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## Nutritive value of forage legumes used for grazing and silage

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**Legume forages have an important position in ruminant production in Western Europe and with further development can play an even larger role. Red clover for silage and white clover in grazed swards lead to enhanced growth rate and milk yield in comparison with pure grasses. Much of the production benefit of these legumes relates to enhanced intake since digestibilities are not markedly different to grasses. The higher intake of legume silages reflects differences in the cell structure of legume plants which combined with high fermentation rates means that they break down into small particles in the rumen, and leave the rumen more rapidly than perennial ryegrass. Ease of ingestion leads to high rates of intake, which explains higher intakes for grazed legumes. A further benefit of legumes is the reduced rate of decline in digestibility with advancing maturity. Whilst legumes have limited effects on gross milk composition or carcass characteristics, there are marked increases in levels of beneficial n-3 PUFA. Legumes have often led to a reduction in methane production from the rumen and again, this relates to both physical and chemical differences between forage species. The high rates of release of soluble protein and of breakdown to small particles from clovers and lucerne is associated with susceptibility to bloat, which is a limitation to further exploitation in grazing systems. The high concentration of rapidly degraded protein in legumes also leads to inefficient utilisation of dietary N and increased urinary N output. Research with tanniniferous forages, such as birdsfoot trefoil and sulla, demonstrates the potential for future legumes with reduced environmental and health effects, though these particular forage legumes are not well adapted to temperate regions of Western Europe that are the focus of this review.**

*Keywords:* animal health; fertility; forage legume; nutrient utilisation; product composition; rumen function

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### Introduction

This short review paper focuses on the main legumes used in temperate regions of Western Europe. White clover (*Trifolium repens*; WC) is the most important forage legume for grazing, whether as a spontaneous component of natural or permanent pastures or sown in association with grasses such as perennial ryegrass (*Lolium perenne*; PRG). Red clover (*Trifolium pratense*; RC) is occasionally used for grazing, pure or in mixtures, but its contribution to biomass production declines rapidly after the first 2–3 years under grazing (Delaby *et al.*, unpublished) and so it is used more for silage production (Le Gall, 1993). We will make some comparisons with lucerne (*Medicago sativa*; LUC), which has similar uses to RC, but which has been used to a much greater extent in North America. The review touches on some other legumes which may not be well adapted to grow in this region, but which have interesting nutritional attributes that could be incorporated into future legumes. The paper covers plant composition/characteristics that affect production of milk and meat (digestibility and intake), product quality (fatty acids and taints), environmental effects (Nitrogen-use efficiency and methane production) and fertility, as well as effects on bloat – a specific health issue that is closely related to ingestion of certain legumes.

### Effects of legumes on animal production

The beneficial effects of legume forages on animal production have been recognised for a long time and so will be mentioned only briefly in this paper, which focuses on underlying mechanisms. Many earlier studies showed the production benefits of legumes silages (e.g., Castle, Reid and Watson, 1983; Thomas, Aston and Daley, 1985; Hoffman *et al.*, 1998) and white clover in pasture (Ulyatt, 1970). Results from some more recent studies showing the beneficial effects of increasing the level of white clover in pasture are summarised in Tables 1–3 (Harris *et al.*, 1998; Ribeiro Filho, Delagarde and Peyraud, 2003; Philips *et al.*, 1998, 2000). Increased performance has been observed in dairy cows (e.g., Dewhurst *et al.*, 2003a), steers (e.g., Thomas, Gibbs and Tayler, 1981), ewes in late pregnancy (Orr *et al.*, 1990; Table 4) and finishing lambs (e.g., Fraser *et al.*, 2004). Higher intakes are pivotal to higher performance with legume forages since effects on feed efficiency are inconsistent (e.g., Table 3). Ulyatt (1970) suggested that 50–75% of the animal production response is related to variation in herbage intake. Legume forages generally lead to higher intakes and animal production than grass silages of comparable digestibility. This is true whether as silages (Dewhurst *et al.*, 2003a; Table 5) or grazed herbage (Fraser *et al.*, 2004; Table 6). Harris *et al.*

**Table 1. Effect of increasing the proportion of white clover in the diet on intake, feed conversion and milk yield (Harris *et al.*, 1998)**

	White clover in the diet (g/kg DM)					
	Experiment I			Experiment II		
	200	500	800	200	500	800
DM intake (kg/day)	10.9	12.3	12.7	10.9	12.2	12.0
Milk yield (l/cow)	9.9	12.9	13.2	8.5	10.0	9.8
Milk solids (kg/cow)	0.90	1.19	1.24	0.92	0.95	0.93
Efficiency (MJ ME/l milk)	9.73	8.57	8.79	10.4	10.6	10.3

**Table 2. Effect of white clover inclusion in pasture on herbage intake and milk production at two stages of regrowth (Ribeiro Filho *et al.*, 2003)**

	Age of regrowth x Pasture type <sup>†</sup>			
	19 days		35 days	
	PRG	PRG/WC	PRG	PRG/WC
Biomass (above 5 cm; kg DM/ha)	2160	1150	4500	3260
OM intake (kg/day)	12.3	13.7	10.5	13.0
Grazing time (mins/day)	510	517	460	503
Rate of intake (g OM/min)	24.2	26.7	23.0	26.1
Milk yield (kg/cow)	16.3	17.7	13.8	16.0
Fat content (g/kg)	39.2	37.7	39.2	37.9
Protein content (g/kg)	30.5	31.4	30.2	30.7

<sup>†</sup>PRG: Perennial ryegrass; WC: White clover.

**Table 3. Effect of including white clover in perennial ryegrass swards on milk production and composition**

	Study x Pasture type <sup>†</sup>			
	Phillips and James (1998)		Phillips <i>et al.</i> (2000)	
	Pure PRG	WC mixed	Pure PRG	WC mixed
Stocking rate (cow/ha)	4.54	3.85	2.90	2.10
Grazing time (mins/day)	518	545	371	386
Milk yield (kg/cow)	18.9	22.1	11.5	13.0
Fat content (g/kg)	37.0	34.6	43.1	42.2
Protein content (g/kg)	33.1	33.4	36.1	38.4

<sup>†</sup>PRG: Perennial ryegrass; WC: White clover.

**Table 4. Ewe stocking rate and lamb performance at grazing on continuous high fertilised PRG or WC/PRG mixed sward (means over 3 years) (Orr *et al.*, 1990)**

	Pasture type <sup>†</sup>	
	Pure PRG	WC mixed
Stocking rate (ewes/ha) <sup>1</sup>	12.8	16.7
Lamb growth rate (g/day)	298	294
Lamb live-weight output (kg/ha)	875	1136

<sup>1</sup>Before weaning with twin lambs per ewes.

<sup>†</sup>PRG: Perennial ryegrass; WC: White clover.

(1998) showed that the benefit of including white clover in zero-grazed herbage was lost when feed intake was restricted. Red clover silage can often be of lower digestibility than grass silage, but perfor-

mance is maintained as a result of higher DM intake (Steen and McIlmoyle, 1982; Thomas *et al.*, 1985).

Grazing of forage legumes (RC, LUC or *Lotus corniculatus* in comparison with PRG) led to increased growth rates and a significant reduction in time to slaughter of lambs (Speijers *et al.*, 2004). These gains were achieved without negative effect on carcass characteristics; indeed, carcass weight and killing out % were increased significantly for lambs grazing RC. Vipond *et al.* (1993) showed a similar reduction in days to slaughter and improved conformation for lambs grazing ryegrass/white clover. Effects of forage legumes on gross milk composition are usually small. Inclusion of WC has some-

**Table 5. Effect of legume silages on DM intake, milk yield and composition**

	Study x Pasture type <sup>†</sup>					
	Experiment I			Experiment II		
	PRG	RC	WC	PRG	RC	WC
DM intake (kg/day)	11.4	13.4	12.9	12.6	15.2	15.9
Milk yield (kg/cow)	24.9	28.1	31.5	27.5	30.2	33.2
Milk fat (g/kg)	44.5	45.2	43.9	41.0	37.4	35.2
Milk protein (g/kg)	32.6	31.4	32.0	30.4	29.7	31.7
Milk N / Feed N (g/g)	0.256	0.210	0.205	0.235	0.197	0.204
18:3 n-3 (g/100 g milk fatty acids)	0.43	0.81	0.91	0.40	1.28	0.96

<sup>†</sup>PRG: Perennial ryegrass; RC: Red clover; WC: White clover.

All cows received 8 kg/day of a standard concentrate (Dewhurst *et al.*, 2003a).

**Table 6. Intake and production performance of grazing lambs finished on red clover or perennial ryegrass swards (Fraser *et al.*, 2004)**

	Pasture type <sup>†</sup>	
	PRG	RC
Herbage intake (kg DM/day)	1.16	2.06
Lamb growth rate (g/day)	184	305
Days to slaughter	66	38
18:2 n-6 (g/100 g fatty acids) <sup>1</sup>	2.91	4.47
18:3 n-3 (g/100 g fatty acids) <sup>1</sup>	2.07	2.86

<sup>1</sup>Of *longissimus dorsi* muscle.

<sup>†</sup>PRG: Perennial ryegrass; RC: Red clover.

times led to reductions in milk fat concentration, whether animals are grazing (e.g., Ribeiro Filho *et al.*, 2003 (Table 2); Phillips and James (1998; Table 3) or fed silage (Dewhurst *et al.*, 2003a). However, in each case there are parallel studies showing no significant effect (Phillips, James and Nyallu, 2000; Dewhurst *et al.*, 2003a; Tables 3 and 5). In some studies, WC has led to a small increase in milk protein concentration (e.g., Ribeiro Filho *et al.*, 2003), though others showed no effect (Phillips and James, 1998).

### Fertility

Red clover contains a number of isoflavones – particularly formononetin (Cox

and Braden, 1974) – but also daidzein, genistein and biochanin A (Saviranta *et al.*, 2008). These can exert oestrogen-like effects within animals, disrupting reproductive cycles and impairing fertility in sheep (Newton and Betts, 1968; Thomson, 1975). In cattle, however, there is no evidence for a negative effect of RC phyto-oestrogens on fertility – indeed first service conception rates were higher for cows offered RC silage rather than grass silage in the studies of Austin *et al.* (1982) and Thomas *et al.* (1985). Whilst the majority of problems were encountered with sheep grazing RC, it appears not to be an effect of the ensiling process since problems were encountered with sheep offered RC silage in the study of Thomson (1975). Reasons for possible improvements in fertility when feeding RC silage could include the general improvement in nutritional status as a result of increased intakes, or a specific effect such as effects of fatty acids on ovarian function. It has already been noted that RC silage leads to reduced rumen biohydrogenation of 18:3 fatty acid, leading to an increased supply of this n-3 fatty acid. Studies with other n-3 sources, notable linseed oil, have shown effects on prostaglandin metabolism and fertility in cattle (Petit *et al.*, 2001, 2002).

### Chemical composition and nutritive value of forage legumes

Chemical composition and nutritive values for pure swards of WC and RC, grown in France, have recently been summarised by INRA (2007) and some main results are presented in Table 7. Nutritive values for silages produced in the UK from 3 cuts each of WC and RC over 2 years are presented in Table 8. Red clover, and particularly WC, are rich in protein and minerals such as calcium, but contain low levels of sugar and fibre. The particularly low fibre concentration of WC reflects the absence of structural components such as stems and sheaths (Ayres, Nandra and Turner, 1998; Giovanni, 1990). As a result of these

morphological and chemical differences, WC has a high digestibility (0.80) and a high energy value (1.00 UFL/kg of DM). These values are usually higher than for RC and PRG.

An additional benefit of legumes is that the rate of decline in digestibility with advancing maturity is less than for grasses. This has been known for many years in both RC (Thomas *et al.*, 1981) and white clover (Ulyatt, 1970). Whilst the digestibility of PRG can decline by 20 g/kg DM per week in May, WC declines by 10 g/kg DM per week at most. In later rotations (cycles 2–4), the digestibility of WC did not decline between 28 and 42 days of regrowth, whilst the digestibility of

**Table 7. Chemical composition (g/kg DM) and nutritive value of pure white and red clover (Giovanni, 1990; Steg *et al.*, 1994; INRA, 2007)**

Growth stage	Composition				Nutritive value			
	OM	CP	NDF	ADF	OMd	UFL	PDIE	PDIN
	<i>White clover</i>							
1 <sup>st</sup> cycle Vegetative	880	249	294	176	0.83	1.09	106	161
1 <sup>st</sup> cycle Early flowering	887	229	320	193	0.80	1.03	102	147
2 <sup>nd</sup> cycle	875	220	308	186	0.79	0.96	95	139
	<i>Red clover</i>							
1 <sup>st</sup> cycle Early budding	868	196	437	270	0.76	0.88	93	125
1 <sup>st</sup> cycle Early flowering	883	166	476	312	0.69	0.74	86	106
2 <sup>nd</sup> cycle	868	245	417	244	0.76	0.88	97	156

OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; OMd: Organic matter digestibility; UFL: Unité Fourragère Lait (1 UFL=1,700 kcal NEL); PDIE and N: Protein truly digestible in the intestine.

**Table 8. Mean chemical composition of silages prepared from perennial ryegrass and white and red clover silages; WSC values are for the herbage as ensiled (Dewhurst *et al.*, 2003a)**

Silage type	OM	WSC (herbage)	CP	NDF	ADF
Perennial ryegrass	925	166	140	526	309
White clover	901	107	237	283	264
Red clover	900	92	189	413	321

OM: Organic matter; WSC: Water-soluble carbohydrates; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre.

PRG declined by 20 g/kg DM (Giovanni, 1990). The stability in nutritive value of PRG/WC swards according to season and age of regrowth makes them easier to manage than pure grass swards in terms of maintaining quality. This flexibility of use makes legume forages particularly attractive for sustainable livestock production systems.

White clover is almost always grown in association with grass and so it is most interesting to look at the nutritive value of the components of this mixture. This has been evaluated in a study with PRG/WC grown without N fertiliser at an INRA experimental farm in Normandy (Le Pin au Haras – 48.44° N–0.09° E) over 6 years. Material was harvested during the second to the fifth grazing rotations after a first-cut for silage and separate analyses conducted for PRG and WC (Table 9; Delaby *et al.*, unpublished). The species maintained the differences in crude protein (+62 g/kg DM) and crude fibre (–77 g/kg DM) noted above, though OM concentrations were similar. The CP concentration of both species increased in autumn, as a

consequence of the high N soil mineralization and N availability at this time.

#### Rumen degradation of forage legumes

The rumen degradation of forage legumes has been evaluated in comparison with perennial ryegrass in nylon bag studies with fresh herbage (Beever *et al.*, 1986), dried herbage (Hoffman *et al.*, 1993) and dried silage (Dewhurst *et al.*, 2003b). Degradability estimates for DM (or OM) and Nitrogen are given in Tables 10 and 11 respectively. A rumen outflow rate of 5%/hour was assumed to provide a common basis for these comparisons – but note the discussion (below) about the higher rumen passage rates with forage legumes.

Mean estimated degradability of DM (OM) was 0.63, 0.75, 0.70 and 0.65 for PRG, WC, RC and LUC respectively. This overall ranking of forages closely matches differences in total tract digestibilities, though again the greater variability with PRG (range 0.55–0.78) and relatively narrow range of values for WC (all greater than 0.72) are evident. Degradability

**Table 9. Evolution of the chemical composition (g/kg DM) and the nutritive value of white clover and perennial ryegrass stemming from a same mixed pasture (42 days of age of regrowth) (Delaby *et al.*, unpublished)**

Month	Species	Composition			Nutritive value			
		OM	CP	CF	OMd <sup>(1)</sup>	UFL	PDIE	PDIN
June	PRG	898	143	268	0.70	0.83	88	93
	WC	896	195	196	0.77	0.94	101	127
July	PRG	897	128	266	0.68	0.80	83	83
	WC	896	196	197	0.76	0.92	100	128
August	PRG	899	148	263	0.66	0.77	85	96
	WC	902	213	181	0.77	0.95	104	140
September	PRG	887	197	252	0.68	0.79	93	128
	WC	896	250	170	0.78	0.96	110	165
October	PRG	887	196	236	0.70	0.82	95	128
	WC	888	268	158	0.79	0.98	113	177

PRG: Perennial ryegrass; WC: White clover; CF: Crude fibre.

<sup>(1)</sup> calculated according to the pepsin-cellulase digestibility method (INRA, 2007).

**Table 10. Estimates of DM (or OM for Beever *et al.*, 1986) fermentability of forage legumes based on nylon bag incubations and assuming a rumen outflow rate of 5%/hour**

	Species			
	Perennial ryegrass	White clover	Red clover	Lucerne
Fresh herbage: early season <sup>1</sup>	0.60	0.80	–	–
Fresh herbage: mid season <sup>1</sup>	0.57	0.75	–	–
Fresh herbage: late season <sup>1</sup>	0.56	0.73	–	–
Dried herbage: vegetative <sup>2</sup>	0.78	–	0.80	0.75
Dried herbage: bud/boot <sup>2</sup>	0.72	–	0.71	0.62
Dried herbage: flowering <sup>2</sup>	0.55	–	0.65	0.57
Dried silage: mixed cuts <sup>3</sup>	0.61	0.72	0.64	–

<sup>1</sup>Beever *et al.*, 1986; <sup>2</sup>Hoffman *et al.*, 1993; <sup>3</sup>Dewhurst *et al.*, 2003b.

**Table 11. Estimates of Nitrogen degradability of forage legumes based on nylon bag incubations and assuming a rumen outflow rate of 5%/hour**

	Species			
	Perennial ryegrass	White clover	Red clover	Lucerne
Fresh herbage: early season <sup>1</sup>	0.70	0.83	–	–
Fresh herbage: mid season <sup>1</sup>	0.67	0.79	–	–
Fresh herbage: late season <sup>1</sup>	0.67	0.75	–	–
Dried herbage: vegetative <sup>2</sup>	0.89	–	0.88	0.85
Dried herbage: bud/boot <sup>2</sup>	0.87	–	0.82	0.79
Dried herbage: flowering <sup>2</sup>	0.69	–	0.73	0.73
Dried silage: mixed cuts <sup>3</sup>	0.76	0.83	0.77	–

<sup>1</sup>Beever *et al.*, 1986; <sup>2</sup>Hoffman *et al.*, 1993; <sup>3</sup>Dewhurst *et al.*, 2003b.

estimates for N in these forages were all higher than for DM (OM) in the same samples. However, differences between forages were much smaller with similar means for both clovers and LUC (0.79–0.80), with PRG only slightly lower (0.75). Beever *et al.* (1986) showed that the higher fermentation rate of WC in comparison with PRG, led to high concentrations of volatile fatty acids in the rumen of steers, even when DM intake was equalised.

#### Intake of legume silages

When feed intake is not constrained by herbage allowance or sward structure (e.g., with silages or zero-grazing), the higher intake of legume forages relative

to grass forages is linked to their chemical degradation and physical breakdown within the rumen. These operate through effects on rumen fill. Differences have been attributed to both faster rates of fermentation (Beever and Thorp, 1996) and more rapid particle breakdown and clearance from the rumen (Moseley and Jones, 1984; Waghorn *et al.*, 1989; Jamot and Grenet, 1991). Dewhurst *et al.* (2003b) suggested that fermentation rate may be more important for WC, whilst rapid particle breakdown may be more important for LUC. Clearly there will be an inter-relationship between chemical degradation and particle breakdown. Differences in particle breakdown and rumen passage rate are partly related to plant anatomy.

The vein structure of perennial ryegrass leads to long fibres that are retained within the rumen, whilst the reticular vein structure of legumes breaks down into small particles more readily (Wilman, Mtengeti and Moseley, 1996; Wilson and Kennedy, 1996).

#### **Intake of grazed legumes**

Herbage intake in the grazing situation depends firstly on daily herbage allowance and, to a lesser extent, on sward structure (Prache and Peyraud, 1997; Peyraud *et al.*, 1996). The leaves of legumes, and particularly WC, are more favourable for prehension than those of grasses, particularly during the spring heading period. There have been relatively few detailed studies of the mechanisms for effects of incorporating WC alongside PRG on herbage intake. Ribeiro Filho *et al.* (2003) compared PRG and PRG/WC (40–45% WC) and showed the anticipated increased intake and milk production responses (Table 2). The higher herbage intake of PRG/WC was a result of a higher intake rate and a longer time spent grazing. Penning *et al.* (1995) showed that the increased DM intake for sheep grazing WC rather than perennial ryegrass was associated with increased times spent eating and ruminating – notably the ‘handling time’. The higher intake rate of PRG/WC can be related to the morphological characteristics of WC that favour prehension. The increased time spent grazing on PRG/WC can be explained by the smaller proportion of sheaths to defoliate (Ribeiro Filho *et al.*, 2003).

Another interesting feature of grazing on mixed PRG/WC swards is the opportunity for diet choices, with animals expressing their preference for grass or clover. In a recent review, Rutter (2006) showed that cattle and sheep both eat

mixed diets, showing a partial preference of approximately 70% for clover. Rutter *et al.* (2004) showed that dairy cattle chose diets containing 63 and 84.5% WC when given choices from monocultures in which the area of WC made up 25% or 75% of the area respectively. It is suggested that the preference for WC is driven by animals seeking to maximise the nutrient benefit achieved per unit energy expended on grazing. It is noted that animals maintain a mixed diet despite their preference for WC (Rutter *et al.*, 2004) and this may be related to maintaining effective rumen function.

#### **Fatty acids in milk and meat**

The relationships between dietary fat and the incidence of human disease, particularly coronary heart disease are well established and medical authorities have developed guidelines in relation to fat in the diet (WHO, 2003). Recent research has focussed on the primary targets of reducing saturated fatty acids (SFA) and increasing n–3 polyunsaturated fatty acids (PUFA) – particularly the longer carbon chain eicosapentaenoic acid (EPA; 20:5n–3) and docosahexaenoic acid (DHA; 22:6n–3), as well as the isomers of conjugated linoleic acid (CLA).

Plants are the primary source of n–3 PUFA in terrestrial ecosystems. Forages such as grass and clover contain a high proportion (50–75%) of total fatty acids as  $\alpha$ -linolenic acid (Dewhurst *et al.*, 2006) which is the precursor of EPA and DHA through elongation and desaturation in the body. The plethora of CLA isomers in milk, beef and lamb derive ultimately from rumen biohydrogenation of PUFA. Exploiting herbage as a source of n–3 PUFA is therefore an important nutritional strategy for enhancing the concentration of n–3 PUFA and CLA. The comparative



effects of feeding a grass-based or concentrate-based ration on the fatty acid composition of muscle have been recently reviewed (Scollan *et al.*, 2006).

Dewhurst *et al.* (2006) reviewed a series of studies from his group that evaluated effects of clover silages on fatty acids in milk. In comparison with milk from cows fed grass silages, clover silages had only small and inconsistent effects on proportions of the various SFA, as well as CLA, in milk. In contrast, both RC and WC silages led to highly significant increases in the proportion of the n-3 fatty acid  $\alpha$ -linolenic acid (18:3n-3) in milk. In each of two comparisons with WC silage and four with RC silage, there was at least a doubling in 18:3n-3. Values were increased three-fold when RC silage made up a high proportion of the diet (concentrate level was 4 kg/day).

When compared to grazed grass, cattle grazing a WC-rich pasture generally had a higher concentration of PUFA in total muscle lipids which contributed to an increase in the PUFA:SFA ratio (Scollan *et al.*, 2002a, Moloney, McGilloway and French, 2007). A similar effect was observed in lambs by Vipond *et al.* (1993) and Lourenço *et al.* (2007a). Increasing the deposition of 18:3n-3 in meat on forage-based rations is dependent on increasing the level of 18:3n-3 in the forage, increasing forage consumption and reducing the extent of ruminal biohydrogenation. These factors have recently been reviewed by Palmquist *et al.* (2005) and Dewhurst *et al.* (2006) and contribute, to a varying extent, to the differences observed due to clover feeding. With respect to WC, an increase in intake and a faster rate of passage from the rumen are most likely the main factors involved. The CLA concentration was either unaffected (Scollan *et al.*, 2002a,b) or decreased by WC consumption (Moloney *et al.*, 2007) which, in

the latter case at least, is consistent with a decrease in rumen biohydrogenation due to an increase passage rate.

Muscle from cattle consuming red-clover rich pasture had a higher PUFA:SFA ratio than cattle grazing perennial ryegrass or a WC-rich pasture but the CLA concentration was unaffected (Scollan *et al.*, 2002a). A similar observation was made for lambs by Fraser *et al.* (2004; Table 6). While the concentrations of both C18:2n-3 and C18:2n-6 were increased in all cases, the increase was greater for the latter resulting in an increase in the n-6 to n-3 PUFA ratio. Nevertheless, this ratio was always less than the recommended ratio of 4:1. Similar, albeit non-significant, trends were reported by Lourenço *et al.* (2007b) for a comparison of ensiled RC and intensive ryegrass fed to lambs. For steers or cull cows fed ensiled RC or grass, similar effects were seen as for grazed forage on the PUFA:SFA ratio and CLA concentration (no effect) but the n-6 to n-3 PUFA ratio was decreased (Richardson *et al.*, 2005 and Lee *et al.*, 2009, respectively). Companion studies have revealed that the RC response is related to a direct reduction in ruminal biohydrogenation of PUFA, which is possibly related to the protective effects of the enzyme polyphenol oxidase (Lee *et al.*, 2004). Fraser *et al.* (2004) also compared LUC with a grass sward but observed little difference in lamb muscle fatty acid composition.

#### **Shelf-life of beef and lamb**

The bright red colour of beef and lamb is important for consumers when making purchasing decisions. Meat containing greater contents of the highly unsaturated lipids is more prone to lipid oxidation which contributes to loss of redness and a decrease in shelf-life. Nevertheless, pasture-fed beef is generally more resis-

tant to lipid oxidation than grain-fed beef, despite its higher PUFA content (O'Sullivan *et al.*, 2003). This is most likely due to increased antioxidants, such as  $\alpha$ -tocopherol and  $\beta$ -carotene, in the meat.

In the study of Scollan *et al.* (2002b), muscle from cattle that grazed grass or a mixture of grass and RC or grass and WC had similar colour and lipid stability. In contrast, in a study using similar rations Enser *et al.* (2001) reported an increase in lipid oxidation in muscle from cattle grazing the grass and RC mixture which appeared to be related to a lower concentration of vitamin E in muscle. There was no difference between muscle from cattle that grazed grass or a grass and WC. A similar increase in muscle lipid oxidation due to consumption of RC silage was reported by Lee *et al.* (2009). In none of these studies was colour stability significantly affected. In the study of Richardson *et al.* (2005), cattle fed RC silage, grass silage, or a 50:50 mix of the two, had an increasing concentration of PUFA in muscle with increasing RC silage proportion in the diet but vitamin E concentration decreased as did colour and lipid oxidative stability. When a fourth group of animals was fed the RC silage supplemented with vitamin E, vitamin E content of muscle, colour and lipid stability was the same as that in the muscle of grass silage-fed animals. Differences in colour stability between the above studies appear to relate to the extent of lipid oxidation and the absolute difference in muscle vitamin E concentration. In the study of Fraser *et al.* (2007), Welsh Black cattle grazed permanent pasture (PP) or a *Molinia*-dominated semi-natural pasture (SNP). In the winter they were fed silage from the permanent pasture whilst one group received RC silage (PP+RC). Upon slaughter after a second grazing season, loin steaks from the SNP-grazed animals

had significantly more vitamin E than those from PP-grazed animals and this was reflected in the lower fat oxidation.

Data on the effects of consumption of WC on lipid oxidation in sheep muscle are equivocal with an increase (Shorland *et al.*, 1970) or no effect being observed (Petron *et al.*, 2007). Fraser *et al.* (2004) reported an increase in lipid oxidation in muscle from lambs that grazed RC or LUC.

### **Sensory characteristics of beef and lamb**

Tenderness is the most important eating quality attribute of meat in determining its acceptability, but there is little evidence that forage type influences muscle tenderness when other potentially confounding influences are removed. When tenderness is increased or acceptable to consumers, flavour and juiciness increase in relative importance. The flavour of red meats, developed during cooking, derives from the Maillard reaction between amino acids and reducing sugars and the thermal degradation of lipid. The former produces roasted/meaty flavours and the latter the species differences in flavour (Mottram, 1998; Gandemer, 1999). Strategies which can alter the fatty acid composition of the lipid fraction of meat could also alter the amount and type of volatiles produced and hence its aroma and flavour (Elmore *et al.*, 1999) and type of forage has been associated with pastoral flavour in ruminant lean and fat (see Young *et al.*, 2003).

Since flavour is usually subjectively assessed by a trained or consumer sensory panel, differences in methodology make achieving a consensus across studies difficult. Thus, Enser *et al.* (2001) and Scollan *et al.* (2002b) found no difference in flavour intensity between muscle from cattle fed grass or mixtures of grass and WC or grass and RC. The same taste panel failed to detect major differences in the meat

from the three treatments in the study of Fraser *et al.* (2007) above. In contrast, using a descriptive flavour approach, Lee *et al.* (2009) reported that muscle from cows fed RC silage was less acidic, more greasy and more fishy than muscle from cows fed grass silage. Larick *et al.* (1987) observed that steers finished on WC had a higher “grassy” flavour than those fed grass (*Festuca arundinacea*). They attributed these differences in flavour to both the increased content of PUFA, particularly  $\alpha$ -linolenic acid, its lower oxidative stability, and to odoriferous compounds stored in the depot fats.

The effect of forage type on flavour appears to be more intensive in lamb and is better documented for sheep. In general, meat from sheep that consumed WC or LUC alone had a more intense flavour and odour than meat from grass-fed sheep (Schreurs *et al.*, 2008). Duckett and Kuber (2001) concluded, based on their review of the literature, that the intensity of flavour is increased with grazing of WC or LUC when compared to grass but that these differences can be lessened by grazing grass for 2–3 weeks before harvest. These authors also noticed that certain times of the year and season influenced the intensity of the flavour observed. In contrast, Vipond, Marie and Hunter (1995) failed to demonstrate an effect of clover in the diet of lambs on flavour. They suggested that differences between the experiments may be due to the proportion of clover in the diet, in that many studies have used pure swards in their comparisons, which are unlikely to be used in commercial practice. The effects of different pasture species on sheep flavour were compared by Young *et al.* (1994). Six month old lambs were allowed to graze for six weeks single species swards of ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*), cocksfoot (*Dactylis glomerata*), Phalaris

(*Phalaris aquatics*), Lucerne (*Medicago sativa*), chicory (*Chicorium intybus*) and prairie grass (*Bromus willdenowii*). Animals grazing on Phalaris gave meat samples with the highest intensity of foreign flavours and cocksfoot was most favoured but in general, meat from lambs fed seven different pastures did not differ in acceptability.

Other compounds, not derived from fatty acids, may also contribute to flavour. Skatole (3-methyl indole) is commonly associated with boar taint in pigs but has been found in high concentrations in the fat of cattle and sheep that had been fed on grass (Lane and Frazer, 1999; Young *et al.*, 2003). Schreurs *et al.* (2008) suggest that the differences in “pastoral” flavour observed with different forages may reflect their varying propensity to form skatole during rumen fermentation in particular of dietary crude protein. These authors suggest a role for plants, rich in condensed tannins, in ameliorating the development of undesirable flavours in fat of cattle or sheep consuming WC or LUC.

#### **Utilisation of dietary protein and urinary N output**

Whilst the crude protein content of grass with moderate levels of N fertiliser is often relatively close to animal requirements (130 to 170 g/kg DM), legumes often contain much higher levels (180 to 300 g/kg of DM), much in excess of requirements. In terms of protein feeding values for legumes, the PDIE value (which depends on OM digestibility) is lower than the PDIN value (which depends on the crude protein content and the protein degradability). In fact, the ratio PDIE/UFL for legumes is excellent, close to the level of 100 g of PDIE/UFL recommended for optimal feeding of dairy cows (INRA, 2007). However, in these terms the ratio

PDIN/UFL is too high (150–160 PDIN/UFL). The difference between the PDIE and PDIN values reflect the high rumen degradability of the nitrogen (N) contained in legumes (Table 11; Peyraud, 1993; Steg *et al.*, 1994; Cohen, 2001).

The high level of protein in legumes and extensive degradation during ensilage means that legume silages contain high levels of quickly degradable N. This leads to inefficient utilisation (Dewhurst *et al.*, 2003a; Table 5) and particularly high urinary N output (Cohen, Stockdale and Doyle, 2006; Dewhurst, Davies and Kim, 2009). These problems have both been resolved by offering low protein supplements, such as barley (Cohen *et al.*, 2006) or maize silage (Margan, Moran and Spence, 1994; Auldust *et al.*, 1999; Dewhurst *et al.*, 2009) alongside legume silages. This appears to be driven entirely by imbalance in the levels of energy and N in the diet. There is no evidence that the inefficiency is driven by asynchronous supply of energy and N to rumen micro-organisms since meal patterns had no effect on N utilisation (Cohen *et al.*, 2006).

As a result of the imbalance between fermentable energy and rumen degraded N (RDN), there is much interest in forages that exhibit a reduced N degradability. Condensed tannins bind to protein and so tanniniferous forage legumes such as birdsfoot trefoil (*Lotus corniculatus*) and sulla (*Hedysarum coronarium*) have reduced RDN and increase N-use efficiency. Unfortunately, these legumes are not well adapted to most temperate regions of Western Europe and so there has been recent interest in mechanisms that might lead to variability in RDN within legumes species that are grown widely. Considerable attention has been focussed on the very low efficiency of utilisation of N from LUC silage, a major forage in US dairy rations.

Jones, Muck and Hatfield (1995) identified a fraction within RC that inhibits proteolysis and this has subsequently been shown to be polyphenol oxidase (PPO). PPO produces quinones which bind to proteins and reduce N degradability in the rumen. The degradability of N in RC herbage is less than for LUC (Broderick and Albrecht, 1997; Cassida *et al.*, 2000). Similarly, RC silage contains approximately 30–40% less non-protein-N than LUC silage (Owens, Albrecht and Muck, 1999; Albrecht and Muck, 1991). This may explain observations of increased N-use efficiency, that is, conversion of feed N into milk N, with RC silage (e.g., Broderick, Walgenbach and Maignan, 2001). Broderick *et al.* (2004) also found that the CP degradability for RC was lower than for LUC, when both forages were freeze-dried. This work evaluated a series of 133 RC entries and found significant variation in the rate of CP degradation (0.085–0.145 per hour) and undegraded dietary protein (280–400 g/kg DM), both evaluated using an *in vitro* system.

### Methane production

Globally, animal agriculture is the largest contributor of anthropogenic greenhouse gas production with the largest proportion being methane (CH<sub>4</sub>) (Castel *et al.*, 2006). Energy lost as enteric CH<sub>4</sub> also represents an energy loss to the animal which ranges from 2% to 12% of gross energy intake (Johnson *et al.*, 1995). Ruminant livestock produce approximately 80 million tonnes of CH<sub>4</sub> annually (Beauchemin *et al.*, 2008), predominantly as a byproduct of anaerobic digestion within the rumen, which serves as a sink for hydrogen and preserves homeostasis within the rumen. As such methane production can not be eliminated from the ruminant production

system but it is open to dietary manipulation. Numerous methane mitigation strategies have been proposed and tested with varying success ranging from increasing the concentrate portion of the diet (Lovett *et al.*, 2006), to adding plant oils (Petrie *et al.*, 2009). However in forage based production systems the mitigation focus must fall on the forage portion of the diet to maintain the competitiveness of such systems.

Frequently  $\text{CH}_4$  emissions, when expressed on a proportion of gross energy intake (Waghorn *et al.*, 2006) or unit intake basis are lower for forage legume fed animals (McCaughey, Wittenberg and Corrigan, 1999; Waghorn, Tavendale and Woodfield, 2002), compared to animals receiving a predominantly grass diet, although this is not always the case (Van Dorland *et al.*, 2007). In a review of dietary strategies to reduce  $\text{CH}_4$  production Beauchemin *et al.* (2008) proposed that the lower emissions for legume fed animals is a result of a combination of factors including the presence of condensed tannins (CT), lower fibre content, higher dry matter intakes and an increased passage rate from the rumen. These latter authors noted that although differences in  $\text{CH}_4$  emissions reflect compositional differences between grasses and legumes, maturity at the time of harvest may be confounded with the impact of forage type on  $\text{CH}_4$  emissions.

There are substantial differences in the gross morphology and cellular structures of grasses, WC, RC and LUC, which affect patterns of ingestion, mastication, rumination and particle size reduction. These are discussed earlier in this review in relation to feed intake, but also affect  $\text{CH}_4$  production. The higher passage rate of legumes from the rumen restricts the opportunity for methanogens to colonise the feed substrate leading to reductions

in  $\text{CH}_4$  production. Additionally higher digestibility and a faster rate of passage (Minson and Wilson, 1994) results in a shift toward high propionate production (an alternative hydrogen sink) in the rumen and reduced methane production. With low quality forages, modest inclusion of legumes in the sward led to approximately 22% higher calf gains for each unit of  $\text{CH}_4$  produced (McCaughey *et al.*, 1999).

Further to the impact of fibre concentration, passage rate and digestibility of legumes on  $\text{CH}_4$  emissions, the presence of secondary plant compounds, particularly condensed tannins (CT), have a major role to play in the  $\text{CH}_4$  mitigating effects of legumes. Condensed tannins are flavonoid polymers which complex with soluble proteins and render them insoluble in the rumen; yet release them under the acidic conditions found in the small intestine, reducing bloat and increasing amino acid absorption. Condensed tannins are present in the leaves of some legumes such as *Lotus corniculatus* however they are not present in the leaves of white or RC but are present in the inflorescences of these species (Abberton *et al.*, 2007) which predominate in temperate climates.

Condensed tannin mediated  $\text{CH}_4$  suppression is source dependent with *Schinopsis quebracho* CT failing to reduce  $\text{CH}_4$  emissions in cattle (Beauchemin *et al.*, 2007), whilst several other sources reduced  $\text{CH}_4$  emissions (Hayler, Steingass and Drochner, 1998; Carulla *et al.*, 2005). The inhibition of methanogenesis by CT may arise from a suppression of fibre degradation (Hess *et al.*, 2006). However, a direct effect of condensed tannins on rumen methanogens as proposed by Field, Kortekaas and Lettinga (1989) cannot be totally excluded. Waghorn *et al.* (2002) have attributed 16% reductions in methane output to the direct action of CT.

There are still numerous obstacles to the widespread adoption of CT containing legumes in temperate grasslands which present significant challenges for plant breeders. Many CT containing legumes cannot compete with traditional forage crops in temperate regions, high CT concentrations can have negative effects on animal performance and variation in content and response to source of CT all indicate problems that require focus in the future. In the long term and for many parts of the world where livestock production is important, plant breeding strategies to reduce methane emissions are likely to become of increasing utility (Abberton *et al.*, 2007).

### Bloat

The danger of ruminants developing legume bloat (also known as pasture bloat) limits the greater use of legumes for grazing. Legumes such as RC, WC and LUC can cause bloat (Majak, Hall and McCaughey, 1995), whilst others, such as sainfoin (*Onobrychis viciifolia*), birdsfoot trefoil (*Lotus corniculatus*) and cicer milkvetch (*Astragalus cicer*), do not (McMahon *et al.*, 2000; Sarkar, Howarth and Goplen, 1976). Bloat occurs due to a build-up of gas in the rumen and can lead to acute abdominal distension (Howarth *et al.*, 1991), with the rate of gas production being greater than the animal's ability to expel the gas from the rumen. Legume bloat is a form of 'frothy' bloat in which bubbles of gas, about 1 mm in diameter, get trapped in the rumen fluid and the resulting froth fills the rumen cavity, with the normal layering of the rumen contents not apparent (Cole and Boda, 1960; Howarth, 1975; Garry, 1990). The digesta from bloating animals is usually more viscous than that from non-bloating animals (Clarke and Reid, 1974; Howarth *et al.*,

1984). The froth inhibits the nerve endings that control the cardia opening into the oesophagus.

Howarth *et al.* (1978) found that the mesophyll cells of bloat-safe legumes were more resistant to mechanical rupture than those of bloat-causing legumes and subsequently found that whole leaves of bloat-safe legumes were consistently less digested than bloat-causing legume leaves (Howarth *et al.*, 1979). Thompson *et al.* (2000) showed that the early stages of LUC growth were the most likely to cause bloat. This was associated with a higher CP concentration and a lower DM and fibre concentration. Majak *et al.* (1995) demonstrated an increase in the probability of bloat with increasing soluble protein concentration. The rapid release of soluble protein into ruminal fluid promotes the formation of a polysaccharide slime that traps the rumen gases (Clarke and Reid, 1974; Howarth *et al.*, 1991; Pinchak *et al.*, 2005). Indeed, Waghorn *et al.* (1989) demonstrated that the rate of release of soluble protein may be as important as the amount of soluble protein in the development of bloat. More recently, Min *et al.* (2006) cultured a number of rumen bacteria *in vitro* with soluble protein extract from wheat forage. *S. bovis* exhibited the greatest specific growth rate and produced more slime than other bacteria monocultures tested. These findings are also applicable to legume forage-fed animals, as *S. bovis* is one of the dominant microbes in the rumen of animals offered high quality fresh forage (Attwood and Reilly, 1996).

Other studies have demonstrated the importance of small particles (e.g., chloroplast fragments from immature LUC; Thompson *et al.*, 2000) in the development of pasture bloat. The rumen bacteria attached to these particles have an abundance of carbohydrates, both internal (as storage granules) and external (as

slime) which contribute to froth formation (Majak *et al.*, 2003). The small particles themselves also play an important role in froth formation as they become part of the slime matrix in which the gas bubbles get trapped (Majak *et al.*, 2003).

A further aspect of pasture bloat is the variation between animals in susceptibility. Majak *et al.* (1983) closely monitored animals that were, and were not, bloaters. They found that, in cattle that subsequently bloated, pre-feeding rumen chlorophyll concentrations were higher. This suggested increased flotation and slower disappearance of suspended particles from the rumen in these animals. Indeed, these animals also had larger volumes of buoyant particles pre-feeding. Majak *et al.* (1986) subsequently confirmed the theory that rate of passage was slower in bloat-susceptible than in non-bloating animals. This theory is supported by the results of Mendel and Boda (1961), who had previously showed that bloat-resistant cattle secreted more saliva than bloat-susceptible cattle.

As mentioned above, some legumes are bloat-causing whilst others are bloat-safe; this is often related to the presence of CT. Stable CT:protein complexes are formed under rumen conditions (pH 4.0–7.0; Jones and Mangan, 1977) and ruminal protein degradation is thus reduced (Frutos *et al.*, 2004; Min *et al.*, 2003), as is froth production. Other CT remain free in the rumen (Barry and Forss, 1983) and may react with enzymes secreted by the rumen bacteria and thus inhibit bacterial activity (Barry and Manley, 1986). Min *et al.* (2000, 2002a,b) reported that the action of CT in birdsfoot trefoil reduced rumen proteolytic bacterial populations and rumen proteolytic activity *in vivo*, reduced protein solubilisation *in sacco* and reduced rumen protein degradation *in vitro*.

## Conclusions

The chemical and physical composition of temperate forage legumes, particularly red and white clover, confer many benefits in terms of nutritive value. These legumes lead to enhanced growth rate or milk yield in comparison with grass, whether grazed or fed as silage. Increased feed intake, related to more rapid fermentation and physical breakdown in the rumen, is a major source of this advantage. The clovers have other nutritional effects, both positive (increasing levels of polyunsaturated fatty acids in milk or meat and reducing methane production) and negative (increasing urinary N output). Further insight into rumen function will form the basis for refinement or amelioration of these effects.

Despite the nutritional advantages of these legume forages, there are problems with slow uptake by the industry. Amongst the nutritional constraints, bloat remains a concern, though perceptions are often worse than reality and a wide range of management guidelines for control are available. Much more of the reticence relates to agronomic issues, such as lower yield, a shorter growing season, lack of persistence, and variability in legume contribution from year to year. These constraints are discussed in greater detail in other papers in this issue (Black *et al.*, 2009; Woodfield and Clark, 2009).

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