

Irish Journal of Agricultural and Food Research **48**: 115–135, 2009

Potential food production from forage legume-based-systems in Europe: an overview

J.L. Peyraud^{1,2,3,†}, A. Le Gall^{3,4} and A. Lüscher⁵

¹INRA, UMR 1080, Production du Lait, F-35590 Saint Gilles, France

²Agrocampus Ouest, UMR 1080, Production du Lait, F-35590 Saint Gilles, France

³Institut de l'Élevage, Site de Rennes, F-35652 Le Rheu Cedex, France

⁴UMT Recherche et Ingénierie en Élevage Laitier, F-35590 St Gilles, France

⁵Agroscope Reckenholz-Tanikon Research Station ART, Reckenholzstrasse 191, CH-8046 Zurich, Switzerland

Intensification of EU livestock farming systems has been accompanied by the development of maize silage and intensively fertilised grasses at the expense of forage legume crops. However in the new context of agriculture, the development of forage legumes constitutes one of the pillars for future livestock farming systems with high environmental and economical performances. Yield benefits of grass-clover mixtures are equivalent fertiliser N inputs of 150 to 350 kg/ha, and productive grass-clover mixtures can fix 100 to 380 kg N per hectare symbiotically from the atmosphere. Animal intake of legumes is high and the rate of decline of legume nutritional quality with advancing maturity is less than for grasses, especially in the case of white clover, which makes mixed pastures easier to manage. Animal performances at grazing are identical or higher on clover-enriched pastures. Due to their high protein concentration, conserved forage legumes fit well with maize silage. Forage legumes increase the concentration of beneficial α -linolenic acid in ruminant products. Environmental balance of forage legumes is positive. Increasing the proportion of white clover at the expense of mineral N fertilisation can reduce the risk of nitrate leaching. Because forage legumes only require solar energy to fix N from the air, they also reduce energy consumption and associated impacts. They contribute to reduce the global warming potential of livestock systems by reducing emission of enteric methane and nitrous oxide from pasture and crop production. As an element of arable crop rotations, grass-clover leys suppress pests, diseases and weeds, improve soil structure and prevent soil erosion and nitrate leaching. Nevertheless, forage legumes have some limitations: expensive to harvest, difficulties

[†]Corresponding author: jean-louis.peyraud@rennes.inra.fr

of conservation, management of the associations. To take full advantage of forage legumes in the future, new research and development are required as well as financial support from the EU.

Keywords: environmental impact; forage legumes; forage production system; ruminant nutrition

Introduction

The area of pure forage legume crops has been decreasing for several decades in many European countries associated with the development of maize silage and the low price of purchased soybean meal. For example, in France, the acreage of lucerne (LUC) and red clover (RC) decreased by 75% during the last 30 years. These forage legumes covered 1.0 M hectares in 1970 but only 321,000 in 2000 (Pflimlin *et al.*, 2003) whereas in the same time maize silage increased from 350,000 to 1.4 million ha. Mixed pastures have been more resistant to this decline. The development of pastures based on mixtures of white clover (WC) and perennial ryegrass (PRG) in the west of France can be regarded as emblematic of a certain renewed interest in forages legumes. Today 50% of sown pastures are mixed grasses and white clover in the west part of France compared to less than 10% in 1985. In temperate grasslands WC is by far the predominant forage legume in grazing pastures but is rarely used for silage production because its dry matter and sugar concentrations are low. Red clover is occasionally used for grazing in multi-species pastures but its persistency is poor and its contribution to the annual yield decreases rapidly after 2 to 3 years. It is most often use for silage production (Le Gall, 1993). Lucerne is mainly used for silage and hay making but is also used as dehydrated forage in France.

In contrast to most European countries grass-clover mixtures have a long tradition in Switzerland and, more importantly, they have always formed the backbone of

forage production on cropland. In the last 30 years the area under grass-clover leys even increased by 15% and today is four times greater than the area of maize for silage. Leys are almost exclusively sown with grass-clover mixtures while pure legume leys or heavily N-fertilised pure grass leys are not found in Switzerland. A system of standard mixtures was introduced in 1955, when the Federal Research Stations published the first prescriptions for grass-clover mixtures (Frey, 1955). Since then, the standard mixtures have been revised and published every 4 years (Suter *et al.*, 2008) based on systematic variety testing and mixture development programmes (Kessler and Suter, 2004). The large and sustained success of the Swiss standard mixtures is not only due to the numerous advantages of grass-clover mixtures described in this paper, but also due to the very high quality of the mixtures. These are distinguished with a quality label of the Swiss Grassland Society and are due to an exemplary collaboration between research, extension and industry. In Switzerland important efforts such as modified fertilisation and management systems and overseeding are undertaken to also increase legume proportions in permanent grasslands.

Legumes can make an important contribution to the future sustainability of ruminant production systems in Western Europe. They have significant potential to reduce inputs of purchased mineral N and concentrate N, given their ability to use atmospheric N for producing home grown proteins and their high nutritional value.

They also have potential for improving the environment and to some extent the quality of the animal products. It is also important to consider that 55 MJ are required to produce, transport and spread 1 kg of mineral N while legumes only require solar energy to fix N from the air. Thus, legumes can positively contribute to the energy balance of the agricultural sector, to reducing its contribution to the global warming and to limiting the utilisation of non-renewable energy.

Our objective in this paper is to review different aspects of the potential of legumes to increase sustainability of ruminant production systems in the future. The issues covered are agronomic value, animal production of milk and meat and environmental effects of legumes. We will consider both animal responses, forage system and whole farm levels because the potential of forage legume utilisation lies at these different levels. Besides practical considerations our objective is also to focus on underlying mechanisms.

Agronomic value of forage legumes

Higher yield and less unsown species with grass-clover mixtures: A pan-European experiment carried out at 28 sites in 17 countries across Europe (Figure 1) showed strong benefits of grass-clover mixtures containing four species were compared to these species sown in monoculture (Kirwan *et al.*, 2007; Kirwan *et al.*, 2009; Lüscher *et al.*, 2008). At each site, the two most important forage grasses and the two most important forage legumes were tested and the management of the swards followed local recommendations for best agricultural practice. The species varied according to the growth conditions at the site. At mid-European sites (15 sites) the species examined were *Lolium perenne*, *Dactylis glomerata*, *Trifolium pratense* and

Trifolium repens. Eleven four-species mixtures were sown and varied widely in their species proportions: four mixtures were dominated in turn by each species (sown proportions of 70% of dominant and 10% of each other species), six mixtures were dominated in turn by pairs of species (40% of each of two species and 10% of the other two) and the centroid mixture contained equal proportions of each species (for details, see Kirwan *et al.*, 2007).

Compared to the mean of the four species in monoculture, the increased yield of the centroid mixture (overyielding) was 47% when averaged over all 28 sites (Kirwan *et al.*, 2007). There was even significant transgressive overyielding (higher yield than best monoculture yield) for most of the mixtures in the first harvest year (Figure 2). For the mid-European and north-European sites, all the mixtures yielded more than the best monoculture (Figure 2). This occurred even though mixtures were sown with widely varying species proportions (10 to 70% for each species). Preliminary analysis of mid-European sites indicated that this result persisted over the 3 years of the experiment, with transgressive overyielding being 6%, 20% and 16% in years 1 to 3, respectively. The performance of the mixture depended on its clover proportion. This is shown in Figure 3 for three different levels of fertiliser N (50, 150 and 450 kg ha⁻¹ y⁻¹) for the Swiss site. The maximum yield of the mixtures was reached at a clover percentage in the sward between 36 and 70% depending on the year and the fertiliser N treatment (Nyfeler *et al.*, 2009). More importantly, at the low and the moderate N levels, the annual dry matter (DM) yield of the mixture was higher or at least as high as that of the highest yielding monoculture (11.8 t/ha) over a wide range of about 15 to 90% clover percentage in the sward (Figure 3; Nyfeler *et al.*, 2009).



Figure 1. Location of the 28 experimental sites in 17 European countries. Sites with the same symbol show the same set of species examined: (■) mid-European, (●) north-European, (▲) moist Mediterranean, (◆) dry Mediterranean and (★) others (from Kirwan *et al.*, 2007).

Over the three years of the pan-European experiment, unsown species contributed progressively more to the total yield of monocultures, but in

the mixtures, invasion of unsown species was minimal (Figure 4 for monocultures and the centroid mixture). All these findings clearly demonstrate that grass-clover

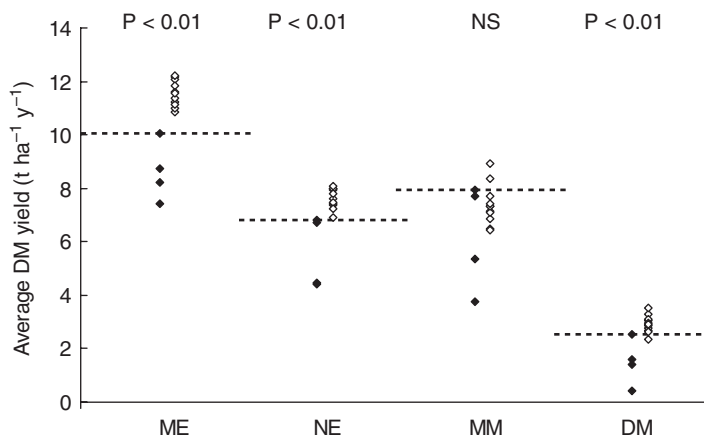


Figure 2. Total dry matter (DM) yield across sites of four monocultures (◆) and 11 grass-legume mixtures with strongly varying species proportions (◇) for harvest year 1 within each of four groups of species examined and significance of differences between monocultures and mixtures: ME = mid-European (15 sites), NE = north-European (5 sites), MM = moist Mediterranean (3 sites), DM = dry Mediterranean (2 sites) (taken from Kirwan et al., 2007).

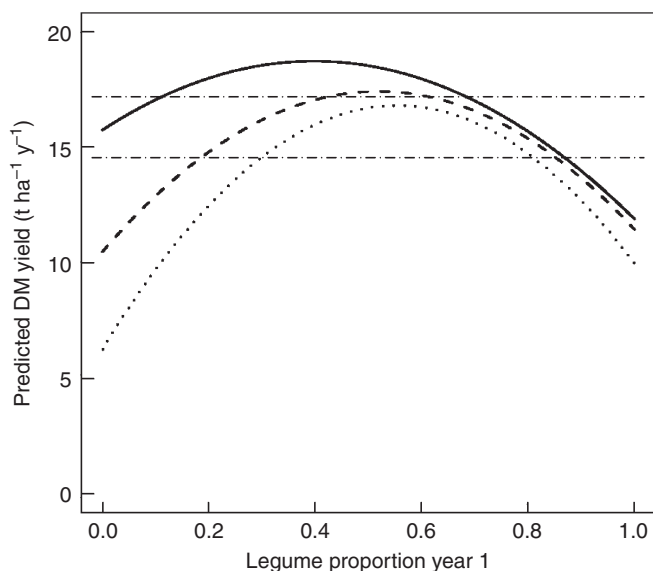


Figure 3. Predicted annual dry matter (DM) yield of mixtures with increasing clover proportions in the sward and three fertiliser N levels in the second experimental year at the Swiss site in Zurich. Predictions are based on regression analysis from 86 experimental plots (for details see Nyfeler et al., 2009). Mixture yields are for N inputs of 50 (.....), 150 (-----), and 450 (—) kg ha⁻¹ y⁻¹. The DM yield of two grass monocultures fertilised with N (450 kg ha⁻¹ y⁻¹) are indicated with the horizontal lines: *Lolium perenne* (14.3 t/ha) and *Dactylis glomerata* (17.2 t/ha).

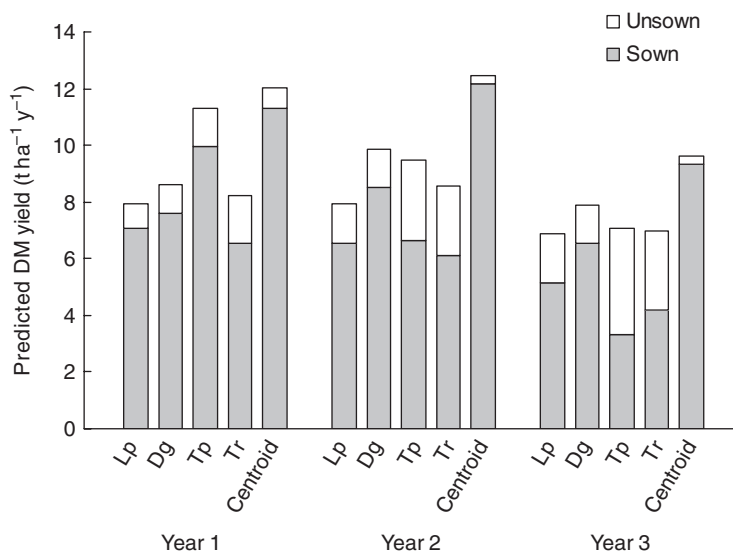


Figure 4. Predicted dry matter (DM) yield of sown and unsown species for each monoculture and the centroid mixture for each year from the combined analysis across mid-European sites. (Lp = *Lolium perenne*, Dg = *Dactylis glomerata*, Tp = *Trifolium pratense*, Tr = *Trifolium repens*, Centroid = mixture with 25% sown proportion of each of the four species) (from Lüscher *et al.*, 2008).

mixtures have significant advantages when compared to their monocultures that are managed at the same cutting frequency and fertiliser input. Hence, it does not matter whether the clover-grass-mixtures are compared to the clover or to the grass monocultures: they were better than any of the monocultures. These advantages of the mixtures were surprisingly robust: they occurred over the whole gradient of climatic conditions from the Mediterranean to the Arctic, and they occurred in every experimental year and over a wide range of clover proportions in the mixture.

Yield benefits of grass-clover mixtures are equivalent to 150–350 kg/ha fertiliser N: Comparing the yield of heavily N-fertilised grass swards with the yield of moderately fertilised grass-clover mixtures illustrates the potential N fertiliser savings associat-

ed with grass-clover mixtures. At the Swiss site in Zurich, additional plots were sown alongside the pan-European experiment to investigate the effect of three fertiliser N levels (50, 150 and 450 kg N ha⁻¹ y⁻¹; for details see Nyfeler *et al.*, 2009). Grass-clover mixtures fertilised with 50 or 150 kg ha⁻¹ y⁻¹ attained annual DM yields in the range of the heavily fertilised (450 kg N per hectare per year) monocultures of the highly productive grass species *Lolium perenne* (14.3 t/ha) and *Dactylis glomerata* (17.2 t/ha) if the mixture's clover percentage was between 30 and 80% (Figure 3). In this experiment, amounts of nitrogen harvested with the sward that derived from symbiotic atmospheric N₂ fixation up to 300 kg/ha if fertiliser N input was low or moderate. This is in line with the range of N derived from symbiosis of 100 to 400 kg ha⁻¹ y⁻¹ measured in other field

experiments under comparable conditions (Boller and Nösberger, 1987; Zanetti *et al.*, 1996; Zanetti *et al.*, 1997; Lüscher *et al.*, 2000; Lüscher and Aeschlimann, 2006). High levels of symbiotic N₂ fixation by legumes was not only found in leys but also in permanent grasslands on different soils and up to high altitudes (Jacot *et al.*, 2000a,b). Symbiotic N₂ fixation is a major reason for the yield advantage of grass-clover mixtures over grass monocultures. There are other reasons, however, including: (i) characteristic within-season growth patterns favouring the grasses in spring during reproductive growth (Menzi, Blum and Nösberger, 1991; Daepf, Nösberger and Lüscher, 2001) and the legumes in summer when temperatures are high (Lüscher, Fuhrer and Newton, 2005), (ii) deep tap roots of *Trifolium pratense* which may be relevant for nutrient (e.g. mineral N) and water uptake from deeper soil horizons in contrast to the shallow-rooting grasses (Boller and Nösberger, 1988; Nyfeler *et al.*, 2009) and (iii) lower variability in mixture yield between years resulting in an advantage in total DM yield over the whole 3 year experimental period (Nyfeler *et al.*, 2009).

The potential of mixed pastures compared to pure grass stands in different soil and climatic conditions in the western part of France was investigated in a study of more than 400 fields in commercial farms over several years (Pflimlin *et al.*, 1993; ITEB, 1997; Institut de l'Élevage, 2004). The study confirmed that the productivity of mixed pastures is directly related to the contribution of clover. It was established that the DM production of mixed pastures increased by 7.2 to 7.9 and 9.2 t/ha for clover contributions of, respectively, less than 20, 20 to 40 and 40 to 60% in summer (Le Gall, 1999). On good and deep soils and with a sufficient water supply in summer, mixed pastures with low or no fertiliser N

input produce almost as much DM as the pure grass pastures receiving N inputs of 200 to 250 kg/ha (9.6 vs. 9.8 t/ha). Under dry conditions, mixed pasture produced less than the pure stands because the reduction or arrest of growth during the summer, does not make it possible to compensate for the late spring start of production for mixed pastures. Mixed pastures also produce less DM (500 kg/ha) than pure grasses on soils susceptible to water-logging (Pflimlin *et al.*, 1993). Production of mixed pasture starts less quickly at the end of winter than the production of pure grass pastures. This effect is all the more marked in cold soils. Indeed the soil temperature must be about 9 °C at least before clover nodules start to absorb atmospheric N. On the other hand, the WC grows at a faster rate than PRG under relatively high temperatures. Therefore mixed pastures generally produce more biomass in summer than pure grass pastures.

Difficulties in maintaining well balanced grass-legume mixtures and their tendency to lose key species (Guckert and Hay, 2001) may be a main reason for the preference of pure grass swards. Indeed in the pan-European experiment legume proportion strongly decreased in the third year. Adaptive management practices with reduced N fertilisation and/or increased cutting frequencies can contribute to increase clover proportion (Schwank, Blum and Nösberger, 1986; Hebeisen *et al.*, 1997; Nyfeler *et al.*, 2009). Optimised composition of the seed mixture (which species included, which cultivar included, which proportions of the species) is another option to ameliorate the stability of grass-clover mixtures. This is evident from the Swiss site of the pan-European experiment where besides the experimental four-species mixtures (described above) also Swiss Standard Mixtures (Suter *et al.*, 2008) were examined. While the decrease

of clover percentage in the sward from the first to the third year was from 35 to 10% for the experimental mixtures, it was only from 43 to 30% for the Swiss Standard Mixtures. Selection of species and cultivars may contribute to maximise gains of mixtures by two ways (Lüscher and Jacquard, 1991): selection for 'combining ability' would result in better resource exploitation through niche differentiation (Hill, 1990), while selection for more balanced 'competitive ability' would result in more balanced and stable mixtures (Lüscher, Connolly and Jacquard, 1992).

Utilisation of forage legumes by ruminants

The nutritional value of forage legumes is high, particularly that of WC: The nutritional advantage of WC over grasses is well established (Thomson, 1984; INRA, 2007). A series of experiments conducted in Rennes with fistulated dairy cows (Peyraud, 1993 and unpublished) has shown that WC increases organic matter (OM) digestibility (0.80 vs. 0.78 kg/kg) and the amount of non-ammonia N entering the intestine (28.9 v 24.3 g/kg DM intake) which reflected the supply of metabolisable protein (expressed by the digestible protein in the small intestine in the French system of feed evaluation (PDI; INRA, 2007) compared to perennial ryegrass (PRG). With WC, both alimentary N flow and efficiency of microbial protein synthesis are increased. These results reflect the absence of structural components which are less digestible than cells contents.

Red clover and LUC are less digestible and their net energy concentration (expressed in UFL in the French system of feed evaluation where 1 UFL = 7.1 MJ; INRA, 2007) is lower than for WC at a similar stage of growth, the difference being more important for LUC (respec-

tively 0.78; 0.86 and 1.01 UFL per 1 kg DM for LUC, RC, and WC; INRA, 2007). These values are further reduced in silage and hay. The low energy concentration may limit the utilisation of LUC in the diet of high producing dairy cows. Therefore LUC, and to a lesser extent RC, should be cut at an early stage of growth. By contrast their protein feed value remains near to 100 g PDIE (PDI when energy is limiting) per 1 UFL when fed as fresh forages (Figure 5), which is close to the recommended level for optimal feeding of dairy cows (INRA, 2007) and higher than recommendations for low producing animals. Hay making allows maintaining the high protein level but ensiling reduces the protein value (-30 g PDIE per 1 UFL).

Voluntary DM intake of legumes measured using sheep at maintenance is 10 to 15% greater than that of grasses of similar digestibility and this is true whether forage legumes are fed as silage, hay or fresh (INRA, 2007). This is illustrated in

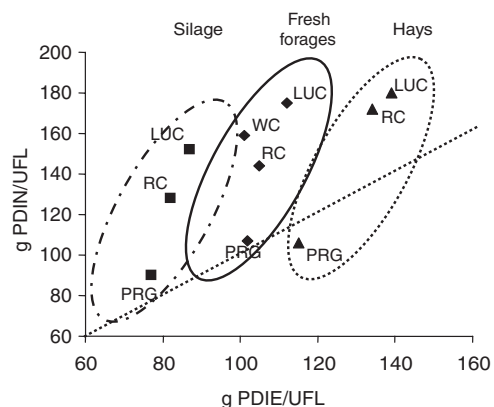


Figure 5. Protein feeding value of legumes compared to perennial ryegrass and fed as fresh forage, silages and hay (adapted from INRA, 2007). WC = white clover, LUC = lucerne; RC = red clover, PRG = perennial ryegrass.

Figure 6 for WC and RC. These differences are attributed to both a lower resistance of legumes to chewing and a higher rate of particles breakdown, digestion and clearance from the rumen (Waghorn, Shelton and Thomas, 1989; Steg *et al.*, 1994). In a recent experiment, Dewhurst *et al.* (2003) reported that silage dry matter intake is increased by 2 to 3 kg when cows are fed RC or WC silage compared to PRG silage. Because WC is often used in mixture with PRG, the question of the optimal proportion of WC in the mixture arises. Harris *et al.* (1998) showed that DM intake was at its maximum when the proportion of WC reached 60% in housed dairy cows.

At grazing, herbage intake is markedly higher (+15 to +20%) with pure legume relative to pure grass pastures (Alder and Minson, 1963). The beneficial effects of WC on animal intake and performance within a WC-grass pasture have been demonstrated by Wilkins *et al.* (1994), the difference increasing with the clover content. In the studies conducted in Rennes (Ribeiro-Filho, Delagarde and Peyraud, 2003; 2005), mixed pastures steadily increased DM intake and milk

yield (on average 1.5 kg/day) whatever the level of herbage allowance. In addition to the positive effect of legumes on voluntary intake, it is also probable that leaves of legumes are more favourable for prehension than stems and sheaths of grasses. Thus Ribeiro-Filho *et al.* (2003) have reported that higher intake on WC-grass pastures is mediated through a higher rate of intake on mixed pastures compared to pure PRG pastures.

One of most decisive advantages of white clover is that the rate of decline of nutritional quality throughout the plant-ageing process is far less than for grasses. Digestibility and voluntary DM intake of grasses decrease by 20 g/kg and 0.2 kg/day per week, respectively, whilst their decline was two times less for WC. In particular, Peyraud (1993) and Delaby and Peccatte (2003) reported digestibility higher than 0.75 kg/kg after 7 weeks regrowth or at flowering stage during the first growth. At grazing the difference in DM intake between pure grass pastures and WC-grass pasture increases with increasing age of regrowth. Ribeiro-Filho *et al.* (2003) showed that herbage DM intake declined

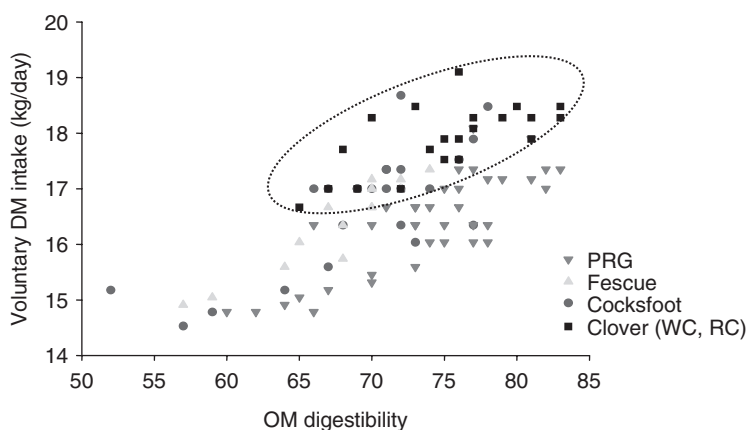


Figure 6. Relationship between organic matter (OM) digestibility and voluntary dry matter (DM) intake.

by 2.0 kg/day on PRG pasture compared to 0.8 kg/day on mixed pastures (Figure 7). This makes mixed pastures easier to manage than pure grass pastures. Age of regrowth can be increased without adverse effects on quality. For LUC and RC, the decline of nutritional quality with advancing maturity is intermediate between WC and grasses (INRA, 2007).

Forage legumes can sustain high animal performances: Several trials have shown that pure legume silage and legume-dominated silage can increase milk production compared to pure grass silage (Castle, Reid and Watson, 1983; Thomas, Aston and Daley, 1985; Dewhurst *et al.*, 2003). Chenais (1993) summarised 10 French experiments on the effect of a mixed diet based on maize silage and RC silage or LUC silage compared to pure maize silage based diets. The mixed diets led to similar dairy performance when the legume silages are of good quality and in particular when their DM concentration is higher than 30%. The LUC silage led to lower milk production than RC silage and reduced body condition score recovery of the cows. In the same way

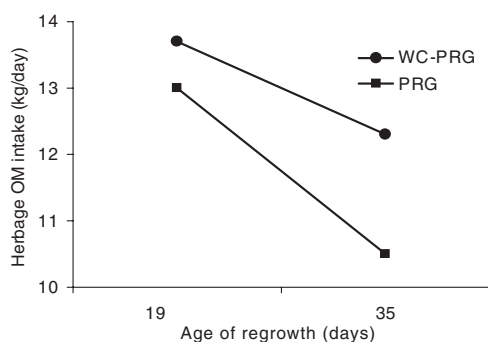


Figure 7. Effect of the age of regrowth on herbage organic matter (OM) intake by dairy cows on mixed (WC-PRG) or pure grass (PRG) pastures (adapted from Ribeiro-Filho *et al.*, 2003).

for beef production, the RC silage makes it possible to obtain growth identical to the maize silage when it is well preserved (Weiss and Raymond, 1993). It should be pointed out that difficulties of conservation (quality of silage; losses of leaves during hay making) are often a hazard for the quality of conserved legumes and that special care must be taken to produce conserved legumes (Arnaud, Le Gall and Pflimlin, 1993).

Increasing the content of WC in pasture increased milk yield by 1 to 3 kg per cow per day in several short term trials when stocking rate was reduced on the WC-grass pastures so that the same amount of herbage DM was on offer ($\text{kg cow}^{-1} \text{day}^{-1}$) in the two systems (Philips and James, 1998; Philips, James and Nyallu, 2000; Ribeiro-Filho *et al.*, 2003). The difference increases with clover content and reaches a maximum when WC content averages 50 to 60% (Harris *et al.*, 1998). As a consequence of higher energy intake, milk protein concentration tends to increase on WC-grass pastures. However, in trials carried out over several years, with well managed pure grass pasture and WC content between 20 to 40% the performances are comparable between the two types of pastures (Figure 8). For example, in the trials carried out in Brittany (Pflimlin, 1993), the milk yield was identical between PRG pastures receiving fertiliser N input of 350 kg/ha and WC-grass pastures with no fertiliser N and grazed with a 10 day longer regrowth period to achieve similar biomass per hectare in the two systems. Milk yield was reduced when the proportion of clover was low (<20%, Institut de l'Elevage, 2004). As mixed pastures are managed with very low fertiliser N inputs, the biomass per hectare is often lower compared with highly fertilised PRG pastures at a same age of regrowth. To allow the same amount of herbage the

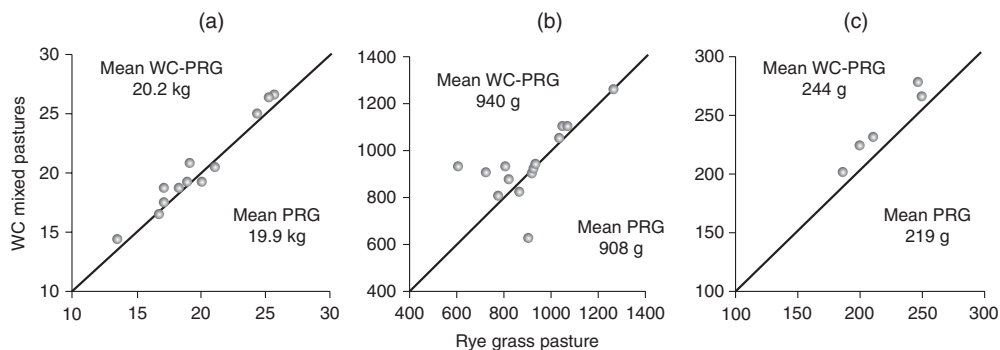


Figure 8. Comparison of animal performance (a = milk yield; b = growth rate of cattle, c = growth rate of lambs) grazing on pure perennial rye grass pasture or on WC-rich pasture (adapted from Institut de l'Élevage, 2004).

stocking rate should be reduced on WC-grass pastures thus penalizing productivity. Another alternative is to extend the regrowth period. The main advantage of WC-grass pastures is their good flexibility which allows intervals between two successive grazing of more than 4 to 6 weeks in summer for compensating lower productivity without penalizing the performances of the cows.

Growth rate of growing cattle (heifers, beef) do not largely differ with the type of pasture (Figure 8). Nonetheless on set stocked swards maintained at a similar height growth rate tends to be higher on WC mixed sward. The pasture with legume grass mixtures led to a little higher growth of lambs than fertilised grass pastures (Figure 5 and Speijers *et al.*, 2004). Orr *et al.* (1990) showed that continuously grazed WC mixed swards managed at 6 cm allowed the same lamb growth rate as highly fertilised PRG pastures. WC-grass pastures increased the lamb growth rate when they were managed at 9 cm but reduced live weight output per hectare as stocking rate was reduced. Red clover is rarely used for grazing. However, one trial reported a significantly higher live weight gain for finishing lamb grazing of

RC mixed pastures compared to PRG pastures (Fraser *et al.*, 2004).

Forage legumes improve nutritional quality of lipids in ruminant products: Reducing the intake of saturated fatty acids (SFA) and increasing the intake of omega-3 polyunsaturated fatty acids (omega-3 PUFA) are highly recommended to reduce cardiovascular risk (WHO, 2003). Because ruminant products are rich in SFA and poor in omega-3 PUFA compared to oils, fish, eggs and others meats, there is huge interest in decreasing SFA in ruminant products and increasing omega-3 PUFA. The dominant omega-3 PUFA in ruminant product is α -linolenic (C18:3 n-3). The dominant conjugated linoleic acid (CLA) in ruminant products is the cis-9, trans 11 isomer (rumenic acid, RU) and it has been shown to exert anti-carcinogenic properties in a range of human cell lines (De la Torre *et al.*, 2006). There is also interest in increasing this fatty acid.

Feeding fresh PRG compared to maize silage diets results in higher concentration of linolenic acid (0.7 vs. 0.2%), lower proportion of SFA (29 v 36%) and higher proportion of RU (1.7 v 0.5%) in milk (Couvreur *et al.*, 2006). Botanical

composition of herbage is another factor of variation. Grazing WC or RC mixed pastures relative to PRG pastures increases the deposition of α -linolenic acid in muscle lipids and subcutaneous fat of cattle and sheep (Scollan *et al.*, 2002; Lourenço *et al.*, 2007) but hardly affects SFA and rumenic acid deposition. It also increases the deposition of linoleic acid (C18:2 n-6) which is not considered as a positive effect from a nutritional point of view. However the increase is greater for C18:3 n-3; thus the ratio n-6/n-3 is improved when forage legumes are fed. Feeding mixture of grass and clover silages (both WC and RC) relative to grass silage alone increased the deposition of linolenic acid in milk fat (Dewhurst *et al.*, 2006) and in muscle of cattle (Scollan *et al.*, 2006) but again rumenic acid deposition is unaffected and C18:2 n-6 deposition increases slightly. Feeding dehydrated LUC in a maize-based diet also increases linolenic acid concentration in milk (Peyraud and Delaby, 2002). Among legumes, RC seems to be more efficient than WC for increasing α -linolenic concentration in animal product (Dewhurst *et al.*, 2006).

The presence of forage legumes in the diet of ruminants has been associated with an increased rumen PUFA outflow which is likely due to a faster rate of passage limiting forage PUFA hydrogenation (Dewhurst *et al.*, 2006). For RC this also might be associated with the presence of polyphenol oxidase, which reduces lipolysis during ensiling (Lourenço, Van Ranst and Fievez, 2005) and rumen fatty acid hydrogenation (Lee *et al.*, 2007).

But utilisation of N from forage legumes by the ruminants is inefficient: Ruminal N losses in ruminants that are fed legumes are always high due to the unbalanced level of degradable N and fermentable

energy in the forage. This leads to an inefficient utilisation of forage N and high urinary N excretion. This is clearly illustrated by the studies conducted in Rennes with fistulated cows (Peyraud, 1993). White clover increases N excretion relative to PRG from 20.1 to 29.8 g/kg DM intake and duodenal N is always far below N intake, averaging 75% of N intake with WC while it averaged 93% for PRG. Therefore N excretion at grazing should increase on mixed pasture. From the data of Ribeiro-Filho *et al.* (2005) it could be calculated that N excretion increased on mixed pastures compared to ryegrass pastures from 17.0 to 20.7 g/kg milk.

Lucerne and RC also contain high levels of rapidly degradable N and this is reflected in the difference between PDIN and PDIE values (INRA, 2007, see Figure 2). The situation is worse when considering silages, as the difference between PDIN and PDIE still increases compared to the fresh forages (Figure 5). The supplementation with energy-rich concentrate which is required to overcome the relatively low energy concentration of legume silage will reduce urinary N losses (Cohen, Stockdale and Doyle, 2006). Legume silages or dehydrated Lucerne (Peyraud, Delaby and Marquis, 1994) might be good companions of maize silage in mixed diets as they may provide both degradable and undegradable protein and they offer some potential to substitute purchased soybean meal with home grown proteins. The selection of legumes with high concentration of polyphenol oxidase, which reduces ruminal proteolysis (Jones, Muck, and Hatfield, 1995), or the use of some essential oils (McIntosh *et al.*, 2003; Newbold *et al.*, 2004) are promising ways to reduce the imbalance between degradable N and fermentable energy in diets based on legume-dominated silage.

Forage legume based systems have potential for reducing negative effects of livestock systems on the environment

Despite an apparently negative effect on N excretion by the ruminants, legumes provide opportunities for reducing N losses at the system level. For example at grazing, it should be kept in mind that a molecule of N can be ingested by the ruminant, then excreted on the pasture, re-incorporated in the forage (and/or reorganised in soil OM) and ingested again without being lost outside of the system. Thus high urinary losses at the animal level do not necessarily imply high levels of nitrate leaching because a lot of factors may interact at larger scales. Legumes also provide opportunities for reducing the consumption of non-renewable energy and associated greenhouse gas emissions.

Grass-clover pastures reduce the risk of nitrate leaching compared to highly fertilised pure grass pastures: Increasing the proportion of WC in PRG pastures at the expense of mineral N fertilisation has been suggested as an important component of low-input sustainable systems for livestock production (Thomas 1992; Pflimlin *et al.*, 2003).

Milk yield and the quantities of N excreted per grazed hectare can be expressed as the product of daily milk yield or daily N excretion per cow and the number of grazing days (GD) per hectare, where GD represents the number of grazing days realised per hectare over the grazing season. The N surplus at field level can be calculated as the difference between total N input on the field (i.e. fertilizer, concentrate, symbiotic fixation, atmospheric deposition) and total N output (i.e. milk, harvested forages and transfer of N from the field to the lanes and milking shed via excreta) (Farrugia *et al.*, 1997). The transfer of N from the

field to the lanes and milking shed via cow excreta corresponds to a removal from the pasture of 15% (Ledgard, Penno and Sprosen, 1999) to 20% (Delaby *et al.*, 1997) of the N excreted. The internal N flows (i.e. N produced in grass and grazed by the cows, N excreted on the field and N falling from grass herbage in litter) are not taken into account in the calculation of the N surplus at field level.

Ledgard *et al.* (1999) have measured the N inputs and outputs and N flows over 3 years in a trial involving three dairy farmlets (i.e., the herd and the experimental area required to feed the herd). Each farmlet received a specific input of fertilizer N (Table 1). The grass-clover pastures were grazed throughout the year. N fertilisation reduced white clover content by 70%. On the 0 N farmlet, N₂ fixation by WC was the sole source of N input and total N inputs were only 40% of those in the 400 N farmlet. N₂ fixation decreased with increasing level of N fertilisation as clover proportion in the pasture decreased. Milk N represented the major form of N output. Compared to white clover, fertiliser input resulted in a small increase in removal of N via milk. The response was lower than that reported by Delaby and Peyraud (1998) mainly because the stocking rate was kept constant in this experiment. The results clearly indicate that from an environmental point of view, intensively managed WC-grass pastures are relatively efficient in terms of conversion of N inputs from N₂ fixation into milk and to reduce N surplus at the field level.

However, total N inputs in WC-grass pastures are very dependant on N₂ fixation by WC as the sole main source of N. In the study of Ledgard *et al.* (1999), the contribution from this source varied by up to 2 fold between years (101 to 235 kg/ha) mainly due to climatic differences.

The challenge of these mixed pastures will be to maintain a clover content of around 30 to 40% to allow sufficient grass production and stability of the system from year to year. Indeed, the production of mixed pastures is most often lower than that observed on highly fertilised grass pastures.

In their study, Ledgard *et al.* (1999) have quantified the different routes of N surplus. They have shown that nitrate-N is the major form of N loss to the environment. Leaching of nitrate-N was minimal for the WC based system and increased very rapidly with the level of fertilisation and decreased proportion of clover (Table 1). Losses of N by denitrification remained small but were reduced on WC-grass pastures. Hooda *et al.* (1998) has also reported lower annual nitrate leaching losses from intensively managed WC-grass pastures compared to pure PRG pastures receiving fertiliser N input of 250 kg/ha although both pastures annually received more than 150 kg/ha slurry N. In the study of Ledgard *et al.* (1999), the amount of nitrate-N leached varied greatly (20 to 74 kg/ha) in the WC-grass pastures and these variations are linked to the N₂ fixation by WC. Loiseau *et al.* (2001) have reported higher leaching losses from lysimeters, when pastures were sown with pure WC compared to PRG (28 to 140 kg/ha N) whereas the losses from WC-grass pastures were lower than 20 kg/ha over the 6 years of the experiment. Indeed leaching under WC-grass pastures would rise with increasing legume content as shown by Schils (1994) because level of N₂ fixation per hectare, dry matter yield and stocking rate can increase. Mixed pastures reduce the risk on nitrate leaching primarily because they can not sustain the same level of stocking than highly fertilized grasses pastures.

Table 1. Annual N inputs and outputs (kg/ha) for dairy farmlets varying in N fertilizer input and white clover content¹

Item	Fertiliser N input (kg/ha)		
	0	200	400
Stocking rate (cow/ha)	3.3	3.3	3.3
N input from			
Fertiliser	0	215	413
N ₂ fixation	174	117	40
Purchased feed	3	4	3
Atmospheric deposition	2	2	2
N output via			
Milk and meat	80	95	98
Harvested forage	1	15	28
Transferred N excreta ²	57	78	84
N surplus ³	41	150	248
Denitrification	5	15	25
Volatilisation	16	38	61
Leaching	40	79	150
N balance ⁴	-20	18	12

¹ Adapted from Ledgard, Penno and Sprosen (1999) – mean of 3 years with 3.3 cow/ha.

² 20% of total N excreted is excreted outside.

³ Calculated as N in fertiliser + N in feed + N deposition + N fixation – N in products – N transferred from the field to the lanes and milking shed – N harvested as grass.

⁴ Calculated as N surplus – N losses.

Forage legumes can contribute to reduce global warming potential and energy consumption from livestock production systems: A report by FAO in 2006 stressed the issue of greenhouse gas (GHG) production from livestock systems. Even if its conclusions are debatable, it is extremely probable that the limitation of GHG emissions (and/or development of mitigation strategies) will be a new constraint for livestock systems in the future. In addition, the expected increased price for fossil energy (and thus of N fertiliser) could strongly modify ruminant systems and in particular dairy systems.

Methane produced in the rumen is a large contributor to the GHG emissions of dairy systems. Methane produced in the rumen represents 8% of the gross

energy intake (Vermorel, 1988). Many nutritional strategies are currently being explored to reduce ruminal methanogenesis. Utilisation of plant secondary compounds (tannins, saponins, essential oils) produces highly variable responses (Martin, Doreau and Morgavi, 2008). Actually, the most promising alternative is the addition of dietary fats and special attention should be paid to linoleic acid (Beauchemin *et al.*, 2008; Martin *et al.*, 2008), which also contributes to improved quality of fatty acids in ruminant products. Many data suggest that a high proportion of concentrates in the diet leads to a sharp reduction in methane production (Martin *et al.*, 2008), however, because other factors affect the total GHG emissions (i.e. extra concentrate needs to be grown, processed and associated GHG emissions need to be accounted for) this strategy needs to be evaluated at the whole farm level. Legumes might contribute to reduced ruminal methane production. Animals fed legume forages emit less methane compared to emission from grass-fed animals (Beever *et al.*, 1985; Waghorn, Tavendale and Woodfield, 2006). This may be due to the high digestibility (in the case of WC) and thus a modified ruminal fermentation pattern toward propionate (which is a hydrogen

carrier) combined with an increased passage rate of legume particles.

However it is at the level of the whole system that the strength of forage legumes lies. Global warming potential (GWP) of livestock systems include methane emission, N₂O emission and CO₂ emission. GWP of methane and N₂O are respectively 24 and 310 times higher than that of CO₂. Basset-Mens, Ledgard and Carran (2005) have compared, using life cycle assessment (LCA) and emission coefficients, the GWP for some European milk production systems, in Sweden (Cederberg and Mattsson, 2000), Southern Germany (Hass, Wetterich and Köpke, 2001) and the New Zealand dairy farm system (Table 2). The New Zealand system relies essentially on permanent WC-grass pastures grazed all around the year with a fertiliser N input of 100 kg/ha per year and less than 10% of the feed requirement of the cows provided by feed supplements. They showed that global warming potential per 1 kg milk is 30 to 80% lower in the New Zealand system. The breakdown of the three main emissions are similar between New Zealand and European organic system and extensive systems, 60% of the GWP was due to methane emission during digestion and 40% was associated with the production of pasture and crops for feed

Table 2. Comparison of global warming potential (GWP) and energy use consumption for different milk production systems¹

	Production system			
	NZ	Organic (Sweden)	Conventional extensive (Germany)	Conventional intensive (Sweden, Germany)
GWP (CO ₂ -equivalent g/kg milk)	718	950	1000	1200
Percentage due to				
Digestive CH ₄	57		60	50
Pasture and crop production	40 ²		40 ²	30
As CO ₂	9		10	20
Energy use (MJ/kg milk)	1.5	2.5	1.3	3.1

¹ Adapted from Basset-Mens, Ledgard and Carran (2005).

² Mainly due to N₂O which constitute 85% of the total.

supplement. In intensive European dairy farms, where utilisation of legumes is rare and cows are offered high amounts of concentrates, GWP is high, the contribution of methane is reduced in proportion at the expense of CO₂ contribution, which dramatically increased (3.7 times higher than for New Zealand system) due to the production and transport of concentrated feed and mineral fertiliser and also linked to effluent management. Schils *et al.* (2005) compared the GWP of dairy systems in Netherlands, either based on fertilised ryegrass or on grass-clover pastures (i.e. mineral N inputs of 208 and 17 kg ha⁻¹ year⁻¹), using IPCC emission factors. Calculated N₂O emissions and total GWP per 1 kg of milk were 20% lower for the system based on grass-clover pastures. Similarly, under New Zealand conditions with grazing as the sole feed source for dairy cows, GWP per kilogram of milk was reduced by 15% for grass-clover pasture receiving zero N compared with a system receiving annual fertiliser N input of 139 kg/ha (Basset-Mens, Ledgard and Boyes, 2009). In these two trials the lower GWP for grass-clover pasture receiving low/zero N fertiliser inputs was mainly due to the reduction in N₂O emissions.

Ruminant livestock production systems relying on grazing and legume utilisation can reduce energy consumption because in such pasture-based systems, cows ingest their feed from, and apply their excreta directly on, pasture. This reduces the energy consumption, and all associated impacts, for manure management and feed distribution as well as to produce, transport and spread mineral N. Energy efficiency, calculated as herbage UFL produced per 1 MJ of energy consumed is 3 times higher for WC-grass pastures compared to fertilised grasses pastures (2.5 v 0.8 UFL/MJ, Besnard, Montarges-Lellahi and Hardy, 2006). It is also noticeable

that total energy consumption per 1 kg of milk is two times less in the New Zealand system than in intensive systems (Table 2). Similarly, Le Gall (unpublished) has shown that energy consumption decreases from 5.0 MJ/kg milk for intensive dairy farm in Netherlands to 4.0 MJ/kg for French farms using maize silage and fertilised grasses and to 3.1 and 1.4 MJ/kg for systems based on grazing in Ireland and in New Zealand, respectively.

The higher energy consumption in Irish grassland-based systems appears to be linked to the utilisation of high amounts of N fertiliser on pure PRG pastures. Data in literature are still relatively scarce and it is worthy of further investigation to precisely quantify the benefits of forage legumes used for grazing or as conserved forages in comparison to pure grasses and maize silage based systems.

Conclusions

The previous and new interest in forage legumes are numerous and should become more important during the next years. New opportunities have emerged in recent years to match in a positive way the environmental stakes of livestock farming. The reduction in the consumption of fossil energy and in its effects on greenhouse gas emissions, the limitation of N losses, the protein self-sufficiency at farm and country levels, the significant development of organic farming and the emergence of consumer demand for quality and image of livestock products are factors which open new prospects for development of forage legumes. The development of forage legumes undoubtedly constitutes one of the pillars for the development of future livestock farming systems with high environmental and economic performances. But they still suffer from a number of limitations. The har-

vest of forage legume is expensive and it is still difficult to produce conserved forages of high quality. In several European countries maize silage is a major competitor because of its ease of production and conservation. Finally, bloat remains a specific health problem related to the use of legumes. All aspects of forage legume production and utilisation should be re-examined such as selection of varieties adapted to production in mixed pastures, management of these pastures (fertilisation, weed control, etc.), animal nutrition and management and evaluation of the whole system.

References

- Alder, F.E. and Minson, D.J. 1963. The herbage intake of cattle grazing lucerne and cocksfoot pasture. *Journal of Agricultural Science, Cambridge* **60**: 359–369.
- Arnaud, J.D., Le Gall, A. and Pflimlin A. 1993. Evolution des surfaces en légumineuses fourragères en France. *Fourrages* **134**: 145–154.
- Basset-Mens, C., Ledgard, S. and Carran, A. 2005. First life cycle assessment of milk production from New Zealand dairy farm systems. www.anzsee.org/anzsee2005papers/Basset-Mens_LCA_NZ_milk_production.pdf.
- Basset-Mens, C., Ledgard, S.F. and Boyes, M. 2009. Eco-efficiency of intensification scenarios for milk production in New Zealand. *Ecological Economics* **68**: 1615–1625.
- Beauchemin, K.A., Kreuzer, M., O'Mara, F. and McAllister, T.A. 2008. Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture* **48**: 21–27.
- Beever, D.E., Thomson, D.J., Ulyatt, M.J., Cammell, S.B. and Spooner, M.C. 1985. The digestion of fresh perennial ryegrass (*Lolium perene* L. cv. Melle) and white clover (*Trifolium repens* L. cv. Blanca) by growing cattle fed indoors. *British Journal of Nutrition* **54**: 763–775.
- Besnard, A., Montarges-Lellahi, A. and Hardy, A. 2006. Systèmes de culture et nutrition azotée. Effets sur les émissions de GES et le bilan énergétique. *Fourrages* **187**: 311–320.
- Boller, B.C. and Nösberger, J. 1987. Symbiotically fixed nitrogen from field-grown white and red clover mixed with ryegrasses at low levels of ¹⁵N-fertilization. *Plant and Soil* **104**: 219–226.
- Boller, B.C. and Nösberger, J. 1988. Influence of dissimilarities in temporal and spatial N-uptake patterns on ¹⁵N-based estimates of fixation and transfer of N in ryegrass clover mixtures. *Plant and Soil* **112**: 167–175.
- Castle, M.E., Reid, D. and Watson, J.N. 1983. Silage and milk production: studies with diets containing white clover silage. *Grass and Forage Science* **38**: 193–200.
- Cederberg, C. and Mattsson, B. 2000. Life cycle assessment of milk production – a comparison of conventional and organic farming. *Journal of Cleaner Production* **8**: 49–60.
- Chenais, F. 1993. Ensilage de légumineuses et production laitière. *Fourrages* **134**: 258–265.
- Cohen, D.C., Stockdale, C.R. and Doyle, P.T. 2006. Feeding an energy supplement with white clover silage improves rumen fermentation, metabolisable protein utilisation and milk production in dairy cows. *Australian Journal of Agricultural Research* **52**: 415–425.
- Couvreur, S., Hurtaud, C., Lopez, C., Delaby, L. and Peyraud, J.L. 2006. The linear relationship between the proportion of fresh grass in the cow diet and milk fat characteristics and butter properties. *Journal of Dairy Science* **89**: 1956–1969.
- Daepf, M., Nösberger, J. and Lüscher, A. 2001. Nitrogen fertilization and developmental stage alter the response of *Lolium perenne* to elevated CO₂. *New Phytologist* **150**(2): 347–358.
- Decau, M.L., Delaby, L. and Roche, B. 1997. Azopât: une description quantifiée des flux annuels d'azote en prairie pâturée par les vaches laitières. II- Les flux du système sol-plante. *Fourrages* **151**: 313–330.
- Delaby, L., Decau, M.L., Peyraud, J.L. and Accarie, P., 1997. AzoPât: une description quantifiée des flux annuels d'azote en prairie pâturée par les vaches laitières. I. Les flux associés à l'animal. *Fourrages* **151**: 297–311.
- Delaby, L. and Peccatte, J.R. 2003. Valeur alimentaire des prairies d'association ray grass anglais/trèfle blanc utilisées entre 6 et 12 semaines de repousse. *Rencontres Recherches Ruminants* **10**: 389.
- Delaby, L. and Peyraud, J.L., 1998. Effet d'une réduction simultanée de la fertilisation azotée et du chargement sur les performances des vaches laitières au pâturage. *Annales de Zootechnie* **47**: 17–39.
- De la Torre, A., Debiton, E., Juanada, P., Durand, D., Chardiny, J.M., Barthomeuf, C., Bauchart, D. and Gruffat, D., 2006. Beef conjugated linoleic acid isomers reduce human cancer cell growth even when associated with other beef fatty acids. *British Journal of Nutrition* **95**: 346–352.

- Dewhurst, R.J., Fisher, W.J., Tweed, J.K.S. and Wilkins, R.J. 2003. Comparison of grass and legume silages for milk production. 1. Production response with different levels of concentrate. *Journal of Dairy Science* **86**: 2598–2611.
- Dewhurst, R.J., Shingfield, K.J., Lee, M.R.F. and Scollan, N.D. 2006. Increasing the concentration of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* **13**: 168–206.
- Farrugia, A., Decau, M.L., Vertès, F. and Delaby, L. 1997. En prairie, la balance azotée à l'échelle de la parcelle. *Fourrages* **151**: 281–296.
- Food and Agriculture Organisation (FAO). 2006. Livestock's Long Shadow, Environmental issues and options. United Nations Food and Agriculture Organisation, Rome, 390 pages.
- Fraser, M.D., Speijers, M.H.M., Theobald, V.J., Fychan, R. and Jones, R. 2004. Production performance and meat quality of grazing lambs finished on red clover, lucerne or perennial ryegrass swards. *Grass and Forage Science* **59**: 345–356.
- Frey, E. 1955. Neue Standardmischungen für den Futterbau. *Mitteilungen für die Schweizerische Landwirtschaft* **3(9)**: 129–142.
- Gately, T.F. 1982. Evolution of the role of white clover for milk production. *Grass and Forage Science* **37**: 171–172.
- Guckert, A. and Hay, R.K.M. 2001. The overwintering, spring growth, and yield in mixed species swards, of white clover in Europe. *Annals of Botany* **88**: 667–668.
- Harris, S.L., Auld, M.J., Clark, D.A. and Jansen, E.B.L. 1998. Effect of white clover content in the diet on herbage intake, milk production and milk composition of New Zealand dairy cows housed indoors. *Journal of Dairy Research* **65**: 389–400.
- Hass, G., Wetterich, F. and Köpke, U. 2001. Comparing intensive, extensive and organic grassland farming in southern Germany by process life cycle assessment. *Agriculture, Ecosystems and Environment* **83**: 43–53.
- Hebeisen, T., Lüscher, A., Zanetti, S., Fischer, B.U., Hartwig, U.A., Frehner, M., Hendrey, G.R., Blum, H. and Nösberger, J. 1997. Growth response of *Trifolium repens* L. and *Lolium perenne* L. as monocultures and bi-species mixture to free air CO₂ enrichment and management. *Global Change Biology* **3**: 149–160.
- Hill, J. 1990. The 3 Cs – competition, coexistence and coevolution – and their impact on the breeding of forage crop mixtures. *Theoretical and Applied Genetics* **79**: 168–176.
- Hooda, P.S., Moynagh, M., Svoboda, I.F. and Anderson, H.A., 1998. A comparative study of nitrate leaching from intensively managed monoculture grass and grass-clover pastures. *Journal of Agricultural Science, Cambridge* **131**: 267–275.
- Institut de l'Élevage. 2004. Associations graminées – Trèfle blanc, Le pâturage gagnant. Collection Synthèses, 64 pages.
- Institut National de la Recherche Agronomique (INRA). 2007. Alimentation des bovins, ovins et caprins, Quae Eds, Versailles, France, 330 pages.
- ITEB – EDE de Bretagne. 1997. Des pâtures riches en trèfle blanc: pourquoi, comment? Technipel, Paris, France, 50 pages.
- Jacot, K.A., Lüscher, A., Nösberger, J. and Hartwig, U.A. 2000a. Symbiotic N₂ fixation of various legume species along an altitudinal gradient in the Swiss Alps. *Soil Biology and Biochemistry* **32**: 1043–1052.
- Jacot, K.A., Lüscher, A., Nösberger, J. and Hartwig, U.A. 2000b. The relative contribution of symbiotic N₂ fixation and other nitrogen sources to grassland ecosystems along an altitudinal gradient in the Alps. *Plant and Soil* **225**: 201–211.
- Jones, B.A., Muck, R.E. and Hatfield, R.D. 1995. Red clover extract inhibits legume proteolysis. *Journal of the Science of Food and Agriculture* **67**: 329–333.
- Kessler, W. and Suter, D. 2004. The role of grass-clover mixtures in Swiss agriculture. In: "Adaptation and Management of Forage Legumes – Strategies for Improved Reliability in Mixed Swards" (eds. B.E. Frankow-Lindberg, R.P. Collins, A. Lüscher, M.T. Sébastia and A. Helgadottir), SLU Service/Repro, Uppsala, Sweden, pages 176–182.
- Kirwan, L., Connolly, J., Finn, J.A., Brophy, C., Lüscher, A., Nyfeler, D. and Sebastia, M.T. 2009. Diversity-interaction modelling: estimating contributions of species identities and interactions to ecosystem function. *Ecology* **90(8)**: 2032–2038.
- Kirwan, L., Lüscher, A., Sebastia, M.T., Finn, J.A., Collins, R.P., Porqueddu, C., Helgadottir, A., Baadshaug, O.H., Brophy, C., Coran, C., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B.E., Golinski, P., Grieu, P., Gustavsson, A.M., Höglind, M., Huguenin-Elie, O., Iliadis, C., Jørgensen, M., Kadziulienė, Z., Karyotis, T., Lunnan, T., Malengier, M., Maltoni, S., Meyer, V., Nyfeler, D., Nykanen-Kurki, P., Parente, J., Smit, H.J., Thumm, U. and Connolly, J. 2007. Evenness drives consistent diversity effects in an intensive grassland system across 28 European sites. *Journal of Ecology* **95(3)**: 530–539.
- Ledgard, S.F., Penno, J.W. and Sprosen, M.S. 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitro-

- gen fertilizer application. *Journal of Agricultural Science, Cambridge* **132**: 215–225.
- Le Gall, A. 1999. Le trèfle blanc; un moyen économe d'assurer la nutrition azotée des prairies. Journée technique Fertilisation azotée des prairies dans l'Ouest, Rennes.
- Le Gall, A. 1993. Les grands légumineuses: situation actuelle, atouts et perspectives dans le nouveau paysage fourrager français. *Fourrages* **134**: 121–144.
- Lee, M.R.R., Parfitt, L.J., Scollan, N.D. and Minchin, F.R. 2007. Lipolysis in red clover with different polyphenol oxidase activities in the presence or absence of rumen fluid. *Journal of the Science of Food and Agriculture* **87**: 1308–1314.
- Loiseau, P., Carrere, P., Lafarge, M., Delpy, R. and Dublanquet, J. 2001. Effect of soil-N and urine-N on nitrate leaching under pure grass, pure clover and mixed grass/clover pastures. *European Journal of Agronomy* **14**: 113–121.
- Lourenço, M., Van Ranst, G. and Fievez, V. 2005. Difference in extend of lipolysis in red or white clover and rye grass silage in relation to polyphenol oxidase activity. *Communications in Agriculture and Applied Biological Science* **70**: 169–172.
- Lourenço, M., Van Ranst, G., De Smet, S., Raes, K. and Fievez, V. 2007. Effect of botanical composition of silages on rumen fatty acid metabolism and fatty acid composition in longissimus muscle and subacute fat of lamb. *Animal* **1**: 911–921.
- Lüscher, A. and Aeschlimann, U. 2006. Effects of elevated [CO₂] and N fertilization on interspecific interactions in temperate grassland model ecosystems. *Ecological Studies* **187**: 337–350.
- Lüscher, A. and Jacquard, P. 1991. Coevolution between interspecific plant competitors. *Trends in Ecology and Evolution* **6**: 355–358.
- Lüscher, A., Connolly, J. and Jacquard, P. 1992. Neighbor specificity between *Lolium perenne* and *Trifolium repens* from a natural pasture. *Oecologia* **91**: 404–409.
- Lüscher, A., Finn, J.A., Connolly, J., Sebastia, M.T., Collins, R., Fothergill, M., Porqueddu, C., Brophy, C., Huguenin-Elie, O., Kirwan, L., Nyfeler, D. and Helgadottir, A. 2008. Benefits of sward diversity for agricultural grasslands. *Biodiversity* **9**: 29–32.
- Lüscher, A., Fuhrer, J. and Newton, P.C.D. 2005. Global atmospheric change and its effect on managed grassland systems. In: "Grassland: A global resource" (ed. D.A. McGilloway), Wageningen Academic Press, Wageningen, The Netherlands, pages 251–264.
- Lüscher, A., Hartwig, U.A., Suter, D. and Nösberger, J. 2000. Direct evidence that symbiotic N₂ fixation in fertile grassland is an important trait for a strong response of plants to elevated atmospheric CO₂. *Global Change Biology* **6**: 655–662.
- Martin, C., Doreau, M. and Morgavi, D.P. 2008. Methane mitigation in ruminants: from rumen microbe to the animal. In "Livestock and Global Climate Change" (Eds. P. Rowlinson, M. Steele and A. Nefzaoui), Cambridge University Press, Cambridge, UK, pages 130–133.
- McIntosh, F.M., Williams, P., Losa, R., Wallace, R.J., Beever, D.A. and Newbold, C.J. 2003. Effects of essential oils on ruminal microorganisms and their effects on protein metabolism. *Applied and Environmental Microbiology* **69**: 5011–5014.
- Newbold, C.J., McIntosh, F.M., Williams, P., Losa, R. and Wallace, R.J. 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. *Animal Feed Science and Technology* **114**: 105–112.
- Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J. and Lüscher, A. 2009. Strong mixture effects among four species in fertilised agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* **46**: 683–691.
- Orr, R.J., Parson, A.J., Penning, P.D. and Treacher, T.T. 1990. Sward composition, animal performance and the potential production of grass/white clover swards continuously stocked with sheep. *Grass and Forage Science* **45**: 325–336.
- Peyraud, J.L. 1993. Comparaison de la digestion du trèfle blanc et des graminées prairiales chez la vache laitière. *Fourrages* **135**: 465–473.
- Peyraud, J.L. and Delaby, L. 2002. Introduction of dehydrated Lucerne in long form or straw into diets of high producing dairy cows. In "Multi Function Grassland, Quality Forages, Animal Products and Landscapes", (Eds. J.L. Durand, J.C. Emile, Ch. Huyghe and G. Lemaire). Proceedings of the 19th General Meeting of the European Grassland Federation, La Rochelle, France, pages 224–225.
- Peyraud, J.L. and Delaby, L. 2006. Grassland management with emphasis on N flows. In: "Fresh Herbage for Dairy Cattle, the Key to a Sustainable Food Chain", (Eds. A. Elgersma, J. Dijkstra and S. Tamminga). Wageningen UR Frontis Seris, Wageningen, The Netherlands, pages 103–124.
- Peyraud, J.L., Delaby, L. and Marquis, B. 1994. Intérêt de l'introduction de luzerne déshydratée en substitution de l'ensilage de maïs dans les rations des vaches laitières. *Annales de Zootechnie* **43**: 91–104.
- Pfimlin, A. 1993. Conduite et utilisation des associations graminées-trèfle blanc. *Fourrages* **135**: 407–428.

- Pflimlin, A., Annezo, J.F., Le Gall, A., Boscher, A., Bayon, D., Henot, A.Y., Kerouanton, J., Le Viol, F. and Lymes, T. 1993. Intérêt des prairies de ray-grass anglais-trèfle blanc dans les exploitations laitières bretonnes. *Fourrages* **135**: 389–398.
- Pflimlin, A., Arnaud, J.D., Gautier, D. and Le Gall, A. 2003. Les légumineuses fourragères, une voie pour concilier autonomie en protéines et préservation de l'environnement. *Fourrages* **174**: 183–203.
- Philips, C.J.C. and James, N.L. 1998. The effects of including white clover in perennial ryegrass pastures and the height of mixed pastures on the milk production, pasture selection and ingestive behaviour of dairy cows. *Animal Science* **67**: 195–202.
- Philips, C.J.C., James, N.L. and Nyallu, H.M. 2000. The effects of forage supplements on the ingestive behaviour and production of dairy cows grazing ryegrass only or mixed ryegrass and white clover pastures. *Animal Science* **70**: 555–559.
- Ribeiro-Filho, H.M.N., Delagarde, R. and Peyraud J.L. 2003. Inclusion of white clover in strip-grazed perennial ryegrass pastures: herbage intake and milk yield of dairy cows at different ages of pasture regrowth. *Animal Science* **77**: 499–510.
- Ribeiro-Filho, H.M.N., Delagarde, R. and Peyraud, J.L. 2005. Herbage intake and milk yield of dairy cows grazing perennial ryegrass pastures or white-clover/perennial rye grass pastures at low and medium herbage allowance. *Animal Feed Science and Technology* **119**: 13–27.
- Schils, R.L.M. 1994. Nitrates losses from grazed grass and clover pasture on clay soil. *Mestoffen*.
- Schils, R.L.M., Verhagen, A., Aarts, H.F.M. and Sebek, L.B.J. 2005. A farm approach to define successful mitigation strategies for GHG emission from ruminant livestock systems. *Nutrient Cycling in Agroecosystems* **71**: 163–175.
- Schwank, O., Blum, H. and Nösberger, J. 1986. The influence of irradiance distribution on the growth of white clover (*Trifolium repens* L.) in differently managed canopies of permanent grassland. *Annals of Botany* **57**(2): 273–281.
- Scollan, N.D., Costa, P., Hallett, K.G., Nute, G.R., Word, J.D. and Richardson, R.J. 2006. The fatty acid composition of muscle fat and relationships to meat quality in Charolais steers: influence of the level of red clover in the diet. In: "Proceedings of the British Society of Animal Science", page 23.
- Scollan, N.D., Enser, M., Richardson, R.I. and Wood, J.D. 2002. Effects of forage legumes on the fatty acid composition of beef. *Proceedings of the Nutrition Society* **61**: 99A.
- Speijers, M.H.M., Fraser, M.D., Theobald, V.J. and Haresign, W. 2004. The effects of grazing forage legumes on the performances of finishing lambs. *Journal of Agricultural Science, Cambridge* **142**: 483–493.
- Steg, A., Van Straalen, W.M., Hindle, V.A., Wensink, W.A., Dooper, F.M.H. and Schils, R.L.M. 1994. Rumen degradation and intestinal digestion of grass and clover at two maturity levels during the season in dairy cows. *Grass and Forage Science* **49**: 378–390.
- Suter, D., Rosenberg, E., Frick, R. and Mosimann, E. 2008. Standardmischungen für den Futterbau: Revision 2009–2012. *Agrarforschung* **15**(10): 1–12.
- Thomas, R.J., 1992. The role of the legume in the nitrogen cycle of productive and sustainable pastures. *Grass and Forage Science* **47**: 133–142.
- Thomas, C., Aston, K. and Daley, S.R. 1985. Milk production from silage. 3. A comparison of red clover with grass silage. *Animal Production* **41**: 23–31.
- Thomson, D. 1984. The nutritive value of white clover. In: "Forage legumes", Occasional Symposium of the British Grassland Society. N°16, (Ed. D.J. Thomson), Hurlley, UK: British Grassland Society, pages 78–92.
- Vermorel, M. 1988. Nutrition énergétique. In: "Ruminant Nutrition: Recommended Allowances and Feed Tables", (Ed. R. Jarrige), INRA, John Libbey, Londres, Paris, pages 57–70.
- Waghorn, G.C., Shelton, I.D. and Thomas, V.J., 1989. Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne* L.) and Lucerne (*Medicago sativa* L.) fed to cows during a restricted feeding period. *British Journal of Nutrition* **61**: 409–423.
- Waghorn, G.C., Tavendale, M.H. and Woodfield, D.R. 2006. Methanogenesis from forages fed to sheep. *Proceedings of the New Zealand Grassland Association* **64**: 159–165.
- Weiss, P. and Raymond, F. 1993. Utilisation de l'ensilage de trèfle violet pour l'engraissement des taurillons. *Fourrages* **134**: 283–286.
- Wilkins, R.J., Gibb M.J., Huckle C.A. and Clements A.J. 1994. Effect of supplementation on production by spring calving dairy cows grazing pastures of differing clover content. *Journal of Agricultural Science, Cambridge* **77**: 531–537.
- World Health Organization (WHO). 2003. "Diet, Nutrition and Prevention of Chronic Diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series 916, Canada.

- Zanetti, S., Hartwig, U.A., Lüscher, A., Hebeisen, T., Frehner, M., Fischer, B.U., Hendrey, G.R., Blum, H. and Nösberger, J. 1996. Stimulation of symbiotic N₂ fixation in *Trifolium repens* L. under elevated atmospheric pCO₂ in a grassland ecosystem. *Plant Physiology* **112**: 575–583.
- Zanetti, S., Hartwig, U.A., van Kessel, C., Lüscher, A., Hebeisen, T., Frehner, M., Fischer, B.U., Hendrey, G.R., Blum, H. and Nösberger, J. 1997. Does nitrogen nutrition restrict the CO₂ response of fertile grassland lacking legumes? *Oecologia* **112**: 17–25.