Journal of Animal and Veterinary Advances 13 (16): 1009-1013, 2014
ISSN: 1680-5593

Effects of Different Proteins and Carbohydrates on Rumen Microbial Protein Degradation and Synthesis

[1,2]Hongbo Zhao, [1,2]Fachun Wan, [1,2]Xiaomu Liu, [1,2]Qingkai Sheng and [1,2]Xingling Wang
[1]Shandong Academy of Agricultural Sciences, Institute of Animal Science and Veterinary Medicine,
[2]Shandong Key Lab of Animal Disease Control and Breeding, Sangyuan Road No. 8,
Ji'nan City, 250100 Shandong Province, China

Abstract: Optimisation of diets in cows is essential to achieve the best possible performance from these animals. Here, researchers sought to investigate the effects of diet on ruminal microbial protein degradation and synthesis in cows. Three ruminally fistulated Chinese Yellow heifers were used in a 3×3 Latin square trial to study the kinetics of rumen degradation of corn stover. Three diets were formulated to contain corn plus soybean meal (diet 1), corn plus pig blood meal (diet 2) and wheat plus soybean meal (diet 3). Each heifer was allowed a 30 days adaptation following by a 7 day a rumen evacuation *in situ* during which rumen content was evacuated 0, 2, 4, 8, 16 or 24 h after feeding. Dry Matter (DM) intakes were similar for all diets. The result of rumen evacuation showed that rumen NH_3-N pooling peaked 8 h after feeding for diets 1 and 3. Moreover, rumen Volatile Fatty Acid (VFA) pooling varied depending on DM retention for all 3 diets and rumen DM, Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) poolings were lowest for diet 1 at 4, 8 and 16 h after feeding, respectively ($p<0.05$). Therefore, diet 1, containing corn plus soybean meal, resulted in low rumen DM, NDF and ADF pooling but high DM, NDF and ADF degradability in the rumen of Chinese Yellow heifers. These results shed light on ruminal processes and will aid in the development of optimal diets in cows.

Key words: Large-diameter rumen fistula, dietary combination, rumen evacuation, cows, heifers

INTRODUCTION

Maximising rumen microbial protein synthesis requires the timely availability of nitrogen and carbohydrate sources suitable for rapid microbial growth (Sniffen and Robinson, 1987). Nonstructural Carbohydrates (NSCs) and proteins from feedstuffs are degraded in the rumen of cattle at different rates and to different extents and these are the main factors controlling the availability of energy and nitrogenous compounds for microbial growth (Hoover and Stokes, 1991). If energy in the rumen is limited, microorganisms degrade feed protein to ammonia and ammonia uptake by ruminal microorganisms is inhibited (Nocek and Russell, 1988; Hristov *et al*., 1997). Since, microbial proteins synthesised in the rumen supply the majority of absorbable amino acids to the small intestine (NRC, 2001), it is important to maintain optimal microbial activity in the rumen. Corn and barley, the main cereal grains used in high-concentrate beef cattle diets, vary in NSCs and therefore may affect the activity of the ruminal microbiota. Barley has a lower NSC content but a greater rate and extent of ruminal degradation than corn (Herrera-Saldan *et al*., 1990), however, barley is less energy dense. Corn is a main energy source in ruminant diets due to its high starch content. The efficiency of starch utilization is important to improve performance of lactating dairy cows (Zhong *et al*., 2008).

Although, there is extensive research on the effects of these NSC sources on microbial fermentation and animal performance using dairy cattle diets (Nocek and Russell, 1988; Herrera-Saldan *et al*., 1990; Casper *et al*., 1999; Nombekela and Mushy, 1995; Rotger *et al*., 2006) few efforts have been made to improve ruminal fermentation with high-concentrate beef cattle diets by synchronising these energy sources with protein supplements having similar degradation characteristics. High-concentrate diets are rapidly fermented in the rumen, leading to high concentrations of Volatile Fatty Acids (VFAs) in ruminal fluid and relatively low ruminal pH (Beauchemin *et al*., 2001). Therefore, further investigation of the effects of high-concentrate diets on characteristics of ruminal processes is required to facilitate the optimisation of dietary nutrition in cattle.

Corresponding Author: Xingling Wang, Shandong Academy of Agricultural Sciences,
Institute of Animal Science and Veterinary Medicine, Sangyuan Road No. 8, Ji'nan City,
250100 Shandong Province, China

The objective of this research was to investigate the effects of different NSCs and protein sources on ruminal fermentation mechanisms after evacuation of total rumen content in order to further optimise the structural parameters of dietary nutrition.

MATERIALS AND METHODS

Heifers and animal management: Six Chinese Yellow heifers weighing an average of 326±32 kg were fitted with ruminal fistulas under local anaesthesia and sterile conditions 3 weeks prior to the beginning of the experiment and were treated in a 3×3 Latin square design. The heifers were fed 1 of 3 treatment diets (n = 2 heifers each group) consisting of Soybean Meal (SBM) plus corn (diet 1), pig blood mean plus corn (diet 2) or SBM plus wheat (diet 3). BM was used as the low ruminal degradable protein source and wheat was used as the high ruminal degradable energy source. Feedstuff compositions are presented in Table 1. All other components were similar among the 3 diets (Table 1). Heifers were fed the diet twice daily at 8:00 and 16:00 and were given free access to fresh water at all times. The dietary intake was as 1% live weight for each animal during the 3 experimental periods. Dry corn stover intake was as the same as during the same experimental period. Heifers were fed the experimental diets for a 30 days adaptation period followed by a 7 days sample collection period consisting of the experimental sub period.

Sample collection and analysis: Rumen evacuation was performed on 3 rumen-fistulated heifers at the same time after day 31 of each experimental period. Total rumen content including liquids and solids was collected at 2, 4, 8, 16 or 24 h after feeding. After sampling, extra ruminal liquids and solids were returned to the rumen. Ruminal liquid and solid contents were separated, weighed and measured to determine the amount of Dry Matter (DM) and the ruminal pH value. The pH of the ruminal fluid was measured immediately and 2 subsamples were collected each time. The ruminal solid and liquid contents were sampled and kept frozen until later analysis. Solid rumen samples were measured for nitrogen, Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) (AOAC, 1990) while liquid rumen samples were measured for VFAs (Fussell and McCalley, 1987) ammonia nitrogen (NH_3-N) (Crook and Simpson, 1971), liquid nitrogen and liquid microbial nitrogen. The VFA content of each sample was determined by gas chromatography as described by Murphy *et al.* (2000) and ammonia and total α-amino nitrogen were determined simultaneously on a Technicon AutoAnalyzer (Broderick and Kang, 1980) (Table 2).

Table 1: Concentrate formulation for the 3 cattle with rumen fistulas (%)

Diets	SBM+corn (diet 1)	BM+corn (diet 2)	SBM+wheat (diet 3)
Corn	55.00	55.00	8.00
Wheat			55.00
SBM	35.00		32.00
BM		22.00	
Wheat bran	5.00	18.00	
$CaHPO_4$	1.50	1.50	1.50
Salt	1.50	1.50	1.50
Premix	2.00	2.00	2.00
DM (%)	87.43	86.87	88.63
CP (%)	18.80	21.10	19.60
NDF (%)	9.52	9.61	8.84
ADF (%)	3.43	4.83	5.70

*SBM: Soybean Meal; BM: pig Blood Meal; CP: Crude Protein; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre

Table 2: Chemical analysis of trial feedstuffs (% DM)

Diets	DM	CP	NDF	ADF
Corn stover	91.10	6.00	72.69	42.04
SBM	90.08	46.21	13.83	10.07
BM	85.73	83.02		
Wheat	91.32	12.46	11.06	5.19
Corn	89.11	7.98	7.25	4.82

*DM: Dry Matter; CP: Crude Protein; MDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; SBM: Soybean Meal; BM: pig Blood Meal

Statistical analysis: Data were analysed by Analysis of Variance (ANOVA) for the Latin square design using the GLM procedure of SAS Software to examine the effects of animal, period, treatment and different times of rumen evacuation on rumen pooling of DM, nitrogen, NDF, ADF, VFAs, NH_3-N and liquid microbial nitrogen. Comparisons of treatment means were conducted by contrast t-tests following determination of significance in the ANOVA Model.

RESULTS AND DISCUSSION

Rumen pooling of various dietary DM contents and detergent fibres: The differential pooling of rumen content is shown in Table 3. With the same intake, diet 1 resulted in the lowest total pooling of ruminal DM, liquids and NDF ($p<0.05$) while similar total pooling of ruminal ADF was observed ($p>0.05$, although the total pooling of ruminal ADF tended to be lower than the other 2 diets after different times of rumen evacuation). The combination of SBM and corn in diet 1 resulted in lower ruminal content pooling at 2-8 h after feeding ($p<0.05$) and similar pooling at 16 h after feeding was observed for all diets ($p>0.05$). Diet 1 allowed for the least retention of rumen content which may have been caused by the promotion of ruminal protein degradation and utilisation by diet 1, improving the fibre degradation rate and disappearance of the of solid/liquid content. Additionally, cattle fed diet 1 exhibited increased degradation of NDFs from straw than cattle fed diets 2 and 3, improving NDF degradation by 11.9 and 13.9%, respectively at 4 h after feeding; similarly, ADF was improved by 10.4 and 14% by diets 2 and 3, respectively. Moreover, in cattle fed diet 1,

Table 3: DM and detergent fibre pooling of the 3 rations in the rumen (kg)

	DM				NDF				ADF			
Time (h)	Diet 1	Diet 2	Diet 3	SEM	Diet 1	Diet 2	Diet 3	SEM	Diet 1	Diet 2	Diet 3	SEM
2	4.46^{b}	4.66^{a}	5.09^{a}	0.16	2.93^{b}	3.12^{ab}	3.35^{a}	0.07	1.56	1.67	1.74	0.05
4	4.13^{b}	4.52^{a}	4.63^{a}	0.15	2.67^{b}	3.03^{a}	3.10^{a}	0.06	1.47	1.64	1.71	0.05
8	3.88^{b}	4.35^{a}	4.37^{a}	0.19	2.53^{b}	3.09^{a}	3.03^{a}	0.67	1.49^{b}	1.85^{a}	1.78^{ab}	0.04
16	3.27	3.85	3.59	0.22	2.23^{b}	2.82^{a}	2.54^{ab}	0.13	1.34^{b}	1.70^{a}	1.47^{b}	0.07
24	2.99	3.23	3.25	0.19	2.09	2.31	2.36	0.18	1.22	1.38	1.36	0.04

*DM: Dry Matter; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; SEM: Standard Error of the Mean; For each time point, values with superscript a are significantly different than values with superscript b but not when compared to values with superscript ab ($p<0.05$)

Table 4: Nitrogen fraction and bacterial nitrogen pooling of the 3 rations in the rumen (g)

	Total nitrogen				Liquid NH_3-N				BN			
Time (h)	Diet 1	Diet 2	Diet 3	SEM	Diet 1	Diet 2	Diet 3	SEM	Diet 1	Diet 2	Diet 3	SEM
2	137.5^{ab}	125.7^{b}	157.6^{a}	3.01	8.06^{a}	4.00^{b}	10.10^{a}	0.07	19.10^{a}	12.3^{b}	16.5^{a}	0.43
4	134.3^{a}	106.4^{b}	134.2^{a}	3.23	8.35^{a}	3.93^{b}	9.03^{a}	0.21	15.02^{a}	10.0^{b}	14.1^{a}	0.25
8	108.7^{b}	119.9^{b}	143.0^{a}	0.76	8.24^{a}	6.11^{b}	7.66^{ab}	0.32	17.70^{ab}	15.3^{b}	18.6^{a}	0.16
16	96.4	97.3	89.6	0.45	5.70	4.67	5.49	0.53	17.50^{a}	13.7^{b}	12.9^{b}	0.14
24	78.9	77.5	72.7	0.56	5.14	5.70	4.94	0.24	11.50	10.0	10.4	0.17

*BN: Bacterial Nitrogen; SEM: Standard Error of the Mean; For each time point, values with superscript a are significantly different than values with superscript b but not when compared to values with superscript ab ($p<0.05$)

NDF disappearance increased by 18.1 and 16.5% at 8 h after feeding as compared to diets 2 and 3, respectively while ADF disappearance increased by 19.4 and 16.3%, respectively. These results suggested that diet 1 (i.e., SBM plus corn) promotes the digestion of straw fibres, allowing the maintenance of an ideal environment for straw degradation in the rumen.

Ruminal pooling of various dietary nitrogen fractions and ruminal microbial nitrogen: Rumen liquid NH_3-N concentrations reflect the dietary protein degradation rate in the rumen and the effect of rumen microbes on ammonia utilisation (Devant *et al*., 2000). Ruminal ammonia concentrations have been shown to be inversely related to carbohydrate availability (Russell *et al*., 1992; Hristov *et al*., 1997; Heldt *et al*., 1999). Thus, researchers next investigated the accumulation of ammonia in the rumen. The pooling of ruminal liquid NH_3-N is shown in Table 4. The total production of ruminal liquid NH_3-N peaked for diets 1 and 3 ($p<0.05$) at 2 h after feeding but decreased rapidly for diet 3 at 2 h after feeding and decreased for diet 1 at 8 h after feeding. Production of microbial and liquid nitrogen was similar between diets 1 and 3 but higher than diet 2 between 2 and 8 h after feeding ($p<0.05$). Cattle fed diets 1 and 3 exhibited increased ruminal pooling of total nitrogen compared to diet 2 between 2 and 4 h after feeding ($p<0.05$) and similar ruminal pooling of total nitrogen was observed at 16 h after feeding ($p>0.05$). It is possible that protein degradation in the rumen of cattle fed diet 2 was too low to allow for ruminal microbial synthesis, leading to fermentation and improvement in NH_3-N concentrations at 8 h after feeding. Until 24 h after ingestion, all diets resulted in the same amount of NH_3-N. In cattle fed diet 1, rumen NH_3-N peaked at 2-8 h after feeding, suggesting that rumen microorganisms were able to maintain an active degradation function for a prolonged period.

Rumen microbial protein synthesis mainly depends on the efficiency of ruminal carbohydrates and nitrogen. Ruminal ammonia concentrations have been shown to be inversely related to carbohydrate availability (Russell *et al*., 1992; Hristov *et al*., 1997; Heldt *et al*., 1999. Too fast or too slow of a ruminal protein degradation rate will cause energy deficiency or deficiency of nitrogen availability. The degradation of nitrogen and energy in the rumen is asynchronous, causing a reduction in the amount of microbial protein synthesis occurring in the rumen. As shown in Table 4, the amount of bacterial nitrogen and liquid nitrogen was nearly equal at 2-8 h after feeding in cattle fed diets 1 and 3 and both were higher than corresponding values in cattle fed diet 2 ($p<0.05$). The amount of bacterial nitrogen was highest at 2 h after feeding and reduced at 4 h after feeding while the amount of liquid nitrogen unchanged. At 8 h after feeding, bacterial nitrogen in the rumen fluid increased again until 16 h after feeding and straw fibre in the rumen was degraded, allowing ruminal microbes to make full use of the nutrient. Diets 2 and 3 followed the same trend as diet 1 for bacterial nitrogen synthesis at 2-8 h after feeding, however at 16 h after feeding, bacterial nitrogen synthesis decreased for both diets 2 and 3 ($p<0.05$). Moreover, at 2-8 h after feeding, bacterial and liquid nitrogen levels were consistently lower in diet 2 than diets 1 and 3 ($p<0.05$) possibly due to the slow degradation of the BM, leading to nitrogen deficiency.

At 2 h after feeding, total nitrogen retention volume was increased in cattle fed diet 3 than in cattle fed diet 2 ($p<0.05$). At 2-4 h after ingestion, cattle fed diets 1 and 3 exhibited an increase in total dietary rumen nitrogen retention volume as compared to cattle fed diet 2 ($p<0.05$)

Table 5: Rumen liquid VFA pooling and production for each of the 3 rations (%)

	pH				Acetic				Propionic				Butyric			
Time (h)	Diet 1	Diet 2	Diet 3	SEM	Diet 1	Diet 2	Diet 3	SEM	Diet 1	Diet 2	Diet 3	SEM	Diet 1	Diet 2	Diet 3	SEM
2	6.8	6.9	6.7	0.03	1.51	1.49	1.55	0.05	0.60	0.57	0.55	0.03	0.39	0.28	0.38	0.01
4	6.8	6.9	6.6	0.03	1.51	1.45	1.90	0.08	0.57	0.57	0.73	0.07	0.42	0.31	0.46	0.05
8	6.9	7.0	6.9	0.04	1.30	1.56	1.31	0.04	0.39	0.71	0.49	0.04	0.24	0.32	0.33	0.06
16	7.2	7.2	7.2	0.03	0.79	1.24	1.05	0.08	0.30	0.49	0.40	0.03	0.13	0.22	0.22	0.02
24	7.3	7.3	7.3	0.02	0.76	0.84	0.88	0.04	0.31	0.31	0.34	0.02	0.11	0.09	0.11	0.01

*VFA: Volatile Fatty Acid; SEM: Standard Error of the Mean

at 16 h after feeding, there was no difference in nitrogen retention ($p>0.05$). The rumen total nitrogen retention and the dry nitrogen retention in cattle fed diet 3 was highest at 2 h after feeding. This may be related with the rumen DM retention shown in Table 3. The rumen total nitrogen retention of diet 2 was the lowest, potentially resulting from the low the dry nitrogen retention and liquid retention.

VFA production and pooling in the rumen liquid for the 3 diets: Rumen pH is the basic index of valuating rumen fermentation conditions and reflects on the fermentation of substrate utilisation degree by rumen microbial populations (NRC, 2001) when the rumen pH is <6.2, microbial proteins are synthesised slowly (Van Houtert, 1993).

Cows with rumen fluid pH values above 5.8 are considered normal while those with rumen fluid pH values between 5.0 and 5.8 may be suffering from subclinical acidosis (Wang *et al*., 2007). In the current study, rumen pH was maintained between 6.7 and 7.3 and did not affect the growth of the rumen microbiota. The rumen pH value in cattle fed diet 3 decreased at 2 h after feeding and reached a minimum at 4-8 h after feeding. Khorasani *et al*. (2001) found that the source of corn did not affect rumen pH, however in the current study, rumen pH values of heifers fed barley were higher than those of cattle fed corn. At 2 h after feeding, cattle fed diet 1 exhibited the lowest ruminal pH which then rose in a slow, linear fashion. This slow recovery may demonstrate the buffer capacity for rumen microbial activity. Additionally, the rumen pH value in cattle fed diet 2 at 2-8 h after feeding was basically unchanged.

Most studies in dairy cattle have reported greater VFA concentrations in barley-based diets due to the greater rate of NSC degradation for barley (McCarthy *et al*., 1989; Khorasani *et al*., 2001), in contrast, other studies have reported no differences between barley and corn-based diets (Casper and Schingoethe, 1989) and greater VFA concentrations for corn-based diets (Casper *et al*., 1999). Surber and Bowman (1998) reported a greater VFA concentration in barley-based diets compared with corn-based diets in beef cattle. Molar proportions of individual VFAs were not affected by the NSC or protein source (Casper *et al*., 1999; Rotger *et al*., 2006). Other studies with dairy cattle observed a greater concentration of propionate in barley-based diets, possibly because of enhanced degradation of starches in these diets (Casper and Schingoethe, 1989; McCarthy *et al*., 1989). VFA production and pooling in cattle fed each of the 3 diets is also shown in Table 5. Total VFA production (mM) was higher for diet 1 than for diets 2 and 3 at 2 h after feeding ($p<0.05$) and higher for diets 1 and 3 than for diet 2 at 4 h after feeding ($p<0.05$). Total VFA pooling (M) was similar among cattle fed the 3 diets ($p>0.05$) but individual total VFA pooling (M) for each diet trended toward being the same as the total VFA production (mM).

CONCLUSION

Diet 1 consisting of SBM and corn, supplied the ideal ruminal fermentation environment for the degradation of corn stover fibre and improved the disappearance of ruminal DM, NDF and ADF as well as ruminal pH, VFAs and ruminal content after different feeding times. The synchronisation of protein and energy for high-concentrate diets must allow for the optimisation of the dietary combination, benefitting ruminal microbial fermentation and degradation of roughage fibre with optimum nutrient sources and subsequently improving the utilisation of roughage.

ACKNOWLEDGEMENTS

This project is funded by the National Basic Research Program of China (973 Program) (2011CB100805) and National Natural Science Foundation of Shandong Province (2011ZRC02028). Hongbo Zhao and Fachun Wan contributed equally in this research.

REFERENCES

AOAC, 1990. Official Methods of Analysis. 13th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Beauchemin, K.A., W.Z. Yang and L.M. Rode, 2001. Effects of barley grain processing on the site and extent of digestion of beef feedlot finishing diets. J. Anim. Sci.. 79: 1925-1936.

Broderick, G.A. and J.H. Kang, 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci., 63: 64-75.

Casper, D.P. and D.J. Schingoethe, 1989. Lactational response of dairy cows to diets varying in ruminal solubilities of carbohydrate and crude protein. J. Dairy Sci., 72: 928-941.

Casper, D.P., H.A. Maiga, M.J. Brouk and D.J. Schingoethe, 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. J. Dairy Sci., 82: 1779-1790.

Crook, W.M. and W.E. Simpson, 1971. Determination of ammonium in Kjeldahl digests of crops by an automated procedure. J. Sci. Food Agric., 22: 9-10.

Devant, M., A. Ferret, J. Gasa, S. Calsamiglia and R. Casals, 2000. Effects of protein concentration and degradability on performance, ruminal fermentation and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100-230 kg body weight. J. Anim. Sci., 78: 1667-1676.

Fussell, R.J. and D.V. McCalley, 1987. Determination of volatile fatty acids (C_2-C_5) and lactic acid in silage by gas chromatography. Analyst, 112: 1213-1216.

Heldt, J.S., R.C. Cochran, G.L. Stokka, C.G. Farmer, C.P. Mathis, E.C. Titgemeyer and T.G. Nagaraja, 1999. Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by beef steers. J. Anim. Sci., 77: 2793-2802.

Herrera-Saldan, R., R. Gome-Alarcon, M. Torabi and J.T. Huber, 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. J. Dairy. Sci., 73: 142-148.

Hoover, W.H. and S.R. Stokes, 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci., 74: 3630-3644.

Hristov, A.N., T.A. McAllister and K.J. Cheng, 1997. Effect of carbohydrate level and ammonia availability on utilization of á-amino nitrogen by mixed ruminal microorganisms *in vitro*. Proc. Western Sect. Am. Soc. Anim. Sci., 48: 186-189.

Khorasani, G.R., E.K. Okine and J.J. Kennelly, 2001. Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield and milk composition of Holstein cows. J. Dairy Sci., 84: 2760-2769.

McCarthy, Jr. R.D., T.H. Klusmeyer, J.L. Vicini, J.H. Clark and D.R. Nelson, 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci., 72: 2002-2016.

Murphy, M., M. Akerlind and K. Holtenius, 2000. Rumen fermentation in lactating cows selected for milk fat content fed two forage to concentrate ratios with hay or silage. J. Dairy Sci., 83: 756-764.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Edn., National Academy Press, Washington, DC., USA., ISBN-13: 9780309069977, Pages: 408.

Nocek, J.E. and J.B. Russell, 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci., 71: 2070-2107.

Nombekela, S.W. and M.R. Mushy, 1995. Sucrose supplementation and feed of dairy cows in early lactation. J. Dairy Sci., 78: 880-885.

Rotger, A., A. Ferret, S. Calsamiglia and X. Manteca, 2006. Effects of nonstructural carbohydrates and protein sources on intake, apparent total tract digestibility and ruminal metabolism *in vivo* and *in vitro* with high-concentrate beef cattle diets. J. Anim. Sci., 84: 1188-1196.

Russell, J.B., J.D. O'Connor, D.G. Fox, P.J. Van Soest and C.J. Sniffen, 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci., 70: 3551-3561.

Sniffen, C.J. and R.H. Robinson, 1987. Microbial growth and flow as influenced by dietary manipulations. J. Dairy Sci., 70: 425-441.

Surber, L.M. and J.G. Bowman, 1998. Monensin effects on digestion of corn or barley high-concentrate diets. J. Anim. Sci., 76: 1945-1954.

Van Houtert, M.F.J., 1993. The production and metabolism of volatile fatty acids by ruminants fed roughages: A review. Anim. Feed Sci. Technol., 43: 189-225.

Wang, S., W. Wang, J. Wang, Z. Tan and Y. Gong, 2007. Effects of dietary concentrate-to-forage ratio on rumen fermentation and performance of dairy cows. J. Northwest A&F Univ. (Nat. Sci. Edn.), 35: 44-50.

Zhong, R.Z., J.G. Li, Y.X. Gao, Z.L. Tan and G.P. Ren, 2008. Effects of substitution of different levels of steam-flaked corn for finely ground corn on lactation and digestion in early lactation dairy cows. J. Dairy Sci., 91: 3931-3937.