CHAPTER II
REVIEW OF LITERATURE

2.1 An overview of milk production

Livestock is vital to the economies of many developing countries. Animals are the source of food, more specifically protein for human diets, income, employment and possibly foreign exchange. For low income producers, livestock can serve as a store of wealth; provide draught power and organic fertilizer for crop production and a means of transport. Consumption of animal products developing countries, though starting from a low base, is growing rapidly. In the last decade, the Thai Government has implemented key policy and market adjustments to enable its relatively young dairy sector to take off. From 1996 to 2003, the Thai milk production doubled the milk yield per dairy animal per year increased by a factor of 1.7, the number of dairy animals rose by 10 percent higher while the Buffalo population shrank to 60 percent of its 1996 level (Otto et al., 2006). In January 2011, the total number of dairy cows in the country was 535,986 head, an increased by 40,576 heads or 7.6 percent from 2009 (OAE, 2011). The average farm size is estimated at 20 dairy animals per farm, which is just above 70 percent of the average size of dairy herd in Germany. With an average herd size of 20 heads, Thai dairy farms achieve a milk yield of above 3,000 kg per dairy animal per year, which is 95 percent of the averge yield of New Zealand dairies.

Thailand's domestic milk production covers about 40 to 50 percent of the national ready-to-drink dairy products. This situates the country as one of the biggest importers representing 0.12 percent of total world milk production. In another perspective, Thailand reached about 0.50, 0.84 and 0.95 percent of the European Union, India and USA total milk productions respectively. Thailand' milk yield of 3,000 kg per cow is relatively low when compared to Germany with 6,000 and US with 8,000 kg. Interestingly, the Thai yield is very close to that in New Zealand. When compared within Asia, Thai farms are high producers due to mainly higher concentrate use and better genetics (Otto et al., 2006). In 2006 to 2010, total

production of raw milk was increased by 2.8 percent per year or 862,000 tons in 2010 (OAE, 2011). The world milk production forecast for 2011 is reduced slightly from last year. Relatively high milk prices are being offset by high feed costs and only slight growth is expected in the herd for the remainder of the year (USDA, 2011).

Thailand contribution to the world milk output is a mere 0.1 percent in 2003. Protected from international competition, in 2011, the Thai farm gate price of 0.57 US$/litre was 1.8 times higher than in New Zealand, for instance. The costs of milk production are slightly above those of New Zealand milk price. Therefore, lowering the milk production cost would mean that dairy producers could compete with imports of dairy products and also to produce milk for export, provided international quality standards can be achieved and the dairy chain being internationally competitive.

2.2 Ruminant nutrition

Virtanen (1966) showed that dairy cows could produce over 4,000 kg milk per year, when fed a diet solely consisting of urea (nitrogen source), cellulose, starch and sugar (carbohydrate sources), vegetable oil, minerals, and fat soluble vitamins. A large proportion of dietary nutrients are made available to ruminants in the form of end-products of rumen fermentation, primarily volatile fatty acids (VFA) and microbial protein. VFA are used by the host as a major source of metabolizable energy. Microbial protein is the major source of metabolizable amino acids for maintenance and milk synthesis. The removal of fermentation products from the rumen and the outflow of microbial biomass have a direct impact on the nutritional status of ruminants. Consequently, it is imperative to manage the rumen to optimize microbial growth and fermentation.

The rumen is a complex environment inhabited by many different microbial species, having different nutrient requirements and metabolism. Therefore, considering the nutrient requirements of ruminal microorganisms is crucial to understanding protein and carbohydrate metabolism in the rumen as well as the factors that may modify it self. Carbohydrates are the main energy sources for bacteria. Ruminal microbial protein synthesis depends on supply of adequate amounts and type of carbohydrate as an energy sources. Readily fermentable carbohydrates such as starch or sugars are more effective than other sources such as cellulose in term of promoting microbial growth (Stern and Hoover, 1979). Several *in vitro* and *in vivo* studies demonstrated that increasing amounts

of readily fermentable carbohydrate decreased ammonia-N concentrations due to improved N uptake by ruminal microbes (Casper and Schingoethe, 1989; Henning et al., 1991). In addition, to the amounts of nutrient supplied, the synchrony of which nutrients become available is also important. When the rate of protein degradation exceeds the rate of carbohydrate fermentation, large quantities of N can be lost as ammonia. When the rate of carbohydrate fermentation exceeds the rate of protein degradation, microbial protein synthesis can be restricted (Nocek and Russell, 1988). Protein and carbohydrate sources are required to maximize microbial growth. Not only is the quantity of nutrients but also simtaneously ruminal degradation rate necessary for optimal fermentation and microbial growth.

2.3 General aspects of rumen microbial metabolism

Dietary carbohydrates mainly polysaccharides are degraded to hexoses and pentoses. Pentoses proceed mainly through the transketolase and transaldolase reactions of the pentose cycle, which yield hexose and triose phosphate. As the majority of the carbohydrates are in the form of hexose, they are metabolized to pyruvate, almost exclusively by the Embden-Meyerhof-Parnas (EMP) glycolytic pathway (France and Siddons, 1993). The EMP pathway is the most common pathway of hexose metabolism in both aerobic and anaerobic microorganisms (Gottschalk, 1979). This is advantageous to anaerobic bacteria, because it maximizes the yield of ATP. Pyruvate proceeds to acetate or butyrate with acetyl CoA as an intermediate. Propionate is formed mainly via succinate (randomizing pathway) but an alternative pathway (direct reductive pathway) involves acrylate (Russell and Wallace, 1988; France and Siddons, 1993). The acrylate pathway only yields 2 ATP per hexose, as does the fermentation to lactate, ethanol or valerate.

According to Russell and Wallace (1988), the fermentation of one hexose to acetate, propionate or butyrate, yields 4, 4 and 3 ATP, respectively. The microbial ATP produced by rumen fermentation is required for microbial growth. Bauchop and Elsden (1960) found a correlation between the amount of ATP which could be derived from catabolism, and the yield of cell mass (Y_{ATP}, g/mol ATP). The values ranged from 8.3 to 12.5 with an average of 10.5 from studies of several bacterial species. However, some researchers have shown a much greater range (Stouthammer and Bettenhaussen, 1973)

with many rumen bacteria have Y_{ATP} above 20 (Russell and Wallace, 1988). Approximately two-thirds of the ATP is needed for polymerization reactions, and transport activity constitutes most of the remainders of energy costs (15 to 27 % of the total). Energy costs for synthesizing specific cell constituents differ and are, for example, threefold greater for protein than for polysaccharide (Stouthammer, 1973). Russell (1983) studied the growth of *Prevotella ruminicola* (formerly *Bacteriodes*) on a medium with ammonia as the sole nitrogen source. He found that Y_{ATP} increased when protein hydrolysate was added, and that little glucose was used as carbon source. Maeng and Baldwin (1976a; 1976b) found that the yield of microbial protein per mole ATP was higher when the nitrogen came both from amino acids and ammonia, rather than only from one of these sources. The stimulatory effects of amino acids upon microbial growth appeared to be related closely to the amounts of starch remaining in the rumen, suggesting that the growth of amylolytic bacteria is especially stimulated by amino acids (Maeng and Baldwin, 1976a). Maeng et al. (1976) found that the specific growth rate of rumen microbes as well as Y_{ATP} was highest when 25% of urea-N was replaced by amino acid-N in the incubation medium. A very important concept was shown by Stouthammer and Bettenhaussen (1973) that a lower proportion of ATP is used for maintenance at high specific growth rates, and consequently, Y_{ATP} is improved. Differences in the proportional redistribution of organic matter into microbial protein and fermentation end-products are considerable with respect to providing available nutrients for absorption by the animal. Beever (1993), gave an examples of three different diets; high forage, high cereal and high molasses, stimulating acetate, propionate and butyrate production, respectively

Acetate-induced fermentation is the most efficient for the microbes, as it results in the greatest amount of ATP produced per mole hexose. The amount of VFA metabolzable energy to the animal is greatest with propionate induced fermentation. This type of fermentation pattern is more efficient in transferring carbohydrate energy to metabolizable energy from fermentation end-products (VFA and ATP; Table 2.1), in line with lesser methane production.

Table 2.1 End-product formation and energetic efficiency resulting from fermentation of hexose by different pathway

	High forage	High cereal	High molasses
Moles end-product from 1 mole hexose:			
Acetate	1.34	0.90	0.94
Propionate	0.45	0.70	0.40
Butyrate	0.11	0.20	0.33
Methane	0.61	0.38	0.54
ATP	4.62	4.38	4.50
Total VFA	1.90	1.80	1.67
Energetic efficiency:			
VFA energy (MJ/mol hexose)	2.11	2.31	2.16
VFA as proportion of hexose energy	0.73	0.80	0.75
VFA + ATP as proportion of hexose energy	0.85	0.92	0.87

Source: Beever (1993)

2.4 Importance of fermentation products to ruminant

Volatile fatty acids (VFA) such as acetate, propionate, butyrate, and etc. are end products of rumen microbial fermentation (Stevens, 1969). The energy supplied from the production of VFA has been estimated to make up as high as 70-80 percent of the gross energy required by ruminants (Van Houtert, 1993). Pyruvate is the predominant common intermediate molecule in the fermentation pathways from carbohydrate to VFA (Van Houtert, 1993). It is from pyruvate that the different VFA are derived. Pyruvate is converted to acetate through an enzymatic pathway that results in the cleavage of pyruvate to form equi-molar amounts of acetate and formate (Leng, 1970). Propionate can be derived from the conversion of pyruvate to propionate via succinate or lactate (Bergman, 1990). When the pyruvate is converted to succinate, it undergoes carboxylation, first to form oxaloacetate or malate, then fumarate and finally succinate. From there, the succinate is available for other bacteria

to use and convert it to propionate through decarboxylation steps (Van Houtert, 1994). Propionate is derived from lactate or acrylate, usually when concentrate is included in higher proportions of the diet. Providing excess rapidly fermenting carbohydrates alters the metabolic pathways of the starch, such as in *Streptococcus bovis*. Although total ATP production was conventionally considered the limiting factor for microbial growth, it is now believed that ATP production per unit time is more critical. *S. bovis* will normally produce acetate and propionate under normal conditions but when excess starch is included in the diet it will switch from producing acetate and propionate to lactate. In order to sustain growth under excess supply of starch, production of lactate results in more ATP production per unit time.

The VFA are absorbed through the rumen wall via microvilli into the rumen epithelium before going into the bloodstream (McAnally, 1944). The pH within the rumen has an inverse effect on the absorption rate. As the rumen pH declines there is formation of non-dissociated formed VFA which absorb through the wall easier. This absorption occurs as a passive gradient since the pH of the bloodstream is more alkaline than that in the rumen (Dijkstra et al., 1993; Van Soest, 1994). When the VFA are absorbed into the rumen epithelium, some metabolism occurs before they proceed to the circulatory system. Acetate does not undergo extensive metabolism in the rumen epithelial tissue and will pass through the rumen wall intact until it reaches the peripheral tissues of the body. There is some evidence that metabolism of propionate to carbon dioxide occurs in the rumen epithelia tissue as observed in the study of Weekes and Webster (1975). Propionate can go through oxidation for tissue use to form carbon dioxide and lactate (Bergman, 1990). Other studies observed almost no metabolism of propionate in rumen epithelium due to high butyrate concentrations inhibiting the function of proponyl-CoA synthetase and that propionate remains predominantly intact until transported and converted in the liver (Ash and Baird, 1973). It has been found that a higher metabolism of propionate in rumen epithelium of sheep than cattle (Bergman, 1990). Among the VFA, butyrate seems to undergo the most extensive metabolism during absorption through the rumen epithelium. Most of the butyrate is absorbed and transformed to the ketones acetoacetate and beta-hydroxybutyrate within the epithelium (Van Houtert, 1993).

The main source of metabolic glucose supply in the ruminant comes from propionate (Van Soest, 1994). Propionate is converted to glucose by gluconeogenesis in the liver and is the only VFA that results in net glucose production. Glucose produced is then available for use by select tissues. Some amounts of acetate as well as any butyrate not metabolized by the rumen epithelium are metabolized by adipose and mammary tissue to form ATP and long chain fatty acids (LCFA) and cholesterol (Bell, 1981; Bergman, 1990). The majority of acetate is utilized by peripheral tissue for energy and is the main lipogenic fatty acid (Van Soest, 1994). The muscle and adipose tissue are the main peripheral locations where acetate is consumed. In the ruminant, acetate is primarily oxidized by the muscle for energy purposes predominantly when at rest (Bergman, 1990). In other places such as the mammary tissue however, acetate is lipogenic, contributing to the fat content in milk.

2.5 Importance of microbial protein to ruminants

Microbial crude protein has been reported to supply from 34 to 89% of the total amino acid nitrogen entering the small intestine of ruminants (Owens and Bergen, 1983; Clark et al., 1992). Microbial crude protein is considered to have a good amino acid balance relative to the animal's requirements (Clark et al., 1992) with a mean true digestibility of 84.7% (Storm et al., 1983). The microbial mass that flows from the rumen to the small intestine forms a major part of the metabolizable nutrient supply to the ruminant animal. The theoretical contribution of microbial protein to the total protein requirement of the lactating dairy cow, calculated at three efficiencies of microbial protein synthesis and their levels of milk production, is presented in Table 2. Contribution of microbial protein to total protein requirement was determined using NRC (2001) values for a 680-kg lactating dairy cow producing 25, 35, or 45 kg/d of 4% fat-corrected milk (FCM). At these three milk yields, microbial protein would contribute 51, 49 and 48%, respectively of the total protein required by the cow when microbial synthesis in the rumen was 30 g of N/kg of organic matter truly digested (OMTD). When milk yield is 45 kg/d, the contribution of microbial protein to total protein required by the cow would increase from 32 to 63% as efficiency of microbial protein synthesis increases from 20 to 40 g of N/kg of OMTD. Stern and Hoover (1979) reviewed and reported that approximately 30 g of N

was synthesized per kilogram of OMTD in the rumen; values ranged from 10 to 50 g. Efficiencies of microbial protein synthesis (Table 2.2) fall into the range of reported values.

Table 2.2 Contribution of microbial protein to total protein requirement of lactating dairy cattle[a]

Microbial synthesis, g of N/kg of OM truly digested[b]	Contribution of microbial protein when daily milk production (kg) equals:		
	25 (55 lb)	35 (77 lb)	45 (100 lb)
	%		
20	34	33	32
30	51	49	48
40	68	65	63

[a]Requirements determined using NRC (2001).

[b]Assumed that 55% of OM intake is truly digested in the rumen.

Source: Stern et al. (2006).

Therefore, theoretical contributions of microbial protein clearly depict the importance of optimizing microbial protein synthesis in the rumen of high producing dairy cows. In addition, these calculations demonstrate that, as milk yield increases, a substantial quantity of RUP from protein supplements must leave the rumen to meet the protein requirement of the cow. However, it is clear that substantial quantities of RUP from protein supplements must be incorporated into the diet of high producing ruminants.

The composition of the microorganisms determines the potential specific nutrient contribution to the small intestine. Bacteria typically contain 50% protein, 20% RNA, 3% DNA, 9% lipid and 18% carbohydrate, but this composition can change substantially (Nocek and Russell, 1988). The two main components of microbial mass that contribute to the metabolizable nutrient supply in the small intestine are MCP and microbial storage carbohydrate (α- glucan). Bacterial amino

nitrogen as a percentage of total nitrogen has been considered as relatively constant, but it can range from 54.9 to 86.7%, with an average of 66.5% (Clark et al., 1992). Large changes may also be seen in microbial glycogen content, especially when cultures are starved for nutrients other than energy (Nocek and Russell, 1988). There are many of factors that can affect microbial growth, of which supply of carbohydrate and nitrogen (Clark et al., 1992) appears to be the most important. Another factor that may affect the efficiency of microbial growth is pH (Russell et al., 1992).

Under practical conditions, efficiency of microbial protein synthesis (EMPS) remains relatively constant within a wide range of pH. To assess the potential effect of ruminal pH on EMPS, a meta analysis as described by St-Pierre (2001) was conducted with literature providing *in vivo* data (n = 187) shows the results of this meta-analysis with the observations adjusted for the average study effect and illustrates no relationship between ruminal pH and EMPS. These observations agree with *in vitro* studies (de Veth and Kolver, 2001; Calsamiglia et al., 2002). In contrast, total bacterial N flow is negatively related to pH. Low ruminal pH is the result of fermentation of large amounts of available OM. When the quantity of OM fermented increases, microbial protein synthesis also increases (Hoover and Stokes, 1991). As a result, the negative relationship between pH and bacterial nitrogen flow is a consequence of the increased supply of energy with highly fermentable rations (low pH). Changes in dilution rate of liquid and solids fractions of the ruminal content can also exert an important effect on ruminal fermentation and microbial growth (Russell et al., 1992). Solids and liquid dilution rates depend on various factors including level of intake, proportion of forage in the ration, and particle size of the ration (Uden, 1988; Rode and Satter, 1988; Faichney, 1993). In general, *in vitro* studies with pure or mixed cultures of rumen bacteria indicated a greater synthesis and EMPS with increases in liquid dilution rate, solids dilution rate or both (Isaacson et al., 1975; Shriver et al., 1986; Schadt et al., 1999). However, as dilution rates increase, ruminal degradation of OM and energy availability for microbial growth decrease, reducing the expected flow of bacterial N. Meng et al. (1999) reported that, in single-flow continuous cultures, when dilution rate increased from 0.03 to 0.20/h, EMPS increased 2.2-fold, whereas microbial N flow increased only 1.5- fold, likely because

of a reduction of OM truly digested, which decreased from 62.5 to 44.0%. The increase in microbial protein synthesis and EMPS that is obtained with high dilution rates has been attributed to the selection of microbial species with greater rates of growth, a higher proportion of the microbial population in the exponential phase of growth, and a dilution of the maintenance requirements of microbes. In addition, high dilution rates are associated with shorter retention times in the rumen, which reduce bacterial lysis and bacterial predation by protozoa (Firkins et al., 1992).

2.6 Microbial requirements for substrate

Many species of ruminal bacteria produce polysaccharide, and some can store large amounts as an intracellular reserve (Lou et al., 1997). McAllan and Smith (1974) reported higher microbial α-glucan content for animals on a diet with more than 70% concentrates (barley and flaked corn). A change in microbial α-glucan content with time after feeding also occurs in that particle associated microbial populations had higher α-glucan content compared to the liquid associated populations in the rumen (Craig et al., 1987). Ruminal microorganisms may incorporate and store carbohydrate as α-glucan under conditions of excess available carbohydrate (shortly after feeding) and potentially limiting nitrogen supply. It is possible that when the supply of available dietary carbohydrate diminishes, microorganisms will utilize the storage carbohydrate as a source of energy. This stored carbohydrate can also become available to other microorganisms upon cell lysis or it can pass to the small intestine and supply glucose to the animal (Russell, 1998).

Russell et al. (1992) proposed a simplified model to describe energy and protein requirements of microbial subpopulations. Microbes that degrade structural carbohydrate (cellulolytic) have low maintenance requirements, grow slowly, and use NH_3-N as their main N source, whereas microorganisms that degrade nonstructural carbohydrate (amylolytic) have higher maintenance requirements, grow rapidly, and use ammonia, peptides, and AA as N sources. Certain strains of *Butyrivibrio fibrisohens* can ferment both starch and cellulose and produce ammonia, but they degrade cellulose at a much slower rate than do other cellulolytic bacteria (Bryant, 1973). However, bacterial growth has been shown to increase with addition of AA and (or) peptides in cellulolytic and amylolytic bacteria (Maeng and Baldwin, 1976;

Argyle and Baldwin, 1989). Similarly, fiber digestion was reported to increase with the supply of AA and peptides to pure cellulolytic bacteria (Cruz Soto et al., 1994; Griswold et al., 1996; Carro and Miller, 1999. Atasoglu et al. (2001) demonstrated with pure cultures of cellulolytic bacteria, that the incorporation of NH_3-N into microbial cell N decreased as the proportion of AA increased in the medium, suggesting that cellulolytic bacteria would use AA if available. Similar findings were reported with increasing concentrations of peptides, although Atasoglu et al. (2001) reported a greater preference of cellulolytic bacteria for incorporating AA-N compared with peptide-N into their cell. However, at typical ruminal peptide and AA concentrations, about 80% of the cell, N is derived from NH_3-N.

Addition of branched chain AA that will ferment to BCVFA, and addition of peptides to ruminal fluid has increased fiber digestion, microbial protein production, and microbial growth efficiencies (Russell and Sniffen, 1984). The increase in microbial growth observed with addition of AA and (or) peptides may be due to direct incorporation of AA into microbial protein and (or) to increased availability of carbon skeletons (from AA deamination), which can be used for energy production or as carbon skeletons for new microbial AA (Bryant, 1973). Russell et al. (1983) reported that microorganisms that ferment NFC derived up to 66% of their protein from peptides or AA, and the rest from NH_3-N. Those researchers claimed that this proportion was not influenced by rate of microbial growth and that, in the absence of carbohydrate, all peptide N would be converted to NH_3-N. However, the optimum concentration of peptides in the rumen needed to maximize microbial protein synthesis has not been determined (NRC, 1996). Assuming that bacteria transform available peptides into microbial protein with an efficiency of 80% (Russell et al., 1983) and that NFC fermenting bacteria may use up to 66% of the available N in the form of peptides. Atasoglu et al. (1999) reported that the proportion of bacterial N derived from ammonia decreased as the ratio of NH_3-N: total available N decreased. Firkins et al. (1987) also reported a negative relationship between NH_3-N concentration and the percentage of microbial protein derived from NPN. These observations suggest that NH_3-N accumulation in the rumen is the result of preferential use of peptides or AA by microbes, either as a source of N or as a source of energy. Therefore, the proportion of bacterial N derived from NH_3-N is not a fixed value, and

the proposed value of 1.2 g of peptide N/kg of OM fermented may not apply to all rations. Atasoglu et al. (2004) studied the fate of N and carbons from AA in ruminal mixed microorganisms. Results showed that several AA were synthesized by rumen microorganisms with greater difficulty than others. In general, it is believed that rumen microbes do not have an absolute requirement for any AA; however, Atasoglu et al. (2004) suggested that some AA may be limiting growth. They also confirmed the theory that ruminal bacteria have difficulty synthesizing phenylalanine (Phe), leucine (Leu) and isoleucine (Ile) (Oltjen et al., 1971; Amin and Onodera, 1997) and proposed that Lys is a potential AA limiting growth of rumen bacteria. *In vitro* growth rate of the mixed ruminal bacteria was inhibited when the 3 inhibitory AA (Ile, Phe, Thr (threonine)) were each (1 m*M*) added to individual control treatments in which an ammonium salt was included as a sole N source (Kajikawa et al., 2005). Therefore, ensuring generous supplies of specific AA might result in greater microbial growth. Consideration of the inhibitory effects of some AA and the antagonism of other AA to these inhibitions may improve the precision for estimating the amount of microbial yield and metabolizable protein in such a nutritional model.

In order to obtain maximal microbial protein synthesis, the N requirement of the rumen bacteria has to be met first. Even though microbial protein synthesis can occur in the rumen of animals fed semi-purified diets containing urea as the only N source, N sources also must include amino acids and peptides in addition to NPN to maintain optimal microbial protein synthesis. It seems that diets containing a mixture of structural and NFC sources increase the yield and efficiency of microbial protein synthesis because of an improved ruminal environment for more diverse ruminal bacteria species and increased amounts and type of substrates, available for microbial protein synthesis (Karsi and Russell, 2002). Additionally other nutritional factors, such as sulfur supply, other non-nutritional factors, such as ruminal pH and dilution rate, also play an important role in microbial protein synthesis.

2.7 Protein utilization in the ruminant

Ruminants make efficient use of diets that are poor in true protein content because microbes in the rumen are able to synthesize a large proportion of the animal's required protein. The AA pattern of this protein is of better quality than

nearly all of the dietary ingredients commonly fed to domestic ruminants (Broderick, 1994; Schwab, 1996). Generally, animal feedstuffs are analysed for total N and by multiplying the CP content reported by the factor 6.25. This approach assumes that; 1) all N in the feed derives from proteins, and 2) the N content in all proteins is 160 g/kg protein. Regarding the first assumption, there are large differences between feeds. In silage, due to extensive proteolysis, more than half of the N can be NPN in the form of peptides, free amino acids, amines, ammonia, and nitrate. Regarding the second assumption, the N content in the 20 standard amino acids of proteins ranges from 80 (tyrosine) to 270 (arginine). Thus, depending on the amino acid composition, every protein has its individual N content. Several protein evaluation systems have been developed to describe and predict the amino acid uptake in dairy cows. Examples of these are: the NRC (2001), the Dutch DVE/OEB system (Tamminga et al., 1994) and the Nordic AAT/PBV system (Madsen et al., 1995). Although there are differences among the systems, they all attempt to estimate the contribution of feed and microbes to the amino acid supply of the animal.

The quantity and degradability of dietary protein affects rumen fermentation (Nocek and Russell, 1988), which ultimately affects net efficiency of absorbed nutrients. The NRC (2001) classifies dietary CP into two components, rumen degradable protein (RDP) and rumen undegradable protein (RUP), each of which possesses separate and distinct functions in ruminant diets (Figure 2.1). The Protein fraction are used in the Cornell Net Carbohydrate and Protein System (CNCPS) subsequently RDP is the sum of protein fractions A (non protein nitrogen, NPN) and B (true protein, B_1, B_2, and B_3) with degradation and passage rates as noted in the NRC (2001). Soluble protein (A and B_1 fractions) is assumed to be immediately available for utilization by rumen microbes and chemically defined as protein fractions dissolved in a borate-phosphate buffer (Sniffen et al., 1992). Potentially RDP (B_2 and B_3 fractions) is not as immediately available for utilization as soluble protein but is fractionally degraded with time. Not all B_2B_3 may be rumen-degraded due to lignin-bound proteins, passage rates, and feeding management (Tamminga, 1979). Potentially RDP is not chemically determined but can be calculated as RDP minus soluble CP. Given degradation differences between fractions of RDP (AB_1 and B_2B_3), it is apparent the type and amount of each fraction may affect overall

utilization of protein and other nutrients. Natural feed sources vary in their level of ruminal degradability, whereas most NPN sources are 100 % degreaded (Gleghorn et al., 2004). RDP is required for ruminal fermentation because it provides the mixture of peptides, free AA, and NH_3-N required for microbial growth, activity, and synthesis of microbial protein.

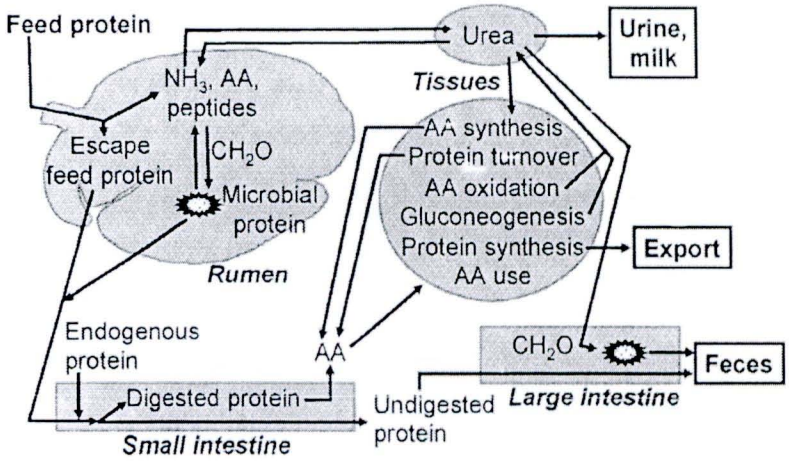

Figure 2.1 Interplay of dietary, ruminal, and extraruminal sources of N and amino acid (AA); CH_2O = carbohydrates; Export = milk, conceptus, scurf, secretions

Source: Hall and Huntington, 2008

Optimum utilization of dietary CP requires selection of complementary feed protein sources that provide the types and amounts of RDP and RUP that will meet, but not exceed, the N requirement of both rumen microbes and the animal (NRC, 2001). RUP provides a direct source of digestible AA to the animal. The animal's requirement for RUP in dietary DM in a given feeding situation is a function of need for metabolizable protein (MP) from RUP and the quality (i.e., digestibility and AA composition) of the RUP. Therefore, efficient utilization of dietary protein is importance due to dietary cost and environmental concerns. It is essential to meet requirements for both rumen degradable undegradable proteins.

2.7.1 Ruminally undegradable protein, RUP

Undegraded protein from the diet is classified as bypass and is a way to preserve higher quality protein for the host animal by inhibiting its fermentation and digestion by the rumen microbes. Protein sources are manipulated in order to obtain bypass ability, most commonly with heat to reduce the water-soluble form of the protein in the diet (Van Soest, 1994). The treatment of protein ingredient may not in fact render the protein as true bypass, but may decrease the digestibility of the protein in the rumen enough to result in most of it passing through gastro intestinal tract. Protein requirements of a lactating dairy cow are met from the supply of essential and non-essential AA reaching the small intestines in microbial and undegraded feed protein. Common protein supplements that are high in RUP and are used in ruminant diets are fish meal, meat and bone meal, feather meal, blood meal, corn gluten meal, distillers dried grains, and brewers dried grains (Santos et al., 1998). Soybean meal (SBM) is the most commonly used protein supplement in developed countries. Possible reasons for a lack of response to increased RUP are 1) microbial synthesis in the rumen decreased (Clark et al. 1992; Schingoethe, 1991) the RUP source had a poor essential AA (EAA) profile (Chandler, 1991) RUP sources in the SI had low digestibility (Schingoethe, 1991) control diets already were sufficiently high in RUP (NRC, 1989). Some studies (Clark et al., 1992; Chen et al., 1993) have suggested that the source of RUP should have an AA profile that would complement the profile of microbial protein. Infusion trials have indicated that lysine (Lys) and methionine (Met) are probably the first- and second limiting AA, respectively, for milk yield and milk protein synthesis (Schwab et al., 1992) in diets of dairy cattle. The amounts of Lys and Met as percentages of total EAA in duodenal digesta that were recommended for maximizing milk and milk protein yields were 15 and 5%, respectively (Schwab et al., 1992). Therefore, RUP supplements that are low or unbalanced in Lys and Met might result in no increase or a decrease in yields of milk and milk protein. The recommended level of RUP ranges from 32 to 39 percent of crude protein and is highly dependent on the level of milk production.

2.7.2 Rumen degradable protein, RDP

The degradation of dietary feed CP in the rumen is important because it supports rumen microbial growth. On average 59% of the non ammonia-N (NAN) that reaches the duodenum is supplied by microbial CP (Clark et al., 1992). Insufficient RDP

could lead to a ruminal NH_3-N deficiency that would depress microbial growth. However, this does not always lead to a reduction in metabolizable protein (MP) availability to the animal because reductions in microbial N flow can be offset by increases in RUP flow (Santos et al., 1998). However, an RDP deficiency can also precipitate depressed fiber digestion, which can lead to reduced DMI and energy supply to the animal (Firkins et al., 1986; Allen, 2000). Thus, it is critical to provide enough RDP to meet the requirements of ruminal microbes. RDP requirements for dairy cows generally range from 9.5 to 10.5% of dietary DM depending on diet, animal characteristics, and production level (NRC, 2001).

2.7.3 Soluble protein

Soluble protein is the portion of RDP immediately available for microbial utilization in the rumen. Krishnamoorthy et al. (1982) reported soluble N varied from 4.0% in dried brewer's grain to 53.1% of total N in oats. N fractions based on solubility in bicarbonate-phosphate buffer are in Table 2.3. Such variation is not uncommon because of inherent characteristics of feed ingredients and various treatments applied to some feed ingredients during processing. Six solvents (bicarbonate- phosphate buffer, McDougall's artificial saliva, Burrough's mineral mixture, 0.15*M*NaCI, borate-phosphate buffer and autoclaved rumen fluid) were used to compare for nitrogen solubility. Borate-phosphate buffer was found to be more stable than other solvents. Insoluble nitrogen determined with borate-phosphate buffer had a correlation coefficient of 0.92 with insoluble nitrogen obtained with autoclaved rumen fluid.

Table 2.3 Nitrogen a fractions [b] based on solubility in bicarbonate-phosphate buffer

Feedstuff	Total CP[a]	Insoluble N[a]	Soluble N[a]	Soluble true protein[c]	Soluble nonprotein nitrogen[d]
	(% DM)		(% total nitrogen)		
Corn grain	9.9	88.9	11.1	3.4	7.7
Brewers dried grain	27.9	95.9	4.1	1.2	2.9
Corn gluten feed	24.0	60.8	39.2	0.2	39.0
Beet pulp	10.2	73.5	26.5	0.7	25.8
Corn gluten meal	69.0	95.8	4.2	0.5	3.8
Oats	14.0	46.9	53.1	43.3	9.8
Distillers grains with solubles	25.1	88.8	11.3	1.1	10.2
Soybean meal, solvent extracted	54.8	79.7	20.4	9.1	11.3
Rapeseed meal, solvent extracted	42.3	67.6	32.4	9.4	23.0
Peanut meal, solvent extracted	47.5	67.1	32.9	24.3	8.6
Dried corn silage	7.9	47.8	52.2	4.0	48.2
Guinea grass hay, mature	7.1	61.2	38.8	3.3	35.5
Timothy hay, mature	8.1	74.2	25.8	0.8	25.1
Tall fescue hay, mature	11.3	74.1	25.9	0.5	25.4
Rice straw	3.9	59.2	40.8	6.6	34.2
Corn stover	4.4	57.1	42.9	3.0	39.9

[a]Expressed as N × 6.25.

[b]Average of 2 replicates. All duplicate measurements were within ± 5% of mean except soluble true protein. Duplicate measurements of soluble true protein were within ± 10% of mean.

[c]Determined by Lowry analysis.

[d]Soluble N is soluble true protein.

Source : Krishnamoorthy et al. (1982)

Chaudhry and Webster (2001) used a gel electrophoresis technique to assess ruminal degradation of soluble crude protein (CP) of several feeds; resistance to, or escape from, rumen degradation of soluble CP varied with class of feed and with type and molecular weight of soluble CP. Soluble protein from feeds such as cotton seed meal can provide adequate protein to the animal and are found in most dairy rations. The ruminal microbes utilize non-protein nitrogen which supplies the necessary NH_3-N for microbial protein synthesis. One of the more common feed ingredients in this category is urea. It gives the microbial population N in the form of ammonia, once broken down. Schwingel and Bates (1996) used electrophoresis and showed that approximately 30% of soluble CP from SBM incubated with mixed ruminal microorganisms was not degraded after 9 h. Hedqvist and Uden (2006) measured degradation of soluble CP of 20 feedstuffs *in vitro* and *in vivo* found that drastic differences in degradation of soluble CP among various protein sources. These results indicate that a fraction of soluble dietary CP might not be ruminally degraded and can, therefore, supply the animal with amino acids. However, most dairy feeding models (NRC, 2001) assume that all soluble CP is instantaneously degraded in the rumen. Bach et al. (2008) reported that the extent of degradation of the soluble CP fraction from fish meal, soybean meal, and canola meal was determined to be 99, 30, and 37% of soluble CP, respectively. These results indicate that the soluble CP fraction is not 100% degraded in all feeds and that assuming a high degradation coefficient of the soluble CP fraction from soybean meal and canola meal may result in an underestimation of the supply of undegradable protein from these protein sources. Addition of protected individual amino acids to the diet is another possible way to supply protein to the ruminant especially for the limiting amino acids such as Met and Lys. These can be added separately or linked together as peptides. This supplies the bacteria with high quality amino acids that can be used to synthesize other types or be incorporated into the bacterial metabolism (Leng and Nolan, 1984).

If there is insufficient rumen available energy or the degradation rates of RDP and carbohydrates are not synchronized, then excess NH_3-Nitrogen will be absorbed into portal blood and transported to the liver where it is converted to urea (Hoover and Stokes, 1991). Depending on prevailing dietary conditions, 40 to 60% of liver urea output is excreted in urine (Huntington, 1989), which represents an irretrievable

loss of N to the animal and is also a source of pollution that has become an environmental concern. Soluble protein should be between 30 to 32 percent of total protein or about half of the RDP level (NRC, 1989).

2.7.4 RDP and soluble protein in diets of dairy cattle

Matching the supply of rumen degradable N provided by mixtures of sources with the quantity require by microbes will maximize the capture of the degradable N as microbial protein. NRC (2001) ties RDP requirements to dietary energy intake where microbial N (g) is equivalent to 20.8 × TDN. Assuming the maximal efficiency of RDP use for microbial N synthesis is 85%, the RDP requirement would be 24.5 g per g of TDN intake.

Boucher et al. (2007) reported that the amount of RDP increased with the addition of urea to lactating Holstein cows' diets (0.3, 0.6, or 0.9% of urea in diet DM). A quadratic effect of treatment on microbial protein flow and efficiency of microbial protein synthesis was observed with maximum responses at dietary RDP concentrations of 10.8 and 10.0% of DM, respectively (RDP concentration of control diet was 9.2% of diet DM). They conclude that the optimum ruminal NH_3-N concentration required to support maximum flow of microbial protein to the duodenum was 12.8 mg/dl. The results of this study indicate that there were some positive effects of adding urea to the lactating dairy cow diet. However, in non lactating Holstein cow, Kang-Meznarich and Broderick (1981) reported that 8.5 mg/dl of NH_3-N in rumen fluid was sufficient for maximum content of microbial protein (measured by ruminal content of diaminopimelic acid) in the rumen. Satter and Slyter (1974) determined the NH_3-N requirements of mixed ruminal bacteria for microbial protein synthesis in continuous culture using a variety of substrate mixtures and tungstic acid-precipitable-N as a marker for microbial protein. Reynal and Broderick (2005) fed diets varying in RDP concentrations (7.7, 9.2, 10.9, and 12.5% of diet DM) to lactating cows with urea mixed into the TMR. They observed a linear increase in microbial NAN flow to the omasal canal in response to increasing dietary RDP. The greatest concentration of RDP (increased dietary RDP concentrations by the addition of a combination of urea and true protein from solvent SBM), fed was 12.6% of diet DM and corresponded to the greatest observed mean ruminal NH_3-N concentration of 12.3 mg/dl.

Gable and Heinrichs (2003) reported heifers were fed treatment rations containing 62.1 ± 0.8 g CP /Mcal ME at 2.0% BW with altered soluble CP fractions (AB_1) (33.6 or 40.6% of CP) and potentially rumen degradable protein fractions (B_2B_3) (20.9 or 28.2% of CP). Urinary excretion of allantoin and uric acid was not affected by increased intake of AB_1 and B_2B_3. Urinary excretion of allantoin constituted 94% of total purine derivatives (PD) excreted in this study, which is consistent with previous studies (Devant et al., 2000). However, Haig et al. (2002) determined the effects of dietary protein solubility in multiparous Holstein cows fed one of three dietary treatments that were similar in crude protein (17.7%) content but differed in their content of soluble intake protein (SIP). Dietary contents of SIP, as % of total CP were 30, 36, and 48%. As dietary content of SIP increased, excretion of urinary N increased quadratically, and it was the primary route of N excretion. Urinary excretion of PD responded quadratically as dietary SIP content increased.

Griswold et al. (2003) also observed an increase in total VFA concentrations in continuous culture when urea was added to the artificial saliva. Urea addition to the cultures resulted in increases in NH_3-N concentrations from 0.26 to 6.01 and 0.21 to 6.11 mg/dl. Increasing the level of urea (0, 0.3, 0.6, and 0.9% of diet DM) to lactating Holstein cows; urea was manually top dressed and incorporated into the ration. The basal diet, without urea addition, contained 9.2% RDP in DM and had a predicted RDP balance of -167 g/d (NRC, 2001). There were no effects of dietary treatment on ruminal true digestibility of organic matter or ruminal apparent digestibility of neutral detergent fiber and acid detergent fiber. Total ruminal volatile fatty acid concentrations increased linearly with increasing urea level. Feeding increasing amounts of urea quadratically increased rumen NH_3-N concentrations (9.0, 11.9, 12.8, and 17.4 mg/dl at 0, 0.3, 0.6, and 0.9% urea supplementation, respectively). There was no effect of urea supplementation on ruminal digestibility of DM, OM, starch, NDF, or ADF. They reported that the lowest mean concentration of ruminal NH_3-N for the control diet (9.0 mg/dl) may have been adequate, or more than adequate, for maximum digestibility of diet OM (Boucher et al., 2007). Kang-Meznarich and Broderick (1981) observed that a ruminal NH_3-N concentration of 3.3 mg/dl was adequate to support maximum ruminal digestion of DM in nonlactating cows fed a pelleted diet (75 % corn, 19.5% cottonseed hulls, and 2.8% vitamins and minerals). The pellets were fed hourly and contained increasing levels of urea. Ruminal

NH_3-N concentrations were 1.3, 3.3, 8.5, 13.8, 22.8, and 28.9 mg/dl, and the respective ruminal DM digestibility values were 61, 69, 65, 69, 67, and 68% (Kang-Meznarich and Broderick, 1981).

Altering the soluble CP and potentially rumen degradable protein fractions of diets affect rumen fermentation. Four prepubertal Holstein heifers, average 169.1 kg body weight (BW) were fed diets containing 16% CP and 71.5 % TDN with altered soluble CP fractions (AB_1) (33.6 or 40.6% of CP) and potentially rumen degradable protein fractions (B_2B_3) (20.9 or 28.2% of CP). Increased intake of AB_1 increased rumen ammonia, but decreased total volatile fatty acid concentrations and molar proportions of isovalerate and isobutyrate. Increased intake of B_2B_3 tended to increase volatile fatty acid concentrations, increased molar proportions of propionate, and decreased the acetate to propionate ratio. Nitrogen utilization was not affected by increased intake of AB_1 or B_2B_3. Feeding with increased potentially rumen degradable protein fractions (B_2B_3) affected rumen fermentation but did not affect DM digestibility or N utilization (Gable and Heinrichs, 2003). Zanton et al. (2007) also reported that dry matter, organic matter, and neutral detergent fiber digestibility were not different when postpubertal Holstein heifers (455 kg BW) were fed diets containing low or high levels of soluble protein (SP, 30 or 40% of CP) and low or high RUP (38 or 46% of CP). Excretion of urinary nitrogen was highest for diets with low SP and low RUP and with high SP and high RUP, which resulted in these rations being the least efficient in retention of apparently digested nitrogen. The proportion of consumed or absorbed nitrogen retained was not significantly different between treatments. Responses to alterations in crude protein degradability are observable in postpubertal heifers; however, the level of response may depend on the diet in which protein degradability is altered. This results concur with the findings of Gabler and Heinrichs (2003), who reported that apparently digested DM was unaffected by altering the solubility and degradability of CP provided to prepubertal Holstein heifers (147 kg initially). Likewise, when the degradability of dietary CP was altered in diets fed to Friesian crossbred heifers (101 kg initially) in a high-concentrate diet apparent total tract digestibilities of both DM and OM were unaffected by the treatments used (Devant et al., 2000). However, these results contrast with the results of Amos (1986), in which rations that contained a higher proportion of soluble CP resulted in the greatest total tract digestibility of DM, energy, and NDF. Reported responses of ruminal concentrations of

VFA to increasing ruminal NH_3-H concentrations are inconsistent (Griswold et al., 2003; Reynal and Broderick, 2005). Slyter et al. (1979) observed an increase in ruminal VFA concentrations when ruminal NH_3-H concentrations of steers were increased from 2.2 to 4.5 mg/dl, but did not observe a further increase at greater (up to 22.5 mg/dl) ruminal NH_3-H concentrations. The steers were fed diet contained 30:70 forage: concentrate, NH_3-H concentrations were altered by continuous intraruminal infusions of different amounts of urea. Although there is a difference in the range of observed NH_3-H concentrations, the findings of Slyter et al. (1979); Griswold et al. (2003) and Boucher et al. (2007) found that urea addition resulted in an increase in total VFA concentrations.

Renal and Broderick (2005) studies on twenty-eight lactating Holstein cows were fed diets with different levels of RDP at 13.2, 12.3, 11.7, and 10.6% of DM. Intake of DM and yield of milk, fat-corrected milk, and fat were not affected by treatments. Dietary RDP had positive linear effects on milk true protein content and non ammonia-N flow at the omasal canal and a quadratic effect on true protein yield, with maximal protein production at 12.3% RDP. Other research indicates that microbial synthesis may be improved when RDP is greater than 10.4% (Stokes et al., 1991). Cyriac et al. (2008) reported that fourty mid-lactation cows (36 Holstein and 4 Jersey × Holstein cross-breds) were fed diet formulated to contain 11.3, 10.1, 8.8, or 7.6% RDP; whereas, ruminally undegradable protein remained constant at 7.1% of DM. Dietary RDP had no effect on body weight or milk fat, protein, and lactose contents. Milk protein yield was not affected by RDP level; however, milk fat yield decreased linearly as dietary RDP was reduced. However, the studies by Kalscheur et al. (2006), Thirty-two multiparous and 16 primiparous Holstein cows in midlactation averaging 126 day in milk were used to determine the effects of RDP concentration on lactation performance. Diets were formulated to provide 4 concentrations of dietary RDP at 6.8, 8.2, 9.6, and 11.0% of DM while rumen-undegraded protein remained constant (5.8% of DM). Dry matter intake was not affected by treatment. Milk yield, fat yield, and protein yield all increased linearly when cows were fed diets with greater RDP as compared to lower controls. Milk fat and protein concentration each increased by 0.16 percentage units for cows fed 11% RDP compared with 6.8% RDP. Milk protein yield increased by 0.19 g/d for every 1 g/d increase in crude protein supplied mainly as RDP. As RDP increased, the efficiency of N use declined linearly. Milk urea N increased linearly when cows were fed increasing

amounts of RDP, indicating increased losses of N via urine. Feeding deficient RDP diets to dairy cows can decrease N excretion, but it also decreases lactation performance.

2.8 Carbohydrates utilization in the ruminant

The main component of the ruminant diet is carbohydrates and provides the majority of the animal's energy required by rumen fermentation. The uptake and hydrolysis of polysaccharides by microbe results in hexoses and pentoses are readily fermented and support microbial growth. These sugars are rapidly fermented into VFA and can provide up to 70% of the energy requirements of the cow (Van Soest, 1994). The first system that partitioned carbohydrates was the proximate analysis or Weende system developed by Henneberg at the Weende experiment station in Germany (Maynard and Loosli, 1975). The proximate analysis system divides carbohydrates into crude fiber (CF) and nitrogen-free extract (NFE) fractions. However, in the rumen, portions of the CF are sometimes digested and portions of the NFE are indigestible. These failings of the proximate analysis system in partitioning carbohydrates led to the development of analyses that are more nutritionally relevant.

The detergent system, originally developed at the USDA (Goering and Van Soest, 1970), divides plant carbohydrates by their solubility in detergent solutions. The feed components that are insoluble in a neutral detergent solution (pH ~ 7.0) are NDF (include cellulose, hemicellulose, and lignin). The feed components that are insoluble in an acid detergent solution (pH ~ 2.0) are ADF (include cellulose and lignin). These two measures of fiber differ by their hemicelluloses content. Providing adequate levels of fiber is also critical to stimulate rumination, saliva production and maintenance of normal milk fat and protein composition. Forage should depends on forage quality or comprise no less than 40 to 50 percent of the total ration DM for corn silage. Minimum ADF and NDF levels should be 20 and 26 percent, respectively. At least 75 percent of the NDF should come from a forage source (NRC, 2001).

The feed components that are soluble in a neutral detergent solution with heat-stable, α-amylase are labeled nonfiber carbohydrates (NFC). These include mono-, di-, and oligosaccharides (sugars), starches, organic acids, fructans, and pectic substances and other carbohydrates of the appropriate solubility. The NFC fraction of feedstuffs is

estimated from the following calculation: NFC = 100 - CP - EE - ash - NDF (NRC, 2001). The terms NSC and NFC have at times been used interchangeably for the fraction derived by this calculation. However, NSC refers to cell contents, and includes organic acids (which are not carbohydrates), mono- and oligosaccharides, starch and fructans, whilst NFC also includes soluble fiber: pectic substances, β-glucans and galactans (Van Soest et al., 1991). The NRC (2001) applies a total digestible nutrient content of 98% to the NFC fraction and it can therefore play a major role in the nutrient supply to the animal. Synchronization of rates of digestion among sources of NFC and NDF is important to provide a continuous supply of available carbohydrates. Appropriate NFC levels can improve both milk fat and protein while overfeeding often leads to milk fat depression. The detergent system is preferred over the proximate analysis system for feed analysis because improved separation of the most and least digestible fractions of feeds is achieved. However, carbohydrates in the NFC fraction are not uniform in their nutritional characteristics and partitioning of the NFC as related to digestion characteristics. The potential end products formed during fermentation of NFC are present in Table 2.4.

2.8.1 Starch

Starch is a polymer of glucose molecules and is the major storage carbohydrate in most cereal grains. It consists of amylose, a predominantly linear α-(1→4) linked polymer, and amylopectin, α-(1→4) linked polymer with α-(1→6) linked branches, which can be present in various ratios. Amylopectin comprises 70 to 80% of most cereal starches and amylose 20 to 30% (Rooney and Pflugfelder, 1986). The proportions of these two polysaccharides appear to affect the digestion characteristics of starch. According to Piva and Masoero (1996) amylose is slowly degraded in the rumen, whereas amylopectin is more rapidly degraded. Nonetheless, starch fermentation in the rumen is considered to be extensive, but can vary from 40 to 90% (NRC, 2001) depending on factors such as structure (amylose/amylopectin ratio; plant source and processing or physical form (Rooney and Pflugfelder, 1986; Piva and Masoero, 1996). Starch can be degraded by ruminal microbial enzymes as well as enzymes in the small intestine of the animal. A series of enzymes are required to degrade amylose and amylopectin to glucose in both the rumen and small intestine. These include randomly acting endo-α-amylases releasing maltodextrins from amylose

and β amylases removing maltose units from the non-reducing end of the chain. Approximately 50% of amylopectin can be degraded by β-amylase to maltose.

Table 2.4 Neutral detergent soluble carbohydrate of selected feedstuffs and their fermentation end products

Feed	Major NFC factions	intermediates
Alfalfa hay	Pectin and sugar	Acetate and lactate
Alfalfa silage	Pectin, sugar, and organic acids	Acetate, lactate, and butyrate
Corn silage	Starch and organic acids	Lactate
Grass hay	Fructan and sugar	Lactate
Grass silage	Organic acids and sugar	Lactate
Corn grain	Starch	Lactate
Barley grain	Starch and b-glucans	Lactate and acetate
Wheat grain	Starch and b-glucans	Lactate and acetate
High moisture corn	Starch and organic acids	Lactate
Hominy	Starch	Lactate
Molasses	Sugar	Lactate
Whey	Sugar	Lactate
Beet pulp	Pectin	lactate
Citrus pulp	Pectin and sugar	Acetate and lactate
Almond hulls	Pectin and sugar	Acetate and lactate
Soy hulls	Pectin	Acetate
Wheat midds	Starch	Lactate

(Varga, 2003)

The residue is hydrolyzed by glucoamylase (cleaving α-(1→4) linkages), and α-dextrin-6-glucanohydrolase and isoamylase (cleaving at the α-(1→6) linked branch points). Maltose and maltodextrins are degraded to glucose by α-glucosidase (Chesson and Forsberg, 1997). There are numerous amylolytic ruminal bacteria, which include *Ruminobacter amylophilus*, *Prevotella ruminicola*, *Succinimonas amylolytica*, *S. bovis*, *S. ruminantium*, *B. fibrisolvens*, *E. ruminantium* and *Clostridium spp.* (Cotta, 1988).

Corn, wheat, oats, barley, sorghum, cassava tuberous root and many by-products such as hominy, bakery waste and cassava pulp are common feedstuffs that have high starch contents. Corn contains an average of 72% of DM as starch (Huntington, 1997), which has the potential to be highly digestible and rapidly fermented in the rumen. Total tract digestibility of corn ranges from 91.2 to 98.9% depending on the processing method and grain type, with ground corn averaging 93.5% (Huntington, 1997). Cassava starch is the high amylopectin content (70%) making it a particularly suitable energy source for ruminants, particularly when combined with nonprotein nitrogen in feeds (Mailer, 1977). If a starch source is rapidly fermented and represents a large portion of the diet, ruminal acidosis can develop, which can cause digestive upset and a depression in intake and digestibility of other nutrients (Nocek, 1997). Management practices that promote consumption of large meals in a short time frame and heat stress conditions also may contribute to ruminal acidosis due to fermentation and respiration, respectively. Huntington (1997) proposed that almost all of the adversities associated with feeding high-grain diets are caused by excessively rapid fermentation of starch to VFA. These include acetate, propionate, and butyrate, as well as lactate. High starch diets have been associated with relatively increased propionate and decreased butyrate concentrations (Strobel and Russell, 1986; Friggens et al., 1998; Heldt et al., 1999). Moreover, rate of starch availability and rates of passage for starch and OM from the rumen are important determinants of microbial protein synthesis *in vivo*. Starch that is not degraded and utilized by ruminal microbes may be digested in the intestines. The pancreas secretes α-amylase which breaks down amylose and amylopectin into linear oligosaccharides and limit dextrans. The amount of starch digested post-ruminally can vary from 5 to 20% of starch from the diet with most of that being digested in the small intestine (Streeter et al., 1991; Hill et al., 1991; Zinn, 1991).

2.8.2 Sugars

The term "sugars" is used collectively to describe mono-, di-, and oligosaccharides. These are comprised of one, two, or less than twenty monosaccharide (typically hexose or pentose) residue molecules, respectively (Zubay, 1998). The sugar content of most of the common feeds fed to lactating cow can range from less than 1 to over 20% of DM (Hoover and Miller-Webster, 1998). Glucose and fructose are the most

common monosaccharides found in plants. Sucrose, the most common disaccharide found in plants, sucrose, fructose, and glucose are found in relatively high amounts in molasses, a common cattle feed. Another feed that can have a high content of sugars is citrus pulp, but the content is quite variable. Measured values of sugars in citrus pulp range from 12% to 40% on a dry matter (DM) basis (Hall, 2002). As with other fermentable carbohydrates, fermentation of sugars can alter the ruminal environment as well as the supply of metabolizable nutrients to the cow. Bacteria and protozoa are known to convert a portion of sugars to glycogen (α-linked storage polysaccharide in bacteria) (Thomas, 1960). The fermentation of sugars *in vitro* or *in vivo* shows variation among sugar sources in the yield of products and effects on the ruminal environment, and differences with other NFC sources.

Sucrose and its constituent monosaccharides glucose and fructose are the predominant saccharides of the mono- and oligosaccharide component of NFC and are found in byproduct feeds such as molasses (Kunkle et al., 2000), sugar beet pulp (Hall, 2002) and citrus pulp (Ben-Ghedalia et al., 1989). Sucrose is a disaccharide consisting of single glucose and fructose monomers linked through an α-1→2 linkage. It is considered to be 100% degradable in the rumen (Sniffen et al., 1992) and is reported to be fermented at a rate as high as 300%/h (Sniffen et al., 1983). Sucrose is hydrolyzed to glucose and fructose by the enzyme sucrase (Van Soest, 1994). Ruminal bacteria that ferment sucrose include *Streptococcus bovis, Lachnospira multiparus, Lactobacillus ruminis, Lactobacillus vitulinis, Clostridium longisporum, Eubacterium cellulosolvens,* and some strains of *Eubacterium ruminantium, Butyrivibrio fibrisolvens, Ruminococcus albus, Ruminococcus flavefaciens, Megaspaera elsdenii, Prevotella spp., Selenomonas ruminantium* and *Succinivibrio dextrinosolvens* (Stewart et al., 1997).

2.8.3 Pectic substances and pectin

Pectic substances are found in the middle lamella and other cell wall layers (Van Soest, 1994), but are not covalently linked to the lignified portions. They are almost completely digested (90-100%) in the rumen (Nocek and Tamminga, 1991; NRC, 2001). Pectic substances are a family of complex molecules that contain a great variety of monomers and potential branch-points. The pectin backbone consists of galacturonic acid monomers linked via α-(1→4) linkages, and rhamnose inserts. With the addition of neutral sugar side chains, made up largely of arabinose

and galactose, bound to the rhamnose inserts, these complex molecules are referred to as pectic substances (Jarvis, 1984). Various degrees of methoxylation and acetylation of the galacturonic acid backbone are possible (Jarvis, 1984; Marounek and Duskova, 1999). The acid groups in the backbone can also be associated with calcium ions (Van Soest, 1994). Animals do not have the enzymes to digest pectin, but microorganisms in the rumen do (McDonald et al., 1995). At least two enzymes, a methylesterase and polygalacturonidase, are required for pectin hydrolysis (Baldwin and Allison, 1983). Pectin-utilizing bacteria include some of the prominent ruminal populations such as *Fibrobacter succinogenes, P. ruminicola, B. fibrisolvens*, *S. bovis* and *Lachnospira multiparus* (Czerkawski and Breckenridge, 1969; Gradel and Dehority, 1972; Baldwin and Allison, 1983).

Pectin is degraded by three groups of bacteria (Kasperowicz, 1994). First, there are bacterial species that degrade pectin and/or use the degradation products. Second are the bacterial species that degrade pectin but can only use the simple sugars. Finally, the third group consists of bacteria that cannot degrade pectin, but utilize the oligogalacturonides and galacturonic acid from the degradation of pectin. *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, and *Lachnospira multiparous* make up the first group of bacteria (Stewart and Bryant, 1988). *Streptococcus bovis* falls in the second group, while *Selenomonas ruminantium* and *Fusobacterium* belong to the third group (Ziolecki et al., 1972). Gradel and Dehority (1972) showed that pure strains of *P. ruminicola*, *L. multiparous*, and *B. fibrisolvens* were able to ferment up to 80% of citrus pectin.

2.8.4 Effects of NFC on microbial fermentation products

The NFC fraction is considered a good source of readily available energy for microbial growth (Ariza et al., 2001). It has been suggested that microbial growth is directly proportional to the rate of carbohydrate degradation (Russell et al., 1992). As documented in the Cornell Net Carbohydrate and Protein System model, simple sugars are considered to have a fast degradation rate, but starch and pectin an intermediate rate (Sniffen et al., 1992). Differences among NFC components regarding microbial fermentation may also imply that the complement of NFC in a particular feedstuff is important when predicting animal response.

Hall and Herejk (2001) compared the MCP yield from sucrose, starch and pectin in an *in vitro* fermentation with mixed ruminal microorganisms. Microbial growth was initiated most rapidly on sucrose, followed by pectin and starch. Maximal yield was greatest for starch compared to sucrose and pectin ($P<0.01$). An explanation offered for the proportional difference between maximal yields of MCP for sucrose and starch was the difference between the NFC source in the amount of hexose, and relative amounts of carbon available to the microorganisms. However, this approach still overestimated the theoretical maximal yield of MCP from sucrose. In an *in vivo* study, Cameron et al. (1991) found no effect on microbial nitrogen flow to the small intestine and efficiency of microbial crude protein synthesis in response to starch and dextrose supplementation to mid-lactation Holstein cows. Contrastly, Oba and Allen (2003) study in eight ruminally and duodenally cannulated Holstein cows (55 ± 15.9 days in milk). Experimental diets contained either ground high moisture corn (HM) or dry ground corn (DG) at two dietary starch concentrations (32 vs. 21%). Diets were formulated for 18% CP. They found that microbial N flow was greater for high starch diets compared with low starch diets, but was not affected by corn grain treatment. Microbial efficiency was lower for HM compared with DG treatment (39.7 vs. 48.4 g of microbial N/kg of true ruminally degraded OM), but was not affected by dietary starch concentration. Microbial efficiency was positively correlated with rate of passage for OM and starch ($r=0.77$ and 0.75, respectively). Ribeiro et al. (2005) showed that bacterial OM production in continuous culture increased linearly from 12.3 to 14.4 g/d as the concentration of sucrose increased from 0 to 8%. Rapid passage rate may have decreased microbial turnover in the rumen, enhancing microbial efficiency. Microbial efficiency was negatively correlated with rate of starch digestion ($r=-0.55$), consistent with the energy spilling theory. However, energy spilling did not appear to be from lack of ammonia or low ruminal pH. Microbial efficiency was not related to ruminal NH_3-N concentration, mean of ruminal pH, or minimum ruminal pH. Rate of starch availability and rates of passage for starch and OM from the rumen are important determinants of efficiency of microbial protein synthesis *in vivo*.

2.8.5 Effects of NFC on ruminal pH and fiber digestion

NFC supplementation has the potential to decrease ruminal pH. Low pH in turn may decrease MCP efficiency (Russell et al., 1992), which may be related to energy spilling strategies of ruminal microorganisms to cope with excess available carbohydrate and low pH (Russell and Strobel, 1993). One example of an energy spilling strategy involves the ability of *S. bovis* to ferment glucose to lactate which only yields 2 ATP molecules per glucose molecule as opposed to acetate, formate and ethanol which yield approximately 3 ATP molecules per glucose molecule (Russell and Baldwin, 1979). At a low pH, *S. bovis* decreases its intracellular pH, which favors lactate production (Russell and Hino, 1985). Low pH has also been shown to increase the maintenance energy cost of ruminal microorganisms and thus decrease microbial cell yield (Shi and Weimer, 1992). It appears that cellulolytic bacteria are especially sensitive to low ruminal pH. However, a moderate decrease in pH from 6.8 to 6.0 does not always affect cellulolytic numbers (Van der Linden et al., 1984) and isolated fibrolytic enzyme activity remains high in this range (Smith et al., 1973). On the other hand, a decrease in pH below 6.0 has been reported to result in loss of fibrolytic activity and decreased numbers of cellulolytic bacteria *in vitro* and *in vivo* (Slyter et al., 1970; Mould and Ørskov, 1984). At a pH between 4.5 and 5.0 there is virtually complete inhibition of fiber digestion (Hoover et al., 1984; Mould et al., 1984). Russell and Dombrowski (1980) observed washout of cellulolytic bacteria in continuous culture fermentations at a pH below 6.0. Huhtanen and Khalili (1992) reported a decrease in cellulolytic and hemicellulolytic enzymes at decreased ruminal pH, when sucrose was supplemented to cattle on grass-silage based diets. It is thus not surprising that one of the major results of decreased ruminal pH has been a decrease in fiber digestion, reported both *in vitro* and *in vivo*.

The effect of NFC supplementation (especially sucrose and starch) on fiber digestion, Piwonka and Firkins (1996) suggested that there might be a carbohydrate effect related to microbially produced inhibitors, which is independent from pH. Substitution of dried, pelleted beet pulp for high moisture corn did not affect ruminal pH and increased both extent and rate of NDF digestion (Voelker and Allen, 2003b) when fed to Holstein cows on an alfalfa and corn silage diet in early lactation. In several other studies increased NDF digestion as a result of supplementing sugar beet pulp or citrus pulp (Zinn and Owens, 1993; Miron et al., 2002) for barley or corn has been reported. In Penner and Oba

(2009) study was undertaken to investigate the effect of feeding diets varying in sugar concentration to postpartum transition cows. Fifty-two Holstein cows, including 28 primiparous and 24 multiparous cows were used. Feeding high sugar tended to increase nadir (5.62 vs. 5.42), mean (6.21 vs. 6.06), and maximum pH (6.83 vs. 6.65). Moreover, the digestibility of DM, OM, NDF, and starch were not affected by treatment, averaging 63.3, 65.2, 43.2, and 93.5%, respectively. *In vitro* and *in vivo* studies (Heldt et al., 1999; Broderick and Radloff, 2004; Vallimont et al., 2004; Broderick et al., 2008) have reported no effect of sucrose on rumen pH. Penner and Oba (2009) and Penner et al. (2009) reported that sucrose tended to improve ruminal pH. There are several possible explanations for why the replacement of starch by sucrose improved ruminal pH status. Disappearance of carbohydrates from the rumen may not necessarily result in fermentation acid production if OM is converted to microbial N (Allen, 1997) or stored as glycogen (Hall and Weimer, 2007).

Decreased fiber digestion *in vivo* is often associated with supplementation of forage diets with readily fermentable carbohydrate sources. Cameron et al. (1991) reported decreases in ruminal NDF and ADF digestion for lactating dairy cows receiving supplements of starch and dextrose (glucose). Heldt et al. (1999) reported a decrease in total tract NDF digestion relative to control diet-fed animals in steers supplemented at 0.3% BW of DM/d with starch, sucrose, glucose or fructose with low-quality, tall grass-prairie hay. The diets in this study were supplemented with degradable intake protein at 0.031% BW of DM/d, which may have been below the amount needed to meet ruminal microbe requirements for a degradable nitrogen source. In a second study, with the same NFC sources, but with supplemental degradable intake protein of 0.122% BW of DM/d, total tract NDF digestion increased with NFC supplementation (Heldt et al., 1999). Also, ruminal pH decreased more in animals consuming the starch diet, and these animals had a lower total tract NDF digestion compared to animals fed the sugar (sucrose, glucose and fructose) diets. Some of the decreases noted for fiber digestion may be the result of competition between NFC and fiber utilizing microorganisms for the nitrogen supply. A decrease in ruminal fiber digestion is often attributed to a decrease in ruminal pH caused by rapid fermentation of NFC and production of VFA by ruminal microorganisms (Hoover, 1986). Several studies have contradicted this concept and reported no effect on pH and varying effects on fiber digestion as a result of

supplementation with starch or sucrose (Aldrich et al., 1993; Casper et al., 1999). In a two-part study by Khalili and Huhtanen (1991), a decrease in both pH and NDF digestion was reported in animals fed a grass silage and barley-based diet with supplementation of sucrose at 1 kg DM/day. However, Vallimont et al. (2004) observed a quadratic increase in NDF digestibility when sucrose replaced corn starch in continuous culture, the negative effect on pH and NDF digestion was reduced when sodium bicarbonate was supplemented in combination with sucrose.

The potentially greater risk for ruminal acidosis must be considered with inclusion of sugar in the diets for postpartum transition cows because they are susceptible to ruminal acidosis (Penner et al., 2007). However, Penner et al. (2009) demonstrated that replacement of cracked corn with sucrose did not decrease ruminal pH. As such, the replacement of starch with sucrose may have the potential to improve nutrient supply and digestibility to transition cows without negatively affecting ruminal fermentation.

2.8.6 Effects of NFC on animal response

1) Dry Matter Intake

According to Nombekela et al. (1994) evaluated the palatability of diets with a sweet, sour, salty, or bitter taste by comparing the voluntary DMI and preferential eating order of each respective diet. They found that diets containing sucrose (sweet) were preferentially eaten 59% follow by control (36%), bitter (4%), NaCl (1%), and sour (0.3%) of the time, respectively. Sucrose inclusion (1.5 % DM basis) increased DMI by 13% compared with the control diet. In a subsequent study, Nombekela and Murphy (1995) fed diets with (1.5% of dietary DM) or without sucrose to cows during the first 12 wk of lactation. They found that inclusion of sucrose did not affect DMI, and small improvements in palatability likely had a marginal impact on DMI during early lactation. Variability in the effects of feeding starch- or pectin-rich diets was illustrated in studies by Leiva et al. (2000). In this study cows were fed one of two diets with the NFC fraction providing predominately either citrus pulp or hominy in eleven multiparous Holstein cows. The average concentrations of CP and NDF for the diets were 17.9 and 36.1% of DM, respectively. Intakes in kg/d of DM, CP, and NDF were all similar with cows on the citrus pulp diet (15.1% starch, 4.8% sugar) and cows on the hominy diet (26.5% starch, 2.5% sugar). Several studies reported no effect of NFC type on forage intake for a variety of forages and animals, including hay for steers, pasture for lactating dairy cows and

silage for sheep (Charmely et al., 1991; Heldt et al., 1999; Delahoy et al., 2003). However, cattle consuming diets containing pectin-rich feeds have been shown to increase intake (Lees et al., 1990; Chester-Jones et al., 1991), while including sugars in diets occasionally has resulted in increased intake (Maiga et al., 1995; Broderick et al., 2002). Broderick and Radloff (2004) fed dried or liquid molasses as a source of sugar in replacement for high-moisture corn grain, and found increased DMI with additional sugar. These results indicated that replacement of corn with sucrose increases DMI, and demonstrate a potential nutritional strategy for increasing DMI during early lactation. In Penner and Oba (2009) study found that cows fed high sugar (8.4% of DM) had increased dry matter intake compared with those fed low sugar (4.7% of DM) (18.3. vs. 17.2 kg/d; $P<0.05$). Some studies have reported increased NDF passage to the omasum with increased dietary sucrose concentration (Broderick et al., 2008), whereas others have reported increases in the solid or liquid passage rates with sucrose (Rooke et al., 1987; Sutoh et al., 1996).

Broderick et al. (2008) have reported a linear increase in DMI as the proportion of sugar increased from 0 to 7.5%. Dietary modifications that increase DMI during the postpartum phase of the transition period should also increase energy intake and may improve lactation performance. The varying responses in forage and DMI among studies may be due to differences in the amount, source and combination of NFC supplemented. However, it would appear that in general the different NFC sources do not differ from each other in their effect on dry matter intake, and potentially also forage intake. DMI and feed efficiency have varied when replacing starch with soluble fiber (primarily pectin) or sugar sources.

2) Volatile fatty acids

Total volatile fatty acids: Total volatile fatty acid (VFA) production is generally similar among different NFC sources both *in vitro* and *in vivo* (Chamberlain et al., 1993; O'Mara et al., 1997; Leiva et al., 2000; Sannes et al., 2002; Voelker and Allen, 2003c). Total VFA concentration increased ($P<0.05$) or tended to increase ($P<0.10$) in lactating dairy cows fed a total mixed ration (TMR) containing dried citrus pulp and high moisture ear corn in a 50:50 ratio compared to cows receiving a TMR containing high moisture ear corn or cracked shelled corn, respectively (Broderick et al., 2002b). Acetate, propionate and butyrate are the major VFA included in the total VFA

concentration. Despite giving relatively similar total VFA yields there may be differences in the relative proportions of individual VFA from different NFC sources.

Acetic acid: Acetate is a lipogenic nutrient, a precursor of fatty acid synthesis and ultimately of milk fat synthesis in the mammary gland (Van Soest, 1994). Starch and sucrose have been associated with relative decreases in ruminal acetate concentration (Chamberlain et al., 1993; Moloney et al., 1994; Heldt et al., 1999), whereas pectin had either no effect (Van Vuuren et al., 1993; Leiva et al., 2000) or increased acetate in the rumen (Broderick et al., 2002b; Voelker and Allen, 2003c). *In vitro* fermentations of different carbohydrates showed a greater acetate production from pectin compared to starch and sucrose ($P<0.05$). The effect of sugars on the ruminal molar proportion of acetate *in vivo* may depend on the amount of sucrose or glucose included in the diet Sucrose supplementation at 10% of silage DM intake did not affect ruminal acetate molar proportion for sheep compared to those on the silage control diet ($P>0.05$; Charmely et al., 1991). When sucrose was substituted for corn at 3.2% of diet DM in a diet for lactating dairy cows, acetate production was also not affected ($P>0.10$; Sannes et al., 2002). Dextrose (glucose) at 5.6% of diet DM did not affect ruminal acetate proportions in heifers compared to a medium concentrate diet containing 39.7% ground barley, or the control diet containing 10% ground barley ($P>0.05$; Piwonka et al., 1994). When cane molasses was fed to steers at 61% of DM intake, ruminal acetate proportions was decreased compared to steers fed a diet with the same amount of barley, a starch source ($P<0.01$; Moloney et al., 1994).

Pectin is reported to ferment primarily to acetate (Czerkawski and Breckenridge, 1969). When citrus pectin was fermented in cultures of *B. fibrisolvens* 787 and *P. ruminicola* AR29, 73.7 and 57.3% of metabolite carbon was captured in acetate, respectively (Marounek and Duskova, 1999). When increasing concentrations of citrus pulp substituted for high moisture corn in lactating dairy cow diets results in a linear increase ($P<0.01$) in the ruminal acetate proportion (Voelker and Allen, 2003c). It would appear that fermentation of pectin in general increases the molar proportion of acetate compared to fermentation of sugars and starch, while sugars often decrease the acetate proportion when compared to starch.

Propionic acid: Propionate is a precursor for glucose synthesis in the liver and thus important for the glucogenic energy supply to the ruminant. The effect of sugars compared to starch on ruminal propionate proportion varies among *in vivo* studies. In some studies ruminal molar proportions of propionate did not differ between sugars and starch, whether small amounts (5.6% dextrose; Piwonka et al., 1994) or larger amounts of sugar (61% molasses) were added to the diet (Moloney et al., 1994). In contrast, ruminal propionate molar proportions in sheep on a starch-supplemented diet were similar to that of sheep fed the control ryegrass silage diet ($P > 0.05$), whereas ruminal propionate proportions increased ($P<0.05$) for sheep fed a sucrose supplementation (Chamberlain et al., 1993). In another contrasting study, ruminal molar proportions of propionate tended to increase with starch supplementation ($P>0.05$) compared to supplementation of sugars (sucrose, glucose and fructose) when a low amount of ruminally degradable protein (RDP; 0.031% BW/d) was supplemented to steers, and increased ($P<0.01$) ruminal propionate proportions when a higher amount (0.122 % BW/d) of RDP was supplemented (Heldt et al., 1999). It may be that other components of the diet such as protein alter the yield of propionate from NFC.

Pectin yielded less ($P <0.05$) propionate compared to starch and sucrose when fermented *in vitro* with mixed ruminal bacteria (Strobel and Russell, 1986). Broderick et al. (2002b) also reported higher ruminal propionate proportions in lactating dairy cows fed high moisture ear corn ($P<0.01$) and cracked shelled corn ($P<0.05$) compared to cows fed a diet in which citrus pulp substituted for 50% of high moisture ear corn. However, when beet pulp was substituted for 50% of the corn in a continuous culture study no difference was found for the molar proportion of propionate ($P>0.05$, Mansfield et al., 1994). Other researchers also reported no effect on ruminal propionate proportions in lactating dairy cows when replacing beet pulp for ground corn (O'Mara et al., 1997) and replacing dried citrus pulp for corn hominy (Leiva et al., 2000). The varied propionate response when feeding citrus and beet pulp could be a result of the variation in composition of these feedstuffs.

Butyric acid: Butyrate supplies energy to the animal, mainly to the heart and skeletal muscle, in the form of β-hydroxybutyrate ,(a ketone body; McDonald et al., 1995). It is ketogenic and can be used for the production of fat. Ruminally produced butyrate is converted to β-hydroxybutyrate in the ruminal epithelial cells, and is

considered more effective than propionate or acetate in enhancing development of ruminal papillae (Van Soest, 1994). Overall, it would appear that sucrose yields more butyrate than other NFC. Several *in vivo* studies also reported increased butyrate production from sucrose compared to starch. Ruminal butyrate proportions in cannulated steers increased with sugar (sucrose, glucose and fructose) supplementation compared to supplementation with starch (Heldt et al., 1999). Khalili and Huhtanen (1991) reported greater ruminal molar proportions of butyrate for bulls consuming a sucrose-supplemented diet compared to grass silage and barley-based diet. Steers fed a molasses-based diet also had increased ruminal butyrate proportions compared to those fed a barley-based diet (Moloney et al., 1994). Studies that have evaluated the fermentation of pectin or feeds that are reported to contain substantial amounts of pectin have shown differences among microorganisms in the yield of butyrate. Pectin fermentation in a *B. fibrisolvens* 787 culture yielded a small amount of butyrate (2.6 mmol/l), while no butyrate production was detected in a culture with *P. ruminicola* AR29 (Marounek and Duskova, 1999). *In vivo* comparisons of the fermentation of feeds high in starch and those that typically contain a high proportion of pectin (citrus and beet pulps) have shown no difference (Ben-Ghedalia et al., 1989; Leiva et al., 2000) or an increase in ruminal butyrate concentration (Broderick et al., 2002b; Voelker and Allen, 2003) for animals consuming diets containing pulps. Citrus pulp can contain between 12.5 and 40.2% sugars, and sugar beet pulp between 12.8 and 24.7% (Hall, 2002). The increase in the proportion of butyrate in these studies may be a result of the fermentation of sugar rather than of the soluble fiber content.

Branched chain volatile fatty acids: The branched chain volatile fatty acids (BCVFA), isobutyric, iso-valeric and 2-methylbutyric acid result from the deamination of valine, leucine and iso-leucine, respectively (Van Soest, 1994). Branched chain VFA serve as carbon skeletons to ruminal microorganisms for the synthesis of MCP from ammonia. In fact, the value of amino acids to cellulolytic organisms that have an obligate need for BCVFA appears to be mainly as a source of BCVFA (Stern, 1986). Sheep fed diets supplemented with sucrose or starch showed decreased proportions of ruminal BCVFA compared to those fed a silage control diet (Chamberlain et al., 1993), The ruminal concentrations of BCVFA for lactating dairy cows were greater for animals fed a corn control diet compared to those receiving a diet with sucrose ($P<0.05$)

substituted for corn at 3.2% of diet DM (Sannes et al., 2002). The apparently consistent thread here is that BCVFA concentrations are less for diets with more sucrose relative to starch.

Lactic acid: Compared to acetate, butyrate and propionate (average p*K*a = 4.8), lactate (lactic acid, p*K*a = 3.1) is a 10-fold stronger acid (Dawson et al., 1997). An increase in lactate concentration therefore has a greater potential to decrease ruminal pH. The fermentation of sugars and starch can yield lactate (Strobel and Russell, 1986), whereas pectin fermentation is generally not associated with lactate production (Strobel and Russell, 1986; Hatfield and Weimer, 1995). *In vitro* fermentations of sucrose with mixed ruminal microorganisms gave a higher lactate concentration compared to fermentations with starch ($P<0.05$; Strobel and Russell, 1986). Heldt et al. (1999) also reported higher ruminal proportions of lactate for steers fed sugar supplements (sucrose, glucose, fructose) compared to those fed starch. However, in a study with cannulated steers, animals fed a barley-based diet tended to have higher ruminal concentrations of L-lactate compared to those receiving a molasses-based diet ($P<0.10$; Moloney et al., 1994). This difference in lactate production response may have been a result of a difference in the source of starch (corn starch vs. barley) and sugar (sucrose, glucose and fructose vs. molasses) supplemented in the two studies. Although pectin is generally not associated with the production of lactate, pectin fermentation has been shown to yield small amounts of lactate (Czerkawski and Breckenridge, 1969). *In vivo* studies showed no effect on lactate production in animals fed diets containing citrus pulp ($P>0.05$; Leiva et al., 2000) or sugar beet pulp ($P>0.05$; Voelker and Allen, 2003) compared to those fed corn hominy and high moisture corn supplements, respectively. In general, pectin is not expected to yield lactate as compared to sugar and starch.

3) Milk Production and Composition

The effect of different NFC sources on milk yield and milk composition is inconsistent. When compared to performance on diets containing more starchy feeds, cows consuming pectin-rich feeds had decreased milk yield and milk protein percentage (Leiva et al., 2000; Solomon et al., 2000; Broderick et al., 2002b) while increasing milk fat concentration (Lees et al., 1990; Mansfield et al., 1994). In three studies, substituting beet pulp for ground corn (Delahoy et al., 2003) and citrus pulp for corn (Solomon et al., 2000) had no effect on milk production. In other studies, substituting citrus pulp (100%)

for high moisture ear corn or cracked shelled corn or corn meal decreased milk production (Leiva et al., 2000; Broderick et al., 2002b; $P<0.01$). Varied responses to NFC supplementation in these studies may be due to differences in carbohydrate composition of feedstuffs such as beet pulp and citrus pulp, and also variation in composition within a particular feedstuff.

The influence of sugar and starch feeding on milk composition was demonstrated by the work of Sannes et al. (2002). The diets contained 17% CP (DM basis), fed with corn at 20% of dietary DM or a combination of corn and sucrose (13.5 and 3.2% of dietary DM, respectively). Diets were based on corn silage and alfalfa. Milk fat and protein yields were greater for the cows consuming the corn as compared to those consuming the sucrose treatment ($P<0.05$). Additionally, milk urea N concentration (MUN) tended ($P<0.10$) to be greater for the sucrose treatment compared to the corn. The authors concluded that achieving a beneficial response to sucrose supplementation may require additional dietary RDP to avoid ammonia-N limitation. Broderick and Radloff (2002) reported there was a significant quadratic response for 3.5% fat-corrected milk (FCM) and fat yield with the maximum at 3.5% dried molasses in the diet ($P<0.05$). The prepartum feeding of sucrose had small effects prepartum but no detectable carryover effects in lactation. In the study of Ordway et al. (2002), thirty-four multiparous lactating Holstein cows were fed diets of 0 or 2.7% sucrose (DM basis) with sucrose partially replacing ground corn (11.5% of dietary DM as ground corn). Feeding sucrose did not affect insulin and blood urea N pre- or postpartum or milk production and milk composition postpartum. While sucrose supplementation increased blood glucose concentrations prepartum, suggested absorption of additional glucogenic precursors. Although not statistically significant, cows supplemented with sucrose appeared to have less periparturient health problems and less incidence of ketosis (4 of 16 in the control group and 1 of 18 in the sucrose group).

Maiga et al. (1995) demonstrated that feeding of sugar sources with fat altered lactation response. Forty Holstein cows (28 primiparous and 12 multiparous) were fed one of the following diets; control, fat (tallow at 2% of dietary DM), molasses plus fat (8.3% of diet as liquid feed containing molasses plus 19% fat of DM), or dried whey plus fat (whey at 5.4% and tallow at 2.0% of dietary DM). Diets contained similar concentrations of CP, NDF and total nonstructural carbohydrates. Milk fat and protein

percentage tended to be greater for the cows fed the tallow diet without sugar as compared to those fed the molasses plus fat and the dried whey plus fat diets (fat: 3.65 vs. 3.53 and 3.40%, respectively, protein: 2.98 vs. 2.91 and 2.86%, respectively, P <0.10). There were no differences in yields of milk, 3.5% FCM between the fat-supplemented diets. Nombekela and Murphy (1995) have reported cows fed a diet with sucrose at 1.5% of dietary DM, milk yield and 3.5% FCM were not affected by dietary treatment of sucrose supplementation. Milk protein concentration was greater for the cows consuming the control diet as compared to the sucrose supplemented diet (3.51 vs. 3.28%, P<0.01). Milk fat yield tended to be greater for the sucrose supplemented cows as compared to those consuming the control diet (P<0.10). Feed efficiency (3.5% FCM:DM intake, kg/kg) was not affected by dietary treatment. Several other studies also reported no effect of sugar supplementation on milk yield (Cherney et al., 2003; Broderick et al., 2008). However, Broderick and Radloff (2004) found quadratic effects of dietary sugar inclusion on milk yield in which low dietary sugar concentration (up to 7%) increased milk yield but diets exceeding 7% sugar decreased milk yield. They further concluded that the optimal dietary sugar concentration was approximately 5% diet DM. Penner and Oba (2009) found that milk fat yield tended to be higher for cows fed high sugar (8.4%) compared with low sugar (4.7%) diets. The increase in milk fat yield may be attributed to increased mobilization of adipose tissue for cows fed high sugar compared with those fed low sugar diets because milk fat yield was positively related to concentrations of plasma NEFA (r=0.38, P<0.001). In addition, milk fat yield was positively related to plasma BHBA concentration (r=0.31, P<0.001). Kessel et al. (2008) also reported that cows with elevated levels of BHBA in plasma had greater milk fat yield. These results are supported by research demonstrating a linear increase in milk fat yield with increasing dietary sucrose concentration (Broderick et al., 2008).

Changing the dietary source of NFC fed to lactating dairy cows has been shown to alter the ruminal environment. Though not always consistent, effects have been observed on proportions of VFA, concentration of rumen ammonia-N, and extent of fiber digestion with feeding starch, sugars or pectin-rich feeds. These changes in ruminal characteristics likely altered the flow of potentially metabolizable nutrients to the cow throughout the digestive tract. Altering the nutrients provided to the cow may have the potential to change milk production and composition. With the effects of NFC on animal

and microbial responses reported in the literature, and the variation in these responses. It is necessary to consider factors that may, in combination with NFC supplementation, affect substrate utilization, microbial product yield and nutrient supply to the ruminant animal.

2.9 Matching of protein and carbohydrate in ruminant diet

Microbial growth and protein output is dependent on the carbohydrate and protein supplied in the diet (Hoover and Stokes, 1991). Feeding a diet that is balanced for optimal release of energy and nitrogen in the rumen may provide the ruminant with more microbial protein and VFA. Microbial protein provides a variety of amino acids and can supply the ruminant with all of them, essential as well as non-essential. It is beneficial to maximize fermentation in the rumen in order to minimize nutrient loss. By quantifying optimal fermentation and maximal synthesis of microbial protein in the rumen, it may be possible to more accurately determine microbial nutrients requirements and the remaining nutrient requirements for the ruminant (Firkins, 1996). There are some dietary factors to consider when trying to optimize the rumen supply of nutrients. A feed high in NFC content will be a good source of rapidly fermentable energy but it is necessary to compliment it with a rapidly degradable protein source as well. In a study by Stokes et al. (1991), maximum microbial growth occurred with the narrowest ratio of NSC and degradable intake protein (DIP) in the diet that included 50 to 55% of the total carbohydrate as NSC and 13 to 18% of the total DM as DIP. Stokes et al. (1991) also noted that based on results from other studies high NSC in the diet does not result in maximal microbial production. Sniffen and Robinson (1987) suggested that energetic uncoupling with high levels of NSC in the diet may be responsible for the decrease in microbial growth. Hoover and Stokes (1991) determined that the carbohydrate digestion and microbial efficiency were correlated with the level of DIP in the diet as well. This suggests that combining rapidly or slowly degraded carbohydrate and protein sources in order to synchronize the degradation, leads to the greatest increase in microbial yield (Herrera-Saldana et al., 1990). Rate of carbohydrate degradation can affect microbial growth without affecting microbial efficiency (Hoover and Stokes, 1991). In reviewing numerous studies observed that the synthesis of microbial protein improved when the carbohydrate level increased due to the greater availability of energy. It was

noted by Stokes (1991) that greater carbohydrate in the diet led to greater fermentation overall and specifically better CP digestion. Likewise, it has also been noted that increased degradable protein in the diet enhances microbial efficiency and hence, microbial growth. If synchrony of energy and protein fermentation does not occur within the rumen, a portion of the available energy or protein will be wasted and not used by either the microbial population or the host animal. Asynchrony can occur when either the carbohydrate or protein source is in short supply for use by bacteria. In a study by Newbold and Rust (1992), it was observed that reducing sugar concentrations declined after 2 hours of feeding. It was proposed that this resulted in limiting the available carbohydrate supply for microbial growth when compared to the experiment where soybean meal was included. The authors suggested that degradable protein might have contributed additional carbohydrate for bacteria to use.

Carbohydrate availability has an influence on the end products of amino acid metabolism (Russell et al., 1992). The conversion of peptides to amino acids and ammonia is regulated by the release of energy from carbohydrates. When energy is limiting in the rumen, peptide nitrogen will be converted to ammonia and absorbed through the rumen wall (Russell et al., 1992). Kim et al., (1999) altened the synchrony of carbohydrate and protein digestion in dairy cattle by offering maltodextrin and urea at different times during feeding. It was observed that if the maltodextrin was not incorporated into the bacteria and stored when it was not synchronized with urea content, the ATP generated by the rapid fermentation would be divented to bacterial maintenance rather than growth and microbial protein synthesis would not be sustained. A study by Matheron et al. (1999) looked at this possibility in *Fibrobacter succinogenes,* a fiber digesting bacteria. When they studied the relationship between the storage of polysaccharides and the degradation of ammonia, they noticed that the bacteria changed its metabolism when nitrogen was added to the culture. The bacteria decreased the formation of the glycogen storage and in fact started to reverse it in the presence of ammonia. The microorganism, *F. succinogenes*, seemed able to increase microbial protein synthesis using its stored glycogen reserves when a nitrogen source was present within the culture.

2.9.1 Effects of RUP and NFC source in ruminant diets

Santos et al. (1998) reviewed 88 lactation studies from 1985 to 1997 and found inconsistent animal production responses to RUP supplementation. Only 17% of the studies reported greater milk yield by cows fed diets of greater RUP concentration. Of these, cows fed fish meal or treated soybean meal showed the most positive milk yield response. Although it is clear that varying the intake of readily fermentable carbohydrates affects the supply of protein to the small intestine, little research has been done that has addressed the relationship of dietary concentration of RUP with that of different NFC sources. Microbial CP yield is influenced by carbohydrate source, nitrogen source, rate of carbohydrate fermentation, bacterial growth rate, dilution rate and pH (Van Kessel and Russell, 1996). Varga et al. (1988) reported a decrease in microbial growth and depressed fiber and protein digestibilities *in vitro* with substrates having NSC: RDP ratios greater than 6.0. Supplying more microbial crude protein to the small intestine may decrease the need to supplement a diet with additional RUP. If NFC source differ in their ability to fuel microbial yield, it may be related to the level of RUP needed to optimize rumen microbial function.

Companion studies that specifically evaluated the effect of NFC type and RUP supplementation were carried out by Broderick et al. (2002b). They fed 48 multiparous, lactating Holstein cows. The diets included one of three carbohydrate sources (high-moisture ear corn, HMEC; cracked shelled corn, CSC; and a 50:50 mixture of high-moisture ear corn plus dried citrus pulp, HCP) fed with or without expeller soybean meal (ESBM). All diets contained 38.2 to 43.7% of DM as total NFC. ESBM replaced urea to supply the ruminally undegradable protein. This study found that DMI, yields of milk, 3.5% FCM, fat and protein and concentrations of MUN and plasma urea N (PUN) were all greater for the diets containing ESBM. Milk and 3.5% FCM yields were both greater for the cows fed the two corn diets as compared to the diet containing citrus pulp ($P<0.05$). Plasma glucose was only greater for the cows consuming CSC compared to HCP ($P< 0.05$).

In late lactation cow study, ruminal pH and NH_3-N concentration were greater for the cows fed diets containing ESBM ($P<0.05$). Molar proportions of acetate and butyrate were decreased for cows not supplemented with ESBM ($P< 0.05$). Molar proportions of propionate were greatest for HMEC followed by CSC and then HCP

($P<0.05$). Acetate to propionate ratio was greatest for the HCP diets both with and without ESBM (3.42 and 3.45). It was concluded that the NH_3N and VFA patterns suggested that the carbohydrate fermentation decreased in the order of HMEC > CSC > HCP, proposing that site of starch digestion may have played a role. While effects of ESBM were detected in this study, concentration of total CP differed in the no supplement vs. the ESBM supplemented diets may have affected production (Broderick et al. 2002b). Solomon et al. (2000) fed twenty lactating Holstein cows diets of high starch (corn starch) or high pectin (dry citrus plup pellets) with (6.0% of DM as ether extract) or without (3.3% of DM as ether extract) the addition of full fat extruded soybeans. Diets contained similar concentrations of CP, NDF, and total NSC. DMI was greater for the cows fed high starch ($P<0.01$) and the extruded soybeans ($P<0.05$). Milk yield was greater for the extruded soybean diets compared to those without the beans ($P<0.01$). Milk protein concentration was greater for cows consuming more starch without the extruded soybeans ($P<0.01$). Milk fat yield was greater for cows consuming the extruded soybean diets ($P<0.01$). Elevated MUN concentrations were detected with the addition of extruded soybeans to the diets ($P<0.01$).

With animal by-products as the RUP source, Mansfield et al. (1994) compared the animal response to supplementation with starch from corn or pectin and sugars from sugar beet pulp. Forty-six Holstein cows were assigned one of four dietary treatments comparing corn and dried sugar beet pulp with either soybean meal (more RDP) or animal by-products (more RUP from meat and bone meal, feather meal, and blood meal). Milk yield and 3.5% FCM were not affected by dietary treatment. Milk fat concentration was greater for the cows fed the beet pulp (3.82 vs. 3.64%) but this did not translate into an increased milk fat yield. Milk protein percentage (3.01 vs. 2.90%) and yield were decreased for the cows fed the beet pulp diet compared to the corn diet. Feed efficiency (3.5% FCM/DMI, kg/kg) was greater for the cows consuming beet pulp as compared to those consuming corn (1.67 vs. 1.55). Milk protein concentration was greater for the cows fed the soybean meal diet as compared to the RUP animal by-product diet (3.00 vs. 2.91%, respectively). DMI and feed efficiency were not affected by protein type.

Feeding a commercial sugar product and two protein sources, Thirty-two multiparous Holstein cows were fed the following diets; solvent SBM (SSBM), SSBM plus 5% brown sugar food product, ESBM, or ESBM plus 5% brown sugar food product. All diets contained ryegrass, ground corn and a mineral mix. Intake of DM was not affected by protein source, sugar, or the interaction of the two. While milk yield numerically increased with the addition of sugars to the SSBM diet and decreased with the addition of sugars to the ESBM diet. Milk fat percentage was numerically greater for the ESBM diets compared to the SSBM (3.39 and 3.53 vs. 3.24 and 3.25%, respectively; $P>0.05$). Yields of 3.5% fat-corrected milk, fat and protein were not affected by dietary treatment. Plasma urea N concentration was greater for cows fed the sugar supplemented diets ($P<0.05$) (McCormick et al., 2001). Although the information is limited, it appears that NFC source together with RUP supplementation have the potential to change ruminal fermentation characteristics, nutrient supply and consequently production response. The most common strategy is to increase the escape of high quality dietary protein by minimizing proteolysis, peptidolysis and deamination. It is well accepted however, that increasing the undegradable intake protein fraction must not be at the expense of lowering the degradable intake protein in the diet.

2.9.2 Effect of RDP and NFC Source in ruminant diets

Microbial synthesis in the rumen provides most of the protein used by the lactating ruminant for maintenance and milk production, so increasing microbial protein formation is an ideal way to improve utilization of dietary CP. Matching ruminal carbohydrate fermentation with RDP availability should improve N efficiency. There are substantial differences among starch sources (Herrera-Saldana et al., 1990), and within grains due to processing, in the rates of energy release in the rumen. Effects of processing on extent of ruminal digestion of corn starch are much greater than on total tract digestibility (Owens et al., 1986).

Ruminal organisms fermenting NFC, particularly soluble sugars and pectins, were thought to make greater contribution to microbial protein synthesis per unit of fermented carbohydrate (Russell et al., 1992). Chamberlain et al. (1993) found that sugar supplements were more effective than starch in grass silage diets for increasing urinary excretion of purine derivatives in sheep; the order of carbohydrate effectiveness was sucrose > lactose > fructose > xylose > wheat starch. Some research indicated that

ruminal infusions of sucrose (Kim et al., 1999a) or maltodextrin (partially digested starch; Kim et al., 1999b), to supplement of grass silage diets in dairy cows, stimulated microbial protein synthesis in the rumen. Trevaskis et al. (2001) reported that sucrose infusion into the rumen was more effective for stimulating microbial protein formation when it was synchronized with the ammonia peak occurring 1-2 hours after feeding. Kim et al. (2000) also showed a positive effect of sucrose infusion into the rumen but no advantage of synchrony with ruminal ammonia. Molasses and a number of other byproduct feeds can serve as economical sugar sources. Corn starch was replaced with sucrose (Broderick et al., 2000), or dried molasses or liquid molasses (Broderick and Radloff, 2004), in 3 feeding studies in which the basal diets contained 2.6% total sugars. An overall analysis of the data from these trials indicated maximum feed intake at (DM basis) 6.8% total sugars and maximum milk protein yield at 4.8% total sugars. However, the positive production effects of sugar feeding in these trials were at least partly driven by increased feed intake. Harvesting forages in late afternoon, just after maximal photosynthetic activity, increases forage sugar and NFC contents (Owens et al., 1999). Trevaskis et al. (2004) reported that managing grazing cows such that they consumed foliage largely in late-afternoon was effective for improving milk production. Kim et al. (2005) observed a linear increase in microbial protein yield with increased ruminal infusions of sucrose-urea mixtures in sheep but there was a linear decline in microbial efficiency (g microbial protein/kg ruminal fermented organic matter). However, most evidence, including that from many studies (Cabrita et al., 2003; Richardson et al., 2003; Trevaskis et al., 2004) show little or no production benefit from direct manipulations to synchronize protein degradation and energy fermentation in the rumen. Nevertheless, when feeding reduced CP diets, there is a more substantial period of the day when RDP limiting and an opportunity to improve microbial protein formation by synchronizing energy fermentation with N release in the rumen. Optimal protein supply to the animal depends on adequate degradable protein to maximize capture of organic matter in microbial biomass. Synchronizing the rate of nitrogen hydrolysis with the rate of energy release will increase the rate of assimilation of ammonia by microbes and maximize nitrogen use by the animal. Only part of the dietary protein can be replaced by urea or other NPN sources because of a limitation in the ability of ruminal microbes to utilize the resulting ammonia as their sole source of RDP. Thus, enhancing the value of NPN supplementation on diets

based on tropical forages is problematic. Ammonia is used best on diets with greater amounts of NFC and/or higher digestible fiber. Feeding more extensively processed concentrate, if adequate effective fiber is maintained in the diet, will help maximize utilization of dietary NPN for microbial protein formation in the rumen. Adding molasses or another sugar source to the diet stimulates intake and, thus, helps improve production.

2.10 Techniques measuring digestibility for the nutritional evaluation of feeds

The feeding systems need to be founded on the mechanisms that govern the response of animals to nutrients, dealing with quantitative aspects of digestion and metabolism in the ruminant animal. Digestibility and rumen degradability have been recognized as the main sources of variation of the energy and protein value of feeds, respectively. For the quantitative description of digestive and metabolic processes, appropriate biological data are required and can be obtained using *in vivo*, in situ and *in vitro* methods. Information obtained *in vivo* is the most reliable and should be the reference to evaluate other methods, because it represents the actual animal response to a dietary treatment. However, *in vivo* digestion trials are expensive, laborious, time-consuming and not readily applicable to large numbers of feeds or when only small quantities of each feedstuff are available. *In vivo* results are restricted to the experimental conditions under which measurements are carried out, such as level of feeding and associative affects between feeds (Kitessa et al., 1999).

2.10.1 *In vivo* techniques

In vivo techniques to determine rumen degradability or intestinal digestibility require animals to be surgically modified, and measurements of digesta flows and of microbial and endogenous contributions of nutrients may be needed, resulting in digestibility and degradability estimates subject to large variability and additional errors associated with use of digesta flow rate markers, microbial markers and inherent animal variation

1) Total collection technique

The total collection (conventional digestion trial) is the most reliable method of measuring a feed's digestibility. Unfortunately, however, it is somewhat time consuming, tedious, and costly. Basically, the feed in question is fed in known quantities to an animal. Usually, the animal is restrained in an individual cage so that a quantitative

collection of feces can be made. Accurate records of feed intake, refusals and fecal output are kept, and a sub sample of each (usually 10% of daily output in the case of feces) is retained for analysis. When estimates of nitrogen balance are desired, urine output is also measured. Three animals per feed are required as a minimum. The animals are usually allowed from 7 to 21 days to adjust to the feed, followed by a collection. Samples can then be dried, ground, and analyzed for the nutrients of interest. The most common arrangement for collecting the excreta of animals for digestibility experiments is through the use of metabolic crates. A metabolic crate is actually a stall or box large enough for the animal set on legs from 50 cm to 1 m high. It is so planned as to permit the quantitative collection of feces and urine. However, a common criticism of digestion estimation by total collection technique is that feed intake by animals is sometimes abnormally low and erratic. This lack of appetite is in many cases attributable to the fact that the animal may be too nervous or frightened to eat, resulting from the close confinement made necessary by the very nature of the equipment used. It is important that the experimental animals must be sufficiently comfortable during the adjustment period. The space allowed to the animal must be large enough to permit considerable freedom of movement. But conducting a digestion experiment may normally entail appreciable annoyance to the animal. Some individual animals are temperamentally unsuited to be used in such experiments and are too nervous to be used in digestion trials. Even though conventional digestion trials are the standard with which all other measures of digestibility are compared, the values obtained still vary ± 1 to 4% as a result of animal-to-animal variation, sampling procedures and analytical errors.

2) Marker technique

There have been considerable interest among animal nutritionists in methods of reducing the time and expense involved in digestion experiments by the use of methods where total feces are not collected and weighed but are merely analyzed. This departure from the former method of determining digestibility gravimetrically has been designated as the indicator or index method (Kotb and Luckey, 1972). In this method, in addition to the chemical analysis of the usual proximate nutrients, the content in the feed and feces of an indigestible reference substance is determined. The substance may be a natural constituent of the feed (internal indicator) or it may be added to the feed (external indicator). Substances used for this purpose include Ytterbium oxide, chromic

oxide, lignin, silica, chromogen, acid-insoluble ash (Van Keulen and Young, 1977) and indigestible acid detergent fiber (Waller et al., 1980). Chromic oxide (Cr_2O_3) has been studied and widely used for a long time (Faichney, 1975). Cr_2O_3 is known to behave independently of both the liquid and particulate phases of digesta in in the gastro-intestinal tract. Chromium is always given to animals in the trivalent state, which is non-toxic for humans and animals. Ytterbium oxide used as a digestive marker have the same accuracy as chromic oxide for estimating daily faecal DM output variations in dairy cows fed a total mixed ration (Delagrade, et al., 2010). A good marker must be strictly non absorbable, must not affect or be affected by the gastrointestinal tract or its microbial population, must be physically similar to or intimately associated with feed material and its method of estimation in digesta samples must be specific and sensitive and not interfere with other analyses. A characteristic of this method is that the digestibility is calculated from the relation between the nutrients and the indicator substance in the feed and in the feces. The digestion coefficient is computed by using the change in the ratio of each nutrient with reference to the special indigestible substance in the feed and in the feces. By chemical analysis of a suitable feed sample, the ratio of the concentration of the inert substance to that of any nutrient in the feed can also be established. A similar ratio can be determined in the feces and the digestibility can be calculated without weighing either the feed consumed or the feces produced. It is assumed that the reference substance passes through the alimentary tract at a uniform rate. If its rate of excretion during the day is inconsistent, special sampling plans is followed to adjust for diurnal variation. On the other hand, the ratio of the indicator and the nutrients is the same throughout 24 h period, only a small amount of feces collected at any time of the day or night should be sufficient to give an estimate of digestibility. This method of determining digestibility will hopefully avoid much of the time, labor and expense involved in conducting digestion trials.

2.10.2 *In situ* technique

The Dacron polyester or nylon bag technique has been used widely for estimating ruminal nutrient degradation because it is a relatively simple, low-cost method compared with methods involving intestinally cannulated animals. The technique involves suspending bags containing different feedstuffs in the rumen and measuring nutrient disappearance at various time intervals. Hence, it also may provide an advantage

compared with laboratory methods because it involves digestive processes that occur in the rumen of a living animal; however, several factors affect estimates of nutrient digestion and need to be controlled for this technique to be standardized. These factors include porosity of bag material, ratio of sample weight to bag surface area, particle size of sample, method of bag placement in the rumen, diet of the animal, frequency of animal feeding, and degree of bacterial attachment to feed residues remaining in the bag (Nocek, 1988; Michalet-Doreau and Ould-Bah, 1992). The in situ Dacron bag technique has been used to estimate ruminal DM and carbohydrate digestion, but it has been most commonly used to estimate microbial protein degradation in the rumen. Dewhurst et al. (1995) suggested that the in situ technique may not be as precise with forages as with concentrates or protein supplements because of the high proportion of water-soluble materials that can leave the bag unfermented. The Dacron bag technique was compared with the Tilley-Terry *in vitro* procedure, with a strong curvilinear relationship noted between carbohydrate composition of feeds and extent of overestimation of OM degradation using the Dacron bag technique (Dewhurst et al., 1995). Herold and Klopfenstein (1996) suggested that the in situ technique overestimates ruminal protein degradation of animal proteins compared with the ammonia release technique because of food particle losses from the bags during washing.

Mathematical models have been proposed to combine estimates of degradation rate with outflow rates to estimates protein degradation (Ørskov and McDonald, 1979; Mertens and Loften, 1980). Nonlinear models (Mertens and Loften, 1980) have been used more extensively to predict rate and extent of degradation of NDF than of protein, whereas the logarithmic-linear transformation has been more extensively used for protein. Broderick et al. (1991) emphasized that, although the in situ technique is imperfect in ways that cannot be fully compensated for, it is rapid, fairly reproducible, and requires minimal apparatus. However, this technique requires surgical preparation of an animal with a ruminal cannula and facilities for animal maintenance, which may be inconvenient and expensive.

2.10.3 *In vitro* technique

In vitro digestibility techniques provide a quick, inexpensive, and precise prediction of *in vivo* or conventionally determined digestibility in ruminants. The *in vitro* procedure does a better job of prediction than chemical composition because it accounts

for all factors affecting digestibility, whether known or unknown, which is not possible with current chemical methods. As indicated previously, the *in vitro* procedure is quite simple, but nonetheless subject to a number of variables that may influence the results obtained.

1) Tilley and Terry Method

The technique first described by Tilley and Terry (1963) has been the most commonly used *in vitro* method for predicting digestibility and as a selection tool for improving the nutritional quality of forages. Several modifications of the original procedure have been used to maximize the digestion process because *in vitro* systems that do not maximize digestion kinetics may not detect differences in substrate digestion (Grant and Mertens, 1992b). Maximizing *in vitro* digestion depends on several factors, including dilution of the ruminal inoculum, type of buffer used, particle size of the sample, type of mill used for grinding, and type of diet the donor animal is fed. Ruminal inoculum is typically strained through several layers of cheesecloth and diluted (20:80) in saline solution, artificial saliva, or various buffers. Craig et al. (1984) suggested that strained ruminal fluid alone was not as effective as strained ruminal fluid plus an inoculum of particulate-associated bacteria for simulating ruminal fermentation of fiber from different feedstuffs. Varel and Kreikemeier (1995) compared the in situ and the Tilley and Terry techniques using alfalfa or brome grass as substrates. Differences in lag time, rate of digestion, and extent of digestion were noted between the two techniques. Differences were attributed to a lower microbial concentration with the *in vitro* technique compared with microbial concentrations in the rumen of the animal. Attempts to increase the microbial concentration *in vitro* have not been successful because of a rapid accumulation of end products and a subsequent decrease in pH. The decrease in pH might be of major concern when using the *in vitro* technique to study fiber digestion because cellulolytic bacteria are more sensitive to low pH than are amylolytic species (Therion et al., 1982). However, Terry et al. (1969) demonstrated a minimal decrease in cellulose digestion with an addition of 40% glucose when pH was maintained at 6.8. Grant and Mertens (1992a) developed an *in vitro* buffering system capable of pH control between 5.8 and 6.8 that has been successfully used to study fiber digestion *in vitro* (Grant and Mertens, 1992c). Starch digestion also seems to be affected by pH *in vitro*. When conducting *in vitro* techniques, maintenance of anaerobic conditions is important. Grant

and Mertens (1992b) tested several factors that could affect fiber digestibility values using the *in vitro* technique, including maintenance of anaerobic conditions. Purging tubes with CO_2 but not gassing continuously resulted in a 56% increase in lag time for NDF digestion and a 69% decrease in rate of NDF digestion. These authors also suggested the use of nutritional additives such as microminerals, vitamins, and tryptone to ensure that nonfiber factors did not limit fermentation, especially with substrates low in protein and microminerals.

Compared to conventional methods, the ANKOM filter bag method (ANKOM Technology, Macedon, New York, USA) simplifies the measurement of *in vitro* digestibility by eliminating the requirement for filtering samples after digestion, which is often one of the most labor intensive steps in the conventional procedure. The incubation of several samples within a jar in the ANKOM incubator also reduces the need for individual inoculation of samples in tubes. Several studies have also demonstrated that the ANKOM method produces comparable digestibility values to traditional procedures for many feeds (Holden, 1999; Mabjeesh et al., 2000; Wilman and Adesogan, 2000). However, some of the limitations of the ANKOM method include the potential for losses of undigested, soluble or fine particulate material through the pores of the bags which may overestimate digestibility. However, different digestibility estimates are obtained when alternatives to ANKOM F57 bags (pore size, 25μm) are used in ANKOM DaisyII incubators. ANKOM bags gave more precise predictions of tube based, conventionally determined digestibility estimates (Adsogan, 2005).

2) Gas Production

The gas production technique was developed to predict *in vivo* digestibility by simulating the *in vivo* fermentation of feedstuffs. The gas production technique and its variants are superior to digestibility and degradability techniques because they account for contributions from soluble and insoluble feed fractions while providing information on the dynamics of forage fermentation. Anaerobic digestion of carbohydrates by ruminal microbes produces VFA, CO_2, CH_4, and traces of H_2; hence, measurement of gas production *in vitro* can be used to study the rate and extent of digestion of feedstuffs (Hungate, 1966). Theodorou et al. (1991) developed an *in vitro* method to measure head space gas accumulation that does not require expensive glass syringes like the gas production method described by Menke et al. (1979). The gas

production method requires the use of an inoculum or medium with low fermentable energy, so that gas accumulation is low in the blank control fermentation. The use of gas production to study carbohydrate digestion presents an advantage over the traditional gravimetric method because it accounts for both soluble and insoluble substrates (Pell and Schofield, 1993). High correlations between gas production and NDF disappearance, $r^2 = 0.99$ (Pell and Schofield, 1993) or gas production and DM disappearance, $r^2 = 0.95$ (Prasad et al., 1994) have been reported. Pell and Schofield (1993) suggested that under conditions in which nutrients are not limiting, gas production is a direct measure of microbial growth, and in some respects is a better index for predicting forage ME than the indirect measure based on NDF disappearance. Total gas production is the result of various fractions being fermented at the same time, but at different rates, which leads to complex multiple rates that are difficult to partition. Schofield and Pell (1995) suggested that plotting rates of gas production as a function of time, calculated by subtracting gas volumes at adjacent times, can be used to identify pools that are digested at different rates. One of the most challenging problems associated with using gas production methods is that the amount of gas produced varies with different molar proportions of VFA. For example, a higher propionate concentration is associated with lower gas production because an extra carbon atom in propionate would otherwise have appeared as CO_2 (Wolin, 1960). Schofield and Pell (1995) suggested that it is important to monitor the molar proportions of VFA to correct for such differences.

Prasad et al. (1994) compared digestibility values of millet straw measured *in vivo* with the gas production method and concluded that the best fit was obtained with 45 to 52 h of *in vitro* gas production. However, extent of digestion for some samples was higher *in vivo* compared with the gas production method. Khazaal et al. (1993) reported different correlations, depending on the length of incubation time, ranging from 0.58 to 0.84 between the volume of gas produced and *in vivo* apparent digestibility of several forage samples. Most research using *in vitro* gas production has been conducted with forages. Further investigation is required to evaluate the ability of *in vitro* gas production to predict digestibility of highly fermentable substrates.

3) Continuous culture fermenters

Various continuous culture fermentation systems have been designed to simulate the ruminal environment, enabling the study of factors affecting microbial ecology and digestion of nutrients (Hoover et al., 1976). Advantages of these systems compared with *in vivo* measurements include decreased time, and variation among experimental units. Furthermore, there are no complications from endogenous sources, and digesta flow rate markers are not required because passage rates are regulated and measured directly. However, similar to *in vivo* measurements, reliable techniques are required for isolation of microbial cells and for differentiation of effluent digesta into microbial and dietary N fractions. Most of these techniques are based on determination of a single chemical marker that is thought to characterize microbial components. The two most commonly used *in vitro* continuous culture fermenter systems for measuring nutrient digestion by ruminal microbes are the Rusitec system (Czerkawski and Breckenridge, 1977) and dual-flow continuous culture (Hoover et al., 1976). The Rusitec system has a single outflow, and residence time in the rumen is simulated by placing feedstuffs into nylon bags and suspending these bags inside the reaction vessel for 48 h. Prevot et al. (1994) evaluated the Rusitec system and found that in its present form this system cannot reproduce the *in vivo* state of conventionally reared animals. Other differences observed between fermenters and the rumen can be attributed to lack of absorptive capacity and defaunation *in vitro*. Because continuous culture systems are elaborate, expensive, and require inoculation with ruminal digesta, the technique is not suitable for routine analysis of microbial digestion for individual feed ingredients.

4) Enzymes

Enzymatic techniques have the advantage of being completely independent of the animal, which should result in less variation, thereby making this technique relatively simple to standardize. Conversely, the biological validity of the results can be limiting as a result of incomplete enzymatic activity compared with the ruminal environment. Mahadevan et al. (1987) found large differences when comparing digestion of different protein sources using protease from *Streptomyces griseus* with an extract of ruminal microbial enzymes. They concluded that the use of nonruminal enzymes in an *in vitro* system for predicting dietary protein degradation may be of limited value, or even misleading because nonruminal enzymes may not have the same action as

those of ruminal origin. When enzymatic techniques are used to predict microbial fermentation in the rumen, it is crucial that the enzyme concentration is sufficient to saturate the substrate. When enzyme concentration is limiting, accumulation of end-products during incubation can lead to a progressive inhibition of the enzyme activity. Krishnamoorthy et al. (1983) attempted to simulate ruminal proteolysis *in vitro* by choosing a protease enzyme concentration (0.07units/ml) that would provide a proteolytic activity similar to that of whole ruminal fluid. Feed samples were subjected to proteolysis for 18 or 48 h to resemble mean retention times in the rumen of grain and roughages, respectively. Krishnamoorthy et al. (1983) indicated that the technique could be used to evaluate feedstuffs on a relative basis, and that it also offered the potential to allow assessment of the influence of dynamic ruminal criteria, such as proteolytic activity and retention of feed, on the predicted undegraded CP by manipulation of enzyme concentration and incubation time.

Enzymatic techniques also have been used to determine carbohydrate degradation in the rumen. Marten et al. (1988) used a fungal cellulase to predict fiber digestibility of a large set of forage samples (n = 499) and reported a high correlation ranging from 0.93 to 0.98 and similar ranking between the *in vitro* Tilley and Terry technique and the fungal cellulase method. Lila et al. (1986) combined amylolytic, cellulolytic, and proteolytic enzymes to estimate the digestibility of forages *in vitro*. However, correlations between these enzymes and *in vivo* results were low, ranging from 0.11 to 0.49.

5) Near infrared reflectance spectroscopy

Near infrared reflectance spectroscopy (NIRS) has proven to be a rapid, inexpensive, and fairly accurate method for estimating chemical composition of various feedstuffs. This technique also has potential for estimating DM and protein degradation of feedstuffs in the rumen. Marten et al. (1988) developed prediction equations for estimating ruminal degradation of different forages, reporting coefficients of multiple determinations that ranged from 0.87 for birdsfoot trefoil to 0.97 for alfalfa. Mentink et al. (2006) heve reported the CP, NDF, starch, and *in vitro* DM contents of TMR were predicted by NIRS with good degrees (R^2>0.85) of accuracy with proportionally low standard errors of prediction. However, difficulty was observed using NIRS in predicting CP fractions and *in vitro* NDF digestibility. Todorov et al. (1994)

compared degradability of DM and CP for 34 forages, consisting of grasses, legumes, straw, haylage, and dehydrated alfalfa, using NIRS and in situ procedures. The relationship between NIRS and CP degradability was lower than between NIRS and DM digestibility. The authors concluded that NIRS has potential for predicting DM and CP degradability of forages, which could save a considerable amount of time and money.

2.11 Microbial protein synthesis measurement methods

A wide range of approaches have been used to identify microbial protein in rumen contents (both *in vitro* and *in vivo*) and in digesta flowing at the abomasum, omasum or duodenum, though all have limitations.

2.11.1 External marker

Radioactive isotopes are an external marker use to determine microbial protein synthesis. Inorganic ^{32}phosphorus (^{32}P) has been used as a microbial marker by incorporating it into microbial phospholipids (Bucholtz and Bergen, 1973). However, ^{32}P has limitation that its radioactivity presents a greater environmental hazard. Thus, ^{32}P cannot be recommended for widespread application with out the substantial precautions that attend radioactive tracers (Broderick and Merchen, 1992). Inorganic ^{35}sulphur has been also used as a microbial marker. Inorganic sulphur (^{35}S) is incorporate into bacterial protein via de novo synthesis of the sulphur containing amino acids, cysteine and methionine or by incorporation into substances such as coenzyme A (Marais, 2000). However radioactivity accumulates in tissue and milk, rendering it unsuitable for consumption. Thus, routine use of ^{35}S as a microbial trace in lactating dairy cows would be inconvenient because of waste disposal problems (Broderick and Merchen, 1992). Inorganic nitrogen (^{15}N) has been used extensively as a tracer for labeling BCP *in vivo* (Broderick and Merchen, 1992) and *in vitro* (Hristov and Broderick, 1994; Blummel and Lebzien, 2001).

2.11.2 Internal marker

1) Diamino pimelic acid (DAPA)

DAPA is an amino acid occurring in varying concentrations in bacteria cells (Marais, 2000). Since the DAPA to protein ratios of Mixed bacteria are relatively constant (Clark et al., 1992), it has become to the most commonly used internal marker for estimating microbial protein synthesis (Marais, 2000). Presence of DAPA

only in the bacterial cell wall implies that its concentration in total ruminal bacterial protein would vary with growth conditions that alter mean cell size (Broderick and Merchen, 1992). Moreover, amounts of DAPA in feedstuffs can be alteration DAPA: protein ratio (Rahnema and Theurer, 1986). Therefore, abomasal DAPA flows should be corrected for dietary DAPA intake. Disproportionately high DAPA out flow from the rumen may lead to over estimates of bacterial protein yields (Marais, 2000).

2) Amino acid profiles

A method for estimating quantities of microbial and dietary proteins in duodenal digesta based upon differences in the amino acid content of the protein reaching the duodenum was reported by Evans et al. (1975). Individual proteins passing to the duodenum were identified by their characteristic amino acid profiles. It was assumed that the profile of digesta was the weighted sum of the various profiles contributing to it. The method depends upon the computer generation of several profiles which represent mixtures of different proportions of the known profiles of the dietary and endogenous components that may be arriving at the duodenum. At present, this method is limited by a lack of knowledge relative to the differential degradation rates of different proteins present in conventional diets (Nikolic and Jovanovic, 1973).

3) D-alanine

D-Alanine is more widely distributed among bacterial than DAPA and form part of the oligopeptides cross-linking bacterial cell wall peptidoglycan (Marais, 2000). Garertt et al. (1987) suggested D-alaninne to estimate post ruminal flow of bacterial protein. D-alanine was used successfully by that worker to estimate bacterial protein flow for *in vivo* measurement of bacteria crude protein (BCP) synthesis and dietary protein escape. However, reports of bacterial protein flow values exceeding total protein flow when estimates were base on D-alanine quotation its used fullness as a bacterial protein marker (Quigley and Schwab, 1988).

4) Nucleic acids (DNA, RNA and purine)

The high concentrations of DNA and especially RNA in unicellular organisms led to recognition of their potential as markers for ruminal bacteria cell protein (BCP) (Broderick and Merchen, 1992). There are many nucleic acids useful for determine microbial protein synthesis such as RNA, total purine, and cytosine. Purine procedure is the best to quantify microbial protein yield (Firkins et al., 1987). On the

other hand, Broderick and Merchen (1992) recommended the use of purine or ^{15}N as microbial marker. The technique for analyzing purines is simple, fast and inexpensive; however, the purines: N ratio may change with time after feeding (Cecava et al., 1990). Total purine technique should be follow in Zinn and Owens (1986). However for this technique Broderick and Merchen (1992) mention that the purine:N ratio for BCP be determine using mixed bacteria isolated from ruminal contents, except that the ruminal content from which the microbes are isolated should be treated with 1 % (w/v) formaldehyde. However a major limitation of nucleic acid procedures for estimating microbial protein is requiring fistulated animals. Recently, non-invasive procedure base on the urinary excretion of purine derivatives have been developed (Chen et al., 1995).

5) Estimation of microbial protein supply to cattle based on urinary excretion of purine derivatives

The methods generally use for determining microbial protein synthesis depend on the use of internal marker such as RNA and DAPA or external markers ^{35}S, ^{15}N or ^{32}P as describes, previously. However, these methods involve surgical intervention such as post-rumen cannulation and complex procedures that require accurate and quantitative information on both digesta and microbial marker flow. Since researchers known that purine in diet and endogenous materials are rapidly degrade by microbial enzymes in rumen, it is highly likely that negligible concentrations of purines are found in digesta leaving the rumen. A low efficiency could be indicative of nutrient deficiencies affecting microbial activity in the rumen (Nolan and Kahn, 2004). Rumen microbes are rich in nucleic acids: around 18 % of the total nitrogen is present in nucleic acids or 11 % in purines. The purines from the rumen microbes are metabolized and excreted in the urines as their end products: hypoxanthine, xanthine, uric acid and allantoin. The excretion of these metabolites can potentially be used as parameter to quantitatively estimate the supply of rumen microbial protein to ruminant. The term purine derivatives (PD) refer to the sum of allantoin, uric acid, xanthine and hypoxanthine. All compounds are excreted in the urine of sheep, goat, llamas, red deer and camels; but xanthine and hypoxanthine are virtually absence from urine of cattle, buffaloes and yaks. In ruminant, xanthine and hypoxanthine are converted to uric acid by xanthine oxidase, and uric acid is futher converted to allantoin by uricase. The presence of high activities of xanthine oxidase in cattle and buffalo plasma leads to the complete

conversion of hypoxanthine and xanthine to uric acid. The nucleic acids in the feed would not contribute significantly to the nucleic acid content in the rumen, and the nucleic acids entering the duodenum of ruminants were essentially of microbial origin.

Urinary PD has been used as a non-invasive method to estimate the microbial protein synthesis in the rumen (Topps and Elliott, 1965). Martin-Orue et al. (2000) reported that microbial N estimated based on PD excretion in in growing Holstein heifers (initial BW 306 kg) closely matched, but were consistently lower than, the direct measurements based on intestinal flow of purine bases. However, there been few comparative studies conducted in cattle, but the values of microbial N synthesis estimated by the PD excretion for European cattle were within the range as expected from the fermentable energy intake. Obviously, a direct comparison would be useful. However, few of such comparative studies have been conducted due to the complexity of conducting direct measurements of intestinal flow of microbial markers and of the flow rate of digesta in to the duodenum using cannulated animals. Indeed, all methods have limitation and there are inconsistencies in results depending on the choice of marker.

The method to estimate the intestinal flow microbial N from PD excretion was described by Chen et al. (1992). Use of purine derivatives excretion to calculate microbial protein supply

Step 1: the absorption of purines (X mmol/d) is estimated from the in the urine (Y mmol/d)

- For European cattle $Y = 0.85X + (0.385\ W^{0.75})$

  Thus, $X = (Y - 0.385 \times W^{0.75} / 0.85$

- For Zebu cattle, $Y = 0.85X + (0.147\ W^{0.75})$

  Thus, $X = (Y - 0.147 \times W^{0.75}) / 0.85$

Step 2: Microbial nitrogen (N) yield is then calculated using:

$$\text{Microbial N (g N/d)} = \frac{X\ (\text{mmol/d}) \times 70}{0.116 \times 0.83 \times 1000} = 0.727X$$

When digestibility of microbial purines assum to be 0.83. This is taken as the mean digestibility value for microbial nucleic acids based on observations reported in the literature. The N content of purines is 70 mg N/mmol. The ratio of purine-N: total-N in mixed rumen microbes was measured as 11.6:100 (Chen, 1986)

The use of urinary PD excretion as an indicator of rumen microbial protein synthesis has been extensively applied to ruminant nutrition research. The technique has become a useful tool to aid understanding how dietary factors affect microbial protein synthesis. The variation of PD excreted could be up to 10%, thus treatment effects should be viewed in respective of the size of variability in PD measurements. In order to minimize the day-to-day variability, measurements should be made with sufficient duration of continuous urine collection (at least 4-5 day). The possibility of using plasma and milk concentration of allantoin (or total PD) as an alternative index of microbial protein supply has been investigated (Chen et al., 1992; Chen et al., 1995). However, plasma PD and concentration of PD in milk is not sensitive parameter.

(1) PD in spot urine measurements: The daily excretion of PD in urine has been used as a parameter to estimate the supply of microbial protein to the animal. This method provides a simple and non-invasive tool to indicate the nutritional status of farm animals. Due to the need for complete urine collection, the potential application of the method under farm conditions is restricted. Spot urine sampling technique to estimate the urinary PD excretion. Valadares et al. (1999) and Chizzotti et al. (2008) related that a single spot urine sample and total urine collection result nearly the same estimation of urinary PD output. Oliveira et al. (2001) and Silva et al. (2001) reported no differences in the PD excretion estimated by the spot urine sampling technique and that observed in the total urine collection. Chen et al. (1995) concluded that the spot urine sampling could be utilized to estimate the microbial production in steers. The PDC index had been show to be linearly correlated with daily PD excretion in sheep (Chen et al., 1992) and European cattle (Vagnoni, 1997) and thus provide a practical indicator of microbial protein supply using a spot sampling procedure. Chen et al. (2004) proposed to use a term PDC index as floow:

$$\text{PDC index} = \frac{[\text{PDmolar concentration}]}{[\text{Creatinine molar concentration}]} \times \text{kg } W^{0.75}$$

Where, W is the body weight, [PD] and [Creatinine] are PD and creatinine concentration, respectively, in mmol/l

$$\text{PD excretion (mmol/d)} = (\text{PDC index}) \times C$$

Where, C is the daily creatinine excretion (mmol/kgW$^{0.75}$) for specific breed of animals. From this equation, total daily excretion of PD can be calculated from the PDC index, yet with out the need for total collection. From the estimated daily PD excretion data, calculate the microbial N supply based on previously established models. For practical applications, a banding system was suggested (Chen et al, 1995). The PDC index and the corresponding microbial N supply are divided into several (3-5) bands. A preliminary system for sheep suggested by Chen et al. (1995) is provided in Table 2.5. With Friesian cattle, six bands are proposed on research carried out with dairy cattle in Chile (Orellana et al., 2004). The six bands (Table 2.6) are equivalent to energy intake of <0.5, 0.5-1, 1-1.5, 1.5-2, 2-2.5, and 2.5-3 maintenance energy requirement, respectively.

Table 2.5 The corresponding values for the daily PD excretion and microbial N supply in sheep at four different ranges of the PDC index

Band	PDC index	PD excretion (mmol/d)	Estimated Microbial N (g/d)
1	< 15	< 6	< 5
2	15-30	6-14	6-12
3	3-45	14-23	12-20
4	>45	>23	>20

Source: Chen et al. (1995)

Table 2.6 The corresponding values for the daily PD excretion and microbial N supply at six different ranges of the PDC index for Friesian cattle

Band	PDC index	PD excretion (mmol/d)	MN supply (g/d)	DOMI(kg/d)
1	<35	<42	0	1.82
2	35-65	42-83	0-31	3.64
3	65-100	83-119	31-62	5.46
4	100-130	119-155	62-92	7.27
5	130-160	155-190	92-123	9.09
6	160-190	190-226	123-153	10.91

Source: Orellana et al. (2004)

(2) Creatinine: The estimation of microbial protein synthesis using PD excretion, total urine collection is involved. Total urine collection is laborious and difficult in grazing animals or under farm conditions and generates discomfort in the animals due to the presence of catheters or collecting funnels. As an alternative to total urine collection, several authors (Chen et al., 1992; Valadares et al., 1999; Cetinkaya et al., 2006) have proposed the use of spot sampling technique to assess the excretion of urinary nitrogenous compounds. The PD: creatinine concentration ratio in the spot urine sample is a good indicator of the daily urinary PD excretion if the daily creatinine excretion is known (Valadares et al., 1999). Creatinine excretion is a little affected by dietary factors such as protein intake (Kertz et al., 1968), and non fiber carbohydrates (Renno et al., 2000) and nonprotein nitrogen content in the diet (Susmel et al., 1995; Oliveira et al., 2001; Silva et al., 2001). Whittet (2004) reported a diurnal variation on creatinine excretion of crossbred heifers on a 2-h interval collection, but did not find differences on daily variation. This finding suggested that the rate of creatinine excretion in lactating Hosltein cows was constant during the day. Moreover, Chizzotti et al. (2008) finding the level of milk production did not influence creatinine excretion. The stage of maturity might affect the creatinine excretion (per unit of BW), when an animal is fed a diet containing adequate energy, the percentage of protein decreases whereas the percentage of fat increases in the empty body, as its BW approximates to the mature BW. Thus, in growing animals, the percentage of muscle tissue varies according to the BW of the animal consequently creatinine excretion in mmol/kg BW can be altered. Therefore, once we can estimate the daily creatinine excretion from animal's body weight (BW), the daily urine volume can be estimated from the creatinine concentration in a urine sample collected during the day (spot sample) and, from the estimated urinary volume, the daily excretion of uric acid and allantoin could be estimated.