The effect of secondary compounds of birdsfoot trefoil and red clover on dairy cow grazing preferences

C.H.E.C. POLI, J. HODGSON, G. C. ARNOLD AND G.P. COSGROVE[1]

College of Sciences, Massey University, Palmerston North, New Zealand

ABSTRACT

The responses of grazing animals to specific plant secondary compounds were examined in trials in which dairy cows grazed spaced plants of either birdsfoot trefoil *(Lotus corniculatus L.)* with high and low concentration of extractable condensed tannins (2.54 and 0.62 % in the DM respectively for high and low tannin genotypes), or red clover *(Trifolium pratense L.)* with high and low formononetin concentration (0.68 and 0.29 % in the DM respectively for high and low formononetin genotypes). Plants were established in 4 linear sequences of 26, each providing three blocks (replicates) of balanced sets of 2 plant species, 2 concentrations of test components, and plants either not trimmed or trimmed to minimise physical differences between genotypes within species. The sequences were grazed by four trained cows and selective behaviour was determined by the number of grazing bites per plant. Observations were repeated in March (P1) and April (P2). On average, animals grazed more on non-trimmed than on trimmed plants (7.6 vs 3.1 ± 0.711 bites/plant in P1, P=0.0001; and 8.4 vs 3.6 ± 0.858 bites/plant in P2, P=0.0002), but did not show clear discrimination between red clover and lotus (6.3 vs 4.4 ± 0.711 bites/plant in P1, P=0.053; and 6.3 vs 5.7 ± 0.858 bites/plant in P2, P=0.620). However, though the distribution of bites between red clover plants with high and low formononetin was similar (7.2 vs 5.5 ± 0.76 bites/plant in P1, P= 0.130; and 7.3 vs 5.3 ± 1.08 bites/plant in P2, P= 0.191), they showed strong preference for low condensed tannin concentration in lotus (2.1 vs 6.6 ± 1.11 bites/plant in P1, P= 0.0068; and 3.9 vs 11.1 ± 1.35 bites/plant in P2, P= 0.069).

Keywords: selective grazing; red clover *(Trifolium pratense)*; birdsfoot trefoil *(Lotus corniculatus)*; extractable condensed tannin; formononetin.

INTRODUCTION

Condensed tannin and formononetin are important secondary compounds in legumes. Condensed tannin (CT) may protect dietary protein from degradation in the rumen (Barry *et al*.,1986; Barry and Blaney,1987), but at high concentration can also inhibit forage intake (Barry, 1989; Waghorn *et al*., 1990). Formononetin may promote severe abnormalities in animal fertility (Marshall, 1973). However the influence of formononetin in red clover *(Trifolium pratense L.)* and CT in birdsfoot trefoil *(Lotus corniculatus L.)* on diet selection has not been defined. The experiment described here investigated the specific effects of these two secondary compounds on diet selection by cattle following a preliminary field study (Poli *et al*., 1997) of the preferential grazing of areas of birdsfoot trefoil and red clover.

MATERIALS AND METHODS

Design

The experiment was carried out at the Dairy Cattle Research Unit, Massey University, Palmerston North. Dairy cows grazed sequences of spaced plants of two genotypes of birdsfoot trefoil *(Lotus corniculatus L.)*, providing low (Goldie) and high (accession PI 273938) concentration of condensed tannin (CT) and two of red clover *(Trifolium pratense L.)* providing low (G27) and high (Pawera) concentration of formononetin. Within each sequence, plants were either left untrimmed or were trimmed similar to the size of the alternative genotype in the same species (±trimming). Plants were spaced at one meter intervals in sequences of 26, each providing three blocks (replicates) of the eight treatments (2 species x 2 cultivars x ± trimming). There were four sequences balanced so that each treatment was followed by every other treatment at least once, and all were present with equal frequency in each sequence. The first and the last plant in each sequence were not considered in the assessment. The plants were originally sown in a glasshouse and transplanted to the field on 30th October 1996.

Four mature lactating Friesian cows were used, each required to graze one sequence of 26 plants at a time within a raceway 2 m wide formed by temporary electric fencing. Within periods, each cow was randomly allocated to a single sequence. The experiment was repeated on the same plant sequences in two periods, 19th March and 24th April 1997.

Plant measurements

Measurements of plant height, diameter, and leafiness were made before and after grazing. The highest point of each plant was measured with a sward stick in half centimetre increments. Two diameters (perpendicular and parallel to the sequence) of each plant were measured with a ruler in centimetre units. The percentage of leaf per plant material was visually estimated (Real, 1997). Before each grazing, the flowers were removed by hand from all plants.

Chemical analysis

[1]AgResearch Grasslands, PB 11008, Palmerston North, New Zealand

Two intact stems were harvested from each plant before grazing and bulked across blocks within each sequence according to cultivar and trimming. Red clover samples were analysed for formononetin (fluorimetric assay described by Gosden and Jones,1978 and modified by Anwar,1994), and birdsfoot trefoil samples were analysed for extractable condensed tannin (ECT) content using a modification of DMACA-HCl protocol described by Li *et al.*, 1996. Negligible concentrations of formononetin and ECT were found in samples of birdsfoot trefoil and red clover, respectively.

Grazing behaviour

During grazing the behaviour of each cow was assessed from manual and video camera records in terms of the number of bites taken from each plant (Griffiths *et al.*, 1996).

Statistical analysis

Analyses of variance were carried out to compare the treatment factors, species, secondary compounds and trimming, and their interactions. Analyses were made within periods, and also between periods to check treatment interactions with period. Plant chemical composition was analysed using sequences as replicates. The data were analysed using statistical package SAS (SAS, 1990).

RESULTS

Plant characteristics

Birdsfoot trefoil (BT) plants had greater height and greater area than red clover (RC), but RC had a higher percentage of leaves (Table 1). Untrimmed plants were bigger and with higher percentage of leaf than trimmed plants of the same genotype. Plants were generally larger in Period 1 than Period 2.

There was a significant interaction in secondary compound concentration between period and genotype in absolute but not in proportional terms: Pawera had on average 2.3 times more formononetin than G-27, and PI273938 had on average 4.2 times more tannin than Goldie (Table 2). There was no significant difference between trimmed and untrimmed BT plants in ECT content but there was a significant interaction between trimming and genotype effects in formononetin content in RC, Pawera having higher concentration of formononetin (0.75 vs 0.62 ± 0.0203 %DM) when trimmed.

TABLE 2: Genotype contrasts in extractable condensed tannin (ECT) (in BT) and formononetin (in RC) concentration (%DM), in the Periods 1 and 2.

	Period 1		*Period 2*			
	low	high	low	high	P-value*	SEM*
ECT	0.72	3.28	0.47	1.81	0.0007	0.151
Formononetin	0.30	0.72	0.30	0.65	0.0460	0.020

*P-value and SEM refers to the interaction: secondary compound concentration x period effect.

Selective behaviour

The overall effects of period and species on selective behaviour were not significant, though the number of bites per plant was marginally greater for RC than BT in period 1 (6.3 vs 4.4 ± 0.711 bites/plant, P=0.0526). In BT there was a significant difference in the number of bites between trimmed and untrimmed plants, and between high and low ECT concentration, though in Period 2 the significance was marginal (Table 3). In RC trimming had a major influence on bite number, but formononetin concentration had no effect. There was no significant interaction between secondary compounds and trimming.

TABLE 3: Average of number of bites in birdsfoot trefoil (BT) and red clover (RC), in relation to secondary compound concentration (Sec.Comp.Conc.) and trimming (trim. and ntrim.) characteristics in Period 1 and Period 2.

		Sec. Comp. Conc.			*Trimming*			SEM
	species	low	high	P-value	trim.	ntrim.	P-value	
Period 1	BT	6.6	2.1	0.0068	1.9	6.8	0.0035	1.113
	RC	5.5	7.2	0.1305	4.3	8.3	0.0007	0.760
Period 2	BT	7.5	3.9	0.0693	3.0	8.4	0.0086	1.350
	RC	5.3	7.3	0.1912	4.2	8.4	0.0096	1.082

TABLE 1: Characteristics of trimmed (trim) and untrimmed (ntrim) plants of birdsfoot trefoil Goldie (Low Tannin) and accession PI273938 (High Tannin), and red clover cultivars G27 (Low Form) and Pawera (High Form) in Periods 1 and 2.

	Birdsfoot trefoil(BT)				*Red clover(RC)*				*SEM*
	Low Tannin		High Tannin		Low Form		High Form		
	ntrim	trim	ntrim	trim	ntrim	trim	ntrim	trim	
Height (cm)									
period1	18.9	15.1	13.7	13.7	15.6	12.9	15.0	13.5	0.90
period2	14.1	11.0	15.5	11.0	12.7	10.2	11.2	10.2	0.76
Plant area (cm2)									
period1	7050	4090	5740	3990	2280	1540	2880	1580	483
period2	4030	2250	4380	2220	1830	1230	2450	1230	334
Leafiness (%)									
period1	50	42	40	37	75	72	75	74	1.7
period2	47	43	42	40	75	69	73	69	1.7

DISCUSSION

Trimming had an important influence on distribution of grazing activities in both birdsfoot trefoil and red clover. Trimmed plants were smaller and with a lower percentage of leaves (Table1). As in several other studies (O'Reagain and Mentis, 1989, O'Reagain, 1993), plants with high proportion of stem were avoided. The modification of behaviour according to trimming shows that structural differences between plants need to be considered in studies of grazing preference.

Condensed tannin (CT) had a substantially greater effect on diet selection than formononetin. The concentration of ECT in genotype Goldie (Table 2) agrees with previous findings (Terrill *et al*., 1992). The reduced significance of the CT effect in Period 2 (Table 3) is apparently a consequence of the lower concentration of CT in this period (Table2). Condensed tannins have been thought to decrease forage intake by inhibition of digestion (Barry, 1989). However, this result indicates that they also have an immediate effect on selection (Provenza and Malechek, 1984)

Cows were apparently not directly influenced by the formononetin concentration in red clover, which were similar to levels reported in other studies (Kelly *et al*., 1979, Anwar, 1994). Francis (1973) postulated that unhydrolyzed glycosides in subterranean clover could contribute to unpalatability, but Harborne (1993) argued that isoflavones are not sufficiently repellent in taste to deter feeding.

CONCLUSIONS

Selective grazing behaviour was significantly affected by both morphological and biochemical plant characteristics.

Discrimination response to extractable condensed tannin in birdsfoot trefoil was sensitive to concentration in the plant within the range 0.5-3.3%, and may therefore be expected to change with seasonal changes in ECT concentration.

In red clover, morphological characteristics were more important than formononetin concentration in influencing selection.

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