Journal of Animal and Veterinary Advances 10 (13): 1724-1730, 2011
ISSN: 1680-5593

The Effects of Soluble Protein and Sugar Concentration on Ruminal Fermentation and Nutrient Digestibility in Crossbred Steers

[1]Sirirat Buaphan, [1]Virote Pattarajinda, [2]Mark A. Froetshel,
[1]Monchai Duangjinda and [3]Yanin Opatpattanakit
[1]Department of Animal Science, Faculty of Agriculture, Khon Kaen University,
P.O. Box 40002, Khon Kaen, Thailand
[2]Department of Animal and Dairy Science, The University of Georgia, P.O. Box 30602, GA, USA
[3]Faculty of Animal Science and Technology, Maejo University, P.O. Box 50290, Chiangmai, Thailand

Abstract: This study investigated the effects of varying the Soluble Protein (SP) and sugar concentration in total mixed rations. Four crossbred Thai native steers, 241±26 kg BW, fitted with rumen cannulae were arranged in a 4×4 Latin square design with a 2×2 factorial arrangement of treatments. The steers were fed treatment rations with varied SP (60.0 or 80.0% of CP) and sugar (11.0 or 22.0% of DM) levels. Steers fed a high SP diet tended to exhibit a decrease in their DMI ($p<0.10$) as compared with those fed a low SP diet. The high sugar-level diet decreased the ADF intake ($p<0.05$). The rumen pH, NH_3-N, BUN and BG levels were not significantly different after increasing the intake of SP or sugar. Increasing the SP and sugar levels did not affect the total VFA concentration in the rumen however, feeding a high SP diet tended to decrease the acetate concentration ($p<0.10$; 55.5 and 64.8%). The high SP-level diet decreased the total tract ADF ($p<0.05$), DM and OM ($p<0.10$) digestibility. This study indicated that a low SP and sugar level diet had more positive effects on the intake, ruminal fermentation and nutrient digestibility in crossbred Thai steers.

Key words: Nutrient digestion, rumen fermentation, soluble protein, sugar, steer, Thailand

INTRODUCTION

The major nutrients both in quantity and simultaneous ruminal degradability, required by rumen microbes are protein and carbohydrates which are necessary for optimal microbial protein synthesis (Hoover and Miller-Webster, 1998; Sinclair *et al*., 1995). The optimum utilization of dietary Crude Protein (CP) requires a selection of complementary feed protein sources that provide the type and amounts of Rumen Degradable Protein (RDP) that will meet the nitrogen requirement of rumen microbes. Soluble Protein (SP) is the portion of the RDP that is presumed to be immediately available for utilization by rumen microbes (NRC, 2001). The amounts of SP can affect the ammonia nitrogen (NH_3-N) that escapes microbial capture, depending on the availability of readily fermentable carbohydrate sources. Therefore, carbohydrate availability for ruminal fermentation is the key factor for improving the efficiency of ruminal ammonia and the overall dietary Nitrogen (N) utilization in ruminants. Feeding supplementary sugar has been shown to decrease ruminal ammonia and increase fiber digestibility (Sannes *et al*., 2002; Vallimont *et al*., 2004). Hall and Herejk (2001) found that sucrose initiated rapid *in vitro* microbial growth in order of induction, sucrose was followed by pectin, starch and isolated neutral detergent fiber. Moreover, the Cornell net carbohydrate and protein system (NRC, 1996) has indicated that the organisms that ferment soluble sugar could contribute approximately 18% more of the microbial protein synthesis than the organisms that ferment starch. Several reports have studied the effects on fermentation products of adding sugar to the diet of lactating cows (McCormick *et al*., 2001; Sannes *et al*., 2002; Broderick *et al*., 2008; Penner and Oba, 2009) in which the sugar added was in proportions not exceeding 3-8.4% of the dietary Dry Matter (DM). Buaphan *et al*. (2008) have reported an *in vitro* study that showed that replacing cassava with sugar at a 25% level improved the DM and NDF digestion linearly. If sugars improve microbial growth and fiber digestibility through a better synchronization between the rapidly available nitrogen and carbohydrate then SP and sugar may be used at a higher level and enhance nutrient digestion and utilization in ruminants fed low-quality roughage. The objective of this study was to investigate the effects of

Corresponding Author: Virote Pattarajinda, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, P.O. Box 40002, Khon Kaen, Thailand

varying the concentrations of SP and sugar on the Dry Matter Intake (DMI), ruminal fermentation, blood metabolites and total tract digestibility of nutrients in crossbred Thai native steers.

MATERIALS AND METHODS

Animals, experimental design and diets: All of the experimental procedures were pre-approved by the Faculty Animal Policy and Welfare Committee at Khon Kaen University before the initiation of the research. Four crossbred Thai native steers (average age 24±2 months, 241±26 kg of body weight) fitted with ruminal cannulae were used in a 4×4 Latin square design. The steers were treated for internal and external parasites at the beginning of the experiment and kept in individual pens of approximately 9 m^2. Treatments diets were in a 2×2 factorial arrangement with the main effects being the level of SP (60 or 80% of the total CP) and the level of sugar (11 or 22% of the DM) with similarly calculated total CP (14% of DM) and Total Digestible Nutrient (TDN; 70% of the DM) contents. Sugarcane powder, the by-product of fruit-flavored instant drink mix (Kraft Foods, Thailand) was used as the main source of sugar; the nutrient composition was 98% DM and the total sugar concentration was approximately 96% of the DM, consisting mostly of sucrose (Table 1). The experiment was conducted for 72 days (divided into 4 periods). Each experimental period was run for 18:11 days for adaptation and 7 days for data collection and sampling. Steers were individually fed *ad libitum* intake, twice daily at 08:00 and 16:00 h. Diets were fed as a Total Mixed Rations (TMR) in which rice straw and concentrate (previously mixed) were weighed and mixed before feeding. The orts were collected and weighed once daily and diets were adjusted daily to yield orts of approximately 5-10% of the total feed offered. Steers had free access to clean drinking water.

Sample collection and analysis: Feed samples were pooled within each collection period, dried in a forced-air oven at 60°C for 48 h and ground through a 1 mm screen. Samples were analyzed for DM, Organic Matter (OM), CP, ash and Acid Detergent Fiber (ADF) (AOAC, 1990), SP (Krishnamoorthy *et al*., 1982), sugar (AOAC, 2000) and Neutral Detergent Fiber (NDF) as determined using heat-stable, α-amylase and sodium sulfite (Van Soest *et al*., 1991).

On day 12th of each experimental period, the ruminal content was obtained at 0, 2, 4 and 8 h after the morning feeding and was subsequently strained through two layers of cheesecloth. The pH was measured immediately by using a pH meter (Electrochemical analyzer, Consort model C933P). The ruminal fluid was preserved by adding 5 mL of 1M H_2SO_4 to 45 mL of rumen fluid and stored at -20°C for the analysis of ammonia nitrogen (NH_3-N) and Volatile Fatty Acids (VFA). Samples were thawed and centrifuged at 3,500 rpm for 15 min at 4°C; the NH_3-N was analyzed by using the micro-Kjeldahl method and the VFA level was analyzed by using an HPLC (Instruments by controller water model 600 E; water model 484 UV detector) according to the method of Zinn and Owen (1986).

Table 1: Ingredients of the feed and the chemical compositions of the experimental diets

	L-SP		H-SP	
Items	L-S	H-S	L-S	H-S
Ingredients (DM%)				
Rice straw	30.0	30.0	30.0	30.0
Cassava chips	40.2	27.7	49.0	36.5
Ground corn	5.0	5.0	5.0	5.0
Soybean meal	11.0	11.5	1.0	2.5
Sugarcane powder	11.0	22.0	11.0	22.0
Urea, 46 (%) N	2.4	2.4	3.6	3.6
Vitamins and minerals	0.4	0.4	0.4	0.4
Analyzed contents				
DM (DM%)	92.1	92.9	92.3	93.8
OM (DM%)	90.3	91.0	91.4	91.6
CP (DM%)	13.7	13.5	13.5	13.0
NDF (DM%)	28.3	29.0	28.9	26.6
ADF (DM%)	20.1	18.5	18.3	18.0
NFC[a] (DM%)	47.8	48.0	48.5	51.4
Sp[b] (total protein%)	61.2	59.9	82.9	81.6
Total sugar (DM%)	12.0	22.6	11.3	22.0

[a]NFC, Non-Fiber Carbohydrate = 100- (CP (%)+NDF (%)+EE (%)+Ash (%)); [b]SP: Soluble Protein; L-SP: Low Soluble Protein; H-SP: High Soluble Protein; L-S: Low Sugar; H-S: High Sugar

Blood samples were collected at 0, 1, 2 and 4 h after feeding on day 12th from the jugular vein of each steer. Blood samples were collected into 10 mL serum tubes and allowed to clot for 60 min and then centrifuged at 2,500 rpm for 15 min.

Serum aliquots were stored at -20°C until further analysis for Blood Urea-N (BUN) and Blood Glucose (BG) concentrations with the Automated Chemistry analyzer (HITACHI, 912).

The total tract digestibility of the DM, OM, CP, ADF and NDF was determined during the last week (day 13-18th) of each period. Chromium oxides (Cr_2O_3) were used as an indigestible marker. The TMR diets were mixed to contain 1 g chromium oxide kg^{-1} DM and fed to the steers for 4 consecutive days before collecting feces samples every 4 h, daily.

The Cr 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 nutrients and chromium oxide in the diet and feces (Maynard *et al*., 1979).

Statistical analyses: The dry matter intake, nutrient intake, Average Daily Gain (ADG), VFA and total tract digestibility of nutrients were analyzed using the SATTHERH model statement in the MIXED procedure of SAS (1996) for a 4×4 Latin square design with a 2×2 arrangement of treatments. The model included effects for the SP and sugar level and the interaction between these factors with repeated experimental periods. Mean separations were determined using the PDIFF statement in PROC MIXED. Treatment differences were considered to be significant when $p<0.05$ and were considered to indicate a trend at $0.05<p<0.10$. The statistical model was the following:

$$Y_{ijkl} = \mu+\pi_i+P_j+\delta_k+\alpha_l+\delta_k*\alpha_l+\varepsilon_{ijkl}$$

Where:

Y_{ijkl} = The measured variable
μ = The overall mean
π_i = The random effect of the ith steer
P_j = The fixed effect of the jth period
δ_k = The fixed effect of the kth SP level
α_l = The fixed effect of the lth sugar level
$\delta_k*\alpha_l$ = The interaction term for SP and sugar level
ε_{ijkl} = The residue error

The rumen fluid pH, NH_3-N, BUN and BG concentration data collected over time were analyzed using the MIXED procedure of SAS (1996) with the model described above except the repeated option was used for time after feeding instead of period.

RESULTS AND DISCUSSION

The ingredients and nutrient composition of the diets are shown in Table 1. Diets were formulated to be isonitrogenous (14% of the CP) however, analyses produced values that ranged from 13-13.7% of CP. The level of SP increased with the inclusion level of urea in the TMR diets, averaging 60.8 and 83.5% of the CP for the low- and high-SP diets, respectively. The diets with low and high sugar levels contained 11.7 and 22.3% of sugar, respectively. There were no interactions detected between the SP and the sugar concentration in any of the variables.

The daily intakes of nutrients and the performance of the steers are shown in Table 2. There were no significant differences ($p>0.05$) in the daily intakes of OM among the treatments. However, the DMI and the DMI (as a percentage of body weight) were comparatively lower ($p<0.10$) for steers fed a diet high in SP. These decreases in the DMI were also reflected in the daily intake of CP and NDF ($p<0.05$) for steers fed high-SP diets. The ADF intake decreased ($p<0.05$) with an increase in both dietary SP and sugar. The intake of SP was significantly greater ($p<0.01$) for steers fed a high-SP diet compared to those fed a low-SP diet (0.8 and 0.6 kg day^{-1}). The intake of sugar was significantly greater ($p<0.05$; 1.6 kg day^{-1}) for steers consuming a high-sugar diet, whereas the Average Daily Gain (ADG) was not significantly different among the dietary treatments. Mean responses in the levels of pH, NH_3-N, BG and BUN were not significantly different among the treatments (Table 3). After increasing the SP and sugar levels, there were no significant differences in the production of total VFA, the molar proportions of

Table 2: Effects of SP and sugar concentration on the nutrient intake of crossbred steers

	L-SP		H-SP			p value		
Items	L-S	H-S	L-S	H-S	SEM	SP	S	SP×S
Intake (kg day^{-1})								
DM	7.41	7.37	7.13	6.84	0.20	0.08	0.43	0.54
OM	6.69	6.70	6.51	6.26	0.19	0.14	0.53	0.48
CP	1.01	0.99	0.96	0.89	0.03	0.02	0.11	0.36
NDF	2.10	2.13	2.06	1.82	0.06	0.02	0.11	0.42
ADF	1.49	1.36	1.30	1.23	0.03	<0.01	0.03	0.45
SP	0.61	0.61	0.79	0.76	0.02	<0.01	0.44	0.57
Sugar	0.89	1.66	0.81	1.50	0.04	0.03	<0.01	0.34
Intake (BW%)								
DM	2.79	2.79	2.66	2.59	0.14	0.08	0.70	0.66
Steer performance	1.01	0.99	0.86	0.92	0.09	0.11	0.76	0.57
[a]ADG (kg day^{-1})								

SP: Soluble Protein; S: Sugar; L-SP: Low Soluble Protein; H-SP: High Soluble Protein; L-S: Low Sugar; H-S: High Sugar; [a]ADG: Average Daily Gain

Table 3: Effects of SP and sugar concentration on the rumen fermentation, blood metabolite levels and total tract digestibility in crossbred steers

	L-SP		H-SP			p value		
Items	L-S	H-S	L-S	H-S	SEM	SP	S	SP×S
pH min.	6.27	5.99	6.16	6.38	0.16	0.44	0.86	0.20
pH max.	6.95	6.78	6.84	6.85	0.14	0.72	0.68	0.28
pH mean[a]	6.65	6.40	6.53	6.65	0.17	0.67	0.71	0.23
NH_3-N[b] (mg dL^{-1})	22.96	22.37	20.71	23.70	2.09	0.75	0.40	0.22
Total VFA (mM)	97.20	97.95	91.24	82.45	5.87	0.12	0.52	0.45
Individual VFA, molar proportion								
Acetate	65.48	64.11	57.53	53.41	4.91	0.07	0.52	0.79
Propionate	18.57	20.37	21.56	16.47	3.05	0.87	0.56	0.24
Butyrate	12.80	13.46	12.15	12.57	1.31	0.60	0.71	0.93
A:P ratio[e]	3.94	3.19	2.94	3.35	0.61	0.32	0.79	0.27
Blood metabolites								
BG[c] (mg dL^{-1})	53.29	45.07	52.68	52.58	5.94	0.45	0.36	0.37
BUN[d] (mg dL^{-1})	15.51	14.65	15.18	17.48	1.12	0.29	0.54	0.18
Digestibility (%)								
DM	89.35	89.42	88.79	88.12	0.44	0.07	0.50	0.41
OM	91.19	91.03	90.66	90.23	0.34	0.08	0.39	0.69
CP	91.56	90.10	90.67	90.53	0.37	0.56	0.08	0.14
NDF	77.15	77.35	76.90	74.08	1.14	0.16	0.27	0.21
ADF	76.69	75.99	74.10	71.39	1.41	0.02	0.21	0.44

L-SP: Low Soluble Protein; H-SP: High Soluble Protein; L-S: Low Sugar; H-S: High Sugar. [a]Treatment x time interaction ($p = 0.02$); [b]Treatment x time interaction ($p = 0.02$); [c]Treatment x time interaction ($p = 0.11$); [d]Treatment x time interaction ($p = 0.40$); [e]Acetate: propionate

propionate and butyrate or the acetate to propionate ratio. However, steers fed a diet high in SP tended to exhibit a decrease ($p<0.10$) in the molar proportion of acetate as compared with steers fed low-SP diets (55.5 vs. 65.0%). The total tract DM and OM digestibility tended to decrease in steers fed high-SP diets ($p<0.10$) and an increasing SP intake was also associated with a low total tract ADF digestibility ($p<0.05$). However, diets with low sugar levels tended to show an increase in the digestibility of the CP ($p<0.10$).

In this study, the effects of the soluble protein and sugar levels on the intake, ruminal fermentation, blood metabolite levels and nutrient digestibility in steers were investigated. Urea was the main source of nitrogen in this study (2.4 or 3.6% of the DM). The DM intake tended to decrease for steers fed a high-SP diet compared to steers fed low-SP diets. Huber and Kung Jr. (1981) have reported that the observed decrease in the DMI was due to the bitter taste of the urea in the feed. Similarly, Casper and Schingoethe (1986) have reported that the lowest DMIs were found in cows fed urea and Milton *et al.* (1997) have reported that a maximal DMI was observed in steers that consumed only 1.1% urea. In a more recent study, Broderick *et al.* (2008) reported a linear increase in the DMI as the proportion of sugar increased from 0 to 7.5%. In the present study, however diets containing high sugar levels (11.3-22.6% of the DM) did not result in a difference in the DMI of the steers. There were no differences in the ADG among the dietary treatment results which is similar to the results of Chizzotti *et al.* (2008) in which no differences were reported for the ADGs among steers fed diets containing non-protein nitrogen (NPN) up to 66.3% of the total nitrogen (urea up to 2.0% of DM). Additionally, Gleghorn *et al.* (2004) found no differences in the ADGs among steers fed TMR diets (90% of concentrate) containing different CP concentrations (11.5, 13 or 14.5% of dietary CP) and degradability (100:0, 50:50, 0:100% of urea: cottonseed meal). In the current study, the concentration of SP was 60.8 or 83.5% of CP and the NPN from urea was 49.2 and 78.0% of the SP for the low- or high-SP diets, respectively.

This resulted in proportionally higher dietary soluble nitrogen levels in both the low and high-SP diets as compared to NRC (1996) recommendations; therefore the intake data and weight gain of the steers might have been influenced by the amount of the SP, rather than the increase in the sugar level.

Penner *et al.* (2007) were of the view that the inclusion of higher levels of sugar in a ruminant diet might promote and lead to acidosis. However in the current study, the average daily pH across treatments ranged from 6.4-6.7 which is generally considered suitable for fiber digestion (Mould and Orskov, 1983). Surprisingly, the daily minimum, maximum and mean data of the ruminal pH did not differ among the dietary treatments. A recent study by Penner and Oba (2009) demonstrated that the replacement of cracked corn with sucrose (8.4% of the DM) did not decrease the ruminal pH. In contrast, the pH was reported to have decreased and the lactic acid production increased 3-fold when diet containing 16% of sucrose was fed (6.3 kg day^{-1}) to male Friesian cattle (Khalili and Huhtanen, 1991). Furthermore, Penner *et al.* (2009) reported that the ruminal pH for cows fed high-sugar (5.7%) diets was higher than those fed low-sugar (2.8%) diets and that this may have indicated that the disappearance of sugar from the rumen did not necessarily increase the fermentation acid production in the rumen.

Hoover and Miller-Webster (2001) have suggested that a high proportion of sucrose leaves the rumen with the liquid fraction before fermentation and Henning *et al.* (1993) have reported that the disappearance rate of sugar was 69% h^{-1}. However if sucrose supplementation increased, the microbial-cell yield, the ruminally degraded OM available for fermentation acid production would be reduced (Allen, 1997). Lactic acid production was observed for the 1st period of the present study in steers fed the high-sugar diet, 2.3 mM on average and it remained at a normal range (0-5 mM) (Nagaraja and Titgemeyer, 2007). Satter and Slyter (1974) have reported that when the NH_3-N concentration exceeded 5 mg dL^{-1}, the microbial efficiency was maximized in continuous-culture. In the present study, the mean daily concentrations of rumen NH_3-N were above this level for all of the diets. Excess NH_3-N in the rumen fluid is absorbed through the rumen wall and transported to the liver where it is metabolized to urea. The increased intake of SP did not affect the BUN concentration and it appears that the NH_3-N concentration in the rumen was not high enough to elevate the BUN levels. However, this lack of BUN response may be due to the high level of sugar in the diets. Sannes *et al.* (2002) have reported that sucrose tended to reduce ($p<0.10$) the total urinary N excretion and Milk Urea Nitrogen (MUN) of lactating Holstein cows and decrease the plasma urea nitrogen (PUN) in sheep (Obara and Dellow, 1993).

These effects suggest a potential for improved nitrogen utilization by adding sucrose; however effects on either NH_3-N or BUN by the diet sugar levels were not observed in this study. It is possible that the concentration of the dietary SP might be above the requirement of the ruminal microbes; therefore, the BUN seemed to increase with time after feeding. Increasing the sugar intake did not have a significant difference on the BG concentration among the treatments; it remained within a normal range (45-75 mg dL^{-1}; Kaneko, 1997). It

was observed that feeding a high-SP diet tended to cause the molar proportion of acetate to decrease as compared to steers fed a low-SP diet; this might have been due to the decreased ADF digestibility in the current study. Steers fed low-SP (8.3% of DM) diets tended to show an improvement in the total tract digestibility of DM and OM and had higher values of total tract apparent ADF digestibility as compared with steers fed high-SP (11.1% of the DM) diets.

The decrease in the ADF digestibility with the high-SP diet may have been affected by the diet containing low amounts of true protein or there may have been too much protein substrate which was supplied more quickly than the microbes could utilize. According to Merry *et al.* (1990) and Griswold *et al.* (1996), the digestibility of ADF was observed to increase with a true protein source as compared with a diet of 100% urea in an *in vitro* study. Indeed, the relationship between fiber digestibility and protein supplementation with respect to sugar diets may be related to the competition for NH_3-N between fiber- and NFC-fermenting bacteria (Jones *et al.*, 1998). Moreover, Russell and Sniffen (1984) have reported that the addition of Branched-Chain volatile Fatty Acids (BCFAs) enhanced cellulose digestion.

On the contrary, another study reported that the addition of sucrose caused BCFAs to decrease linearly (Ribeiro *et al.*, 2005).

However in the study, we found that the 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.

CONCLUSION

The results of the present study demonstrate that the SP has more of an influence on the intake, digestibility and performance of steers than the sugar level in the diet. 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 12% of the DM. Feeding excess SP (>60% of CP) did not improve animal performance and may cause excessive nitrogen excretion and ammonia volatilization into the environment.

However when a diet is incorporated with low SP and sugar, it does not necessarily mean that the diet has a very low content of urea and sugar. Therefore with a proper ratio of SP and sugar, the urea and sugar ingredients can be used at higher levels to reduce the cost of the feed. The results of the current study indicate that the productivity of steers fed low-quality roughage can be improved when the total sugar:SP ratio is 1.4:1 as a percentage of the DM.

ACKNOWLEDGEMENT

The researchers would like to especially thank the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0127/2006) for financial support.

REFERENCES

AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Arlington, Virginia, USA.

AOAC, 2000. Official Methods of Analysis. 19th Edn., Association of Official Agricultural Chemists. Washington, DC. USA.

Allen, M.S., 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci., 80: 1447-1462.

Broderick, G.A., N.D. Luchini, S.M. Reynal, G.A. Varga and V.A. Ishler, 2008. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. J. Dairy Sci., 91: 4801-4810.

Buaphan S., V. Pattarajinda, M.A. Froetschel, M. Duangjinda and Y. Opatpattanakit, 2008. Effects of replacing dietary starch with sugar on nutrient digestibility and gas production technique. Proceedings of the 13th Animal Science Congress of the Asian-Australasian Association of Animal production Societies, Sept. 22-26, Hanoi, Vietnam, pp: 68-68.

Casper, D.P. and D.J. Schingoethe, 1986. Evaluation of urea and dried whey in diets of cows during lactation. J. Dairy Sci., 69: 1346-1354.

Chizzotti, F.H.M., O.G. Pereira, L.O. Tedeschi, S.C.V. Filho, M.L. Chizzotti, M.I. Leao and D.H. Pereira, 2008. Effects of dietary nonprotein nitrogen on performance, digestibility, ruminal characteristics and microbial efficiency in crossbred steers. J. Anim. Sci., 86: 1173-1181.

Gleghorn, J.F., N.A. Elam, M.L. Galyean, G.C. Duff, N.A. Cole and J.D. Rivera, 2004. Effects of crude protein concentration and degradability on performance, carcass characteristics and serum urea nitrogen concentrations in finishing beef steers. J. Anim. Sci., 82: 2705-2717.

Griswold, K.E., W.H. Hoover, T.K. Miller and W.V. Thayne, 1996. Effect of form of nitrogen on growth of ruminal microbes in continuous culture. J. Anim. Sci., 74: 483-491.

Hall, M.B. and C. Herejk, 2001. Differences in yields of microbial crude protein from *in vitro* fermentation of carbohydrates. J. Dairy Sci., 84: 2486-2493.

Henning, P.H., D.G. Steyn and H.H. Meissner, 1993. Effect of synchronization of energy and nitrogen supply on ruminal characteristics and microbial growth. J. Anim. Sci., 71: 2516-2528.

Hoover, W.H. and T.K. Miller-Webster, 1998. Role of sugars and starch in ruminant fermentation. Proceedings of the Tri-State Dairy Nutrition Conference, (TSDNC'89), Wayne, pp: 1-13.

Hoover, W.H. and T.K. Miller-Webster, 2001. The contribution of sugars to rumen fermentation and milk production. Proceedings of the 42nd Annual New England Dairy Feed Conference, (ANEDF'01), West Lebanon, New Hampshire, pp: 1-24.

Huber, J.T. and L. Kung Jr., 1981. Protein and nonprotein nitrogen utilization in dairy cattle. J. Dairy Sci., 64: 1170-1195.

Jones, D.F., W.H. Hoover and T.K. Miller-Webster, 1998. Effects of concentrations of peptides on microbial metabolism in continuous culture. J. Anim. Sci., 76: 611-616.

Kaneko, J.J., 1997. Carbohydrate Metabolism and its Diseases. In: Clinical Biochemistry of Domestic Animals, Kaneko, J.J., J.W. Harvey and M.L. Bruss (Eds.). 5th Edn., Academic Press, San Diego, pp: 45-81.

Khalili, H. and P. Huhtanen, 1991. Sucrose supplements in cattle given grass silage based diet. 1. Digestion of organic matter and nitrogen. Anim. Feed Sci. Technol., 33: 247-247.

Krishnamoorthy, U., V. Muscato, C.J. Sniffen and P.J. van Soest, 1982. Nitrogen fractions in selected feedstuffs. J. Dairy Sci., 65: 217-225.

Maynard, L.A., J.K. Loosli, H.F. Hintz and R.G. Warner, 1979. Digestive Processes in Different Species. In: Animal Nutrition, Maynard, L.A., J.K. Loosli, H.S. Hintz and R.G. Warner (Eds.). McGraw-Hill Inc., New York, pp: 21-46.

McCormick, M.E., D.D. Redfearn, J.D. Ward and D.C. Blouin, 2001. Effect of protein source and soluble carbohydrate addition on rumen fermentation and lactation performance of Holstein cows. J. Dairy Sci., 84: 1686-1697.

Merry, R.J., A.B. McAllan and R.H. Smith, 1990. *In vitro* continuous culture studies on the effect of nitrogen source on rumen microbial growth and fibre digestion. Anim. Feed Sci. Technol., 31: 55-64.

Milton, C.T., R.T. Jr. Brandt and E.C. Titgemeyer, 1997. Urea in dry-rolled corn diets: Finishing steer performance, nutrient digestion and microbial protein production. J. Anim. Sci., 75: 1415-1424.

Mould, F.L. and E.R. Orskov, 1983. Manipulation of rumen fluid pH and its influence on cellulolysis in sacco, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. Anim. Feed Sci. Technol. 10: 1-14.

NRC, 1996. Nutrient Requirements of Beef Cattle. 7th Edn., National Academy Science, Washington, DC. USA.

NRC., 2001. Nutrient Requirements of Dairy Cattle. 7th Edn., National Academies Press, Washington, DC. USA., ISBN: 0309069971, pp: 381.

Nagaraja, T.G. and E.C. Titgemeyer, 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. J. Dairy Sci., 90: E17-E38.

Obara, Y. and D.W. Dellow, 1993. Effects of intraruminal infusions of urea, sucrose or urea plus sucrose on plasma urea and glucose kinetics in sheep fed chopped lucerne hay. J. Agric. Sci., 121: 125-130.

Penner, G.B. and M. Oba, 2009. Increasing dietary sugar concentration may improve dry matter intake, ruminal fermentation, and productivity of dairy cows in the postpartum phase of the transition period. J. Dairy Sci., 92: 3341-3353.

Penner, G.B., K.A. Beauchemin and T. Mutsvangwa, 2007. Severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. J. Dairy Sci., 90: 365-375.

Penner, G.B., L.L. Guan and M. Oba, 2009. Effects of feeding fermenten on ruminal fermentation in lactating Holstein cows fed two dietary sugar concentrations. J. Dairy Sci., 92: 1725-1733.

Ribeiro, C.V.D.M., S.K.R. Karnati and M.L. Eastridge, 2005. Biohydrogenation of fatty acids and digestibility of fresh alfalfa or alfalfa hay plus sucrose in continuous culture. J. Dairy Sci., 88: 4007-4017.

Russell, J.B. and C.J. Sniffen, 1984. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria *In vitro*. J. Dairy Sci., 67: 987-994.

SAS, 1996. The SAS System. Version 6.2, SAS Institute Inc., Cary, NC. USA.

Sannes, R.A., M.A. Messman and D.B. Vagnoni, 2002. Form of rumen-degradable carbohydrate and nitrogen onmicrobial protein synthesis and protein efficiency of dairy cows. J. Dairy Sci., 85: 900-908.

Satter, L.D. and L.L. Slyter, 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. Br. J. Nutr., 32: 199-208.

Sinclair, L.A., P.C. Garnsworthy, J.R. Newbold and P.J. Battery, 1995. Effects of synchronizing the rate of dietary energy and nitrogen release in diets with a similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci., 124: 463-472.

Vallimont, J.E., F. Bargo, T.W. Cassidy, N.D. Luchini, G.A. Broderick and G.A. Varga, 2004. Effects of replacing dietary starch with sucrose on ruminal fermentation and nitrogen metabolism in continuous culture. J. Dairy Sci., 87: 4221-4229.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Zinn, A.R. and F.N. Owen, 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci., 66: 157-166.