

**Table 1.** Botanical composition (% of dry matter) of 2<sup>nd</sup> and 3<sup>rd</sup> cut alfalfa (ALF)-grass and red clover (RC)-grass mix swards

Item	2 <sup>nd</sup> Cut		3 <sup>rd</sup> Cut	
	ALF-grass	RC-grass	ALF-grass	RC-grass
Legume, %	65	80	84	96.5
Timothy, %	17	15	1	0.3
Meadow fescue, %	0.3	0.4	2	2
Broadleaf weeds, %	12	1	2	1
Grass weeds, %	5.7	3.6	11	0.2

**Table 2.** Nutritional composition of baleages fed to lactating dairy cows

Item	Baleages			
	Alfalfa-grass mix, 2 <sup>nd</sup> cut	Alfalfa-grass mix, 3 <sup>rd</sup> cut	Red clover-grass mix, 2 <sup>nd</sup> cut	Red clover-grass mix, 3 <sup>rd</sup> cut
	-----% of dry matter (unless otherwise noted)-----			
Dry matter, % as fed	42.7	48.9	75.7	32.3
Crude protein (CP)	20.9	21.8	20.1	20.5
Soluble CP, % of CP	63.0	62.0	25.5	40.5
aNDFom <sup>1</sup>	41.0	41.3	41.6	42.1
Acid detergent fiber	31.7	33.1	29.9	33.7
Lignin	6.05	7.25	5.05	7.95
Starch	2.60	1.75	1.40	0.85
Sugars	4.95	3.05	6.90	4.05
Crude fat	3.20	3.65	3.75	3.35
NE <sub>L</sub> , <sup>2</sup> Mcal/kg	0.63	0.60	0.68	0.57
Ca	0.91	0.89	1.12	1.07
P	0.40	0.41	0.36	0.35

<sup>1</sup>Amylase treated, ash free neutral detergent fiber.

<sup>2</sup>NE<sub>L</sub> = net energy of lactation.

**Table 3.** Nutritional composition of the concentrate mash used in the feeding trial

Item	
Dry matter, % as fed	89.7
Crude protein (CP)	13.9
Soluble CP, % of CP	21.0
aNDFom <sup>1</sup>	9.40
Acid detergent fiber	5.00
Lignin	1.20
Starch	45.2
Sugars	7.20
Crude fat	5.60
NE <sub>L</sub> , <sup>2</sup> Mcal/kg	2.03
Ca	0.87
P	0.52

**Table 4.** Ingredient composition of the experimental diets

Item	Experimental diets	
	Alfalfa-grass mix	Red clover-grass mix
	-----% of diet dry matter-----	
Alfalfa-grass mix, 2 <sup>nd</sup> cut	32.5	-
Alfalfa-grass mix, 3 <sup>rd</sup> cut	32.5	-
Red clover-grass mix, 2 <sup>nd</sup> cut	-	32.5
Red clover-grass mix, 3 <sup>rd</sup> cut	-	32.5
Grain mash	35.0	35.0

**Table 5.** Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on OTU count, bacteria:archaea ratio, and bacterial phylum relative frequency (%)

Item	Week 4		Week 7		SEM	<i>P</i> -value		
	ALF-grass	RC-grass	ALF-grass	RC-grass		Treatment (T)	Week (W)	T × W
OTU count	77.4	60.2	154	157	8.76	0.41	<0.001	0.27
Bacteria:Archaea ratio	41.2	42.7	47.6	41.2	4.63	0.59	0.58	0.37
Bacterial phylum, %								
Bacteroidetes	51.6	54.6	47.6	46.2	1.87	0.66	<0.01	0.24
Firmicutes	32.2	27.4	36.5	38.1	2.03	0.43	<0.01	0.13
Proteobacteria	7.33	7.01	4.74	4.61	1.46	0.88	0.10	0.95
Spirochaetes	1.90	1.86	2.69	2.48	0.43	0.78	0.11	0.84
Tenericutes	1.88	1.54	2.30	2.71	0.24	0.90	0.00	0.13
Fibrobacteres	1.16 <sup>b</sup>	3.00 <sup>a</sup>	1.48	1.76	0.34	<0.01	0.19	0.03
Kiritimatiellaeota	0.87 <sup>b</sup>	2.49 <sup>a</sup>	0.94	1.14	0.33	<0.01	0.06	0.03
Actinobacteria	0.20	0.21	0.39	0.54	0.09	0.35	<0.01	0.45
Planctomycetes	0.25	0.17	0.32	0.36	0.07	0.74	0.08	0.42
Cyanobacteria	0.25	0.31	0.40	0.39	0.10	0.83	0.28	0.73
Chloroflexi	0.36	0.18	0.36	0.24	0.09	0.09	0.76	0.75
Others <sup>1</sup>	2.03	1.16	2.28	1.53	0.19	<0.001	0.23	0.52

<sup>a,b</sup>Means with different superscripts within week of sampling differ significantly.

<sup>1</sup>Includes unclassified phyla and phyla with relative frequencies lower than 0.1%.

**Table 6.** Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on methanogenic/archaea genus relative frequency (%)

Item	Week 4		Week 7		SEM	<i>P</i> -value		
	ALF-grass	RC-grass	ALF-grass	RC-grass		Treatment (T)	Week (W)	T × W
Methanobrevibacter, <sup>1</sup> %	8.78	8.55	17.6	7.53	3.28	0.11	0.22	0.13
Methanobrevibacter, <sup>2</sup> %	45.6	39.8	39.3	43.3	6.65	0.89	0.83	0.45
Methanobrevibacter, <sup>3</sup> %	34.9	44.6	28.5	33.5	7.33	0.30	0.22	0.74
Methanosphaera, <sup>4</sup> %	10.8	7.07	14.6	15.1	1.96	0.39	<0.01	0.27
Methanosphaera, <sup>5</sup> %	0.00	0.00	0.00	0.61	0.21	0.16	0.16	0.19

<sup>1</sup>Uncultured Methanobrevibacter sp.

<sup>2</sup>Uncultured archaeon.

<sup>3</sup>Unclassified.

<sup>4</sup>Uncultured archaeon.

<sup>5</sup>Unclassified.

**Table 7.** Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on ruminal fermentation profile in organic Jersey cows

Item	Week 4		Week 7		SEM	<i>P</i> -value		
	ALF-grass	RC-grass	ALF-grass	RC-grass		Treatment (T)	Week (W)	T × W
Ruminal total VFA, <sup>1</sup> mM	100	100	91.3	98.9	6.05	0.60	0.35	0.51
Ruminal acetate, %	73.6	75.5	73.2	74.8	0.28	<0.001	0.03	0.54
Ruminal propionate, %	13.4	12.7	13.6	13.4	0.18	0.05	0.02	0.19
Ruminal butyrate, %	10.3	9.92	10.6	9.68	0.22	0.02	0.80	0.19
Ruminal isobutyrate, %	0.94 <sup>a</sup>	0.61 <sup>b</sup>	0.79 <sup>a</sup>	0.58 <sup>b</sup>	0.02	<0.001	<0.001	<0.01
Ruminal valerate, %	1.32 <sup>a</sup>	0.98 <sup>b</sup>	1.33 <sup>a</sup>	1.23 <sup>b</sup>	0.04	<0.001	<0.001	<0.001
Ruminal isovalerate, %	0.49	0.27	0.43	0.26	0.02	<0.001	0.10	0.19
Ruminal A:P ratio <sup>2</sup>	5.54	5.97	5.41	5.61	0.08	<0.01	<0.01	0.19

<sup>a,b</sup>Means with different superscripts within week of sampling differ significantly.

<sup>1</sup>VFA = volatile fatty acids.

<sup>2</sup>A:P = acetate:propionate.

**Table 8.** 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

Item	Week 4		Week 7		SEM	P-value		
	ALF-grass	RC-grass	ALF-grass	RC-grass		Treatment (T)	Week (W)	T × W
DMI, <sup>1</sup> 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, <sup>2</sup> kg/d	27.9	25.0	25.5	24.4	0.72	0.05	0.02	0.15
ECM yield, <sup>3</sup> 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, <sup>4</sup> mg/dL	14.4 <sup>a</sup>	11.6 <sup>b</sup>	11.7 <sup>a</sup>	10.4 <sup>b</sup>	0.24	<0.001	<0.001	<0.001

<sup>a,b</sup>Means with different superscripts within week of sampling differ significantly.

<sup>1</sup>DMI = dry matter intake

<sup>2</sup>FCM = fat-corrected milk.

<sup>3</sup>ECM = energy-corrected milk.

<sup>4</sup>MUN = milk urea nitrogen.



**Table 9.** Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on the plasma concentration ( $\mu\text{M}$ ) of essential amino acids in organic Jersey cows

Amino acids	Week 4		Week 7		SEM	<i>P</i> -value		
	ALF-grass	RC-grass	ALF-grass	RC-grass		Treatment (T)	Week (W)	T $\times$ W
Arginine	82.7	77.2	76.1 <sup>b</sup>	89.2 <sup>a</sup>	4.36	0.41	0.54	0.05
Histidine	38.2 <sup>b</sup>	52.8 <sup>a</sup>	35.4 <sup>b</sup>	57.4 <sup>a</sup>	4.00	<0.01	0.55	0.02
Isoleucine	152	153	135	155	9.05	0.37	0.30	0.18
Leucine	150	173	134	184	11.3	0.03	0.76	0.11
Lysine	96.5	90.0	89.6	100	6.13	0.76	0.76	0.14
Methionine	24.6	20.3	22.8	24.1	1.74	0.47	0.51	0.07
Phenylalanine	59.0	59.9	54.9 <sup>b</sup>	66.2 <sup>a</sup>	2.79	0.08	0.65	0.04
Threonine	110	103	98.6	99.9	8.44	0.81	0.13	0.42
Tryptophan	44.7	40.6	40.9	43.8	2.06	0.81	0.84	0.03
Valine	269	289	237 <sup>b</sup>	300 <sup>a</sup>	15.3	0.06	0.24	0.03
Total BCAA <sup>1</sup>	572	615	506	639	34.9	0.07	0.38	0.07
Total EAA <sup>2</sup>	1,027	1,059	924 <sup>b</sup>	1,120 <sup>a</sup>	53.1	0.11	0.59	0.05

<sup>a,b</sup>Means with different superscripts within week of sampling differ significantly.

<sup>1</sup>BCAA = branched-chain amino acids.

<sup>2</sup>EAA = essential amino acids.

**Table 10.** Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on the plasma concentration ( $\mu M$ ) of nonessential amino acids in organic Jersey cows

Amino acids	Week 4		Week 7		SEM	<i>P</i> -value		
	ALF-grass	RC-grass	ALF-grass	RC-grass		Treatment (T)	Week (W)	T $\times$ W
Alanine	235	242	231	257	14.2	0.39	0.51	0.30
Asparagine	52.2	47.9	49.6	50.3	3.43	0.66	0.99	0.38
Aspartic acid	1.43	1.30	1.62	1.37	0.16	0.34	0.23	0.59
Citrulline	79.3	85.1	75.8 <sup>b</sup>	92.8 <sup>a</sup>	3.14	0.01	0.30	0.01
Cystine	22.7	22.3	22.2	22.8	1.15	0.92	0.99	0.47
Glutamine	246	217	235	207	13.2	0.09	0.39	0.97
Glutamic acid	57.3	53.6	59.8	55.5	1.86	0.11	0.12	0.81
Glycine	302	327	300	306	22.2	0.60	0.56	0.51
Hydroxyproline	7.45	7.44	7.47	6.62	0.35	0.37	0.07	0.05
Ornithine	46.6	50.7	44.3	54.6	2.65	0.07	0.61	0.06
Proline	85.5	90.8	78.6	95.6	4.88	0.10	0.74	0.08
Serine	99.8	85.9	94.7	87.6	7.16	0.28	0.68	0.42
Taurine	35.3	36.0	34.5	39.1	2.41	0.36	0.57	0.33
Tyrosine	64.9	57.3	56.7	67.2	3.82	0.71	0.82	0.03
Total NEAA <sup>1</sup>	1,335	1,324	1,292	1,344	53.6	0.76	0.76	0.42

<sup>a,b</sup>Means with different superscripts within week of sampling differ significantly.

<sup>1</sup>NEAA = nonessential amino acids

**Table 11.** 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

Fatty acids (FA)	Week 4		Week 7		SEM	P-value		
	ALF-grass	RC-grass	ALF-grass	RC-grass		Treatment (T)	Week (W)	T × W
4:0	5.63	5.59	5.61	5.55	0.15	0.81	0.64	0.86
6:0	3.00	3.00	2.95	2.94	0.05	0.90	0.02	0.74
8:0	1.63	1.62	1.56	1.57	0.03	1.00	<0.001	0.66
10:0	3.68	3.61	3.47	3.42	0.10	0.67	<0.001	0.78
<i>cis</i> -9 10:1	0.33	0.32	0.31	0.30	0.01	0.56	<0.01	0.77
12:0	4.24	4.01	3.94	3.77	0.13	0.29	<0.001	0.57
14:0	11.7	11.7	11.7	11.4	0.10	0.20	0.10	0.10
<i>cis</i> -9 14:1	0.85	0.81	0.84	0.79	0.05	0.52	0.45	0.83
16:0	32.2 <sup>a</sup>	30.4 <sup>b</sup>	32.9 <sup>a</sup>	30.3 <sup>b</sup>	0.53	0.02	0.01	<0.001
<i>cis</i> -9 16:1	0.98	0.90	0.98	0.92	0.06	0.38	0.33	0.63
18:0	11.4	12.6	11.2	12.8	0.44	0.04	0.95	0.18
<i>trans</i> -10 18:1	0.15	0.19	0.19	0.21	0.01	0.01	<0.001	0.38
<i>trans</i> -11 18:1	1.12	1.15	1.20	1.20	0.07	0.92	0.02	0.58
<i>cis</i> -9 18:1	13.8	14.3	13.7	14.7	0.35	0.12	0.27	0.21
<i>cis</i> -9, <i>cis</i> -12 18:2	1.80	2.00	1.75	2.05	0.07	0.03	0.95	0.08
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.67 <sup>b</sup>	0.85 <sup>a</sup>	0.61 <sup>b</sup>	0.87 <sup>a</sup>	0.03	<0.001	0.05	<0.01
<i>cis</i> -9, <i>trans</i> -11 18:2 CLA	0.42	0.39	0.46	0.42	0.03	0.37	<0.01	0.67
Σ <i>trans</i> -18:1 FA	1.90	1.99	2.05	2.07	0.09	0.66	<0.01	0.35
Σ <i>cis</i> -18:1 FA	14.4	15.0	14.4	15.5	0.35	0.12	0.22	0.24
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 <sup>b</sup>	0.93 <sup>a</sup>	0.67 <sup>b</sup>	0.95 <sup>a</sup>	0.03	<0.001	0.08	<0.01
ω-6/ω-3 ratio	3.04 <sup>a</sup>	2.62 <sup>b</sup>	3.22 <sup>a</sup>	2.62 <sup>b</sup>	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 <sup>a</sup>	31.3 <sup>b</sup>	33.9 <sup>a</sup>	31.2 <sup>b</sup>	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

<sup>a,b</sup>Means with different superscripts within week of sampling differ significantly.

**Table 12.** Effect of feeding diets containing alfalfa-meadow fescue-timothy grass (ALF) or red clover-meadow fescue-timothy grass (RC) baleage on methane (CH<sub>4</sub>) production, CH<sub>4</sub> yield, CH<sub>4</sub> intensity, and urinary N excretion in organic Jersey cows

Item	Week 4		Week 7		SEM	<i>P</i> -value		
	ALF-grass	RC-grass	ALF-grass	RC-grass		Treatment (T)	Week (W)	T × W
CH <sub>4</sub> production g/d	424 <sup>a</sup>	378 <sup>b</sup>	421	410	14.6	0.17	0.05	0.02
CH <sub>4</sub> yield <sup>1</sup> , g/kg DMI	19.8	18.9	19.7	21.2	1.15	0.82	0.08	0.06
CH <sub>4</sub> intensity <sup>2</sup> , 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 <sup>a</sup>	157 <sup>b</sup>	200	182	11.2	0.02	0.63	<0.01

<sup>a,b</sup>Means with different superscripts within week of sampling differ significantly.

<sup>1</sup>DMI = dry matter intake.

<sup>2</sup>ECM = energy-corrected milk.