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Development and evaluation of low-phytate wheat germplasm to reduce diffuse phosphate pollution from pig and poultry production units

by

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1. ABSTRACT

The aim of this project was to reduce phosphate pollution from monogastric farm animals by changing the availability of phosphate from wheat. Currently a lot of wheat derived phosphate is stored in a non-available form, as phytate. The approaches taken in this research project were to: i) determine the effect of high available phosphate (HAP) wheat on phosphorous excretion in pigs and poultry ii) develop a model for quantifying the effect of HAP wheat on diffuse pollution reduction iii) develop adapted wheat germplasm, iv) development of a toolkit for marker-assisted breeding of the HAP trait and v) determine the effect of P fertiliser treatment on the growth and phosphate metabolism of HAP wheat.

Five feeding trials were undertaken using both pigs and poultry to demonstrate the potential reduction in phosphate excretion when HAP wheat was used in formulated diets. The animal performance was not as anticipated, with poultry showing an apparent increase in the availability of P when conventional wheat was fed but with a reduced total P content in the diet (as a result of reducing the amount of inorganic P added to the diet). However, the results were confounded by differences in the protein contents of the HAP and conventional wheat. Specialised diets were therefore produced to start understanding the potential mechanisms involved. The results demonstrated that under certain conditions, P excretion could be reduced in both pigs and poultry using HAP wheat. This can also be achieved (in poultry) using conventional wheat, and reducing the amount of dicalcium phosphate added to the diet. However, when this was done, the growth rate of the birds was reduced.

Using the data from our feeding trials it can be calculated that by replacing conventional feed with a low phytate alternative it would be possible to reduce P load to GB waters by 0.53% (321 tonnes P per year) and the agricultural contribution to the total P load to GB waters by 2.73%. As the low availability of phytate P is a feature of digestion in all monogastric animals, it could be envisaged that the development of HAP wheat would also have an impact on P excretion in both the industrial fish farming and human nutrition sectors.

Adapted wheat germplasm was developed using three different techniques. This has provided germplasm with several different mutations resulting in the reduction of phytate and potentially an increased uptake of phosphate. Within the period of the project, full characterisation has not been possible but adapted material has been developed. The development of the toolkit for marker-assisted breeding will improve the efficiency of this process.

There was no evidence for altered performance or need for altered agronomy with HAP wheat. This will need to be confirmed with the UK adapted material and the new mutations. However,

since phytate is associated with Fe, Zn etc., the micronutrient levels should be monitored when new germplasm is developed as changes were seen in the analysis conducted on the US variety.

Although significant advantages were shown with the use of low-phytate wheat (LPW) in this project the value of such a commercial development has been superseded by other technologies. The production cost and efficacy of phytase enzymes has improved, such the cost of adding these to formulated feeds for monogastrics is very cheap. In addition, the pig breeding industry has developed GM pigs modified to produce phytase in their saliva. The plant breeding industry therefore considers that the development of a commercial LPW variety would provide no competitive advantage and that the development of other traits would be a better use of their efforts.

However the lines developed in this program are still of interest in some third world countries where human diets are generally poor and changing the availability of micronutrients has significant health benefits.

2. SUMMARY

The purpose of this project was to reduce diffuse phosphate pollution through the production of new improved wheat lines. The consortium developed UK-adapted wheat germplasm with a lower phytate content in the grain, thus increasing bioavailability of phosphate to monogastric animals. This in turn reduced the level of phosphates entering the environment from animal wastes. The precise effect of changes in feed quality was determined through chicken and pig feeding studies using University of Idaho material. The consortium has started mapping the genes involved in this pathway to understand the plant physiology underlying changes in phytate content, together with related mineral nutrition. In addition to the original source of low phytate germplasm, a structured mutation population was evaluated to identify potential new candidates for low phytate lines. Finally, the net effect on the environment, industry and diffuse pollution has been modelled in this project.

The major form in which phosphorus (P) occurs in plants is myo-inositol-1,2,3,4,5,6-hexakisphosphate, commonly referred to as phytic acid, or InsP6; it is an important anti-nutritional factor to farm animals due to its ability to complex micro-nutrients such as iron and zinc. It occurs in different tissues but accumulates in high amounts in the seeds where it serves as a storage form of *myo*-inositol and phosphorus for utilisation during seed germination and seedling growth. Phytic acid biosynthesis in developing seeds is not very well understood although much progress has been made in understanding the biosynthesis pathway from the analysis of mutant lines exhibiting a low phytic acid (*lpa*) phenotype. Studies on maize, barley, rice and soybean in particular have shown that genetic lesions in several distinct gene classes can lead to lower levels of seed phytate.

Production of microbial-derived phytase, which can be added to the diets of pigs and poultry, is undertaken on a commercial scale as part of a feed enzyme market that is now extremely large. Exogenous phytase is an effective means of breaking down a proportion of the phytate in plant-derived feeds and thereby rendering the P, as well as other minerals and certain proteins, available to the animal. By using a careful balance of nutrients combined with phytase (together with other measures), the livestock industry in the Netherlands has halved the amount of P excreted by growing and finishing pigs over the last 20 years (Lenis and Jongbloed, 1999). However, these beneficial effects of microbial phytase are adversely affected by a high ratio of calcium: total phosphorus (Brady *et al.*, 2002). Since the calcium content of layer hen diets is high (and the Ca:P ratio is also high), microbial phytases would not be efficacious and so are not normally added to their ration.

Total pig output in the UK in terms of number of pigs is 9.5m/annum (down from 14+ m in 1998) and they consume 2.7 m tonnes of feed comprising 50–55% wheat. It is estimated that 9,720

tonnes of P₂O₂ is excreted by pigs each year in the UK. The impact that low P availability has on the total P contents in pig diets is illustrated in Table 1. Pig manure is generally utilised as efficiently as possible, however, where pig manure is spread, soil content of P has increase over a number of years. This is exacerbated by the 150,000 outdoor sows where waste output diffuses across land that is un-cropped for up to two years.

Table 1. The maximum potential loss of P from pigs to the environment in the UK.

	Kg Feed per pig produced (MLC 2004 Pig Year book)	Digestible P required g/kg (Whittemore <i>et al.</i> , 2003)	Total P supplied in typical diets g/kg.	Max. potential loss to the environment kg/pig
Sow feed	66.5	2.75	8	0.35
Weaner feed	48.5	3.4	9	0.27
Finisher feed	168.0	2.4	8	0.94
Total	283.0	NA		1.56

Following the removal of bone meal as a traditional source of P, there is now a greater reliance on adding phosphates in the form of dicalcium phosphate or the less digestible rock phosphates. An increasing number of supplement manufacturers include phytase in pig diets, which results in a combination of reduced total P in the diet and increased P availability (up to 60% available across a number of studies; Jongbloed *et al.*, 2004; Pallauf *et al.*, 1993; Hoppe *et al.*, 1993). Inclusion of phytases have been shown to reduce faecal output of P by up to 30% (down from 625 g P/pig finished to 428 g P/pig finished). These data indicate that, while exogenous phytases can significantly reduce P excretion by pigs, there is scope, by the use of high available phosphate (HAP) wheat in the diet, to further reduce diffuse P pollution from pig enterprises.

2.1. Programme of work

The work in this proposal was divided into 5 work packages; only after preliminary work in WP1 had confirmed that HAP wheat demonstrates the same value as seen for HAP maize and barley, was considerable effort in WP2-5 justified. These latter work packages developed commercial germplasm (WP3), markers for the breeding industry (WP4); determined the effects of P fertiliser treatment on the growth and phosphate metabolism of HAP wheat (WP5). Data from these investigations was used to develop a model (WP2) to predict and quantify phosphorous budgets in terms of metabolism in the animal and subsequent losses into the environment. The output from WP1&2 will be important in informing policy regarding measures to reduce pollution that can be used to offset livestock number reductions which may otherwise be required to effect reduced pollution reduction.

2.1.1. Work Package 1. Effect of HAP wheat on phosphorus excretion in pigs and poultry

Samples of HAP wheat were compared with conventional feed wheat in the presence or absence of exogenous phytase. Diets containing these wheats (and phytases) were fed to groups of monogastric livestock. In the first instance, broilers were used and this study informed the planning for other trials. Feed intakes, growth rates, feed conversion efficiencies and phosphorus balances were determined in each case. The effect of wheat type, added phytase and interactions between wheat type and added phytase were considered on each of these parameters. This was then used to define and initiate quantification of the potential value of HAP wheat for the industry in terms of the reduction in P excretion that it brings about. The University of Reading and Harper Adams University College led this work with technical assistance from Frank Wright, ABN and MLC.

2.1.2. Work Package 2. Develop a model for quantifying effect of HAP wheat on diffuse P pollution reduction.

The data from Work Package 1, was used to develop a model to predict phosphorus losses by pigs and poultry in the UK and estimate the contribution that HAP wheat may make (compared with exogenous phytase) in ameliorating these losses. This model took into account the relative cost of different amelioration strategies to enable the development of a decision support system that could be used for selecting appropriate strategies in different situations and be important in informing policy. The University of Nottingham and the Scottish Crop Research Institute led this work, with inputs from Harper Adams University College, The University of Reading and NIAB. The industry partners had a key input into this work package, including Anglian Water and the Environment agency.

2.1.3. Work Package 3. Development of Germplasm; Three types of material were developed in this work package for three distinct purposes.

- 1. Trait introgression:** In close co-operation with Limagrain UK Ltd (formally Advanta Seeds), a crossing program was undertaken to transfer the low phytate trait from the University of Idaho low phytate wheat line Js-12-Mu-6 into Northern European adapted material. Nine of the most competitive new lines from the Limagrain crossing programs and a small selection of established feed wheat varieties were used as parental material. This was backcrossed for three generations and the progeny assayed from the F₂ generation onwards to confirm the presence of the low phytate trait. By backcrossing with these elite lines we hoped to speed up the development of genetically uniform commercially competitive elite lines containing the low phytate trait.

2. **Development of a DH mapping population for the Js-12-Mu-6 HAP wheat:** one of the Js-12-Mu-6 x UK elite crosses described above was chosen for generation of a large (min. 200 lines) doubled haploid mapping population to facilitate the objectives of WP4.
3. **Development of a mutant population:** in order to prospect for new sources of reduced phytic acid, Paragon M6 seed stocks, developed through the Wheat Genetic Improvement Network (WGIN) and held at John Innes Centre (JIC), were assayed using the wheat flour colorimetric test. Candidates identified using the colorimetric assay have been partially characterised biochemically to determine possible effects on pathway intermediates and complexed ions.

2.1.4. **Work Package 4. Development of a toolkit for marker-assisted breeding of the HAP trait.**

The objectives of this WP were:

1. **To fine map the genes responsible for the low phytic acid phenotype of the Js-12-Mu-6 donor line** utilised in this programme. This activity was guided by pre-existing knowledge supplied by the University of Idaho, and screening for marker polymorphisms, facilitated by large marker sets available through ongoing wheat diversity and mapping work in NIAB and Limagrain.
2. **To characterise in detail the myo-inositol 3-phosphate synthase (MIPS) family of genes from the hexaploid wheat genome.** This provided markers to rule in or out candidates affected in the Js-12-Mu-6 and other mutant lines generated de novo in the programme.
3. **To isolate a panel of new HAP mutants from the EMS mutant population using phenotypic screens.** This work utilised a new mutant population in a high-yielding spring feed wheat background (Cadenza) for isolation of mutants with high HAP levels, which may offer different advantages and disadvantages for breeders as well as being able to test the hypothesis that particular MIPS genes are critical in conditioning overall phytate levels in the wheat grain. The mutant lines can then be characterised for MIPS gene expression levels, subjected to quantitative HPLC analysis of levels of InsP6 as well as the range of phosphorylated intermediates.
4. **To relate all new mutations identified to candidate genes and to each other.** This work involved sequencing candidate genes from a series of mutant lines to determine if there are suspect lesions that might explain those phenotypes.

Ultimately, the overall aim via the above tasks was to generate novel molecularly tagged phenotypic variation to underpin more effective breeding for HAP wheat. This work evolved significantly during the program due to the rapid changes in technology and new resources becoming available to the project.

2.1.5. Work Package 5. Determine the effects of P fertiliser treatment on the growth and phosphate metabolism of HAP wheat

Existing specialist field sites were used to study the growth and phosphate metabolism of the natural (spring-sown) low phytate wheat germplasm. The original spring-sown variety, from which the low-phytate germplasm was developed, was used as a control. Three replicate P-response gradients (P Indices between 3 and 9; MAFF, 2000) were established using broadcast triple superphosphate (TSP) in the low P-fertility Wharf Ground Field at Wellesbourne, the University of Warwick (Greenwood *et al.*, 2005).

Overall Conclusions

The feeding of LPW in combination with phytase enzymes had a significant additive effect in pigs. The increased availability of the plant derived phosphate, reduction the total excreted phosphate and the need for adding some of the phosphate to the diet. A model was developed to show the possible environmental benefits of this work.

New wheat germplasm was developed for this project and has been made available to the plant breeding industry. The detailed work necessary for the development of marker based selection requires further investment to complete the work. This is still technically quite difficult and expensive as the wet chemistry necessary for this work is still not completely reliable.

However, since this project started there have been other technical developments within the feed industry which have started to provide more cost effective solutions. These include the development of pigs with phytase enzymes in their saliva and the feed industry has taken advantage of cheaper and better phytase enzyme available for use in formulated feeds. There is also evidence that the availability of rock phosphate may be less critical as a finite resource due the development of new reserves.

Therefore, for plant breeders the development of LPW is no longer attractive as a solution for the availability of phosphate to monogastric animals or for the reduction in phosphate pollution. However there is still interest in effects of LPW on the availability of certain micronutrients as the human health benefits could be quite significant in certain regions of the world.