CHAPTER V

EXPERIMENT 3: OPTIMIZING THE RATIOS OF SOLUBLE PROTEIN AND CARBOHYDRATE ON TOTAL TRACT DIGESTIBILITY, MICROBIAL PROTEIN SYNTHESIS AND MILK PRODUCTION OF DAIRY COWS

5.1 Introduction

Current feeding standards for dairy cows focus on providing protein as a nutrient for both ruminal microbes and the animal (NRC, 2001). Nevertheless, feeding dairy cow excess protein contributes to environmental nitrogen pollution (Kebreab et al., 2002) and can result in unnecessary feeding expenses due to the high costs of protein sources. Soluble protein is the portion of rumen degradable protein (RDP) including free amino acids and NPN that is immediately available for microbial utilization in the rumen (NRC, 2001). Haig et al. (2002) found nitrogen (N) excretion increased for lactating cow as dietary soluble N level increased from 47.9 to 59.7% of CP ($P<0.05$) by adding at urea 0.5 - 2.7% of diet an as fed basis. This might indicate of inefficient capture of ruminal NH_3-N for microbial protein synthesis, likely attributable to an insufficient supply of readily fermentable energy substrate. When high roughage diets are fed, the rate of ruminal energy fermentation may be too slow and limiting the ruminal microorganisms ability to synthesize protein from the rapidly available RDP (Kim et al., 1999). Under these circumstances, increasing the rate of carbohydrate fermentation could result in more effective capture of RDP and improved supply of metabolizable protein to the dairy cow.

Sugar is more rapidly fermented in the rumen than starch (Chamberlain et al., 1993). The Cornell Net Carbohydrate and Protein System (NRC, 1996) indicated that the microorganisms fermenting soluble sugars could contribute approximately 18% more microbial protein than those fermenting starch with high-moisture corn diets. Ribeiro et al. (2005) showed that bacterial production in continuous culture increased linearly from 12.3 to 14.4 g/d as the concentration of sucrose increased from 0 to 8 %. Microbial protein production increased linearly and total organic acids (sum of

acetate, propionate, butyrate and lactate) tended to increase linearly with increasing sucrose (Hall and Weimer, 2007). Lean et al. (2005) showed that the combination of sugar with a source of soluble protein such as ammonia, amino acids and peptides increased more microbial protein production than starch alone. Gerbler and Heinrichs (2003) indicated that the differences in microbial production might due to RDP or specific RDP fractions. 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, a recent study by Penner et al. (2009) demonstrated that replacement of cracked corn with sucrose did not decreased ruminal pH. The replacement of starch with sucrose may have the potential to improve nutrient supply and digestibility to lactating cows without negatively affecting ruminal fermentation. Based on the results of the experiment 2, the experiment 3 was conducted as a follow up with the objective of investigating a protein and sugar fraction in TMR diet, with increased soluble protein at 30% to 60% of total CP and sugar level fed to lactating cows on productivity, ruminal fermentation, and nutrient digestibility.

5.2 Materials and Methods

5.2.1 Experimental design, Cows and diets

Twelve Crossbred Holstein (>93.75%) lactating cows (9 primiparous and 3 multiparous) with 99 ± 63 days in milk (DIM), were blocked into 3 groups of 4 by DIM in a randomized complete block design. All cows were injected with mixture of vitamins (A, D and E). Before starting experimental all cows were fed the same diet for a week (week) and the experiment was conducted for 56 day (d). Cows within blocks were then randomly assigned to 1 of the 4 diets and fed only that diet during 8-week experimental of period.

Rice straw were chopped into a theoretical length of 1.3 cm and used as roughage source. The four experimental diets were incorporated with rice straw, cassava chip, cassava pulp, palm meal, cane sugar powder waste, soybean meal (SBM), urea and vitamin and mineral mixed (Table 5.1). The cane sugar powder waste (approximately 96% sucrose) in diets ranged from 8 to 17% and urea ranged from 0.9 to 2.9%. The level of soluble protein (SP) ranged from 4.8, 6.4, 8.0, or 9.6%

of the diet DM (30, 40, 50, or 60% of total CP, respectively) by using KCF 2006 Program (Pattarajinda and Duangjinda, 2006). SBM in the diet were replaced by urea in order to increase dietary soluble protein. Cows were housed in separated stalls and had free access to water during the trial. All diets were fed as a total mixed ration (TMR) for *ad libitum* intake, with feed offered 4 times a day (06.00, 10.00, 14.00, 18.00 h). Cows were milked twice a day at 05.30 and 15.30 h.

5.2.2 Data and Sample Collection

Maximum and minimum dairy barn temperature was recorded throughout the experiment. Body weights were measured on 2 consecutive days at the start and end of the 8 week experiment to compute BW change. The feed offered was adjusted daily to yield orts of about 5 to 10% of intake. The feed bunks were cleaned out on each morning and ort was collected and weighed throughout the experiment. Weekly composites of the TMR, orts, were collected from daily samples of about 0.5 kg and stored at - 20°C. Milk production was measured throughout the experimental period, and individual milk yields were recorded at each milking. Milk samples were collected and preserving with 2-Bromo-nitropropane-1, 3-diol at 2 consecutive (p.m. and a.m.) through week 2, 4, 6, and 8 of experimental period, and were analyzed for fat, protein, lactose, ash and solid non fat (SNF).

On the last day of week 2, 4, 6, and 8 of experimental period, blood samples were collected from the coccygeal vein at 0 h and 3 h after the morning feeding in 10 ml tubes containing sodium heparin, immediately placed on ice until centrifugation at 3,500 rpm for 15 min and the plasma was stored at - 20°C before analysis for glucose, urea, and insulin. Rumen fluid samples were collected at the same time of blood was collected, via stomach tube from each cow. Ruminal fluid samples were obtained by straining ruminal contents through 2 layers of cheesecloth; pH was immediately determined by using pH meter (Electrochemical Analyzer, Consort model C933P) and then preserved by addition of 5 ml of 1*M*H_2SO_4 solution to 50 ml of rumen fluid and stored at - 20°C.

Total tract digestibility of DM, OM, CP, ADF, NDF, and EE was determined during the week 7 and 8 of experiment. Chromium oxide (Cr_2O_3) was used as an indigestible marker. The TMR diets were mixed to contain 1 g chromium-oxide/kg of diet DM and fed to the steers for 4 consecutive days before collecting

feces samples every four hours, daily. Then feces sample were composited from each time point and frozen at -20°C until analysis. Spot urine samples were collected from all cows at 6 and 18 h after feeding, by manual stimulating of the cow to urinate on 2 days during the first 2 day of week 8. A 5 ml aliquot of urine was diluted immediately with 45 ml of 0.036*N* H_2SO_4 and was stored at - 20°C until analysis for purine derivatives and creatinine.

5.2.3 Sample Analysis

Diets TMR, orts, and fecal samples were dried at 60°C and ground to pass through a 1-mm screen using a Wiley mill (Thomas-Wiley, Philadelphia, PA). Ground diet samples were analyzed for DM; OM was calculated as the difference between the DM content and ash content. Ash content was determined after placing samples in a muffle furnace for 4 h at 550°C (AOAC, 2002), and crude protein was obtained by total N determination using micro-Kjeldahl technique and a fix conversion factor (6.25). Ether extract (EE) was determined gravimetrically after extraction using petroleum ether in a Soxhlet instrument (AOAC, 1990). ADF (AOAC, 1990) and NDF (Van Soest et al., 1991) were determined by the fiber analyzer (ANKOM$^{200/220}$, ANKOM Technology Corp., Fairport, NY, USA). Sodium sulfite and α-amylase were used for NDF analysis. Soluble protein was determined with a sodium borate, sodium phosphate buffer procedure (Krishnamoorthy et al., 1982). Total sugar was determined according to AOAC (2000). Non-fiber carbohydrate (NFC) was calculated by subtraction of % CP, NDF, EE, and ash (Sniffen et al., 1992). Daily DMI was computed based on the 100°C basis for TMR and orts. Individual milk samples were analyzed for concentrations of fat, CP, lactose, mineral and SNF (AOAC, 1997) by using Milk analyzer (MILKO SONIC S-L90). Plasma samples were analyzed for plasma urea concentrations, glucose and insulin by using Automated Chemistry (HITACHI 912).

Ruminal fluid, immediately prior to analysis samples were thawed and centrifuged at 3,500 rpm for 15 minutes at 4°C and supernatants were collected to determine ammonia nitrogen (NH_3-N) by using Kjeldahl method and volatile fatty acids (VFA) concentration by using a high-performance liquid chromatography (HPLC, Instruments by controller water model 600E; water model 484 UV detector), according to the method of Zinn and Owens (1986). Composited fecal samples were analyzed for DM, OM, ash, CP, EE, ADF, and NDF as described for feeds analysis previously. The Cr in

fecal and diets was measured by atomic absorption spectrometry at a wavelength of 357.9 nm, using potassium dichromate as a standard. The total tract digestibility was calculated by using the concentrations of the individual nutrients and chromium oxide in the diet and feces (Maynard et al., 1979). Spot urine samples were pooled (equal volume daily) for each cows and analyzed for total N (Kjeldahl method), uric acid, creatinine (IAEA, 1997) and allantoin content in urine was determined by HPLC as described by Chen and Gomes (1995). The estimated daily purines derivative (PD, uric acid plus allantoin) excretion from spot urine samples and PDC index is as an indicator of microbial growth in the rumen was calculated (Makkar and Chen, 2004) as follows:

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

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

$$\text{Daily urinary PD (mmol)} = (\text{PDC index}) \times C$$

Where "C" is the overall daily creatinine excretion in the urine in mmol/kg$W^{0.75}$/d. Since Friesian crossbred cattle were used in experiment, a value of 0.89 mmol/kg$W^{0.75}$/d based on the average value reported for European cattle (Chen et al., 1995; Vagnoni et al., 1997; Pimpa and Liang, 2004) was used. Purine absorption (X, mmol/d) and PD excretion (Y, mmol/d), can be calculated (for European cattle) as:

$$Y = 0.85X + (0.385\ W^{0.75})$$

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

Then, microbial nitrogen production was calculated from PD excretion in urine using the method proposed by Chen and Gomes (1997) as:

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

When digestibility of microbial purine is assumed to be 0.83. The N content of purines is 70 mg N/mmol. The ratio of purine N: total N in mixed rumen microbes is taken as 11.6:100.

5.2.4 Statistical analysis

Body weight, nutrient intake, total tract digestibility of nutrients, VFA, urinary metabolites, and microbial N estimated from urinary excretion of PD were analyzed as a randomized complete block design using the MIXED procedure (SAS Institute, 1996). The model included the fixed effects of treatment and the random effects of block. Treatment differences were considered to be significant when $P< 0.05$ and were considered to indicate a trend at $P<0.10$. The statistical model was:

$$Y_{ijk} = \mu + b_i + t_j + \varepsilon_{ijk}$$

Where:

Y_{ijk} = the measured variable,

μ = the overall mean,

b_i = the block effect (i= 1, 2, 3),

t_j = the treatment effect (j= 1, 2, 3, 4),

ε_{ijk} = residue error.

Dry matter intake (DMI), NH_3-N concentration, plasma urea nitrogen and glucose, and milk yield and composition were analyzed using the MIXED procedure of SAS with the model described above except the 'repeated' option was used for time (week) of sampling. The statistical model was:

$$Y_{ijkl} = \mu + b_i + t_j + time_k + b_j*time + t_j*time_k + \varepsilon_{ijkl}$$

Where:

Y_{ijk} = the measured variable,

μ = the overall mean,

b_i = the block (i= 1, 2, 3, 4),

t_j = the treatment (j= 1, 2, 3, 4),

$time_k$ = the week of sampling (k= 2, 4, 6, 8),

ε_{ijkl} = residue error.

5.3 Results and Discussion

Maximum and minimum means of daily barn temperature were 33.2 ± 0.6 and 25.3 ± 1.3°C. Ingredient and nutrient composition of diets are shown in Table 5.1. Diets were formulated to be isonitrogenous (16.0%), however, analyses ranged from 16.3% to 16.5% CP. SP increased from 29.9% to 60.3% of CP, sugar increased from 10.0% to 17.7% of diet DM. Rice straw was the main source of fiber in this study contained 2.9% protein, 68.4% NDF, and 41.1 % ADF. Cane sugar powder waste was 96% sugar concentration and consisted mostly of sucrose. The total mixed experimental diets were relatively similar in NDF, ADF and NFC.

Table 5.1 Ingredients and composition of TMR diets

Diet	T1	T2	T3	T4
SP, % of DM	4.8	6.4	8.0	9.6
Sugar, % of DM	8	11	14	17
SP:sugar ratio	1:1.67	1:1.72	1:1.75	1:1.77
Ingredient, % of DM				
Rice straw	28.0	28.0	28.0	28.0
Cassava chips	11.8	13.3	14.3	15.8
Cassava pulp	15.0	15.0	15.0	15.0
Palm seed meal	10.0	10.0	10.0	10.0
Soybean meal	25.9	20.7	16.0	10.8
Cane sugar powder waste	8.0	11.0	14.0	17.0
Urea 46 % N	0.9	1.6	2.2	2.9
Vitamin-mineral premixed	0.5	0.5	0.5	0.5
Feed cost, baht/kg	6.73	6.11	5.54	4.92
Analyzed content, % of DM				
DM	92.04	92.12	92.40	92.55
CP	16.25	16.33	16.54	16.52
SP	4.79	6.44	7.98	9.63
SP, % of total CP	29.91	40.29	49.86	60.28
EE	1.49	1.56	1.33	1.12
NDF	36.80	35.89	34.32	33.43
ADF	24.20	24.55	22.11	21.49
NFC[a]	38.83	39.85	40.30	42.18
Total sugar	9.98	12.53	15.11	17.66
Ash	6.62	6.36	7.51	6.76
TDN	70.32	69.67	69.09	68.45
NE for lactation [b]Mcal/kgDM)	1.603	1.587	1.573	1.557

[a] NFC 100 – (% Protein + % EE + % NDF + % ash)

[b] $NE_L = (0.0245 \times TDN) - 0.12$

5.3.1 Intake and total tract digestibility

The DMI, nutrients intake, and BW are presented in Table 5.2. Dietary treatment did not affect on DMI and organic matter intake (OMI) (12.3 and 11.4 kg/d, respectively). The average amount of NPN (from urea) that fed for T1, T2, T3 and T4 were 112.2, 200.3, 269.1 and 349.7 g/d, respectively. Poos et al (1979) reported that inclusion rates of 1 to 2% urea in diet DM can be use in high grain diet without any adverse effect. The highest level of urea that used in this study is 2.9% of diet DM, however, no sign of urea toxicity was observed might possible due to the amount of sugar was high (9-18%; Table 5.1). Increasing SP and sugar level in TMR diets did not affect intake of CP, NDF, ADF, NFC and NE_L of dairy cows ($P>0.05$). However, EE intake was lower for cow fed 9.6% SP and 17% sugar (T4) as compared to those fed other dietary treatments. Rodriguez et al. (1997) and Cabrita et al. (2003) reported that sugar was likely responsible for reductions in feed intake in dairy cattle fed higher levels of citrus pulp. Thus the numerical reduction in DMI in the present study may have resulted from higher sugar levels.

Dietary treatment did not affected apparent total tract digestibility of DM, OM, CP, EE, NDF or ADF (Table 5.2). Digestibility of DM, OM, CP, EE, NDF and ADF had an average 77.26, 75.48, 78.39, 86.77, 62.54 and 59.85%, respectively. Broderick and Radloff (2004) reported adding 2.4 to 7.2% sugar to diet resulted in linear increases in apparent DM and OM digestibility. These researchers estimated of overall optimum for total dietary sugar based on yield of milk and milk components to be about 5% total sugar. Over feeding of sugar above this level appeared to reduce performance.

Table 5.2 Effects of increasing SP and sugar level in TMR diets on intake and total tract digestibility of nutrient

Diet	T1	T2	T3	T4		
SP, % of DM	4.8	6.4	8.0	9.6		
Sugar, % of DM	8	11	14	17		*P*-
SP:sugar ratio	1:1.67	1:1.72	1:1.75	1:1.77	SEM	value
Intake, kg/d						
DM, kg/d	12.47	12.52	12.23	12.06	0.24	0.52
DM, %BW	3.24	3.04	3.28	3.25	0.19	0.81
OM	11.57	11.65	11.24	11.18	0.54	0.90
CP	2.03	2.04	2.02	1.99	0.10	0.99
SP	0.60^{d}	0.81^{c}	0.98	1.16^{a}	0.04	<0.01
EE	0.19^{ab}	0.20^{a}	0.16^{bc}	0.13^{c}	<0.01	0.01
NDF	4.59	4.49	4.20	4.03	0.21	0.30
ADF	3.02	3.07	2.71	2.59	0.14	0.13
NFC	4.48	4.99	4.93	5.09	0.23	0.89
Sugar	1.24^{d}	1.57^{c}	1.85^{b}	2.13^{a}	0.08	<0.01
NE_L (Mcal/d)	19.98	19.87	19.62	19.33	0.38	0.52
Total tract digestibility, %						
DM	77.55	80.51	77.71	73.27	2.58	0.34
OM	75.84	79.09	75.77	71.21	2.79	0.34
CP	77.75	82.42	76.73	76.67	2.35	0.24
EE	86.52	90.83	83.42	86.31	1.76	0.11
NDF	64.55	68.68	62.84	54.08	4.68	0.26
ADF	61.84	68.03	59.01	50.55	5.30	0.23

[a, b, c] Means within a row different superscripts differ ($P<0.05$).

5.3.2 Characteristics of ruminal fermentation

1) Ruminal pH

Rumen fluid pH is shown in Table 5.3. The pH of rumen fluid collected 0 h and 3 h after feeding were not affected by dietary treatments (P>0.05) (6.7 and 6.8, respectively). Garrett (1996) reported that cows had rumen fluid pH above 5.8 were consider normal, while those between 5.0 - 5.8 may be suffering from subclinical acidosis. Mean ruminal pH was maintained at normal level at 3 h after

feeding in animals fed the TMR diet, suggesting that no ill effects of lowered pH resulted from feeding diets that averaged 40.3% NFC and 9 to 18% sugar, moreover lactic acid was not observed at detectable in this study. *In vitro* (Vallimont et al., 2004), and *in vivo* studies (Held et al., 1999; Broderick, 2004; Broderick et al., 2008) had reported no effect of sucrose on rumen pH, which is similar to these results. Recently Penner et al. (2009) reported that sucrose tended to improve ruminal pH. Mean ruminal pH was normal at 3 h after feeding in present study; one explanation may be that urea provides buffering through its conversion to ammonium bicarbonate (NH_4HCO_3) in the rumen (Van Soest, 1994; Chenost et al., 2001). According to Kertz et al. (1982) has been reported that reticulum pH was elevated when cows were fed urea-containing diets. Therefore, it was indicated that the level of SP in the TMR diets can be maintain ruminal pH in this study.

2) Ruminal NH_3-N

Ruminal NH_3-N levels depend on CP content of the diet, the rate of degradation of feed protein, the feed intake level, feeding pattern and the synchrony between the degradation of protein and carbohydrate (Tamminga, 2006). In the current study ruminal NH_3-N concentration increased at 3 h after feeding with increasing SP and sugar level in diets, but there was no statistically significant differences by treatments and had average at 18.3 and 25.0 mg/dl on 0 h and 3 h after feeding, respectively (Table 5.3). The optimum ruminal NH_3-N concentration has been defined as the minimum concentration of NH_3-N necessary to support the maximum synthesis of microbial protein (Satter and Slyter, 1974; Kang-Meznarich and Broderick, 1981) and the maximum ruminal degradability of DM (Mehrez et al., 1977; Kang-Meznarich and Broderick, 1981) that range from 2 to 13 mg/dl and from 3 to 25 mg/dl, respectively. Boucher et al. (2007) studied in lactating cow fed 16.5% CP diet found that microbial protein synthesis was maximized at average ruminal NH_3-N concentration of 12.8 mg/dl. In ruminants fed low-quality roughages, critical ruminal NH_3-N levels for microbial activities range from 5 to 20 mg/dl (Boniface et al., 1986). Perddok and Leng (1989) showed that higher level of ruminal NH_3-N (15-30 mg/dl) improved intake and feed digestibility. Wanapat and Pimpa (1999) also found NH_3-N level of 13.6 to 34.4 mg/dl improved rumen fermentation by increasing digestibility and intake of straw in swamp buffaloes. However, increasing ruminal

NH_3-N up to 30 mg/dl significantly decreased acetate plus butyrate:propionate, increasing rumen fungal zoospores as well as increasing microbial protein synthesis (17 - 47%) (Kanjanapruthipong and Leng, 1998). It was suggested that optimum ruminal NH_3-N level for ruminants fed lower quality roughage would be higher than 15 mg/dl. In present study, DM and nutrient digestibility were not different and may be due to the concentration of NH_3-N was substantially more than minimum levels that would limit microbial digestion activity; therefore the response of feed digestibility was not different among dietary treatments.

3) VFA production

(1) Total VFA

Total VFA and individual VFA are shown in Table 5.3. At week 2 of experimental period concentrations of total VFA was not different among dietary treatments. There tended to higher total VFA ($P<0.05$) 3 h post feeding for cows fed 9.6% SP and 17% sugar diet (T4) than those fed other diets ($P<0.10$). The last week of experiment (week 8), at 0 h (before feeding) cow fed T4 diet tended to have higher total VFA ($P<0.10$) than those fed other diets. Moreover, at 3 h post feeding, total VFA concentration were highest in for cows fed T4 diet compared to cows fed T1 and T3 diets ($P<0.05$). In contrast, Bach et al. (1999) reported an increase ($P>0.05$) in total VFA concentration for cracked corn compared to beet pulp and molasses in a continuous culture study. VFAs are the main products of microbial fermentation of carbohydrates in the rumen, therefore increased ruminal VFA concentrations are often assumed to be a result of increased fermentation of carbohydrates in the rumen. Although, carbohydrate digestibility (NDF, ADF) was not significant different among dietary treatments, cows fed T4 diet had highest in total VFA concentration.

(2) Acetate

Acetate is a lipogenic nutrient, a precursor of fatty acid synthesis and ultimately of milk fat synthesis in the mammary gland (Van Soest, 1994). Molar proportion of acetate was not significant difference among dietary treatments before feeding neither week 2 nor week 8 of experimental period (Table 5.3). However, at 3 h post feeding molar proportion of acetate tended to increase in week 2 ($P<0.10$) for cow fed T4 diet more than cows fed other diets, but acetate was also higher for cows fed T4 ($P<0.05$) during week 8 of experiment period compared to cow fed T1 and T3

diets. Therefore, the current study, higher in proportion of acetate was found for cow fed the highest level of sugar diet. However, Charmely et al. (1991) reported that sucrose supplementation at 10% of silage intake (DM) did not affect molar proportion of acetate in the rumen ($P>0.05$). Sucrose has been associated with relative decreases in ruminal acetate concentration (Sutton, 1979; Khalili and Huhtanen, 1991; Chamberlain et al., 1993; Moloney et al., 1994; Heldt et al., 1999). Chamberlain et al. (1993) found that when sucrose was supplemented at 200g/d (approximately 5% of daily diet DM) to a grass silage diet, ruminal acetate proportions was decreased in sheep ($P<0.05$). When cane molasses, a refinery by-product source of sugars, was fed to steers at 61% of DMI, ruminal acetate proportion decreased as compared to steers fed a diet with the same amount of barley ($P<0.01$; Moloney et al., 1994). However, when sucrose was substituted for corn at 3.2% of diet DM in a diet for lactating dairy cows, acetate production was not affected ($P>0.10$; Sannes et al., 2002). The effect of sugars on the ruminal molar proportion of acetate in vivo may depend on the amount of sucrose included in the diet.

(3) Propionate

Increasing SP and sugar level in TMR diets did not affected molar proportion of propionate both before and 3 h post feeding on week 2 and week 8 ($P>0.05$; Table 5.3). In contrast, ruminal propionate proportions increased ($P<0.05$) for sheep fed a sucrose supplemented diet (Chamberlain et al., 1993). In some studies ruminal molar proportions of propionate did not differ whether small amounts (5.6% dextrose; Piwonka et al., 1994) or larger amounts of sugar were added (61% molasses; Moloney et al., 1994) to the diet. 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 (Heldt et al., 1999). It may be that other components of the diet such as protein alter the yield of propionate from NFC. Propionate is a precursor for glucose synthesis in the liver and thus important for the glucogenic energy supply to the ruminant. The effect of sugar on ruminal propionate proportion varies among in vivo studies the reason for this variation remains to be determined.

(4) Butyrate

Butyrate supplies energy to the animal, mainly to the heart and skeletal muscle, in the form of β-hydroxybutyrate (a ketone body). It is ketogenic and can be used for the production of fat (McDonald et al., 1995). Data in this study showed that before feeding, molar proportion of butyrate was not affected statistically by dietary treatments in week 2 and week 8 of experimental period ($P>0.05$; Table 5.3). At 3 h post feeding, there was tended to higher ($P<0.10$) in molar proportion of butyrate for cows fed 9.6% SP and 17% sugar (T4) diet compared to cow fed other diets in week 2 of experimental period. However, in week 8 of experiment it was numerically increased ($P>0.05$) at 3 h post feeding for cows fed T4 diet ($P<0.10$) compared with cow fed other diets. According to Heldt et al. (1999) studied, it would appear that sucrose yields more butyrate than glucose and fructose. 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). However, *in vivo* comparisons of the rumen fermentation of feed high in citrus and beet pulps (contain 12.5 - 40.2% and 12.8 - 24.7% sugar, respectively) 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).

Increasing SP and sugar level diets, lactate concentration was not observed in the present study. It is possible that bacteria convert dietary sugar to microbial glycogen as a short-term storage of energy that can be utilized later, thereby temporarily reducing fermentation acid production. According to Hall and Weimer (2007) *in vitro* that addition of 65, 130, or 195 mg of sucrose to 130 mg of isolated NDF resulted in increased glycogen at 0 and 4 h but found that glycogen concentration decreased thereafter. Other studies have demonstrated that inclusion of sugar may result in increased solid or liquid passage rate from the rumen (Rooke, 1987; Sutoh et al., 1996). If feeding sucrose increased the passage rate, it may also have decreased the amount of carbohydrate fermented in the rumen and thereby reduced fermentation acid accumulation. Nocek (1997) have reported that lactate accumulation in dairy cows was rare. Penner et al. (2007) found ruminal lactate less

than 5 m*M* when ruminally cannulated transition cows were fed prepartum diets vary in the forage to concentrate ratio. In contrast, *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.

The ratio of acetate:propionate before feeding (0 h) was not significant difference among dietary treatments either week 2 or week 8 of experimental period. However, at 3 h post feeding acetate to propionate ratio was lower for cows fed T1 diet (4.8% SP; 8% sugar) in week 2 ($P<0.05$) and also tended to be low in week 8 ($P<0.10$) as compared to cows fed other diets. Acetate to propionate ratio of 3.0 - 4.1 was reported in vivo (DePeters et al., 1997; Brown et al., 2002). Higher acetate to propionate ratio is an indication of proportionally higher digestible NDF; however NDF digestibility was not significantly different among diets in current study.

Table 5.3 Effects of increasing SP and sugar level in TMR diets on ruminal fermentation characteristics

	Diet	T1	T2	T3	T4		
	SP, % of DM	4.8	6.4	8.0	9.6		
	Sugar, % of DM	8	11	14	17		*P*-
	SP:sugar ratio	1:1.67	1:1.72	1:1.75	1:1.77	SEM	value
Rumen pH	0 h before feeding	6.77	6.71	6.75	6.77	0.09	0.96
	3 h-post feeding	6.80	6.86	6.82	6.78	0.07	0.66
NH3-N, mg/dl	0 h before feeding	17.83	19.39	18.16	17.78	1.57	0.87
	3 h-post feeding	21.91	23.80	26.68	27.53	2.02	0.21
VFA (Week 2)							
Total VFA, mM	0 h before feeding	81.07	92.33	81.94	86.03	6.66	0.64
Acetate		51.91	59.78	53.90	56.73	4.92	0.72
Propionate		18.17	18.76	18.00	16.10	1.11	0.41
Butyrate		10.95	13.79	10.04	13.20	1.30	0.17
Acetate: propionate		2.74	3.18	3.03	3.51	0.21	0.12
Total VFA, mM	3 h post feeding	79.96	91.70	93.50	102.74	5.02	0.09
Acetate		51.02	58.91	63.62	68.18	3.65	0.06
Propionate		18.75	19.28	18.86	19.18	0.66	0.68
Butyrate		10.20	13.50	11.02	14.75	1.08	0.07
Acetate: propionate		2.72^{b}	3.05^{ab}	3.37^{a}	3.44^{a}	0.12	0.02
VFA (Week 8)							
Total VFA, mM	0 h before feeding	82.30	84.38	83.61	105.98	4.81	0.06
Acetate		54.09	56.02	53.45	67.50	3.71	0.17
Propionate		16.81	16.73	18.16	18.87	1.03	0.42
Butyrate		11.40	11.63	12.01	14.43	1.06	0.34
Acetate: propionate		3.24	3.35	2.94	3.56	0.26	0.49
Total VFA, mM	3 h post feeding	85.50^{b}	97.95^{ab}	88.50^{b}	107.52^{a}	4.59	0.04
Acetate		54.64^{c}	64.87^{ab}	57.10^{bc}	69.81^{a}	3.00	0.03
Propionate		19.19	19.68	19.43	21.13	1.01	0.56
Butyrate		11.67	13.40	11.98	16.58	1.41	0.15
Acetate: propionate		2.85	3.30	2.94	3.32	0.12	0.06

[a, b, c] Means within a row different superscripts differ ($P<0.05$).

Individual VFA expressed as molar proportion.

5.3.3 Plasma metabolites

Increasing the SP concentration in diets increased plasma urea nitrogen (PUN) concentration (Table 5.4). PUN concentration was lower for cow fed 4.8% SP and 8% sugar (T1) diet than those fed other diets ($P<0.01$) at 0 h and 3 h post feeding. There was no differing in PUN for cow fed diets contain SP of 6.4 to 9.6% and sugar of 11 to 17% of DM ($P>0.05$). The decreased PUN with diets contain glucose and starch (Hristov et al., 2005), reflected increased capture of NH_3-N in the rumen and seem to related to the amount of ruminally fermentable energy introduced to rumen with glucose and starch. Huhtanen and Robertson (1988) and Sannes et al. (2002) also observed decreased urinary N losses with sugar supplementation. PUN was decreased with maltodexrin (Kim et al., 1999), or glucose supplement to dairy cows (Osbone et al., 2002), but no effect was reported by Sannes et al. (2002) and Ordway et al. (2002). Other report had observed increased PUN (McCormick et al., 2001) with sucrose supplementation. The high level of PUN at 3 h after feeding in this study may be possible due to the concentration of nitrogen solubility being higher than the ability of ruminal microbes to capture it. The lack of effect of sucrose supplementation on nitrogen utilization may be possible due to the high rate of sucrose outflow from the rumen that prevented it to be utilized by ruminal bacteria as an energy source (Cherney et al., 2003). Hoover and Webster (2001), in a review of *in vitro* and *in vivo* data on soluble carbohydrate, suggested that a high proportion of a very soluble carbohydrate such as sucrose may leave the rumen before being fermented with the liquid fraction and thus it lowers its contribution to microbial protein synthesis. According to Henning et al. (1993) that indicated that the disappearance rate of sugar was 69% /h.

However, cow fed diet with increasing sugar level between 9-18% of DM were no detectable significant treatment affects on plasma concentration of glucose or insulin ($P>0.05$) average 60.93 mg/dl, or 13.53 μIU/ml at 3 h after feeding, respectively. Cabrita et al. (2007) reported plasma concentration of glucose (56.0 - 57.3 mg/dl) and insulin (1.15 - 2.3 μIU/ml) were lower for cow fed low starch diets (high sugar level) compared to high starch (low sugar) diet, reflecting the low supply of glucogenic substrates. The result of present study indicates increasing sugar level

diets did not difference supply of glucogenic nutrients; it remained within a normal range (45-74 mg/dl; Kaneko et al, 1997).

Table 5.4 Effects of increasing SP and sugar level in TMR diets on blood metabolites levels

Diet	T1	T2	T3	T4		
SP, % of DM	4.8	6.4	8.0	9.6		
Sugar, % of DM	8	11	14	17		*P*-
SP:sugar ratio	1:1.67	1:1.72	1:1.75	1:1.77	SEM	value
Plasma urea N, mg/dl						
0 h before feeding	12.42[b]	18.08[a]	18.00[a]	18.58[a]	0.78	<0.01
3 h-post feeding	13.75[b]	19.33[a]	20.41[a]	21.83[a]	0.97	<0.01
Plasma glucose, mg/dl						
0 h before feeding	63.00	62.25	62.50	59.92	1.04	0.18
3 h-post feeding	59.83	60.23	61.08	62.25	0.88	0.26
Plasma insulin, μIU/ml						
0 h before feeding	15.41	9.27	12.62	10.03	2.38	0.28
3 h-post feeding	13.13	15.88	11.25	13.87	2.20	0.56

[a, b] Means within a row different superscripts differ ($P<0.05$).

5.3.4 Estimation of microbial protein production

In ruminant the measurement of urinary purines derivatives (PD; uric and allantoin) used as an indicator of ruminal microbial protein production (Chen et al., 1990; Chen and Gomes, 1997). Chizzotti et al. (2008) reported the PD excretion estimated by the spot sampling was not different from that observed using the total urine collection (r^2=0.82) in lactating cow; it was not significantly different between morning and afternoon samples (Pimpa and Liang, 2004). Several authors (Chen et al., 1992; Valadares et al., 1999; Cetinkaya et al., 2006) have proposed the used of spot sampling technique to access the excretion of urinary nitrogenous compounds. Maximizing synthesis of microbial protein as a relatively inexpensive source of readily digestible protein in the small intestine is desirable; however, inefficiency of protein use within the animal increases as RDP increases from 6.8, 8.2, 9.6, and 11.0% of DM, causing concern

of increased N excreted as waste (Kalscheur et al., 2006). The present study was hypothesized that optimizing the ratio of soluble protein and carbohydrate would be improving microbial protein synthesis. Results of the study showed that, the concentration of allantoin, total PD, creatinine, and total N in urine were not significantly different by the concentration of SP and sugar in dietary treatment ($P>0.05$). Allantoin has been reported to be the major form of PD excreted in cattle urine (Chen et al., 1990). This is in agreement with the results of this study (Table 5.5). Moreover, daily PD excretion (mmol/d) increased with digestible organic matter intake (DOMI, kg/d). This is in agreement with observations of Thanh et al. (2004) who studies in local cattle of Vietnam, cattle were fed the diets at 40, 60, 80, or 90% of *ad libitum* intake. The PDC index calculated as the molar concentration ratio of PD: creatinine time the metabolic live weight ($kgW^{0.75}$) of the cow were higher for cow fed T2 ($P<0.05$) than those fed T1 and T4 diets. Similarly, the estimated microbial crude protein (MCP) supplies for cows using the model developed for Holstein cattle (Chen and Gomes 1995) were 46.3, 72.7, 62.1, and 48.1 g N/d ($P<0.05$) in cow fed T1, T2, T3, and T4 diets, respectively. The ruminal NH_3-N concentration has been defined as the minimum concentration of NH_3-N necessary to support the maximum synthesis of microbial protein (Satter and Slyter, 1974; Kang-Meznarich and Broderick, 1981) range from 2 to 13 mg/dl. However, it has been reported that increasing ruminal NH_3-N up to 30 mg/dl increased microbial protein synthesis (17 - 47%) (Kanjanapruthipong and Leng, 1998). In this study the ruminal NH_3-N concentration was not affected by dietary treatments, ranged from 21.9 - 27.5 mg/dl at 3 h post feeding, however, the greatest of MCP was observed in cow fed T2 diet as the ruminal NH_3-N was 23.8 mg/dl). It was suggested that optimum ruminal NH_3-N level for ruminants fed lower quality roughage would be higher than 13 mg/dl. The increase in lactate production with increasing sucrose treatment is in accord with reports that, in some microbial species (*Streptococcus bovis*; *Selenomonas ruminantium*) rapid growth at high sugar concentration, with a resultant decrease in catabolic efficiency (ATP produced per unit hexose consumed). Therefore, a decrease in energy available to the microbes could limit the synthesis of macromolecules including protein, thus limiting the yield of microbial mass and MCP from fermented sugar (Russell and Hino, 1985; Melville et al., 1988). Moreover, it is envisaged that MCP will be maximized by balancing the availability of fermentable energy and degradable nitrogen in the rumen

(Dewhurst et al., 2000). The results of this study indicate the optimum ratio of SP: sugar is 1: 1.7 or when dietary SP and sugar at 6.4% and 11.0% of the DM, respectively, improved of microbial growth in the rumen, then supplies higher MCP for cow fed rice straw based diet

Table 5.5 Effects of increasing SP and sugar level in TMR diets on concentration of urinary metabolite and estimated microbial crude protein

Diet	T1	T2	T3	T4		
SP, % of DM	4.8	6.4	8.0	9.6		
Sugar, % of DM	8	11	14	17		*P*-
SP:sugar ratio	1:1.67	1:1.72	1:1.75	1:1.77	SEM	value
Uric acid, mmol/l	2.44	2.56	1.98	2.54	0.38	0.65
Allantoin, mmol/l	10.72	16.02	18.24	18.83	3.28	0.37
Creatinine, mmol/l	12.28	12.96	14.91	18.27	2.13	0.29
PD[1], mmol/l	13.17	18.58	20.22	21.37	3.37	0.40
PD:C[2]	1.08	1.43	1.34	1.16	0.09	0.09
PDC index[3]	101.13^{b}	137.24^{a}	120.74^{ab}	101.92^{b}	6.12	0.02
PD excreted, mmol/d	90.00^{b}	122.14^{a}	107.46^{ab}	90.71^{b}	5.45	0.02
PD absorbed, mmol/d	63.75^{b}	99.97^{a}	85.43^{ab}	66.13^{b}	6.47	0.02
Total N in urine, g/l	12.15	13.14	14.90	17.88	2.94	0.33
$BW^{0.75}$, kg	93.02	96.54	90.50	89.60	5.19	0.64
DOMI[4], kg/d	9.45	9.71	9.53	8.66	0.57	0.55
MCP[5], g N/d	46.35^{b}	72.68^{a}	62.11^{ab}	48.07^{b}	4.70	0.02

$^{a, b}$ Means within a row different superscripts differ ($P<0.05$).

[1]PD, total purines derivatives (uric acid + allantoin).

[2]PD:C, total purines derivatives:creatinine.

[3]PDC index, [total purines derivatives] / [creatinine] × $kgBW^{0.75}$ (metabolic weight).

[4]DOMI, digestibility of organic matter intake (kg).

[5]MCP, microbial crude protein.

5.3.5 Milk production

Milk production per cow continues to be a major factor in determining dairy farm sustainability/profitability. Seymour et al. (2005) have been reported milk yield of cows was positively correlated to DMI (r=0.83). 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). In the current study NFC content was similar in all diets (average of 40.3%); it was found that milk yield tended to higher for cow fed diet contained 6.4% and 11% of SP and sugar (T2), respectively than those fed other diets ($P<0.10$). However, significant differences in yield of 4% fat corrected milk (4% FCM) were not observed among dietary treatments ($P>0.05$) (Table 5.6). DePeter and Cant (1992) reported that increasing the protein concentration above requirements had no effect on yield of milk except that NPN content increased. However, Sannes et al. (2002) found that milk yield was unaffected by the level of protein and source of RDP (soybean meal or urea) and Mertens (1992) have been reported that carbohydrates indirectly affect milk production by altering microbial protein production and amino acid supply. Sannes et al. (2002) have reported that milk yield was decreased by replacement of some dietary corn with sucrose. 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). The results from this study indicated that the T2 diet provides the balance of nitrogen and NFC (sugar and starch) to enhance microbial yield, results in increased milk yield. In the current study found that interaction between dietary treatment and week of milk sampling were detected for milk yield and composition (Figure 5.2).

1) Milk fat

Milk fat concentration tended to higher in cow fed T4 diet than those fed other diets ($P<0.10$) (Table 5.6). These results supported by Wings et al. (1988) and Murphy (1999) study demonstrated that cow fed dietary molasses or sucrose increased in milk fat percentages. A linear increased in milk fat yield with increasing dietary sucrose (2 to 7.5%) concentration (Broderick et al., 2008). Penner and Oba (2009) found that milk fat yield tended to high for cows fed high sugar (8.4%) compared to low sugar (4.7%) diets. In contrast, Nombekela and Murphy (1995),

using cows during the first 12 week of lactation, found no effect of sucrose on milk fat yield for cows fed 1.5% sucrose. Increased fat production due to sugar feeding has been increased butyrate production in the rumen (Khalili and Huhtanen, 1991; Lee, 2003). Golombeski et al. (2006) have reported that cows fed fermentable sugar had a significant higher milk fat percentage. However, milk fat percentage was unaffected by the addition of slow release urea and agreed with other studies in which feeding urea had no significant affect on milk fat content (Van Horn et al., 1967; Galo et al., 2003). However, Pan et al. (2003) have reported urea infusion (5.7g/h) enhance fibolytic enzyme activity of liquid-associated microbes at early stage after feeding and those of pacticle-associated microbes at a later stage, through stimulating growth of cellulolytic microbes in steers fed low quality grass hay. According to Highstreet et al. (2010) have reported that feeding a slowly released urea increased milk fat yield, perhaps due to a shift in the profile of VFA or a changed proportion of rumen cellulolytic microorganism. The current study an increase in milk fat percentage may be attributed to observe of increases in molar proportions of ruminal acetate and butyrate (Table 5.3). Both acetate and butyrate are important precursor for de novo fatty acid synthesis for milk fat formation (Bauman and Griinari, 2001).

2) Milk protein

In this experiment milk protein concentration and yield were higher for cow fed 6.4% SP and 11% sugar (T2) diet than those fed other diets ($P<0.01$). The decreased in protein yield can be attributed to the trend for decreased milk yield ($P<0.10$) in those cows. The lowest in milk protein concentration and yield was observed for cow fed 9.6% SP and 17% sugar (T4) diet. Sutton (1989) reported that milk protein concentration increased from 3.1 to 3.5% as the amount of molasses inclusion was increased to 48% of the diet DM; therefore, the level of sugar may not be influence the milk protein in this study. However, the amount and type of dietary protein can influence the protein content of milk. Milk protein yield increased with increasing dietary protein, protein content of milk increased 0.02% for each 1% increase in dietary CP when the CP was not derived from urea (DePeters and Cant, 1992). Sannes et al. (2002) have reported that microbial protein synthesis was increased 8% by soybean meal relative to urea. This suggests that when increasing SP level by urea, the level of soybean meal that provide true protein fraction was

decreased and then affect the profile of AA in the feed protein leaving the rumen. Moreover, estimation of microbial protein production was low for cows fed T4 diet compared with cows fed T2 diet (48.1 vs. 72.7 g N/d), therefore, low milk protein concentration for cows fed T4 diet, it would be unsatisfied of protein or AA for cow requirement to supported milk production.

3) Milk lactose

The percentage of lactose in milk from cow fed 9.6% SP and 17% sugar diet was lower ($P<0.01$) than cows fed other dietary diets, whereas, cows fed SP and sugar at 6.4 % and 11% diet, respectively had the highest lactose concentrations (Table 5.6). Reported effects of sugar on milk lactose concentrations are few, and previous research on feeding sugar such as lactose (DeFrain et al., 2004; 2006) did not demonstrate similar effects. 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). Generally negative relationship between milk fat and lactose concentration has been noted (Jenness and Patton, 1959), and this relationship appears to be supported by increased milk fat concentrations in this experiment. Furthermore, this study was not different in proportion of propionate among treatments, since, lactose provides as a precursor for glucose and subsequent lactose synthesis. However, production of acetate was higher for cow fed diet with 9.6% of SP and 17% of sugar level ($P<0.01$). Lactose is commonly considered to be the major compound regulating milk osmolality and related to the milk yield, therefore the lowest in milk yield were observed in cows that lowest in milk lactose concentration.

Milk ash concentration was higher in cow fed T2 and T3 diets than those fed other diets ($P<0.01$). The lowest concentration of solid not fat (SNF) were detected in milk of cow fed T4 than those fed other diets ($P<0.01$) (Table 5.6). Nonetheless, concentration of total solid were not significant differences among treatments ($P>0.05$), average 13.12% across treatments. In present study were superior in all of component as compared to the standard levels set of the raw milk (3.5% fat; 2.8% protein; 4.5% lactose; 8.25% solids not fat; 12% total solids) by the Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand (Yooyuenyoung et al., 2003). The greatest change of milk composition from

the standard was observed in the concentration of milk fat. Seymour et al. (2005) have reported that milk fat was positively correlated with rumen acetate. Therefore, increasing SP and sugar level tended to increase percentage of fat possible due to a changed proportion of rumen cellulolytic microorganism, resulted in an increase acetate production. Milk yield and component data in this study demonstrated that the level of SP and sugar level at 6.4% and 11% of the diet DM, respectively in TMR diet suitable for lactating cows.

4) Energy corrected milk (ECM) and feed efficiency

The mean values of ECM and feed efficiency (ECM per unit of DMI) are shown in Table 5.6. ECM yield were similar for all 4 diets with average of 13.97 kg/d. Although fed diet containing 9.6% SP and 17% sugar tended to produce less milk; however, ECM yield was similar to the other diets as a result of increased milk fat percentage ($P<0.10$). Feed is the largest single cost to dairy producers and its efficient use will improve net income and reduce potentially negative impacts on the environment. In this study increasing SP and sugar level affected feed efficiency, cows fed T2 and T3 diet had higher in feed efficiency compared to cow fed T1 diet ($P<0.05$). This result indicated that insufficient of nutrient delivery to support lactation then milk production decreased, and feed efficient decreased for cow fed T1 diet.

5.3.6 Body weight

There were not significantly different among treatments in body weight (Table 5.6). However, cow fed high SP and high sugar (T4) diets had a greater decreased in body weight than those fed other diets ($P>0.05$). Penner and Oba (2009) observed that cows fed high sugar (8.4% of diet DM) had 23 and 67% increase in plasma concentration of non esterifies fatty acid (NEFA) and BHBA, respectively compared with cow fed low sugar diet (4.7% of diet DM). Although we did not measure NEFA and BHBA content, it is possible that cow fed the highest sugar level diets had increased mobilization of body reserves and might possible due to body weight loss.

Table 5.6 Effects of increasing SP and sugar level in TMR diets on weight change, milk production, and feed efficiency

Diet	T1	T2	T3	T4		
SP, % of DM	4.8	6.4	8.0	9.6		
Sugar, % of DM	8	11	14	17		*P*-
SP:sugar ratio	1:1.67	1:1.72	1:1.75	1:1.77	SEM	value
Body weight, kg						
Initial weight	416.3	448.3	409.3	412.3	28.25	0.65
Final weight	421.8	443.0	407.0	402.0	31.12	0.64
Weight change	5.0	-5.0	-2.3	-10.3	9.81	0.38
ADG, g/d	95.2	-89.3	-41.7	-184.5	175.11	0.38
Milk production						
Milk, kg/d	13.54	14.63	14.16	13.29	0.26	0.07
4.0 %FCM, kg/d	13.80	15.15	14.89	14.65	0.47	0.24
Fat, %	4.16	4.23	4.45	4.74	0.15	0.06
Fat, kg/d	0.56	0.62	0.61	0.62	0.03	0.32
Protein, %	3.00^{b}	3.05^{a}	3.03^{b}	2.96^{c}	0.02	<0.01
Protein, kg/d	0.40^{b}	0.44^{a}	0.42^{b}	0.39^{c}	0.008	<0.01
Lactose, %	4.60^{b}	4.67^{ab}	4.64^{b}	4.53^{c}	0.02	<0.01
Lactose, kg/d	0.62^{bc}	0.68^{a}	0.65^{b}	0.60^{c}	0.01	<0.01
Ash, %	0.75^{b}	0.76^{a}	0.76^{a}	0.75^{b}	0.002	<0.01
Ash, kg/d	0.102^{bc}	0.112^{a}	0.105^{ab}	0.097^{c}	0.002	<0.01
Solid not fat, %	8.21^{b}	8.34^{a}	8.29^{ab}	8.09^{c}	0.04	<0.01
Solid not fat, kg/d	1.11^{b}	1.22^{ab}	1.16^{b}	1.08^{c}	0.02	<0.01
Total solid, %	12.87	13.06	13.24	13.33	0.13	0.11
Total solid, kg/d	1.73^{b}	1.91^{a}	1.84^{ab}	1.76^{b}	0.04	0.03
ECM[1], kg/d	13.24	14.62	14.21	13.81	0.39	0.12
Feed efficiency[2]	1.06^{b}	1.17^{a}	1.17^{a}	1.14^{ab}	0.03	0.04

[a, b, c] Means within a row different superscripts differ ($P<0.05$).

[1]ECM, energy corrected milk (kg/d) = ((41.63 × milk fat %) + (24.13 × milk protein %) − (21.60 × milk lactose %) × milk (kg/d))/ 340 (Tyrell and Reid, 1965).

[2]Feed efficiency = ECM/ DMI.

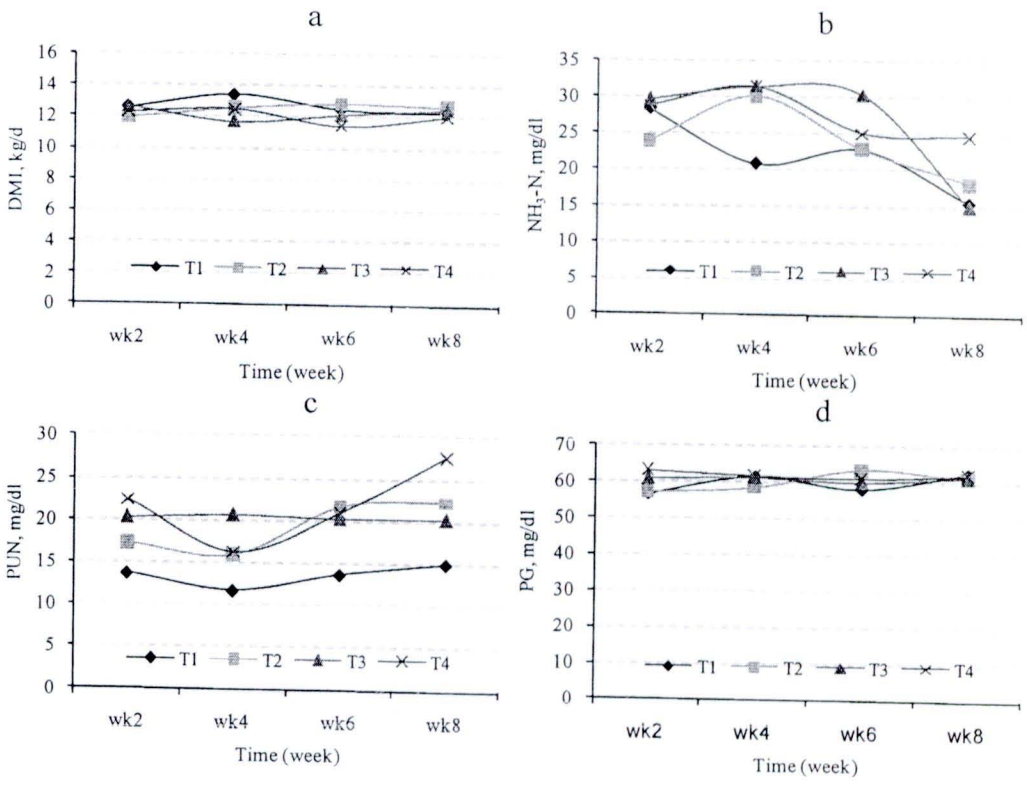

Figure 5.1 Effects of increasing SP and sugar level in TMR diets on DMI (a), ruminal NH_3-N (b), plasma urea nitrogen (c), and glucose (d) within time of sampling

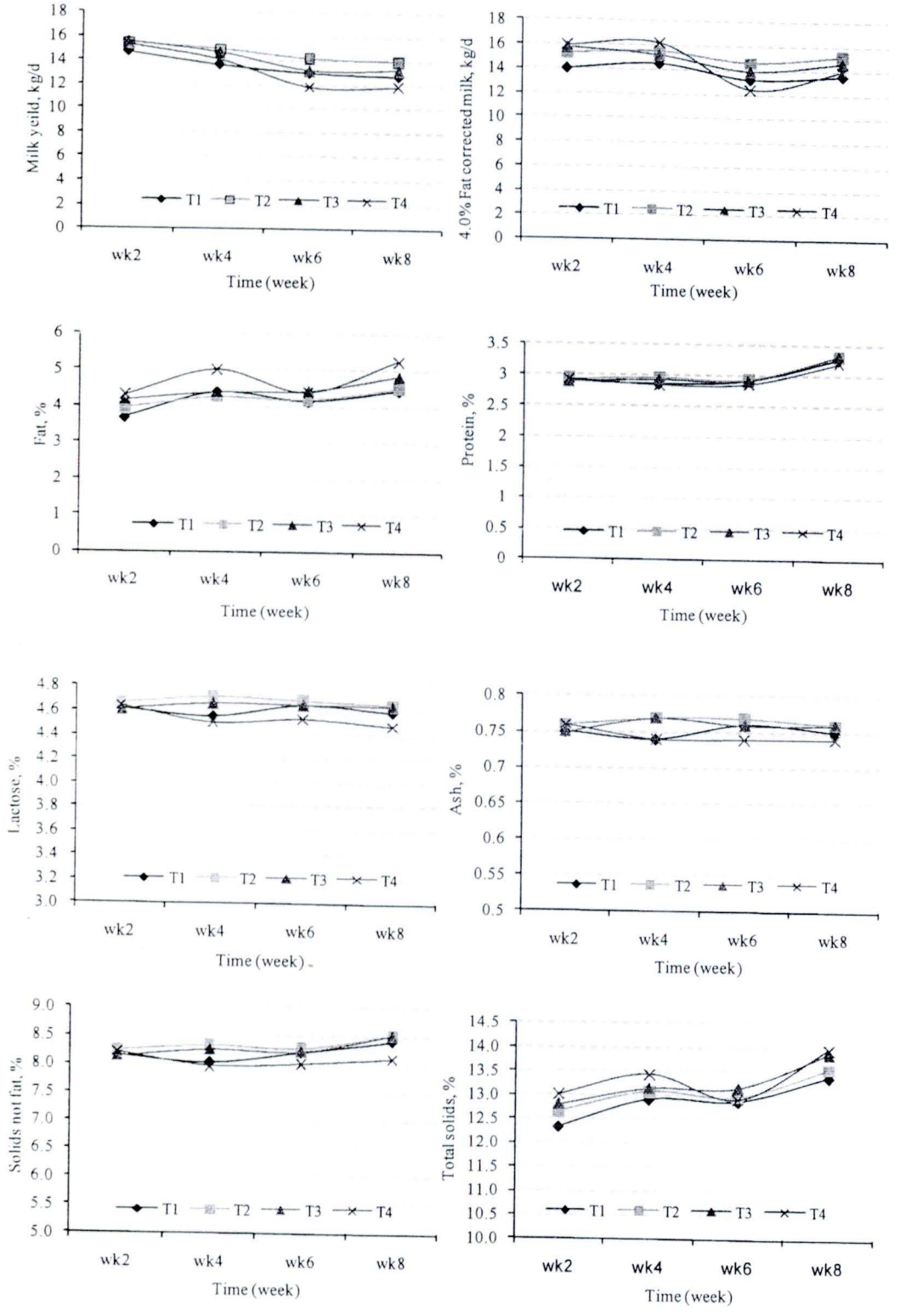

Figure 5.2 Effects of increasing SP and sugar level in TMR diets on milk production and composition within time of sampling

5.4 Conclusions

This study show that to increase the SP concentration of diet for lactating dairy cow, the requirement of rumen microbes for true protein and the cow for glucogenic nutrients must be satisfied, to not limit feed intake and to direct amino acids use toward milk production. The results of the current study indicate that feed intake, ruminal fermentation, microbial protein synthesis and milk production and composition of lactating cows fed low-quality roughage can be improved as dietary SP and sugar increased to 6.4% (40% of total CP) and 11% of the DM, respectively or when the total SP:sugar ratio is 1: 1.7, as a percentage of the DM.