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The drawing on the cover is from Kjell Aukrust's *«Guttene på broen»*.

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ACCURACY OF NEAR INFRARED REFLECTANCE SPECTROSCOPY IN THE DETERMINATION OF IVDMD IN FORAGE

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In modern forage breeding the use of Near Infrared Reflectance Spectroscopy is becoming more and more common for estimating quality parameters like IVDMD and protein. In this paper a systematic study of the method is made and compared with the conventional method described by Tilley & Terry (1963). The results show that there is good agreement between the method based on the NIRS technique and the conventional method. However, compared with the conventional method the NIRS method gives much more stable and repeatable results (std = 1.24 for the conventional method, but std = 0.30 for the NIRS-based method).

Key words: Fodder grasses, IVDMD, NIRS-technique.

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It is usual in Norway for In Vitro Dry Matter Digestibility (IVDMD) of forage to be estimated by the method described by Tilley & Terry (1963), but with a few modifications. In our paper this is called the conventional method. It is observed that the standard error is relatively high from this analysis. Templeton et al. (1983) reported standard errors of about 3.5% from the conventional analysis of IVDMD and Norwegian experiments show similar results (e.g. Aastveit & Aastveit,1988). In larger breeding trials with fodder grasses we have noticed that the total variation between clones or families in a population may range from 68% to 80% IVDMD, with most of the families in the range from 73% to 77%. It is therefore easy to understand how the conventional method for estimating IVDMD does not give results which are reproducible. The Tilley & Terry method is also very time-consuming, and for this reason we cannot accommondate their conventional method in plant breeding trials.

Marum et al. (1979) reported the first use of Near Infrared Spectroscopy (NIRS) to measure quality (six constituents) in a genetic breeding programme. The correlation coefficients (r) between laboratory and NIRS estimates were 0.90 or greater for two fibre fractions and crude protein, and r = 0.73 or greater for lignin, silica

and IVDMD. Standard errors of calibration were usually less than 1% for all constituents except IVDMD (1.7 %) and were lower than those commonly reported for conventional laboratory assays. When Marum et al. (1979) used the calibration equation based on samples from one year to predict the forage quality of samples taken the following year, however, they found that the standard errors of prediction more than doubled. We have therefore based our calibration equation on samples from different years, species and cuts.

The conventional methods for estimating forage quality are expensive and laborious in practice. Since the NIRS method has been successfully used for quality estimation of other substances, e.g. meat, this method should also be useful for fodder. It is much faster than the conventional methods and is therefore more economic. The problem could be that the standard errors will be large, or that the method will give biased estimates of the forage quality. For plant breeders the relative rather than absolute forage quality values are much more important than systematic bias. Templeton et al. (1983) compared the standard errors associated with conventional and NIRS analyses of 30 cold- and warm-season forage grasses at six laboratories of the NIRS Forage Quality Research Project Network. Their results show that the NIRS analyses are very good for estimating IVDMD compared with conventional methods. For other quality traits the results vary, but in most cases NIRS was at least as good as the conventional methods.

With these facts in mind, the Norwegian Forage Research Project installed equipment for NIRS analyses of forage quality (in 1982). For several years, parallel measurements of both NIRS and conventional analyses were carried out in some experiments, the results showing that the correlation between the two methods is sometimes relatively low (r = 0.1 - 0.2). We have also noticed that in some cases differences between the two methods have been up to 3-4%. As far as we are aware, however, there is no systematic bias between the results from the two methods. Sometimes the differences are negative, other times they are positive. In order to study the bias and the random errors of the two methods, we have carried out a systematic experiment with 39 different samples of timothy.

MATERIAL AND METHODS

In 1986 five samples of timothy from each of eight State Agricultural Experiment Stations in Norway were collected (Figure 1). Only four samples were harvested from the State Agr. Exp. Sta.

Figure 1: Location of the different State Agr. Exp. Sta.
1. Særheim, 2. Fureneset, 3. Løken, 4. Apelsvoll, 5. Kvithamar, 6. Tjøtta, 7. Vågønes, 8. Holt

Apelsvoll. Altogether, then we had 39 samples of timothy from 8 different sites.

All 39 samples were dried at 60 °C for two days at the research station where they were harvested and then sent to the State Agr. Exp. Sta. Løken. The samples were cut into pieces of approximately 1-3 cm and then ground with a cyclone mill fitted with a 1 mm screen. Each of the ground samples was homogenized by shaking and then divided into 20 subsamples. Great care was taken to obtain representative subsamples. From each sample, subsamples were selected at random, forming 20 replications. The 39 samples from replicate number one were first analysed by the NIRS technique at Løken and then sent to the State Agr. Exp. Sta. Vågønes. At Vågønes the samples were analysed due to the conventional method. The following week replicate number two was analysed, and so on.

For the NIRS analyses two parallel estimates of the IVDMD were made on two consecutive days. The calibration equation (LIGN87) used in this study is based on approximately 600 samples from a wide range of different grass spcies and varieties grown at different sites and in different years. The coefficient of determination from the least square regression of known quality values on NIRS values is 0.75, while the standard error of calibration is 1.93. The number of wavelengths needed for best prediction equation of IVDMD is 7. In our opinion this calibration is good, even although there are fairly large errors in both the regressors and the regresand in the calibration equation.

The method described by Tilley & Terry (1963) has also been used with two parallel analyses being made simultaneously. Only the average estimates of the two parallels are used.

The data were analysed according to the usual randomized block model for the two estimates based on the NIRS technique and the conventional analysis respectively. In order to study the correspondence between the results from the two methods the ordinary correlation coefficient was calculated. To find a bias between the two methods, the differences between the estimates were taken. The variances between the different subsamples of the same sample were taken too, in order to study the random errors of the respective methods.

RESULTS

An analysis of variance for both methods was carried out, and for the NIRS an analysis was made for parallels and for the average of parallels. The results of these analyses are given in Table 1.

It can be seen from this table that the residual mean squares are much higher for the data based on the conventional method. Since the residual mean square is an estimator for the random variation, this means that the random errors are much larger for the conventional estimation procedure compared with the NIRS techniques ($p < 0.001$ by use of an

Table 1: The analyses of variance of IVDMD using conventional and NIRS methods

Source	Degrees of freedom	M.Square for NIRS1	M.Square for NIRS2	M.Square for NIRS	M.Square for LAB
Samples	38	209.78	207.21	208.46	252.55
Replicates	19	1.80	1.69	1.09	32.80
Error	722	0.131	0.134	0.074	0.789

NIRS1 = data from NIRS parallel 1. NIRS2 = data from NIRS parallel 2. NIRS = analysis based on the means from NIRS1 and NIRS2. LAB = data based on the conventional analysis.

F-test between the two mean squares). Also the mean square resulting from replicates is much larger in the conventional method ($p < 0.001$ by use of an F-test with 19 and 19 degrees of freedom). This means that the mean value of the 39 samples varies much more in the conventional method than in the NIRS method. Highly significant differences exist between the means for the different samples. There are, however, no significant differences between mean squares

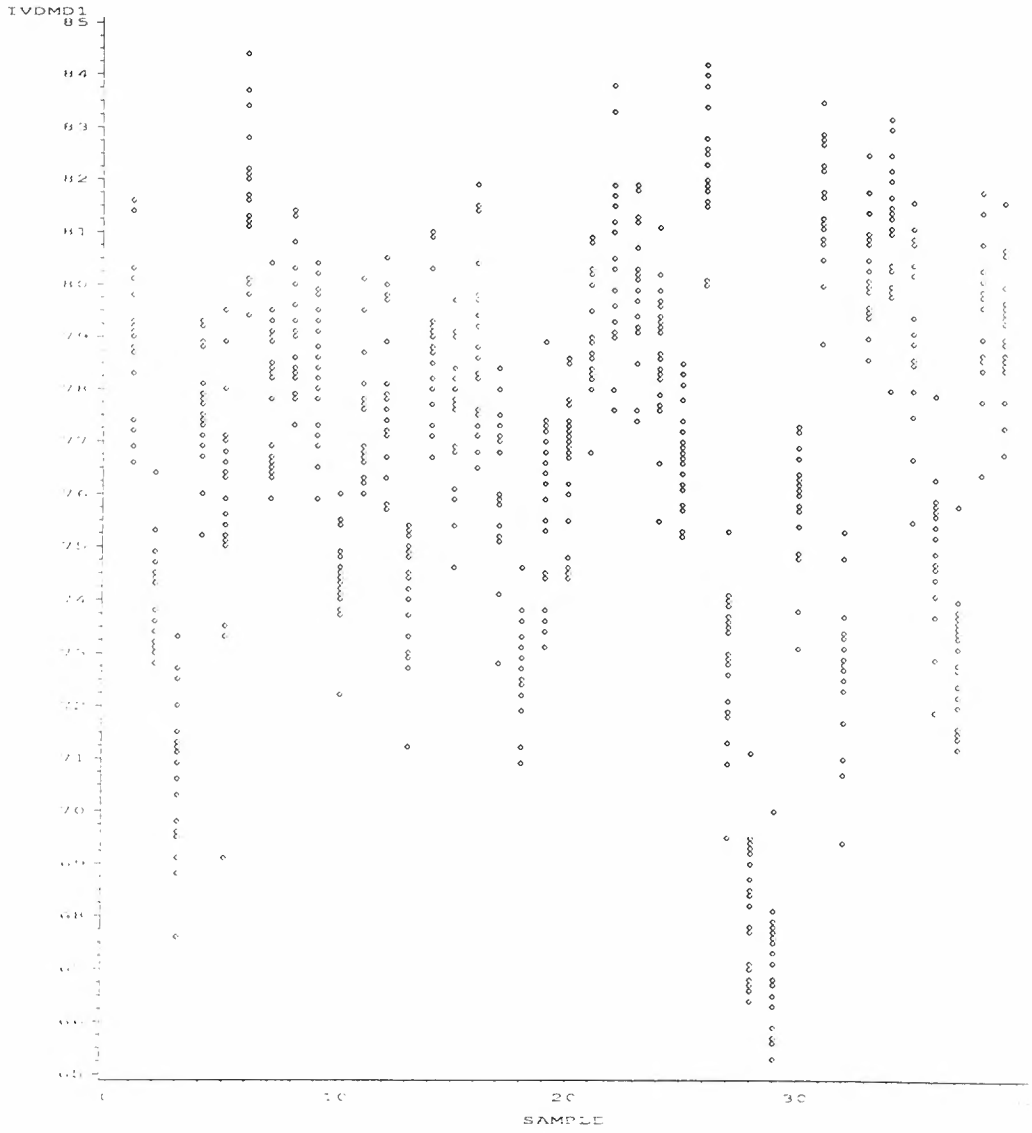

Figure 2: The random variation of the 20 estimates of IVDMD from each of the 39 samples. The estimates are based on the conventional method described by Tilley & Terry (1963)

for samples when comparing LAB and NIRS in Table 1.

From Figure 2 and Table 3 it is apparent that the different estimates of the standard deviations vary considerably from sample to sample, both for the NIRS-based estimates and the estimates based on the conventional method. A Bartlett test of homogeneity of variances resulted in significantly different variances both for the conventional method ($p < 0.05$) and for the NIRS-based method

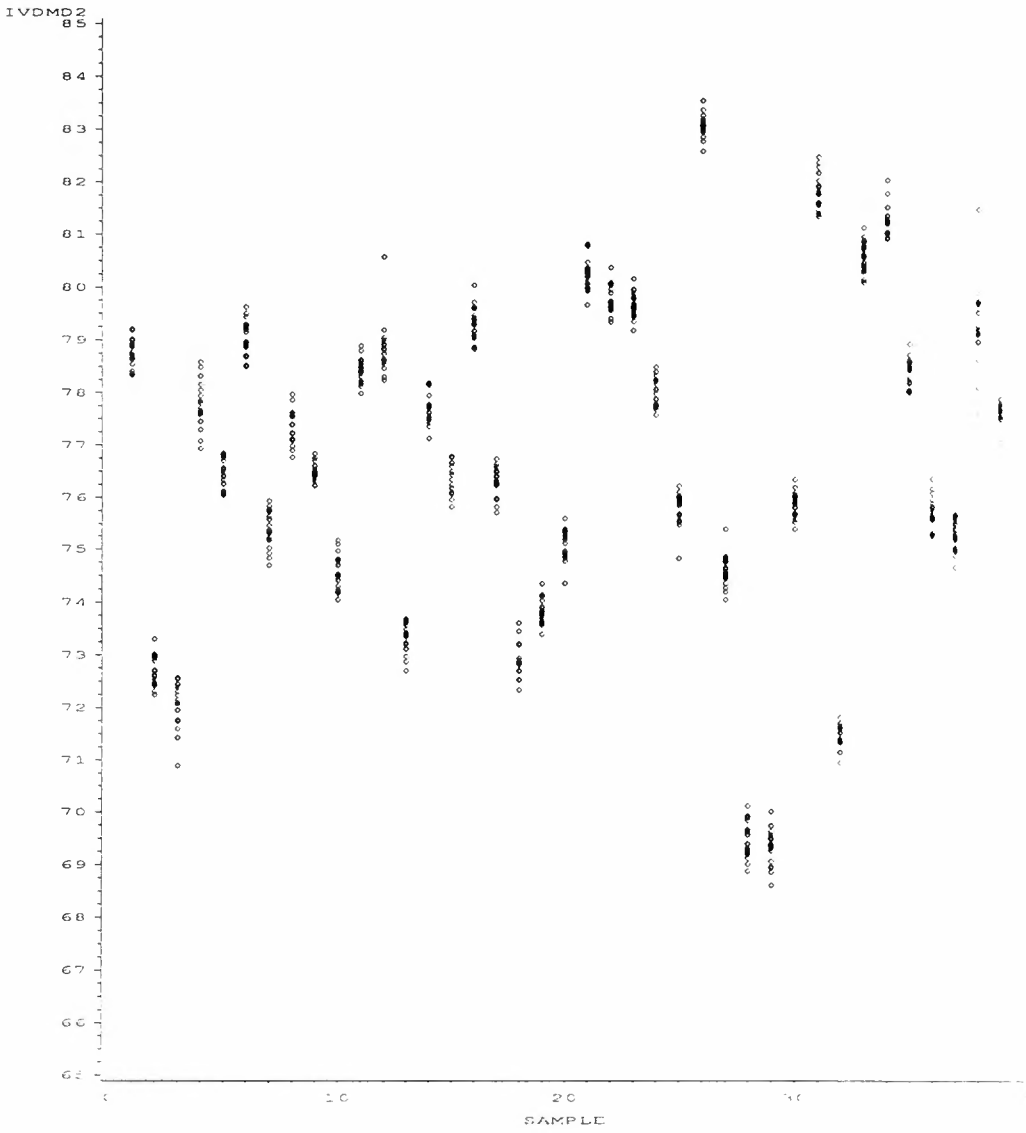

Figure 3: The random variation of the 20 estimates of IVDMD from each of the 39 samples. The estimates ere based on the NIRS method

($p<0.001$). The results show that there are relatively greater differences between samples in variability for the NIRS-based method. On examining Figure 3, it can be seen that the instability of the NIRS-based estimates is because of sample 38, which seems to be unique, differing dramatically as it does from the others.

Figure 4 shows a plot of the means of the 39 samples in each replication. It can be seen from the figure that the estimates based on the conventional method vary much more from replicate to replicate. In theory, each replicate should give the same result, since all the subsamples have been given the same handling. The fact that the means range from 75.5 to 78.9% on average over 39 samples does not favour the conventional method. The corresponding range for the NIRS-based method is from 76.3 to 76.9%. This means that the NIRS method gives results which are more readily repeatable than the conventional method.

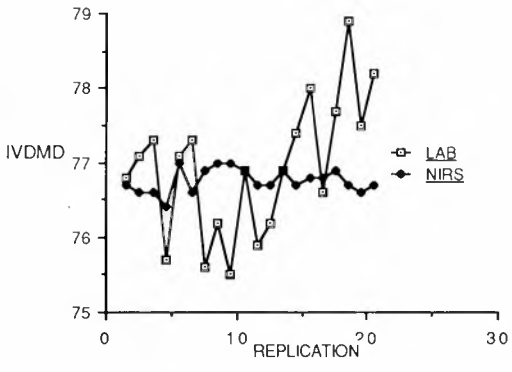

Figure 4: The average estimates of IVDMD for each of the 20 replications

In order to study possible systematic biases, the differences between every estimate based on NIRS and the conventional analysis were taken for each of the parallels and the means for the NIRS estimates. In all cases the effect of samples was significant (the means for the different samples are given in Table 2). From the table it can be seen that there are differences between the estimates. It is, however, not possible to find a systematic bias. Sometimes the difference between the two estimates is negative, sometimes it is positive. The results seem to be the same for both parallels of the NIRS techniques. From a plant breeder´s point of view, the ranks between the samples are important. When examining the ranks, it will be recognized that there is good agreement between the conventional analysis results and the NIRS-based results ($r=0.92$). These results, however, are based on the means of 20 subsamples. The correlation coefficient between the conventional method and the NIRS method is $r = 0.87$, while the correlation between the two parallels for the NIRS data is $r = 0.99$. If we examine the correlations for each replication separately, the correlation between the NIRS data and the estimates from the conventional method vary considerably ($r = 0.87$-0.95), while the correlation between the two parallels from NIRS was always greater than 0.987. All correlation coefficients are highly significant ($p<.001$).

With regard to the repeatability of the data from the two methods, the random errors are important. In Table 3 the standard deviations of the 20 subsamples within each sample are given for the conventional method, each of the two parallels from the NIRS method and for the mean of the two parallels. It can be seen from the table that the random errors are much higher for the conventional method. The proportions between the two variances are F-distributed with 19 and 19 degrees of freedom. In nearly all cases the variance is significantly larger for the conventional method than for the NIRS method. In Figures 2 and 3 we have plotted the estimates from the two methods for each sample. These two figures also illustrate that the random

Table 2: The means over 20 replications and the ranks for the two different methods

Sample	LAB	RANK1	NIRS1	NIRS2	NIRS	RANK2
1	79.04	28	78.73	78.78	78.76	28
2	74.00	8	72.89	72.88	72.89	5
3	70.60	3	71.99	71.98	71.99	4
4	77.58	20	77.77	77.71	77.74	24
5	75.90	14	76.44	76.45	76.44	19
6	81.69	38	78.99	79.13	79.07	30
7	77.87	22	75.42	75.37	75.39	13
8	79.25	31	77.31	77.33	77.32	21
9	78.42	23	76.57	76.41	76.49	20
10	74.51	10	74.80	74.63	74.62	10
11	77.44	18	78.43	78.43	78.43	27
12	77.78	21	78.93	78.76	78.84	29
13	74.04	9	73.26	73.26	73.31	7
14	78.77	25	77.62	77.67	77.65	22
15	77.47	19	76.36	76.31	76.33	18
16	78.89	26	79.32	79.30	79.31	32
17	76.43	15	76.31	76.30	76.30	17
18	72.92	7	72.83	72.83	72.83	6
19	75.67	12	73.83	73.85	73.84	8
20	76.56	16	75.03	75.16	75.10	11
21	79.01	27	80.17	80.23	80.20	35
22	80.39	35	79.73	79.75	79.74	34
23	79.85	33	79.73	79.58	79.65	33
24	78.73	24	78.00	78.10	78.05	25
25	76.75	17	75.80	75.79	75.80	15
26	82.29	39	83.10	83.04	83.07	39
27	72.80	5	74.62	74.60	74.61	9
28	68.18	2	69.45	69.49	69.46	2
29	67.07	1	69.26	69.46	69.36	1
30	75.86	13	75.93	75.81	75.87	16
31	81.53	37	81.80	81.87	81.84	38
32	72.61	4	71.51	71.42	71.46	3
33	80.37	34	80.57	80.58	80.57	36
34	81.12	36	81.20	81.30	81.25	37
35	79.04	29	78.36	78.45	78.40	26
36	75.00	11	75.71	75.74	75.72	14
37	72.84	6	75.26	75.29	75.27	12
38	79.38	32	79.52	79.01	79.26	31
39	79.14	30	77.65	77.65	77.65	23

LAB = the means from data based on the conventional analysis.
NIRS1 = data from NIRS, parallel 1. NIRS2 = data from NIRS, parallel 2. NIRS = analysis based on the means from NIRS1 and NIRS2. RANK1 = the ranks from LAB. RANK2 = the ranks from NIRS.

error is much larger for the conventional method than for the method based on the NIRS technique. For the NIRS method, only sample 38 had a relatively high random variation.

DISCUSSION

The results from this study indicate that the estimates of IVDMD have much lower standard deviation for the NIRS-based method, compared with the conventional method described by Tilley & Terry (1963). This result is similar to the results presented by Templeton et al. (1983) on this point. The correlation between the NIRS estimates and that from the Tilley & Terry method of IVDMD is very high and is similar to the result of Marum et al. (1979). Also, when looking at the ranks for the different samples, we

Table 3: The standard deviations and the fractions of the variances of the conventional methods compared with the NIRS-based method

Sample	LAB	NIRS1	FRACT1	NIRS2	FRACT2	NIRS	FRACT
1	1.35	0.34	16.1	0.35	15.1	0.26	27.1
2	0.91	0.37	5.9	0.46	3.9	0.28	10.2
3	1.44	0.51	7.8	0.46	9.6	0.44	10.9
4	1.01	0.48	4.3	0.48	4.4	0.43	5.5
5	2.22	0.40	31.0	0.27	68.6	0.27	67.0
6	1.28	0.41	9.6	0.42	9.5	0.33	15.6
7	1.30	0.48	7.3	0.58	5.0	0.35	14.0
8	1.22	0.40	9.3	0.40	9.5	0.32	14.0
9	1.26	0.35	12.8	0.39	10.5	0.19	43.4
10	0.84	0.29	8.3	0.52	2.6	0.33	6.4
11	1.09	0.28	15.2	0.37	8.7	0.22	24.8
12	1.42	0.82	3.0	0.29	24.4	0.47	9.1
13	1.08	0.42	6.4	0.29	14.1	0.26	16.8
14	1.17	0.35	11.2	0.29	15.6	0.25	21.5
15	1.27	0.42	9.3	0.39	10.8	0.30	18.1
16	1.57	0.40	15.4	0.34	21.9	0.29	29.8
17	1.41	0.45	9.9	0.33	18.8	0.27	28.2
18	0.88	0.35	6.2	0.37	5.7	0.33	7.4
19	1.58	0.33	22.9	0.50	9.9	0.24	44.6
20	1.27	0.34	14.0	0.46	7.6	0.28	20.5
21	1.03	0.30	12.1	0.40	6.6	0.28	14.0
22	1.58	0.36	19.5	0.30	27.4	0.26	38.0
23	1.21	0.30	16.3	0.34	12.5	0.23	27.8
24	1.28	0.36	12.9	0.31	17.6	0.28	21.7
25	0.95	0.35	7.2	0.46	4.2	0.31	9.2
26	1.13	0.31	13.4	0.28	15.9	0.21	30.1
27	1.30	0.38	12.0	0.30	18.8	0.28	20.8
28	1.22	0.42	8.5	0.37	10.8	0.33	13.7
29	1.09	0.45	6.0	0.46	5.6	0.34	10.4
30	1.09	0.34	10.2	0.27	16.8	0.23	21.9
31	1.09	0.37	8.9	0.41	7.1	0.32	11.4
32	1.37	0.34	16.0	0.36	14.5	0.22	40.3
33	0.96	0.32	8.8	0.34	8.0	0.28	11.6
34	1.25	0.32	15.7	0.38	10.7	0.29	18.0
35	1.63	0.35	21.5	0.42	15.2	0.26	38.9
36	1.27	0.36	12.7	0.44	8.5	0.30	17.7
37	1.17	0.31	14.5	0.40	8.7	0.28	17.5
38	1.23	0.84	2.1	1.02	1.4	0.76	2.6
39	1.13	0.30	14.6	0.33	12.1	0.20	33.7
Mean	1.24	.39		.40		.30	

LAB = the standard deviation from data based on the conventional analysis. NIRS1 = data from NIRS, parallel 1. NIRS2 = data from NIRS, parallel 2. NIRS = analysis based on the means from NIRS1 and NIRS2. FRACT1 = $(LAB/NIRS1)^2$, FRACT2 = $(LAB/NIRS2)^2$, FRACT = $(LAB/NIRS)^2$

found that there is good agreement between the conventional method and the method based on the NIRS technique. For some of the samples we obtained significant different estimates of the IVDMD, but it was not possible to find a systematic bias.

With regard to the stability of the two methods, it will be seen from Figure 4 that the method based on NIRS seems to give much more stable and repeatable results than the conventional method (std = 1.24 for the conventional method, but std = 0.30 for the NIRS-based method (from Table 3)). This concurs with the fact that the standard deviation

for the NIRS-based method is much smaller compared with the conventional method. The results from our study indicate that the quality of the IVDMD estimates is better when based on the NIRS techniques than when based on the conventional method described by Tilley & Terry (1963). From this fact and the fact that the NIRS method is cheaper to use, we find the NIRS method much more viable for forage breeding programmes. We recommend this method for practical use.

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ESTABLISHMENT OF CRITICAL LEVELS OF ZINC FOR MAIZE IN SOILS OF THE HIGH RAINFALL AREAS OF ZAMBIA

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Banda[1], D.J. & B.R. Singh[2] 1989. Establishment of Critical Levels of Zinc for Maize in Soils of the High Rainfall Areas of Zambia. Norwegian Journal of Agricultural Sciences 3: 221-227. ISSN 0801-5341.

The objectives of this study were to determine the critical levels of zinc and to evaluate various extractants for predicting zinc availability to maize in the soils of the high rainfall areas of Zambia. Hybrid maize (MM 752) was grown in pots on some Ultisols (Katito, Kasama, Misamfu, Mpongwe, and Mufulira), Oxisols (Konkola, Maheba, and Kasempa), Alfisol (Malashi) and Inceptisol (Chinsali).

Lime (1.65 x Exch. Al) and basal fertilizers were applied to all the pots. Zn as zinc sulphate was applied to each soil at the rate of 0, 2.5, 5.0, and 10.0 ppm. Moisture in the pots was maintained at near field capacity.

Zinc application significantly increased both the dry matter yields and the Zn concentration in the plant tissue. Critical levels of zinc for maize on these soils were established at 0.7, 2.0, and 1.5 ppm by 0.005M DTPA, 0.1N HCl, and NH_40Ac-EDTA, respectively. In the plant tissue 15 ppm was found to be the critical zinc concentration.

A comparison of the three extractants showed that 0.005M DTPA and 0.1N HCl were superior than NH_40Ac-EDTA in relating zinc concentration in the plant tissue with the extractable zinc in the soil. The 0.005M DTPA, however, gave the highest correlation between extractable zinc and the plant tissue zinc. These results therefore indicate that 0.005M DTPA and 0.1N HCl are suitable in predicting zinc deficiency problems for maize in most soils of the high rainfall areas of Zambia.

Based on the DTPA-extractable zinc values it has been shown that the native available zinc content in most soils of the high rainfall areas of Zambia is generally below the critical level for maize. The Inceptisols, Oxisols, and Ultisols tended to have lower values than the Alfisols and Entisols.

Key words. Benchmark soils, critical levels, extractants, high rainfall areas, maize, zambia, zinc availability, zinc.

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Zinc deficiency in agricultural crops is one of the most common and widespread micronutrient deficiencies (Lindsay 1972). Although there has never been a systematic assessment of the areal extent of the micronutrient deficiencies in tropical Africa, most studies have shown that zinc and boron deficiencies are commonly observed throughout the region (Kang & Osiname 1985). Maize has long been identified as one of the most susceptible crops to zinc deficiencies (Viets et al. 1954). It is the main food crop and is extensively grown in Zambia. Major con-

straints to crop production in this area are soil acidity and the low fertility status of soils (Njøs 1983).

The success of a fertilizer programme largely depends upon the existing knowledge about the nutrient status of the soils for which fertilizer is intended. In Zambia, this kind of information on Zn is lacking. The main objectives of this study were to find out the response and to establish critical levels of zinc for maize grown on the soils of the high rainfall areas of Zambia. Different extractants were also evaluated as a means of selecting the most suitable for assessing available soil zinc.

MATERIALS AND METHODS

Soil sampling

The high rainfall areas of Zambia comprise those with an annual rainfall above 1000 mm. Twenty-three major soil series were selected throughout this zone (Table 1). Composite soil samples, each consisting of 30 subsamples, were collected from 0-20 cm of each soil series. Ten of these, mostly benchmark soils, were used in the pot experiment while the rest were just tested for their native available Zn. Some chemical characteristics of the soils used in the pot experiment are given in Table 2.

Pot experiment

Composite samples from each of the ten soil series were thoroughly mixed, divided into 4 kg portions (oven-dry basis) and filled into 5 dm^2 plastic pots. All pots were limed with chemical grade $CaCO_3$ equivalent to 1.65 x Exch. Al. (Sanchez 1976). Lime was allowed to incubate with the soil kept moist at near field capacity for ten days, after which basal fertilizers were applied. Four levels of Zn, 0.0, 2.5,

Table 1. The extractable zinc status of the selected soil series

	Soil series	Classification (SOIL TAXONOMY)	EXTRACTANT DTPA	HCl	$NH_4$0AcEDTA
	Chansongo	Aquic Ustorthent	0.61	1.76	0.85
	Ifisa	Ultic(kandi)ustalf	0.36	0.85	0.50
*	Malashi	Oxic Rhodustalf	0.32	1.68	0.50
	Munkumpu	Ultic(kandi)ustalf	0.36	0.90	0.50
*	Katito	Oxic/Rhodic Paleustult	0.26	1.32	0.50
*	Kasama	Oxic/Rhodic Paleustult	0.18	1.04	0.20
*	Misamfu	Oxic/Rhodic Paleustult	0.14	0.88	0.20
*	Mufulira	Oxic Paleustult	0.26	1.44	0.50
*	Mpongwe	Ustoxic Palehumult	0.16	0.64	0.50
	Lubu	Typic Haplustult	0.09	0.48	0.25
	Kateshi	Rhodic (kandi) Ustult	0.10	0.45	0.35
	Kungu	Aquic (kandi) Ustult	0.09	0.48	0.35
	Kawambwa	Typic (kandi) Ustult	0.09	0.32	0.20
	Shilende	Typic (kandi) Ustult	0.31	0.69	0.70
	Mulobola	Xanthic (kandi) Ustult	0.18	0.61	0.55
*	Konkola	Rhodic/Acric Haplustox	0.10	0.40	0.20
*	Meheba	Rhodic/Acric Haplustox	0.12	0.88	0.20
*	Kasempa	Typic Haplustox	0.20	1.08	0.70
	Mutwale	Typic Haplustox	0.17	1.00	0.55
	Mayondo	Aquic Haplustox	0.20	0.52	0.33
*	Chinsali	Ustoxic Dystropept	0.14	0.72	0.40
	Luano	Typic Tropaquept	0.12	0.57	0.50

* = Soil used in the pot experiment.

Table 2. Some chemical characteristics of the soils for the pot experiment (0-20 cm). TR = Trace. - = data not available

SOIL SERIES	OC %	N %	K	Ca	Mg	Na	Al	CEC	BS %	pH (0.01M $CaCl_2$)
			<----------meq/100g soil---------->							
Malashi	1.84	0.10	0.50	4.6	1.50	TR	-	10.0	66	5.6
Katito	1.20	0.07	0.20	TR	0.30	TR	1.2	7.4	11	4.5
Kasama	1.22	0.07	0.10	1.5	0.80	TR	0.8	6.6	53	4.8
Misamfu	1.50	-	0.20	1.3	1.30	TR	1.1	7.9	51	4.6
Konkola	2.50	0.13	0.14	0.8	0.68	TR	1.5	10.0	16	4.5
Mufulira	0.85	0.05	0.20	0.2	0.40	TR	1.0	4.9	22	4.3
Meheba	1.90	-	0.30	0.3	0.20	TR	1.5	10.2	7	4.2
Chinsali	1.48	-	0.05	3.4	0.90	TR	-	12.5	64	5.4
Mpongwe	3.00	0.18	0.60	8.0	4.60	TR	0.8	21.2	89	5.4
Kasempa	1.09	-	0.35	0.4	0.09	TR	1.6	20.0	14	4.5

5.0, and 10.0 ppm applied as zinc sulphate were tested on each soil series with three replications.

Six seeds of a hybrid maize (variety MM752) were planted in each pot and thinned to three after germination. Soil moisture was maintained at near field capacity by regular watering with distilled water. Eight weeks after planting, all the mature leaves were removed for the tissue zinc and phosphorus analysis and the plants were harvested for dry matter determination. After harvesting the maize, soil in the pots was remixed and representative samples were collected for the determination of pH and available zinc.

Soil and plant analysis

Three extraction procedures, the 0.005M DTPA+0.01M $CaCl_2$, (Lindsay & Norvell 1978), the 0.5M CH_3C00NH_4 + 0.5M CH_3COOH +0.02M Na_2EDTA buffered at pH 4.65 (Sillanpaa 1982), and the 0.1N HC1 (Wear & Evans 1968) were used in assessing available soil Zn. Soil pH was measured in 0.01M $CaCl_2$ (Schofield & Taylor 1955).

The fresh leaves were washed in distilled water, dried at 65 °C for 48 hours, ground (0.1 mm), and digested in a nitric acid perchloric acid mixture (Zasoski & Barau 1977). The Zn content in the digest was read on an atomic absorption spectrophotometer. To determine tissue P, 2 ml of the digest was reacted with 8 ml of ammonium molybdate-ascorbic acid solution (Olsen et al. 1954), diluted to 50 ml with deionized water and read at 882 nanometres on a Bausch and Lomb Spectronic 21 colorimeter.

Statistical Analysis

A 2-way ANOVA was used to test the main effects and interactions of applied zinc and soil type on the dry matter yield and plant tissue zinc concentration. Regression analysis, with and without soil pH, was undertaken to test the relationship between the plant tissue zinc concentration and the post-harvest extractable soil zinc.

RESULTS AND DISCUSSION

Native available soil. Zn

A few differences were discernible among the various soils (Table 1). An Entisol (Chansongo) exhibited the highest extractable soil zinc, followed by the Alfisols. The Inceptisols, Ultisols, and Oxisols had lower available soil zinc. These results have shown that the order of extractability was HCl > $NH_4$0Ac-EDTA> DTPA and support the earlier findings by Gupta & Mittal (1981), who observed a zinc extractability order as

1N HCl > 0.1N HCl > $(NH_4)2CO_3$-EDTA > NH_40Ac-EDTA > DTPA > 1M $MgCl_2$. Similarly, Lauer (1971) also observed that zinc extraction values by 0.1N HCl were higher than those by the DTPA.

Pot experiment

Zinc deficiency symptoms were observed in many of the pots without Zn application. Plant growth in these was generally slower than in pots with added zinc, and leaves developed chlorotic bands on both sides of the midrib. Some growth anomalies in maize grown on Konkola and Meheba soil series was observed and hence results from these series were not included in the subsequent statistical analyses.

The response of maize to zinc application is shown in Table 3 and for some selected soils in Fig. 1. The effects of Zn application and soil type on dry matter yields and the Zn concentration in the plant tissue were significant ($P < 0.05$). The interaction effect between these factors, however, was not significant.

Correlations between plant tissue and extractable soil Zn were positive and highly significant (Table 4). When results from the eight soils shown in Table 4 were combined, the resulting significant correlation coefficients between plant tissue and extractable zinc were 0.56, 0.50 and 0.36 for the DTPA, HCl, and NH40Ac-EDTA, respectively. An inclusion of soil pH in the regression analysis did not improve these correlation coefficients.

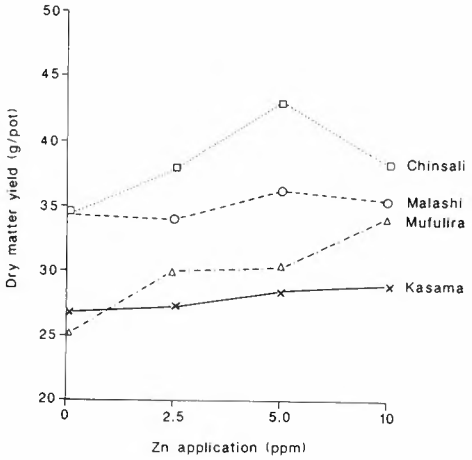

Figure 1. Maize response to zinc on four representative soils

Better correlation for the DTPA procedure than the others is in line with the observations of many other workers who have acknowledged the effectiveness of DTPA in predicting Zn deficiency problems (Brown et al. 1971; Korcak & Fanning 1978; Gupta & Mittal 1981; Madziva, 1981). However, in cases of limited availability of DTPA, HCl, the extractant showing second highest correclation coefficient, can be used for predicting Zn deficiency in soils of the high rainfall areas of Zambia.

Critical levels of zinc were determined using a graphical method developed by Cate & Nelson (1965); this method has proved equally reliable when compared with the conventional quadratic, logarithmic and Mitscherlich models (Cate & Nelson 1971).

The resulting critical levels of zinc for maize in this study were around 0.7, 2.0, and 1.5 ppm for the DTPA, HCl, and NH_40Ac- EDTA extractants, respectively. Based on these limits, it can be seen that the available Zn status of most soils of the high rainfall areas of Zambia is low (Table 1) and much below the critical levels mentioned above. The critical concentration of zinc in maize leaves was established at 15 ppm, which is within the range found by other workers (Viets et al. 1953; Boehle & Lindsay 1969).

Extractable Zn content after harvest in the different soils is given in Table 3 and, combined for all the soils, is plotted against zinc application in Fig. 2. As already observed from the results in Table 1, the order of extractability was again HCl > NH_40Ac-EDTA > DTPA. However, due to the higher correlation coefficient by DTPA extractable soil Zn than the other extractants with dry matter and

Table 3. Extractable soil Zn at harvest and maize response of Zn

Soil series and (pH)	Zn applied ppm	Soil Zn extracted with HCl	DTPA	$NH_4$0Ac-EDTA	Maize response in terms of Dry matter yield (g/pot)	Leaf tissue Zn (ppm)	Leaf tissue P (%)
			ppm				
Kasama (4.8)	0.00	0.57	0.07	1.40	26.74	6.67	0.60
	2.50	3.33	0.57	2.17	27.27	10.00	0.50
	5.00	6.85	1.39	4.60	28.57	13.33	0.70
	10.00	13.36	3.51	7.10	29.07	28.33	0.50
Malashi (5.0)	0.00	1.28	0.27	2.00	34.20	13.33	0.43
	2.50	4.03	0.95	2.90	34.95	20.00	0.61
	5.00	7.65	1.88	4.33	36.27	25.00	0.60
	10.00	14.77	3.90	7.97	35.55	30.00	0.47
Mpongwe (4.9)	0.00	1.31	0.09	1.03	38.35	11.67	1.50
	2.50	3.36	0.62	2.13	39.73	16.67	1.01
	5.00	6.77	1.06	3.37	41.45	20.00	1.01
	10.00	11.84	2.15	6.23	35.90	31.67	0.90
Katito (5.0)	0.00	1.31	0.41	0.77	30.82	10.67	0.70
	2.50	4.40	1.25	3.50	32.34	13.67	0.67
	5.00	7.65	2.20	4.87	33.18	12.67	0.63
	10.00	16.11	4.48	9.07	36.48	18.33	0.60
Kasempa (4.7)	0.00	1.04	0.10	1.20	21.81	7.33	0.56
	2.50	3.79	0.29	2.47	24.73	12.67	0.54
	5.00	6.67	1.12	5.13	26.34	15.33	0.50
	10.00	14.72	3.22	6.00	24.91	28.00	0.50
Konkola (5.0)	0.00	0.91	0.12	0.90	12.25	15.67	0.09
	2.50	3.81	0.66	3.83	10.34	26.33	0.14
	5.00	7.88	1.13	4.27	10.74	19.67	0.14
	10.00	13.25	2.53	10.57	11.46	22.67	0.11
Chinsali (5.2)	0.00	1.12	0.24	1.30	34.33	3.00	0.83
	2.50	4.32	0.94	4.70	37.87	5.33	0.80
	5.00	8.11	2.14	4.13	43.09	11.67	0.80
	10.00	15.71	4.57	9.07	38.37	18.00	0.80
Maheba (5.5)	0.00	0.80	0.28	2.90	21.87	3.00	0.40
	2.50	4.48	0.87	2.23	26.55	8.33	0.31
	5.00	8.56	1.52	3.23	25.00	7.00	0.27
	10.00	16.85	3.37	8.67	22.81	9.33	0.48
Misamfu (4.9)	0.00	5.41	0.08	3.00	24.72	9.33	0.52
	2.50	3.44	0.61	3.10	28.15	6.33	0.55
	5.00	6.27	1.21	3.63	26.37	12.00	0.56
	10.00	9.68	2.82	5.33	27.84	17.67	0.55
Mufulira (5.0)	0.00	0.45	0.33	0.56	25.16	6.00	0.70
	2.50	3.81	0.97	2.00	29.99	9.99	0.65
	5.00	6.56	1.25	3.93	30.31	11.33	0.80
	10.00	14.45	3.40	7.10	34.22	20.00	0.60

plant tissue Zn concentration, it is considered superior for predicting the Zn deficiency problems of the studied soils. Earlier, Lauer (1971) concluded that

Table 4. Correlations between plant tissue and the post-harvest extractable zinc

Soil series	0.005M DTPA	0.1N HCl	NH40Ac-EDTA
Kasama	0.96	0.94	0.93
Malashi	0.85	0.84	0.72
Mpongwe	0.93	0.91	0.82
Katito	0.67	0.65	0.71
Kasempa	0.94	0.97	0.80
Chinsali	0.95	0.93	0.74
Misamfu	0.83	0.79	0.65
Mufulira	0.98	0.94	0.84

0.1N HCl extracted zinc which was neither available to the plants nor extractable by DTPA. Similarly comparing 0.1N HCl and dithizone, Martens et al. (1966) concluded that much of the zinc extracted by 0.1N HCl over dithizone was not available to plants. The steady increase in extractable zinc in Fig. 2 suggests a correspondingly steady increase in Zn availability as zinc application levels increase and very little zinc fixation in the soils under study.

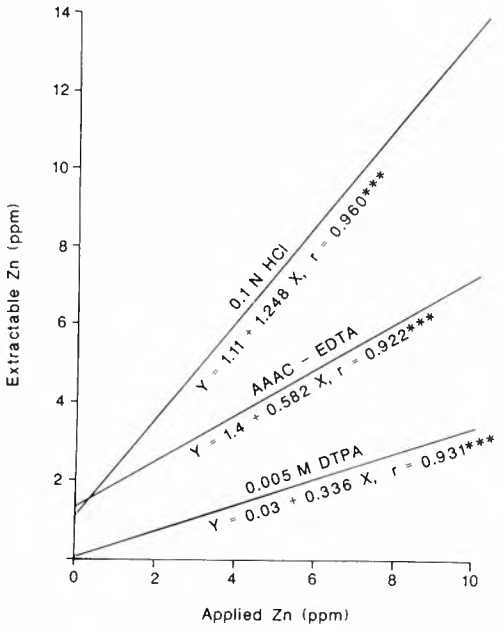

Figure 2. Correlation between applied zinc and that extracted by different extractants

CONCLUSIONS

The following conclusions can be drawn from the results of this study.

1. Maize is highly responsive to zinc application in the soils studied.

2. Critical levels of zinc for maize in the major soils of the high rainfall areas of Zambia are 0.7, 2.0, and 1.5 ppm by 0.005M DTPA, 0.1N HCl, and NH40Ac-EDTA, respectively. The critical zinc concentration in maize leaves is established at 15 ppm. Based on these results, the available zinc content of most soils in the high rainfall areas of Zambia is below the critical level for maize.

3. A comparison of the three extractants shows that 0.005M DTPA is more suitable for predicting zinc deficiency problems but in cases of limited availability of DTPA, 0.1N HCl is the suitable alternative.

4. The Alfisols and Entisols show somewhat higher available zinc values than the Inceptisols, Oxisols, and the Ultisols.

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UNSATURATED FREE FATTY ACIDS AS A SUBSTRATE OF LIPID OXIDATION IN MILK

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The linoleic acid content of free fatty acid shows the largest variation and the strongest correlation among milk lipids with oxidized flavour.

Key words: Milk, free fatty acids, oxidized flavour.

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The free fatty acids of milk and milk products have recieved much attention due to the correlation of their content with the rancid taste defect in milk and milk products. The flavour of short chain fatty acids has a low threshold of detection. The free fatty acids are sometimes classified according to their origin, and at the point when off flavour is discovered in milk, free fatty acids deriving from enzymatic hydrolysis of the triglycerides contribute about 0.5 - 1% of the total milk lipids. However, freshly secreted milk also contains free fatty acids in amounts assessed to make less than 0.5% of the total milk lipids (Chilliard & Lamberet 1984). Total free fatty acid composition has been studied, but little attention has been given to the composition of the original fraction of free fatty acids in milk. From our experience with unsuccessful efforts in casein - protection of free fatty acids (Astrup et al. 1972), we noted very rapid oxidation of the free fatty acids. Since oxidation is the cause of another flavour defect, the oxidized flavour, the fatty acid composition of milk fraction lipids was compared with the development of the oxidized flavour defect. Oxidized flavour has already been found to correlate with the total milk lipid content of linoleic acid (Astrup 1966, Astrup & Sivertsen 1971).

Milk from 64 animals in the university herd was sampled in February in 500 ml milk bottles. Milk samples, 4 x 20 ml, were poured into 16 mm code-labelled test tubes in a rack, stored for 48 h at 5°C, brought to room temperature in a waterbath and tasted, using a stainless steel spoon. The evaluation was made by the experienced judge, chief consultant Hans Jetlund. The tubes were arranged randomly. The rest of the milk was frozen immediately after sampling and kept in the milk bottles at -20°C till the result of the tastings arrived. Seventeen bottles of milk ranging in degree of oxidized flavour were then selected for further investigation. The frozen milk was tawn to give a homogeneous slurry at 0°C, and 100 ml was transferred to 1 liter round bottom flasks which were immidiately mounted on the freeze drier (Christ).

Two-gram samples of the dried milk were then extracted in new timbles in a Soxleth apparatus with a mixture of chloroform and methanol (2:1 v/v). The extracted lipids were then isolated in vacuum in a rotation evaporator. The lot was redissolved in petrol ether (bp. 60°C) and transferred to a micro column containing silica. Using solvents of increasing polarity, fractions were eluated in the following order: 1. cholesterol esters, 2. triglycerides, 3. free fatty acids, diglycerides, free cholesterol and 4. phospholipids.

1. 1% ethyl ether in petrol ether (20 ml)
2. 10% ethylacetate in petrol ether (27 ml)
3. ethyl ether (25 ml) and
4. acetic acid, methanol, ethyl ether 4:9:27 (v/v/v) (30 ml), respectively. The separation procedure was adopted from Brown and Stull (1966), who analyzed the same components in blood serum. Separation of the lipid fraction was followed on thin layer chromatography on silica and with petrol ether, ethyl ether, acetic acid in ratio 90:10:1 (v/v/v).

The solvent of each fraction was removed in vacuum in the rotation evaporator and the residue was saponified with alcoholic kali and finally methylated with diazometan in ethyl ether. The ether solution was applied to the FM gas chromatograf DEGS column at 195°C with argon as carrier gas and with hydrogen flame detector.

RESULTS AND DISCUSSION

Mean values of fatty acid composition of the lipid fractions is given in Table 1. The two most volatile fatty acids, butyric and caproic acids are not reported on because of their inferior separation from the solvent.

The first two fractions are similar, and there is a similarity between fractions 3 and 4 too.

The phospholipid fraction (no. 4) had low levels of fatty acids with chain length up to C16, but increased level of acids of higher chain lengths. The four longest chain lengths does also appear in the free fatty acid fraction, but not in the triglycerides. Compared with fatty acid

Table 1. Average fatty acids distribution in various milk lipids from 17 cows

Fatty acids,%	Total	1 Cholesterol ester	2 Triglyc.	3 Free fatty acids	4 Phospho-lipids
8	2.8	0.8	0.8	0.1	0.2
10	4.9	2.5	2.4	0.3	0.2
12	5.4	3.3	3.6	1.0	0.6
14	12.5	11.7	12.1	7.8	3.3
14:1	1.9	1.9	1.8	0.7	0.7
16	26.1	27.5	28.9	27.2	16.3
16:1	2.9	3.1	3.1	5.1	4.1
18	13.1	14.3	12.5	14.7	18.3
18:1	22.7	25.4	22.3	22.0	29.1
18:2	3.2	3.9	3.7	5.8	9.3
x1	0.7	0.7	0.9	2.2	2.5
x2	1.1	1.8	1.1	2.7	2.9
x3	0	0	0	1.4	3.1
x4	0	0	0	0.6	2.2
x5	0	0	0	1.4	2.3
x6	0	0	0	1.7	0.4

composition of the blood serum as reported by Brown & Stull, milk lipid fractions 3 and 4, but not fractions 1 and 2, are similar to the same fractions in blood. This might suggest that these lipids are transfered from the blood.

The result of thin layer chromatography, showed fraction 1 to be present in trace amounts, and contaminated slightly by fraction 2. Fraction 3, however, was completely separated from fractions 2 and 4.

The linoleic acid content of the various fractions was correlated with the oxidized flavour scores of the milk. The coefficients turned out to be 0.43, 0.24, 0.68 and 0.19 for fractions 1, 2, 3 and 4, respectively. The standard deviations of linoleic acid fractions were 2.7, 2.7, 3.7 and 2.2, suggesting that lipid oxidation in milk may be regulated by the appearance of unsaturated free fatty acids in the milk.

In an early study (Astrup 1961) it was discovered that oxidized flavour in milk had a peak value in cows estreous. When Aurand et al. (1968) analyzed milk from cows given estrogens they noted a resulting rise in TBA values in the milk. This is in accordance with the oxidized flavour increase in milk seen during heat.

Since membrane permeability is assumed to be increased by estrogens, the hormone may facilitate uptake of the unsaturated free fatty acids from the blood and as a result produce more oxidized flavour in milk. Interesting observations have been made by Garm et al. (1963). A rancid flavour and high level of butyric acid occasionally appeared in milk when estrogens were injected intramuscularly to the cow. The milk butyric acid derives from the triglycerides, and thus lipolysis in milk had been triggered by the hormone. The conclusion must be that estrogens are non-specific in their effect on milk flavour. Thomson (1987) suggests that the increase in udder cell permeability in late lactation might favour transfer of lipase activator from the blood. This is in agreement with our hypothesis (Astrup et al. 1980) that the animals' choise of milk flavour takes place in the blood. Flavours are regulated by the kind of lipids present. Unsaturated fat emulsion readily extracts blood lipoprotein lipase activator in vitro (Astrup & Bengtsson 1982). Unsaturated fat in vivo may dilute and reduce activator impact of the udder cell and thus give less milk lipolysis. The milk, however, will contain more unsaturated fat and become oxidized.

SUMMARY

Lipids in fresh milk from the university herd was fractionated and analysed for composition of fatty acids. The fatty acid distribution of the free fatty acids fraction was similar to the distribution in the phospholipids. Linoleic acid content of the free fatty acid fraction showed the highest variation and the strongest correlation with oxidized flavour of the milk.

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IRRIGATION ROUTINES AND LEACHING IN GLASSHOUSE TOMATOES

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The effects of different irrigation routines on leaching in glasshouse tomatoes grown on rockwool were investigated. Recirculation with the nutrient solution renewed every fourth week gave the lowest amount of leaching. In ordinary drip irrigation, leaching increased with an increasing number of irrigations when the same total amount of water was given. In the four treatments without recirculation the total leaching varied from 42 to 61% of applied nutrient solutions. The content of nutrients in the rockwool slabs was negatively correlated with the irrigation frequencies. Despite this fact, the content of nutrients in the leaves was not affected by the irrigation frequencies. No significant differences were recorded in marketable yield between the different types of irrigation, but both grading and fruit size were affected.

Key words: Irrigation routines, nutrient leaching, nutrient uptake, tomato.

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The pollution of the environment from plant production in the field and from animal husbandry has induced extensive discussion, research and practical measures over the last few years. Very little has been done, however, on pollution from protected plant production.

Modern glasshouse crop production uses very small amounts of growth media per plant. The growth medium normally has a low water and nutrient capacity and makes heavy demands on the grower to maintain a well-balanced water and nutrient supply. Surplus irrigation is the normal routine in maintaining a stable EC level and in ensuring a good oxygen supply to the roots. It is equally important that plants at different positions in the glasshouse are ensured sufficient irrigation (Burg & Hamaker 1985, 1986). Water requirement in glasshouses is estimated to be approximately 1000 litres per m^2 per annum.

Differing opinions about the plants' irrigation and nutrient requirements, together with the limited possibilities for adjusting the nutrient solution, often lead to an oversupply of water and fertilizer (Hamaker & Burg 1982, 1983, 1984, Krohnstad 1988). The oversupply involves higher costs for the grower and may result in pollution of the environment. The aim of the present investigation is to evaluate leaching losses from glasshouses using different irrigation frequencies and production systems.

MATERIALS AND METHODS

Seeds of the tomato varieties Abunda, Criterium and WW 240 were sown in the glasshouse, in 5 x 5 x 5 cm rockwool cubes in Week 1 and transferred to 6.5 x 10 x 10 cm blocks 17 days later. The temperature during germination was kept at 21 ± 0.5°C. After sowing, the rockwool cubes were watered with a complete nutrient solution, EC level 1.0 mS cm^{-1}.

The solution, composed of red Superba, calcium nitrate and potassium sulphate, contained: 160 N, 42 P, 240 K, 40 Mg, 114 Ca, 53 S, 2.0 Fe, 1.1 Mn, 0.20 Cu, 0.30 Zn, 0.33 B and 0.025 Mo mg per litre. The EC level of the nutrient solution was gradually increased during growth and one month after sowing reached 2.3 mS cm^{-1}.

After emergence, the seedlings were irradiated with 12W m^{-2} (Philips TL 33) for one month, after which irradiation was increased to 20W m^{-2} (PAR). The duration of supplemental irradiation was 16 hours daily with CO_2 concentration throughout the growth period maintained at 500 vpm.

In Week 7 the young plants were selected for uniformity and placed directly on rockwool slabs (Grodan 20 x 6.5 x 100 cm), 2.5 plants per m^2 and two nozzles to each plant. The slabs had free drainage to one side. The leaching was collected and measured once daily.

Five different irrigation methods were compared (Table 1). In all treatments normal drip irrigation was used. In treatment 4 the nutrient solution was recirculated and renewed every 4 weeks. The irrigation was timed to occur during the day (0700 - 1900 h depending on the season). In treatment 5 additional water was given during the night.

The minimum air temperature was 18°C (night) and 20°C (day) with ventilation at 23°C. Vegetative growth was controlled in later stages by varying the night temperature between 16 and 20°C. The EC level of the nutrient solution was also used to regulate vegetative growth.

The glasshouse atmosphere was enriched to 600vpm CO_2 from sunrise to sunset each day except during ventilation periods. Mechanical vibration for enhanced pollination was used three times a week from flowering till the end of May. The global radiation during the experimental period reached a daily average of 10.5 MJ m^{-2}.

The yield was harvested and graded three times a week according to Norwegian Standard No. 2815. The first harvesting was taken in Week 16. The experiment was terminated in Week 32.

Every fourth week, from Week 10 to Week 26 and then Week 32, samples taken from fully developed leaves (MacLean et al. 1968) were sent to Landbrukets analysesenter, Ås for analysis. The samples were collected as an average

Table 1. Irrigation treatments, irrigation frequencies and amount of nutrient solution per plant in litres per day. In treatments 1, 2, 3, and 5 the nutrient solution was given by normal drip irrigation while it was recirculated and renewed every 4 weeks in treatment 4. In treatment 5 additional water was given during the night

Irrigation treatments	Time period		
	16 Febr.-15 March	19 March- 15 May	16 May-15 Aug.
1	3 water. á 0.22 l	6 water. á 0.22 l	9 water. á 0.22 l
2	4 water. á 0.17 l	8 water. á 0.17 l	12 water. á 0.17 l
3	5 water. á 0.13 l	10 water. á 0.13 l	15 water. á 0.12 l
4	3 water. á 0.22 l	6 water. á 0.22 l	9 water. á 0.22 l
5	3 + 1 water. á 0.22 l	6 + 2 water. á 0.22 l	9 + 3 water. á 0.22 l

of the three varieties included in the experiment. Total nitrogen is analysed as Kjeldahl nitrogen. Chemical analyses of the nutrient solution in the rockwool slabs and the drainage liquid were performed at the same laboratory weekly until Week 26 inclusively and then every fortnight.

The experiment was conducted in a 250 m^2 section of a N-S single-span house with a total floor area of 600 m^2. The 15 possible treatment combinations were randomly arranged and sufficiently replicated so as to minimize positional effects. Each plot include 13 plants. The data were subjected to analyses of variance. Significant levels are given as follows: *** = $P \leq 0.001$, ** = $P \leq 0.01$, * = $P \leq 0.05$, ns = $P > 0.05$.

RESULTS

Yield and quality

No significant yield differences were found between the various irrigation methods (Table 2). There is some indication, though, that irrigation method 4 (recirculation) gives a somewhat lower yield than other methods ($P = 0.07$). The yield reduction was due to both fewer and smaller fruits. Quality, on the other hand, was clearly superior when recirculation was compared to other irrigation methods.

There was no difference in yield between the three varieties (Table 2). The mean fruit weight, however, was approximately 9 g higher for 'W 240' than for 'Abunda' and 'Criterium'. 'W 240', however, was of a considerably lower quality than the other two varieties.

Yield regarded as unmarketable because of misgrowth such as puffiness, cracking, and fluted or generally misshapen fruits or blossom-end rot was lower with recirculation than the other irrigation methods ($P \leq 0.01$). Ripening disorders clearly occurred more often ($P \leq 0.01$) in 'W 240' (2.2 kg per m^2) than in 'Abunda' (0.7 kg per m^2) and 'Criterium' (0.9 kg per m^2).

No significant interaction was observed between irrigation method and variety in relation to yield and quality parameters.

Content of different nutrients in leaves and growth medium

The nutrient content in the growth medium was distinctly influenced by the irrigation method (Table 3). The nutrient

Table 2. Yield (kg/m^2), % class 1, fruit weight (g) and % of class 1 in different size groups

Irrigation treatments	Marketable yield	% class 1	Fruit weight	% of class 1 in different size groups		
				40-47 mm	47-57	≥ 57 mm
1	17.8	74.0	71.6	11	57	32
2	16.6	72.2	70.4	12	56	32
3	17.2	70.7	72.2	11	57	32
4	15.1	79.6	68.2	13	61	26
5	17.6	71.6	71.4	10	58	32
Signif.	ns	*	**	**	ns	**
Variety						
Abunda	16.7	81.3	68.7	13	60	27
Criterium	16.9	78.4	69.1	12	62	26
WW 240	17.1	60.8	77.9	9	48	43
Signif.	ns	***	***	***	***	***

Table 3. The EC level, pH and content of nutrients (mg per litre) in the growing medium for different irrigation treatments as an average during the research period

Irrigation treatment	EC	pH	NO_3-N	P	K	Ca	Mg	SO_4-S	Na	Fe	Cu	Mn	Zn	B	Mo	Al
1	3.6	5.8	376	59	389	413	96	172	17.6	3.0	1.09	0.74	0.64	0.53	0.052	0.45
2	3.0	5.8	309	52	355	318	74	133	12.5	1.7	0.77	0.70	0.44	0.43	0.038	0.39
3	3.2	5.8	319	53	369	332	77	140	13.7	1.9	0.82	0.68	0.46	0.45	0.040	0.41
4	4.2	6.3	452	50	342	571	127	222	26.2	4.1	1.77	0.51	1.00	0.62	0.049	0.47
5	3.1	5.6	300	56	367	337	76	134	13.4	2.0	0.78	0.72	0.48	0.43	0.035	0.45
Signif.	**	***	**	*	ns	***	***	***	***	*	***	ns	***	***	***	ns

contents in the rockwool slabs were highest after recirculation, except for P, K, Mn, and Mo, which were highest after irrigation method 1. The nutrient contents in the slabs will normally vary throughout the season, as was the case in this experiment ($P \leq 0.01$). No significant interaction was found, however, between irrigation method and the time of sampling. The nutrient contents in the leaves were not influenced by the irrigation method. The content of specific nutrients varied, however, throughout the growth season (Table 4).

The contents of tot.-N, P and K in the leaves were gradually reduced throughout the growth season. In Week 32 the contents of N, P, and K were reduced by 30, 27, and 28 %, respectively, compared to the first sampling in Week 10. The contents of copper and zinc in the leaves also tended to diminish throughout the season, decreasing gradually until Week 26 to the level at which they remained for the rest of the experimental period. Sodium, iron, manganese, boron and aluminium on the whole, followed the copper and zinc pattern until Week 26, after which they increased considerably. No significant interactions were found between time of sampling and the irrigation method with respect to the nutrient contents of the leaves. The contents of some nutrients in the leaves were clearly influenced by the contents of others (Table 5). The contents of certain nutrients and the pH of the growth medium in several cases apparently influenced the plants' uptake of various nutrients (Table 6).

In addition to the above correlations, the analysis showed an apparent relation between the iron content in the growth medium and that of the leaves.

Leaching and nutrient contents in the drainage liquid

With the exception of irrigation method 4, where recirculation was applied, there

Table 4. The content of different nutrients in leaves during the season. Means of three varieties and five irrigation treatments

Week No	per cent of dry matter							ppm of dry matter						
	Tot. N	P	K	Ca	Mg	S	Na	Fe	Cu	Mn	Zn	B	Mo	Al
10	4.68	0.76	5.43	1.62	0.41	0.73	0.05	143	30	113	31	41	4	90
14	4.62	0.78	5.80	1.74	0.40	0.98	0.05	134	27	135	32	40	4	67
18	4.60	0.72	5.62	1.63	0.38	0.82	0.05	151	22	129	30	31	4	45
22	3.52	0.60	3.68	1,72	0.33	0.66	0.05	77	14	110	22	35	4	53
26	3.33	0.58	3.47	1.38	0.30	0.72	0.04	76	13	115	18	31	3	39
32	3.25	0.55	3.93	1.92	0.30	0.89	0.07	103	15	159	20	47	5	66
Signif.	***	***	***	ns	ns	ns	**	***	***	ns	***	***	ns	**

Table 5. Significant coefficients of correlation ($P \leq 0.01$) between the content of some nutrients in the leaves

	P	K	Mg	Fe	Cu	Zn
Tot. N	0.84	0.92	0.57	0.79	0.89	0.80
P	-	0.82	0.70	0.56	0.81	0.78
K	-	-	0.66	0.70	0.81	0.86

Table 6. Correlation coefficients between nutrients in the leaves and nutrient content and pH in the growth medium

Leaf	Growth medium pH	NO^3-N	P	K
Tot. N	-0.42*	0.56**	0.55**	0.47*
P	-0.49**	0.39*	0.42*	0.42*
K	-0.42*	0.43*	0.40*	0.33 ns
Fe	-0.51**	0.61**	0.62**	0.52**
Cu	-0.50**	0.42*	0.57**	0.56**

was a significant difference ($P \leq 0.01$) in leaching between the various irrigation methods. Average leaching for irrigation method 1 was 12.7 litres per m^2 per week. For irrigation methods 2,3, and 5, the respective leaching was 17.2, 18.2, and 17.7 litres per m^2. Total leaching for the entire growth season amounted to 42, 57, 61, and 44% of applied nutrient solution, respectively, for those four irrigation methods.

With irrigation method 4, where the nutrient solution was renewed every fourth week, leaching per week was 1.7 litres per m^2. The leaching clearly varied throughout the season ($P \leq 0.001$), with a distinct maximum at midsummer (Fig. 1).

Only a slight difference appeared between the nutrient content of the growth medium and that of the drainage liquid. Average salinity in the leachate was 0.1 units lower than the salinity of the growth medium ($P \leq 0.05$); average pH was 0.4 units lower ($P \leq 0.001$). The contents of iron, manganese and molybdenum were also somewhat lower in the

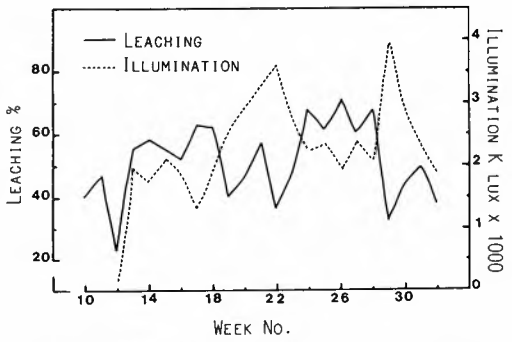

Figure 1. The variation in illumination and leaching during the growing season

leaching than in the mats ($P \leq 0.05$), though the aluminium content was higher ($P \leq 0.001$). No significant difference was obtained with the other nutrients.

The nutrient contents in the leaching varied distinctly with the irrigation method (Table 7). Nutrient leaching through the drainage liquid clearly increased with irrigation frequency. By increasing the number of irrigations (method 1 to method 3), weekly loss of nitrate increased by 13%, while the leaching of phosphorus increased in excess of 20%. With the exception of manganese and aluminium, there was an increased leaching of all other nutrients. Additional irrigation at night did not lead to increased nutrient leaching on a per cent basis connection with irrigation method 1.

Recirculation led to minimal nutrient leaching. The nitrate loss amounted to only 14%, and in the case of phosphorus 11%, of the leaching after irrigation method 1.

Leaching over the entire range of free drainage irrigation methods showed a marked relationship to several climatic factors, the average radiation measured in K.lux ($r = -0.23^*$), the humidity saturation deficit ($r = -0.41^*$) and the temperature in the growth medium ($r = 0.40^*$).

Table 7. Weekly leaching for the different types of irrigation (mg per m^2)

Irrigation treatments	Nutrient elements													
	NO_3-N	P	K	Ca	Mg	SO_4-S	Na	Fe	Cu	Mn	Zn	B	Mo	Al
1	4676	796	5022	5149	1157	2096	209	24.4	12.1	8.4	7.1	6.5	0.56	6.1
2	4941	891	6190	5606	1273	2344	233	24.6	13.0	10.1	7.3	7.4	0.64	7.3
3	5345	1006	6902	6178	1408	2597	253	31.0	14.7	11.2	9.0	8.2	0.69	8.5
4	768	85	581	971	216	377	45	3.4	3.0	0.9	1.7	1.1	0.08	0.8
5	5355	982	6389	5878	1328	2374	238	29.9	13.4	11.0	8.2	7.6	0.55	8.5
Signif.	***	*	*	***	***	***	***	**	***	ns	***	***	***	ns

1) Irrigation No. 4 is not included in the analysis of variance

DISCUSSION

For technical reasons the irrigation in this experiment was carried out with a given amount of water per plant each day; maximum 2 litres during the summer season. This irrigation regime may easily lead to excess irrigation during dull periods, while the opposite will be the case in periods with strong radiation. Following the same irrigation regime throughout the growth season (from August 16 to October 10) as from March 15 until May 15, the total irrigation amount on a yearly basis would have been 890 litres per m^2. The actual total amount given in the experiment was somewhat lower than what is considered normal.

The average EC level in the growth medium, in excess of 3 mS cm^{-1} throughout the season, also indicates that the experimental plants might have been given more water (Bjelland 1984). It is not evident whether the high salinity level in the nutrient solutions after irrigation methods 1, 2, 3, and 5 may have influenced the yield level in general, by reducing the fruit weight. There are, on the other hand, ample indications that the reduced fruit weight found after recirculation may have been due to a high salinity (Burg et al. 1986). High electrical conductivity reduces the water uptake and thereby contributes to increased leaching seen in relation to a fixed application of nutrient solution. The nutrient contents in the leaves seem, with the exception of calcium and sulphur, to fall clearly within the range of recommended values (Roorda van Eysinga & Smilde 1981). The low calcium content together with the somewhat high salinity may explain the occasional occurrence of fruits infested with blossom-end rot (Adams & Ho 1985). Reduced contents of a number of nutrients in the leaves throughout the season have also been stated in previous experiments (Bradley & Fleming 1960, MacLean et al. 1968).

In the pH range where this experiment was carried out (most recordings in the 5.3 - 6.2 area), an increased pH appears to lower the contents of certain nutrients in the leaves. There seems to be no relationship between this trend, however, and the high contents of the same nutrients during the early part of the experimental season.

In this experiment leaching varied between 42 and 61% of the applied water, depending on irrigation frequency. After nocturnal irrigation 49% of applied water was recorded as leaching. This supports previous observations that the plants also produce considerable transpiration during the night (Graaf 1983, 1984, Christensen 1986). No positive influence of nocturnal irrigation was noted in terms of increased yield or improved fruit quality.

Excluding irrigation method 4, where recirculation was applied, phosphorus leaching varied from 796 to 1006 mg per

m^2 and week, corresponding to an amount of 27 to 34 kg P per daa over the entire growth season. The NO_3-N content in the leachings also varied between the various irrigation methods, from 4676 to 5355 mg per m2 and week, corresponding to a yearly discharge of 159 to 182 kg per daa. No significant difference was found in the leaf content of phosphorus and total -N in this experiment, which was apparently due to an equal uptake with all irrigation methods. The higher salinity in the slabs after irrigation method 1 may suggest slightly drier periods between irrigations under this method than under the others. A possible explanation of the lower phosphorus and nitrogen in the leaching after irrigation method 1 may be accumulation because of slight periodic drying of the slabs. Information from the literature suggests that uptake of phosphorus by tomato plants during a season varies considerably, from 3.5 kg (White 1964) to 10.5 (Lucas et al. 1960) per daa. With regard to the relatively high yield levels in ordinary tomato production, one would presume the total phosphorus uptake in the plants to be somewhat higher, probably around 12 - 15 kg per daa annually (Adams & Winsor 1979). Information on the N-uptake varies too (Kidson et al. 1953, White 1964, Maher 1976). The plants' N-uptake is strongly influenced by the light availability, increasing with irradiance (Adams 1986). The uptake may thus vary from one day to the next, but also from one season to the next. A normal uptake, however, seems to fall somewhere close to 60 - 70 kg per daa (Adams 1986).

On enquiry a number of tomato growers in Rogaland specified yearly fertilizer applications corresponding to 40 - 70 kg P and 190 - 280 kg N per daa. These are surprisingly high figures which indicate a leaching in excess of 60% .

There are several ways of reducing glasshouse leaching. In the first instance, better planned irrigation practices would undoubtedly reduce the leaching considerably. The amount of nutrient solution applied at any given time should as far as possible correspond to the uptake of the plants. Using other cultivation systems, such as recirculation and exchanging the nutrient solution at regular intervals, leaching can be reduced to a minimum, as demonstrated by the present results. This cultivation system does, however, raise a number of problems concerning diseases, the nutrient balance, and the accumulation of various chemical compounds. These problems therefore require further investigation before recirculation can be recommended on a large scale.

The accumulation of leaching for recirculation in the open field is an alternative which demands further scrutiny. In this connection a reconsideration of the content of phosphorus and nitrogen in the fertilizers would seem a natural consequence.

SUMMARY

The effect of different irrigation methods on leaching in tomato plants grown on rockwool was investigated. Distinct differences were recorded between the various irrigation methods despite considerably varied leaching throughout the season, culminating at midsummer. Minimum leaching was obtained after recirculation, averaging 1.7 litres per m^2 per week. The leaching was due to the renewal of the nutrient solution every fourth week. Traditional drip irrigation increased the leaching with increasing irrigation frequency. A relative increase of the frequency from 9 to 15 applications per day increased the leaching from 12.7 to 18.2 litres per m2 weekly, corresponding to 42 and 61% of applied liquid, respectively.

The nutrient contents in the growth medium varied with the irrigation methods, reaching a clear maximum after recirculation. The leaf nutrient contents were identical, however, regardless of ir-

rigation method. Different irrigation methods showed no significant effect on the yield amount, but influenced both quality and fruit size.

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THE EFFECT OF SILAGE TO CONCENTRATE RATIO ON THE NYLON BAG DEGRADATION OF FEEDSTUFFS IN THE RUMEN OF DAIRY COWS

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Bøe, U.B. The effect of silage to concentrate ratio on the nylon bag degradation of feedstuffs in the rumen of dairy cows. Norwegian Journal of Agricultural Sciences 3: 241-249. ISSN 0801-5341

1. The nylon bag technique was used to examine the rumen degradability of organic matter in different feedstuffs.
2. Increasing amounts of concentrates in the diet had a marked negative effect on rate and extent of rumen degradation of all the roughage samples tested. The depression in organic matter degradation varied between test samples: hay < NaOH-treated straw < grass silage < untreated straw < NH_3-treated straw.
3. Barley was degraded to the same extent regardless of diet composition.

Key words: concentrate supplementation, nylon bags, rumen degradability, rumen pH, rumen VFA.

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Roughage diets supplemented with concentrates containing readily fermentable carbohydrates depress rumen cellulolysis and dry matter digestion (Hamilton 1942, El-Shazly et al. 1961, Henning et al. 1980). The depression of rumen cellulolytic degradation is composite in nature; it is due partly to the decreased rumen pH and partly to the adaptations in the rumen microflora (Mould et al. 1984). The extent of this depression depends on a number of factors, including the level of feeding (Ørskov 1984), the nitrogen content of the diet (Andrews et al. 1972), and the nature and processing of the carbohydrate supplement (Ørskov & Frazer 1975, Ørskov et al. 1978). The quality of the roughage itself is possibly conducive to the effect of readily fermentable carbohydrates on the roughage digestibility attained. Some workers have found that the rumen degradability of roughages with low digestibility is depressed most when supplemented with readily fermentable carbohydrates (Mould et al. 1984, Dixon 1985). Others have found the greatest suppressive effect to be on the high quality roughages (Vadiveloo & Holmes 1979).

This study was carried out to investigate the effect of varying proportions of concentrate on the degradability of organic matter from different roughage sources in the rumen of dairy cows. Rumen fluid parameters were observed as well.

MATERIALS AND METHODS

Animals, diets and feeding procedures
Two dairy cows fitted with permanent rumen cannulas (inner diameter 120 mm) were used in the experiment. Four diets with different proportions of pelleted concentrates (Kufôr A, a commercial feed to dairy cows, with 12,5% digestible crude protein) to grass silage were offered. The proportions of grass silage to concentrates were 100:0, 80:20, 60:40 and 40:60 on a dry matter (DM) basis.

The cows were fed twice daily (at 0600 and 1500 hours) in two equal portions. Table 1 shows the daily amounts offered from each diet.

Experimental design and procedures
The all-grass silage diet (100:0) was given to the cows just before calving. The diet containing 60% concentrates (40:60) was given around the peak yield of lactation, and the 60:40 and 80:20 diets successively thereafter.

The cows were fed in accordance with the standard feeding covering maintenance and production requirements (Ekern & Vik-Mo 1983). The diet was given for three weeks before the nylon bags were incubated in the rumen. Samples of rumen fluid were taken on the final day of each incubation period.

Ruminal degradation of feed samples
The feed samples used for nylon bag incubations were grass silage, hay, untreated barley straw, barley straw treated with ammonia (Sundstøl et al. 1978), barley straw "dip-treated" with NaOH (Sundstøl 1981), and ground barley. The chemical compositions are shown in Table 2.

The grass silage preserved with formic acid was taken from the same batch as fed to the cows. All samples were dried at 70°C and ground in a laboratory hammer mill through a 1 mm screen. One gram of the test samples was weighed into nylon bags with a pore size of 36

Table 1. Daily intake of dry matter (DM) and composition of diets offered

Diet silage:consentrate	DM intake, (kg)		$g \cdot kg^{-1}$ DM	
	Silage	Concentrates	Crude protein	Crude fibre
100:0	7.9	0	159	364
80:20	11.2	2.8	158	312
60:40	9.5	6.3	157	260
40:60	6.9	10.2	154	208

Table 2. Chemical composition of the different oven-dried feedstuffs incubated in the rumen

	Dry matter $g \cdot kg^{-1}$	$g \cdot kg^{-1}$ DM				
		Organic matter	Crude fibre	Crude protein	Ether extr.	N-free extr.
Grass silage	916	929	364	159	52	354
Barley, ground	905	976	52	114	18	792
Hay	933	912	320	122	25	445
Untreated straw	948	930	412	45	22	451
NH_3-treated straw	944	931	438	86	18	389
NaOH-treated straw	912	857	413	113	14	317

µm. In relation to bag size, the sample incubated equalled 10 mg per cm^2 bag surface area. Handling of the bags and arrangements for incubation were as described by Vik-Mo & Lindberg (1985).

Duplicated bags for each sample in each cow were removed after 6, 12, 24 and 48 h for determination of organic matter (OM) residues, expressed as a percentage of incubated feed sample. The disappearance rate of the beginning of incubation was found according to the first order kinetic equation (Nocek & English 1986):

$\ln Y_o = \ln Y - kt$

where Y = percent residuals for OM (t>0)
t = time (h)
k = disappearance rate coefficient (slope)
Y_o = residual OM at t=0, found from bags with test samples washed using the usual procedure (Y intercept)

Rumen fluid samples

Rumen fluid (100 ml) was obtained through the cannula by suction through a rigid tube which secured sampling from the ventral rumen. Samples were taken 14 times between 0530 hours (before the first feeding) and 2300 hours.

The pH was determined immediately after the sample was obtained, using a portable pH-meter. Fluid was strained through a double layer of cheese cloth, and 10 ml was added to 0.5 ml formic acid. The samples were stored at 5°C until analysis of volatile fatty acids (VFA), lactic acid, and ammonia-N.

Chemical analysis

Chemical composition of the feed samples was determined according to the Weende procedure (A.O.A.C. 1977). The incubated replicantes from each cow were pooled for analysis of dry matter (DM) and ash content.

Ammonia in the stored rumen fluid was determined by the automated colorimetric indophenol reaction (Logsdon 1960).

Rumen fluid samples were analysed for volatile fatty acids (VFA) using a PYE - Unicam GGD gas chromatograph with a hydrogen flame ionization detector. The VFA was separated on a glass column (186x0.40 cm) packed with Chromosorb 101, 60-80 mesh (Johns-Manville, USA). The column temperature was 175°C.

L-lactic acid was determined spectrophotometrically using lactate dehydrogenase (Sigma diagnostics, Procedure No 826-UV).

Statistical analysis

The data obtained from the nylon bags were analysed using the general linear model (GLM) procedure (SAS Institute Inc. 1985). No significant differences were found between animals, and diffe-

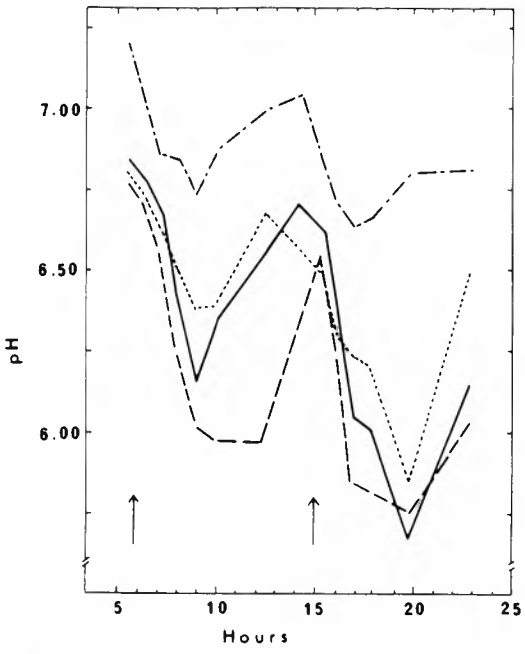

Figure 1. Circadian changes in rumen pH. Average of two cows fed grass silage and concentrates in different proportions.
—— diet 100:0
- - - - - - - - " 80:20
———— " 60:40
—— —— —— " 40:60
↑ = feeding time

rences between diets were therefore analysed for each feed on the pooled data.

RESULTS

Rumen fermentation

The diurnal fluctuations in rumen pH became greater as the level of concentrate supplementation increased (Figure 1).

The characteristics of rumen pH are given in Table 3. Both the length of time pH was below 6.0 and the summated pH-depression increased with increasing levels of concentrate supplementation.

Rumen ammonia (NH_3) concentration varied greatly throughout the day on all diets, with peaks 2-3 h after feeding (Figure 2). The average value of NH_3-concentration was lowest for the all-roughage diet, with only small differences between the other diets (Table 4).

The influence of the different diets on the concentration of total and relative proportions of rumen VFA are presented in Table 4. The concentration of total VFA was higher for the diets containing concentrates than for the all-roughage diet, but there was no relationship be-

Table 3. Some characteristics of the variation in rumen pH during the day in two cows fed different amounts of grass silage and concentrates

	Diet: silage:concentrates				
	100:0	80:20	60:40	40:60	SEM
Average pH	6.85[a]	6.44[b]	6.39[bc]	6.21[c]	0.08
Lowest pH	6.57	5.80	5.65	5.55	
pH < 6 (h)	0	2	4	10	
Summated pH-depression[1)]	0	0.15	0.70	0.89	

a-c Means in the row with the same superscript are not significantly different ($p > 0.05$).

1) Summated pH-depression was calculated as the area on the pH curve (Figure 1) where pH was below 6. The change in pH was measured in pH-units and time in hours (Istasse et al. 1986).

Table 4. Rumen fluid volatile fatty acids (VFA) and NH_3-N. Mean values of 14 samples withdrawn throughout the day from cows fed grass silage and concentrates in different proportions

	Diet silage:contentrates				
	100:0	80:20	60:40	40:60	SEM
NH_3-N, mmol/l	9.9[a]	14.9[b]	14.3[b]	15.5[b]	1.5
Total VFA, mmol/l	82.3[a]	117.9[b]	106.3[c]	108.9[bc]	1.9
Molar proportions:					
Acetate (C2)	71.6[a]	65.6[b]	66.6[b]	66.8[b]	0.5
Propionate (C3)	17.1[a]	18.2[b]	18.4[b]	19.1[c]	0.3
Butyrate (C4)	6.9[a]	13.0[b]	11.5[b]	10.9[b]	0.2
iso-Butyrate (IC4)	1.0	0.8	0.9	0.9	0.03
Valerate (C5)	1.4	1.1	1.2	1.1	0.05
iso-Valerate (IC5)	1.9[a]	1.4[b]	1.3[b]	1.2[b]	0.1
C2 + C4:C3	4.6[a]	4.4[b]	4.3[bc]	4.1[c]	0.1

a-c: Means in each row with the same superscript are not significantly different ($p > 0.05$).

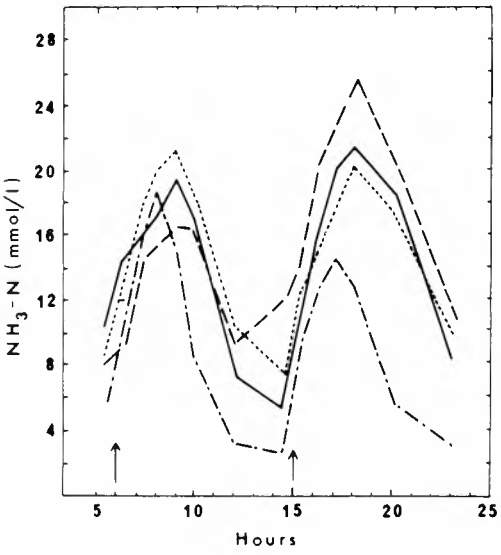

Figure 2. Circadian changes in rumen NH_3.N (mmol/l. Average of two cows fed grass silage and concentrates in different proportions.
— — — — — diet 100:0
- - - - - - - - " 80:20
———————— " 60:40
— — — — — — " 40:60
↑ = feeding time

tween VFA-concentration and the level of concentrates added.

A significant negative correlation was observed between pH and VFA concentrations ($r^2 = 0.67$) for all diets ($p < 0.05$). The change in the relative proportion of VFA between diets was small. No differences could be seen for acetic acid, but the proportion of propionic acid increased ($p < 0.05$) and butyric acid tended to decrease with increased supplementation of concentrates. Mean pH did not significantly correlate with total concentration of VFA, but a high correlation was found between pH and VFA-ratio (mol % ratio of $C_2 + C_4/C_3$) ($r^2 = 0.96$, $p < 0.05$).

The concentration of L-lactic acid in the rumen was almost negligible throughout the day, with slightly higher concentrations 2-3 h after feeding (up to 0.7 mM) for the diets containing concentrates.

Disappearance of organic matter from nylon bags

Organic matter disappearance (OMD) of the incubated feeds is shown in Table 5. For ground barley, the OMD decreased with increasing levels of concentrates after 6 h (OMD6) and 12 h (OMD12) incubation ($p < 0.05$), but after 24 h (OMD24) and 48 h (OMD48) incubation there was no effect of the diet.

For the roughage samples, the decline in OMD between the all-roughage diet and the diet containing 60% concentrates was highly significant for all incubation times ($p<0.001$). The depression was greatest after 6 h and 12 h incubation. At these incubation times, inclusion of 20% concentrates also depressed the degradability significantly ($p<0.01$, means of all-roughage samples). When incubated for 24 h and 48 h, the OMD tended to decline with increasing amounts of concentrates, but the depression did not become significant until 60% concentrates were included in the diet. There was one exception, however. The OMD48 for NH_3-treated straw was significantly depressed ($p<0.001$) with only 20% concentrate inclusion, and the degradation was reduced to a level below the degradation of untreated straw.

The depression in OMD48 varied among test samples. With 60% concentrates, the depressions between this diet and the all-silage diet were 5.5% for hay, 6.1% for NaOH-treated straw, 7.8% for grass silage, 9.3% for untreated straw and 24.1% for NH_3-treated straw.

The initial rate of degradation of the test samples is given as organic matter disappearance rate ($k \cdot h^{-1}$) in Table 6. The OMD rate declined for all samples when concentrates were added to the diet ($p < 0.01$).

Barley OM disappeared at a much faster rate than the roughage samples. Grass silage had the highest disappearance rate of the roughage test samples,

Table 5. Disappearance of organic matter (OMD, g·100 g^{-1}) of different feeds from nylon bags in the rumen of cows fed grass silage and concentrates in different proportions

Feed sample	Diet silage:concentrate	Incubation time (h) 6	12	24	48
	100:0	43.6[a]	56.4[a]	66.7[a]	74.7[a]
	80:20	33.9[b]	51.1[ab]	60.4[a]	71.9[ab]
Grass silage	60:40	35.1[a]	53.1[ab]	59.2[a]	72.3[a]
	40:60	31.3[b]	48.0[b]	60.5[a]	68.9[b]
	100:0	36.7[a]	53.8[a]	68.6[a]	77.8[a]
	80:20	28.6[b]	44.4[b]	61.4[a]	75.2[ab]
Hay	60:40	27.2[bc]	46.5[b]	60.5[a]	75.3[ab]
	40:60	23.6[c]	41.7[c]	62.2[a]	73.5[b]
	100:0	19.1[a]	29.1[a]	44.4[a]	55.9[a]
Untreated	80:20	15.9[a]	28.2[a]	41.2[a]	55.4[ab]
straw	60:40	17.4[a]	26.0[a]	37.3[b]	53.7[ab]
	40:60	9.9[b]	21.8[b]	39.7[c]	50.7[c]
	100:0	21.5[a]	32.8[a]	49.3[a]	63.2[a]
Ammonia	80:20	16.3[a]	25.9[b]	39.0[b]	51.9[b]
treated	60:40	15.8[ab]	26.3[b]	44.2[ab]	51.4[b]
straw	40:60	12.1[b]	22.0[b]	32.5[b]	48.0[c]
	100:0	43.2[a]	61.2[a]	76.3[a]	88.3[a]
NaOH-	80:20	39.0[ab]	55.5[ab]	70.9[a]	84.4[ab]
treated	60:40	40.1[ab]	55.0[b]	67.7[ab]	85.3[b]
straw	40:60	35.0[b]	50.6[c]	69.7[a]	82.9[b]
	100:0	87.6[a]	88.7[a]	90.6[a]	93.3[a]
Barley	80:20	80.3[ab]	87.6[a]	89.9[a]	91.6[a]
	60:40	79.9[ab]	87.8[a]	89.4[a]	92.1[a]
	40:60	70.6[b]	82.8[b]	89.3[a]	91.2[a]
SEM		0.7	0.6	1.1	0.9
C.V.		8.0	5.4	5.8	3.7

a-c: Means in each column with the same superscript are not significantly different ($p > 0.05$).

Table 6. Organic matter disappearance rate ($k \cdot h^{-1}$) of the test samples incubated in nylon bags in the rumen of cows fed grass silage and concentrates in different proportions

Feed sample	Diet silage:concentrates 100:0	80:20	60:40	40:60
Grass silage	0.077	0.050	0.053	0.042
Hay	0.047	0.032	0.033	0.027
Untreated straw	0.020	0.013	0.013	0.012
Ammonia treated straw	0.027	0.017	0.020	0.013
NaOH-treated straw	0.052	0.042	0.042	0.033
Barley	0.295	0.218	0,215	0.152

followed by NaOH-treated straw, hay, NH_3-treated straw and untreated straw.

The coefficients of correlation between rate and extent of OMD for roughage feed samples and the different rumen pH characteristics were calculated.

OMD was not correlated to the duration of the depression of pH below 6 and summated pH-depression. A better correlation was found with the lowest pH-value during the day ($p<0.05$) and with the average pH ($p<0.01$).

DISCUSSION

Many workers have found a high correlation between dry matter disappearance after 48 h incubation in nylon bags in the rumen (DMD48) and in vivo OM digestibility of the feed (Sauvant et al. 1985). The DMD48 value has therefore been used for predicting the OM digestibility or energy value of the feed.

For a similar level of DMD48 or OMD48, however, the disappearance rate at the beginning of the incubation may be very different from one feed to the other (Sauvant et al. 1985). These two parameters have therefore been used to describe the kinetics of OMD in nylon bags.

The results of the present study clearly show that both the initial rate and extent of OMD in roughage samples depend on the basal diet fed, and they decrease with increasing levels of concentrates in the diet.

As was also found by Lindberg (1981), rumen degradability of roughage in nylon bags was more susceptible to changes in the diet than the concentrate tested (in this experiment barley). Although there were differences between barley degradation rates at the beginning of incubation for the different diets, there were no differences in the extent of degradation after 24 or 48 h incubation. The overall effect is that ground barley is degraded to the same extent regardless of the diet composition.

NH_3-treated straw was most affected by concentrate supplementation of the roughages tested. The DMD48 of NH_3-treated straw was reduced to a level below the degradation of untreated straw even with 20% concentrate in the diet. Fahmy & Sundstøl (1985) compared the rumen degradability of NH_3-treated, NaOH-treated and untreated straw. They also found that increasing proportions of concentrate in the diet reduced the degradability of NH_3-treated straw to the greatest extent, although the degradability was higher than for untreated straw.

Disregarding the chemically treated straw, the degradation of the other roughage samples is in accordance with results from Mould et al. (1984) and Dixon (1985), who found that roughages with low digestibility will be depressed to the greatest extent when supplemented with concentrates. It seems that some of the positive effects on ruminal degradation of chemical treatment of straw are lost when the diet contains readily fermentable carbohydrates. Treatment of straw with NH_3 is most vulnerable in such a situation.

Ørskov (1984) pointed out that the level of feeding will to a large extent determine the amount of easily digestible supplement that can be tolerated before cellulolysis is inhibited. Therefore, in testing the effect of diet on degradability it is important that the animals are fed at the same level as would be applied in practise. In this experiment, the cows were fed the different diets at levels which were likely to follow the lactation cycle. Therefore the increased level of feeding, along with supplementation of concentrates, can explain the considerable rise in total VFA concentration and the fall in pH from the all-roughage diet to the 20% concentrate diet. Murray et al. (1978) found that production of VFA increased linearly with intake.

The importance of an adequate N supply in the rumen for fibre digestion has long been recognized (Sutton 1986).

A decrease in fibre digestion is often found in diets low in nitrogen, and may be due to a decline in the number of cellulolytic bacteria in the rumen, resulting from their inability to compete with faster growing amylolytic bacteria (El-Shazly et al. 1961). The concentration of NH_3 found by various authors to be limited for the microbial cellulolytic activity in the rumen, ranges from less than 3.6 mM (Satter & Slyter 1974) to as high as 17 mM (Mehrez et al. 1977). Most values, however, are below 9 mM (Mercer & Annison 1976). Since the average concentration of NH_3 in the rumen of the cows in this experiment was between 10 and 15 mM, it is unlikely that NH_3-concentration would be limiting for microbial activity.

Branch-chained VFA, such as isobutyric and isovaleric acids, along with valeric acid, are also required for growth of cellulolytic bacteria, but in very small amounts (Hoover 1986). Dehority et al. (1967) found that the amount of isobutyric acid required for optimum growth of cellulolytic bacteria is less than 0.3 mM. Concentrations above 0.8 mM for the branch-chained VFA in all diets should not be limiting for fibre digestion in this study.

The length of time and the extent to which pH falls below 6 is important in relation to fibre digestion, because the cellulolytic acticity in the rumen is strongly inhibited at this pH level (Stewart 1977, Mould et al. 1984). According to Henning et al. (1980), the 'pH-hours below 6.0' is a convenient method of evaluating the data from this point of view. Istasse et al. (1986) found that the average pH did not reflect the conditions for fermentation, and hence the DM disappearance of roughage. They found the strongest correlation with the summated pH depression. The reason why we could not find this association in the present study is probably that extreme pH-values were not observed over extended periods.

It can be concluded that inclusion of starchy concentrates in the basal diet has a negative effect of roughage degradation in the rumen. This effect is not a result of nitrogen limitations, lack of branch-chained VFA, or extreme pH-values alone. There must be other factors in addition to, or along with, pH and nutritional restrictions which influence the activity of cellulolytic microbes when starch is present in the rumen. Further investigations are needed to examine whether the effects observed in the nylon bags are reflected in the digestibility and net energy content of the roughage for feed evaluation purpose.

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UTILIZATION OF GRASS SILAGE BY SHEEP: EFFECTS OF SUPPLEMENTATION WITH DIFFERENT AMOUNTS OF GROUND BARLEY

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Bøe, U.B. 1989. Utilization of grass silage by sheep: Effects of supplementation with different amounts of ground barley. Norwegian Journal of Agricultural Sciences 3: 251-263. ISSN 0801-5341.

Inclusion of barley in a grass silage diet depressed rumen degradation and dry matter digestibility of the silage. Up to 35% barley could be added to the silage before fibre digestibility of the diet was affected to any great extent. When the animal is fed above maintenance level, this depression in fibre digestibility may reduce the net energy value of the diet.

Key words: barley supplementation, fibre digestibility, grass silage, energy utilization, feeding level, rumen degradation, rumen pH, rumen VFA.

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Associative effects of individual feeds in mixed rations were first described by Forbes et al. (1931), who reported that the net energy of a food was not constant but varied with the ration to which it was added.

Today, there is still controversy regarding the associative effects of individual feeds in mixed rations. Vance et al. (1972) found that net energy values for maintenance of a feed remain constant with varying proportions of concentrate in the ration, but that net energy value for weight gain varies depending on the diet composition. Byers et al. (1976) found negative associative effects on both net energy values for maintenance and gain. Lofgreen & Garrett (1968) and Garrett (1979) could not find any associative effects on the net energy content of mixed feed, and stated that a system of feed evaluation based on assigning a specific net energy value for each feedstuff should be appropriate.

However, many workers have reported that supplementation of roughage with concentrates with a high starch content reduces the extent and rate of fibre digestion (Hamilton 1942, El-Shazly et al. 1961, Henning et al. 1980, Mould et al. 1984). The extent of the depression varies with both the type of forage and the concentrate (Sutton 1986); perhaps the controversy in the literature can be explained by this fact.

In Norway, more than 70% of the harvested grass in conserved as direct cut silage (Sundstøl & Ekern 1980). It is therefore of special interest to study how barley supplementation affects the utilization of grass silage. Bøe (1989) observed that the extent and rate of degradation of grass silage in the rumen dairy cows decreased when concentrates were added to the diet.

The objective of this study was to examine the influence of barley on rumen degradation, digestibility and energy utilization of grass silage fed to sheep at two levels of intake.

MATERIALS AND METHODS

Animals, diets and experimental design

Four mature Dala x Texel rams fitted with permanent rumen cannulas (inner diameter 40 mm) were used. The rams were 2 or 3 years old and weighed 58-80 kg at the beginning of the experiment. They were kept in metabolism cages during the experimental periods, and were fed four different diets of grass silage and ground barley in the proportions (silage:barley): 100:0, 85:15, 65:35 and 45:55 on dry matter (DM) basis. Chemical composition of the dietary ingredients and composition of the different diets offered are shown in Tables 1 and 2.

Silage was made from regrowth grass harvested in the late autumn. Formic acid was added at the time of harvest at a rate of 3 l per tonne of fresh grass.

The silage needed for the whole experimental period was handmixed with a fork, weighed into daily rations, and stored at -20°C until 2 days before use. A single batch of ground barley was used.

The animals were fed twice daily (at 0800 and 1500 hours) in two equal portions. Supplements of 10 g salt and 10 g of a mineral mixture were given daily, and water intake was freely available.

The experiment was carried out according to a double 4 x 4 latin square design at two levels of intake. The four diets consisting of the low level of feeding were intended to meet the animals' metabolizable energy requirement for maintenance. At the higher level of feeding, the silage diet was given close to ad libitum to achieve positive energy balances.

Each experimental period lasted 21 days. After a 10-day adjustment period, faeces and urine were collected for 10 days. The animals were kept in respiration chambers for the last 7 days of the collection period. In the final day of each period, samples of rumen fluid were taken. Nylon bags were placed in the rumen of the sheep before the collection period started.

Table 1. Chemical composition of direct cut grass silage and barley (means of eight samples of silage and four of barley)

	Grass silage	Barley
pH	4.6	-
DM ($g.kg^{-1}$)	203	875
Components of DM (kg):		
Gross energy (MJ)	18.2	18.6
Organic matter (g)	919	973
Crude protein (g)*	144	114
Ammonia-N ($g.kg^{-1}$-total N)	94	-
Ether extract (g)	36	18
Crude fibre (g)	376	70
ADF (g)	459	64
N-free extracts (g)	363	770
Lactic acid (g)	32	-
Formid acid (g)	9	-
Proponic acid (g)	7	-
Butyric acid (g)	1.9	-
Acetic acid (g)	48	-

* N·6.25

Rumen degradation of feeds

The nylon bag technique was used to estimate degradation of dry matter and acid detergent fibre (ADF) in the rumen. Grass silage for the nylon bags was dried at 70°C and ground in a laboratory hammer mill through a 1 mm screen.

Two grams of the dried silage was weighed into nylon bags and incubated as described by Bøe (1989). Duplicated bags were removed after 6, 12, 24, 48 and 72 h.

The fitted values for DM degradation were calculated using the equation (Ørskov & McDonald 1979).

Table 2. Daily intake of DM and the composition of diets offered

Diet silage:barley	DM intake (g) Low level	DM intake (g) High level	g·k⁻¹DM CP	g·k⁻¹DM CF	g·k⁻¹DM ADF	g·k⁻¹DM NFE
100:0	1049	1718	144	376	459	363
85:15	921	1435	140	330	400	433
65:35	834	1221	134	268	321	509
45:55	714	1151	128	214	242	582

$$p = a + b(1 - e^{-ct}),$$

where

p = the actual degradation after time t.
a = the intercept of the degradation curve at time zero (the very rapidly disappearing fraction).
b = the potential degradability of the component which will, in time, be degraded.
c = degradation rate of the b fraction.

Nylon bags with barley were removed after 48 h incubation.

Rumen fluid samples

Rumen fluid samples were withdrawn after 0, 1, 2, 3, 4 and 6 h after feeding and handled as described by Bøe (1989).

Calorimetric procedures

Two open-circuit respiration chambers used for the determination of heat and methane production were described by Sundstøl et al. (1974). The animals were placed in the chambers for a 2-day adjustment period before gaseous exchange was measured at 18°C for two 24-h periods. They were then moved to the other chamber, and after a 1-day adjustment period in this chamber, gas exchange was measured for two new 24-h periods.

Photocells were placed in the chambers to record activity. Heat production (HP) was calculated from the gaseous exchange and urinary nitrogen loss according to the factors of Brouwer (1965).

Fasting heat production (FHP) and ME required for energy equilibrium (EE) was calculated using the regression method (Lofgreen & Garrett 1968, ARC 1980), relating log HP to ME intake, where HP and ME are in kJ per metabolic body weight ($W^{0.75}$). Partial net energy for maintenance (NE_m) and fattening (NE_f) was determined after Vance et al. 1972.

Efficiency of ME utilization for maintenance (k_m) and fattening (k_f) was estimated by:

$$k_m = \frac{\text{Net energy for maintenance (FHP)}}{\text{ME required for EE}}$$

$$k_f = \frac{\text{Energy retention}}{\text{ME intake - ME required for EE}}$$

Chemical analysis

Samples of feed, urine and fresh excreta were analysed for nitrogen by the Kjeldahl method, and energy content was determined by means of an adiabatic bomb calorimeter, urine first being freeze-dried and feed and faeces oven-dried.

The chemical composition of feed and feaces was determined according to the Weende procedure (A.O.A.C. 1977), and the acid detergent fibre fraction (ADF) to Goering & Van Soest (1970). Rumen fluid samples were analysed as described by Bøe (1989). The composition of the air going in and out of the respiration chambers was analysed volumetrically with a Haldane apparatus for O_2 and CO_2 contents, and CH_4 with an infrared analyser (Hartmann & Braun).

Statistical analysis

Statistical analysis was performed using the general linear model (GLM) procedure (SAS Institute Inc. 1985). Means are presented with their standard errors

(SEM). The significance for difference between diets or between feeding levels is indicated by: *** $p<0.001$; ** = $p<0.01$; * = $p<0.05$; NS = not significant.

RESULTS

Rumen fluid measurements

A depression in rumen pH was observed as the proportion of barley in the diet was increased (Figure 1). The highest level of feeding gave the lowest pH values, the differences between the two feeding levels being greatest for the diet containing 55% barley. The minimum pH measured was above 6.2 for all diets except for the diet containing 55% barley at the high feeding level, where pH was 5.8 four hours after feeding.

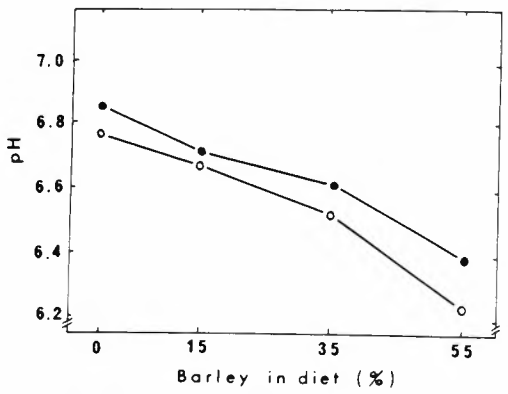

Figure 1. Rumen fluid pH as influenced by the level of barley: low level of feeding (● ——— ●) and high level of feeding (o ——— o). Samples are taken at feeding, 1, 2, 3, 4, & 6 h after feeding

The concentration of ammonia and total VFA and the proportion of VFA in the rumen are presented in Table 3.

There were no differences in ammonia and VFA concentrations between diets at the same feeding level, but the VFA concentrations were highest for the high level of feeding.

At the low level of feeding, the molar proportion av acetic acid (C_2) was significantly depressed by 35% and 55% barley supplementation ($p<0.001$). For butyric acid (C_4), the molar proportion increased with increasing level of barley in the diet ($p<0.001=$. The proportion of propionic acid (C_3) did not change with the diets. At the high level of feeding, the amount of acetic acid decreased, and propionic and butyric acid increased as the barley supplementation increased. The proportions of branched-chain fatty acids (IC_4 and IC_5) changed very little with the diets and feeding level.

Rumen degradation

There was little variation in the potential degradable DH-fraction (a + b) in the grass silage when the silage was given along with different amounts of barley, but the time taken to reach this value varied between the diets (Table 4). Increasing the feeding level or the barley inclusion decreased the degradation rate of the grass silage (c). For the diets containing 0% and 15% barley, the potential degradability was reached after 48 h incubation. The diet containing 55% barley at the high level of feeding was incubated for 120 h before an asymptote was found.

The loss of the ADF fraction of the grass silage followed a similar pattern to that of DM, apart from lower final values for ADF degradation. Degradation of ADF after 48 h incubation is presented in Figure 2. At the high level of feeding there was a large decrease in ADF-degradability when the diet consisted of 55% barley.

Digestibilities

Apparent digestibilities of dry matter (DMD), organic matter (OMD), crude fibre (CFD) and acid detergent fibre (ADFD) of the diets are given in Table 5.

There was no significant differences between the two feeding levels, so the results are pooled.

The DMD and OMD of the mixed diets increased as the amount of barley concumed increased. However, the digestibility of the fibre fraction declined significantly with increasing barley supp-

Table 3. Average rumen fluid NH_3-N and volatible fatty acids as influenced by the diet offered

Diet silage barley	NH_3-N (mmol/l)	Total VFA (mmol/l	Molar proportions C_2	C_3	C_4	IC_4	C_5	IC_5
Low level of feeding:								
100:0	14.7	53.7	73.6[a]	15.6	6.2[a]	2.1[a]	1.9	0.7
85:15	14.5	59.3	73.4[a]	15.8	7.0[b]	1.9[ab]	1.4	0.7
65:35	13.0	57.0	70.9[a]	16.9	8.3[c]	1.8[bc]	1.4	0.6
45:55	14.0	56.3	70.3[b]	15.6	10.7[d]	1.6[c]	1.3	0.6
SEM	4.8	11.1	2.3	2.0	1.2	0.4	0.8	0.4
Diff. between diets	NS	NS	***	NS	***	***	NS	NS
High level of feeding:								
100:0	15.2	68.2	73.9[a]	16.7[a]	5.8[a]	1.6	1.6	0.4
85:15	15.1	64.7	72.9[a]	15.8[a]	8.1[a]	1.5	1.4	0.4
65:35	14.1	66.1	68.4[b]	20.0[b]	8.4[b]	1.6	1.2	0.4
45:55	14.4	64.6	66.1[b]	20.5[b]	9.9[c]	1.4	1.6	0.5
SEM	5.0	12.8	5.4	4.4	2.1	0.6	0.6	0.2
Diff. between diets	NS	NS	***	***	***	NS	NS	NS
levels	NS	***	**	***	NS	***	NS	***

a-d: means in each column with the same superscript are not significantly different ($p<0.05$).

Table 4. Rate of extent of disapperance of DM from grass silage incubated in the rumen

Diet silage:barley:	Potential degradation (a + b)	Rate of degradability (c)[1)]	RSD	DMD48
Low level of feeding:				
100:0	73.7	0.081	1.05	73.3[a]
85:15	75.0	0.071	2.32	72.7[a]
65:35	74.0	0.067	1.86	72.0[a]
45:55	74.9	0.051	3.10	68.6[b]
SEM				1.3
Difference between diets				***
High level of feeding:				
100:0	74.4	0.071	1.60	72.0[a]
85:15	71.6	0.054	1.48	70.4[a]
65:35	72.8	0.054	2.54	63.9[b]
45:55	71.9	0.020	1.76	55.8[c]
SEM				3.1
Difference between diets				***

a-c: means in column with the same superscript are not significantly different ($p<0.05$).

1) a, b and c are constants in the exponential equation $p = a+b(1-e^{-ct})$, where p is degradability at time t.DMD48 is dry matter degradability after 48 h incubation (p when t = 48).RSD = residual standard deviation.

lements, with regard to both CFD ($p<0.001$) and ADFD ($p<0.05$). Quadratic expressions ($p<0.001$) accounted for a significantly greater percentage of the

Table 5. Digestibility of dry matter (DM), organic matter (OM), crude fibre (CF) and acid detergent fibre (ADF) as influenced by the diet offered. Means of both feeding levels

Diet silage barley	Digestibility of the diet (g·100 g^{-1}) DM	OM	CF	ADF	DM digestibility of grass silage[1)]	Depression in digestib. of silage, %
100:0	66.5^{a}	69.4^{a}	76.8^{a}	70.7^{a}	66.5^{a}	-
85:15	68.8^{b}	71.3^{a}	75.4^{a}	70.0^{a}	65.4^{a}	1.7
65:35	71.9^{c}	74.5^{b}	72.7^{b}	67.6^{b}	64.3^{ab}	3.3
45:55	74.9^{d}	77.6^{c}	69.2^{c}	63.6^{c}	61.6^{b}	7.4
SEM	1.9	2.2	2.3	3.2	3.4	
Diff. between diets	***	***	***	**	*	

a-d: means in each column with the same superscript are not different ($p<0.05$).
1) Calculated by difference from assumed digestibility of barley (based om barley degradation in nylon bags after 48 h incubation).

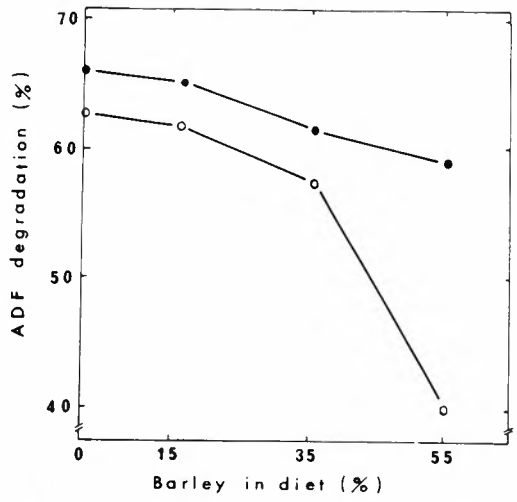

Figure 2. Degradation of acid detergent fibre (ADF) in grass silage after 48 h incubation in nylon bags in the rumen, as influenced by the level of barley: low level of feeding (● ——— ●) and high level of feeding (o ——— o)

variation than the linear regressions ($p<0.05$):

CFD = 76.17 - 0.0024 B^2 ($p<0.001$, $r^2 = 0.62$)
ADFD = 70.50 - 0.0023 B^2 ($p<0.001$, $r^2 = 0.50$)

where B represents the percentage of barley in the diets.

If the assumption is made that the digestibility of barley is not greatly affected by the diet, as found by nylon bag incubation for 48 h, where the barley DM degradation was 870 g.kg^{-1} for all diets (no significant differences between diets), this value of barley DMD can be used to calculate the apparent digestibility of grass silage by difference. The DMD of grass silage decreased with increasing amounts of barley ($p<0.05$).

The digestion and utilization of dietry energy

There were no differences between animals or between diets in the time the animals spent standing or lying in the respiration chambers. Lying time during 24 h varied between 12.0 and 15.9 h. Correction for energy cost of movement was therefore not taken into account.

Intake of gross energy (GE) and losses of energy in faeces, urine and methane are given in Table 6. Supplementation of grass silage with barley reduced the proportion of GE lost in faeces and urine, but there was no significant difference between feeding levels. Methane energy losses increased as the barley supplementation increased, and the losses in percentage of GE intake were significantly lower with the high feeding level ($p<0.01$).

The digestible (DE) and metabolizable energy (ME) levels of the diets are presented in Table 7.

Table 6. Partitioning of dietry energy as influenced by the diet offered

Diet silage:barley	GE intake (MJ.day $^{-1}$)	Proportion of GE intake in: Faeces	Urine	Methane
Low level of feeding:				
100:0	19.67	36.5	4.9^{a}	7.6
85:15	16.44	34.9	3.7^{a}	8.4
65:35	15.05	31.4	2.3^{b}	9.5
45:55	13.11	25.2	2.7^{c}	9.6
SEM		2.1	1.6	1.0
Difference between diets		***	NS	NS
High level of feeding:				
100:0	30.39	38.1^{a}	4.2	6.4^{a}
85:15	26.10	32.2^{b}	3.4	7.6^{ab}
65:35	23.13	27.8^{bc}	3.4	8.2^{b}
45:55	21.42	25.7^{c}	3.0	8.3^{c}
SEM		2.4	0.5	0.6
Difference between diets		**	NS	*
levels		NS	NS	**

a-c: means in each column with the same superscript are not significantly different ($p < 0.05$).

Table 7. Digestible (DE) and metabolizable energy (ME) contents of the diets

Diet silage:barley	DE (% GE)	ME (% GE)	DE in dietary DM (MJ.kg^{-1})	ME in dietary DM (MJ.kg^{-1})
Low level feeding:				
100:0	63.5^{a}	51.0^{a}	11.52^{a}	9.25^{a}
85:15	65.1^{a}	52.9^{a}	11.83^{a}	9.63^{a}
65:35	68.6^{b}	57.8^{b}	12.54^{b}	10.39^{b}
45:55	74.8^{c}	61.6^{c}	13.75^{c}	11.31^{c}
SEM	1.5	2.2	0.4	0.4
Difference between diets	***	***	***	***
High level of feeding:				
100:0	61.8^{a}	51.2^{a}	11.16^{ab}	9.24^{b}
85:15	67.8^{b}	56.7^{b}	12.46^{ab}	10.43^{b}
65:35	72.2^{bc}	60.7^{bc}	13.82^{bc}	11.72^{bc}
45:55	74.3^{c}	63.0^{c}	13.82^{c}	11.72^{c}
SEM	2.4	2.4	0.7	0.6
Difference between diets	***	**	**	**
levels	NS	*	NS	**

a-c: means in each column with the same superscript are not significantly different ($p < 0.05$).

DE increased as the amount of barley consumed increased, but there were no significant differences between feeding levels. DE contents of the DM were closely related to the digestibility of DM

and OM in the diets ($p<0.001$, $r^2 = 0.81$ and 0.85, respectively)

The ME contents of the dietary DM, however, were significantly higher for the high level of feeding ($p<0.001$).

Net availability of ME for maintenance (k_m) and for fattening (k_f) is given in Table 8. Linear regressions relating k_m and k_f to the percentage of barley in the diet were highly significant ($p<0.001$):

$k_m = 55.0 + 0.215\ B$ ($r^2 = 0.98$, mean values)
$k_f = 32.8 + 0.446\ B$ ($r^2 = 0.98$, mean values)

Table 8. Net availability of ME in the diets for maintenance (k_m) and fattening (K_f).

Diet silage:barley	% k_m	k_f
100:0	54.5	31.6
85:15	58.4	40.4
65:35	63.4	50.1
45:55	66.2	56.1

Figure 3 shows the relationship between determined net energy values and the percentage of barley in the diet. Net energy for maintenance (NE_m) increased linearly as the amount of barley consumed increased, and the linear regression relating NE_m to the percentage of barley in the diet was highly significant ($p<0.001$):

NE_m (kJ/g DM) $= 4.94 + 0.048\ B$ ($r^2 = 0.99$, mean values)

Regression analysis on NE_f and percentage of barley in the diet showed a significant ($p<0.01$) overall quadratic effect:

NE_f (kJ/g DM) $= 4.16 + 0.00086\ B^2$ ($r^2 = 0.97$, mean values).

DISCUSSION

The effect of barley supplementation on fibre degradation

Supplementation of grass silage with

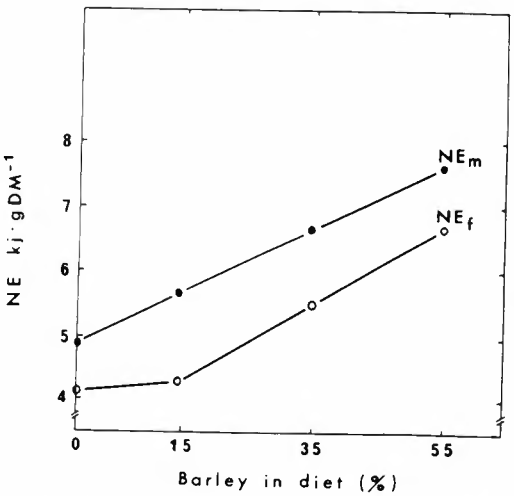

Figure 3. Net energy value of the diets as influenced by the level of barley: net energy for maintenance (● ——— ●) and net energy for fattening (o ——— o)

ground barley depressed the degradation of fibres, analysed as crude fibre or acid detergent fibre, the two methods of analysis giving the same results. For the diet with the highest barley content, depression in fibre digestibility was 10% in relation to the control diet where grass silage was offered alone, with the corresponding reduction in DM digestibility of grass silage at 7.4% ($p<0.001$).

When readily fermentable carbohydrates are added to a roughage diet, the subsequent depression in fibre digestibility is often associated with nutritional limitations or a decrease in pH which can influence the activity of cellulolytic bacteria in the rumen.

A lack of essential nutrients, like NH_3 and branched-chained VFA, in the rumen fluid may result in a decline in the number of cellulolytic bacteria, and hence may depress fibre digestibility (El-Shazly et al. 1961; Dehority et al. 1967). The concentrations of these nutrients in this study were far above the concentrations accepted as limiting for microbial cellulolytic activity, as discussed in a previous paper (Bøe 1989).

The rumen pH is of importance to rumen fibre digestion since pH-optimum of fibre degradation is in range 6.6-7.0 (Dixon 1985). It has been shown in vitro (Stewart 1977) and in vivo by infusing acids into the rumen (Mould & Ørskov 1984) that cellulase activity and hence fermentation of fibre decreases with a reduction in pH, and is negligible when pH is less than 6.0.

In general the rumen pH of an animal that consumes roughage will be in the range 6.2-7.0, and consequently too high for any reduction fibre digestion to take place (Dixon 1985).

With one exception, rumen pH did not fall below 6.2 with the given diets in this study. The depression in fibre digestion caused by pH reduction from 6.8 to 6.0 is not readily explainable. The results of some studies suggest that attachment of the cellulolytic bacteria to the roughage substrate may be involved in the depression of fibre digestion associated with moderate decreases in pH (Hoover 1986).

Mould et al. (1984) thought that fibre depression which could not be alleviated by the maintenance of the rumen pH above 6.0 was due to adaptations in the rumen microflora, such that some of the cellulolytic bacteria grew on the readily fermentable carbohydrates, rather than to the relatively complex structure of the cellulose. As such they designated this depression the «carbohydrate-effect».

For the diet containing 55% barley at the high feeding level, however, rumen pH fell to 5.7 four hours after feeding. This drop in pH below the level known to inhibit cellulolysis was found to affect the rumen fibre degradation considerably. Relative to the control diet without addition, the ADF degradability after 48 h incubation was reduced by 36.0%, compared with 10.2% for the low feeding level.

The VFA-pattern for the two feeding levels also showed that the microflora had changed at the high feeding level, with an increased proportion of propionic acid and a lowered proportion of acetic acid, which is typical for starch fermentation.

This marked reduction in fibre degradation was not observed in the digestibility of fibre in the entire digestive tract, as the apparent digestibility of grass silage was depressed to the same extent for both low and high feeding levels.

It seems probable, therefore, that a decrease in roughage content of the diet at the high feeding level leads to an increase in the retention time of fibrous residues in the rumen. Eng et al. (1964) reported that the mean retention time of hay increased as the roughage content of the diet of sheep was reduced from 100% to 25%.

It has also been shown that as cellulose degradation in the rumen is decreased by readily fermented carbohydrates, the proportion of cellulose digestion occurring in the caecum and colon increases (MacRae & Armstrong 1969), and this may also partly explain why a depression in fibre degradation in the rumen may not be observed over the entire digestive tract. Ternrud & Neergaard (1986) found that by increasing the feeding level from 740 to 880 g DM, the amount of cellulose digested in the hind gut increased from 1.9% to 17.7% of the overall digestion for sheep fed NaOH-treated straw supplemented with 40% barley. Such a situation, with a large reduction in fibre degradability in the rumen, although not observed to the same extent in the apparent digestibility, can have detrimental effects of roughage intake (Lamb & Eadie 1979). Hovell (1985) stated that rumen degradation characteristics are the main determinant of roughage intake.

At the low feeding level there was a good correlation between dry matter degradation of grass silage incubated in nylon bags for 48 h (DMD48) and calculated apparent digestibility of the grass silage. As just discussed, this was not the case at the high feeding level.

DMD48 has often been used to predict DM digestibility of feedstuffs, and many workers have found high correlation between these two parameters (Sauvant et al. 1985).

This study has shown that in a mixed diet this correlation for digestion of the roughage part of the diet depends to a great extent on the feeding level. It supports the statement that the nylon bag technique can only be used as a qualitative indicator of general principles (Ørskov et al. 1980).

Energy partitioning

Barley supplementation increased the digestibility of total dietary energy corresponding to the DM and OM digestibility, and the digestibility was not affected by the feeding level. This was also found by Kelly and Thomas (1978) for late-cut autumn silage.

The ME content, however, was higher at the high level of feeding for the diets containing barley. In general, the metabolizability declines with increasing feeding level. The rise in ME content in the present experiment was caused by a significant decline in energy loss from methane for the high feeding level. Blaxter & Wainman (1964) also found that less GE was lost as methane when the amounts of hay and flacked maize offered to sheep were increased.

The DE and ME content of the dietary DM increased as the amount of barley consumed increased, and linear regressions relating DE and ME values to the percentage of barley in the diet were highly significant ($p<0.001$, $r^2 = 0.73$ and 0.71, respectively).

Including the quadratic component of barley (% of DM) in the equation also gave a significant relationship ($p<0.001$), but accounted for less of the variation ($r^2 = 0.62$ and 0.64 for DE and ME, respectively) than the linear regression. In the interpretation of these regressions, it must thus be taken into consideration that the depressed fibre digestibility of grass silage when supplemented with barley may be reflected in the DE and ME contents of the mixed diets.

To estimate the net energy content of the diets, the fasting heat production of the animals has to be known. Since the proportion of roughage to concentrate was the same of both feeding levels, all the data were used to estimate the heat production at zero intake (Lofgreen & Garrett 1968, Rattray et al. 1973). The animals used in this experiment varied in weight. At our Institute, McNiven (1983) found that metabolic body size can be used to predict fasting heat production in sheep with varying body fat content. The calculated average fasting heat production (263 $kJ/kg^{0.75}$) is in good agreement with the value stipulated by ARC (1980), which is set to 253 $kJ/W^{0.75}$ for 3-year-old rams.

The linear relationship found between net energy values for maintenance (NE_m) and percentage barley in diet supports the concept that the net energy value of a feed used for maintenance is constant and can be considered to be independent of the proportion of other feeds in the ration (Lofgren & Garrett 1968, Vance et al. 1972). However, Byers et al. (1976) found this relationship to be curvilinear for mixtures of corn and corn silage.

For the diet net energy values for fattening (NE_f), a significant quadratic influence of the percentage barley in the diet was found. These values suggest that the ration NE_f values are not additive, i.e. that the net energy content used for fattening of each feed ingredient is not constant. Similar results were found by Vance et al. (1972) and Byers et al. (1976) with corn silage and corn fed to steers.

The efficiency of ME utilization for fattening varied linearly with levels of barley in the diet. Thus, the associative effects on the net energy values for fattening found in this study are likely to be due to depressions in energy digestibility rather than to depressions in ME utilization.

In practical terms, these results show that up to 35% barley can be added to the silage diet before fibre digestibility is affected to any great extent. This depression in fibre digestibility may reduce the net energy value of the diet when the animal is fed above maintenance.

SUMMARY

1. The influence of barley on rumen degradation, digestibility and energy utilization of grass silage was studied.

2. Four sheep fitted with permanent rumen cannulas were used. They were fed four different diets containing late cut grass silage of regrowth and ground barley at two feeding levels. The proportions of silage to barley were 100:0, 85:15, 65:35 and 45:55 on DM basis.

3. Calorimetric experiments were performed using the open circuit type of respiration chamber.

4. The fibre digestibility of the diets, measured as crude fibre or acid detergent fibre, were depressed when barley was added to the silage. Significantly lower digestion coefficients were found when 35% barley was included in the diet compared with silage alone. The depression was similar at both feeding levels.

5. The DM digestibility of the silage, calculated by difference from an assumed digestibility of barley, was significantly depressed when 55% barley was added to the diet. The depression was 7.4% for both feeding levels.

6. The rate and extent (DMD48) of degradation of silage incubated in nylon bags in the rumen decreased with increasing feeding level and with the proportion of barley in the diet. At the low level of feeding there was a good correlation between DMD48 and calculated apparent digestibility of the silage. At the high level of feeding, the DMD48 was much lower than the silage DM digestibility.

7. A linear relationship was found between net energy content of the diets used for maintenance (NE_m) and percentage of barley in the diet. This indicates that the net energy value of the silage used for maintenance is constant, regardless of the proportion of barley in the diet.

8. A significant quadratic influence of the percentage barley in the diet was found on the net energy content of the diets used for fattening (NE_f). This curvilinear relationship indicates that NE_f of each feed ingredient is not constant, but depends on the remaining diet composition.

9. The efficiency of ME utilization for fattening (K_f) increased linearly with the levels of barley in the diet. Thus, the associative effects on the net energy value for fattening are likely to be due to depressions in the energy digestibility rather than to depressions in ME utilization.

10. It was concluded that up to 35% barley could be added to the silage diet before fibre digestibility was affected to any great extent. This depression in fibre digestibility may reduce the net energy value of the diet when the animal is fed above maintenance.

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II. A LITERATURE REVIEW CONCERNING ROOT DEATH IN CUCUMBER AND OTHER CROPS

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In the literature concerning physiological root death, competition for assimilates between fruits and roots has often been proposed as the cause of root death. An analysis of this problem was attempted by considering assimilate production, transport and distribution in normal plants and in plants in which the balance between source and sinks has been changed, with special reference to the cucumber. Fruit production in the cucumber is cyclic; in periods of little fruit growth, root growth is vigorous and vice versa. The distribution of assimilates in the vegetative plant depends on light and temperature and may affect root morphology as well. In the generative plant long-term distribution seems to be adequate, but short- term studies have revealed a possible distribution shortage for the roots.
Reduction of the fruit load did not prevent root death, nor did reducing the root mass aggravate it. Reduction of the leaf area increased photosynthetic efficiency in the remaining leaves, but did not affect root death. It would seem that competition for assimilates might be a problem during some phases of fruit growth, but not the cause of root death. Further research, specifically on root death, is needed. It is suggested that plant hormones also play a part in this.

Key words: cucumber, *Cucumis sativus*, root death

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Production of early greenhouse cucumbers (*Cucumis sativus* L.) is often accompanied by problems with the roots. Thus the term «root death» has been coined though it is not strictly accurate. The roots become brown, later the cortex decays and the roots may become slimy. However, when root growth is restarted, new roots form as laterals from the «dead» roots. The period between root death and the formation of new roots may vary and is considered a characteristic of root death. Plants may wilt on sunny days when they are affected with root death. Root death occurs in all seasons and in all growing media, but is more severe in spring.

The phenomenon has been known for a long time, but not until recently has there been an attempt to gain more insight into the problem. A typical feature of root death of cucumber and other crops is its timing. Root death symptoms always occur a short time (dependent on the crop) after fruit set; in the case of cucumber 12-14 days. Therefore, it has been suggested that root death is caused

by competition for assimilates between roots and fruits.

In this paper, first a review is given of research on fruit production in cucumber in which indications of root death are pointed out. Secondly, assimilate production and distribution are reviewed for cucumber and some other crops. Thirdly, experiments in which assimilate distribution and/or transport and competition between roots and fruits are changed are discussed in connection with root death of cucumber and other crops.

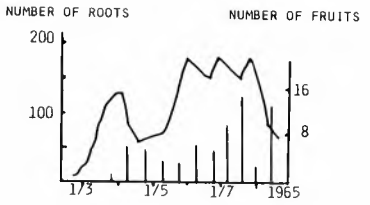

Figure 1. Number of healthy roots and yield of fruits of cucumber cultivated in concrete containers (Van der Post, 1968)

Root death and fruit production

Root death as such has only been mentioned in relatively recent papers. However, in earlier reports one may find indications of root death, and also studies of root growth can be found.

Root growth of the cucumber follows a cyclic pattern (Fig. 1). At the onset of fruit growth, root growth decreases and eventually stops. The size of the root mass reaches a minimum when the fruit load is maximal. This prevents the setting of new fruits which in turn makes root growth possible. When the roots have regained a certain size, fruit set increases inhibiting root growth, and so on. The first cycle displays the most severe root death. This is probably because the plants have a small leaf area and are growing under poor light conditions, as in practical growing in spring (Van der Post, 1968). Hence fruit production also follows a cyclic pattern, which is not desirable from the growers' point of view.

As early as 1934 McCollum noticed that fruit growth inhibited vegetative growth. He used a monoecious, non-parthenocarpic cultivar and observed that vegetative growth was inhibited until seed development was completed. Parthenocarpic fruits also inhibited vegetative growth to some extent, but complete inhibition was only obtained with seeded fruits. McCollum (1934) assumed that there was competition for assimilates between vegetative and generative parts. He expected, but did not find, an accumulation of assimilates in some part of the plant before the onset of fruit growth.

Carlsson (1973b) noticed that yield was reduced by seeded fruits in a parthenocarpic cultivar. The earlier the development of seeded fruits, the greater the reduction in yield.

In monoecious cultivars grown for parthenocarpic fruits, cyclic fruit production was observed by Von Hösslin and Sieber (1958). When big fruits were harvested the number of fruits was lower than with smaller fruits. Besides, fruit quality decreased and abortion increased with bigger fruits. The plant was more vigorous, with more side-shoots, when small fruits were harvested than when the fruits were left on the plant.

In another experiment Carlsson (1973a) compared production in number of fruits and yield in kilogram when the fruits were harvested at specific weights. The number of fruits decreased with increased fruit weight. The maximum kilogram yield was reached at different fruit weights for different cultivars.

De Stigter (1969) used plants in which there was just one leaf to each fruit. He observed competition between fruits and roots. The roots that «died» first took the longest time to recover. He assumed that these were roots connected to the same vascular bundles as the first fruit. There was also competition between fruits. The first fruit inhibited growth of the younger fruits, when it was removed the second fruit became domi-

nant. Fruits might be inhibited 3-4 times in their development.

The cyclic fruit production pattern has been observed in gynoecious cultivars too (De Lint & Heij, 1982). Root death has become a problem in gynoecious, parthenocarpic cultivars also, probably because vegetative growth is very much restricted in modern growing techniques, as compared with McCollum's experiments.

In all the above cases a high early production was combined with a low total production. This could have been caused by more severe root death in plants with a bigger fruit load, especially in early spring. In seeded fruits, seed growth is also an important sink; the competition with the roots might be even more severe. It was also observed that when the root mass is reduced, fruit set is very low, and a lot of ovaries will abort. Besides, bigger fruits will compete more among themselves.

This same cyclic fruit production was also observed for tomato (Leonard & Head, 1958), sweet pepper (Kato & Tanaka, 1971) and melon. Van der Post (1968) showed that in all these crops root growth is opposed to fruit growth.

Assimilate production and distribution

Vegetative plants

In a vegetative plant, mature leaves will be sources of assimilates; the apex, young leaves and roots will be sinks. Under low light conditions the net assimilation rate (NAR) of cucumber was highest at 18°C, compared with 12°C and 24°C air temperature, with root growth maximal as well (Kleinendorst & Veen, 1983). Ikeda (1978) showed that the highest rate of photosynthesis occurred between 10°C and 20°C. The transition from sink to source in the leaves of cucumber depends on light and temperature conditions (Pharr & Sox, 1984). In the tomato the first leaf does not become a source until 30% of its full size is reached (Ho et al., 1984). The authors compared this result with other studies on tomatoes, cucumbers and other crops and found that usually the leaf was 30-50% of its full size before it became a source.

In the cucumber equal amounts of assimilates are transported during day and night (Kanahama & Hori, 1980). In the tomato Ho et al. (1984) found more export during the day than during the night (80% vs. 20%).

In cucumbers, transport was reduced at 5°C compared to higher temperatures and distribution too was affected by temperature (Kanahama & Hori, 1980). A low air temperature would decrease the shoot/root ratio (S/R); this was also observed by Murakami & Inayama (1974) who compared 14°C and 20°C night temperatures. Challa & Brouwer (1978) observed that this was because of a reduction of root respiration, the dry matter content being unchanged. The same was observed in other crops, e.g. tomato (Hori & Shishido, 1978).

In addition, Moorby & Graves (1980) noticed that root morphology of tomatoes changed with the temperature of the roots. At 15°C the roots became thick and brown, and at slightly higher temperatures a mat of coarse roots was observed. At 23°C they observed thin, white, healthy roots with a great number of laterals. At 30°C, root morphology was the same as at 15°C.

Root growth was closely related to NAR, because the roots are completely dependent on assimilate import and have few reserves. An important question with regard to root death is which is the most important character: S/R, root surface area (morphology), root dry weight or root activity.

Generative plants

Photosynthesis of cucumbers increased when fruit growth started, and 80% of the exported assimilates were transported to the fruit. The absolute amount available to the vegetative parts remained the same (Barrett & Amling, 1978). On the other hand it was observed that

95% was exported to the fruit by the leaves closest to it. The demand of the fruit was highest after 4-6 days (about 0.5 g dry weight) of fruit growth. Source capacity seemed to be a limiting factor rather than sink strength of the fruits (Murakami et al.,1982).

The relative sink strength of the roots was greatest at 12°C air temperature, when the relative sink strength of the fruit was minimal. At both higher and lower temperatures root sink strength decreased and fruit sink strength increased. Transport of assimilates to the roots occurred mainly at night (Kanahama & Hori, 1980).

In contrast to the vegetative phase there was no accumulation of starch in the leaves of generative plants. Fruit growth depleted the leaves during the night. The leaves avoided competition partially by growing as much during the day as during the night. The maximum growth rate of the fruit was calculated to be 3.3 g assimilate/day. This amount could be supplied by a 14 dm^2 leaf area. In addition, maintenance and growth of other plant parts must be provided for (Pharr et al.,1985).

Using lower light intensities than Pharr et al. (1985), Schapendonk & Challa (1980) found a maximum growth rate of 3.4 g CO_2/day for a fruit weighing 6 g dry matter. Maximum growth rate was reached after 12-14 days. The difference with the Japanese results might be caused by the use of different final fruit weights (Ito, pers. comm. 1985). Schapendonk & Challa calculated the leaf area required for growth per fruit including plant growth, to be 20 dm^2. The assimilation capacity of the fruit itself seemed to be sufficient to provide for its maintenance respiration.

In the tomato, distribution of assimilates from the first 10 leaves was studied by Russell & Morris (1983). All the leaves exported assimilates to all plant parts, the oldest leaves (1-4) mainly acropetally, the younger leaves (5-9) mainly basipetally. The truss, formed after the ninth leaf, was placed between the orthostichies of leaves 1 and 6 and 3 and 8. It received its assimilates mainly from these leaves. The maximum growth rate of tomato fruits was reached 25 days after pollination (Ho, 1984). Hurd et al. (1979) observed that the S/R of the vegetative parts remained constant throughout the season, with the exception of one period when the fruit load was maximal. Although the overall distribution of assimilates to the fruits was about 80% of the total export, it was 90% at the time of maximal fruit load.

Comment: The fruit load of both cucumber and tomato was maximal at the time of the first occurrence of root death, at which time the plant was relatively small, and several fruits were growing. It seemed that source capacity limited growth, especially as root activity must be higher at this time to meet the demands of the growing fruits and shoot. The same absolute amount of assimilates to the vegetative parts might be too small at this time. If, even for a short time, the fruit receives more than 80% of assimilates, which is the mean for the whole season, competition is unavoidable.

Changing the competition

So far only the ordinary development of the plants has been discussed. In order to study root death's connection with fruit production measures had to be taken of changing root mass, leaf area and/or fruit load. These experiments will be discussed later. Very little research has been done, so studies not involving the roots will also be discussed. Most research has been done with tomatoes.

Changes in fruit load

Liebig (1978) compared cucumber plants with and without fruits and noticed that the leaf area, especially of gynoecious cultivars, was reduced in plants with fruits. Photosynthetic efficiency was increased but probably not enough to avoid competition.

Also the root mass became bigger in plants without fruits than in plants with fruits. Root death was not prevented, although plants without fruits had less severe root death than plants with fruits (Van der Vlugt, 1986).

In the tomato, the effects of truss removal and truss thinning have been studied with regard to photosynthesis, translocation and distribution of assimilates. In a long-term experiment Hurd et al. (1979) observed that root growth was reduced before flowering and ceased completely about 1 month after flowering. When the fruit load was reduced to 3 fruits per truss, reduction and cessation of root growth were also observed, but the final root mass was bigger than in plants with a full fruit load. They assumed that there was a functional balance between vegetative and generative growth independent of fruit load. They suggested that the vegetative mass at the beginning of fruit production determined the occurrence and degree of root death.

Every other week, starting at flowering, Starck et al. (1979) studied the photosynthesis in tomato plants with and without fruits. In some cases the fruits were kept in the dark to prevent photosynthesis. The total dry matter production (roots not included) was the same for all treatments, fruits reducing the growth of the vegetative parts. After 2 weeks, photosynthesis and dark respiration were the same in plants with and without fruits. After 4 weeks, photosynthesis was lower in plants without fruits than in the other treatments. At the end of their experiment, photosynthesis was also lower in those plants with fruit in the dark, than in plants with fruits exposed to light. Their conclusion was that photosynthesis was not directly related to sink demand.

When the first truss of tomato plants was removed NAR decreased (Tanaka & Fujita, 1974). Translocation from the lower leaves was reduced, but their assimilates were redistributed to the other trusses. The total dry matter accumulation was the same in plants with and without the first truss. Tanaka & Fujita (1974) observed that assimilate transport to the roots was adequate in high light conditions, but decreased in low light conditions.

Quast (1977), too, found an unchanged dry matter production in tomato plants with and without fruits. Plants without fruits were found to have more carbohydrate reserves in all plant parts. The dry matter content of the roots was highest in plants without fruits.

Ho et al. (1983) carried out experiments with plants in which the source sink system was reduced to one truss and one leaf. Assimilate transport to the roots was stopped. In low light intensity, photosynthesis did not change with the removal of the truss, in contrast to high light intensity. More carbohydrates were accumulated in leaf and stem after truss removal, irrespective of light conditions.

Hall (1977) shaded or removed the first fruit on sweet pepper plants. Without the fruit the root system continued to grow after it had stopped in plants with fruit. Eventually a reduction in root mass was also observed in plants without fruit. The dry matter content of the roots decreased after flowering in both treatments, but more abruptly and severely so in plants with fruit. The rate of photosynthesis was influenced by the source-sink balance. Photosynthesis was reduced by 30% in high light conditions and less than this in low light conditions. Compared with plants with fruit, more carbohydrates were accumulated in plants without fruit in leaf and stem after fruit removal (Hall & Milthorpe, 1978).

In bean plants, where the pods were the strongest sinks, the amount of assimilates available to the roots decreased. The nearest leaf was the main assimilate supplier to the pods. When the pods were removed this leaf redistributed its assimilates to all plant parts (Olufajo et al., 1982).

Total dry matter content of the plants was the same in plants with and without fruits in all the crops investigated. Reduction in vegetative growth was observed and must be due to competition between fruits and vegetative parts.

In low light conditions photosynthesis also remained unaffected, showing that dry matter production was below maximum. The roots would have been more affected than the shoot if, as in vegetative plants, transport to the roots had been reduced under low light intensities.

Changes in leaf area

Defoliation in cucumbers was often carried out in order to reduce vegetative growth. In monoecious cultivars this had the additional advantage of increasing the number of female flowers, and thereby probably increasing production (Liebig, 1978). Liebig compared a monoecious (cv M) and a gynoecious (cv G) cultivar in several deleafing treatments. Cv M gave a better yield with deleafing compared with non-deleafing. Cv G showed a tendency to decreasing yield with more severe defoliation. The reduction of the leaf area was not completely compensated by increased photosynthetic efficiency.

Van Uffelen & Bulthuis (1984) compared plants with one and two shoots. The two-shoot plants gave a low early yield, but the total yield was increased when both shoots came from the lowest nodes.

Hurd & Mountifield (1980) compared a compact and a vigorous tomato cultivar. Both were grown with and without an extra side-shoot. The S/R was bigger in the vigorous cultivar and was not changed by the extra shoot. The compact cultivar gave a very high early yield and a relatively low total yield, the side-shoot having no influence on the number of fruits per truss. The leaf area decreased after flowering. This cultivar had more severe root death than the vigorous cultivar; this was not reduced by the extra side-shoot. In the vigorous cultivar, early yield was low, but total yield was high. The side-shoot reduced the number of fruits per truss. The leaf area remained the same throughout the season. Hurd & Mountifield suggested that more vigorous cultivars should be used in hydroponics instead of trying to manipulate the vegetative/generative ratio.

The effect of the leaf area, decreased by more vigorous defoliation than is normal in a tomato crop, was compared with the effect of the leaf area increased by allowing one leaf of each side-shoot to develop. A large leaf area decreased the early yield but the total yield was similar to that of a normally defoliated crop. Rigorous defoliation did not influence early yield but reduced total yield (Lamm, 1956).

Tanaka & Fujita (1974) also removed leaves in their experiments, either the oldest, below the first truss, or the youn gest, above the first truss. When all lea ves were removed, transport of assi mi lates to the roots was stopped. Removal of the oldest leaves had little effect on the trusses. Removal of the youngest leaves decreased truss weight, especially of truss numbers 2, 3 and 4. Removal of 30% of the area of each leaf had no effect on dry matter in trusses or stem, but the dry matter in the leaves decreased.

In defoliated sweet pepper plants fewer carbohydrates were found in leaf lamina and stem than in normal plants. The effect was stronger when the rate of photosynthesis was higher (Hall & Milthorpe, 1978).

Comment: Again the light conditions proved to be very important. The production of assimilates must be maximal if good and regular production is to be maintained.

Increasing the leaf area with an extra shoot disturbed the balance between vegetative and generative growth and resulted in a low early yield. However, the total yield was equal to or higher than that of the control plants of

all crops studied, i.e. the roots suffered less.

Changes in root mass

Carlsson (1963) showed that cultivars with a large root mass gave higher yields than cultivars with a small root mass. He managed to increase yield by grafting a little productive scion on a vigorous root system. It seemed that a large root mass was needed to compete with the fruits.

In our grafting experiments we did not find any differences in yield (Van der Vlugt, 1986, yield not published). The root masses were not significantly different. Grafting on *Cucurbita ficifolia* prevented root death.

Tachibana (1982) studied the cucumber/*Cucurbita* graft at different temperatures. *Cucurbita* itself, or as a rootstock, had a lower temperature optimum than cucumber. It was assumed that *Cucurbita* roots were a better sink than cucumber roots because their respiration was higher at lower temperatures.

De Stigter (1971) compared cucumber and melon on their own roots or grafted on *Cucurbita ficifolia*. In intact plants the starch content of the lower stem decreased in the order *Cucurbita* - cucumber - melon. The graft with melon on *Cucurbita* showed more starch above the graft than the graft with cucumber, but less in the rootstock. The starch content of the rootstock in the graft cucumber/*Cucurbita* was less than in *Cucurbita*. De Stigter assumed that the *Cucurbita* roots were a stronger sink than cucumber or melon roots.

Scanlan & Morgan (1982) increased the root mass of tomatoes by increasing the nutrient supply. They increased vegetative growth relative to generative growth. Root death was not observed.

Although more root death might be expected in root pruning experiments, our experiments showed that the plants had recovered from root pruning at the time of root death. It was concluded that root age was not important in connection with root death (Van der Vlugt, 1986).

Kobza (1977) also observed increased root growth after root pruning of cucumber. After 20-30 days the original shoot/root ratio was restored and root growth rate was reduced to maintain this ratio.

In vegetative bean plants, transport of assimilates was studied in plants with intact roots and in plants with 1/2 root mass. Acropetal transport rate was reduced in plants with 1/2 root mass. Basipetal transport rate was the same for both plants. The total transport was reduced after root pruning, but not to the same extent as the root mass. It has been suggested that the roots supply hormones to the shoot, sending fewer hormones from a pruned root system, thereby reducing shoot growth and giving relatively more transport to the roots (Ghobrial, 1983).

It would seem that a larger root mass is a better competitor. *Cucurbita ficifolia* roots might make good competitors because they accumulate more assimilates for some reason or other. In our root pruning experiment the recovery period before root death was expected, was almost as long as the one observed by Kobza (1977). Root death of some roots might possibly be prevented if root pruning was carried out later.

DISCUSSION

The literature concerning root death is very limited. One might draw conclusions from the observed changes in asssimilate production, transport and distribution, but the connection with root death remains to be proved.

In commercial cucumber growing, yield is somewhat reduced by root death but equally important is the reduction in quality of the fruits. De Stigter (1969) showed that the third and fourth fruit need a longer time to develop and the storage quality of these fruits is low.

Since root death is more of a problem in the cucumber than in the tomato,

because of the stronger dominance of the above-ground parts, research on root death should be carried out on the cucumber.

Commercial cucumber growers start the growing season in early winter, when the light conditions are very poor. Under low light conditions the plants are not able to increase photosynthesis in order to meet the increased demand for assimilates. The roots are in a special situation because they have to import all assimilates and have very few reserves. Transport to the roots occurs mainly at night , when also the fruits demand a lot of assimilates. In addition, Tanaka & Fujita (1974) showed that transport to the roots is low under poor light conditions. All in all, this explains why root death occurs and is most severe in spring.

It is obvious then that a high early production severely inhibits root growth and the suggestion by Hurd et al. (1979), that the promoting of a sturdy vegetative plant should be attempted before fruit production, seems logical.

So far, increased root mass has not given any results. The root surface area is the most important factor for uptake of water and nutrients. Research should be carried out to find out whether the balance between root surface and root dry weight is different before and after root death.

The distribution and transport of assimilates is also affected by plant hormones and the onset of fruit growth might alter the hormonal balance in the plants (Lenton, 1984). In general, plants change their hormonal balance when changing from vegetative to generative growth, and it might therefore be difficult for the plants to have both flowers and fruits at the same time, possibly also because of different hormonal requirements.

CONCLUSION

Root death may be caused by competition for assimilates between roots and fruits and in commercial cucumber growing it is aggravated by the light conditions in early spring. However, competition does not seem to be the only cause of root death because it is not prevented by keeping the plants vegetative.

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III. THE EFFECTS OF PLANT DENSITY, AN EXTRA SIDE-SHOOT AND REDUCED FRUIT SET ON ROOT DEATH IN THE CUCUMBER

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An attempt was made to prevent physiological root death by lowering the plant density, thus reducing the extent of the plants shading each other, and allowing more assimilates to become available to the roots. An alternative approach was the growing of an extra side-shoot, which would also increase the assimilate supply to the roots. The size of the root mat was dependent on treatment. Root death occurred after the first fruits had been harvested. The recovery period after root death was longest in the highest shoot density.
In another experiment, the number of leaves per fruit was varied by pinching flowers from specified nodes in order to increase assimilate supply to the roots. Root death seemed to occur earlier in the relatively more vegetative plants than in the more heavily loaded plants. More vegetative plants also needed a longer time to recover than heavily loaded plants.
The effect of vigorous vegetative growth on root exudation and its relation to root death was discussed.

Key words: Cucumber, *Cucumis sativus*, Fruit load, Plant density, Pruning, Root death

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According to one hypothesis, physiological root death of cucumber (*Cucumis sativus*) is caused by competition between roots and fruits for assimilates (Van der Vlugt, in press). Thus, variation of the plant density may influence root death by changing the assimilate supply, through changes in photosynthesis rates for the plants.

Plant density and spatial arrangement have been found to be correlated with fruit weight and quality (Bakker & Van de Vooren, 1984).

Carlsson (1973) observed that gynoecious cultivars could be grown at greater density than monoecious ones. With increasing plant density the plants will shade each other more. Liebig (1983) investigated the relation of the leaf area index (LAI) with plant density and found that up until LAI = 1 plants do not interact with each other. With LAI = 2 plants begin the generative growth phase. The maximum LAI reached by cucumbers in a greenhouse was LAI = 3, independent of plant density.

With a high plant density the mutual shading will be considerable with very few leaves per plant performing at maximum photosynthetic rate. In winter, in particular, the light conditions near the ground being so poor that they may lead to leaf-fall (Nederhoff, 1984).

In Hurd & Mountifield's (1978) comparison of a vigorous and a compact tomato cultivar, with and without a side-shoot, no beneficial results were obtained with a side-shoot, possibly because the plant density was not changed.

In Norway, cucumber fruit production is started in the 4th-6th node, and plants will be affected with root death. A cucumber fruit needs the assimilate production of 5 leaves at maximum photosynthesis (Challa & Schapendonk, 1984), and the plants themselves also have to grow. It was assumed that production was being started too early, but a complete removal of fruits did not prevent root death (Van der Vlugt, 1986). The effect of reducing the fruit load was investigated.

With regard to root death, maximal photosynthesis was considered necessary. A lower plant density would improve the situation for the individual plant, but would also lead to decreased production. Therefore it was decided that some plants with extra side-shoots should also be grown in order to raise productivity. At the same time, the extra leaf area from the side-shoot might improve the assimilate supply to the roots and thereby prevent root death.

MATERIALS AND METHODS

General

Seedlings were raised in 7.5 cm rockwool cubes and set out in NFT gullies 3 weeks after sowing. The gullies were 4.5 m long, 20 cm wide and 1 m apart. The nutrient solution was recirculated continuously. The E.C. was measured daily and adjusted to 2.0 - 2.5 mS. The pH was measured every other work-day and when necesssary adjusted with KOH or HNO_3 to pH 5.5 - 6.5.

In a preliminary experiment the gynoecious cucumber cultivars Farbio, Farbiola, Saskia and Corona were compared with regard to vigour and root death, but no significant differences were found. In the present experiments the cultivar Farbio was used.

Plant density experiments

The number of plants per gully was 2, 4, 8 or 10. When there were only 2 plants per gully 2 side-shoots of each plant were grown, giving a total number of 3 shoots and a shoot density of 0.86/m^2. With 4 m^2 plants/gully one side-shoot was grown, giving a shoot density of 1.1 shoot/m^2. In the other treatments, plant densities were 1.1 and 1.4 plants/m^2. The shoots were taken out as soon as possible. Both main stems and shoots were trained according to the umbrella system. There were 2 replicate gullies of each treatment in each experiment, planted on 9 April and 29 July 1985.

Fruit load experiment

The plant density was 1.4 plants/m^2 planted on 3 and 10 June 1985 respectively. In the control treatment (1) all the fruits which developed spontaneously were left on the plant (1 leaf/fruit). In the other treatments flowers were removed at anthesis, leaving every 3rd, 5th or 7th fruit. In this way each fruit had a constant number of leaves. After harvesting a fruit, leaves were removed, leaving only the required number of leaves for the next fruit.

Root size was measured on a scale from 1 to 5, with 5 being an exceptionally big root mass. Root death symptoms - browning, root death and formation of new roots - were recorded in days after planting.

Table 1. Early yield of plants grown at different plant densities (means of two gullies). A: planted in April, yield from days 27 to 51. B: planted in July, yield from days 25 to 65

Plant density/ shoots per plant	Number of 1st class fruit		Total number of fruits		kg/m^2	
	A***	B**	A***	B**	A***	B*
2/3	33,5	40,5	58,0	80,0	3,436	4,055
4/2	60,5	60,0	95,0	108,0	5,430	5,475
8/1	81,5	79,5	130,5	145,5	7,188	7,570
10/1	95,0	62,5	172,0	118,5	9,464	5,655

* = $p<0.05$ ** = $p<0.025$ *** = $p<0.01$

RESULTS

Plant density

Harvesting started at days 27 and 25 for the April and July plantings respectively. Early yield included mainly fruits from the main stems, and a very few from the side-shoots. Regression analysis of the results based on the number of plants gave a better explanation than regression analysis based on number of shoots. Early yield (24 days) increased with increased density in the first experiment (Table 1). In the second experiment the greatest yield after 40 days was obtained with 8 plants/m^2.

The size of the root mats was assessed several times during the experiments. An analysis of variance was carried out with treatments and days of observation as the main factors. In the first experiment the effects of time and plant density were both significant ($p<0.05$). In the second experiment only the effect of time was significant (Table 2). At all densities maximum root size was reached on the day of the first harvest. Afterwards root size decreased a little and then fluctuated. Browning of

Table 2. Mean size of the root mat during the whole experiment (arbitrary scale). A: planted in April, B: planted in July

Plant density/ shoots per plant	A	B
2/3	3,2	3,2
4/2	3,6	3,5
8/1	3,8	3,6
10/1	3,9	3,4

Table 3. Root death symptoms (in days after planting) in plants grown at different plant densities (means of two gul- lies). A: planted in April, B: planted in July

Plant density/ shoots per plant	Browning		Root death R		Formation of new roots N		N-R (days)	
	A***	B*	A	B	A	B	A	B
2/3	21,0	22,0	40,5	33,5	46,5	37,0	6,0	3,5
4/2	22,0	22,0	41,0	30,0	48,0	59,0	7,0	29,0
8/1	32,5	23,0	42,0	28,0	47,5	34,5	5,5	6,5
10/1	32,5	23,0	40,5	30,0	51,0	46,5	11,5	16,5

* = $p<0.05$ ** = $p<0.025$ *** = $p<0.01$

Table 4. Yield of plants with different numbers of leaves per fruit (means of two gullies with 10 plants each). A: planted 3rd June, yield from days 24 to 44. B: planted 10th June, yield from days 23 to 39

Leaves/ fruit	Number of 1st class fruits		Total number of fruits		kg/m^2	
	A***	B***	A***	B***	A***	B***
1	47,0	48,5	79,0	91,0	4,798	4,644
3	36,5	37,0	53,0	58,5	3,880	3,804
5	23,5	31,0	35,0	42,5	2,248	2,881
7	20,5	20,5	30,0	34,0	1,947	2,157

* = $p<0.05$ ** = $p<0.025$ *** = $p<0.01$

the roots was delayed in both experiments with increased plant density (Table 3). The trend seemed to be the same in both experiments, with delayed formation of new roots at greater plant density. The treatment 4/2 in the second experiment behaved anomalously.

Fruit load

Regression analysis was carried out with the number of leaves per fruit as the independent variable. Harvesting started on day 23 and lasted for 16 days in experiment A, and on day 24, lasting for 20 days in experiment B. In both experiments significant increases in yield were found with the heavier fruit load (Table 4).

Only in the first experiment were significant differences in recovery period (N-R) found, the treatments with less fruit actually requiring a longer recovery period (Table 5). The tendency seemed to be the same in both experiments: earlier and more severe root death with less fruit load.

DISCUSSION

Although root size in the treatments with the lowest plant densities may have been underestimated, there was a strong correlation between root size and yield, as observed by Carlsson (1963). The differences in root death symptoms between the April and July plantings may have been caused by the different growing seasons, as is also indicated by the yields. The side-shoots did not produce fruit in the topical period for root death. They may have served as assimilate suppliers to the roots after their leaves were fully developed.

Table 5. Root death symptoms (in days after planting) in plants with different numbers of leaves per fruit (means of two gullies). A: planted 3rd June, B: planted 10th June

Leaves/ fruit	Browning		Root death R		Formation of new roots N		N-R (days)	
	A	B	A	B	A	B	A	B
1	27,5	23,0	37,0	28,0	40,5	40,5	3,5	12,5
3	34,5	26,5	37,0	30,0	40,5	37,0	3,5	7,0
5	23,0	24,0	35,0	28,0	40,5	43,0	5,5	15,0
7	23,0	24,0	35,0	26,5	40,5	35,0	5,5	8,5

Root size and root death correlated better with plant density than with shoot density, indicating that the number of shoots is not important provided photosynthesis conditions are satisfactory.

The number of leaves per fruit was increased by the sideshoots, which had a beneficial effect on recovery after root death, in contrast to the fruit load experiment.

In the fruit load experiment, plants with a high number of leaves per fruit showed more severe root death than plants with a low number of leaves per fruit. However, these plants were all grown at a high plant density.

In a closed system like NFT, root exudates will remain in the root zone. It has been suggested that the plants produce some substances which are toxic to themselves at high concentrations (Van der Vlugt, 1986). With more plants per gully a build-up of toxic substances will be more rapid than with fewer plants per gully.

Pegg (1986) showed that more vigorous tomato plants had a more active root system and also exudated to a greater extent than less vigorous plants. This concurs with the results presented here. Fewer plants or more heavily loaded plants produce less exudates than vigorous plants at high densities.

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VARIABILITY AMONG POTATO CLONES DERIVED FROM IRRADIATED CALLUS

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Production of regenerants from irradiated callus of the potato cultivars Kerrs Pink and Pimpernel is described. In the first generation of clones, (M_1), 16.2 and 4.3 % are tuber colour mutants in the two respective cultivars. The variations in the characters - number of stems per plant, plant height, tuber yield and dry matter content - are studied in the M_3 and M_4 generations in field experiments. A great continuous variation is observed for all these characters, and estimates of heritabilities in the broad sense are high (0.57 - 0.87). From a practical point of view only a small proportion of the mutants could be regarded as positive.

Key words: Potato, tissue culture, mutations

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While the variation created by induced mutations in sexually propogated crops can be regarded as a raw material for recombination, it may in addition be utilized more directly in vegetatively propagated plants. The clones in such crops are usually very heterozygous, and in order to avoid breakdown of the whole genotype of a good cultivar by crossing, it should be possible to achieve genetic gain by changing one or a few characters by induced mutations, provided stable mutants can be obtained. This would be possible by in vitro technique if mutated cells are produced - spontaneously or by mutagenic treatment - before regeneration from callus. The extent to which the mutants are stable if more than one cell participates in bud initiation depends on the number of cell generations the mutated cells have passed.

Numerous reports have shown that plants regenerated from callus display great variation also without mutagenic treatment. Reviews of the somaclonal variation in general have been given by Scowcroft & Larkin (1982), Scowcroft (1985), Karp & Bright (1985) and by Ahloowalia (1986). Regarding potato, somaclonal variation in regenerants from protoplasts has been reported by several authors including Secor & Shephard (1981), who have described variation in 22 morphological characters. Karp et al. (1982), Ramulu et al. (1983, 1984) and Creissen & Karp (1985) attributed a large part of the variation partly to aneuploidy and partly to polyploidy. Somaclonal variation in regenerants from potato callus which have not been through a protoplast phase, has been reported by Wheeler et al. (1985) and by Evans et al. (1986). The former authors reported only a small frequency of the regenerants to be aneuploids, in contrast to the result of the protoplast method mentioned above.

Van Harten et al. (1981, 1986) increased the degree of variation by irradiating the explant before incubation. Sonnino et al. (1986, 1987) studied the variation in plants derived from protoplasts compared with those obtained from irradiated buds propagated by microcuttings. They concluded that the mutation spectrum obtained by the two methods was largely similar. The irradiation method, however, gave a smaller number but less dramatic mutations. In the present work, which was carried out during the years 1979-82, the variation among regenerants from irradiated callus has been studied.

MATERIAL AND METHODS

Two cultivars, Kerrs Pink and Pimpernel, and two clones of breeding material were used to spot the proper culture media for callus growth and for regeneration. Later on, a total of 22 clones - including breeding material and commercial cultivars - were added. Callus was formed by all clones, while only 12 of them regenerated plantlets which were propagated and used in field experiments. The variation obtained in the cultivars Kerrs Pink and Pimpernel will be described here.

Most of the material was grown from 4 mm long, longitudinally split pieces of petioles. These seemed to give better results than the 4 x 4 x 2 mm pieces of tuber which were used to begin with. The petioles were sterilized in 1 % silver nitrate, while sodium hypochlorite was used for the tubers before cutting. The material was inoculated on an agar medium and kept under a Philips LT 33 fluorescent tube, giving an effect of 6.75 W/m^2,16 hours daylength, and a temperature of 26°C.

The callus was transferred to a fresh medium at intervals of 2-8 weeks, according to the vigour of the callus growth. The optimal culture medium was as follows: Inorganic compounds according to Murashige & Skoog (1962), organic compounds according to Nitsch & Nitsch (1969), and caseinhydrolysate (1g/1). In addition, for callus growth: NAA (5-10 mg/1), K(0.05- 0.5 mg/1), BAP(0-0.4 mg/1), and for regeneration: IAA(0.4 mg/1), K(0.8 mg/1), BAP(0.4 mg/1), GA(0.4 mg/1). The callus was exposed to gamma radiation at doses of 3000 R, 1-3 times at intervals. When this treatment was started at an early stage in the callus development - before any differentiation had taken place - regeneration was very difficult to obtain. Therefore, irradiation was given to the callus when regeneration had started, but where differentiated tissue as far as possible had been removed.

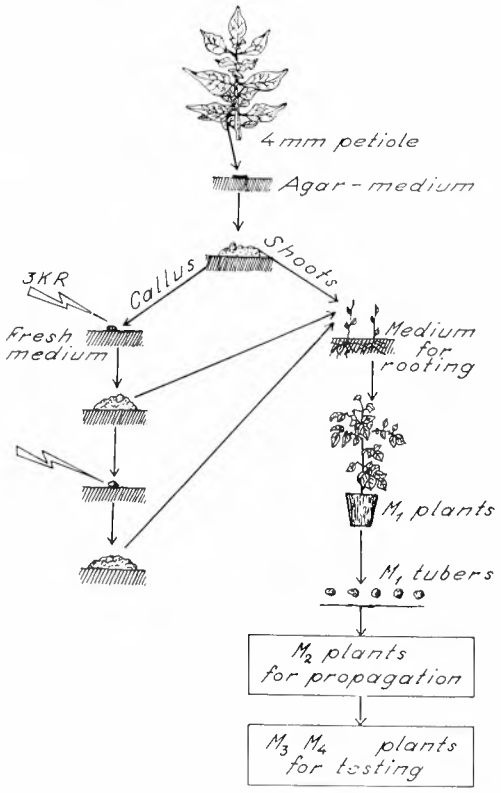

Figure 1. Induction of mutations in potato by means of gamma irradiation of callus

The regenerants were planted in soil and placed in a glasshouse (M_1 generation). Tubers produced by these plants were observed for colour, and propagated in the field (M_2 generation). In the M_3 and M_4 generations, field experiments were carried out to study mutations in quantitatively inherited characters. The procedure is shown in Fig. 1.

The field experiments were laid out according to a special type of incomplete block design. Replications were divided into incomplete blocks, each of which comprised several control plots, i.e. the original cultivars. In order to adjust for some of the environmental variations, the observations of each clone were corrected in relation to the mean values of the control clones in each block, the control clones being common for all blocks. The details for each experiment are given in Table 1. An estimate of the dry matter content was based on the specific weight of the tubers.

RESULTS

Tissue culture

The 26 cultivars which were inoculated showed a great variation in their ability to produce callus as well as to regenerate. While one cultivar produced embryoids, most of the others, including Kerrs Pink and Pimpernel, produced shoots which formed roots later on. Irradiation given to the callus at a very early stage seemed to inhibit regeneration, while given after differentiation had started the callus might go on producing plantlets for months.

Mutations in tuber colour

The colour mutants were observed in the M1 generation. Kerrs Pink and Pimpernel, which are both red, produced blue and blue-spotted mutants; Kerrs Pink also produced whites (Table 2).

Table 2 shows great differences in response between the two cultivars regarding mutant frequencies, which may be explained in various ways. As the potato is at least partly an autotetraploid (Lunden 1937, 1960), the difference may simply be due to differences in the number of dominant alleles at one or more loci. According to Lunden (1937, 1974) Kerrs Pink is simplex for a gene necessary for tuber colour production, while Pimpernel is duplex, which can easily explain the absence of white mutants in Pimpernel. Factors like this may be responsible for great differences between the cultivars in response to irradiation.

An increased number of irradiations from 1 to 3 had only a slight effect on the frequency of colour mutants. The increased number of irradiations has probably caused a greater loss of cells, a fraction of which might have had a high mutant frequency.

It may be noted that 8 colour mutants appeared out of 22 non-irradiated clones of Kerrs Pink. However, conside-

Table 1. Details describing the layout of the field experiments with the M_3 and M_4 generation clones

	Kerrs Pink M_3 1981	Kerrs Pink M_4 1982	Pimpernel M_4 1982
No of clones to be tested	276	48	48
Replications (complete blocks)	2	4	4
Incomplete blocks/replication	6	4	4
Test clones/incomplete block	46	12	12
Control plots/incomplete block	5	3	3
Plants/plot	2-4	16	10
Plot size, m_2	0.6-1.2	3,4	2,4

Table 2. Frequencies (in per cent) of colour mutants obtained from irradiated callus

Clone	No. of irridiations	No of surviving clones in M_1	Colour mutants: white No.	white %	blue No.	blue %	blue spotted No.	blue spotted %	total No.	total %
Kerrs Pink	0	22	1	4.5	4	18.2	2	9.1	7	31.8
	1	780	46	5.9	4	0.5	64	8.2	114	14.6
	2	341	7	2.1	11	3.2	47	13.8	65	19.6
	3	248	0	0	12	4.8	28	11.3	40	16.1
	Total	1391	54	3.9	31	2.2	141	10.1	226	16.2
Pimpernel	1	159	0	0	0	0	5	3.1	5	3.1
	2	110	0	0	1	0.9	1	0.9	2	1.8
	3	643	0	0	6	0.9	26	4.0	32	5.0
	Total	912	0	0	7	0.8	32	3.5	39	4.3

ring the small number of non-irradiated clones, no general conclusion regarding the frequency can be drawn.

One group of mutants is called blue spotted. Observations in later generations indicate that some of these are stable blue spotted, giving blue-spotted offspring irrespective of which part of the tuber the next generation originates. A large fraction of the blue-spotted mutants seem, however, to be chimeras, most likely the result of differentiated zones in the callus during irradiation.

Mutations in quantitatively inherited characters

As many of the clones were small and weak, they did not produce enough tubers in the M_2 generation to allow for the testing of quantitatively inherited characters. The number of M_3 clones from Kerrs Pink in the 1981 field experiment had therefore to be reduced to 276. (The Pimpernel experiment of 1981 had to be discontinued because of damage by flooding.) The 1982 experiments included 48 M_4 clones of each cultivar. These clones had previously been selected, mainly for tuber yield and partly for dry matter content. The characters scored were as follows:

Plant height
Number of stems per plant
Total yield
Yield of tubers exceeding 50 grams each
Per cent dry matter, based on specific weight
Gram dry matter per plant

Frequency distributions for these characters in the two experiments are presented in Figs. 2-4.

The clone means over complete blocks (replications) and the variation among clones are compared with the corresponding means and variations of the control clones. The interval of confidence of the control means is also given in the figures. As expected, most of the mutants were inferior to the control. However, for all characters some of the mutant clones showed a variation beyond the range of the parental clones, some of the mutants being significantly superior to the control as far as each single character was concerned. It is noteworthy that tuber yield was generally higher in 1981 than in 1982, which can be seen by comparing the mean yields of Kerrs Pink control clones between the two years. Estimates of heritability in the broad sense (Table 3) for 4 characters in each population gave fairly high and consistent values over years and cultivars,

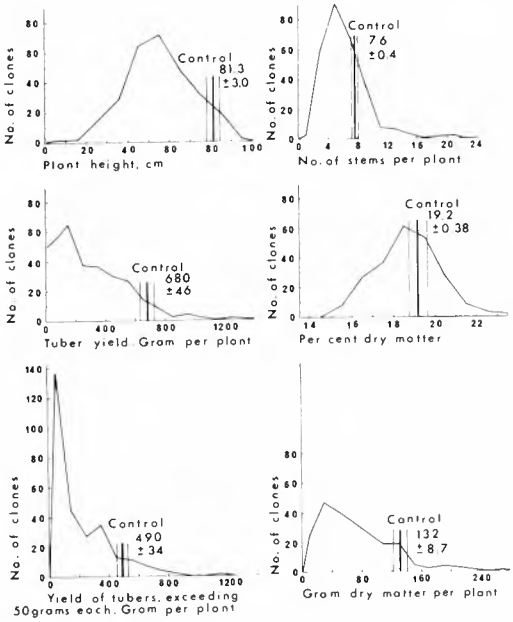

Figure 2. Frequency distribution of 6 quantitatively inherited characters. Kerrs Pink, M_3, 1981. 276 clones

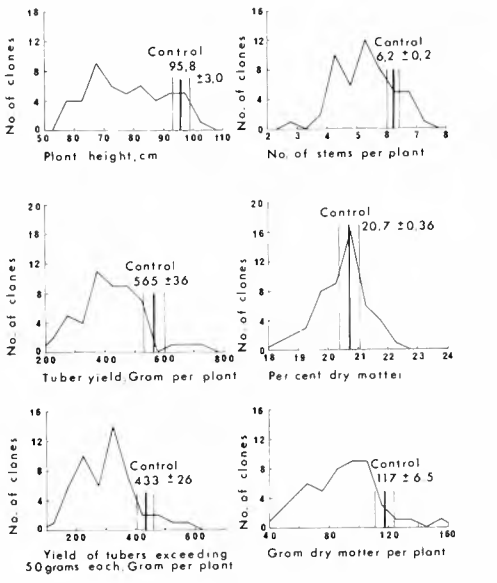

Figure 3. Frequency distribution of 6 quantitatively inherited characters. Kerrs Pink, M_4, 1981. 48 clones

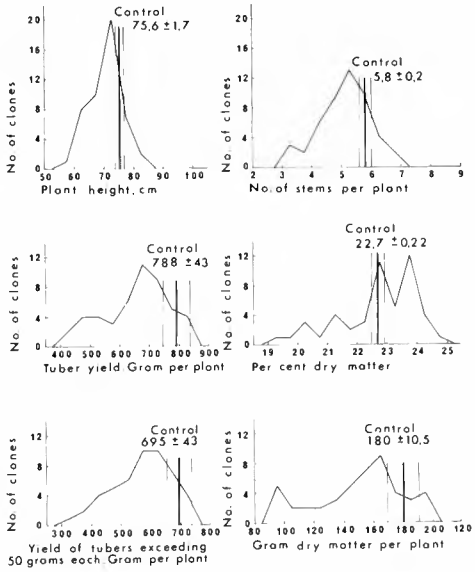

Figure 4. Frequency distribution of 6 quantitatively inherited characters. Pimpernel, M_4, 1982. 48 clones

showing that a great part of the variation is genetic.

Some of the relationships between the observed characters have been studied by means of phenotypic coefficients of correlation (Table 4).

As can be seen from Table 4 the relationship between tuber yield and plant size is fairly close in Kerrs Pink, but less obvious in Pimpernel. Dry matter content showed no correlation with tuber yield among M_3 clones of Kerrs Pink, while this correlation is highly significant in the selected M_4 clones of both cultivars.

DISCUSSION AND CONCLUSIONS

Regarding the quantitatively inherited characters, it may be concluded that the method has created a great genetic variation, including some favourable variants. In both fields of selected clones in 1982 there was a positive correlation between

Table 3. Heritability in the broad sense (h^2)

			Plant height	Number of stems	Total yield of tubers	% dry matter
Kerrs Pink	M_3	1981	0.78	0.71	0.70	0.62
" "	M_4	1982	0.82	0.87	0.71	0.57
Pimpernel	M_4	1982	0.70	0.57	0.68	0.66

Table 4. Coefficients of correlation between the various charcters. The asteriks show the level of significance (0.05 - 0.001). NS: not significant

			Plant height	Number of stems per plant	Yield of tubers total	Yield of tubers exceeding 50 g each
Total tuber yield	Kerrs Pink	M_3	0.59***	0.27***	-	0.83***
	" "	M_4	0.55***	0.73***	-	0.91***
	Pimpernel	M_4	0.20^{NS}	0.37**	-	0.98***
% dry matter	Kerrs Pink	M_3	0.08^{NS}	0.09^{NS}	-0.05^{NS}	0.05^{NS}
	" "	M_4	0.35*	0.49***	0.56***	0.56***
	Pimpernel	M_4	0.37**	-0.20^{NS}	0.39**	0.38**

the characters tuber yield and dry matter content, both of which show a fairly high heritability.

However, as chimeras were observed for tuber colour, chimeras are to be expected for the quantitatively inherited characters as well. Such chimeras will show up as a within clone variation in the succeeding generations. No such visual variation was observed. However, in order to detect the small variation caused by the genes behind these characters, the within clone variation would have to be calculated in a larger material.

A certain frequency of chimeras seems to be common among most regenerants from callus, whether developing directly from the explant or after passing a protoplast phase, regardless of irradiation (Van Harten et al. 1981, Ramulu 1986, Sonnino et al. 1987). This fact indicates that more than one cell participates in the bud initiation. In the present material the chimeras may partly be due to small undetected differentiated zones at the time of irradiation. By irradiating at an earlier stage, there was an obvious inhibition of regeneration. Such inhibition has been shown before in potatoes (Roest & Bokelmann 1980) as well as in other species. In order to reduce the frequency of chimeras this inhibition has to be overcome, probably by reducing the irradiation to a dose less harmful to regeneration as well as to the regenerants. With this precaution it should be possible to take advantage of the great variation which can be created by the method described here.

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DECOMPOSITION OF DAIRY MANURE IN THREE SOIL TYPES: INFLUENCE OF TEMPERATURE, LIMING AND COMPACTION

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Hanssen, J.F. & K. Myhr 1989. Decomposition of dairy manure in three soil types: influence of temperature, liming and compaction. Norwegian Journal of Agricultural Sciences 3: 289-299. ISSN 0801-5341.

The decomposition of dairy manure was observed in three different soil types (loamy sand, silt loam and organic soil) in greenhouse at 6, 12 and 18°C. The manure, enclosed in nylon litterbags, was embedded in double-walled lysimeter vessels. The soils were limed and compacted at two levels. 'Tevera' ryegrass was planted in each vessel.
Temperature was the most important factor influencing the extent of microbial decomposition in this experiment. Cellulose and hemicellulose decreased in all soil types, the maximum decrease being in organic soil. No decrease in lignin content was observed. The respiratory CO_2 evolution showed good correlation with the content of remaining organic material in the decomposed manure determined as COD (soluble) and sugar. Only minor influences of soil compaction and liming were observed on the decomposition of the dairy manure.
The experiments indicated that soil compaction in combination with manure application could harm plant growth when aeration was limited. This can be a practical problem in a cold and wet climate.

Key words: decomposition, manure, microbial activity, soil types, temperature.

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During recent decades an efficient and advanced animal husbandry has been built up in many coastal and mountain districts in Norway. Most of the concentrates, and some hay and straw, are shipped in from other parts of the country and from abroad. Suitable tilled land for manure application is in demand. On many farms large quantities of animal manure have to be spread on cold and wet compacted and acid soils of poor recipient capacity. The pores in the upper part of the soils have been sealed by manure and compaction caused by heavy machinery. The fields have become waterlogged, winter-killing has prevailed in grassland, and arable land has become difficult to manage because of enhanced soil wetness (Myhr 1984). Other soil properties, such as consistency, plasticity, strength, compactibility, penetrability, stickiness, and trafficability depend very much on the soil water content, especially in areas where the cli-

mate is cold and humid. Success in animal husbandry depends on adequate solutions for manure management and decomposition in the soil (McCalla et al. 1970). Water pollution must also be avoided (Bischoff 1984).

This investigation was conducted to elucidate the capability of different soil types to decompose fibre structures in cattle manure at varying temperatures, compaction and liming. The characteristics of the remaining material after a period of decomposition are used as a measure of degradation. This report deals with microbiological and chemical analyses of dairy manure before and after decomposition in the soil. Yields and chemical composition of plants are registered to illustrate the conditions for plant growth.

MATERIALS AND METHODS

Manure and litterbags

Faeces from dairy cows, later called manure, was sampled on two occasions (in 1986 and 1987). The excrement in each sample was thoroughly mixed and portions of 500 g were put into 150 x 150 mm bags made of 1mm mesh nylon cloth. Chemical characteristics of the original, untreated manure are referred to in Table 1 as an average for the two years. Total solids were 16.7% and the pH value was 6.5.

Table 1. Chemical characteristics of dairy manure at the start of the experiment, % of total solids. COD and sugar in g/kg TS

Ether extract	6.3	Ash	17.6
Lignin	7.6	Kjeldahl-N	3.4
Hemicellulose	24.1	P	1.2
Cellulose	17.7	K	1.1
Crude fibre	21.2	Ca	1.6
		Mg	0.6
COD (total)	910		
COD (soluble)	366		
Sugar (soluble)	43		

Differences in the chemical composition of the manure in the two separate samples were negligible, so are not dealt with in this report. Some of the experimental parameters were analysed for only one of the samples.

Experimental design and description

The individual experiments were carried out in greenhouse during wintertime and the following factors were investigated:

3 soil types; sand, loam and organic soil,
3 temperatures; 6, 12 and 18°C,
2 levels of soil acidity,
2 levels of soil compaction.

Each experiment was run in duplicate and consisted of 72 units.

Double-walled lysimeter vessels with 7.5 l volume for growth medium were applied for cultivation. The vessel height was 245 mm, lower diameter 180 mm and upper diameter 225 mm. The water-level could be checked through a transparent tube fitted at the bottom level. One litterbag with manure was embedded horizontally in each vessel 120 mm above the bottom, approximately in the middle of the soil volume. The temperature levels were obtained in separate chambers in a greenhouse.

The soil types were: loamy sand with 4% ignition loss, silt loam with 8% ignition loss, and organic soil with 44% ignition loss; the mineral fraction was loam.

To reduce soil acidity, ground limestone was mixed into the soil in one-half of the cultivation vessels before the experiments started. At termination, the pH values (measured in distilled water) for the untreated and treated soils were: loamy sand 6.0 and 6.8, silt loam 6.3 and 6.8, and organic soil 5.7 and 6.5.

A vibrator was used to secure even soil bulk density for the separate treatments. To obtain even soil compaction in the vessel a 5 kg piston was placed on top of the soil, layer for layer, while it was shaken mechanically. This treatment in-

creased air dry soil bulk density from 1.40 to 1.55 g/cm^3 for the loamy sand, from 1.30 to 1.45 g/cm^3 for the silt loam, and from 0.60 to 0.75 g/cm^3 for the organic soil.

Conditions for plant growth and manure decomposition
No fertilizer was applied in addition to the manure in the bags. Ten sprouting seeds of 'Tevera' ryegrass were planted in each vessel at the start of the experiment. After 10 and 20 days the plants were cut to promote tillering. Water was supplied in adequate quantities for plant growth, but never in such excess as to risk causing anaerobic zones in the lower part of the vessels. Artificial light for plant growth was supplied for 18 hours per day. In 1987 the experiment ended after 98 days, and in 1988 after 94 days. The ryegrass plants were cut 5 cm above the soil surface for dry matter registration and chemical analyses of the whole plants. The litterbags were taken out of the soil and were cleaned outside mechanically. The microbiological analyses were performed immediately. The material for chemical determination was kept at a low temperature until analysed. Ryegrass roots which had penetrated the nylon bags were removed from the manure and dried and weighed.

Chemical analyses
The fibre types lignin, hemicellulose and cellulose were analysed, according to Goering & Van Soest (1970), with Fibertec System equipment, for determining NDF (neutral detergent fibre), ADF (acid detergent fibre), and ADL (acid detergent lignin). Hemicellulose was calculated as the difference between NDF and ADF, and cellulose as the difference between ADF and ADL. Ether extractables, crude fibres, Kjeldahl-N and inorganic components were determined in accordance with common laboratory methods.

Plate counts
The viable bacterial number in the decomposed manure was determined by the plate count technique in two agar types - nutrient agar (Merck) and CMC-agar (20 g carboxymethylcellulose (Merck), 5 g peptone, 5 g yeast extract, 5 g NaCl, 1 g KH_2PO_4, 5 g $NaNO_3$, 15 g agar, 1000 ml distilled water). A quantity of decomposed manure was diluted to 1/100 in Winogradsky's salt solution (0.25 g K_2HPO_4, 0.13 g $MgSO_4$, 0.13 g NaCl, 2.5 mg $Fe_2(SO_4)_3$, 2.5 mg $MnSO_4$, 1000 ml distilled water), homogenized, and diluted further. Three replicates from each dilution were prepared and incubated at 20°C. The bacterial number was recorded after 1 and 2 weeks. On CMC-agar the numbers indicate bacteria producing cellulolytic enzymes.

Visualization of cellulolytic activity (Teather & Wood 1982)
Pure cultures of isolated strains from decomposed manure were inoculated on CMC-agar in Petri dishes. After incubation at 28°C for 3 or 5 days, the agar was flooded for 30 min with a solution of 1% Congo red in distilled water. The Congo red was poured off and the plates treated with 1 M NaOH for 15 min. The zones of CMC hydrolysis around the colonies were measured in millimetres.

Chemical oxygen demand (COD)
A mixture of decomposed manure and distilled water was homogenized and centrifuged at 15,000 g for 30 min. COD was determined in the supernatant. Ten millilitre samples were digested with dichromate, according to APHA (1971).

Sugar determination
The samples for sugar determination were prepared as for the COD-analyses. One millilitre of diluted supernatant was boiled for 15 min with anthrone reagent (APHA 1971). The absorption was measured at 620 nm. The sugar was calculated as glucose equivalents.

Respiratory CO_2 evolution from decomposed manure

A small pot containing 10 g decomposed manure (TS) was placed, along with a vial with 10 ml 1M NaOH, in a sealed 2 l glass jar. Before use the manure was equilibrated for one day in laboratory conditions.

At the start of incubation 10 ml of distilled water was added to each sample. Two replicates from each sample were incubated at 15°C. After 1, 2 and 4 weeks the jars were opened, the NaOH renewed, and the jar was left open for about 10 min to ensure replacement of air. The CO_2 evolution was observed over a period of 30 days. The CO_2 was analysed in an IR gas analyser (ADC, Hoddesdon, England). The NaOH was diluted to 100 ml with distilled water, 0.2 ml samples were acidified with 2 N H_2SO_4, and the CO_2 evolved was pumped into the measuring cell. For calibration a standard gas from Norsk Hydro with 300 ppm CO_2 was used. The CO_2 evolved during the respiration period was calculated as mg CO_2 - C per g decomposed manure (TS), and is a measure of the respirability of the remaining material.

RESULTS

Degradation of total and volatile solids

The degradation of total (TS) and volatile solids (VS) in dairy manure in different soil types at increasing temperatures and liming is reported in Table 2. TS and VS are given on the basis of weights per cultivation vessel.

The values were calculated in relation to a number of frozen check samples where no degradation had occurred. The ash content in the manure was not supposed to be reduced, but recycled by the micro-organisms present. In these experiments, however, the ryegrass plants were able to utilize the mineral nutrients in the manure as fertilizer. However, calculations showed that the average amount of minerals in the manure samples was kept relatively constant during the growth experiments.

Rising temperatures increased TS and VS reduction significantly in all three soil types. The reduction was signi-

Table 2. Effects of soil type, temperature and liming on degradation of total (TS) and volatile solids (VS) of dairy manure. Relative values for different treatments calculated at termination of experiments. Check denotes corresponding frozen samples in g per litterbag

Temperature and liming	Loamy sand		Silt loam		Organic soil	
	TS	VS	TS	VS	TS	VS
Check, g per bag	84	69	84	69	84	69
Check, relative	100	100	100	100	100	100
6°C	96	93	87	83	78	78
12°C	90	84	80	75	62	62
18°C	78	74	66	59	56	55
LSD 5% 1)	6	5	6	5	6	5
Non-limed soil	90	84	82	76	68	67
Limed soil	88	83	75	69	63	62
LSD 5% 1)	5	4	5	4	5	4

1) Check not included in the analyses of variance

ficantly greater in organic soil than in mineral soils, and significantly greater in silt loam than in loamy sand. Liming increased TS and VS reduction significantly in silt loam and organic soil. In loamy sand, too, there was a tendency to enhanced reduction of these fractions after liming. Compaction did not cause any significant change in the TS and VS contents of the litterbags.

Chemical composition of decomposed manure

The relative contents of some manure components before and after decomposition at different soil conditions are given in Table 3.

Ether extractable materials were significantly reduced in all types of soil. Furthermore, increasing temperatures increased the degradation significantly. Soil type, liming and soil compaction had no effect on the relative content of the ether extracts.

Lignin had a significant rise in relative content during the period of decomposition. The effect of temperature was significant for all soil types. In silt loam and organic soil the relative lignin content was higher than in loamy sand. Liming resulted in higher relative lignin content, whereas soil compaction had no such effect.

The relative content of hemicellulose was kept approximately constant during the degradation period. The relative cellulose content was significantly reduced in all types and at all temperatures. Rising temperatures increased the reduction of cellulose. The cellulose content was lowest in silt loam. Liming was shown to increase the decomposition of cellulose. Compaction had no effect. Figure 1 shows how the absolute values of the cellulose and hemicellulose fractions decrease during the experiment. No similar reduction in lignin content was found. The reduction in crude fibres is a result of cellulose and hemicellulose reduction. The relative content of ash increased when the volatile solids in the manure were degraded.

Bacterial numbers

The plate count numbers in nutrient agar of the decomposed manure in the litterbags are given in Table 4. Compared with newly collected manure higher numbers of bacteria were found. On comparing the different soils and tem-

Table 3. Effects of soil type and temperature on some components of dairy manure, % of dry matter at termination of experiments. Check denotes corresponding frozen samples

Soil type	Temp. °C	Ether extr.	Lignin	Hemi-cellul.	Cellu-lose	Crude fibre	Ash	Kjeldahl N
Check	<0	6.3	9.2	26.0	17.7	21.2	17.6	3.4
Loamy sand	6	2.3	9.8	26.2	16.0	20.8	19.9	2.7
	12	2.2	12.2	25.2	14.6	20.7	23.7	2.8
	18	1.5	13.5	25.0	13.1	19.7	22.8	3.0
Silt loam	6	2.1	11.8	24.6	13.4	19.1	21.6	3.1
	12	1.8	14.0	24.3	12.1	18.9	22.5	3.2
	18	1.5	15.0	23.5	11.0	18.1	23.6	3.2
Organic	6	3.0	10.8	25.9	16.2	21.7	18.9	2.8
	12	2.0	13.9	26.5	13.5	20.7	19.7	2.9
	18	1.5	14.5	24.9	12.7	20.0	20.9	3.0
LSD 5% 1)		0.2	1.2	1.4	1.1	0.8	1.0	0.3

1) Check not included in the analyses of variance

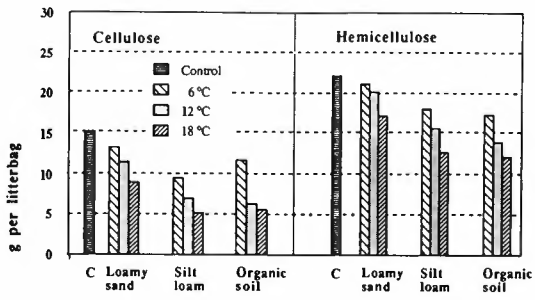

Figure 1. Total contents of cellulose and hemicellulose in dairy manure after decomposition in three soil types at 6, 12 and 18 °C. Control (C) - value at start

perature treatments only small variations in bacterial numbers could be seen. However, the number of bacteria seemed to be highest in the material after incubation in organic soil. The highest values of viable bacteria were generally found at the lowest temperature.

No regular differences dependent on soil and temperature were found on CMC-agar (Table 5). It is possibly indicated that the cellulose decomposing bacteria are present in lowest numbers in the manure embedded in organic soil. Table 6 shows the CMC-ase activity detected by the Congo red method from bacteria isolated from the litterbags. The clearing zone around the colony shows the activity of extracellular CMC-ase. A larger proportion of CMC-ase positive organisms, as well as the highest number of the most active organisms, were found in the manure embedded in organic soil.

Respiratory CO_2 evolution

The respiration activities of the decomposed manure are a measure of the degradability of the remaining material in the litterbags. The values are given in Table 7. In all series the lowest activity was found after treatment at 18°C, indicating that the most limited amount of material was left for respiration at the highest degradation temperature. The respiration activity of the remaining material increased regularly with de-

Table 4. Number of viable bacteria in decomposed manure from litterbags (per g TS, grown on *nutrient agar* at 20°C)

Temperature °C	Loamy sand	Silt loam	Organic	Mean
6	$6.7 \cdot 10^9$	$8.1 \cdot 10^9$	$7.1 \cdot 10^9$	$7.3 \cdot 10^9$
12	$3.1 \cdot 10^9$	$4.9 \cdot 10^9$	$8.8 \cdot 10^9$	$5.6 \cdot 10^9$
18	$4.3 \cdot 10^9$	$4.3 \cdot 10^9$	$6.2 \cdot 10^9$	$4.9 \cdot 10^9$
Mean	$4.7 \cdot 10^9$	$5.8 \cdot 10^9$	$7.4 \cdot 10^9$	

Table 5. Number for viable bacteria in decomposed manure from litterbags (per g TS, grown on *CMC agar* at 20°C)

Temperature °C	Loamy sand	Silt loam	Organic soil	Mean
6	$2.6 \cdot 10^9$	$2.0 \cdot 10^9$	$0.4 \cdot 10^9$	$1.7 \cdot 10^9$
12	$1.1 \cdot 10^9$	$1.0 \cdot 10^9$	$0.5 \cdot 10^9$	$0.9 \cdot 10^9$
18	$1.7 \cdot 10^9$	$4.3 \cdot 10^9$	$0.3 \cdot 10^9$	$2.1 \cdot 10^9$
Mean	$1.8 \cdot 10^9$	$2.4 \cdot 10^9$	$0.4 \cdot 10^9$	

Table 6. Frequency of CMC-ase producing bacteria detected by Congo red test. Bacteria picked up randomly from CMC-agar plates

Soil type	No. of strains tested	CMC-ase positives zone diameter			CMC-ase positives / Total viable count
		4-8 mm	8-12 mm	>12 mm	
Loamy sand	36	5	5	3	0.36
Silt loam	36	17	4	2	0.64
Organic soil	36	16	4	6	0.72

Table 7. Respiratory CO_2 evolution, COD and sugar content of decomposed dairy manure embedded in different soil types

Soil type	Temp. °C	Resp. mg CO_2-C per g TS	COD mg/g TS	Sugar mg/g TS
Loamy sand	6	24	29	13
	12	22	18	11
	18	16	21	8
Silt loam	6	16	30	16
	12	13	24	14
	18	9	20	10
Organic	6	28	36	14
	12	26	24	9
	18	20	17	4
LSD 5%		3	10	6

creasing temperatures in the three soil types.

The mean values for all soils and temperature treatments are given for some parameters in Table 8. Concerning respiratory CO_2 evolution, significant decreasing values were found with increasing temperatures.

COD

The COD-value of the soluble organic material from undecomposed manure was very high (Table 1). During decomposition the values decreased by more than 90% of the start value in all three soil types (Table 7). The highest value was found with decomposed manure embedded in organic soil at 6°C. During the experimental period soluble organic substrates were utilized as carbon and energy sources by the heterotrophic microflora.

There was a positive correlation between increasing respiratory CO_2 evolution and COD values in the water extracts from all three soil types (Table 8). This indicated that the soluble organic material was the main source for the respiration activity of the micro-organisms.

Sugar

Total sugar was measured in water extracts from the decomposed manure. For the sugar analyses, too, the highest values were found at the lowest experimental temperatures (Table 7). No sig-

Table 8. Mean respiratory CO_2-evolution. COD and sugar content in decomposed dairy manure embedded in soil at different temperatures

		6°C	12°C	18°C	LSD (5%)
Respiration	(mg CO_2-C/g TS)	28	20	15	2
COD	(mg O_2/g TS)	31	22	17	4
Sugar	(mg glucose eqv./g TS)	15	11	7	3

nificant differences were found between the three soil types regarding the content of free sugars in the decomposed manure. The mean values for all experiments (Table 8) revealed an increased sugar content as the temperature decreased.

Plant growth and root infiltration

Ryegrass was grown in the soil where the manure decomposition took place. The plants appeared to be healthy and vigorous during the experimental period. Growth and development were highly dependent on the temperature. At 6°C vegetative growth dominated, and no head had appeared by the time of harvest. At 12 and 18°C the plants were taller and most had heads at the time of termination. Those ryegrass roots which had invaded the litterbags were separated manually from the manure, cleaned and dried for TS registration. The results are presented in Table 9.

Ryegrass TS yield was significantly higher at 12°C than at 6 or 18°C. At the 6°C level the yield was significantly lower on silt loam than on loamy sand and organic soil. Soil compaction harmed plant growth and reduced TS yield on silt loam, but not on the other two soil types represented in the investigation. The highest yields of root mass in the litterbags were registered at 12°C. In silt loam only small root quantities were found in the litterbag.

The chemical composition of the ryegrass TS reflected plant development and yield level. The significantly highest concentrations of N, P and K were found in plants grown at 6°C.

DISCUSSION

The decomposition of manure in soil is a complex biological process in which different types of heterotrophic microorganisms participate. In the dairy manure used (faeces without urine and litter), hemicellulose, cellulose and lignin constituted nearly 60% of the organic material, whereas the contribution of soluble sugars was approximately 5.4%. These sugars are excellent carbon- and energy sources, and will initiate a rapid and significant microbial activity in the soil. When this supply is exhausted, further development depends on degradation of biopolymers. The protein content was calculated to be ca. 24% of the organic material. This amount seems to be reduced by approximately 1/10 during the degradation period, and

Table 9. Effects of soil type and temperature on ryegrass yield, ryegrass roots in litterbags, and chemical composition of ryegrass plants

Soil type	Temperature °C	Kg TS per hectare		Per cent of TS		
		Ryegrass	Roots	N	P	K
Loamy sand	6	3680	54	3.10	0.42	4.53
	12	6830	148	1.93	0.34	3.87
	18	5760	124	2.00	0.39	4.32
Silt loam	6	2510	18	3.31	0.41	4.66
	12	6780	50	2.12	0.31	3.98
	18	5760	32	2.19	0.32	4.26
Organic	6	3500	32	2.72	0.41	4.38
	12	6800	104	1.80	0.37	3.98
	18	6270	92	1.88	0.38	4.11
LSD 5%		570	15	0.34	0.03	0.31

will of course contribute as a substrate for micro-organisms.

The amount of ether extractables was greatly reduced. From composting at higher temperatures of wastes from slaughterhouses (Molland 1982) lipid degradation was found to occur rapidly. Faecal wastes contain food lipids and also lipids from intestinal bacteria and these can amount to some 5-10% of the bacterial weight (Hobson et al. 1981). Probably a common feature of organisms associated with faeces degradation is that they produce lipases and lipoxydases.

The crude fibre part of the dry matter decreased during the experiment. Results showed that the decrease was mainly due to degradation of cellulose and hemicellulose, whereas the amount of lignin was probably unaffected.

A significant difference was found between the soil types in their ability to affect the degradation of the added manure. Organic soil showed the greatest overall activity (Table 2). This is consistent with the fact that organic soil generally has a higher biological activity than more inorganic soils. Improved microbiological activity in the soil consequently seems to be advantageous for manure degradation. In practical agriculture, where the manure is more evenly distributed in the soil, this effect will probably be even bigger. However, in many coastal and mountain districts the cultivated organic soil is often wet and acid (pH below 5), cold and compacted. Under such conditions microbial activity is reduced. Manure particles clog the pores and the production of bacterial slime closes the natural waterways through the soil profile. Water from rain and melting snow infiltrates the topsoil slowly, and the field becomes watersoaked.

The soil temperature strongly influenced the degradation of the organic material in the litterbags. By increasing the temperature from 6 to 18°C the degradation of organic materials in all three soils was increased by approximately 20%. At 12°C the increase was halved. This, of course, clearly points out the difficulties that can arise after application of cow manure to agricultural land at low temperatures (McCalla et al. 1970).

Some influence of liming on decomposition was indicated (Table 2). Jenkinson (1977) found that decomposition of organic matter was reduced in soil when pH was below 4. In our experiments a reduction was already seen in the pH range 6.3-5.7, depending on the soil type.

The microbial activity in the rumen digestive system is anaerobic. The solubilization of plant cells in the rumen is accomplished by the concerted action of a wide range of different microbial species including bacteria, protozoa and anaerobic fungi (Chesson et al. 1986, Gijzen et al. 1988, Orpin 1984). On leaving the animal, the faeces is inoculated with micro-organisms from the environment. In this rich substrate facultative anaerobic and aerobic bacteria proliferate at the expense of the organic matter in manure. Relatively small variations were found on comparing the bacterial counts in the different litterbags. However, the numbers were significantly higher than those normally found for untreated soil on the same medium (10^6). Godden et al.(1983) found that during composting of cattle manure viable counts of bacteria were 10^8, and 10^7 for actinomycetes. In soil, fungi are responsible for a great part of the degradation processes. In our investigations the development of fungal biomass was not followed up.

Cellulolytic micro-organisms are common in manure and soil. Among them are organisms active in highly different environmental conditions with regard to temperature, oxygen and pH. Godden et al.(1983) studied cellulase activity during composting of cattle manure and found an increasing activity during the mesophilic period. We found that degradation of cellulose increased signi-

ficantly when the temperature increased from 6 to 18°C.

Aerobic bacteria able to grow on CMC-agar show different patterns in the Congo red test because of quantitative differences in extracellular enzyme production. A positive test shows that the cells produce extracellular endocellulase that hydrolyse ß-(1,4)- D-glucans (Teather & Wood 1982). Typical colonies of actinomycetes were observed on CMC-agar.

Well-known lignin-degrading microorganisms are aerobic and belong to the fungi. Some anaerobic bacteria are able to degrade parts of the lignin molecules (Coldberg & Young 1985, Zeikus et al. 1982). In recent years ruminal fungi have been shown to be significant colonizers of fibre (Akin & Rigsby 1987). In our experiments no decrease in lignin content was observed.

The respiratory CO_2 evolution of the decomposed manure indicated that different amounts of substrates were available after decomposition. The largest amounts were found at the lowest temperature. Comparing COD and sugar in the soluble part of the manure with the CO_2 evolution, a good correlation was observed. If we assume that almost all the COD (soluble) is a possible substrate for respiration, water soluble organics like sugars, amino acids and volatile fatty acids can be considered as substrates. Obviously not all the CO_2 evolved from the respiration is caught in the CO_2 trap. Some CO_2 is fixed by the cells, and some is dissolved in the soil water. However, the values of COD (soluble) indicate that non-soluble compounds are also respired during the incubation period of 30 days. During degradation of the manure in the litterbags we found a decrease in both cellulose, hemicellulose and ether extractables. For all three soil types the plant growth temperature at 18°C gave the highest decrease in fibre content. By comparing the values of respiratory CO_2 evolution at different temperatures with corresponding COD and sugar values, the relationships between soluble organics and respiration were clearly demonstrated.

Nutrient cycling in soil is closely related to organic matter turnover. Microbial activity plays a key role in the mineralization and immobilization processes of plant nutrients. Application of large quantities of untreated liquid manure on fields with poor recipient capacities obviously causes reduced plant growth. Our decomposition experiments in greenhouse clearly demonstrated that decomposition of manure in organic soil worked well in aerated, compacted soil in spite of low pH. However, we know that compacted organic soil in coastal districts, with pH below 5 and when watersoaked, behaves differently under natural conditions.

Generally the amount of the roots in the litterbags reflected the plant green mass. However, in silt loam this root mass was greatly reduced compared with other soils. This emphasizes the point that dairy manure can be prohibitive to root development during soil conditions, leading to restricted air supply. It seems a reasonable recommendation that there should be some kind of degradation of liquid manure before application to fields. We assume that microbial growth on easily decomposable organics in the manure causes an oxygen shortage for plant roots, especially in organic soil in districts with a combination of low mean summer temperature and wet climate.

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