Comparison of white clover, perennial ryegrass and the high tannin containing forage *Lotus pedunculatus* as finishing diets: Effect on sheepmeat quality

M.M. FAROUK, M. TAVENDALE, G. LANE, D. PULFORD and J. WALLER

AgResearch Ruakura Research Centre, Hamilton, New Zealand.

ABSTRACT

Rams were finished for 22 days on *Lotus pedunculatus* (LP), perennial ryegrass (*Lolium perenne*) (RG), or white clover (*Trifolium repens*) (WC). WC sheep had higher carcass weight ($P < 0.001$), muscle fat ($P < 0.01$) and back fat ($P < 0.03$) depths compared to those finished on LP or RG. Muscle glycogen at slaughter was lower ($P < 0.01$) in RG compared to LP and WC. Loin p-Cresol was higher ($P < 0.001$) in LP animals compared to WC or RG. Meat from RG tended to have a more stable retail colour display life compared to LP or WC ($P < 0.01$). Meat from LP had higher loss of weight during cooking ($P < 0.05$) compared to WC. The animal diet did not affect the flavour of their meat. There was a strong negative correlation between loin p-Cresol and sheepy odour ($r = -0.7$; $P < 0.05$). Within the parameters of this study, finishing lambs on a high tannin containing forage diet did not improve the quality attributes of their meat. The digestibility of the forage diet seems to have exerted a stronger effect on meat quality.

Keywords: Sheep; pasture; tannins; meat quality; flavour.

INTRODUCTION

Meat exporters want to differentiate their products from those of competitors by naturally changing meat quality attributes, particularly flavour and colour stability. One way this might be achieved is through altering rumen fermentation of forage protein through feeding a high tannin diet for a period before slaughter. *Lotus corniculatus,* a tannin-containing forage, reduced the rumen formation of indole and skatole in sheep relative to white clover (*Trifolium repens*) (WC) or ryegrass (*Lolium perenne*) (RG) with marginal effects on the meat odour and flavour (Schreurs *et al.,* 2007a). It was postulated that feeding sheep *Lotus pedunculatus* (LP) would produce a more dramatic effect due to its higher level of condensed tannin relative to *Lotus corniculatus* (Terrill *et al*., 1992; Meagher *et al*., 2004). This study determined the effect on meat quality of finishing sheep on LP, a high tannin containing diet relative to the common New Zealand sheep finishing pastures of WC and RG.

MATERIALS AND METHODS

Animals and pasture treatments

Thirty nine, 6-month old Romney x Suffolk rams (initial weight range 38-44.5 kg) were divided into three treatment groups (13 per group) and grazed on a monoculture of RG, LP or WC for 22 days during March 2006 at AgResearch, Aorangi farm, Manawatu. Sheep were allocated a fresh break of pasture every two days to provide a daily allowance of 2.4 kg DM/sheep/d. Samples of each forage were collected twice weekly during the course of the study. The samples were hand plucked to represent the herbage being eaten from the break of grass about to be allocated to each treatment group. At the end of the grazing period, sheep were weighed using electronic scales (Trutest, Auckland New Zealand) and transported to AgResearch, Ruakura Abattoir and processed according to established practices. Carcass weights were recorded off the abattoir scales. A proportion of the loin muscle from one side of the carcass was sub-sampled approximately 10 minutes post-slaughter, frozen in liquid nitrogen and stored at -75°C for glycogen analysis. The remaining lamb carcasses were held for 24 hours at 10°C until they were in rigor. Loin muscles were then removed. From one loin, subcutaneous fat was removed from the lean and stored at -20°C for chemical analysis and the lean sampled for meat quality, vacuumed packaged and stored at -1.5°C. Samples were analysed for pH, colour, expressible water, cook loss and tenderness (shear force) after 48 hours, 3 and 9 weeks storage. The remaining intact loin (subcutaneous fat and lean) were stored at -1.5°C prior to sensory analysis.

Chemical analyses

Samples of forage were dried at 60°C and ground for analysis using near-infrared reflectance spectroscopy (NIRS) (Feedtech, AgResearch). The tannin content of LP was analysed by the HCl-butanol assay (Terrill *et al.*, 1992). Thawed fat was sub-sampled (5-10 g) and analysed for indole, skatole and p-Cresol by simultaneous distillation

extraction and gas chromatograph mass spectrometry as described by Lane & Fraser, (1999). Glycogen content was determined using the iodine method (Krisman, 1962).

Meat quality measurements

The pH of samples was measured at each time point by inserting a calibrated pH probe (Mettler Toledo MP 125 pH meter with an Inlab 427 probe) directly into the meat. Duplicate readings were taken for analysis of each sample.

Meat colour measurements were taken on one steak per treatment at each time point. Steaks were tray over-wrapped with an oxygen permeable plastic film and allowed to bloom for 2 hours of aerobic display at 4°C before colour was measured. The same steak was measured daily for 14 days using a HunterLab MiniScan spectrophotometer (MiniScan 45/0 XE, Hunter Associates Laboratory Inc., Virginia, USA). CIE L* (lightness), a* (redness) and b* (yellowness) values were measured (D65, 10°) through the package film at two random locations on each steak, averaged and hue angle (arctan b*/a*) calculated.

To determine percent expressible water, 0.5 g sample from each treatment was held under a pressure (60 kg). The area of expressed fluid was measured using imaging software (Image Pro Plus, Version 4.0, Media Cybernetics, Maryland, USA). The amount of expressed water is inversely proportional to the meat's water holding capacity.

The weight of the meat was recorded before and after cooking. After cooking the samples were blotted dry and re-weighed. Cook loss was calculated as amount of weight lost expressed as a percentage of the original sample weight.

At each time point, individual samples were cooked in bags submerged in boiling water until the internal temperature of the sample reached 75°C. A digital thermometer (KM 20 Foodcheck, Kane-May Ltd, Swallowfield, Herts, UK) was used to measure the temperature at the centre of the sample during cooking. When the temperature was reached, the samples were immediately cooled on ice. Ten 1 cm x 1 cm cross-section slices (bites) were prepared from the cooked sample with the muscle fibres running longitudinally along the slice. Each sample was then sheared with the long axis of the fibres running perpendicular to the blade, using a MIRINZ tenderometer. The results were expressed as shear force (kgF).

The odour and flavour of lamb samples were assessed by a trained panel. Due to unforeseen circumstances measurements were obtained for a subset of samples only (6 LP, 5 WC, 8 RG). Samples were cooked, and the sensory panel assessed the odour and flavour of the cooked lamb as described by Schreurs *et al*. (2007b).

Statistical Analysis

The design was a completely randomised design. The data were analysed using GenStat (2005).

RESULTS AND DISCUSSION

Sheep finished on white clover (WC) had significantly higher live and carcass weights and fat depths compared to those finished on LP and RG (Table 1). The higher soluble sugar and crude protein and lower fibre contents of WC compared to RG (Table 2) is the reason for the higher weight and fat depths of WC relative to RG. The difference observed in the live and carcass weights and fat depth of sheep finished on WC and LP is attributable to the effects of the condensed tannins in LP rather than differences in energy and protein content of the forages. The inhibitory effects of the condensed tannins of LP on protein degradation in the rumen of sheep have been shown to be offset by inhibition of essential amino acid absorption in the small intestine (Waghorn *et al.,* 1994). The difference in forage composition and associated digestibility for WC and LP compared to RG (Table 2) resulted in higher glycogen content at kill in the meats of animals finished on these two diets compared to RG ($P < 0.01$).

Table 1: Effect of finishing diet on live sheep, their carcasses and meat attributes. Weight gains and carcass weights were adjusted for live weights. LP = Lotus pedunculatus; RG = Rye grass; WC = white clover; LSD = least significant difference at $P < 0.05$ for comparing means within a column; *, **, *** indicates statistical significance at $P < 0.05$, 0.01 & 0.001 respectively.

Finishing diets	Live weight gain (kg)	Carcass weight (kg)	Muscle+fat depth (mm)	Back-fat depth (mm)	Glycogen at kill (mg/g)
LP	4.8	18.2	11.8	5.4	8.7
RG	4.8	17.3	9.8	5.0	6.2
WC	6.3	19.8	14.4	6.9	8.9
LSD	1.7	1.0	2.8	1.5	1.7
Treatment effect	*	***	**	*	**

Table 2: The mean concentrations (g/100g DM) of crude protein (CP), soluble sugars and starch (SSS), acid (ADF) and neutral (NDF) detergent fibre lipid, ash and condensed tannin (CT) of white clover (WC), perennial ryegrass (RG) and Lotus pedunculatus (LP) grazed by lambs. LSD = least significant difference at $P < 0.05$ for comparing means within a column; ND = not determined; **, *** indicates statistical significance at $P < 0.01$ & 0.001 respectively; NS = not statistically significant.

Finishing diets	CP	SSS	ADF	NDF	Lipid	Ash	CT
Lotus	21.2	12.8	32.5	54.1	1.9	9.6	8.3[†]
Ryegrass	16.8	6.5	30.9	62.2	4.2	10.3	ND
WhiteClover	22.5	10.7	25.8	45.8	3.5	11.0	ND
LSD	2.5	2.3	2.6	4.3	0.4	0.8	
Treatment effect	***	***	***	***	***	**	

[†]SD = 1.0, ND not determined.

Table 3: Effect of the finishing diet on meat quality attributes. LP = Lotus pedunculatus; RG = Rye grass; WC = white clover; EW = Expressible water; CL = Cook loss; SF = Shear force; *, **, *** indicates statistical significance at $P < 0.05$, 0.01 & 0.001 respectively; NS = not statistically significant.

Finishing diets	Storage time (weeks)	pH	Hue angle (°) Display time (days) 1	7	8	EW (%)	CL (%)	SF (KgF)
LP	0	5.52	40.9	46.3	47.0	10.4	33.4	8.0
	3	5.61	42.2	49.3	50.5	10.8	31.2	4.4
	9	5.41	44.4	55.7	56.3	7.5	30.4	3.7
RG	0	5.55	41.3	43.7	44.5	9.5	31.9	7.4
	3	5.66	40.0	47.3	50.5	9.7	30.9	4.2
	9	5.46	42.6	47.6	48.0	5.6	30.3	3.0
WC	0	5.46	40.5	47.9	50.0	9.6	30.8	4.9
	3	5.59	41.9	55.7	58.5	10.1	28.4	3.7
	9	5.42	45.3	55.0	55.3	6.7	29.8	2.5
Treatment effect		NS	NS	***	***	NS	*	NS
Storage effect		***	***	***	***	***	**	***

The difference in the glycogen contents of the muscles at kill (Table 1) did not affect ($P > 0.05$) their pH or water holding capacity (Table 3), indicating that there was enough glycogen in the meats of all the animals to lower the pH of their meats to a normal post-rigor level. Finishing diets had no effect on the brownness (hue angle) of freshly fabricated meats (day 1) on simulated retail display ($P > 0.05$). However, by the 7th and 8th day of simulated retail display meat from sheep finished on RG were less brown (lower hue angle) compared to meat from sheep finished on to LP and WC (Table 3). The reason for the lower hue angle of meat from RG sheep could be because of the lower glycogen content of the meats from RG sheep compared to the others. Rigor temperature is positively correlated to hue angle in stored meats (Farouk & Swan, 1998) and higher glycogen content of meat is associated with poorer meat colour with reduced redness and increased yellowness (Immonen *et al*., 2000). In the present study, glycogen at kill had a strong positive correlation with hue angle at various storage times and display periods ($r = + 0.7 - 0.8$; $P < 0.05$) indicating that meat was browner with higher glycogen content of meat at kill.

Cooked meat from sheep finished on the three diets did not differ significantly in tenderness (Table 3). Cooked meat from LP had higher cook losses ($P < 0.05$) compared to RG and WC meat (Table 3). This may relate to differences in muscle and fat distribution. Vacuumed chilled sheepmeat quality attributes were more significantly affected by storage time compared to finishing diets (Table 3). Meat became browner ($P < 0.001$), lost less water under pressure ($P < 0.001$) and on cooking ($P < 0.01$ and became more tender over the 9 weeks storage time. The higher waterholding capacity with storage could be because of the breakdown of muscle proteins with ageing and the resulting changes in the muscle structure (Huff-Lonergan & Lonergan, 2005). The effect was unlikely to due to biochemical changes as the pH of the samples decreased ($P < 0.001$) with storage time. With the decrease in pH with storage, the waterholding capacity of the meat should have decreased too had biochemical rather than physical structural changes being the reason for the change in the waterholding capacity.

Data on the effect of the finishing diet on flavour metabolites is given in Table 4. The differences between the diets used in this study did not

Table 4: Effect of finishing diet on flavour metabolites in lamb. LP = Lotus pedunculatus; RG = Rye grass; WC = white clover; LSD = least significant difference at P < 0.05 for comparing means within a row. *** and NS described in Table 1.

	Finishing diet			LSD	Treatment effect
Metabolites	**LP**	**RG**	**WC**		
Loin skatole, (ng/g fat)	49.5	63.8	52.5	47.5	NS
Loin indole, (ng/g fat)	8.5	9.2	6.7	2.9	NS
Loin p-cresol, (ng/g fat)	122.3	96.7	57.3	32.4	***

significantly affect the concentrations of the flavour metabolites, indole and skatole in the fat at slaughter (Table 4). The expert sensory panellists used in the study were also unable to detect any difference in the flavour and odour notes in the cooked meats from the sheep finished on the diets. Schreurs *et al*. (2007b) reported higher indole and skatole in the rumen and plasma of lambs fed WC compared to those fed RG but did not find any effect of diet on the indole and skatole in the fat or the odour and flavour notes similar to the ones assessed by the trained sensory panellists in our study. Loin skatole in this study had a positive correlation with sheepy flavour (r = +0.7; P < 0.05) and a negative correlation with musty flavour (r = - 0.7; P < 0.05). While "sheepy" odours and flavours are usually associated with branched-chain fatty acids, positive associations with skatole have also been reported (Young *et al*., 2006).

Lambs finished on LP had higher (P < 0.001) *p*-Cresol in their meat compared to lambs finished on WC or RG (Table 3). The *p*-Cresol content of the meats were in the order LP > RG > WC (P < 0.001). *p*-Cresol can be derived from the rumen digestion of the amino acid tyrosine or plant polyphenolics including condensed tannins (Lane & Fraser, 1999). The higher content of condensed tannins in LP compared to the other two forages (Meagher *et al*., 2004) could be the reason for the higher content of *p*-Cresol in the meat of lambs finished on LP. The higher *p*-Cresol in the fat of the LP lambs could also be an indication of a higher level of phenolic antioxidants in that meat compared to meats from the other two diets. However there was no evidence of this in effects on the colour stability of the meat. Loin *p*-Cresol had a negative correlation with sheepy odour described as the odour of cooked lamb (r = -0.7, P < 0.05).

The lack of a significant effect of feeding a high tannin containing diet on the indole and skatole content of the fat at slaughter may simply indicate the sheep needed to be finished longer on the diets for any significant difference in meat flavour chemistry to be obtained. The required reduction in rumen formation of indole and skatole to affect a reduction in their accumulation in the fat needs to be established if meat quality attributes are to be altered through high tannin containing diets.

Implications

Within the parameters of this study, finishing sheep on a high tannin containing forage diet for 22 days did not improve the quality of lamb as hypothesised. However, data in this study confirmed what was previously known on tenderness of lamb and contributed new insights into the waterholding capacity of lamb stored chilled for nine weeks. The improvement in the tenderness and waterholding capacity of chilled vacuum-packaged sheepmeat with ageing at -1.5°C would be advantageous for the New Zealand meat industry considering the distance of chilled meat markets and the time it takes for products from this country to reach the end user in those markets. However, it is important to balance this improvement in tenderness and waterholding capacity with the maintenance of optimum meat texture and display colour shelf life in order to avoid supplying over tenderised meat that lost its texture or one that could not meet the retailers display colour shelf-life requirements.

The higher colour stability of meat from the RG diet provided further evidence of a link between glycogen content of muscle at kill and the colour and colour stability of vacuum packaged chilled meat in storage suggesting this could be used as a predictive tool. With the availability of technologies – such as NIR spectroscopy – for the rapid determination of the glycogen content of meat, the meat industry could use these technologies to estimate the display shelf life of their meat before shipment to customers who are now beginning to demand guarantees of the colour display life of meat supplied to them.

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REFERENCES

Farouk M.M.; Swan J.E. 1998: Effect of rigor temperature and frozen storage on functional properties of hot-boned manufacturing beef. *Meat Science* **49**: 233-247.

GENSTAT. 2005: GenStat - Eighth Edition. Version 8.1.0.156. VSN International Ltd., Oxford.

Huff-Lonergan E.; Lonergan S. 2005: Mechanisms of water-holding capacity of meat: The role of post-mortem biochemical and structural changes. *Meat Science* **71**: 194-204.

Immonen K.; Ruusunen M.; Puolanne, E. 2000: Some effect of residual glycogen concentration on the physical and sensory quality of normal pH beef. *Meat Science* **55**: 33-38.

Krisman, C.R. 1962: A method for the colorimetric estimation of glycogen with iodine. *Analytical Biochemistry* **4**: 17-23.

Lane G.A.; Fraser K. 1999: A comparison of phenol and indole flavour compounds in fat, and of phenols in urine of cattle fed pasture or grain. *New Zealand Journal of Agricultural Research* **42**: 289-296.

Meagher L.P.; Lane G.A.; Sivakumaran S.; Tavendale M.H.; Fraser K. 2004: Characterization of condensed tannins from *Iotus* species by thiolytic degradation and electrospray mass spectrometry. *Animal Feed Science Technology* **117**: 151-163.

Schreurs N.M.; McNabb, W.C.; Tavendale M.H.; Lane G.A.; Barry T.N.; Cummings T.; Fraser K.; Lopez-Villalobos N.; Ramırez-Restrepo C.A. 2007a: Skatole and indole concentration and the odour of fat from lambs that had grazed perennial ryegrass/white clover pasture or *Lotus corniculatus*. *Animal Feed Science and Technology* (In Press).

Schreurs N.M.; Tavendale M.H.; Lane G.A.; Barry T.N.; McNabb, W.C.; Cummings T.; Fraser K.; Lopez-Villalobos N. 2007b: The effect of supplementation of a white clover or perennial ryegrass diet with grape seed extract on indole and skatole metabolism and the sensory characteristics of lamb. *Journal of the Science of Food and Agriculture* (In Press).

Terrill, T.H.; Rowan, A.M.; Douglas, G.B.; Barry, T.N. 1992: Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereals grains. *Journal of the Science of Food and Agriculture* **58**: 321-329.

Waghorn, G.C.; Shelton, I.D.; McNabb, W.C.; McCutcheon, S.N. 1994: Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. J*ournal of Agricultural Science* **123**: 109-119.

Young O.A.; Lane G.A.; Podmore C.; Fraser K.; Agnew M.J.; Cummings T.L.; Cox N.R. 2006: Changes in composition and quality characteristics of ovine meat and fat from castrates and rams aged to 2 years. *New Zealand Journal of Agricultural Research* **49**: 419-430.