1 INTRODUCTION

Nutritionists and dairy producers have a challenge to meet two primary goals namely, high milk production with healthy animals and increase production efficiency. At the same time they should also pay more attention to the non-animal aspects, as it is a high concern to the environmental pollutions caused by an intensive animal production system (e.g.: manure disposal, nitrates in ground water, and high methane and ammonia losses). Therefore the future of a modern dairy industry will depend on how to realize a balance of these goals.

Although the high producing dairy cows are more efficient converting dietary nitrogen (N) in the feed to milk protein and contribute less emissions per kg milk to the environment than those who produce less milk (Flachowsky, 2002; 2003), they are not particularly efficient compared with other animals, especially pigs where the efficiency of N utilization could reach 40% (Nieto et al. 2002) and 48% in broilers (Nguyen et al. 2003). In dairy cows, the efficiency of conversion of feed N into milk N, ranging from 23 to 32% (Børsting et al. 2003; Castillo et al. 2000; Dewhurst et al. 1996; Jonker et al. 2002a), depends largely on the stage of lactation. The N efficiency of cows at daily production levels of 30 - 50 kg milk, is generally around 30%, whereas cows with lower milk yields, cows in late lactation or dry cows have lower N efficiency (Kalscheur et al. 1999).

Low efficiency of N utilization in dairy cows is largely due to the losses of N in urine and faeces (Castillo et al. 2001; Tamminga and Verstegen 1996; Van Soest, 1994). Losses of N in urine are mainly caused by an oversupply of crude protein and/or an imbalance in the supply of amino acids. The amount of N excreted in faeces, however, is reported to be relatively constant in proportion to dry matter intake (DMI), about 7.5 g/kg DMI (Castillo et al. 2001) or 0.6% of dietary DMI (Van Soest, 1994). In some cases, increase of N in faeces might be a result of high amounts of undigestible feed protein (UDP) or undigested microbial N (microbial protein : 6.25) from the large intestine. Moreover, in normal practical rations for dairy cows both true digestibility of UDP and of microbial protein (MP) are high, suggesting that reduction of N excretion in the faeces does not appear to be a promising way for substantially reducing N losses from ruminants (Tamminga, 1992). Therefore, a more

promising way to reduce N output is by reducing urinary N excretion (Castillo et al. 2000; Monteils et al. 2002a; Smith et al. 1995).

Feeding N in excess of requirements, feeding excessive amounts of rumen degradable protein (RDP) or feeding diets not properly balanced for degradable or undegradable protein, amino acids, or energy in the rumen may increase urinary N excretion (NRC, 2001). Hence, such factors that reduce urinary N excretion would improve efficiency of N utilization in dairy cows.

Synchronization of rumen available protein and energy is one of the conceptual methods to increase the efficiency of utilization of nutrients by the ruminants. Sinclair et al. (1993) reported that the formulation of diets that are synchronous for energy and nitrogen release in the rumen has been shown to increase the efficiency of MP synthesis in the rumen. Aldrich et al. (1993) and Herrera-Saldana et al. (1990) have also reported the same results. Contrary to these results, Henning et al. (1993), Kim et al. (1999b), Mabjeesh et al. (1997), Sauvant and Van Milgen (1995), reported unaffected MP synthesis by the synchronization of energy and nitrogen release in the rumen. These contradictory findings may be the result of the different experimental methods and animals. There are at least three methods in which the synchronization experiments were usually conducted, (1) changing dietary ingredients (2) dosing the specific form of energy and nitrogen directly into the rumen, and (3) altering the relative times of feeding the different ingredients. Some researchers (Blümmel et al. 2001; Richardson et al. 2003; Sinclair et al. 1993;1995; Witt et al. 1999a) used a term of index to determine the degree of the synchrony of energy and protein supply for MP synthesis. An index of 1.0 means a perfect synchrony of energy and protein availability in the rumen for MP synthesis and an index close to 0.0 means imperfect synchronization. Until now, it has not been clear whether the observed or not observed effects of synchronization with respect to the index were associated with the synchrony of feed degradation of the ingredients or to the methods used.

The objectives of these experiments were therefore:

- 1. To alter the release of energy and nitrogen in the rumen by feeding energy and N yielding concentrates in 3 different sequences
- 2. To calculate the synchronization index of each feeding sequence from the *in sacco* degradation characteristics for each feedstuffs
- 3. To prove whether the synchronization index is related to the efficiency of microbial protein synthesis in the rumen and/or to the urea content in the milk

The experiments tested the hypothesis that synchronization of energy and protein as well as the degree of their synchrony (given by the index) calculated by the *in sacco* method would affect microbial protein synthesis and milk urea N.

2 REVIEW OF LITERATURE

2.1 Carbohydrate degradation in the rumen

Carbohydrates are the main components in the dairy ration, comprising roughly 60 - 80% of total dry matter (DM) and could supply to 70% net energy lactation (NEL) for high yielding dairy cows. Therefore understanding carbohydrates features and applying their characteristics for the formulation of dairy rations are important for improving milk production and animal health.

As the main reservoir of photosynthetic energy in plants, plant carbohydrates composed of 50 - 80% of the DM of forages and cereals (Van Soest, 1994). The carbohydrates in the feed can be divided into two fractions, namely fibre and non-fibre fractions. Fibre fraction, commonly referred to structural carbohydrates (SC) include cellulose, and hemicelluloses. Non structural carbohydrates (NSC) include starch, pectin, and sugars (Van Soest, 1994). In ruminants, nearly all carbohydrate digestion (> 90%) occurs in the rumen (Armstrong and Smithhard 1979; Sutton, 1979), although in some cases, such as at a high rate of passage, a significant portion of NSC digestion can occur in the small intestine (Nocek and Tamminga 1991). The simple sugars found in plant cells are glucose, fructose, and sucrose. They are rapidly degraded in the rumen to yield short chain fatty acids (SCFA), which are absorbed into the blood through the rumen wall. Polysaccharides must be hydrolysed into simple sugar before being utilized. Starch as a NSC is easily degraded in the rumen while degradation of structural carbohydrates, such as celluloses and hemicelluloses, varies considerably (Baldwin and Allison 1983). Cellulose degradability of forages varies from 30 – 90% while hemicelluloses digestibility varies from 45 - 90% (Van Soest, 1994). This broad range of cellulose and hemicelluloses digestibility from the different feeds is mainly due to lignification. Therefore the rate and extent of digestion of cellulose and hemicelluloses are related to the lignin content (Allen and Mertens 1988; Van Soest, 1994).

2.2 **Protein degradation in the rumen**

Dairy cows meet their protein requirement from two major sources (1) from ruminally synthesized MP which have an optimal amino acids profile, (2) from feed protein that escapes microbial degradation in the rumen, and to a much lesser extent from endogenous CP. Digestion of MP, undegradable feed protein and endogenous protein in the intestines contribute to a total yield of protein available for dairy cows (Broderick et al. 1991).

Feed protein are degraded by microorganism in the rumen via amino acids into ammonia and branched chain fatty acids. Non-protein nitrogen (NPN) from feed and urea recycled from saliva and from the blood across the rumen wall also contribute to the ammonia pool (Figure 2.1). Because of the central role of ammonia in the N transactions in the rumen, research has focused for long time on ammonia content in rumen fluid. If ammonia levels in the rumen are too low, there will be a shortage of N for microbes. Failure to meet the N requirement of rumen microbes can have serious consequences on the feed utilization and protein synthesis.

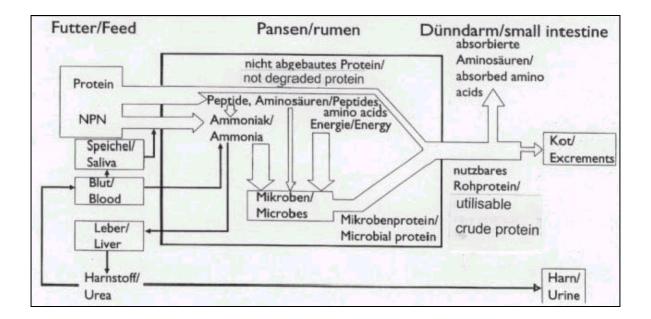


Figure 2.1. Schematic representation of nitrogen conversion in dairy cows (Lebzien, 1996).

The rate and extent by which feed protein is degraded in the rumen depend on many factors. Factors such as characteristics of feedstuffs, level of proteolysis activity, mean retention time of feed particles in the rumen and rumen environments affect the rate and extent of feed protein degradation (Broderick et al. 1991; Morrison, 2000; Nolan, 1993; Wallace et al. 1999).

2.3 Rumen microbial protein synthesis

2.3.1 Factors affecting microbial protein synthesis

2.3.1.1 Energy supply

Rumen MP synthesis is dependent upon the availability of N and carbohydrates (Hoover and Stokes 1991). When N is adequate in the rumen, microbial protein synthesis is a function of carbohydrates availability. Therefore it is widely accepted that energy produced from carbohydrates fermentation in the rumen is the first limiting factor to MP synthesis.

Fermentation of nutrients (carbohydrates) in the rumen provides adenosine triposphate (ATP) which is utilized for maintenance and growth of microbial population (Obara et al. 1991). The fermentation of carbohydrates is dependent on its degradability and rate of degradation. Microbial protein synthesis is therefore mainly determined by the total digestible nutrient intake (Sniffen and Robinson 1987) or organic matter (OM) fermented in the rumen (Clark et al. 1992; Dewhurst et al. 2000). Clark et al. (1992) reviewed 41 experiments in which cows were fed 161 different diets and they found a positive correlation between OM intake (OMI) (kg/d) and microbial N flowing at the duodenum (g/d) with the following equation :

Y = 14.69 OMI + 21.94; $r^2 = 0.62$

where Y = (g) microbial N and OMI = (kg) organic matter intake. Fitting a linear and a quadratic relationship between OM truly digested (OMTD, kg/d) in the rumen and microbial N (g/d) resulted in a coefficient correlation (r²) of 0.39 and of 0.49, when OMTD in the rumen was increased from 2 kg/d to 14 kg/d, suggesting that amount of the OM fermented in the rumen is an important factor that contributes to the amount of microbial N flow at the duodenum. But the fact that OMI (kg/d) was better correlated to the microbial N flow at the duodenum (g/d) than OMTD in the rumen either linearly or quadratically, suggests that

estimation of OMI is more exact than estimation of OMTD, and/or factors other than amount of OM digested in the rumen affect microbial N synthesis. Factors such as supply of other nutrients, rumen environment and synchronization of the degradation rate of energy and protein supply in the rumen could contribute to the degree of MP production. All these factors will be reviewed in the following subsections.

Not only the quantity of OM but also the source of OM affects MP synthesis. Lebzien et al. (1983) conducted an experiment to investigate the effect of feeding different cereal grains (wheat vs. corn) on the passage of non ammonia nitrogen (NAN), microbial N and amino acid profiles at the duodenum of dairy cows. Cows fed 11 kg OM/day of the wheat or corn based diets that contained 67% grass hay and 33% concentrates. Apparent total tract digestibility of OM was the same for both diets but the total of microbial N flow at the duodenum was 41 g/d greater in cows fed the wheat based diet than corn based diet. However, the undigestible feed protein was 144 g/d higher in cows fed the corn based diet than the wheat based diet which contributed to the same amount of total N and NAN flow at the duodenum on both diets. At the same time fermented OM (FOM) (calculated as the difference of OMI and OM flow at the duodenum and corrected for microbial OM) was 1.4 kg/d higher for wheat based diet than for corn based diet, therefore the efficiency of microbial N synthesis calculated as g microbial N/kg FOM was the same for wheat and corn based diets. This type of experiment suggests that the difference between wheat and corn is explained by the difference in OMTD (kg FOM). In accordance with these results, McCarthy et al. (1989), who examined the effects of different carbohydrates sources (corn vs. barley) and protein sources (fishmeal vs. soybean meal) on the passage of nutrients in dairy cows, found that OMTD in the rumen was 1.5 kg/d higher for the barley based diet than for the corn based diet (10 kg/d vs. 8.5 kg/d). They also reported a greater amount of non-ammonia non microbial N (NANMN) flow at the duodenum for corn based diets, suggesting a less extensive degradation of protein in corn than in barley. In contrast to Lebzien et al. (1983), in this experiment cows fed the barley based diet had only 20 g/d more microbial N than those fed the corn based diet, resulting in a higher efficiency of microbial N synthesis as well as a higher amount of NAN flow at the duodenum in the corn based diet than in the barley based diet. Garcia et al. (2000), Kung et al. (1992), Rode and Satter (1988), and Spicer et al. (1986) have also reported that there was an effect of different