**PROJECT REPORT No. 273**

# NUTRITIVE VALUE OF WHEAT FOR RUMINANTS: AN INDEX FOR RANKING WHEAT VARIETIES

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# NUTRITIVE VALUE OF WHEAT FOR RUMINANTS: AN INDEX FOR RANKING WHEAT VARIETIES

by

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# Contents

## Abstract

1. Traditionally, the nutritive value of wheat for ruminants has been described almost entirely in terms of an energy and protein value and the former in particular has been regarded as more or less constant. In wheat, the greatest proportion of its dry matter is starch. The role of starch in the diets of ruminants has become the subject of considerable debate because it appears to have a role in enhancing milk protein content. It is vital to know not only how much starch a feed provides but also the proportion of that starch which is likely to be fermented in the rumen.

2. The extent to which wheat varies in starch content and nature, together with the factors which affect these is not known with any certainty. The objective of this study was therefore to examine the effect of year, site and variety on chemical composition, endosperm texture and nutritive value of wheat for ruminants.

3. A total of 66 wheats harvested in 1997, 1998 and 1999 from six RL sites (Cockle Park, Cambridge, Norfolk, Bridgets, Harper Adams and Cornwall) with six different varieties (Hereward, Reaper, Rialto, Consort, Madrigal and Riband) were obtained and studied. An *in vitro* gas production (GP) system was used to simulate rumen fermentation to assess the proportion of the starch in the wheat fermented in the rumen (RDS). Near infrared reflectance spectroscopy (NIRS) was examined as a possible rapid means of predicting starch quality and other factors.

4. The wheats showed a wide range of nitrogen and starch contents and notably, these two fractions were negatively related. Since it is known that nitrogen content can be influenced by nitrogen fertiliser this offers the possibility that starch content may be manipulated by agronomic management.

5. RDS disappearance for wheat grains is an important characteristic of the nutritive value of wheat for ruminants. This value is influenced by year of harvest, site of growth and variety. There was no site x variety interaction. The varieties were ranked in 1997 as Hereward<Consort<Reaper, in 1998 there were no variety effects and in 1999 as Riband<Rialto< Hereward<Madrigal<Consort<Reaper.

6. There were no significant correlations between any of the GP parameters and either the chemical composition or the grain quality parameters. Nitrogen content was highly negatively correlated with starch content ($r = ^{-}0.55$). NIRS was able to predict grain hardness, N content and endosperm texture, but was unable to reliably predict any of the *in vitro* parameters.

7. This work has highlighted the fact that the ability to supply starch to the rumen or post-ruminally is a key aspect of the nutritional quality of wheat and that it can vary substantially with variety and site of growth. It was not possible to develop a rapid method of assessment for the nutritive value of wheat. It is recommended that RDS determined using the *in vitro* GP technique is carried out for samples from the RL variety testing programme and this parameter along with starch content and endosperm texture be incorporated into the Recommended Variety lists.

# 1.0 Introduction

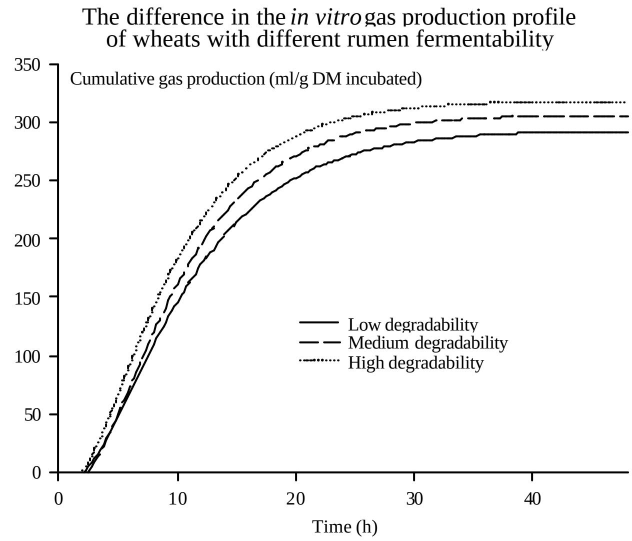
Current HGCA-funded UK Recommended Lists for cereals include grain quality parameters for each variety, by region, indicating their potential value to millers, maltsters, bakers and exporters. However, the information provided in the UK Lists provide relatively little indication of the nutritive value of wheat as a feed for ruminant livestock. MAFF statistics indicate that wheat often constitutes less than 10% of raw materials used in the manufacture of animal feeding stuffs for ruminants, a level which is well below the maximum that may safely be incorporated into ruminant diets. A better description of the nutritional value of wheat would identify greater opportunities for the incorporation of wheat into ruminant diets, at the expense of alternative ingredients, many of which are imported.

UK ruminant livestock farmers are facing increasing economic and environmental pressures. To counter these pressures, feed formulation needs to focus on maximising the utilisation of dietary nutrients. To this end, wheat is increasingly being looked to as a means both of improving dietary nitrogen capture by rumen micro-organisms and providing additional energy (in the form of rumen by-pass starch) to high yielding dairy cows.

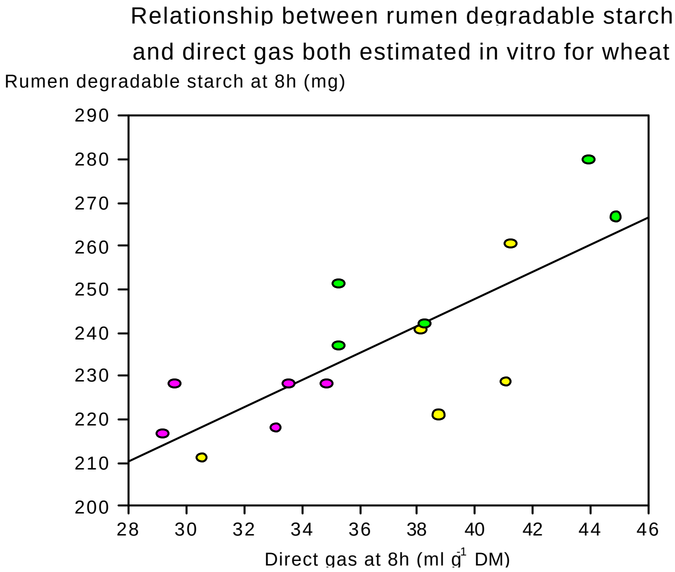
Recent HGCA-funded studies (Factors Affecting the Nutritive Value of Wheat for Ruminants, Project Report No. 182) undertaken at the then ADAS Feed Evaluation and Nutritional Sciences (FENS) examined the nutritive value of wheat and maize using an *in vitro* gas production technique. These studies demonstrated that:

- Current measures of energy and protein do not adequately describe the nutritional value of wheat in ruminant diets

- The *in vitro* gas production technique can differentiate between wheat samples in terms of the amount of starch available for rumen fermentation, as illustrated below:



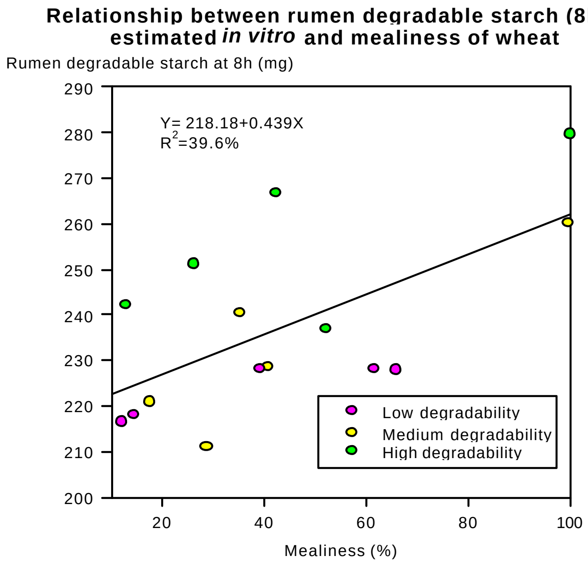

As illustrated below, the studies also demonstrated the ability of the gas production technique to predict the rumen degradability of wheat starch.




- Most of the grain comprises starch. The amount of starch present, coupled with extent of starch fermentation in the rumen are critical determinants of starch 'quality' and largely determine the nutritive value of wheat for ruminants.

- Nutritive value measured was not related to any of the grain quality parameters routinely reported for wheat e.g. specific weight, thousand grain weight, Hagberg falling number, Zeleny.

- The nutritive value of wheat for ruminants (defined as starch fermentability in the rumen) was related to starch content, grain hardness and endosperm texture. The figure below illustrates the relationship observed between the texture of the endosperm and degradability of the starch in the rumen. The low correlation reflects the relatively small number of samples.




- Specific weight is a poor measure of nutritive value and is highly variety dependent.

Starch content can be chemically determined while grain hardness, which is mainly genetically determined, can be determined using near infra-red reflectance spectroscopy (NIRS). These studies have further confirmed that endosperm texture can be determined using light transflectance, but this currently only provides a subjective assessment of texture, and additional work is required to provide a more objective and quantifiable measure of texture.

A key finding of the HGCA funded study "Factors Affecting the Nutritive Value of Wheat for Ruminants" (Project Report No. 182) was, that the rumen degradable starch content of wheat could be estimated from *in vitro* gas production directly and that this relationship was improved when grain hardness and/or endosperm texture were included. Because of the nature of the sample set, which did not come from specifically designed experiments, the effect of variety *per se* on nutritive value could not be examined. There is a large volume of literature reporting chemical composition of grains and variation in the nutritive value, but the effects due to genotype and environment from which the samples were sourced can not be differentiated (O'Brien, 1999). Opatpatanakit *et al.* (1994) showed significant variety effects using the gas production technique for total gas production and significant site effects and no interaction. Garnsworthy and Wiseman (1999) used near-isogenic lines of wheat that varied in only one or two known characteristics and grown under the same agronomic conditions. There was no effect on the rumen digestion of starch when only one characteristic differed (i.e. Hard v Soft, 1B/1R v non-1B/1R) but rumen starch degradability was significantly lower for Soft non-1B/1R and Hard 1B/1R. For many farmers, variety is the single most important factor in deciding which wheat to grow and therefore it is essential that the effect of variety on nutritive value is understood, and how this may be influenced by harvest year. Additionally, because of the effects of variety on hardness and environment on endosperm texture, the objective of the project is to study the effects of variety, site and harvest year on the nutritive value of wheat.

# 2.0 Materials and Methods

## 2.1 Samples

### *2.1.1 In vitro gas production*

A total of 66 samples was obtained from existing from HGCA/NIAB Recommended List trials, from the 1997, 1998 and 1999 harvest. The material was selected to cover a wide range in terms of both genotype (varieties) and environments (site). Details of the samples collected are shown in Table 1. Samples were stored fresh in airtight containers at room temperature.

Table 1. Year, site, variety and identification number of wheats used.

| Year: | 1997 | | | 1998 | | | | | 1999 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site*: | CP | Br | Cor | CP | Ca | Br | HA | Nor | CP | Ca | Br | HA | Nor |
| a)Hard wheat | | | | | | | | | | | | | |
| Reaper | AB59[1] | AB54 | AB64 | CA3 | CA26 | CA19 | CA17 | CA11 | CB99 | CB37 | CB92 | CB27 | CC43 |
| Rialto | | | | CA5 | CA25 | CA24 | | CA9 | CB97 | CB41 | CB96 | CB31 | CC40 |
| Hereward | AB55 | AB50 | AB60 | CA1 | CA27 | CA22 | CA15 | CA10 | CC2 | CB39 | CB93 | CB21 | CC44 |
| b) Soft wheat | | | | | | | | | | | | | |
| Consort | AB58 | AB53 | AB63 | CA2 | CA7 | CA20 | CA18 | CA13 | CC3 | CB38 | CB95 | CB25 | CC42 |
| Riband | | | | CA6 | CA8 | CA21 | | CA12 | CC1 | CB36 | CB94 | CB29 | CC39 |
| Madrigal | | | | CA4 | CA28 | CA23 | CA16 | CA14 | CB98 | CB40 | | CB23 | CC41 |

* CP = Cockle Park
Ca = Cambridge
Br = ADAS Bridgets
Cor = Cornwall
Nor = Norfolk
HA = Harper Adams
[1] Sample identification number

## 2.2 Measurement of gas production *in vitro*

A sub-sample (100g) of the 66 wheat samples was milled through a hammer mill (Christy Norris, UK) with a 3 mm screen size, to produce a particle size distribution representative of that used in the compound feed industry and incubated for measurement of gas production *in vitro*. The feedstuffs were weighed in duplicate (1g dry matter (DM)) and pre-wetted in 10 ml of distilled water, prior to addition of 70 ml of buffer (Schofield and Pell, 1995), inoculated with 20ml of strained rumen fluid (taken from four mature wether sheep, two hours post feeding with a grass hay plus concentrate (60:40 DM basis) diet, and incubated with agitation (50 rpm) for 48 h at 39 $^{O}$C). The number of pressure releases was logged at 15 minute time intervals (as Cone, 1994). At 48 h, an organic matter (OM) degradation assessment was made using ultra-centrifugation (20,000 g for 30 min at 4° C). The model of France *et al*. (1993) was used to fit the gas accumulation profiles from each of the samples. For comparison, fermentable OM (FOM) was estimated from volatile fatty acid (VFA) production at 8h (VFOM) according to Demeyer (1991).

Additionally, all the wheat samples were incubated again and their gas production values at 8 h (equivalent to the typical rumen retention time of wheat grain in a high yielding dairy cow) were measured as described above and starch disappearance (by completely drying the incubation medium and residue) determined, together with VFA production. Starch content was determined by its enzymatic conversion to glucose using amyloglucosidase, glucose then being measured using glucose oxidase. Volatile fatty acids were determined using a gas chromatograph fitted with a flame ionisation detector.

## 2.3 Chemical analysis

Sub-samples of each wheat sample were analysed for dry matter and nitrogen (MAFF, 1986), neutral detergent fibre with an amylase pre-treatment (NDFa; Van Soest *et al*., 1991) and starch by the enzymatic method. Acid ether extract was measured as described by Alderman (1985) and neutral detergent-cellulase plus gamannase digestible organic matter content (NCGD) was determined as described by MAFF (1986).

## 2.4 The light transmission method for distinguishing endosperm texture

Whole wheat grains (sub-samples of 100 grains) were assessed using the light transflectance meter developed by Brewing Research International (Chandra *et al.*, 2001) , whereby light is shown onto each individual grain and the light transflected is recorded. The grains are grouped according to absorbance bands <400, >400<800, >800 mv for mealy, piebald and steely respectively.

## 2.5 Perten Single Kernel Characterisation wheat hardness tester

Whole wheat grains (sub-samples of 300 individual grains) were assessed using the Perten Single Kernel Characterisation wheat hardness tester which determines individual grain hardness, grain moisture, diameter and weight.

## 2.6 Near infrared reflectance spectroscopy (NIRS)

Three physical forms (whole grain, 3mm grind and 1mm grind) of the 66 wheat samples were scanned over the infrared region covering wavelengths from 1100 to 2300 nm with the spectral data collected as log 1/R (reflectance) values and subjected to the standard normal variate and detrending transformation (SNV-D; Barnes *et al*., 1989). The milled grains were scanned using small reflectance cells (capacity approximately 2g), whilst the whole grains were scanned using a larger rectangular cell (capacity approximately 50g). Calibrations were developed using the modified partial least squares approach for dry matter, nitrogen, starch, NDFa, effective OM disappearance, asymptote, lag, rate (8h), half life (h) and rate at half life for the gas production data and endosperm texture, hardness, weight and diameter for the quality data. The maths treatment found to be optimal was 1.4.4.1 (as described by Baker *et al*., 1994).

## 2.7 Statistical analysis

### *2.7.1 Experimental design*

The analytical data was produced from apparatus which was generally nested, though not herein, within each replicate of a randomized trial design of six varieties of wheat for six sites in the U.K. which was conducted for three consecutive years, though neither all sites nor all varieties were complete for all years.

### *2.7.2 Nature of the data*

Notwithstanding the unbalanced design, data were removed by the experimenter from the dataset originally collected on the following scientific basis that one of the gas production vessels consistently gave outlying measurements, one gas production run gave significantly low readings and any OM recoveries greater than 100% were disregarded. The remaining data were then submitted for statistical analysis.

### *2.7.3 Statistical analysis*

The trial was unbalanced (*vide supra*) and could only be comprehensively analysed using either REML or General Linear Modelling(GLM). To facilitate customer comprehension of statistical analysis, the GLM approach was taken as output was similar to ANOVA. A statistical analysis of a significant fraction of the data, manipulated to reduce the imbalance of the trial, with concomitant loss of information, was previously analysed by ANOVA to gauge the main likely outcomes of the trial. The present analysis was conducted on a year-by-year basis, for 1998 and 1999 combined where the data were more comprehensive. A restricted subset of all years combined for only those varieties investigated in 1997 was also undertaken. The model used chose replicate and machine as random factors and year, variety and site as fixed factors, no intercept was used and data were analysed using STATISTICA v5.5 (1999).

The interpretation of the statistical analysis is divided into 3 main parts as follows:-

a) ANOVA table.

This comprised output, *inter alia,* in the form of the sums-of-squares, mean squares, F-ratio and attendant p-value for each effect, where appropriate, and relevant interactions.

b) Tables of Means.

These are of two categories, the weighed means (*i.e.* weighted for the number of observations in each ANOVA cell as the trial is unbalanced). Secondly, the least squares means which are the predicted means (*i.e.* fitted response) from which the residuals are produced and may be plotted to judge the compatibility of the model and data. Tables of weighted and least squares means have attendant standard errors and 95% confidence bounds. These means are produced for all relevant main effects and interactions, the former are used to report overall effects and the later for model evaluation. Thirdly, a random distribution of said residuals around zero when plotted against the predicted means indicates a good fit of the data and appropriateness of the model and acceptability of the p-values for each effect, and the converse. As with any analysis of variance of such a factorial type of design, significant interactions will necessitate careful interpretation of the main effects.

The data from all the samples was used for regression analysis, to identify predictors of nutritive value. Agglomerative hierachical clustering of observations was carried out using Minitab statistical package. Agglomerative clustering of observations begins with all observations separate, each forming its own cluster. In the first step, the two observations closest together are joined. In the next step, either a third observation joins the first two, or two other observations join together into a different cluster. Each step results in one less cluster than the step before until, at the end, all classes are combined in one cluster. Once two observations are combined in a cluster, they may join with other observations, but they will always remain together. The linkage measure used in this analysis were 'single' (the distance between two clusters is the minimum distance between an observation in one cluster and an observation in the other cluster). The distance matrix used with this was Euclidean method. The final grouping of clusters was determined by studying the similarity and distance values in the output for each amalgamation step. The step below the one where these values changed abruptly was the point where the cluster groupings were chosen.

# 3.0 Results

## 3.1 Chemical composition

The chemical composition of the grains by year, for variety and site as factors are shown in Table 2a and 2b respectively. The wheat samples from the 1997, 1998 and 1999 harvest had a mean DM content of 890, 876 and 882 g $kg^{-1}$ fresh respectively and mean nitrogen content of 20.8, 20.3 and 20.7 g $kg^{-1}$ DM respectively. For DM content there was no significant effect of site or variety for the 1997 harvest, but in 1998 Harper Adams had a significantly ($P<0.001$) lower DM content and in 1999 Cockle Park had a significantly ($P<0.01$) lower DM content than the other sites. Cambridge had the highest DM content in 1998 and Harper Adams in 1999. There was no variety effect for DM content. There was a significant ($P<0.001$) variety and site effect for nitrogen content in all years with Hereward and Rialto > Reaper > Consort, Madrigal and Riband and a significant ($P<0.001$) site effect with Cambridge and Norfolk > Cockle Park and Harper Adams > Bridgets in 1998 and Cambridge and Norfolk > Bridgets and Harper Adams > Cockle Park. The wheats had a mean NDFa content of 93.6 g $kg^{-1}$ DM (range 64 to 155). NDFa content was significantly ($P<0.001$) lower in the 1999 harvest and there was a significant ($P<0.01$) site effect in 1999 with Cambridge, Norfolk and Harper Adams > Cockle Park and Bridgets. Starch content ranged from 658 to 781 g $kg^{-1}$ DM (mean 722) and was inversely related to nitrogen content (Figure 1). There were separate relationships for the 1997/1998 harvest years and the 1999 harvest year. There were no significant interaction for any of the chemical components for site x variety.

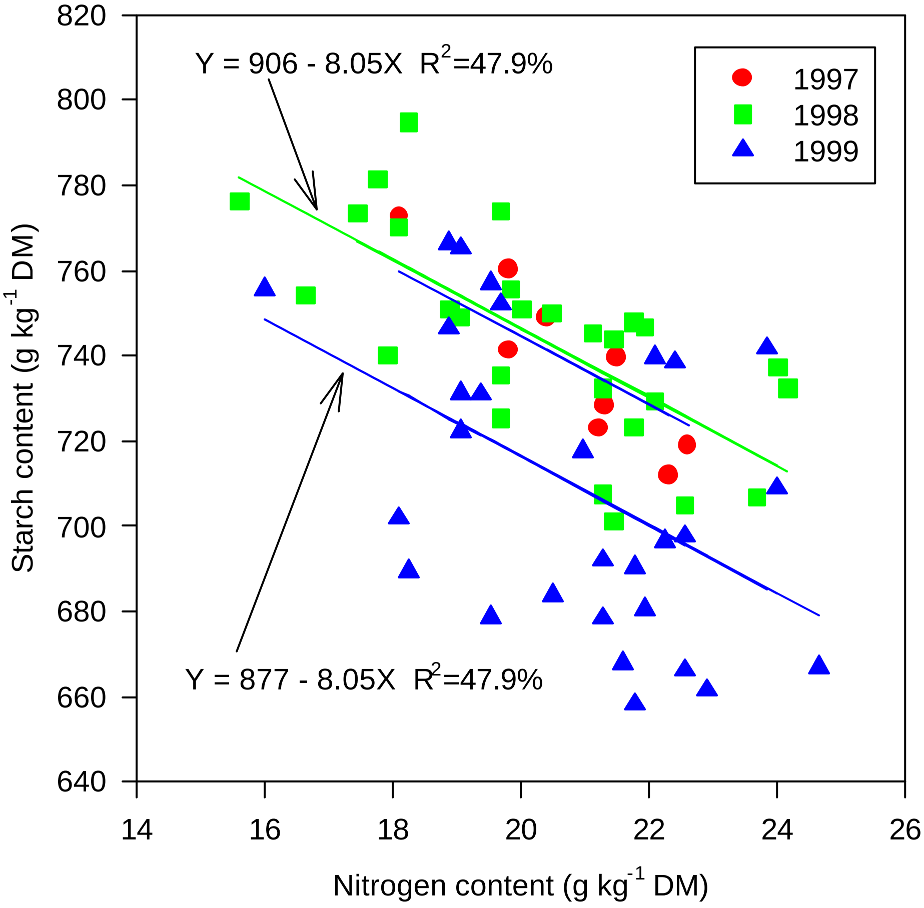
Table 2a Chemical composition of wheat grain as affected by variety for three different harvest years (all as g kg$^{-1}$ DM unless stated otherwise)

| Analysis / Year | Variety | | | | | | Variety mean by Yr | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| | Hereward | Reaper | Rialto | Consort | Madrigal | Riband | | Variety | Variety x Site |
| DM (g kg$^{-1}$ fresh) | | | | | | | | | |
| 1997 | 889 | 891 | | 891 | | | 890 | 0.500 | |
| 1998 | 873 | 876 | 878 | 875 | 880 | 875 | 876 | 0.234 | |
| 1999 | 884 | 884 | 883 | 880 | 880 | 883 | 882 | 0.588 | |
| Total ash | | | | | | | | | |
| 1997 | | | | | | | | | |
| 1998 | 15.0 | 15.6 | 17.5 | 14.4 | 16.0 | 16.0 | 15.8 | 0.011* | |
| 1999 | 15.4 | 16.6 | 15.8 | 13.4 | 15.0 | 16.6 | 15.5 | 0.023* | |
| Nitrogen | | | | | | | | | |
| 1997 | 22.1$^{a}$ | 20.8$^{b}$ | | 19.4$^{c}$ | | | 20.8 | 0.002** | |
| 1998 | 22.0$^{a}$ | 20.3$^{b}$ | 21.8$^{a}$ | 19.0$^{cd}$ | 19.6$^{bd}$ | 18.9$^{cd}$ | 20.3 | 0.000*** | |
| 1999 | 21.6$^{a}$ | 20.2$^{b}$ | 22.1$^{a}$ | 20.3$^{b}$ | 20.0$^{b}$ | 20.0$^{b}$ | 20.7 | 0.002** | |
| Starch | | | | | | | | | |
| 1997 | 723$^{a}$ | 737$^{b}$ | | 754$^{c}$ | | | 738 | 0.004** | |
| 1998 | 734$^{bc}$ | 739$^{ac}$ | 714$^{b}$ | 756$^{ac}$ | 764$^{a}$ | 755$^{ac}$ | 744 | 0.003** | |
| 1999 | 712$^{ac}$ | 698$^{ac}$ | 676$^{bc}$ | 736$^{a}$ | 737$^{a}$ | 714$^{ac}$ | 712 | 0.042** | |
| Oil | | | | | | | | | |
| 1997 | | | | | | | | | |
| 1998 | 22.0 | 23.0 | 26.5 | 26.2 | 23.0 | 26.8 | 24.6 | 0.011* | |
| 1999 | 22.2 | 22.8 | 23.6 | 25.8 | 24.3 | 23.4 | 23.7 | 0.103 | |
| NDFa | | | | | | | | | |
| 1997 | 100 | 117 | | 78 | | | 98 | 0.384 | |
| 1998 | 100 | 91 | 101 | 100 | 101 | 114 | 101 | 0.368 | |
| 1999 | 85 | 85 | 95 | 81 | 89 | 91 | 88 | 0.495 | |
| NCGD | | | | | | | | | |
| 1997 | | | | | | | | | |
| 1998 | 934 | 945 | 926 | 942 | 940 | 940 | 938 | 0.000*** | |
| 1999 | 932 | 935 | 918 | 936 | 932 | 931 | 931 | 0.001*** | |

Table 2b Chemical composition of wheat grain as affected by site for three different harvest years (all as g kg$^{-1}$ DM unless stated otherwise)

| Analysis / Year | Site | | | | | | site mean by Year | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| | Cockle Park | Cambridge | Norfolk | Bridget | Harper Adams | Cornwall | | Site | Variety x Site |
| DM (g $kg^{-1}$ fresh) | | | | | | | | | |
| 1997 | 888 | | | 890 | | 892 | 890 | 0.234 | |
| 1998 | $870^{cd}$ | $886^{a}$ | $879^{b}$ | $876^{bc}$ | $866^{d}$ | | 875 | 0.000*** | |
| 1999 | $877^{d}$ | $882^{bc}$ | $881^{bcd}$ | $884^{ac}$ | $888^{a}$ | | 882 | 0.001*** | |
| Total ash | | | | | | | | | |
| 1997 | | | | | | | | | |
| 1998 | 15.7 | 16.2 | 15.3 | 16.2 | 14.8 | | 15.6 | 0.434 | |
| 1999 | 16.0 | 16.3 | 14.5 | 14.4 | 16.0 | | 15.4 | 0.088 | |
| Nitrogen | | | | | | | | | |
| 1997 | $21.4^{a}$ | | | $19.8^{b}$ | | $21.1^{a}$ | 20.8 | 0.010** | |
| 1998 | $19.5^{b}$ | $22.1^{a}$ | $22.0^{a}$ | $17.7^{c}$ | $20.0^{b}$ | | 20.3 | 0.000*** | |
| 1999 | $19.0^{c}$ | $22.6^{a}$ | $22.1^{a}$ | $20.5^{b}$ | $19.4^{bc}$ | | 20.7 | 0.000*** | |
| Starch | | | | | | | | | |
| 1997 | $730^{a}$ | | | $758^{b}$ | | $727^{a}$ | 738 | 0.003** | |
| 1998 | $754^{a}$ | $729^{b}$ | $738^{ab}$ | $755^{a}$ | $745^{ab}$ | | 744 | 0.058 | |
| 1999 | 724 | 704 | 700 | 706 | 719 | | 711 | 0.629 | |
| Oil | | | | | | | | | |
| 1997 | | | | | | | | | |
| 1998 | 25.5 | 26.3 | 25.8 | 23.2 | 19.8 | | 24.1 | 0.001*** | |
| 1999 | 27.0 | 26.5 | 23.2 | 24.4 | 17.3 | | 23.7 | 0.000*** | |
| NDFa | | | | | | | | | |
| 1997 | 118 | | | 104 | | 73 | 98 | 0.286 | |
| 1998 | 104 | 95 | 113 | 93 | 98 | | 101 | 0.151 | |
| 1999 | $71^{b}$ | $101^{a}$ | $91^{ac}$ | $79^{bc}$ | $95^{a}$ | | 87 | 0.001*** | |
| NCGD | | | | | | | | | |
| 1997 | | | | | | | | | |
| 1998 | 937 | 936 | 940 | 937 | 943 | | 939 | 0.425 | |
| 1999 | 936 | 928 | 933 | 930 | 927 | | 931 | 0.029* | |

Figure 1. Relationship between the starch and nitrogen content of the wheat




### 3.3 Measurement of gas production *in vitro*

The mean gas production data from the grains by year with site and variety as factors are shown in Tables 3a and 3b for variety and site respectively. (A definition of gas production data is included in Appendix 2). The OM disappearance determined by ultracentrifugation after 48 h incubation ranged from 77.8 to 82.8 % (mean 80.4). There was only a significant effect of variety on OM disappearance in 1997 with Consort having a significantly ($P<0.068$) higher OM disappearance than Hereward. There was a significant site effect in both 1997 and 1999 ($P<0.05$ and $P<0.001$ respectively) with Bridgets having the lowest OM disappearance in 1997 and Norfolk and Harper Adams having the lowest in 1999. The ranking of the varieties for OM disappearance was different at each site with Rialto having the highest OM disappearance at the Bridgets site and the lowest at the Cambridge and Norfolk sites. The asymptotic value for gas production from the model of France *et al.* (1993) ranged from 281 to 331 ml $g^{-1}$ DM and the lag time ranged from 0.9 to 2.1 h. The samples with the longer lag period (time to produce gas) were related to grain hardness (correlation coefficient = 0.54, "Hard" or "Soft"), with the "Soft" varieties tending to have the longer lag times. For the asymptotic value of gas production, there was only a significant effect of variety in 1999. Reaper and Riband had significantly ($P<0.05$) greater values than the other varieties. There was a significant ($P<0.05$) site effect in 1998 with Cambridge having significantly lower gas production than the other sites. The calculated effective degradability of organic matter (EOMD) and the combined fractional rate of gas production at 6% $h^{-1}$ rumen outflow rate ranged from 40.9 to 48.3 % and 0.080 to 0.110 $h^{-1}$ respectively. There was no significant effect ($P>0.05$) of either variety or site nor any interactions on EOMD and the combined fractional rate of gas production. There was a tendency for Rialto to have the lowest EOMD and combined fractional rate of gas production and for the Cambridge and Norfolk sites to record the lowest values for all the varieties with the exception of Consort where the lowest values were recorded at Cockle Park.

Table 3a Gas production and associated data of wheats studied by year for variety

| Analysis / Year | Variety Hereward | Reaper | Rialto | Consort | Madrigal | Riband | Variety mean by Yr | p Values Variety | Variety x Site |
|---|---|---|---|---|---|---|---|---|---|
| pH at 48h | | | | | | | | | |
| 1997 | 6.22 | 6.19 | | 6.22 | | | 6.21 | 0.573 | 0.072 |
| 1998 | 6.20[acd] | 6.23[a] | 6.22[ac] | 6.18[bce] | 6.22[ac] | 6.16[de] | 6.20 | 0.030* | 0.000*** |
| 1999 | 6.22[bc] | 6.27[a] | 6.26[ac] | 6.23[ac] | 6.21[bc] | 6.24[ac] | 6.24 | 0.078 | 0.233 |
| OMD at 48h (%) | | | | | | | | | |
| 1997 | 79.9[b] | 80.1[ab] | | 81.4[a] | | | 80.4 | 0.068 | 0.568 |
| 1998 | 80.5 | 80.7 | 79.3 | 80.0 | 79.9 | 80.7 | 80.2 | 0.184 | 0.086 |
| 1999 | 79.8[bc] | 80.8[ac] | 80.2[ac] | 79.9[bc] | 80.2[ac] | 80.8[a] | 80.2 | 0.126 | 0.373 |
| Asymptote (ml $g^{-1}$ DM) | | | | | | | | | |
| 1997 | 296 | 309 | | 302 | | | 302 | 0.440 | |
| 1998 | 298[bc] | 310[bc] | 298[bc] | 299[ac] | 306[ac] | 306[ac] | 303 | 0.116 | |
| 1999 | 298[bc] | 309[ac] | 296[bc] | 303[ac] | 302[ac] | 313[a] | 304 | 0.046* | |
| Underlying rate ($h^{-1}$) | | | | | | | | | |
| 1997 | 0.16 | 0.15 | | 0.17 | | | 0.16 | 0.314 | |
| 1998 | 0.16 | 0.16 | 0.14 | 0.16 | 0.16 | 0.16 | 0.16 | 0.345 | |
| 1999 | 0.17[ac] | 0.16[ac] | 0.16[ac] | 0.18[a] | 0.17[ac] | 0.16[bc] | 0.17 | 0.278 | |
| Time dependent rate ($h^{-1/2}$) | | | | | | | | | |
| 1997 | -0.323 | -0.278 | | -0.319 | | | -0.307 | 0.163 | |
| 1998 | -0.287[ad] | -0.374[a] | -0.256[bde] | -0.320[ad] | -0.327[bcd] | -0.309[ace] | -0.312 | 0.121 | |
| 1999 | -0.343[ac] | -0.308[bc] | -0.311[bc] | -0.363[a] | -0.333[ac] | -0.307[bc] | -0.328 | 0.048* | |
| Lag (h) | | | | | | | | | |
| 1997 | 1.7 | 1.5 | | 1.8 | | | 1.7 | 0.237 | |
| 1998 | 1.5[ac] | 1.4[bc] | 1.3[bc] | 1.8[a] | 1.8[a] | 1.7[a] | 1.6 | 0.012* | |
| 1999 | 1.9[ac] | 1.6[bd] | 1.6[bd] | 2.0[a] | 1.8[ad] | 1.7[bcd] | 1.8 | 0.018* | |
| Time to half asymptote (h) | | | | | | | | | |
| 1997 | 9.4 | 9.4 | | 9.3 | | | 9.4 | 0.848 | |
| 1998 | 9.3 | 9.0 | 9.7 | 9.4 | 9.7 | 9.2 | 9.4 | 0.306 | |
| 1999 | 9.4 | 9.1 | 9.1 | 9.3 | 9.4 | 9.6 | 9.3 | 0.651 | |

Table 3a Continued

| Analysis / Year | Variety | | | | | | Variety mean by Yr | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| | Hereward | Reaper | Rialto | Consort | Madrigal | Riband | | Variety | Variety x Site |
| Combined rate at 8h | | | | | | | | | |
| 1997 | 0.106 | 0.104 | | 0.109 | | | 0.106 | 0.591 | |
| 1998 | 0.105 | 0.108 | 0.097 | 0.106 | 0.104 | 0.109 | 0.105 | 0.416 | |
| 1999 | 0.110 | 0.110 | 0.110 | 0.113 | 0.108 | 0.104 | 0.109 | 0.604 | |
| Combined rate at $t^{1/2}$ | | | | | | | | | |
| 1997 | 0.111 | 0.108 | | 0.113 | | | 0.111 | 0.491 | |
| 1998 | 0.109 | 0.111 | 0.101 | 0.111 | 0.109 | 0.112 | 0.109 | 0.406 | |
| 1999 | 0.114 | 0.113 | 0.113 | 0.117 | 0.113 | 0.109 | 0.113 | 0.510 | |
| Combined rate at rumen outflow 0.06 $h^{-1}$ ($h^{-1}$) | | | | | | | | | |
| 1997 | 0.090 | 0.090 | | 0.093 | | | 0.091 | 0.718 | |
| 1998 | 0.091 | 0.095 | 0.084 | 0.091 | 0.088 | 0.094 | 0.091 | 0.339 | |
| 1999 | 0.093 | 0.095 | 0.094 | 0.095 | 0.092 | 0.089 | 0.093 | 0.676 | |

Table 3b Gas production and associated data of wheats studied by year for site

| Analysis / Year | Site | | | | | | site mean by Year | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| | Cockle Park | Cambridge | Norfolk | Bridget | Harper Adams | Cornwall | | Site | Variety x Site |
| pH at 48h | | | | | | | | | |
| 1997 | 6.27[a] | | | 6.21[ac] | | 6.15[bc] | 6.21 | 0.002** | 0.072 |
| 1998 | 6.19 | 6.20 | 6.19 | 6.22 | 6.23 | | 6.21 | 0.220 | 0.000*** |
| 1999 | 6.20[b] | 6.27[a] | 6.27[a] | 6.25[a] | 6.23[ab] | | 6.24 | 0.004** | 0.233 |
| OMD at 48h (%) | | | | | | | | | |
| 1997 | 81.2[a] | | | 79.8[b] | | 81.3[a] | 80.7 | 0.045* | 0.568 |
| 1998 | 79.8 | 80.3 | 80.2 | 80.3 | 80.6 | | 80.2 | 0.881 | 0.086 |
| 1999 | 80.9[a] | 81.2[a] | 79.5[b] | 80.4[a] | 79.5[b] | ui | 80.3 | 0.000*** | 0.373 |
| Asymptote (ml $g^{-1}$ DM) | | | | | | | | | |
| 1997 | 306 | | | 301 | | 300 | 302 | 0.770 | |
| 1998 | 305[a] | 293[b] | 308[a] | 303[a] | 308[a] | | 303 | 0.025* | |
| 1999 | 307 | 303 | 303 | 302 | 304 | | 304 | 0.856 | |
| Underlying rate ($h^{-1}$) | | | | | | | | | |
| 1997 | 0.17 | | | 0.17 | | 0.15 | 0.16 | 0.092 | |
| 1998 | 0.16 | 0.16 | 0.15 | 0.16 | 0.16 | | 0.16 | 0.482 | |
| 1999 | 0.16 | 0.16 | 0.17 | 0.17 | 0.17 | | 0.17 | 0.313 | |
| Time dependent rate ($h^{-1/2}$) | | | | | | | | | |
| 1997 | -0.321[ab] | | | -0.331[a] | | -0.269[b] | -0.307 | 0.074 | |
| 1998 | -0.290 | -0.300 | -0.270 | -0.306 | -0.328 | | -0.299 | 0.327 | |
| 1999 | -0.311 | -0.321 | -0.353 | -0.334 | -0.319 | | -0.328 | 0.194 | |
| Lag (h) | | | | | | | | | |
| 1997 | 1.7 | | | 1.7 | | 1.6 | 1.7 | 0.556 | |
| 1998 | 1.4[bc] | 1.6[ac] | 1.4[bc] | 1.6[ac] | 1.8[a] | | 1.6 | 0.152 | |
| 1999 | 1.7[ac] | 1.7[ac] | 1.9[a] | 1.8[ac] | 1.6[bc] | | 1.7 | 0.083 | |
| Time to half asymptote (h) | | | | | | | | | |
| 1997 | 9.2 | | | 9.3 | | 9.7 | 9.4 | 0.373 | |
| 1998 | 9.1 | 9.6 | 9.5 | 9.3 | 9.6 | | 9.4 | 0.269 | |
| 1999 | 9.5 | 9.5 | 9.4 | 9.1 | 8.9 | | 9.3 | 0.231 | |

Table 3b Continued

| Analysis / Year | Site | | | | | | site mean by Year | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| | Cockle Park | Cambridge | Norfolk | Bridget | Harper Adams | Cornwall | | Site | Variety x Site |
| Combined rate at 8h | | | | | | | | | |
| 1997 | 0.110 | | | 0.109 | | 0.100 | 0.106 | 0.168 | |
| 1998 | 0.109 | 0.102 | 0.101 | 0.108 | 0.106 | | 0.105 | 0.411 | |
| 1999 | 0.105 | 0.106 | 0.111 | 0.112 | 0.113 | | 0.109 | 0.290 | |
| Combined rate at $t^{1/2}$ | | | | | | | | | |
| 1997 | 0.114 | | | 0.113 | | 0.104 | 0.110 | 0.139 | |
| 1998 | 0.112 | 0.107 | 0.105 | 0.112 | 0.111 | | 0.109 | 0.451 | |
| 1999 | 0.109 | 0.111 | 0.115 | 0.116 | 0.115 | | 0.113 | 0.326 | |
| Combined rate at rumen outflow 0.06 $h^{-1}$ ($h^{-1}$) | | | | | | | | | |
| 1997 | 0.094 | | | 0.093 | | 0.087 | 0.091 | 0.263 | |
| 1998 | 0.095 | 0.088 | 0.088 | 0.093 | 0.090 | | 0.091 | 0.320 | |
| 1999 | 0.090 | 0.090 | 0.093 | 0.096 | 0.098 | | 0.093 | 0.247 | |

The gas production, OM and starch disappearance, pH and VFA composition from the *in vitro* incubations after 8 h are given in Tables 4a and 4b for the grain samples by year for variety and site respectively. There were significant effects of site, variety, and variety x site interactions on total gas production at 8h (P<0.001, P<0.001, and P<0.01 respectively). The total gas for variety was ranked as Riband = Madrigal = Consort =Hereward <Reaper =Rialto in 1998 and as Consort = Hereward = Riband = Madrigal = Rialto < Reaper in 1999. The total gas was lowest at the Cambridge and Norfolk sites in both 1998 and 1999 and significantly greater at the Bridgets site in both years. Cockle Park had the highest total gas of all the sites in 1997 and 1998 but had low total gas in 1999, whereas Harper Adams had the lowest total gas in 1998 and the highest in 1999. There was a significant variety x site effect for total gas in 1997 and 1998 harvest years (P<0.05 and P<0.001 respectively).

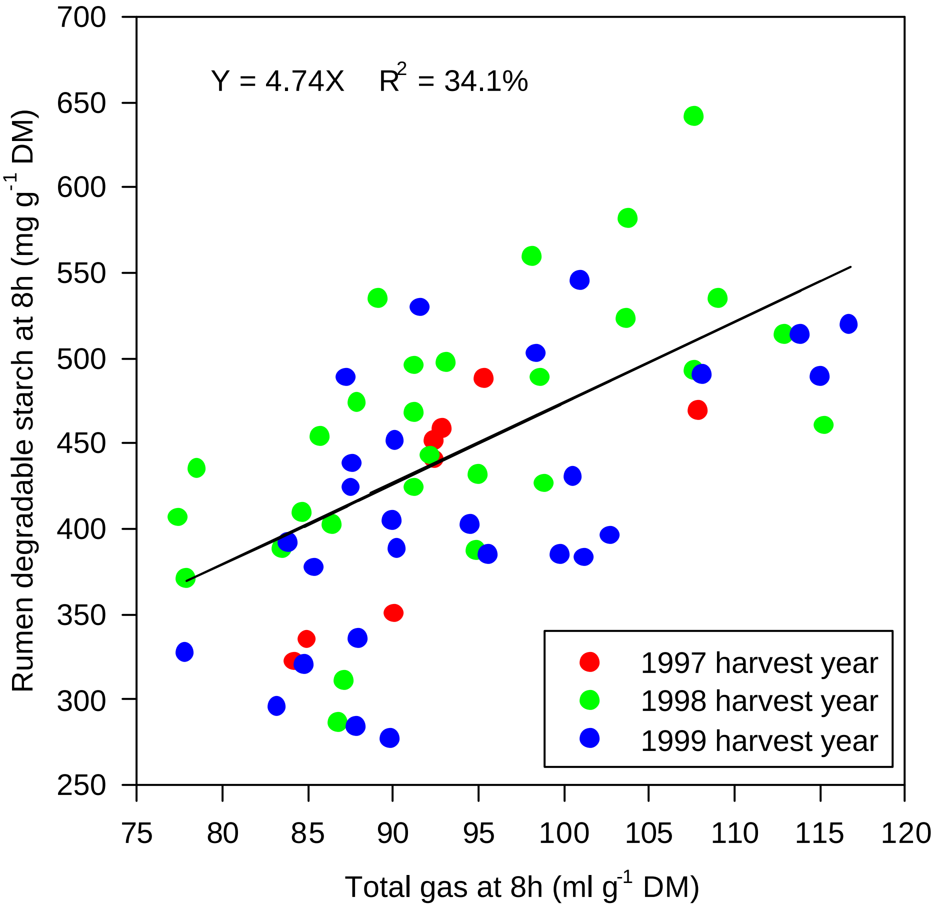
There were significant site and variety effect, for starch disappearance at 8h. Cockle Park had significantly (P<0.001) higher starch disappearance than all the other sites in 1998, and there was no effect of site in the other harvest years. Reaper had significantly higher starch disappearance than Hereward and Consort in 1997, and Reaper also had the highest starch disappearance in 1999, whereas in 1998 there was no significant effect of variety on starch disappearance. Overall Riband had significantly lower starch disappearance than Rialto, Hereward and Madrigal and these were lower than Consort and Reaper.

There was a variety and variety x site interaction for total VFA production (P<0.001, P<0.01, P<0.01 respectively). Consort, Madrigal and Riband had the lowest total VFA and Reaper had significantly higher total VFA in all harvest years. Cockle park had significantly higher total VFA concentration than all the other sites in 1997 and 1998 whereas in 1999 Bridgets had significantly higher total VFA concentration than all the other sites. There was a site effect for both the molar proportion of acetate and n-butyrate (P<0.001 and P<0.001 respectively) in both 1998 and 1999 harvest years. For the molar proportion of acetate in 1998 Bridgets and Harper Adams had a significantly greater molar proportion than Norfolk, whereas in 1999 all the sites had a significantly greater molar proportion of acetate than Cambridge. For the molar proportion of n-butyrate Cambridge had significantly higher molar proportion than all the other sites in 1998 and for 1999 both

Cambridge and Norfolk had significantly higher molar proportion of n-butyrate than the other sites. There was a variety effect on the molar proportion of acetate and n-butyrate ($P<0.05$ and $P<0.001$ respectively) with Reaper having significantly greater molar proportion of acetate than the other varieties in the 1999 harvest year. The opposite was true for the molar proportion of n-butyrate. The mean volume of total gas at 8 h increased with increasing degradability as defined by both OM and starch disappearance (%) at 8 h. The total VFA concentration increased with increasing degradability, and there was a negative relationship between starch disappearance and the molar proportion of n-butyrate.

The amount of OM and starch degradation at 8 h are also given in Table 4a and 4b for the wheat samples by year for variety and site respectively and the responses were similar to the respective OM and starch disappearance. The mean OM degraded for each sample was low compared with the starch degraded, due to the methodology used which resulted in the microbial biomass being included in the residue for the OM measurement. The difference between the amount of OM degraded at 8 h and the calculated FOM from the VFA production, provides an estimation of microbial biomass generated within the system. When more degradable carbohydrate was available for fermentation, there was a significant relationship ($P<0.001$) with more being diverted into fermentation products and less directly into microbial synthesis. The starch degraded at 8 h was positively correlated with the 8 h total gas production ($r = 0.593$, Figure 2),

Figure 2. Relationship between rumen degradable starch and total gas both estimated *in vitro* for wheats from all harvest years




### 3.4 The light transmission method for distinguishing mealy grains from steely grains

Tables 5a and 5b show the proportion of mealy and steely grains for the wheat samples from the 1997, 1998 and 1999 harvest years by variety and site respectively. There was a significant effect of variety and site (P<0.001, P<0.001 respectively) for steeliness in all the harvest years. For variety Rialto had the greatest proportion of steely grains and was significantly greater than Hereward and Reaper which were all significantly greater than

Consort and Riband. The opposite was true for mealiness. Cambridge site gave the greatest proportion of steely grains with the remaining sites ordered as follows Cockle Park>Norfolk=Bridgets=Harper Adams in the 1999 harvest year. The same was true in the 1998 harvest year with the exception that Cockle Park had the lowest level of steely grains. Again the opposite was true for the mealiness. There was a positive correlation between nitrogen content and steeliness (r = 0.392) and the relationship was negative for nitrogen content and mealiness (r = -0.482).

There was a poor relationship between rumen degradable starch at 8h and the degree of mealiness ($R^2$ = 2.6%), this was improved when the samples from the 1998 harvest were investigated separately ($R^2$=16.7%).

Table 4a Gas production, organic matter and starch disappearance, pH and volatile fatty acid production at 8h for the wheat samples by year with the effect of variety.

| Analysis | Variety | | | | | | Variety | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| / Year | Hereward | Reaper | Rialto | Consort | Madrigal | Riband | mean by Yr | Variety | Variety x Site |
| Total gas (ml g$^{-1}$ DM) | | | | | | | | | |
| 1997 | 87.2 | 92.3 | | 92.6 | | | 90.7 | 0.208 | 0.022* |
| 1998 | 92.7$^{ac}$ | 96.3$^{a}$ | 99.3$^{a}$ | 94.7$^{ac}$ | 92.0$^{ac}$ | 88.2$^{bc}$ | 93.9 | 0.035* | 0.001*** |
| 1999 | 90.0$^{b}$ | 108.3$^{a}$ | 95.3$^{b}$ | 88.8$^{b}$ | 91.6$^{b}$ | 91.8$^{b}$ | 94.3 | 0.000*** | 0.441 |
| Organic matter disappearance at 8h (%) | | | | | | | | | |
| 1997 | 32.3 | 33.9 | | 34.8 | | | 33.7 | 0.598 | 0.423 |
| 1998 | 38.0$^{ab}$ | 37.6$^{ab}$ | 42.1$^{a}$ | 37.9$^{ab}$ | 33.8$^{b}$ | 40.2$^{a}$ | 38.3 | 0.212 | 0.955 |
| 1999 | 34.0$^{b}$ | 39.3$^{a}$ | 38.7$^{a}$ | 36.2$^{ab}$ | 37.5$^{ab}$ | 36.0$^{ab}$ | 37.0 | 0.066 | 0.017* |
| Organic matter degraded at 8h[3] (mg) | | | | | | | | | |
| 1997 | 274 | 289 | | 300 | | | 288 | 0.536 | 0.450 |
| 1998 | 321$^{ab}$ | 319$^{ab}$ | 357$^{a}$ | 320$^{ab}$ | 386$^{b}$ | 342$^{a}$ | 341 | 0.213 | 0.928 |
| 1999 | 286$^{b}$ | 332$^{a}$ | 327$^{a}$ | 306$^{ab}$ | 317$^{ab}$ | 305$^{ab}$ | 312 | 0.074 | 0.033* |
| Starch disappearance at 8h (%) | | | | | | | | | |
| 1997 | 51.2$^{b}$ | 66.1$^{a}$ | | 56.3$^{b}$ | | | 57.9 | 0.039* | 0.298 |
| 1998 | 65.0$^{a}$ | 60.6$^{ac}$ | 66.0$^{a}$ | 61.5$^{ac}$ | 61.7$^{ac}$ | 56.2$^{bc}$ | 61.8 | 0.082 | 0.186 |
| 1999 | 56.3$^{bc}$ | 68.1$^{a}$ | 54.8$^{bc}$ | 61.2$^{ac}$ | 57.2$^{bc}$ | 53.2$^{bc}$ | 58.5 | 0.011* | 0.501 |
| Starch degraded at 8h[1] (mg) | | | | | | | | | |
| 1997 | 320$^{c}$ | 419$^{a}$ | | 370$^{b}$ | | | 370 | 0.027* | 0.172 |
| 1998 | 408 | 383 | 405 | 398 | 406 | 363 | 394 | 0.368 | 0.191 |
| 1999 | 338$^{bcd}$ | 406$^{a}$ | 318$^{bd}$ | 388$^{ac}$ | 360$^{ad}$ | 328$^{bcd}$ | 356 | 0.005** | 0.208 |
| Total VFA (mmol 100 ml$^{-1}$) | | | | | | | | | |
| 1997 | 20.63 | 23.66 | | 20.30 | | | 21.53 | 0.007** | 0.067 |
| 1998 | 22.25$^{ac}$ | 24.40$^{a}$ | 22.92$^{ac}$ | 21.62$^{bc}$ | 21.70$^{bc}$ | 22.81$^{ac}$ | 22.62 | 0.302 | 0.008** |
| 1999 | 21.97$^{bc}$ | 24.70$^{a}$ | 23.11$^{ac}$ | 20.96$^{bc}$ | 21.80$^{bc}$ | 21.52$^{bc}$ | 22.34 | 0.016* | 0.094 |
| Molar proportions of VFA - Acetate | | | | | | | | | |
| 1997 | 53.69 | 53.48 | | 54.71 | | | 53.96 | 0.349 | 0.524 |
| 1998 | 53.65 | 53.06 | 53.52 | 53.53 | 52.62 | 53.66 | 53.34 | 0.061 | 0.088 |
| 1999 | 53.90$^{b}$ | 55.52$^{a}$ | 53.17$^{b}$ | 53.64$^{b}$ | 52.77$^{b}$ | 53.53$^{b}$ | 53.76 | 0.001*** | 0.095 |

Table 4a Continued

| Analysis / Year | Variety | | | | | | Variety mean by Yr | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| | Hereward | Reaper | Rialto | Consort | Madrigal | Riband | | Variety | Variety x Site |
| Molar proportions of VFA - Propionate | | | | | | | | | |
| 1997 | 33.51[ac] | 32.54[a] | | 31.29[bc] | | | 32.45 | 0.060 | 0.940 |
| 1998 | 33.67[ac] | 34.32[a] | 32.88[ad] | 32.42[bcd] | 32.07[bd] | 32.30[bcd] | 32.94 | 0.004** | 0.007** |
| 1999 | 31.85[bc] | 32.84[ac] | 33.44[ac] | 32.00[bc] | 33.79[a] | 32.49[ac] | 32.74 | 0.081 | 0.065 |
| Molar proportions of VFA - n-butyrate | | | | | | | | | |
| 1997 | 7.60[bc] | 7.65[a] | | 8.57[ac] | | | 7.94 | 0.000*** | 0.254 |
| 1998 | 7.46[b] | 7.46[b] | 8.08[a] | 8.43[a] | 8.48[a] | 8.21[a] | 8.02 | 0.001*** | 0.043* |
| 1999 | 8.01[bcd] | 7.07[b] | 7.84[bd] | 8.38[ac] | 8.23[ad] | 8.53[a] | 8.01 | 0.000*** | 0.028* |

Table 4b Gas production and associated data of wheats studied by year for site

| Analysis / Year | Site: Cockle Park | Cambridge | Norfolk | Bridget | Harper Adams | Cornwall | site mean by Year | p Values: Site | Variety x Site |
|---|---|---|---|---|---|---|---|---|---|
| Total gas (ml g$^{-1}$ DM) | | | | | | | | | |
| 1997 | $97.8^{a}$ | | | $85.1^{b}$ | | $89.3^{b}$ | 90.7 | 0.001*** | 0.022* |
| 1998 | $102.7^{a}$ | $88.8^{b}$ | $89.8^{b}$ | $98.7^{a}$ | $88.0^{b}$ | | 93.6 | 0.000*** | 0.001*** |
| 1999 | $91.2^{bc}$ | $92.9^{bc}$ | $87.6^{b}$ | $97.7^{ac}$ | $103.0^{a}$ | | 93.7 | 0.000*** | 0.441 |
| Organic matter disappearance at 8h (%) | | | | | | | | | |
| 1997 | 36.4 | | | 33.5 | | 31.3 | 33.7 | 0.179 | 0.423 |
| 1998 | 40.6 | 38.1 | 35.7 | 38.4 | 37.0 | | 38.0 | 0.394 | 0.955 |
| 1999 | 35.7 | 37.1 | 35.9 | 38.6 | 38.0 | | 37.1 | 0.243 | 0.017* |
| Organic matter degraded at 8h$^{1}$ (mg) | | | | | | | | | |
| 1997 | 311 | | | 288 | | 267 | 289 | 0.172 | 0.450 |
| 1998 | 344 | 325 | 301 | 325 | 312 | | 321 | 0.338 | 0.928 |
| 1999 | 297 | 316 | 302 | 328 | 323 | | 313 | 0.166 | 0.326* |
| Starch disappearance at 8h (%) | | | | | | | | | |
| 1997 | $64.1^{a}$ | | | $57.9^{ac}$ | | $54.0^{bc}$ | 58.7 | 0.300 | 0.298 |
| 1998 | $72.5^{a}$ | $54.5^{b}$ | $61.4^{b}$ | $61.2^{b}$ | $58.4^{b}$ | | 61.6 | 0.001*** | 0.186 |
| 1999 | 56.5 | 59.6 | 48.3 | 58.4 | 60.0 | | 56.6 | 0.235 | 0.501 |
| Starch degraded at 8h$^{3}$ (mg) | | | | | | | | | |
| 1997 | $407^{a}$ | | | $378^{a}$ | | $339^{b}$ | 375 | 0.190 | 0.172 |
| 1998 | $467^{a}$ | $341^{cd}$ | $387^{bc}$ | $394^{b}$ | $371^{bd}$ | | 392 | 0.001*** | 0.191 |
| 1999 | 346 | 363 | 286 | 416 | 373 | | 357 | 0.180 | 0.208 |
| Total VFA (mmol 100 ml$^{-1}$) | | | | | | | | | |
| 1997 | 22.88 | | | 20.81 | | 21.27 | 21.65 | 0.087 | 0.067 |
| 1998 | $24.41^{a}$ | $22.11^{bc}$ | $22.55^{ac}$ | $22.19^{bc}$ | $20.67^{bc}$ | | 22.39 | 0.025* | 0.008** |
| 1999 | $21.41^{b}$ | $22.65^{b}$ | $20.81^{b}$ | $24.97^{a}$ | $22.33^{b}$ | | 22.43 | 0.002** | 0.094 |
| Molar proportions of VFA - Acetate | | | | | | | | | |
| 1997 | 54.61 | | | 53.45 | | 53.87 | 53.98 | 0.229 | 0.524 |
| 1998 | $53.46^{ac}$ | $52.97^{ac}$ | $52.58^{bc}$ | $53.93^{a}$ | $53.92^{a}$ | | 53.37 | 0.026* | 0.088 |
| 1999 | $53.59^{ac}$ | $52.59^{bc}$ | $54.01^{a}$ | $54.52^{a}$ | $54.39^{a}$ | | 53.82 | 0.015* | 0.095 |

Table 4b Continued

| Analysis | Site | site mean | p Values |
|---|---|---|---|

| / Year | Cockle Park | Cambridge | Norfolk | Bridget | Harper Adams | Cornwall | by Year | Site | Variety x Site |
|---|---|---|---|---|---|---|---|---|---|
| Molar proportions of VFA - Propionate | | | | | | | | | |
| 1997 | 32.30 | | | 32.41 | | 32.81 | 32.51 | 0.884 | 0.940 |
| 1998 | 33.08[a] | 33.04[a] | 33.57[a] | 33.47[a] | 30.94[b] | | 32.82 | 0.000*** | 0.007** |
| 1999 | 33.09 | 33.13 | 31.63 | 32.50 | 33.13 | | 32.70 | 0.179 | 0.065 |
| Molar proportions of VFA - n-butyrate | | | | | | | | | |
| 1997 | 7.72 | | | 8.08 | | 7.89 | 7.90 | 0.408 | 0.254 |
| 1998 | 7.86[bc] | 8.33[a] | 8.29[ac] | 7.64[bd] | 7.99[acd] | | 8.02 | 0.039* | 0.043* |
| 1999 | 7.72[b] | 8.54[a] | 8.48[a] | 7.77[b] | 7.41[b] | | 7.98 | 0.000*** | 0.028* |

[1] mg $g^{-1}$ fresh weight

Table 5a The proportions of mealy, steely and piebald grains and the SKS values in the 1997, 1998 and 1999 wheat samples

| Analysis | Variety | | | | | | Variety | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| / Year | Hereward | Reaper | Rialto | Consort | Madrigal | Riband | mean by Yr | Variety | Variety x Site |
| Weight (mg) | | | | | | | | | |
| 1997 | 50.3 | 48.7 | | 51.6 | | | 50.2 | 0.353 | |
| 1998 | 50.1 | 49.9 | 45.9 | 49.3 | 48.9 | 50.0 | 49.0 | 0.879 | |
| 1999 | 50.2 | 54.6 | 53.6 | 53.0 | 50.5 | 56.5 | 53.1 | 0.037* | |
| Moisture (%) | | | | | | | | | |
| 1997 | 13.9[a] | 13.5[bc] | | 13.8[ac] | | | 13.7 | 0.073 | |
| 1998 | 14.2 | 14.1 | 13.8 | 13.7 | 13.9 | 13.7 | 13.9 | 0.633 | |
| 1999 | 12.9 | 13.1 | 13.2 | 13.4 | 13.5 | 13.3 | 13.2 | 0.261 | |
| Diameter (mm) | | | | | | | | | |
| 1997 | 3.0 | 3.0 | | 3.1 | | | 3.0 | 0.390 | |
| 1998 | 3.1 | 3.0 | 2.9 | 3.0 | 3.0 | 3.0 | 3.0 | 0.748 | |
| 1999 | 3.1 | 3.2 | 3.2 | 3.1 | 3.0 | 3.3 | 3.2 | 0.008** | |
| Hardness | | | | | | | | | |
| 1997 | 35.6 | 48.4 | | 47.0 | | | 43.7 | 0.793 | |
| 1998 | 34.8 | 44.5 | 41.7 | 44.7 | 44.5 | 38.5 | 41.5 | 0.939 | |
| 1999 | 48.8 | 47.8 | 58.6 | 15.4 | 19.5 | 17.8 | 34.7 | 0.000*** | |
| Steely (light transflectance, population(%)) | | | | | | | | | |
| 1997 | 33.3[a] | 37.3[a] | | 20.9[b] | | | 30.5 | 0.036* | |
| 1998 | 32.7[b] | 23.9[bc] | 49.1[a] | 16.6[bc] | 53.1[a] | 14.3[c] | 31.6 | 0.000*** | |
| 1999 | 32.4[ac] | 34.0[ac] | 43.6[a] | 20.4[bc] | 43.9[a] | 18.6[bc] | 32.2 | 0.045* | |
| Piebald (light transflectance, population(%)) | | | | | | | | | |
| 1997 | 30.7 | 23.5 | | 25.6 | | | 26.6 | 0.350 | |
| 1998 | 25.5 | 22.8 | 28.5 | 15.3 | 20.6 | 17.7 | 21.7 | 0.248 | |
| 1999 | 20.1 | 9.9 | 16.9 | 13.5 | 20.2 | 13.0 | 15.6 | 0.369 | |
| Mealy (light transflectance, population(%)) | | | | | | | | | |
| 1997 | 36.0[bc] | 39.2[ac] | | 53.6[a] | | | 42.9 | 0.060 | |
| 1998 | 41.9[b] | 51.2[b] | 22.4[c] | 68.1[a] | 26.3[c] | 68.0[a] | 46.3 | 0.000*** | |
| 1999 | 39.6[ac] | 48.6[ac] | 57.8[a] | 31.7[bc] | 52.0[ac] | 48.5[ac] | 46.4 | 0.082 | |

Table 5b The proportions of mealy, steely and piebald grains and the SKS values in the 1997, 1998 and 1999 wheat samples

| Analysis / Year | Site | | | | | | site mean by Year | p Values | |
|---|---|---|---|---|---|---|---|---|---|
| | Cockle Park | Cambridge | Norfolk | Bridget | Harper Adams | Cornwall | | Site | Variety x Site |
| Weight (mg) | | | | | | | | | |
| 1997 | $50.8^{a}$ | | | $55.0^{a}$ | | $44.9^{b}$ | 50.2 | 0.012* | |
| 1998 | $42.4^{b}$ | $47.9^{ab}$ | $52.9^{a}$ | $52.7^{a}$ | $49.8^{a}$ | | 49.1 | 0.019* | |
| 1999 | 54.1 | 49.4 | 52.8 | 58.1 | 52.3 | | 53.3 | 0.002** | |
| Moisture (%) | | | | | | | | | |
| 1997 | $13.7^{ac}$ | | | $14.0^{a}$ | | $13.5^{bc}$ | 13.7 | 0.057 | |
| 1998 | $13.2^{b}$ | $13.9^{a}$ | $14.3^{a}$ | $14.1^{a}$ | $14.2^{a}$ | | 13.9 | 0.017* | |
| 1999 | 14.1 | 12.8 | 13.1 | 12.8 | 13.3 | | 13.2 | 0.000*** | |
| Diameter (mm) | | | | | | | | | |
| 1997 | $3.0^{a}$ | | | $3.2^{a}$ | | $2.8^{b}$ | 3.0 | 0.011* | |
| 1998 | $2.8^{b}$ | $2.9^{ab}$ | $3.2^{a}$ | $3.1^{a}$ | $3.0^{a}$ | | 3.0 | 0.017* | |
| 1999 | 3.2 | 3.0 | 3.1 | 3.4 | 3.1 | | 3.2 | 0.000*** | |
| Hardness | | | | | | | | | |
| 1997 | 54.1 | | | 25.6 | | 51.3 | 43.7 | 0.383 | |
| 1998 | 47.9 | 40.2 | 45.0 | 39.2 | 32.4 | | 40.9 | 0.749 | |
| 1999 | 37.5 | 50.7 | 27.9 | 34.7 | 25.1 | | 35.2 | 0.000*** | |
| Steely (light transflectance, population(%)) | | | | | | | | | |
| 1997 | $17.9^{b}$ | | | $34.1^{a}$ | | $39.6^{a}$ | 30.5 | 0.014* | |
| 1998 | $10.4^{c}$ | $59.6^{a}$ | $37.1^{b}$ | $20.1^{cd}$ | $30.5^{bd}$ | | 31.5 | 0.000*** | |
| 1999 | $49.5^{b}$ | $74.9^{A}$ | $11.4^{c}$ | $6.24^{c}$ | $8.9^{c}$ | | 30.2 | 0.000*** | |
| Piebald (light transflectance, population(%)) | | | | | | | | | |
| 1997 | 26.6 | | | 25.7 | | 27.5 | 26.6 | 0.925 | |
| 1998 | $12.3^{b}$ | $24.8^{ac}$ | $31.3^{a}$ | $19.6^{bc}$ | $19.4^{bc}$ | | 21.5 | 0.015* | |
| 1999 | 19.2 | 12.9 | 18.8 | 13.9 | 12.0 | | 15.4 | 0.424 | |
| Mealy (light transflectance, population(%)) | | | | | | | | | |
| 1997 | $55.5^{a}$ | | | $40.2^{b}$ | | $33.0^{b}$ | 42.9 | 0.310 | |
| 1998 | $77.3^{a}$ | $15.6^{d}$ | $30.0^{c}$ | $60.2^{b}$ | $50.1^{b}$ | | 46.6 | 0.000*** | |
| 1999 | $70.2^{a}$ | $32.6^{b}$ | $8.7^{c}$ | $35.0^{b}$ | $77.3^{a}$ | | 44.8 | 0.000*** | |

### 3.5 SKS determinations

Tables 5a and 5b also show the mean grain weight, moisture, grain diameter and hardness value for the wheat samples from the 1997, 1998 and 1999 harvest years by variety and site respectively. For grain weight there was a significant site effect ($P<0.01$). Grain weight was higher in 1999 compared with 1998 (54 v 49 mg respectively) and this was a result of the Cockle Park and Bridgets sites having greater grain weights in 1999. The Bridgets site produced the highest grain weight in 1997 and 1998 and in 1998 grain weights at Bridgets was =Norfolk =Cambridge =Harper Adams > Cockle Park. In 1999 Bridgets again produced the highest grain weight and was significantly higher than all the other sites. The same was true for grain diameter as there is a significant positive correlation between grain weight and grain diameter ($r= 0.945$). There was a significant effect of year and year x site interaction for grain moisture ($P<0.001$ and $P<0.01$ respectively) with lower moisture for 1999 harvest and this was a reflection of the Cambridge, Norfolk and Bridgets sites having lower moisture in 1999. There was a significant effect of variety on grain hardness ($P<0.05$) with Hereward, Reaper and Rialto having a greater hardness value than Consort, Madrigal and Riband.

### 3.6 NIR spectroscopy

Calibration and cross-validation performance data (standard error of calibration (SEC) and cross validation (SECV)) are given in Table 6 for the whole grain for all the samples.

Table 6. Calibration and cross-validation statistics for different chemical, quality and *in vitro* gas production characteristics of 66 whole wheat grain samples.

| | Calibration | | Cross-validation | |
|---|---|---|---|---|
| Predicted term | SEC | $R^2$ | SECV | $R^2_{CV}$ |
| Steely proportion | 6.817 | 0.936 | 8.058 | 0.912 |
| Mealy proportion | 10.681 | 0.856 | 11.907 | 0.823 |
| Nitrogen content | 0.081 | 0.839 | 0.098 | 0.765 |
| Starch/Nitrogen ratio | 2.068 | 0.815 | 2.54 | 0.722 |
| Dry matter content | 0.441 | 0.644 | 0.462 | 0.605 |
| Mean grain diameter | 0.154 | 0.444 | 0.165 | 0.353 |
| Mean grain weight | 4.116 | 0.427 | 4.446 | 0.322 |
| Organic matter disappearance 8h (%) | 3.294 | 0.377 | 3.929 | 0.136 |
| Starch | 2.589 | 0.374 | 2.717 | 0.323 |
| NCGD | 0.682 | 0.373 | 0.707 | 0.335 |
| Asymptote | 6.874 | 0.324 | 7.854 | 0.119 |
| NDFa content | 1.405 | 0.309 | 1.575 | 0.145 |
| Organic matter disappearance 8h | 0.03 | 0.308 | 0.034 | 0.091 |
| Combined rate of gas production 8h | 0.006 | 0.257 | 0.006 | 0.251 |
| Combined rate at half gas production | 0.006 | 0.236 | 0.006 | 0.235 |
| Gas production 8h | 7.278 | 0.233 | 7.761 | 0.129 |
| SKS Hardness | 18.032 | 0.153 | 18.584 | 0.117 |
| Starch disappearance 8h | 0.072 | 0.011 | 0.078 | 0 |

There was a good calibration and cross-validation for the proportions of steely and mealy grains and nitrogen content of the grains ($R^2_{CV}$ = 0.912, 0.823 and 0.765 respectively). This is noteworthy as it eliminates the need to grind samples prior to nitrogen estimation, which would be extremely useful for routine screening of grain samples. Prediction of starch content was poor ($R^2_{CV}$ = 0.323). There was however a reasonable prediction of starch to nitrogen ratio ($R^2_{CV}$ = 0.815). None of the gas production parameters were well predicted by NIRS with the best prediction of combined rate of gas production at 8h ($R^2_{CV}$ = 0.251). This was disappointing but the ranges for these parameters were narrow. NIRS radiation does not penetrate too far into the grain which may explain why the prediction of starch content is poor as the starch is held in the endosperm. Since starch is the main factor influencing gas production a better prediction of starch is required before the prediction of the gas production parameters can be improved.

### 3.7 Use of NIRS to indicate hardness of wheat grains

With NIRS, the reflectance of the sample is recorded as log 1/R and the magnitude of this depends on the concentration of the absorbing species, their absorption constants and the degree of scattering. The scatter in turn is related to particle size of the sample, so that coarser samples have higher log 1/R across the spectrum and finer samples have lower log 1/R across the spectrum. Consequently, if wheat samples are ground under standard conditions the log 1/R values will be higher the harder the wheat, and log 1/R at any wavelength can be used as a measure of hardness (Osborne, 1991). Since NIRS measurement of hardness is based on the relationship between scatter and particle size and not on the concentration of constituents in the samples, there is no need to calibrate against another method.

The log 1/R values at 1680 and 2230 nm, which have previously been reported as optimum wavelengths for wheat hardness measurement by NIR reflectance (Norris *et al*., 1989), for all the wheat samples by year and variety are shown in Table 7 and visually in Figure 3. The ranking for the degree of hardness varied at the two wavelengths. The wheats identified as being Soft wheats (Consort, Madrigal and Riband) tended to have the lowest log 1/R values and the opposite was true for the Hard wheats. Additionally, there was a significant negative relationship between hardness as estimated by NIR and the degree of mealiness determined using light transflectance ($r = -0.840$). This suggests that Soft wheats will tend to be more mealy than Hard wheats but that both are influenced by site (environment). This confirms the earlier work (HGCA Project Report No. 182).

Figure 3. NIRS Log 1/R values for wheat over the wavelength range 1100 to 2500 nm highlighting the wavelengths for wheat hardness

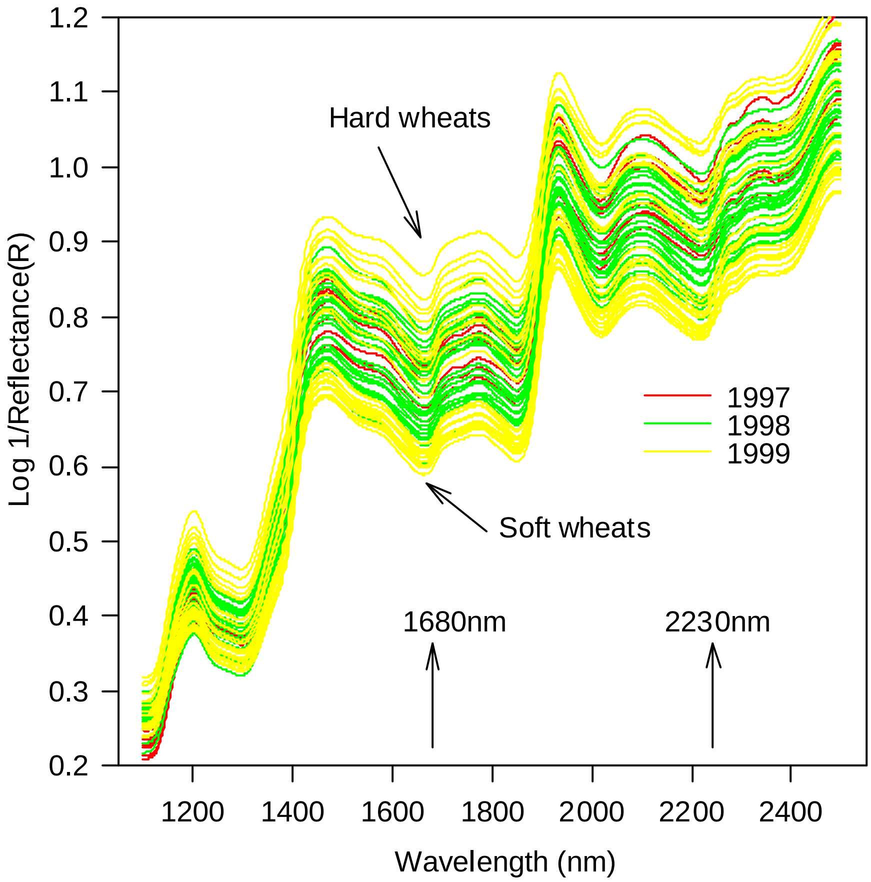


Table 7. NIRS log 1/R at 1680 and 2230 nm as a measurement of wheat hardness.

| Variety | Log 1/R at | |
|---|---|---|
| | 1680 nm | 2230 nm |
| 1997 | | |
| Hereward | 0.723 | 0.940 |
| Reaper | 0.716 | 0.915 |
| Consort | 0.707 | 0.938 |
| | | |
| 1998 | | |
| Hereward | 0.720 | 0.902 |
| Reaper | 0.710 | 0.884 |
| Rialto | 0.710 | 0.869 |
| Consort | 0.711 | 0.920 |
| Madrigal | 0.720 | 0.893 |
| Riband | 0.670 | 0.860 |
| | | |
| 1999 | | |
| Hereward | 0.696 | 0.885 |
| Reaper | 0.684 | 0.855 |
| Rialto | 0.704 | 0.869 |
| Consort | 0.681 | 0.889 |
| Madrigal | 0.701 | 0.884 |
| Riband | 0.646 | 0.840 |

### 3.8 Hierachial cluster analysis using chemical composition and grain characteristic parameters

Hierachial cluster analysis of observations based on chemical composition data (DM, N, NDF (g kg$^{-1}$ DM)) and grain characteristic parameters (mealy, steely, piebald, grain weight, moisture, diameter and hardness) gave rise to four clusters comprising:

Group 1 - n=31

Group 2 - n=19

Group 3 - n=9

Group 4 - n=7

The cluster centroids are given in Table 8. Cluster group 1 comprised wheats from all three harvest years and similar amounts of varieties of Soft and Hard genotypes. The endosperm texture was equally steely, mealy and piebald, and the kernels had low mean weight, medium N and NDF content and the highest starch content. Group 2 comprised wheats from all three harvest years and two-thirds of the varieties were Hard genotypes and the rest Soft. The endosperm texture was low steely, low/medium piebald and medium/high mealy, and the kernels had medium mean weight, medium N and starch content and high NDF content. Group 3 comprised wheats from the 1998 and 1999 harvest years only and all the varieties were of the Soft genotype. The endosperm texture was both low for steely and piebald and the highest for mealiness. The kernels had the highest weight and the lowest measurement for hardness and they were also low in N and NDF content with a correspondingly high starch content. Group 4 comprised wheats from the 1998 and 1999 harvest years only and the varieties were mostly of the Hard genotype. The endosperm texture was the highest level of steeliness, the kernels had a medium weight and had the highest measurement for hardness. The samples had the highest N content, high NDF and the lowest starch content.

Table 8 Cluster centroids

| Variable | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Grand centroid |
|---|---|---|---|---|---|
| Dry matter (g $kg^{-1}$ fresh) | 880 | 879 | 882 | 886 | 881 |
| Nitrogen (g $kg^{-1}$ DM) | 21 | 20 | 19 | 22 | 20.6 |
| NDF (g $kg^{-1}$ DM) | 93 | 100 | 88 | 93 | 94.4 |
| Starch (g $kg^{-1}$ DM) | 735 | 720 | 740 | 709 | 728.3 |
| Steely grains | 40.1 | 9.6 | 7.0 | 81.5 | 31.23 |
| Piebald grains | 26.2 | 16.7 | 7.2 | 14.1 | 19.60 |
| Mealy grains | 33.3 | 73.7 | 85.8 | 4.4 | 49.01 |
| Kernel weight (mg) | 49.9 | 51.3 | 53.8 | 51.9 | 51.04 |
| Kernel diameter (mm) | 3.01 | 3.11 | 3.15 | 3.13 | 3.07 |
| Kernel moisture (g $kg^{-1}$ fresh) | 13.9 | 13.3 | 13.3 | 13.3 | 13.58 |
| Kernel hardness | 32.6 | 52.4 | 14.1 | 63.2 | 39.0 |

Regression analysis was carried out for starch disappearance (at 8h) (Y variate) of the population of wheats sub-divided into the four groupings against the chemical components and the grain characteristic parameters. Sub-dividing the population of wheats according to both the chemical and grain derived characteristics improved the variance accounted for in starch disappearance for all groups compared with the population as a whole. The best derived relationships were as follows:

Group 1

Rumen degradable starch (mg $g^{-1}$ fresh) = 2237 + 1.01NDF (g $kg^{-1}$ DM) - 2.23 DM (g $kg^{-1}$ fresh)

SEP = 53.3, $R^2$ = 15.7%

Group 2

Rumen degradable starch (mg $g^{-1}$ fresh) = 1036 - 17.8 N (g $kg^{-1}$ DM) - 5.44 Weight (mg)

SEP = 41.9, $R^2$ = 57.3%

Group 3

Rumen degradable starch (mg $g^{-1}$ fresh) = 2.55 Starch (g $kg^{-1}$ DM) - 114 Hardness

SEP = 32.5, $R^2$ = 90.9%

Group 4

Rumen degradable starch (mg $g^{1}$ fresh) = 2298 - 508 Diameter (mm) - 3.84 NDF (g $kg^{-1}$ DM)

SEP = 50.0, $R^2$ = 66.5%

## 4.0 Discussion

The six varieties investigated in this study were chosen to represent both Hard and Soft wheats. Hereward, Reaper and Rialto are the Hard wheats and Consort, Madrigal and Riband are the Soft wheats. Both Rialto and Madrigal possess the 1B/1R gene (substituted from Rye to increase yield). The wheat samples chosen had a wide range in chemical composition, and this was a reflection of variety (genotype) and site and year (environment) effects. The Hard wheat varieties had higher nitrogen content than the Soft wheat varieties across all environments. Similarly there were no environment x variety interactions for any of the other chemical components. Therefore the ranking of varieties could be combined for all RL sites and years but it should be noted that the absolute levels will vary from trial to trial. With the gas production parameters there was apparently less variation between samples than for chemical composition, but when it is considered that each sample had essentially the same carbohydrate source then the range was acceptable and good. The calculated EOMD at 6% $h^{-1}$ rumen outflow ranged from 40.9 to 48.3%. There was no significant effect of either year, site (environment) or variety on calculated EOMD at 6% $h^{-1}$ rumen outflow nor for the combined fractional rate at that rumen outflow. There were no significant correlations between any of the gas production parameters and the chemical composition. Nitrogen content was negatively correlated with starch content ($r = -0.56$). This is to be expected as in under fertilised crops nitrogen accumulation will be affected to a greater degree than starch accumulation, and in over fertilised crops there will be luxury uptake of nitrogen. Similarly, plants growing in cool conditions may have higher starch contents because the rate of development is more temperature dependent than the rate of growth (HGCA, 1997). This was also noted in the previous HGCA study (Project Report No. 182) where the relationship was stronger ($r = -0.74$) due to a wider range in values and that the samples came from only one harvest year. Cockle Park, Bridgets and Harper Adams tended to have higher starch contents than Norfolk and Cambridge sites. Therefore although it is possible to manipulate the relative concentrations of starch and nitrogen in the grain through nitrogen fertiliser management, which could be of significant value to the feed manufacturing industry, the effects on crop yield *per se* must also be considered.

The further investigation of the samples after 8 h incubation showed that the total gas production was directly related to OM disappearance at 8 h (r = 0.403) and this has been shown by other workers for barley (r = 0.95; Trei *et al*., 1970). This was to be expected as rumen microbes degrade plant carbohydrates, both non-structural and structural polysaccharides, to provide energy. The main end-products of carbohydrate fermentation in the rumen are VFA and gas ($CO_2$ and methane). In this study, there was also a strong relationship between starch degraded and gas produced (r = 0.574) which provides confirmation that the technique can be used to reflect starch utilisation. This is of particular importance as gas production was not related to starch content. A good relationship (r = 0.93) between gas production after 7 h incubation and wheat starch degraded has also been noted by Opatpatanakit *et al*. (1994).

The volume of gas produced per gram of starch degraded at 8h was 396ml. There was also a significant but poor negative relationship between the calculated rumen degradable starch (at 8h, mg $g^{-1}$ fresh) and the nitrogen content of the grains as follows:

Rumen degradable starch at 8h (mg $g^{-1}$ fresh) = 566 - 9.31 N(g $kg^{-1}$ DM)

SEP = 70.46 $R^2$ = 5.3%

The varieties described as "Hard" in the UK Recommended list tended to require higher N content than the "Soft" varieties to provide the same low level of rumen degradable starch. There was a significant relationship between rumen degradable starch at 8h and grain starch content as follows:

Rumen degradable starch at 8h (mg $g^{-1}$ fresh) =0.515 Starch (g $kg^{-1}$ DM)

SEP = 66.0 $R^2$ = 17.4%

this relationship was significantly improved when the Soft wheats were considered alone as follows:

Rumen degradable starch at 8h (mg $g^{-1}$ fresh)= -1015+ 1.87 Starch (g $kg^{-1}$ DM)

SEP = 52.3 $R^2$ = 51.6%

There was no significant relationship between rumen degradable starch and starch content when the Hard grains were considered alone and as starch and nitrogen content were negatively correlated for all grains, this was not expected, but indicates that N content particularly for Hard grains influences starch degradability in some way other than by a purely dilution effect.

For rumen degradable starch, Cambridge, Norfolk and Harper Adams had significantly lower contents than Cockle Park and Bridgets and this corresponded with lower grain nitrogen and higher grain starch contents for the last two sites compared with the others. The varieties were ranked for rumen degradable starch Riband<Rialto=Hereward=Madrigal<Consort and Reaper for 1998 and 1999 harvests combined.

The increased level of N and NDFa in the Cambridge and Norfolk site samples may be indicative of plant stress. Water stress during the grain filling period can lead to serious loss of yield, due to premature senescence of the crop canopy limiting availability of carbohydrates for grain filling. Water availability can be affected by a number of factors including; drought, soil structure and rotation. The amount of soluble carbohydrate stored in the stem varies between varieties and, varieties with a high level of soluble carbohydrate are better able to withstand the effects of late season drought stress (Foulkes and Scott, 1998) . The carbohydrate stored in the stems can act as an alternative source for grain filling when direct supply from photosynthesis is limited by stress during grain filling. Foulkes and Scott, (1998) reported that Rialto had the highest soluble stem carbohydrate reserves and Riband and Spark the lowest suggesting that Rialto is a drought resistant variety. Rialto's relative insensitivity to drought stress appeared to be reflected in the rumen degradable starch at 8h which was unaffected at the Cambridge and Norfolk sites (where drought stress occurred) relative to the Cockle Park and Bridgets sites (unstressed). Conversely Riband which is reported to be more susceptible to drought stress, had lower rumen degradable starch at 8h at the Norfolk site compared with the Cockle Park and Bridgets sites.

The wheat samples were characterised by the proportion of mealy and steely grains using the light transmission method and by degree of hardness using NIRS. There was a positive correlation between nitrogen content and both steeliness and hardness and these relationships were negatively correlated with starch content. Both degree of hardness and mealiness were significantly negatively correlated (r = - 0.84) with each other. Of particular note was the fact that different wheat grains from the same sample (and therefore the same variety and agronomic treatment etc.) could be either mealy or steely or intermediate.

Endosperm cells contain starch granules embedded in a protein matrix. In Soft wheats, air spaces and discontinuities in the matrix make it friable, whereas in Hard wheats the endosperm cells are tightly packed with starch granules held firmly within the matrix. In terms of milling technology, Hard wheats fracture differently to Soft wheats producing larger, coarser starch-protein particles with a high degree of starch damage caused by disruption of the crystalline structure (Mattern, 1988). Soft wheats produce less free-flowing, smaller particles, with more free starch and protein with less starch damage (Neel and Hoseney, 1984).

From this it can be surmised that wheat which is Hard and steely in nature will contain larger particles when ground, which have a significant protein matrix and hence these may be more resistant to microbial attack but the higher level of starch damage in Hard wheats may counteract this. Whereas Soft wheat that is mealy in nature contains smaller particles, within a more open structure and lower association with the protein matrix and hence may be more open to microbial attack, but the greater association of the cell wall with the endosperm may counteract this. Cone *et al.* (1989) showed that the percentage degradation of starch from wheat grain, sieved to provide samples of varying particle sizes prior to incubation in rumen fluid *in vitro*, decreased linearly with increasing particle size.

The amount of starch degraded after 8 h was related to total gas production at 8 h ($R^2$ = 20.5 %). The prediction of the amount of starch disappearance at 8 h was further improved by the addition of starch content together with total gas production at 8 h. The relationship was as follows:

Starch degraded at 8 h (mg $g^1$ fresh) = -741 +3.71total gas at 8h (ml $g^1$ DM) + 1.05 starch (g $kg^{-1}$ DM)

$R^2$ = 42.5 % ; Standard error of prediction = 54.9

It can be concluded that the year and site of growth influence the amount of starch degraded in the rumen (at least as determined *in vitro*) for different varieties of wheat. This implies that bread making wheats grown with high rates of fertiliser nitrogen will lead to a lower starch supply to the rumen. In addition, the characteristics of the starch in terms of mealiness and steeliness and any variety effect on hardness or softness will further influence the amount of starch available to rumen microbes. From other work (HGCA Project Report No. 182) it appears that mealiness and steeliness are predominantly an effect of nitrogen content of the grain and hence related to the environment/crop management and nutrition.

A recent HGCA funded study (Project report No. 253) 'The nutritional value of wheat for poultry: Analysis of gene effects using isogenic lines' reported on the nutritional consequences of key genetic characteristics of wheat. The use of isogenic lines allows the study of single genetic characteristics. Starch digestibility in poultry was investigated and it was concluded that the presence of the 1B/1R gene is associated with depressed starch digestibility and that Soft wheats tended to have higher starch digestibility than Hard wheats. There was an interaction between the 1B/1R gene and grain Hardness, as follows: starch digestibility was ranked with 1B/1R Hard < 1B/1R Soft < no 1B/1R Soft < no 1b/1R Hard. The effect of dough extensibility on starch digestibility was determined in Soft wheats and there was an increase in digestibility with dough extensibility. Nutritionists have identified that poultry require wheat varieties of high starch digestibility whereas, ruminants require wheat which is of low rumen degradability and high whole tract digestibility.

In this study Rialto and Madrigal (Hard and Soft wheat respectively) contained the 1B/1R gene. From the isogenic work it would be expected that Rialto would have lower starch digestibility than Madrigal and overall this was confirmed for rumen degradable starch. It would also be expected that the varieties containing the 1B/1R gene would have lower starch digestibility than the non-1B/1R varieties and overall this was also confirmed for rumen degradable starch.

Comparing Riband and Consort within a harvest year (but regardless of site) the rumen degradable starch was always lower for Riband compared with Consort. Consort is very closely related to Riband (Consort's pedigree = Riband 'sib' x Fresco x Riband). However, it is higher yielding and has a slightly different glutenin sub-unit composition which makes its protein slightly more extensible. As recorded in the isogenic work dough extensibility in Soft wheats gave rise to higher starch digestibility as determined in poultry. This may partly explain why Consort consistently had higher rumen degradable starch than Riband. It has been suggested that Consort has a slightly harder endosperm and higher level of starch damage, under identical milling conditions, but this is not likely to be a big effect. In this study overall the Hardness as determined using the SKS machine was not significantly different between the two varieties, there may, however, have been an influence of milling on starch damage but this was not measured in this study. Consort is thought to be higher yielding due to longer persistence of the canopy for grain filling (Roger Sylvester-Bradley, Personal communication). This means that the pattern of deposition of starch granules may be different i.e. if allowed to go to full maturity, Consort may have more of the smaller B-type granules than Riband which would increase the degradability of the starch in the rumen. Overall the starch content for Consort was 12 g $kg^{-1}$ DM higher than for Riband.

The use of NIRS to develop calibrations for this wheat population was successful for nitrogen content and endosperm texture on the whole grains. Garnsworthy *et al.* (2000) used NIRS to accurately predict the chemical composition of wheat ($r^2$ = 0.51 for DM, 0.90 for crude protein, 0.78 for starch). The coefficient of determination was similar in the current study for protein, but lower for starch. In the current study whole grains were scanned as opposed to milled grain in the study of Garnsworthy *et al.* (2000). NIRS radiation does not penetrate far into the grain which may explain why the prediction of starch content is poor as the starch is held in the endosperm. Calibrations of NIRS with any of the gas production parameters were unsuccessful. This may be a result of the insufficient spread of data within this population. Since starch is the main factor influencing rumen degradability a better prediction of starch is required before the prediction of the gas production parameters can be improved. Garnsworthy *et al.* (2000) were unable to successfully predict the nutritive value of wheat for pigs and poultry using either NIRS or chemical composition and agronomic characteristics.

Rumen degradable starch disappearance for wheat grains is an important characteristic of the nutritive value of wheat for ruminants. This value is influenced by year of harvest, site of growth, agronomy and variety. There was no site x variety interaction. The varieties were ranked in 1997 as Hereward<Consort<Reaper, in 1998 there was no significant varietal effect detected and in 1999 as Riband<Rialto< Hereward<Madrigal<Consort<Reaper. There were no significant regression relationships between simple measures of grain quality and gas production techniques that could be recommended for routine use. Over all three years Reaper had the highest RDS and Riband the lowest. The information from these varieties were used in the ration formulation programme produced from the FIM consortium to formulate dairy diets. The model was used to formulate a ration for a 650 kg cow producing 30 l milk per day, losing 0.5 kg liveweight per day and 15 weeks into its lactation. The model was offered grass silage (max. 30 kg fresh weight) rapeseed meal, soya bean meal (Hipro), molassed sugar beet feed and a minimum of 4.5 kg DM of either of the two wheats. The FIM model has a rumen sub-model and both diets were formulated to provide a balanced rumen pH and to meet the nutrient requirements of the cow. The details of the rations, the dry matter intake and the diet composition are given in the Table below:

| | Reaper | Riband |
|---|---|---|
| Ration (kg DM $d^{-1}$) | | |
| Grass silage | 8.99 | 8.98 |
| Rapeseed meal | 2.55 | 2.46 |
| Soya bean meal | 0.24 | 0.00 |
| Molassed sugar beet feed | 2.33 | 1.80 |
| Wheat | 5.30 | 5.87 |

| | | |
|---|---|---|
| Dry matter intake (kg $d^{-1}$) | 19.4 | 19.1 |
| Diet composition (g $kg^{-1}$ DM) | | |
| Crude protein | 169 | 162 |
| Starch | 209 | 236 |
| Sugar | 84 | 76 |
| Neutral detergent fibre | 308 | 306 |
| Oil | 36 | 38 |
| ME (MJ $kg^{-1}$ DM) | 12.2 | 12.2 |

The ration contains 0.57 kg wheat DM $d^{-1}$ more of the Riband compared with Reaper due to its slower fermentation rate for starch. This equates to inclusion rates of 27.3 and 30.7% for Reaper and Riband respectively. For a 100 cow herd using this diet for 100 d, the farmer would require an additional 5700 kg of Riband compared to Reaper. In monetary terms, the diets were costed at £84.54 and £82.20 per tonne of DM, for Reaper and Riband respectively, a differential of £2.34 per tonne DM. This differential is due entirely to the differences in fermentation rate between the two varieties of wheat, hence it would be possible to increase the price of Riband wheat up to a maximum of £7.62 per tonne DM wheat.

Hierachial cluster analysis using chemical composition and grain characteristics, sub-divided the population of wheats into four clusters. Two small clusters (clusters 3 and 4) were identified to contain varieties displaying extreme characteristics of endosperm texture and hardness. Cluster 3 contained wheat with the majority of the endosperm as mealy and Cluster 4 steely and respectively the two groups had the lowest and highest N content and hardness. For the mealy group rumen degradable starch was influenced most by starch content and hardness, increasing and decreasing rumen degradable starch respectively. Whereas for the steely group, grain diameter and NDF content had the greatest negative effect on rumen degradable starch and suggests that larger grains with a higher cell wall content help to counteract the negative effects of reduced particle size and increased starch damage from steeliness, hardness and lower N content.

The remaining two clusters contained a higher proportion of the population and differed from the other clusters. Cluster 2 had low steely and high mealy but differed from cluster 3 in that the grains had a high level of hardness. This cluster had the highest mean rumen degradable starch and this was negatively related to both N content and the kernel weight. The negative relationship of rumen degradable starch with N content for this cluster was noted in HGCA project No. 182. Cluster 1 contained almost equal proportions of steely, piebald and mealy grains and was intermediate for hardness. This cluster contained samples from all the variety and site groups but this varied with harvest year. It is thought that it is difficult to predict the nutritive value of these wheat samples due to the heterogeneous nature of the endosperm texture. There may also be a stronger influence of milling on particle size distribution in these samples which was not determined in this study.

# 5.0 Conclusions

1. A total of 66 contrasting wheat samples harvested in 1997, 1998 and 1999 from six RL sites (Cockle Park, Cambridge, Norfolk, Bridgets, Harper Adams and Cornwall) with six different varieties (Hereward, Reaper, Rialto, Consort, Madrigal and Riband) were obtained and studied. They were subjected to chemical analysis, two tests of grain hardness, tests to distinguish steely from mealy endosperm textures. An *in vitro* gas production system was used to simulate rumen fermentation to assess the proportion of the starch in the wheat which would be fermented in the rumen. Near infrared reflectance spectroscopy (NIRS) was examined as a possible rapid means of predicting starch quality and other factors.

2. The wheats showed a wide range of nitrogen and starch contents and notably, these two fractions were negatively related. Since it is known that nitrogen content can be influenced by nitrogen fertiliser this offers the possibility that starch content may be manipulated by agronomic management.

3. Detailed study of the grains confirmed earlier work that gas production was a good indicator of starch degradation. Rumen degradable starch disappearance for wheat grains is an important characteristic of the nutritive value of wheat for ruminants. This value is influenced by year of harvest, site of growth and variety. There was no site x variety interaction. The varieties were ranked in 1997 as Hereward<Consort<Reaper, in 1998 there was no variety effect and in 1999 as Riband<Rialto< Hereward<Madrigal<Consort<Reaper. There were no regression relationships that accounted for a significant amount of the variance that could be recommended for routine use.

4. For chemical composition e.g. nitrogen, starch and NDFa content the year effect was significant but small in comparison to the effects of variety and site on these parameters. The chemical composition parameters were equally affected by variety and site.

5. Endosperm texture characterised by the proportion of steely and mealy grains was unaffected by year and was effected by variety and site with a bigger emphasis on the site effect, with year x site interactions which confirms that endosperm texture is strongly linked to environmental factors.

6. There was a significant site x variety effect for rumen degradable starch at 8h and for gas production which makes the understanding of what factors influence these parameters very difficult to interpret. Varieties which were sensitive to stress were more variable in their rumen degradable starch than those which contain physiological characteristics which buffer against adverse environmental conditions. Lower rumen degradable starch was recorded in 1999 compared with 1998 which corresponds with higher grain nitrogen. The rumen degradable starch for the samples of wheat by year of harvest are shown in Figure 4.

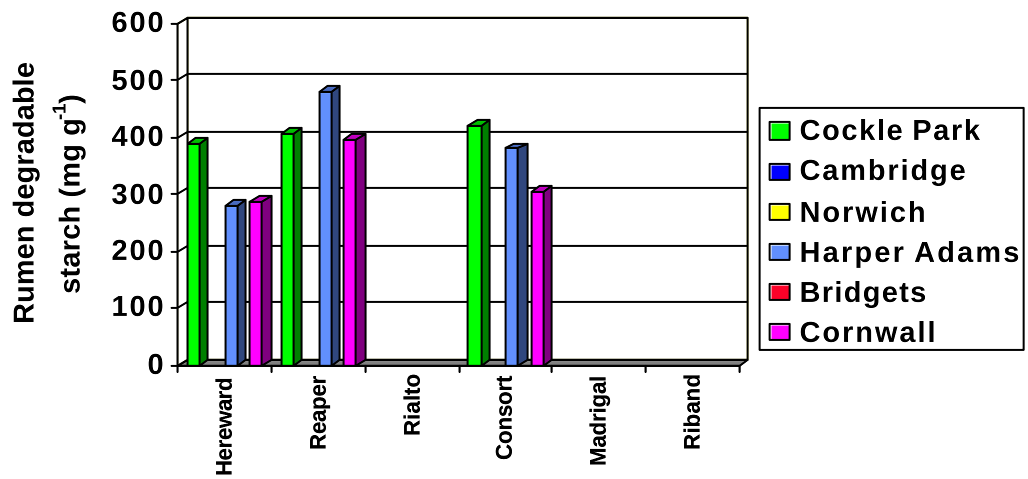
7. There were no significant correlations between any of the gas production parameters and either the chemical composition or the grain quality parameters. Nitrogen content was highly negatively correlated with starch content ($r = ^{-}0.55$). NIRS was able to predict grain hardness, N content and endosperm texture, but was unable to reliably predict any of the *in vitro* parameters.

8. This work has highlighted the fact that the ability to supply starch to the rumen or post-ruminally is a key aspect of the nutritional quality of wheat and that it can vary substantially with variety and site of growth. It was not possible to develop a rapid method of assessment for the nutritive value of wheat.

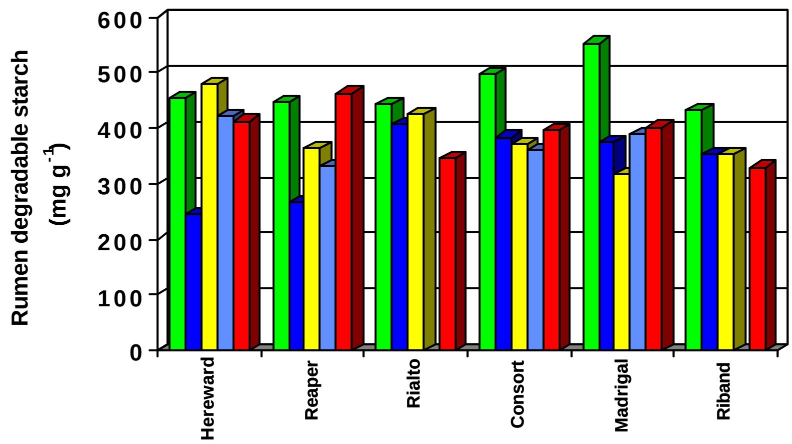
9. Including the low RDS variety Riband compared to the high RDS variety, in the ration of dairy cows on a grass silage based ration allows an increased inclusion rate of 0.57 kg DM per day and a reduction in the price of the ration of £2.34 per tonne DM, equivalent to £7.62 per tonne of wheat DM

Figure 4 Rumen degradable starch for all the varieties of wheat by site and year.

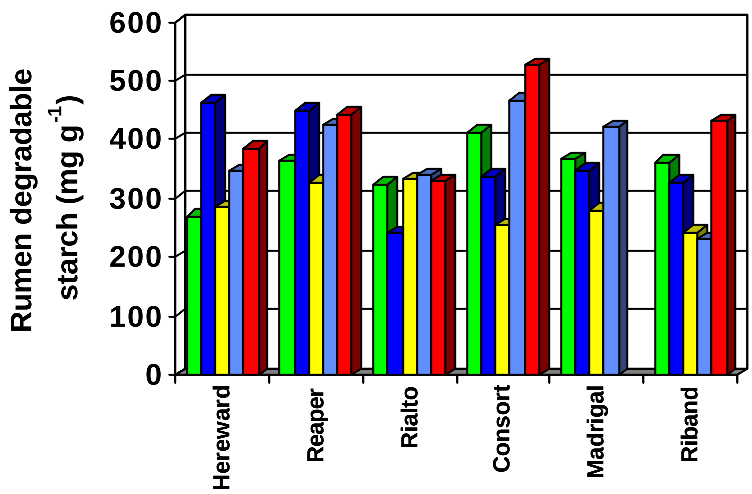
**1997**




**1998**




**1999**



## Recommendations

Significant genotype differences were shown to exist for chemical composition and for nutritive value as determined *in vitro* by the gas production technique. Scope exists to enhance the nutritive value of wheat by breeding through modification of endosperm composition, association of nitrogen with the endosperm and starch damage during milling.

Further understanding of what determines nutritive value of feed wheat for ruminants is required prior to undertaking plant breeding programmes.

It is recommended that rumen degradable starch determined using the *in vitro* gas production technique is carried out for samples from the RL variety testing programme and this parameter along with starch content and endosperm texture be incorporated into the Recommended Variety lists.

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## Appendix 1

The utilisation of feeds by ruminants is dependent upon microbial degradation within the rumen and the description of feeds in terms of their degradation characteristics would provide a useful bases for their evaluation. Kinetics of the fermentation of feedstuffs can be determined from fermentative gas and the indirect gas released from the buffering of the short chain fatty acids produced during fermentation. Kinetics of gas production is dependent on the relative proportions of soluble, insoluble but rumen degradable and undegradable fractions of the feed. Mathematical descriptions of gas production profiles allows analysis of data, evaluation of substrate-related differences and fermentability of soluble and slowly fermentable components of feeds. Various models have been used to describe gas production models.

The model used to fit to the gas production data from this study was that described by France *et al*. (1993). The model of France *et al*. (1993) is based on a generalised Mitscherlich equation as follows:

$$\mu = b + (c/\,(2\sqrt{t})) \text{ and } t{=}T$$

Where $\mu$ = combined rate of gas production at time t

b = underlying rate

c = time dependent rate

T = lag phase prior to gas production

$y = A\,(1\text{-}\ \exp\,[^{-}b(t\ \text{-}T)\text{-}C(\sqrt{t}\text{-}\sqrt{T})]\ )$

Gas production = y

A = asymptote

This information, combined with the undegraded fraction of the feed can be used to calculate an effective degradability of OM at a pre-stated rumen outflow rate.