
NEW ZEALAND SOCIETY
ANIMAL PRODUCTION
VOLUME 62 2002

Ruminal utilisation of pasture nitrogen in response to inclusion of dietary starch

E. S. KOLVER, L. D. MULLER[1] AND G. A. VARGA[1]

Dairying Research Corporation, Private Bag 3123, Hamilton, New Zealand.

ABSTRACT

Four diets based on high quality pasture were formulated using additions of starch to contain 12.8, 19.6, 26.3, or 33% total nonstructural carbohydrate and were evaluated using four continuous culture fermenters during four 10 day periods. The true ruminal digestibility of organic matter was not changed by diet but the digestibility of neutral detergent fibre and crude protein linearly decreased, and the digestibility of total nonstructural carbohydrate linearly increased, as the level of starch inclusion increased. Production of NH_3 N was reduced linearly from 35.2 to 14.2 mg/dl ($P<0.001$) and the flow of non-NH_3 N as a proportion of total N flow increased linearly from 66% to 83% ($P<0.001$) as inclusion of starch increased. The quantity of microbial N and non-NH_3 N flowing from continuous culture was unchanged. The experiment indicated that if the amount of organic matter digested is not appreciably increased by the inclusion of nonstructural carbohydrate then the linear increase in ruminal utilisation of pasture N arises more from a reduced pasture N intake than an improvement in microbial protein synthesis.

Keywords: Pasture; carbohydrate; microbial protein synthesis; nitrogen utilisation; continuous culture.

INTRODUCTION

A possible key limitation to milksolids production from high quality pasture is the poor utilisation of pasture N as a result of a highly degradable protein content coupled with a concentration of nonstructural carbohydrate which is low compared with international feeding standards (Beever, 1993; Edwards and Parker, 1994; Moller *et al*., 1993; Poppi and McLennan, 1995). Overseas, total mixed rations for dairy cows are formulated to supply approximately 35% of the diet as nonstructural carbohydrates to provide energy to the dairy cow and to maximise the incorporation of NH_3 N, peptides, and amino acids into microbial protein in the rumen (Hutjens, 1996).

To investigate how these concepts might apply to a diet based on pasture, Carruthers *et al*., (1997) fed dairy cows with an isocaloric, isonitrogenous pasture diet which contained 10% more total nonstructural carbohydrate (TNC) than an all pasture diet. Microbial protein synthesis was not increased, but when energy intake was increased by providing an additional 10% more TNC, microbial protein synthesis was increased. This suggested that if manipulation of the TNC content did not change energy intake, the proportion of NH_3 N captured as microbial protein would not be influenced.

This paper reports on an experiment which extended these findings by investigating the ruminal utilisation of pasture N in response to an incremental increase in the level of starch inclusion when spring pasture was fermented in continuous culture.

MATERIALS AND METHODS

Orchardgrass *(Dactylis glomerata* L.*)* which had been freeze dried and ground to 4 mm was supplemented with a ruminally available energy supplement (corn starch; Sigma Chemical Co., St Louis, MO) to formulate four diets with three levels of starch inclusion: pasture only (12.8% TNC), pasture including 5.9 g of starch (Starch 1; 7.9% starch, 19.6% TNC), pasture including 11.7 g of starch (Starch 2; 15.6% starch, 26.3% TNC), and pasture including 17.5 g of starch (Starch 3; 23.3% starch, 33% TNC). Intake of dry matter (DM) was held constant across diets (75 g DM/d) and diets formulated so that crude protein (CP), degradable CP, ruminal NH_3 N, and neutral detergent fibre (NDF) were not expected to limit microbial growth and nutrient digestion. Minerals and vitamins were included according to NRC (1989) requirements for a dairy cow producing 35 kg milk/d.

The experiment was conducted during four 10-day periods; 7 days for adaptation followed by 3 days for sample collection. The dual flow continuous culture system used was similar to that described by Hannah *et al*., (1986). The system comprised of four fermenter units with a mean working volume of 1037 ml. Liquid dilution rate was maintained at 12%/h and solids dilution rate at 5%/h. Ruminal innoculum was obtained from a nonlactating, ruminally cannulated Holstein cow grazing pasture containing predominantly orchardgrass. Each fermenter was fed a total of 75 g of DM/d in equal portions at 0800, 1300, 1800 and 2200 h with the effluent weighed at 1230 h each day.

The solid and liquid effluent portions (which were the equivalent of duodenal digesta) were homogenised and a 600-ml subsample collected during the last 3 days of each period. After each 3-day collection these subsamples were composited for each fermenter and DM content determined. The remaining digesta was freeze dried and ground through a 1-mm sieve. In addition, a 50-ml sample of effluent was collected for NH_3 N and VFA analysis on each sample day as described by Kolver (1997). On the last day

[1] Department of Dairy and Animal Science, The Pennsylvania State University, PA 16802, United States of America

of each period, bacteria were isolated from the fermenter contents (see Kolver 1997), freeze-dried, and ground through a 1-mm screen.

Samples of pasture and effluent were analysed for ash, CP, NDF, acid detergent fibre (ADF) and TNC. The soluble CP and degradable CP content of the pasture samples were determined by colourimetric analysis and starch was analysed for TNC and ash. Bacterial samples were analysed for ash, CP, and purine content. These analyses were conducted according to procedures cited by Kolver (1997).

Data were analysed using the general linear models procedure of SAS according to a randomised complete block design. Linear and quadratic treatment relationships were evaluated, but none of the quadratic relationships were significant and are not presented. All means presented are least squares means tested with the PDIFF option in SAS.

RESULTS

Pasture was of high quality (Table 1). The concentration of TNC increased linearly from 12.8% to 33% TNC across the four dietary treatments. The diet with the highest rate of starch inclusion contained 1.3% more organic matter (OM) and 0.07 Mcal/kg of DM more net energy for lactation (NE_L) than the all-pasture diet. The concentrations of CP, NDF and ADF of all diets were above the minimum levels recommended by NRC (1989).

TABLE 1: Chemical composition of four pasture-based diets formulated with an incremental addition of starch.

Composition (%DM)	Pasture	Starch 1	Starch 2	Starch 3
Organic matter	90.1	90.5	90.9	91.4
Total nonstructural carbohydrate	12.8	19.6	26.3	33.0
NEL (Mcal/kgDM)[1]	1.68	1.70	1.72	1.75
Crude protein	24.4	22.3	20.3	18.4
Soluble crude protein	8.7	7.9	7.2	6.5
Degradable crude protein	18.0	16.5	15.0	13.5
Neutral detergent fibre	46.9	43.1	39.2	35.4
Acid detergent fibre	24.8	22.7	20.7	18.7
Fat	5.7	5.2	4.8	4.3

[1] Net energy for lactation

When the apparent ruminal digestibility of DM and OM was corrected for bacterial DM and OM, no significant differences in true ruminal digestibility of DM and OM between treatments were detected (Table 2). The apparent ruminal digestibility of TNC increased linearly with inclusion of starch, but the treatment differences in apparent ruminal digestibility of NDF, while numerically lower for pasture diets with a high level of starch inclusion, were not sufficient to reach statistical significance. Total VFA concentrations increased linearly in response to higher levels of dietary starch; similarly, concentrations of acetate increased but statistical significance was not reached (Table 2).

TABLE 2: Nutrient digestibility and VFA production in response to increased level of starch inclusion in a pasture diet fermented in continuous culture.

Item	Pasture	Starch 1	Starch 2	Starch 3	SED	P <[2]
True digestibility (% OM[1])						
Dry matter	66.1	66.7	67.7	70.4	1.9	0.16
Organic matter	70.2	70.7	72.6	74.4	2.3	0.20
Apparent digestion (%)						
Total nonstructural carbohydrate	40.8[a]	60.5[b]	71.5[c]	76.5[d]	1.0	0.001
Neutral detergent fibre	43.5	38.8	35.8	36.8	2.8	0.12
Volatile fatty acids (μmol/ml)						
Total volatile fatty acids	82.6[ab]	82.1[a]	86.2[ab]	89.1[b]	2.6	0.08
Acetate	55.1	54.4	57.2	59.9	2.0	0.11
Propionate	14.6	13.5	12.7	13.2	0.7	0.17
Butyrate	8.4[a]	9.5[b]	11.4[c]	11.2[c]	0.2	0.001

[1] Organic matter
[2] Linear relationship between dietary responses
[a,b,c,d] Values on the same line with different superscripts are significantly different, P<0.10

Intake of N decreased linearly as starch made up a greater proportion of the pasture-based diet (Table 3). A similar linear decrease in the concentration of NH_3 N was evident, with the fermentation of the all-pasture diet producing approximately 2.5 times more NH_3 N than the pasture diet containing the highest level of starch. Digestibility of CP decreased in a linear fashion as the level of starch inclusion increased. Fermentation of the all pasture diet resulted in a ratio of 50.5 g of rumen degradable N per kg of rumen degradable OM (RDN:RDOM), which lin-

TABLE 3: Nitrogen metabolism in response to increased level of starch inclusion in a pasture diet fermented in continuous culture.

Item	Pasture	Starch 1	Starch 2	Starch 3	SED	P <[2]
Total N intake (g/d)	3.47[a]	3.24[b]	2.98[c]	2.74[d]	0.008	0.001
NH_3 N (mg/d*l*)	35.2[a]	30.7[b]	20.5[c]	14.2[d]	0.98	0.001
True CP digestibility (%)	68.7[a]	66.5[ab]	61.4[b]	61.3[b]	2.01	0.02
RDN:RDOM[1] (g/kg)	50.5[a]	44.8[b]	37.0[c]	33.0[d]	0.84	0.001
N Flows (g/d)						
Total N	3.07[a]	2.92[b]	2.66[c]	2.44[d]	0.01	0.001
NH_3 N	1.04[a]	0.92[b]	0.60[c]	0.41[d]	0.03	0.001
Non-NH_3 N	2.04	2.0	2.06	2.03	0.03	0.72
Bacterial N	1.12	1.10	1.12	1.17	0.06	0.50
Dietary N	0.92	0.90	0.94	0.86	0.05	0.58
N Flows (% of total N flow)						
NH_3 N	33.8[a]	31.6[a]	22.6[b]	16.9[c]	1.07	0.001
Non-NH_3 N	66.2[a]	68.4[a]	77.5[b]	83.1[c]	1.07	0.001
Bacterial N	36.5[a]	37.7[a]	42.0[a]	48.1[b]	2.13	0.01
Dietary N	29.8[a]	30.7[ab]	35.4[b]	35.0[b]	1.77	0.04

[1] g of N digested in the rumen per kg of organic matter truly digested in the rumen
[2] Linear relationship between dietary responses
[a,b,c,d] Values on the same line with different superscripts are significantly different, P<0.10

early decreased to 33.0 g of RDN per kg of RDOM at the highest level of starch inclusion. Flow of total N and NH_3 N decreased linearly as starch inclusion increased (Table 3). Diet did not influence flow of non-NH_3 N, bacterial N, or dietary N. When expressed as a proportion of total N flow, NH_3 N flow resulting from fermentation of the all-pasture diet was twice that of Starch 3. Concomitantly, addition of incremental levels of starch increased the proportion of non-NH_3 N, bacterial N, and dietary N in the total N flow to the duodenum.

DISCUSSION

When pasture diets with increased levels of starch were fermented *in vitro*, concentrations of NH_3 N were lower than those measured from the fermentation of diets containing all pasture but the quantity of microbial protein and total protein available for absorption in the small intestine was unchanged. This feeding scenario is similar to that where dairy cows grazing high quality pasture exhibit a high rate of substitution when supplementary carbohydrate is fed. The present experiment suggests that when the rate of substitution is high (e.g., 1 kg of pasture per 1 kg of supplement), loss of N during digestion of high quality pasture can be reduced with inclusion of TNC. This is more a result of the dilution of N intake than an improvement in the capture of ruminal N by ruminal micro-organisms. Synthesis of microbial protein is a function of the ratio RDN:RDOM. The RDN:RDOM ratio of the diets fed in the present experiment linearly decreased from 50.5 for the all pasture diet to 33 for the pasture diet with the highest rate of starch inclusion. Thus, the 4 diets contained CP levels greater than required by NRC (1989) and were fermented in an environment of excess N relative to energy supply (Poppi and McLennan, 1995). The lack of a diet effect on the quantity of non-NH_3 N flowing from continuous culture supports the contention of Poppi and McLennan (1995) that, at a given intake of pasture DM (or energy intake), the supply of CP to the duodenum is unlikely to be changed when fermentation is operating in this range of N excess relative to energy supply.

The need to balance the supply of ruminally available energy and N is well recognised in the formulation of dairy cattle rations (Hutjens, 1996). Intensively managed grazing systems can supply pasture that frequently has a CP content of 20-30 % (Beever, 1993). The data from this experiment indicate that care must be taken when ratios such as RDN:RDOM and recommended levels of TNC are applied to a ruminal environment of excess N such as that resulting from the digestion of high quality pasture. The ratio RDN:RDOM can be decreased by changing either RDN or RDOM and each manipulation has a somewhat different impact on the utilisation of N. If RDN is reduced, but RDOM is unchanged, quantity of microbial protein flowing to the small intestine is unlikely to be changed, but ruminal NH_3 N will likely be reduced. This was the case in the present experiment and represents the feeding situation whereby intake of high quality pasture by dairy cows is largely substituted for supplemental carbohydrate, with little change in total DMI. Loss of N is reduced, and thus utilisation of pasture N is increased.

If, however, the quantity of RDN remains unchanged and RDOM is increased, microbial protein will likely increase and ruminal NH_3 N decrease as a result of increased incorporation of NH_3 N for microbial protein synthesis. This represents the feeding situation where the total DMI of a grazing dairy cow is increased with supplemental feeding of carbohydrate. Utilisation of ruminal N is increased in this scenario, as well as the supply of CP to the duodenum. This clarification of the conditions required for a microbial protein response when high quality pasture is supplemented with nonstructural carbohydrate is well illustrated by the results of Carruthers *et al*., (1997). Feeding isonitrogenous and isocaloric diets based on pasture, an increase in the TNC content of a high quality pasture diet from 22.5% (all pasture) to 28.7% (pasture with ruminal infusion of starch/dextrose solution) did not increase the yield of microbial protein available for absorption by lactating dairy cows. These diets were compared with a third, isonitrogenous pasture treatment supplemented with a starch/dextrose infusion to supply an additional 10% more metabolisable energy and increase TNC content to 28.2%. Approximately 15% more microbial protein was produced on this diet compared to the all pasture diet.

The relationship between non-NH_3 N flow (g of non-NH_3 N per g of N intake excluding buffer N; Y) and N content of the diet (g of N excluding buffer N per kg of OM; X) in the present experiment was described by the equation: $Y = 1.58 (\pm 0.073) - 0.0206 (\pm 0.0019)X$ (mean ± SE; $R^2 = 0.98$). From this equation it can be calculated that an incomplete transfer of dietary N to the duodenum would occur when the N content of the diet exceeded 28 g of N/kg of OM (a dietary CP content of approximately 19.5% DM). For a cow fed 15 kg of OM/d, the predicted supply of non-NH_3 N would be maximised at a dietary CP of approximately 21.3% and be associated with a 20% net loss in dietary N. This relationship is very similar to the equations developed by Ulyatt *et al*., (1988) and van Vuuren *et al*., (1992) for diets containing only pasture.

The linear decrease in NDF digestibility in the present experiment was not statistically significant, but did reflect the reduced digestibility of NDF reported by others when dairy cows fed pasture have been supplemented with starchy feeds (Carruthers *et al*., 1997; van Vuuren *et al*., 1993). Fibre digestion is reduced when ruminal pH is less than 6.2, but based on the reports of Mould *et al*., (1983) fibre digestion can also be reduced at a higher pH when supplemental nonstructural carbohydrates are degraded by microbes in preference to structural carbohydrate.

CONCLUSION

This study demonstrated that the in vitro ruminal utilisation of N from high quality pasture, when expressed as the proportion of N intake flowing to the duodenum, increased linearly in response to an incremental increase in the level of starch inclusion. Production of NH_3 N was reduced by 60% and the flow of non-NH_3 N as a proportion of total N flow was increased from 66% to 83% as inclusion of starch increased. The actual quantity of microbial N and non-NH_3 N flowing from continuous culture was unchanged. The implication of these results for dairy cows grazing high quality pasture ad libitum and expressing a high rate of pasture substitution is that supplementation with nonstructural carbohydrate will not necessarily improve the capture of ruminal NH_3 N, largely because of the concomitant decrease in NDF digestibility. In this situation the improved efficiency with which dairy cows use pasture N is more a result of a dilution of N intake, which may subsequently impact energy requirements for N excretion as well as the N balance of the whole farm system.

REFERENCES

Beever, D.E. 1993. Ruminant animal production from forages: present position and future opportunities. *Proceedings of the XVII International Grassland Congress* 535-542.

Carruthers, V.R., Neil, P.G., and Dalley, D.E. 1997. Effect of altering the non-structural:structural carbohydrate ratio in a pasture diet on milk production and ruminal metabolites in cows in early and late lactation. *Animal Science* **64:** 393-402.

Edwards, N.J. and Parker, W.J. 1994. Increasing per cow milksolids in a pasture-based dairying system by manipulating the diet: A review. *Proceedings of the New Zealand Society of Animal Production* **54:** 267-273.

Hannah, S.M., Stern, M.D., and Ehle, F.R. 1986. Evaluation of a dual flow continuous culture system for estimating bacterial fermentation in vivo of mixed diets containing various soya bean products. *Animal Feed Science and Technology* **16:** 51-62.

Hutjens, M.F. 1996. Practical approaches to feeding the high producing cow. *Animal Feed Science and Technology* **59:** 199-206.

Kolver, E.S. 1997. Supplemental feeding strategies to increase the utilisation of pasture nitrogen by high producing dairy cows. Ph.D. Diss., The Pennsylvania State University, PA.

Moller, S., Matthew, C., and Wilson, G.F. 1993. Pasture protein and soluble carbohydrate levels in spring dairy pasture and associations with cow performance. *Proceedings of the New Zealand Society of Animal Production* **53:** 83-86.

Mould, F.L., Orskov, E.E., and Mann, S.O. 1983. Associative effects of mixed feeds. 1. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. *Animal Feed Science and Technology* **10:** 15-30.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle, 6th rev. ed. National Academy of Science, Washington, DC.

Poppi, D.P. and McLennan, S.R. 1995. Protein and energy utilisation by ruminants at pasture. Journal of Animal Science 73: 278-290.

Ulyatt, M.J., Thomson, D.J., Beever, D.E., Evans, R.T., and Haines, M.J. 1988. The digestion of perennial ryegrass (Lolium perenne cv. Melle) and white clover (Trifolium repens cv. Blanca) by grazing cattle. *British Journal of Nutrition* **60:** 137-149.

van Vuuren, A.M., Krol-Dramer, F., van der Lee, R.A., and Corbijn, H. 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh Lolium perenne with different nitrogen contents. *Journal of Dairy Science* **75:** 2215-2225.

van Vuuren, A.M., van der Koelen, C.J., and Vroons-de Bruin, J. 1993. Ryegrass versus corn starch or beet pulp fiber diet effects on digestion and intestinal amino acids in dairy cows. *Journal of Dairy Science* **76:** 2692-2700.