Effect of white clover containing either high or low concentrations of water-soluble carbohydrate on metabolic indicators of protein degradation in the rumen of dairy cows

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ABSTRACT

White clover has high nutritive value, but contains excess protein relative to energy, leading to the wasteful excretion of nitrogen (N) by animals. Five cycles of divergent selection has resulted in white clover lines having high (HS) or low (LS) concentrations of water-soluble carbohydrate. A grazing trial was carried out to evaluate the effect of this selection on protein utilization, using skatole and indole concentrations in the milkfat as metabolic indicators for nitrogen utilisation in the rumen. Skatole and indole arise in the rumen from the degradation of the amino acid tryptophan, and rapidly accumulate in milkfat of lactating animals fed diets rich in soluble and degradable protein. Skatole and indole concentrations were both significantly ($P < 0.001$) lower in cows fed HS clover (356 and 84 ng/g milkfat for skatole and indole, respectively) compared to those fed the LS white clover diet (800 and 420 ng/g milkfat, respectively) suggesting protein utilisation of the HS clover was improved. Although not statistically significant in this short-term study, the trends to lower concentrations of milk urea-nitrogen and urinary-N, and the nitrogen:creatine ratio for the HS clover were in the direction expected and warrant further investigation under longer-term feeding.

Keywords: white clover; water soluble carbohydrate; protein; skatole; indole.

INTRODUCTION

Livestock production in New Zealand is predominantly based on perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pastures (Burke *et al.*, 2002; Waghorn & Barry, 1987). There are, however, limitations associated with pasture feeding, particularly regarding the utilisation of protein (Ulyatt, 1997). A significant proportion of dietary nitrogen (N) is lost from the rumen as ammonia. This is partly because pasture proteins are highly soluble and rapidly degraded by plant and microbial proteases after ingestion. In addition, the microbial population is unable to capture all the non-protein nitrogen (NPN) released during the proteolysis of plant proteins (Beever *et al.*, 1986; Kingston-Smith & Theodorou, 2000). Rumen microbes need energy in the form of adenosine triphosphate (ATP) to be able to capture the ammonia and use it as a nitrogen source for growth. Although carbohydrate fermentation is the main source of ATP, microbes will use amino acids as an energy source when carbohydrates are deficient. This leads to excessive ammonia accumulation in the rumen (Nocek & Russell, 1988).

The protein in white clover is more soluble and more degradable than that of perennial ryegrass (Min *et al.,* 2000) making it an important contributor to the inefficient use of protein on pasture-based diets. The aromatic compounds skatole and indole are formed in the rumen from the microbial degradation of the amino acid tryptophan (Deslandes *et al.*, 2001; Schreurs *et al.*, 2003). Their concentrations in milkfat have been used in previous studies as indicators of nutrient acquisition and rumen protein metabolism (Cosgrove *et al.*, 2006; Pacheco *et al*., 2008).

Plant breeders have selected white clover for high water-soluble carbohydrate (WSC) concentration to improve the supply of readily available energy and enable the more efficient use of the high protein concentrations. The objective of this study was to determine if the difference in WSC concentration between two divergent selections of white clover was sufficient to change protein utilisation, using the concentrations of skatole and indole in the milkfat, and nitrogenous compounds in milk and urine of lactating dairy cows as indicators of ruminal protein degradation.

MATERIALS AND METHODS

This study was conducted during March and April 2006, on the Massey University No. 1 Dairy Unit in Palmerston North, on Manawatu sandy loam soils.

Experimental design and treatments

A cross-over design was used with two treatments and two periods (two days in Period 1 and three days in Period 2). The treatments consisted of high (HS) and low (LS) WSC white

clover (*Trifolium repens*) grazed during the day between the morning and afternoon milking. For each clover treatment, cows grazed a ryegrass monoculture (RG) (*Lolium perenne*) at night between the afternoon and next morning milking. A four-day adaptation period preceded the experiment during which the cows were fed RG at night and "normal" white clover during the day.

Animals

Ten spring-calving Friesian cows (521 ± 39 kg live weight; 3.6 ± 0.3 condition score; Mean ± Standard deviation) were selected from the main herd and allocated to two treatment groups of five, balanced for age, production worth and milk yield. Bloat protection was administered to all cows in the form of a Rumensin® antibloat rumen capsule releasing 320 mg monensin sodium per day (Elanco Animal Health, Manukau, New Zealand), for five days prior to beginning the pre-trial adjustment period. In addition, cows were dosed at each milking, and water troughs treated twice daily with Bloateze containing 700g/l alcohol ethoxylate and 70g/l ethylene/propylene co-polymer (Farmers Industries Ltd., Mount Maunganui, New Zealand).

Pasture allocation and measurement

Cows were split into their respective treatment groups during the morning milking, and allocated to their assigned clover treatment for the day between 8:00 h and 15:00 h. The two groups were joined as a single group following afternoon milking, and grazed RG at night from 17:00 h to 6:00 h the next day. All cows were offered an allowance of 37 kg dry matter (DM)/d with target intakes of 18 kg DM/cow/d. The total daily allocation of DM was offered in equal portions of 50% after the morning milking (white clover) and 50% after the afternoon milking (ryegrass). This was shown by Cosgrove *et al.* (2006) to be the way cows distribute their grazing when offered clover-only during the day and grass-only at night. The area of each break was derived by assessing pre-grazing pasture mass, and adjusting the area allocated to give the desired allowance per cow, whilst maintaining target post-grazing residual pasture mass of 1800 kg DM/ha for grass, and 1500 kg DM/ha for white clover. Due to a shortage of grass during the study period, cows were supplemented at night with 4 kg DM/cow of pasture silage (9 MJ ME/kg DM). This continued the practice of supplementation that the trial cows had received prior to separation from the main herd.

An electronic rising-plate meter (Farmworks Precision Farming Systems, Feilding, New Zealand) was used at each grazing to estimate pre- and post-grazing herbage mass (kg DM/ha), from 50 readings taken randomly throughout the area allocated for grazing. Ten 0.25 m^2 quadrats were cut to ground level pre- and post-grazing from each plot, once each period for both grass and clover.

Pasture species composition

Herbage samples of HS and LS clover were taken at approximately 8:00 h each morning and RG at 17:00 h each afternoon. Samples were hand-plucked from 10 to 15 sites around each plot to represent the herbage being consumed by the cows, frozen in the field using liquid nitrogen and stored at -20ºC. They were then freeze dried, ground to pass a 1 mm sieve, and concentrations of soluble sugars and starch, crude protein, fibre fractions, organic matter digestibility and metabolisable energy predicted using near-infra red reflectance spectroscopy (FeedTech, AgResearch Grasslands, Palmerston North, New Zealand).

Milk yield measurements and sample collection

Milk yield was recorded at each milking using Metatron™ (Westfalia, Oelde, Germany) meters and proportional in-line samplers. Sub-samples for each cow were retained at each milking for analysis of milkfat, protein and lactose (FT 6000 Fourier Transform infrared analyser, Foss Electric, Hillerød, Denmark). Further sub-samples of approximately 100 mL were retained and frozen at -20ºC for subsequent analysis of milkfat metabolites and milk urea-nitrogen (MUN). Mid-stream urine samples were collected during voluntary urination of cows immediately prior to the afternoon and following morning milking of the final 24 hours of each period. These samples were then divided into 20 mL aliquots for the analysis of creatinine and 75 mL aliquots for the analysis of urinary nitrogen. The 75 mL aliquot was reduced to pH $\leq$2 using approximately 3 mL of 6 mol/L hydrochloric acid and subsequently freeze dried. Both samples were stored at -20ºC prior to analysis.

Milkfat metabolites

Milkfat was separated from the whole milk by centrifugation in a Thermo Scientific Sorvall Evolution RC super speed refrigerated centrifuge (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) (4ºC). Samples were weighed (55 g) into test tubes and spun for 30 minutes at 10,800 *g*. The milkfat and skim-milk were subsequently separated and stored frozen at -20ºC prior to analysis. Skatole and indole concentrations in the milkfat were estimated using high performance liquid chromatography (HPLC) with fluorescence detection (excitation 275 nm, emission 345 nm) and followed the procedure as per Lane *et al*. (2002).

The skim-milk fraction was used to determine the concentration of MUN, following methods described by Pacheco *et al*. (2007). The samples were analysed on a Flexor E clinical chemistry analyser, (Vital Scientific, Dieren, The Netherlands), using a

TABLE 1: The mean concentration and standard error of the mean of soluble sugars and starch (SSS), crude protein (CP), neutral detergent fibre (NDF) (hemicellulose, cellulose and lignin), acid detergent fibre (ADF) (cellulose and lignin), organic matter digestibility (OMD) and metabolisable energy (MJ/kg DM) of white clover containing either high (HS) or low (LS) concentrations of water-soluble carbohydrate offered during the day, and perennial ryegrass offered at night. DEM = Standard error of the mean.

Treatment		SSS (g/kg DM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	OMD (g/kg DM)	ME (MJ/kg DM)
Clover	HS	168	219	311	236	836	12.3
	LS	129	247	286	230	825	12.0
	SEM	1	3	1	3	2	0.1
	P value	0.03	0.09	0.04	0.33	0.15	0.22
Perennial ryegrass		171	195	464	259	801	12.0
	SEM	5	10	13	5	11	0.1

TABLE 2: Mean concentration of skatole and indole in the milkfat and yield of fat, milksolids, and milk in the afternoon and morning milk of cows grazing white clover containing either high (HS) or low (LS) concentrations of water-soluble carbohydrate during the day, and perennial ryegrass at night. SED = Standard error of difference.

Component	Time of milking	Clover treatment		SED	P value
		HS	LS		
Skatole (ng/g)	Afternoon	356	800	68	<0.001
	Morning	15	23	2	<0.001
Indole (ng/g)	Afternoon[1]	84	420	0.1	<0.001
	Morning	1.7	2.4	0.9	0.44
Fat yield (kg)	Afternoon	0.35	0.36	0.03	0.75
	Morning	0.47	0.45	0.02	0.53
Milksolids yield (kg)	Afternoon	0.63	0.61	0.03	0.62
	Morning	0.88	0.88	0.02	0.90
Milk yield (kg)	Afternoon	6.6	6.5	0.2	0.59
	Morning	10.9	10.7	0.3	0.60

[1]Log transformed for analysis. Means are back-transformed data. SED applies to transformed data.

TABLE 3: The concentrations of milk urea nitrogen (MUN), urinary nitrogen (N urine) and the urinary nitrogen:creatinine ratio (N:C ratio) in samples collected at the afternoon and morning milking of cows fed white clover containing either high (HS) or low (LS) concentrations of water-soluble carbohydrate during the day, and perennial ryegrass at night. SED = Standard error of difference.

Component	Time of milking	Clover treatment		SED	P value
		HS	LS		
MUN (mmol/L)	Afternoon	7.7	8.2	0.3	0.24
	Morning	5.3	5.6	0.3	0.33
N urine (g/100g)	Afternoon	0.40	0.46	0.05	0.28
	Morning	0.49	0.51	0.09	0.81
N:C ratio	Afternoon	0.31	0.35	0.04	0.32
	Morning	0.16	0.17	0.03	0.76

commercial diagnostic kit (Roche Diagnostic NZ Ltd., Auckland, New Zealand).

Urinary nitrogen

Urinary nitrogen was determined using the instrumental combustion method (Carlo Erba Nitrogen Analyser, Milan, Italy), following methods described by Pacheco *et al*. (2008). Creatinine concentrations in the urine were analysed by a spectrophotometric assay (Hitachi 902 automatic analyser; New Zealand Veterinary Pathology Ltd, Palmerston North, New Zealand) using a compensated Jaffe reaction (Husdan & Rapoport, 1968). The nitrogen:creatinine ratio was derived by

dividing the concentration of nitrogen in the fresh urine by the creatinine concentration, accounting for the effect of urinary volumes on nitrogen concentration.

Statistical analysis

For analysis of variance of pasture data, PROC GLM of SAS (2003) was used. The model included the effects of treatment and period, tested against the treatment × period interaction. Differences between treatments were considered significant at $P < 0.05$ while trends were considered present at $P < 0.1$. Raw data was tested for normality using the Shapiro-Wilk test with values greater than $P = 0.05$ considered normal. Data are presented as least squares means ± the standard error of the mean.

To overcome within-cow-within-period correlation, means were calculated for each cow within each period separately for concentrations in morning and afternoon milkings and for daily totals for yields, as described by Rowell and Walters (1976). As the numbers of observations making up each mean varied, these were included as weights in a residual maximum likelihood analysis to get best estimates of the means for each diet using morning data alone, afternoon data alone and daily totals. Variation around the mean is reported as the standard error of difference. Wald tests gave approximate tests of significance for the differences between the diet means (SAS, 2003).

RESULTS

Dry matter intake

Cows grazing both the HS and LS clover treatments had total daily intakes, including silage, of approximately 15.5 kg DM/cow/d with 55% of the intake being made up of white clover. Pre-grazing herbage mass was significantly higher ($P < 0.05$) in the HS treatment. Plate meter and quadrat cut measurements estimated 2,850 and 2,900 kg DM/ha, respectively, for the HS treatment, and 2,600 and 2,750 kg DM/ha, respectively, for the LS treatment. Post-grazing herbage mass was approximately 1,400 kg DM/ha for both clover treatments. Pre- and post-grazing ryegrass masses were 2,770 and 2,200 kg DM/ha, respectively, as measured by the rising-plate meter, and 2,650 and 2,100, respectively, as measured by quadrat cuts.

Nutrient composition of pasture species

The HS selection had higher concentrations of WSC ($P < 0.05$) and neutral detergent fibre (NDF) ($P < 0.05$) but lower crude protein (CP) concentrations ($P < 0.1$) (Table 1). Acid detergent fibre (ADF) concentration, metabolisable energy concentration and organic matter digestibility did not differ significantly between treatments. Compared with the clover treatments, the perennial ryegrass offered at night contained less CP, considerably higher NDF concentration, and was similar to the clovers in metabolizable energy (12.0 MJME/kg DM).

Milk production and milkfat metabolites

With the exception of 0.9 g/kg milk more protein ($P < 0.05$) in the afternoon milk, and 0.9 g/kg milk less fat ($P < 0.001$) in the morning milk of cows grazing the HS clover, milk production and composition of major nutrients were similar between treatments. Cows produced 17 to 17.5 kg milk/cow/d and approximately 1.5 kg MS/cow/d (Table 2). In the afternoon milk samples, milkfat concentrations of skatole were over two fold lower, and indole close to five fold lower for cows grazing the HS treatment compared to those grazing the LS treatment ($P < 0.001$). For the morning milkfat samples, skatole concentration was lower in the HS treatment ($P < 0.001$), however, the indole concentrations were similar for each treatment (Table 2).

Nitrogen components in milk and urine

There were no significant differences between treatments in MUN and urinary-N concentration, or the nitrogen:creatinine ratio (Table 3).

DISCUSSION

The protein in white clover is highly degradable and products of amino acid breakdown such as skatole and indole rapidly appear in the blood stream and accumulate in milk and urine (Schreurs *et al.*, 2003). This rapid appearance and then clearance when cows are switched from eating clover to grass with less degradable protein (Cosgrove *et al.*, 2006), suggested that these compounds could be sensitive metabolic indicators for evaluating the effects of changes in the WSC concentration of white clover.

The HS and LS clovers differed in both structural and non-structural carbohydrates. Water-soluble carbohydrate was approximately 40 g/kg DM higher in the HS compared to the LS treatment providing more fermentable energy in the rumen. The similar ADF concentrations between the two clover lines suggests the higher NDF concentration in the HS treatment was due to more hemicellulose, which is also an important energy source in the ruminant diet (Coen & Dehority, 1970). Associated with the higher concentration of WSC, the HS clover had approximately 28 g/kg DM less CP than the LS treatment. The lower CP concentrations coupled with the increase in structural and non-structural carbohydrates provided cows with a more-balanced protein and energy supply.

The reduction of skatole and indole on the HS compared to the LS clover in this study is consistent with our hypothesis that high WSC should improve protein utilisation. Lane *et al.* (2002) proposed that skatole and indole are primarily derived from excess

dietary protein and should show a positive association with CP concentrations in the feed. Therefore, differences between clovers may also be linked to the lower CP supply in the HS sward reducing the availability of amino acids. Interactions between carbohydrate and protein metabolism are particularly strong in the rumen and the higher energy:protein ratio in the HS treatment may have improved both the balance, and the temporal release of N and energy in the rumen, which on forage-based diets are generally out of synchrony (Miller *et al.*, 2001). If this is the case, a positive association could also be expected with MUN and urinary N, as amino acid degradation in the rumen leads to high ammonia concentrations that are subsequently metabolised to urea in the liver (Nocek & Russell, 1988; Lapierre & Lobley, 2001).

Milk urea-nitrogen, urinary-N and the nitrogen:creatinine ratio were all numerically lower in the HS treatment, consistent with the effects of this treatment on skatole and indole concentrations, although the effects were smaller and statistically not significant. The greater sensitivity of these metabolites to differences between clovers is consistent with a subsequent study (Pacheco *et al.*, 2008) that showed skatole and indole were more closely correlated with rumen degradable protein balance than was MUN. In the study reported here there was also a trend for higher apparent efficiency of dietary protein utilisation for milk production in the HS treatment (0.22 kg milk N/kg N intake) compared with the LS treatment (0.19 kg milk N/kg N intake; see Higgs (2007) for calculations). Determining dry matter intake in grazing trials is difficult and these data are estimates only, but the trend suggests more of the protein consumed on the HS diet was used for milk production. Milk protein yield was similar between treatments meaning the additional CP consumed by cows grazing the LS treatment was probably excreted in the urine. Increased energy supply on forage-based diets has reduced nitrogen components in the blood, urine and milk in other studies (Kolver *et al.*, 1998; Miller *et al.*, 2001; Lane *et al.*, 2002; Lee *et al.*, 2002; Moorby *et al.*, 2006). The comparatively short period of exposure to the contrasting clovers during the day may have been insufficient to establish differences in these less-sensitive parameters. Further assessment of treatment effects on nitrogen excretion by cows offered these treatments day and night would, therefore, be beneficial.

CONCLUSION

This study indicates that the accumulation of skatole and indole is very sensitive to energy and protein supply in the cow's diet, and provides preliminary evidence that selection for high WSC has a positive effect on protein utilisation. Further research relating the changes in metabolites reported here, to changes in N excretion or possible production responses would be valuable. The current pressure on farmers to reduce nutrient loss to waterways, and the continual drive for higher production makes nitrogen utilisation very important. This study indicates that improving protein retention through plant breeding may be possible.

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REFERENCES

Beever, D.E.; Losada, H.R.; Cammell, S.B.; Evans, R.T.; Haines, M.J. 1986: Effect of forage species and season on nutrient digestion and supply in grazing cattle. *British Journal of Nutrition* **56(1)**: 209-225.

Burke, J.L.; Waghorn, G.C.; Chaves, A.V. 2002: Improving animal performance using forage-based diets. *Proceedings of the New Zealand Society of Animal Production* **62**: 267-272.

Coen, J.A.; Dehority, B.A. 1970: Degradation and utilization of hemicellulose from intact forages by pure cultures of rumen bacteria. *Applied Microbiology* **20**: 362-368.

Cosgrove, G.P.; Burke, J.L.; Death, A.F.; Lane, G.A.; Fraser, K.; Pacheco, D.; Parsons, A.J. 2006: Clover-rich diets and production, behaviour and nutrient use by cows in late lactation. *Proceedings of the New Zealand Society of Animal Production* **66**: 42-49.

Deslandes, B.; Gariepy, C.; Houde, A. 2001: Review of microbiological and biochemical effects of skatole on animal production. *Livestock Production Science* **71(2/3)**: 193-200.

Higgs, R.J. 2007: Effect of low- or high-water-soluble carbohydrate white clover on aspects of protein degradation in dairy cows. B.Appl.Sci. (Hons.) Thesis, Massey University, Palmerston North, New Zealand. 60 pp.

Husdan, H.; Rapoport, A. 1968: Estimation of creatinine by the Jaffe reaction - A comparison of three methods. *Clinical Chemistry* **14(3)**: 222-238.

Kingston-Smith, A.H.; Theodorou, M.K. 2000: Post-ingestion metabolism of fresh forage. *New Phytologist* **148(1)**: 37-55.

Kolver, E.; Muller, L.D.; Varga, G.A.; Cassidy, T.J. 1998: Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *Journal of Dairy Science* **81(7)**: 2017-2028.

Lane, G.A.; Fraser, K.; Kolver, E.S.; Rowan, D.D.; Allen, J.M.; Mills, O.E.; Abraham, A.S.; Olney, S.D. 2002: Effect of a total mixed ration diet on the concentration of amino acid-derived volatiles in milk. *Proceedings of the New Zealand Society of Animal Production* **62**: 242-245.

Lapierre, H.; Lobley, G.E. 2001: Nitrogen recycling in the ruminant: A review. *Journal of Dairy Science* **84**: 233-236.

Lee, M.R.F.; Harris, L.J.; Moorby, J.M.; Humphreys, M.O.; Theodorou, M.K.; MacRae, J.C.; Scollan, N.D. 2002: Rumen metabolism and nitrogen flow to the small intestine in steers offered Lolium perenne containing different levels of water-soluble carbohydrate. *Animal Science* **74(3)**: 587-596.

Miller, L.A.; Moorby, J.M.; Davies, D.R.; Humphreys, M.O.; Scollan, N.D.; MacRae, J.C.; Theodorou, M.K. 2001: Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass and Forage Science* **56(4)**: 383-394.

Min, B.R.; McNabb, W.C.; Barry, T.N.; Peters, J.S. 2000: Solubilization and degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (*EC* 4.1.1.39; Rubisco) protein from white clover (*Trifolium repens*) and *Lotus corniculatus* by rumen microorganisms and the effect of condensed tannins on these processes. *Journal of Agricultural Science* **134(3)**: 305-317.

Moorby, J.M.; Evans, R.T.; Scollan, N.D.; MacRae, J.C.; Theodorou, M.K. 2006: Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. *Grass and Forage Science* **61(1)**: 52-59.

Nocek, J.E.; Russell, J.B. 1988: Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *Journal of Dairy Science* **71(8)**: 2070-2107.

Pacheco, D.; Burke, J.L.; Death, A.F.; Cosgrove, G.P. 2007: Comparison of models for estimation of urinary nitrogen excretion from dairy cows fed fresh forages. *In*: Meeting the Challenges for Pasture-based Dairying. *Proceedings of the Australasian Dairy Science Symposium, 18-20 September 2007, University of Melbourne, Victoria, Australia*. p. 417-423.

Pacheco, D.; Lane, G.A.; Fraser, K.; Macdonald, K.A.; Back, P.J. 2008: Indole and skatole as markers for nitrogen utilisation in dairy cows. *Proceedings of the New Zealand Society of Animal Production* **68**: 23-26.

Rowell, J.G.; Walters, D.E. 1976: Analysing data with repeated observations on each experimental unit. *Journal of Agricultural Science, Cambridge* **87(2)**: 423-432.

SAS. 2003: SAS User's Guide. SAS Institute Inc, Cary, North Carolina, USA.

Schreurs, N.M.; Tavendale, M.H.; Lane, G.A.; Barry, T.N.; Marotti, D.M.; McNabb, W.C. 2003: Postprandial indole and skatole formation in the rumen when feeding white clover, perennial ryegrass and Lotus corniculatus. *Proceedings of the New Zealand Society of Animal Production* **63**: 14-17.

Ulyatt, M.J. 1997: Livestock Improvement Corporation lecture. Can protein utilisation from pasture be improved? *Proceedings of the New Zealand Society of Animal Production* **57**: 4-8.

Waghorn, G.C.; Barry, T.N. 1987: Pasture as a nutrient source. *In*: Feeding livestock on pasture, A.M. Nicol. ed. New Zealand Society of Animal Production, Occasional Publication No. 10. p. 21-37.