

To determine the effect of the *in vitro* fermentation technique on the fibre fraction of five samples of temperate hay and straw.

#### Introduction:

The *in vitro* fermentation technique has been used extensively in the Livestock Section for the last eighteen months. A lot of information has been obtained on a wide range of subjects ranging from the effects of anti-nutritive factors on fermentation to the supplementation of poor quality feeds with concentrates. However we still know very little about the kinetics of the fermentation, which fractions of the feed are being digested and to what extent. The aim of this experiment was to analyse the five samples before and after fermentation and thereby determine the effects of the fermentation on the individual feed fractions.

#### Methods:

Barley straw, Lucerne hay, Meadow hay, Rye hay, and Timothy hay were analysed (according to Livestock Section methodologies) for CP, Ash, Moisture, NDF, ADF, Lignin, CP in NDF, and CP in ADF. Hemicellulose was calculated as the difference between NDF and ADF. Cellulose was calculated as the dry weight loss in the muffle furnace after lignin determination. These analyses were performed sequentially on each sample. Dry matter digestibility (DM deg 1) was determined by Tilley and Terry and apparent digestibility (DM Deg 2) and gas pool size by *in vitro* fermentation.

F20: The fermentation technique was carried out according to the Chris Wood methodology. Three replicates of each sample were taken. Sample bottles were autoclaved immediately after the final gas reading had been taken and then kept warm until they could be filtered through porosity 2 sintered glass crucibles. Filtrates were washed three times with water and three times with acetone and dried overnight at 103 °C. After weighing, dried filtrates were removed from crucibles, pooled and ground in a mortar and pestle to break up the samples and ensure homogeneity. It was assumed that the residue was 100% dry matter.

The residue was subjected to NDF, ADF, Lignin, Cellulose and Hemicellulose analysis using the same methods as above.

#### Results:

Analytical data obtained before fermentation is shown in Table 1. Data obtained after fermentation is shown in Table 2.

Fermentation curves showing cumulative gas produced over the assay period are shown in Figure 1. Rates of gas production are shown in Fig 2 and the relationship between total gas pool and apparent dry matter digestibility is illustrated in Fig 3.

#### Discussion:

A number of interesting points may be observed from the data in Table 1. The percentage lignin was highest in Lucerne and Timothy (11.11% and 9.34% respectively). Both were higher than Barley straw (7.13%), which was not expected. Cellulose content was similar for all four hays and ranged from 28.36% for Lucerne to 32.73 for Timothy, the Barley straw being higher at 39.64%. Hemicellulose was highest in Barley (37.18%) and lowest in Lucerne (14.37%).

Lucerne hay would appear to be the odd one out having the highest lignin, crude protein, ash and moisture and the lowest cellulose and hemicellulose contents. The fact that it also has the highest digestibility by the Tilley and Terry method (64.02%) may indicate that digestibility by this method is strongly influenced by protein content of the feed. Although the data presented are very limited, Tilley and Terry digestibility does appear to vary with CP content (possibly something to do with the secondary stage - acid pepsin digestion ?)

Results from the Hurley *in vitro* technique are substantially higher than those obtained by the previously discussed method. Due to the extended incubation period it is possible that adaptation of certain bacterial species has allowed a greater proportion of the fibre fraction to be broken down. Barley straw has the highest digestibility by this method (and also has the highest cellulose+hemicellulose !).

Table 4 shows the percentage loss in fibre fractions during fermentations when expressed as a % of the original fractions. Except for lignin, the % loss of each fraction is relatively constant for each sample species. Again, Lucerne hay is different to the others. Since the % loss of fibre during fermentation is considerably lower in Lucerne than the other samples it should follow that its overall digestibility would also be lower which is not the case. Possible explanations of this are

- (1) high lignin content interfering with fibre digestion
- (2) compensation by the high CP which may itself be fermentable
- (3) fermentation of feed components not accounted for i.e. soluble sugars etc.

Table 5 shows a mass balance on the five samples prior to fermentation. The six analyses illustrated account for 100% of Barley straw (just over !). For the four hays, there remains between 9.52% and 12.58% still unaccounted for. It is suspected that the majority of the remainder may be soluble sugars and starch although a small amount of fat (ether extract) may also be present. In future, these sol sugars will have to be estimated as they are likely to have an influential effect on the early hours of fermentation. To support this idea Figure 2 shows rates of gas production. The initial few hours of fermentation are interesting in that all samples have a sharp high peak which should represent the utilisation of rapidly fermentable sugars. The peak height for each sample would appear to be directly related to the remainder after mass balance i.e. soluble sugars and starch.

Table 6 shows that the majority of dry matter remaining after fermentation may be accounted for by lignin + cellulose + hemicellulose. The remainder, between 2.5% and 3% of the original DM (except Luceřne) may be residual protein or ash.

**Table 1: Analytical data from five samples of hay and straw (before fermentation)**

	% NDF	% ADF	%Lignin	% Cellulose	% Hemicellulose	% CP	% Ash	%Moisture
Barley straw	83.78	46.6	7.13	39.64	37.18	5.15	4.71	7.5
Leucerne hay	53.27	38.9	11.11	28.36	14.37	14.88	8.62	10.28
Meadow hay	63.68	36.21	6.05	30.21	27.47	7.93	6.81	8.95
Rye hay	64.71	37.07	5.93	31.39	27.64	8.58	7.26	9.68
Timothy hay	71.48	42.83	9.34	32.73	28.65	5.28	4.03	8.59

	% CP in NDF	% CP in ADF	DM deg 1*	DM deg 2*	Gas pool 2
Barley straw	2.77	2.35	50.45	75.24	292
Leucerne hay	3.47	2.69	64.02	69.58	268
Meadow hay	2.82	2.06	58.13	73.87	286
Rye hay	2.93	1.92	57.79	74.95	282
Timothy hay	1.99	1.76	51.87	66.68	268

**Table 2: Analytical data from five samples of hay and straw (after fermentation)**

	% NDF	% ADF	% Lignin	% Cellulose	% Hemicellulose
Barley straw	22.27	13.39	5.13	8.15	8.88
Leucerne hay	34.48	26.5	9.74	13.73	7.98
Meadow hay	23.67	14.48	4.91	9.45	9.19
Rye hay	22.37	13.63	4.83	8.56	8.74
Timothy hay	30.79	19.97	6.82	13.09	10.82

\*1=Tilley and Terry

\*2=Hurley fermentation technique

**Table 3: Percentage loss in fibre fractions during fermentation (expressed as % of total)**

	% NDF	% ADF	% Lignin	% Cellulose	% Hemicellulose
Barley straw	61.51	33.21	2	31.49	28.3
Leuceme hay	18.79	12.4	1.37	14.63	6.39
Meadow hay	40.01	21.73	1.14	20.76	18.28
Rye hay	42.34	23.44	1.1	22.83	18.9
Timothy hay	40.69	22.86	2.52	19.64	17.83

**Table 4: Percentage loss in fibre fractions during fermentation (expressed as a % of original fraction)**

	% NDF	% ADF	% Lignin	% Cellulose	% Hemicellulose
Barley straw	73.42	71.27	28.05	79.44	76.12
Leuceme hay	35.27	31.88	12.33	51.59	44.47
Meadow hay	62.83	60.01	18.84	68.72	66.55
Rye hay	65.43	63.23	18.55	72.73	68.38
Timothy hay	56.93	53.37	26.98	60.01	62.23

**Table 5: Mass balance before fermentation**

	Barley	Leucerne	Meadow	Rye	Timothy
Moisture	7.5	10.28	8.95	9.68	8.59
Ash	4.71	8.62	6.81	7.26	4.03
CP	5.15	14.88	7.93	8.58	5.28
Lignin	7.13	11.11	6.05	5.93	9.34
Cellulose	39.64	28.36	30.21	31.39	32.73
Hemicellulose	37.18	14.37	27.47	27.64	28.65
<b>Total</b>	<b>101.31</b>	<b>87.62</b>	<b>87.42</b>	<b>90.48</b>	<b>88.62</b>
Remainder	-1.31	12.38	12.58	9.52	1.38

**Table 6: Mass balance after fermentation**

	DM remaining after fermentation %	Hemicellulose + cellulose + lignin %	Remainder
Barley	24.76	22.16	-2.6
Leucerne	30.42	31.45	1.03
Meadow	26.13	23.55	-2.58
Rye	25.05	22.13	-2.92
Timothy	33.32	30.73	-2.59

**Figure 1: Cumulative gas produced during in vitro fermentation of five samples of straw and hay**

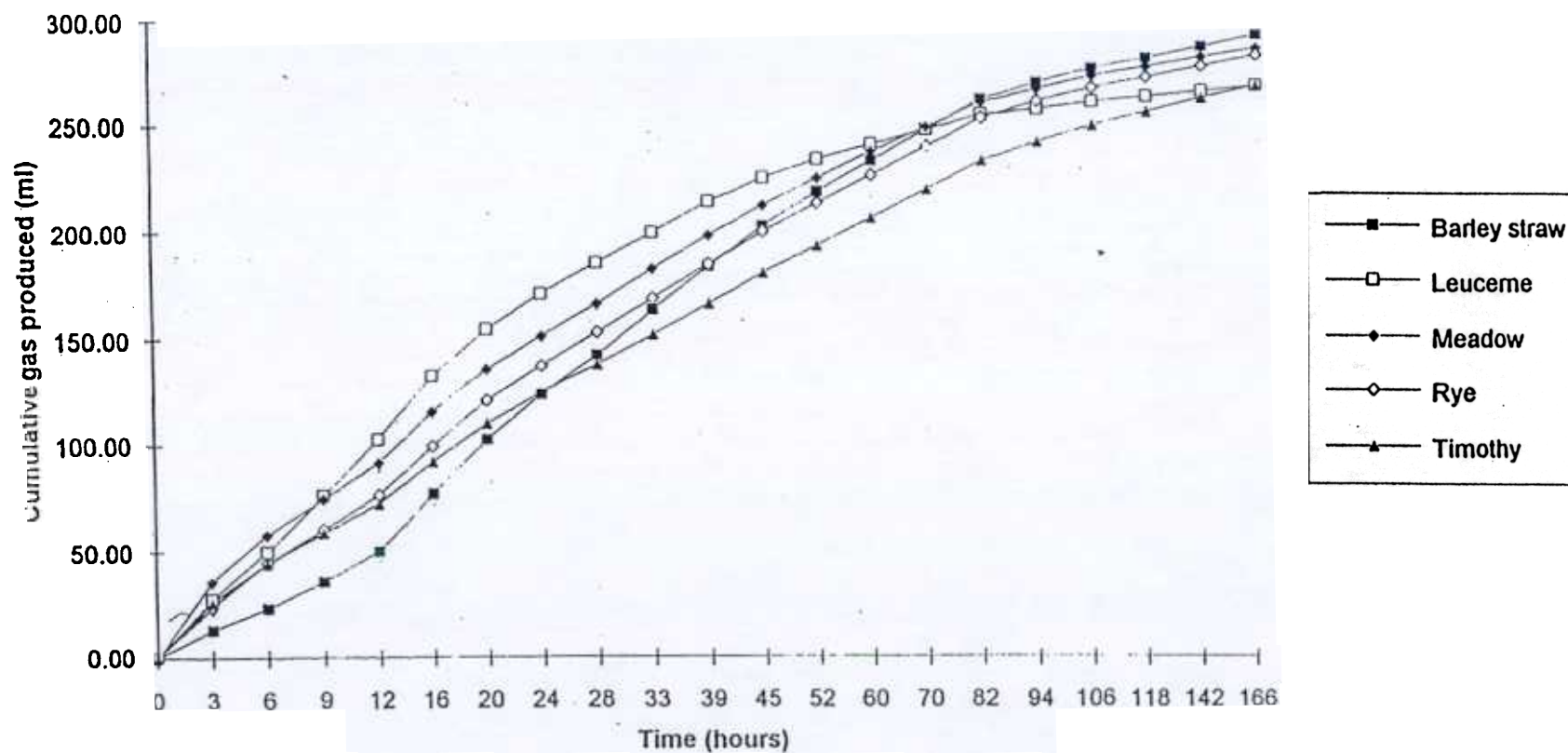


Figure 2: Rate of gas production (ml/hr) during in vitro fermentation of five samples of hay and straw

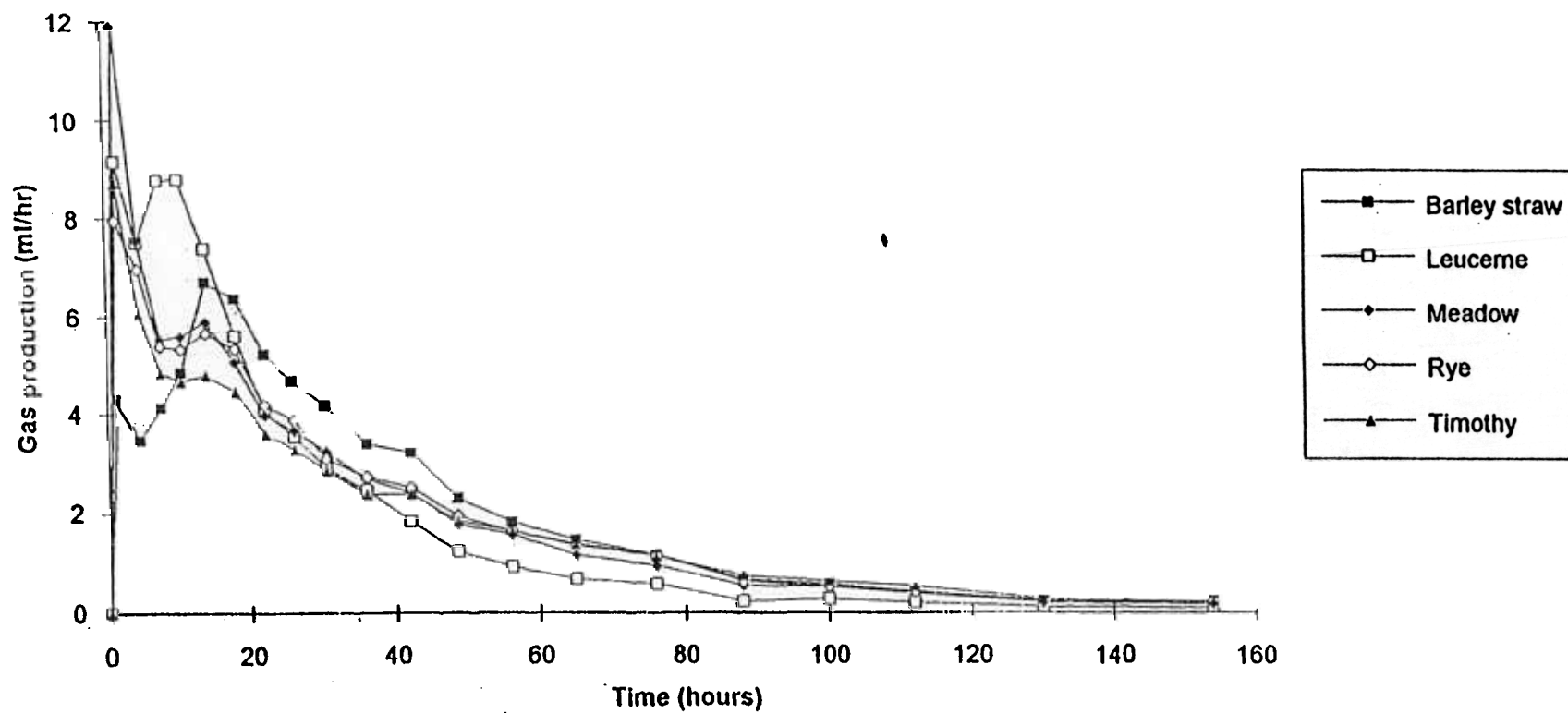




Figure 3: Changes in total gas pool size with apparent DM digestibility

