HGCA

PROJECT REPORT No. 182

FACTORS AFFECTING THE NUTRITIVE VALUE OF WHEAT FOR RUMINANTS

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by

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This is the combined final report of two HGCA-funded projects. Project 0069/01/96 (1801) started in February 1997, lasted for sixteen months and was funded by a grant of £95,129. Project 0071/01/95 (2029) started in February 1998, lasted for five months and was funded by a grant of £16,859. The work is complementary to research funded by the Milk Development Council.

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PREFACE

This work has studied a wide range of factors concerning the nutritive value of wheat for ruminants with emphasis on the availability of starch to the rumen microbes. To aid the reader the findings are presented in this report as three parts each of which represents a distinct area of research.

Part 1, which comprised part of HGCA Project 0069/1/96, studied the effect of wheat variety and crop management on starch availability to rumen microbes whilst Part 2 (also part of HGCA Project 0069/1/96) examined the agronomic implications of the findings presented in Part 1 and based on these implications made recommendations for future research. Part 3 (HGCA Project 0071/01/97) went on to study the effect of specific weight on the chemical composition and energy value of wheat. Also as a natural extension of the findings in Part 1, Part 3 also presents the results of a comparison of starch availability to rumen microbes in maize and wheat.

Because of the rather different nature of work presented in Parts 1, 2 and 3, the executive summaries for each Part is presented separately at the beginning. References cited in all three parts are given at the end of the report.

EXECUTIVE SUMMARIES

Part 1: Effect of variety and crop management on starch availability to rumen microbes

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. Sustained increases in the genetic potential of dairy cows has focused attention on the need to carefully balance the cows dietary requirements using starch and protein provided by compound feeds. Furthermore the animal feed industry must ensure that the supplementary feeding regime does not lead to an excessive environmental impact (e.g. excessive nitrogen excretion) as a consequence of imbalances in the feeding regime.

2. 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. Much of this effect is probably because it provides a very useful form of energy for rumen microbial protein synthesis, although starch which escapes rumen fermentation may be absorbed later as glucose and contribute to the tenuous glucose economy of the animal. It is therefore 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.

3. 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 a number of these important issues.

4. A total of 61 contrasting wheat samples harvested in 1996 from various agronomic experiments were obtained and studied. They were subjected to chemical analysis, two tests of grain hardness, tests to distinguish steely from mealy endosperm textures and a selection of endosperms were also studied using light microscopy. 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. In addition, traditional measures of wheat quality (e.g. specific weight, Hagberg falling number) were obtained from the agronomic experiments that provided the wheat. Near infrared reflectance spectroscopy (NIRS) was examined as a possible rapid means of predicting starch quality and other factors.

5. 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.

6. Detailed study of a selected sub-set (15) confirmed earlier work that gas production was a good indicator of starch degradation. Gas production results from the whole sample population indicated that the effective degradability of organic matter and combined fermentation rate (both primarily reflecting attributes of starch) varied from 50.6 to 55.6 % and 0.088 to 0.104 h^{-1}. This variability particularly when combined with the variability in starch content is likely to result in nutritionally very important differences in starch supply to the rumen.

7. None of the traditional measures of wheat quality commonly used in the milling and baking industries were directly related to the gas production values but the relationship between gas production and the quantity of starch degraded was significantly improved by the inclusion of an index of endosperm mealiness/steeliness. This confirms work in other cereals that endosperm structure and protein deposition therein has an important bearing on starch availability in the rumen. Whilst Hard wheats had a greater tendency to steeliness than Soft wheats, there were exceptions to this. 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 mealy, steely or intermediate in texture. A small additional study carried out on some separated grains from the same sample confirmed that steely grains contain more nitrogen and are fermented more slowly than mealy grains. These findings raise the question as to what controls this characteristic within the plant and whether it can be manipulated by

agronomic treatments. Clearly more work is required in this area, both from the plant and animal perspectives.

8. Satisfactory NIRS relationships were achieved for grain hardness, dry matter and nitrogen contents and specific weight could be predicted by scanning whole grains.

9. Overall, the study has shown that wheats exhibit significant variability in the amounts of starch they contain and although variability in the availability of that starch for rumen fermentation was not as great, the combination of these factors resulted in variability in starch supply which could be nutritionally important in dairy cow rations. The importance of endosperm structure in mediating starch availability to the rumen microbes was an important finding of this study which merits further investigation. This should also include an examination of interactions between the degree of grain processing before feeding and endosperm structure.

10. 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. It has also shown that the measurement of specific weight is not a good guide to this. Accordingly, work is needed to develop other rapid methods for assessing this and other important nutritional characteristics of wheat.

Part 2: Agronomic implications of the findings in Part 1 and recommendations for future research.

1. Wheat is a nutritious feed and a major source of both energy and protein, with approximately 40% of UK production destined for the animal feed sector. Most feed wheat is fed to pigs and poultry, but there is significant potential for increased sales into the dairy market as that industry strives to feed increasingly high yielding cows with ever greater metabolic demands. The current highly competitive wheat prices provide an added incentive to consider wheat in ruminant rations.

2. Traditionally, wheat inclusion rates in the diets of dairy cows have been limited to 1-2 kg/day, but up to 7 kg/day have been fed to high yielding dairy cows at ADAS

Bridgets. Wheat can be rapidly fermented in the rumen leading to acidosis and the complete breakdown of starch in the rumen rather than the small intestine is metabolically less efficient. Maize has been considered a more appropriate source of starch than wheat for dairy cows as it is less readily degraded in the rumen and therefore contains a higher proportion of 'by-pass starch'. Ration formulation is a complex topic in all forms of farm livestock and in practice it is the balance and behaviour of quantifiable ingredients that is critical to efficient feed utilisation and in the dairy cow, manipulation of final milk quality. Rapid fermentation of wheat in the rumen is not necessarily a negative character in the context of ration formulation, as grass based diets may need fast fermenting types to encourage protein breakdown of the grass component, whilst a diet high in lucerne or maize silage would need slow fermenting types.

3. To improve understanding of how UK wheat behaves when fed to dairy cows and how 'quality' from the cereal farmer perspective might be defined, a collaborative study between the HGCA and the MDC was proposed. Initial funding from HGCA was secured for a laboratory evaluation of a range of wheat samples at ADAS FENS, which were sourced from existing experiments. MDC funding is supporting two further studies, firstly a starch sites of digestion study and secondly a feed study with dairy cows.

4. The study reported in Part 1 identified relationships between the proportion of rumen degradable starch and measurable parameters in wheat grain, although, because of the nature of the sample set which did not come from specifically designed experiments, some of these were weak. These relationships have led to the development of several hypotheses as to how feed quality could be manipulated by genotype and environmental factors and these are presented in this report. Further testing of these hypotheses via laboratory and field research is required and a programme of work to achieve this objective is proposed.

5. Wheat destined for sale into the dairy sector has for many years been considered simply as a market for lower grade, perhaps in some way sub-standard, grain. The only specified quality parameter has been grain specific weight, but such concepts are

now being challenged. A number of issues have led to this debate. These include more rigorous controls on livestock ration formulation (quality assurance), tighter management (metabolic) requirements when feeding high performing livestock e.g. dairy cows, fragmentation in the milling and baking cereal market - highlighting the need for specific qualities parameters for each definable market sector and finally economics, encouraging higher wheat inclusion rates in the ration, which highlights technical features (both advantages and disadvantages) of the wheat product as a feed.

6. No price premiums are paid for wheat destined for the animal feeding market. Indeed it is often a scenario of minimising price reductions. Nevertheless, to maintain market share there will be increasing pressure on producers to meet buyer requirements and this pressure will intensify, even at the low prices currently achieved in the UK market place. Improved understanding of how UK growers can meet 'quality' feed wheat targets can only benefit the cereal sector and maximising value and returns. The perception that feed wheat and quality are incompatible terms should be addressed and knowledge gained within this project will contribute to this debate.

Part 3: Effect of specific weight on the chemical composition and energy value of wheat and comparison of starch availability in maize and wheat.

1. Specific weight of wheat is used as an indicator of nutritional quality for trading purposes despite there not being much firm evidence that such a relationship exists. An earlier HGCA-funded project (Part 1) examined some aspects of specific weight but the population of wheats used did not contain samples with low specific weights. The first objective of the present study was therefore to obtain a set of low specific weight samples, to add these to the earlier set and then select sub-population of about 30 wheats to represent a wide range of specific weight values. These were analysed for a range of nutritional characteristics and any relationships with specific weight examined.

2. Starch content and its degradability in the rumen is a key aspect of the nutritional quality of wheat. An earlier HGCA-funded project (Part 1) showed that both of these

aspects exhibited considerable variability. It is often stated that maize grain represents a type of cereal, the starch in which is substantially more resistant to rumen degradation than wheat although comparisons have often been only made between individual samples. The second objective of this study was therefore to examine six selected samples of maize grain in terms of composition and *in vitro* starch degradability/fermentibility for comparison with the values obtained for wheat in the earlier project.

3. The population of 31 wheats selected had dry matter, nitrogen, starch, predicted metabolisable energy and specific weight values which ranged from 852 to 876 g kg^{-1}, 17.0 to 25.9, 627 to 792 g kg^{-1} DM, 13.5 to 14.0 MJ kg^{-1} DM and 61.4 to 84.4 kg hl^{-1} respectively. The objective of including wheat samples with low specific weights was therefore achieved, the lowest value observed in the previous study being 68.7 kg hl^{-1}.

4. Relationships between specific weight and all of the nutritional parameters measured were generally weak although a positive correlation was seen for dry matter (r = 0.56) and starch contents (r = 0.62) although the relationship with dry matter in particular was strongly influenced by variety (particularly Soisson and one sample of Mercia). There was no relationship between specific weight and predicted metabolisable energy value although the variability in the latter was low. Because of the findings in relation to specific weight and dry matter and starch contents, the 31 samples were added to the remaining samples from the earlier study (Part 1) and the same relationships examined for the expanded population (84 samples). In this enlarged set specific weight was also positively correlated (r= 0.51) with dry matter although starch content had less influence.

5. The maize samples had lower nitrogen (13.6 to 14.7 g kg^{-1} DM) and higher starch (729 to 753 g kg^{-1} DM) contents than wheats. In addition, during *in vitro* fermentation, maize grain had 55 5 % longer lag phases, slower fermentation rates (range, maize: 0.052 to 0.058; wheat: 0.088 to 0.104 h^{-1}) and lower effective degradability of organic matter than the wheat. In addition starch degradation after 8h incubation was lower in maize (mean 28.8 %) than wheat (mean 43.5%).

6. The results provide further evidence that specific weight is a poor indicator of nutritive value and although wheat with low specific weights are likely to be associated with high moisture contents this seems to be variety dependant. A new rapid means of predicting nutritive value is required both for trading and diet formulation purposes. It seems likely that near infrared reflectance spectroscopy will play a substantial role in this.

7. The results confirm the contention that maize starch is more slowly and less extensively degraded than wheat starch. For highly productive animals this will often confer an advantage to maize although in terms of starch degradation some wheat did have values almost as low (37.7 %) as the highest starch degradability of the maize (33.5 %). There does therefore seem to be scope for further selection of wheat with characteristics which approach those of maize although for substantial changes in wheat it is likely that some form of processing / treatment will be needed. The current price differential between wheat and maize (about £40 / t) gives wheat a large advantage and if relatively small changes to the characteristics of wheat could be produced with consistency then the opportunity for further market expansion in dairy cow diets in particular would be substantial.

8. Research should now be directed at the replacement of specific weight and on more detailed studies related to factors affecting starch / endosperm quality. This will need to be coupled with an evaluation of possible novel approaches to wheat processing and treatment.

Part 1: The nutritive value of wheat for ruminants: Effect of variety and crop management on starch availability

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Introduction

The current UK Recommended Lists For Cereals (Anon., 1997) which are financially supported by the HGCA, include grain quality information on each variety outlining its potential value to the miller, baker or maltster. These grain quality measures, such as those for grain protein content, Hagberg falling number (HFN), specific weight, Zeleny and endosperm texture are used as a basis for premiums paid to producers. However, no such standards exist for feed grains with the exception that contracts often indicate a minimum specific weight. This is in spite of the fact that feed grains account for 41% of wheat and 50% of barley sales from UK produced cereals.

Wheat is already used by compound feed manufacturers as an energy source for ruminants and dairy cows in particular. Usage would be increased if its nutritive value was better defined to include information such as the proportion of the starch that is either rumen degradable or rumen undegradable (so called by-pass starch) and the rate of degradation of this starch in the rumen. It is vital to know the quantity of starch available to the rumen since it is a major source of energy for microbial protein synthesis (see Reynolds *et al.*, 1997). It has also been shown that increasing the quantity of by-pass starch fed to dairy cows can increase milk protein content (see Reynolds *et al.*, 1997), bringing financial benefits to producers. In terms of starch resistant to breakdown in the rumen, grain maize is the most competitive ingredient to wheat.. Grain maize is also favoured due to the lack of digestive upsets when fed in large quantities. Recent research on maize suggests that there are differences between maize varieties in the amount of rumen degradable and by-pass starch and that this can be manipulated through plant breeding and variety selection (Michalet-Doreau and Champion, 1995).

With the complexities of diet formulation, it is simplistic to describe a 'single ruminant wheat type' for all purposes. Grass-based diets for example, may require fast fermenting types to enable the rumen microbes to utilise the large amounts of soluble nitrogen present in the rumen from the feed, whilst diets high in maize silage or lucerne may require slow fermenting types.

Cereal grains for use as a ruminant feed have traditionally been characterised by their metabolisable energy content, which has been considered to be relatively constant within cereal species . It has however, been shown in barley and maize grain, that the rate of rumen fermentation of the starch is dependent on particle size and the protein matrix within which the starch granules are held. McAllister *et al.* (1993) showed that small particles (0.25 to 0.89 mm) lead to more extensive degradation of the starch than large particles (2 to 3 mm) in both barley and maize grain and that barley starch was more extensively degraded than that in maize. When isolated starch granules from barley and maize were compared, there was no difference in their rumen degradation. The authors concluded that the differences observed in the rumen degradation of barley and maize starch was due mainly to the differences in the protein matrix in the endosperm of the different species.

Cereal quality for other purposes is already measured routinely using established procedures and these quality factors can be manipulated to a greater or lesser extent by plant breeding, environmental conditions and agronomic management. The relative importance of these quality parameters on ruminant feed value, or the interrelationships between them is unknown. In order to determine the rate and extent of starch fermentation in the rumen there are three main possible approaches. These include determining starch flow *in vivo*, using the polyester fibre bag technique *in situ* (Ørskov and MacDonald, 1979) and using the *in vitro* gas production technique (Pell and Schofield, 1993). The first two methods are expensive and time consuming and additionally the *in situ* technique has previously been shown to be a poor estimator of starch disappearance due to problems with fine particle loss from the bags (Weisbjerg *et al.*, 1990). Measurement of gas production with a mixed suspension of rumen micro-organisms has been used to study the influence of grain processing on starch fermentation by rumen micro-organisms (Trei *et al.*, 1970). These workers showed the technique to be sufficiently sensitive and able to determine small differences in utilisation or fermentation of grain substrates by rumen micro-organisms. Gas production was highly correlated with *in vitro* dry matter disappearance, total volatile fatty acid production and starch degradation. With wheat, grain starch represents the largest portion of the carbohydrate substrates and the major portion of the gas produced can be

expected to be from starch, although cell wall carbohydrates would also in part be responsible. It was therefore proposed in this study to use the *in vitro* automated gas production technique to screen a large population of wheat grains obtained from wide ranging management and environmental conditions.

1.1 The programme of work

The programme of work is to be undertaken in four phases and the entire programme is funded by both the HGCA and the Milk Development Council (MDC) and as a result will be of direct benefit to both cereal producers and dairy farmers. This research programme involves nutritional characterisation, agronomic and milk production studies. A schematic plan of the proposed research activities is shown in Appendix 1. This report details the research undertaken in Phase 1. Phase 1 involved *in vitro* nutritional/chemical characterisation of wheat at ADAS Feed Evaluation & Nutritional Sciences (FENS) department, data analysis and in collaboration with other organisations, an assessment of the implications of the *in vitro* study leading to the development of key hypotheses to be evaluated by future cereals research (phase 2).

Phases 3 and 4 (which are to be funded by the MDC) are designed to confirm that variability in starch availability measured in Phase 1 by the *in vitro* fermentation technique was translated in to differences in the sites of digestion of starch in the dairy cow. Phase 4 will evaluate the impact of variation in starch availability on animal performance in the high producing dairy cow.

The scientific objectives of this project were:

Phase 1

1. To determine within wheat, whether there is nutritionally significant variability in starch availability to rumen microbes and hence in starch availability post-ruminally.

2. To determine the degree of variation in wheat starch quality for ruminants which could be attributable to (a) genotype and (b) environmental factors.

The large population of wheat grains were investigated over a 48 h period for gas production profile and terminal organic matter disappearance. A smaller sub-set of the population were used for further studies. An assessment of the implications of the *in vitro* study leading to the development of a 'key hypothesis' to be evaluated for future cereals research will be studied by the Nottingham University/ADAS cereal biochemistry and crop physiology group and reported separately to this report.

Phase 2

Phase 2 will be dependent on the findings and conclusions from Phase 1. It was anticipated that it would be possible to rank samples based upon genetic or environmental traits. Field experiments would be required to test hypotheses from Phase 1 and to provide material with appropriate extremes in terms of their value in ruminant feeding.

Phase 3

It is necessary to confirm the region of the digestive tract in which cereal starch is lost since this has a major bearing on the efficiency of utilisation. This knowledge will also allow the data from the gas production technique to be used for diet formulation. The design of modern diets (and hence compound feeds) requires knowledge of site of digestion in order to tailor the diet for optimum output of milk protein, fat etc. This study will be undertaken using a 4 x 4 Latin square design with four lactating dairy cows fitted with both rumen and duodenal cannulae, four diets and over four time periods. Starch intake from four contrasting wheats will be measured and starch flow to the duodenum will be estimated with the aid of digestion flow markers. Apparent digestibility of starch will also be determined.

Phase 4

A feeding study will be carried out at ADAS Bridgets using a 3 x 2 factorial design with twelve cows per treatment to test the concepts developed in phases 1 to 3. Milk protein output will be compared at one of two levels of by-pass starch supplied from either standard wheat, improved wheat or maize grain. The study will involve a total of 72 Holstein cows in early lactation and will run for a total of twelve weeks. During this period intake, milk yield, milk quality, milk component yield, liveweight and condition score will be recorded for each cow at regular intervals throughout the study.

This report only describes the results from Phase 1 of the study.

2.0 Materials and Methods

2.1 Samples

2.1.1 Screen size study

It was essential that before the study commenced that the gas production screening procedure was carried out on material that represented the processing that is used routinely within the feed manufacturing industry. As a result, samples of wheat were requested from four major feed suppliers/manufacturers to establish the range in screen sizes used in ruminant compound feeds. Three were received as follows:

1) Wheat (1mm screen)
2) Wheat (3mm screen)
3) Wheat (10mm screen)

2.1.2 In vitro gas production

Wheat material was obtained from existing HGCA and MAFF funded studies being undertaken nationally, and by the University of Nottingham and ADAS, and from HGCA/NIAB Recommended List trials, all from the 1996 harvest. The material was selected to cover a wide range in terms of both genotype (varieties) and environments (site and crop management). This provided material of a wide range but the samples did not come from experiments designed to provide wheat samples for this research project. The samples collected are shown in Table 1.

Table 1. Treatment number, nomenclature, site and sample type of wheats used.

Sample number	Sample code	Site	Sample Type
1	AB1	Rosemaund	Mid September 500 seeds/m^2 Nil pgr Mercia
2	AB2	Rosemaund	Mid September 500 seeds/m^2 CCC+Terpal
3	AB3	Rosemaund	Mid September 250 seeds/m^2 Nil pgr
4	AB4	Rosemaund	Mid September 250 seeds/m^2 CCC+Terpal
5	AB5	Rosemaund	Early November 500 seeds/m^2 Nil pgr
6	AB6	Rosemaund	Early November 500 seeds/m^2 CCC+Terpal
7	AB7	Rosemaund	Early November 250 seeds/m^2 Nil pgr
8	AB8	Rosemaund	Early November 250 seeds/m^2 CCC+Terpal
9	AB9	HGCA (0037/1/91)	First Wheat Brigadier
10	AB10	HGCA (0037/1/91)	First Wheat Rialto
11	AB11	HGCA (0037/1/91)	First Wheat Riband
12	AB12	HGCA (0037/1/91)	First Wheat Soissons
13	AB13	HGCA (0037/1/91)	First Wheat Spark
14	AB14	HGCA (0037/1/91)	Third Wheat Brigadier
15	AB15	HGCA (0037/1/91)	Third Wheat Rialto
16	AB16	HGCA (0037/1/91)	Third Wheat Riband
17	AB17	HGCA (0037/1/91)	Third Wheat Soissons
18	AB18	HGCA (0037/1/91)	Third Wheat Spark
19	AB19	Gleadthorpe	Unirrigated Riband
20	AB20	Gleadthorpe	Unirrigated Rialto
21	AB21	Gleadthorpe	Unirrigated Spark
22	AB22	Gleadthorpe	Unirrigated Mercia
23	AB23	Gleadthorpe	Unirrigated Haven
24	AB24	Gleadthorpe	Irrigated Riband
25	AB25	Gleadthorpe	Irrigated Rialto
26	AB26	Gleadthorpe	Irrigated Spark
27	AB27	Gleadthorpe	Irrigated Mercia
28	AB28	Gleadthorpe	Irrigated Haven
29	AB29	Terrington	Slejpner Crop Unshaded
30	AB30	Terrington	Slejpner GS 31-39
31	AB31	Terrington	Slejpner GS 39-59
32	AB32	Terrington	Slejpner GS 59-61
33	AB33	Terrington	Slejpner GS 61-71
34	AB34	Terrington	Slejpner GS 71-87
35	AB35	Boxworth	Riband N 0
36	AB36	Boxworth	Riband N 40 kg/ha
37	AB37	Boxworth	Riband N 90 kg/ha
38	AB38	Boxworth	Riband N 135 kg/ha
39	AB39	Boxworth	Riband N 180 kg/ha
40	AB40	Boxworth	Riband N 240 kg/ha
41	AB41	Boxworth	Riband N 300 kg/ha
42	AB42	Boxworth	Riband N 360 kg/ha
43	AB43	Boxworth	Riband N 420 kg/ha
47	AB47	Rosemaund	Multi site Mercia
48	AB48	Boxworth	Multi site Mercia
49	AB49	Sutton Bonington	Multi site Mercia
50	AB50	Bridgets NIAB	Hereward

51	AB51	Bridgets NIAB	Cadenza
52	AB52	Bridgets NIAB	Caxton
53	AB53	Bridgets NIAB	Consort
54	AB54	Bridgets NIAB	Reaper
55	AB55	Cockle Park NIAB	Hereward
56	AB56	Cockle Park NIAB	Cadenza
57	AB57	Cockle Park NIAB	Caxton
58	AB58	Cockle Park NIAB	Consort
59	AB59	Cockle Park NIAB	Reaper
60	AB60	Cornwall NIAB	Hereward
61	AB61	Cornwall NIAB	Cadenza
62	AB62	Cornwall NIAB	Caxton
63	AB63	Cornwall NIAB	Consort
64	AB64	Cornwall NIAB	Reaper

2.2 Determining screen size for sample preparation

The three samples of wheat from the major compound feed manufacturers (milled using 1, 3 and 10 mm screens) were sub-sampled to provide three 100g samples which were sieved for 10 minutes over the following screens: 8mm, 4mm, 2mm, 1mm, 500μm, 250μm, 150μm, 90μm and 45μm. The weight retained on each screen was determined and the above procedure was repeated for each replicate and each sample.

Additionally, a sample of wheat grain was obtained and milled through the Christy Norris hammer mill at ADAS FENS over three screen sizes (1mm, 3mm and 8mm). The above sieving procedure was carried out on this sample for comparison with the commercially prepared samples.

2.3 Measurement of gas production *in vitro*

A sub-sample (100g) of the 61 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 filtration. 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).

Following statistical analysis of the gas production results, 15 wheat samples (Table 2) of contrasting carbohydrate fermentation were identified. 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.

2.4 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.

Table 2. Treatment number, nomenclature, site and sample type of wheats used for starch disappearance.

Sample number	Sample code	Site	Sample Type, Variety and Treatment
4	AB4	Rosemaund	Mid September 250 seeds/m^2 CCC+ Terpal Mercia
14	AB14	HGCA (0037/1/91)	Third Wheat Brigadier
16	AB16	HGCA (0037/1/91)	Third Wheat Riband
21	AB21	Gleadthorpe	Unirrigated Spark
22	AB22	Gleadthorpe	Unirrigated Mercia
23	AB23	Gleadthorpe	Unirrigated Haven
29	AB29	Terrington	Slejpner Crop Unshaded
35	AB35	Boxworth	Riband N 0
36	AB36	Boxworth	Riband N 40 kg/ha
51	AB51	Bridgets NIAB	Cadenza
52	AB52	Bridgets NIAB	Caxton
53	AB53	Bridgets NIAB	Consort
54	AB54	Bridgets NIAB	Reaper
58	AB58	Cockle Park NIAB	Consort
63	AB63	Cornwall NIAB	Consort

2.5 The light transmission method for distinguishing mealy grains from steely grains

Whole wheat grains (sub-samples of 100 grains, for each of the 15 samples further studied) were placed on a glass plate and illuminated from below. The Soft white mealy portions of the endosperm appeared dark and opaque whereas the Hard grey steely portions of the endosperm appeared translucent and the transmitted light "glowed" orange. The proportions of mealy, steely and piebald (mixed mealy and steely grains) grains in each sample were determined (Tillett *et al.*, 1996) using the visual appearance as described and the whole process was repeated three times.

2.6 The sedimentation test for classification of Hard and Soft wheat

The sedimentation test for wheat hardness was developed from the method of Palmer and Harvey (1977) for the 15 samples further studied. The principle of the test is that when milled, the rigid structure of Hard wheat yields flour with larger particles and with less free starch than Soft wheat. Therefore, the Hard wheat flour drops out of suspension faster than the Soft wheat flour. Wheat (16g) was milled for 5 seconds in a

Braun coffee mill. The milled wheat was sieved and the flour with a particle size less than 250 μm was collected. To 0.4g of this flour was added 20 ml of cold (4°C) 70% (v/v) ethanol in a graduated boiling tube. After shaking for 5 seconds, the tube was allowed to stand for 5 minutes. If the ethanol was cloudy then the wheat was Soft, if the ethanol was clear then the wheat was Hard.

2.7 Light microscopy

Transverse sections (approximately 1 to 2 mm thick) of a selection of mealy/steely/piebald types of wheat grain from the 15 samples studied, were examined using a Nikon Labophot biological light microscope at 20x magnification and each of these were photographed.

2.8 Near infrared reflectance spectroscopy (NIRS)

Three physical forms (whole grain, 3mm grind and 1mm grind) of the 61 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 thousand grain weight, specific weight and Hagberg falling number for the quality data. The maths treatment found to be optimal was 1.4.4.1 (as described by Baker *et al.*, 1994).

2.9 Statistical analysis

2.9.1 Experimental design

This was an investigative study, not a formally designed experiment. It used 61 different samples of wheat from a wide range of sites and varieties which had been selected from other experiments and had therefore been subjected to different treatments. This approach provided a wide spectrum of different wheat characteristics which could have influenced gas production parameters. The 61 samples of wheat were randomised across 21 consecutive gas production runs utilising two x twelve place units, to provide two replicates per sample per run plus two blanks per unit, resulting in six replicates per sample.

2.9.2 Statistical methods

The *in vitro* gas production data was fitted to the model of France *et al.* (1994). Descriptive statistics were then calculated for all variables to ascertain the location and variability of each variable. A matrix was produced of the Pearson product moment correlation for each pairwise comparison, this indicated the extent of linear relationships between the variables. Background information about the variable and the results of the above analysis were used to select variables. Heirarchical cluster analysis was used to try and identify groupings in the wheat samples, the results were also expressed as dendograms.

3.0 Results

3.1 Determining screen size for sample preparation

The particle size distribution for the three samples of wheat grain from commercial sources and the sample of grain prepared using the mill at ADAS FENS are shown in Figure 1. For the 1mm screen samples the sample prepared at FENS had a much greater proportion of particles below 250 μm than the commercially milled sample and therefore was not comparable. The 3 mm screen prepared samples were similar in their particle size distribution profiles with 50% of the sample with a particle size greater than 1 mm. The 10 and 8 mm screen samples had 60 to 70% of the sample with particle size greater than 1 mm and were very heterogeneous in nature. It was therefore concluded that a standard particle size distribution was not normally achieved by commercial mills and that for the purposes of this study, which required a homogenous sample that had not had its endosperm structure completely destroyed by the milling process, that the 3 mm screen would most meet these criteria. Additionally, previous work undertaken for the HGCA investigating rumen nitrogen degradation (Project No. 0014/1/92; Givens *et al.*, 1997) had been carried out with samples milled through a 3 mm screen, thus assisting comparison and interpretation of results.

3.2 Chemical composition

The chemical composition of the grains and their traditional grain quality parameters are shown in Tables 3 and 4 respectively.

Figure 1 Particle size distribution for three wheats from commercial feed mills and one wheat milled through three screen sizes at ADAS

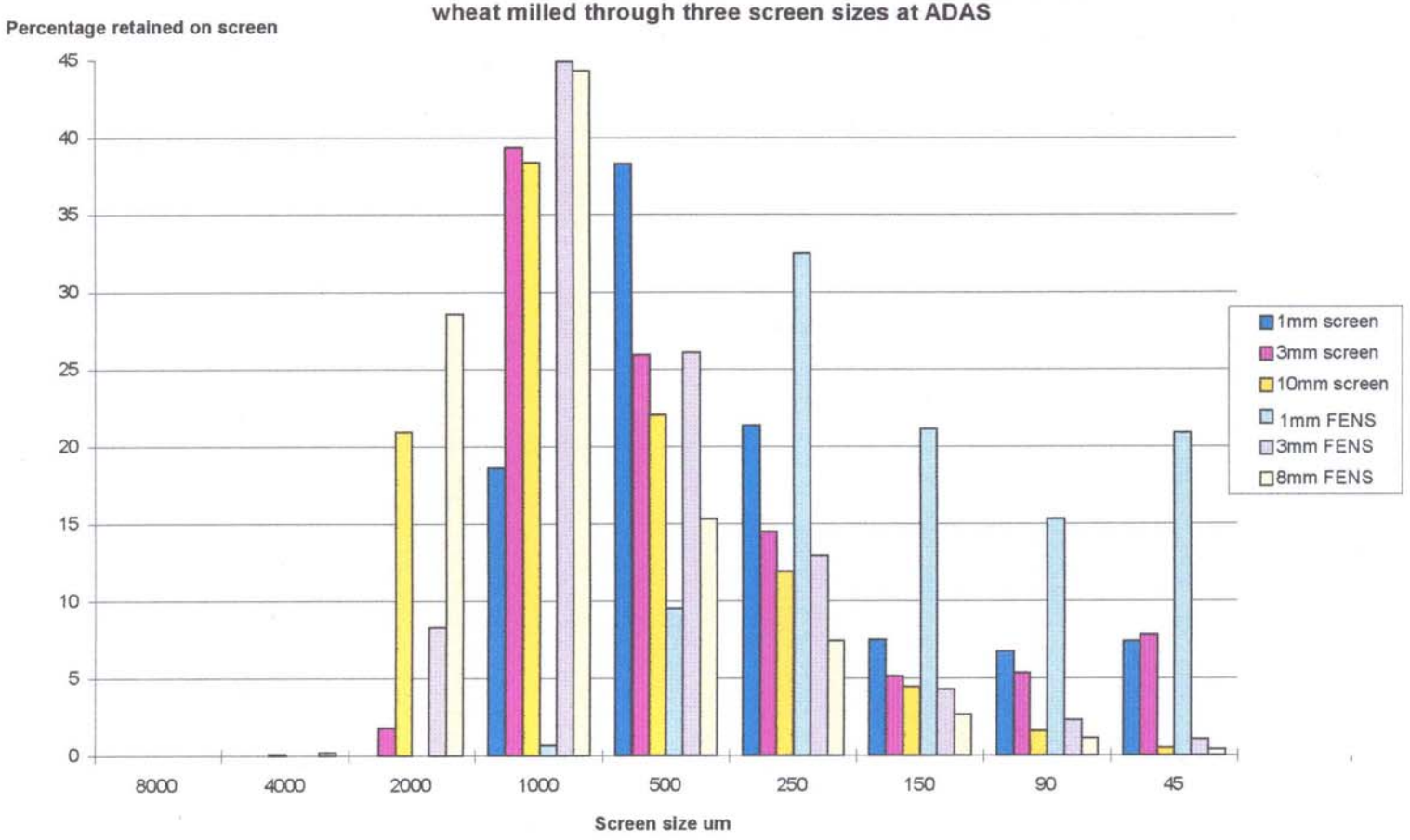


Table 3 Chemical composition of 61 samples of wheat grain (g kg^{-1} DM or as stated).

Sample code	Site	Sample type, variety and treatment	Dry matter (g kg^{-1} fresh)	Nitrogen	NDFa	Starch
AB1	Rosemaund	Mid September 500 seeds/m2 Nil pgr Mercia	867	20.5	73	697
AB2	Rosemaund	Mid September 500 seeds/m2 CCC+ Terpal	872	20.5	83	716
AB3	Rosemaund	Mid September 250 seeds/m2 Nil pgr	871	20.8	64	714
AB4	Rosemaund	Mid September 250 seeds/m2 CCC+ Terpal	870	21.3	93	702
AB5	Rosemaund	Early November 500 seeds/m2 Nil pgr	873	20.6	69	705
AB6	Rosemaund	Early November 500 seeds/m2 CCC+ Terpal	873	20.7	81	702
AB7	Rosemaund	Early November 250 seeds/m2 Nil pgr	881	21.2	78	706
AB8	Rosemaund	Early November 250 seeds/m2 CCC+ Terpal	878	22.1	68	690
AB9	HGCA (0037/1/91)	First Wheat Brigadier	876	22.7	96	718
AB10	HGCA (0037/1/91)	First Wheat Rialto	876	23.0	102	700
AB11	HGCA (0037/1/91)	First Wheat Riband	864	20.5	69	751
AB12	HGCA (0037/1/91)	First Wheat Soissons	859	22.3	99	745
AB13	HGCA (0037/1/91)	First Wheat Spark	865	24.2	80	651
AB14	HGCA (0037/1/91)	Third Wheat Brigadier	873	21.5	91	670
AB15	HGCA (0037/1/91)	Third Wheat Rialto	872	22.9	109	641
AB16	HGCA (0037/1/91)	Third Wheat Riband	877	22.2	104	683
AB17	HGCA (0037/1/91)	Third Wheat Soissons	870	22.5	106	735
AB18	HGCA (0037/1/91)	Third Wheat Spark	876	23.6	114	706
AB19	Gleadthorpe	Unirrigated Riband	869	20.8	105	734
AB20	Gleadthorpe	Unirrigated Rialto	860	23.4	128	697
AB21	Gleadthorpe	Unirrigated Spark	858	25.8	118	680
AB22	Gleadthorpe	Unirrigated Mercia	857	25.7	108	675
AB23	Gleadthorpe	Unirrigated Haven	862	21.3	101	703
AB24	Gleadthorpe	Irrigated Riband	890	15.8	92	702
AB25	Gleadthorpe	Irrigated Rialto	891	16.8	87	714
AB26	Gleadthorpe	Irrigated Spark	893	17.6	75	703
AB27	Gleadthorpe	Irrigated Mercia	892	17.4	80	710
AB28	Gleadthorpe	Irrigated Haven	893	16.5	104	750
AB29	Terrington	Slejpner Crop Unshaded	870	19.4	88	740
AB30	Terrington	Slejpner GS 31-39	865	21.4	81	725
AB31	Terrington	Slejpner GS 39-59	862	20.1	104	702
AB32	Terrington	Slejpner GS 59-61	863	20.1	70	704
AB33	Terrington	Slejpner GS 61-71	865	20.2	75	711

Table 3 Continued...

Sample code	Site	Sample type, variety and treatment	Dry matter (g kg^{-1} fresh)	Nitrogen	NDFa	Starch
AB34	Terrington	Slejpner GS 71-87	867	22.3	81	682
AB35	Boxworth	Riband N 0	874	10.3	95	814
AB36	Boxworth	Riband N 40 kg/ha	878	10.6	95	806
AB37	Boxworth	Riband N 90 kg/ha	880	12.3	98	807
AB38	Boxworth	Riband N 135 kg/ha	879	14.3	91	801
AB39	Boxworth	Riband N 180 kg/ha	881	15.3	95	790
AB40	Boxworth	Riband N 240 kg/ha	880	17.8	115	787
AB41	Boxworth	Riband N 300 kg/ha	876	18.7	40	772
AB42	Boxworth	Riband N 360 kg/ha	875	19.8	89	768
AB43	Boxworth	Riband N 420 kg/ha	880	20.6	101	750
AB47	Rosemaund	Multi site Mercia	871	17	122	783
AB48	Boxworth	Multi site Mercia	858	18.2	122	792
AB49	Sutton Bonington	Multi site Mercia	886	24.2	105	719
AB50	Bridgets NIAB	Hereward	889	21.5	117	749
AB51	Bridgets NIAB	Cadenza	889	19.4	52	723
AB52	Bridgets NIAB	Caxton	890	18.6	107	748
AB53	Bridgets NIAB	Consort	888	18.1	60	772
AB54	Bridgets NIAB	Reaper	892	19.8	136	759
AB55	Cockle Park NIAB	Hereward	888	22.6	136	720
AB56	Cockle Park NIAB	Cadenza	887	21.8	114	709
AB57	Cockle Park NIAB	Caxton	889	21.3	56	717
AB58	Cockle Park NIAB	Consort	890	20.4	84	749
AB59	Cockle Park NIAB	Reaper	887	21.2	134	730
AB60	Cornwall NIAB	Hereward	889	22.3	47	740
AB61	Cornwall NIAB	Cadenza	888	24.4	133	699
AB62	Cornwall NIAB	Caxton	888	22.3	61	710
AB63	Cornwall NIAB	Consort	894	19.8	91	742
AB64	Cornwall NIAB	Reaper	893	21.3	81	721
Mean			877.2	20.2	92.7	726.9
Standard deviation			10.88	3.18	22.33	38.50
Min.			857	10.3	40	641
Max.			894	25.8	136	814

Table 4. Grain quality data of 61 samples of wheat grain.

Sample code	Site	Sample type, variety and treatment	Hardness	Yield (t ha^{-1})	Thousand grain weight (g)	Specific weight (kg hl^{-1})	HFN
AB1	Rosemaund	Mid September 500 seeds/m2 Nil pgr Mercia	Hard	8.12	37.5	81.9	312
AB2	Rosemaund	Mid September 500 seeds/m2 CCC+ Terpal	Hard	9.45	38.7	82.2	362
AB3	Rosemaund	Mid September 250 seeds/m2 Nil pgr	Hard	9.42	38.9	83.1	352
AB4	Rosemaund	Mid September 250 seeds/m2 CCC+ Terpal	Hard	9.70	38.9	82.9	343
AB5	Rosemaund	Early November 500 seeds/m2 Nil pgr	Hard	10.05	42.5	83.5	334
AB6	Rosemaund	Early November 500 seeds/m2 CCC+ Terpal	Hard	9.87	39.6	82.3	342
AB7	Rosemaund	Early November 250 seeds/m2 Nil pgr	Hard	10.07	44.8	83.5	352
AB8	Rosemaund	Early November 250 seeds/m2 CCC+ Terpal	Hard	9.53	43.0	82.8	340
AB9	HGCA (0037/1/91)	First Wheat Brigadier	Hard	9.52	42.9	75.3	336
AB10	HGCA (0037/1/91)	First Wheat Rialto	Hard	10.83	44.4	77.1	368
AB11	HGCA (0037/1/91)	First Wheat Riband	Soft	9.20	42.7	74.1	265
AB12	HGCA (0037/1/91)	First Wheat Soissons	Hard	9.92	40.4	78.3	324
AB13	HGCA (0037/1/91)	First Wheat Spark	Hard	9.02	33.6	78.9	330
AB14	HGCA (0037/1/91)	Third Wheat Brigadier	Hard	8.81	41.9	74.9	368
AB15	HGCA (0037/1/91)	Third Wheat Rialto	Hard	9.62	42.8	75.8	335
AB16	HGCA (0037/1/91)	Third Wheat Riband	Soft	8.32	40.6	74.2	313
AB17	HGCA (0037/1/91)	Third Wheat Soissons	Hard	9.00	40.0	78.4	323
AB18	HGCA (0037/1/91)	Third Wheat Spark	Hard	8.36	30.9	77.8	381
AB19	Gleadthorpe	Unirrigated Riband	Soft	6.76	48.7	72.9	336
AB20	Gleadthorpe	Unirrigated Rialto	Hard	6.06	45.2	73.6	279
AB21	Gleadthorpe	Unirrigated Spark	Hard	6.11	33.7	70.8	373
AB22	Gleadthorpe	Unirrigated Mercia	Hard	5.22	39.0	73.7	383
AB23	Gleadthorpe	Unirrigated Haven	Hard	6.30	47.5	68.7	369
AB24	Gleadthorpe	Irrigated Riband	Soft	10.42	50.4	73.4	238
AB25	Gleadthorpe	Irrigated Rialto	Hard	10.88	49.0	80.6	298
AB26	Gleadthorpe	Irrigated Spark	Hard	10.07	40.0	78.7	273
AB27	Gleadthorpe	Irrigated Mercia	Hard	9.80	46.1	79.4	296
AB28	Gleadthorpe	Irrigated Haven	Hard	11.47	50.7	75.0	248
AB29	Terrington	Slejpner Crop Unshaded	Hard	12.43	47.1	80.7	343
AB30	Terrington	Slejpner GS 31-39	Hard	10.40	50.8	81.6	341

Table 4 Continued...

Sample code	Site	Sample type, variety and treatment	Hardness	Yield (t ha^{-1})	Thousand grain weight (g)	Specific weight (kg hl^{-1})	HFN
AB31	Terrington	Slejpner GS 39-59	Hard	11.62	52.7	81.5	364
AB32	Terrington	Slejpner GS 59-61	Hard	12.07	46.6	80.7	349
AB33	Terrington	Slejpner GS 61-71	Hard	11.00	48.1	80.4	372
AB34	Terrington	Slejpner GS 71-87	Hard	9.78	35.2	76.1	365
AB35	Boxworth	Riband N 0	Soft	5.34	47.2	71.9	264
AB36	Boxworth	Riband N 40 kg/ha	Soft	7.08	47.3	72.3	222
AB37	Boxworth	Riband N 90 kg/ha	Soft	7.94	43.8	72.7	258
AB38	Boxworth	Riband N 135 kg/ha	Soft	8.69	42.6	73.8	256
AB39	Boxworth	Riband N 180 kg/ha	Soft	9.32	42.1	73.6	276
AB40	Boxworth	Riband N 240 kg/ha	Soft	9.43	40.3	74.8	307
AB41	Boxworth	Riband N 300 kg/ha	Soft	9.34	38.9	75.5	335
AB42	Boxworth	Riband N 360 kg/ha	Soft	9.62	39.6	74.7	351
AB43	Boxworth	Riband N 420 kg/ha	Soft	9.50	38.1	74.3	322
AB47	Rosemaund	Multi site Mercia	Hard	9.87	43.3	81.8	346
AB48	Boxworth	Multi site Mercia	Hard	9.43	43.8	84.4	374
AB49	Sutton Bonington	Multi site Mercia	Hard	8.22	39.6	80.4	390
AB50	Bridgets NIAB	Hereward	Hard	11.42	36.84	82.42	287
AB51	Bridgets NIAB	Cadenza	Hard	12.53	36.14	81.72	332
AB52	Bridgets NIAB	Caxton	Hard	12.04	36.7	80.56	230
AB53	Bridgets NIAB	Consort	Soft	12.53	40.43	81.86	153
AB54	Bridgets NIAB	Reaper	Hard	13.03	34.66	79.88	280
AB55	Cockle Park NIAB	Hereward	Hard	9.51	34.98	79.1	271
AB56	Cockle Park NIAB	Cadenza	Hard	9.61	37.54	77.66	274
AB57	Cockle Park NIAB	Caxton	Hard	10.41	36.77	76.96	260
AB58	Cockle Park NIAB	Consort	Soft	9.91	38.19	76.48	291
AB59	Cockle Park NIAB	Reaper	Hard	10.31	38.16	76.64	352
AB60	Cornwall NIAB	Hereward	Hard	9.51	36.61	82.57	360
AB61	Cornwall NIAB	Cadenza	Hard	*	38.4	79.3	347
AB62	Cornwall NIAB	Caxton	Hard	9.23	34.08	81.6	327
AB63	Cornwall NIAB	Consort	Soft	*	37.51	79.37	292
AB64	Cornwall NIAB	Reaper	Hard	10.19	40.9	79.07	366
Mean				9.55	41.30	78.04	319
Standard deviation				1.706	4.91	3.811	48.03
Min.				5.22	30.91	68.69	153
Max.				13.03	52.67	84.43	390

The wheat samples had a mean DM content of 877 g kg^{-1} fresh (range 857 to 894), nitrogen content ranged from 10.3 to 25.8 g kg^{-1} DM (mean 20.2) with wheat from the nitrogen application experiment at Boxworth having the lowest nitrogen content. The irrigated varieties at Gleadthorpe and the NIAB varieties grown at ADAS Bridgets also had low nitrogen contents (< 20 g kg^{-1} DM). The wheats had a mean NDFa content of 92.7 g kg^{-1} DM (range 40 to 136). NDFa content tended to be lower in first wheats compared with third wheats ($P<0.068$) and was also lower for the irrigated wheats compared with unirrigated ($P<0.008$). **Starch content ranged from 641 to 814 g kg^{-1} DM (mean 727) and was inversely related to nitrogen content** (Figure 2). This was particularly notable for the samples from the nitrogen application experiment at ADAS Boxworth.

Amongst the wheat samples obtained there were thirteen different varieties of which eleven are classified as "Hard" varieties and the remaining two (Riband and Consort) are defined as "Soft". The yield of grain recorded for each of the treatments ranged from 5.22 to 13.03 t ha^{-1} with the unirrigated and low nitrogen application treatments having the lowest yields. The NIAB varieties at ADAS Bridgets tended to have the highest yields. **Thousand grain weight and specific weight ranged from 30.9 to 52.7g and 68.7 to 84.4 kg hl^{-1} (respectively) and there was a poor relationship between these two parameters.** The seed population experiment at ADAS Rosemaund (var. Mercia), the multi-site experiment (var. Mercia) and the NIAB varieties at ADAS Bridgets had the highest specific weights. **The unirrigated samples had the lowest specific weights**. Hagberg falling number ranged from 153 to 390, but this was skewed as only one sample was at 153 and the rest were greater than 222. The low HFN was recorded for the Soft wheat (var. Consort) from the NIAB site at ADAS Bridgets. **The mean HFN for all the samples of "Soft" wheat was 280 (SD=50.2) and that of the "Hard" wheats was 325 (SD=59.5). It is therefore possible that HFN may be related to wheat hardness.**

3.3 Measurement of gas production *in vitro*

The mean gas production data are shown in Table 5. (A definition of gas production data is included in Appendix 2). The OM disappearance determined by filtration after 48 h incubation ranged from 75.2 to 80.3 % (mean 78.1). The asymptotic value for gas production from the model of France *et al.* (1993) ranged from 293 to 341 ml g^{-1} DM and the lag time ranged from 1.35 to 2.77 h. The samples with the longer lag period (time to produce gas) were related to grain hardness (correlation coefficient = 0.53, "Hard" or "Soft"), with the "Soft" varieties tending to have the longer lag times. 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 50.6 to 55.6 % and 0.088 to 0.104 h^{-1} respectively.

When cluster analysis was performed on the data set using EOMD and the combined rate of gas production at 6 % rumen outflow as parameters, four clusters were identified. The four clusters contained 18, 33, 5 and 5 samples and had mean EOMD values of 52.5, 53.9, 51.1 and 55.3 % respectively and mean rates of 0.093, 0.094, 0.091 and 0.097 h^{-1} respectively. **The low EOMD cluster had the lowest combined rate and the high EOMD cluster had the highest combined rate.** The samples from clusters 3 and 4 were identified as samples to be studied further.

Figure 2 Relationship of starch with nitrogen content for wheat

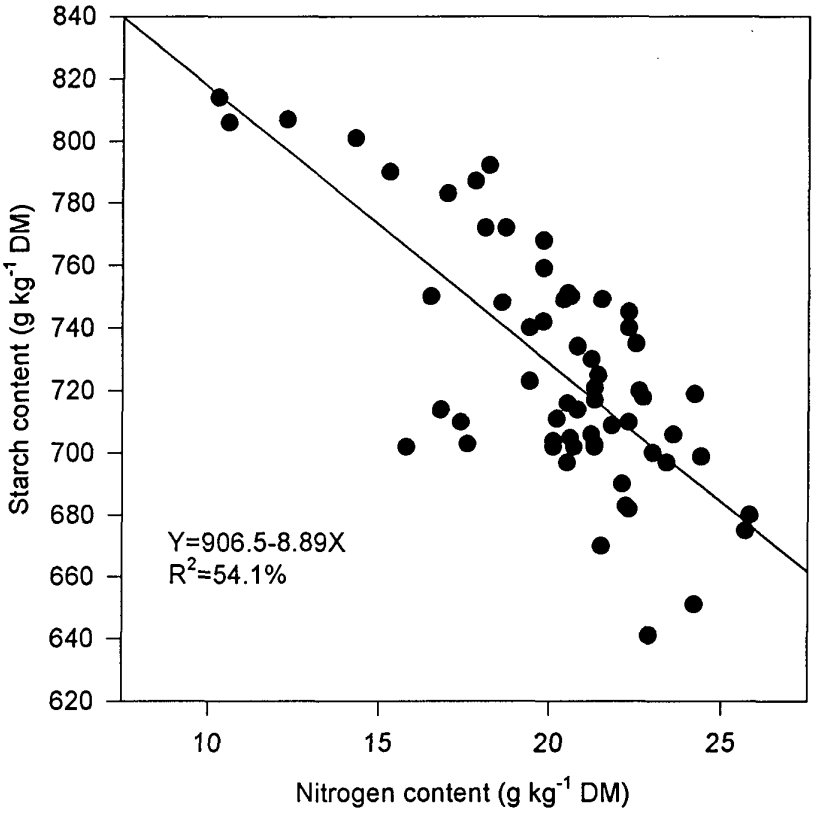


Table 5 Gas production and associated data of all wheats studied.

Sample code	pH at 48h	OMD at 48h (%)	Asymptote (ml g^{-1} DM)	Underlying rate (h^{-1})	Time dependent rate ($h^{-1/2}$)	Lag (h)	Time to half asymptote (h)	Combined rate at 8h (h^{-1})	Combined rate at $t^{1/2}$ (h^{-1})	Effective degradability of organic matter[1] (%)	Combined rate at rumen outflow 0.06 h^{-1} (h^{-1})
AB1	6.2	79.7	313	0.203	-0.527	2.49	10.1	0.109	0.120	52.4	0.092
AB2	6.2	79.4	311	0.205	-0.509	2.28	9.6	0.115	0.123	53.7	0.095
AB3	6.2	78.9	302	0.191	-0.451	1.77	9.5	0.112	0.118	54.3	0.092
AB4	6.2	79.1	294	0.192	-0.456	1.98	9.6	0.112	0.119	54.0	0.093
AB5	6.2	79.2	317	0.191	-0.491	2.28	10.2	0.105	0.115	51.9	0.089
AB6	6.2	79.3	309	0.190	-0.459	1.90	9.8	0.109	0.117	53.8	0.091
AB7	6.2	78.5	302	0.209	-0.528	2.24	9.6	0.116	0.129	53.2	0.095
AB8	6.2	79.1	305	0.192	-0.452	1.80	9.5	0.112	0.119	54.5	0.093
AB9	6.2	78.6	301	0.198	-0.495	2.08	9.8	0.110	0.119	53.0	0.092
AB10	6.3	77.3	302	0.186	-0.443	1.64	9.7	0.108	0.115	52.9	0.090
AB11	6.2	78.9	305	0.202	-0.510	2.32	9.9	0.111	0.120	52.7	0.093
AB12	6.2	77.0	309	0.241	-0.623	2.56	9.1	0.131	0.138	53.1	0.104
AB13	6.3	76.6	318	0.226	-0.568	2.23	9.1	0.126	0.132	53.3	0.100
AB14	6.2	77.7	317	0.198	-0.491	2.10	9.8	0.111	0.119	52.4	0.092
AB15	6.2	76.4	309	0.205	-0.479	1.81	9.1	0.120	0.126	53.7	0.097
AB16	6.2	79.7	332	0.197	-0.530	2.77	10.5	0.104	0.116	50.6	0.090
AB17	6.2	76.7	307	0.237	-0.594	2.40	9.0	0.132	0.137	53.6	0.104
AB18	6.2	76.9	309	0.219	-0.541	2.05	9.2	0.123	0.129	53.6	0.098
AB19	6.2	77.7	321	0.199	-0.505	2.13	9.9	0.110	0.119	52.0	0.091
AB20	6.2	75.5	308	0.207	-0.502	1.87	9.3	0.118	0.125	52.5	0.096
AB21	6.3	77.0	293	0.192	-0.482	2.05	10.0	0.107	0.116	51.4	0.089
AB22	6.3	76.9	315	0.187	-0.466	1.99	10.0	0.105	0.114	51.3	0.088
AB23	6.2	77.3	308	0.227	-0.538	1.84	8.7	0.132	0.135	55.6	0.103
AB24	6.1	78.9	312	0.212	-0.529	1.98	9.4	0.119	0.126	54.5	0.095
AB25	6.2	77.1	300	0.188	-0.432	1.54	9.5	0.111	0.117	53.6	0.092
AB26	6.2	77.7	321	0.198	-0.474	1.43	9.4	0.115	0.121	54.4	0.092
AB27	6.2	78.3	307	0.199	-0.463	1.83	9.3	0.117	0.122	54.6	0.096
AB28	6.1	76.4	317	0.191	-0.456	1.61	9.6	0.110	0.117	52.7	0.091
AB29	6.2	79.2	315	0.183	-0.426	1.35	9.6	0.108	0.115	54.9	0.089
AB30	6.3	79.4	308	0.189	-0.455	1.76	9.8	0.108	0.116	54.1	0.090

[1] at rumen outflow of 0.06 h^{-1}

Table 5 Continued....

Sample code	pH (at 48h)	OMD (48h)	Asymptote (ml g^{-1} DM)	Underlying rate (h^{-1})	Time dependent rate ($h^{-1/2}$)	Lag (h)	Time to half asymptote (h)	Combined rate at 8h (h^{-1})	Combined rate at $t^{1/2}$ (h^{-1})	Effective degradability of organic matter[1] (%)	Combined rate at rumen outflow 0.06 h^{-1} (h^{-1})
AB31	6.2	78.8	314	0.190	-0.461	1.82	9.8	0.109	0.117	53.7	0.090
AB32	6.2	79.6	320	0.192	-0.464	1.82	9.7	0.110	0.117	54.4	0.091
AB33	6.2	78.3	307	0.185	-0.446	1.74	9.9	0.106	0.114	53.0	0.088
AB34	6.2	79.0	316	0.202	-0.488	1.90	9.4	0.116	0.123	54.7	0.095
AB35	6.1	75.3	315	0.236	-0.581	2.11	8.8	0.133	0.138	53.5	0.104
AB36	6.2	77.5	318	0.221	-0.526	1.86	8.8	0.128	0.132	55.2	0.101
AB37	6.1	78.0	341	0.210	-0.525	2.12	9.5	0.117	0.125	53.4	0.095
AB38	6.2	78.3	320	0.192	-0.452	2.01	9.5	0.112	0.119	53.6	0.094
AB39	6.1	78.1	307	0.217	-0.556	2.32	9.5	0.119	0.127	53.1	0.097
AB40	6.2	79.0	324	0.195	-0.499	2.25	10.1	0.107	0.116	52.2	0.090
AB41	6.2	79.7	318	0.205	-0.537	2.57	10.1	0.110	0.120	52.2	0.093
AB42	6.2	80.3	319	0.213	-0.553	2.40	9.8	0.115	0.124	53.8	0.095
AB43	6.2	79.8	324	0.190	-0.487	2.26	10.3	0.104	0.114	52.3	0.089
AB47	6.1	78.0	313	0.191	-0.440	1.70	9.4	0.114	0.119	54.3	0.094
AB48	6.2	79.8	317	0.176	-0.407	1.74	9.9	0.104	0.111	53.9	0.088
AB49	6.2	79.3	316	0.198	-0.479	1.94	9.5	0.113	0.121	54.4	0.093
AB50	6.1	77.1	300	0.213	-0.552	2.38	9.7	0.115	0.124	51.8	0.095
AB51	6.2	78.1	326	0.195	-0.456	1.61	9.3	0.115	0.121	54.5	0.094
AB52	6.2	79.0	311	0.200	-0.470	1.68	9.3	0.117	0.123	55.3	0.095
AB53	6.2	77.5	322	0.209	-0.553	2.47	10.0	0.111	0.122	51.2	0.093
AB54	6.1	78.6	305	0.211	-0.499	1.75	9.0	0.123	0.128	55.6	0.098
AB55	6.2	77.9	301	0.225	-0.558	2.22	9.1	0.126	0.132	54.3	0.101
AB56	6.2	78.0	312	0.183	-0.437	1.42	9.8	0.106	0.113	53.5	0.088
AB57	6.2	75.2	314	0.211	-0.514	1.91	9.2	0.120	0.126	52.4	0.096
AB58	6.2	77.0	305	0.209	-0.553	2.45	10.0	0.111	0.121	50.9	0.092
AB59	6.2	76.9	304	0.209	-0.513	1.79	9.3	0.119	0.125	53.7	0.095
AB60	6.2	77.7	306	0.194	-0.460	1.71	9.51	0.113	0.120	53.8	0.093
AB61	6.2	79.0	304	0.182	-0.432	1.64	9.9	0.105	0.113	53.6	0.088
AB62	6.2	78.1	304	0.191	-0.460	1.45	9.6	0.110	0.117	54.0	0.090

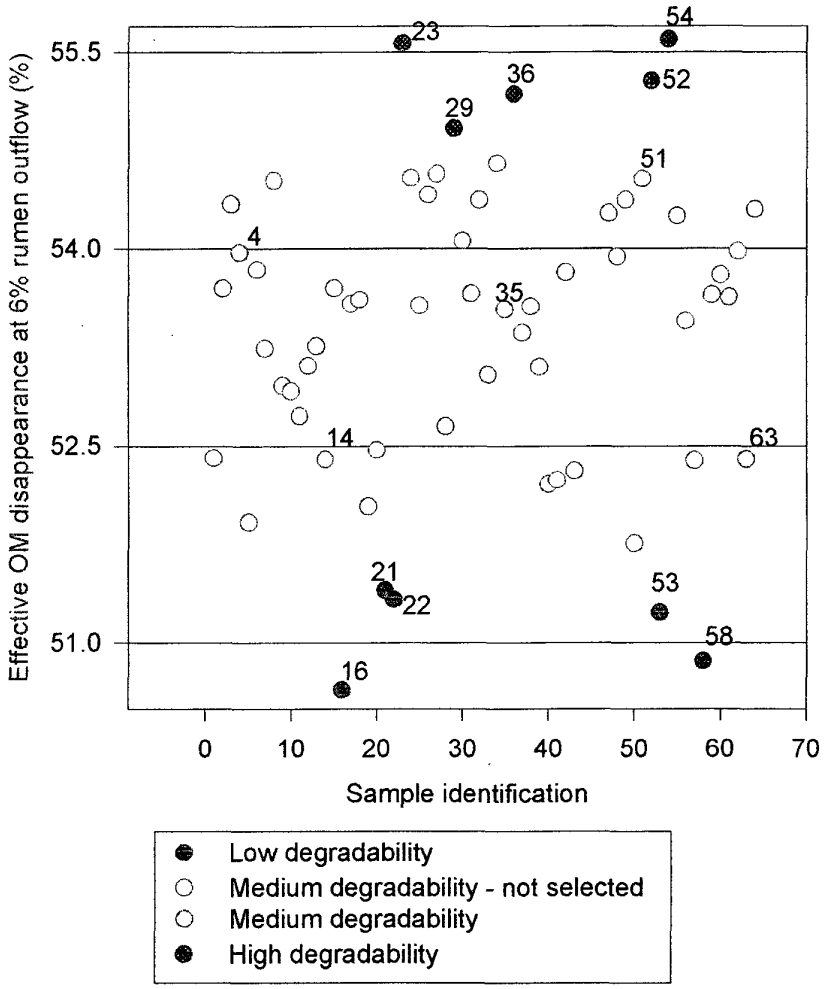
Table 5 Continued....

Sample code	pH (at 48h)	OMD (48h)	Asymptote (ml g^{-1} DM)	Underlying rate (h^{-1})	Time dependent rate ($h^{-1/2}$)	Lag (h)	Time to half asymptote (h)	Combined rate at 8h (h^{-1})	Combined rate at $t^{1/2}$ (h^{-1})	Effective degradability of organic matter[1] (%)	Combined rate at rumen outflow 0.06 h^{-1} (h^{-1})
AB63	6.2	77.7	306	0.218	-0.563	2.48	9.6	0.119	0.127	52.4	0.097
AB64	6.2	78.5	320	0.219	-0.555	2.01	9.3	0.120	0.129	54.3	0.096
Mean	6.2	78.1	311	0.2022	-0.4976	1.99	9.577	0.1142	0.1217	53.4	0.094
SD	0.05	1.18	8.8	0.01444	0.04742	0.325	0.3848	0.00741	0.00645	1.14	0.0041
Min	6.1	75.2	293	0.1756	-0.6230	1.35	8.656	0.1036	0.1111	50.6	0.088
Max	6.3	80.3	341	0.2408	-0.4068	2.77	10.525	0.1332	0.1378	55.6	0.104

[1] At rumen outflow of 0.06 h^{-1}

The remaining five samples selected for further study were chosen from clusters 1 and 2 to represent the mean EOMD and to cover a range of starch concentrations. The fifteen samples were identified and described in Table 2 and are shown in Figure 3 by EOMD value.

Figure 3 The ranking of effective organic matter degradability at 6% rumen outflow determined *in vitro* for wheat



The gas production, OM and starch disappearance, pH and VFA composition from the *in vitro* incubations after 8 h are given in Table 6 for the fifteen samples. The mean

volume of direct gas at 8 h increased with increasing degradability as defined by OM disappearance (%) at 8 h whilst the starch disappearance (%) was more variable with the medium group having a lower mean than the low degradability group. The total VFA concentration increased with increasing degradability, although the molar proportions of the individual acids were similar between samples of wheat.

The amount of OM and starch degradation and the estimated fermentable organic matter (FOM) calculated from the volatile fatty acid production at 8 h are given in Table 7 for the 15 samples. 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 tendency for more to be diverted into fermentation products and less directly into microbial synthesis.** However this relationship was not significant ($P>0.05$). The amounts of starch degraded at 8 h increased with the degradability groups and were similar in value to the calculated FOM content. **The starch degraded at 8 h was positively correlated with the 8 h total gas production ($r = 0.822$),** direct gas volume ($r = 0.752$, Figure 4) and also to a lesser degree with the amount of EOMD at 6 % h^{-1} rumen outflow ($r = 0.632$, Figure 5).

Figure 4 Relationship between rumen degradable starch and direct gas both estimated in vitro for wheat

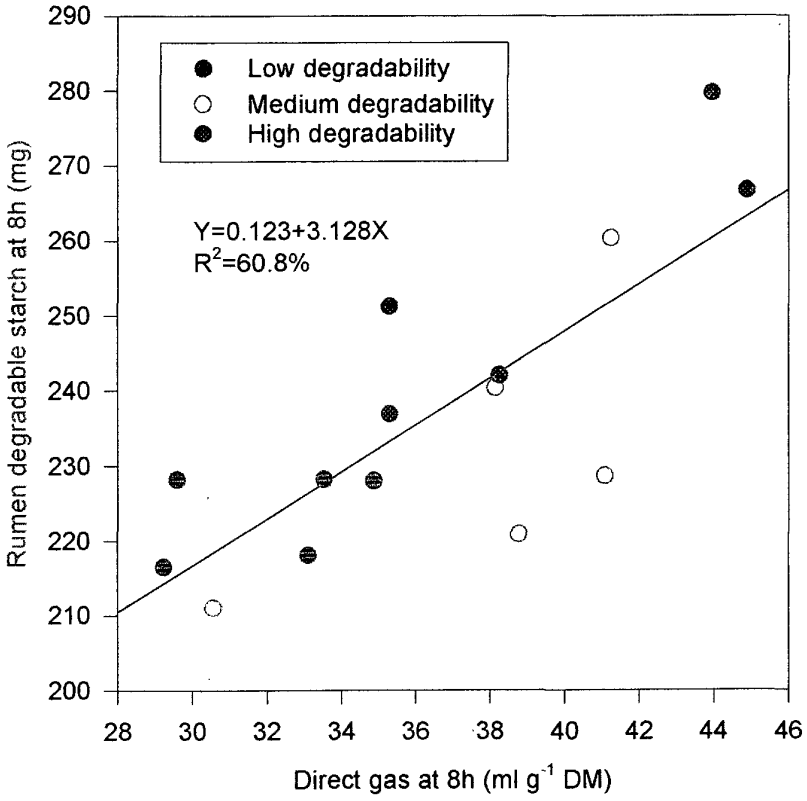
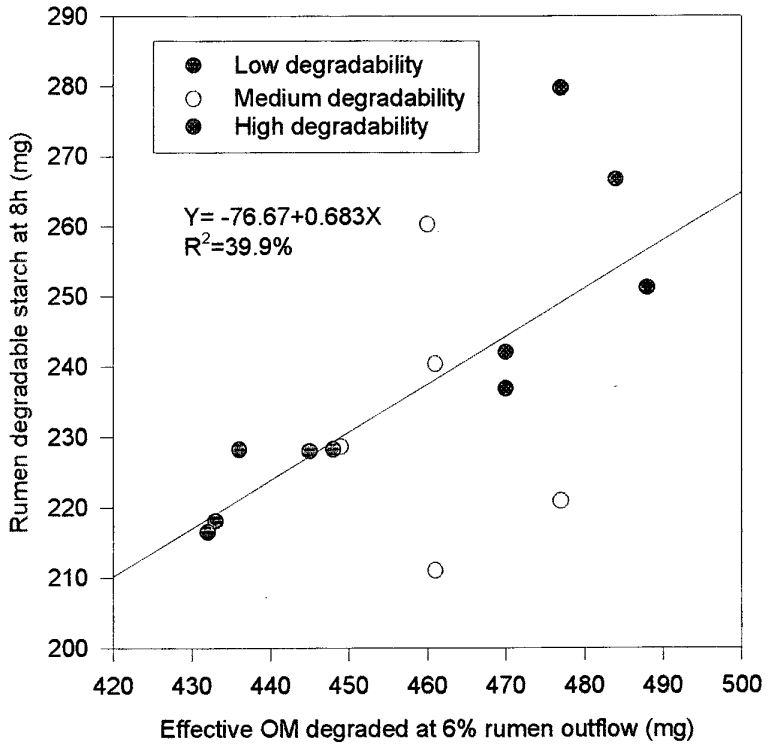


Figure 5 Relationship between rumen degradable starch (8h) and effective organic matter degraded at 6% rumen outflow both estimated *in vitro* for wheat



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

Table 8 shows the proportion of mealy and steely grains for the fifteen wheat samples. The two samples of Riband (Boxworth site) which had low nitrogen fertiliser application and hence low grain nitrogen contents (mean nitrogen = 10.45 g kg^{-1} DM) had 100 % of mealy grains, whereas the sample of Riband (not Boxworth site) which had standard nitrogen application (nitrogen content = 22.2 g kg^{-1} DM) had only 61 % of mealy grains. **There was a positive correlation between nitrogen content and steeliness (r = 0.686) and the relationship was negative for starch content and steeliness (r = -0.588).** The two samples of the variety Mercia which had contrasting nitrogen contents (25.7 vs

21.3 g kg^{-1} DM; unirrigated at Gleadthorpe and early sowing and low seed rate at Rosemaund respectively) also had contrasting degrees of steeliness (74 vs 36 %).

There was a good positive relationship between rumen degradable starch at 8h and the degree of mealiness (Figure 6).

Figure 6 Relationship between rumen degradable starch (8h) estimated *in vitro* and mealiness of wheat

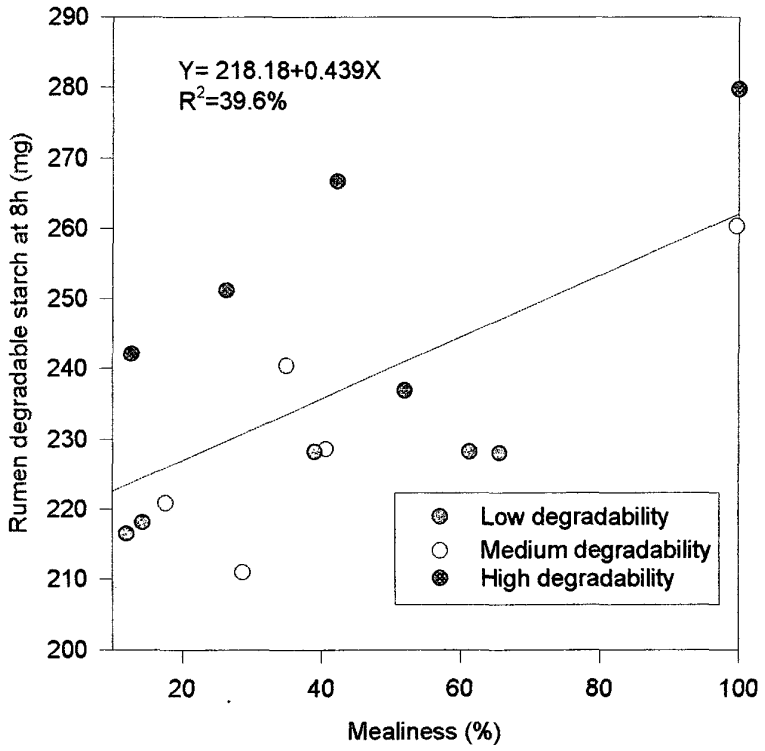


Table 6. Gas production, organic matter and starch disappearance, pH and volatile fatty acid production at 8h for the selected fifteen wheat samples.

Sample code	Total gas (ml g^{-1} DM)	Direct gas[1] (ml g^{-1} DM)	Indirect gas[2] (ml g^{-1} DM)	Organic matter disappearance at 8h (%)	Starch disappearance at 8h (%)	Total VFA (mmol l^{-1})	Molar proportions of VFA			
							Acetate	Propionate	n-butyrate	n-valerate
Low degradability										
AB16	71.2	29.6	41.6	16.6	43.9	20.0	0.54	0.34	0.09	0.014
AB21	76.0	33.1	42.9	19.0	42.2	20.6	0.54	0.35	0.09	0.013
AB22	73.0	29.2	43.8	19.9	44.5	21.0	0.54	0.35	0.09	0.013
AB53	77.2	33.5	43.7	18.1	41.4	21.0	0.54	0.35	0.09	0.011
AB58	78.9	34.9	44.0	15.6	42.9	21.2	0.54	0.35	0.09	0.011
Medium degradability										
AB4	88.4	38.1	50.3	20.3	42.5	24.2	0.55	0.36	0.07	0.009
AB14	85.7	42.6	43.1	17.1	41.9	20.7	0.53	0.37	0.08	0.009
AB35	93.0	41.2	51.8	25.4	44.7	24.9	0.55	0.35	0.08	0.013
AB51	86.2	38.8	47.4	16.7	39.7	22.8	0.55	0.36	0.07	0.011
AB63	73.1	30.6	42.5	20.4	39.8	20,5	0.53	0.35	0.09	0.012
High degradability										
AB23	82.4	35.3	47.1	19.1	45.8	22.6	0.55	0.35	0.08	0.010
AB29	84.4	38.3	46.1	20.7	42.2	22.2	0.54	0.37	0.08	0.013
AB36	91.3	44.0	47.3	21.7	48.0	22.7	0.55	0.36	0.08	0.010
AB52	97.6	44.9	52.7	24.7	48.7	25.3	0.54	0.37	0.07	0.011
AB54	86.5	35.3	51.2	24.0	44.7	24.6	0.54	0.36	0.08	0.013

[1] Direct gas calculated from subtraction of indirect gas from total gas

[2] Indirect gas calculated assuming that one mmol TVFA gives rise to 20.8 ml gas indirectly from the buffer (Rymer and Moss, 1997).

Table 7. The amount of organic matter and starch degraded and the estimated microbial biomass and fermented organic matter calculated from volatile fatty acid production at 8 h for the selected fifteen wheat samples.

Sample code	Total gas (ml g^{-1} DM)	Direct gas (ml g^{-1} DM)	Organic matter degraded at 8h (mg)	Starch degraded at 8h (mg)	Starch escaping rumen degradation at 8h (mg)	Fermented organic matter at 8h (mg)	Microbial biomass at 8h (mg)	Effective organic matter disappearance at 0.06 h^{-1} rumen outflow (mg)
Low degradability								
AB16	71.2	29.6	138.0	228.2	291.5	208.2	70.2	436
AB21	76.0	33.1	156.2	218.1	298.8	216.5	60.3	433
AB22	73.0	29.2	164.7	216.5	269.7	219.4	54.7	432
AB53	77.2	33.5	155.2	228.2	323.3	212.5	57.3	448
AB58	78.9	34.9	134.0	228.0	303.3	213.3	79.3	445
Medium degradability								
AB4	88.4	38.1	170.6	240.4	324.8	245.6	75.0	461
AB14	85.7	41.1	141.9	228.6	317.2	220.2	78.3	449
AB35	93.0	41.2	215.2	260.3	321.4	252.9	37.7	460
AB51	86.2	38.8	143.3	220.9	335.9	226.6	83.3	477
AB63	73.1	30.6	174.9	211.0	319.0	207.8	32.9	461
High degradability								
AB23	82.4	35.3	158.6	236.9	280.4	233.1	74.5	470
AB29	84.4	38.3	172.9	242.1	331.3	228.1	55.2	470
AB36	91.3	44.0	183.8	279.7	302.6	229.9	46.1	477
AB52	97.6	44.9	211.0	266.7	280.9	253.4	42.4	484
AB54	86.5	35.3	207.0	251.2	311.3	246.8	39.8	488

Table 8. The proportions of mealy, steely and piebald grains in the sub-population of fifteen wheat samples and their categorisation into Hard and Soft by the sedimentation test.

Sample code	Variety	Nitrogen (g kg^{-1} DM)	Starch (g kg^{-1} DM)	Population (%) Mealy	Steely	Piebald	Sedimentation test
Low degradability							
AB16	Riband	22.2	683	61	34	5	Hard/Soft
AB21	Spark	25.8	680	14	50	36	Hard
AB22	Mercia	25.7	675	12	74	14	Hard
AB53	Consort	18.1	772	39	11	50	Soft
AB58	Consort	20.4	749	66	1	33	Soft
Medium degradability							
AB4	Mercia	21.3	702	35	36	29	Hard
AB14	Brigadier	21.5	670	41	10	49	Hard
AB35	Riband	10.3	814	100	0	0	Soft
AB51	Cadenza	19.4	723	18	31	51	Hard
AB63	Consort	19.8	742	29	18	53	Soft
High degradability							
AB23	Haven	21.3	703	52	19	29	Hard
AB29	Slepjner	19.4	740	13	15	72	Hard/Soft
AB36	Riband	10.6	806	100	0	0	Soft
AB52	Caxton	18.6	748	42	15	43	Hard/Soft
AB54	Reaper	19.8	759	26	23	51	Hard

3.5 The sedimentation test for determining between Hard and Soft wheat

The sedimentation test was used to further categorise the fifteen wheat samples into Hard wheat and Soft wheat. The results of the sedimentation test are shown in Table 8. The variety Riband is known to be a Soft wheat but within the three samples included in this sub-population, one sample was defined by the test as not being completely Soft and it had the highest N content of the three. This sample had 34 % of steely grains compared with no steely grains for the other two samples. The varieties Slepjner and Caxton are both Hard varieties but in the sedimentation test were shown to be not completely Hard. This corresponded with high levels of either mealiness and/or piebald grains, but low steeliness (15 %).

3.6 Light microscopy

Examples for steely and mealy grains from both Hard and Soft varieties are shown in Plates 1 and 2 respectively. The steely Hard grain transverse section shows more prominent cellular patterns which are indicative of fracturing along endosperm cell walls. This is in contrast to the mealy Soft grain transverse section which shows the characteristically loose association of small starch granules to the protein matrix material of the endosperm. This loose structure facilitates fracturing across endosperm cells leaving the starch more accessible to microbial attack in the rumen.

3.7 NIR spectroscopy

Calibration and cross-validation performance data (standard error of calibration (SEC) and cross validation (SECV)) are given in Tables 9, 10 and 11 for the whole grain, 3mm and 1mm grind samples respectively.

Table 9. Calibration and cross-validation statistics for different chemical, quality and *in vitro* gas production characteristics of 61 whole wheat grain samples.

	Calibration		Cross-validation	
Predicted term	SEC	R^2	SECV	R^2_{CV}
Nitrogen	0.044	0.980	0.054	0.971
Specific weight	0.426	0.976	0.821	0.910
Dry matter	0.411	0.858	0.424	0.849
HFN	25.32	0.644	26.39	0.616
Starch	2.202	0.639	2.239	0.631
Lag	0.229	0.504	0.268	0.325
Thousand grain weight	3.510	0.489	4.125	0.289
Combined rate at half life	0.005	0.422	0.006	0.204
Combined rate at 8 h	0.006	0.254	0.006	0.168
Effective degradability of organic matter at 6 % h^{-1} outflow	1.029	0.149	1.079	0.073
NDFa	2.112	0.106	2.244	0.023
Asymptote (gas volume)	7.541	0.017	7.820	-0.011

There was a good calibration and cross-validation for nitrogen content of the grains regardless of sample preparation (R^2_{CV} = 0.971, 0.971 and 0.966 for whole grain, 3 mm and 1 mm grind 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 DM content was best with the 1mm grind preparation (R^2_{CV} = 0.870) and prediction of starch content was best for the 3mm grind (R^2_{CV} = 0.746). **Specific weight was well predicted using NIRS on the whole grain samples (R^2_{CV} = 0.910) only**, as was also true for HFN (R^2_{CV} = 0.616), though the latter relationship would not be good enough for routine purposes. None of the gas production parameters were well predicted by NIRS regardless of sample preparation. This was disappointing but the ranges for these parameters were narrow.

Table 10. Calibration and cross-validation statistics for different chemical, quality and *in vitro* gas production characteristics of 61 wheat grain samples milled through a 3 mm screen.

	Calibration		Cross-validation	
Predicted term	SEC	R^2	SECV	R^2_{CV}
Nitrogen	0.033	0.989	0.055	0.971
Dry matter	0.442	0.837	0.461	0.821
Starch	1.805	0.775	1.930	0.746
HFN	26.73	0.619	28.90	0.565
Lag	0.222	0.535	0.263	0.351
Thousand grain weight	3.281	0.452	3.496	0.379
Asymptote (gas volume)	6.291	0.380	7.597	0.132
Effective degradability of organic matter at 6 % h^{-1} outflow	0.973	0.211	1.028	0.136
Combined rate at half life	0.005	0.212	0.005	0.122
Specific weight	8.609	0.180	9.480	0.031
Combined rate at 8 h	0.007	0.068	0.008	-0.066
NDFa	2.199	0.026	2.355	-0.080

Table 11. Calibration and cross-validation statistics for different chemical, quality and *in vitro* gas production characteristics of 61 wheat grain samples milled through a 1mm screen.

	Calibration		Cross-validation	
Predicted term	SEC	R^2	SECV	R^2_{CV}
Nitrogen	0.053	0.972	0.060	0.966
Dry matter	0.331	0.908	0.392	0.870
Specific weight	0.964	0.884	1.599	0.682
Thousand grain weight	2.225	0.795	3.885	0.369
Starch	1.873	0.766	2.329	0.640
Lag	0.184	0.679	0.248	0.420
HFN	27.05	0.594	28.26	0.550
NDFa	1.847	0.257	1.963	0.187
Combined rate at 8 h	0.006	0.216	0.006	0.157
Asymptote (gas volume)	6.963	0.207	7.360	0.150
Combined rate at half life	0.005	0.206	0.005	0.175
Effective degradability of organic matter at 6 % h^{-1} outflow	1.091	0.086	1.146	-0.002

3.8 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 the fifteen wheat samples (3 mm grind) are shown in Table 12 and visually in Figure 7. The ranking for the degree of hardness was the same whether log 1/R values were used from the 1680 or the 2230 nm regions, with the exception that the samples

ranked seventh and eighth were transposed at the higher wavelength. From the sedimentation test data the 2230 nm ranking seemed more appropriate, so this data set was investigated further. The wheats identified by the sedimentation test as being Soft wheats tended to have the lowest log 1/R values and the opposite was true for the Hard wheats. There was a range in log 1/R values of 0.4504 to 0.6551, with individual varieties, for example Riband (Soft wheat), having the range 0.4504 to 0.5287 for its three samples. **Additionally, there was a significant negative relationship between hardness as estimated by NIR and the degree of mealiness determined using light transflectance (r = -0.890). This suggests that Soft wheats will tend to be more mealy than Hard wheats but that both are influenced by environment/crop management.**

Figure 7 NIRS log 1/R values for wheat over the Wavelength range 1100 to 2500, highlighting the wavelengths for wheat hardness

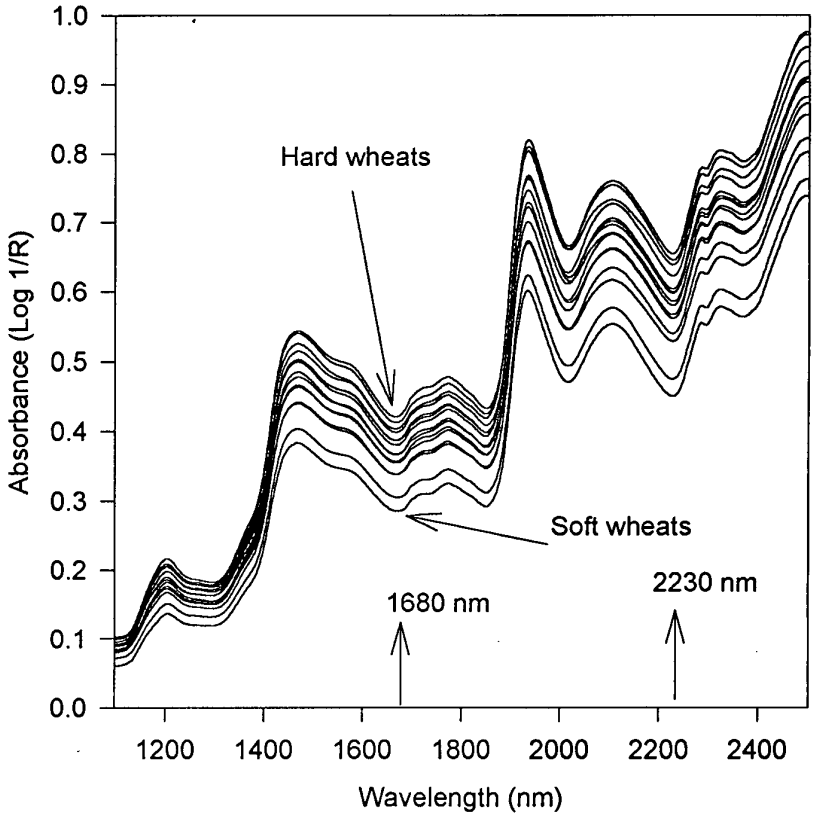


Table 12. NIRS log 1/R at 1680 and 2230 nm as a measurement of wheat hardness.

Sample code	Variety	Log 1/R at		Sedimentation test
		1680 nm	2230 nm	
Low degradability				
AB16	Riband	0.3415	0.5287	Hard/Soft
AB21	Spark	0.3702	0.5858	Hard
AB22	Mercia	0.4083	0.6385	Hard
AB53	Consort	0.3712	0.5801	Soft
AB58	Consort	0.3420	0.5401	Soft
Medium degradability				
AB4	Mercia	0.4167	0.6454	Hard
AB14	Brigadier	0.3842	0.5980	Hard
AB35	Riband	0.2868	0.4504	Soft
AB51	Cadenza	0.4255	0.6551	Hard
AB63	Consort	0.3609	0.5670	Soft
High degradability				
AB23	Haven	0.3580	0.5615	Hard
AB29	Slepjner	0.3903	0.5997	Hard/Soft
AB36	Riband	0.3065	0.4750	Soft
AB52	Caxton	0.3918	0.6041	Hard/Soft
AB54	Reaper	0.4028	0.6226	Hard

4.0 Discussion

The wheat samples chosen had a wide range in both chemical composition and traditional grain quality measurements, and it is likely that this was largely a reflection of the environment/crop management imposed. With the gas production parameters there was an apparently narrower range, 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 50.6 to 55.6%. There were no significant correlations between any of the gas production parameters and either the chemical composition or traditional grain quality parameters. Nitrogen content was highly negatively correlated with starch content ($r = - 0.74$) which was to be expected because plants with low nutrient status tend to accumulate less protein and more sugars than those adequately supplied, so if nitrogen is deficient, starch will accumulate. Similarly, plants growing in cool conditions may have higher starch contents because the rate of growth is more temperature dependent than the rate of photosynthesis (V. Breeze, personal communication). It may therefore be possible to manipulate both the starch and nitrogen content of the grains through nitrogen fertiliser management. The ability to manipulate starch content in particular could be of significant value to the feed manufacturing industry.

When cluster analysis was performed, low and high degradability groups were identified. The fifteen wheat samples selected were grouped into low, medium and high degradability according to EOMD. The further investigation of these samples after 8 h incubation showed that the total gas production was directly related to OM disappearance at 8 h ($r = 0.712$) 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 ($r = 0.822$) which provides confirmation that the technique can be used to reflect starch utilisation. A good relationship ($r = 0.93$) between gas production after 7 h incubation and wheat starch degraded has also noted by Opatpatanakit *et al.* (1994).

The volume of gas produced per gram of starch degraded at 8h was 350ml. It is therefore possible to use this relationship to calculate the rumen degradable starch at 8h for the 61 wheat samples. For the 61 wheat samples there was, as previously suggested, a good negative relationship between the starch content of the grains and the nitrogen content. **There was also a 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) = 390 - 3.33 N(g kg^{-1} DM)

SEP = 2.41 R^2 = 15.0%

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 (mg g^{-1} fresh). There was no relationship between rumen degradable starch at 8h (mg g^{-1} fresh) and grain starch content and as starch and nitrogen content were negatively correlated this was not expected but indicates that N content influences starch degradability in some way other than a purely dilution effect.

In experiment 1, where Mercia was grown on one site at two sowing rates and dates, and with and without growth regulator, only growth regulator had a significant effect ($P<0.06$) on rumen degradable starch at 8h (301 v 321 mg rumen degradable starch g^{-1} fresh without and with growth regulator respectively). There was a tendency for high seed rate and no growth regulator to give rise to the lowest level of rumen degradable starch ($P<0.125$). In experiment 2, where first and third wheats were compared, there was no effect of this on rumen degradable starch at 8h, but there was an effect of variety. The varieties grown had rumen degradable starch at 8h of 282, 307, 334, 337 and 338 mg g^{-1} fresh ($P<0.068$) for Riband, Brigadier, Rialto, Soissons and Spark respectively.

In the irrigation experiment at ADAS Gleadthorpe there was no effect of either irrigation or variety on level of rumen degradable starch at 8h. There appeared to be an interaction between irrigation and variety for rumen degradable starch at 8h. The unirrigated samples tended to have lower rumen degradable starch at 8h for Riband, Spark and

Mercia; Rialto was unaffected and Haven had higher rumen degradable starch when unirrigated. Both N, NDFa, and HFN were significantly higher in the unirrigated samples whilst specific weight was significantly lower.

The increased level of N and NDFa in the unirrigated samples is indicative of plant stress. Stress during the period between flagleaf emergence and grain filling caused by lack of water when stem reserves are being mobilised for use in the developing grain can lead to serious loss of yield. Water availability can be affected by drought, soil structure and rotation. Work performed during an HGCA funded physiology study of wheat at the University of Nottingham, in conjunction with NIAB has shown that the amount of soluble carbohydrate stored in the stem varies between varieties and that varieties with a high level of soluble carbohydrate are better able to withstand the effects of late season drought stress. In this experiment Rialto had the highest soluble stem carbohydrate and Riband and Spark the lowest. This suggests that Rialto is a drought resistant variety which corresponds to the fact that the rumen degradable starch at 8h was unaffected by irrigation whereas Riband and Spark are more susceptible to drought stress and the unirrigated samples had lower rumen degradable starch at 8h compared with the irrigated samples.

For the variety Slepjner there was no effect of shading on rumen degradable starch. For the variety Riband increasing N application from 0 to 420 kg ha^{-1} linearly decreased the rumen degradable starch at 8h accordingly to the following relationship:

Rumen degradable starch at 8h (mg) = 374 - 0.24 N rate (kg ha^{-1})

$R^2 = 81.5\%$

or Rumen degradable starch at 8h (mg) = 471 - 9.27 N content (g kg^{-1})

$R^2 = 90.1\%$

The latter relationship is better than that previously described for the entire population with a different slope and intercept. There was no significant effect of growth site on rumen degradable starch at 8h for the three NIAB sites but there was a significant effect of variety ($P<0.08$) and rumen degradable starch was 287, 319, 330, 332 and 340 mg g^{-1}

fresh for Consort, Hereward, Cadenza, Caxton and Reaper respectively. There was no relationship between rumen degradable starch at 8h and N content for these 15 samples, but when the three samples of "Soft" wheat were removed from the sub-population more variation in rumen degradable starch was accounted for by N content (Y = 513 - 8.55 N (g kg^{-1}), R^2 = 36.0%). This reinforces the earlier findings that "Soft" wheats with a high N content have lower rumen degradable starch (at 8h) than "Hard" wheats of similar N content.

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 and the sedimentation test. 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 = - 0.89) 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. A small additional study (see Appendix 3) carried out on some separated grains from the same sample confirmed that steely grains contain more nitrogen and are fermented more slowly than mealy grains. These findings raise the question as to what controls this characteristic within the plant and whether it can be manipulated by agronomic management. Clearly more work is needed in this area both from the plant and animal perspective.

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. As a result, upon milling of Soft wheats the bran remains associated with the endosperm. Hard wheats fracture during milling along cell-cell interfaces giving relatively large particles which consist of groups of cells, while in the case of Soft wheats the cells are torn open and the cell contents spill out. In terms of milling technology Hard wheats may be defined as those which yield coarser, freer flowing flour with higher levels of starch damage and Soft wheats as those which yield finer flour with lower levels of starch damage.

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. 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. 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 = 64.9 %) and with direct gas at 8 h (R^2 = 57.8 %). The improved relationship with total gas compared with direct gas is likely to be a result of the total gas accounting for both end-products of fermentation (gas and VFA), whereas direct gas ignores VFA production. The prediction of the amount of starch disappearance at 8 h was further improved by the addition of either NIRS hardness or mealiness as predictors together with total gas production at 8 h. The relationships were as follows:

Starch degraded at 8 h (mg) = 0.145 - 0.123NIR2230 + 0.00196 total gas at 8 h (ml)

R^2 = 77.4 % ; Standard error of prediction = 0.0094

Starch degraded at 8 h (mg) = 0.0839 + 0.000265mealy (%) + 0.00171 total gas at 8 h (ml)

R^2 = 76.5 % ; Standard error of prediction = 0.0096

The relationship between starch disappearance (%) and gas production (R^2 = 20.8 %) and other predictors were significantly poorer than for the above relationships which was disappointing, but implies that the starch content of the grains has a strong influence on the amount of starch fermented in the *in vitro* system at least and probably also *in vivo*.

Table 13 shows the sub-population of wheats sub-divided as Hard or Soft wheats and then ranked within those groups according to rumen degradable starch at 8h (mg g^{-1} fresh weight of wheat).

Table 13 Ranking of wheat samples according to starch fermentation at 8h

Variety	Experiment	Hard/Soft by sedimentation test	N (g kg^{-1})	Mealy (%)	Rumen degradable starch (mg g^{-1} fresh)
Soft wheats					
Consort	NIAB Cornwall	Soft	19.8	29	211
Riband	3rd Wheat	Hard/Soft	22.2	61	228
Consort	NIAB Cockle Park	Soft	20.4	66	228
Consort	NIAB Bridgets	Soft	18.1	39	228
Riband	Zero N	Soft	10.3	100	260
Riband	40 kg/ha N	Soft	10.6	100	280
Hard wheats					
Mercia	Unirrigated	Hard	25.7	12	217
Spark	Unirrigated	Hard	25.8	14	218
Cadenza	NIAB Bridgets	Hard	19.4	18	221
Brigadier	3rd Wheat	Hard	21.5	41	229
Haven	Unirrigated	Hard	21.3	52	237
Mercia	Sowing date/rate/ PRG	Hard	21.3	35	240
Slepjner	Unshaded	Hard/Soft	19.4	13	242
Reaper	NIAB Bridgets	Hard	19.8	26	251
Caxton	NIAB Bridgets	Hard/Soft	18.6	42	267

The "Soft" wheats had the widest range of starch degradable at 8h which was negatively correlated with N content and was also influenced by the mealiness of the grains. The "Hard" wheats had a narrower range of starch degradable at 8h (217 -267 mg g^{-1} fresh). The low level of degradable starch was associated with very high grain N content and samples which were likely to have been drought stressed during grain filling and these were varieties (ie. Spark, Cadenza) which have been shown to have low soluble stem carbohydrate and hence more susceptible to late season drought stress.

It can be concluded that the nitrogen fertiliser application will have a substantial influence on the amount of starch degraded in the rumen (at least as determined *in vitro*) due to its negative affect on starch content. 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 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 fertiliser conditions. The use of NIRS to develop calibrations for this wheat population was successful for DM and nitrogen content and the calibrations were equally good regardless of sample preparation (whole grain, 3mm or 1mm grind). Specific weight and grain nitrogen was predicted well by the whole grain sample scans only. This information would be worthy of further development, as it would provide a rapid, non-invasive screening technique.

Calibrations of NIRS with any of the gas production parameters were unsuccessful regardless of sample preparation prior to scanning. This may be a result of the insufficient spread of data within this population.

Conclusions

The *in vitro* gas production technique can be used to identify wheat grains (3 mm grind) of varying rumen degradability. Total gas volume after 8 h was strongly correlated with the amount of rumen degradable starch ($r = 0.822$), more strongly so than the derived EOMD value ($r = 0.632$) and percentage OM disappearance ($r = 0.688$). Hence the total gas method may be used to identify wheats providing different amounts of rumen degradable starch.

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 ($r = -0.74$). NIRS was able to predict grain hardness, N content and specific weight, but was unable to reliably predict any of the *in vitro* parameters. Specific weight was not well correlated with any of the *in vitro* determined parameters but was very strongly influenced by variety and this requires further work.

The amount of starch degraded was influenced by the starch content of the grain and this was mediated by both the hardness and mealiness of the grain. This work confirms that it is the physical structure of the grain and the starch within the endosperm along with starch content that are the main factors which determine the amount of rumen degradable starch available from a particular sample of wheat. These findings provide the possibility that starch content and quality in wheat may be manipulated by crop management although further work is required on the factors which control protein deposition in the endosperm and hence the mealy/steely characteristic. Varieties apparently vary in their resistance to late season drought stress which influences the amount of degradable starch.

Recommendations

1. Further investigate the factors (variety, environment/management) which influence the physical structure of the grain and the starch within the endosperm.

2. Further investigate the impact of endosperm structure/composition on the availability of starch to rumen microbes and to examine any interactions with grain processing.

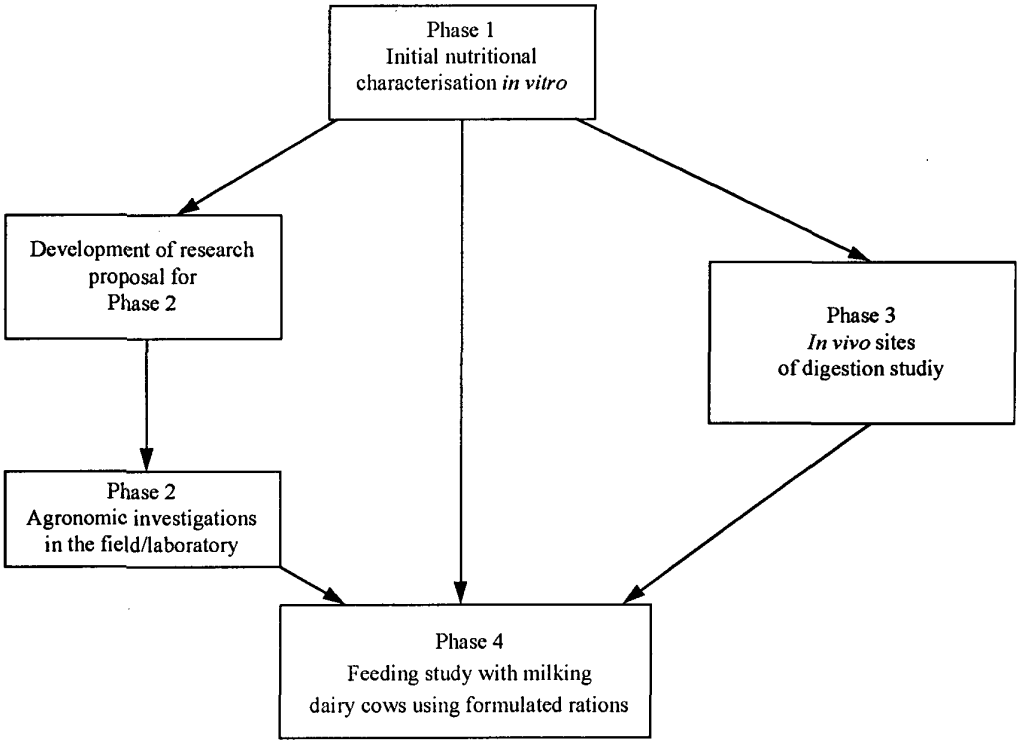
3. Expand the data set to enable multivariate analysis and to enable development of NIRS calibrations.

4. To develop rapid techniques to estimate the nutritive value of wheat for livestock to replace or supplement the measurement of specific weight.

APPENDICES

Appendix 1

Project timetable and integration of multi-disciplinary effort



Appendix 2

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 two models used to fit to the gas production data form this study were those described by France *et al.* (1993) and Groot *et al.* (1996). The model of France *et al.* (1993) is based on a generalised Mitscherlich equation as follows:

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

Where μ = combined rate of gas production at time t

b = underlying rate

c = time dependent rate

T = lag phase prior to gas production

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

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.

The tri-phasic model of Groot *et al.* (1996) is based on a generalised Michealis - Menten equation and can differentiate between soluble, insoluble but fermentable feed fractions and microbial turn-over. When the wheat data was fitted to this model, only a single phase was determined indicating that the carbohydrate source in wheat was essentially of one type.

Appendix 3

Chemical composition and gas production parameters of a sample of wheat that was categorised into mealy, steely and piebald grains using light transflectance and a sample of Durum wheat.

The sample of AB4 when separated using light transflectance into steely, mealy and piebald grains was 40.5, 34.1 and 25.4 % respectively. The sample of Durum wheat was 96.6, 0.7 and 2.7% steely, mealy and piebald respectively.

Table 1 shows the chemical composition of the samples. The steely grains had a higher nitrogen content than both the mealy and piebald grains. The nitrogen content of the steely grains was higher than the original samples nitrogen content and the mealy and piebald grains had a lower nitrogen content. The starch content tended to be higher for the mealy grains which is consistent with the negative correlation between starch and nitrogen content in wheat grains. The Durum wheat sample had high nitrogen content and low starch content.

Table 1. Chemical composition of two samples of wheat grain (g kg^{-1} DM or as stated).

	Dry matter (g kg^{-1} fresh)	Nitrogen	NDFa	Starch
AB4 Mealy	873	19.1	67	694
AB4 Steely	890	22.0	73	683
AB4 Piebald	872	19.4	103	684
AB4	870	21.3	93	702
BB15 Durum	875	23.7	68	660

Table 2 shows the gas production data and the associated data, after they were fitted to the model of France *et al.* (1993). There were no significant differences between the samples in terms of the gas production curves. The rate of gas production was slowest

for the steely grains and the sample of Durum wheat, with the mealy grains having the fastest rate of gas production. Conversely the mealy grains had a longer lag than the steely grains and the combination of this with the faster rate gave rise to little differences between the samples for EOMD at 6% h^{-1} rumen outflow rate.

Table 3 shows the gas production and organic matter disappearance measured after 8 h incubation. There was little difference in gas production at 8 h but there was a reduction in OM disappearance with increasing steeliness.

Table 2. Gas production and associated data of two wheats studied.

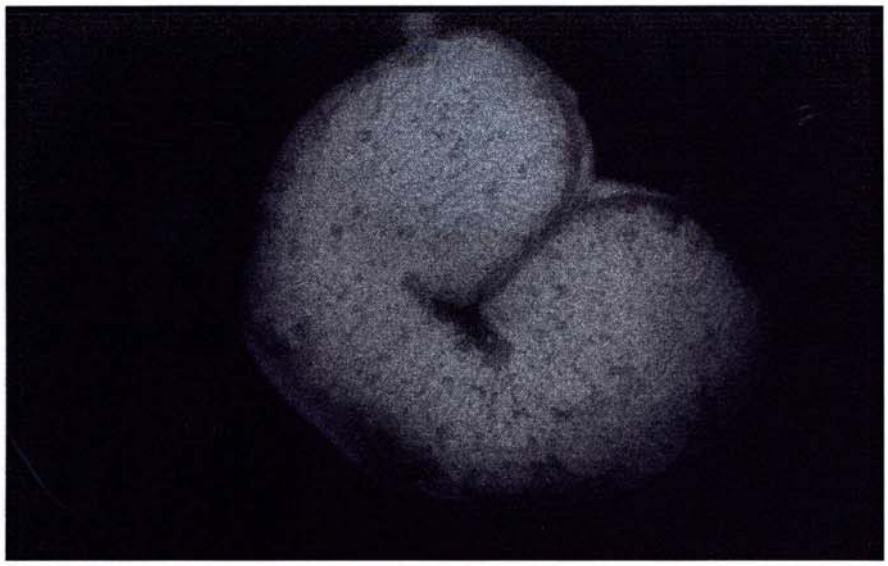
	pH at 48h	OMD at 48h (%)	Asymptote (ml g^{-1} DM)	Underlying rate (h^{-1})	Time dependent rate ($h^{-1/2}$)	Lag (h)	Time to half asymptote (h)	Combined rate at 8h (h^{-1})	Combined rate at $t^{1/2}$ (h^{-1})	Effective degradability of organic matter[1] (%)	Combined rate at rumen outflow 0.06 h^{-1} (h^{-1})
AB4 Mealy	6.19	80.8	344	0.220	-0.6026	2.65	10.00	0.1138	0.1250	53.1	0.084
AB4 Steely	6.24	81.6	330	0.194	-0.5113	2.09	10.32	0.1036	0.1144	53.7	0.079
AB4 Piebald	6.20	81.0	321	0.210	-0.5558	2.34	9.96	0.1113	0.1215	53.8	0.084
BB15 Durum	6.27	82.1	351	0.182	-0.4832	2.32	10.84	0.0963	0.1083	52.3	0.073

Table 3. Gas production and organic matter disappearance at 8 h for the two wheat samples.

	Total gas (ml g^{-1} DM)	Organic matter disappearance at 8h (%)
AB4 Mealy	98.6	10.0
AB4 Steely	98.5	7.7
AB4 Piebald	97.4	8.6
BB15 Durum	88.7	12.4

Plate 1. Steely (S) and mealy(M) endosperms in hard wheat cv. Mercia

S



M

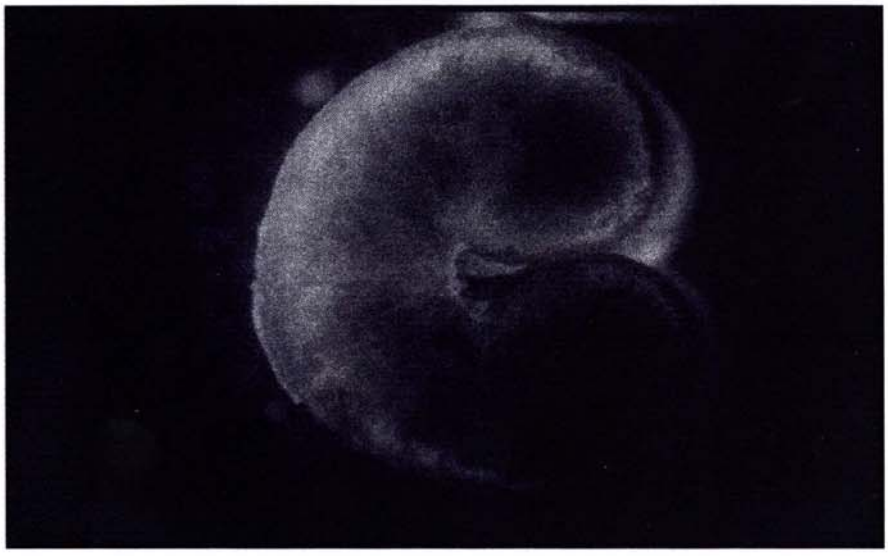
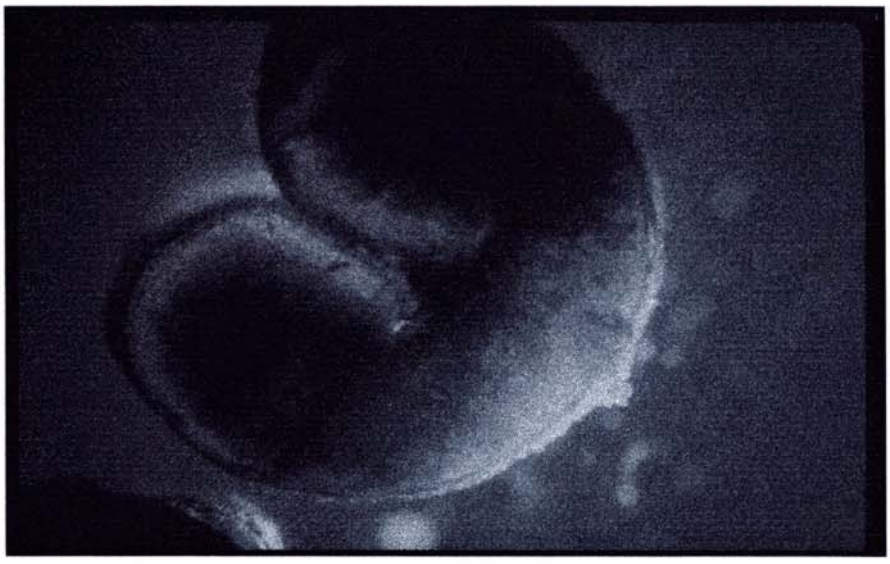


Plate 2. Steely (S) and mealy (M) endosperms of soft wheat cv. Consort

S

M



Part 2: The nutritive value of wheat for ruminants: Agronomic implications of the findings in Part 1 and recommendations for future research

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Period of study:: July-September 1998

Date of issue of report: 30 October 1998

1.0 Background

This report is supplementary to the work described in Part 1. In Part 1, which was completed in August 1998, wheat samples were drawn from existing HGCA funded experiments managed by ADAS, Nottingham University, Sutton Bonington and NIAB. These were subsequently evaluated at the Feed Evaluation and Nutritional Sciences Unit (FENS) of ADAS using a range of laboratory methods including an *in-vitro* gas production technique. This approach was used to mimic rumen function, thereby allowing comparison of wheat samples in terms of relative performance when fed to ruminant livestock.

This initial FENS study (phase 1) was the first step of a collaborative project jointly funded with the MDC (Milk Development Council) - see Appendix I. Phase 3 of this research project, an *in-vivo* sites of digestion study, funded by the MDC, has just begun at FENS. Within the original proposal to the HGCA, a review of the implications of the results of the laboratory analysis and proposals for further agronomic research underpinning future MDC studies were included.

This document summarises the discussions and thoughts of a team of researchers from both ADAS and the University of Nottingham, Sutton Bonington who met to consider the results of the initial evaluation of wheat samples. This team comprised of both crop physiologists, agronomists, cereal biochemists and livestock nutritionists. It summarises the key conclusions from the FENS study which have implications for the cereal farming industry, postulates on general and specific hypotheses, and recommends and prioritises a programme of future research for consideration by HGCA for funding under phase 2 of this collaborative programme.

2.0 Introduction

2.1 Study described in Part 1 and associated collaborative research funded by the Milk Development Council

In order to address the issue of quantifying the nutritive value of wheat as a feed for ruminants, a collaborative research effort between the HGCA and MDC was proposed linking expertise in both crop agronomy and animal nutrition. This research programme was structured into four phases. These are shown in Appendix II.

The initial phase, phase 1, was funded by the HGCA (96K) and involved an initial evaluation of wheat samples from existing experiments, using a range of laboratory techniques including an in-vitro gas production method. Phases 3 and 4 are being funded by the MDC and the phase 3 study is already underway (approx. 150K). Funding for phase 2 was not sought in the original proposal, as the results of phase 1 needed to be studied in order to formulate hypotheses for further research. This report defines the scope of the phase 2 agronomic research and recommends a programme of future laboratory and field research.

2.2 Phase 1 research study at FENS

In this project (see Part !) a total of 61 contrasting wheat samples harvested in 1996 from various agronomic experiments were analysed. They were subjected to chemical analysis, two tests of grain hardness, tests to distinguish steely from mealy endosperm textures and in a selection of samples, endosperm structure was also studied using light microscopy. 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. In addition, traditional measures of wheat quality (e.g. specific weight, Hagberg falling number) were obtained from the agronomic experiments from which the wheat samples were obtained. Near infrared reflectance spectroscopy (NIRS)

was examined as a possible rapid means of predicting starch quality and other factors. The results and conclusions from this work are summarised in section 4.0 of this report.

2.3 Role and purpose of this report in the research programme schedule

The aim of this report is to review the key findings of the laboratory evaluation undertaken at FENS and consider these results, together with those from related research studies to determine their agronomic significance to UK growers. As part of this process, opportunities for manipulation of wheat quality, in terms of ruminant feed quality, will be proposed together with a research programme to consider the importance of both genotype and environmental factors.

3.0 The role of wheat in ruminant feeding

3.1 Wheat in the ration of dairy cows

Use of wheat in dairy cow diets has been steadily increasing during recent years, but from a relatively low base level. The genetic merit of the UK dairy herd is increasing rapidly and higher genetic merit animals tend to produce more milk. In order to sustain higher yields, these cows have to consume more food and/or need diets which contain higher levels of energy. Feed compounders formulate compound feeds to a detailed nutrient specification to minimise cost within a defined specification and have tended to use by-product feeds such as wheat feed, sugar beet pulp, oil seed cakes and maize gluten as cost effective energy sources. Of the 2.94 million tonnes of dairy feed produced by the compound feed industry in 1992, wheat accounted for only 4%. Since 1995 the percent inclusion rate of wheat in animal feedstuffs has risen from approximately 25% to 33% according to MAFF statistics. Most of this increase is attributable to increased demand from the dairy sector, as wheat inclusion rates in the pig and poultry sectors were already high and have limited potential for expansion. With the low prices currently being experienced by the wheat industry inclusion rates in most dairy compound feeds are in the 15-20% range. Not only are compounders including more wheat in the ration because of its high energy content, but dairy farmers themselves are feeding whole wheat as part of the ration.

In addition, milk producers are paid on the basis of protein and fat content of milk, with each unit of protein being worth 168% more than a unit of fat. Consequently, producers are striving to improve milk protein content and, although some improvements can be achieved through breeding, work at the ADAS Dairy Research Centre and elsewhere (Sutton *et al.* 1989; Mansbridge *et al.* 1994, 1995) has shown that milk protein content can be increased by feeding high levels of cereal starch (see Table 1). This has added to the increasing use of wheat in dairy cow diets.

Table 1. The effect of increasing wheat starch intake on milk protein content (%) and protein yield (kg/day).

	Starch intake (kg/day)				
	2.0	3.5	5.0	F prob.	SEM
Protein content	3.23	3.33	3.44	0.003	0.042
Protein yield	0.74	0.80	0.84	<0.001	0.013

Source: Mansbridge et al., 1995

3.2 Economics of including wheat in the ration

UK wheat prices are currently at a 20 year low, making it a highly competitive feed for dairy cows, both for feeding as whole wheat or as part of a compound feed. At current prices (October 98), there is a £40-45/tonne price difference between imported European grain maize (£110-115/t) and UK wheat (£70/t), both of which can be used to provide starch and energy within the ration. Whilst a small part of this additional cost of maize at the farm gate reflects its slightly higher, on average, energy content over wheat (13.8 vs 13.7 MJ ME/kg DM), the majority of the price differential is accounted for by market supply and the perceived value of maize realtive to wheat.

Historically, dairy farmers seeking to maximise protein output in the dairy herd (via increasing milk yield or protein% in milk) have chosen a maize based diet as the most reliable method of providing rumen by-pass starch. Recent HGCA funded research carried out at ADAS FENS has compared the nature and fermentability of starch in both maize and wheat. Some of the results obtained comparing French maize with UK wheat are shown in Table 2. These indicated that whilst starch fermentability was greater and levels of undegraded starch were less in wheat than maize, the differences between them, were were not as large as had been hypothesised. It was also apparent from the FENS research that there were large range in the performance of different wheats compared, indicating that their value in ruminant rations would also vary, possibly justifying some price differentiation. However, at this stage in the research process it is difficult to ascribe a marginal value to a unit of either rumen degradable starch (RDS) or

undegradable starch (UDS) without clear indications of minimum or maximum levels of inclusion of these contrasting wheats in the final diet. Once this information becomes available (from the MDC funded phase 4 study within the HGCA/MDC programme) it will be possible to estimate the extent of any premiums which wheats with specific starch fermetability characteristics may attract.

Table 2. Starch content and fermentability in samples of maize and wheat.

	Maize		Wheat	
	min-max	Mean	min- max	Mean
Starch content (%)	73.0-73.8	**73.4**	72.1-80.6	**76.4**
Starch fermentability(@ 8 hr)	23.9-33.3	**28.6**	39.8-48.0	**43.9**
Degraded starch (g/kg)	175-251	**213**	287-386	**337**
Undegraded starch (g/kg)	479-563	**521**	420-434	**427**

Source: Moss (unpublished)

3.3 Wheat 'quality' for ruminant feeding

There is little doubt that wheat for animal feeding has for many years been considered simply as a market for lower grade, perhaps in some way sub-standard, grain. This concept is only now being challenged;

- through more rigorous controls on livestock ration formulation (quality assurance)

- because of tighter management (metabolic) requirements when feeding high performing livestock e.g. dairy cows

- because of fragmentation in the milling and baking cereal market - highlighting the need for specific quality parameters for each definable market sector

- and finally economics, encouraging higher wheat inclusion rates in the ration, which highlights technical features (both advantages and disadvantages) of the wheat product as a feed

No price premiums are paid for wheat destined for the animal feeding market. Indeed it is often a scenario of minimising price reductions. In recent seasons some growers have been faced with price penalties due to low grain specific weight and the percieved inferior quality of their grain. This view is being challenged using the results of HGCA funded research which has shown poor relationships between grain specific weight and *in-vitro* feed quality measurements. If the importance of grain specific weight as the dominant and almost universal measure of feed quality is to be challenged, then alternative methods which more accurately define feed quality will be required to underpin any marketing efforts.

Nevertheless, to capitalise on expanding markets in the dairy feed sector and maintain market, there will be increasing pressure on producers to meet buyer requirements, however defined and this pressure will intensify, even at the low prices currently achieved in the UK market place. Intervention markets, which historically may have taken lower 'quality' grain, will also provide less attractive market support if Agenda 2000 proposals are implemented.

For the cereal farming industry improved understanding of how UK growers can meet 'quality' feed wheat targets can only benefit the cereal sector and maximising value and returns. The perception that feed wheat and quality and incompatible terms should be addressed and knowledge gained within this project will contribute to this debate. The

concept of FEED GRAIN +, with the plus emphasising notable and definable quality attributes may ultimately be worthy of consideration.

4.0 Agronomic significance of results of from Part ! and suggestions for further research

4.1 Feed wheat quality and the potential for agronomic manipulation

The preceding sections emphasised that feed wheats have traditionally been considered a bulk, low-quality, low-value product providing a relatively constant energy value to an animal's diet. The only specification in most feed wheat contracts is for a specific weight > 72 kg/hl and freedom from excessive impurities. Currently, the major problems limiting greater inclusion of wheat in ruminant rations are poor characterisation of its nutritive value and a tendency for it to cause digestive upsets (acidosis), due to rapid fermentation of starch caused by rumen microflora. Therefore, in many cases, diets based upon more expensive grain maize is a preferred feed as it causes less acidosis. A quality feed wheat would thus be one which was less readily fermentable in the rumen, containing less rumen-degradable starch (RDS) than lower quality cultivars, where more degradable starch could cause acidosis. There are indications that as well as improving digestion, increased rumen by-pass starch (RBS) amounts, *i.e.* the residual starch after fermentation of RDS, could lead to favourable increases in milk protein yield (Reynolds *et al.*, 1997).

The recent ADAS research (Part 1), using *in-vitro* simulation of rumen conditions ('gas production tests'; Cone, 1994) demonstrated variation of about 10% in organic matter degradability (50.6-55.5% organic matter digested) and about 20% variation in rumen degradable starch (210-280 mg g^{-1}, 39.7-48.9% of total starch) and rumen by-pass starch (270-335 mg g^{-1}, 51.1-60.3% total starch) in the sample set analysed. This variation in digestibility could be of significance for the quality of the wheat for the animal's diet, which will be tested in a feeding trial funded by the Milk Development Council, in collaboration with the HGCA funded work described in Part 1.

The 61 wheats studied were sourced from ADAS/University of Nottingham and NIAB trials with widely varying cultivars and husbandry. Several significant relationships of

the amount of RDS to specific wheat grain properties, which could be modified by agronomic decisions (cultivar, husbandry choice, *etc.*), were found. Thus, the increased understanding of the factors controlling wheat fermentibility afforded by the gas production method means that it could ultimately be possible to design husbandry protocols, select cultivars and initiate breeding programmes to define 'quality feed wheats' of specified fermentibility for ruminants. This could increase utilisation of wheat for animal feeding, without adverse side-effects. However, the relationships indicated in Part 1, which were in some cases relatively weak, need to be confirmed by a designed experiment and ranked in order of priority before sensible attempts at growing 'designer feed wheats' can be made.

4.2 Wheat grain properties governing rumen fermentibility of starch - conclusions from Part 1

The ADAS research described in Part 1 demonstrated variability in the chemical composition and digestibility of the 61 wheats studied, which were then divided into high, medium and low digestibility groups for further detailed analysis of 15 samples. The *in-vitro* gas production technique showed a strong correlation between gas volume released from fermentation of the 15 sample sub-set and the amount of RDS (also noted by Oparparanakit *et al.*, 1994). Thus RDS values, determined from gas volumes, could be compared with agronomic factors and wheat grain properties, for the parent samples as well as the subset. The amount of rumrn by-pass starch was not well correlated with gas volume, or with any of the agronomic factors correlated with RDS amounts. Thus the conclusions below and the recommendations for agronomic research refer to the availability of RDS rather than of rumen by-pass starch.

The main results of agronomic significance were:

- A strong significant negative correlation between starch and nitrogen content, determined by chemical analysis, with high nitrogen content causing lower starch content

(*Figure 1*). This relationship would be expected from the literature (Wrigley and Bietz, 1988). Further analysis of the data for this report showed the relationship was maintained for percentage protein (% protein = 5.7*nitrogen content (g kg^{-1} dry matter)*100/1000) and percentage starch. When nitrogen:starch ratio or protein:starch ratio was analysed, an improved significant positive correlation of the ratio to nitrogen or protein content (thus a negative correlation to starch content) was identified (*Figure 2*).

- A negative correlation between nitrogen content (or percentage protein) and the amount of RDS was found, in the whole 61 sample set and more strongly in the 15 sample set studied in more detail (*Figure 3*). This was also reflected in the negative relationship between nitrogen fertilisation and RDS found for the nitrogen management trial with the cultivar Riband. There was a positive relationship of RDS to starch content (or percentage) in the 15 sample set, but this was not found in the 61 sample set. A negative correlation between RDS and protein:starch ratio was also found by Lunn (unpublished).

- A correlation between endosperm texture (either percentage mealy grains or percentage steely grains, positive and negative respectively, *Figure 4*) and RDS could be demonstrated. Lunn (unpublished) found that slightly more variance could be explained by weighting the percentage mealiness or steeliness to also include the percentage of mixed texture (piebald) grains.

- Positive correlations were found between nitrogen (protein) content and grain hardness and protein content and grain steeliness. A negative correlation was found between hardness and percentage mealiness.

- Fractionation of grains of the same sample into steely and mealy groups showed that steely grains contained more nitrogen (protein) than mealy grains and were fermented more slowly.

- There was more limited evidence that grain hardness was also an important character determining the amount of RDS. There was a positive correlation between

RDS and log reciprocal NIR reflectance at 2230 nm (*Figure 5*), a measure of wheat hardness (Osborne, 1991). Also, in the overall nitrogen/RDS relationship there were indications that a higher N (equivalent to protein) content was required to cause a similar low RDS value to soft wheats of lower N content.

- There were no significant relationships of RDS or digestibility to any other chemical composition or quality parameters (yield, specific weight, thousand grain weight and Hagberg falling number, *etc.*). Of particular note was the lack of any significant relationship of digestibility to specific weight, traditionally used as a specification in feed contracts.

- The relatively small sample size and absence of material from specifically designed experiments made it difficult to isolate the effects of all agronomic treatments. No effect of sowing date or sowing rate was found, although use of a growth regulator regime (chlormequat + Terpal) increased RDS content. With comparison of rotational position (mainly take-all pressure), no effect of a first versus a third wheat was observed. A significant cultivar effect was found, with least RDS in the soft cultivar Riband. No significant effect of irrigation or cultivar was seen in a drought tolerance experiment, although there may have been an irrigation x cultivar interaction, with compensation for loss of yield due to drought stress via high stem soluble carbohydrate reserves in the cultivar Rialto. No effect on RDS was found for shading the cultivar Slejpner at different growth stages. A strong negative correlation between nitrogen fertilisation and RDS was found for the cultivar Riband, caused by the effect of nitrogen fertilisation on protein content as described earlier. For cultivars grown by NIAB, no site effect was identified, although a significant cultivar effect was found, with the soft cultivar Riband showing the lowest RDS.

Figure 1: Starch content versus nitrogen content
(61 sample set)

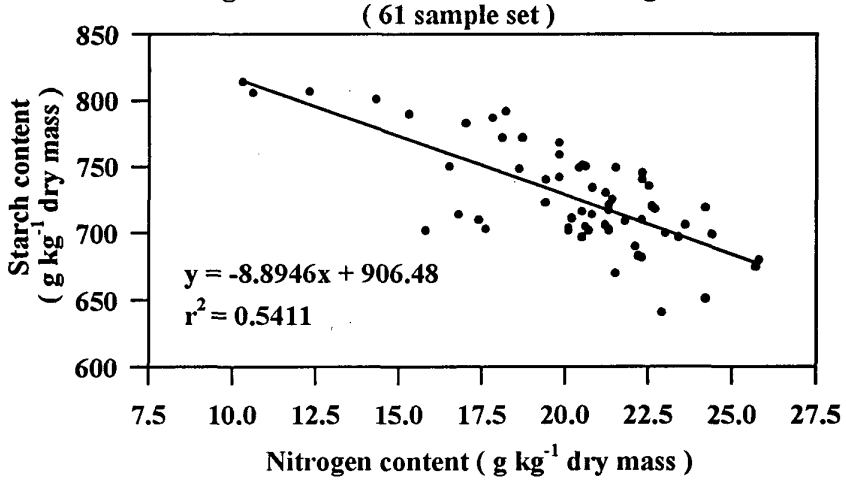


Figure 2: Protien-starch ratio versus nitrogen content
(61 sample set)

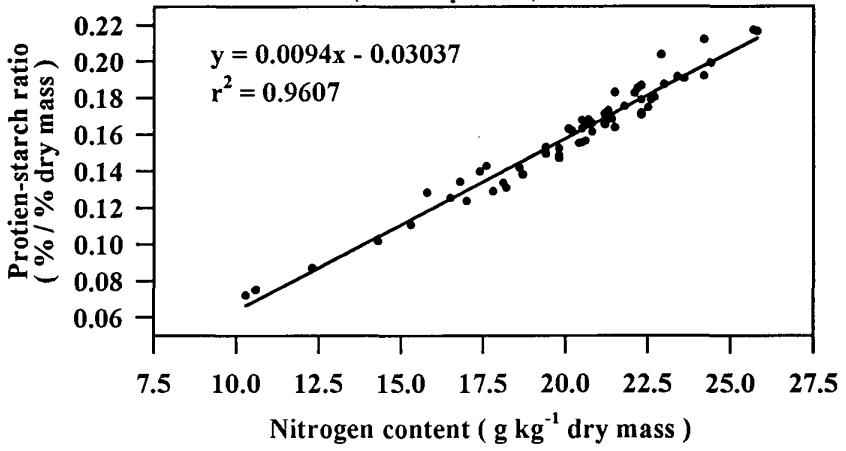


Figure 3: Rumen degradable starch content versus nitrogen content (15 sample set)

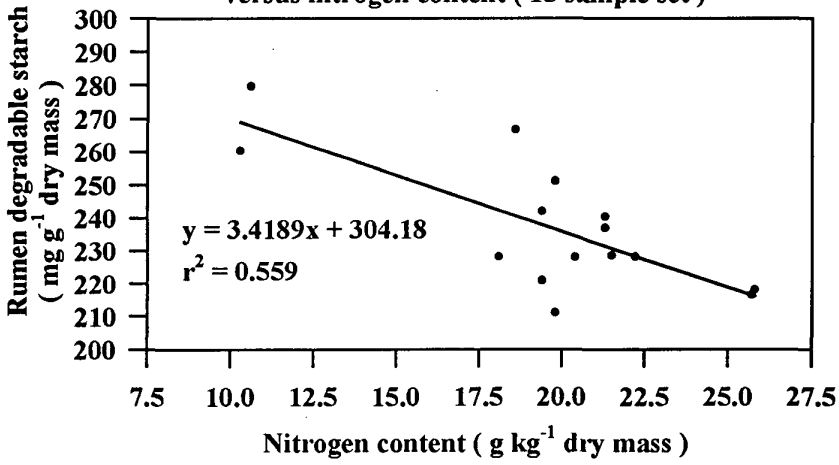
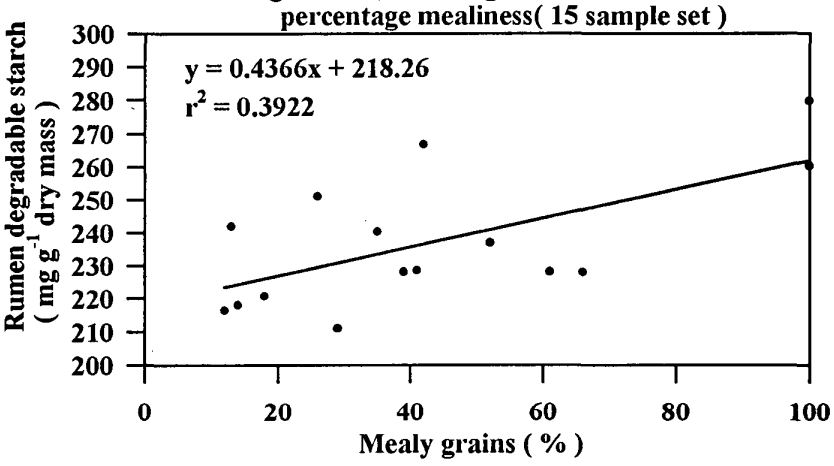
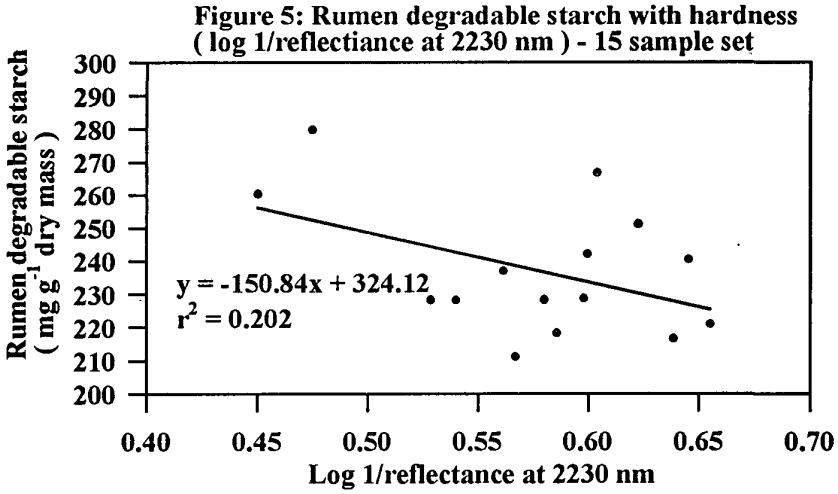


Figure 4: Rumen degradable starch with percentage mealiness(15 sample set)





4.3 Key influencers of rumen degradable starch (RDS) in wheat

In summary, the *in-vitro* gas production work identified several factors possibly responsible for governing RDS variation in wheat, namely:

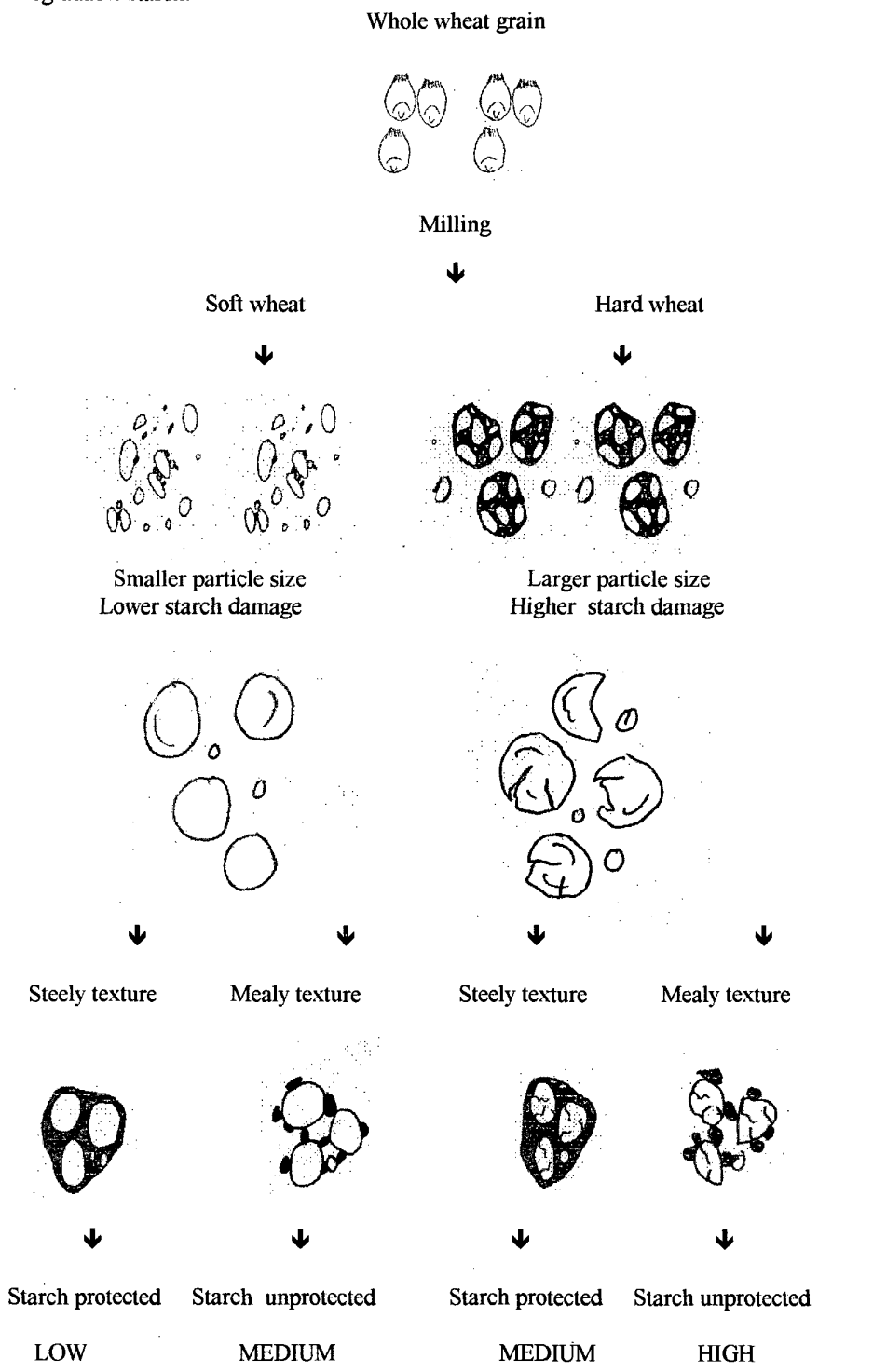
a) Protein and starch content, protein:starch ratio
b) Endosperm texture (mealy, steely or mixed)
c) Endosperm hardness (soft or hard)

Multiple regression analysis by Lunn (unpublished) showed that up to 50% of the variance in the RDS data could be explained by measurements related to the above three properties (*e.g.* protein:starch ratio, percentage steeliness and log 1/R (2230 nm)). The factors are listed above in order of their importance in explaining the variation in the data used in Part 1. Of these, hardness is primarily controlled by genetic factors, whilst protein:starch ratio and endosperm texture are more strongly influenced by environmental conditions.

4.4 A hypothetical model to explain the effects of hardness, protein content, and endosperm texture on the amount of RDS in wheat

Identification of the important grain quality parameters described above allows a hypothesis to be proposed to explain the variation of RDS in wheat, shown schematically in *Figure 6*. Due to the inverse relationship between starch and protein content, part of the negative effect of N or protein content on amount of RDS could be explained by a simple concentration effect. On milling, 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). Thus, although there is the possibility of a particle size effect with less starch digestion in large particles (in hard wheats) due to a smaller surface area:volume ratio effect, high starch damage in hard wheats (Farrand, 1969) allows greater water absorption and easier microbial enzyme access for fermentation. Cone *et al.* (1989) showed that the percentage degradation of wheat starch decreased linearly with increasing particle size after fractionation by sieving. This would lead to the hypothesis that hard (coarsely ground wheats) would be less digestible than soft wheats due to the particle size effect. This was not reflected by experimental results, where soft wheats had the lowest RDS. This necessarily led to the hypothesis that starch damage (greater in hard wheats than soft wheats) has a more important effect on the amount of RDS than particle size. This was reflected in the correlation between RDS and NIR hardness measurements. However, neither particle size nor starch damage were measured in Part 1. As these properties could account for some of the remaining 50% of the variance in RDS it will be important to consider them in future research. Antagonistic to the effect of starch damage, envelopment of starch with protein in high protein-content, steely-textured grains appears to offer some protection from digestion, as shown by the relationships of RDS to endosperm texture and protein content in the previous section. *In-vivo* grain hardness, endosperm texture, protein content, milling and compounding procedures will all interact to vary the relative effects of particle size, starch damage and protein encapsulation on digestibility and RDS amounts. The relative importance of each of these factors was not evident from the initial trial. However,

Figure 6: Hypothetical model for the possible effects of wheat endosperm properties on rumen degradable starch.



Without interaction with other factors, increased RDS correlated with

- Decreasing particle size
- Increasing starch damage
- Decreasing starch encapsulation

from the identified properties it is possible to hypothesise that an ideal 'quality feed wheat' (with low amounts of RDS) would be a soft, high-protein content wheat with a high proportion of steely grains, a large particle size and little starch damage.

4.5 *Agronomic effects on protein/starch content, hardness and endosperm texture*

All three important factors are controlled to varying extents by genetic and environmental parameters which could be manipulated by the producer to provide a quality feed wheat of low RDS. Grain hardness is more completely controlled by genotype (cultivar) than protein content and endosperm texture, which are affected far more strongly by environmental and husbandry conditions.

Protein:starch content

To a certain extent, potential starch and protein content is genetically determined (Law and Krattiger, 1989). Soft, biscuit-making cultivars traditionally have a lower protein content and more starch (and are thus higher-yielding) than hard, bread-making cultivars. Some recent genetic developments associated with improved yield such as the 1B/1R rye translocation, increasing stem soluble carbohydrate stores, *etc.*, might act to dilute protein, cause a lower protein:starch ratio (Foulkes, personal communication), thereby increasing RDS. This could be assessed by study of an appropriate range of genetic material by selection of suitable cultivars.

Protein content is also greatly affected by nitrogen fertilisation, with increased protein associated with higher levels of nitrogen fertilisation, as shown for example in the Riband/nitrogen experiment analysed in Part 1. In addition to this, protein and starch deposition in the wheat grain are affected differently by various environmental and grain development factors. Starch and protein biosynthesis have different temperature optima, with starch deposition depressed by high temperature more than protein deposition (Bhullar and Jenner, 1985). High rainfall during grain filling reduces protein deposition (Halverson and Zeleny, 1988). High irradiance would favour starch deposition, which could be reduced in dull seasons. Length of the grain filling period affects starch/protein ratio, with higher protein contents produced by short grain filling periods and more

starch accumulation in longer grain filling (Jenner *et al.*, 1991). These factors could be varied by using trial sites at various locations with recognised climatic differences. The source/sink balance of the crop (the ratio of the canopy green area producing photosynthate to the number of grain sites depositing starch) could also affect the protein/starch ratio in grain. This could be affected by sowing date, sowing rate, canopy management, disease pressure, *etc.*, and thus manipulated by these conditions. These aspects are being studied in various HGCA research projects and greater value could be obtained by closer liaison.

Hardness

Grain hardness appears to be controlled by a single major gene (*Ha*) located on Chromosome 5A (Symes, 1965), although with the possibility of modifier genes. Initially, hardness was though to be associated with high protein content. Although hard wheats do tend to have a higher protein content than soft wheats. Miller *et al.* (1984) showed that this was an empirical rather than causative relationship. Research has shown no difference between the intrinsic hardness of the starch and protein components from hard and soft cultivars (Barlow *et al.*, 1973). Continuity of the endosperm matrix has been forwarded as an explanation for grain hardness (Stenvert and Kingswood, 1977). Although an initially attractive theory, grain texture is too easily influenced by environment to account for the strongly genetically-determined grain hardness (Parish and Halse, 1968). Although most hard grain also have continuous (steely) starch-protein matrices their appears to be no genetic linkage and the phenomena are considered to be completely separate (Anjum and Walker, 1991). Current theories favour the degree of starch-protein adhesion, since it was noticed that starches prepared from hard grains had more adhering 'wedge' protein (Simmonds *et al.*, 1973). Adhesion was originally though to be mediated by the presence of water-soluble 'glue' proteins at the surface of the starch granule (including purothionins). One small water-soluble protein, friabilin, present in soft but not hard wheats has been postulated as a 'non-stick' protein (Greenwell and Schofield, 1989).

Over and above the genotype, environmental and agronomic conditions have a relatively small effect on hardness. However, moisture content is negatively correlated with hardness (with higher moisture content causing softer grain) and weathering (repeated

wetting and drying) of wheat grain in the field can lead to softening (Pomeranz *et al.*, 1985) as well as changes in endosperm texture.

Endosperm texture

Endosperm texture (Hoseney and Seib, 1973), ranges from mealy (with airspace's and a discontinuous protein matrix surrounding starch, *Figure 7*) to steely (with a continuous protein matrix enveloping starch, *Figure 8*). Endosperm texture appears to be controlled mainly by drying conditions (Parish and Halse, 1965). Although endosperm steeliness is usually empirically associated with grain hardness and high protein content, it is possible to produce low-protein steely wheats and vice versa (Simmonds, 1974, Anjum and Walker, 1991). Endosperm texture varies considerably within a sample of grain and division of the grain of one sample into mealy and steely grains showed a higher protein content in steely grains, with mealy grains showing more RDS. Therefore, in-field weather conditions during grain maturation, as well post-harvest drying management, can significantly influence endosperm texture.

Figure. 7: Scanning electron micrograph of mealy grain texture

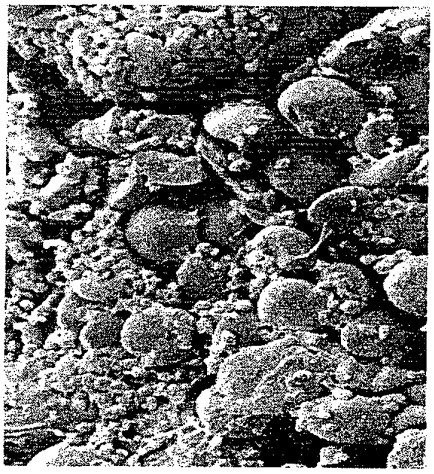
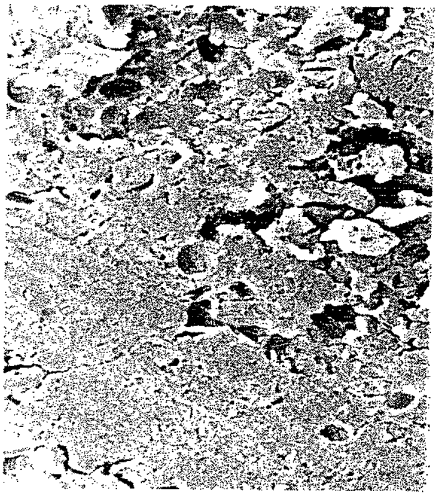


Figure 8: Scanning electron micrograph of steely (vitreous) grain texture



5.0 Key issues for future research

5.1 Programme summary

As described above, all the candidate wheat endosperm properties affecting RDS could be varied by judicious cultivar choice, a range of growth locations, husbandry and input management. The final physical properties of the grains thus produced would then interact with milling and processing, to determine the final amounts and degrabability of RDS in the grain. However, several of the 'agronomic' relationships identified in Part 1 were weak. It is therefore proposed that a short period of laboratory experimentation would be necessary to confirm and prioritise the hypotheses that could be most usefully test in the subsequent programme of field research. It should be possible to achieve this within 6 months. Specific hypotheses to be challenged in the programme of field research could then be refined in the light of the conclusions from the laboratory studies.

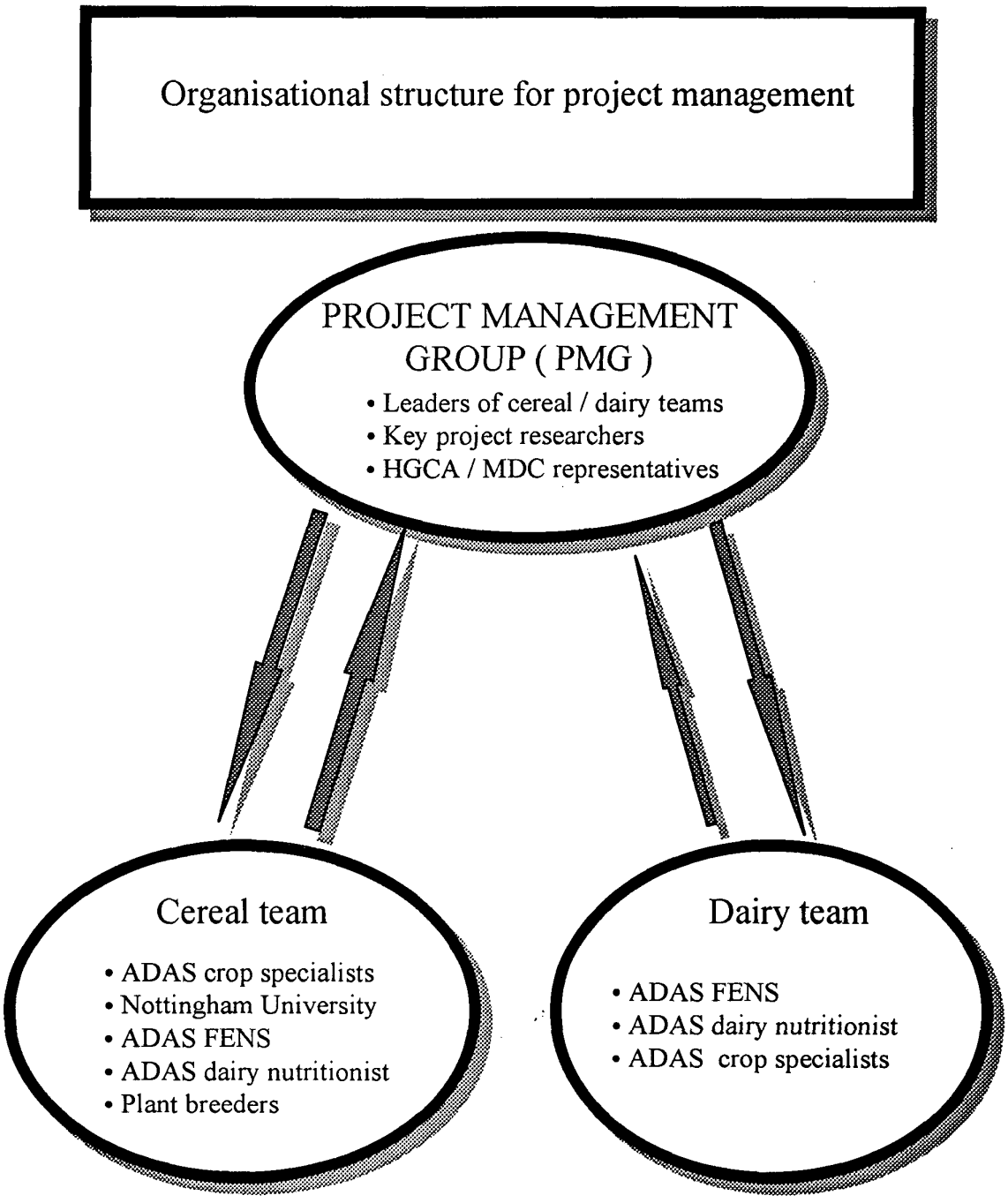
The field research should test the general hypothesis that **RDS availability is controlled by starch:protein ratio (or protein content), endosperm hardness and endosperm texture which can be manipulated agronomically**.

Completion of the field trials will provide a data set to show the extent to which RDS and 'feed wheat quality' could be agronomically manipulated. As part of this programme of research, combine harvest samples of varying properties, but with standardised agronomy, could be evaluated using both the gas production method and lower cost physical evaluation techniques.

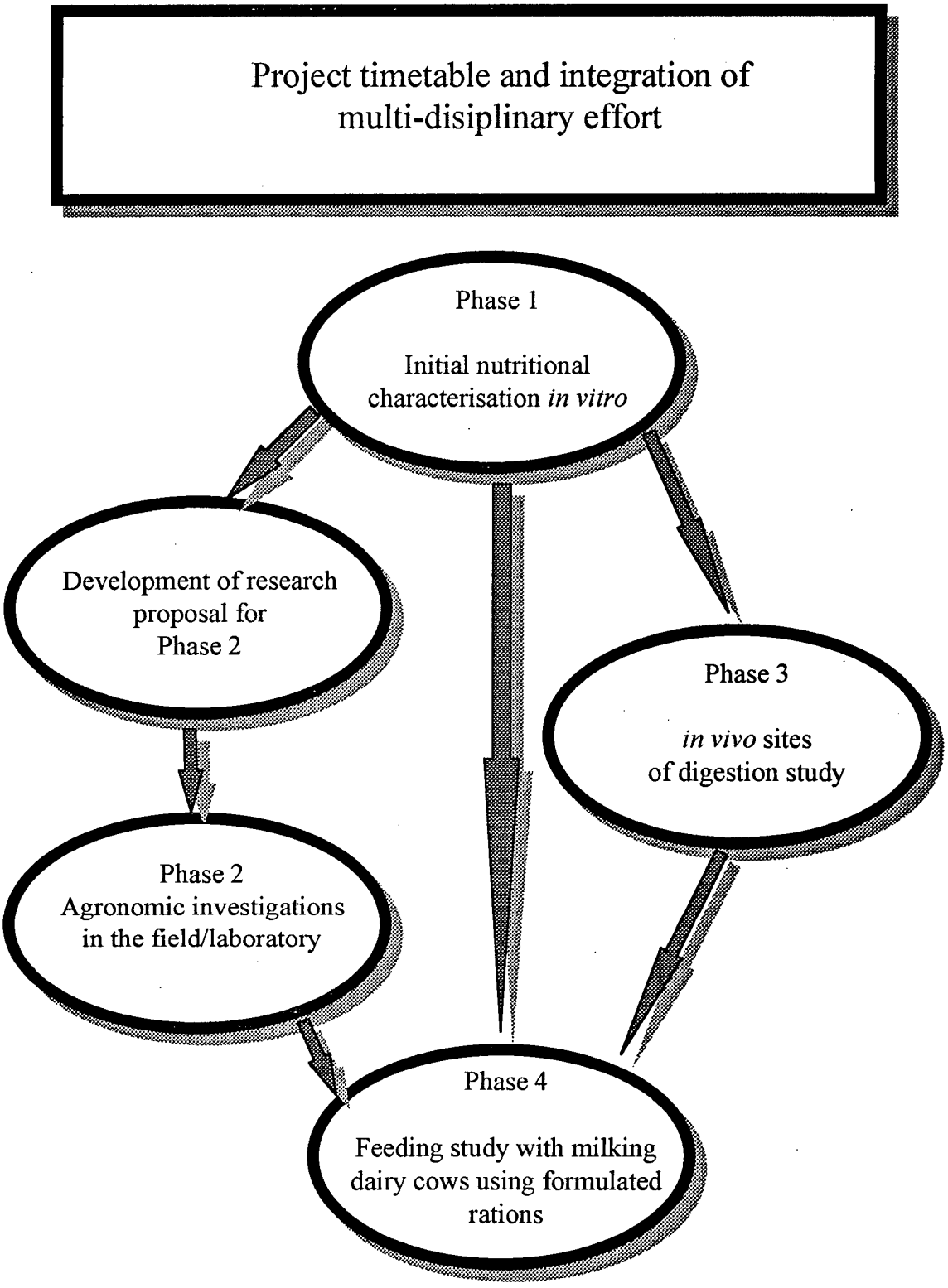
Results from this research programme, in both laboratory and field would complement the continuing programme of MDC funded research on the feeding value of wheat, and account should be taken of their on-going work in refining agronomic priorities.

APPENDICES

Appendix I: Organisational structure of project teams



Appendix II: Research programme, phases 1-4



Appendix III: Glossary of terms

Grain hardness

A physical property of the wheat grain related to milling quality and largely governed by interaction of the starch and protein components of the endosperm. Hard wheats require more milling energy than soft wheats, producing coarser free-flowing flowers with more starch damage compared to soft wheats which produce smaller particles, with more free starch and less starch damage with flows and sieves less well than a hard flour. Grains for bread making need to be hard and for cake and biscuit making soft. General feed wheat varieties can be hard or soft

Endosperm texture

The appearance of the endosperm when cut or sectioned. *Mealy* (or floury) grains appear white, opaque and friable, due to diffraction of light by the rough surface of free starch grains and airspace's. *Steely* (glassy or vitreous grains) appear translucent as the continuous protein matrix does not scatter light. Grains containing a mixture of the two types of endosperm texture may be called piebald, mitidene or yellowberry grains.

Starch-protein matrix

The association between the starch and protein storage reserves of the dead starchy endosperm of mature wheat grains. The starch-protein matrix can range from discontinuous, with the starch and protein disrupted by air spaces, to continuous, with starch grains completely enveloped by the neighbouring unbroken protein storage reserves.

Protein:starch ratio

A measure of the relative amounts of protein and starch (%/% or g/g) and thus of the likelihood of encapsulation of starch by protein.

Starch damage

Disruption of the crystalline structure of the starch grain (caused by compression during milling) allowing more water absorption and easier enzymatic digestion than in non-starch damaged grains. Caused by milling and grain hardness.

Rumen digestible starch (RDS)

The fraction of starch in *in-vitro* gas production tests digested in 8 hours

Rumen by-pass starch

The remaining starch left after 8 hours digestion in *in-vitro* gas production tests

In vitro *gas production tests*

A test simulating the conditions of an animals rumen, in order to measure the amount of starch digested (in relation to the amount of gas produced from fermentation).

Part 3: The nutritive value of wheat for ruminants: Effect of specific weight on its chemical composition, energy value and economic value and comparison of starch availability in maize and wheat

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Period of Investigation: April 1998 - September 1998

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1. Introduction

The current UK Recommended Lists For Cereals (Anon., 1997) which are financially supported by the HGCA, include grain quality information on each variety outlining its potential value to the miller, baker or maltster. These grain quality measures, such as those for grain protein content, Hagberg falling number (HFN), specific weight, Zeleny and endosperm texture are used as a basis for premiums paid to producers. However, no such standards exist for feed grains with the exception that contracts often indicate a minimum specific weight. This is in spite of the fact that feed grains account for 41% of wheat and 50% of barley sales from UK produced cereals.

Wheat is already used by compound feed manufacturers as an energy source for ruminants and dairy cows in particular. For many years purchasers of wheat to be utilised in animal feeding have relied on the determination of specific weight as a guide to nutritional quality. Wheat from the 1997 harvest, much of which was of low specific weight, was discounted on price due to its specific weight (<72 kg hl^{-1}) and perceived reduced nutritive value. Evidence in the scientific literature for a firm relationship between specific weight and either chemical composition or energy value (particularly when fed to ruminants) is scarce. One recent study (Stewart *et al.*, 1997) with pigs and poultry found that with wheat specific weight as low as 51 kg hl^{-1} had no effect on growth rate or feed conversion efficiency. Most data related to specific weight for ruminants is based on studies with barley and the data of Mathison *et al.* (1991) showed that there was no effect on the growth rate of steers fed barley with specific weights as low as 59 kg hl^{-1}.

The objective of this part of the study was to obtain a set of low specific weight wheats, to add these to the sample set from Phase 1 of an earlier study (Part 1) and then select a set of some thirty wheats from the total to represent as wide as possible a range of specific weights. This set would be analysed for a wide range of nutritional characteristics and then any relationships between specific weight and chemical composition and predicted metabolisable energy (ME) content would be examined.

For many years it has been believed that maize grain represented a type of cereal which contained starch that is substantially more resistant to rumen degradation than wheat or barley, although in most studies individual samples of maize have been compared with individual samples of wheat (e.g. Herrera-Saldana *et al.*, 1990). If maize was shown to have consistently lower starch degradability than wheat, it would therefore be the cereal of choice if a diet was designed to deliver substantial amounts of starch to the small intestine. However few attempts have been made to compare the range of starch quality possible in the two cereals.

The results from Phase 1 of Part 1 identified significant variability between wheats in the extent to which starch was fermented in the rumen *in vitro,* although the source of this variability is still unclear. Earlier studies with maize (Nordin and Campling, 1976) have shown it to be less extensively degraded/fermented than other cereals. Considering the variability observed in wheat starch measured in Phase 1, it is important to compare the data obtained from wheat with that from typical examples of maize grain. If it can be shown that some wheats have starch characteristics approaching that of maize then this would be of value to cereal producers when competing for access to animal feed markets.

The objective of the second part of this study was to examine six samples of selected maize grain prepared in exactly the same way as the wheat in Phase 1 of Part 1 with the gas production method for assessing starch degradability and fermentibility in the rumen. Starch degradation will also be directly measured, as will the contents of starch, protein and cell walls (fibre). These data will then be compared with the values obtained for the wheat in Phase 1 (Part 1).

2.0 Materials and Methods

2.1 Samples

2.1.1 Specific weight study

Wheat samples were obtained (23 samples) from existing HGCA and MAFF funded studies being undertaken nationally, and by ADAS, all from the 1997 harvest. The material was selected to cover a wide range of specific weight values. These samples were combined with a further eight samples chosen from the earlier HGCA funded project (Part 1) to provide the widest and most complete range in specific weight. This approach provided material of a wide range but the samples did not come from experiments designed to provide wheat samples for this research project. The samples collected are shown in Table 1.

2.1.2 In vitro gas production

Six maize grain samples were obtained from a range of commercial sources to reflect maize available to compound feed manufacturers in the UK at the time. Details of the samples collected are shown in Table 1.

2.2 Chemical analysis

Sub-samples of each wheat and maize sample were analysed for dry matter (DM) and nitrogen (MAFF, 1986), neutral detergent fibre with an amylase pre-treatment (NDFa; Van Soest *et al.*, 1991) and starch by the enzymatic method. Additionally, the wheat samples were analysed for acid ether extract (AEE) as described by Alderman (1985) and the digestible organic matter (OM) in the DM was determined *in vitro* using the neutral detergent-cellulase and gamannase technique (NCGD) described by Dowman (1993). Metabolisable energy content was calculated using the equation E_3 (Thomas *et al.*, 1988) described as follows:

ME (MJ kg^{-1} DM) = (0.014 * NCGD) + (0.025 * AEE)

with NCGD and AEE expressed as g kg^{-1} DM.

Table 1. Treatment number, nomenclature, site and type of wheat (samples 1 to 31) and maize samples used.

Sample number	Sample code	Site	Sample Type
1	AB3	Rosemaund	Mid September 250 seeds/m2 Nil pgr
2	AB6	Rosemaund	Early November 500 seeds/m2 CCC+ Terpal
3	AB12	HGCA (0037/1/91)	First Wheats Soissons
4	AB16	HGCA (0037/1/91)	Third Wheats Riband
5	AB31	Terrington	Slejpner GS 39-59
6	AB34	Terrington	Slejpner GS 71-87
7	AB47	Rosemaund	Multi site Mercia
8	AB48	Boxworth	Multi site Mercia
9	BA66	Bridgets	MAS MAP New Hampshire
10	BA67	Bridgets	MAS MAP Mississippi
11	BA68	Bridgets	Bounder 1
12	BA69	Bridgets	Bounder 2
13	BA70	Bridgets	Bounder 3
14	BA71	Bridgets	Bounder 4
15	BA72	Bridgets	Bounder 6
16	BA73	Bridgets	Bounder 7
17	BA79	Rosemaund	Spark, September sown
18	BA80	Rosemaund	Cadenza, September sown
19	BA81	Rosemaund	Soissons, November sown
20	BA82	Rosemaund	Spark, November sown
21	BA83	Rosemaund	Soissons, September sown
22	BA84	Rosemaund	Cadenza, November sown
23	BA85	Rosemaund	Cadenza, September sown
24	BA86	Rosemaund	Buster, October sown
25	BA87	Rosemaund	Cadenza, November sown
26	BA88	Rosemaund	Spark, November sown
27	BA89	Rosemaund	Consort, September sown
28	BA90	Rosemaund	Spark, September sown
29	BA91	Rosemaund	Brigadier, September sown
30	BA92	Rosemaund	Soissons, September sown
31	BA93	Rosemaund	Soissons, November sown
32	BA94	Dalgety	Maize
33	BA95	Cargill	Maize
34	BA96	Pye Farm Feeds	Maize
35	BA97	J. Bibby Agriculture	Maize
36	BA98	Dalgety Oldacres	Maize
37	BA99	Maizecor Foods Ltd.	Maize

2.3 Measurement of gas production *in vitro*

A sub-sample (100g) of the six maize 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 fresh) 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 assessment of OM degradation was made using filtration. 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).

The above was then repeated stopping the incubations after 8 h (equivalent to the typical rumen retention time of maize grain in a high yielding dairy cow) and starch disappearance (by completely drying the incubation medium and residue) was determined, together with VFA production. Starch 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.

2.4 Near infrared reflectance spectroscopy (NIRS)

Three physical forms (whole grain, 3mm grind and 1mm grind) of the 23 additional wheat samples and the six maize 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).

2.5 Statistical analysis

2.5.1 Experimental design

This was an investigative study, not a formally designed experiment. It used 31 different samples of wheat from a wide range of sites and varieties which had been selected from other experiments and had therefore been subjected to different treatments. This approach provided a wide range of specific weight values which could have influenced the composition and energy value of the wheat.

The six samples of maize were randomised across three consecutive gas production runs utilising two x twelve place units, to provide two replicates per sample per run plus two blanks per unit, resulting in six replicates per sample.

2.5.2 Statistical methods

To study relationships between the specific weight of wheat and its nutritive value as determined by chemical composition and predicted ME content, linear regression analysis was undertaken.

The *in vitro* gas production data from the maize samples were fitted to the model of France *et al.* (1993). The parameters determined were compared with the same parameters for the wheat samples investigated in the earlier HGCA funded study .

3.0 *Results*

3.1 Chemical composition

The chemical composition of the grains and their specific weight values are shown in Table 2. The wheat samples had a mean DM content of 863 g kg^{-1} fresh (range 852 to 876) and nitrogen contents ranged from 17.0 to 25.9 g kg^{-1} DM (mean 21.7). The wheats had a mean NDFa content of 87.8 g kg^{-1} DM (range 64 to 122) whilst starch content ranged from 627 to 792 g kg^{-1} DM (mean 682). There was little variation amongst the wheats in AEE and NCGD, hence there was also little variation in estimated ME content (range 13.5 to 14.0 MJ kg^{-1} DM). Specific weight ranged from 61.4 to 84.4 kg hl^{-1}, this being greater than the range in the previous study (Part 1) of 68.7 to 84.4 kg hl^{-1}.

The chemical composition for all the maize grain samples was similar, with maize grain having a lower mean nitrogen content, similar NDFa and higher mean starch content than the population of wheat grains in either this or the previous study.

Table 2 Chemical composition and specific weight of 31 samples of wheat grain and the chemical composition of the six maize grains (g kg^{-1} DM or as stated).

Sample code	Site	Variety	Dry matter (g kg^{-1} fresh)	Nitrogen	NDFa	Starch	NCGD[1]	AEE[2]	ME (MJ kg^{-1} DM)	Specific weight (kg hl^{-1})
AB3	Rosemaund	Mercia	873	20.8	64	714	922	26	13.6	83.1
AB6	Rosemaund	Mercia	876	20.7	81	702	924	25	13.6	82.3
AB12	HGCA (0037/1/91)	Soissons	852	22.3	99	745	922	26	13.6	78.3
AB16	HGCA (0037/1/91)	Riband	872	22.2	104	683	932	28	13.8	74.2
AB31	Terrington	Slejpner	868	20.1	104	702	934	23	13.7	81.5
AB34	Terrington	Slejpner	870	22.3	81	682	925	25	13.6	76.1
AB47	Rosemaund	Mercia	871	17.0	122	783	929	26	13.7	81.8
AB48	Boxworth	Mercia	856	18.2	122	792	921	28	13.6	84.4
BA66	Bridgets	New Hampshire	857	22.1	113	665	929	23	13.6	67.3
BA67	Bridgets	Mississippi	863	22.7	82	661	921	26	13.5	72.9
BA68	Bridgets	Bounder 1	861	19.4	91	665	922	26	13.6	65.5
BA69	Bridgets	Bounder 2	863	19.4	84	669	929	26	13.7	70.0
BA70	Bridgets	Bounder 3	858	18.9	96	683	925	26	13.6	67.2
BA71	Bridgets	Bounder 4	867	21.4	88	648	916	27	13.5	72.3
BA72	Bridgets	Bounder 6	866	18.9	78	686	933	27	13.7	66.5
BA73	Bridgets	Bounder 7	863	18.7	96	674	925	29	13.7	66.1
BA79	Rosemaund	Spark	871	22.1	94	677	934	29	13.8	72.9
BA80	Rosemaund	Cadenza	870	20.5	71	648	938	33	14.0	80.7
BA81	Rosemaund	Soissons	868	22.6	87	663	931	27	13.7	75.7
BA82	Rosemaund	Spark	871	23.8	83	669	928	30	13.7	79.7
BA83	Rosemaund	Soissons	864	23.0	76	697	932	25	13.7	78.9
BA84	Rosemaund	Cadenza	872	22.2	74	689	936	32	13.9	77.1
BA85	Rosemaund	Cadenza	858	25.9	86	655	906	32	13.5	65.2

Table 2 Continued

Sample code	Site	Variety	Dry matter (g kg^{-1} fresh)	Nitrogen	NDFa	Starch	NCGD[1]	AEE[2]	ME (MJ kg^{-1} DM)	Specific weight (kg hl^{-1})
BA86	Rosemaund	Buster	857	23.5	85	639	920	29	13.6	68.2
BA87	Rosemaund	Cadenza	858	23.7	66	650	927	33	13.8	68.0
BA88	Rosemaund	Spark	860	25.8	77	658	920	29	13.6	68.7
BA89	Rosemaund	Consort	856	21.8	72	685	915	27	13.5	65.0
BA90	Rosemaund	Spark	858	23.5	80	627	914	31	13.6	67.5
BA91	Rosemaund	Brigadier	859	23.7	85	656	914	26	13.5	61.4
BA92	Rosemaund	Soissons	853	24.3	104	677	924	27	13.6	68.2
BA93	Rosemaund	Soissons	854	22.7	76	706	922	26	13.6	72.7
Mean			**863**	**21.7**	**87.8**	**682**	**925**	**27.5**	**13.64**	**72.9**
SD			**6.82**	**2.18**	**14.80**	**37.1**	**7.3**	**2.64**	**0.120**	**6.51**
Min			**852**	**17.0**	**64.0**	**627**	**906.0**	**23**	**13.45**	**61.4**
Max			**876**	**25.9**	**122.0**	**792**	**938.0**	**33**	**13.96**	**84.4**
BA94	Dalgety	Maize	878	14.1	96	746				
BA95	Cargill	Maize	866	13.6	95	753				
BA96	Pye Farm Feeds	Maize	866	14.1	97	738				
BA97	J. Bibby Agriculture	Maize	863	14.7	85	729				
BA98	Dalgety Oldacres	Maize	874	14.4	86	735				
BA99	Maizecor Foods Ltd.	Maize	874	13.9	86	730				
Mean			**870.2**	**14.1**	**90.8**	**738.5**				
SD			**5.95**	**0.38**	**5.71**	**9.40**				
Min			**863**	**13.6**	**85**	**729**				
Max			**878**	**14.7**	**97**	**753**				

3.2 Relationship between specific weight and the nutritive value of wheat

Relationships between the specific weight values of the 31 samples of wheat and all the chemical parameters and estimated ME contents were investigated. The Pearson's correlation coefficients for these relationships are shown in Table 3. None of the correlations with specific weight were highly significant although values greater than 0.5 were obtained between specific weight and either DM (0.56) or starch content (0.62). The relationships between specific weight and DM, starch and ME are shown in Figures 1 to 3 respectively. There werepositive relationships between specific weight and both DM and starch content and the relationship between specific weight with DM content was strongly influenced by variety (particularly Soisson and one sample of Mercia). There was no relationship between specific weight and predicted ME content (calculated from the E3 equation of Thomas *et al.*, 1988).

Table 3. Pearson's correlation coefficient matrix for specific weight and the chemical composition and calculated ME content of the wheat grain.

	Specific weight	Nitrogen	Dry matter	NDFa	Total ash	Oil (acid hydrolysis)	NCGD	Starch
Nitrogen	-0.298							
Dry matter	0.555	-0.272						
NDFa	0.143	-0.397	-0.136					
Total ash	-0.130	0.247	-0.190	0.025				
Oil (acid hydrolysis)	-0.128	0.326	0.008	-0.363	0.338			
NCGD	0.447	-0.371	0.521	0.079	-0.662	-0.045		
Starch	0.622	-0.544	0.064	0.518	0.038	-0.353	0.163	
ME	0.308	-0.145	0.453	-0.135	-0.390	0.511	0.835	-0.056

Figure 4 shows the relationship between measured specific weight and specific weight calculated as a function of DM and starch content for the 31 wheat samples. The variance accounted for is high ($R^2 = 62.8\%$).

Figure 1. Relationship between specific weight and dry matter for 31 wheat grains.

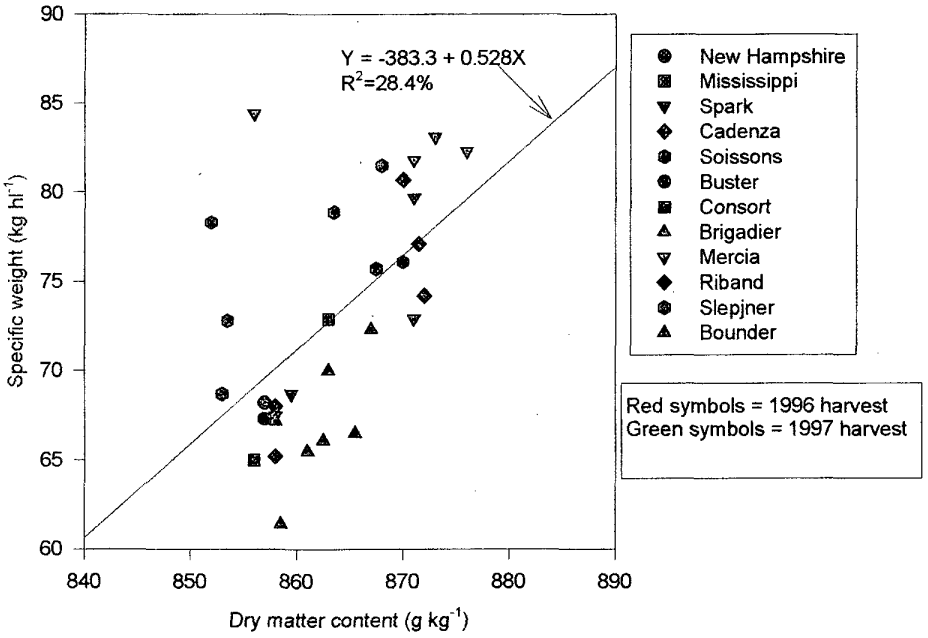


Figure 2. Relationship between specific weight and starch content for 31 wheat grains.

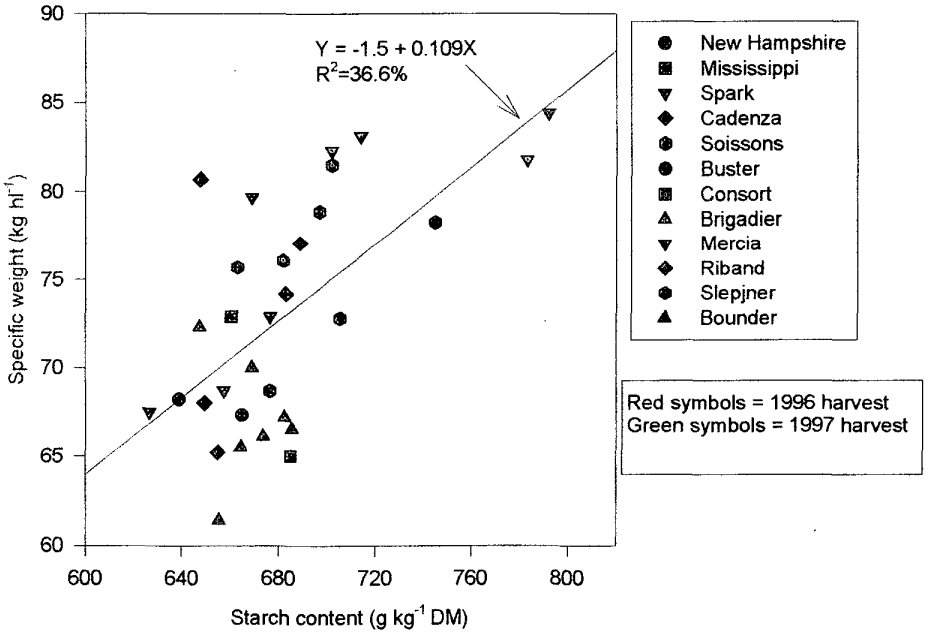


Figure 3. Relationship between specific weight and metabolisable energy content for 31 wheat grains.

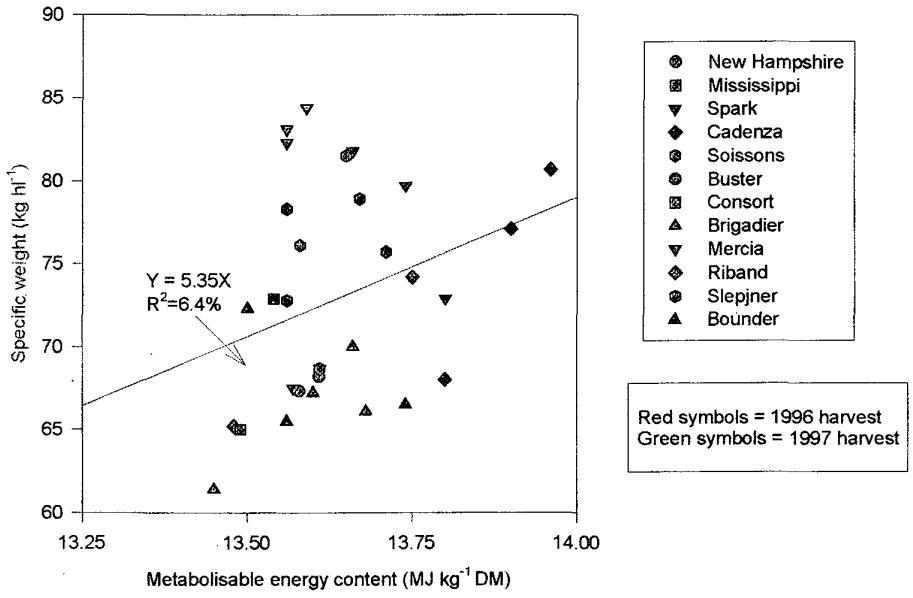


Figure 4. Relationship between specific weight and dry matter and starch content for 31 wheat grains.

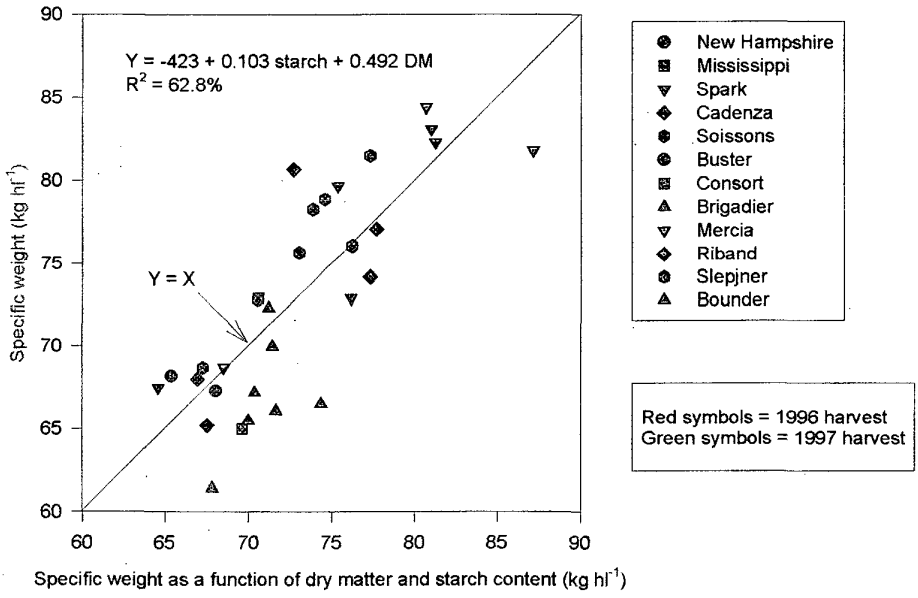
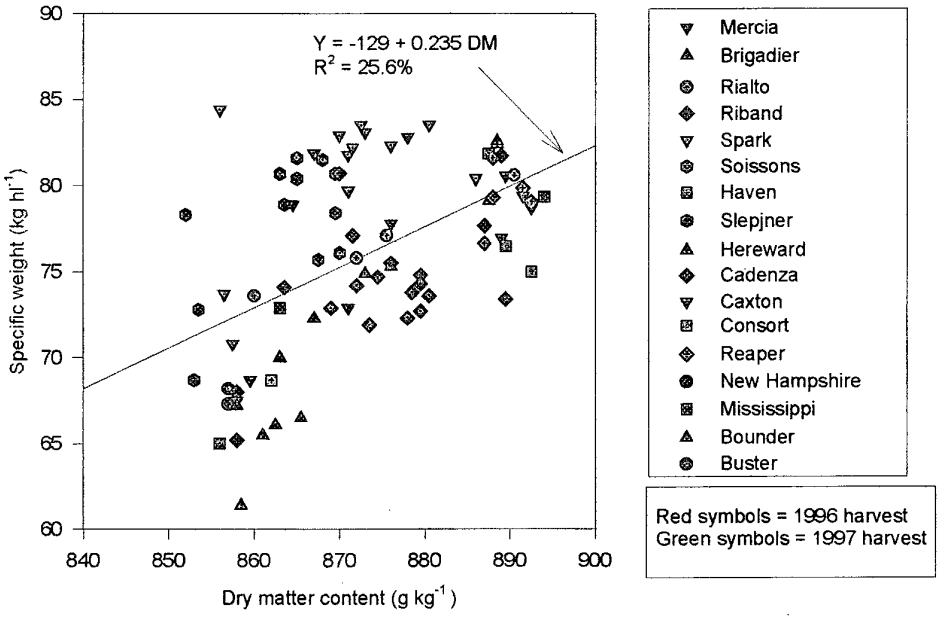


Figure 5. Relationship between specific weight and dry matter content for 84 wheat grains.



As only DM and starch values were correlated significantly with specific weight for the sub-set of 31 wheat samples, it was possible to enlarge the data set by including the remaining wheat samples from the earlier HGCA funded project (Part 1). With this expanded data set (84 samples) specific weight was only significantly correlated with DM (Figure 5) and the addition of starch content to the relationship did not significantly improve the variance accounted for. The variance accounted for in specific weight by DM was similar to that of the smaller data set though the slope and constant were different. The relationship was again strongly influenced by variety.

3.3 Measurement of gas production *in vitro*

The mean gas production data for the six samples of maize grain are shown in Table 4 along with the mean data from the wheat project (Part 1). The OM disappearance determined by filtration after 48 h incubation ranged from 76.4 to 77.1 % (mean 76.8 %) compared with 75.2 to 80.3 % (mean 78.1%) for the wheat samples. The asymptotic value for gas production from the model of France *et al.* (1993) ranged from 318 to 367 (mean 338) and 293 to 341 (mean 311) ml g^{-1} DM and the lag time ranged from 3.0 to

3.2 (mean 3.1) and 1.35 to 2.77 (mean 2.0) h for the maize and wheat samples respectively. The maize grains on average produced 8.6% more total gas and had 55% longer lag time than the wheat grains. The calculated effective degradability of organic matter (EOMD) and the combined fractional rate of gas production at an assumed 6% h^{-1} rumen outflow rate ranged from 40.5 to 42.7% and 0.052 to 0.058 h^{-1} and 50.6 to 55.6 % and 0.088 to 0.104 h^{-1} respectively for maize and wheat grains. The range in values was lower for the maize grain than the wheat samples although this may be a reflection of the small sample set for maize compared with the wheat data set. The lower EOMD for the maize grain compared with the wheat was mainly a reflection of the longer lag phase and the slower combined rate of gas production.

Table 4 Gas production and associated data of the six maize samples and all wheats (n=61) previously studied.

Sample code	pH at 48h	OMD at 48h (%)	Asymptote (ml g^{-1} DM)	Underlying rate (h^{-1})	Time dependent rate ($h^{-1/2}$)	Lag (h)	Time to half asymptote (h)	Combined rate at 8h (h^{-1})	Combined rate at $t^{1/2}$ (h^{-1})	Effective degradability of organic matter[1] (%)	Combined rate at rumen outflow 0.06 h^{-1} (h^{-1})
BA94	6.2	77.0	318.4	0.138	-0.354	3.2	12.8	0.075	0.089	42.7	0.058
BA95	6.2	76.7	336.5	0.130	-0.339	3.2	13.4	0.070	0.084	41.3	0.054
BA96	6.2	76.4	319.3	0.126	-0.327	3.2	13.7	0.068	0.081	40.5	0.052
BA97	6.2	76.8	350.5	0.133	-0.344	3.1	13.2	0.072	0.085	41.9	0.055
BA98	6.2	76.9	366.8	0.129	-0.335	3.1	13.4	0.070	0.084	41.5	0.054
BA99	6.2	77.1	336.7	0.131	-0.333	3.0	13.1	0.072	0.085	42.5	0.056
Mean	**6.2**	**76.8**	**338.0**	**0.131**	**-0.339**	**3.1**	**13.3**	**0.071**	**0.085**	**41.7**	**0.055**
SD	0.01	0.25	18.57	0.0041	0.0094	0.08	0.31	0.0024	0.0026	0.81	0.0020
Min	6.2	76.4	318	0.126	-0.327	3.0	12.8	0.068	0.081	40.5	0.052
Max	6.2	77.1	367	0.138	-0.354	3.2	13.7	0.075	0.089	42.7	0.058
Wheat samples											
Mean	**6.2**	**78.1**	**311**	**0.202**	**-0.498**	**2.0**	**9.6**	**0.114**	**0.122**	**53.4**	**0.094**
SD	0.05	1.18	8.8	0.0144	0.0474	0.33	0.38	0.0074	0.0065	1.14	0.0041
Min	6.1	75.2	293	0.176	-0.623	1.4	8.7	0.104	0.111	50.6	0.088
Max	6.3	80.3	341	0.241	-0.407	2.8	10.5	0.133	0.138	55.6	0.104

[1] At rumen outflow of 0.06 h^{-1}

The gas production, OM and starch disappearance, pH and VFA composition from the *in vitro* incubations after 8 h are given in Table 5 for the six maize samples and the mean data for the wheat samples. The OM disappearance for the six maize samples was more variable than for the wheat samples and the mean value was 9.5 percentage units higher. However the starch disappearance at 8h was lower for the maize samples compared with the wheat samples and the mean value was 14.7 percentage units lower (28.8% compared with 43.5% respectively). The total VFA concentration was lower for the maize samples than the wheats and the molar proportions of acetate and propionate were higher and lower respectively for the maize samples compared with the wheat samples.

The amount of OM and starch degradation and the estimated fermentable organic matter (FOM) calculated from the volatile fatty acid production at 8 h are given in Table 6 for the six maize samples and the mean data for the wheat samples. The amounts of maize starch degraded at 8 h were slightly higher in value than the calculated FOM content, whereas for the wheat samples the two values were similar. The VFA produced per gram of starch degraded was 7.2 mmol for the maize grains and 9.4 mmol for the wheat but they had similar total gas production and lower direct gas from the wheat samples. This was expected as the VFA proportions from the wheat samples showed more propionate and had a lower acetate+butyrate to propionate ratio than the maize samples (1.73 v 2.55 respectively). The formation of propionate in the rumen does not yield gas whereas the formation of acetate and butyrate produces CO_2 and methane.

3.4 Near infrared reflectance spectroscopy (NIRS)

The NIRS spectra from the additional wheat grains were added to the dataset from the previous HGCA funded study (Part 1) for future use.

Table 5. Gas production, organic matter and starch disappearance, pH and volatile fatty acid production at 8h for the six maize samples and the mean data for the selected fifteen wheat samples.

Sample code	Total gas (ml g^{-1} DM)	Direct gas[1] (ml g^{-1} DM)	Indirect gas[2] (ml g^{-1} DM)	Organic matter disappearance at 8h (%)	Starch disappearance at 8h (%)	Total VFA (mmol l^{-1})	Molar proportions of VFA			
							Acetate	Propionate	n-butyrate	n-valerate
BA94	82.0	50.6	31.4	23.3	32.1	15.1	0.60	0.28	0.11	0.016
BA95	85.6	59.2	26.4	20.3	28.0	12.7	0.58	0.28	0.11	0.019
BA96	101.3	73.0	28.3	24.4	33.5	13.6	0.60	0.27	0.10	0.018
BA97	85.5	57.2	28.3	39.1	26.0	13.6	0.59	0.27	0.11	0.017
BA98	76.1	48.4	27.7	34.8	29.5	13.3	0.60	0.27	0.11	0.018
BA99	79.0	51.5	27.5	35.3	23.9	13.2	0.60	0.28	0.10	0.018
Mean	**84.9**	**56.7**	**28.3**	**29.5**	**28.8**	**13.6**	**0.595**	**0.275**	**0.107**	**0.0177**
SD	8.84	9.00	1.69	7.78	3.63	0.81	0.0084	0.0055	0.0052	0.0010
Min	76.1	48.4	26.4	20.3	23.9	12.7	0.58	0.27	0.10	0.016
Max	101.3	73.0	31.4	39.1	33.5	15.1	0.60	0.28	0.11	0.019
Wheat samples										
Mean	**83.0**	**36.6**	**46.4**	**20.0**	**43.5**	**22.3**	**0.54**	**0.36**	**0.082**	**0.012**
SD	7.93	5.06	3.69	3.01	2.62	1.77	0.007	0.009	0.008	0.002
Min	71.2	29.2	41.6	16.6	39.7	20.0	0.53	0.34	0.07	0.009
Max	97.6	44.9	52.7	25.4	48.7	25..3	0.55	0.37	0.09	0.014

[1] Direct gas calculated from subtraction of indirect gas from total gas

[2] Indirect gas calculated assuming that one mmol TVFA gives rise to 20.8 ml gas indirectly from the buffer (Rymer and Moss, 1997).

Table 6. The amount of organic matter and starch degraded and fermented organic matter calculated from volatile fatty acid production at 8 h for the six maize samples and the mean data for the selected fifteen wheat samples.

Sample code	Total gas (ml g^{-1} DM)	Direct gas[1] (ml g^{-1} DM)	Organic matter degraded at 8h (mg)	Starch degraded at 8h (mg)	Starch escaping rumen degradation at 8h (mg)	Fermented organic matter at 8h (mg)	Effective organic matter disappearance at 0.06 h^{-1} rumen outflow (mg)
BA94	82.0	50.6	204	212	449	154	374
BA95	85.6	59.2	175	184	472	131	357
BA96	101.3	73.0	212	218	436	139	351
BA97	85.5	57.2	340	168	478	139	365
BA98	76.1	48.4	302	192	459	137	358
BA99	79.0	51.5	307	152	484	135	370
Mean	**84.9**	**56.7**	**257**	**188**	**463**	**139.2**	**363**
SD	8.84	9.00	67.8	25.3	18.4	7.86	8.69
Min	76.1	48.4	175	152	436	131	351
Max	101.3	73.0	340	218	484	154	374
Wheat samples							
Mean	**83.0**	**36.6**	**168.5**	**237.1**	**307.4**	**227.6**	**459**
SD	7.93	5.06	26.28	19.83	20.00	15.77	18.4
Min	71.2	29.2	134.0	211.0	269.7	207.8	432
Max	97.6	44.9	215.2	279.7	335.9	253.4	488

[1] Direct gas calculated from subtraction of indirect gas from total gas

[2] Indirect gas calculated assuming that one mmol TVFA gives rise to 20.8 ml gas indirectly from the buffer (Rymer and Moss, 1997).

4.0 Discussion

4.1 Relationship between specific weight and the nutritive value of wheat

In the previously HGCA funded study (Part 1) there was no relationship between specific weight of wheat and any nutritive value parameters. The objective of the current study was to extend the dataset to include wheat samples of low specific weight and relate these to nutritive value parameters as wheat samples of low specific weight have previously been thought to have an impaired nutritive value. The evidence for this relationship is scarce particularly for ruminants. In the present study ME was used as a primary indicator of nutritive value. There was no relationship between specific weight and ME (Figure 3) and there was also no apparent cut-off point in specific weight where ME content appeared to decline linearly as has previously been noted with poultry (McNab,1991) although this conclusion had been confounded by moisture content. This shows that for ruminants, specific weight is not a good indicator of nutritive value when this is described in terms of ME although it is noteworthy that in the present work little variability in predicted ME was seen.

Specific weight is a measure of the bulk density of grain, i.e. the weight of grain that can be contained in a unit volume packed in a certain way. It is a poor measure of grain quality as its value can be influenced by many factors. For example, a poor standard of threshing and windrowing would result in the inclusion of unthreshed heads, chaff and pieces of straw in the grain samples, detracting from the value of cereals sold on a volume basis. Specific weight is also dependent on moisture content, grain density, grain size and shape, surface friction of the grains, temperature and also the method used to fill the test vessel (Gooding and Davies, 1997). Gooding and Davies (1997) also suggested that specific weights above 74 kg hl^{-1} did not relate to either the degree of grain shrivelling or flour yield.

In this study specific weight was only related to DM and starch content but this was strongly influenced by variety. Gooding and Davies (1997), when reviewing the literature, showed specific weight was related to grain DM content such that specific

weight falls as DM falls below 880 gkg^{-1}. However the relationship between specific weight and DM content differed between varieties (Gooding and Davies 1997) and specific weights at a given DM content were shown to alter depending on whether the grain was dried or wetted. There are therefore clear reasons why specific weight of grain is not a good measure of grain quality whatever its required end-use. In poultry, McNab (1991) showed in wheat that there was a positive relationship between specific weight and true metabolisable energy (TME) value on a fresh weight basis, although this was to some extent a function of the relationship between specific weight and moisture content. The results from the present study confirmed the influence of moisture content on specific weight but confirmed that any link between specific weight and nutritive value is probably complex and this may also partially explain why in some cases relationships with nutritive value are weak or non-existent.

In the earlier HGCA-funded project (Part 1) samples of wheat were scanned by NIRS in three forms, the whole grain, and the grain milled through 1 and 3 mm screens. NIRS calibrated well for specific weight of the whole grains, but the calibrations were significantly weaker for the milled grains, possibly indicating that specific weight is a poor method of distinguishing between poorly filled grains and those samples that have poor packing characteristics. It is therefore necessary to develop a rapid technique to estimate the nutritive value of grains and grain quality (for milling) other than specific weight. Specific volume of ground grains would eliminate differences in grain packing and is likely to be more related to factors influencing the nutritive value.

4.2 *In vitro* gas production of maize versus wheat grain

The OM disappearance determined by filtration after 48 h incubation was similar for the two grain types. The mean asymptotic value for gas production from the model of France *et al.* (1993) was 338 and 311 ml g^{-1} DM and the lag time ranged was 3.1 and 2.0 h for the maize and wheat samples respectively. The maize grains on average produced 8.6% more total gas and had 55% longer lag time than the wheat grains. It is likely that the mean total gas production was greater for the maize grains compared with the wheat

because on average the maize samples had more total carbohydrate available for fermentation (starch plus cell wall).

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 40.5 to 42.7% and 0.052 to 0.058 h^{-1} and 50.6 to 55.6 % and 0.088 to 0.104 h^{-1} respectively for maize and wheat grains. This compares with effective DM degradability at 6% h^{-1} rumen outflow rate determined *in situ* for a range of wheat grains milled through a 3mm screen of 68.7% (Givens *et al.*, 1997). Herrera-Saldana *et al.* (1990) showed *in situ* the starch in wheat to be more rumen degradable than maize starch at 6% h^{-1} rumen outflow rate when ground through a 1mm screen (95 v 62% respectively). In this case the finer grind explains the higher rate of starch degradation for both cereal types.

The difference between the FOM value at 8h and the OM degraded would normally be attributed to partitioning of degraded carbohydrate directly into microbial biomass production rather than to fermentation. The OM degraded and the starch disappearance at 8h were estimated from the OM or starch in the residue after the entire contents of the *in vitro* tube had been dried, hence the residue contains undegraded feed, any matter that has solubilised but not been fermented and microbial biomass. In the case of the starch residue there may be some starch attributed to the microbes as either engulfed starch granules by protozoa or assimilated polysaccharides. In the case of the wheat samples the FOM and starch disappearance were very similar in value but both greater than the OM disappearance. It can be concluded from these results that most of the FOM was from starch and that some of the degraded OM was directly incorporated into microbial growth without fermentation. However the maize samples had greater starch disappearance than FOM and the OM degraded was greater than both of these values. These results can be interpreted as the difference between OM degraded and FOM being attributed to a portion of the feed being degraded and not subsequently fermented i.e. protein and the difference between starch disappearance and FOM being attributed to some of the starch not being fermented and directly incorporated into microbial biomass. Maize grain differs from wheat in its endosperm structure. Maize endosperm comprises two distinct regions, horny and floury whereas wheat endosperm is homogenous

throughout and in it the starch granules are only loosely associated with the protein matrix. The association of starch granules with protein in the maize floury endosperm is similar to that in wheat but in the horny endosperm, starch granules are tightly embedded in the protein matrix (Hoseney, 1986). Once grain has been partially processed via milling as was the case in this study, the protein matrix and the endosperm cell wall dictate the rate at which bacterial enzymes gain access to the readily digestible starch granules. Many of the bacteria capable of digesting starch (*Streptococcus bovis, Ruminobacter amylophilus, Prevotella ruminicola, Butyrivibrio fibrisolvens, Succinimonas amylolytica and Selenomonas ruminatium)* are unable to digest the endosperm cell wall, which is rich in β-glucan. Cellulolytic bacteria must first penetrate the cell wall so that the amylolytic bacteria can access the starch granules contained in the endosperm cell. Within the endosperm cell the starch granules are surrounded to varying degrees depending on species and variety, by a protein matrix which must be degraded to allow amylolytic attack of the starch granules. In maize, the protein matrix is extremely resistant to microbial invasion and rumen fungi appear to be the only ruminal microorganisms capable of penetrating this structure (McAllister *et al.*, 1990; McAllister *et al.*, 1993). The protein matrix in wheat is readily penetrated by a variety of proteolytic bacteria and digestion of starch granules proceeds rapidly (McAllister *et al.*, 1990). For these reasons the majority of the maize protein will have been degraded and included in the OM degraded at 8h prior to the commencement of starch degradation. This theory is also substantiated by the molar proportions of VFAs at 8h where the fermentation of maize grain produced higher molar proportions of butyrate and n-valerate which are end-products of protein hydrolysis, deamination of the amino acids and the fermentation of the carbon skeletons. *In situ,* Herrera-Saldana *et al.* (1990) showed the protein in maize grain to degrade more slowly than starch and that by 12 and 48h protein was more extensively degraded than starch. This supports previous reports (Hale, 1973; Rooney and Pflugfelder, 1986) that the protein matrix must first be degraded before the degradation of starch can occur whereas for wheat grain there was a distinct parallel between protein and starch degradation. In support of this McAllister *et al.* (1993) showed that maize starch, when pre-treated with a commercial protease, degraded at a similar rate to barley starch.

The starch disappearance at 8h was lower for the maize samples compared with the wheat samples and the mean value was 14.7 percentage units lower (28.8 compared with 43.5% respectively). The range in rumen degradable starch for maize and wheat were 152 to 218 and 211 to 280 mg g^{-1} fresh respectively whilst the mean rumen by-pass starch values were 463 and 307 mg g^{-1} fresh grain. This shows that some wheat grains may approach maize in terms of rumen degradable starch but that in general there is likely to be less rumen by-pass starch from wheat grain. It has been reported (Reynolds *et al.*, 1997) that although more slowly degraded starch in the rumen can reduce acid load and can allow more total starch intake, the recovery of starch reaching the intestines as net glucose absorption is low. This is in part due to limitations to starch digestion in the small intestine and the use of glucose by the portal drained viscera. The former may thus result in a portion of the starch reaching the intestines being fermented in the hindgut. Starch fermented in the hindgut provides VFAs which may be absorbed but microbial protein supply will not benefit (Reynolds *et al.*, 1997). Therefore when considering site of starch digestion consideration should also be given to the site of digestion within the intestines as well as the whole digestive tract.

Another limitation to the use of maize grain as a source of rumen by-pass starch is its relative cost compared with wheat. At current prices (October 1998), there is a £40 to 45/t price difference between imported European grain maize (£110-115/t) and UK wheat (£70/t), both of which can be used to provide starch and energy within the ration. All the above information must be carefully considered when formulating diets, but being able to better characterise the starch in wheat will enable nutrition consultants and the compound feed industry to maximise inclusion rates of home-grown wheat in the diets of UK ruminants.

If more slowly degraded wheat starch is the ultimate goal of the ruminant feed industry then it would be pertinent to investigate the effect of processing or chemical treatment on cereal starch fermentibility. 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. Michalet-Doreau *et al.* (1997) showed formaldehyde to be effective at reducing the

effective starch degradability of wheat grain at 6% h^{-1} rumen outflow rate by 15 percentage units. These processing treatments require further investigation.

Conclusions

The *in vitro* gas production technique has confirmed that maize grain is more slowly fermented in the rumen than wheat and that after 8 h incubation the mean rumen degradable starch was 188 mg g^{-1} fresh weight compared with 237 mg g^{-1} fresh weight of wheat. These values are obviously influenced by starch content of the grains which was lower and much more variable in wheat grain. The difference in starch disappearance at 8h was 28.8% and 43.5% for maize and wheat respectively. The range of values associated with starch degradation was wide for the wheat samples and the results confirm that ME or specific weight do not fully account for these important nutritional differences. In addition, it is concluded that specific weight is a poor indicator of nutritive value and although low specific weight values were associated with high moisture contents, this relationship appeared to be variety dependant.

Recommendations

1. It is recommended that a rapid technique is developed to estimate the nutritive value of grains and grain quality (for milling) other than specific weight. Specific volume of ground grains would eliminate differences in grain packing and is likely to be more related to factors influencing the nutritive value.

2. Grain processing and chemical treatments require further investigation in order to reduce the rate of rumen degradation of wheat starch.

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 fermentibility of soluble and slowly fermentable components of feeds. Various models have been used to describe gas production models.

The two models used to fit to the gas production data form this study were those described by France *et al.* (1993) and Groot *et al.* (1996). 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 μ = combined rate of gas production at time t

b = underlying rate

c = time dependent rate

T = lag phase prior to gas production

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

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.

The tri-phasic model of Groot *et al.* (1996) is based on a generalised Michealis - Menten equation and can differentiate between soluble, insoluble but fermentable feed fractions and microbial turn-over. When the wheat data was fitted to this model, only a single phase was determined indicating that the carbohydrate source in wheat was essentially of one type.

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