' concept, noting that there is only a modest (2 to 5%) gain in efficiency of conversion of dietary CP into milk protein by meeting the requirement for a single limiting amino acid. For example, conversion of rumen-protected methionine into milk methionine might be only about 10% efficient because it is used for other bodily functions. Therefore, any real gain in N balance relative to N input will primarily be accentuated via decreased N intake. Moreover, as discussed in Chapter 2, lysine and methionine supplies might not actually be limiting or might only be near- or co-limiting (Sloan, 1997; Hvelplund *et al.*, 2001; Vanhatalo *et al.*, 1999).

Nitrogen balance probably changes the most during the period from late gestation into the first few weeks of lactation (the 'transition period'). The splanchnic tissues [portal-drained viscera (PDV; the gastrointestinal tract, pancreas, spleen and associated adipose) plus liver], mammary gland, and foetus increase protein synthesis at a time when DMI might be insufficient to meet protein requirements (Bell *et al.*, 2000). Labile protein reserves might be mobilized to balance shortfalls in supply of protein or limiting amino acids but also provide gluconeogenic precursors. In this regard, mRNA and activity for liver pyruvate carboxylase (Greenfield *et al*., 2000) and alanine use for glucose synthesis by hepatocytes *in vitro* (Drackley *et al*., 2001) increase immediately after calving, supporting the concept of increased amino acid use for glucose synthesis. However, body protein deficit is usually relatively modest except in the first days of lactation (Grummer, 1995; Table 1), and the glucogenic requirements for amino acids may be less of a metabolic priority than hypothesized (Reynolds *et al*., 2003). When dietary RUP was increased pre- and post-calving, body protein mobilization (assessed by deuterium oxide dilution) accounted for only about 7% of the energy lost or gained (Komaragiri and Erdman, 1997). In the most comprehensive slaughter balance study conducted in dairy cows of which we are aware (Gibb *et al.*, 1992), the amount of body protein lost in the first 8 weeks postpartum was relatively small (5.6 kg), especially when compared to the amount of body fat loss (37.4 kg), in relatively low producing cows fed grass silage. Much of this body protein loss occurred in the first 2 weeks postpartum (2.7 kg), which equated to a loss of 31 g of N per day. In transition dairy cows catheterized for measurements of splanchnic nutrient flux, the potential gluconeogenic contribution of alanine, as well as lactate and glycerol, was greatest 10 days after calving, but the required contribution of other amino acids was lowest at this time. Indeed,

increases in net liver removal of these glucose precursors and volatile fatty acids between 9 days before calving and 10 days after calving could account for all of the increase in the measured release of glucose by the liver (Reynolds *et al*., 2003).

The NRC (2001) reviewed protein requirements for transition cows, suggesting increases in protein requirements for heifers, but not cows, in late gestation compared with previous requirements. In one study (Putnam and Varga, 1998), increasing dietary CP and RUP concentrations prepartum to multiparous cows did tend ($P = 0.09$) to increase N balance, but even the cows fed diets lower than the NRC (1989) requirements still had positive N balance on days –12 to –5 relative to expected calving, and no response in milk production postpartum was detected. In other studies, feeding supplemental protein before calving increased milk or milk protein yield after calving in heifers (Van Saun *et al*., 1993; Santos *et al.*, 2001) but decreased DMI or milk yield in multiparous cows (Santos *et al*., 2001; Hartwell *et al.*, 2000).

To further account for body energy and protein retention during early lactation, Sutter and Beever (2002) performed a series of weekly total collections of faeces and urine, combined with respiration calorimetry to assess the energy status for multiparous cows. Although too variable for statistical significance, N balance was only negative for the first two weeks of lactation and primarily only in the first week (Table 1), supporting the conclusion that N balance should reach a nadir on about day 7 of lactation (Bell *et al*., 2000). Body tissue energy balance was negative throughout the study but increased linearly, apparently primarily because of a linear decrease in milk energy secretion (except for week 1). The authors concluded that N mobilization by labile reserves might be more important for relocation within body tissues (e.g., gut and liver) than for milk protein and the changes in body weight might not reflect primarily differences in water repletion of tissues or increases in the weight of the gut plus contents. Increases in the crude protein content of splanchnic tissues were relatively small (0.85 kg) compared to body protein loss in the first 8 weeks of lactation (Gibb *et al*., 1992), but a large portion (46%) of this change in splanchnic protein content occurred in the first 2 weeks postpartum. Urinary energy was not affected by week in lactation, and the energy lost in urine accounted for about 4% of digestible energy (Table 1). Therefore, with proper balancing of RDP and RUP, some mobilization and repletion of body protein seems to help transition the cow to lactation, but the impact on tissue N balance must be relatively minor.

Based on preceding results, gut metabolism and whole-body urea transfer probably have potentially large impact on efficiency of whole body N balance, so further attention will be given to these subjects in this review.

2 Whole-Animal Nitrogen Fluxes and Nitrogen Balance

2.1 Nitrogen Exchange Among Tissues

As discussed by Lapierre and Lobley (2001), ruminants have adapted their metabolism to rely on large fluxes of N exchanging between the blood and digestive tract. They calculated that 40 to 80% of the blood urea N (BUN) produced in the liver enters the digestive tract instead of being excreted into the urine. In the rumen, ureolysis, proteolysis, and deamination of amino acids is considerable, as would be expected based on the diversity of microbial enzymes responsible (Wallace *et al.*, 1997). Despite the ruminal pH being considerably lower than the

pK_a of ammonia/ammonium (Satter *et al.*, 2002), causing a low proportion to be in the un-ionized form for absorption (Leng and Nolan, 1984), absorption of NH_3 N from the rumen and intestines is extensive (Parker *et al.*, 1995). Consequently, considerable cycling of BUN back to the digestive tract might be needed for positive N balance for many species, including man (Waterlow, 1999), but particularly for ruminants (Lapierre and Lobley, 2001).

In the past decade, considerable research has been done with the double ^{15}N-urea infusion technique, which has been well described by Lobley *et al.* (2000). Using this approach, Lapierre and Lobley (2001) generalized that approximately one-third of BUN actually gets excreted into the urine, with two-thirds (40 to 80%) being cycled back to the digestive tract. Of the NH_3 N produced from urea that gets transferred to the gut, about 10% is excreted as faecal N, 40% is absorbed and converted back to BUN, and 50% is incorporated into microbial protein in the rumen, which is subsequently absorbed from the small intestine. The latter flux (50% of the two-thirds) is high, in part, because of multiple entry rather than entry via a single pass. Microbial protein synthesized using N from BUN ranges from 8 to 38% (Lapierre and Lobley, 2001). Because of the eventual loss of urinary N from urea, though, these authors calculated that upper limits for N retention as body tissue or milk would, therefore, be 50 to 60% of dietary N or 70 to 90% of apparently digested N. Based on a regression of literature from cattle with indwelling blood catheters for the measurement of splanchnic flux, they reported a prediction of urea N synthesis by the liver (g/d) = 0.80 (N intake, g/d) – 30 ($r^2 = 0.45$). In multicatheterized cattle, regression of net liver removal of NH_3 N and release of BUN on digested N gave slopes of 0.68 and 0.90, respectively (Reynolds, 1995), but in most cases these data came from cattle fed protein well in excess of their requirements. In a more recent integration of these (Reynolds, 1995) and more recent observations from the University of Reading (Figure 1; Reynolds, 2002; 2003), the relationship between daily N intake and liver urea release for 304 individual measurements had a slope of 0.65 ($R^2 = 0.64$). In this case, the data set included observations from dairy cows fed varying levels of dietary protein, at various stages of lactation and levels of production, and receiving abomasal infusions of casein or amino acids. For the same data set, the relationship between N intake and net PDV release of NH_3 N had a slope of 0.42 ($R^2 = 0.84$; Figure 2). Although N intake is a major determinant of PDV absorption of NH_3 N and liver BUN release, other factors are also important. We note that both variables are likely correlated (increasing DMI should be related to N intake and also to overall net flux of all metabolites), so the relatively low R^2 for the prediction of liver urea release (Figure 1) documents the considerable amount of variation remaining to be explained (Lapierre and Lobley, 2001). As emphasized by Reynolds (2002), much of the remaining variation could be attributed to the amount of dietary N absorbed relative to requirements, which ultimately determines the extent of N excretion in urine (Waterlow, 1999).

In contrast to urine N, faecal N excretion is determined by amounts of indigestible N consumed and endogenous N losses, which to a large extent are determined by capture of urea N as microbial protein in the hindgut. In lactating dairy cows, abomasal starch infusion increased faecal N excretion (Reynolds *et al.*, 2001). Concomitant decreases in faecal pH likely reflect increased starch fermentation in the hindgut, which would explain the increase in faecal N concentration and excretion observed. On the other hand, changing steers from a high-concentrate to a high-alfalfa diet, at similar ME intake, markedly increased the transfer of BUN to the mesenteric-drained viscera (Reynolds and Huntington, 1988; Huntington, 1989). This

increase in BUN transfer to the postruminal digestive tract was likely a consequence of increased fermentation of fiber in the hindgut.

Increased absorption of glucose from starch digested in the small intestine may also increase the efficiency of ingested N that is retained as body protein (Reynolds *et al.*, 2001; Obitsu *et al.*, 2000) the latter of which is dependent on insulin (Bergen, 1978). In lactating dairy cows, infusion of starch into the abomasum increased tissue energy balance, and over half of the increase in energy retention was attributable to greater protein deposition (Reynolds *et al.*, 2001). Increasing the amount of starch digested in the rumen or hindgut decreases net absorption of ammonia by the PDV in dairy cows (Reynolds *et al.*, 1998; Delgado-Elorduy *et al.*, 2000a) and, in some studies, increased urea nitrogen transfer from blood to the rumen (Delgado-Elorduy *et al*., 2000b). Presumably, these changes in N cycling reflect increases in ammonia utilization for microbial protein synthesis in the rumen or hindgut.

2.2 Energetic Cost of Urea.

If each mole of urea produced in the liver requires 4 moles of ATP (McBride and Kelly, 1990), then it would be logical that energetic cost of urea production could exert a strong regulatory constraint against BUN fluxes back and forth from the gut, especially for grazing cattle consuming large amounts of RDP (Stockdale and Roche, 2002; Kolver and Muller, 1998). Supplementation of grain should decrease ruminal NH_3 N concentration, in part because of decreased N intake (Bargo *et al.*, 2003), but grain supplementation is markedly recent in evolutionary terms. Waterlow (1999) commented that, although 4 moles of ATP are consumed, 6 moles could be produced per mole of urea synthesis (2 moles of NADH produced from oxidative deamination of glutamate and regeneration of aspartate from fumarate). Therefore, urea synthesis might not be as critical as previously thought, especially because neither of these amino acids is essential. Ammonia infusion into the duodenum increased urinary N excretion but did not affect N balance or yield of any milk components (Moorby and Theobald, 1999). Similarly, feeding steers urea markedly increased net PDV absorption of ammonia and liver urea synthesis, without significant effects on net liver oxygen consumption, glucose release or amino acid metabolism (Maltby *et al*., 1993). In a methodical series of studies in sheep, Lobley and colleagues have explored effects of increased ammonia absorption on liver metabolism and similarly found no significant deleterious effects on liver metabolism of oxygen, glucose or amino acids (see Lobley *et al.*, 1995; 1996; Milano and Lobley, 2001; Milano *et al*, 2000; Reynolds, 2003). Despite ranging from 67 to 102 g/d of urinary N excretion (data not shown), N excretion in urine is a minor proportion of digestible energy intake (Table 1).

The concept of a high 'penalty' for ammonia absorption and urea recycling needs to be evaluated within this context because it goes against the apparent adaptation toward urea cycling (previous section) and the discovery of urea transporters (Waterlow, 1999; Lapierre and Lobley, 2001) in the mammalian gut and other peripheral tissues. Yet, despite their presence, their role in BUN recycling is not clear (Marini and Van Amburgh, 2003). These latter authors suggested that BUN could passively transfer through the epithelial cells, so the transporter's role could also be to efflux urea back into the blood before bacterial hydrolysis in the rumen during times of high N availability. Oba and Allen (2003b) reported that ammonium combined to make propionate more potent to depress feed intake, and such a situation of high NH_3 N and propionate would seem to occur only when N intake was excessive. Although it has been proposed that there is a

deamination cost involved with high urea fluxes (Parker *et al.*, 1995), the energetic cost of ureagenesis appears now to be more a consequence of the metabolism of amino acids absorbed in excess of requirements rather than a cost of ammonia absorption and conversion to urea *per se* (Reynolds, 2003) or possibly only in extremely high availability of ruminal NH_3 N (Milano *et al*., 2000).

Importance of the Rumen for N Capture. Direct quantifiable relationships between ruminal N metabolism and urinary N excretion are limited, but available data support the concept that the rumen is a major mediator of N retention. Al-Dehneh *et al.* (1997) reported that the ratio of ^{15}N enrichments in urinary N and BUN was constant by 40 h after the start of infusion of ^{15}N-urea into the jugular vein, implying inter-related N metabolism. Kennedy and Milligan (1980) reported that the transfer of BUN to ruminal NH_3 N was inversely proportional to the ruminal NH_3 N concentration and was increased with increasing grain or degradable carbohydrate inclusion in the diet. Although this could be a result of increased microbial growth and N capture (Delgado-Elorduy *et al.*, 2000b), greater BUN recycling for higher grain diets also could reflect increased energy for body protein retention, which could reduce the catabolism of absorbed amino acids. Whitelaw *et al.* (1991) added a urease inhibitor to the rumen of maintenance-fed sheep. This decreased the irreversible loss rate of BUN by 33% without affecting N intake or urinary N excretion. This limited work is interpreted to suggest that the eventual trapping of BUN for metabolic usage and not as urinary N will depend largely on N capture as microbial N in the rumen as well as the metabolic requirements for metabolizable protein (Lapierre and Lobley, 2001).

Intraruminal Nitrogen Recycling. Wallace *et al.* (1997) cited a model described by Nolan (1975) to conclude that "ammonia overflow leads to inefficient N retention". The biological importance of such recycling is extensive and is the subject of Chapters 3 and 4, but modeling efforts will be discussed briefly herein within the context of their role in whole body N metabolism.

Firkins (1996) reviewed quantitative studies that characterized flux among either chemical [i.e., nonammonia N (NAN)] or biological (bacterial, protozoal, or combined) pools in the rumen. Biological pools are more mechanistic but might be difficult to repeatably fractionate for subsequent determination of specific activity of a tracer. For instance, protozoa-enriched samples are typically based on sedimentation yet probably are significantly contaminated with bacteria (Sharp *et al.*, 1998). Chemical pools are more systematically differentiated but require appropriate independent biological data collection such that the flux rates among those pools have important mechanistic interpretation. Faichney *et al.* (1997) derived a complicated model evaluating protozoa-mediated turnover based on the abundance of protozoal RNA, which was characterized as the difference of signals from eukaryotic minus fungal probes. More recently, Oldick *et al.* (2000) documented extensive recycling of microbial protein in the rumen, but chemical precipitation techniques could not differentiate the recycling of microbial protein from a slowly turning over compartment as opposed to the exchange of NAN from a rapidly turning over compartment. They suggested that rapidly exchanging NAN probably has a smaller impact on efficiency of microbial growth and N capture for metabolizable protein. Direct experimental approaches quantifying intra-ruminal N recycling typically involved the use of multiple (and often radioactive) tracers, used fractionation procedures that might be difficult to systematically repeat, and (or) incorporated by-difference calculations that compound variation (which was

typically ignored). Therefore, more attention to over-parameterization needs to be given using considerations such as those of Oldick *et al.* (2000), so that models can be used in experimental designs with enough statistical power to explain interactions among treatments. Conversely, Dijkstra *et al.* (2002) recently discussed important modeling considerations with regard to mechanistic models. Given the large importance of microbial N capture (previous section), more quantitative work is needed in this area to decrease variability among feeding conditions in order to stimulate the adoption of lower protein diets in the field to decrease N excretion by cattle.

Meaurement of N Balance. Although objectives of individual researchers might be to compare treatment differences within a study, it is no longer sufficient to ignore known errors in measurement of N balance; the absolute measurements of multiple studies are being used to either derive or evaluate models with increasing frequency. Martin (1966) and Johnson (1986), among others, have clearly identified and quantified losses of N and sources of experimental error in measuring N balance in ruminants. More recently, Spanghero and Kowalski (1997) described major routes of N loss that accumulate to overestimate the by-difference calculation of N balance. From 35 published trials that they surveyed, about 1/5 did not determine N in faeces on a wet basis, leading to underestimation of N excretion. Methods to capture urinary N were variable or not even reported. In some studies that they cited, equivalents of NH_3 excreted might have exceeded the equivalents of acid added in the urine collection vessels. Several studies did not account for non-protein N in milk. Despite corrections that they applied to literature data, tissue N balance still had a median of 10.2 g/d, which they estimated to correspond to about 255 g/d of body weight gain. The median was significantly higher than the mean, indicating a skewed distribution of data. To account for differences among studies, they calculated deviations of individual treatment means compared with the mean from each experiment; from these data, they suggested that N balance was overestimated with increasing N availability for metabolism. Faecal N excretion can vary considerably from day to day, and we note that, although the appropriate number of days is likely variable (Schneider and Flatt, 1975), collection periods in the literature (we have noted some as low as 2 days) might be too short. Moreover, N balance data in Table 1 document variability among weeks, at least in early lactation. Readers are referred to Castillo *et al.* (2000) for a comprehensive review of dietary factors influencing efficiency of N capture in milk relative to excretion in urine and faeces.

Nitrogen balance can be used to evaluate amino acid requirements for growing cattle (Greenwood and Titgemeyer, 2000; Wessels and Titgemeyer, 1997), although the reader is referred to Chapter 2 for a more comprehensive review. Moreover, Iburg and Lebzien (2000) noted that amino acid requirements for dairy cattle really should be calculated at zero tissue N balance, which is an assumption that probably should be verified experimentally in more studies. As diets approach and then exceed the requirements for limiting amino acids, then N balance could be fluctuating from negative to positive. In short term experiments for which milk protein is the response criterion, the degree of response could be mediated in part by tissue protein mobilization. Such reasoning could help explain the variation in metabolizable lysine and methionine requirements determined by break-point analysis (NRC, 2001).

Manipulation of microbial populations can influence N retention. McGuffey *et al.* (2001) reported that ionophores increased N digestibility by about 3.5 percentage units and that several individual studies documented increasing N retention as a percentage of N intake. Besides increasing the efficiency of beef cattle growth, prepartum feeding of ionophores could increase N

retention for dairy cattle (Plaizier *et al.*, 2000), which might positively influence transition to lactation. Defaunation of the rumen had mixed effects on N retention (Jouany, 1996), but the practical importance of elimination or reduction of protozoa in the rumen in actual growing conditions is the subject of Chapter 4.

Models to Balance Supply and Requirements of Protein

Supply Models. Several systems have been developed by leading research institutions in the U.S. and Europe [see review (Dijkstra *et al.*, 1998)]. Although much improved, the new Dairy NRC (2001) still empirically predicts microbial protein flow from the rumen based on intake of total digestible nutrients (TDN), with the TDN concentration being discounted progressively with increasing DMI and with increasing TDN concentration (excluding high-fat diets). In the NRC (2001) system and many others, requirements for RUP are calculated by difference (after accounting for intestinal digestibility) of the animal's estimated protein requirements minus predicted duodenal flows of microbial and endogenous protein, therefore compounding variation associated with the prediction of microbial protein flow.

Prediction of Microbial Protein Supply. Microbial protein is extremely well balanced with amino acids relative to meat or milk protein (NRC, 2001). RDP normally is much cheaper than RUP (St-Pierre and Glamocic, 2000), even if incomplete conversion of RDP to microbial protein (NRC, 2001) is accounted for. Although TDN includes fat and protein that provide relatively little energy to support microbial protein synthesis, this mechanistic problem (Kebreab *et al.*, 2002) probably is of relatively minor importance for empirical prediction by the NRC (2001). Two separate equations were justified for the prediction of microbial protein flow to the duodenum based on net energy for lactation (NEL) intake for cattle fed diets with or without fat (Oldick *et al.*, 1999), yet visual inspection of the fitted lines documents that the use of separate equations makes a relatively modest impact at intakes that would be seen in production situations. Fat should decrease protozoal numbers and increase efficiency of microbial protein synthesis (Doreau and Ferlay, 1995; Firkins, 1996). Also, RDP intake was relatively static in most experiments from which the empirical relationship was determined and for which it would be used.

Although the NRC (2001) system ignores the sites of carbohydrate digestion, again this important mechanistic problem might have a statistically minor impact on prediction of microbial protein flow because microbial efficiency probably decreases with increasing ruminal availability of carbohydrate. Satter *et al.* (2002) logically concluded that "finely ground high moisture shelled corn, through its ability to support microbial growth and protein synthesis, may be the cheapest 'protein source' we have". However, this generalization was not substantiated by experimental data (Firkins *et al.*, 2001; Oba and Allen, 2003a). In fact, when other factors were equalized, cows fed high moisture corn, despite higher ruminal starch degradability, actually had numerically lower microbial protein flow to the duodenum than those fed corn grain processed in other ways and having lower ruminal starch digestibility (Firkins *et al.*, 2001). In a recent study (Harvatine *et al.*, 2002), replacing ground corn with steam-flaked corn increased microbial N flow to the duodenum by 15%; despite the 36% greater true ruminal starch digestibility, ruminal pH did not decrease, apparently because DMI decreased such that intake of truly digestible organic matter only increased by 7% with steam-flaking. However, in the same study, progressive replacement of forage with whole linted cottonseed linearly increased DMI and

microbial N flow; however, ruminal pH and efficiency of microbial protein synthesis were depressed linearly. Clearly, the amount of ruminally available carbohydrate is impacted as much, or more, by changes in total DMI as by the fermentability of the carbohydrate in the diet fed. Increased carbohydrate degradation (g/d) can decrease microbial efficiency by factors directly related to low pH (Russell and Wilson, 1996) or because of increased energy spilling (metabolic wasting of high energy phosphate bonds), particularly if RDP becomes limiting (Wells and Russell, 1996). These results (Harvatine *et al.*, 2002) demonstrate that an empirical prediction using a constant efficiency clearly leads to inaccuracies that contribute to variation. However, they also document the importance of DMI prediction or determination as well as the need to predict carbohydrate fermentation and ruminal pH and its effects on microbial efficiency. Prediction of ruminal pH is very difficult (Allen, 1997) and is interpreted to be a major roadblock for all modeling systems.

Empirically (statistically) speaking, a bigger criticism of the current NRC (2001) procedure to estimate microbial protein flow could be that its evaluation method was biased, leading readers to have a false conclusion regarding its accuracy. When residuals (predicted minus measured) were regressed against measured microbial protein flows to the duodenum, a negative slope bias was detected. Similarly, a negative slope bias was detected for nonammonia nonmicrobial N (NANMN) flows. In both cases, this would mean that microbial N and NANMN are being underpredicted with increasing measured values. Yet, a much smaller response was noted for total NAN (the sum of microbial N and NANMN fractions, which should logically accumulate negative slope bias). St-Pierre (2003) explained this apparent discrepancy as being caused by a biased evaluation procedure; when residuals were properly plotted against predicted values, the actual equation was considerably less biased than presented by the NRC (2001). Therefore, even though the prediction ignored effects of experiment and did not weight treatment means for variation among experiment (St-Pierre, 2001), which both have highly significant effects on regressions and interpretation of microbial protein production (Oldick *et al.*, 1999), the prediction actually appears to be relatively robust over a wide range of conditions, even if it lacks precision.

Microbial protein production is predicted based on a more mechanistic approach than NRC (2001) using the Cornell Net Carbohydrate and Protein System (or its derivative models), which has been evaluated by Alderman *et al*. (2001a; 2001b). An early version of this model predicted average daily gain reasonably well over a wide range (0.7 to 1.5 kg/d) of predictions as assessed by an r^2 of 0.70 for a linear regression of predictions *vs*. measured data (Ainslie *et al*., 1993). O'Connor *et al*. (1993) similarly concluded that the model predicted supply of individual amino acids to the duodenum well based on a high r^2 (0.81 to 0.90 for predicted *vs*. observed) over even larger ranges of approximately ten-fold. Yet, a range in the data approaching 100% of the mean prediction can typically be visualized in their graphs, and Alderman *et al*. (2001b) noted that their data set was actually composed of two clusters, which could bias the interpretation. Besides the limitation in using r^2 (coefficient of determination) or R^2 (multiple coefficient of determination, including effects of trial as in Figures 1 and 2) from a *sample* to extend toward accuracy of a prediction for a *population*, extending the range of X measurements will inflate coefficient of determination as a measure of goodness of model fit for clustered data (Neter *et al.*, 1996). We note that the evaluation also would have been improved by appropriate residuals analysis for fit (see St-Pierre, 2003).

Cotta and Russell (1997) elaborated on the importance of synchronous N and carbohydrate supplies for microbial cell synthesis. Mechanistic prediction of microbial protein flow to the duodenum has been well reviewed by Dijkstra *et al.* (1998). These models tend to emphasize the importance of synchronization of energy from carbohydrate fermentation with availability of RDP, however, which tend not to have been substantiated by direct *in vivo* experimentation (Dewhurst *et al.*, 2000; Castillo *et al.*, 2000; Bateman *et al.*, 2001b) and tended to cause overprediction of microbial protein flow in one evaluation (Bateman *et al*., 2001a). With regard to stimulation of microbial protein production by increasing amino N, the yield of microbial growth was increased by 19 to 77%, depending on the model used (Dijkstra *et al.*, 2002). Such a large range emphasizes the predictive limitations for mechanistic models until further research is available. A sensitivity analysis (Bannink and De Visser, 1997) of the elaborate system described by Dijkstra (1994) indicated that more quantitative data are needed to improve the accuracy of parameters (coefficients) describing protozoal physiology and ecology for model robustness. We note that, although these systems might not be suspect to the errors associated with measuring microbial protein *in vivo*, they still are suspect to errors in measurement (and therefore prediction) of ruminal passage rate, which are also significant (Firkins *et al.*, 1998). Comparative accuracy and precision of virtually all models that are more mechanistic than the NRC (2001) model are difficult to assess at the present time, although mechanistic models probably hold more promise in the future to explain interactions among various dietary factors.

Methodological Issues Contributing to Variability in Estimation of Supply

Microbial Markers. Markers to estimate microbial protein flow to the duodenum have been reviewed (Broderick and Merchen, 1992; Firkins *et al.*, 1998; Shingfield, 2000), and this topic is beyond the scope of this review. However, we note two current potential errors that could promote excessive variation among studies, contributing to the high significance of experiment in regression-based empirical approaches to predict metabolizable amino acid supply. Purines, the most common microbial marker, might have incomplete recovery or contain inhibitors when hydrolyzed using the originally published conditions (Klopfenstein *et al.*, 2001). However, comparisons with ^{15}N (Broderick and Merchen, 1992; Shingfield, 2000) either do not support such large potential responses or indicate that recoveries are similar in both harvested bacteria and in duodenal samples, factoring out the error. Routine recovery checks in the first author's laboratory have documented the concentration of perchloric acid to have minor, if any, impact on purine recovery or concentration. As a result of the large importance of microbial N for capturing BUN as well as its importance in supply/requirement models, we recommend that researchers carefully evaluate marker procedures in their own laboratory conditions prior to continuing further research. Shingfield (2000) documented other sources of error for estimation of microbial N flow using excretion of purine derivatives and also potential escape of purines to the duodenum.

Protein Degradability. Forage protein degradability probably adds considerable variation to prediction of metabolizable amino acid supply. Despite advancements in knowledge gained (Broderick, 1995), protein degradability still is highly variable (Kohn and Allen, 1995). Klopfenstein *et al.* (2001) outlined an improved methodology to estimate RUP of forages. More

kinetics studies evaluating rates of degradation of protein fractions using ^{15}N-fertilized forages will help (Hristov *et al.*, 2001), but questions still remain regarding which fractions pass rapidly with ruminal fluid (Hvelplund *et al.*, 2001).

A fundamental principle of all kinetics studies is that dosing the tracer does not perturb the steady state of the tracee. We note a disturbing trend in current research to simply provide a large, potentially unphysiological bolus dose of some nitrogenous compound(s) into an unadapted rumen. Some published escape values for nitrogenous compounds likely have been inflated using such procedures. Investigators need to remember that 1) a bolus dose must be shown not to affect the true metabolism/dilution of the tracee or else 2) a bolus dose of labeled tracer should replace an equal amount of unlabeled tracee that has been fed long enough to adapt rumen microbes. Interpretation of a log-linear elimination of tracer to document first-order kinetics is insufficient proof of the first assumption (as some authors have claimed). First-order kinetics can include multi-exponential dilutions or can aggregate a mix of heterogeneous rates.

Balancing Supply to Reduce N Excretion

Although more limited for beef cattle, there are several reports of supply models being used to reduce N excretion for lactating dairy cows. Wu *et al.* (1997) summarized five experiments with respect to the Cornell model's ability to predict limiting amino acids and responses in milk production. The authors concluded that the model explained differences in milk yield, particularly for studies in which protein sources were manipulated compared with use of rumen-protected methionine and (or) lysine. Dietary protein could be reduced and milk N efficiency increased without a loss in milk production in one study (Kalscheur *et al.*, 1999). However, a constructive example (Dinn *et al.*, 1998) can demonstrate potential problems when this model is used to balance rations (rather than to evaluate them) to improve N efficiency. Diets were balanced to meet metabolizable lysine and methionine requirements estimated by the Cornell model while progressively decreasing dietary crude protein concentration and concomitantly increasing inputs of rumen-protected lysine and methionine. The partitioning of digestible protein toward milk N and away from urinary N increased progressively, as expected. The authors reported no change in milk N secretion, although it numerically decreased by 8.5%. Dry matter intake and milk production both decreased significantly. St-Pierre and Thraen (1999) used the data of Dinn *et al.* (1998) to estimate that balancing diets for metabolizable amino acids actually would have cost $4.40/kg reduction of N excretion. The Cornell model did not predict retained N well in another study (Haig *et al.*, 2002) and was marginally less effective than a procedure in which diets were balanced to meet predicted requirements of 15 and 5% of essential amino acid flow to the duodenum for lysine and methionine, respectively (Piepenbrink *et al.*, 1998). We note that the researchers' objective was to continue updating this model for field usage (Boston *et al.*, 2000), and on-going efforts should increase its accuracy.

At the University of Reading, a series of recent studies have statistically evaluated dietary factors influencing N excretion. Castillo *et al.* (2000) compiled a database from 580 individual cows fed 90 treatments. They noted that, as N intake exceeded 400 g/d (corresponding to about 15% CP in the dietary DM), excretion of N in the urine increased exponentially. However, the authors noted that these data were from cows producing moderate amounts of milk (most < 35 kg/d). For higher yielding cows under U.S. conditions, Satter *et al.* (2002) recommended upper

limits of about 17.5% CP. Still, both reviews note that the major response in CP intake above those amounts would be to increase urinary N output substantially.

The Reading group specifically investigated various managerial and dietary factors potentially influencing N excretion in urine. Kebreab *et al.* (2000) determined that cows fed early-cut grass silage had lower urinary N excretion but higher faecal N excretion when the grass was fertilized with a lower amount of N. Feeding a fibrous *vs.* starchy concentrate decreased faecal N loss but increased urinary N. Their data can be used to calculate that the starch-based concentrate increased the ratio of milk N:manure N excretion by 13% but only increased the ratio of (milk N plus retained N):manure N excretion by 5%. In another study (Castillo *et al.*, 2001a), cows that were fed highly degradable starch (mostly barley) had much higher N excretions in urine than those fed fibrous concentrate, low degradable starch (mostly ground corn), or soluble sugars (molasses). Numerically, the cows fed highly degradable starch had at least a 20% lower ratio of (milk N plus retained N):manure N excretion than the other groups. However, the group fed fibrous concentrates had an average of 48 g/d of N balance, which would equate to about 1.2 kg/d of body weight gain (Spanghero and Kowalski, 1997), which is probably high even for cows producing < 21 kg/d of milk. In this study, the effects of feeding ground corn on tissue N balance support observations in late lactation cows receiving abomasal starch infusions at this location (Reynolds *et al.*, 2001), which we discussed previously. In another study (Castillo *et al.*, 2001b), concentrates with low or high percentages of crude protein were factorialized with high, medium, or low RDP (soyabean meal replaced by formaldehyde-treated soyabean meal). Decreasing degradability greatly decreased urinary N while increasing tissue N balance. The RUP supply was always in excess of estimated requirements, but RDP became progressively limiting as degradability decreased, which likely would have progressively limited microbial N production.

After constructing a whole-body model to explain the preceding data, Kebreab *et al.* (2002) concluded that the efficiency of conversion of rumen-degradable protein into microbial protein "had a major effect on N excretion especially by way of urine". Similarly, the model predicted that increasing energy concentration (using the U.K. system) in the diet should decrease N losses, particularly in the urine. However, at an average N intake, N excretion in the urine still had a range of measured data about as large as the prediction. The authors stated that the model is a first step toward a mechanistic approach for nutrient modeling. This model, like others that have been reviewed, should be valuable for simulating N emissions from dairy systems, but predictive ability should improve with further development and adaptation to higher producing situations.

In the next few years, more studies should be available to evaluate the NRC (2001). Recently, Noftsger and St-Pierre (2003) balanced diets using the CPM (Cornell-Penn-Miner) model for metabolizable lysine and methionine based on feed samples screened before the study to have either low or high predicted intestinal digestibilities of the RUP. Only the high CP, high digestible RUP treatment was predicted to have a positive metabolizable protein balance (requirement < supply; Table 2). Therefore, selection for highly digestible RUP sources increased milk production, as expected, during the 12-week study. Despite a predicted negative metabolizable protein balance for cows fed both low protein diets, milk production for cows fed the blend of rumen-protected and -unprotected methionine was similar to those fed the diet with

high crude protein/high digestible RUP. The diet with methionine increased efficiency of dietary N conversion into milk N and decreased N excretion (calculated by assuming zero N balance; i.e., dietary N intake – N secretion in milk) relative to N intake. Interestingly, during a 5-day digestibility experiment at the end of the production measurements, methionine addition did not increase N efficiency, perhaps because it might have ceased to be limiting by 16 weeks in lactation. Although demonstrating the difficulty of integrating N balance data with production data among published research, this report does highlight how emerging technology will likely be adapted in the future to improve efficiency of dietary conversion into milk protein.

Models have been developed to integrate dairy production and agronomic practice (Klausner *et al.*, 1998; Rotz *et al*., 1999). We refer readers to Chapter 6 for a more extensive review of whole-farm implications. However, we note here that most, if not all, models ignore variation among cows within groups, nutrients in feeds, and other factors that inflate 'safety factors' for protein intake on working farms. Table 3 estimates how uncertainty drives up crude protein percentage in dairy rations in practical situations (St-Pierre and Thraen, 1999). As can be seen, N efficiency was maximized at 14.9% crude protein, which agrees well with results from models based on individual cows in the U.K. (Castillo *et al.*, 2000; Kebreab *et al.*, 2002). Yet, such a strategy does not include effects of uncertainty, which considerably increased the optimal percentage of dietary protein (Table 3). St-Pierre and Thraen (1999) argued that a strategy to maximize N efficiency while decreasing CP concentration of the diet would decrease N excretion by 24% but would decrease milk production by 10.4%. Thus, this strategy was estimated to cost $1.35 billion for a 'national' dairy herd, or up to $9.55/kg of reduction of N excretion. Despite this uncertainty, the authors' simulations demonstrated that tighter grouping strategy would improve efficiency of N utilization. Yet, Jonker *et al.* (2002) noted that dairy farmers surveyed were not effectively grouping herds to reduce N loss. Clearly, confidence in ration balancing/modeling software needs to increase, including adaptability away from the 'average cow' toward group-feeding dynamics, before efficiency of N usage will be optimized.

Conclusions

The exchange of blood urea N (BUN) with the gut is extensive and is probably an adaptive mechanism to enhance ruminal degradation of poor quality fiber even when N intake is low. When ruminal N increases, then BUN transfer to the urine becomes increasingly dominant and wasteful and potentially harmful to the environment. As much as 20% of the N lost into the environment, particularly from urine, is recoverable in cattle feeding operations. Methods are being refined to measure and predict the amount of microbial protein production in the rumen. Yet, despite the importance of the rumen, its metabolism interacts with the spanchnic and peripheral tissues. Research has documented how shifts in site of digestion and the metabolism by the splanchnic tissues influence whole-body N metabolism and excretion of urinary N. Improved integration of the rumen, gut, and splanchnic tissues will advance the development of various models. Stage and level of production clearly influence metabolism of energy and amino acids, thereby affecting tissue N retention. Combined with better protein supplementation to meet metabolizable amino acid requirements, these systems will allow reduced inputs of dietary N and greater capture of BUN. As the prediction error in models is reduced and environmental regulations toughened, nutritional advisors should be able to use this information to decrease the amount of protein overfed with less risk of significant losses in animal productivity or loss of clientele.

Literature Cited

Ainslie, S.J., Fox, D.G., Perry, T.C., Ketchen, D.J. and Barry, M.C. (1993) Predicting amino acid adequacy of diets fed to Holstein steers. *Journal of Animal Science* 71, 1312-1319.

Al-Dehneh, A., Huber, J.T., Wanderley, R., Theurer, C.B., Pessarakli, M. and DeYoung, D. (1997) Incorporation of recycled urea-N into ruminal bacteria flowing to the small intestine of dairy cows fed a high-grain or high-forage diet. *Animal Feed Science and Technology* 68, 327-338.

Alderman, G., France, J. and Kebreab. E. (2001a) A critique of the Cornell Net Carbohydrate and Protein System with emphasis on dairy cattle. 1. The rumen model. *Journal of Animal and Feed Sciences* 10, 1-24.

Alderman, G., Blake, J.S., France, J. and Kebreab, E. (2001b) A critique of the Cornell Net Carbohydrate and Protein System with emphasis on dairy cattle. 2. The post-rumen digestion model. *Journal of Animal and Feed Sciences* 10, 203-221.

Allen, M.S. (1997) Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *Journal of Dairy Science* 80, 1447-1462.

Bannink, A., and De Visser, H. (1997) Comparison of mechanistic rumen models on mathematical formulation of extramicrobial and microbial processes. *Journal of Dairy Science* 80, 1296-1314.

Bargo, F., Muller, L.D., Kolver, E.S. and Delahoy, J.E. (2003) Invited Review: Production and digestion of supplemented dairy cows on pasture. *Journal of Dairy Science* 86, 1-42.

Bateman, H.G., Clark, J.H., Patton, R.A., Peel, C.J. and Schwab, C.G. (2001a) Accuracy and precision of computer models to predict passage of crude protein and amino acids to the duodenum of lactating cows. *Journal of Dairy Science* 84, 649-664.

Bateman, H.G., Clark, J.H., Patton, R.A., Peel, C.J. and Schwab, C.G. (2001b) Prediction of crude protein and amino acid passage to the duodenum of lactating cows by models compared with in vivo data. *Journal of Dairy Science* 84, 665-679.

Bell, A.W., Burhans, W.S. and Overton, T.R. (2000) Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. *Proceedings of the Nutrition Society* 59, 119-126.

Bergen, W.R. (1978) Nutrient-nutrient, nutrient-hormonal and nutrient-genetic interactions. In: Church (ed.) *Digestive Physiology and Nutrition of Ruminants.* Oxford Press, Portland, OR, pp. 430-441.

Boston, R.C., Fox, D.G., Sniffen, C., Janczewski, E., Munson, R. and Chalupa, W. (2000) The conversion of a scientific model describing dairy cow nutrition and production to an industry tool: the CPM Dairy Project. In: McNamara, J.P., France, J. and Beever, D.

(eds.) *Modeling Nutrient Utilization in Farm Animals*. CABI Publishing, New York, pp. 361-377.

Broderick, G.A. (1995) Desirable characteristics of forage legumes for improving protein utilization in ruminants. *Journal of Animal Science* 73, 2760-2773.

Broderick, G.A., and Merchen, N.R. (1992) Markers for quantifying microbial protein synthesis in the rumen. *Journal of Dairy Science* 75, 2618-2632.

Castillo, A.R., Kebreab, E., Beever, D.B. and France, J. (2000) A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. *Journal of Animal and Feed Sciences* 9, 1-32.

Castillo, A.R., Kebreab, E., Beever, D.E., Barbi, J.H., Sutton, J.D., Kirby, H.C. and France, J. (2001a) The effect of energy supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *Journal of Animal Science* 79, 240-246.

Castillo, A.R., Kebreab, E., Beever, D.E., Barbi, J.H., Sutton, J.D., Kirby, H.C. and France, J. (2001b) The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *Journal of Animal Science* 79, 247-253.

Clark, J.H., and Davis, C.L. (1980) Some aspects of feeding high producing dairy cows. *Journal of Dairy Science* 63, 873-885.

Coleman, S.W., and Wyatt, R.D. (1982) Cottonseed meal or small grains forages as protein supplements fed at different intervals. *Journal of Animal Science* 55, 11-17.

Cotta, M.A., and Russell, J.B. (1997) Digestion of nitrogen in the rumen: A model for metabolism of nitrogen compounds in gastrointestinal environments. In: Mackie, R..I. and White, B.A. (eds) *Gastrointestinal Ecosystems and Fermentations.* Chapman and Hall, New York, NY, vol. 1, pp. 380-423.

Delgado-Elorduy, A., Theurer, C.B., Huber, J.T., Alio, A., Lozano, O., Sadik, M., Cuneo, P., De Young, D., Simas, I.J., Santos, J.E.P., Nussio, L., Webb, Jr., L.K.E. and Tagari, H. (2002a) Splanchnic and mammary nitrogen metabolism by dairy cows fed steam-rolled or steam-flaked sorghum grain. *Journal of Dairy Science* 85, 148 –159.

Delgado-Elorduy, A., Theurer, C.B., Huber, J.T., Alio, A., Lozano, O., Sadik, M., Cuneo, P., De Young, D., Simas, I.J., Santos, J.E.P., Nussio, L., Webb, Jr., L.K.E. and Tagari, H. (2002b) Splanchnic and mammary nitrogen metabolism by dairy cows fed steam-rolled or steam-flaked corn. *Journal of Dairy Science* 85, 160 –168.

Dewhurst, R.J., Davies, D.R. and Merry, R.J. (2000) Microbial protein supply from the rumen. *Animal Feed Science and Technology* 85, 1-21.

Dijkstra, J. (1994) Simulation of the dynamics of protozoa in the rumen. *British Journal of Nutrition* 72, 679-699.

Dijkstra, J., France, J. and Davies, D.R. (1998) Different mathematical approaches to estimating microbial protein supply in ruminants. *Journal of Dairy Science* 81, 3370-3384.

Dijkstra, J., Mills, J.A.N. and France, J. (2002) The role of dynamic modeling in understanding the microbial contribution to rumen function. *Nutrition Research Reviews* 15, 67-90.

Dinn, N.E., Shelford, J.A. and Fisher, L.J. (1998) Use of the Cornell Net Carbohydrate and Protein System and rumen-protected lysine and methionine to reduce nitrogen excretion from lactating dairy cows. *Journal of Dairy Science* 81, 229-237.

Doreau, M., and Ferlay, A. (1995) Effect of dietary lipids on nitrogen metabolism in the rumen: a review. *Livestock Production Science* 43, 97-110.

Drackley, J.K., Overton, T.R. and Douglas, G.N. (2001) Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. *Journal of Dairy Science* 84(E. Suppl.), E100-E112.

Faichney, G.J., Poncet, C., Lassalas, B., Jouany, J.P., Millet, L., Dore, J. and Brownlee, A.G. (1997) Effects of concentrates in a hay diet on the contribution of anaerobic fungi, protozoa and bacteria to nitrogen in rumen and duodenal digesta in sheep. *Animal Feed Science and Technology* 64, 193-213.

Firkins, J.L. (1996) Maximizing microbial protein synthesis in the rumen. *Journal of Nutrition* 126, 1347S-1354S.

Firkins, J.L. and Fluharty, F.L. (2000) Soy products as protein sources for beef and dairy cattle. In: Drackley, J.K. (ed.) *Soy in Animal Nutrition*. Federation of Animal Science Societies, Savoy, IL, pp. 182-214.

Firkins, J.L., Allen, M.S., Oldick, B.S. and St-Pierre, N.R. (1998) Modeling ruminal digestibility of carbohydrates and microbial protein flow to the duodenum. *Journal of Dairy Science* 81, 3350-3369.

Firkins, J.L., Eastridge, M.L., St-Pierre, N.R. and Noftsger, S.M. (2001) Effects of grain variability and processing on starch utilization by lactating dairy cattle. *Journal of Animal Science* 79(E. Suppl.), E218-E238.

Firkins, J.L. and Fluharty, F.L. (2000) Soy products as protein sources for beef and dairy cattle. In: Drackley, J.K. (ed.) *Soy in animal nutrition.* Federation of Animal Science Societies, Savoy, IL, pp 182-214

Gabler, M.T. and Heinrichs, A.J. (2003) Dietary protein to metabolizable energy ratios on feed efficiency and structural growth of prepubertal Holstein heifers. *Journal of Dairy Science* 86, 268-274.

Gibb, M.J., Ivings, W.E., Dhanoa, M.S. and Sutton, J.D. (1992) Changes in body components of autumn-calving Holstein-Friesian cows over the first 29 weeks of lactation. *Animal Production* 55, 339-360.

Greenfield, R.B., Cecava, M.J. and Donkin, S.S. (2000) Changes in mRNA expression for gluconeogenic enzymes in liver of dairy cattle during the transition to lactation. *Journal of Dairy Science* 83, 1228-1236.

Greenwood, R.H., and Titgemeyer, E.C. (2000) Limiting amino acids for growing Holstein steers limit-fed soyabean hull-based diets. *Journal of Animal Science* 78, 1997-2004.

Grummer, R.R. (1995) Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science* 73, 2820-2833.

Haig, P.A., Mutsvangwa, T., Spratt, R. and McBride, B.W. (2002) Effects of dietary protein solubility on nitrogen losses from lactating dairy cow and comparison with predictions from the Cornell Net Carbohydrate and Protein System. *Journal of Dairy Science* 85, 1208-1217.

Harmon, D.L. and McLeod, K.R. (2001) Glucose uptake and regulation by intestinal tissues: Implications and whole-body energetics. *Journal of Animal Science* 79(E. Suppl.), E59-E72.

Hartwell, J.R., Cecava, M.J. and Donkin, S.S. (2000) Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. *Journal of Dairy Science* 83, 2907-2917.

Harvatine, D.I., Firkins, J.L. and Eastridge, M.L. (2002) Whole linted cottonseed as a forage substitute fed with ground or steam-flaked corn: Digestibility and performance. *Journal of Dairy Science* 85, 1976-1987.

Hristov, A.N., Huhtanen, P., Rode, L.M., Acharya, S.N. and McAllister, T.A. (2001) Comparison of the ruminal metabolism of nitrogen from ^{15}N-labeled alfalfa preserved as hay or as silage. *Journal of Dairy Science* 84, 2738-2750.

Huntington, G.B. (1989) Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a concentrate diet. *Canadian Journal of Animal Science* 69, 215-223.

Hvelplund, T., Misciatelli, L. and Weisbjerg, M.R. (2001) Supply of the dairy cow with amino acids from dietary protein. *Journal of Animal and Feed Sciences* 10, 69-85.

Iburg, M. and Lebzien, P. (2000) Requirements of lactating dairy cows for leucine and methionine at the duodenum. *Livestock Production Science* 62, 155-168.

James, T., Meyer, D., Esparza, E., DePeters, E.J. and Perez-Monti, H. (1999) Effects of dietary nitrogen metabolism on ammonia volatilization from manure from Holstein heifers. *Journal of Dairy Science* 82, 2430-2439.

Johnson, D.E. (1986) Fundamentals of whole animal calorimetry: use in monitoring body tissue deposition. *Journal of Animal Science* 63(Suppl. 2), 111-114.

Jonker, J.S., Kohn, R.A. and High, J. (2002) Dairy herd management practices that impact nitrogen utilization efficiency. *Journal of Dairy Science* 85, 1218-1226.

Jouany, J.P. (1996) Effect of rumen protozoa on nitrogen utilization by ruminants. *Journal of Nutrition* 126, 1335S-1346S.

Kalscheur, K.F., Vandersall, J.H., Erdman, R.A., Kohn, R.A. and Russek-Cohen, E. (1999) Effects of dietary crude protein concentration and degradability on milk production responses of early, mid, and late lactation dairy cows. *Journal of Dairy Science* 82, 545-554.

Kebreab, E., Castillo, A.R., Beever, D.E., Humphries, D.J. and France, J. (2000) Effects of management practices prior to and during ensiling and concentrate type on nitrogen utilization in dairy cows. *Journal of Dairy Science* 83, 1274-1285.

Kebreab, E., France, J., Mills, J.A.N., Allison, R. and Dijkstra, J. (2002) A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact on N excretion on the environment. *Journal of Animal Science* 80, 248-259.

Kennedy, P.M. and Milligan, L.P. (1980) The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: A review. *Canadian Journal of Animal Science* 60, 205-221.

Klausner, S.D., Fox, D.G., Rasmussen, C.N., Pitt, R.E., Tylutki, T.P., Wright, P.E., Chase, L.E. and Stone, W.C. (1998) Improving dairy farm sustainability I: An approach to animal and crop nutrient management planning. *Journal of Production Agriculture* 11, 225-233.

Klopfenstein, T.J. and Erickson, G.E. (2002) Effects of manipulating protein and phosphorus nutrition of feedlot cattle on nutrient management and the environment. *Journal of Animal Science* 80(E. Suppl. 2), E106-E114.

Klopfenstein, T.J., Mass, R.A., Creighton, K.W. and Patterson, H.H. (2001) Estimating forage protein degradation in the rumen. *Journal of Animal Science* 79(E. Suppl.), E208-E217.

Kohn, R.A. and Allen, M.S. (1995) Effect of plant maturity and preservation method on in vitro protein degradation of forages. *Journal of Dairy Science* 78, 1544-1551.

Kohn, R.A., Dou, Z., Ferguson, J.D. and Boston, R.C. (1997) A sensitivity analysis of nitrogen losses from dairy farms. *Journal of Environmental Management* 50, 417-428.

Kolver, E.S. and Muller, L.D. (1998) Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science* 81, 1403-1411.

Komaragiri, M.S. and Erdman, R.A. (1997) Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein. *Journal of Dairy Science* 80, 929-937.

Krehbiel, C.R., Ferrell, C.L. and Freetly, H.C. (1998) Effects of frequency of supplementation on dry matter intake and net portal and hepatic flux of nutrients in mature ewes that consume low-quality forage. *Journal of Animal Science* 76, 2464-2473.

Kuipers, A., Mandersloot, F. and Zom, R.L.G. (1999) An approach to nutrient management on dairy farms. *Journal of Dairy Science* 82(Suppl. 2), 84-89.

Lapierre, H. and Lobley, G.E. (2001) Nitrogen recycling in the ruminant: A review. *Journal of Dairy Science* 84(E. Suppl.), E223-E236.

Leng, R.A. and Nolan, J.V. (1984) Nitrogen metabolism in the rumen. *Journal of Dairy Science* 67, 1072-1089.

Lobley, G.E., Connell, A., Lomax, M.A., Brown, D.S., Milne, E., Calder, A.G. and Farringham, D.A.H. (1995) Hepatic detoxification of ammonia in the ovine liver: possible consequences for amino acid catabolism. *British Journal of Nutrition* 73, 667-685.

Lobley, G.E., Weijs, P.J.M., Connell, A., Calder, A.G., Brown, D.S. and Milne, E. (1996) The fate of absorbed and exogenous ammonia as influenced by forage or forage-concentrate diets in growing sheep. *British Journal of Nutrition* 76, 231-248.

Lobley, G.E., Bremner, D.M. and Zuur, G. (2000) Effects of diet quality on urea fates in sheep as assessed by refined, non-invasive [$^{15}N^{15}N$] urea kinetics. *British Journal of Nutrition* 84, 459-468.

Maltby, S.A., Reynolds, C.K., Lomax, M.A. and Beever, D.E. (1993) The effect of increased absorption of ammonia and arginine on splanchnic metabolism of beef steers. *Animal Production* 56, 462.

Marini, J.C. and Van Amburgh, M.E. (2003) Nitrogen metabolism and recycling in Holstein heifers. *Journal of Animal Science* 81, 545-552.

Martin, A.K. (1966) Some errors in the determination of nitrogen retention of sheep by nitrogen balance studies. *British Journal of Nutrition* 20, 325 - 337.

McBride, B.W. and Kelly, J.M. (1990) Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. *Journal of Animal Science* 68, 2997-3010.

McGuffey, R.K., Richardson, L.F. and Wilkinson, J.I.D. (2001) Ionophores for dairy cattle: Current status and future outlook. *Journal of Dairy Science* 84(E. Suppl.), E194-E203.

Meyer, D. and Mullinax, D.D. (1999) Livestock nutrient management concerns: Regulatory and legislative overview. *Journal of Dairy Science* 82(Suppl. 2), 51-62.

Milano, G.D., Hotston-Moore, A. and Lobley, G.E. (2000) Influence of hepatic ammonia removal on ureagenesis, amino acid utilization and energy metabolism in the ovine liver. *British Journal of Nutrition* 83, 307-315.

Milano, G.D. and Lobley, G.E. (2001) Liver nitrogen movements during short-term infusion of high levels of ammonia into the mesenteric vein of sheep. *British Journal of Nutrition* 86, 507-513.

Moorby, J.M. and Theobald, V.J. (1999) Short communication: The effect of duodenal ammonia infusions on milk production and nitrogen balance of the dairy cow. *Journal of Dairy Science* 82, 2440-2442.

National Research Council. (1989) Nutrient Requirements of Dairy Cattle. 6th rev. ed.: Update 1989. *National Academy of Science*, Washington, DC.

National Research Council. (2000) Nutrient Requirements of Beef Cattle. 7th rev. ed.: Update 2000. *National Academy of Science*, Washington, DC.

National Research Council. (2001) Nutrient Requirements of Dairy Cattle. 7th rev. ed. *National Academy of Science*, Washington, DC.

Nelson, C.J. (1999) Managing nutrients across regions of the United States. *Journal of Dairy Science* 82(Suppl. 2), 90-100.

Neter, J., Kutner, M.H., Nachtscheim, C.J. and Wasserman, W. (1996) *Applied Linear Regression Models*. 3rd Edition. McGraw-Hill Co., Inc., Chicago, IL.

Noftsger, S.M. and St-Pierre, N.R. (2003) Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. *Journal of Dairy Science* 86, 958-969.

Nolan, J.V. (1975) Quantitative models of nitrogen metabolism in sheep. In: McDonald, I.W. and Warner, A.C.I. (eds.) *Digestion and Metabolism in the Ruminant*. University of New England Publishing Unit, Armidale, Australia, pp. 416-431.

Oba, M. and Allen, M.S. (2003a) Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. *Journal of Dairy Science* 86, 195-207.

Oba, M. and Allen, M.S. (2003b) Hypophagic effects of ammonium are greater when infused with propionate compared with acetate in lactating dairy cows. *Journal of Nutrition* 133, 1100-1104.

Obitsu, T., Bremmer, D., Milne, E. and Lobley, G.E. (2000) Effect of abomasal glucose infusion on alanine metabolism and urea production in sheep. *British Journal of Nutrition* 84, 157-163.

O'Connor, J.D., Sniffen, C.J., Fox, D.G. and Chalupa, W. (1993) A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *Journal of Animal Science* 71, 1298-1311.

Oldham, J.D. and Smith, T. (1980) Protein – energy interrelationships for growing and for lactating cattle. In: Miller, E.L.,Pike, I.H. and Van Es, A.J.H. (eds.) *Protein Contribution of*

Feedstuffs for Ruminants: Application to Feed Formulation. Butterworth Scientific, London, pp. 103-130.

Oldick, B.S., Firkins, J.L. and St-Pierre, N.R. (1999) Estimation of microbial nitrogen flow to the duodenum of cattle based on dry matter intake and diet composition. *Journal of Dairy Science* 82, 1497-1511.

Oldick, B.S., Firkins, J.L. and Kohn, R.A. (2000) Compartmental modeling with nitrogen-15 to determine effects of degree of fat saturation on intra-ruminal N recycling. *Journal of Animal Science* 78, 2421-2430.

Parker, D.S., Lomax, M.A., Seal, C.J. and Wilton, J.C. (1995) Metabolic implications of ammonia production in the ruminant. *Proceedings of the Nutrition Society* 54, 549-563.

Piepenbrink, M.S., Schingoethe, D.J., Brouk, M.J. and Stegeman, G.A. (1998) Systems to evaluate the protein quality of diets fed to lactating cows. *Journal of Dairy Science* 81, 1046-1061.

Plaizier, J.C., Martin, A., Duffield, T., Bagg, R., Dick, P. and McBride, B.W. (2000) Effect of a prepartum administration of monensin in a controlled-release capsule on apparent digestibilities and nitrogen utilization in transition dairy cows. *Journal of Dairy Science* 83, 2918-2925.

Putnam, D.E. and Varga, G.A. (1998) Protein density and its influence on metabolite concentration and nitrogen retention by Holstein cows in late gestation. *Journal of Dairy Science* 81, 1608-1618.

Reynolds, C.K. (1995) Quantitative aspects of liver metabolism in ruminants. In: Engelhardt, W.V., Leonhard-Marek, S., Breves, G. and Giesecke, D. (eds.) *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction: Proceedings of the Eighth International Symposium on Ruminant Physiology*. Ferdinand Enke Verlag, Stuttgart, Germany, p. 351.

Reynolds, C.K. (2000) Forage evaluation using measurements of energy metabolism. In: Givens, D.I., Owen, E., Axford, R.F.E. and Omed, H.M. (eds.) *Forage Evaluation in Ruminant Nutrition*. CAB International, Wallingford, U.K., pp. 95-111.

Reynolds, C.K. (2002) Economics of visceral tissue metabolism in ruminants. Toll keeping or internal revenue service? *Journal of Animal Science* 80(E. Suppl.), E74-E84.

Reynolds, C.K. (2003) Nitrogen Metabolism by Splanchnic Tissues of Ruminants. In: Burrin, D. and Merssman, H. (eds.) *Biology of Metabolism of Growing Animals*. Elsevier Science, Oxford, England, submitted.

Reynolds, C.K. and Huntington, G.B. (1988) Partition of portal-drained visceral net flux in beef steers. 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post-stomach tissues. *British Journal of Nutrition* 60, 539-551.

Reynolds, C.K., Humphries, D.J., Cammell, S.B., Benson, J., Sutton, J.D. and Beever, D.E. (1998) Effects of abomasal wheat starch infusion on splanchnic metabolism and energy balance of lactating dairy cows. In: McCracken, K.J., Unsworth, E.F. and Wylie, A.R.G. (eds.) *Energy Metabolism of Farm Animals.* Proceedings of the 14th Symposium on Energy Metabolism, CAB International, pp 39-42.

Reynolds, C. K., Cammell, S.B., Humphries, D.J., Beever, D.E., Sutton, J.D. and Newbold, J.R. (2001) Effects of post-rumen starch infusion on milk production and energy metabolism in dairy cows. *Journal of Dairy Science* 84, 2250-2259.

Reynolds, C.K., Aikman, P.C., Lupoli, B., Humphries, D.J. and Beever, D.E. (2003) Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *Journal of Dairy Science* 86, 1201-1217.

Rotz, C.A., Mertens, D.R., Buckmaster, D.R., Allen, M.S. and Harrison, J.H. (1999) A dairy herd model for use in whole farm simulations. *Journal of Dairy Science* 82, 2826-2840.

Russell, J.B. and Wilson, D.B. (1996) Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *Journal of Dairy Science* 79, 1503-1509.

Santos, J.E.P., DePeters, E.J., Jardon, P.W. and Huber, J.T. (2001) Effect of prepartum dietary protein level on performance of primigravid and multiparous Holstein dairy cows. *Journal of Dairy Science* 84, 213-224.

Satter, L.D., Klopfenstein, T.J. and Erickson, G.E. (2002) The role of nutrition in reducing nutrient output from ruminants. *Journal of Animal Science* 80(E. Suppl. 2), E143-E156.

Schneider, B.H. and Flatt, W.P. (1975) *The Evaluation of Feeds Through Digestibility Experiments*. University of Georgia Press, Athens, GA, pp. 168-178.

Sharp, R., Ziemer, C.J., Stern, M.D. and Stahl, D.A. (1998) Taxon-specific associations between protozoal and methanogen populations in the rumen and a model rumen system. *Federation of European Microbiological Societies Microbiology Ecology* 26, 71-78.

Shingfield, K.J. (2000) Estimation of microbial protein supply in ruminant animals based on renal and mammary purine metabolite excretion. A review. *Journal of Animal and Feed Sciences* 9, 169-212.

Sloan, B.K. (1997) Developments in amino acid nutrition of dairy cows. In: Garnsworthy, P.C. and Wiseman, J. (eds.) *Recent Advances in Animal Nutrition*. Nottingham University Press, Nottingham,UK, pp. 167-198.

Spanghero, M. and Kowalski, Z.M. (1997) Critical analysis of N balance experiments with lactating cows. *Livestock Production Science* 52, 113-122.

St-Pierre, N.R. (2001) Integrating quantitative findings from multiple studies using mixed model methodology. *Journal of Dairy Science* 84, 741-755.

St-Pierre, N.R. (2003) Reassessment of biases in predicted nitrogen flows to the duodenum by NRC 2001. *Journal of Dairy Science* 86, 344-350.

St-Pierre, N.R. and Glamocic, D. (2000) Estimating unit costs of nutrients from market prices of feedstuffs. *Journal of Dairy Science* 83, 1402-1411.

St-Pierre, N.R. and Thraen, C.S. (1999) Animal grouping strategies, sources of variation, and economic factors affecting nutrient balance on dairy farms. *Journal of Dairy Science* 82(Suppl. 2), 72-83.

Stern, M.D., Bach, A. and Calsamiglia, S. (1997) Alternative techniques for measuring nutrient digestion in ruminants. *Journal of Animal Science* 75, 2256-2276.

Stockdale, C.R. and Roche, J.R. (2002) A review of the energy and protein nutrition of dairy cows through their dry period and its impact on early lactation performance. *Australian Journal of Agricultural Research* 53, 737-753.

Sutter, F. and Beever, D.E. (2002) Energy and nitrogen metabolism in Holstein-Friesian cows during early lactation. *Animal Science* 70, 503-514.

Vanhatalo, A., Huhtanen, P., Toivonen, V. and Varvikko, T. (1999) Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. *Journal of Dairy Science* 82, 2674-2685.

Van Horn, H.H. and Hall, M.B. (1997) Agricultural and environmental issues in the management of cattle manure. In: Recheigl, J.E. and MacKinnon, H.C. (eds.) *Agricultural Uses of By-Products and Wastes*. American Chemical Society, Washington, DC, pp. 91-109.

Van Horn, H.H., Newton, G.L. and Kunkle, W.E. (1996) Ruminant nutrition from an environmental perspective: Factors affecting whole-farm nutrient balance. *Journal of Animal Science* 74, 3082-3102.

VanSaun, R.J., Idleman, S.C. and Sniffen, C.J. (1993) Effect of undegradable protein amount fed prepartum on postpartum production in first lactation Holstein cows. *Journal of Dairy Science* 76, 236-244.

Wallace, R.J., Onodera, R. and Cotta, M.A. (1997) Metabolism of nitrogen-containing compounds. In: Hobson, P.N. and Stewart, C.S. (eds.) *The Rumen Microbial Ecosystem*. Chapman and Hall, New York, NY, pp. 283-328.

Waterlow, J.C. (1999) The mysteries of nitrogen balance. *Nutrition Research Reviews* 12, 25-54.

Wells, J. E. and Russell, J.B. (1996) Why do many ruminal bacteria die and lyse so quickly? *Journal of Dairy Science* 79, 1487-1495.

Wessels, R.H. and Titgemeyer, E.C. (1997) Protein requirements of growing steers limit-fed corn-based diets. *Journal of Animal Science* 75, 3278-3286.

Whitelaw, F.G., Milne, J.S. and Wright, S.A. (1991) Urease (*EC* 3.5.1.5) inhibition in the sheep rumen and its effect on urea and nitrogen metabolism. *British Journal of Nutrition* 66, 209-225.

Whitlock, B.K., VandeHaar, M.J., Silva, L.F.P. and Tucker, H.A. (2002) Effect of dietary protein on prepubertal mammary development in rapidly growing dairy heifers. *Journal of Dairy Science* 85, 1516-1525.

Wu, Z. and Huber, J.T. (1994) Relationship between dietary fat supplementation and milk protein concentration in lactating cows: A review. *Livestock Production Science* 39, 141-155.

Wu, Z., Polan, C.E. and Fisher, R.J. (1997) Adequacy of amino acids in diets fed to lactating dairy cows. *Journal of Dairy Science* 80, 1713-1721.

Table 1. Nitrogen and metabolizable energy (ME) in dairy cows during the first eight weeks of lactation[a].

	Week								
	1	2	3	4	5	6	7	8	SE
N intake, g/d[b]	394	440	462	458	447	435	428	426	6.5
Milk N, g/d[L]	183	183	186	182	172	168	165	164	2.1
Urinary N, g/d	87	102	90	96	81	76	76	67	3.7
N retained, g/d	-19	-1	15	4	23	17	10	20	5.0
ME intake, MJ/d[b]	164	191	199	199	202	198	199	200	2.9
Milk energy, MJ/d[L]	104	104	106	103	97	95	94	93	1.2
Energy retained, MJ/d[L]	-64	-36	-36	-33	-24	-22	-21	-21	2.8
Urinary energy, MJ/d	8.1	9.1	9.3	9.5	8.0	8.3	8.2	8.5	0.28
Urinary energy, % DE	4.24	4.22	4.13	4.16	3.49	3.64	3.60	3.68	NA

[a]Data from Sutter and Beever (2002). Their data were used to calculate urinary energy as a percentage of digestible energy (DE), so a SE was not available.
[L]Linear effect of week in lactation ($P < 0.01$).
[b]Week 1 < week 2 ($P < 0.05$).

Table 2. Least square means for performance measures for diets that vary in crude protein and digestibility of rumen-undegraded protein[1].

	High CP[2]		Low CP[3]		
	DRUP	HDRUP	HDRUP	HDRUP + Met	SEM
	Experiment 1 (n = 60; 12 wk)				
DMI, kg/d	21.7[a]	23.3[b]	23.2[b]	23.6[b]	0.49
Milk yield, kg/d	40.8[a]	46.2[b]	42.9[a]	46.6[b]	0.72
N intake, g/d	641[a]	690[b]	645[a]	651[a]	14.2
MP balance, g/d[4]	-84	20	-58	-257	--
Milk N production, g/d	188	214	203	228	3.9
Gross N efficiency[5]	29.5[a]	31.1[b]	31.7[b]	35.0[c]	0.60
Environmental efficiency[6]	2.47[a]	2.25[b]	2.19[b]	1.89[c]	0.06
	Experiment 2 (n = 24; 5 d)				
N intake, g/d	770[a]	735[a,b,c]	682[b,c]	679[b]	27.9
Fecal N, g/d	279	271	257	263	10.9
Urine N, g/d	268[a]	259[a,c]	216[b,d]	224[c,d]	19.3
Apparent N retention, g/d	-1	-13	-16	-23	18.3
Productive N, % of N intake[7]	29.1	28.0	30.8	28.5	1.9
Environmental efficiency[8]	2.43[a]	2.44[a]	2.09[b]	2.24[a,b]	0.10

[1]From Noftsger and St-Pierre (2003).
[2]High CP diets contained 18.3% crude protein. Diets had protein sources with low (LDRUP) or high (HDRUP) digestible rumen-undegraded protein.
[3]Low CP diets had 16.9% (LDRUP) or 17.0% (HDRUP) crude protein. The latter diet had extra supplemental methionine that was partially protected from ruminal degradation.
[4]Metabolizable protein balance (requirement – supply) from actual data using the NRC (2001) model.
[5]Calculated as milk N/N intake x 100.
[6]Calculated as kg N excreted/kg N in milk; N excreted calculated as N intake – milk N, assuming zero N balance.
[7]Productive N = milk N + retained N.
[8]Calculated as kg N excreted/kg N in milk; actual N excretion data were used.
[a,b,c]Treatment means in the same row with different superscripts are different ($P < 0.05$).

Table 3. Crude protein percentages required to optimize milk production, N efficiency, or income over feed costs (IOFC) with or without uncertainty of model parameters.[a]

Scenario	Milk	N Efficiency	IOFC
	---------- Crude Protein % required ----------		
No uncertainty	18.5	17.0	17.7
With uncertainty	18.6	14.9	18.0

[a]Adapted from St-Pierre and Thraen (1999). Simulations are for a herd with high milk production potential (11,350 kg/year). N efficiency = kg milk/kg N excreted.

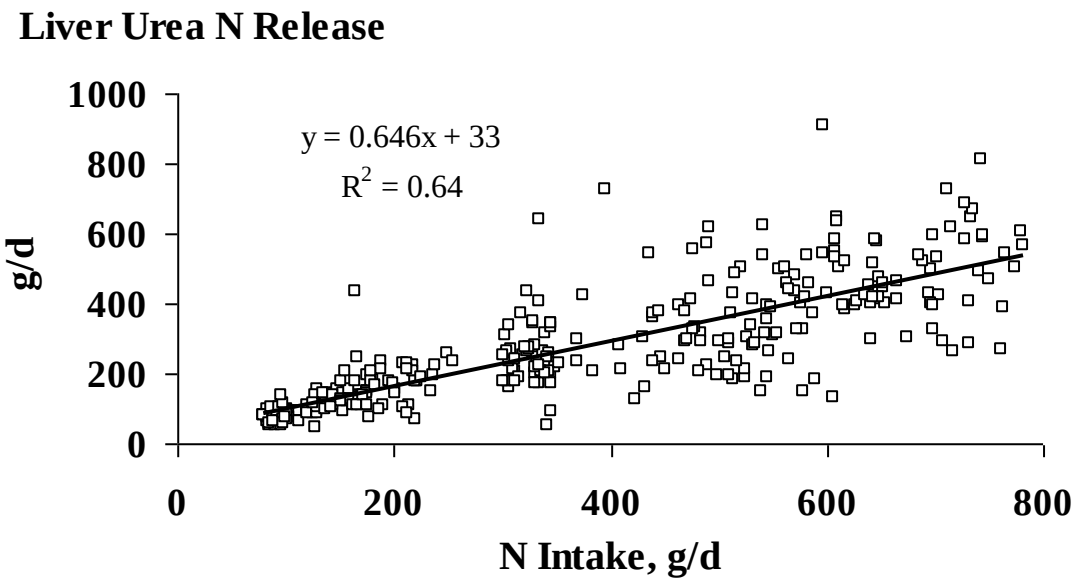

Figure 1. Relationship between N intake and net liver release of urea N in cattle (corrected for random effects of study as described by St-Pierre, 2001; n = 304; for sources of the original data, see Reynolds, 2003).

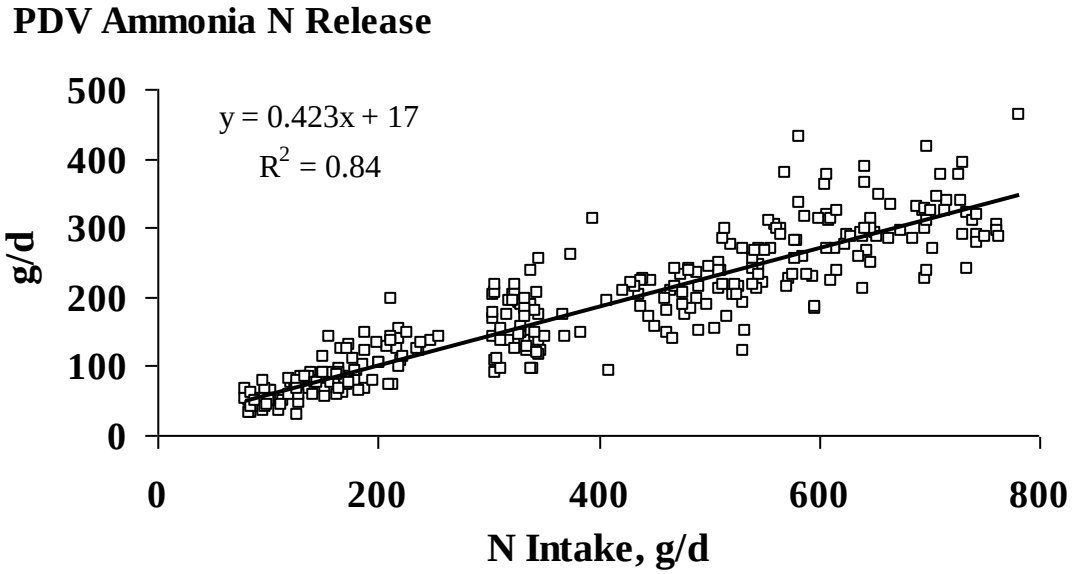

Figure 2. Relationship between N intake and net PDV release of ammonia N in cattle (corrected for random effects of study as described by St-Pierre, 2001; n = 308; for sources of the original data, see Reynolds, 2003).