Effect of Feeding Bypass Protein on Rumen Fermentation Profile of Crossbred Cows

V. H. Kalbande* and C. T. Thomas¹

Department of Animal Nutrition, Kerala Agricultural University, Mannuthy, Trichur 680 651, India

ABSTRACT: The effect of three varying ratios (high, medium and low) of Rumen Degradable Protein (RDP) to Undegradable Dietary Protein (UDP) of 37:63, 52:48 and 70:30 in iso-nitrogenous and iso-caloric concentrate mixtures on rumen fermentation profile was studied using rumen fistulated Jersey crossbred cows. Rumen pH and ammonia nitrogen concentration were found to be lower with a concentrate mixture containing a higher UDP level of 63.38% when compared with those having medium and low UDP levels of 47.55 and 29.75%, respectively, at all post feeding intervals. Total volatile fatty acid concentration as well as concentrations of individual fatty acids viz., acetate, propionate and butyrate were also found higher in animals fed concentrate mixture with the highest UDP level. (Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 7: 974-978)

Key Words: By-Pass Protein, Crossbred Cows, Rumen Fermentation Profile

INTRODUCTION

Change in the rumen fermentation profile and fermentation characteristics as influenced by quantity and solubility of dietary proteins have been well documented by many workers (Thomas and Hodson, 1979; Miller, 1982; Wanpath et al., 1982; Hoover and Stokes, 1990). Lough et al. (1983) suggested a synergistic relationship between acetate and post ruminal protein availability on milk fat synthesis. In view of the variable reported responses, the work was planned to study the influence of the three varying ratios of Rumen Degradable Protein (RDP) and Undegradable Dietary Protein (UDP) in a concentrate mixture on the rumen fermentation profile viz., rumen pH, ammonia nitrogen, total volatile fatty acid (TVFA) and individual volatile fatty acid concentration.

MATERIALS AND METHODS

Three fistulated, adult, healthy, non producing Jersey crossbred cows weighing on average 275 kg ± 2.5 fitted with large rumen cannulae were selected for the study and were fed three concentrate mixtures A, B and C with varying levels of rumen degradable protein (table 1), and maintained for a period of one month under similar conditions of management. All the animals were fed the same type of roughage ad libitum. Each concentrate mixture was then fed to the experimental cows for a period of 10 days, the last two days being employed for the collection of rumen

Received February 2, 2000; Accepted February 2, 2001

liquor.

Rumen liquor was collected on two consecutive days from each of experimental cow at 0, 3, 6 and 12 h after feeding on each day. The rumen liquor collected from different sites in the rumen was strained through four layers of muslin cloth. The pH and ammonia nitrogen concentration were determined immediately after collection, while estimation of total volatile fatty acid concentration was done on samples preserved by using a saturated solution of mercuric chloride (1 ml/50 ml strained rumen liquor). Strained rumen liquor (SRL) was preserved by adding 0.5 ml of 50% formic acid to 9.5 ml SRL and was then mixed and centrifuged at 3,000 rpm for 10 min. The supernatant was used for individual fatty acid determination.

The ammonia nitrogen (NH₃-N) concentration in the rumen liquor was estimated by the microdiffusion method of Conway (1957) using mixed indicator. Total volatile fatty acid concentration was estimated following the procedure of Barnett and Reid (1957). The concentrations of individual fatty acids viz., acetate, propionate and butyrate were estimated using gas liquid chromatograph AIMIL Nucon 5700 (Bernard and Boucque, 1968). The data were statistically analysed (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

Rumen pH

From the results presented in table 2, it can be seen that the pattern of changes in rumen pH in animals fed the three iso-nitrogenous and iso-caloric concentrate mixtures (A, B and C) varying in ratios of RDP to UDP was found to be almost similar with increase in post feeding intervals. There was increase in rumen pH from 0 to 3 h post feeding and

^{*} Corresponding Author: V. H. Kalbande. Tel: +91-24-333902, Fax: +91-2452-23582

¹ Dept. of Animal Nutrition, College of Veterinary & Animal Sciences, Manunuthy, TRICHUR-680 651, Kerala, India.

Table 1. Percentage ingredient composition and nutritive value of concentrate mixtures A, B and C

Particulars	Concentrate mixtures					
	A	В	С			
Groundnut cake (deoiled)	5	5	5			
Gingelly cake	5	15	17			
Cotton seed cake (decorticated)	10	5	5			
Coconut cake (solvent extracted)	25	8	5			
Yellow maize	32	29	20			
Wheat bran	20	35	45			
Mineral mixture	2	2	2			
Common salt	1	1	1			
	100	100	100			
% Crude protein (estimated)	20.24	20.71	20.19			
% RDPdo	7.48	10.76	14.13			
% UDPdo	12.76	9.94	6.05			
RDP:UDP ratiodo	37:63	52:48	70:30			
Nutritive value (calculated)						
% DCP	15.44	15.32	15.85			
% TDN	72.25	70.21	68.66			
ME kcal/kg	2639.6	2627.7	2594.6			

thereafter a linear decline up to 12 h post feeding in all cases. The results further indicate that the rumen pH was higher with increase in protein degradability rates though a significant (p<0.01) difference was noted only between the concentrate mixtures A and B with RDP levels of 36.62 and 52.45%, respectively. However, the rumen pH values in animals fed the three concentrate mixtures with varying levels of degradable protein were found to range from 6.30 to 6.92 at all post feeding intervals and all were within the normal range. Similar observations were made by other workers (Sampath and Sivaraman, Khorasni et al., 1994). Leng and Nolan (1984), stated that at lower ruminal pH, the efficiency of microbial protein synthesis was increased because of decreased protozoa content in the rumen. Mertens (1979) observed that crude fiber digestion decreased greatly when ruminal pH declined below 6.0.

Rumen ammonia nitrogen

Ammonia nitrogen concentration (NH₃-N mg/100 ml SRL) in rumen liquor was found to increase with increasing levels of degradable protein in the concentrate mixture, the differences being significant (p<0.01) at all post feeding intervals (table 2). It can

be seen that with concentrate mixture A the peak ammonia nitrogen level was reached at 6 h post feeding and the levels were found to decline thereafter. With concentrate mixture B and C, a higher level of ammonia was reached even at 2 h post feeding and the peak levels were reached much later, being at 9 h post feeding. With concentrate mixtures B and C, higher ammonia nitrogen levels persisted even up to 12 h post feeding, while there was a slight decline in ammonia nitrogen levels after 6 h with concentrate mixture A. The highest level of ammonia nitrogen concentration of 19.49 mg/100 ml SRL was obtained at 9 h post feeding in animals receiving concentrate mixture C with a RDP level of 70.25%. These observations of higher ammonia nitrogen level with increased levels of degradable protein are in agreement with all other studies (Wholt et al., 1976; Santos et al., 1984; Sampath and Sivaraman, 1985; Rao et al., 1987). In practical situations. rumen ammonia nitrogen concentrations increases after feeding and the extent of increase is determined by the solubility of the protein and the form of energy substrate which governs the rate at which ammonia is converted to microbial protein. Annexstad et al. (1987) recorded lower ammonia nitrogen concentrations in the rumen fluid of cows fed extruded soybean meal and corn gluten meal, as compared to soybean meal which is highly degradable in the rumen. Leonard and Block (1986) stated that lowering dietary nitrogen solubility from 53 29% significantly (p<0.01) depressed rumen ammonia nitrogen levels. Several in vitro fermentation studies have suggested a rumen ammonia nitrogen level of 5 to 8 mg/100 ml of rumen fluid as optimum for maximum rumen microbial protein synthesis (Allison, 1970; Satter and Slyter, 1974; Satter and Roffler, 1975; Annison, 1975). Hume et al. (1970) through in vitro studies observed that microbial growth attained a maximum level when rumen ammonia nitrogen concentration reached approximately 9 mg/100 ml rumen liquor. It is evident that the nature and extent of degradation in the rumen of both nitrogen and carbohydrate sources are quite important in determining the efficiency of microbial growth, which ultimately may affect the rumen fermentation profile and production performance in ruminants. The results of present study revealed that concentrate mixture A containing 36.62% RDP was found to be optimum in respect of rumen ammonia nitrogen levels.

Total volatile fatty acid (TVFA) concentration

From the summarised data (table 2) on TVFA concentration in the rumen liquor in cows fed three iso-nitrogenous and iso-caloric diets (A, B and C), it appears that protein degradability affects the formation of the end products of ruminal fermentation.

Table 2. The pH, ammonia (NH₃-N mg/100 ml) and total volatile fatty acids (TVFA mEq/1000 ml) concentration in rumen liquor of Jersey cross bred cows fed concentrate mixtures (A, B and C) of varying protein degradability, at different hours after feeding (means of 12 observations)

Parameter	Concentrate	Mint	Hours after feeding					
		Mixture —	0	3 .	6	9	12	
рН	Α	Mean	6.43ª	6.52ª	6.40°	6.37 ^a	6.30°	
		SE±	0.16	0.03	0.03	0.05	0.03	
	В	Mean	6.80	6.85 ^b	6.83 ^b	6.72 ^b	. 6.71°	
		SE±	0.04	0.02	0.02	0.02	0.05	
	С	Mean	6.80	6.92°	6.89 ^b	6.71 ^b	6.69 ^b	
		SE±	0.07	0.02	0.03	0.15	0.08	
		CD	0.067	0.073	0.024	0.079		
NH3-N	Α	Mean	5.60 ^a	7.22*	8.09 ^a	7.79 ^a	7.38ª	
		SE±	0.18	0.22	0.22	0.18	0.12	
	В	Mean	6.82 ^b	9.55 ^b	11.63 ^b	12.12 ^b	12.05 ^b	
		SE±	0.22	0.16	0.45	0.44	0.30	
	С	Mean	11.15°	15.70°	16.73°	19.49°	19.25°	
		SE±	0.29	0.37	0.48	0.40	0.31	
		CD	0.18	0.78	1.33	0.85	1.60	
TVFA	Α	Mean	81.30 ^a	89.76 ^a	101.31°	112.69ª	109.08ª	
		SE±	0.61	0.82	1.82	3.01	1.82	
	В	Mean	79.95 ^{ab}	85.51 ^b	86.31 ^b	92.31 ^b	93.97 ^b	
		SE±	1.11	0.77	1.31	0.88	1.19	
	C	Mean	78.08 ^b	81.76°	84.98 ^b	91.43 ^b	89.36°	
		SE±	0.84	0.65	0.70	1.12	1.24	
		CD	2.28	2.55	2.95	3.22	3.72	

Means bearing a, b and c superscript in a column differ significantly (p<0.01)

TVFA levels in the rumen liquor in animals fed concentrate mixture A with a protein degradability level of 36.62% was found to be significantly (p<0.01) higher at all post feeding intervals as compared to those in cows fed concentrate mixture B and C with protein degradability levels of 52.45% and 70.25% respectively. Though the concentration of TVFA (mEq/1000 ml SRL) was found to decrease with increase in degradability rates of protein, a significant (p<0.05) difference was observed between concentrate mixture B and C only at 3 and 12 h post feeding.

The TVFA concentration was found to increase linearly with increase in post feeding intervals reaching a peak at 9 h and declining thereafter, except for concentrate mixture B where the concentration was found to increase even up to 12 h post feeding. Sampath and Sivraman (1985) recorded mean TVFA levels of 90.31, 83.63 and 85.38 mEq/1,000 ml SRL with RDP levels of 45.30, 64.70 and 76.90%, respectively, in animals maintained under an almost

similar feeding regimens. They have attributed higher TVFA production with less degradable protein to increased cellulolytic bacteria, resulting from low proteolysis. Khorasani et al. (1994) recorded no significant changes in TVFA concentration in rumen liquor of cows fed iso-nitrogenous diets but differing in insoluble protein contents.

Concentrations of individual volatile fatty acids

Results indicated that the concentrations of all individual volatile fatty acids, viz. acetate, propionate and butyrate followed almost the same trend as that of TVFA level at all post feeding intervals. Results for VFA concentrations in rumen liquor (table 3) further indicated that the molar percentages of individual volatile fatty acids increased with a decrease in solubility rates corresponding to the higher TVFA levels, more so for acetate and propionate than butyrate. Concentrations of all the three volatile fatty acids were found to reach maximum at 3 to 6 h post

Table 3. Acetate, propionate and butyrate concentration (mole/100 mole) in the rumen liquor of Jersey crossbred cows at different hours after feeding concentrate mixtures A, B and C (means of 12 observations)

Parameter	Concentrate	3.62	Hour after feeding				
		Mixture	0	3	6	9	12
Acetate	A	Mean	57.66 ^a	63.66ª	62.16ª	62.00°	62.83ª
		SE±	1.20	1.45	1.49	0.73	1.01
	В	Mean	51.66 ^b	54.00 ^b	55.83 ^b	55.00 ^b	53.33 ^b
		SE±	1.20	2.08	2.04	2.48	1.61
	С	Mean	47.50°	51.50°	51.83°	49.50°	49.50°
		SE±	1.02	0.56	0.65	1.02	0.72
		CD	3.76	2.30	3.65	1.76	3.52
Propionate	A	Mean	22.33°	24.00 ^a	25.16 ^a	24.83°	24.73 ^a
		SE±	1.71	1.67	. 1.08	1.31	1.16
	В	Mean	21.50 ^a	21.66 ^a	21.16 ^b	21.03 ^b	20.50 ^b
		SE±	1.52	1.28	1.49	1.60	1.26
	С	Mean	18.00 ^b	18.50 ^b	22.83 ^b	22.66 ^b	17.83 ^b
		SE±	0.77	0.96	0.87	1.96	1.40
		CD	4.63	3.99	3.14	0.00	4.07
Butyrate	Α	Mean	10.66ª	11.50°	11.83 ^a	11.16 ^a	10.66ª
		SE±	0.49	0.50	0.49	0.47	0.21
	В	Mean	8.33 ^b	8.83 ^b	9.33 ^b	8.16 ^b	8.16 ^b
		SE±	0.33	0.47	0.66	0.60	0.60
	С	Mean	10.00^{a}	9.00 ^b	8.66 ^b	8.33 ^b	8.16 ^b
		SE±	0.63	0.63	0.55	0.55	0.54
		CD	1.16	1.94	2.20	1.94	1.63

Means bearing a, b and c superscript in a column differ significantly (p<0.01).

feeding with all three concentrate mixtures. Blauwiekel and Kin Caid (1986) also recorded significantly (p<0.01) higher acetate in the rumen fluid of cows fed low solubility diets when compared to cows fed high solubility diets. Similar results were obtained by Zimmerman et al. (1992) and Herrera-Saldana and Huber (1989). Christensen et al. (1993) reported lower molar proportions of acetate and butyrate and a higher level of propionate with low rumen undegradable protein diets resulting in low acetate to propionate ratio.

From the overall results on TVFA concentration it can be seen that though the TVFA concentration as well as molar percentages of individual volatile fatty acids appeared to be affected by the protein degradability levels, all the values were within the normal range recorded for animals maintained on standard diets. As such, the differences observed may be more related to the composition of the fermentable organic matter and its influence on kind of microbes rather than on protein degradability rate, which is also

evidenced by the almost normal rumen ammonia nitrogen levels.

ACKNOWLEDGEMENT

The senior author is grateful to the Indian Council of Agricultural Research, New Delhi, for the award of Senior Fellowship and the Dean, College of Veterinary and Animal Sciences, KAU, Mannuthy, Trichur, for providing the facilities during prosecution of the research work.

REFERENCES

Allison, M. J. 1970. Nitrogen metabolism of ruminal micro-organisms. In: Physiology of Digestion and Metabolism in Ruminants. (Ed. A. T. Phillipson). Oriel Press Ltd., England.

Annexstad, R. J., M. D. Stern, D. E. Otterby, J. G. Linn, and W. P. Hansen. 1987. Extruded soyabeans and corn gluten meal as supplemental protein sources for lactating dairy cattle. J. Dairy Sci. 70:814.

- Annison, E. F. 1975. Microbial protein synthesis in relation to amino acid requirements. In: Tracer Studies on Non-protein Nitrogen for Ruminants-II. FAO/LAED, p. 141.
- Barnett, A. J. G. and R. L. Reid. 1957. Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen. I. The volatile acid production from fresh grass. J. Agric. Sci. 48:315.
- Bernard, G. C. and C. V. Boucque. 1968. Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid, J. Agric. Food Chem. 16:192.
- Blauwiekel, R. and R. L. Kin Caid. 1986. Effect of crude protein and solubility on performance and blood constituents of dairy cows. J. Dairy Sci. 69:P2091.
- Conway, E. J. 1957. Microdiffusion Analysis and Volumetric Error. 4thed. Crossby and Lockwood and Son. Univ. Press, Glasgow.
- Cristensen, R. A., G. L. Lynch, J. H. Clark and Y. Yu. 1993. Influence of amount and degradaility of protein on production of milk and milk components by lactating Holstein cows. J. Dairy Sci. 76:3490.
- Herrera-Saldana, R. and J. T. Huber. 1989. Influence of varying protein and starch degradabilities on performance of lactating cows. J. Dairy Sci. 72:1477.
- Khorasani, G. R., G. Deboer, B. Robinson and J. J. Kennelly. 1994. Influence of dietary protein source on the amino acid profiles available for digestion and metabolism in lactating cows. J. Dairy Sci. 77:813.
- Lough, D. E., E. C. Prigge, W. H. Hoover and G. A. Varga. 1983. Utilization of ruminally infused acetate or propionate and abomasally infused casein by lactating goats. J. Dairy Sci. 66:756.
- Mertens, D R. 1979. Effect of buffers upon fiber digestion. In: Regulation of Acid-Base Balance. (Ed. W. H. Hale and Manhardt). Church and Dwight Co. Inc. Nutley, NI.
- Miller, E. L. 1982. Methods of assessing protein for ruminants, including laboratory methods. In: Protein

- Contribution of FeedStuffs for Ruminants. (Ed. Miller, E. L., I. H. Pike, E. S. Van and A. J. H.), London. Butterworths. p. 26.
- Rao, E. R., D. A. Prasad and N. Krishna. 1987. *In-vitro* and *in-vivo* evaluation of concentrate mixtures varying in nitrogen solubility. Indian J. Dairy. Sci. 40:339.
- Santos, K. A., M. D. Stern and L. D. Satter. 1984. Protein degradation in the rumen and amino acid absorption in the small intestine of lactating dairy cattle fed various protein sources. J. Anim. Sci. 58:244.
- Satter, L. D. and R. E. Roffler. 1975. Nitrogen requirement and utilization in dairy cattle. J. Dairy Sci. 58:1219.
- Satter, L. D. and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in-vitro. Br. J. Nutr. 32:199.
- Sampath, K. T. and E. Sivaraman. 1985. In-situ dry matter disappearance and protein degradability of yellow maize, wheat bran and guinea grass (Panicum maximum) in the rumen of cattle. Ph. D. thesis submitted to Kerala Agricultural University, Trichur.
- Snedecor, G. W. and W. S. Cochran. 1968. Statistical Methods Oxford and IBH. Pub. Co., Calcutta.
- Thomas, P. C. and J. C. Hodson. 1979. The clearance rate of rumen liquid and rumen fermentation patterns in sheep receiving forage diets. J. Agric. Sci. 92:683.
- Wanpath, M., D. O. Erickson and W. D. Slanger. 1984. Nitrogen metabolism in sheep fed protein sources of various solubilities with low quality roughages. J. Anim. Sci. 54:625.
- Wholt, J. E., C. J. Sniffen, W. H. Hoover and L. L. Johnson. 1976. Nitrogen metabolism in weathers as affected by dietary protein solubility and amino acid profile. J. Anim Sci. 42:1280.
- Zimmerman, C. A., A. H. Rakes, T. E. Daniel and B. A. Hopkins. 1992. Effect of total and rumen undegradable protein on the performance of cows fed low fiber diets. J. Dairy Sci. 75:1954.