Characteristics of in vitro Dry Matter Digestibility of *Phleum pratense* L. as a Basis of Breeding for High Quality Cultivars

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As a part of the study on breeding for high quality cultivars of timothy, the author has carried out a number of experiments on various aspects of dry matter digestibility (hereafter referred to DMD) of timothy[5] and the results obtained are presented briefly in this paper.

Applicability of simple *in vitro* methods of determining DMD to timothy plants

1) *The Tilley and Terry in vitro digestion method*[9]

Mean values of DMD of 10 species of forages including timothy analysed using the Tilley and Terry *in vitro* method were similar to the values obtained with the *in vivo* digestion method. The correlation coefficient between the two methods was highly significant ($r=0.956$, $p<0.01$), and the regression equation $Y=7.92+0.88X$ was calculated, where $Y=$*in vivo* DMD and $X=$DMD determined with the Tilley and Terry *in vitro* method.

2) *The simple enzyme digestion method*

The simplified cellulase digestion method of estimating DMD was tried to apply to timothy plants. The DMD values of 10 forages including timothy analysed using the shaking digestion method[4] were lower than those of the *in vivo* method. The correlation coefficient between DMD values obtained with this method and those of the *in vivo* method was highly significant ($r=0.902$, $p<0.01$), and the regression equation $Y=41.64+0.58X$ was calculated, where $Y=$*in vivo* DMD and $X=$DMD by the enzyme digestion method (Fig. 1). It is concluded that the simple cellulase digestion method is very useful for the selection of high digestible plants or strains.

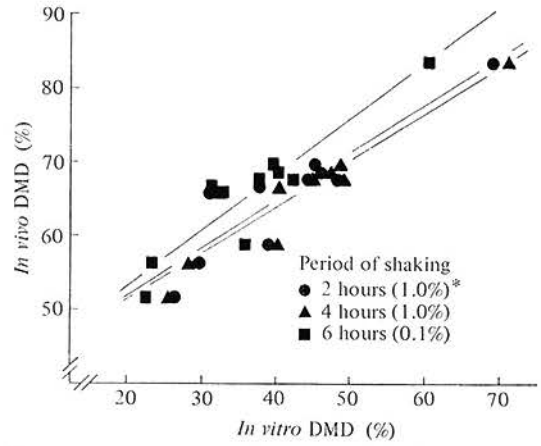

Fig. 1. Relation of DMD estimated with the shaking cellulase digestion method to *in vivo* DMD
* Cellulase concentration.

3) *Near-infrared reflectance spectrophotometer analysis*[2]

The difference between DMD analysed with this method and that of the *in vivo* method

was very small for 10 forages. The correlation between DMD values obtained with this method and with the *in vivo* method was highly significant ($r=0.985$, $p<0.01$), and the regression equation $Y=-4.06+1.07X$ was calculated, where $Y=$*in vivo* and $X=$*in vitro* values.

Thus, it is concluded that these *in vitro* methods of analyses are applicable as the accurate and rapid method of estimating DMD of timothy plants.

Variability of DMD of timothy plants

1) Variation of DMD among cultivars or strains

DMD of 50 cultivars and 2 strains was determined with each of the 1st, 2nd, and 3rd crops using the Tilley and Terry *in vitro* digestion method. As given in Table 1, a large variation of DMD was observed among 50 cultivars and 2 strains for each of three successive crops[7].

2) Changes in DMD with the advance of the initial growth of plants

Changes of DMD with the advance of the initial growth of 50 cultivars and 2 strains were examined using the Tilley and Terry *in vitro* digestion method. As shown in Table 2, the range of variation in DMD among 50 cultivars and 2 strains observed during the period of about one month of the initial growth was greater than 10.5%. The tendency that the average DMD of all cultivars decreased with time, but the range of variation increased in the later half of the period indicates that the DMD of early, medium early, and medium late maturing cultivars and strains decreased rapidly, while those of late maturing cultivars and strains decreased slowly.

3) Variation of DMD of leaves and stems among cultivars and strains

It was examined using 50 cultivars and 2 strains during the same growth period as above. DMD value was determined with the Tilley and Terry *in vitro* digestion method. A result is shown in Fig. 2. Leaf blades showed higher DMD, lower coefficients of variation and smaller ranges of variation than stems.

Table 1. Variability of DMD among 52 timothy cultivars and strains

Items	DMD (%)			
	lst crop	2nd crop	3rd crop	Average
Average	59.1	64.2	67.7	63.7
Standard deviation	1.17	2.20	1.99	2.60
Coefficient of variation	2.0	3.4	2.9	4.1
Range	13.8	12.3	16.5	13.5
Significance	**	**	**	**

** Significant at 1 % level.

Table 2. Changs in DMD with the advance of the initial growth of 52 timothy cultivars and strains

Items	DMD (%)					
	June 5	June 12	June 19	June 26	July 3	July 10
Average	78.6	73.1	69.2	66.2	59.4	55.7
Standard deviation	2.23	3.02	2.41	3.07	3.38	3.64
Coefficient of variation	2.8	4.1	3.5	4.6	5.7	6.5
Range	10.7	11.7	10.5	14.6	13.8	18.8

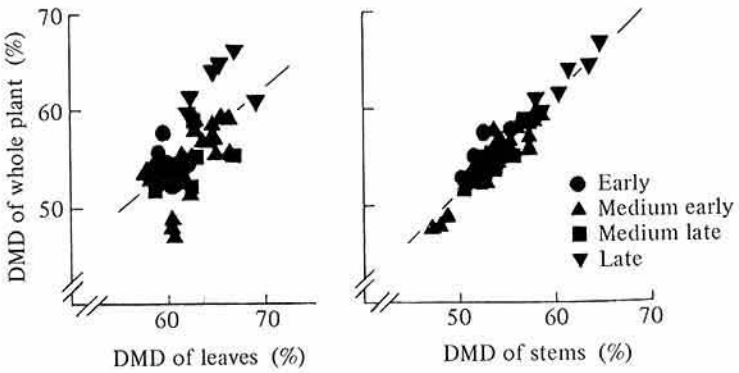

Fig. 2. Relation between whole plant DMD and leaf or stem DMD of the lst crop of timothy

Relationship between DMD and morphological or physiological characteristics of plants

1) Relation between DMD and leaf weight ratio

Relation of DMD to leaf weight ratio (percentage of leaf blade weight to whole plant weight; dry matter basis) of 50 cultivars and 2 strains was examined with three successive crops using the Tilley and Terry *in vitro* digestion method. The leaf weight ratio for the 1st and 2nd crops showed larger variation among 52 cultivars and strains than that for the 3rd crop. The correlation coefficient between DMD and the leaf weight ratio for the 1st crop was highly significant, but not for the 2nd and 3rd crops.

2) Relation between DMD and crude protein contents

It was examined using 216 clones. DMD and crude protein content value were determined with a near-infrared reflectance spectrophotometer. Crude protein contents of the 1st and 2nd crops showed a large variation among 216 clones. The correlation coefficients between DMD and crude protein contents for the 1st and 2nd crops were highly significant.

3) Relation between DMD and plant disease

It was studied with three successive crops of 50 cultivars and 2 strains using the Tilley and Terry *in vitro* digestion method. It was found out that DMD values were negatively correlated to the susceptibility of cultivars and strains to the natural infection of purple spot in the field. This correlation was highly significant in the 1st crops, but not in the 2nd and 3rd crops.

A negative correlation ($r=0.873$, $p<0.01$) between leaf blades DMD analysed using the

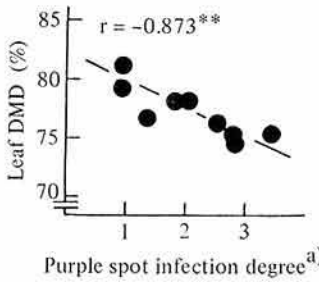

Fig. 3. Relation between DMD and purple spot susceptibility of the lst crop of timothy inoculated in a greenhouse
a) : Rating scale=1; least infection to 5; the most serious infection.
** Significant at 1% level.

Table 3. Relation of DMD to dry matter yields of 52 timothy cultivars and strains

Items	1st crop					2nd crop	3rd crop
	Total	E	ME	ML	L		
Correlation coefficient	−0.553**	0.029	−0.003	−0.477**	−0.557**	−0.233	−0.207
Genetic correlation (r_g)	−0.809	−0.282	−0.659	−0.938	−1.020	—	—
Environmental correlation	0.323	0.230	0.198	0.267	−0.120	—	—

E, ME, ML and L : Early, medium early, medium late and late maturing cultivars, respectively.
** Significant at 1 % level.

Tilley and Terry *in vitro* digestion method and susceptibility[6] was also shown when purple spot was inoculated to the 1st crop in a greenhouse (Fig. 3).

4) Relation between DMD and dry matter yields

This relation among 50 cultivars and 2 strains was examined with three successive crops using the Tilley and Terry *in vitro* digestion method. It was found out that negative genetic correlation between DMD and dry matter yields was very high (r_g= −0.809) for the 1st crop, but not in the 2nd and 3rd crops[1]. However, the negative genetic correlation for 8 early-maturing cultivars and strains was low (r_g=−0.282) for the 1st crop (Table 3).

Selection for DMD in timothy plants

1) Heritability estimates for DMD

The DMD of 24 parents and their polycross progenies was determined with the 1st crop using the Tilley and Terry *in vitro* digestion method. The mean DMD of the 1st crop of 24 parents was 59.0%, and that of the 1st crop of 24 polycross progenies was 55.8%. The range of DMD was 15.4% for the parent and 9.2% for the polycross progeny. The DMD of the 1st crop showed significant variation among both the 24 parents and their polycross progenies.

The correlation coefficient between the DMD of the parent and that of the polycross progenies was highly significant. The heritability estimates for DMD derived from the parent-polycross progeny correlation was 0.891 (Table 4). This result suggests that genetic variation of DMD exist among timothy plants, and can be utilized for the breeding[3].

Table 4. Relation between DMD of 24 parents and that of their polycross progenies of the lst crop of timothy

Heritability	Correlation coefficient	Regression equation
0.891	0.606**	Y=29.53+0.45X

Heritability : 2 Cov PO/σ_T^2.
X and Y : DMD of parent and polycross progeny.
** Significant at 1% level.

2) Breeding for high DMD

A high DMD strain and a low DMD strain were bred by synthetic methods using 3 clones derived from 24 unselected early maturing clones. The difference of DMD analysed using the shaking cellulase digestion method between the high and low synthetic strains was significant for all of the crops, although the difference itself was small (Table 5). However, the difference of dry matter yields between them was not significant for all of the crops.

A high DMD strain was bred by the synthetic method using 5 clones derived from 28 early maturing clones (selected for DMD of the 1st crop). The difference of DMD analysed using the shaking cellulase digestion method between the high DMD strain and Nosappu (check cultivar) was significant for the 1st crop of the 2nd year. The DMD of the former was 3.6% higher than that of the latter. However, there was no difference in dry matter yields between them.

Table 5. DMD of timothy strains selected for high or low DMD

Strain and cultivar	DMD (%)					
	1977			1978		
	lst crop	2nd crop	3rd crop	lst crop	2nd crop	3rd crop
High-strain	64.2	66.7	71.9	65.7	69.1	75.0
Low-strain	63.1	64.5	70.5	64.4	66.8	72.4
Senpoku	62.7	64.4	71.1	63.4	66.7	73.7
l.s.d. (5%)	1.02	1.49	1.20	1.26	1.20	1.35

3) Seedling selection for high DMD

Selection at the seedling stage was tested using the shaking cellulase digestion method. The DMD showed a large variation among 44 seedlings tested, although the plants in the field showed only a small variation in all of the crops. The correlation between DMD of the seedlings in the greenhouse and that of plants in the field was not significant.

Effect of environmental conditions on DMD of timothy plants

1) Effect of air-temperature on DMD

Effect of air-temperature on DMD was examined using 5 cultivars. DMD value was determined with the shaking cellulase digestion method. The DMD of leaf blades and herbage was highest under the lowest air-temperature regime. No significant interaction between temperatures and cultivars in DMD was recognized (Table 6).

2) Effect of soil moisture on DMD

Effect of soil moisture on DMD was examined using 5 cultivars. DMD value was determined with using the shaking cellulase digestion method. The mean DMD of leaf blades and stems of the 1st crop of all cultivars used was highest under the lowest soil moisture regime (i.e. retarded growth). To the contrary, DMD of the 2nd crop was highest under the highest soil moisture regime. The interaction between soil moistures and cultivars in DMD showed significance for the 1st and 2nd crops[8] (Table 7).

Table 6. Effect of air temperature on DMD of the lst crop of timothy cultivars

Cultivar	DMD (%)			Significance		
	T_1	T_2	T_3	T	C	T×C
Kunpū	68.2	70.7	73.8			
Senpoku	66.9	70.1	72.4			
Nosappu	66.6	66.8	72.7	*	**	ns
Erecta RvP	67.1	68.1	72.4			
Hokushū	67.5	70.0	73.7			
Average	67.2	69.5	73.0			

T_1, T_2 and T_3 : 31/26, 23/18 and 15/10°C air temperature (day, 12hr/night).
T : Temperature, C : Cultivar.
*, **, ns : Significant at 5% and 1% level, non-significant, respectively.

Table 7. Effect of soil moisture on DMD of the lst crop of timothy cultivars

Cultivar	DMD (%)			Significance		
	M_1	M_2	M_3	M	C	M×C
Kunpū	64.8	64.3	67.6			
Senpoku	65.5	64.4	71.6			
Nosappu	65.7	64.6	65.1	*	**	**
Erecta RvP	64.5	62.7	74.7			
Hokushū	68.4	66.8	69.2			
Average	65.8	64.5	69.6			

M_1, M_2 and M_3 : 82%, 63% and 39% of maximum soil water-holding capacity, respectively.
M : Moisture, C : Cultivar.
DMD of (stems + leaf sheaths + heads) was measured.
*, ** Significant at 5% and 1% level, respectively.

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