

CHAPTER VI

GENERAL DISCUSSION AND CONCLUSION

This thesis shows soluble macro-nutrient strategies that can improve ruminal fermentation, nutrient digestion, microbial protein synthesis and milk production. When availability of macro-nutrient substrates are lacking, the microbial growth is limited, impairing digestion rate and either intake or nutrient utilization. Limiting nutrients influence the rumen microbes and this affects the nutrition of the animal. The most important factor influencing rates of digestion are the intrinsic properties of the feed carbohydrates and protein. Microbial growth may be improved by causing a better synchronization between available nitrogen and carbohydrate.

The results in the Experiment 1.1 showed that supplementation of soluble N and NFC improved *in vitro* DM digestibility (IVDMD) of cellulose and rice straw than supplemented with only NFC. Ribeiro et al. (2005) reported that addition of sucrose (4% of DM) did not affect fiber digestion of alfalfa hay in which concentration of sucrose in the hay was 6.8% of DM. In the current study, substrate that was supplemented with N plus sugar improved IVDMD (70.5%) followed by soluble N plus starch (70.4%) and soluble N plus starch and sugar (68.3%). It appears that soluble N and either starch or sugar was better for enhancing microbial growth than soluble N and a mixture of starch and sugar (50:50). Ruminants have the ability to utilize NPN as source of protein through rumen microbial protein synthesis. The amount of non-protein N, such as urea used in diets was restricted and of limited benefit due to rapid hydrolysis to ammonia in the rumen. Increasing the supply of ruminally fermentable starch reduced ruminal $\text{NH}_3\text{-N}$ concentration, probably through increased microbial protein synthesis (Vagnoni and Broderick, 1997). Others have shown a reduction in ruminal $\text{NH}_3\text{-N}$ concentration and increase microbial protein synthesis with added sugar (Broderick and Radloff, 2004; Hristov et al., 2005). Broderick and Radloff (2004) reported that cows had linear increases in dry matter and fiber digestibility when high moisture shell corn was replaced with dry molasses. In experiment 1.2 trypticase could be replaced with urea at 25 to 50% of the CP without affecting IVDMD. Maeng et al. (1976) found that even higher levels

of NPN substitution and suggested that the optimum ratio of urea-N to amino acid-N for microbial growth in a purified diet was 75% urea-N and 25% amino acid-N. The combination of urea: trypticase (50:50) as soluble N source plus sugar (100% of NFC) with rice straw as substrate had higher IVDMD as compared lower urea:trypticase supplementation. Vallimont et al. (2004) reported that incremental replacement of starch with sucrose (7.5% of DM) in a continuous culture system with diets containing 31-33% of NSC had higher NDF digestibility compared with 2.5 and 5% of sucrose. Similarly, Hoover et al. (2006) reported that addition of sugar (2.9 to 9.5%) in 28 and 33% of NSC diets increased fiber digestion. However, in current study IVDMD of rice straw did not differ by providing mixtures of sugar and starch as supplements. It appears that fiber digestion in the high NDF rice straw (76% of NDF) may not respond to addition of mixtures of sugar and starch. However, a mixture of soluble N and NFC source provided a more sufficient nutrient balance to promote bacterial growth and enhanced feed digestion. The effects of soluble protein (5, 8, or 11% of the DM) and sugar (8, 16, or 24% of the DM) in TMR diets by increasing soluble N from urea at 25, 50 and 75% of the CP were conducted in the Experiment 1.3. Increasing soluble protein (5 to 11% of the DM) and sugar level (8 to 24% of the DM) led to improve IVDMD and gas production. At ≤ 8 h of incubation increased SP level appeared to improve microbial growth and activity because IVDMD, IVNDFD and IVADFD were all increased. Hoover and Weimer, (2007) found sucrose to be the predominant substrate at ≤ 8 h of fermentation and also tended to increase lactic acid production that would have greater impact on pH because of its lower dissociation constant (pKa). Thus, the quadratic decrease in IVADFD in the TMR diet containing increased sugar level at 8 h, in the current study, may be due to a reduction in pH by the lactic acid fermentation of sugar and its impact on reducing the cellulolytic bacterial population and activity. Griswold et al. (1996) reported that digestibility of ADF was increased by supplying a true protein source compared to urea in an *in vitro* study. Dryhurst and Wood (1998) reported that when more rapidly fermentable carbohydrate was supplied, more N (NPN, amino acids and peptide) was required to meet bacterial growth requirement. This research demonstrates that diets containing 11% of SP are too rapidly hydrolyzed to $\text{NH}_3\text{-N}$ before the N can be captured and efficiently utilized by microbes, and results in less available N at a later hour of

fermentation. Lower supplemental SP (5%, high true protein concentration) and low sugar (8%) level diet had higher NDF and ADF digestibility (38.7 and 49.2%, respectively).

There was trend for linear increase in potential gas production with increasing SP level, but this and gas production from fibrous substrate were linearly decreased with increased sugar level. These results are similar to Hall and Weimer (2007) who reported that gas production from the insoluble fraction decreased linearly with increasing sucrose, suggesting a decrease in NDF fermentation. It shows that gas production is a more sensitive tool for detecting differences in carbohydrate fermentation than is measurement of small quantities of unfermented diets. Positive correlation between IVDMD and cumulative gas production ($r=0.82$) in present study were in accordance with other researchs (Blummel and Orskov, 1993; Apori et al., 1998). There were high correlations observed between IVDMD and *in vitro* gas production indicating that feed digestibility evaluation using ANKOM method was simple and just as useful as the gas production technique. *In vitro* systems is less expensive and more rapid alternative to screening animal feeding trials, however, *in vivo* studies is essential to confirm results. Therefore, the effects of varying the concentrations of SP (8 or 11% of the DM; 60 or 80% of the CP) and sugar (11 or 22% of the DM) on the dry matter intake, ruminal fermentation, blood metabolites, and total tract digestibility of nutrients were measured in crossbred Thai native steers in the Experiment 2 (Chapter IV). DMI was comparatively lower in steers fed high-SP diet. Milton et al. (1997) reported that a maximal DMI was observed in steers that consumed only 1.1% urea, which supplied all supplemental N. Researchers suspect that urea decreased DM intake because of its impact on palatability due to its bitter taste (Huber and Kung (1981). However, in this research there were no differences in weight change among treatments that contained soluble protein from urea at 50-75% of the total CP. This finding was similar to Chizzotti et al. (2008) who fed steers with diets containing NPN up to 66.3% of N. Diurnal rumen pH (minimum, maximum and mean) did not differ in cattle fed dietary treatments with the sugar levels at either 11 or 22% of the diet DM. *In vivo* studies (Heldt et al., 1999; Broderick and Radloff, 2004; Broderick et al., 2008) reported no effect of sucrose on rumen pH, and Penner et al. (2009) reported that sucrose tended to improve ruminal pH. Supplemental sugar may not impact rumen pH like other soluble carbohydrates for several reasons. One possibility is that disappearance of sugar from the rumen may not necessarily increase fermentation acid

production in the rumen if more of its OM is converted to microbial N (Allen, 1997) or stored as microbial glycogen (Hall and Weimer, 2007). Secondly, a high proportion of sucrose reportedly leaves the rumen with the liquid fraction bypassing fermentation (Hoover and Webster, 2001). Henning et al. (1993) reported that the rumen disappearance rate of sugar was as much as 69%/h. Thirdly, it is possible that urea provides a buffering effect through its conversion to ammonium bicarbonate (NH_4HCO_3) in the rumen (Van Soest, 1994; Chenost et al., 2001).

Although, reduced ruminal $\text{NH}_3\text{-N}$ concentrations were reported in many studies with dietary sugar supplementation (Broderick and Radloff, 2004; Hristov et al., 2005), $\text{NH}_3\text{-N}$ or BUN were not affected by sugar level in this study. It is possible that the concentration of the dietary SP used may exceed the ruminal microbial requirement. Accordingly BUN did appear to increase with time after feeding. Moreover, the blood glucose concentration among treatments remained within a normal range (45-75 mg/dl). Feeding a high-SP diet tended to cause the molar proportion of acetate to decrease, as compared to feeding steers a low-SP diet; this might have been related to the decreased ADF digestibility in the current study. Decreased ADF digestibility with high-SP diet may be a consequence of insufficient true protein, as much of the protein substrate was rapidly degraded to ammonia and supplied at a rate that exceeded rumen microbe's ability to utilize. Digestibility of ADF was increased supplementing a true protein source, as compared with a diet of 100% urea in an *in vitro* study (Merry et al., 1990; Griswold et al., 1996). In present study, sugar level had no significant effect on fiber digestion. Therefore, a TMR diet that contains 8.3% of SP (60% of the CP) and 12.0% of total sugar may be a viable strategy for improving the intake, ruminal fermentation, digestibility and performance of steers. The findings suggest that dietary SP (up to 60% of the CP) can be fed to crossbred Thai steers receiving sugar at a level of 11% of the DM. Feeding excess SP (>60% of CP) did not improve animal performance and causes excessive nitrogen excretion and ammonia volatilization into the environment.

Optimizing the ratios of soluble protein and sugar in TMR diet, with increased soluble protein (30, 40, 50, or 60% of the total CP; 4.8, 6.4, 8, or 9.6% of diet DM) and sugar level (8, 11, 14, or 17% of diet DM) on total tract digestibility, microbial protein synthesis and milk production of dairy cow were conducted in Experiment 3 (Chapter V). Increasing SP and sugar level in TMR diets did not affect DMI, total tract digestibility

of DM, OM, CP, EE, NDF or ADF. Rumen lactic acid was not detected, contributing to ruminal pH being in the suitable range 3 h after feeding (>6.7). This research suggests that feeding diets 40.3% NFC consisting of 9 to 18% sugar could be acceptable in commercial practice. It has been reported that cows fed urea-containing diets, that reticulum pH was elevated (Kertz et al., 1982); one explanation may be that urea provides buffering through its conversion to ammonium bicarbonate in the rumen (Van Soest, 1994; Chenost et al., 2001). This was also apparent in this research in that the level of SP in the TMR diets maintained a ruminal pH that was more near neutral. Perddok and Leng (1989) showed that higher level of ruminal $\text{NH}_3\text{-N}$ (15-30 mg/dl) improved intake and feed digestibility. Microbial protein synthesis was maximized at average ruminal $\text{NH}_3\text{-N}$ concentration of 12.8 mg/dl in lactating cow fed 16.5% CP diet (Boucher et al., 2007). In the present study, DM and nutrient digestibility were affected by dietary treatments; it was suspected that concentrations of $\text{NH}_3\text{-N}$ may have been higher than minimum levels for optimal digestibility and microbial growth, averaging 18.3 and 25.0 mg/dl at 0 h and 3 h after feeding, respectively.

Increased ruminal VFA concentrations are often assumed to be a result of increased fermentation of carbohydrates in the rumen (Bach et al., 1999). It was noticed that, at 3 h post feeding total VFA tended to increase (in week 2) and significantly increased (in week 8) for cows fed 9.6% SP and 17% sugar diet. This indicates that higher sugar levels improved total VFA concentration, without affecting structural carbohydrate digestibility (NDF, ADF). In addition, the molar proportion of acetate was higher in cow fed the highest level of sugar (17%), that contributed to it increased total VFA. In contrast, supplementing sucrose was associated with a decrease in ruminal acetate concentration (Sutton, 1979; Khalili and Huhtanen, 1991; Chamberlain et al., 1993; Moloney et al., 1994; Heldt et al., 1999). The effect of sucrose on the ruminal molar proportion of acetate may depend on the amount of it included in the diet. It is also possible that, soluble protein provided buffering through its conversion into ammonium bicarbonate causing a shift in towards proportions of the population of microbial species that produce acetate. More rumen acetate production is related to a ketogenic metabolism in dairy cattle and is conducive to a greater milk fat percentage.

Milk fat (g/100g) is positively correlated with the acetate:propionate proportion (Seymour et al., 2005). In the current study acetate:propionate ratio increased with SP and sugar supplementation of TMR, due to a change in molar proportion of acetate without being affected by the molar proportion of propionate. It is reported that the ruminal molar proportions of propionate did not differ between sugars and starch, whether 5.6% dextrose or 61% molasses were added to the diets (Moloney et al., 1994; Piwonka et al., 1994). It may be due to the influence of other component of the diet such as soluble protein, which could alter the yield of propionate from NFC. In this study, at 3 h post feeding, there was a tendency ($P < 0.10$) in molar proportion of butyrate to increase, in cows fed diet containing 9.6% and 17% SP and sugar, respectively. Khalili and Huhtanen, (1991); Moloney et al., (1994) and Heldt et al. (1999) reported that sucrose appears to yield more butyrate. Whereas, Ben-Ghedalia et al., (1989); Leiva et al., (2000); Broderick et al., (2002) and Voelker and Allen, (2003) reported that the feeding soluble carbohydrates in the form of citrus and beet pulps (containing 12.5 - 40.2% and 12.8 - 24.7% sugar, respectively) had no effect on n butyrate concentration.

Cows fed diets with increasing sugar levels between 9-18% of DM showed no detectable treatment effects on circulating concentrations of insulin and plasma glucose, average 13.53 μ IU/ml and 60.9 mg/dl, respectively at 3 h post feeding. These values were higher than that reported by Cabrita et al. (2007) who fed cows low starch diets (7.5% of sugar). The result of present study indicates that increasing sugar level in diets does not alter plasma glucose from its normal range (45-74 mg/dl). Decreased plasma urea nitrogen (PUN) with diets containing glucose and starch (Hristov et al., 2005), reflect increased rumen metabolism of $\text{NH}_3\text{-N}$ and relates to supplying more ruminally fermentable energy with glucose and starch. In the current study increasing the SP concentration in diets increased PUN concentration at 3 h post feeding; there was higher PUN for cow fed diets contained SP of 6.4 - 9.6% of DM compared to cow fed 4.8% of SP diet (30% of total CP) (20.5 vs. 13.8 mg/dl). The high level of PUN in this study may has been caused due to higher concentration of nitrogen and its higher solubility that exceeded the ruminal microbial requirement, or may be related to the higher rates of sucrose outflow from the rumen (Cherney et al., 2003). Henning et al. (1993) reported that the sugar disappeared from the rumen at a relatively fast rate ($\sim 69\%/h$), suggesting that a high proportion of sucrose may be leaving the rumen with liquid fraction without

being fermented and contributing as a source of energy for microbial protein synthesis (Hoover and Webster, 2001). Sannes et al. (2002) reported that there was increase (8%) in microbial protein synthesis on using soybean meal relative to urea. However, the PDC index and estimated microbial crude protein were higher in cow fed 6.4% of SP and 11% of sugar diets. This suggests that the level of soybean meal at 20.7 % and urea at 1.6% of diet DM was sufficient to satisfy the cow's protein requirement for maintenance and milk production without supplementing additional urea.

The inclusion of NFC in the range of 35 to 42% of dietary DM is seen as a popular way to increase energy density and thus milk production (Lykos et al., 1997). Balance of carbohydrates in the diet impacts milk production because it affects amount and ratios of ruminal VFA produced, which in turn alters metabolism and partitioning of nutrients (Mertens, 1992). Seymour et al., (2005) reported that milk yield of cows were positively correlated to DMI and similar trend was observed in the current study, where NFC content was similar in all diets (average of 40.3%); it was found that milk yield tended to be higher in cow fed diet containing 6.4% of SP and 11% of sugar, these diets improved DMI, resulting in increased milk production; in addition there was increased milk protein and lactose concentration as well. Decreases in protein yield were attributed to trends for decreased milk yield. Both the amount and type of dietary protein can influence the protein content of milk (DePeters and Cant, 1992), therefore, high milk protein for cow fed 6.4% of SP diet (40% of total CP) may be possible due to higher supply of microbial crude protein for the lactating cow than those fed other diets. It is generally considered that the concentration of lactose cannot be changed by dietary means apart from severe underfeeding. However, Cherney et al. (2003) found percentage of milk lactose was higher in high NFC (40%) diets and also slightly higher with sucrose diet (sucrose substituting for 10% of corn). In the current study, cows fed 6.4% SP and 11% sugar diet had the highest lactose content. Lactose is commonly considered to be the major compound regulating milk osmolality and related to the milk yield, therefore the lowest milk yield was observed in cows that had lowest content of lactose in milk.

In conclusion, the results from the present study showed that supplementation of soluble N plus NFC source improved IVDMD of cellulose and rice straw, moreover, 25-50% of total soluble N from urea can be used to enhance feed digestion. Conversely, IVDMD of rice straw did not differ by varying the concentration of starch: sugar as

percentage of NFC. Increasing the level of soluble protein at 5% of diet DM (38% of total CP) and sugar level at 8% of the DM can alter digestibility and gas production. The SP and sugar at 5% and 8% of diet DM respectively had higher NDF and ADF digestibility. By increasing sugar level the potential extent of gas production and gas production from insoluble fraction was linearly decreased. The findings in feeding trial suggest that dietary SP (up to 60% of the CP) can be fed to crossbred Thai steers receiving sugar at a level of 11% of the DM. Feeding excess SP (>60% of CP) does not cause further improvements in animal performance. As dietary SP and sugar increased to 6.4% (40% of CP) and 11% of diet DM, respectively, there were improvement in feed intake, nutrient digestion, microbial protein synthesis and milk production and composition. The results of the current study indicate that the productivity of lactating cows fed rice straw as roughage can be improved when the SP: sugar ratio is 1:1.7, as a percentage of the DM. However, there is a requirement for further studies to be conducted, particularly with diets containing high soluble fraction of SP and sugar on the rumen microbial population profiles and the impact of ketogenic metabolites produced on energetic and reproductive efficiency of dairy cows.