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Effect of forage legumes on feed intake, milk production and milk quality – a review*

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Literature data from experiments with lactating dairy cows offered silage-based diets was reviewed to evaluate the effects of the grassland legume species *Trifolium repens* (WC, white clover), *Trifolium pratense* (RC, red clover) and *Medicago sativa* (M, lucerne) on feed intake, milk production and milk quality. Seven data sets were created to compare grass silage (G) with grassland legumes in general (L), G with RC, G with WC, G with M, RC with WC, RC with M and different silage proportions of RC. Daily dry matter intake and milk yield were on average 1.6 and 1.6 kg, respectively, higher and milk fat content 1.2 g/kg milk lower on L than on G based diets. Similar differences were found when G was compared with RC or WC diets. Cows offered WC yielded 1.1 kg/d more milk than RC, and milk produced on WC and M contained 0.7 g more protein per kg than milk from RC diets. Increasing the silage diet RC proportion from 0.5 to 1.0 also decreased the milk protein content by 0.8 g/kg milk. RC increased the level of poly-unsaturated fatty acids, particularly C18:3n-3, and isoflavones, particularly equol, in milk. Effects are discussed in relation to plant cell wall characteristics, plant chemical constituents and changes in rumen digestion to explain the origin of the differences in intake, milk yield and milk composition.

KEY WORDS: Forage legumes / milk / fat/ protein / fatty acids / phytoestrogens

Grassland legumes are essential in organic agriculture due to their significant/ atmospheric nitrogen (N) fixing capacity, which to a large extent determines grassland

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yield and thus the productivity of the farming system [Steinshamn 2001, Younie 2001]. In organic grassland livestock farming, e.g. dairy production, forage legumes not only ensure the N input, they also provide high quality fodder. Grassland legumes are regarded to have superior feeding value, as higher intake and animal production on legumes than on grasses has generally been observed [Frame *et al*. 1998]. White clover (*Trifolium repens* L.), lucerne (*Medicago sativa* L.), and red clover (*Trifolium pratense* L.) are the most widely cultivated grassland legumes, with white clover as the most important species in Europe [Frame *et al*. 1998]. In recent years, the interest for these grassland legumes has increased, and new research has improved our understanding of legumes and their effect on milk production and milk quality. The objective of this review was to summarize the effect of grassland legumes in silage based diets on feed intake, milk production and milk composition by using data both from previously and more recently published literature.

Material and methods

Data from published experiments with dairy cows on silage based diets was gathered to evaluate the effects of grassland legumes on dry matter intake (DMI), milk production and milk composition. Seven datasets were created (Tab. 1): 1) legume based silage diets (L, average across legume species) were compared with grass only silage (G) in 20 experiments, 2) red clover (RC) was compared with G in 11 experiments, 3) white clover (WC) was compared with G in 8 experiments, 4) lucerne (M) was compared with G in 7 experiments, 5) WC and RC were compared in 6 experiments, 6) M was compared with RC in 8 experiments, and 7) different silage RC proportions (0.34-0.5 or 1.0 on DM basis) were compared in 5 experiments. Only experiments with *ad libitum* feeding of silage were included in the data sets. An exception was made for the quality trait milk fatty acid (FA) composition, where an experiment published by Vanhatalo *et al*. [2006] with fixed rate of DMI was included. Perennial ryegrass (*Lolium perenne* L.) was the most widely used grass species [Castle *et al*. 1983, Thomas *et al*. 1985, Wilman & Williams 1993, Hoffman *et al*. 1998, Broderick *et al.* 2002, Bertilsson and Murphy 2003, Dewhurst *et al*. 2003b, Al Mabruk *et al*. 2004, van Dorland 2006, van Dorland *et al*. 2008, Moorby *et al*. 2009], but timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis*), orchard grass (*Dactylis glomerata* L.) or tall fescue (*Festuca arundinacea* Schreb.) were also utilized in some experiments [Weiss & Shockey 1991, Randby 1992, Orozco-Hernandez *et al.* 1997, Cherney *et al.* 2004, Vanhatalo *et al*. 2007, Vanhatalo *et al*. 2008, Linton and Allen 2008, Steinshamn and Thuen 2008, Linton and Allen 2009, Vanhatalo *et al*. 2009]. In most cases, pure crops of legumes and grasses were used and mixed at feeding if legume proportion was included as a treatment. However, in some experiments the legume silages were prepared from mixed stands of legumes and grasses [Castle *et al*. 1983(experiment 1), Randby 1992, Wilman and Williams 1993, Vanhatalo *et al*. 2006, Steinshamn and Thuen 2008]. In addition to forage species, other experimental

Table 1. Overview of the studies used and the data sets created for the statistical analysis of dry matter intake, milk yield, and milk and protein contents from dairy cows fed silages of either grass (G), legumes on average (L), red clover (RC), white clover (WC), lucerne (M) or different proportions of RC (0.34-0.5 *vs.* 1.0)

Source (Experiment)	Legume species	Data sets						
		1 G *vs* L	2 G *vs* RC	3 G *vs* WC	4 G *vs* M	5 RC *vs* WC	6 RC *vs* M	7 RC prop.
Castle *et al*. [1983] (1)	WC	X		X				
Castle *et al*. [1983] (2)	WC	X		X				
Thomas *et al*. [1985]	RC	X	X					
Randby [1992]	RC	X	X					
Weiss & Shockey *et al*. [1991]	M	X			X			
Wilman & Williams [1993]	WC	X		X				
Hoffman *et al*. [1997] (1)	RC, M						X	
Hoffman *et al*. [1997] (2)	RC, M						X	
Orozco-Hernandez *et al*. [1997]	M	X			X			
Hoffman *et al*. [1998]	M	X			X			
Broderick *et al*. [2000]	RC, M						X	
Broderick *et al*. [2001] (1)	RC, M						X	
Broderick *et al*. [2001] (2)	RC, M						X	
Broderick *et al*. [2002]	M	X			X			
Bertilsson & Murphy [2003] (1)	RC, WC	X	X	X		X		X
Bertilsson & Murphy [2003] (2)	RC,WC	X	X	X		X		X
Dewhurst *et al*. [2003b] (1)	RC, WC, M	X	X[1]	X	X	X[1]	X	X
Dewhurst *et al*. [2003b] (2)	RC, WC	X	X[1]	X		X[1]		X
Al-Mabruk *et al*. [2004]	RC	X	X[1]					
Cherney *et al*. [2004]	M	X			X			
Broderick *et al*. [2007] (1)	RC, M						X	
Broderick *et al*. [2007] (2)	RC, M						X	
Vanhatalo *et al*. [2006]	RC		[1]					
Vanhatalo *et al*. [2007, 2009], Kuoppala *et al*. [2009]	RC	X	X[1]					
Linton and Allen [2008, 2009]	M	X			X			
Van Dorland [2006], Van Dorland *et al*. [2008]	RC, WC	X	X[1]	X		X[1]		
Vanhatalo *et al*. [2008]	RC	X	X[1]					
Steinshamn & Thuen [2008]	RC, WC					X[1]		
Moorby *et al*. [2009	RC	X	X[1]					X
Total number of comparisons		20	11	8	7	6	8	5

[1] Milk fatty acid composition analysed.

factors included were concentrate supplementation, growth stage, cut and vitamin supplement, but in the present review the mean value across these additional treatments was calculated before being subjected to statistical analysis. All studies reported dry matter intake (DMI), milk yield, milk fat and protein content, whilst milk FA composition was only reported in a few studies (Tab. 1). Thus, the comparison of milk FA profile was restricted to G *vs*. RC and WC *vs*. RC. Other milk quality traits, like oxidative stability, organoleptic quality or phytoestrogen content, were only examined in a few studies and are presented without any statistical analysis. Before statistical analysis, the average for each treatment within experiment was calculated, thus giving only one observation for each treatment and experiment in each data set. Statistical analysis was performed using the t test procedure of [SAS 2004].

Results and discussion

Dry matter intake and milk production

Grassland legumes (L) increased on average the total DMI by 1.6 kg, RC by 1.2 kg and WC by 1.3 kg relative to G based diets, and the milk yield was 1.6, 1.5 and 2.2 kg/d higher on L, RC and WC, respectively, than on G (Tab. 2). There was also a tendency ($P<0.1$) that M fed cows had higher DMI and milk yield than G. Cows on RC or WC diets were similar in DMI, but WC containing diets yielded 1.1 kg more milk than RC (Tab. 2). DMI tended to be higher (0.8 kg, $P = 0.054$) on M than on RC. This difference in DMI was, however, not manifested in milk production. Increasing the proportion of red clover had no effect on total DMI or milk production (Tab. 2).

The results confirm previous findings of the higher DMI potential of legume silage and the higher milk production this allows compared to grass silage [Frame *et al*. 1998]. Differences in cell wall fibre content (NDF) and fibre chemical structure are likely the reasons for the superiority of legume silage. Legumes have a lower concentration of NDF but higher proportions of indigestible NDF and lignin than grasses. Due to lower NDF concentration and higher rate of rumen degradation rate of the digestible NDF in legumes than in grass, legumes have similar *in vitro* organic matter digestibility at much lower level of potentially digestible NDF than grasses [Weisbjerg and Søegaard 2008]. Only xylem is lignified in legumes, and in a way causing the cell walls to be completely indigestible, whereas in other legume tissues there is no lignin and the cell walls are almost completely digestible [Wilson and Kennedy 1996]. In grasses, on the other hand, the lignin is distributed throughout all tissues except the phloem. Consequently, the smaller amount of lignin in grass protects a larger amount of cell walls from digestion and makes the rate of cell wall digestion slower than in legumes. It is also possible that lignin chemical constituents may account for differences in inhibition to digestion between grass and legume lignin [Buxton and Russell 1988].

Rumen fermentation studies have shown that the above described differences in cell wall content and structure result in lower rumen fill and higher rumen passage rate, thereby explaining higher intake capacity on legumes than on grasses [Dewhurst *et al*. 2003a, Kuoppala *et al*. 2009]. For WC, lower rumen fill is a result of higher fermentation rate, whilst higher rate of particle breakdown is the mechanism in M [Dewhurst et al. 2003a]. Increased particle density shortens the rumen retention time, and it is likely that the rate of increase in rumen particle size gravity is faster in legumes than in grasses because of the faster digestion of potentially digestible NDF [Allen, 1996]. Cows fed a mixture of RC and grass had higher silage DMI than when these silages were fed alone as pure crops in a study by Kuoppala *et al*. [2009]. Even though feed intake on pure clover was lower than on mixed feeding, the milk yields were similar, indicting a higher feed nutrient utilization on RC than on grass silage diets. Dewhurst *et al*. [2003a] found no difference in digestion kinetics between pure grass silage and RC-containing silages. Kuoppala *et al*. [2009], however, found clear

Table 2. Dry matter intake (DMI, kg/d), milk yield (kg/d), milk fat and protein content (g/kg) and milk fat and protein production (g/d) of dairy cows fed silages of grass, legumes in general, red clover, white clover or lucerne

Data set	Control[1]	Treatment[2]	Difference	SED[3]	P-value
1: Grass *vs*. legumes (n=20)					
DMI (kg/d)	18.3	20.0	-1.6	0.43	0.001
milk (kg/d)	26.2	27.9	-1.6	0.34	<0.001
milk fat (g/kg)	40.1	38.9	1.2	0.47	0.019
milk protein (g/kg)	31.1	31.0	0.1	0.20	0.546
milk fat (g/d)	1047	1075	-29	20.0	0.167
milk protein (g/d)	817	866	-49	14.2	0.003
2: Grass *vs*. red clover (n=11)					
DMI (kg/d)	18.4	19.6	-1.2	0.39	0.014
milk (kg/d)	25.7	27.1	-1.5	0.34	0.002
milk fat (g/kg)	41.8	39.9	2.0	0.40	<0.001
milk protein (g/kg)	31.4	30.9	0.5	0.27	0.092
milk fat (g/d)	1076	1084	-8	21.7	0.711
milk protein (g/d)	806	838	-32	12.7	0.033
3: Grass *vs*. white clover (n=8)					
DMI (kg/d)	17.7	18.9	-1.3	0.43	0.021
milk (kg/d)	24.7	26.9	-2.2	0.76	0.023
milk fat(g/kg)	41.7	40.0	1.8	1.37	0.236
milk protein (g/kg)	30.9	31.1	-0.1	0.39	0.733
milk fat (g/d)	1038	1072	-34	42.3	0.445
milk protein (g/d)	765	836	-71	27.1	0.034
4: Grass *vs*. lucerne (n=7)					
DMI (kg/d)	20.2	22.4	-2.3	1.12	0.092
milk (kg/d)	29.5	31.2	-1.7	0.82	0.085
milk fat (g/kg)	38.1	37.9	0.3	0.85	0.735
milk protein (g/kg)	31.6	31.8	-0.1	0.22	0.546
milk fat (g/d)	1106	1164	-58	45.0	0.245
milk protein (g/d)	929	989	-59	34.5	0.135
5:White clover *vs*. red clover (n=6)					
DMI (kg/d)	20.2	20.1	0.1	0.32	0.881
milk (kg/d)	28.7	27.6	1.1	0.33	0.024
milk fat (g/kg)	39.8	40.9	-1.1	1.22	0.422
milk protein (g/kg)	31.8	31.2	0.7	0.19	0.020
milk fat (g/d)	1141	1132	9	27.1	0.741
milk protein (g/d)	911	859	52	13.1	0.011
6:Lucerne *vs*. red clover (n=8)					
DMI (kg/d)	22.6	21.8	0.8	0.36	0.054
milk (kg/d)	30.6	30.4	0.2	0.35	0.632
milk fat (g/kg)	39.4	38.5	0.9	0.51	0.124
milk protein (g/kg)	32.4	31.6	0.7	0.13	0.001
milk fat (g/d)	1197	1166	32	20.9	0.175
milk protein (g/d)	985	961	24	10.6	0.057
7: 0.34-0.5 *vs*. 1.0 red clover (n=5)					
DMI (kg/d)	20.6	20.9	-0.3	0.57	0.672
milk (kg/d)	28.4	28.5	-0.1	0.56	0.973
milk fat (g/kg)	41.2	40.9	0.3	0.47	0.534
milk protein (g/kg)	31.1	30.3	0.8	0.26	0.033
milk fat (g/d)	1176	1166	11	30.5	0.747
milk protein (g/d)	885	862	23	17.0	0.240

[1]Control is the first mentioned treatment in the comparison.
[2]Treatment is the second mentioned treatment in the comparison.
[3]SED = Standard error of difference.

differences in digestion and passage kinetics, with accumulation of indigestible NDF in the rumen and higher digestion and passage rate of potentially digestible NDF in cows fed red clover silage and accumulation of potentially digestible NDF in cows fed grass silage. Kuoppala *et al*. [2009] suggested that the discrepancies between their study and that of Dewhurst *et al*. [2003a] could be due to the use of different cuts; Kuoppala *et al*. [2009] used silages prepared from the primary growth, whereas Dewhurst *et al*. [2003a] used proportional mixtures of three cuts. The effect of growth period, i.e. cuts within season, on digestion kinetics warrants further research.

The legume effect on DMI is also dependent of the cows' nutrient demand, as the difference between legumes and grasses increases with greater preliminary voluntary dry matter intake (pVDMI). This was demonstrated by Linton and Allen [2008] for lucerne and orchard grass; DMI on lucerne became increasingly higher than DMI on orchard grass with increasing pVDMI. pVDMI is an index of nutrient demand, which is measured pre-experimentally on a common diet.

The reason for the difference between the effect of RC and WC on milk yield is likely the higher organic matter digestibility of WC [Bertilsson and Murphy 2003]. Apparent digestibility of DM, organic matter, NDF, and hemicellulose were larger in diets with RC than M, and Broderick *et al*. [2000] estimated that RC gave 10% greater net energy lactation than M. Higher net energy value of RC likely explains the similarity in milk yield despite higher DMI on M, as was found in both in the study of Broderick *et al*. [2000] and in the present review using data from eight experiments.

Milk protein and fat content and fatty acid composition

Milk fat content was on average 1.2 and 2.0 g/kg milk lower on L and RC, respectively, than on G (Tab. 2). But due to higher milk yield on L and RC than on G, the daily milk fat yields were similar. RC diets resulted in 0.7 g less protein per kg milk than both WC and M based diets. Consequently, as the milk yields were similar, daily milk protein yield was lower on RC than on WC and M. Cows on RC diets also tended ($P<0.10$) to yield milk with lower protein content than cows on G, and increasing dietary silage RC dry matter proportion also decreased the milk protein content by 0.8 g/kg milk (Tab. 2).

The lower milk fat content on legume based silage diets, and particularly RC, than on grasses may be due to fatty acid isomers produced during rumen biohydrogenation. Some intermediates on the biohydrogenation pathway inhibit milk fat synthesis [Bauman and Griinari 2003, Bauman *et al*. 2008], and some authors have reported higher concentrations of these intermediates in milk from cows fed legumes [Vanhatalo *et al*. 2007]. Vanhatalo *et al*. [2007] suggested that lower milk fat content on legume than on grass silage based diets may also be due to increased supply of long-chain FA to the mammary gland, like C18:3n-3 on RC diets. Long-chain FA inhibit *de novo* synthesis of milk fatty acids and thereby contribute to lower milk fat content. In another study by Vanhatalo *et al*. [2009], a lower molar proportion of butyric acid was observed in the rumen of RC fed cows compared to grass fed, which may also explain reduced milk fat with RC diets. Thus, different mechanisms may possibly cause the reduced milk fat content on RC. This issue needs to be further examined.

Milk FA proportion was only determined in a few studies, and consequently the statistical comparisons were limited. However, in eight experiments RC based diets were compared with grass diets (Tab. 3). Red clover had no effect on most fatty acids, but substantially increased the proportion of the polyunsaturated fatty acids C18:2n-6 and C18:3n-3 (Tab. 3). The proportion of C18:3n-3 increased relatively more than C18:2n-6, and the C18:2n-6/C18:3n-3 FA ratio decreased from 2.7 on grass to 1.9 on RC. No significant differences were found between the effect of WC and RC based diets on milk fatty acid composition, but RC diets tended ($P<0.1$) to yield milk with higher proportions of C18:1c-9 and C18:1t-11 than WC diets.

Table 3. Fatty acid composition of milk from dairy cows fed either silages of grass (G), red clover (RC) or white clover (WC)

Fatty acid	G *vs.* RC				WC *vs* RC			
	G	RC	SED[1]	P-value	WC	RC	SED[1]	P-value
n	8	8			4	4		
C12:0	3.51	3.60	0.141	0.536	4.09	4.03	0.329	0.867
C14:0	11.64	11.51	0.258	0.640	11.98	11.46	0.178	0.064
C16:0	31.93	30.39	0.924	0.146	28.56	30.15	2.23	0.537
C18:0	11.17	10.98	0.152	0.248	9.72	10.30	0.363	0.211
C18:1c-9	20.39	20.19	0.610	0.763	19.14	19.90	0.285	0.076
C18:1t-11	1.52	1.42	0.086	0.303	1.44	1.61	0.045	0.065
C18:2n-6	1.39	1.67	0.059	0.002	1.41	1.42	0.061	0.925
C18:2n9t11	0.55	0.52	0.029	0.362	0.72	1.45	0.017	0.225
C18:3n-3	0.54	0.91	0.066	0.001	0.89	0.89	0.053	0.965
C18:2/C18:3	2.74	1.91	0.197	0.004	1.64	1.68	0.126	0.793

[1]SED – Standard error of difference.

Rumen biohydrogenation of poly-unsaturated FA is reduced on RC diets due to reduced plant-mediated lipolysis, a prerequisite of rumen microbial hydrogenation of unsaturated fatty acids [Lee *et al*. 2003]. Reduced lipolysis is caused by the action of the enzyme polyphenol oxidase (PPO), abundant in RC [Lee *et al.* 2004]. This explains the enhanced levels of polyunsaturated fatty acids, particularly C18:3n-3, in milk from dairy cows fed RC silage.

Hoffman *et al.* [1997] suggested that the action of PPO also may produce phenols that bind to amino acids and alter the amino acid flow to the small intestine, thereby affecting milk protein synthesis. Vanhatalo *et al.* [2009] found a higher supply of amino acids (AA) to the intestine and increased supply of absorbed AA in cows offered RC than in cows offered G silage. However, the AA profile in the digesta out of rumen on RC was not balanced. Red clover gave relatively less sulphur containing AA, Cysteine and Mehionine. Replacing M with RC silages reduced the microbial non ammonia nitrogen flow but increased the ruminal undegradable protein flow to the intestine [Brito *et al*. 2007]. Imbalanced AA supply [Vanhatalo *et al.* 2009] and greater supply of nutritionally available AA from microbial protein [Brito *et al*. 2007] may explain reduced milk protein content on RC relative to M and WC. However, this warrants further research.

Milk oxidative stability and sensory quality

Al Mabruk *et al*. [2004] found that both RC and M based diets increased milk oxidative deterioration, measured as increased appearance of thiobarbituric acid reactive substances and reduced content of α-tocopherol content, during storage as compared to grass. Milk from clover diets, and particularly RC, deviated more often from what was regarded as "good quality milk" than milk from grass based diets in the study by Bertilsson and Murphy [2003], while Moorby *et al*. [2009] found only minor effects of RC proportion on milk flavor. The tendency to lower milk oxidative stability observed on RC diets may be due to a higher degree of lipid oxidation resulting from an elevated proportion of C18:3n-3, as observed on other diets that increase the level of C18: 3n-3 [Havemose *et al.* 2006]. The problem may be lessened by supplementing cows with extra vitamin E [Al Mabruk *et al*. 2004].

Milk phytoestrogen content

It is believed that dietary intake of isoflavones may have a health benefit [Tham *et al*. 1998], and that this claimed benefit is associated with the isoflavone metabolite equol [Setchell *et al*. 2002]. Equol is superior to other isoflavones in its antioxidative activity [Arora *et al*. 1998]. It has far greater affinity to estrogen receptors than daidzein [Setchell *et al*. 2002] and can hinder the binding of dihydrotestosterone to its receptor and thereby alter growth and physiological hormone responses that are regulated by androgens [Lund *et al*. 2004]. Red clover based diets yield milk with higher

Table 4. Effects of legume silages on equol concentrations in milk (μg/l)

Study	Legume prop.[1]	Legume species	Equol
Steinshamn *et al*. [2008]	0.30	*T. repens*	75
	0.50	*T. pratense*	319[a]
Andersen *et al*. [2009]	0.67	*Medicao sativa L.*	1.8
	0.03	*T repens/T. pratense*	186[b]
Mustonen *et al*. [2009]	0	Grass, primary growth	229*
	1.0	*T. pratense*, primary growth	473
	1.0	*T. pratense,* secondary growth	613[c]

[1]Legume proportion in silage/pasture on DM basis.
*Differences (P<0.05) reported between grass and legumes (within experiment and column).
[a]Differences (P<0.05) reported between clover species (within experiment and column).
[b]Differences (P<0.05) reported between *M. sativa* and clover (within experiment and column).
[c]Differences (P<0.05) reported between primary and secondary growth (within experiment and column).

concentrations of phytoestrogens from the isoflavone group (formononetin, daidzein, equol, biochanin A, genistein and prunetin) [Steinshamn *et al*. 2008, Andersen *et al*. 2009, Mustonen *et al*. 2009]. Particularly, the concentration of equol, produced in the digestive tract from its precursors formononetin and daidzein, is high relative to grass and grass-white clover based diets (Tab. 4). Daidzein, found in soy and soy products, is in humans metabolised to equol by intestinal bacteria. However, it is estimated that only 30-50% of the population is capable to produce equol from daidzein [Lampe *et al*. 1998]. Therefore, if equol is demonstrated to have the suggested health beneficial effect, milk produced on RC based diets with high concentrations of equol may be an interesting dietary source.

Conclusion

Cows offered legume silage had higher feed intake and milk production than cows on grass silage. Cows on RC diets yielded milk with lower milk fat and protein contents than cows fed grasses and other legumes. Milk produced on RC had higher contents of poly-unsaturated fatty acids, particularly C18:3n-3, than milk produced on grass, and higher contents of phytoestrogens, particularly equol, than milk produced on the other diets. The mechanism for the negative effect of RC diets on milk fat and protein contents needs to be explored.

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