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Reduction in diffuse pollution of poultry operations through selection of wheat cultivars of high and consistent nutritional quality

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1. ABSTRACT

The objectives of the project were to characterise precisely defined genetic stocks of wheat for nutritional value so that it can be predicted accurately, leading to reduced variability in broiler performance and greater confidence in the use of this raw material. Such improvements in bird performance would also reduce diffuse pollution associated with poor-quality diets fed to poultry.

Wheats can be classified as either hard or soft, depending on their milling properties, although gradations of 'hard' and 'soft' exist and, as such, a discrete numeric classification should be used. A range of wheats were bred for nutritional and physico-chemical analysis based on the cultivar crosses 'BS'; Beaver (soft) x Soissons (hard) and 'RIL'; Avalon (hard) x Hobbit (soft). There is much genetic variation possible within the classifications 'hard' and 'soft' and there are also seasonal and environmental effects. Plant breeders can adjust texture using marker assisted selection or phenotypic selection.

Subsequent nutritional assessment showed that there is a significant correlation between total pentosan content of the wheat and Avicheck viscosity (an *in vitro* assessment used to predict the nutritional value of wheat for poultry and how wheats will respond positively to the addition of exogenous dietary enzymes). With 2007 wheats, there was a significant effect of wheat on Apparent Metabolisable Energy (AME). When wheats were grouped into hard or soft categories 'hard' wheats had increased AME and Dry Matter Digestibility (DMD). However with 2008 samples, feed conversion ratio (FCR) improved with diets containing 'soft' wheats. A non-linear effect of hardness on Coefficient of Apparent Digestibility (CAD) of starch in BS was obtained; hardness 34 and 73 having the lowest ileal digestibility compared to 41 and 63. No other significant effects on nutritional parameters were found.

There appears to be no relationship between hardness and pasting potential when measured using Rapid Visco-Analysis (RVA). Viscosities appeared to be higher and more consistent in 2007 than 2008. This could suggest that there was less amylase damage in 2007, supporting the conclusion of breeding work. The RVA could predict nutritional quality, and specifically nitrogen (N) retention, of wheat for poultry.

As N retention is inversely correlated with diffuse pollution potential, this development is of considerable importance to the overall project objectives. At this stage, it is not possible to quantify changes in diffuse pollution. Further studies, to involve both the poultry sector but also the feed industry (in formulating more accurate diets), would be necessary in generating further data to confirm the ability of RVA as a predictor of diffuse pollution.

2. SUMMARY

The HGCA R&D strategy published in January 2004 following extensive stakeholder consultation, included as a research priority ‘To more accurately determine the end-use characteristics for specific markets’. An important market for UK grain is the poultry feed industry which is the biggest market for home-grown wheat. To further define research needs specifically in relation to the poultry industry, HGCA held an industry stakeholder meeting in November 2004 at which representatives of the poultry sector were provided with an update on relevant current R&D and asked to consider future research needs. A full report of this meeting is available from HGCA.

The research needs identified by the industry group were considered by the HGCA R&D Advisory Committee, which issued a call for expressions of interest to meet some of these needs. The industry group met again in April 2005 to review the submitted proposals and assisted the Advisory Committee in selecting and focusing the most worthwhile (report available from HGCA).

Thus the background to and purpose of the current project (that was also successful in obtaining LINK funding under the ‘Sustainable Arable Programme’) was to examine the causes of and solutions to variability in the nutritional value of wheat for poultry. A fundamentally important consequence of lower nutritional value is greater environmental impact through higher waste production leading to increased diffuse pollution which, by definition, will be reduced by improved nutritional value of diets and raw materials contained within them.

Sustainable Arable LINK listed as one of its priorities ‘Biotechnology, breeding and agronomy for specific end-users’. The current project was designed to develop plant breeding solutions to the problem of variability in nutritional quality of wheat. Such variability reduces the continued use of high levels of wheat in UK broiler and turkey diets, which is having a negative impact on wheat growers as the feed industry actively seeks alternative, more reliable, raw materials. This variation is transferred into variation in bird performance, increasing the proportion of birds that fall outside the weight range that attracts a premium price, the net result of which is a reduction in profitability. The current project was planned to assure a market for UK feed wheat, to improve sustainability of the arable sector and reduce diffuse pollution associated with wastes arising from the UK poultry sector associated with poorly digested diets.

There has been much work on the factors that may be responsible for the variation in nutritional value of wheat; earlier work had identified the negative impact of the 1B/1R rye translocation that is now being bred out of wheats. A second characteristic is endosperm texture, and it was established that soft wheats tend to be of better nutritional value than their hard counterparts. However, it is crucial to appreciate that endosperm texture exists as a continuum between very hard and very soft, not simply hard or soft, as has been assumed. This ‘proof of principle’ of

relating endosperm texture to nutritional value will inform future developments in providing definitive answers to the quantitative effects of endosperm texture by using wheat lines of precisely defined genetic constitution varying from ‘soft-softs’ to ‘hard-hards’.

Wheat varieties may be characterised as being hard or soft, on the basis of the particle size produced on milling. The major genetic component of this difference is allelic variation at the *Ha* (hardness) locus on chromosome 5D. Recent evidence suggests that this difference is a consequence of amino acid changes in proteins, termed puroindolines, now thought to be responsible for the hardness phenotype. Two linked genes at the *Ha* locus (the *Pin* genes, named *Pin a* and *Pin b*) are involved, and varieties can be classified using a polymerase chain reaction (PCR) test as whether they carry a ‘soft’ or ‘hard’ allele. However, the situation is more complicated than varieties being simply characterised as ‘hard’ or soft’ and gradations exist because (i) there appear to be multiple alleles for the *Pin a* and *Pin b* proteins at this locus, giving minor variations in texture and (ii) there are other modifier genes affecting texture independent of *Ha*.

All UK soft varieties tested have the *Pina-D1a/ Pinb-D1a* alleles, and hard varieties *Pina-D1a/ Pinb-D1b* alleles, indicating that the hard wheat phenotype is due to mutations in *Pinb-D1*. However, certain hard varieties have different ‘hard’ mutations in *Pinb-D1,* such as the *Pinb-D1c* allele in Cadenza. It is possible, therefore, now to relate feed or other quality differences to this allelic variation using precise genetic stocks, particularly recombinant inbred lines and doubled haploids between contrasting parental hardness types available at JIC. However, puroindoline allelic variation is not the whole explanation of grain texture variation and progress has been made towards identifying other genes contributing to grain texture differences. Variation at these loci can modulate the major effects resulting in a spectrum of differences which can be characterized from ‘soft-softs’ to ‘hard-softs’ to ‘soft-hards’ to ‘hard-hards’. Populations have been developed that vary for this spectrum of variation and, in the current programme, these populations were characterised genetically for *Pin* gene allelic composition and using analytical procedures, particularly NIR, for grain texture. Lines with characterised genotype and phenotype were grown in the field in controlled experiments and harvested to provide seed for nutritional and physico-chemical analyses at the University of Nottingham, Danisco and SAC.

A summary of the approaches adopted in the project and the partners involved are now presented. The main objectives of the John Innes Centre (JIC) were to develop and characterize precise genetic stocks differing in texture across the spectrum of textures available in UK wheats, from soft-softs to hard-hards.

In previous work, JIC classified a range of UK wheats for their alleles at the *Pina* and *Pinb* loci using specific PCR primers. Advanced breeding lines obtained from the John Innes Centre were

assessed in parallel with the defined genetic stocks in order to ascertain the current level of variation available in UK-sourced wheat varieties. PCR primers for the Puroindoline genes on chromosome 5D of wheat were used in this project in molecular marker analysis to characterise individual recombinant lines for whether they carry 'hard' or 'soft' alleles for grain texture. Field plots of all the populations were grown for phenotypic characterization and selected lines were grown in large plots to provide seed multiplication for feeding trials. NIR techniques with appropriate calibrations were used to characterise the spectrum of genetic variation within each population for grain texture and identify a range of lines from 'soft-softs' to 'hard-hards' for multiplication for nutritional evaluation. Texture values from the recombinant populations were subject to Quantitative Trait Loci (QTL) analysis to identify the genes that modify the effect of the *Ha* locus. In collaboration with Nickerson Seeds, field trials to provide the kg quantities of grain needed for nutritional assessment with collaborators were carried out.

Limagrain (formerly Nickersons) were responsible for multiplication of lines produced by JIC into quantities suitable for subsequent evaluation.

The University of Nottingham considered that variations in nutritional quality of wheat are attributable essentially to the range of digestibilities obtained for gross energy and starch (*in vivo* assessments) and these were examined, both within the small intestine (to assess rate of starch digestion at different points) but also throughout the whole digestive tract, in a number of lines of wheat of known and precise genetic composition (specifically 'hardness' as the major variable). Although *in vivo* assessments are, ultimately, the most accurate means of determining nutritional value, there is considerable interest in developing rapid *in vitro* measurements. A number of tests based on rheological properties were developed.

The Scottish Agricultural College examined wheats of differing characteristics and the influence this would have on performance (including uniformity of performance), nutrient utilisation, microflora in the gastro-intestinal tract (GIT), litter quality and, as a result, bird health. The functional characteristics of wheat samples was described to improve the quality and consistency of bird performance, including establishing a balanced microflora, and reducing environmental outputs in line with IPPC. SAC will investigate the effects of wheat and treatment on nutrient utilisation, health and welfare, and microflora within the GIT and litter.

Viscosity of wheats was measured by DANISCO Animal Nutrition using an *in vitro* digestion method to mimic the conditions within the gastrointestinal tract and then quantified using a Brookfield viscometer. The results of the viscosity assay were compared to global wheat viscosity results from the Danisco database, which contains in excess of 4000 wheats over the last 10 years. Endogenous xylanase concentration and xylanase inhibitor concentration were measured

using a modified colormetric Megazyme assay; *in vitro* digestibility of wheat starch can be measured with an *in vitro* digestion method using amyloglucosidase and amylase, with glucose release being measured over 60 minutes. Exogenous enzymes were sourced from within Danisco Innovations or Danisco Genencor including a range of carbohydrases, proteases and/or phytase.

The ultimate aim of the project is to quantify the sources of variability in wheat and to develop a practical way of monitoring this at the feed mill. This information is only of any value if the feed manufacturer can adjust raw material inclusions or adjust formulations to allow for such variability. These adjustments must result in better financial solutions for the feed manufacturer and both ways along the chain to the livestock producer and the raw material supplier. BOCM PAULS ensured through its formulation package (raw material analysis and costs) that proposed changes are economically viable and will provide the financial benefits. The raw material database would need to be updated for the different wheat types.

Wheat was included in all commercial broiler (Grampian Country Food Group) and turkey (Bernard Matthews) diets in ground and pelleted form or as a whole grain at varying levels. Summaries of bird performance data such as FCR, liveweight and mortality were supplied.

The Project Summary will now consider further elements of the programme.

Three years of field experiments using selected recombinant doubled haploid lines from three separate crosses have confirmed the hypothesis that allelic variation at the *Ha* locus on chromosome 5D, classified using diagnostic molecular makers for the puroindoline genes at this locus, has the major effect on determining the classification of varieties into ‘soft’ or ‘hard’ grained categories. However, different alleles have differential effects on texture and it was shown that different *Pinb* alleles, such as the *Pinb-D1c* allele in Cadenza, give a harder texture than the ‘normal’ *Pinb-D1b* allele carried by most UK hard wheats. This has consequences for breeding for animal feed in determining the most appropriate alleles to use.

However, this research has also shown that the classification of grain texture of varieties is much more complicated, and modifier genes affecting texture independent of *Ha* have been identified. By introducing different alleles of these modifier genes, it has been possible to produce characterised sets of lines covering the hardness spectrum from ‘soft-softs’ to ‘hard-softs’ to ‘soft-hards’ to ‘hard-hards’. The consequences of this manipulation for breeding for varieties suitable for animal feed were studied.

Experiments of the same precise genetics stocks over three years have shown that the effects of the growing environment can be quite strong in changing texture phenotype. In this respect 2007

was shown as being a ‘hard’ year and 2006 and 2008 ‘as soft’ years. However, importantly, the texture differences between the lines are maintained over all harvests and are stable genetically, implying that breeding can provide varieties tailored for the grain texture range most suitable for animal feed.

In order to start the project in 2005, Limagrain UK Limited (formally Nickerson) sent a batch of 68 wheat samples from two different sites along with relevant data (hard/soft, HFN, protein) in order to highlight the possible differences from environmental effects on the wheat. This was for assessment by University of Nottingham. This initial batch demonstrated that there is a wide range of ‘hardness’ scores confirming that endosperm texture is by degree (varying hardness), not absolute (hard or soft).

Certain lines from this initial set of material were selected as having differing hardness characteristics and these were then grown on for a second year (sown 2006 for harvest 2007) for further assessment by collaborators. These lines were also sown in 2007 but were no longer required for the project.

The main contribution of Limagrain UK Ltd was to multiply lines, in 2007, supplied by the JIC for larger scale nutrition studies by other collaborators. Twelve lines were chosen for multiplication, 4 coming from the Beaver x Soissons doubled haploid collection and 8 from the Dwarf A Avalon x Hobbit ‘sib’ recombinant inbred line population. These lines were sown with the aim of producing between 0.5 and 1 tonne of grain. This was achieved with all lines, except a slight shortfall with RIL49.

Overall, the data collected during the current project appears to confirm the negative effect of grain hardness on broiler performance and nutrient utilisation, previously reported in the scientific literature. Unfortunately, most of the hardness-related effects observed in the different *in vivo* studies that were carried out were weak, or not significant. This was most likely due to the limited number of replicates per dietary treatment, as well as the feeding method used by the trial site (precision feeding, otherwise referred to as gavage).

Physico-chemical analyses of wheat grains suggested that greater amounts of coarse particles in hard cultivars could be responsible for nutrient entrapment, leading to reduced accessibility for digestive secretions and feed enzymes. Wheat samples from 2005 showed a trend towards soft wheats having improved Dry Matter Digestibility (DMD; $P<0.1$). In 2008, Feed Conversion Ratio (FCR) deteriorated with hard wheats compared to soft ($P<0.05$) and there was a trend towards decreased Coefficient of Apparent Nitrogen Retention (CAR; $P<0.1$). Laboratory results also

seemed to confirm the existence of a positive correlation between wheat viscosity (measured by Avicheck™ method, Danisco Animal Nutrition) and soluble pentosan content in the grain.

In most cases, feed supplementation with exogenous xylanase (provided by Danisco Animal Nutrition) resulted in improved nutrient utilisation (Apparent Metabolisable Energy, True Metabolisable Energy, digestibility coefficients) and better growth performance (Feed Conversion Ratio). However, it is interesting to note that the benefits provided by the enzyme were more pronounced with hard than with soft wheat cultivars. This may be linked to enzyme accessibility issues.

The endosperm hardness (EH) of the 55 wheat samples was determined using SKCS (Single Kernel Classification System) and varied between 9 – 85. There was no relationship between EH and any of the digestibility parameters. Previous experiments observed a positive relationship between wheat EH and Hagberg Falling Number and bird growth performance. They did not find a relationship between the EH and any of the other measurements of the wheat, such as CP digestibility.

It can be concluded that there was a positive relationship between the AME and DMD and N retention in wheat when precision fed (gavage) to broiler chickens. The information is of particular importance to plant breeders who may be able to incorporate improved nutrients/dry matter digestibility traits in their development of new feed wheat cultivars. The AME of a wheat is often used as the main criteria to evaluate the feeding quality. The relationship between the AME and the DMD in these wheat samples suggests that the DMD values alone can be used as a relatively good estimate of the feeding quality of wheat. Determining DMD is easier and less expensive and time consuming compared to the AME and N digestibility/retention. Thus breeders may be able to incorporate DMD in their programmes although future work needs to establish whether this parameter can be predicted *in vitro.*

When Beaver x Soissons wheats were analysed, the difference in Coefficient of Apparent Digestibility (CAD, of starch) between gut regions (ileal CIAD and total tract CTTAD) depended on the hardness of the wheat and year of harvest; two of the wheats (SKCS 34 and 73) had a bigger increase in CTTAD than the others, in 2007. The variation between CIAD (nitrogen) and Nitrogen retention depends on the year of harvest; CIAD was higher in 2007, but retention was lower. There were no significant effects of hardness and year on uric-acid corrected nitrogen retention. When Hobbit x Avalon wheats were analysed, there were no significant effects of hardness on starch or nitrogen digestibility, nitrogen retention or nitrogen retention corrected for uric acid excretion.

There appears to be no relationship between hardness and pasting potential as analysed by Rapid Visco Analysis (RVA). Viscosities appeared to be higher and more consistent in 2007 than 2008. This could suggest that there was less amylase damage of starch in 2007; endogenous alpha amylase can damage pasting profiles of wheat. The 2007 samples were a mixture of wheats with different genetic backgrounds. This could suggest that the decreased pasting potential in 2008 was a factor of the environment in that growing season, rather than genetic background because the pasting was higher in 2007 for all wheats despite being mixtures. This is supported by increased amylase estimates in 2008, which could be a result of environmental differences, it should be noted that the 2008 season was particularly wet from flowering right through to harvest. However, the difference in pasting potential between 2007 and 2008 could also be interpreted that any genetic anomaly that was causing decreased pasting in 2008 was more spread out across samples in 2007.

Linear regression analysis was performed to determine any relationship between the Rapid Visco Analyser (RVA) and chick bioassay parameter data. It is hypothesised that the RVA could be used as a predictor of the nutritional quality of wheat for poultry. It is evident from this analysis that certain parameters that can be measured using the RVA have a relationship with certain indicators of nutritional value for poultry.

None of the RVA parameters measured showed any significant relationship with chick parameters related to starch digestibility. Coefficient of apparent nitrogen retention (CAR) had significant positive relationships with PV (peak viscosity) when amylase activity was negated (use of silver nitrate) (R^2=0.5351; P <0.001); PV in water (R^2=0.2703; P= 0.009); EV (end viscosity) in water (R^2=0.6432; P= <0.001) and a trend towards a relationship with EV with silver ions (R^2=0.1271; P= 0.087). This suggests that these RVA measurements may be able to predict Nitrogen retention; as PV in silver nitrate increases, for example, so does nitrogen retention. Similarly, nitrogen retention corrected for uric acid excretion (CARu) is positively related to PV in silver nitrate (R^2=0.2261; P= 0.019) and EV in water (R^2=0.3518; P= 0.002). Coefficient of ileal apparent digestibility of nitrogen is positively related to EV in silver nitrate (R^2=0.3555; P= 0.002).

As N retention is inversely correlated with diffuse pollution potential, this development is of considerable importance to the overall project objectives. At this stage, it is not possible to quantify changes in diffuse pollution and further studies, involving both the poultry sector and the feed industry (in formulating more accurate diets), would be necessary in generating further data to confirm the ability of RVA as a predictor of diffuse pollution.

3. TECHNICAL DETAIL

3.1. Introduction

The John Innes Centre (JIC) has been involved in breeding wheat cultivars. Wheat varieties are characterised as being hard or soft, on the basis of the particle size produced on milling. The major genetic component of this difference is allelic variation at the *Ha* (hardness) locus on chromosome 5D. Recent evidence suggests that this difference is a consequence of amino acid changes in proteins, termed puroindolines, now thought to be responsible for the hardness phenotype. Two linked genes at the *Ha* locus (the *Pin* genes, named *Pin a* and *Pin b*) are involved, and varieties can be classified using a polymerase chain reaction (PCR) test as whether they carry a 'soft' or 'hard' allele. However, the situation is more complicated than varieties being simply characterised as 'hard' or soft' and gradations exist because (i) there appear to be multiple alleles for the *Pin a* and *Pin b* proteins at this locus, giving minor variations in texture and (ii) there are other modifier genes affecting texture independent of *Ha*. The main objectives of the JIC studies in this project were to develop and characterize precise genetic stocks differing in texture across the spectrum of textures available in UK wheats, from soft-softs to hard-hards.

In previous work, JIC classified a range of UK wheats for their alleles at the *Pina* and *Pinb* loci using specific PCR primers. All UK soft varieties tested have the *Pina-D1a/ Pinb-D1a* alleles, and hard varieties *Pina-D1a/ Pinb-D1b* alleles, indicating that the hard wheat phenotype is due to mutations in *Pinb-D1*. However, certain hard varieties have different 'hard' mutations in *Pinb-D1,* such as the *Pinb-D1c* allele in Cadenza. It is possible, therefore, now to relate feed or other quality differences to this allelic variation using precise genetic stocks, particularly recombinant inbred lines and doubled haploids between contrasting parental hardness types available at JIC. However, puroindoline allelic variation is not the whole explanation of grain texture variation and progress has been made towards identifying other genes contributing to grain texture differences (Turner *et al.* 2004; Weightman *et al,* 2008). Variation at these loci can modulate the major effects resulting in a spectrum of differences which can be characterized from 'soft-softs' to 'hard-softs' to 'soft-hards' to 'hard-hards'. Populations have been developed that vary for this spectrum of variation and, in this work, these populations were characterised genetically for *Pin* gene allelic composition, and using analytical procedures, particularly NIR, for grain texture.

Lines with characterised genotype and phenotype were grown in the field in controlled experiments by Limagrain and harvested to provide seed for nutritional and physico-chemical analyses at the University of Nottingham, Danisco and SAC. In addition, larger-scale commercial trials were undertaken by Grampian (broiler chickens) and Bernard Matthews (turkeys).

3.2. Materials and methods

The major objective of the study was to examine whether variations in endosperm texture would influence physico-chemical and nutritional value of wheat when fed to birds; changes in the latter would impact on diffuse pollution. The John Innes Centre, in collaboration with partners, chose three populations for study. These experimental lines are from crosses involving varieties that have been used extensively in UK wheat breeding programmes and, as such, are relevant to varieties currently in the market or under development.

3.2.1. Beaver x Soissons recombinant doubled haploid lines

This population of 65 doubled haploid lines was developed by JIC in collaboration with ADAS and the University of Nottingham to study genetic variation in characteristics that affect yield and quality under drought and non-drought conditions (Defra funded projects CE0370, AR0908). Beaver is soft-textured and Soissons hard-textured and, in previous field experiments, the individual lines varied considerably for hardness in measured phenotypes from 10 to 76 on an arbitrary scale of 1 – 90 (J Alava, CCFRA using the single kernal characterisation system, SKSC) where lines having a value of >40 are regarded as soft wheats, and lines having a value <45 as hard wheats. The soft lines varied from 8-38, and the hard lines 42-76, giving a full spectrum of variation for texture.

3.2.2. Avalon x Hobbit 'sib' recombinant inbred lines

This population of 97 recombinant inbred lines was developed at JIC for studies of grain texture and grain protein content in work funded by HGCA (HGCA Project Number 2233/L001A). Avalon is hard, and Hobbit 'sib' soft. This population was used for QTL analysis and, in addition to the major effect of *Ha*, genes that modify texture independent of 5D were mapped to chromosomes 1B, 5A and 5BS/7BS. These gave rise to variation in the level of 'softness', using an appropriate NIR calibration, from 3 to 37 (on an arbitrary scale of 1-100, Hobbit 'sib' = 23), and hardness of 44 to 83 (Avalon = 71).

3.2.3. Avalon x Cadenza recombinant doubled haploid lines

This population is the UK wheat reference mapping population under the Defra Wheat Genetic Improvement Network, and allows different 'hard' alleles to be evaluated for effects on animal feed quality. This population of 202 doubled haploid lines was originally developed by JIC in collaboration with ADAS and the University of Nottingham to study variation in physiological characteristics that affect disease escape and tolerance (Defra project CE05321). The parents of this population are both hard, but differ in their *Pin* gene alleles; Avalon has the genotype *Pina-D1a/ Pinb-D1b*, but Cadenza is *Pina-D1a/ Pinb-D1c.* It is interesting to evaluate if these different

hard texture alleles affect nutritional value, as evidence from Australia (H Eagles, pers. comm.) indicates that they affect bread-making quality differentially.

In order to start the project promptly in 2005, Limagrain UK Limited (formally referred to as Nickerson) sent a batch of 68 wheat samples from two different sites along with relevant data (hard/soft, HFN, Protein). Two sites were sent in order to highlight the possible differences from environmental effects on the wheat. This was for physico-chemical and nutritional assessment by University of Nottingham.

Certain lines out of this initial set of material were selected as having differing hardness characteristics and these were then grown on for a second year (sown 2006 for harvest 2007) for further assessment by collaborators. These lines were also sown in 2007 but were no longer required for the project.

The main contribution of Limagrain UK Ltd was to multiply lines, in 2007, supplied by the JIC for larger scale nutrition studies by other collaborators. 12 lines were chosen for multiplication, 4 coming from the Beaver x Soissons doubled haploid collection and 8 from the Dwarf A Avalon x Hobbit 'sib' recombinant inbred line population. These lines were sown with the aim of producing between 0.5 and 1 tonne of grain. This was achieved with all lines, except a slight shortfall with RIL49 (see Appendix D).

Danisco was responsible for conducting a number of assessments that were conducted at their laboratory in Brabrand, Denmark. Wheats from both 2005, 2006, 2007 and 2008 harvests were evaluated.

3.2.4. Exogenous enzyme products

Enzymes were provided, blended and prepared.

3.2.5. Nutritional quality of raw materials and feeds

The carbohydrate fraction of the raw materials and animal feeds was analysed for the determination of total and soluble pentosan content (Rouau and Surget 1994), viscosity (Bedford and Classen, 1993), and rate of starch digestion (RSD60; modified from Englyst et al 1992). The protein fraction was analysed for the determination of endogenous xylanase (Xylanase Assay Kit, Megazyme, Ireland). Levels of endogenous xylanase inhibitors were also determined using a method modified from Bonin *et al.* (1995). The measurement was based on the use of 2 xylanase enzymes: GPU (from Aspergillus) and TXU (from Bacillus). GPU xylanase is inhibited by both TAXI and XIP xylanase endogenous inhibitors, whereas TXU is only inhibited by TAXI xylanase

endogenous inhibitor. The inhibitor Unit (U) is defined as the amount of extract (wheat sample was mixed with buffer before assay) that halves the xylanase activity. Results from GPU and TXU cannot be compared but, for each assay, wheat samples can be ranked against each other.

Study A (2005) examined three wheat cultivars (Claire, Gladiator and Mascot), which were selected for their different levels of endogenous xylanase inhibitors. They were tested in a broiler trial, with or without the addition of xylanase activity at different doses (Xylanase 1 and 2, Danisco Animal Nutrition), using the precision feeding method (McNab and Blair, 1988; Ferraz de Oliveira *et al.,* 1994) in generating apparent metabolisable energy (AME) data in collaboration with the Scottish Agricultural College (SAC – details of the methodology employed by SAC are presented below); AME is of fundamental importance as it is the key measurement of nutritional value of raw materials employed in diet formulation for poultry. The objective of this study was to evaluate the influence of grain endogenous xylanase inhibitors on the feeding value of wheat for poultry, and their potential effect on the efficacy of exogenous xylanase products. Study B (2005) assessed 24 wheat samples for viscosity, total and soluble pentosan content, rate of starch digestion, endogenous xylanase, endogenous xylanase inhibitors, and particle size distribution; all these assessments are useful indicators of nutritional value and were supplemented with further AME data from SAC. The objective was to investigate the variability of the physico-chemical properties of different wheat cultivars (especially the parameters related to viscosity and hardness), and assess their influence on the feeding value of this cereal for poultry.

A total of 78 wheat samples from 2006 were analysed for total and soluble pentosan content. A total of 12 wheat samples were collected from the 2007 harvest. Hardness single kernel characterisation system (SKCS) values ranged from 40 to 78. They were analysed for: viscosity, total and soluble pentosan content, and endogenous xylanase activity. Ten of the 12 wheat samples were used in a broiler nutrition trial carried out at SAC. The nutritional value of the wheat samples, with or without supplementation of a commercial xylanase (provided by Danisco Animal Nutrition) was determined using the precision feeding method. Nine wheat samples were evaluated from the 2008 harvest. Hardness SKCS values ranged from 10 to 68. They were analysed for: viscosity, total and soluble pentosan content, and endogenous xylanase activity. The nine wheat samples were used in a broiler nutrition trial carried out at SAC to determine bodyweight gain, feed conversion ratio (FCR), nitrogen (N) digestibility, litter quality (dry matter content, pH and score) and hock score with or without addition of exogenous xylanase (an enzyme that can improve the nutritional value of diets based on wheat).

Amino acid and total nitrogen content of the wheat samples from the 2008 harvest were determined by BOCM Pauls. These analyses are of crucial importance in assessing nutritional value of raw materials employed in formulation of diets for poultry. Amino acid content was

determined using ion exchange chromatography and spectrophotometry at BOCM Pauls' laboratory. Firstly, the sample was oxidised with a Hydrogen Peroxide / Formic acid / Phenol mixture. Excess oxidation reagent was decomposed with Sodium metabisulphite. The oxidised sample was hydrolysed with 6 M Hydrochloric acid for 24 hours. The hydrolysate was adjusted to pH 2.20, centrifuged and filtered. The amino acids were separated by ion exchange chromatography and determined by reaction with ninhydrin using photometric detection at 570 nm (440 nm for Proline). Total nitrogen was determined using the Dumas method, according to the AOAC method 968.06. Crude protein was calculated by multiplication of total nitrogen by 6.25 (AOAC 1990).

Wheat is one of the major raw materials formulated into UK poultry diets. While it may provide a substantial proportion of the amino acids required by poultry, wheat is a source of metabolisable energy (ME) and there its main attribute lies. Although often scrutinised, the ME of wheat is still widely used to predict its nutritive quality for poultry. However, the ME of wheat is variable and no rapid test that accurately predicts ME of wheat samples has yet been developed. In addition, the empirical measurement of ME cannot be performed quickly enough to allow the data of individual wheat samples to be used before the wheat batches are included in compound feed. The aim of this experiment was to determine the content of ME, dry matter digestibility coefficient and nitrogen retention of fifty-five UK wheat cultivar samples when precision fed to forty-two days old broiler chickens. The relationship between dietary ME, dry matter digestibility coefficient and nitrogen retention was determined by regression analysis.

SAC employed an adapted precision feeding technique (McNab and Blair, 1988; Ferraz de Oliveira *et al.,* 1994) for nutritional assessment of wheats from the 2005 harvest. Five-hundred-thirty-one male (Ross 308) chickens in total fed fifty-six dietary treatments were involved in the study. The fifty-six treatments used were fifty-five wheat cultivar samples and glucose. Each of the wheat samples was coarsely milled and fed by gavage to nine single caged birds, spread over nine time periods, following a randomised block design. The glucose treatment was used for the estimation of endogenous losses and was given to four birds each time, thirty-six in total. From 1 day old the birds were fed an enzyme free wheat- or maize-soya based diet following the breeders' recommendations. At approximately 42 days old, weighing between 2.5 and 3kg, the birds were placed on a raised slatted floor pen with no access to feed, litter or excreta. Water was supplied *ad libitum* throughout the study via a suspended nipple drinker line. To alleviate the stress of feed deprivation, after 24 h the birds were given by gavage 50 ml of a glucose solution (600g/l). After a further 24 h the birds were fed 50 grams of the appropriate wheat treatment by gavage, as the birds used for endogenous losses determination were given another 50ml of glucose solution. All birds were placed in individual cages (0.5m x 0.8m floor area) designed for excreta collection. The

temperature was maintained at about 20°C and birds were provided with 23 hours of light per day. Total excreta collection was made over a 48 h period.

Excreta were collected, oven-dried, weighed and milled to pass through a 0.75 mm mesh. The gross energy (GE) of excreta was determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA) and the metabolisable energy, apparent (AME) and true (TME), of each wheat sample was calculated (McNab and Blair, 1988). The nitrogen (N) in the excreta was determined by the method of Sweeney (1989) using an FP-200 nitrogen analyser (LECO®, St. Joseph, MI, USA). Dry matter (DM) in feed and excreta was determined by drying at 100°C for 24 hours (AOAC 925.10). The daily retention of N was calculated as a difference between the intake and excretion of N. Dry matter digestibility coefficient (DMD) was determined as the difference between the DM intake and excretion, divided by the DM intake.

At the University of Nottingham two experiments were undertaken using the same protocol in further studies to assess the nutritional value of different wheat cultivars that varied in hardness. Day-old, male, Ross strain broilers were sourced (PD Hook Hatcheries Ltd, Thirsk, UK). The birds were housed in pairs, within 10g in weight (at 13-days) of each other. Each treatment was fed to 6 cages. Cages were 37cm wide by 42cm tall by 30cm deep, contained a roost and were wire bottomed, with provision for collection of excreta. Prior to the adaptation and trial period chicks were fed Chick Starter Crumb (Dodson and Horrell Ltd, Northamptonshire, UK). At day 19, the birds began an adaptation period, where they were fed the assigned trial diet. The trial period then took place between days 23 and 27, a total of 96 hours. During this time, feed intake was measured and excreta collected. At all times, feed and water were provided on an *ad libitum* basis. During the trial period, temperature was maintained at 21ºC and the birds were kept under artificial light for 23 hours per day, with one hour of dark. The air in the metabolism room was continuously circulated and humidity monitored.

The birds were culled on day 28 of the bioassay by asphyxiation with carbon dioxide and cervical dislocation to confirm death. The weight of each carcass was recorded. The ileal region of the gut was dissected out from the duodenal-ileal junction to the ileal-caecal junction. Subsequently, this region shall be referred to as the ileum.

Experimental diets were formulated using the ingredients described in Table 1 with wheat as the only variable. All diets were manufactured on site at the University of Nottingham, Sutton Bonington Campus. Wheat was ground using a Pulverisette 15 cutting mill (Fritsch GmbH, Idar-Oberstein, Germany) fitted with a 4mm screen and then mixed using a commercial planetary dough mixer. All wheat was refrigerated prior to use and after manufacture, diets were stored at ambient temperature.

Table 1. Basal dietary composition

Component	Amount (g/kg diet)
Wheat	750
Starch (from maize)	70
Glucose	70
Soya Oil	50
Vitamin and Mineral Premix	50
Titanium Dioxide	10

The identity of the samples used in digestibility trials at Nottingham is described earlier (page 14). In brief, wheat of two genetic backgrounds (Beaver x Soisson; 'BS' and Avalon x Hobbit; RIL) were used, harvested in 2007 and 2008. The BS samples are comparable year on year whereas the RIL samples are not. As such, samples are identified in summary statistics, by their hardness score.

For samples of wheat and diets, dry matter (DM) was determined in triplicate samples weighing 500mg that were dried at 100°C in a forced air convection oven. The dry matter content of excreta was determined in a similar way, although the whole sample was dried. The temperature used was 75°C to avoid cooking the excreta sample and potentially damaging the starch structure. Due to their small sample size and collection directly into plastic containers, digesta samples were frozen and then freeze-dried when determining dry matter.

The Total Starch Assay Kit (Megazyme International, County Wicklow, Ireland) was used to determine starch content in wheat, diet, digesta and excreta samples.

The concentration of titanium dioxide (employed as an inert marker) was determined in diets, digesta and excreta samples using the method described by Short *et al.* (1996).

Total nitrogen was determined using the Dumas method, according to the AOAC method 968.06. Crude protein was calculated by multiplication of total nitrogen by 6.25 (AOAC 1990).

Uric acid was quantified using the method of Pekic *et al.* (1989), whereby uric acid is extracted in lithium carbonate buffer before being quantified using High Performance Liquid Chromatography.

Wheat endosperm hardness was analysed using the Single Kernel Classification System (SKCS) using the SKCS 1400 (Perten Instruments, Sweden). The system test evaluates wheat kernel texture characteristics by measuring the weight and force needed to crush a minimum of 300 individual kernels.

The pasting properties of the wheat samples were analysed using a Rapid Visco Analyser (RVA) (Newport Scientific Pty Ltd, New South Wales, Australia) and a computer program used to integrate the data, Thermocline for Windows version 2 (Newport Scientific Pty Ltd). RVA is used widely in the human food industry as a means of generating physico-chemical data that can differentiate between wheat samples. The intention was to assess whether RVA data would be a useful *in vitro* method of separating wheats on the basis of their nutritional value.

The recommendation for precise data from the RVA is for samples to be sieved before analysis. This was carried out for some experimental work on the RVA and an automatic sieve with a pan and 125µm and 250µm screens (Endecotts Ltd, London, UK) was used. The fraction between 125 and 250µm was used. However, all the test wheat samples were prepared for the RVA by using a single pass though a bench top laboratory mill. The duration of milling per amount of sample was equal, and this method was employed to utilise the potential difference in milling characteristics between the samples.

Milled samples of 3g (dry basis) were mixed with 25g of water immediately before the start of the test (Deffenbaugh and Walker 1989). The RVA was programmed as given in Table 2. The programs were 40 minutes in duration. After ten seconds of stirring at high speed the speed was reduced to a constant speed of 160rpm. The temperature was initially 25°C, but was raised to 95°C before returning to 25°C as indicated in Table 2. The equipment used a paddle to stir the sample and water slurry at the set speed. The torque necessary to maintain this speed was converted to a viscosity in Poise (P) (Ross *et al.* 1987; Deffenbaugh and Walker 1989). The resulting starch pasting profile allowed determination of a peak viscosity (PV) and end viscosity (EV). The test was then repeated using silver nitrate solution (5mM), a known alpha amylase inhibitor, instead of the distilled water as the suspending media (Greenwood and Milne 1968a; Collado and Corke 1999).

All samples were analysed in duplicate, as in the protocol of Becker *et al*. (2001a).

Table 2. RVA program details

Time	Speed (rpm)	Temperature (°C)
0.00.00	960	25
0.00.10	160	25
0.06.00	160	25
0.12.30	160	95
0.19.00	160	95
0.25.00	160	25
0.40.00	160	25

3.3. Results

The results of quality analyses on seed from the field trials of the three populations were analyzed by JIC to validate previous tests and to identify the lines giving greatest differences in grain texture profiles, so as to provide seed multiplication of these lines for more detailed tests of texture differences on animal feed performance.

3.3.1. Beaver x Soissons doubled haploid lines

Over the growing season 2005/06, JIC grew 14 of the Beaver x Soissons lines in 5m x 1m plots in each of three replicate blocks (JIC Experiment D306). These lines were chosen on the basis of the results of the previous project (Defra funded projects CE0370, AR0908) as potentially covering the texture spectrum from soft-softs to hard-hards. These were also selected such that they did not have the 1B/1R translocation and were not double-dwarfs. Three replicate plots of each line were grown and kg quantities of each line harvested. The harvested seed was distributed to partners as follows:

600g of each line, plus Beaver & Soissons, sent to SAC

300g of each line, plus Beaver & Soissons, sent to DANISCO

200g of each line, from each replicate, plus Beaver & Soissons, sent to the University of Nottingham

Both JIC and Nottingham carried out texture measurements on the seed from this harvest, JIC using NIR and Nottingham using SKCS. These results and the mapping codes of the lines chosen are shown in Table 4. There was a close correspondence between the scores from the two laboratories, as shown in Figure 1.

Table 3. Beaver x Soissons DH lines. Summary of textures scores on field grain over years.

		Mean Texture (NIR) measurements			
BS line	**Category**	**2006**	**2007-1**	**2007-2**	**2008**
BS19	SS (1)	11	34	46	10
BS17	SH (2)	22	41	49	23
BS42	HS (3)	49	63	58	37
BS38	HH (4)	60	73	64	52

Table 4. Beaver x Soissons DH lines. Mapping codes and mean endosperm texture characterisation scores for the 2006 harvest seed; comparison of data from JIC and Nottingham.

Mapping Code	**JIC**	**Nott**	**Texture Classification**
BS19	9.1	9.7	SS
BS25	15.4	8.1	SS

BS32	15.8	14.3	
BS21	18.1	16.8	
BS17	25.9	19.9	HS
BS9	50.3	55.8	
BS42	51.1	48	SH
BS14	53.5	56.2	
BS28	54.7	51.3	
BS15	54.9	52	
BS22	55.3	45	
BS12	56.7	47.5	
BS39	57.2	58.3	
BS38	60.2	61.7	HH

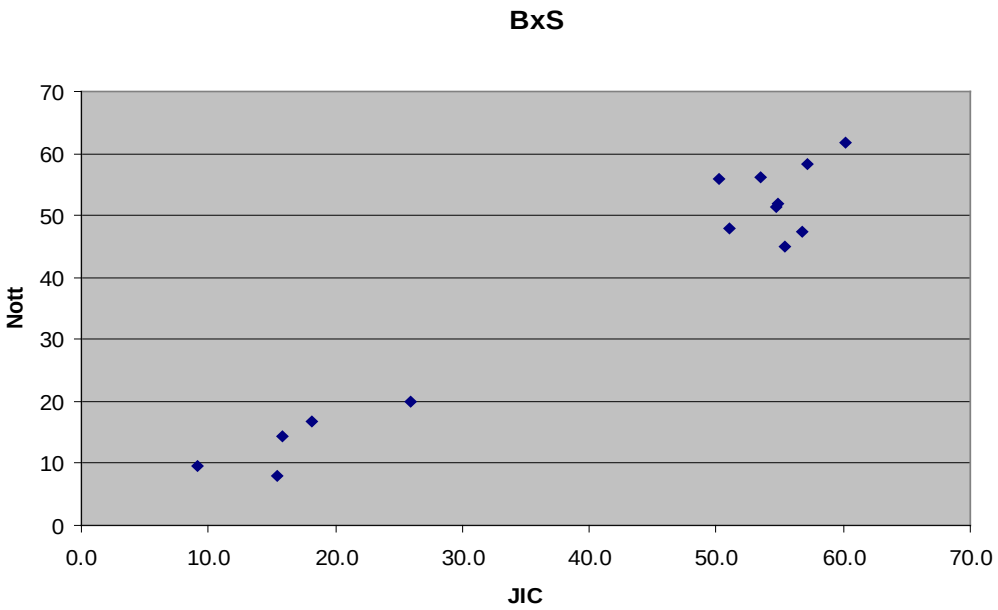

Figure 1. Beaver x Soissons DH lines. Plot of mean JIC v Nottingham endosperm texture measurement scores (mean of 3 reps) for 2006 harvest seed.

From Figure 1, it can be seen that the lines chosen can be clearly categorized into hard and soft groups on the basis of their segregation for the *Ha* gene, but also that there are texture differences within these groups.

A plot was also made for the JIC data of the scores for the first two replicates, Figure 2 and, on this basis, four lines were chosen for future detailed analysis: BS19 (soft-soft), BS17 (hard-soft), BS42 (soft-hard) and BS38 (hard-hard).

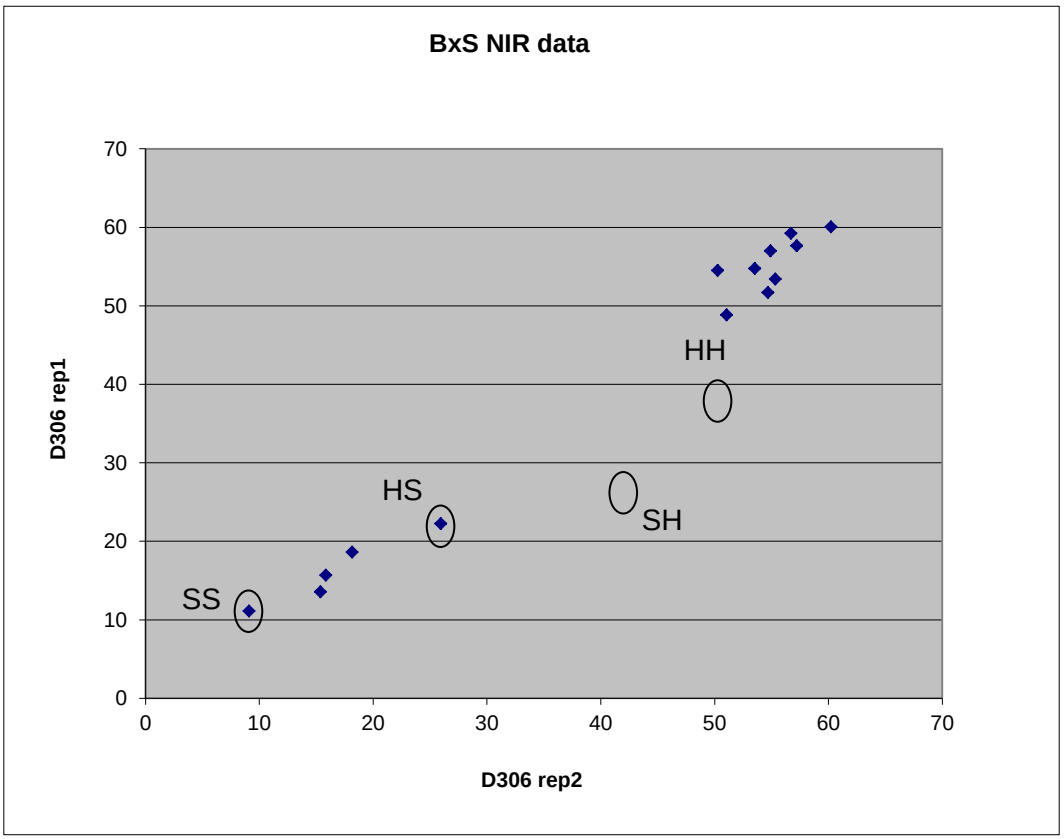

Figure 2. Beaver x Soissons DH lines. Plot of NIR measurements of 2006 harvest seed to identify texture classes.

These lines were re-sown in replicated trials for seed multiplication over the 2006/07 (JIC Experiment D107) and 2007/08 (Experiment D508) seasons and seed for the 2007 harvest distributed to partners for testing for nutritional evaluation. Seed amounts distributed are shown in Table 4. NIR analysis was also carried out on each harvest to check the texture scores. The SKCS analysis for the 2007 harvest was carried out on two occasions, using the same equipment.

The summary of the results over years is shown in Table 3, and plotted in Figure 3 from which it can be seen that there is a significant year effect with 2007 being a ‘hard’ year and 2006 and 2008 ‘soft’ years. However, importantly, the texture differences between the four lines are maintained over all harvests and are stable genetically.

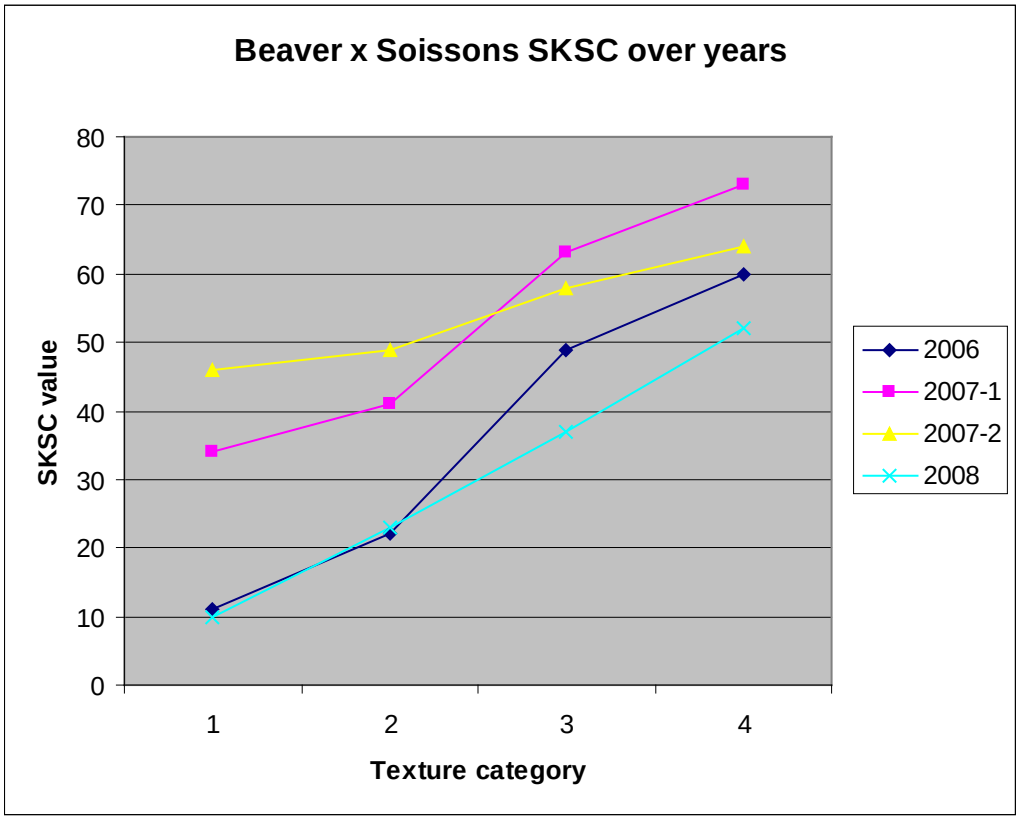

Figure 3. Beaver x Soissons DH lines. Summary of texture scores over years.

3.3.2. Avalon x Hobbit 'sib' recombinant inbred lines

Over the growing season 2005/06 (Experiment H1006), JIC grew 16 of the Avalon x Hobbit 'sib' recombinant inbred lines in 1m x 1m multiplication plots. These lines had been classifed in previous studies as falling into four texture categories, soft-softs, hard-softs, soft-hards and hard-hards. Four replicates of each of the 16 lines were drilled yielding an approximate total of 8kg over the 4 reps at harvest.

This seed was distributed as follows to the project partners:

200g from each replicate of the 16 lines pooled and sent to SAC

200g from each replicate of the 16 lines sent to the University of Nottingham

100g from each replicate of the 16 lines (totalling 400g) sent to DANISCO

JIC also tested the texture of the harvested seed using NIR and the distributions of scores is shown in Figure 4, indicating that they do, in fact, cover the hardness spectrum, as anticipated. However, it can be seen that the difference between soft-hards and hard-hards is not as distinct as between soft-softs and hard-softs. The texture scores by SKCS at Nottingham, on the seed they were sent and analyzed, were compared to the JIC scores, and a very close correspondence observed, as shown in Figure 5.

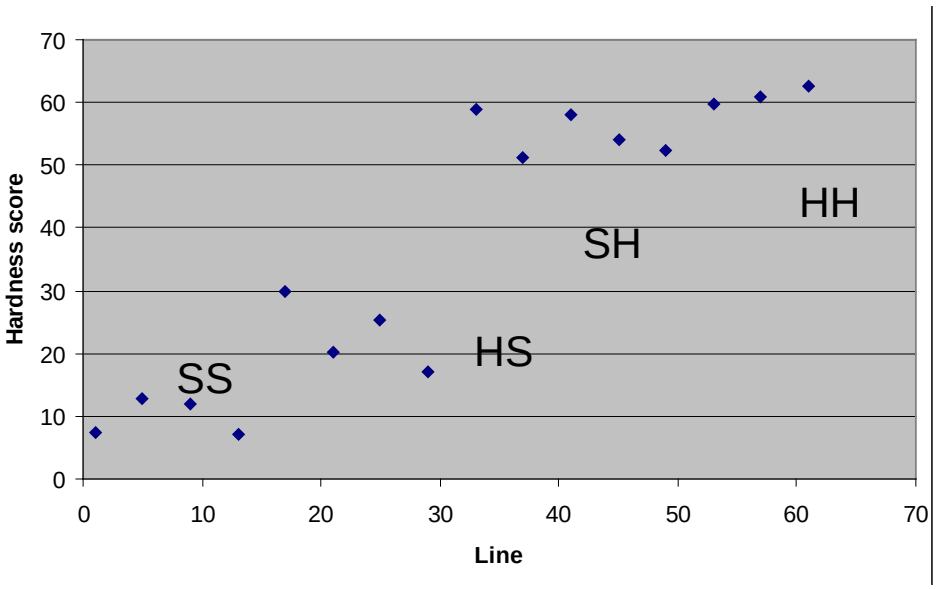

Figure 4. Avalon x Hobbit 'sib' selected RIL lines. Distribution of NIR texture scores (means over four replicates) for the 16 lines of the 2006 harvest.

Residual seed of each individual replicate of the 16 lines (=64 plots) was re-sown in the autumn of 2006 (Experiment D207) into 1m x 5 m plots for further multiplication, testing and seed distribution.

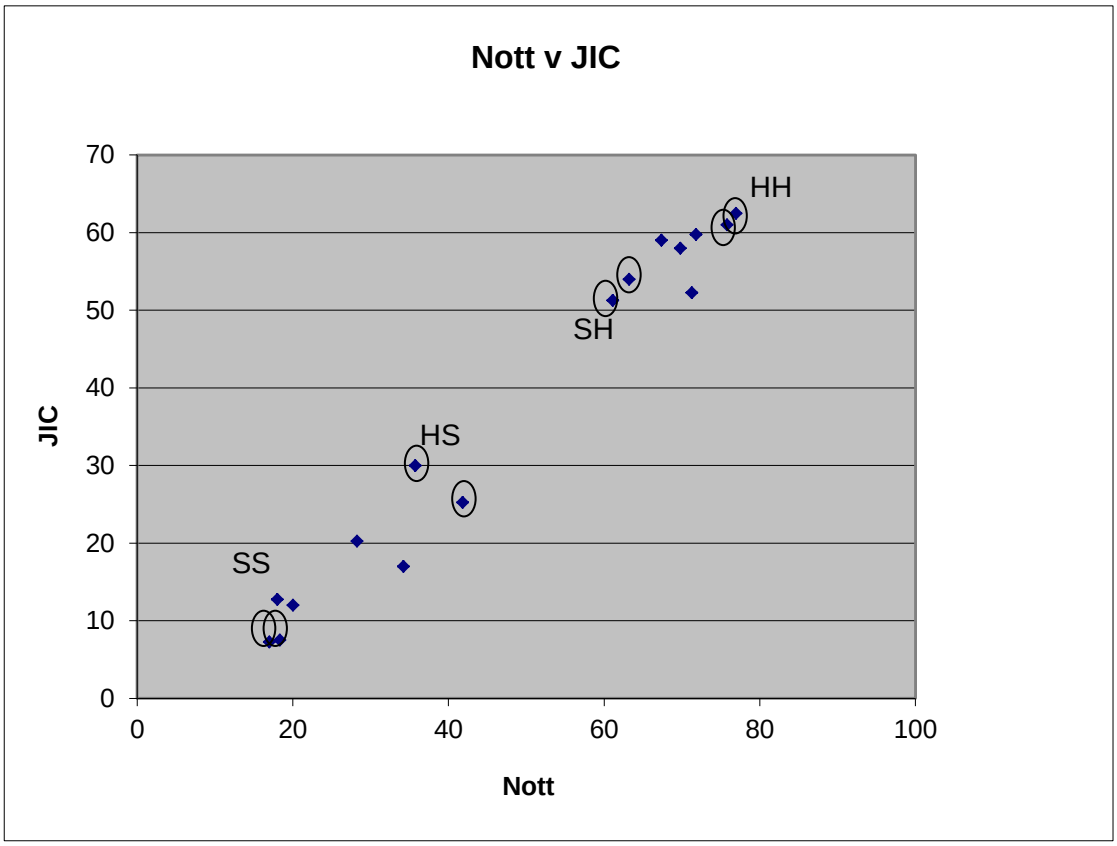

Figure 5. Avalon x Hobbit 'sib' selected RIL lines. Correlation of mean JIC NIR textures scores with mean Nottingham SKSC scores for the 16 lines of the 2006 harvest.

Eight lines of the Avalon x Hobbit ‘sib’ population and four lines of the Beaver x Soissons population from the 2007 harvest (Experiment D207) (circled in Figure 5) were agreed upon for further nutritional evaluation. The seed amounts obtained and distributed from the 2007 harvest are shown in Table 5. These lines were also sown ‘plot to plot’ in 2008 (Experiment D108).

Table 5. Seed harvest amounts and distribution of seed to partners from the 2007 JIC harvest of the selected Beaver x Soissons DH lines and Avalon x Hobbit ‘sib’ RIL lines (Amounts in kg).

Genotype	Classification	Yield	JIC	Nottingham	SAC	Danisco	Nickersons
BS 19	SS	23.825	0.2	10	1		12.125
BS 17	HS	24.901	0.2	10	1	0.5	13.201
BS 42	SH	27.578	0.2	10	1	0.5	15.578
BS 38	HH	25.636	0.2	10	1	0.5	13.936
Hobb/AV RIL 41	SS	17.703	0.4	10	1	0.5	5.803
Hobb/AV RIL 22	SS	21.358	0.4		1	0.5	9.458
Hobb/AV RIL 64	HS	16.726	0.4	10	1	0.5	4.826
Hobb/AV RIL 28	HS	14.684	0.4	10	1	0.5	2.784
Hobb/AV RIL 46	SH	20.947	0.4	10	1	0.5	9.047
Hobb/AV RIL 80	SH	21.617	0.4	10	1	0.5	9.717
Hobb/AV RIL 49	HH	13.895	0.4	10	1	0.5	1.995
Hobb/AV RIL 95	HH	18.937	0.4	10	1	0.5	7.037

The texture of all 64 plots (16 lines x 4 replicate plots) of the 2007 harvest of Avalon x Hobbit ‘sib’ RILs were measured at JIC using NIR, and means for lines calculated. Unfortunately, this revealed a poor correlation and significant anomalies when these data were correlated with the 2006 means and with the expected phenotypes, as shown in Figure 6. Two lines, in particular, RIL 46 and RIL 22, were significantly different from expectations. This suggests that the harvested seed from the 2007 field trial did not correspond to the expected genotypes, giving doubts as to the seed bag designations of all lines. The conclusion drawn was that the field trial had, in some way, been sown in a different order from the designated field plan but it was not possible, initially, to discover how and why this had occurred.

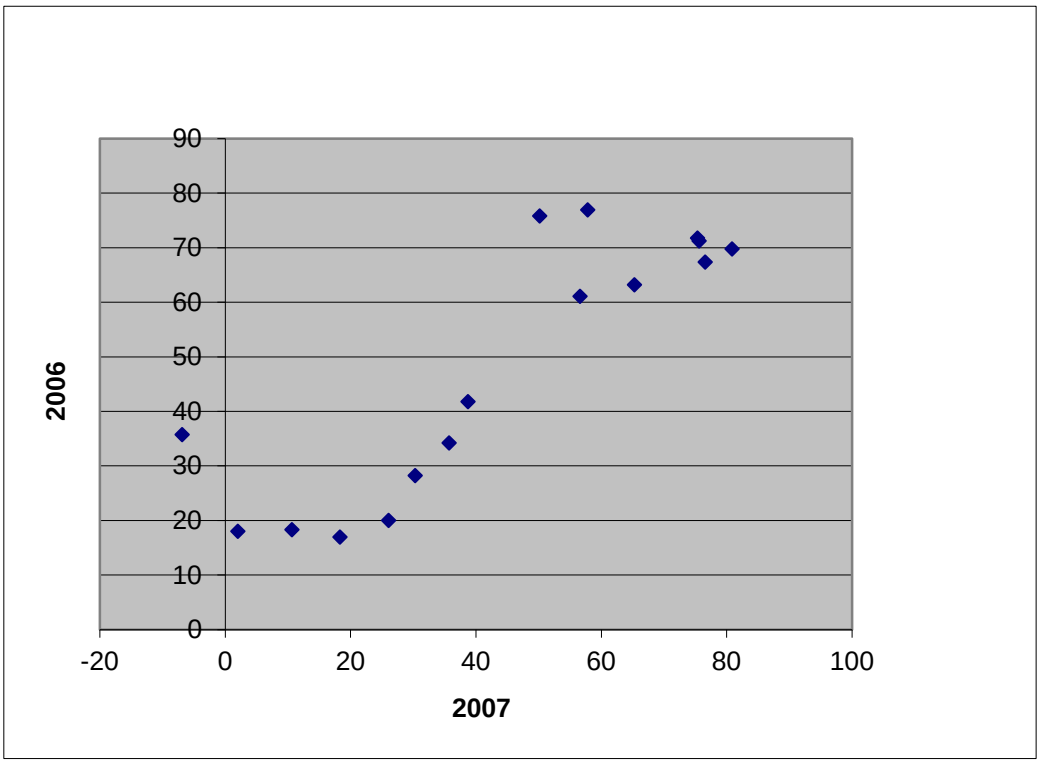

Figure 6. Avalon x Hobbit 'sib' RIL. A comparison of 2006 and 2007 NIR means at JIC.

In an attempt to establish the relationship between the 2006 and 2007 harvests and the correct genotypes of the 2007 seed bags, extensive genotyping of the 2006, 2007 and, later, the 2008 (Experiment D108) harvests was carried out using molecular markers at JIC. Primer sets for several markers were used and six were used on all samples, namely, markers for *Pinb-D1* (5DS), detecting variation at the *Ha* hardness locus, and other, random primers, on different chromosomes over the genome PSP3027 (1A), GWM388 (2B), BARC168 (2D), GWM493 (3BS), GWM285 (3BL). These data were also compared to the original genotyping scores carried out at JIC in a previous HGCA funded project.

An example of the results of this screening for the soft-soft set of lines is shown in Table 6. It can be seen that for the 2006 harvest, there was an identical correspondence between the original, expected, scores and the genotyping of seed from H1006, the 2006 harvest. All the lines had the expected genotypes at the *Ha* (puroindoline) locus, having the Hobbit 'sib' allele conferring softness. This was also true of all other lines apart from RIL 64 which showed a difference for two markers, indicating that the 2006 or original classification was incorrect for this line. There was also a complete correspondence between the 2007 and 2008 harvest genotypes. However, all of the lines apart from the first, RIL 41, differed in genotype between the original 2006 classifications and the 2007 and 2008 harvested seed. This confirms that the mistake occurred in the 2007 field trial.

Table 6. Results of molecular marker screening on the soft-soft samples from harvests 2006 (Experiment H1006), 2007 (Experiment D207), and 2008 (Experiment D108). A = Avalon allele, b = Hobbit 'sib' allele, h = heterozygote.

			Molecular markers					
Genotype	Type	Experiment	PinB-D1b	psp3027	gwm388	barc168	gwm493	gwm285
RIL 41	SS	Original	b	a	b	a	b	b
		2006 harvest	b	a	b	a	b	b
		2007/8 harvests rep 1	b	a	b	a	b	h
RIL 13	SS	Original	b	b	b	a	b	a
		2006 harvest	b	b	b	a	b	a
		2007/8 harvests rep 1	b	a	b	b	a	b
RIL 42	SS	Original	b	b	b	b	b	b
		2006 harvest	b	b	b	b	b	b
		2007/8 harvests rep 1	a	b	a	a	a	a
RIL 22	SS	Original	b	b	b	a	a	a
		2006 harvest	b	b	b	a	a	a
		2007/8 harvests rep 1	a	b	b	a	b	a

The anomalies in Figure 6 can also be explained from the genotyping. RIL 22 is expected to be soft, but the 2007 harvest seed clearly carried the Avalon *Pinb-D1b* (hard) allele, whereas 46 is expected to be hard but carries the *Pinb-D1a* (soft) allele. The genotyping was performed on the individual seed of all 64 plots. However, for seed distribution, seed of the first three replicate plots of each presumed line were pooled. Thus, each bag of the seed distributed and analysed for nutritional parameters at Nottingham from the 2007 harvest is, unfortunately, a mixture of different genotypes. To de-convolute the individual genotypes for each plot in each of the 16 pooled samples, an attempt was made to match the genotypes of the 2006 samples with those of the 2007/2008 samples using the genotyping data. This was fairly successful. For example, Table 7 shows the de-convoluted genotypes based on the marker genotypes for the hard-hard lines RIL 49 and RIL 95.

Table 7. De-convoluted genotypes for plots in 2008 (Experiment D108) Avalon x Hobbit sib field trial, using markers to identify the correct genotype. a= Avalon allele, b = Hobbit 'sib' allele.

DRILLING CODE D108	Supposed genotype D108	Actual genotype	SSR Markers evaluated					
			psp3027	gwm388	barc168	gwm493	gwm285	barc56
15	22	49	a	b	b	a	a	a
31	12	49	a	b	b	a	a	a

47	80	49	a	b	b	a	a	a
63	95	49	a	b	b	a	a	a
16	22	95	b	a	b	b	a	b
32	12	95	b	a	b	b	a	b
48	80	95	b	a	b	b	a	b
64	95	95	b	a	b	b	a	b

This analysis enabled the 64 plots to be identified on marker genotype, within a high degree of accuracy, and hence the composition of the mixtures distributed to partners to be unravelled. This was also checked by correlating the hardness scores of the de-convoluted plots, shown in appendix A. This also presents the means of the four texture groups (SS, HS, SH, HH) and shows that, as with the Beaver x Soissons lines, in all years, the overall genotype classifications are consistent. Figure 7 also shows these data graphically for the correlation between the hardness scores for the 2006 and de-convoluted 2008 data.

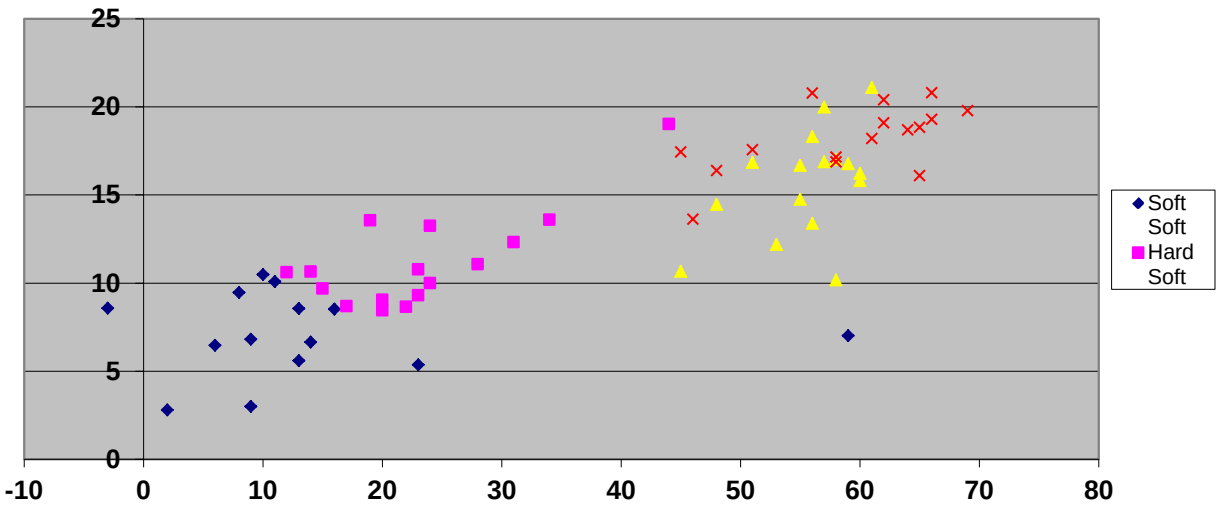

Figure 7. Correlation between hardness scores for de-convoluted genotypes for plots of the Avalon x Hobbit sib RILs in the 2006 (x) and 2008 (y) field trials.

Although the individual plots for the 2007 and 2008 experiments can be de-convoluted in terms of genotype and texture scores, the problem remained that pooled samples sent to collaborators are a mixture of genotypes and not pure genotypes as anticipated. Appendix B provides a list of the pooled samples and the expected texture type of the sample used for nutritional studies.

3.3.3. Avalon x Cadenza DH population

The parents of this population are both hard, but differ in their *Pin* b alleles; Avalon has the genotype *Pina-D1a/ Pinb-D1b*, but Cadenza is *Pina-D1a/ Pinb-D1c*. The 203 lines of the Avalon x

Cadenza DH population were grown in a replicated field trial over the 2005/06 season and grain harvested for testing for texture using NIR, and also for DNA analysis.

The *Pin-D1b* and *Pin-D1c* alleles can be distinguished using a specific PCR test with specific *Pin-D1* marker primers, as shown in Figure 8. The population showed clear segregation for the bands matching the parents, and thus each line could be classified as carrying either the *Pin-D1b* or *Pin-D1c* allele.

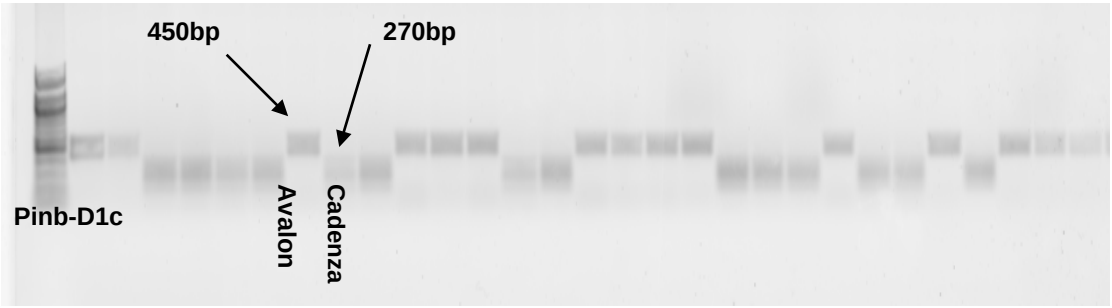

Figure 8. Gel showing differences in band size of the parents and recombinant Avalon x Cadenza *Pin-D1* allele products.

Each line of the population was also tested for texture and the distribution of scores is shown in Figure 9. As expected, all lines could be classified as hard, but variation was evident within this category. These differences in texture may relate to variation between the 'b' and 'c' alleles or to other modifier effects. To test whether the difference between the *Pinb-D1b* and *Pinb-D1c* alleles contributed to texture variation, a simple one-way ANOVA was carried out on difference within and between the 'b' and 'c' groups for hardness scores. Details are given in Appendix C.

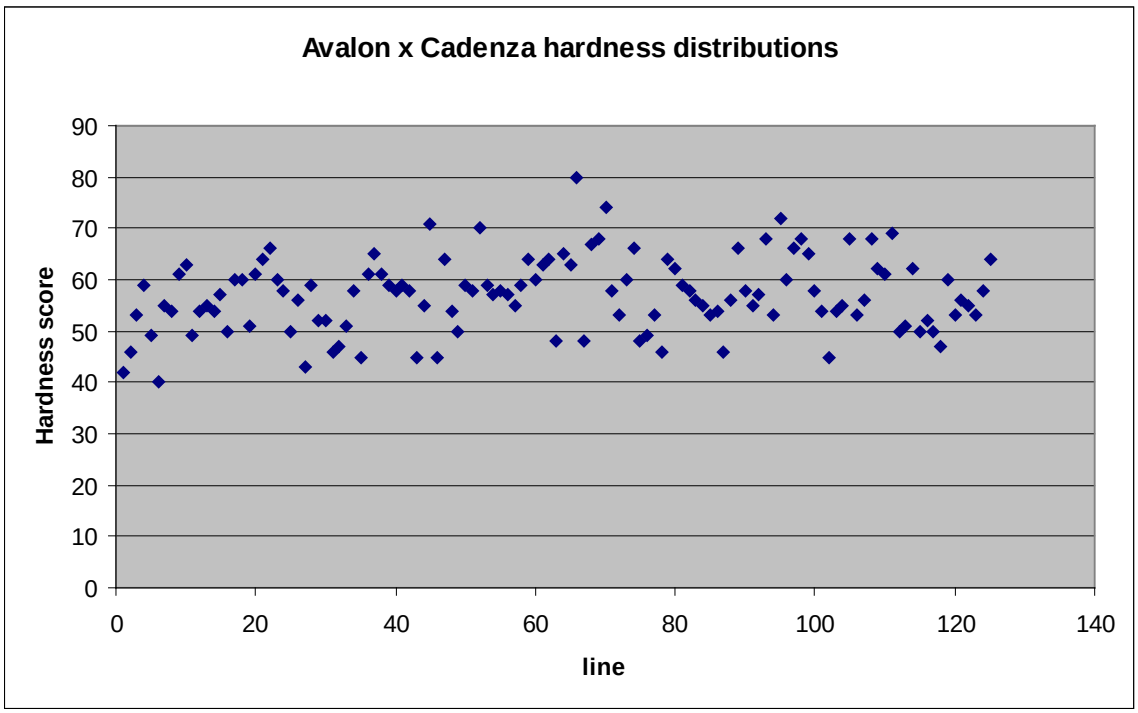

Figure 9. Distribution of hardness scores for the Avalon x Cadenza DH lines, 2006 harvest seed.

Appendix C shows that the 'c' (Cadenza) allele gives a harder texture than the 'b' (Avalon) allele, and this could have knock on effects on nutritional value and, indeed, bread-making quality. However, this difference is only about 4-5 points on the scale used whereas the range is >30 scale points. Thus other modifier genes must also be segregating in this population.

The Avalon x Cadenza population was also grown over the 2006/07 season and the seed that was harvested in 2007 was again characterised using NIR (Figure 10). Significant genetic variation for hardness was again observed in the population and ANOVA again confirmed that the 'c' allele gives a harder endosperm than the 'b' allele. The whole population was also subjected to QTL analysis to identify the gene locations of the modifier genes seen as mediating the additional phenotypic variation. QTL were detected, in particular on chromosomes 4A and 6B, as modifiers of the hardness phenotype.

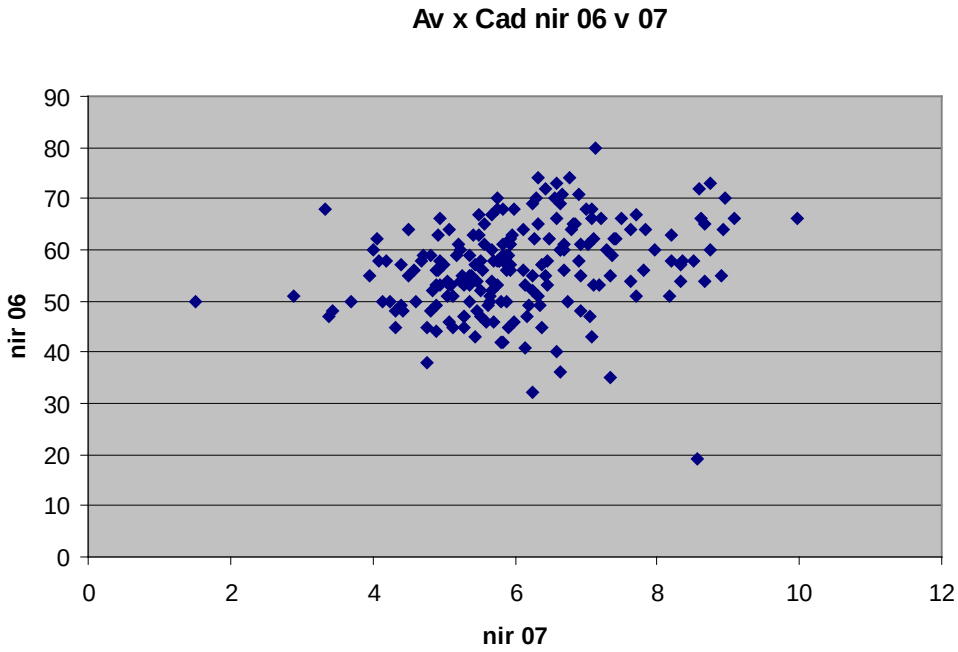

Figure 10. Plot of 2006 and 2007 harvest hardness scores for the Avalon x Cadenza DH lines.

Finally, to confirm that the hardness differences were consistent over seasons, the 2006 and 2007 data were correlated as shown in Figure 10. A significant correlation of r=0.263 was observed (P<0.001). This also indicates a significant year x genotype effect on the hardness scores.

For the 2007/08 season, 60 5m x 1m plots of 20 random genotypes (10 x 'b', 10 x 'c') x 3 replicates were grown to supply seed for future studies on the effect of the 'c' v 'b' allele difference on nutritional quality.

Study A by Danisco had evaluated three samples from the 2005 harvest. Results are presented in Tables 8 and 9. No significant effect (P<0.05) of wheat cultivar and enzyme supplementation was observed for dry matter digestibility or diet apparent metabolisable energy (AME).

Table 8. Effect of cultivar on the nutritional value of 3 wheat samples selected for different levels of endogenous xylanase inhibitors.

Wheat cultivar	**Coefficient of dry matter digestibility**	**AME (MJ/kg, DM)**
Claire	0.6005	14.693
Gladiator	0.6178	15.243
Mascot	0.6206	15.278
P value	*0.50*	*0.12*

Table 9. Effect of xylanase supplementation on the nutritional value of 3 wheat samples selected for different levels of endogenous xylanase inhibitors. Xylanases 1 and 2 are produced by different bacterial strains.

Feed enzyme	**Coefficient of dry matter digestibility**	**AME (MJ/kg, DM)**

None	0.5993	14.955
Xylanase 1	0.6063	14.893
Xylanase 2	0.6243	15.288
P value	*0.41*	*0.35*

Study B had evaluated 24 wheats from 2005 harvest for a number of *in vitro* measurements that may link to biological data. Results are reported in Appendix E. Wheat hardness values ranged from 15 to 85 (SKCS method; AACC Method 53-31, 1989). 12 cultivars exhibited hardness values below 50 (from 15 to 41) and were classified as soft, whereas 12 cultivars exhibited hardness values above 50 (from 52 to 85) and were classified as hard (although endosperm texture is a continuum, not simply hard or soft). Mean particle size of wheat, as well as the ratio between coarse and small particles, was greater ($P<0.05$) for hard than for soft cultivars. There was a positive correlation ($P<0.05$) between SKCS hardness value and mean particle size. These findings are in accordance with previous observations (Abécassis *et al.,* 1997; Hruskova *et al.,* 2004). Despite showing higher ($P<0.05$) soluble pentosan content, there was no significant difference in viscosity between hard and soft cultivars. This could be due to the fact that hard cultivars had higher ($P<0.05$) endogenous xylanase activity. However, the activity of endogenous xylanase inhibitors also tended to be higher with hard cultivars. There was no significant difference in the rate of starch digestion between hardness classes, unlike previous data reported by Pirgozliev *et al.* (2001).

The 24 wheat samples were also used in a broiler nutritional trial carried out at SAC. The nutritional value of the wheat samples was determined using the precision feeding method. A summary of the data can be found in Table 10 and Figure 11a and 11b. When offered to 42 days-old broiler chickens, hard wheat samples tended ($P<0.10$) to show lower dry matter digestibility, and resulted in numerically lower AMEn and TMEn values (average reduction of 0.27 MJ/kg, DM basis) when compared to soft cultivars. The lack of statistical significance may be due to the feeding method (precision feeding or gavage) which did not allow sufficient time for the animal to adapt to the feed. It could also be associated with high variability between birds, as the ability of broilers to digest wheat is strongly influenced by genetics (Mignon-Grasteau *et al*., 2004). The negative effect of grain hardness on wheat nutritional value is supported by previous studies (Carré *et al.,* 2002 & 2005; Péron *et al*., 2006) and can be linked to increased number of coarse particles in the digestive tract and reduced starch digestibility due to enzyme accessibility issues (Péron *et al.* 2005, 2007).

Table 10. Summary of grain nutritional value, 24 wheat samples from the 2005 harvest year.

Grain nutritional value	Unit	Mean	SD	Min.	Max.
Dry Matter digestibility	Coefficient	0.656	0.0108	0.330	0.852
AMEn (DM basis)	MJ/g	12.93	1.52	7.70	17.69

TMEn (DM basis)	MJ/g	15.69	1.35	11.31	20.26

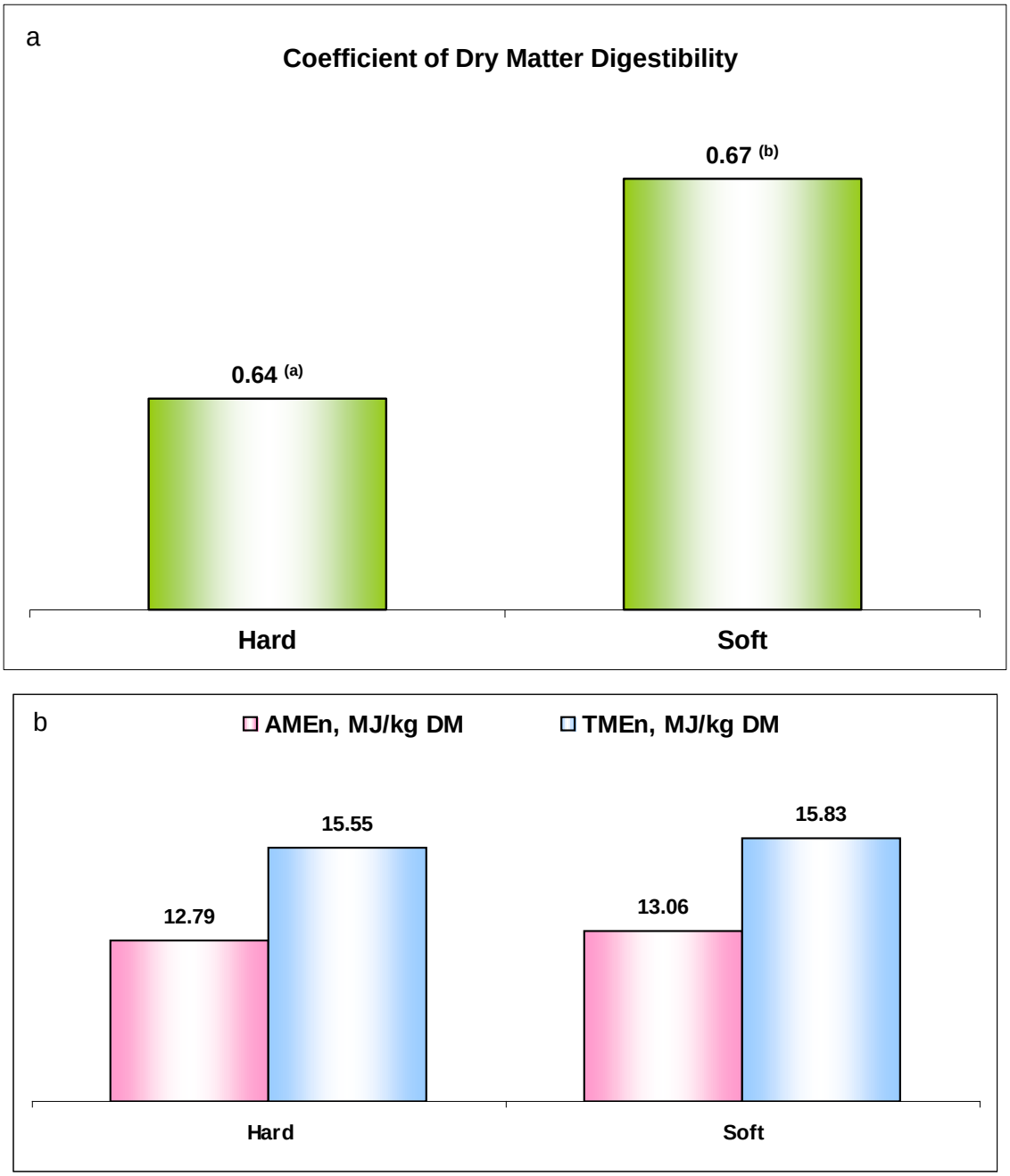

Figure 11a and 11b. Effect of hardness class (Hard vs. Soft) on the DMD (a) and Metabolisable Energy (b) of 24 wheat samples from the 2005 harvest year. (a),(b): $P<0.10$

A total of 78 wheat samples were received at the Danisco laboratory from the 2006 harvest. They were analysed for total and soluble pentosan content. Results are reported in Appendix F. The average total pentosan content was 47.6g/kg, and the average soluble pentosan content was 4.9g/kg (as fed basis). These values were higher than for the previous harvest analysis (30.2g/kg for total pentosan and 3.2 g/kg for soluble pentosan, respectively).

Results for wheats harvested in 2007 are reported in Appendix G. Unlike the 2005 harvest samples, there was a significant positive correlation (R^2=0.82) between soluble pentosan content and viscosity value (Figure 12). The average total pentosan content was 49.0g/kg, and the average soluble pentosan content was 3.8g/kg (as fed basis). This soluble pentosan content was low compared with the previous harvest (4.9g/kg), but relatively similar to the 2005 harvest year (3.2g/kg). However, these differences may be due to the choice and number of samples selected for measurement.

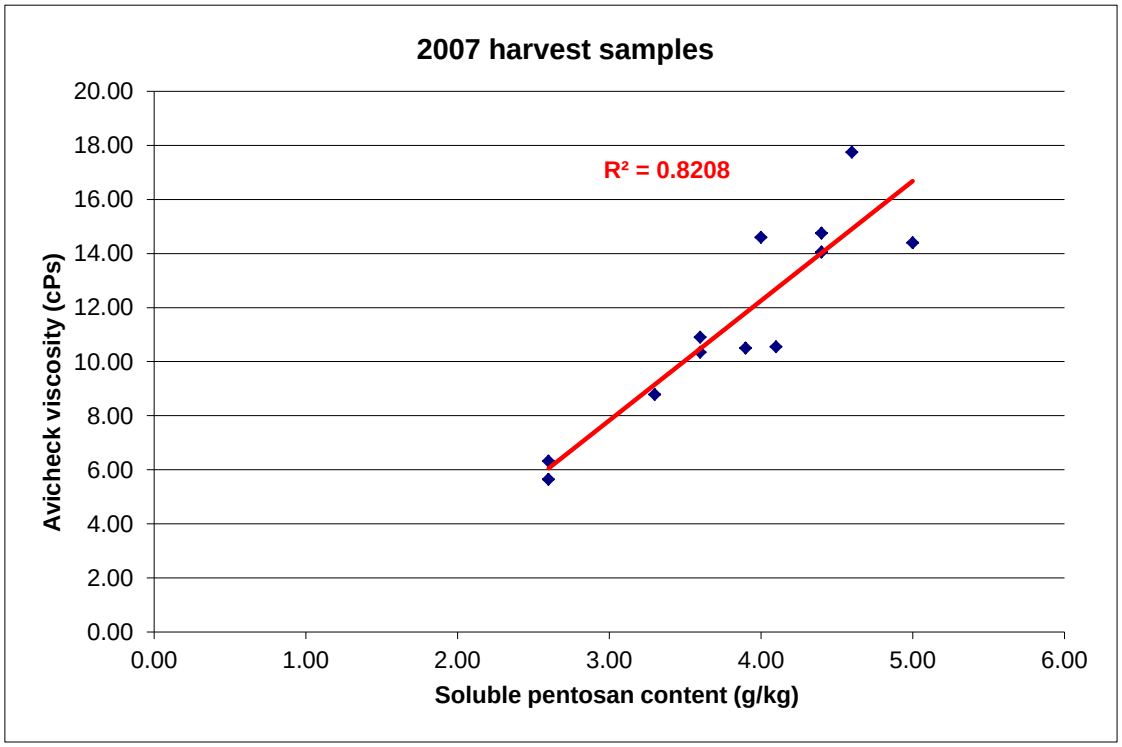

Figure 12. Correlation between soluble pentosan content and Avicheck viscosity value in 12 wheat samples from the 2007 harvest year.

The nutritional value of the ten wheat samples from 2007 harvest, with or without supplementation of a commercial xylanase (provided by Danisco Animal Nutrition) was determined using the precision feeding method. A summary of the data can be found in Appendix H. Results showed that cultivar significantly (P<0.05) influenced metabolisable energy value (AME and TME) of wheat samples. For dry matter digestibility, there was a strong trend for an effect of cultivar (P=0.06). When the wheat samples were divided into 2 hardness classes (Hard = SKCS>50, and Soft = SKCS<50), and the data re-analysed, it appeared that Hard cultivars resulted in greater AMEn, TMEn and dry matter digestibility (P<0.05). This observation was difficult to explain because it went against several previous observations showing that Soft cultivars resulted in higher nutritional value (Carré *et al*., 2002 & 2005; Péron *et al.,* 2006). This unusual finding is likely to be due a bias in the trial design:

- 4 Soft vs. 6 Hard cultivars were tested

- the 4 Soft cultivars were very close to being ranked as Hard, their SKCS values being all between 41 and 48

For both Hard and Soft wheat-based diets, feed supplementation with exogenous xylanase did not affect any of the measured variables (AME, TME and dry matter digestibility). However, previous authors have reported a positive effect of xylanase supplementation on the nutritional value of hard wheat cultivars (Amerah *et al.,* 2008). The lack of enzyme response could be related to the feeding method used in this trial (precision feeding or gavage), with some authors having suggested that it is not an accurate method for assessing the value of feed additives (Cowieson et al., 2006).

The nine wheat samples from the 2008 harvest had Hardness SKCS values ranging from 10 to 68. They were analysed for: viscosity, total and soluble pentosan content, and endogenous xylanase activity. Results are reported in Appendix I. As with the 2007 harvest samples, there was a significant positive correlation (R^2=0.78) between soluble pentosan content and viscosity value (Figure 13). The average total pentosan content was 58.7g/kg, and the average soluble pentosan content was 4.7g.kg. The total pentosan content was higher than for the previous harvest years (30.2g/kg in 2005, 47.6g/kg in 2006 and 49.0g/kg in 2007, as fed basis). The endogenous xylanase activity in 2008 wheat samples was very low, with an average value of 49U/kg (compared to 383 and 86U/kg in 2005 and 2007, respectively).

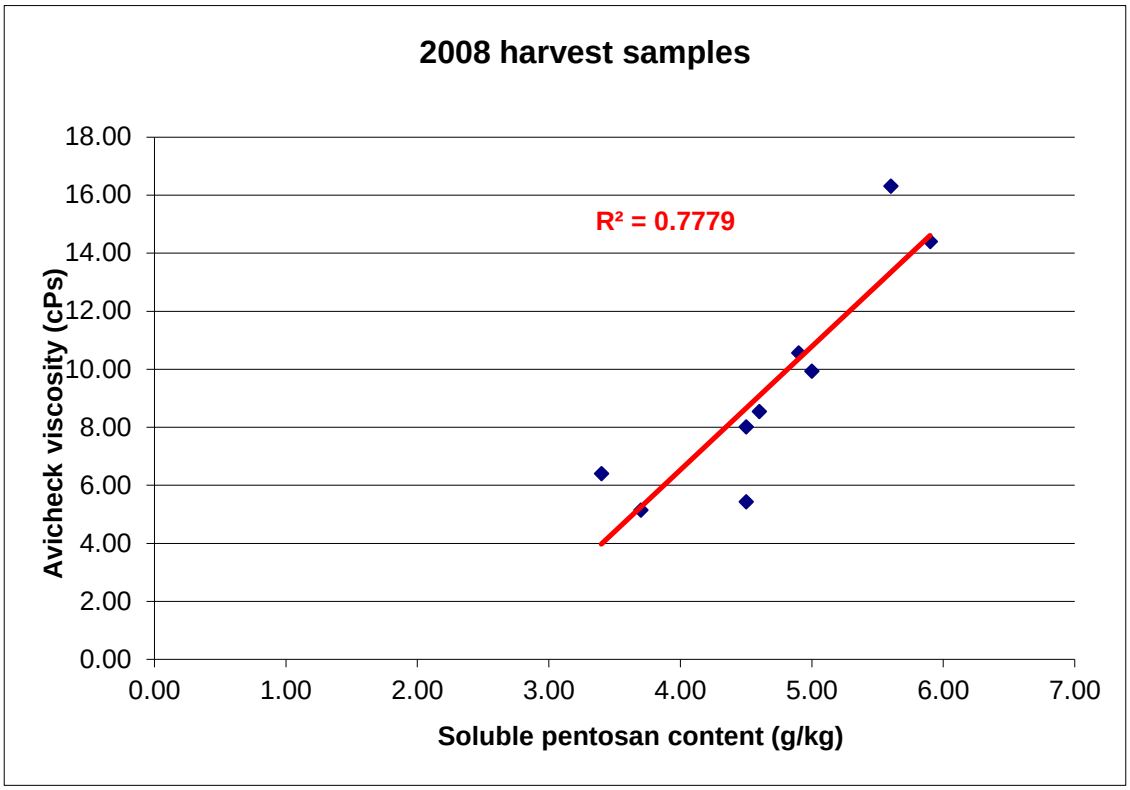

Figure 13. Correlation between soluble pentosan content and Avicheck viscosity value in 9 wheat samples from the 2008 harvest year.

A summary of the biological data with the nine wheat samples from the 2008 harvest can be found in Appendix J. Wheat cultivar significantly ($P<0.05$) influenced bodyweight gain, Feed Conversion Ratio (FCR), N digestibility, litter quality (dry matter content, pH and score) and hock score. Feed supplementation with xylanase activity significantly ($P<0.001$) improved FCR and reduced excreta viscosity. Litter dry matter content and litter score were also improved ($P<0.05$) when the enzyme was added to the wheat-based diets.

When the wheat samples were divided into 2 classes, hard (SKCS>50) and soft (SKCS<50), hardness class was shown to influence overall broiler performance (Table 8). The FCR from 0 to 42 days of age (corrected for mortality) was significantly improved ($P<0.05$) when soft cultivars were offered to the birds. Trial results also indicated that litter pH value was significantly higher ($P<0.05$) with soft cultivars, even if the difference between hardness classes remained limited (8.35 for hard and 8.50 for soft). If the litter is too acidic or too alkaline it can damage the skin. However, this pH level should not have a negative effect. A trend for better nutrient utilization in broilers fed soft cultivars was noted (appendix K): dry matter digestibility and nitrogen retention were greater for birds fed with soft wheat ($P=0.14$ and $P=0.10$ respectively). Surprisingly, the Protein Efficiency Ratio (weight gain / protein intake) was better ($P<0.05$) for birds fed with hard cultivars. This should be interpreted carefully, because the observation was only driven by 1 treatment where values were much higher than in other groups. Wheat viscosity was shown to be positively correlated with excreta viscosity ($P<0.05$). Wheat viscosity has been long known to have a negative effect on growth performance and nutrient utilization. However, in the present study, no significant effect has been observed for performance or digestibility. This could be due to the limited range of variation in viscosity among the selected cultivars, as well as a limited number of replicates per experimental treatment. Nevertheless, some trends were noted: viscosity appeared to have a negative influence on dry matter digestibility and FCR value (data not shown).

Finally, further statistical analyses revealed that hardness class (hard vs. soft) could influence the response to xylanase supplementation in wheat-based diets (appendix L). The benefits of xylanase addition (e.g. improved FCR, greater litter quality and increased nutrient utilisation) were more pronounced in hard than soft wheat diets. A similar observation was made by Amerah et al. (2008), showing greater response to xylanase when hard wheat cultivars were used instead of soft wheat cultivars.

The RIL (Hobbit x Avalon) crosses have a better protein quality (defined as a better amino acid profile) when compared to the BS (Beaver x Soisson) crosses (Appendix M). In particular RIL 95/85 looks particularly good, whilst BS 17 looks particularly bad. However, it is also apparent that the BS crosses have a higher protein (nitrogen) content and the absolute levels of the amino acids are fairly similar. It may well be, therefore, that some of the measured nitrogen in the BS crosses

may be non-protein nitrogen and this is distorting the picture. If the amount of nitrogen that is unaccounted for by the amino acids (corrected for Tryptophan as we can't analyse for that amino acid) is examined then the BS crosses have approximately twice the level of the RIL crosses.

These CP values were then corrected for digestibility and retention coefficients (data from Nottingham). For example, multiplying the CP concentration by the coefficient of ileal apparent digestibility (CIAD) and coefficient of apparent nitrogen retention (CAR and CARu; corrected for uric acid) will allow the wheats to be compared on the basis of their CP content but also how available that CP is to the bird. This is termed Concentration of CIAD (nitrogen, CCIAD), Concentration of apparent nitrogen retention (CCAR) and Concentration of nitrogen retention corrected for uric acid excretion (CCARu).

These data are shown in Table 11. With respect to BS samples, the wheats with the highest corrected CP contents were the harder wheats. For example, wheat with the highest hardness (52) had the highest CP, CCAID and CCAR. Likewise, wheat with the lower two hardness classifications had the lower concentrations. Making the corrections also decreased standard deviation between the wheats. The relationship was not so clear with RIL line wheats. Wheat with a hardness of 58 had the highest CCAR and CCARu and wheat with a hardness of 59 had the lowest CP, CCAID and CCARu. Wheat with hardness of 32 had the highest CCIAD but the lowest CCAR. However, standard deviation was very low for the CCAR values.

Table 11. Effects of hardness on Concentration of CIAD (CCIAD), concentration of retained nitrogen (CCAR) and concentration of retained nitrogen corrected for uric acid (CCARu) in 2008.

Hardness	CP (g/kg)	CCIAD (g/kg)	CCAR (g/kg)	CCARu (g/kg)
		RIL		
18	119	99	42	73
32	125	100	41	71
32	126	106	44	79
48	129	106	44	77
58	134	104	46	86
59	113	93	44	71
67	134	105	44	82
68	125	103	42	76
Standard Deviation	7.3	4.4	1.7	5.5
		BS		
10	13	107	39.8	74.4

23	128	95	40.1	75.0
37	138	117	46.9	91.4
52	139	121	50.0	88.1
Standard Deviation	5.3	11.8	5.1	8.8

Further studies at SAC determined wheat apparent metabolisable energy (AME), AME corrected for N retention (AMEn), true metabolisable energy (TME), TME corrected for N retention (TMEn), gross energy metabolisability (ME:GE) coefficients, DMD and N retention; data are presented in Appendix N. The results were in the expected range for UK wheat obtained with precision fed broiler chickens. There was no significant difference ($P>0.05$) between the results for metabolisable energy, gross energy metabolisability coefficients or N retention. The only significant difference ($P<0.047$) was in terms of DMD, as the difference between the lowest and the highest DMD values was about 0.120 absolute (appendix O). There was a positive relationship between the DMD of wheat samples and their AME, AMEn, TME and TMEn and also the GE metabolisability coefficients (Appendix P). In terms of determined metabolisable energy, the relationship between wheat AME and DMD was most pronounced compared to all other ways for determination of dietary metabolisable energy ($r=0.746$; $P<0.001$). Regression analysis indicated that the DMD and also the N retention were the variables that significantly related ($P<0.001$) to the AME of the wheat samples, $r^2=0.91$ and $r^2=0.40$, respectively (Appendix Q). The regression equations showed that an improvement of DMD of 0.1 would increase the AME by approximately 1.5 MJ/kg DM, although an improvement of N retention of 0.1 would increase the AME by approximately 0.15 MJ/kg DM. However, an improvement of N retention of 0.1 would increase the DMD by approximately 0.009.

The endosperm hardness (EH) of the 55 wheat samples was also determined using SKCS and varied between 9 and 85. There was no relationship between EH and any of the digestibility parameters (Appendix P). Pirgozliev *et al.* (2003) observed a positive relationship between wheat EH and Hagberg Falling Number and bird growth performance. They did not find a relationship between the EH and any of the other measurements of the wheat, such as CP digestibility.

Nottingham had evaluated the nutritional value of wheat in two experiments

3.3.4. Beaver x Soisson (BS) doubled Haploid lines

The Beaver x Soisson lines were pure in 2007 and 2008, therefore all data from both years were analysed in a general ANOVA. The design was a two-factor split plot ANOVA using cage as the plot and hardness and gut region (i.e. ileal digestibility or total tract digestibility/retention) as the factors. This means that hardness was analysed as a factor, but only WITHIN the years and it was

compared to the between cage variation. The region x hardness interaction was compared to within cage variation. The effects on starch digestibility and nitrogen digestibility and retention are shown in Tables 12a and 12b respectively. The difference in Coefficient of Apparent Digestibility (CAD, of starch) between gut regions (ileal CIAD and total tract CTTAD) depends on the hardness of the wheat and year of harvest; two of the wheats (SKCS 34 and 73) had a bigger increase in CTTAD than the others, in 2007. The variation between CIAD (nitrogen) and nitrogen retention depends on the year of harvest; CIAD was higher in 2007 but retention was lower. There were no significant effects of hardness and year on uric-acid corrected nitrogen retention, shown in Table 12c.

Table 12a. Significant effects of hardness and year on ileal and total tract starch digestibility of Beaver x Soissons lines.

		Region		ANOVA		
Year	Hardness	Ileal	Total Tract	Factor	P	sed
2007	34.3	0.796	0.971	Year x Hardness x	0.033	0.0494
	40.7	0.921	0.970	Region		
	63.2	0.840	0.921	Year x Region	<0.001	0.0247
	72.6	0.804	0.913	Region	<0.001	0.0096
2008	10.0	0.942	0.967	Hardness(.year)	0.401	0.0456
	23.0	0.966	0.939			
	37.0	0.923	0.971			
	52.0	0.877	0.912			
	Mean	0.884	0.945			
2007		0.840	0.944			
2008		0.927	0.947			

Table 12b. Significant effects of hardness and year on nitrogen digestibility and retention of the Beaver x Soissons lines.

	Region		ANOVA		
Year	Ileal	Retention	Factor	P	sed
2007	0.781	0.473	Year x Region	<0.001	0.0207
2008	0.816	0.328	Region	<0.001	0.0129
			Hardness (.year)	0.080	0.0323
Mean	0.799	0.401			

Table 12c. Effects of hardness and year on nitrogen retention (corrected for uric acid excretion) of the Beaver x Soissons lines.

			ANOVA		
Year	Hardness	Retention	Factor	P	sed
2007	34.3	0.678	Year	0.171	0.0120
	40.7	0.631	Year.Hardness	0.143	0.0397
	63.2	0.613			
	72.6	0.634			
2008	10.0	0.566			
	23.0	0.584			
	37.0	0.662			
	52.0	0.632			
2007		0.639			
2008		0.611			

3.3.5. Hobbit x Avalon (RIL) 'sib' recombinant inbred lines

The design was a two-factor split plot ANOVA using cage as the plot and hardness and gut region (i.e. ileal digestibility or total tract digestibility/retention) as the factors. There were no significant effects of hardness on starch or nitrogen digestibility, nitrogen retention or nitrogen retention corrected for uric acid excretion. The data are shown in Tables 13a, b and c, respectively.

Table 13a. Effects of hardness and year on ileal and total tract starch digestibility of the Hobbit x Avalon (RIL) lines.

2007					
Hardness	Region		ANOVA		
	Ileal	Total Tract	Factor	P	sed
39	0.788	0.862	Hardness	0.576	0.0861
42	0.805	0.860	Region	<0.001	0.0174
51	0.886	0.913	Hardness x region	0.529	0.0929
75	0.690	0.856			
76	0.896	0.969			
80	0.809	0.837			
81	0.766	0.874			
86	0.857	0.963			
Mean	0.812	0.892			

2008			
Hardness	Region		ANOVA

	Ileal	Total Tract	Factor	P	sed
18	0.925	0.960	Hardness	0.877	0.0987
32	0.795	0.841	Region	<0.001	0.0093
32	0.904	0.925	Hardness x region	0.833	0.1004
48	0.949	0.962			
58	0.854	0.905			
59	0.861	0.899			
67	0.860	0.891			
68	0.816	0.888			
	0.871	0.909			

Table 13b. Effects of hardness and year on nitrogen digestibility and retention of the Hobbit x Avalon (RIL) lines.

	2007				
Hardness	Region		ANOVA		
	Digestibility	Retention	Factor	P	sed
39	0.807	0.730	Hardness	0.430	0.0290
42	0.825	0.496	Region	<0.001	0.0113
51	0.818	0.498	Hardness x region	0.468	0.0368
75	0.785	0.476			
76	0.755	0.468			
80	0.802	0.526			
81	0.828	0.490			
86	0.797	0.493			
Mean	0.802	0.485			

	2008				
Hardness	Region		ANOVA		
	Digestibility	Retention	Factor	P	sed
18	0.833	0.350	Hardness	0.645	0.0322
32	0.796	0.324	Region	<0.001	0.0110
32	0.481	0.348	Hardness x region	0.756	0.0390
48	0.816	0.343			
58	0.777	0.342			
59	0.826	0.392			
67	0.782	0.325			
68	0.830	0.336			
	0.813	0.345			

Table 13c. Effects of hardness and year on nitrogen retention (corrected for uric acid excretion) of the Hobbit x Avalon (RIL) lines.

2007		ANOVA		
Hardness	Retention	Factor	P	sed
39	0.623	Hardness	0.239	0.0371
42	0.660			
51	0.645			
75	0.662			
76	0.620			
80	0.713			
81	0.679			
86	0.675			
Mean	0.660			

2008		ANOVA		
Hardness	Retention	Factor	P	sed
18	0.615	Hardness	0.336	0.0308
32	0.565			
32	0.625			
48	0.599			
58	0.646			
59	0.627			
67	0.610			
68	0.610			
Mean	0.612			

Nottingham also conducted physico-chemical assessments of wheat using the rapid visco analyser

A standard starch pasting profile, illustrating the parameters of interest, is shown in Figure 14. The peak viscosity (PV) indicates the pasting potential of the wheat starch. The PV with silver nitrate, which excludes the activity of amylase, best describes the pasting ability of the starch. The samples were run in water and silver nitrate solution, as outlined above. The difference between the peak viscosity for the experiment with (PV2) and without (PV1) this alpha amylase inhibitor can be used to calculate the *relative* amylase activity, using the following calculation (Collado and Corke, 1999).

Relative amylase Level = (PV2-PV1)/PV1

By extension, this calculation has also been conducted using End Viscosity (EV) values.

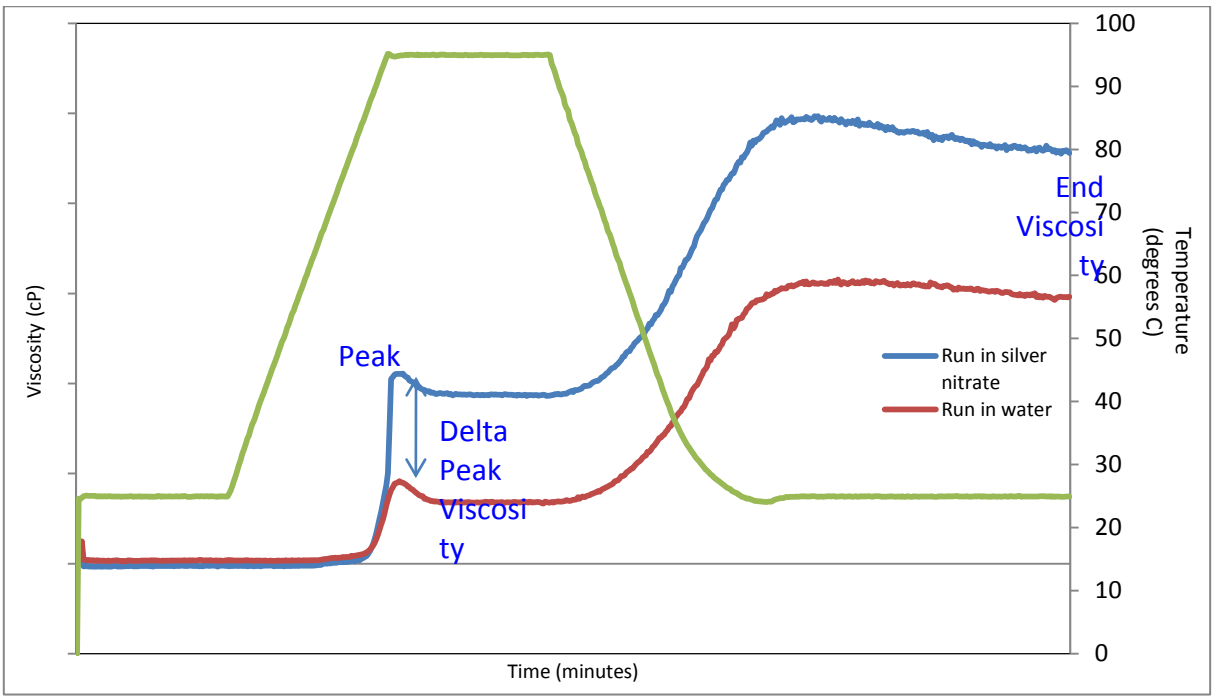

Figure 14. Typical starch pasting profile, showing parameters of interest.

The pasting viscosities (using silver nitrate to exclude any potential amylase activity) of the BS lines and the RIL lines are shown in figures 15a and 15b respectively. The relative amylase levels, using the calculation of Collado and Corke (1999) are shown in tables 14a and 14b.

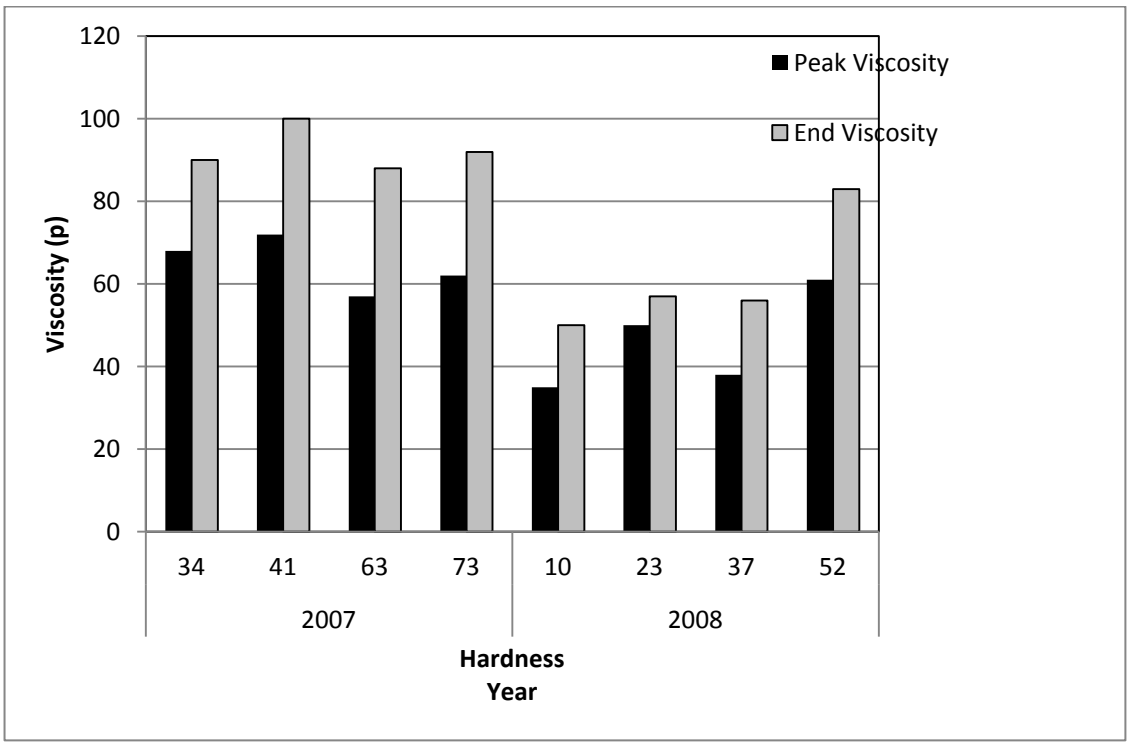

Figure 15a. Pasting viscosities of BS line wheats. Viscosity values given in poise (p).

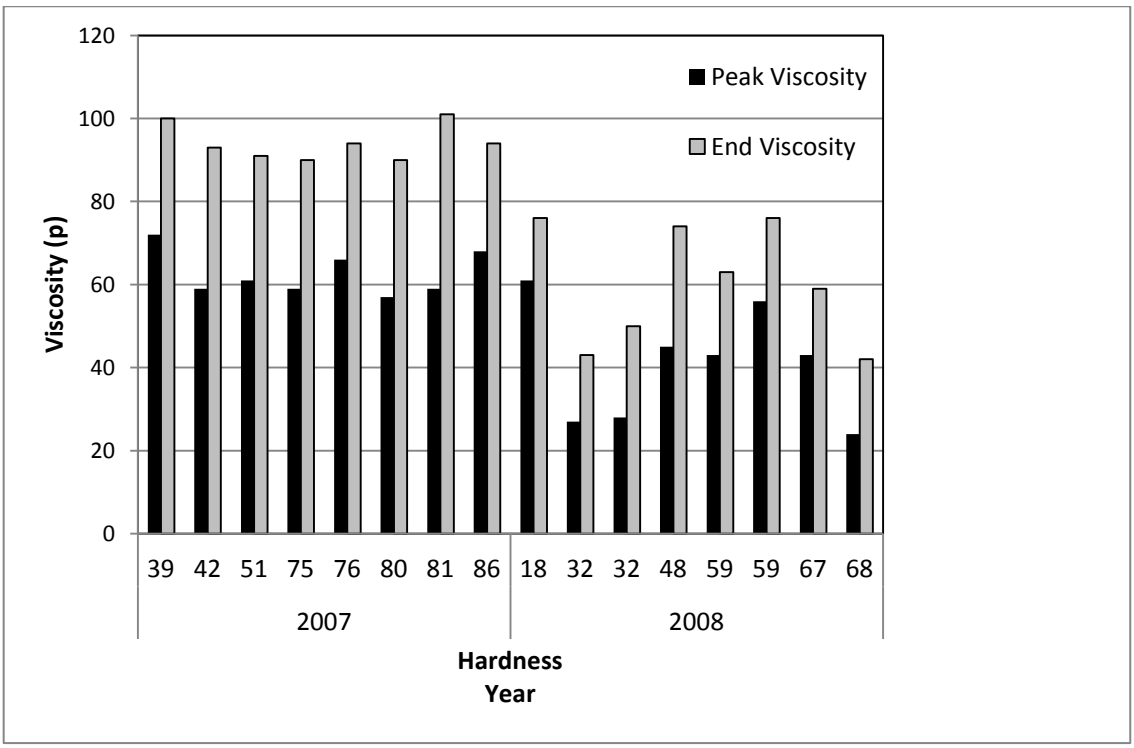

Figure 15b. Pasting viscosities of RIL line wheats. Viscosity values given in poise (p).

Table 14a. Relative amylase level of BS line wheats, estimated as suggested by Collado and Corke (1999).

Hardness	Amylase estimation (PV)	Amylase estimation (EV)
	2007	
34	3.2	1.7
41	0.4	0.2
63	1.8	0.8
73	2.1	0.8
	2008	
10	2.9	3.8
23	1.9	1.0
37	4.3	3.5
52	14.6	236.7

Table 14b. Relative amylase level of RIL line wheats, estimated as suggested by Collado and Corke (1999).

Hardness	Amylase estimation (PV)	Amylase estimation (EV)
	2007	
39	1.8	0.5
42	3.7	1.6
51	1.9	0.8
75	1.1	0.7
76	2.1	0.7
80	1.7	0.9
81	2.7	1.2

86	1.4	0.5
	2008	
18	10.5	7.4
32	3.9	2.7
32	0.2	0.4
48	28.4	99.1
59	21.2	254.5
59	7.8	45.6
67	1.4	0.8
68	2.8	2.7

There appears to be no relationship between hardness and pasting potential (figures 15a and 15b). Viscosities appeared to be higher and more consistent in 2007 than 2008. This could suggest that there was less amylase damage in 2007. The 2007 samples were mixture of wheats with different genetic backgrounds. This could suggest that the decreased pasting potential in 2008 was a factor of the environment in that growing season, rather than genetic background because the pasting was higher in 2007 for all wheats despite being mixtures. This is supported by increased amylase estimates in 2008 (tables 12a and 12b), which could be a result of environmental differences. However, the difference in pasting potential between 2007 and 2008 could also be interpreted as any genetic anomaly that was causing decreased pasting in 2008 was more spread out across samples in 2007.

Linear regression analysis was performed to determine any relationship between the Rapid Visco Analyser (RVA) and chick bioassay parameter data. It is hypothesised that the RVA could be used as a predictor of the nutritional quality of wheat for poultry. The results of this analysis for all data from 2007 and 2008 are shown in Appendix R. Significant values of P for regression analysis are highlighted in bold. The correlation coefficient, R^2, is also given to indicate the strength of the relationship between the two parameters. The interpretation is that, when P is equal to or less than 0.05, the slope of the line is significantly different from zero. Therefore, there is some relationship between *x*, the RVA parameter and *y*, the chick parameter. It is evident from this analysis that certain parameters that can be measured using the RVA have a relationship with certain indicators of nutritional value for poultry.

None of the RVA parameters measured showed any significant relationship with chick parameters related to starch digestibility, despite the starch gelatinization causing the greatest changes in the measured RVA viscosity profile. Coefficient of apparent nitrogen retention (CAR) had significant positive relationships with PV in silver nitrate (R^2=0.5351; P <0.001); PV in water (R^2=0.2703; P= 0.009); EV in water (R^2=0.6432; P= <0.001) and a trend towards a relationship with EV in silver (R^2=0.1271; P= 0.087). This suggests that these RVA measurements may be able to predict

nitrogen retention; as PV in silver increases, for example, so does nitrogen retention. Similarly, nitrogen retention corrected for uric acid excretion (CARu) is positively related to PV in silver (R^2=0.2261; P= 0.019) and EV in water (R^2=0.3518; P=0.002). Coefficient of ileal apparent digestibility of nitrogen is positively related to EV in silver nitrate (R^2=0.3555; P= 0.002).

The final element of the programme involved large-scale commercial studies. Two commercial studies were designed in conjunction with SAC, Bernard Mathews and Grampian Foods to assess the variability in CIAD of nitrogen between flocks of birds fed diets containing high levels of wheat. The first study involved turkeys in a 2x2 factorial design, with location of flock and date being the factors. Five replicate samples of ileal digesta (replicate being one bird) were taken in each case. The second study involved broilers with location of flock being the only factor. Five replicate samples of ileal digesta were taken in from each location. Methods for determining CIAD of nitrogen are described earlier.

Diet composition was commercially sensitive and, accordingly, has not been presented. However, the results have been transmitted back to the companies.

The results are shown in tables 15 and 16. The only significant effect seen in the above studies was the effect of location on CIAD of nitrogen in turkeys. The flock identified as B had significantly reduced CIAD compared to location A.

Table 15. Analysis of variance of variability in CIAD of nitrogen between flocks of turkeys.

	Location		ANOVA		
Date	A	B	Factor	P	sed
1	0.490	0.402	Date	0.598	0.0975
2	0.702	0.296	Location	0.028	0.0975
			Date x location	0.131	0.1379

Table 16. Analysis of variance of variability in CIAD of nitrogen between flocks of broilers.

	Flock		ANOVA		
	1	0.748	Factor	P	sed
	2	0.742	Flock	0.137	0.1054
	3	0.505	Age (.location)	0.100	0.1054
	4	0.613			
	5	0.602			
	6	0.700			

3.4. Discussion

These results of the wheat breeding programme at JIC indicate that there is a great deal of genetic variation for texture differences in wheat within and between different crosses. This, therefore, should reflect differences in feed quality if texture is a major influence on digestibility, and therefore provide new avenues for manipulating feed quality in wheat. Thus, two clear messages come from these studies concerning grain texture in wheat. First, there is considerably more genetic variation in texture than that due only to the *Ha* gene. Secondly, seasonal and environmental effects on texture can be quite considerable as a source of variation for both major texture groups.

With respect to modifiers of grain texture, these results show that plant breeders have considerable leeway in adjusting texture within the major hardness groups determined by *Ha*. QTL controlling these differences have been mapped and could be manipulated by marker-assisted selection, although simple phenotypic selection using NIR would generally be sufficient. The identification of extreme lines in each of the crosses in the current study, as suggested in previous studies, was confirmed and extended in all experiments, so that the materials provide a good test bed for looking at the relationship between texture and nutritional aspects when fed to animals. The finding that the *Pinb-D1b* and *Pinb-D1c* alleles differ in texture is a new finding and will open up possibilities of studying how to modify bread-making quality via texture differences as well as their effect on nutritional characteristics.

Each of the materials was grown in field trials over three years allowing the effect of the environment on texture to be determined on precisely defined genetic material. In each cross there was a highly significant year effect with 2007 being a ‘hard’ year and 2006 and 2008 ‘soft’ years. These differences are as large as the genetics effects such that, in 2006 for the Beaver x Soissons lines, the HS lines in 2007 were harder than the SH lines in 2008. Furthermore, the environment attenuated the differences within and between the groups. Thus, there is a bigger difference between hard and soft in ‘soft’ years, and less variation expressed in ‘hard’ years, particularly in the hard category. Thus year against year comparisons in the nutritional trials are critically important to evaluate the relative influence of genetics and environment of producing the most suitable wheat for animal feed based on texture differences.

Overall, the data collected by Danisco during this project appear to confirm the negative effect of grain hardness on broiler performance and nutrient utilisation, previously reported in the scientific literature. Unfortunately, most of the hardness-related effects observed in the different *in vivo* studies that were carried out were weak, or not significant. This was most likely due to the limited number of replicates per dietary treatment, as well as the feeding method used by the trial site (precision feeding or gavage).

Physico-chemical analyses of wheat grains suggested that greater amounts of coarse particles in hard cultivars could be responsible for nutrient entrapment, leading to reduced accessibility for digestive secretions and feed enzymes. Lab results also seemed to confirm the existence of a positive correlation between wheat viscosity (measured by Avicheck™ method, Danisco Animal Nutrition) and soluble pentosan content in the grain.

In most cases, feed supplementation with exogenous xylanase (provided by Danisco Animal Nutrition) resulted in improved nutrient utilisation (AME, TME, digestibility coefficients) and better growth performance (FCR). However, it is interesting to note that the benefits provided by the enzyme were more pronounced with hard than with soft wheat cultivars. This may be linked to accessibility issues. Thus, because hard cultivars produce more big particles after grinding, they have been associated with lower nutrient (especially starch) utilization in the digestive tract of the animal ultimately leading to reduced performance.

As previously reported (Pirgozliev *et al.,* 2003), the main ingredients that represent the dry matter of UK wheat are starch (about 700g/kg DM wheat), non-starch polysaccharides (about 110g/kg DM) and crude protein (about 130g/kg DM). Starch is the main source of energy in wheat, although the protein would support the growth of the young birds, so it is to be expected that their improved digestibility would have a close relationship to wheat AME. McCracken and Quintin (2000) and Pirgozliev *et al.* (2003) also found a positive relationship between the AME and the starch content of wheat that supports the results from the current experiment.

It can be concluded from the current study that there was a positive relationship between the AME and DMD and N retention in wheat when precision fed to broiler chickens. The information is of particular importance to plant breeders who may be able to incorporate improved nutrients/dry matter digestibility traits in their development of new feed wheat cultivars.

The AME of wheat is often used as the main criteria to evaluate the feeding quality. The relationship between the AME and the DMD in those wheat samples from 2005 suggests that the DMD values can only be used as a relatively good estimate of the feeding quality of wheat. Determining DMD is easier and less expensive and time consuming compared to the AME and N digestibility/retention.

3.5. References

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Appendix A: Hardness scores for de-convoluted genotypes for plots of the Avalon x Hobbit sib RILs in the 2006, 2007 and field trials (JIC data).

Original 1-64 Code	True RIL NUMBER	Hardness Classification	H1006 Hardness	Deconvoluted D207 Hardness	Deconvoluted D108 Hardness	D108 field Code
1	41	SS	9	-0.6	3.00	1
2	41	SS	*	1.1	8.75	17
3	41	SS	6	2.8	6.47	33
4	41	SS	*	0.9	5.91	49
5	13	SS	2	1.6	2.80	2
6	13	SS	16	-0.3	8.52	18
7	13	SS	10	*	10.49	34
8	13	SS	23	-0.7	5.37	50
9	42	SS	59	7.1	7.01	3
10	42	SS	9	2.1	6.82	19
11	42	SS	14	0.5	6.65	35
12	42	SS	13	0.7	5.59	51
13	22	SS	13	4.8	8.56	4
14	22	SS	11	5.1	10.08	20
15	22	SS	8	6.2	9.46	36
16	22	SS	-3	6.5	8.58	52
means			19	0.7	7.1	
17	64	HS	31	0.3	12.32	5
18	64	HS	44	-2.0	19.03	21
19	64	HS	28	-0.9	11.06	37
20	64	HS	17	-0.2	8.69	53
21	66	HS	20	1.3	9.05	6
22	66	HS	15	4.3	9.69	22
23	66	HS	22	3.4	8.65	38
24	66	HS	24	3.2	9.99	54
25	28	HS	23	2.7	10.77	7
26	28	HS	24	2.8	13.25	23
27	28	HS	20	5.0	8.44	39
28	28	HS	34	5.1	13.59	55
29	12	HS	14	4.3	10.65	8
30	12	HS	12	3.8	10.61	24
31	12	HS	19	3.1	13.55	40
32	12	HS	23	3.1	9.31	56
means			23.1	2.5	11.2	
33	50	SH	60	7.8	16.23	9
34	50	SH	57	7.1	16.89	25

35	50	SH	59	7.3	16.77	41
36	50	SH	60	8.4	15.80	57
37	46	SH	45	2.6	10.67	10
38	46	SH	56	1.6	18.31	26
39	46	SH	48	0.9	14.46	42
40	46	SH	56	2.2	13.39	58
41	38	SH	61	7.1	21.10	11
42	38	SH	58	8.1	17.03	27
43	38	SH	58	8.0	10.18	43
44	38	SH	55	9.2	16.69	59
45	80	SH	51	6.7	16.85	12
46	80	SH	55	4.5	14.76	28
47	80	SH	53	7.0	12.20	44
48	80	SH	57	7.9	19.99	60
means			55.6	6.0	15.7	
49	40	HH	58	7.6	16.87	13
50	40	HH	48	7.5	16.39	29
51	40	HH	45	7.5	17.44	45
52	40	HH	58	7.7	17.15	61
53	85	HH	46	4.9	13.63	14
54	85	HH	61	8.5	18.20	30
55	85	HH	66	9.0	20.80	46
56	85	HH	66	7.7	19.29	62
57	49	HH	62	6.1	20.40	15
58	49	HH	62	3.9	19.10	31
59	49	HH	56	5.2	20.79	47
60	49	HH	64	4.8	18.70	63
61	95	HH	69	5.9	19.78	16
62	95	HH	65	5.6	18.85	32
63	95	HH	65	5.6	16.09	48
64	95	HH	51	6.1	17.58	64
means			58.9	6.5	18.2	

Appendix B: The de-convoluted seed samples from the 2007 harvest of the Avalon x Hobbit sib RILs relative to the 2006 field experiment genotypes, and the seed samples sent to co-operators for testing in animal trials, Y = sample sent. (JIC Data)

H1006 Code	RIL number	Classification	D207 CODE As labelled	RIL Number (True)	Classification (True)	Seed sent to (2007)		
						Nott	Danisco	SAC
1-1	41	SOFT-SOFT	1	41	SOFT-SOFT	Y	*	*
1-2	41	SOFT-SOFT	2	13	SOFT-SOFT	Y	*	*
1-3	41	SOFT-SOFT	3	42	SOFT-SOFT	Y	Y	Y
1-4	41	SOFT-SOFT	4	22	SOFT-SOFT	*	*	*
4-1	22	SOFT-SOFT	13	40	HARD-HARD	Y	Y	Y
4-2	22	SOFT-SOFT	14	85	HARD-HARD	Y	*	*
4-3	22	SOFT-SOFT	15	49	HARD-HARD	Y	*	*
4-4	22	SOFT-SOFT	16	95	HARD-HARD	*	*	*
5-4	64	HARD-SOFT	20	22	SOFT-SOFT	*	Y	Y
7-1	28	HARD-SOFT	25	50	SOFT-HARD	Y	*	*
7-2	28	HARD-SOFT	26	46	SOFT-HARD	Y	*	*
7-3	28	HARD-SOFT	27	38	SOFT-HARD	Y	Y	Y
7-4	28	HARD-SOFT	28	80	SOFT-HARD	Y	*	*
10-1	46	SOFT-HARD	37	64	HARD-SOFT	Y	*	*
10-2	46	SOFT-HARD	38	66	HARD-SOFT	*	Y	Y
10-3	46	SOFT-HARD	39	28	HARD-SOFT	Y	*	*
10-4	46	SOFT-HARD	40	12	HARD-SOFT	*	*	*
12-1	80	SOFT-HARD	45	40	HARD-HARD	*	Y	Y
12-2	80	SOFT-HARD	46	85	HARD-HARD	*	*	*
12-3	80	SOFT-HARD	47	49	HARD-HARD	Y	*	*
12-4	80	SOFT-HARD	48	95	HARD-HARD	Y	*	*
15-1	49	HARD-HARD	57	50	SOFT-HARD	*	*	*
15-2	49	HARD-HARD	58	46	SOFT-HARD	Y	Y	Y
15-3	49	HARD-HARD	59	38	SOFT-HARD	Y	*	*
15-4	49	HARD-HARD	60	80	SOFT-HARD	Y	*	*

16-1	95	HARD-HARD	61	40	HARD-HARD	Y	*	*
16-2	95	HARD-HARD	62	85	HARD-HARD	*	*	*
16-3	95	HARD-HARD	63	49	HARD-HARD	Y	*	*
16-4	95	HARD-HARD	64	95	HARD-HARD	*	Y	Y

Appendix C: One-way ANOVA of texture score versus allele for Avalon x Cadenza data 2006 harvest, and allele group means. (JIC data)

Analysis of Variance for texture differences

Source	DF	SS	MS	F	P
Allele	1	908.1	908.1	12.60	<0.001
Error	198	14275.6	72.1		

Individual 95% CIs For Mean

Level	N	Mean	StDev
‘b’	86	54.012	8.741
‘c’	114	58.316	8.298

Appendix D: Limagrain UK Limited (Nickerson); Weight of grain achieved from each line. (Limagrain data)

Line	Weight in kg
BS 17	1146
BS 19	846
BS 38	1325
BS 42	957
RIL 28	700
RIL 95	940
RIL 80	1380
RIL 46	1440
RIL 22	1440
RIL 41	1480
RIL 64	1264
RIL 49	456

Appendix E: Determination of physico-chemical characteristics of 24 wheat samples from the 2005 harvest year. (Danisco data)

Name of cultivar	Replicate location	Hardness class	Avicheck viscosity value (cPs)	SKCS Hardness value	Total pentosans (g/kg, as fed)	Soluble pentosans (g/kg, as fed)	Endogenous xylanase activity (U/kg)	BS2: Xylanase endogenous inhibitors activity (XIU/g)	GPU: Xylanase endogenous inhibitors activity (XIU/g)
Glasgow	Clop	Soft	6.84	14.70	29.5	2.6	82	995	563
Consort	Clop	Soft	6.93	18.80	35	2.9	73	618	399
Glasgow	Mald	Soft	13.40	21.70	24.6	3.2	241	849	551
WW86	Clop	Soft	17.40	25.90	27.4	1.5	81	907	486
Consort	Mald	Soft	7.18	27.80	34.1	4.1	73	762	446
04/7649	Clop	Soft	10.50	28.50	22.6	3.6	58	606	209
04/7649	Mald	Soft	20.30	34.20	23.4	2.6	62	488	307
WW86	Mald	Soft	15.10	34.60	32	3.3	63	884	433
04/8181	Clop	Soft	11.40	36.70	21.9	2.1	57	820	501
04/7482	Mald	Soft	11.60	37.50	30.2	3.4	105	739	423
04/7482	Clop	Soft	7.68	37.90	43.9	4.1	73	800	435
04/8181	Mald	Soft	7.06	40.80	28.7	3.5	48	974	524
Dover	Clop	Hard	10.60	52.00	38.6	3.3	63	762	397
02/4627	Clop	Hard	14.40	52.30	24.6	3.4	88	797	490
02/4627	Mald	Hard	15.60	59.30	20.9	3.7	82	833	496
Dover	Mald	Hard	7.82	64.20	26.3	2.6	77	906	537
03/7631	Clop	Hard	10.80	67.70	45.3	3.5	117	565	475
XI19	Clop	Hard	13.00	68.70	46.2	3.5	91	892	548
03/7631	Mald	Hard	11.60	72.70	26	3.9	83	813	493
03/7444	Mald	Hard	9.71	76.30	20.7	4.1	101	928	449
03/7444	Clop	Hard	8.64	80.30	41.4	4	76	805	406
XI19	Mald	Hard	17.70	82.30	30.1	2.4	88	1021	536
WW85	Clop	Hard	10.10	83.10	30.7	3.1	88	875	492
WW85	Mald	Hard	13.00	84.50	20.9	2.9	91	876	541

Note: the measured values are low (average wheat is generally between 500 and 2000 U/kg), indicating a low contamination of grains.

Determination of physico-chemical characteristics of 24 wheat samples from the 2005 harvest year (part 2/2).

Name of cultivar	Replicate location	Hardness class	Rate of starch Digestion (RSD 60, % DM) *	d(0.1) (µm)	d(0.2) (µm)	d(0.5) (µm)	d(0.8) (µm)	d(0.9) (µm)	Particle Size (µm)
Glasgow	Clop	Soft	55.15	28.1	115.7	603.4	1068.1	1325.5	641.8
Consort	Clop	Soft	55.51	22.7	81.9	530.2	1008.8	1277.6	589.7
Glasgow	Mald	Soft	44.20	32.3	110.9	569.2	1076.9	1342.4	628.5
WW86	Clop	Soft	53.62	30.5	113.9	509.9	958.6	1226.1	575.4
Consort	Mald	Soft	49.80	27.6	134.3	579.6	1071.4	1335.3	636.2
04/7649	Clop	Soft	51.74	36.7	153.5	655.8	1134.8	1387.0	687.1
04/7649	Mald	Soft	48.53	22.9	90.0	528.6	971.5	1229.2	578.9
WW86	Mald	Soft	42.58	28.3	137.3	632.7	1105.0	1359.6	666.9
04/8181	Clop	Soft	52.51	27.7	180.0	731.1	1186.8	1427.3	737.9
04/7482	Mald	Soft	46.39	24.3	124.1	594.0	1063.9	1324.0	638.6
04/7482	Clop	Soft	53.58	26.4	310.4	799.2	1227.6	1457.2	790.5
04/8181	Mald	Soft	45.08	24.4	119.9	582.0	1071.5	1334.9	634.3
Dover	Clop	Hard	55.14	24.1	212.0	766.8	1215.9	1451.1	764.6
02/4627	Clop	Hard	58.16	20.2	185.8	765.4	1216.5	1451.7	760.6
02/4627	Mald	Hard	53.99	23.5	120.0	561.8	1043.6	1307.6	617.2
Dover	Mald	Hard	43.08	28.4	204.8	630.4	1087.5	1342.8	672.6
03/7631	Clop	Hard	54.26	25.8	231.0	654.1	1103.1	1354.9	690.0
XI19	Clop	Hard	55.97	18.1	116.0	581.1	1036.6	1295.2	623.9
03/7631	Mald	Hard	47.20	19.1	139.8	672.2	1138.2	1387.1	691.4
03/7444	Mald	Hard	42.32	23.9	236.6	687.9	1129.1	1374.9	710.6
03/7444	Clop	Hard	57.76	20.5	396.8	865.6	1281.8	1497.8	836.9
XI19	Mald	Hard	45.12	23.9	198.3	685.2	1139.8	1387.3	708.0
WW85	Clop	Hard	58.39	28.1	249.8	680.1	1138.0	1387.3	712.6
WW85	Mald	Hard	52.68	25.6	229.0	703.3	1150.0	1394.4	722.3

Regarding laser particle size analysis, "d" means 10% (0.1), 20% (0.2)... etc... of particles having a diameter inferior than the reported value (µm). * Englyst et al 1992

Analysis summary for the 24 wheat samples from the 2005 harvest year.

Grain measured parameters	**Unit**	**Mean**	**SD**	**Min.**	**Max.**
SKCS Hardness	no unit	50.1	22.4	14.7	84.5
Viscosity	cp	11.6	3.7	6.8	20.3
Total pentosan content	g/kg	30.2	7.8	20.7	46.2
Soluble pentosan content	g/kg	3.2	0.007	1.5	4.1
Endogenous xylanase activity	U/kg	85.9	36.1	48.0	241.0
BS2 xylanase endogenous inhibitors activity	XIU/g	813.1	131.5	488.0	1021.0
GPU xylanase endogenous inhibitors activity	XIU/g	464.0	80.7	209.0	563.0
Rate of Starch Digestion (RSD 60*)	%	50.9	5.2	42.3	58.4
Mean particle size	µm	679.9	66.3	836.9	575.4
Particle size uniformity (D10 / D90)	µm	25.6 / 1360.8	4.2 / 67.0	18.1 / 1226.1	36.7 / 1497.8

Regarding laser particle size analysis, "d" means 10% (0.1), 20% (0.2)... etc... of particles having a diameter inferior than the reported value (µm).

*(Englyst et al 1992)

Effect of hardness class (Hard vs. Soft) on the physico-chemical characteristics of 24 wheat samples from the 2005 harvest year.

GRAIN PARAMETERS	HARD	SOFT	Range of variation (min/max)
SKCS grain hardness value	70 [a]	30 [b]	15 / 85
Avicheck viscosity, cPs	11.9	11.3	6.8 / 20.3
Total pentosan content, g/kg	31.0	29.4	2.07 / 4.62
Soluble pentosan content, g/kg	3.4	3.1	0.15 / 0.41
Endogenous xylanase activity, U/kg	87	85	48 / 241
BS2 endogenous xylanase inhibitor activity, XIU/g	839	787	488 / 1021
GPU endogenous xylanase inhibitor activity, XIU/g	488	440	209 / 563
Rate of Starch Digestion (RSD60*), %	52.00	49.90	42.30 / 58.40
Mean particle size, µm	709 [a]	651 [b]	575 / 837
D_{20}, µm	210 [a]	139 [b]	81 / 397
D_{80}, µm	1140 [(a)]	1034 [(b)]	958 / 1281

a,b: P<0.05

(a),(b): P<0.10

***(Englyst et al 1992)**

Appendix F: Determination of pentosan content in 78 wheat samples from the 2006 harvest year. (Danisco data)

Sample code	Wheat cultivars	Total pentosans g/kg	Soluble pentosans g/kg
H 10 11	DwA x Avalon recombinant inbred lines	51.1	4.6
H 10 12	DwA x Avalon recombinant inbred lines	48.5	4.8
H 10 13	DwA x Avalon recombinant inbred lines	50.3	5.0
H 10 14	DwA x Avalon recombinant inbred lines	34.8	5.1
H 10 21	DwA x Avalon recombinant inbred lines	45.1	5.6
H 10 22	DwA x Avalon recombinant inbred lines	38.1	5.6
H 10 23	DwA x Avalon recombinant inbred lines	46.4	5.4
H 10 24	DwA x Avalon recombinant inbred lines	53.3	5.9
H 10 31	DwA x Avalon recombinant inbred lines	44.8	4.7
H 10 32	DwA x Avalon recombinant inbred lines	51.4	5.5
H 10 33	DwA x Avalon recombinant inbred lines	42.6	5.6
H 10 34	DwA x Avalon recombinant inbred lines	55.5	5.9
H 10 41	DwA x Avalon recombinant inbred lines	45.1	5.5
H 10 42	DwA x Avalon recombinant inbred lines	53.7	5.6
H 10 43	DwA x Avalon recombinant inbred lines	52.5	5.6
H 10 44	DwA x Avalon recombinant inbred lines	52.1	5.6
H 10 51	DwA x Avalon recombinant inbred lines	40.0	5.4
H 10 52	DwA x Avalon recombinant inbred lines	51.8	5.4
H 10 53	DwA x Avalon recombinant inbred lines	43.4	5.6
H 10 54	DwA x Avalon recombinant inbred lines	43.3	5.1
H 10 61	DwA x Avalon recombinant inbred lines	42.6	4.9
H 10 62	DwA x Avalon recombinant inbred lines	50.3	4.9
H 10 63	DwA x Avalon recombinant inbred lines	50.2	5.1
H 10 64	DwA x Avalon recombinant inbred lines	48.0	5.2
H 10 71	DwA x Avalon recombinant inbred lines	4.07	5.0
H 10 72	DwA x Avalon recombinant inbred lines	48.2	5.1
H 10 73	DwA x Avalon recombinant inbred lines	46.2	5.2
H 10 74	DwA x Avalon recombinant inbred lines	49.3	5.2
H 10 81	DwA x Avalon recombinant inbred lines	52.2	5.2
H 10 82	DwA x Avalon recombinant inbred lines	50.5	5.1
H 10 83	DwA x Avalon recombinant inbred lines	50.5	5.3
H 10 84	DwA x Avalon recombinant inbred lines	45.5	5.2
H 10 91	DwA x Avalon recombinant inbred lines	53.0	5.2
H 10 92	DwA x Avalon recombinant inbred lines	51.6	4.4
H 10 93	DwA x Avalon recombinant inbred lines	52.4	4.1
H 10 94	DwA x Avalon recombinant inbred lines	55.6	4.5
H 10 101	DwA x Avalon recombinant inbred lines	54.6	5.5
H 10 102	DwA x Avalon recombinant inbred lines	50.5	5.4
H 10 103	DwA x Avalon recombinant inbred lines	54.8	5.8
H 10 104	DwA x Avalon recombinant inbred lines	61.9	5.6
H 10 111	DwA x Avalon recombinant inbred lines	41.9	4.6
H 10 112	DwA x Avalon recombinant inbred lines	28.6	4.6
H 10 113	DwA x Avalon recombinant inbred lines	46.4	4.8
H 10 114	DwA x Avalon recombinant inbred lines	48.8	4.9

Determination of pentosan content in 78 wheat samples from the 2006 harvest year.

Sample code	Cultivars	Total pentosans g/kg	Soluble pentosans g/kg
H 10 121	DwA x Avalon recombinant inbred lines	46.1	4.8
H 10 122	DwA x Avalon recombinant inbred lines	39.2	4.6
H 10 123	DwA x Avalon recombinant inbred lines	47.5	4.7
H 10 124	DwA x Avalon recombinant inbred lines	49.5	5.1
H 10 131	DwA x Avalon recombinant inbred lines	42.4	4.9
H 10 132	DwA x Avalon recombinant inbred lines	46.6	4.7
H 10 133	DwA x Avalon recombinant inbred lines	43.7	4.8
H 10 134	DwA x Avalon recombinant inbred lines	43.8	4.7
H 10 141	DwA x Avalon recombinant inbred lines	41.1	4.7
H 10 142	DwA x Avalon recombinant inbred lines	39.8	4.9
H 10 143	DwA x Avalon recombinant inbred lines	46.9	4.4
H 10 144	DwA x Avalon recombinant inbred lines	30.5	4.0
H 10 151	DwA x Avalon recombinant inbred lines	45.9	4.7
H 10 152	DwA x Avalon recombinant inbred lines	41.2	4.4
H 10 153	DwA x Avalon recombinant inbred lines	49.0	4.8
H 10 154	DwA x Avalon recombinant inbred lines	54.6	4.6
H 10 161	DwA x Avalon recombinant inbred lines	55.6	5.1
H 10 162	DwA x Avalon recombinant inbred lines	49.7	5.1
H 10 163	DwA x Avalon recombinant inbred lines	42.9	5.4
H 10 164	DwA x Avalon recombinant inbred lines	55.4	5.2
D 3061 10	Beaver x Soisson DH lines	55.3	4.1
D 3061 14	Beaver x Soisson DH lines	64.4	3.9
D 3061 16	Beaver x Soisson DH lines	45.8	4.5
D 3062 17	Beaver x Soisson DH lines	45.9	4.7
D 3062 19	Beaver x Soisson DH lines	42.1	3.1
D 3062 22	Beaver x Soisson DH lines	51.1	4.3
D 3062 24	Beaver x Soisson DH lines	45.9	4.9
D 3062 25	Beaver x Soisson DH lines	47.0	3.5
D 3062 28	Beaver x Soisson DH lines	48.9	4.7
D 3062 31	Beaver x Soisson DH lines	51.4	4.0
D 3062 36	Beaver x Soisson DH lines	46.0	4.0
D 3062 45	Beaver x Soisson DH lines	44.6	3.0
D 3062 46	Beaver x Soisson DH lines	44.6	3.5
D 3062 49	Beaver x Soisson DH lines	51.9	4.4

Appendix G: Determination of the chemical characteristics of 12 wheat samples from the 2007 harvest year. (Danisco data)

Sample name	Origin	SKCS Hardness value	Avicheck viscosity value (cPs)	Total pentosans g/kg (as fed)	Soluble pentosans g/kg (as fed)	Endogenous xylanase activity (U/kg)
BS 17	Beaver x Soisson	48	8.78	55.4	3.3	463
BS 19	Beaver x Soisson	75	10.35	50.4	3.6	337
BS 38	Beaver x Soisson	42	5.65	53.2	2.6	246
BS 42	Beaver x Soisson	72	6.32	55.6	2.6	312
Dwa/ Av RIL 22	Hobbit x Avalon	43	10.90	53.6	3.6	445
Dwa/ Av RIL 28	Hobbit x Avalon	78	14.60	49.8	4.0	585
Dwa/ Av RIL 41	Hobbit x Avalon	69	10.50	46.5	3.9	286
Dwa/ Av RIL 46	Hobbit x Avalon	72	14.05	45.6	4.4	442
Dwa/ Av RIL 49	Hobbit x Avalon	45	14.40	43.3	5.0	531
Dwa/Av RIL 64	Hobbit x Avalon	40	17.75	43.6	4.6	469
Dwa/ Av RIL 80	Hobbit x Avalon	69	10.55	45.6	4.1	265
Dwa/ Av RIL 95	Hobbit x Avalon	61	14.75	48.2	4.4	219

Appendix H: Effect of cultivar and xylanase supplementation on the nutritional value of 12 wheat samples from the 2007 harvest year. (Danisco data)

	AMEn (MJ/kg, DM basis)	TMEn (MJ/kg, DM basis)	DM digestibility coefficient
Wheat sample RIL22			
No enzyme	12.19	16.45	0.611
Enzyme added (xylanase)	12.27	16.52	0.601
Wheat sample RIL28			
No enzyme	12.43	16.70	0.646
Enzyme added (xylanase)	12.27	16.54	0.614
Wheat sample RIL41			
No enzyme	12.24	16.50	0.612
Enzyme added (xylanase)	12.60	16.87	0.620
Wheat sample RIL46			
No enzyme	12.86	17.07	0.659
Enzyme added (xylanase)	12.66	16.88	0.631
Wheat sample RIL49			
No enzyme	12.00	16.22	0.666
Enzyme added (xylanase)	11.72	15.95	0.585
Wheat sample RIL64			
No enzyme	12.44	16.64	0.622
Enzyme added (xylanase)	12.75	16.95	0.617
Wheat sample RIL80			
No enzyme	12.96	17.22	0.659
Enzyme added (xylanase)	13.07	17.33	0.630
Wheat sample RIL95			
No enzyme	12.58	16.87	0.608
Enzyme added (xylanase)	12.74	17.03	0.626
Wheat sample BS17			

No enzyme	12.28	16.45	0.579
Enzyme added (xylanase)	12.43	16.60	0.593
Wheat sample BS38			
No enzyme	12.73	16.98	0.640
Enzyme added (xylanase)	12.82	17.06	0.644
Average for 10 wheat samples			
No enzyme	12.47	16.71	0.630
Enzyme added (xylanase)	12.54	16.78	0.616
P-values			
Effect of wheat cultivar	<0.001	<0.001	0.060
Effect of enzyme addition	0.576	0.576	0.103
Interaction	0.956	0.956	0.309

Appendix I: Chemical characteristics of 9 selected wheat cultivars from the 2008 harvest year. (Danisco data)

Sample	Name	Avicheck viscosity (cPs)	SKCS hardness value	Xylanase endogenous activity (U/kg)	Soluble Pentosan content g/kg (as fed)	Total Pentosan content g/kg (as fed)
1	BS17	6.40	23	0	3.4	60.8
2	BS19	8.01	10	116	4.5	57.3
3	BS38	5.14	52	43	3.7	50.6
4	BS42	5.43	37	114	4.5	52.6
5	RIL22	10.55	67	0	4.9	59.1
6	RIL41	9.93	32	53	5.0	56.9
7	RIL64	14.40	18	65	5.9	64.2
8	RIL80	8.54	68	0	4.6	63.8
9	RIL95	16.30	59	50	5.6	63.1

Appendix J: Effect of cultivar and xylanase supplementation on the nutritional value of 9 wheat cultivars from the 2008 harvest year. (Danisco & SAC data)

	BWG 0-42d (g/b/d)	FCR 0-42d (g:g)	Excreta viscosity (cP)	DM digestibility	N digestibility
Wheat sample RIL22					
No enzyme	72.9	1.758	1.89	0.679	0.255
Enzyme added (xylanase)	69.6	1.738	1.78	0.738	0.392
Wheat sample RIL41					
No enzyme	71.3	1.786	2.61	0.690	0.486
Enzyme added (xylanase)	71.3	1.731	1.40	0.692	0.477
Wheat sample RIL64					
No enzyme	67.4	1.784	3.04	0.654	0.332
Enzyme added (xylanase)	67.9	1.724	1.91	0.625	0.334
Wheat sample RIL80					
No enzyme	67.8	1.778	2.49	0.685	0.412
Enzyme added (xylanase)	67.9	1.728	1.70	0.692	0.438
Wheat sample RIL95					
No enzyme	67.0	1.778	2.68	0.574	0.308
Enzyme added (xylanase)	66.3	1.717	2.38	0.676	0.440
Wheat sample BS17					
No enzyme	68.0	1.731	1.80	0.696	0.360
Enzyme added (xylanase)	68.5	1.712	1.74	0.726	0.460
Wheat sample BS19					
No enzyme	66.5	1.678	2.57	0.691	0.324
Enzyme added (xylanase)	70.2	1.714	1.48	0.684	0.327
Wheat sample BS38					

No enzyme	68.5	1.788	2.35	0.606	0.116
Enzyme added (xylanase)	69.7	1.759	1.84	0.627	0.100
Wheat sample BS42					
No enzyme	67.2	1.788	2.15	0.715	0.411
Enzyme added (xylanase)	69.2	1.723	1.76	0.689	0.351
Average for 9 wheat samples					
No enzyme	68.5	1.763	2.40	0.666	0.334
Enzyme added (xylanase)	68.9	1.727	1.78	0.683	0.369
P-values					
Effect of wheat cultivar	<0.001	0.015	0.205	0.136	<0.001
Effect of enzyme addition	0.373	<0.001	<0.001	0.364	0.310
Interaction	0.110	0.244	0.397	0.823	0.863

Effect of cultivar and xylanase supplementation on welfare parameters of broilers fed with 9 wheat cultivars from the 2008 harvest year (part 2/2).

	Liveability (%)	Litter DM (g/kg)	Litter pH	Litter score	Hock score
Wheat sample RIL22					
No enzyme	90.0	601.1	8.33	2.48	13.00
Enzyme added (xylanase)	88.8	654.3	8.52	1.95	4.75
Wheat sample RIL41					
No enzyme	87.5	550.1	8.50	2.60	17.75
Enzyme added (xylanase)	90.0	576.9	8.28	2.60	13.75
Wheat sample RIL64					
No enzyme	92.5	581.3	8.34	2.38	10.75
Enzyme added (xylanase)	86.3	645.8	8.50	1.79	5.00
Wheat sample RIL80					
No enzyme	93.8	624.8	8.11	2.46	8.00
Enzyme added (xylanase)	88.8	634.1	8.38	1.88	6.00
Wheat sample RIL95					
No enzyme	88.8	624.1	8.36	1.91	8.00
Enzyme added (xylanase)	86.3	668.6	8.42	1.74	6.00
Wheat sample BS17					
No enzyme	86.3	649.6	8.53	1.96	7.00
Enzyme added (xylanase)	91.3	640.0	8.68	2.05	7.25
Wheat sample BS19					
No enzyme	88.8	651.3	8.60	1.90	5.00
Enzyme added (xylanase)	92.5	585.2	8.60	2.48	14.25
Wheat sample BS38					
No enzyme	87.5	551.2	8.34	2.58	12.00

Enzyme added (xylanase)	87.5	587.9	8.38	2.59	12.25
Wheat sample BS42					
No enzyme	90.0	642.7	8.49	1.81	6.25
Enzyme added (xylanase)	90.0	633.1	8.50	1.93	5.25
Average for 9 wheat samples					
No enzyme	89.4	608.5	8.40	2.23	9.75
Enzyme added (xylanase)	89.0	625.1	8.47	2.11	8.28
P-values					
Effect of wheat cultivar	0.887	<0.001	0.002	<0.001	0.001
Effect of enzyme addition	0.746	0.05	0.087	0.047	0.176
Interaction	0.459	0.016	0.229	<0.001	0.033

Appendix K: Effect of hardness class on performance and nutrient utilisation in broilers fed 9 wheat samples from the 2008 harvest year. (Danisco & SAC data)

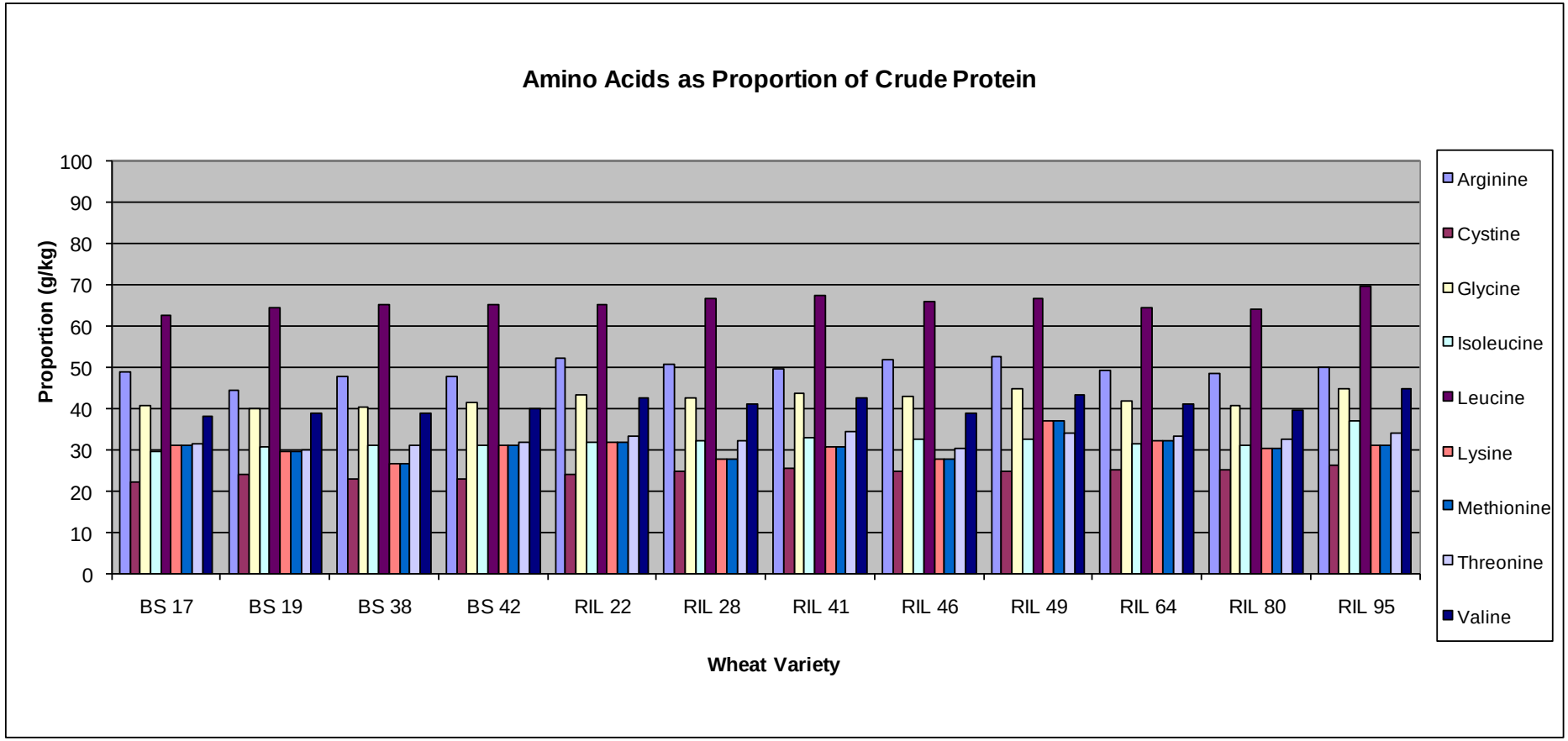
	BWG 0-42d (g/d)	mFCR 0-42d	DM digestibility	N ret. (g/d)	Prot Efficiency Ratio *
Hard wheat (SKCS>50)	68.7	1.770	0.656	1.18	2.76
Soft wheat (SKCS<50)	68.6	1.742	0.686	1.45	2.61
P-value	*0.90*	*0.01*	*0.14*	*0.10*	*0.003*

* PEF = bodyweight gain / protein intake

Appendix L: Influence of hardness class (Hard vs. Soft) on the effect of xylanase supplementation in wheat-based diets (wheat samples from the 2008 harvest). (Danisco & SAC data)

	BWG 0-42d (g/d)	FCR 0-42d	Litter DM (g/kg)	DMD coefficient	N retention (g/d)	Protein Efficiency Ratio
Hard wheat (SKCS>50)						
No enzyme	69.06	1.787	600.3	0.636	1.00	2.72
Xylanase	68.38	1.752	636.2	0.678	1.38	2.79
P-value	*NS*	*0.0158*	*0.0396*	*NS*	*NS (0.15)*	*NS*
Soft wheat (SKCS<50)						
No enzyme	68.07	1.751	615.0	0.689	1.44	2.59
Xylanase	69.24	1.732	616.2	0.683	1.46	2.63
P-value	*NS (0.16)*	*NS (0.19)*	*NS*	*NS*	*NS*	*NS*

Appendix M: Amino acid content of wheat samples from the 2008 harvest (BOCM Pauls data)



Appendix N: Determination of apparent metabolisable energy (AME), AME corrected for nitrogen retention (AMEn), true metabolisable energy (TME), TME corrected for nitrogen retention (TMEn), gross energy metabolisability (ME:GE) and dry matted digestibility (DMD) coefficients, and nitrogen retention (NR) of 55 wheat samples from 2005 harvest year. (SAC data)

Sample N0 as received	2	11	21	22	24	27	40	41	45	47
Sample N0 (SAC)	1	2	3	4	5	6	7	8	9	10
AME MJ/kg DM	13.12	11.95	11.91	11.93	12.21	12.31	12.51	12.11	13.12	13.22
AMEn MJ/kg DM	13.43	12.40	12.46	12.19	12.90	12.82	13.03	13.04	13.51	13.73
AME:GE	0.709	0.646	0.645	0.646	0.661	0.664	0.678	0.653	0.717	0.712
AMEn:GE	0.726	0.670	0.674	0.659	0.698	0.691	0.707	0.703	0.739	0.740
TME MJ/kg DM	15.89	14.71	14.67	14.70	14.96	15.08	15.28	14.88	15.89	15.98
TME:GE	0.859	0.795	0.794	0.795	0.810	0.813	0.829	0.802	0.869	0.861
TMEn MJ/kg DM	16.20	15.16	15.22	14.95	15.66	15.58	15.80	15.81	16.28	16.50
TMEn:GE	0.876	0.819	0.824	0.809	0.848	0.840	0.857	0.853	0.890	0.889
DMD	0.697	0.628	0.629	0.687	0.633	0.649	0.681	0.656	0.700	0.695
NR (g)	-0.39	-0.56	-0.70	-0.32	-0.88	-0.64	-0.66	-0.76	-0.49	-0.65

Sample N0 as received	49	64	70	79	89	90	92	95	108	109
Sample N0 (SAC)	11	12	13	14	15	16	17	18	19	20
AME MJ/kg DM	12.86	12.76	12.07	13.23	12.70	11.56	11.63	12.97	12.00	12.62
AMEn MJ/kg DM	13.39	13.25	12.57	13.65	13.25	12.27	12.46	13.42	12.63	13.13
AME:GE	0.694	0.687	0.655	0.713	0.688	0.625	0.630	0.699	0.654	0.682
AMEn:GE	0.722	0.713	0.683	0.736	0.717	0.664	0.675	0.723	0.688	0.709
TME MJ/kg DM	15.62	15.52	14.84	16.01	15.48	14.33	14.40	15.75	14.78	15.39
TME:GE	0.843	0.835	0.806	0.863	0.838	0.775	0.781	0.849	0.805	0.823
TMEn MJ/kg DM	16.15	16.01	15.35	16.43	16.03	15.04	15.23	16.20	15.41	15.90
TMEn:GE	0.871	0.861	0.833	0.886	0.867	0.813	0.826	0.873	0.839	0.859
DMD	0.675	0.669	0.639	0.702	0.671	0.604	0.609	0.684	0.637	0.661
NR (g)	-0.67	-0.61	-0.63	-0.53	-0.69	-0.88	-0.83	-0.56	-0.78	-0.64

(continued)

Sample N0 (as received)	113	115	117	132	H 1006-1	H 1006-2	H 1006-3	H 1006-4	H 1006-5	H 1006-6
Sample N0 (SAC)	21	22	23	24	25	26	27	28	29	30
AME MJ/kg DM	11.99	11.74	12.34	12.78	12.61	12.34	13.24	12.99	12.85	13.06
AMEn MJ/kg DM	12.64	12.21	12.91	13.37	13.18	12.72	13.62	13.97	13.18	13.40
AME:GE	0.649	0.676	0.664	0.689	0.673	0.660	0.710	0.699	0.672	0.709
AMEn:GE	0.684	0.703	0.695	0.721	0.703	0.681	0.731	0.752	0.708	0.727
TME MJ/kg DM	14.77	14.35	15.11	15.55	15.41	15.15	16.04	16.31	15.31	15.87
TME:GE	0.799	0.827	0.813	0.839	0.823	0.810	0.861	0.850	0.823	0.861
TMEn MJ/kg DM	15.42	14.82	15.68	16.14	15.98	15.52	16.42	16.77	15.99	16.20
TMEn:GE	0.834	0.853	0.844	0.871	0.853	0.830	0.881	0.902	0.859	0.879
DMD	0.628	0.683	0.649	0.671	0.647	0.684	0.684	0.680	0.665	0.694
NR (g)	-0.82	-0.61	-0.72	-0.74	-0.70	-0.46	-0.47	-0.58	-0.41	-0.42

Sample N0 (as received)	H 1006-7	H 1006-8	H 1006-9	H 1006-10	H 1006-11	H 1006-12	H 1006-13	H 1006-14	H 1006-15	H 1006-16
Sample N0 (SAC)	31	32	33	34	35	36	37	38	39	40
AME MJ/kg DM	12.95	12.73	12.30	12.87	11.53	12.83	12.99	11.41	12.64	11.74
AMEn MJ/kg DM	13.35	13.31	12.82	13.30	12.04	13.10	13.45	12.03	12.90	12.16
AME:GE	0.659	0.689	0.659	0.690	0.620	0.684	0.706	0.617	0.683	0.634
AMEn:GE	0.725	0.720	0.687	0.713	0.648	0.699	0.731	0.651	0.697	0.656
TME MJ/kg DM	14.93	15.53	15.10	15.67	14.33	15.64	15.79	14.21	15.44	14.54
TME:GE	0.811	0.841	0.809	0.839	0.771	0.834	0.858	0.769	0.834	0.785
TMEn MJ/kg DM	16.14	16.11	15.62	16.10	14.84	15.91	16.26	14.84	15.70	14.96
TMEn:GE	0.877	0.872	0.837	0.862	0.798	0.849	0.884	0.802	0.848	0.808
DMD	0.692	0.673	0.631	0.670	0.640	0.642	0.722	0.641	0.670	0.644
NR (g)	-0.49	-0.72	-0.65	-0.53	-0.64	-0.34	-0.58	-0.77	-0.32	-0.51

(continued)

Sample N0 (as received)	BS 10	BS 14	BS 16	BS 19	BS 22	BS 24	BS 25	BS 28	BS 31	BS 36
Sample N0 (SAC)	41	42	43	44	45	46	47	48	49	50
AME MJ/kg DM	12.16	12.76	12.79	12.81	11.58	11.38	12.41	12.31	11.93	12.31
AMEn MJ/kg DM	12.39	13.03	13.08	13.23	12.04	11.89	12.82	12.77	12.40	13.33
AME:GE	0.669	0.708	0.689	0.686	0.621	0.615	0.665	0.678	0.672	0.664
AMEn:GE	0.682	0.723	0.705	0.709	0.646	0.643	0.687	0.703	0.699	0.721
TME MJ/kg DM	14.96	15.56	15.58	15.61	14.38	14.18	15.22	15.12	14.74	15.75
TME:GE	0.824	0.863	0.840	0.837	0.772	0.767	0.816	0.833	0.830	0.816
TMEn MJ/kg DM	15.20	15.83	15.88	16.03	14.84	14.70	15.62	15.57	15.21	16.14
TMEn:GE	0.837	0.878	0.855	0.859	0.797	0.794	0.837	0.858	0.857	0.873
DMD	0.693	0.714	0.696	0.658	0.648	0.599	0.647	0.677	0.672	0.656
NR (g)	-0.29	-0.34	-0.36	-0.52	-0.57	-0.63	-0.50	-0.56	-0.58	-0.65

Sample N0 (as received)	BS 45	BS 46	BS 49	BS 66	BS 67		
Sample N0 (SAC)	51	52	53	54	55	LSD	P
AME MJ/kg DM	11.79	13.14	11.83	12.10	11.79	1.3866	0.209
AMEn MJ/kg DM	12.39	13.34	12.35	12.63	12.47	1.2351	0.080
AME:GE	0.637	0.708	0.636	0.654	0.633	0.0769	0.404
AMEn:GE	0.670	0.718	0.664	0.684	0.670	0.0667	0.128
TME MJ/kg DM	14.59	15.94	14.64	14.88	14.59	1.4492	0.250
TME:GE	0.788	0.858	0.787	0.805	0.784	0.0769	0.392
TMEn MJ/kg DM	15.19	16.13	15.15	15.43	15.28	1.2353	0.071
TMEn:GE	0.821	0.869	0.815	0.835	0.821	0.0667	0.125
DMD	0.616	0.699	0.618	0.647	0.605	0.0707	0.047
NR (g)	-0.75	-0.24	-0.64	-0.66	-0.85	0.3996	0.181

Appendix O: Summary statistics for the 55 wheat samples from 2005 harvest year (results based on the average data from 55 wheat samples). (SAC data)

Grain nutritional value	Unit	Mean	SD	Min.	Max.
DM	g/kg	0.856	0.010	0.849	0.913
GE	MJ/kg DM	18.48	0.23	17.36	18.75
AME	MJ/kg DM	12.40	0.538	11.38	13.24
AMEn	MJ/kg DM	12.90	0.508	11.89	13.97
AME:GE	Coefficient	0.670	0.028	0.615	0.717
AMEn:GE	Coefficient	0.698	0.0267	0.643	0.752
TME	MJ/kg DM	15.18	0.555	14.18	16.31
TME:GE	Coefficient	0.820	0.028	0.767	0.869
TMEn	MJ/kg DM	15.68	0.511	14.70	16.77
TMEn:GE	Coefficient	0.849	0.027	0.794	0.902
DMD	Coefficient	0.661	0.030	0.599	0.722
NR	g	-0.590	0.157	-0.880	-0.239
N	g/kg DM	22.3	1.86	19.3	26.2
% VOLUME >2 mm	%	43.52	9.42	22.77	63.22

Key:

DM, GE, AME, AMEn, TME, TMEn, AME:GE, AMEn:GE, TME:GE, TMEn:GE, DMD, NR and %>2mm are the determined dietary dry matter, gross energy, apparent metabolisable energy, apparent metabolisable energy corrected for nitrogen retention, true metabolisable energy, true metabolisable energy corrected for nitrogen retention, gross energy metabolisability coefficients, dry matted digestibility coefficient, nitrogen retention and the % particles bigger than 2 mm of 55 wheat samples from 2005 harvest year.

Appendix P: Correlation matrix for the dietary metabolisable energy, dry matter digestibility and nitrogen retention response criteria of broiler chickens to 55 wheat samples from 2005 harvest year (results based on the average data from 55 wheat samples). (SAC data)

	DM	GE	AME	AMEn	AME:GE	AMEn:GE	TME	TMEn	TME:GE	TMEn:GE	DMD	NR	N	% >2 mm	CP (g/kg DM)	EH
DM	1															
GE	0.557	1														
AME	0.107	0.197	1													
AMEn	0.083	0.238	0.946	1												
AME:GE	0.072	0.089	0.934	0.873	1											
AMEn:GE	0.093	0.077	0.908	0.950	0.925	1										
TME	0.159	0.232	0.953	0.946	0.925	0.897	1									
TMEn	0.140	0.268	0.946	0.998	0.863	0.939	0.949	1								
TME:GE	0.051	0.124	0.923	0.858	0.998	0.921	0.916	0.850	1							
TMEn:GE	0.072	0.115	0.899	0.936	0.925	0.998	0.888	0.926	0.925	1						
DMD	0.070	0.256	0.746	0.630	0.814	0.729	0.698	0.622	0.825	0.742	1					
NR	0.208	0.041	0.472	0.226	0.468	0.246	0.432	0.237	0.482	0.262	0.634	1				
N	0.558	0.211	0.074	-0.009	0.011	-0.076	0.102	0.024	0.030	-0.056	0.060	0.336	1			
% VOLUME >2 mm	0.538	0.234	0.107	-0.198	0.202	-0.278	0.068	0.166	-0.186	-0.261	0.005	0.390	0.509	1		
CP (g/kg DM)	0.558	0.211	0.074	-0.009	0.011	-0.076	0.102	0.024	0.030	-0.056	0.060	0.336	1.000	0.509	1	
EH	0.026	0.077	0.153	-0.199	0.166	-0.229	0.188	0.197	-0.169	-0.233	0.157	0.068	0.050	0.062	-0.050	1

d.f. = 12; Correlation coefficients greater than 0.458, 0.532 and 0.661are statistically significant at 10% (P<0.10), 5% (*P<0.05*) and 1% level (*P<0.001*), respectively. Key:DM, GE, AME, AMEn, TME, TMEn, AME:GE, AMEn:GE, TME:GE, TMEn:GE, DMD, NR and %>2mm are the determined dietary dry matter, gross energy, apparent metabolisable energy, apparent metabolisable energy corrected for nitrogen retention, true metabolisable energy, true metabolisable energy corrected for nitrogen retention, gross energy metabolisability coefficients, dry matted digestibility coefficient, nitrogen retention and the % particles bigger than 2 mm of 55 wheat samples from 2005 harvest year.

Appendix Q: Relationship between wheat apparent metabolisable energy (AME), dry matter digestibility coefficient (DMD) and nitrogen (N) retention (results based on a comparison of individual data obtained with 495 tube fed birds). (SAC data)

Dependant variates	Constant	Explanatory variates	r^2	RSD[1]
		DMD		
AME (MJ/kg DM)	2.25	15.49	0.91	0.521***
	(±0.149)	(±0.222)		
		N retention		
AME (MJ/kg DM)	13.33	1.53	0.40	1.55***
	(±0.087)	(±0.085)		
		N retention		
DMD	0.72	0.094	0.52	0.0746***
	(±0.004)	(±0.0041)		

Statistical significance of regression equation: *** P<0.001.

[1] Residual standard deviation.

Appendix R: Linear regression analysis of the relationships between Rapid Visco Analyser (RVA) and chick bioassay parameter data (Nottingham data)

		CIAD (Starch)	CTTAD (Starch)	CIAD (Nitrogen)	CAR (Nitrogen)	CARu (Nitrogen)
Peak Viscosity (Silver)	R-squared	-0.0346	0.0171	0.0739	**0.5351**	**0.2261**
	P	NS	NS	NS	**<0.001**	**0.019**
Peak Viscosity (Water)	R-squared	-0.0161	0.0065	-0.1078	**0.2703**	0.0684
	P	NS	NS	NS	**0.009**	NS
End Viscosity (Silver)	R-squared	-0.0091	0.0056	**0.3555**	0.1271	0.0898
	P	NS	NS	**0.002**	0.087	NS
End Viscosity (Water)	R-squared	-0.1207	0.0013	0.0579	**0.6432**	**0.3518**
	P	0.096	NS	NS	**<0.001**	**0.002**
Amylase Estimation (PV)	R-squared	0.0865	0.0274	0.0637	-0.117	-0.0208
	P	NS	NS	NS	NS	0.502
Amylase Estimation (EV)	R-squared	0.0183	0.0001	0.0321	0.0786	0.0001
	P	NS	NS	NS	NS	0.0975

CIAD = Coefficient of Ileal Apparent Digestibility; CTTAD = Coefficient of Total Tract Apparent Digestibility; CAR = Coefficient of Apparent Retention; CARu = Coefficient of Apparent Retention corrected for uric acid excretion

Appendix S: Knowledge transfer activities

Péron A, Faurschou-Isaksen M, Acamovic T, Pirzgoliev V, Angus W, Snape J and Wiseman J (2010) Effect of wheat cultivar and xylanase supplementation on broiler performance, nutrient digestibility and litter quality. XIIIth European Poultry Conference, August 2010, Tours, France

Saleem G, Spearks N, Pirgozliev V, Acamovic T, Houdijk J, Wiseman J, Peron A and Snape J (2010) Effect of wheat cultivar, type and xylanase supplementation on *Clostridium perfringens* in broilers. XIIIth European Poultry Conference, August 2010, Tours, France

Péron A, Faurschou-Isaksen M, Acamovic T, Pirzgoliev V, Svihus B, Snape J, Angus W and Wiseman J (2009) Physico-chemical composition and nutritional value of UK wheat cultivars exhibiting a wide range of hardness. Poster presentation at European Symposium on Poultry Nutrition, Edinburgh 2009

Poster summarising the project outcomes displayed on the HGCA stand at Cereals, June 11th and 12th 2009

Elraghig, M and Wiseman J. (2009) Ileal and total tract starch digestibility for broilers of wheat varying in hardness classification *British Poultry Abstracts* **5** (1) 23-24

Acamovic T, Pirgozliev V, Svihus B, Snape J, Angus W, Peron A and Wiseman J. (2008) The effect of grinding of highly characterised wheat samples on particle size and dry matter digestibility coefficients. Oral presentation at XXII World's Poultry Congress, Brisbane, Australia, 2008

Acamovic T, Pirgozliev V, Wiseman J, Snape J and Angus W (2008) The metabolisable energy for chickens of highly characterised wheat samples. Poster presentation at XXII World's Poultry Congress, Brisbane, Australia, 2008

Breeding for nutritional benefits. Crop Science Magazine, September 2007

Reduction in diffuse pollution of poultry operations through selection of wheat cultivars of high and consistent nutritional quality. Society of Feed technologists, January 2011