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**Effect of condensed tannin and fresh forage diets
on the formation of indole and skatole in the
rumen and on the pastoral odour and flavour of
sheep meat**

A thesis presented in partial fulfilment of the requirements for the degree of

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in
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at Massey University
Palmerston North
New Zealand

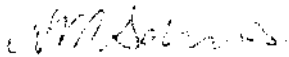
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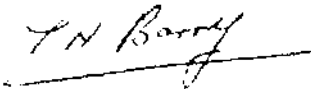
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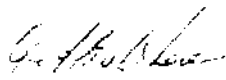
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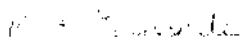
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This thesis is dedicated to Thomas

For my clever Thomas...
Who knew when to be silent
and then what to say
Who knew to provide comfort
and when it was best to stay away

"Dear sir or madam, will you read my book?
It took me years to write, will you take a look?"

Paperback writer
The Beatles

ABSTRACT

Flavour is a factor that has a large influence on meat quality. Pastoral flavour that results from the grazing of pasture is an undesirable characteristic of meat flavour for consumers more accustomed to meat produced by grain and concentrate feeding systems. In New Zealand there is a reliance on grazing systems for sheep meat production, however the resulting meat flavour is one factor that impedes the increase of sheep meat exports to discerning markets.

Correlation of chemical analyses to sensory evaluations of sheep meat has identified that a high concentration of indole and skatole in the fat is associated with pastoral flavours. Indole and skatole are formed in the rumen from the microbial fermentation of tryptophan. New Zealand pasture is high in protein, which is both highly soluble and rapidly degraded in the rumen. As such, pasture diets provide a rich and ready source of tryptophan for the formation of indole and skatole in the rumen. Condensed tannins are known to slow the degradation of protein in the rumen. Therefore, one of the objectives of this study was to establish if dietary condensed tannin can reduce the ruminal biogenesis of indole and skatole and consequently, ameliorate pastoral flavour in sheep meat.

White clover usually comprises up to 30% of the botanical composition of pastures in New Zealand, is highly degradable in the rumen and likely to result in a high availability of tryptophan in the rumen for conversion to indole and skatole. Therefore, another objective of this study was to determine if feeding white clover gave a significant increase in the formation of indole and skatole in the rumen compared to

perennial ryegrass and if this has an effect on pastoral flavour in meat.

These hypotheses were tested using a series of *in vitro* rumen fermentations that incorporated the use of fresh forages (Chapter 3 and 6). *In vivo* experiments were utilised to assess rumen formation of indole and skatole with different forages (Chapter 4) and to assess effects of dietary condensed tannin (CT; Chapter 5 and 7). Meat and fat samples from lambs used in Chapters 5 and 7 underwent sensory evaluation to determine if forage or CT treatments were having an effect on the fat odour or meat flavour.

From the *in vitro* and *in vivo* experiments of (Chapter 3, 4, 6 and 7) it was calculated that the formation of indole and skatole with perennial ryegrass is generally only 6-41% of that formed with white clover. A higher concentration of indole and skatole was also observed in the blood plasma of lambs that were fed white clover compared to those that were fed perennial ryegrass (Chapter 7) and white clover gave an overall more intense flavour in the meat.

Comparison of forages fermented *in vitro* (Chapter 3) showed that with forage legumes of a higher CT concentration, such as *Lotus pedunculatus* (98 gCT kg⁻¹ DM) and *Dorycnium rectum* (122 gCT kg⁻¹ DM), the indole and skatole formed was only 7-21% of that formed with white clover. With forages of an intermediate CT concentration such as sulla (*Hedysarum coronarium*) and *Lotus corniculatus* the indole and skatole concentration formed was 53-68% of that of white clover. From *in vitro* rumen fermentation of mixtures of white clover and *Lotus pedunculatus* it was concluded that

the CT in *Lotus pedunculatus* was not reacting with the protein in white clover. Fermentation of fresh white clover in the presence of an increasing concentration of added CT extract showed that at a higher CT, indole and skatole formation were reduced to low levels. It was inferred that this was due to optimal protein binding and the availability of free condensed tannin to bind other sources of protein, including rumen microbes. However, *in vivo* dosing with a CT extract resulted in only a small reduction in rumen indole and skatole concentration. This indicated that when CT was dosed into the *in vivo* rumen of lambs fed fresh forages the CT probably passed from the rumen before adequate protein release from the forage had taken place. Thus, in the grazing situation it will be optimal to provide CT *in planta* to maximise protein binding and this, in combination with the high CT concentration needed (approximately 80 gCT kg⁻¹ DM), makes *Lotus pedunculatus* or *Dorycnium rectum* the prime candidates for further grazing trials into pastoral flavour amelioration using CT forages.

Grazing *Lotus corniculatus* (40 gCT kg⁻¹ DM) in a field experiment resulted in a lower rumen and blood plasma and fat concentration of indole and skatole in comparison to the grazing of perennial ryegrass/white clover pasture. However, a change in the pastoral odour of the fat was not perceived by the sensory panel when comparing fat samples from lambs that had grazed *Lotus corniculatus* and perennial ryegrass/white clover pasture. The concentration of skatole in the body fat was less variable in the lambs that had grazed *Lotus corniculatus* and resulted in no lambs with a high outlying concentration (>100 ng g⁻¹) of indole and skatole. This finding holds some potential for reducing pastoral flavour for consumers sensitive to high indole and skatole concentration in the fat.

When condensed tannin was dosed to lambs that were fed white clover or perennial ryegrass in the form of a grape seed extract the intermittent supply of CT slightly reduced indole and skatole formation in the rumen and reduced the plasma concentration of indole and skatole. Flavour assessment of meat from the lambs fed white clover or perennial ryegrass with or without CT suggested that CT reduced the intensity of pastoral flavours. However, there were minimal effects on indole and skatole concentration in the body fat. It was possible that other pastoral flavour related compounds derived from the degradation of amino acids, in addition to indole and skatole that were measured, were having an effect on the meat flavour.

It was concluded that dietary condensed tannin is able to reduce the formation of indole and skatole in the rumen and can alter the sensory attributes of sheep meat including reducing pastoral flavours. A higher CT concentration present within the forage plant (approximately 80 g kg⁻¹ DM) will be best to minimise indole and skatole formation in the rumen and reduce pastoral flavours in the meat. Further research is required to confirm this in the grazing situation. Feeding white clover results in a greater rumen biogenesis of indole and skatole compared to perennial ryegrass and therefore, may be the primary contributor to pastoral flavours when ruminants graze conventional pastures. Further research is required to evaluate the flavour attributes that result from feeding white clover to meat producing ruminants in the New Zealand grazing situation.

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ABBREVIATIONS

2MI	2-methylindole
ABPE	acute bovine pulmonary emphysema
ATP	adenosine triphosphate
BCVFA	branched-chain volatile fatty acids
BD	Broadleaf dock
BS	equilibrium buffer solution
°C	degrees Celsius
C	carbon
CHO	carbohydrate
cm	centimetre
CO₂	carbon dioxide
CP	crude protein
CPI	crude protein intake
CT	Condensed tannin
cv.	cultivar
d₃	deuterated
DM	dry matter
DMACA-HCl	4-dimethylaminocinnamaldehyde-hydrochloric acid
DMI	dry matter intake
DR	<i>Dorycnium rectum</i>
EU	European Union
FAD	flavin adenine dinucleotide
Fe	iron
FIA	flow injection analysis
FMN	flavin mononucleotide
g	gram
g	centrifugal force (multiples of earth's gravitational force)
GC/MS	gas chromatography/mass spectrometry
GCO	gas chromatography-olfactory
GSE	grape seed extract (a source of condensed tannin)
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
ID	internal diameter
iu	international units
kg	kilogram
L	litre
LC	<i>Lotus corniculatus</i>
LP	<i>Lotus pedunculatus</i>
M	molar
m²	metre squared
ME	metabolisable energy
MeOH	methanol
mg	milligrams
MJ	megajoules
mL	millilitre
mm	millimetre

mmol	millimole
MW	molecular weight
N	nitrogen
<i>n</i>	number (replicates)
NADPH	nicotinamide adenine dinucleotide phosphate
ND	not determined
NDF	neutral detergent fibre
ng	nanogram
NIRS	near infra-red reflectance spectrometry
nm	nanometre
NZ	New Zealand
OMD	organic matter digestibility
PC	procyanidin
PD	prodelphinidin
PEG	polyethylene glycol
pH	power of hydrogen
ppb	parts per billion
ppm	parts per million
PRG	Perennial ryegrass
®	registered
SL	Sulla
SD	standard deviation
SDE	simultaneous distillation extraction
SEM	standard error of the mean
sp.	species (singular)
SPE	solid phase extraction
spp.	species (plural)
SSS	soluble sugars and starch
USA	United States of America
V	volts
v/v	volume by volume
VFA	volatile fatty acids
W	watt
w/w	weight by weight
WC	White clover
µg	microgram
µL	microlitre
µm	micrometre (micron)