The Legume-Grass Mixture Feeding Guide

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Picture of legume-grass plots at the University of New Hampshire Kingman Farm (Madbury, NH)

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Introduction

Organic dairy is undergoing a transition. After a decade-long “boom” cycle, where demand outpaced supply and organic dairies could not be transitioned at a fast-enough pace to keep product on grocery store shelves, the direction has dramatically turned. Now, with diminished demand for fluid milk and lower pay prices as the potential new reality for organic dairies, the need to enhance production efficiency, feed quality, and milk components becomes even more critical to ensure organic dairy remains profitable. Forage legumes are key to addressing several critical challenges facing the organic dairy industry today, including maximizing forage yields, successfully implementing high-forage diets to capitalize on “grass-fed” and other specialty milk markets, and optimizing forage nutritive value and energy:protein (**E:P**) balance to improve yields of milk and milk components, particularly milk fat and protein. However, legumes are often difficult to establish and maintain in pastures and feeding high-legume diets can be associated with potentially serious tradeoffs such as intake of phytoestrogens, which can negatively impact livestock reproductive health.

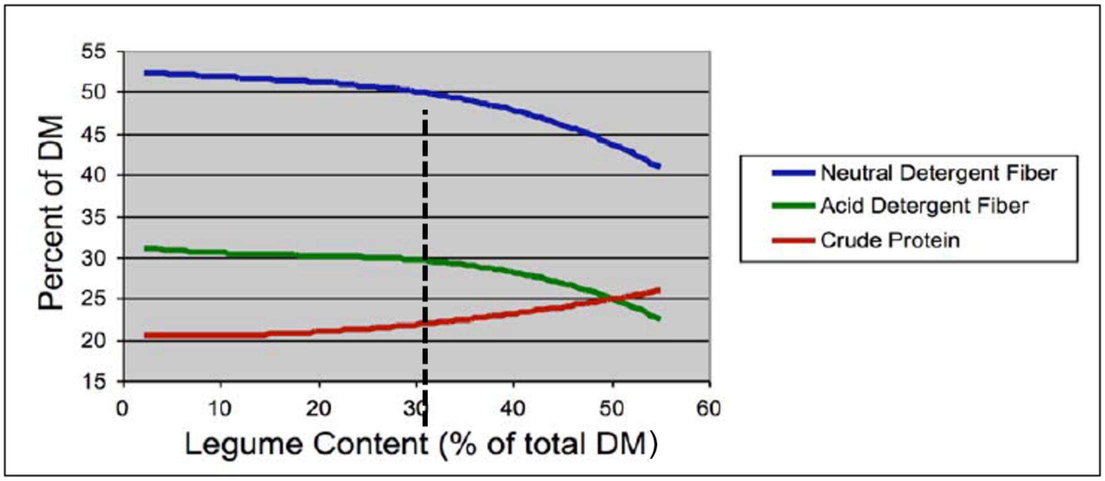
Legume persistence and proper E:P balance in forages are the primary challenges impacting pasture nutritive value and productivity, limiting farmers’ abilities to successfully implement forage-based diets (Rinehart, 2009). Perennial forage legumes provide a crucial, low cost nitrogen (**N**) source in diverse pasture swards and are critical to the E:P balance of forages, which ultimately affects milk productivity, quality, and animal health (Brito et al., 2008, 2009; da Silva et al., 2013, 2014). Yet, we demonstrated that at 14 organic dairies across the Northeast, grasses made up most of the species (67%) used for grazing, whereas legumes only contributed 26% (Hafla et al., 2016). However, maintaining legumes in a pasture sward or hayfield is not simply an agronomic management issue; climate, soil fertility, herd management, and grazing practices interact to impact the longevity and maintenance of the legume composition of the sward as well (Ledgard and Steele, 1992). While managing N for productive pastures or hayfields is paramount, this must be balanced with the need to maintain high-energy forages, as forage-based diets can decrease N utilization in dairy cows due to excess N intake and consequent excretion of N to the environment (Dutreuil et al., 2014; Brito et al., 2017). Another tradeoff of feeding high-forage rations is that increased fiber intake can elevate enteric methane (**CH_4**) emissions, which not only represent an environmental concern (Gerber et al., 2013), but also energy losses that otherwise would be available for producing milk protein and fat (Brito and Silva, 2020). It should be also emphasized that selected legume-grass mixture species have been shown

The goal of this Legume-Grass Mixture Feeding Guide is to cover the importance to increase the proportion of legumes in dairy diets while highlighting specific tradeoffs and best legume-grass mixtures based on results published in the literature and data from grants funded by the USDA Transition to Organics and USDA Northeast SARE programs.

Importance of legumes to improving forage quality and milk production

Lüscher et al. (2014) reported that total dry matter (**DM**) yield of mixed grass and legume pasture was greater than grass-based pasture, and overall feed value was better maintained throughout the grazing season when pastures include legumes. In addition, legume-grass mixtures generally provide more consistent and greater forage yields across a range of environments than grass or legume monocultures (Papadopoulos et al., 2012). However, grazing studies conducted at UNH (Brito et al., 2017; Antaya et al., 2019; Isenberg et al., 2019) revealed that the proportion of legumes in pastures averaged 17.2% (range 7.5 to 25.4%), which agrees with data from 14 northeastern commercial organic dairies (Hafla et al., 2016). Bosworth and Cannella (2007) showed significant positive correlations between legume inclusion in pastures and forage quality traits like crude protein and net energy of lactation, and negative correlations with neutral and acid detergent fiber. They also observed that a minimum of 30% legumes in pasture was needed to promote desirable forage quality characteristics (Bosworth Cannella, 2007) as presented in **Figure 1**. In fact, Johansen et al. (2018) demonstrated in a recent meta-analysis that DM intake (**DMI**;+7%) and production of milk (+6.5%) and energy-corrected milk (+4%) were all greater in dairy cows fed high-forage diets containing legume than grass silages (see **Table 1**). Even though organic matter digestibility was lower in legume silages (**Table 1**), fiber ruminal passage rate is generally faster in legumes resulting in more DMI and milk production.

Figure 1. Relationship between legume proportion in pasture and forage quality traits



DM = dry matter
Source: Bosworth and Cannella (2007)

Table 1. Effect of grasses vs. legumes on production performance and digestibility in dairy cows

	Forage type		
Item	Grasses	Legumes	*P*-value
Dry matter intake, lb/day	40.3	43.2	0.001
Milk production, lb/day	54.0	57.5	<0.001
Energy-corrected milk, lb/day	53.6	55.8	0.006
Feed efficiency[1], lb/lb	1.33	1.30	0.20
OM digestibility[2], %	70.4	67.9	0.01

[1]Feed efficiency = energy-corrected milk/dry matter intake
[2]OM = organic matter
Source: Adapted from Johansen et al. (2018)

Johansen et al. (2017) compared high-forage diets containing different species of grass, legume-grass mixture, and legume silages and concluded that incorporation of legumes improved DMI and production of milk and milk fat and protein relative to grasses-only silage diets (see **Table 2**). Specifically, including at least 50% legume silages (red clover or white clover; RC-LPR or WC-LPR diets; **Table 2**) in the forage mixture was needed to stimulate DMI and milk production in dairy fed high-forage diets. Therefore, legume silages promote feed intake, which in turn, stimulates production of milk and milk components.

Table 2. Effect of silage type on feed intake and milk production in dairy cows fed high-forage diets

	Silage types[1]								
Item	EPR	FEST	TF	LPR	RC-LPR	WC-LPR	RC	WC	SEM
Dry matter intake, lb/d	44.5^{b}	44.8^{b}	41.4^{c}	42.1^{c}	45.9^{ab}	47.4^{a}	47.8^{a}	47.6^{a}	0.73
Milk production, lb/d	66.6^{cd}	67.9^{c}	61.5^{e}	63.3^{de}	68.8^{bc}	72.3^{ab}	73.0^{a}	76.3^{a}	1.72
Milk fat, lb/d	2.93^{de}	3.13^{abc}	2.76^{e}	2.95^{cd}	3.04^{bcd}	3.28^{a}	3.00^{bcd}	3.17^{ab}	0.06
Milk protein, lb/d	2.43^{bc}	2.43^{bc}	2.16^{d}	2.25^{d}	2.40^{c}	2.54^{ab}	2.45^{bc}	2.60^{a}	0.05

^{a,b,c,d,e}Means in the same line with different letters differ at $P < 0.05$

[1]EPR = early perennial ryegrass; FEST = festulolium; TF = tall fescue; LPR = late perennial ryegrass; RC-LPR = red clover + late maturity perennial ryegrass; WC-LPR = white clover + late maturity perennial ryegrass; RC = red clover; WC = white clover

Source: Adapted from Johansen et al. (2017)

Effect of different legume sources on milk production and composition

Alfalfa has become the "gold standard" or reference legume for production of conserved forages including silage, baleage, and hay. Both alfalfa and red clover are commonly used in northeastern organic dairies in mixed pastures and hayfields (Hafla et al., 2016). However, these 2 forage crops have different concentrations of sugars and nonprotein-N. In fact, large proportion of alfalfa protein is broken down to ammonia, amino acids, and peptides (Papadopoulos and McKersie, 1983; Pelletier et al., 2010), whereas that of red clover is protected against proteolysis due to the presence of the enzyme polyphenol oxidase in red clover tissues (Jones et al., 1995). Birdsfoot trefoil is not as prevalent as alfalfa or red clover in the Northeast (Hymes-Fecht et al., 2013), but feeding birdsfoot trefoil as silage or baleage improved milk production and N use efficiency in lactating dairy cows, which are responses likely modulated by birdsfoot's condensed tannins (Hymes-Fecht et al., 2013). Among legume silages, white clover and birdsfoot trefoils appear to be the best for milk production and milk components, while white and red clover resulted in the greatest digestibility of organic matter in dairy cows fed high-forage diets according to meta-analysis of Johansen et al. (2018) presented in **Table 3** below.

Effect of legume-grass mixture on forage biomass yield

Preliminary results from a study conducted at the UNH Kingman Farm where small plots with different binary mixtures of legume-grass showed that red clover-grass yielded the greatest biomass production in 2019. Each plot was planted with 70% legume seed and 30% orchardgrass seeding rate. Compost was applied in the summer, with plots seeded in early September 2018. Plots were organically managed, without additional amendments following compost. We observed a good survival and germination overall, despite region-wide issues with winterkill. Alfalfa '406AP2', red clover 'Freedom', white clover 'Alice', birdsfoot trefoil 'Bruce', and orchardgrass 'Latar' were used. Season forage biomass production is shown in **Figure 2** below.

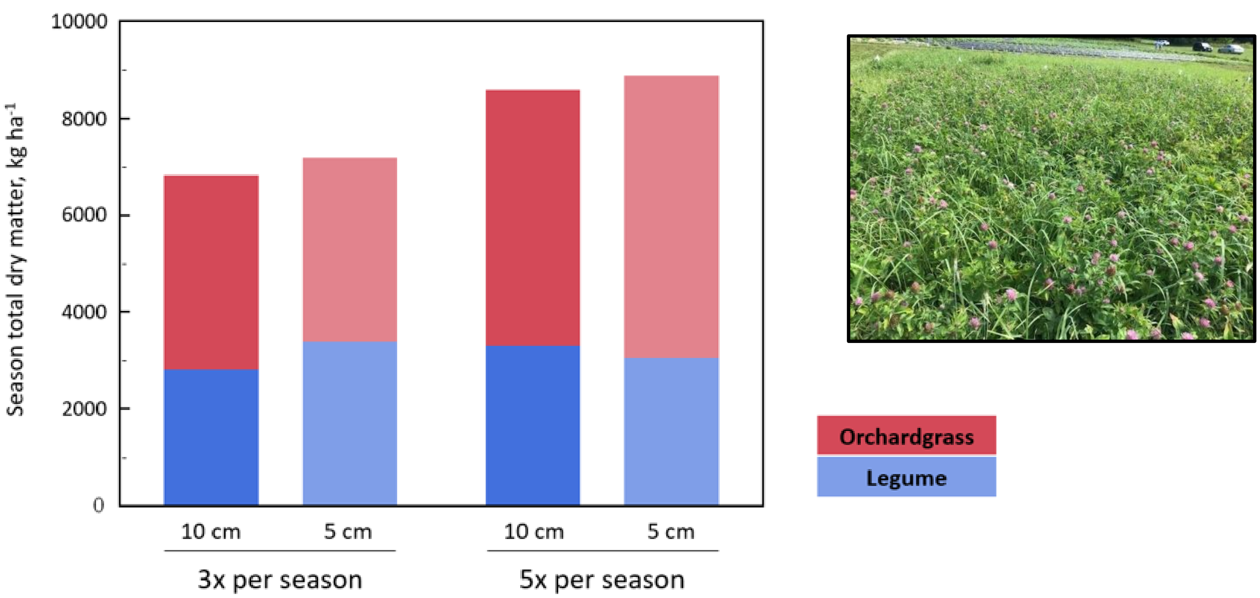
Table 3. Effect of different legume silage sources on feed intake and milk production in dairy cows fed high-forage diets

	Forage type					
Item	Grasses	White clover	Red clover	Alfalfa	Birdsfoot	*P*-value
Dry matter intake, lb/d	41.7^{b}	44.1^{ab}	44.1^{a}	46.3^{a}	48.1^{ab}	<0.001
Milk production, lb/d	57.8^{c}	65.3^{a}	60.2^{b}	61.1^{b}	69.2^{a}	<0.001
Energy-corrected milk, lb/d	56.7^{d}	61.9^{ab}	57.5^{cd}	59.5^{bc}	67.0^{a}	<0.001
Feed efficiency[1], lb/lb	1.35	1.39	1.31	1.30	1.43	0.07
OM digestibility[2], %	71.5^{ab}	73.6^{a}	69.4^{b}	66.0^{c}	67.2^{abc}	<0.001

[a,b,c,d]Values in same line with different letters differ at $P < 0.05$
[1]Feed efficiency = energy-corrected milk/dry matter intake
[2]OM = organic matter
Source: Adapted from Johansen et al. (2018)

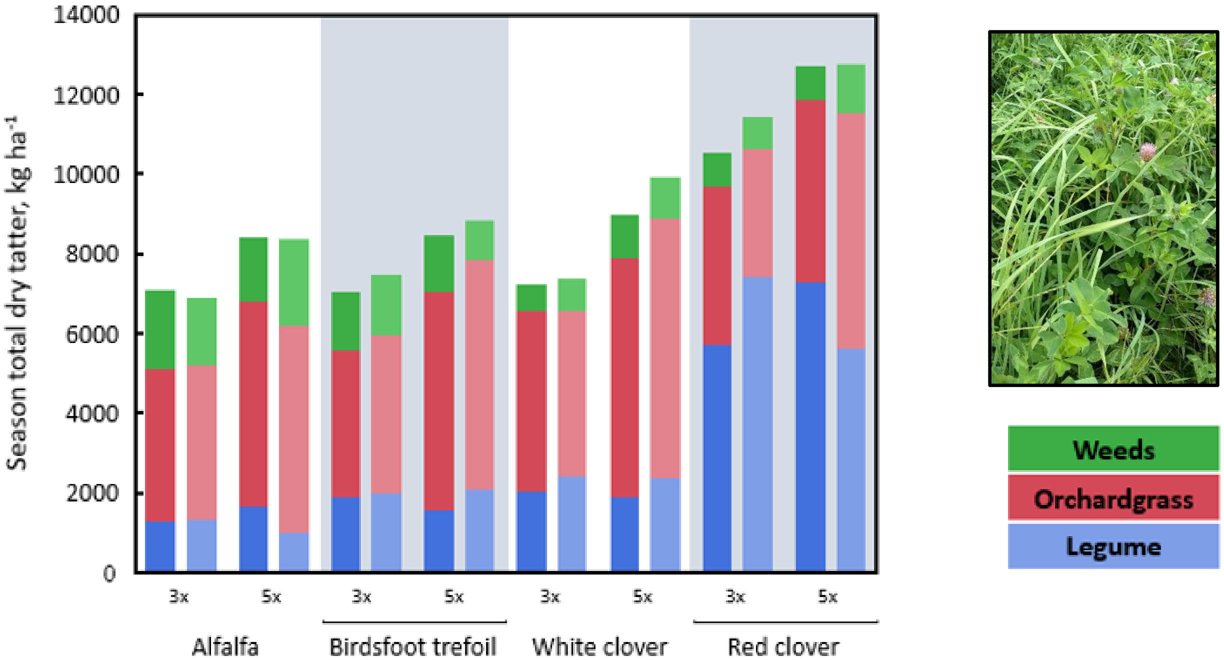
As shown in **Figure 2**, cutting frequency (3x vs. 5x) impacted more legume-grass yield than cutting height. Interestingly, moving from a less frequent (3x/season) to a more frequent (5x/season) cutting management kept legume productivity but increased that of grass. These results suggest that increased cutting frequency, which mimics a grazing rotational schedule lowered the proportion of legumes in the sward mixture and may negatively impact milk production. Further research is needed to better understand the impact of cutting management of legume yield and persistence.

Figure 2. Impact of management on legume-grass mixture production



The season biomass production of different legume-grass mixtures is presented in **Figure 3** below. The red clover-orchardgrass mixture resulted in the best biomass production and greatest proportion of legume relative to grass compared with alfalfa-grass, white clover-grass, and birdsfoot trefoil-grass mixtures independent of cutting frequency (3x vs. 5x per season).

Figure 3. Impact of management on legume-grass mixture production of different legume species.



<u>Effect of legume-grass mixture on milk production and composition, and nutrient use efficiency</u>

A feeding trial was conducted at the UNH Burley-Demeritt Organic Dairy Research Farm (Lee, NH) to investigate the effect of different legume-grass forage mixture on milk production and composition, milk fatty acid (**FA**) profile, plasma concentration of essential amino acids (**EAA**), and nutrient efficiency in Jersey cows. In brief, 18 multiparous and 2 primiparous mid-lactation organic Jersey cows were blocked in pairs by milk yield or parity and, within pair, assigned to treatments in a randomized complete block design. Two fields were planted with alfalfa (ALF)- or red clover (RC)-grass mixture with a 79:14:7 legume:meadow fescue:timothy seeding rate (% total). Forages were harvested as baleage, with second- and third-cut legume-grass mixtures used in the study. The botanical composition (DM basis) of second-cut ALF- or RC-grass swards averaged 65 vs. 80% legume, 17 vs. 15% grasses, and 18 vs. 5% weeds, while that of third-cut ALF- or RC-grass mixture averaged 84 vs. 96.5% legume, 3 vs. 2.3% grasses, and 13 vs. 1.2% weeds, respectively. Diets contained (DM basis) 65% second- and third-cut ALF or RC-grass (32.5% of each cut) and 35% concentrate. The study lasted 9 wk (2-wk covariate) with sample collection done at wk 4 and 7. Data were analyzed with repeated measures in SAS. Diets averaged 18.8 vs. 18.1% CP and 30.5 vs. 31% NDF for ALF- vs. RC-grass, respectively.

No dietary differences were observed for DMI, yields of milk and milk protein and lactose, concentrations of milk fat, protein, and lactose, and feed efficiency (see **Table 4** below). In contrast, 4% FCM (P = 0.05) and ECM (P = 0.06) yields increased with feeding ALF-grass.

Similarly, milk fat yield was greater (*P* = 0.05) in cows fed ALF- vs. RC-grass. A significant treatment by week interaction was observed for milk urea N (**MUN**), with ALF-grass showing greater MUN relative to RC-grass in week 4 (14.4 vs. 11.6 mg/dL) than wk 7 (11.7 vs. 10.4 mg/dL).

Table 4. Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on production performance, milk composition, and feed efficiency in organic Jersey cows

	Week 4		Week 7			*P*-value		
Item	ALF-grass	RC-grass	ALF-grass	RC-grass	SEM	Treatment (T)	Week (W)	T × W
DMI,[1] kg/d	19.8	21.9	19.7	21.5	0.82	0.18	0.30	0.63
Milk yield, kg/d	22.4	20.9	21.1	20.6	0.46	0.11	0.05	0.27
4% FCM yield,[2] kg/d	27.9	25.0	25.5	24.4	0.72	0.05	0.02	0.15
ECM yield,[3] kg/d	29.5	26.6	27.2	26.0	0.76	0.06	0.02	0.16
Milk yield/DMI	1.10	0.99	1.04	1.01	0.04	0.24	0.42	0.15
4% FCM yield/DMI	1.34	1.20	1.24	1.20	0.04	0.13	0.16	0.10
ECM yield/DMI	1.42	1.27	1.33	1.29	0.04	0.12	0.22	0.11
Milk fat, %	5.61	5.32	5.36	5.27	0.10	0.18	0.03	0.13
Milk fat, kg/d	1.26	1.11	1.13	1.08	0.04	0.05	0.02	0.13
Milk protein, %	3.64	3.57	3.73	3.60	0.10	0.48	0.19	0.44
Milk protein, kg/d	0.81	0.75	0.78	0.74	0.03	0.21	0.18	0.40
Milk lactose, %	4.72	4.73	4.66	4.71	0.02	0.23	0.10	0.55
Milk lactose, kg/d	1.05	0.99	0.98	0.97	0.02	0.21	0.03	0.23
MUN,[4] mg/dL	14.4[a]	11.6[b]	11.7[a]	10.4[b]	0.24	<0.001	<0.001	<0.001

[a,b]Means with different superscripts within week of sampling differ significantly.
[1]DMI = dry matter intake
[2]FCM = fat-corrected milk.
[3]ECM = energy-corrected milk.
[4]MUN = milk urea nitrogen.

Significant treatment effects were observed for the milk proportions of individual fatty acids (**FA**) like 16:0, 18:0, *trans*-10 18:1, *cis*-9, *cis*-12 18:2 (linoleic acid), and *cis*-9, *cis*-12, *cis*-15 18:3 (α-linolenic acid) as shown in **Table 5**. Specifically, the milk proportion of 16:0 decreased while that of 18:0 increased with feeding ALF- versus RC-grass, which may be explained by intake differences in 16:0 and 18:0 and reduced ruminal biohydrogenation of unsaturated FA leading to increased 18:0 due to activity of the enzyme polyphenol oxidase present in RC. It is well known that ruminal biohydrogenation of unsaturated FA is reduced by polyphenol oxidase. In fact, cows fed the RC-grass diet had greater proportions of linoleic (*P* = 0.03) acid and α-linolenic acid (*P* < 0.001) in the milk fat likely in response to reduced biohydrogenation of these unsaturated FA. The milk proportion of trans-10 18:1 was greater (*P* = 0.01) in cows fed RC-grass than those offered ALF-grass. *Trans*-10 18:1 is involved in milk fat depression and reduced milk fat yield in cows fed the RC-grass diet may be associated with elevated *trans*-10 18:1 in milk fat. Total milk branched-chain FA, ω-6 FA, and ω-3 FA all significantly increased in cows fed RC- versus ALF-grass, which may be related, at least partially, with less ruminal biohydrogentation of unsaturated FA with the RC-grass diet. The ω-6:ω-3 ratio decreased when cows were fed RC-grass, which together with the increased proportion of ω-3 FA suggest that the RC-grass mixture was better than the ALF-grass mixture to change the milk FA profile towards FA that may be more beneficial to human health. While the sum of 16-carbon FA increased in the milk fat of cows fed ALF-grass, that of 18-carbon increased with feeding RC-grass. Increased 16-carbon FA indicates that the origin of FA in milk fat was a mix between de novo synthesis in the mammary gland and blood extraction (dietary supply and/or mobilization from adipose tissues), whereas increased 18-carbon FA indicates that FA originated from blood extraction only. Significant treatment by week interactions were observed for the milk proportions of 16:0 and α-linolenic acid, as well as total ω-3 FA, ω-6:ω-3 ratio, and total 16-carbon FA (**Table 5**). Overall, while ALF-grass improved yields of FCM and milk fat, RC-grass decreased MUN and elevated FA with potential human-health benefits.

Table 5. Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on the milk proportion (g/100 g) of selected fatty acids in organic Jersey cows

	Week 4		Week 7			*P*-value		
Fatty acids (FA)	ALF-grass	RC-grass	ALF-grass	RC-grass	SEM	Treatment (T)	Week (W)	T × W
16:0	32.2[a]	30.4[b]	32.9[a]	30.3[b]	0.53	0.02	0.01	<0.001
18:0	11.4	12.6	11.2	12.8	0.44	0.04	0.95	0.18
trans-10 18:1	0.15	0.19	0.19	0.21	0.01	0.01	<0.001	0.38
trans-11 18:1	1.12	1.15	1.20	1.20	0.07	0.92	0.02	0.58
cis-9 18:1	13.8	14.3	13.7	14.7	0.35	0.12	0.27	0.21
cis-9, *cis*-12 18:2	1.80	2.00	1.75	2.05	0.07	0.03	0.95	0.08
cis-9, *cis*-12, *cis*-15 18:3	0.67[b]	0.85[a]	0.61[b]	0.87[a]	0.03	<0.001	0.05	<0.01
cis-9, *trans*-11 18:2 CLA	0.42	0.39	0.46	0.42	0.03	0.37	<0.01	0.67
Σ odd-chain FA	1.78	1.76	1.80	1.78	0.03	0.62	0.43	0.92
Σ branched-chain FA	1.10	1.17	1.13	1.20	0.02	0.03	0.05	0.82
Σ ω-6 FA	2.20	2.43	2.14	2.48	0.07	0.02	0.85	0.09
Σ ω-3 FA	0.73[b]	0.93[a]	0.67[b]	0.95[a]	0.03	<0.001	0.08	<0.01
ω-6/ω-3 ratio	3.04[a]	2.62[b]	3.22[a]	2.62[b]	0.03	<0.001	<0.01	<0.01
Σ <16-carbon FA	31.1	30.6	30.4	29.8	0.25	0.11	<0.001	0.64
Σ 16-carbon FA	33.1[a]	31.3[b]	33.9[a]	31.2[b]	0.58	0.02	0.01	<0.01
Σ 18-carbon FA	30.8	32.8	31.1	33.5	0.51	<0.01	0.06	0.49

[a,b]Means with different superscripts within week of sampling differ significantly.

The plasma concentration of leucine increased with feeding RC-grass ($P = 0.03$; 178 vs. 142 µ*M*) as shown in **Table 6**. Significant treatment by week interactions were found for the plasma concentrations of arginine, histidine, phenylalanine, tryptophan, valine, and total EAA. Feeding RC-grass increased ($P \leq 0.05$) plasma arginine, phenylalanine, valine, and total EAA in week 7 but not in week 4. Further, RC-grass enhanced plasma histidine more noticeably in week 7 (+62%) than week 4 (+38%). Compared with ALF-grass, plasma tryptophan decreased in cows fed RC-grass in week 4 and increased in week 7. Even though milk protein yield was not affected by diets, RC-grass seems to be more effective than ALF-grass to elevate plasma EAA.

Table 6. Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on the plasma concentration (µ*M*) of essential amino acids in organic Jersey cows

	Week 4		Week 7			*P*-value		
Amino acids	ALF-GR[1]	RC-GR[1]	ALF-GR[1]	RC-GR[1]	SEM[2]	Treatment (T)	Week (W)	T × W
Lysine, µ*M*	96.5	90.0	89.6	100	6.13	0.76	0.76	0.14
Methionine, µ*M*	24.6	20.3	22.8	24.1	1.74	0.47	0.51	0.07
Histidine, µ*M*	38.2[b]	52.8[a]	35.4[b]	57.4[a]	4.00	<0.01	0.55	0.02
Leucine, µ*M*	150	173	134	184	11.3	0.03	0.76	0.11
Isoleucine, µ*M*	152	153	135	155	9.05	0.37	0.30	0.18
Valine, µ*M*	269	289	237[b]	300[a]	15.3	0.06	0.24	0.03
Total BCAA, µ*M*	572	615	506	639	34.9	0.07	0.38	0.07
Total EAA, µ*M*	1,027	1,059	924[b]	1,120[a]	53.1	0.11	0.59	0.05

[1]ALF-GR = alfalfa-grass mix; RC-GR = red clover-grass mix
[2]SEM = standard error of the mean
[3]BCAA = branched-chain AA
[4]EAA = essential amino acids

Enteric CH_4 emission was measured using the GreenFeed system (**Figure 4**). A significant treatment by week interaction was observed for CH_4 production, with cows fed RC-grass showing lower CH_4 (378 vs. 424 g/d) in week 4 but no change in week 7 (mean = 416 g/d) as shown in **Table 7**. Note that reduced CH_4 production in week 4 with cows fed RC-grass was not related to DMI, which did not change significantly between treatments (**Table 4**). No diet differences were found for CH_4 yield (mean = 19.9 g/kg of DMI) and CH_4 intensity (mean = 15 g/kg of energy-corrected milk) (**Table 7**). A significant treatment by week interaction was also observed for the urinary excretion of N; cows fed RC-grass had decreases urinary N excretion in week 4 despite no difference between treatments in week 7. In general, while the impact of forage sources on CH_4 emissions was small, RC-grass appeared to be more effective than ALF-grass to elevate plasma EAA concentrations.

Figure 4. The GreenFeed solar-powered, automated gaseous measurement unit at the UNH Organic Dairy Research Farm (Lee, NH)

Table 7. Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on methane (CH_4) production, CH_4 yield, CH_4 intensity, and urinary N excretion in organic Jersey cows

	Week 4		Week 7			*P*-value		
Item	ALF-grass	RC-grass	ALF-grass	RC-grass	SEM	Treatment (T)	Week (W)	T × W
CH_4 production g/d	424[a]	378[b]	421	410	14.6	0.17	0.05	0.02
CH_4 yield[1], g/kg DMI	19.8	18.9	19.7	21.2	1.15	0.82	0.08	0.06
CH_4 intensity[2], g/kg ECM	14.6	14.3	15.6	15.6	0.57	0.88	0.01	0.68
Urinary N excretion, g/d	218[a]	157[b]	200	182	11.2	0.02	0.63	<0.01

[a,b]Means with different superscripts within week of sampling differ significantly.
[1]DMI = dry matter intake.
[2]ECM = energy-corrected milk.

Tradeoffs associated with forage legumes and phytoestrogens

While forage legumes can provide many benefits to dairy systems, they can also contain varying types and quantities of phytoestrogens, with potential impacts on animal fertility, milk composition, and human health (Rietjens et al., 2017). Phytoestrogens are plant-derived compounds that are structurally and functionally similar to estrogen, the female sex hormone (Rietjens et al., 2017). Major classes of phytoestrogens include isoflavones, flavones, stilbenes, lignans, and coumestans, and these can vary by plant species; however, in general, they are most abundant in legumes (Reed, 2016). Forage legumes vary in type and concentration of phytoestrogens. For example, red and white clover, both common in the Northeast, can contain flavones and isoflavones, while alfalfa and white clover produce the phytoestrogen coumestans (including coumestrol), which compared to flavones have a much higher binding potential to mammalian estrogen receptors (Reed, 2016). In contrast, phytoestrogen concentrations in birdsfoot trefoil appear to be relatively low (Sarelli et al., 2003; Höjer et al., 2012) and kura clover is purported to produce no phytoestrogens (UMN Extension 2018).

Forage management practices can also affect the concentrations of phytoestrogens. For example, Seguin et al. (2004) found that coumestrol concentrations were higher when alfalfa was harvested at early than late stages of maturity. Similarly, concentrations of coumestrol in alfalfa increased with the number of cuts taken during the season (Cheng et al., 1953). Sarelli et al. (2003) reported that concentrations of phytoestrogens were relatively low in birdsfoot trefoil, and therefore unaffected by management, while phytoestrogen contents of red clover were as high as 1% of DM when harvest occurred in the early stages of growth. Both Kelly et al. (1979) and Sivesind and Seguin (2005) observed decreases in isoflavones of up to 45% when red clover was made into hay. In the case of ensiling, reports are inconsistent due in part to methodological issues. Daems et al. (2016) detected decreases of up to 73% in key isoflavones in unwilted red clover ensiled for as short as 2 weeks (shortest ensiling time), suggesting an active microbial role during the aerobic phase of ensiling. Moreover, red clover ensiled at 40% DM had 9% less total phytoestrogens compared with that ensiled at 25% DM (Sarelli et al. 2003). These results suggest a potential effect of wilting and DM concentration on phytoestrogen concentrations.

While the effects of phytoestrogens on reproduction in sheep have been well documented, ranging from minor reproductive disturbances to complete and irreversible infertility, their impacts on the reproductive health of dairy cows is less studied (Reed, 2016). Researchers have reported links between alfalfa consumption and reproductive abnormalities in cows, including cystic ovaries and temporary infertility (Romero et al., 1997). Mostrom and Evans (2011) reviewed findings from a number of studies reporting reproductive issues in cattle following consumption of a variety of forages, including red clover and alfalfa, and concluded a "washout period" of 4 to 6 weeks in which estrogenic forages are eliminated from the diet usually resulted in a return to normal reproductive cycling, but that more research, particularly in dairy, was necessary. In contrast, Adler et al. (2015) observed no difference in fertility indicators in dairy cows receiving increased proportions of red clover. In a review of the effects of phytoestrogens on the fertility of herbivores, Reed (2016) concluded "coordinated interdisciplinary research and extension is needed to better define the problem, quantify the risk, and improve diagnosis".

Recent work in Europe has shown that a substantive fraction of the phytoestrogens consumed in forages are transferred to the milk of dairy cows and this depends strongly on the composition of the forage (Daems et al., 2016; Bláhová et al., 2016). Höjer et al. (2012) demonstrated it is possible to manage the level and composition of phytoestrogens in milk through careful management of legumes and their proportion in the sward. Adler et al. (2015) observed that milk from organic dairies in Norway contained more isoflavones than did milk from the conventional farms and concentrations were positively correlated with the proportion of red clover in the fields. These results suggest that US "grass-fed" organic dairies may be at increased risk of producing milk with high phytoestrogen concentrations; however, to our knowledge this has not been examined.

Transfer of phytoestrogens to milk has obvious human health implications despite no clear consensus as to whether health effects are largely positive or negative (Mostrom and Evans, 2011; Rietjens et al., 2017). Phytoestrogens are often marketed as natural alternatives to estrogen replacement therapy to lower the risk of menopausal symptoms and osteoporosis, and they have been linked to reduced risks of heart disease, atherosclerosis, and some cancers (Reitjens et al. 2017). Conversely, their potential effects as endocrine disruptors, particularly in infants, has been a source of increasing concern among health professionals (Mostrom and Evans, 2011; Reed, 2016). Compared to soymilk, concentrations of phytoestrogens in fresh milk are relatively lower (Hoerger et al. 2011). Mostrom and Evans (2011) concluded "further research is necessary to determine mechanisms that underlie the impact, detrimental or beneficial, of phytoestrogens on reproductive processes in humans and farm animals."

Conclusions and Recommendations

According to several studies published in the literature, inclusion of legumes in pastures (>30% of diet DM) and total mixed rations (≥ 50% of diet DM) resulted in more production of milk and milk fat compared with grass-based diets. In addition, birdsfoot trefoil and white clover silages generally led to greater milk production and composition compared with silages made from red clover or alfalfa. Preliminary results from agronomic studies at UNH Kingman Farm (Madbury, NH) demonstrated that red clover led to the highest biomass yield and proportion of legume relative to orchardgrass in mixed swards. Results from the feeding trial done at the UNH Organic Dairy Research Farm (Lee, NH) revealed that cows fed the alfalfa-grass diet showed increased production of 4% fat-corrected milk, energy-corrected milk, and milk fat suggesting better dietary energy partition towards milk fat synthesis compared with the red clover-grass diet. However, cows fed red clover-grass had lower milk urea N (MUN) concentration and urinary N excretion than those fed alfalfa-grass indicating reduced ruminal proteolysis of dietary protein and improved N utilization. Moreover, red clover-grass significantly elevated the plasma concentrations of the essential amino acids histidine and leucine, and tended to increase phenylalanine and valine also suggesting reduced ruminal proteolysis and increased passage of dietary protein to the small intestine. While inclusion of red clover-grass in the diet improved the proportion of ω-3 fatty acids in milk and reduced the ω-6:ω-3 ratio, it also increased the proportion of *trans*-10 18:1, which is known to be involved in milk fat depression. Based on the results of UNH research, dairy farmers should feed baleage harvested from alfalfa-grass mixture than that from red clover-grass mixture to maximize milk fat production, which can improve farm profitability. Interestingly, feeding baleage harvested from red clover-grass mixture appears to be more environmentally friendly due to improved N utilization assessed via decreased MUN concentration and urinary N excretion. Red clover-grass mixture also improved ω-3 fatty acids and this is in line with milk fatty acid profile that better match human health. Further research is needed to advance the understanding regarding energy partition in cows fed red clover-grass diets, as well as the impact of red clover on milk *trans*-10 18:1. Research is also needed to assess the impact of legume phytoestrogens on reproductive efficiency of dairy cows fed high-legume diets.

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