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**The feeding value for dairy cows and the
agronomic performance of white clover
(*Trifolium repens* L.) selected for increased
floral condensed tannin**

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of the requirements for the degree of

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Abstract



ABSTRACT

Legumes containing 20 to 40 g of condensed tannin (CT) per kg of dry matter (DM) can improve dairy cow milk production by reducing ruminal protein degradation to ammonia and preventing bloat. White clover (*Trifolium repens* L.) produces CT in its flower heads. High tannin (HT) white clover, bred for increased flowering and increased floral CT concentration, was evaluated under dairy grazing in Hamilton, New Zealand. Its performance in monoculture was compared to that of Grasslands Huia white clover over two years, and five short-term grazing experiments determined its effects on Friesian dairy cows.

Huia and HT had similar floral CT concentrations, ranging from 15 to 77 g/kg DM over two flowering seasons. HT clover had higher flower densities than Huia until the second summer after sowing, resulting in higher clover (leaf plus flower) CT concentrations. Clover CT peaked at 12.1 g/kg DM for HT and 5.7 g/kg DM for Huia. HT swards had lower stolon growing point densities than Huia swards and annual DM yields averaged 10.0 and 11.0 t DM/ha for the respective clovers. The ingress of non-sown white clover genotypes reduced treatment differences in the last 10 months of the experiment.

Mild bloat occurred in cows grazing both clovers. Cows grazing HT white clover had rumen ammonia concentrations 5 to 26% lower than that of cows grazing Huia, indicating less proteolysis in the rumen of HT cows, but there were no consistent effects on rumen soluble protein or volatile fatty acids (VFA). Differences between treatments in dietary CT concentrations were too small to affect milk production or composition.

Minced mixtures of 0, 25, 50, 75 or 100% of DM as white clover flower with the remainder as white clover leaf, were incubated *in vitro* and rumen metabolite concentrations determined at 0, 2, 4, 8, 12 and 24 hours. Polyethylene glycol was added to one of the 50% flower treatments to inactivate CT. Clover flowers had less soluble protein than leaves at 0 hours, and increasing the percentage of flowers from 0 to 100%

reduced the net conversion of plant-N to ammonia-N from 29 to 12%. The contribution of CT to these effects was small. Increasing percentages of clover flowers did not significantly affect total VFA production but increased acetate to propionate (A:P) ratios. White clover CT decreased A:P ratios. In another *in vitro* experiment perennial ryegrass leaf (*Lolium perenne* L.) was incubated either alone or with white clover flowers or birdsfoot trefoil (*Lotus corniculatus* L.). Clover flowers were more effective at reducing proteolysis than birdsfoot trefoil, due largely to less release of soluble protein, but birdsfoot trefoil treatments had the lowest A:P ratios.

In conclusion, HT clover had higher forage CT concentrations than Huia because of increased flowering. Increased flowering reduced the agronomic performance of HT and lowered rumen ammonia concentrations, but did not increase milk production or prevent bloat. White clover flowers reduced rumen proteolysis *in vitro*, but this was mainly a result of their low protein concentration. White clover CT and birdsfoot trefoil forage benefited the molar percentages of VFA, but increasing the proportion of clover flowers did not. Further increases in white clover CT concentrations may benefit ruminant performance, but this should not be implemented through increased flowering.

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**List of abbreviations
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LIST OF ABBREVIATIONS

A	acetate
ADF	acid detergent fibre
a.i.	active ingredient
A+B:P	(acetate plus butyrate) to propionate ratio
A:P	acetate to propionate ratio
Apr 02	April 2002 animal experimental period
Apr 03	April 2003 animal experimental period
B	butyrate
BT	birdsfoot trefoil
°C	degrees celcius
C₃₁	hentriacontane alkane
C₃₂	dotriacontane alkane
C₃₃	tritiacontane alkane
Ca	calcium
CHO	soluble carbohydrate
cm	centimetre (10 ⁻² m)
CO₂	carbon dioxide
CP	crude protein
CT	condensed tannin
Dec 01	December 2001 animal experimental period
Dec 02	December 2002 animal experimental period
dL	decilitre (10 ⁻¹ L)
DM	dry matter
F	white clover flower
Feb 02	February 2002 animal experimental period
g	gram
x g	gravitational field
H	hydrogen
h	hour

ha	hectare (10 000 m ²)
HCl	hydrochloric acid
H₂O	water
HT	high floral tannin white clover
Huia	Grasslands Huia white clover
K	potassium
kg	kilogram (10 ³ g)
kJ	kilojoule
L	litre
LSD	least significant difference
m	metre
ME	metabolisable energy (MJ/kg DM)
Mg	magnesium
mg	milligram (10 ⁻³ g)
MJ	megajoule
mL	millilitre (10 ⁻³ L)
mm	millimetre (10 ⁻³ m)
mM	millimole (10 ⁻³ M)
MUN	milk urea nitrogen
N	nitrogen
NAN	non-ammonia nitrogen
NDF	neutral detergent fibre
NH₃	ammonia
NIRS	near infrared spectroscopy
OH	hydroxide
OMD	organic matter digestibility
P	probability statistic
P	phosphorus (when used in Section 3.3.2)
P	propionate (when reporting on volatile fatty acids)
PEG	polyethylene glycol
R	perennial ryegrass leaf
rpm	revolutions per minute
S	sulphur
SED	standard error of the difference

t	tonne
μL	microlitre (10^{-6} L)
μm	micrometre (10^{-6} m)
VFA	volatile fatty acid
VFI	voluntary feed intake

LIST OF PLANT SPECIES CITED

<u>Common name</u>	<u>Scientific name</u>
Annual poa	<i>Poa annua</i> L.
Birdsfoot trefoil	<i>Lotus corniculatus</i> L.
Broad-leaved dock	<i>Rumex obtusifolius</i> L.
Carob	<i>Ceratonia siliqua</i> L.
Chickweed	<i>Stellaria media</i> L.
Chicory	<i>Cichorium intybus</i> L.
Cicer milkvetch	<i>Astragalus cicer</i> L.
Dandelion	<i>Taraxacum officinale</i> Weber.
Erect dorycnium	<i>Dorycnium rectum</i> L.
Hybrid ryegrass	<i>Lolium x hybridum</i>
Lentil	<i>Lens culinaris</i> Medik.
Lotus major	<i>Lotus pedunculatus</i> Cav.
Lucerne, alfalfa	<i>Medicago sativa</i> L.
Maize, corn	<i>Zea mays</i> L.
Mimosa	<i>Acacia spp</i>
Mulga	<i>Acacia aneura</i> Benth.
Perennial lupin, lupin	<i>Lupinus polyphyllus</i> L.
Perennial ryegrass, ryegrass	<i>Lolium perenne</i> L.
Quebracho	<i>Aspidosperma quebracho</i> Schlecht.
Red clover	<i>Trifolium pratense</i> L.
Redroot	<i>Amaranthus retroflexus</i> L.
Sainfoin	<i>Onobrychis viciifolia</i> Scop.
Sheep's burnet	<i>Sanguisorba minor</i> Scop. ssp. <i>Muricata</i>
Sow thistle	<i>Sonchus olearaceous</i> L.
Sulla	<i>Hedysarum coronarium</i> L.
Summergrass	<i>Digitaria sanguinalis</i> L.
Tamarind	<i>Tamarindus indica</i> L.
Tobacco	<i>Nicotiana tabacum</i> L.
White clover, clover	<i>Trifolium repens</i> L.
Yorkshire fog	<i>Holcus lanatus</i> L.