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## Comparisons of the In Vitro Dry Matter Digestibility of Forage Oats Grown under Different Temperatures and Light Intensities

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Two experiments were conducted with forage oats to find the effects of temperature and light intensity on in *vitro* dry matter digestibility (IVDMD).

The IVDMD of the main shoot and its component leaves and stems, showed declines with the increase in temperature. The differences in the IVDMD of leaves were caused by the temperature effects on **lignification**, on the rate of leaf appearance, accordingly on the age of the leaves, and on the dry matter distribution pattern among leaf positions. Higher temperature seemed to increase slightly the rate of decline in IVDMD of a leaf with aging.

Stem IVDMD was influenced by true stem formation and lignin deposition at higher temperatures.

With the decrease in light intensity the IVDMD of the stem of the main shoot, and, as a result, of that of the whole main shoot and that of the whole top of a plant showed slight declines. CWC content was not affected very much, but nitrogen content increased and also ADF content slightly increased under lower light intensities. The positive effect of light intensity on IVDMD was partly concerned with its effect on ADF content and attributable to its effect on CWC digestibility.

### INTRODUCTION

There are many factors which influence forage digestibility. Differences in digestibility between species or varieties, or changes in digestibility with ontogenetical development, have been studied by many workers as reviewed by Raymond (1969). Some researches have also been done on environmental factors which limit digestibility. High growth temperatures have been reported to lead to a decline in digestibility (Deinum and Dirven, 1974; Minson and McLeod, 1970).

In the present study an attempt is made to examine the effects of temperature and light intensity on the *in vitro* dry matter digestibility of forage oats, and to consider how these environmental factors affect digestibility. There is a discussion of the relations between *in vitro* digestibility on the one hand, and on the other hand, (1) some chemical compositions and (2) ontogenetical development, on which temperature and light intensity have pronounced effects.

In experiment 1 the effect of temperature, and in experiment 2 the effect of light intensity, were determined; in both experiments attention was mostly directed towards the main shoot, which was regarded as the basic unit of growth.

## MATERIALS AND METHODS

### Experiment 1

Forage oats (*Avena sativa* L. cv. Taiho) were grown in glasshouses maintained at constant temperatures of 15°C, 20°C and 25°C. Prior to the treatments three seeds were sown in each pot of ca. 1/10000 a on November 25, 1974. During germination pots were kept in natural conditions and, 5 days after germination, they were transferred to the respective experimental temperatures. The plants were cut at ground level on the 50th day after germination. The main shoot was separated into three portions, namely, green leaf, dead leaf and stem plus sheath. The green leaves were further sorted according to the nodal number of the main shoot on which they were formed and numbered successively from the base to the top. The samples were weighed after drying for 24 hours in a forced-draught oven at 70°C, and were passed through a hammer-mill with a 1 mm screen. *In vitro* dry matter digestibility (IVDMD) was estimated by the method of Goering and Van Soest (1970), which yields true digestibility values, but the CO<sub>2</sub> bubbling during the fermentation was omitted. Acid detergent fiber (ADF) was also determined by their method.

### Experiment 2

This experiment was conducted in the field from April 2 through May 1 in 1975. Preliminary pot management was almost the same as in experiment 1, but the treatment started when the third leaf appeared on the main shoot. Three different light intensity treatments of full, a half of, and a third of, natural light intensity, were produced by covering pots with sheets of shade cloth. The average natural light intensity in the month of April was 218 cal. cm<sup>-2</sup> day<sup>-1</sup>. The plants were cut up as in experiment 1 and, further, tillers were sampled and divided into larger and smaller ones according to whether the 3rd leaf of a tiller was, or was not yet, fully expanded. The *in vitro* digestion trial and the ADF analysis were carried out as in experiment 1, and cell wall constituents (CWC) was also determined following Goering and Van Soest (1970).

In both experiments the *in vitro* digestion trial and chemical analysis were conducted on composite samples of 20 pots, and the data obtained on the ontogenetical development are presented as means of 20 pots in the following tables.

## RESULTS AND DISCUSSION

### Effects of temperature

Plant weight, ADF content, and IVDMD are shown in Table 1. The number of days after full expansion of the respective leaves of the main shoot are presented in parentheses. The plants grew best at 15°C ; the yield was slightly less at 25°C and slightly less still at 20°C. Morphological observations revealed that the plants under 15°C and 20°C remained vegetative throughout the experimental period, and that the stems of these plants consisted only of leaf sheaths, but that some plants under 25°C had slightly elongated true stems.

**Table 1.** Plant weight, ADF content and the IVDMD of the main shoot of forage oats grown under three temperature treatments.

Plant part	Plant weight (mg/pot)			ADF content (%)			IVDMD(%)		
	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
Dead leaves	21	19	30						
Respective green leaves	21	11	2						
1st and 2nd leaves	45(30)	37(31)	21(35)				92.3	88.0	
3rd leaf	99(20)	88(20)	63(26)	17.4	22.2		93.9	88.6	87.4
4th leaf	191(10)	146(10)	107(18)	16.9	21.8	24.2	94.4	89.9	88.5
5th leaf	276 (0)	210 (0)	162( 9)	16.2	20.0	22.2	95.9	92.7	89.6
6th leaf	89	86	200( 1)			23.2	95.4	94.9	91.9
7th leaf			75						92.9
8th leaf			7						
9th leaf									
Combined whole leaves	741	604	667				94.5	<b>91.0</b>	<b>90.8</b>
Stem and sheath	385	328	355	16.5	22.6	25.3	92.5	84.7	81.9
Whole main shoot	1126	932	1022				93.8	88.8	87.7

Figures in the parentheses are the number of days after full expansion.

The plants developed at much the same rate at 15°C and 20°C, reaching the 6th leaf stage, but at 25°C one more leaf had fully expanded during 50 days of growth. The dry matter distribution pattern among leaves showed the highest peak at the highest fully expanded leaf of the 7th leaf at 25°C, and at the 6th leaf at 15°C and 20°C. The leaf weights as percentages of total main shoot weight were about 63 % under all three temperature treatments.

The IVDMD of combined whole leaves at 15°C attained appreciably high values, and showed a decline with the increase in temperature, though the difference between 20°C and 25°C was not considerable. The effect of temperature on the IVDMD of leaves will be discussed in detail later on.

The effect of temperature on stem IVDMD was more evident than on leaf IVDMD, and a marked decline with an increase in temperature was observed. The stems of the plants at 25°C showed slight elongation, and the content of lignified cell walls regarded as ADF increased at the higher temperatures (Table 1). It has been considered that stem formation has a crucial effect on digestibility (Deinum and Dirven, 1975), and that higher temperature stimulates lignification and accordingly may depress digestibility (Deinum *et al.*, 1968; Wilson and Ford, 1973). As a result of the greater negative effect on stem IVDMD, there were larger differences in IVDMD between leaf and stem under the higher temperature treatments.

The IVDMD of the whole main shoot appeared to decline, as temperature increased, as the result of the effects on its component leaves and stems. An obvious decline in stem IVDMD with the increase in temperature probably exerted the major influence on the whole main shoot IVDMD.

Wilson and Ford (1973) reported the same effects of temperature on the entire plant digestibility of some temperate grasses, including *Avena sativa* L. Deinum and Dirven (1975) also observed similar effects on stem and leaf digestibility of two tropical and two temperate grasses, and mentioned that the negative effect on stem digestibility was concerned with the more rapid physiologi-

cal aging at a higher, than at a lower, temperature. Downes *et al.* (1974) also showed higher digestibility in oats under day/night temperature of 21/16°C than at 27/22°C at the 6th, 8th and 10th leaf stages of growth.

The IVDMD of the respective leaves, numbered from the base of the main shoot, are also shown in Table 1. In comparing the IVDMD of leaves among the respective insertion levels of the main shoot, consistent declines from top to bottom were observed at all three temperatures. Burton *et al.* (1964), Hagger and Ahmed (1971) and Wilson (1973) also reported a similar trend in the decline of digestibility according to leaf position. In a glasshouse experiment, as in the present study, where temperature is constant, the difference in the number of days from the appearance of the respective leaves (that is the difference in the age of the leaves) may exert a major influence on the digestibility, showing a decline from top to bottom.

Comparing the IVDMD of leaves of the same insertion levels, among temperature treatments as shown in Table 1, it may be said that there is an obvious effect of temperature on leaf IVDMD, with the lower IVDMD consistently at the higher temperatures. Though the effect of temperature appeared to be clear in the comparison of the IVDMD of respective leaves, the difference in the IVDMD of combined whole leaves at 20°C and 25°C was not considerable, as described earlier. The differences in the dry matter distribution pattern among leaves between the treatments may be one of the reasons why the difference in the IVDMD of combined whole leaves was small. The leaves which had the largest dry matter weight were the 7th leaf at 25°C and the 6th leaf at 20°C, the IVDMD of which were respectively 91.9 % and 92.7 %. So it may be said that temperature affects the dry matter distribution pattern in a plant and that this will affect digestibility to some extent.

There are apparent differences in the IVDMD of leaves of the same insertion levels among different temperature treatments, as mentioned in the previous discussion; however, it is not safe to conclude that these differences are caused directly by the differences in temperature. As shown in Table 1, the number

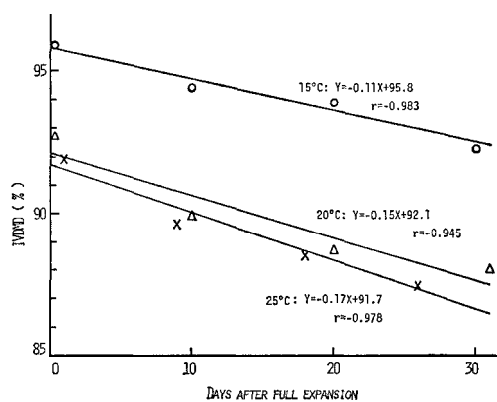


Fig. 1. Changes in the IVDMD of respective leaves of the main shoot with age at three different temperatures.

of days after full expansion differed according to growth temperatures; so the difference might have resulted from the aging process to some extent. To make this point clear, the IVDMD of respective leaves was plotted against the number of days after full expansion as shown in Fig. 1. Linear declines of IVDMD were obtained at the respective temperatures. The IVDMD of leaves at 15°C showed consistently higher values than at the higher temperatures, but appreciable differences were not found in leaves between 20°C and 25°C when compared at the same age. So it may be concluded from Table 1 and Fig. 1 that the difference of IVDMD between the 15°C and the 20°C treatments was a direct effect of temperature on digestibility, possibly in consequence of more lignified cell wall (Table 1) and that the difference in IVDMD between the 20°C and the 25°C treatments was mostly induced by the temperature effect on the rate of leaf appearance which caused the differences in the age of each leaf.

In order to estimate the influence of temperature on the rate of decline of IVDMD with the aging, regression equations were calculated on the assumption that all newly-developed leaves had similar IVDMD at the respective temperatures and declined with the aging. This assumption was made following Schank *et al.* (1973), who showed that the IVDMD of the apical meristem zone was nearly constant over a period of 11 weeks. The regression coefficients indicated slight rates of decline at the higher temperatures.

A significant correlation between the IVDMD and the ADF contents of respective leaves was obtained ( $r = 0.907$ ,  $P < 0.01$ ). But changes in the ADF content of a plant cannot be simply considered as the direct result of changes in temperature; it is partly the result of the aging process because senescence is usually accompanied by reducing cell contents and an increasing relative content of cell wall constituents.

It was confirmed in this trial that environmental temperature has a negative effect on forage quality as the result of its effects on ontogenetical development, chemical composition and the rate of aging. Obviously more detailed research is necessary because temperature in field conditions changes from day to night and from day to day, and so the effect on digestibility must be more complicated.

#### Effects of light intensity

Observed data on growth are presented in Table 2. The dry matter yield increased with the increase of light intensity. This was attributable to the increased growth rate as indicated by the number of leaves formed on the main shoot and the number of tillers produced under the respective treatments. Under full light 25 larger tillers (1.52 g/tiller) per pot were produced in contrast with only about 8 small tillers (0.16 g/tiller) under the lowest light condition.

The *in vitro* digestion trial data are shown in Table 3. No marked differences were found in the IVDMD of whole combined leaves of the main shoot; nor were there found regular patterns of leaf IVDMD among respective insertion levels, as found in experiment 1. These may be the effects of some factors other than light intensity, such as temperature, because the experiment was conducted under field conditions.

**Table 2.** Plant height, plant weight, the number of tillers, and the number of leaves formed on the main shoot of forage oats grown under three different light intensity treatments.

Items	Light intensity		
	Natural	1/2	1/3
Plant height (cm)	33	51	49
Number of tillers per pot	25.0	11.4	7.6
Number of leaves on the main shoot	7.3	6.1	5.8
Plant weight (mg/pot)			
Leaves of the main shoots	633	576	277
Stem of the main shoots	200	256	138
Whole main shoots	833	832	415
Larger tillers	1261	477	
Smaller tillers	640	206	66
Whole top of plants	2734	1515	481

**Table 3.** The IVDMD of forage oats grown under three light intensity treatments.

Plant part	Light intensity		
	Natural	1/2	1/3
Respective leaves of the main shoot	(%)	(%)	(%)
4th leaf	94.5	95.7	95.2
5th leaf	95.9	95.1	94.5
6th leaf	95.8	94.6	95.2
7th leaf	95.7	95.0	
8th leaf	96.2		
Combined whole leaves of the main shoot	95.3	94.2	94.4
Stem of the main shoot	94.4	93.0	90.1
Whole main shoot	95.1	93.8	93.0
Larger tillers	95.8	95.7	
Smaller tillers	95.7	95.6	94.4
Whole top of a plant	95.6	94.6	93.2

The IVDMD of the stem showed a slight decline with decreasing light intensity; as a result, the IVDMD of the whole main shoot revealed a small decline with the decrease in light intensity. It is interesting to note that small differences were found between the IVDMD of the larger tillers and that of the smaller ones, and, in particular, that the tillers affected by the lowest light intensity, were young and small but showed even lower IVDMD. Taking together the yield and the IVDMD of the components of a plant, the IVDMD of the whole top of the plant decreased with a decrease of light intensity at the rate of about 1 % unit in order of the treatments of decreasing light intensity. It is worth noting that the plants at a younger stage of growth under the lower light intensities showed a slightly lower IVDMD than the plants at a more advanced stage of growth under the higher light intensities.

The positive effect of light intensity was also shown by Deinum *et al.* (1968), who showed that the average quantitative effect on the digestibility of herbage would be 0.25 %/100 cal. cm<sup>-2</sup> day<sup>-1</sup>.

The general conclusions about the influence of light intensity on chemical composition are that there is a positive effect on non-structural carbohydrate

**Table 4.** The chemical composition and the *in vitro* CWC digestibility of forage oats grown under three light intensity treatments.

Plant part	Nitrogen (%DM)			CWC (%DM)			ADF (%DM)			<i>In vitro</i> CWC digestibility (%)		
	N	1/2	1/3	N	1/2	1/3	N	1/2	1/3	N	1/2	1/3
Leaves of the main shoot	4.16	4.87	5.06	27.4	27.4	26.7	17.0	18.0	18.7	88.1	84.2	84.3
Stem of the main shoot	2.79	3.62	4.09	34.9	34.6	33.4	19.1	21.0	21.1	87.5	83.6	74.5
Larger tillers	3.89	4.54		31.7	31.4		16.9	18.1		91.3	90.7	
Smaller tillers	3.84	4.52	4.78	32.7	31.0		17.3	18.7		91.0	90.1	

and a negative effect on nitrogen content, and, as for structural constituents, a negative effect on crude fiber content (Alberda, 1965; Deinum *et al.*, 1968; Deinum and Dirven, 1972). In the present experiment an obvious effect on nitrogen content was also found (Table 4). Although the effect on CWC and ADF contents are reported to be comparable to that on crude fiber (a part of the cell walls) (Deinum *et al.*, 1968), distinct effects could not be found on CWC content, and ADF content showed a slight increase with decreasing light intensity in the present experiment (Table 4).

The relations between IVDMD and an individual chemical component cannot fully explain the positive effect of light intensity on IVDMD, although a slight increase in ADF content may explain apart. But the values of *in vitro* cell wall digestibility presented in Table 4 suggested that CWC was tended to be less digestible at the lower light intensities. The differences in CWC digestibility may be associated with the structure of the cell walls and the spatial deposition of lignin within the tissue, though ADF contents stayed at similar levels (Wilson, 1973). Much detailed work will be needed to obtain full understanding of the underlying changes which occur.

Under actual field conditions there are certain situations where light intensity may affect forage quality. For example, plants or parts of plants in the lower layers of a dense sward are shaded more or less and plants grow under seasonal fluctuation of light intensity. Though this trial is only one of simplified cases of these situations, the limited data so far obtained suggest that light intensity has a positive effect on IVDMD and a negative effect on nitrogen content, and a change in IVDMD may result from the effects on CWC digestibility.

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