June 2013

Project Report No. 511

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

by

Dr Helen Masey O'Neil and Prof Julian Wiseman

School of Biosciences

University of Nottingham

Sutton Bonington Campus

LE12 5RD

This is the final report of a 45 month project (RD-2005-3180) which started in April 2006. The work was funded by Defra and a contract for £120,000 from HGCA.

HGCA is the cereals and oilseeds division of the Agriculture and Horticulture Development Board.

CONTENTS

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.