Using Chemical and Biological Approaches to Predict Energy Values of Selected Forages Affected by Variety and Maturity Stage: Comparison of Three Approaches

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ABSTRACT : Two varieties of alfalfa (*Medicago sativa* L cv. Pioneer and Beaver) and timothy (*Phleum pratense* L cv. Climax and Joliette), grown at different locations in Saskatchewan (Canada), were cut at three stages [1=one week before commercial cut (early bud for alfalfa; joint for timothy); 2=at commercial cut (late bud for alfalfa; pre-bloom head for timothy); 3=one week after commercial cut (early bloom for alfalfa; full head for timothy)]. The energy values of forages were determined using three approaches, including chemical (NRC 2001 formula) and biological approaches (standard *in vitro* and *in situ* assay). The objectives of this study were to determine the effects of forage variety and stage of maturity on energy values under the climate conditions of western Canada, and to investigate relationship between chemical (NRC 2001 formula) approach and biological approaches (*in vitro* and *in situ* assay) on prediction of energy values. The results showed that, in general, forage species (alfalfa vs. timothy) and cutting stage had profound impacts, but the varieties within each species (Pioneer vs. Beaver in alfalfa; Climax vs. Joliette in timothy) had minimal effects on energy values. As forage maturity increased, the energy contents behaved in a quadratic fashion, increasing at stage 2 and then significantly decreasing at stage 3. However, the prediction methods-chemical approach (NRC 2001 formula) and biological approaches (*in vitro* and *in situ* assay) had great influences on energy values. The highest predicted energy values were found by using the *in situ* approach, the lowest prediction value by using the NRC 2001 formula, and the intermediate values by the *in vitro* approach. The *in situ* results may be most accurate because it is closest to simulate animal condition. The energy values measured by biological approaches are not predictable by the chemical approach in this study, indicating that a refinement is needed in accurately predicting energy values. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 2 : 228-236)***

Key Words : Energy Values, Truly Digestible Nutrients, Biological and Chemical Approaches, Forage, Varieties, Cutting Stage

INTRODUCTION

Alfalfa (*Medicago sativa* L.) and timothy (*Phleum pratense* L.) are two important forages grown in western Canada for export. Among varieties, Pioneer and Beaver alfalfa and Climax and Joliette timothy are well adapted to climate and soil conditions of the region (Canadian Dehydrator's Association, 1990; Canadian Hay Association, 1999). However, the detailed nutritive values for these varieties affected by cutting stages are lacking. In NRC dairy (2001), there are no energy values for timothy and no effect of varieties and cutting stage on energy values for alfalfa. To produce top quality alfalfa and timothy, the need for this information is crucial.

Numerous studies (Minson and McLeod, 1970; Cox et al., 1994; Lundvall et al., 1994; Jung and Allen, 1995; Mathison et al., 1996; Ayres et al., 1998; Elizalde et al., 1999; Ferdinandez and Coulman, 2001; Lyon et al., 2001) have shown that nutritive values, yield and quality of forages are affected by growth stage, forage species, cultivars, fertilization, soil type, climate condition (e.g. rainfall, temperature), planting conditions (e.g. row spacing, planting rate), and (or) growing conditions.

In NRC dairy (2001), a summative approach is used to derive total digestible nutrients at 1× maintenance (TDN1x). In this approach, the concentrations (% DM) of truly digestible nutrients for each feed are estimated (Weiss et al., 1992) according to the equations in NRC dairy (2001). This is a standard method, also called chemical approach-which means using all chemical compositions to estimate energy values. However, the TDN1x value could also be obtained using biological approaches to the some extent. The truly digestible neutral detergent fibre (tdNDF) value could be determined by using a 48 h rumen *in vitro* (NRC, 2001) or *in situ* assay.

The objectives of this study were: (1) to provide detailed information on energy values for each variety of alfalfa and timothy cut at different maturity stages for Canadian forage export industry; and (2) to investigate relationship between the chemical (NRC 2001 formula) and the biological approaches (*in vitro* and *in situ* assay) on prediction of energy values.

MATERIALS AND METHODS

Forages

The samples of the two varieties and three cutting times

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Table 1. Effect of variety and cutting stage on chemical composition of alfalfa and timothy

Forage variety	Variety	Cutting stage[a]	DM (g/kg)	Ash	EE	CP	ADICP	NDICP	NDF	ADF	ADL	GE (Mcal/ kg DM)
				(g/kg DM)			(g/kg CP)		(g/kg DM)			
Alfalfa	Pioneer	Stage 1	929.0	99.3	33.0	204.4	30.0	400.4	497.8	320.3	113.9	4.404
		Stage 2	935.2	95.6	40.7	187.1	51.8	344.2	471.3	294.1	92.7	4.400
		Stage 3	926.2	94.3	25.9	177.5	173.7	366.1	543.3	320.0	92.9	4.397
	Beaver	Stage 1	934.7	104.9	28.2	199.2	39.5	331.0	487.7	320.6	99.4	4.325
		Stage 2	933.8	100.5	30.2	193.6	52.2	374.4	469.0	293.8	93.2	4.389
		Stage 3	931.7	91.9	28.0	173.8	167.6	330.1	495.9	309.6	105.8	4.363
Timothy	Climax	Stage 1	936.9	71.1	24.9	128.3	55.8	273.4	673.1	365.5	60.2	4.459
		Stage 2	923.3	69.3	28.3	125.0	57.0	260.2	686.1	393.0	57.3	4.529
		Stage 3	930.4	65.9	24.7	96.8	35.2	246.9	705.1	388.0	54.2	4.436
	Joliette	Stage 1	939.4	69.2	20.5	93.6	54.5	264.0	700.6	394.4	47.1	4.389
		Stage 2	926.3	64.6	25.8	78.7	41.8	260.6	725.4	414.5	47.9	4.398
		Stage 3	932.5	62.8	24.0	61.6	65.8	283.5	750.2	426.8	64.2	4.427
SEM[b]			1.22	2.27	3.06	4.62	3.88	18.93	15.34	6.79	4.53	0.0111
Statistical analysis			P values									
Alfalfa vs.Timothy			0.8821	0.0001	0.0063	0.0001	0.0798	0.0001	0.0001	0.0001	0.0001	0.0018
Pioneer vs. Beaver			0.2650	0.3081	0.1422	0.9248	0.9633	0.1366	0.2245	0.6999	0.9425	0.0264
Climax vs. Joliette			0.3844	0.2203	0.3834	0.0002	0.8656	0.5765	0.0294	0.0031	0.4331	0.0007
In alfalfa												
Stage 1 vs. Stage 2			0.1612	0.1156	0.1537	0.3519	0.0206	0.7730	0.2375	0.0218	0.0312	0.3240
Stage 1 vs. Stage 3			0.1370	0.0018	0.2818	0.0428	0.0001	0.4287	0.1650	0.5993	0.2298	0.6086
Stage 2 vs. Stage 3			0.0074	0.0613	0.0181	0.2366	0.0001	0.6116	0.0156	0.0637	0.2886	0.6282
In timothy												
Stage 1 vs. Stage 2			0.0001	0.2098	0.2026	0.4583	0.4099	0.7058	0.3210	0.0370	0.8662	0.2065
Stage 1 vs. Stage 3			0.0018	0.0304	0.6273	0.0164	0.5042	0.8732	0.0408	0.0181	0.3555	0.8088
Stage 2 vs. Stage 3			0.0019	0.3083	0.4183	0.0752	0.8730	0.8271	0.2523	0.7337	0.2778	0.3009

[a] Stage 1=one week before commercial cut (early bud for alfalfa varieties and joint for timothy varieties); 2=at commercially cutting stage (late bud for alfalfa varieties and pre-bloom head for timothy varieties); 3=one week after commercial cut (early bloom for alfalfa varieties and full head for timothy varieties). [b] SEM=standard error of means.

(1=one week before the commercial cut: early bud for alfalfa varieties and joint for timothy varieties; 2=at the commercial cutting stage: late bud for alfalfa varieties and pre-bloom head for timothy varieties; 3=one week after the commercial cut: early bloom for alfalfa varieties and full head for timothy varieties) of alfalfa (Pioneer and Beaver) and timothy (Climax and Joliette), grown at different locations (each) in Saskatchewan in year 2001, were obtained from Elcan Forage Inc. (Saskatchewan, Canada). The detailed locations, growth and climate conditions, estimated maturity stages and sampling procedure were previously described by Yu et al. (2003a,b).

Rumen *in vitro* and *in situ* assay

Rumen *in vitro* digestibility NDF of the forages samples (ground through a 2 mm screen by Hammer Mill) after 48 h incubation was determined using the standard Tilley and Terry *in vitro* procedure (Marten and Barnes, 1980).

Rumen *in situ* digestibility of NDF of the forages samples (ground through a 2 mm screen by Hammer Mill) after a 48 h incubation were determined using the departmental standard *in situ* procedure in dairy cows (McKinnon et al., 1995) with polyester bags (10×20 cm; pore size of 53±10 μm, ANKOM Company, Fairport, NY).

Chemical analysis

The forage samples (ground through 1 mm screen by Retsch ZM-1) were analyzed for DM, ash, EE and N (AOAC, 1990). Acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were analyzed according to the procedures of Van Soest et al. (1991). Acid detergent insoluble N (ADIN) and neutral detergent insoluble N (NDIN) were determined according to the procedures of Licitra et al. (1996).

Estimation of energy values

Gross energy (GE) values were measured, using a Parr adiabatic bomb calorimeter (Model 1200, Parr Instrument Co., Moline, IL). The TDN_{1X}, DE_{1X} (=digestible energy at 1×maintenance), DE_p, ME_p (=digestible energy at production level) and NE_{Lp} (=net energy at production level) values were estimated from the NRC dairy (2001). The ME^{beef96} (=metabolizable energy), NE_m^{beef96} (=net energy for maintenance) and NE_g^{beef96} (=net energy for growth) values were estimated from the NRC beef (1996). The tdNDF content was calculated separately using three approaches as follows:

1) Using NRC (2001) formula (standard method)-chemical approach:

Table 2. Energy values of the forages (alfalfa and timothy varieties cut at three stages) estimated using NRC (2001) formula

Forage	Variety	Cutting stage[a]	NRC-dairy model-2001					NRC-beef model-1996		
			TDN_{1X}	DE_{1X}	DE_p	ME_p	NE_{Lp}	ME^{beef96}	NE_m^{beef96}	NE_g^{beef96}
			(% DM)	(Mcal/kg DM)				(Mcal/kg DM)		
Alfalfa	Pioneer	Stage 1	55.3	2.592	2.381	1.956	1.186	2.126	1.269	0.699
		Stage 2	58.8	2.706	2.485	2.065	1.266	2.219	1.355	0.777
		Stage 3	52.2	2.386	2.192	1.763	1.050	1.957	1.111	0.554
	Beaver	Stage 1	55.2	2.575	2.365	1.939	1.173	2.112	1.257	0.688
		Stage 2	57.2	2.650	2.433	2.008	1.222	2.173	1.313	0.739
		Stage 3	52.8	2.410	2.213	1.785	1.065	1.976	1.129	0.571
Timothy	Climax	Stage 1	56.2	2.523	2.317	1.890	1.139	2.069	1.216	0.651
		Stage 2	56.8	2.543	2.336	1.909	1.152	2.086	1.232	0.665
		Stage 3	56.8	2.509	2.304	1.877	1.129	2.057	1.206	0.641
	Joliette	Stage 1	57.1	2.516	2.310	1.883	1.134	2.063	1.211	0.646
		Stage 2	57.5	2.511	2.306	1.879	1.131	2.059	1.208	0.643
		Stage 3	53.8	2.331	2.141	1.712	1.014	1.911	1.068	0.514
SEM[b]			0.73	0.0328	0.0301	0.0309	0.0223	0.0269	0.0250	0.0230
Statistical analysis			P values							
Alfalfa vs.Timothy			0.1913	0.1552	0.1552	0.1517	0.1474	0.1552	0.1589	0.1607
Pioneer vs. Beaver			0.7668	0.7929	0.7925	0.7692	0.7410	0.7928	0.7995	0.8027
Climax vs. Joliette			0.6925	0.2638	0.2635	0.2670	0.2715	0.2635	0.2595	0.2577
In alfalfa										
Stage 1 vs. Stage 2			0.0048	0.0365	0.0366	0.0352	0.0337	0.0365	0.0399	0.0416
Stage 1 vs. Stage 3			0.0046	0.0003	0.0003	0.0003	0.0004	0.0003	0.0003	0.0003
Stage 2 vs. Stage 3			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
In timothy										
Stage 1 vs. Stage 2			0.5874	0.8452	0.8457	0.8447	0.8450	0.8455	0.8452	0.8443
Stage 1 vs. Stage 3			0.1184	0.0282	0.0281	0.0300	0.0325	0.0282	0.0275	0.0272
Stage 2 vs. Stage 3			0.0417	0.0187	0.0187	0.0199	0.0216	0.0187	0.0182	0.0180

[a] Stage 1=one week before commercial cut (early bud for alfalfa varieties and joint for timothy varieties); 2=at commercially cutting stage (late bud for alfalfa varieties and pre-bloom head for timothy varieties); 3=one week after commercial cut (early bloom for alfalfa varieties and full head for timothy varieties). [b] SEM=standard error of means.

$$tdNDF\ (\%DM)=0.75\times(NDF_n-ADL)\times(1-(ADL/NDF_n)^{0.667})$$

where, NDF_n=NDF-NDICP, in %DM,

2) Using standard *in vitro* approach (NRC, 2001)-biological approach I:

$$tdNDF\ (\%DM)=IVNDFD\ (\%)\times NDF\ (\%DM)$$

where, IVNDFD stands for *in vitro* digestibility of NDF at a 48 incubation.

3) Using standard *in situ* approach-biological approach II:

$$tdNDF\ (\%DM)=ISNDFD\ (\%)\times NDF\ (\%DM)$$

where, ISNDFD stands for *in situ* digestibility of NDF at a 48 incubation.

Statistical analysis

Statistical analyses were performed using the GLM procedure of SAS (1991). The model used for the analysis was:

$$Y_{jk(i)}=\mu+F_i+V_{j(a)}+V_{j(t)}+M_{k(a)}+M_{k(t)}+e_{ijk}$$

Where, $Y_{kl(i)}$ is an observation of the dependent variable for the variety j at maturity k in the forage i; μ is the population mean for the variable; F_i is the forage type i, i=a, t; a is alfalfa, and t is timothy; $V_{j(a)}$ is the effect of forage variety nested within alfalfa; $V_{j(t)}$ is the effect of forage variety nested within timothy; $M_{k(a)}$ is the effect of forage maturity nested within alfalfa; $M_{k(t)}$ is the effect of forage variety nested within timothy; and e_{ijk} is the random error associated with the observation jk(i). Contrasts were used to determine treatment differences (SAS, 1991). Relationships between chemical approach and biological approaches on predicted energy values were also evaluated by correlation and regression analysis with the GLM procedure of SAS (1991). Significance was declared at $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition of the forages

The effects of variety and cutting stage on chemical composition of alfalfa and timothy are presented in Table 1. Alfalfa species contained a higher ($p<0.05$) content of ash (98 vs. 67 g/kg DM), EE (31 vs. 25 g/kg DM), CP (189 vs. 97 g/kg DM), ADICP (86 vs. 52 g/kg CP), NDICP (358 vs. 245 g/kg CP), ADL (100 vs. 55 g/kg DM), but lower

Table 3. Energy values of the forages (alfalfa and timothy varieties cut at three stages) estimated using *in vitro* assay

Forage	Variety	Cutting stage[a]	NRC-Dairy model -2001					NRC-Beef model-1996		
			TDN_{1X}	DE_{1X}	DE_p	ME_p	NE_{Lp}	ME^{beef96}	NE_m^{beef96}	NE_g^{beef96}
			(% DM)	(Mcal/kg DM)				(Mcal/kg DM)		
Alfalfa	Pioneer	Stage 1	62.7	2.901	2.664	2.242	1.387	2.379	1.498	0.906
		Stage 2	64.5	2.946	2.705	2.287	1.423	2.415	1.532	0.937
		Stage 3	55.7	2.534	2.327	1.901	1.146	2.078	1.225	0.660
	Beaver	Stage 1	60.3	2.791	2.563	2.138	1.313	2.288	1.418	0.834
		Stage 2	63.1	2.896	2.660	2.236	1.382	2.375	1.495	0.904
		Stage 3	54.9	2.498	2.294	1.867	1.123	2.048	1.197	0.633
Timothy	Climax	Stage 1	46.2	2.103	1.931	1.501	0.865	1.725	0.886	0.344
		Stage 2	46.0	2.090	1.920	1.489	0.857	1.714	0.875	0.333
		Stage 3	43.4	1.946	1.787	1.355	0.762	1.595	0.757	0.222
	Joliette	Stage 1	38.4	1.727	1.586	1.152	0.620	1.416	0.573	0.046
		Stage 2	44.5	1.968	1.808	1.376	0.777	1.614	0.776	0.240
		Stage 3	37.9	1.666	1.530	1.095	0.580	1.366	0.521	-0.004
SEM[b]			1.75	0.0754	0.0692	0.0701	0.0494	0.0618	0.0579	0.0532
Statistical analysis			P values							
Alfalfa vs.Timothy			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Pioneer vs. Beaver			0.4960	0.5344	0.5346	0.5230	0.5077	0.5344	0.5505	0.5583
Climax vs. Joliette			0.0361	0.0210	0.0210	0.0214	0.0218	0.0210	0.0135	0.0111
In alfalfa										
Stage 1 vs. Stage 2			0.3149	0.4781	0.4781	0.4676	0.4541	0.4782	0.5156	0.5330
Stage 1 vs. Stage 3			0.0121	0.0052	0.0052	0.0052	0.0052	0.0052	0.0086	0.0107
Stage 2 vs. Stage 3			0.0012	0.0010	0.0010	0.0010	0.0010	0.0010	0.0020	0.0027
In timothy										
Stage 1 vs. Stage 2			0.1929	0.2849	0.2849	0.2852	0.2860	0.2848	0.2681	0.2614
Stage 1 vs. Stage 3			0.4697	0.3064	0.3063	0.3073	0.3088	0.3064	0.2937	0.2888
Stage 2 vs. Stage 3			0.0510	0.0450	0.0449	0.0452	0.0457	0.0449	0.0391	0.0370

[a] Stage 1=one week before commercial cut (early bud for alfalfa varieties and joint for timothy varieties); 2=at commercially cutting stage (late bud for alfalfa varieties and pre-bloom head for timothy varieties); 3=one week after commercial cut (early bloom for alfalfa varieties and full head for timothy varieties). [b] SEM=standard error of means.

($p<0.05$) contents in NDF (494 vs. 707 g/kg DM), ADF (310 vs. 397 g/kg DM) and GE (4.38 vs. 4.44 Mcal/kg DM). Generally, these are in agreement with tabular values in NRC (2001). Alfalfa had lower NDF and ADF concentrations than timothy, as expected. The difference in NDF content between alfalfa and timothy can be accounted for by the difference between NDF and ADF, which is primarily hemicellulose (Elizalde et al., 1999). Hoffman et al. (1993) reported that there is a trend of higher NDF accumulation in grasses (i.e., timothy, orchardgrass, perennial ryegrass, quackgrass and bromegrass) compared with legumes (i.e. alfalfa, red clover, and birdsfoot trefoil). Pioneer alfalfa and Beaver alfalfa contained no difference ($p>0.05$) in all chemical compositions except that Pioneer alfalfa had a higher GE content. Climax and Joliette timothy contained the same chemical composition except that Climax timothy had higher levels of CP and GE, but lower fiber content (NDF and ADF) than Joliette timothy. The times of cutting had different effects on the chemical composition in alfalfa and timothy. As plant stage increased (1 to 3), ash decreased in both forages (alfalfa: 102 to 93 g/kg DM; timothy: 70 to 64 g/kg DM); CP greatly decreased in both forages (alfalfa: 202 to 176 g/kg DM; timothy: 111 to 79 g/kg DM); EE, ADL and NDICP were not significantly affected in either forages; there was a minimal effect on alfalfa ADF yet it increased in timothy (380 to 407 g/kg DM); NDF was affected very little in alfalfa, but increased in timothy (687 to 728 g/kg DM); ADICP was highly increased in alfalfa (35 to 171 g/kg CP) but there was no effect on timothy as the plants matured. The different responses of chemical composition to stage of cutting between alfalfa and timothy varieties are due to forage genotype (legume vs. grass) (Minson and McLeod, 1970; Lundvall et al., 1994; Jung and Allen, 1995).

Estimating energy values of alfalfa and timothy using chemical and biological approaches

Using NRC 2001 formula-chemical approach : The normal method to estimate energy value for a feed is to use NRC (2001) equations. This is also called a chemical approach, which is to use all chemical compositions to estimate energy values. The effects of variety and cutting stage on energy content of alfalfa and timothy using this approach are presented in Table 2. The results indicated that across all treatments, there were no significant differences ($p>0.05$) between the two forage species (alfalfa vs. timothy) for all estimated energy values (TDN_{1X}: 55 vs. 56% DM; DE_{1X}: 2.55 vs. 2.49; DE_p: 2.34 vs. 2.29; ME_p:

Table 4. Energy values of the forages (alfalfa and timothy varieties cut at three stages) estimated using *in situ* assay

Forage	Variety	Cutting stage[a]	NRC-Dairy model -2001					NRC-Beef model-1996		
			TDN_{1X}	DE_{1X}	DE_p	ME_p	NE_{Lp}	ME^{beef96}	$NE_m{}^{beef96}$	$NE_g{}^{beef96}$
			(% DM)	(Mcal/kg DM)				(Mcal/kg DM)		
Alfalfa	Pioneer	Stage 1	68.0	3.126	2.871	2.451	1.534	2.563	1.661	1.052
		Stage 2	66.8	3.045	2.797	2.380	1.488	2.497	1.604	1.000
		Stage 3	57.5	2.609	2.396	1.970	1.195	2.139	1.282	0.711
	Beaver	Stage 1	64.3	2.959	2.717	2.294	1.423	2.426	1.541	0.945
		Stage 2	66.3	3.033	2.785	2.363	1.471	2.487	1.595	0.993
		Stage 3	59.8	2.703	2.482	2.058	1.257	2.216	1.353	0.775
Timothy	Climax	Stage 1	60.6	2.707	2.486	2.061	1.259	2.220	1.353	0.774
		Stage 2	57.4	2.568	2.359	1.933	1.169	2.106	1.250	0.682
		Stage 3	52.6	2.333	2.142	1.714	1.015	1.923	1.069	0.515
	Joliette	Stage 1	58.1	2.556	2.347	1.921	1.160	2.096	1.241	0.673
		Stage 2	53.7	2.356	2.164	1.735	1.030	1.932	1.087	0.531
		Stage 3	47.4	2.063	1.895	1.464	0.839	1.692	0.853	0.313
SEM[b]			2.38	0.1018	0.0935	0.0948	0.0671	0.0835	0.0763	0.0693
Statistical analysis			-----P values-----							
Alfalfa vs.Timothy			0.0003	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Pioneer vs. Beaver			0.8236	0.8323	0.8323	0.8212	0.8051	0.8328	0.8505	0.8593
Climax vs. Joliette			0.2001	0.1292	0.1292	0.1300	0.1312	0.1291	0.1194	0.1150
In alfalfa										
Stage 1 vs. Stage 2			0.8580	0.9787	0.9791	0.9928	0.9879	0.9786	0.9809	0.9817
Stage 1 vs. Stage 3			0.0053	0.0023	0.0023	0.0023	0.0024	0.0023	0.0029	0.0033
Stage 2 vs. Stage 3			0.0035	0.0024	0.0024	0.0024	0.0023	0.0024	0.0031	0.0034
In timothy										
Stage 1 vs. Stage 2			0.1307	0.1372	0.1372	0.1388	0.1410	0.1372	0.1371	0.1371
Stage 1 vs. Stage 3			0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0007	0.0007
Stage 2 vs. Stage 3			0.0296	0.0259	0.0259	0.0263	0.0269	0.0259	0.0217	0.0199

[a] Stage 1=one week before commercial cut (early bud for alfalfa varieties and joint for timothy varieties); 2=at commercially cutting stage (late bud for alfalfa varieties and pre-bloom head for timothy varieties); 3=one week after commercial cut (early bloom for alfalfa varieties and full head for timothy varieties). [b] SEM=standard error of means.

1.91 vs. 1.86; NE_{Lp}: 1.16 vs. 1.12; ME^{beef96}: 2.09 vs. 2.04; $NE_m{}^{beef96}$: 1.24 vs. 1.19; $NE_g{}^{beef96}$: 0.67 vs. 0.63 Mcal/kg DM).

There were no differences (p>0.05) between alfalfa (Pioneer vs. Beaver) and between timothy varieties (Climax vs. Joliette) for all estimated energy values.

However, the stage of cutting, in both forages, had significant effects on energy values. In alfalfa, energy values in stage 1, 2 and 3 were significantly different (p<0.05). The energy values at stage 2 were higher than that at stage 1, but as alfalfa advanced to stage 3, all energy values significantly declined (p<0.05). The highest energy values occurred at stage 2 and the lowest at stage 3. Similar results were found in timothy. Although, stage 1 and 2 were not different (p>0.05) for all estimated energy values. As maturity further increased to stage 3, the energy values of timothy were decreased (p<0.05). The above results indicated that as alfalfa and timothy maturity increased, the energy contents behaved in a quadratic fashion, increasing at stage 2 and then significantly decreasing at stage 3.

Using in vitro assay-biological approach 1 : Using a biological approach- *in vitro* assay, the effects of variety and cutting stage on energy content of alfalfa and timothy are presented in Table 3. The results indicated that across all treatments, there were significant differences (p<0.01) between the two forage species (alfalfa vs. timothy) for all estimated energy values (TDN_{1X}: 60 vs. 46% DM; DE_{1X}: 2.76 vs. 1.92; DE_p: 2.54 vs. 1.76; ME_p: 2.12 vs. 1.33; NE_{Lp}: 1.30 vs. 0.74; ME^{beef96}: 2.26 vs. 1.57; $NE_m{}^{beef96}$: 1.39 vs. 0.73; $NE_g{}^{beef96}$: 0.81 vs. 0.20 Mcal/kg DM). These results are completely different from that estimated by NRC 2001 formula- chemical approach, in which no significant differences were found between alfalfa and timothy. The higher energy content in alfalfa species corresponds to higher IVNDFD48 in alfalfa (41 vs. 35%) (Table 5). The estimated energy content for alfalfa in this approach was slightly higher than the tabular values in the NRC (2001). The energy contents for timothy have no tabulated values recorded in NRC (2001).

There were no differences (p>0.05) between alfalfa varieties (Pioneer vs. Beaver), but significant differences (p<0.05) between timothy varieties (Climax vs. Joliette) for all estimated energy values (TDN_{1X}: 45 vs. 40% DM; DE_{1X}: 2.05 vs. 1.79; DE_p: 1.90 vs. 1.64; ME_p: 1.45 vs. 1.21; NE_{Lp}: 0.83 vs. 0.66; ME^{beef96}: 1.68 vs. 1.47; $NE_m{}^{beef96}$: 0.84 vs. 0.62; $NE_g{}^{beef96}$: 0.30 vs. 0.09 Mcal/kg DM for Climax and Joliette, respectively). This is also different from that estimated by NRC 2001 formula.

Table 5. Comparisons of digestibility of NDF and total digestible NDF (tdNDF) obtained from three methods: NRC 2001 formula; *in vitro* and *in situ* assay

Forage	Variety	Cutting stage[a]	Digestibility of NDF (%)			Total degradable NDF[b] (td NDF, % DM)		
			NRC 2001	*In vitro*	*In situ*	NRC 2001	*In vitro*	*In situ*
Alfalfa	Pioneer	Stage1	26.2	41.5	52.1	13.20	20.54	25.89
		Stage2	31.3	43.3	48.7	14.82	20.52	22.90
		Stage3	35.4	41.9	45.2	19.23	22.76	24.53
	Beaver	Stage1	30.7	41.1	49.4	14.96	20.09	24.09
		Stage2	30.0	42.3	49.6	14.11	19.97	23.23
		Stage3	30.8	35.0	44.9	15.27	17.38	22.26
Timothy	Climax	Stage1	51.1	36.2	57.6	34.37	24.38	38.76
		Stage2	52.3	36.3	53.2	35.89	25.10	36.49
		Stage3	54.4	35.4	48.4	38.34	24.94	34.16
	Juliette	Stage1	56.0	29.2	57.4	39.21	20.43	40.18
		Stage2	56.6	38.8	51.5	41.08	28.15	37.38
		Stage3	53.7	32.6	45.2	40.27	24.44	33.89
SEM[c]			1.63	2.92	2.81	1.325	1.732	1.980
Statistical analysis			---P values---					
Alfalfa vs.Timothy			0.0001	0.0023	0.0580	0.0001	0.0007	0.0001
Pioneer vs. Beaver			0.7811	0.2820	0.8319	0.4651	0.1906	0.4627
Climax vs. Juliette			0.1074	0.3134	0.5531	0.0063	0.7687	0.6881
In Alfalfa								
Stage 1 vs. Stage 2			0.2908	0.6144	0.5106	0.8311	0.9717	0.2762
Stage 1 vs. Stage 3			0.0354	0.3447	0.0299	0.0932	0.8954	0.3638
Stage 2 vs. Stage 3			0.2509	0.1554	0.1088	0.1370	0.9235	0.8504
In Timothy								
Stage 1 vs. Stage 2			0.6549	0.1071	0.0498	0.3571	0.0372	0.1563
Stage 1 vs. Stage 3			0.8108	0.6730	0.0003	0.1776	0.2397	0.0052
Stage 2 vs. Stage 3			0.8249	0.2214	0.0337	0.6525	0.3151	0.1064

[a] Stage 1=one week before commercial cut (early bud for alfalfa varieties and joint for timothy varieties); 2=at commercially cutting stage (late bud for alfalfa varieties and pre-bloom head for timothy varieties); 3=one week after commercial cut (early bloom for alfalfa varieties and full head for timothy varieties).

[b] Total degradable NDF: 1) NRC formula: tdNDF (%DM)=0.75× (NDFn-L)×(1-(L/NDFn)$^{0.667}$; 2) *In vitro*: tdNDF (%DM)=IVNDFD48 *NDF; 3) *In situ*: tdNDF (%DM)=*In situ* NDFD48 * NDF. [c] SEM=standard error of means.

The stage of cutting, in both forages, had significant effects on energy values. In alfalfa, energy values in stage 1 and 2 were not different (p>0.05), although the energy values at stage 2 were numerically higher than that at stage 1, but as alfalfa advanced to stage 3, all energy values significantly declined (p<0.05). Similar results were found in timothy. Stage 1 and 2 were not different (p>0.05) for all estimated energy values. As maturity further increased to stage 3, the energy values of timothy were decreased (p<0.05) (for example, TDN_{1x}: 42.3, 45.3, 40.7% for timothy cut at stage 1, 2 and 3, respectively). The above results again indicated that as alfalfa and timothy maturity increased, the energy contents behaved in a quadratic fashion, increasing at stage 2 and then significantly decreasing at stage 3.

Using in situ assay-biological approach II : Using the *in situ* assay - biological approach II, the effects of variety and cutting stage on energy content of alfalfa and timothy are in Table 4. It was found that across all treatments, there were significant differences (p<0.01) between the two forage species (alfalfa vs. timothy) for all estimated energy values (TDN_{1X}: 64 vs. 55% DM; DE_{1X}: 2.91 vs. 2.43; DE_p: 2.67 vs. 2.23; ME_p: 2.25 vs. 1.80; NE_{Lp}: 1.39 vs. 1.08; ME^{beef96}: 2.39 vs. 1.99; NE_m^{beef96}: 1.51 vs. 1.14; NE_g^{beef96}: 0.91 vs. 0.58 Mcal/kg DM). These results are also different from that estimated by NRC 2001 formula- chemical approach, in which no significant difference was found between alfalfa and timothy.

There were no differences (p>0.05) between alfalfa varieties (Pioneer vs. Beaver) and between timothy (Climax vs. Joliette) for all estimated energy values. However, the stage of cutting, in both forages, had significant effects on energy values. In alfalfa, energy values in stage 1 and 2 were similar, but as alfalfa advanced to stage 3, all energy values significantly declined (p<0.05). Similar results were found in timothy. Stage 1 and 2 were not different (p>0.05) for all estimated energy values. As maturity further increased to stage 3, the energy values of timothy were decreased (p<0.05).

In general, using three approaches-no matter chemical approach or biological approaches, all detected that the cutting stage of alfalfa and timothy had significant effect on energy values, which were decreased with increasing maturity stage to 3 (Table 2, 3 and 4). However, using the

Table 6. The Pearson correlation coefficients (R) between NRC 2001 formula and *in vitro* and *in situ* approaches on digestibility of NDF (dNDF), total digestible NDF (tdNDF) and total digestible nutrients at 1x maintenance (TDN1x)

R value	$dNDF^{NRC\ formula}$	$tdNDF^{NRC\ formula}$	$TDN1x^{NRC\ formula}$
$dNDF^{In\ vitro}$	-0.57 (p=0.0039)	-0.58 (p=0.0032)	0.07 (p=0.7437)
$tdNDF^{In\ vitro}$	0.68 (p=0.0002)	0.69 (p<0.0001)	0.18 (p=0.4038)
$TDN1x^{In\ vitro}$	-0.93 (p <0.0001)	-0.94 (p<0.0001)	0.03 (p=0.8834)
$dNDF^{In\ situ}$	0.33 (p=0.1135)	0.31 (p=0.1417)	0.61 (p=0.0017)
$TdNDF^{In\ situ}$	0.91 (p<0.0001)	0.91 (p<0.0001)	0.36 (p=0.0880)
$TDN1x^{In\ situ}$	-0.74 (p<0.0001)	-0.76 (p<0.0001)	0.34 (p=0.1044)

NRC 2001 formula, the energy values were estimated no significant differences between the two species (alfalfa and timothy) (Table 2). However, this was not the case when using biological approaches-*in vitro* and *in situ* assay, which detected significant differences between two species (Table 3 and 4). Using NRC 2001 formula, the energy values were not different between the varieties within each forage species (Table 2). These results were in agreement with the *in situ* results, but were in disagreement with the *in vitro* results (Table 3).

The results indicate that the chemical or biological methods had great influences on estimation of energy values. Comparison of three approaches, the highest prediction values were found by using *in situ* approach and the lowest prediction values by using NRC 2001 formula, the intermediate values by *in vitro* approach (with total average of energy values: TDN_{1X}: 56, 52, 59% DM; DE_{1X}: 2.52, 2.34, 2.67 Mcal/kg DM; DE_p: 2.32, 2.15, 2.45 Mcal /kg DM; ME_p: 1.89, 1.72, 2.02 Mcal/kg DM; NE_p: 1.14, 1.02, 1.24 Mcal/kg DM; ME^{beef96}: 2.07, 1.92, 2.19 Mcal/kg DM; NE_m^{beef96}: 1.21, 1.06, 1.32 Mcal/kg DM; and NE_g^{beef96}: 0.65, 0.50, 0.77 Mcal/kg DM for NRC 2001 formula, *in vitro* and *in situ* approach).

Comparisons of digestibility and total digestible NDF obtained from chemical and biological approaches

Table 5 presents the results of digestibility of NDF and total digestible NDF (tdNDF) obtained from three methods. Using NRC 2001 formula, the estimated digestibility of NDF and tdNDF were higher (p<0.01) in timothy than in alfalfa. However, using biological approach-*in situ* assay, the digestibility of NDF of alfalfa and timothy were not significantly different (p>0.05). Using *in vitro* assay, the digestibility was higher in alfalfa (p<0.05). The results from three methods were not in agreement with each other. Using biological approaches-*in vitro* and *in situ* assay, the SEM for the digestibility was higher, compared with that using NRC 2001 formula (2.9 and 2.8 vs. 1.6).

The tdNDF obtained using three approaches were also different between the methods (Table 5). For alfalfa, the highest tdNDF was measured by the *in situ* assay (24% DM), followed by *in vitro* assay (20% DM). The lowest tdNDF was estimated by NRC 2001 formula (15% DM). For timothy, the highest tdNDF was estimated by *in situ* method (38% DM), followed by NRC formula method (36% DM). The lowest tdNDF was by the *in vitro* assay (24% DM).

The Pearson correlation coefficients (R) between chemical approach (NRC 2001 formula) and biological approaches (*in vitro* and *in situ* assay) on digestibility of NDF, total digestible NDF and TDN1x are presented in Table 6. The R values between NRC 2001 formula and *in vitro* and *in situ* on digestibility of NDF and tdNDF were >0.65. However, the R values between NRC 2001 formula and *in vitro* or *in situ* method on TDN1x were lower (0.03 and 0.34, respectively).

Relationship of energy values predicted from chemical and biological approaches

Regression was used to evaluate the relationship between TDN1x values estimated from NRC 2001 formula and *in vitro* or *in situ* methods. Statistical analysis results show that:

When not considering feed species (alfalfa and timothy) as a covariate in estimated TDN1x, the relationship was very poor (with low R^2<0.15). The results are following:

1) $TND1x^{In\ vitro}$=43.25 (±55.35, p>0.05)+$TDN1x^{NRC\ formula}$×0.15 (±0.99, p>0.05)

Where, P=0.8834, RSD=9.98, R^2=0.001.

2) $TND1x^{In\ situ}$=-0.23 (±35.22, p>0.05)+$TDN1x^{NRC\ formula}$×1.07 (±0.63, p>0.05)

Where, P = 0.1044, RSD=6.35, R^2=0.12

However, when considering feed species (alfalfa and timothy) as a covariate, the relationship was improved with very high R^2>0.70. The predictive equations were:

1) $TND1x^{In\ vitro}$=-37.68(±17.16, p<0.05)+$TDN1x^{NRC\ formula}$×1.43 (±0.30, p<0.01)

[Covariate effect: alfalfa=19.05(±1.25, p<0.001) and timothy=0]

Where, p<0.001, RSD=2.94, R^2=0.92 and TDN1x in % DM.

2) $TND1x^{In\ situ}$=-46.30 (±19.56, $p<0.05$)+$TDN1x^{NRC\ formula}$×1.80 (±0.34, $p<0.01$)

[Covariate effect: alfalfa = 10.84 (±1.43, $p<0.001$) and timothy = 0].

Where, $p<0.001$, RSD=3.35, R^2=0.76, and TDN1x in % DM.

The above results indicate that forage type has profound impact on predictability of energy values of between NRC 2001 formula and the *in situ* or *in vitro* methods. This means that the energy values from the biological approaches were not predictable by chemical approach.

The NRC formula method is to use chemical compositions of a feed to estimate total digestible nutrients and energy values in animals. *In vitro* and *in situ* methods, to some extent, are biological methods. They actually measure total digestible nutrients of a feed, not by estimation. Therefore, biological approaches are more accurate to determine energy values for a feed. Compared *in vitro* and *in situ* results, it is not surprised to find *in situ* results were higher than *in vitro* results. This is because that *in situ* method is closest to simulate animal condition.

However, it needs to be mentioned that in this study only two forage species with two different varieties cut at three different times were evaluated. To make a final conclusion, large feed samples with a wide range of chemical composition are needed to be evaluated by these approaches: chemical and biological.

CONCLUSION

In general, forage species and cutting stage had profound impacts, but the varieties (Pioneer vs. Beaver; Climax vs. Joliette) had minimal effects on energy values. However, the prediction methods-chemical approach (NRC 2001 formula) and biological approaches (*in vitro* and *in situ* assay) had great influences on energy values. The highest prediction values were found by using *in situ* approach, the lowest prediction value by using NRC formula, and the intermediate values by *in vitro* approach. The *in situ* results may be most accurate because it is closest to simulating animal condition. Regression analysis show that the energy values measured by biological approaches are not predictable by chemical approach, indicating that a refinement is needed in accurately predicting energy values.

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