Project Report No. 197E

NUTRITIONAL VALUE OF WHEAT FOR POULTRY: ANALYSIS OF GENE EFFECTS USING ISOGENIC LINES

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NUTRITIONAL VALUE OF WHEAT FOR POULTRY: ANALYSIS OF GENE EFFECTS USING ISOGENIC LINES

by

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CONTENTS

1. Abstract

There is increasing emphasis on wheat quality from the poultry feed industry (who are one of the major end users of UK- grown wheat); poor nutritional quality will result in reduced performance, deteriorating litter conditions and downgrading. Recent developments in plant breeding have produced cultivars which are high-yielding with increased disease resistance and suitable for the quality bread and biscuit markets.

However, little attention has been paid to nutritional quality for poultry. In fact, it is suspected that some of the characteristics which have been bred into modern wheat cultivars are associated with reduced nutritional quality. Both plant breeders and, ultimately growers, are keen to secure markets for their wheat and programmes which seek to maintain quality are accordingly of value.

The report describes a programme of work which was designed to identify the nutritional consequences of key characteristics which are present in wheat. The approach adopted was to use ‘near isogenic’ lines which are those where the only difference is the characteristic under consideration. Thus a ‘check list’ of the effects of specific characteristics can be established.

A major part of the programme concentrated on the nutritional effects of the IB/IR translocation; clear evidence has been obtained which associates this characteristic with reduced nutritional value. The benefits to the industry (grower, plant breeder, poultry feed) are that it is now common to remove this feature for feed wheat breeding programmes, so ‘technology transfer’ from the current programme has been successful.

Further investigations examined the effect of endosperm hardness (with soft endosperm being associated with better quality, although there were interactions with endosperm texture) and the VPM eyespot resistance gene (no effect); degree of waxiness, presence of high molecular weight glutenins and grain colour have all been associated with changes to nutritional value.

2. Summary

The objectives of the experimental programme were to evaluate the nutritional consequences of the introduction of specific genetic features into wheat lines. Individual genes were evaluated using paired near-isogenic lines, where the characteristic under examination was either the only major variable between the two lines within a pair, or was present in a defined background. This is a much more precise methodology than the reliance on evaluating cultivars by name only (which has been a common, but ineffective, approach in the evaluation of the nutritional quality of wheat) which will not allow conclusions to be drawn on the effects of developments in plant breeding.

The approach adopted provides information of value to the breeder, nutritionist and, ultimately, the grower in making informed decisions on further developments in plant breeding, nutritional evaluation and choice of cultivar to grow.

The general conclusions from the current programme were:

- The negative impact of the IB/IR translocation on nutritional quality has in general been confirmed.
- The influence of endosperm texture indicates that the characteristic 'soft' is associated with a superior nutritional value.
- Interactions between IB/IR and endosperm texture indicate, however, that there are situations where the effects are not as predicted, although a combination of 'IB/IR Yes (Present)' and 'Hard Endosperm' did give rise to the lowest nutritional value as might have been thought.
- Endosperm texture further interacts with protein content and quality.

- There are no adverse nutritional consequences of the presence of the VPM eyespot resistance gene Pch1.

- Wheats giving a characteristically more extensible dough are not associated with a reduction in nutritional value.

- The presence of high molecular weight glutenins (as evaluated with Sicco isogenic lines) may be of nutritional significance.

- Degree of waxiness of the endosperm determined by the lowering of amylose content is of nutritional significance.

- Grain colour may give rise to variability in nutritional value.

- Agronomically, wheat grown first in a rotation has a superior nutritional value.

Overall summary figures which describe responses to those characteristics which were evaluated in more than one trial (please see text for explanations) describing both the content of digestible component (e.g. starch / amino acids; for the latter, these are grouped into those which are nutritionally essential and those which are not) and the coefficients of digestibility are presented in subsequent pages.

Each figure identifies the characteristic(s) under consideration, allowing a clear picture of their nutritional effects.

General summary of specific characteristics

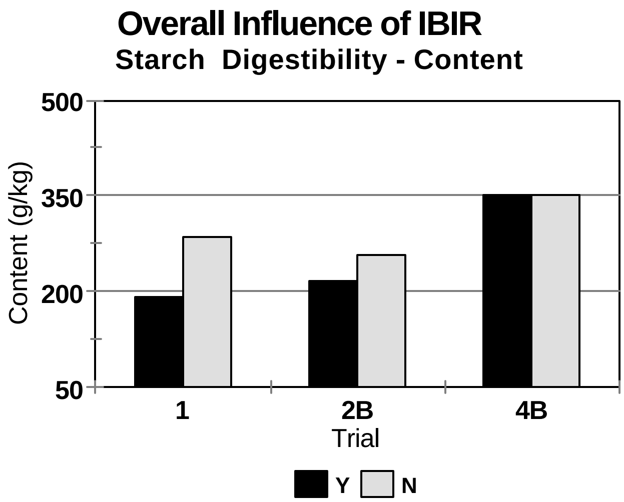
These are presented in figures for the major characteristics that have been associated with variability in nutritional value, including **IB/IR**, **endosperm texture** together with the interaction between **endosperm texture * Protein** over trials (whose number is indicated either within the graph as a label or as an axis title, allowing reference back to the trials in question).

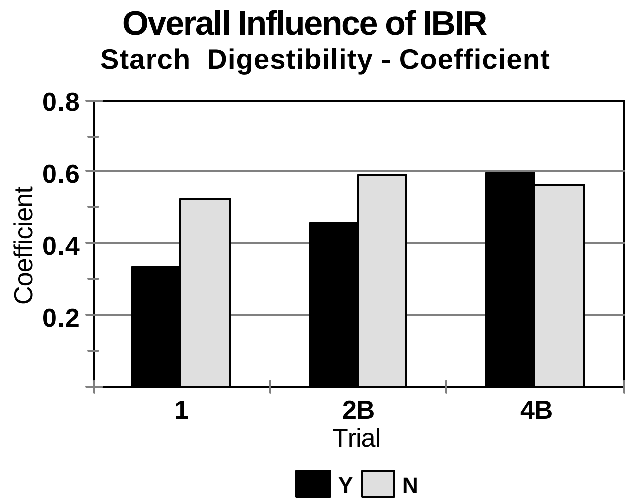
With respect to **IB/IR**, it is evident that it is associated with negative nutritional value, but that its effect may be compensated for by other characteristics, particularly endosperm texture. When considering **endosperm** texture itself, there is evidence that soft leads to higher nutritional value, although not in all cases.

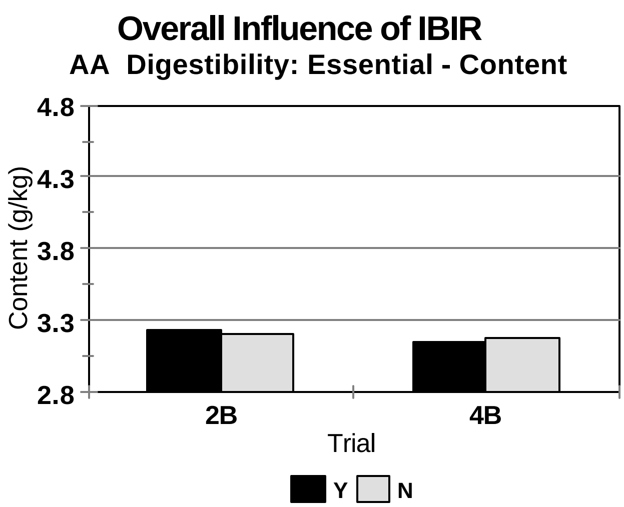
The **endosperm * IB/IR** interaction is seen clearly when comparing 4A and 4C - IB/IR Yes + Hard endosperm is, as would be predicted, of lower value whereas the other three combinations were higher and similar. A further interaction is between **endosperm * protein** from which it may be concluded that the effects are not in a predictable fashion.

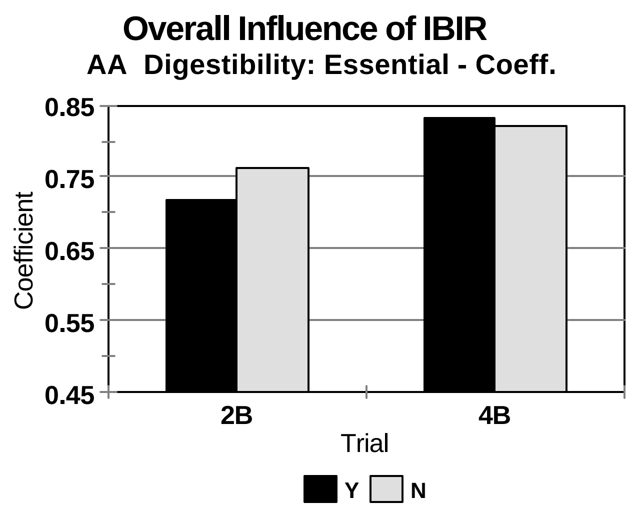
The data presented will be of value to plant breeders who will now be able to breed cultivars of better nutritional value. The poultry feed industry have already embarked on quality control procedures and are identifying those cultivars with which they have greater confidence. The levy payer will benefit in terms of greater awareness of the importance of nutritional quality and decisions on which cultivars to grow in order to secure market outlets for their crops.

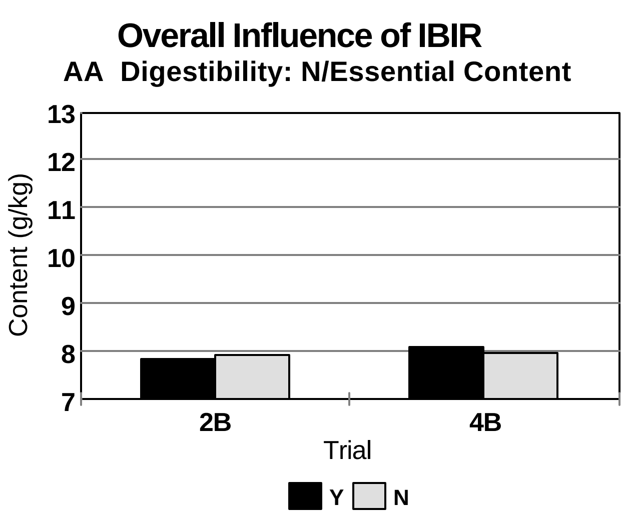
Overall Summary Figures: Effect of IB/IR

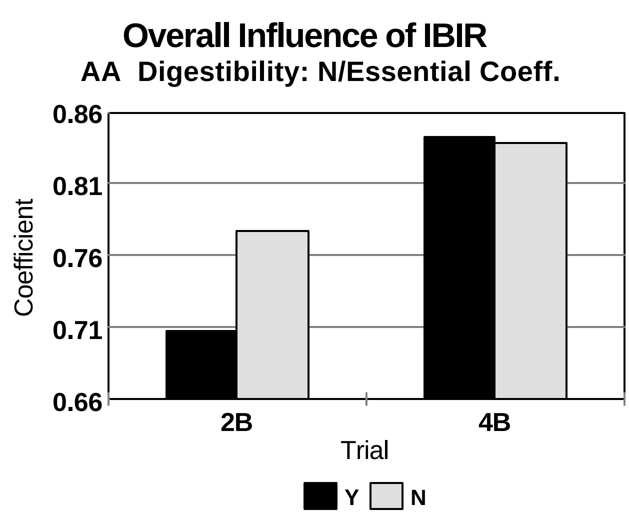




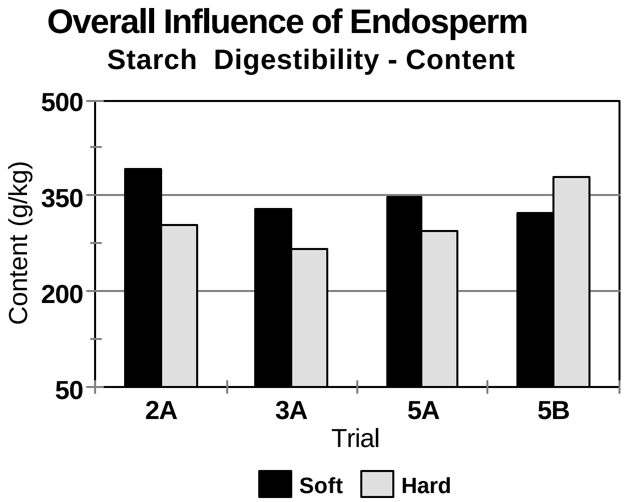


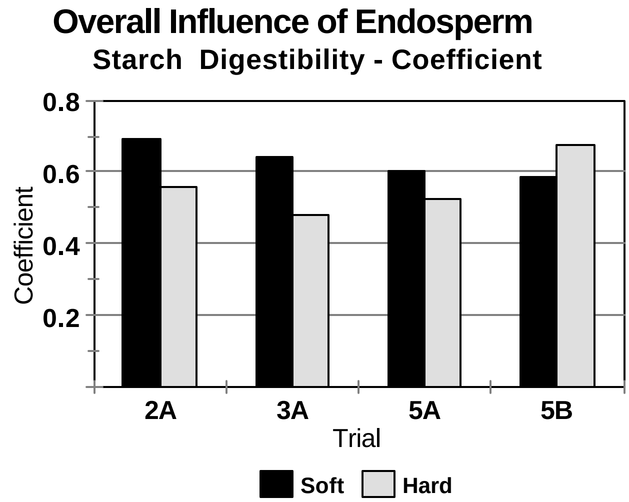


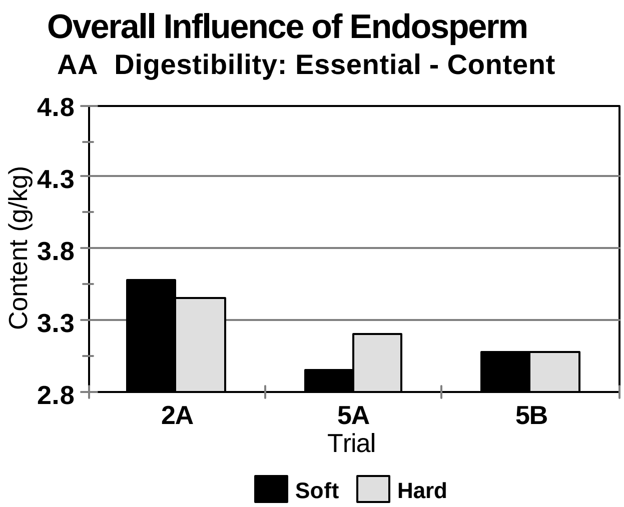


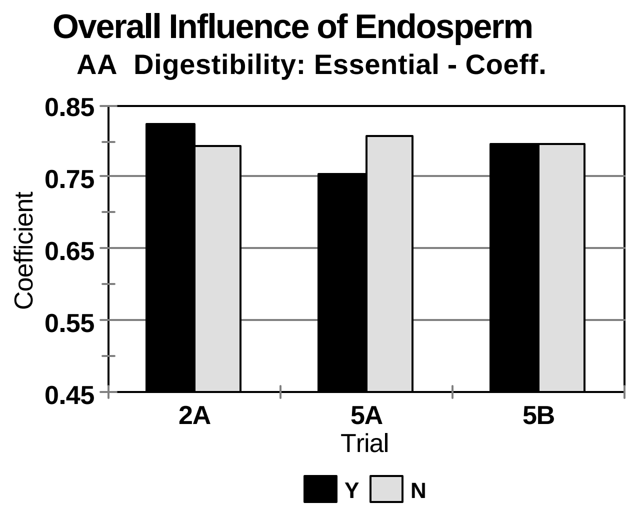


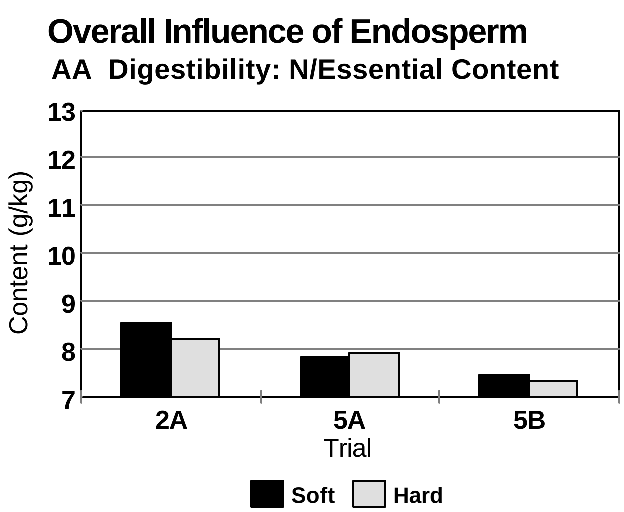
Overall Summary Figures: Effect of Endosperm Texture

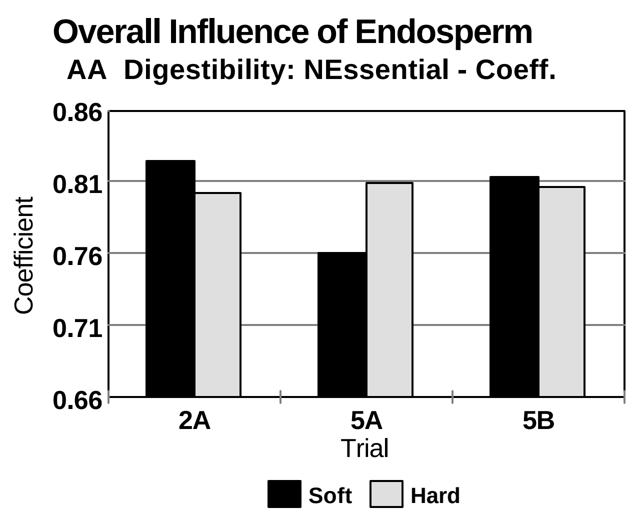




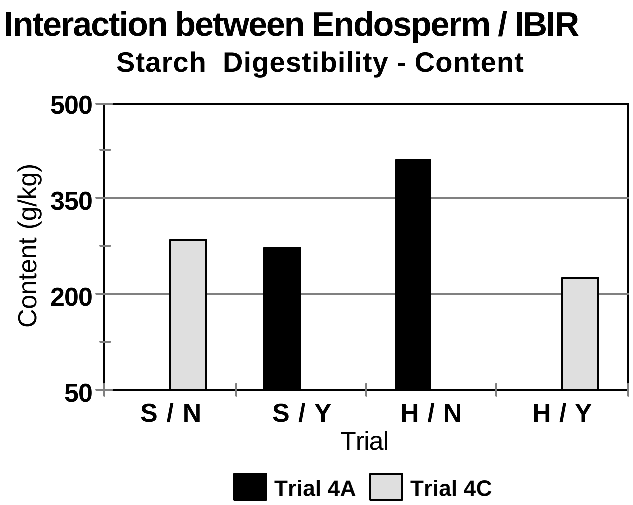


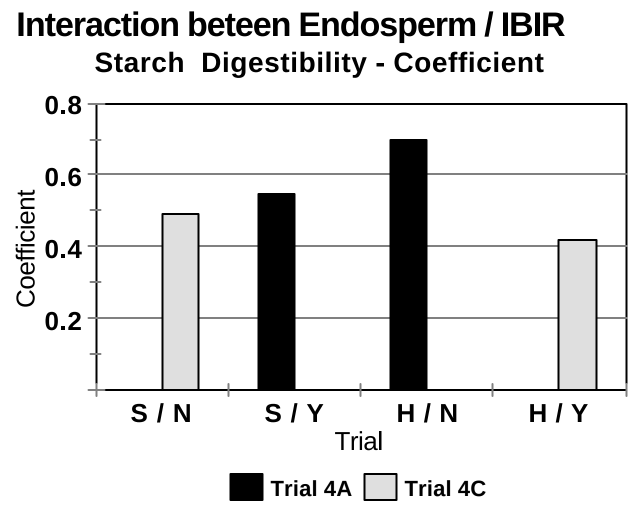


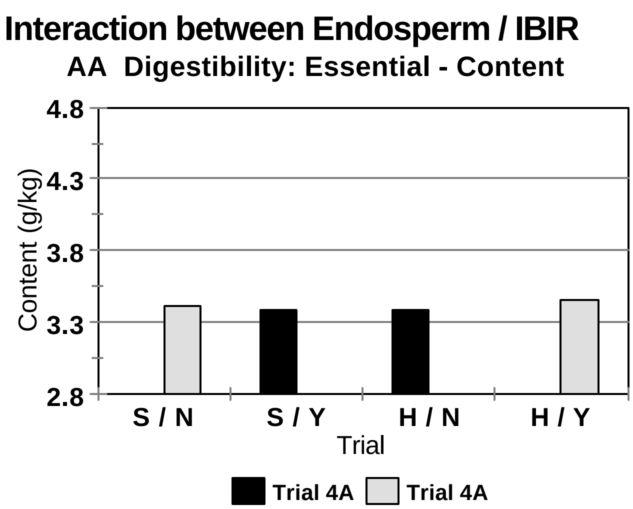


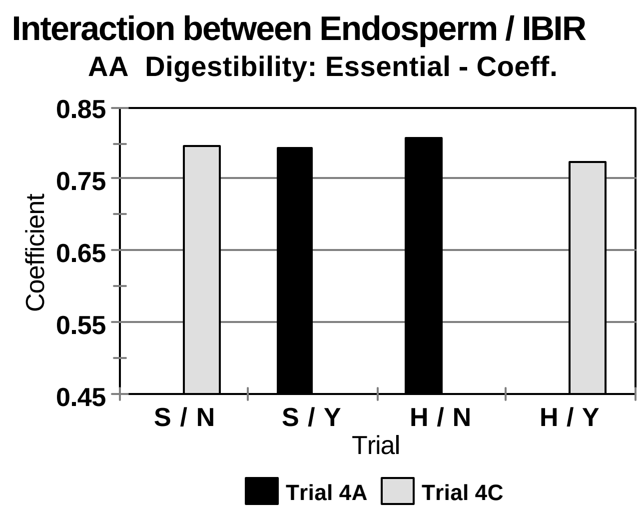


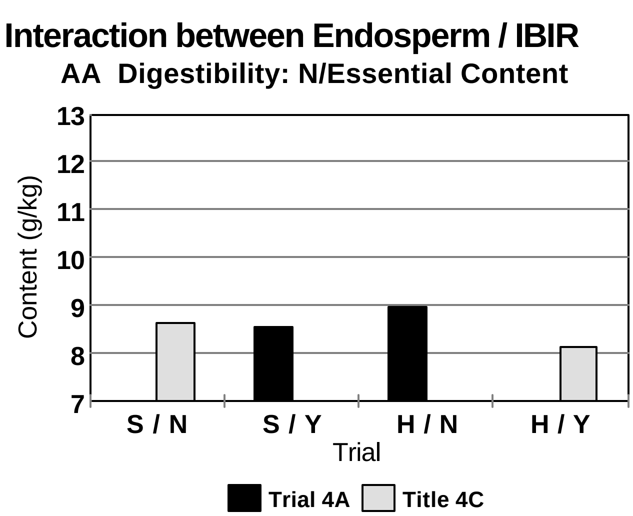
Overall Summary Figures: **Effect of Endosperm Texture * IBIR**

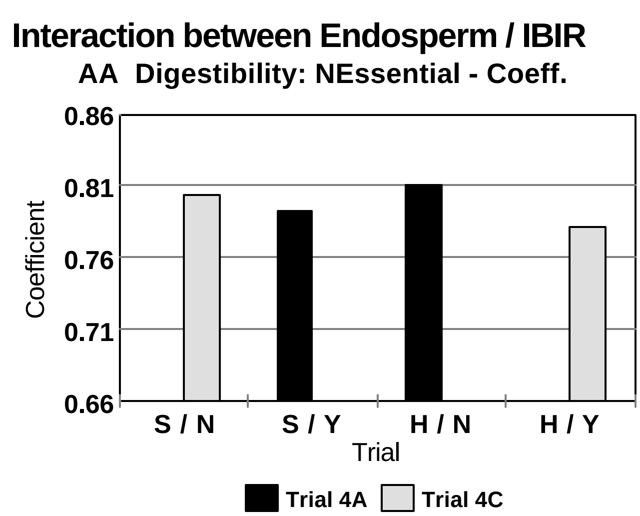






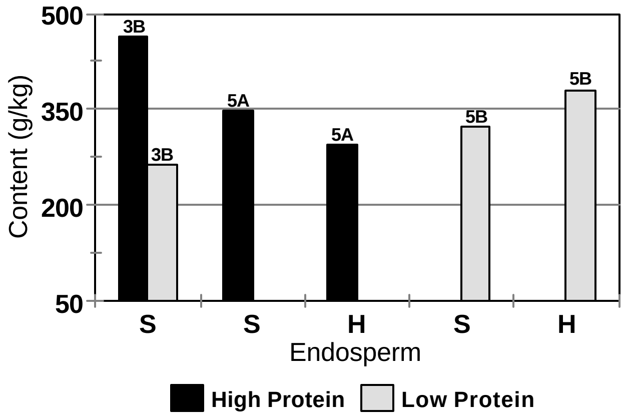




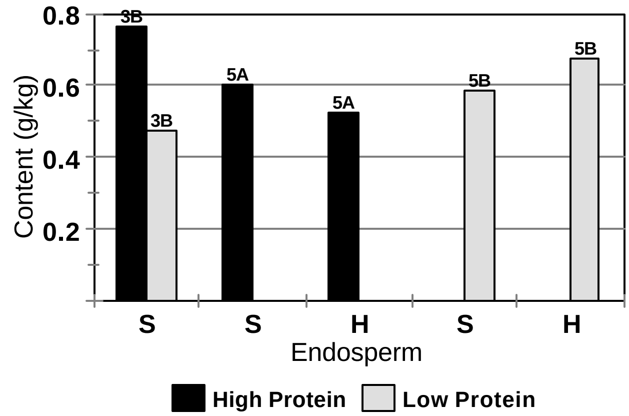


Overall Summary Figures: Effect of Endosperm Texture * Protein interactions

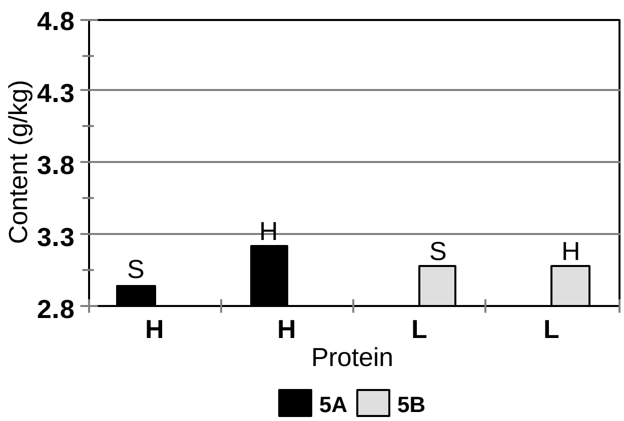
Interaction Endosperm / Protein
Starch Digestibility - Content



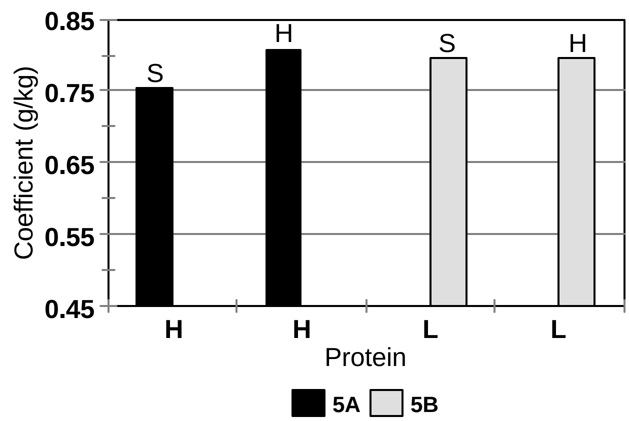
Interaction Endosperm / Protein
Starch Digestibility - Coefficient



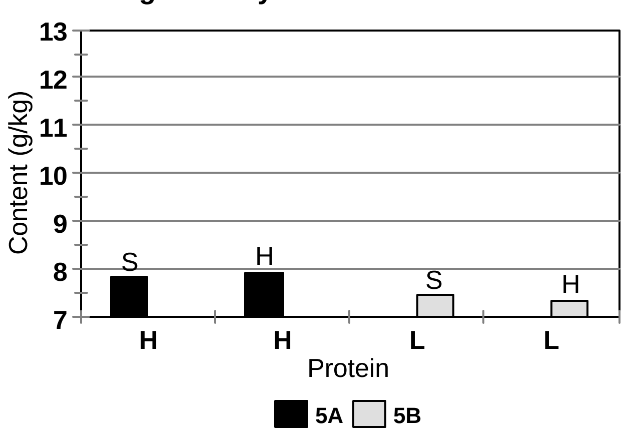
5A/B - Endosperm and Protein
AA Digestibility - Essential: Content



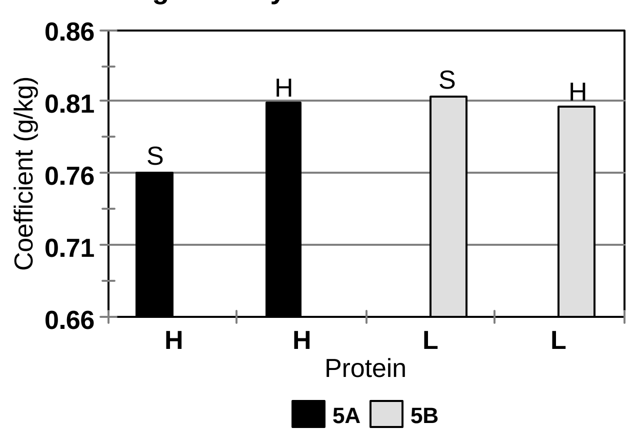
5A/B - Endosperm and Protein
AA Digestibility: Essential - Coeff



5A/B - Endosperm and Protein
AA Digestibility -N/Essential: Content



5A/B - Endosperm and Protein
AA Digestibility -N/Essential: Coeff



3. Scientific Report

3.1. Introduction - Importance of wheat to animals and variability in its nutritional value

The animal feed industry is the largest user of wheat in the UK utilising 5.5 million tonnes of wheat from an annual wheat harvest of 16 million tonnes’; poultry represent the biggest market for feed wheat (over 50%) followed by pigs. Ruminants are a comparatively minor component of the wheat feed market. However both breeders and the feed industry have paid comparatively little attention to the nutritional value of wheat for animal feed. This is in stark contrast to other end users; thus the bread and biscuit making sectors have determined exacting standards for use, been highly influential in establishing which varieties should be grown (together with advising on agronomic inputs) and have, ultimately, been concerned with development of those varieties necessary for their industry.

It is now generally regarded that wheat is of variable nutritional value for poultry (e.g. Wiseman and Inborr, 1990), whilst precise reasons for this remain to be established. Although wheat is considered essentially as the major source of apparent metabolisable energy (AME) in poultry diets it may also make a significant contribution to amino acid requirements. Recent studies (e.g. Short et al., 1999, 2000) from programmes supported by H-GCA have demonstrated that amino acid digestibility is also variable between wheat samples.

Poultry are also particularly sensitive to variations in dietary inputs, thus making them an ideal model for investigating nutritional issues. The relative ease and speed of poultry studies means that data emerging from them may be rapidly disseminated.

A considerable amount of work has been undertaken on assessing nutritional value of wheat in attempts to identify controlling genetic factors. A common approach has been that based on the evaluation of a large number of named cultivars and, then, an attempt to relate data obtained to specific characteristics (e.g. endosperm texture).

However, this approach is basically flawed (as pointed out by Wiseman et al 1996, in a report for H-GCA) as varietal comparison alone is of no value in evaluating genetic potential or otherwise of specific cultivars; varietal comparisons confound the effects of thousands of genes and do not lead to clear genetical interpretations of the value of specific features.

A further observation from previous studies on the nutritional value of wheat (e.g. Wiseman and McNab 1993, in a report for H-GCA, Project number 111)) is that environmental effects may be important. Thus evaluation of nutritional quality must be undertaken with samples grown at the same location under identical conditions unless agronomic features (such as nitrogen fertiliser, place in the rotation) are experimental variables. Obtaining wheat samples for evaluation without regard to location of growth (as would happen if samples were obtained from various regions) is not therefore appropriate.

The major development in understanding the genetics of the nutritional value of wheat has been the use of near isogenic lines. These are lines that can be developed by cross-pollination and backcrossing which are, in principle, identical to the original starting variety except for one key characteristic transferred from a ‘donor' line. Evaluation of the influence of a particular characteristic can therefore proceed in the absence of any other variable.

This led to the observation at the University of Nottingham that the 1B/1R translocation and hard endosperm texture were probably having a negative influence on the nutritional value of wheat for broilers (e.g. Short et al., 2000) in a programme financed by H-GCA (Project number 111).

These observations, however, required confirmation (i.e. the presence of 1B/1R and endosperm texture in various genetic backgrounds) which were two of the objectives of the current collaborative venture; other objectives were the assessment of other characteristics (described below).

The overall objectives of the current programme were an assessment of the nutritional consequences of specific genetic characteristics evaluated with isogenic wheat lines grown under the same conditions (except where agronomic variables were assessed) at the same location.

The intention was to be able to make recommendations to plant breeders as to the value of these characteristics and, ultimately, to be able to advise the grower on those cultivars best suited for the animal feed market.

The fact that one named cultivar has been retained within the NIAB official recommended list (as a result of the current programme) purely on the basis of its nutritional value is an indicator of the success of the approach adopted and also the speed with which information has been disseminated.

3.2 Methods and materials

3.2.1. Developments in plant breeding and production of wheat lines for nutritional evaluation

Wheat is genetically quite complex, being a synthetic species which evolved by the natural hybridization of three wild grasses - *Triticum uratu*, *Aegilops speltoides* and *Triticum tauchii*. Since each of these species had 14 chromosomes, bread wheat, *Triticum aestivum,* is a polyploid species having 42 chromosomes. However, the three ancestors were themselves related so that, effectively, wheat has three sets of related genes.

Current molecular biology studies suggest that wheat has between 25,000- 30,000 unique genes, but only very few of these have so far been identified and their effects understood. The challenge for the future is to identify all the genes that go into making up a wheat plant so that new varieties with high yield, good adaptation, disease resistance, stress tolerance and specifically adapted to a particular end-use, can be bred by plant breeders in a defined way.

The emergence of bread wheat has only occurred in the last 10,000 years, but nevertheless wheat varieties have evolved over this time to be genetically quite variable. This variability extends to end-use and this is clearly illustrated in the classification of varieties into good and poor bread-making, or biscuit making. Several genes controlling these latter attributes have been identified, such as genes controlling protein quality, particularly the high-molecular weight glutenin proteins, and the genes controlling grain hardness.

These discoveries have enabled plant breeders to assemble specific combinations of genes in a defined way to help secure good bread-making quality. However, much less is known about the genetical control of one of the most important other end-uses, namely nutritional quality for animals. Indeed, until recently, very little research had been carried out to identify if there are any specific positive or negative traits that have been introduced by plant breeding in recent years that affect nutritional value. It has been suggested that certain varieties may posses traits that make that variety beneficial in this context, but most of the evidence is circumstantial, and hard data have not been forthcoming. The major reason for this is that the very many genetic differences encountered when comparing named varieties, and the variable effects of environment, often obscure the small number of genetic differences that directly affect quality.

Consequently, an alternative approach to the analysis of genetic influences on nutritional value was necessary; rather than testing named varieties (which may or may not be closely related - name alone is no guide to genetic association) it is more advantageous to evaluate specific qualities within a variety, and evaluate the effects of known, specific genes which, *a priori*, should affect quality.

This is the approach that has been taken in the present investigation, where lines differing by single attributes, so called isogenic lines, have been evaluated.

3.1.2.1. The development of isogenic lines

The development of isogenic lines enables a chosen variety to have, through selective breeding, a single directed change made to its genetic makeup. Isogenics are developed by conventional cross-breeding and backcrossing, where known genes are transferred from one variety, the donor, into the genetic background of another, the recipient.

Sometimes extensive backcrossing is not needed, as advanced sib-lines in a breeding programme can be almost identical, but segregate for a single defined genetic trait. In this case, contrasting near-isogenic lines can be identified in the progeny of such plants directly.

The procedure to develop an isogenic line by backcrossing is shown in Figure 1. Isogenic lines are genetically stable and can therefore be multiplied in the same way as a conventional variety and grown in different environments and tested for different attributes. Any phenotypic difference observed between the isogenic line and its recipient variety must relate to the specific gene transferred. In this way, the genetic effects of any known gene can be precisely defined.

Different genes can be transferred into the same variety so that different alterations are then in a standard background so that a precise comparison can be made of the effects of different genes on a particular trait.

In the series of experiments described in this report, isogenic lines were developed to evaluate the effects on nutritional value for animals of some of the major genetic changes introduced into the UK wheat crop over the last decade. The last twenty years of breeding has seen the introduction of new genes from exotic sources whose effects on agronomic performance as well as quality are, to date, ill defined. Prime examples are:

a. Introduction of 1BL/1RS (rye chromosome arm) chromosome translocation
b. Introduction of semi-dwarf varieties, utilizing the *Rht1, Rht2* genes
c. Change of feed varieties from soft-milling to hard-milling varieties, utilizing the *Ha* gene
d. Introduction of new grain protein genes from Mexico (Moulin), France (Mercia)
e. Introduction of photoperiod insensitivity utilizing the *Ppd-D1*
f. Introduction of alien disease resistance, e.g. eyespot resistance gene, *Pch1*, from *Aegilops ventricosa* (VPM resistance).

Several of these changes are, or could be expected to, affect end-use quality in a positive or negative way. For example, the new grain proteins improve bread-making quality, whilst the 1BL/1RS chromosome results in sticky dough, either due to the presence of the rye secalins on the 1RS arm, or the absence of the wheat gliadins and low-molecular-weight glutenins known to be missing on the 1BS arm. *A priori*, several of these changes in grain constituents would also be expected to affect nutritional value.

The lines developed and multiplied up at the John Innes Centre (JIC) for the nutritional programme at Nottingham are described below:

3.1.2.2. Hard/soft endosperm texture isogenics

Lines were developed at the John Innes Centre to investigate the effects of hard and soft endosperm texture in wheat based on the genetic difference between the varieties Hobbit sib (soft) and Avalon (hard). Genetic data at JIC had shown that the major component of this difference is due to the gene *Ha* on the short arm of chromosome 5D of Avalon which makes it hard. Thus, this gene was transferred by backcrossing into the genetic background of Hobbit sib to create hard isogenic lines of this variety. Four independent hard lines and four soft controls were developed and identified using NIR techniques.

Seed of all lines were multiplied up in the field under normal agronomic conditions, and investigations were also carried out into the effects of normal and high protein (nitrogen) application. For this latter experiment, the eight lines were split into six plots of each. Three plots of each hard and soft endosperm texture isogenic received normal nitrogen application and three received high nitrogen with 425kg (of 34.5% nitrogen) being applied as a standard dressing to both treatments per Ha, and a further 138kg / Ha was then applied to treatment two as a high application rate.

No apparent differences were noted between the treatments during the growing season and this was reflected in the harvest data. The 'normal protein' plots yielded 99.83 kg of seed in total whilst the high protein plots gave 100.71 kg of seed (0.87% difference). Protein analysis results did, however, show significant differences after data were obtained through NIR analysis. The highest protein content was found in the hard lines with high protein application. Lowest protein content was found in the soft lines with normal protein application (see Table A).

Figure 1. Schematic diagram of the development of isogenic lines

Table A. Protein values (g/kg) of hard / soft endosperm textured genotypes grown under high and normal nitrogen application :

		Isogenic line (H=hard, S=soft endosperm texture)							
		1	2	3	4	5	6	7	8
Protein Application	Replicate	H	H	H	H	S	S	S	S
Normal	1	85	90	91	91	77	83	82	73
	2	97	83	95	91	85	85	73	74
	3	93	95	82	91	73	86	81	73
	Mean	92	89	89	91	78	85	79	73
		90				79			
High	1	94	93	93	94	83	82	85	83
	2	97	98	94	93	85	81	82	91
	3	89	95	95	89	83	81	74	81
	Mean	93	95	94	92	84	81	80	85
		94				83			

Hardness scores (on an arbitrary scale using a local calibration) were also obtained using the NIR analyzer and these confirmed the genotypes of the hard and soft lines unambiguously (Table B). These results also indicate that a pleiotropic effect of the *Ha* gene is to give a significant ($P < 0.001$) increase in the protein content of the grain. Additionally, an increase in grain protein content increases the hardness score of the grain. Thus, these isogenics are simultaneously testing the effects of endosperm texture and protein content on nutritional quality, although the different nitrogen treatments will also separate these effects.

These wheats were evaluated in trial 5.

Table B. Hardness values (arbitrary scale) of hard / soft endosperm textured genotypes grown under normal and high nitrogen application (high score = hard, low score = soft)

		Isogenic line (H=hard, S=soft endosperm texture)							
		1	2	3	4	5	6	7	8
Protein Application	Replicate	H	H	H	H	S	S	S	S
Normal	1	159	157	206	152	1	1	39	-19
	2	193	182	199	189	17	-3	-22	5
	3	172	222	152	183	-15	13	23	-4
	Mean	175	187	186	175	1	4	13	-6
		181				3			
High	1	202	203	217	178	47	8	50	23
	2	206	245	204	198	27	16	8	52
	3	197	188	212	183	42	-2	0	17
	Mean	202	212	211	186	39	7	19	31
		203				24			

3.1.2.3. 1BL/1RS translocation (commonly referred to as IB/IR)

One of the most important changes to the genetical constitution of UK winter feed wheats over the last twenty years has been the introduction of the 1B/1R translocated chromosome. This chromosome is different from the equivalent ‘normal’ wheat chromosome, termed 1B, in that the short arm has been replaced by the short arm of rye chromosome 1R. This translocated chromosome can be visualized by the technique of ‘chromosome painting’, as illustrated in Figure 2 where the rye DNA fluoresces a different colour from the wheat DNA because of differential staining. This translocated chromosome is suspected to have arisen originally in German varieties by the spontaneous and natural hybridization between wheat and rye and subsequent selection by breeders because it imparted disease resistance from genes present on the rye arm.

Several high yielding UK feed wheats carry this translocation, for example Brigadier, Hussar, Beaver, Hunter, Haven, Savannah and many of the modern crosses that UK plant breeders make incorporate this germplasm. Thus, advanced sib lines derived from the cross Hunter (with 1BL/1RS) x Buster (lacking 1BL/1RS) were available at Nickersons Seeds, which are very closely related, apart from the presence or absence of the translocation. These were multiplied up in field trials to provide the seed for Nutrition Trial 2B.

Additionally, in preliminary experiments, characterized varieties which reflected these changes in variety profiles were evaluated in Nutrition Trials 2 and 4

Variety	**Hard/ Soft**	**1B/1R**
Hunter	Soft	Yes
Brigadier	Hard	Yes
Riband	Soft	No
Buster	Hard	No

Figure 2 Visualization, by the technique of 'chromosome painting', of the different colour of rye DNA which fluoresces differently from wheat DNA

3.1.2.4. The effect of grain colour

Grain colour can vary between different cultivars of wheat, and red-grain is the result of anthocyanin pigmentation in the pericarp. Anthocyanins could have anti-nutritional properties. Grain colour is simply inherited and is determined by single major genes *R-A1, R-B1* and *R-D1* on chromosomes 3A, 3B and 3D, respectively. The presence of any one gene results in red grains, whilst the absence of all three results in white grains. However, red-grained varieties may have one, two or all three genes. Of 93 red grained wheats representative of the UK gene pool which were tested for R gene constitution, 36 carry a single R gene, 39 carry two and 18 carry all three (Evers and Flintham 1995). No grain colour isogenics based on a UK wheat were available at the beginning of the project, but suitable material was available from a backcrossing programme involving different *R* genes in a Russian white spring wheat background. The variety, Novosibirskaya 67 and the five isogenic lines ANK-1A, ANK-1B, ANK-1C, ANK-1D and ANK-1E were developed by Dr. S. F. Koval at the Institute of Cytology and Genetics Novosibirsk (Koval 1988) and are curated at JIC by Dr F E Flintham. Each isoline carries a single *R* gene from a different red grained donor variety and was selected through a series of nine backcrosses to Novosibirskaya 67.

The six lines were bulked up in field trials to 10 kg for use in Nutrition Trial 7

3.1.2.5. High molecular-weight glutenin proteins

The high-molecular-weight (HMW) glutenins are a major component of the seed storage proteins of wheat. Their inheritance is now well characterized and they are determined by genes on the long arms of chromosomes 1A, 1B, and 1D. Each locus determines the structure of two subunits, the x and y subunits, and several different alleles at each locus have been identified. Further, it has been shown that possession of certain alleles, for example, 5 +10 on chromosome 1D, results in good bread-making properties, and the alternative 2+12 allele in poor bread-making quality, because of the specific structure of the particular proteins they encode. These proteins are thus critical in wheat quality and the different alleles could be expected to be have an influence on nutritional quality. To evaluate this, a series of existing isogenics for different HMW glutenin alleles were multiplied up for evaluation and a series of new isogenics developed.

3.1.2.6. Existing Sicco isogenics

A set of isogenics based on the spring wheat variety Sicco was originally developed by Payne (1987) for their effects on bread making quality and particularly the property that glutenin has on the elasticity of doughs. The lines produced were characterized for differences in their protein banding profiles for the HMW glutenins using sodium dodecyl sulfate (SDS) polyacrylamide-gel electrophoresis (PAGE) as described by Payne (1981b), except that mercaptoethanol was replaced by dithiothreitol. Donor cultivars were used to incorporate varying HMW markers by crossing and backcrossing for five generations. Near-isogenic lines and their corresponding controls were extracted from progeny of the last backcross and selfed to produce foundation grain for multiplication. The three identified lines differed from the control cultivar of Sicco by the presence of different, characterized, alleles at the *Glu-A1, Glu-B1* and *Glu-D1* loci. These are found on chromosomes 1A, 1B and 1D respectively, and in Sicco are represented by the alleles called 1, 7+9 and 5+10.

The 1B isogenic lines developed differed by the inclusion of band 8 (from Chinese Spring), replacing 9 to give an isogenic with bands1, 5+10 and 7+8. This line was labeled Sicco 7+8 isogenic (1B). In another line, bands 2+12 (from Chinese Spring) replaced 5+10 giving an isogenic with 1, 2+12 and 7+9, which was labeled Sicco 2+12 isogenic (1D). In the third line, the 5+10 bands were removed (null identified from a Nap Hal x Gabo cross), to result in a null line having only bands 1 and 7+9, which was labeled Sicco Null 5+10 (also 1D). A further null line was developed which only had bands 5+10 and 7+9, labeled Null 1A isogenic, to complete the set.

A example of a characterization gel is shown in Figure 3. This type of analysis unambiguously identified the three individual lines, which differed from the control lines of the variety Sicco itself. Isogenic lines and their controls were then bulked up.

Figure 3 SDS PAGE showing Sicco Null Isogenic Line recombinants Null 1B (markers &=*) * Null 1D (Markers 5+10)

To ensure against cross pollination, single plants were first grown under glasshouse conditions and their ears bagged before pollination. Later, in field plots, the grain was bulked to gain the required 10 kg for nutritional evaluation

These Single Isogenic lines were assessed in Trials 6 and 7.

All of the above genetic stocks were evaluated in nutrition experiments. However, because the pace of germplasm development exceeded the rate at which these trials could be carried out, several additional genetic stocks were developed and multiplied in the germplasm development programme of the Project, but not tested for nutritional value. These are :

3.1.2.7. New multiple Sicco isogenics

Using the Sicco isogenics described above, inter-crosses were carried out between the single isogenic lines to move towards producing an orthogonal set of single, double and triple isogenic lines for the pairs of HMW sub unit alleles evaluated. Sicco 1B (7+8) was intercrossed with Sicco 1D (in the form of both 2+12 and Null 5+10). The F_1>s produced were grown and selfed, and the F_2 grains were checked for the identification of homozygous double isogenics. This was carried out by dissecting the grains and using the embryo halves for germination, whilst the distill halves were screened by SDS PAGE for genotype. Homozygous lines were identified, selected, grown to maturity and seed sown into field plots for multiplication. A further crossing programme using the Sicco null 1A lines from Payne (1987) were also started a generation behind the previously described material.

3.1.2.8. Effect of alien HMW glutenin subunits

The wild relatives of wheat carry novel genetic variation that could be useful for modifying the end-use quality of wheat. In particular, the A genome diploid wheat species carry novel HMW subunits, since they have two subunits common on chromosome 1A, rather than only the x subunit in cultivated bread-wheats. Two alleles were introgressed into Sicco from two accessions of the diploid wheat species *Triticum boeticum* Boiss. ssp *thaoudar.* The alleles, *Glu-A1r* and *Glu-A1s*, were transferred which encode the high molecular weight subunits 39+40 and 41+42, respectively. The material was developed originally by Rogers (1997) to assess the bread making quality of the alleles. These were bulked in Summer 1999 in anticipation of further nutritional evaluation.

These extra subunit lines were also intercrossed with the original Sicco isogenics from Payne to produce a novel series of recombinants :

Glu A1r x Null 1A, Glu A1r x Null 1B, Glu A1r x Null 1D, Glu A1r x (Null 1B x Null 1D)

Glu A1s x Null 1A, Glu A1s x Null 1B, Glu A1s x Null 1D, Glu A1s x (Null 1B x Null 1D)

All these lines will be multiplied further for possible future experiments.

3.1.2.9. Effects of differences in grain starch composition

The endosperm starch of the wheat grain is composed of two different forms of polymer, amylose and amylopectin. Low amylose content is considered to improve the final texture and quality of flour (Miura 1994). Through the development of precise genetic stocks involving the the spring wheat, Chinese Spring (CS), and substitution lines of chromosomes from the winter wheat Cheyenne (CNN) into CS, it was found that a major decrease in amylose occurred when chromosome 4A replaced 4A from CS and it increased when chromosome 7B from CNN replaced 7B from CS (Miura 1994).

Amylose contents for thge lines used in trial 6C are taken from results obtained from Miura (1994). Mean deviations for amylose content were investigated by the growing of the 21 Chinese / Cheyenne substitution lines over three years of winter-sown trials along with Chinese Spring itself. These were then evaluated for amylose content. The recipient parent Chinese Spring showed 21.01mg amylose / 100mg starch granules. The substitutin lines CS (CNN) 4A deviated by -1.25mg (24.83mg amylose / 100mg starch). The lines showed greatest deviation from the Chinese Spring over the three years and were selected for bulking for nutritional evaluation.

Through genetic manipulation it is possible to manipulate starch composition in wheat. It was thought useful to establish whether these differences in starch composition could be of nutritional importance. To evaluate this, Chinese Spring (Cheyenne) chromosome substitutions involving 4A and 7B, which differ from the recipient variety in amylose / amylopectin ratio were bulked, along with Chinese Spring itself, through single plants in the glasshouse and then on to field plots, to enable the required weight of 10 kg for nutrition trials (trial 6) to be produced.

3.1.2.10. Waxy wheat development

A waxy wheat occurs when amylose content is zero. The production of amylose is encoded by enzymes termed the granule-bound starch synthases, present on chromosomes 7A (*Wx-A1*), 4A (*Wx-B1*) and 7D (*Wx-D1*). Null alleles exist for each gene, and consequently it is possible through selective breeding to remove all three genes and stop the production of amylose. A programme was devised to develop a full waxy wheat using the varieties Bai Huo (null *Wx-D1*) and Kanto 107 (null both *Wx-A1* and *Wx-B1*) as donor varieties of the null alleles and the UK varieties Mercia and Hobbit sib as recipients.

A chromosome substitution line development procedure was used to develop these lines. Monosomics were selected cytologically for both Mercia and Hobbit sib chromosomes 4A, 7A and 7D, and Kanto 107 and Bai Huo were then crossed onto the monosomics . Resultant grain was then checked again for being monosomic. Backcrosses were then made recurrently with the Mercia and Hobbit sib monosomics to produce the substitution lines. At each backcross stage, grain was checked using electrophoresis involving a recently developed SDS PAGE technique to identify the null alleles in the programme. Currently, the programme is at the third backcross stage. When backcrossing is complete the three separately developed null lines will be intercrossed and the triple null isolated, which will be a completely waxy wheat for possible future experiments. Waxy wheats were evaluated in trial 6.

3.2.2. Basis of poultry nutrition trials

Full details of the methodologies adopted are given in Wiseman et al (1998) and Short et al (1999).

Wheat is included into a semi-synthetic diet at a rate of 375 and 750g/kg as the only protein / amino acid- containing component; other dietary constituents are oil (50g/kg to reduce dust and increase palatability), vitamins and minerals in a premix (50g/kg). The total amount is made up to 1000g/kg with the addition of a mixture of purified maize starch and glucose. An inert marker (titanium dioxide) is included at a rate of 5g/kg. Two rates of inclusion of wheat were employed so that both true and apparent digestibility could be determined; apparent digestibility data are reported.

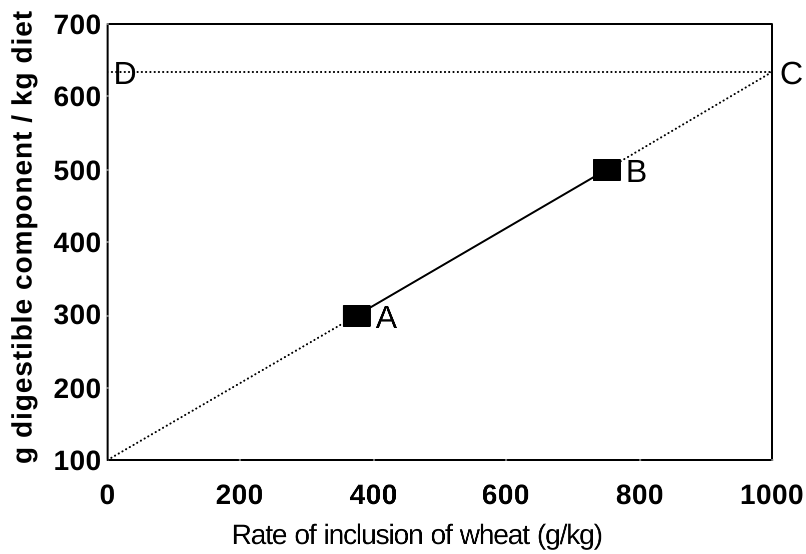
Birds are fed test diets for 3 days at which point digesta samples are removed from two regions, being the upper and lower small intestine. Samples from the former are analyzed for starch and the latter for amino acids; both are analyzed for the internal marker, allowing the calculation of the digestibility of both starch and amino acids. Determination of the nutritional value of wheat (i.e. content of digestible component - e.g. an amino acid - and the coefficient of digestibility of this component) is achieved through regression; rate of wheat inclusion (x) is regressed on dietary content of digestible component (y). Extrapolation of this regression to 1000g wheat/kg gives the content of the digestible component in wheat (in g/kg) which, when divided by the content of the total component, gives the coefficient of digestibility. Extrapolation to 0g wheat /kg (the intercept) gives the content of the digestible component in the remaining portion of the diet.

Both the digestible component (g/kg wheat) and coefficient of digestibility were determined as differences in the former may be a consequence of differences in the total component. Analyses of variance were conducted on the dietary content of digestible component. Tables of results present these analyses together with their solution (giving the digestible content in wheat), the total amount in wheat and the coefficient of digestibility. Figure 4 presents a summary of the methodology employed. The statistical analyses employed were based on assessment of differences between wheats / characteristics in dietary content of digestible component (starch and amino acids).

A full list of trials conducted and wheat samples evaluated is presented in Table C. The nature of the crossing programmes and the need to generate a substantial quantity of each line (around 5kg) for assessment meant that it was often not possible to structure all nutrition trials systematically. It is for this reason that characteristics such as the IB/IR translocation were evaluated over several trials as and when suitable lines became available.

It should be noted that the principal objective of the current programme was to screen as large a number wheat lines as possible to allow the greatest number of characteristics to be evaluated so that future grower decisions, breeding programmes and research work could focus on the more interesting features. Accordingly, this took precedence over the need to establish statistical significance which would have required more replicates per treatment but would have been associated with a less diverse base of data.

Figure 4. Basic methodology in calculating digestibility of dietary components



1. Content of digestible component (g/kg diet) determined (points A, 375, and B, 750 g wheat / kg diet).

   Data at both points A and B are the basis for analysis of variance and presented in tables of results

2. Linear regression relating rate of inclusion of wheat to content of digestible component (as represented by solid line in figure) established.

   y = b + ax

   y = content of digestible component (g/kg diet)
   b = intercept
   x = rate of inclusion of wheat
   a = slope of line

   Linear regression equations presented in tables of results

3. Extrapolation of linear regression to rate of inclusion of wheat of 1000g/kg (point D). This represents the content of digestible component in wheat

   Content presented in tables of results and in figures

4. Value for D (633g / kg in the example) divided by total content of component in wheat (say 800g/kg) to give coefficient of digestibility (633/800=0.791).

   Coefficient presented in tables of results and in figures

3.2.2.1. Description of wheats evaluated in nutrition trials

Table C

Trial 1. Assessment of the IB/IR translocation and VPM eyespot resistance gene

Quality	IB/IR	VPM Eyespot resistance	Origin	Trial
Feed	Yes	Yes		1A
Bread	No	Yes		
Bread	No	No	Spark	1B
Feed	Yes	Yes	Lynx	

In all cases, endosperm texture was 'Hard'.

Trial 2. Assessment of endosperm texture and VPM eyespot resistance gene

Endosperm	Pedigree	Trial
Soft	Advanced sister lines from the cross Wasp x Flame, as near as possible to near-isogenic lines with conventional breeding practices	2A
Hard		

1B/1R	Pedigree	Trial
No	Advanced sister lines from the cross Hunter x Buster.	2B
Yes		

Endosperm texture was 'Hard'

VPM Eyespot resistance	Pedigree	Trial
Yes	Advanced sister lines from the cross Sniper x Rendevous	2C
No		

IB/IR translocation was 'Yes' (present) in both cases

Trial 3. Assessment of endosperm texture - Avalon (Hobbit) chromosome 5A and 5D lines

Endosperm	Protein Content	Trial
5 D - Hard	High	3A
5 D - Soft	Low	
5A - H/P Soft	High	3B
5A - L/P Soft	Low	

For each code, 3 independent near-isogenic sister lines were bulked

Trial 4 Assessment of endosperm texture and protein quality

Endosperm	1B/1R	Other features	Pedigree	Trial
Hard	No			4A
Soft	Yes			
Hard Feed	No			4B
Hard Feed	Yes			
Soft	No		Sister lines from Astron * Beaver	4C
Hard	Yes			
Soft			Galahad vs Galatea	4D
Soft		Very extensible dough	Very closely related, but differ in protein composition	

Trial 5. Comparison of endosperm texture - Hobbit (Avalon) isogenic lines

Code	Endosperm	Protein	Trial
111/2 - 1	Soft	High	5A
107/7 - 8	Soft	High	
84/7 - 3	Hard	High	
116/7 - 5	Hard	High	
111/2 - 2	Soft	Low	5B
107/7 - 7	Soft	Low	
84/7 - 4	Hard	Low	
116/7 - 6	Hard	Low	

Trial 6.Assessment of high molecular weight glutenin subunits and amylose contents

Code	Description	Trial
A	Sicco isogenic null 5 +10 control	6A
B	Sicco isogenic null 5 +10	
A (C)	Sicco isogenic null 2 +12 control	6B
B (D)	Sicco isogenic null 2 +12	
A	Chinese Spring control	6C
B	ACS (CNN 4A) - lower amylose	
C	ACS (CNN 7B) - higher amylose	
D	BSC (CNN 4A) - lower amylose	

Trial 7. Assessment of grain colour and high molecular weight glutenin subunits - Novosibirsaka isogenic lines

Code	Description	Trial
A	Red grain control	7A
B	Red grain ANK 1E	
C	Red grain ANK 1C	
D	Red grain ANK 1A	
E	Red grain ANK 1B	
F	Red grain ANK 1D	
A	Sicco isogenic 7 + 8 control	7B
B	Sicco isogenic 7 + 8	

3.3. Results and discussion

Starch analysis

Following the initial application and funds being awarded, H-GCA asked that additional work be undertaken to examine the digestibility of starch as well as amino acids. It was pointed out that there would be insufficient digesta from the lower region of the small intestine (lower ileum) to allow analyses for both starch and amino acids to be conducted and it was agreed that starch within the upper region of the small intestine should be analyzed. However, it is important to appreciate that the degree of starch digestion within this upper region is not complete and that further digestion would take place in the ileum. Therefor data for starch digestibility would be lower than expected, although useful comparative data would emerge. A further request for additional funding to support the starch work requested by H-GCA was not granted such that the overall programme was not, because of time and resource constraints, able to conduct amino acid analyses on all trials. Whilst comparisons between coefficients of starch and amino acid digestibility within the same trial are possible, this should be undertaken with caution (as digestibilities were determined at different regions of the small intestine)

Data presentation

Results from each trial consist of tables which present (1) the analysis of variance for each digestible component evaluated - starch in all trials, amino acids in some and; an analysis for 'time' is based on the fact that replicates for each trial were separated by one day (2) linear regression equations from which the content of each digestible component in wheat was determined together with the coefficient of digestibility of this component (digestible / total).

Subsequent to these tables are found figures describing the major findings. Rather than present each amino acid separately in graphical form, it was decided to group amino acids into two categories, being "essential" (CYS, MET, THR, VAL, ILEU, LEU, PHE, HIS, LYS, ARG) and "non - essential" (ASP, SER, GLU, GLY, ALA, PRO). This distinction is based on the fact that some amino acids must be provided in the diet of high-performing non-ruminants ("essential") whereas there is no dietary requirement for others ("non - essential").

All figures are drawn with common axes for the three categories of nutrient (starch, essential and non-essential amino acids) so that comparisons between trials may be made, although as each trial was conducted at a different time period, caution should be exercised when making such comparisons.

The data of most importance are those related to content and digestibility of components in wheat. These data are best summarised in histograms which are presented in the results section. All other data are tabulated in appendices.

Trial 1A - Assessment of the presence / absence of the IB/IR translocation

Results (Tables in appendices, figure p37):

Table 1: Mean data / analysis of variance
Table 2: Linear regressions and solutions

The two wheats in this trial had been grown under different conditions - ‘one’ was as first wheat and ‘2' as second wheat in a rotation. The latter would have had less N application and higher disease pressure. This had a significant effect on starch digestibility, although reasons for this are not immediately apparent.

The effect of the IBIR translocation is evident (P=0.056) and has confirmed previous observations that its presence is liable to have a negative impact on nutritional value.

Interactions between the two factors were also evident (P=0.004) suggesting that expression of the effects of the IB/IR translocation may governed by environmental factors such as whether the sample comes from a first or second wheat.

Trial 1B - Assessment of the presence / absence of the Eyespot Resistance Gene Pch1

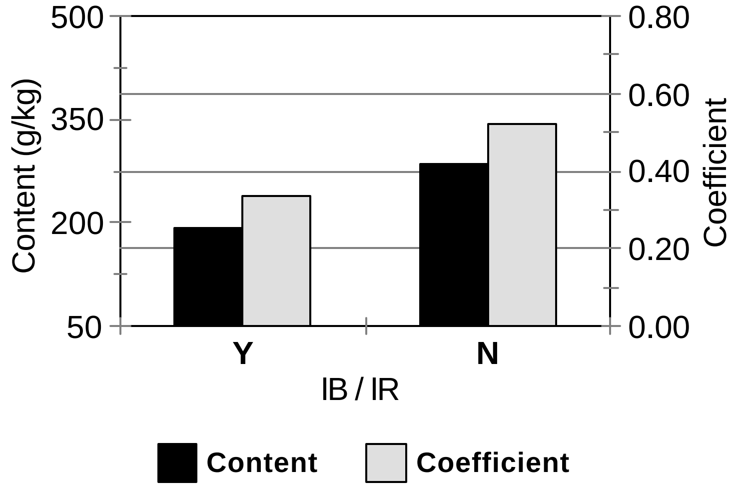
Results (Tables in appendices, figure p37):

Table 3: Mean data / analysis of variance
Table 4: Linear regressions and solutions

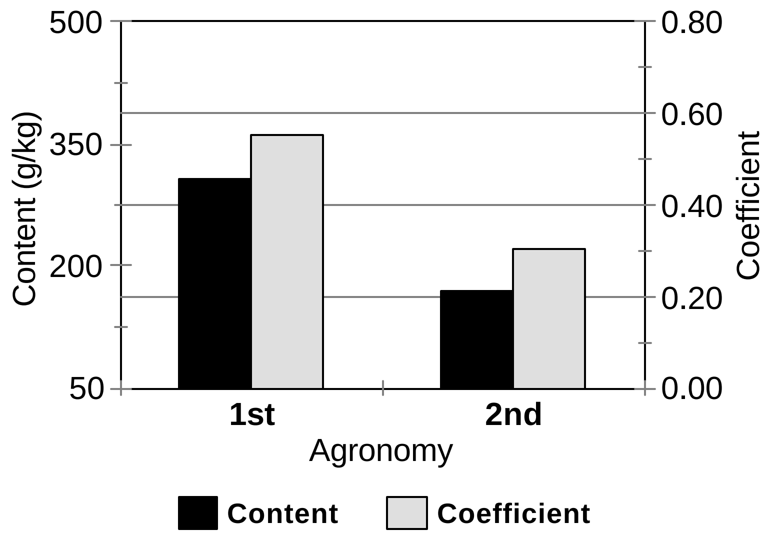
The presence of eyespot resistance appears to have no effect on nutritional value, although there is an apparent interaction (P=0.017) between this effect and agronomy

Trial 1. Figures.

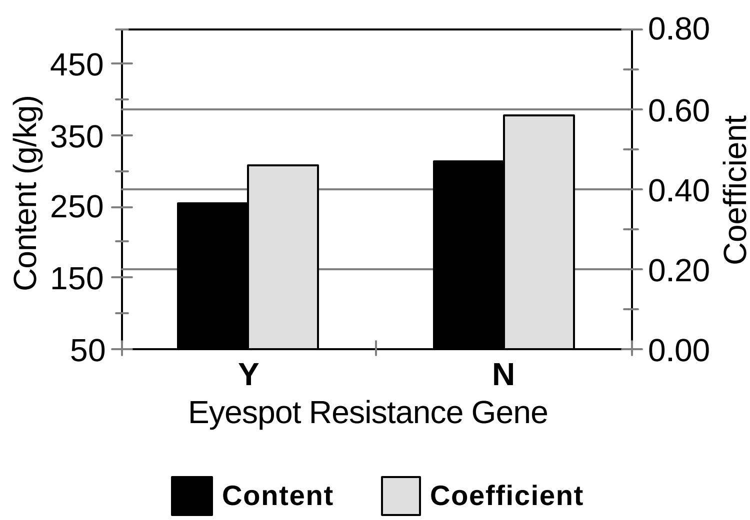
1A: IB/IR
Starch Digestibility



1A: Agronomy
Starch Digestibility



1B: Eyespot Resistance Gene
Starch Digestibility



Trial 2A - Assessment of Endosperm texture

Results (Tables in appendices, figure p39):

Table 5:	Mean data / analysis of variance
Table 6:	Linear regressions and solutions

There were significant differences between wheat samples in content of digestible components for certain components (interestingly with “essential” amino acids including CYS, THR and LYS), although not for others. In addition P values approached significance on some occasions (e.g. P= 0.065 for ASP). Whilst the data suggest that, within this background, endosperm texture is not influencing general nutritional value to a marked extent, it should be pointed out that hard was consistently associated with lower values both for content of digestible component and coefficient of digestibility.

Trial 2B - Assessment of the IB/IR translocation

Results (Tables in appendices, figure p40):

Table 7:	Mean data / analysis of variance
Table 8:	Linear regressions and solutions

Significant differences between the two lines were, as with trial 2A, again confined to a small number of nutrients (MET, GLU, ILEU, PHE) and for these amino acids, the data suggest that their digestible content was higher in the presence of the IB/IR translocation. However this observation was attributable almost entirely to the fact that total amino acid content was higher in the presence of the IB/IR translocation. When this effect was removed (i.e calculation of the coefficient of digestibility - digestible / total) then it is evident that the translocation was associated with reduced nutritional value, confirming previous observations

Trial 2C - Eyespot resistance

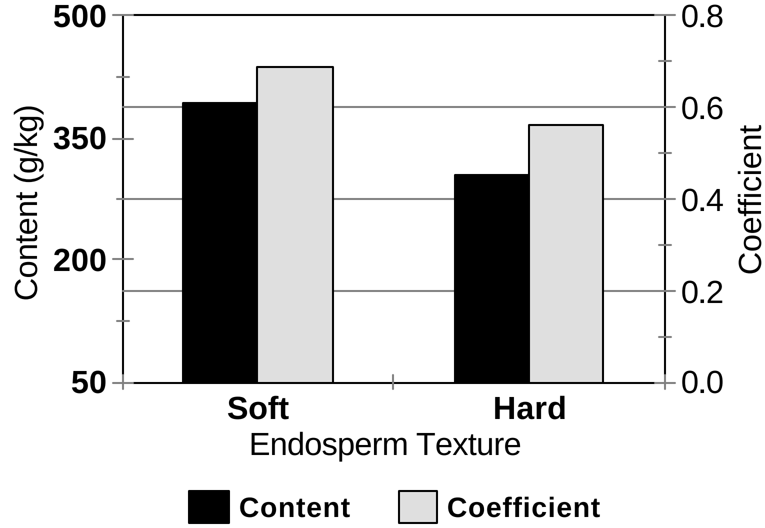
Results (Tables in appendices, figure p41):

Table 9:	Mean data / analysis of variance
Table 10:	Linear regressions and solutions

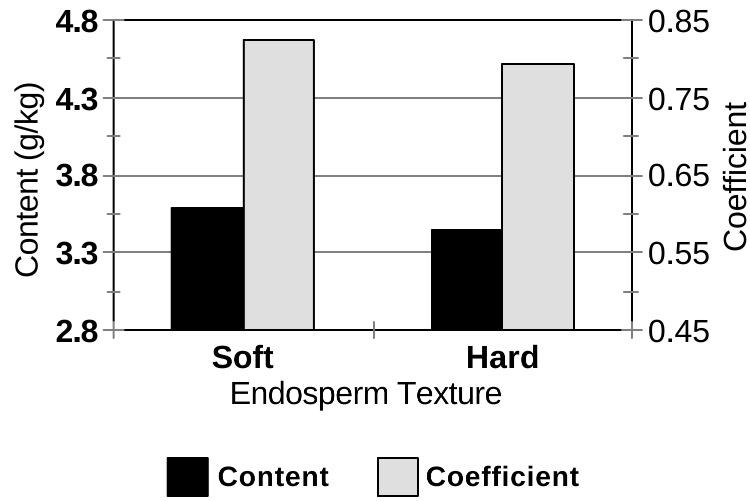
This characteristic appears to have no influence on nutritional value, confirming data generated in Trial 1B. No further assessments of eyespot resistance were therefore considered necessary.

Trial 2 Figures

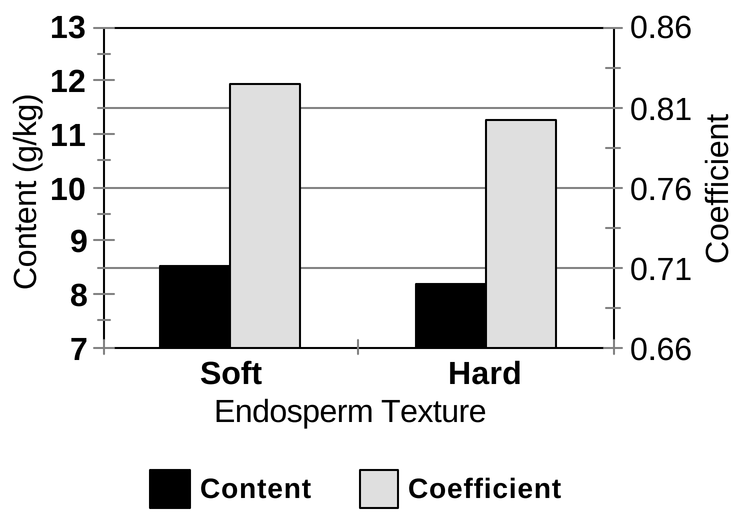
2A: Endosperm
Starch Digestibility



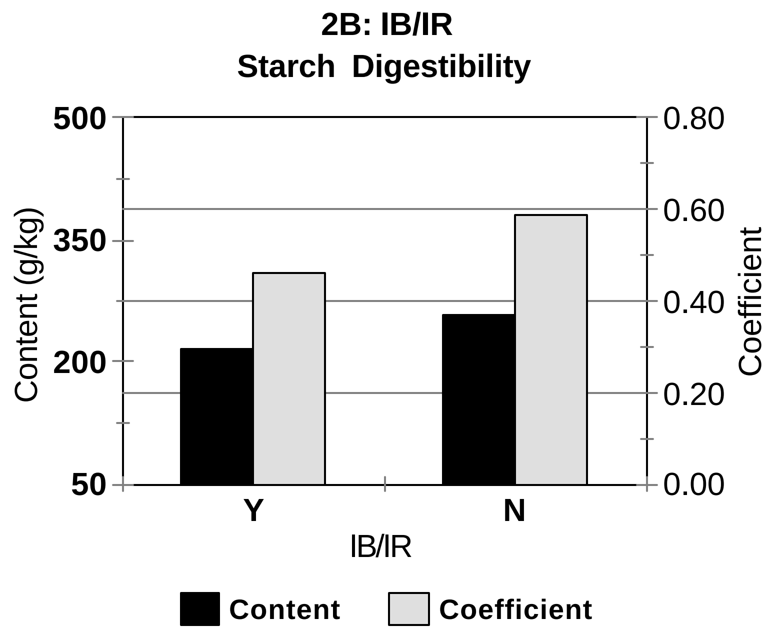
2A: Endosperm
Amino Acid Digestibility -Essential

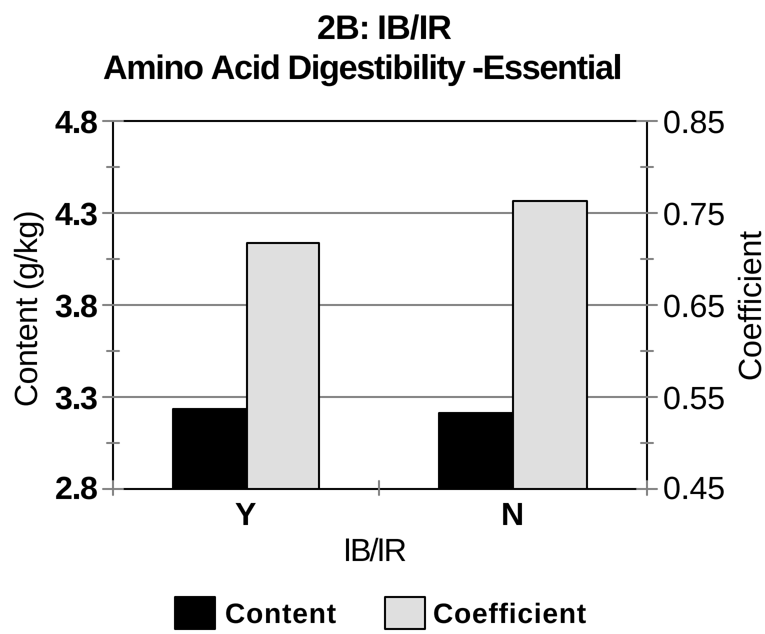


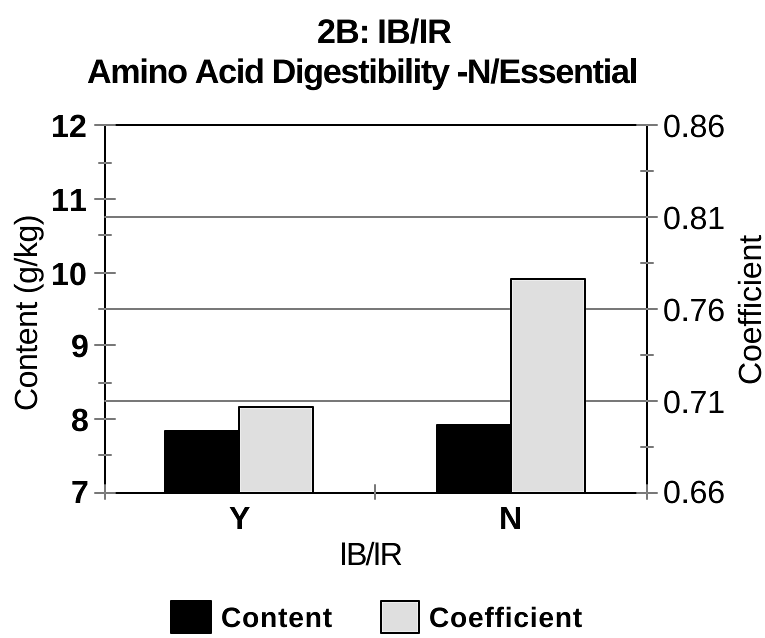
2A: Endosperm
Amino Acid Digestibility -N/Essential



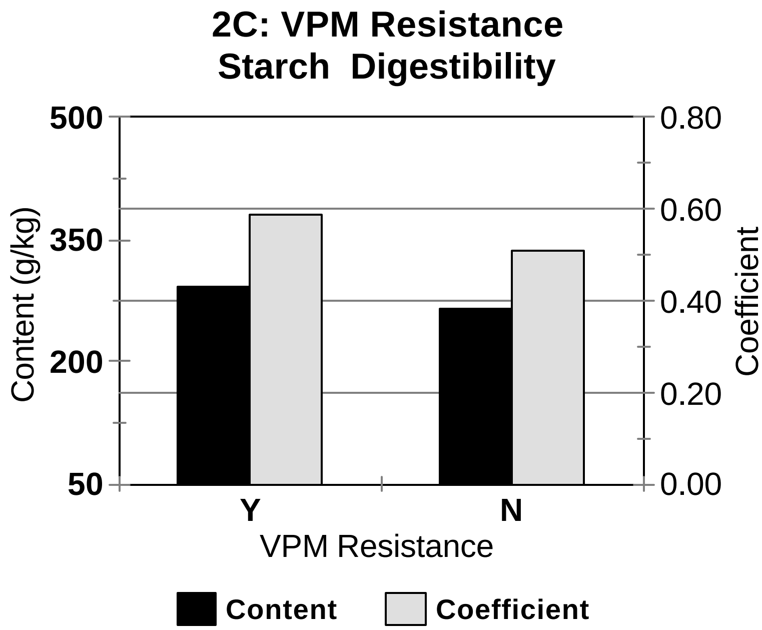
Trial 2 Figures

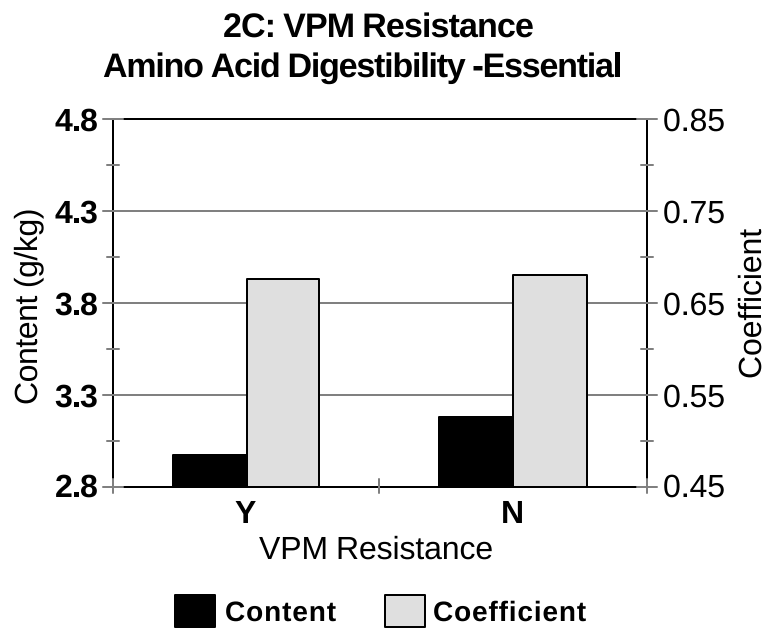


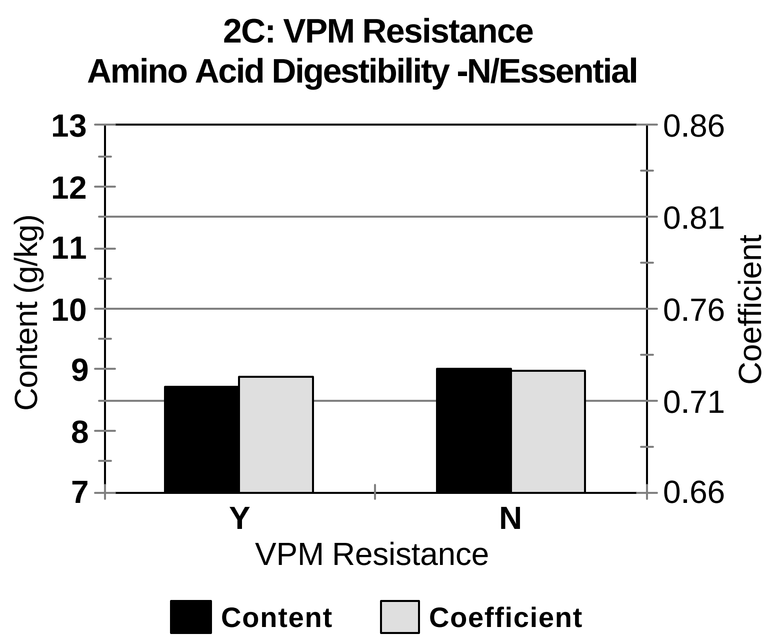




Trial 2 Figures







Trial 3A - Assessment of Endosperm texture

Results (Tables in appendices, figure p43):

Table 11: Mean data / analysis of variance
Table 12: Linear regressions and solutions

Although the data did not demonstrate a significant difference between the two lines evaluated in terms of the dietary levels of digestible starch, the content of digestible starch in wheat and coefficient of digestibility were both higher in the soft line. This confirms the importance of calculating the two latter data sets in any comparisons between lines.

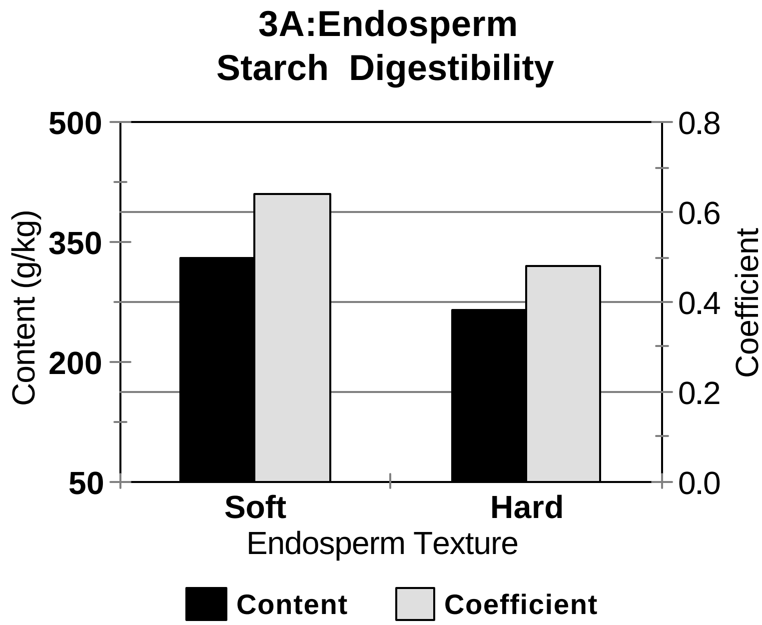
Trial 3B. Assessment of differences in protein content

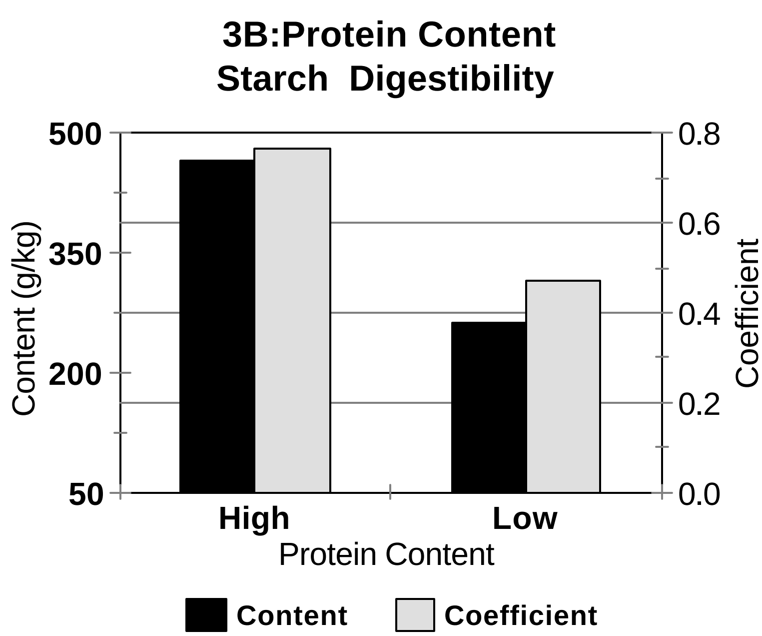
Results (Tables in appendices, figure p43):

Table 13: Mean data / analysis of variance
Table 14: Linear regressions and solutions

Protein content had an effect on both content of digestible starch in wheat and the coefficient of starch digestibility where a higher level of protein was associated with superior nutritional value.

Trial 3 Figures





Trial 4A - Assessment of the interaction between the IB/IR translocation and Endosperm texture

Results (Tables in appendices, figure p45):

Table 15: Mean data / analysis of variance
Table 16: Linear regressions and solutions

This trial was not balanced in that the two factors being assessed were not independent of each other. However previous work and earlier trials in the current programme had indicated that IB/IR was having a negative effect as was hard endosperm structure. Accordingly, it was thought interesting to compare a IB/IR NO + H Endosperm line (wheat A) with IB/IR YES + S Endosperm (wheat B). It could be hypothesized that the negative features of IB/IR might be offset by the possible positive ones of soft endosperm.

There were significant differences between the two lines for a large number of nutrients with the general effect being that IB/IR NO +H Endosperm was superior to IB/IR YES + S Endosperm. Thus the IB/IR effect was generally larger than the endosperm effect. However, data from this trial were seemingly contradictory with a small number of nutrients (e.g. HIS and the nutritionally important LYS) following the reverse of this general trend. There is the suggestion that general nutritional differences may in fact be specific to the nutrient in question.

Trial 4B - Assessment of the IB/IR translocation in a hard endosperm background

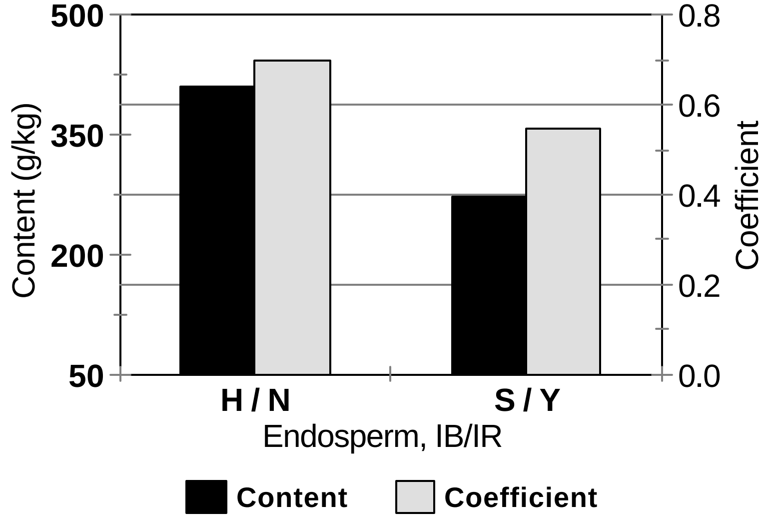
Results (Tables in appendices, figure p46):

Table 17: Mean data / analysis of variance
Table 18: Linear regressions and solutions

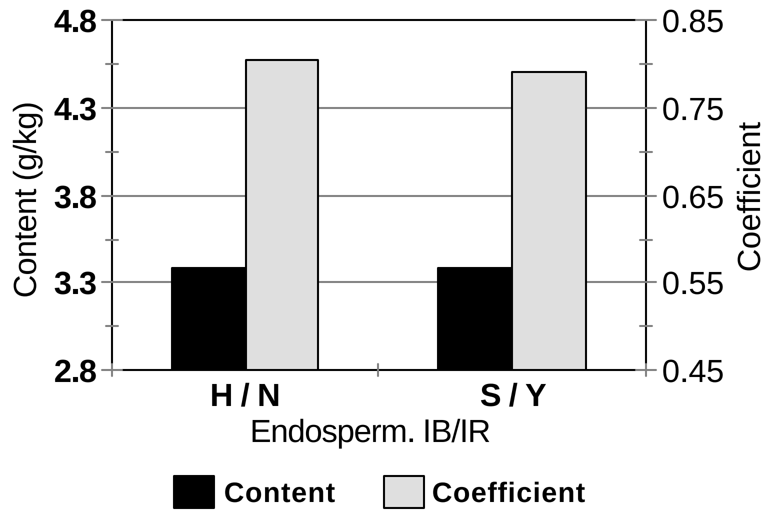
There were no major effects detected in this trial. Having established that IB/IR and, less importantly, endosperm texture are, generally, nutritionally significant, it would also appear that expression of the IB/IR effect is dependent on genetic background such as type of endosperm texture.

Trial 4 Figures

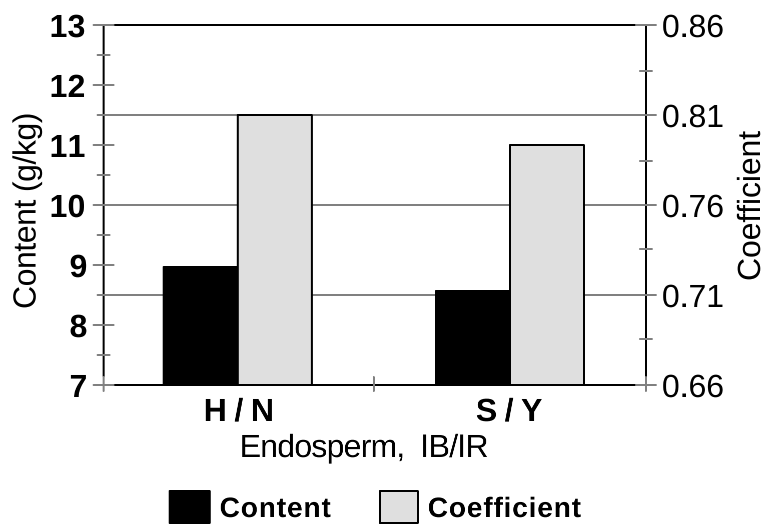
4A: Endosperm, IB/IR
Starch Digestibility



4A: Endosperm, IB/IR
Amino Acid Digestibility - Essential

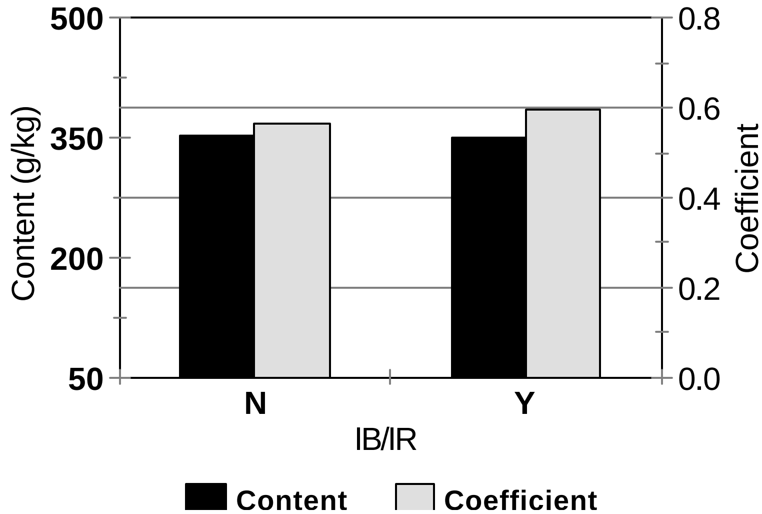


4A: Endosperm/IBIR
Amino Acid Digestibility - N/Essential

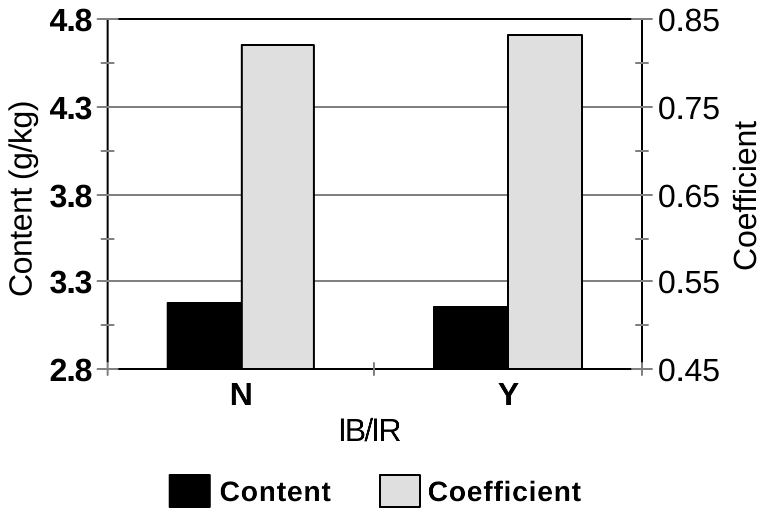


Trial 4 Figures

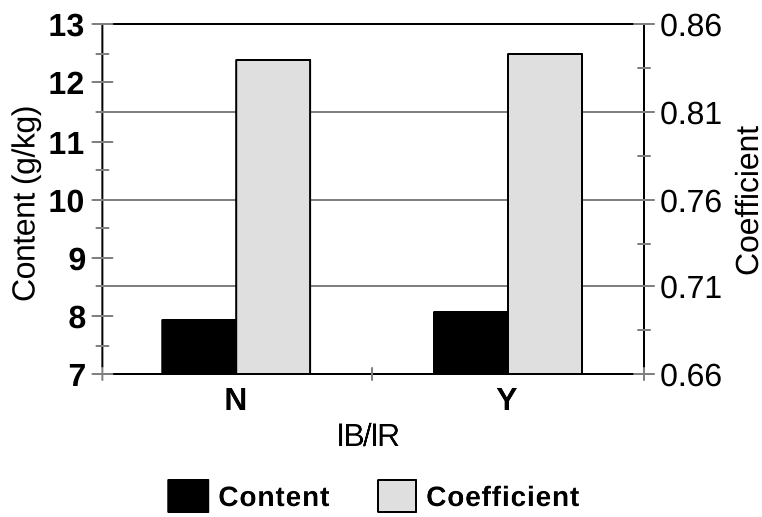
4B: IB/IR
Starch Digestibility



4B: IB/IR
Amino Acid Digestibility - Essential



4B: IB/IR
Amino Acid Digestibility - N/Essential



Trial 4C - Assessment of IB/IR translocation and endosperm texture

Results (Tables in appendices, figure p48):

Table 19: Mean data / analysis of variance

Table 20: Linear regressions and solutions

This trial was of a similar nature to Trial 4A - the two characteristics were not separated; in the current trial, IB/IR NO + Endosperm soft (A) was compared with IB/IR Yes + Endosperm Hard (B) - a hypothesis could be that the former should be nutritionally better than the latter. The data support this hypothesis.

Trial 4D - Assessment of protein composition and dough extensibility

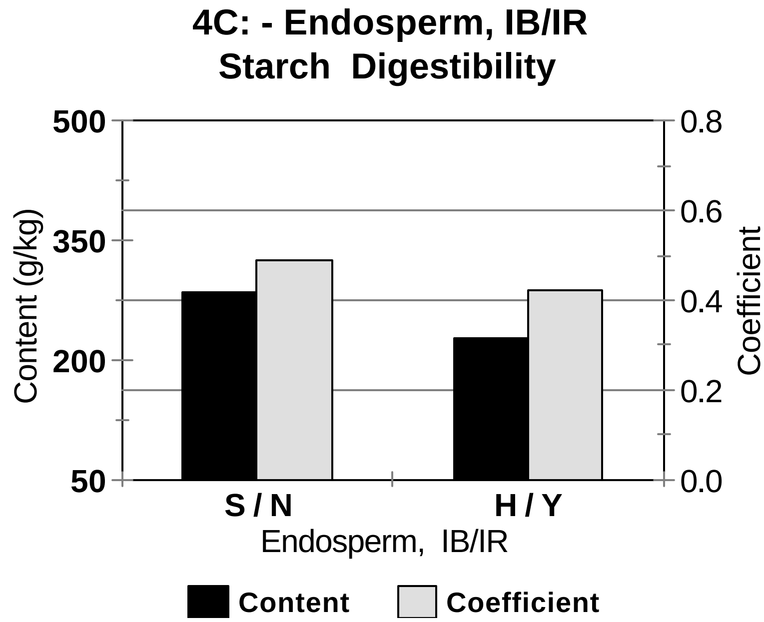
Results (Tables in appendices, figure p49):

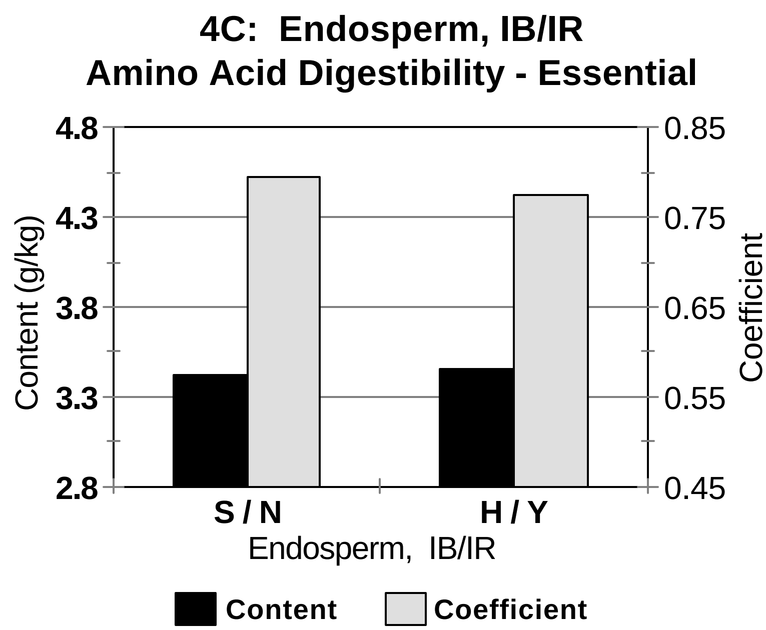
Table 21: Mean data / analysis of variance

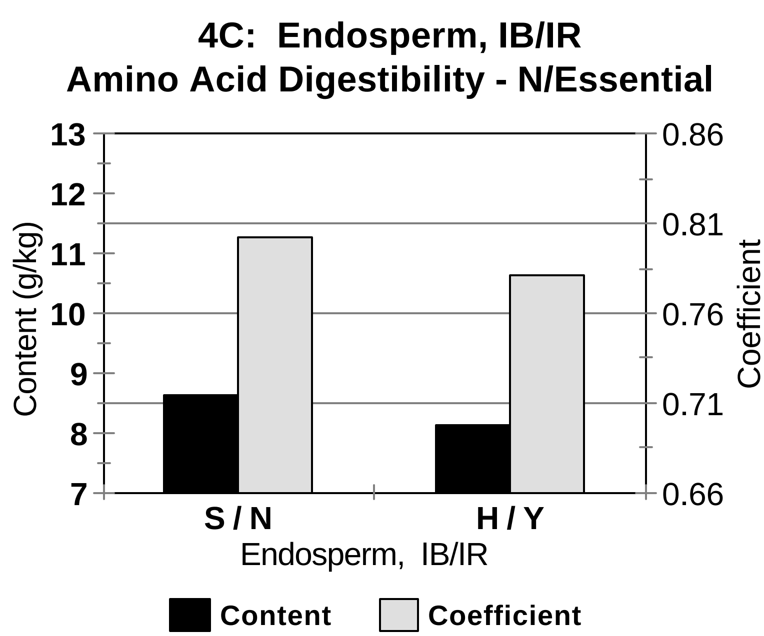
Table 22: Linear regressions and solutions

One of the means through which the nutritional value of wheat may vary is through promoting increased digesta viscosity (which would interfere with digestive enzyme activity). This could arise from soluble non-starch polysaccharides or more extensible dough (having escaped digestion). However the data from the current trial indicate that the latter is not having an effect on nutritional value.

Trial 4 Figures

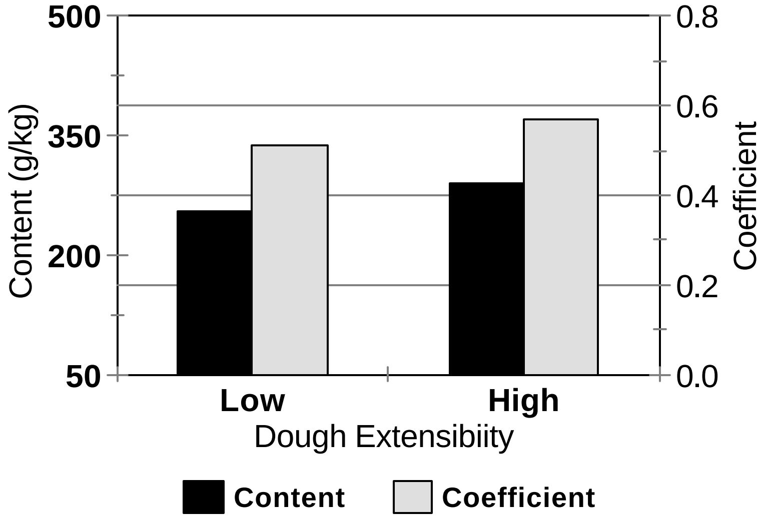




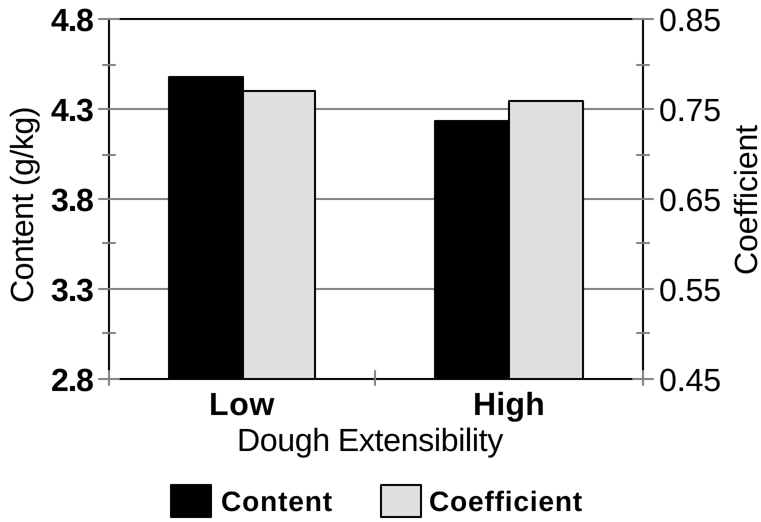


Trial 4 Figures

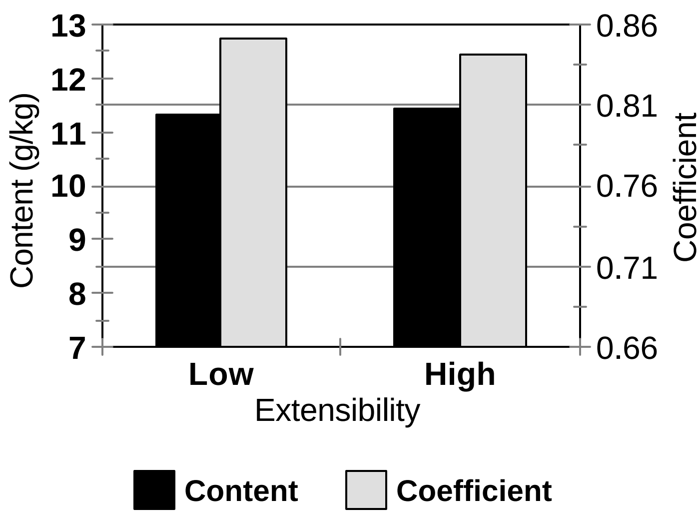
4D: Extensibility
Starch Digestibility



4D: Extensibility
Amino Acid Digestibility - Essential



4D: Extensibility
Amino Acid Digestibility - N/Essential



Trial 5A / 5B - Assessment of endosperm texture in specific protein backgrounds (5A - high, 5B - low)

5A Results (Tables in appendices, figure p51):

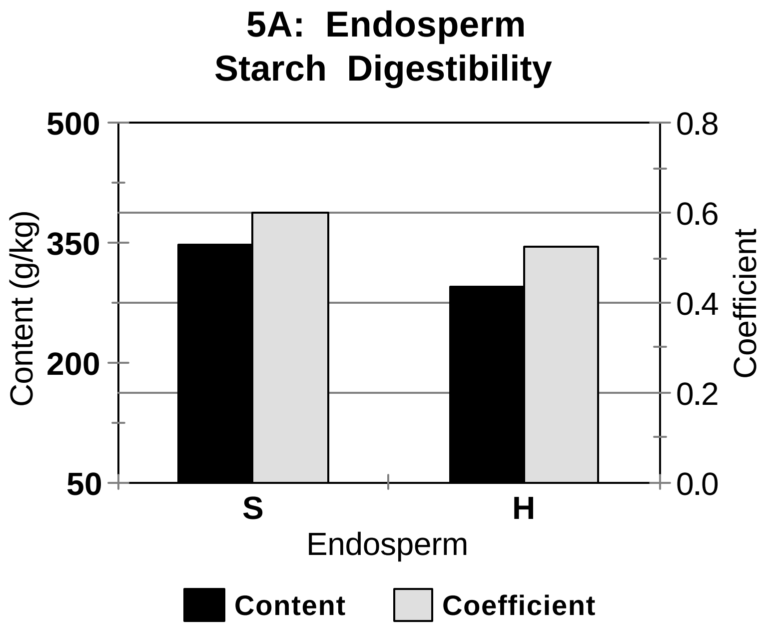
Table 23: Mean data / analysis of variance
Table 24: Linear regressions and solutions

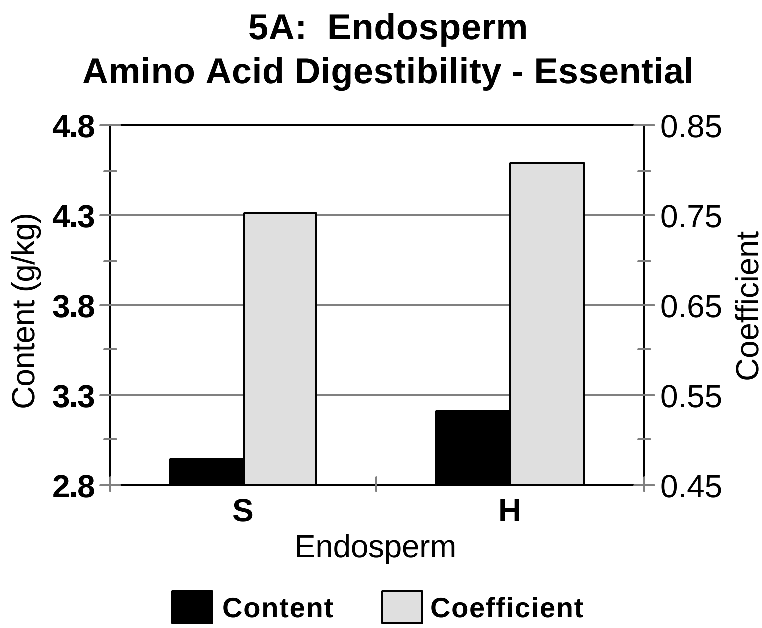
5B Results (Tables in appendices, figure p52):

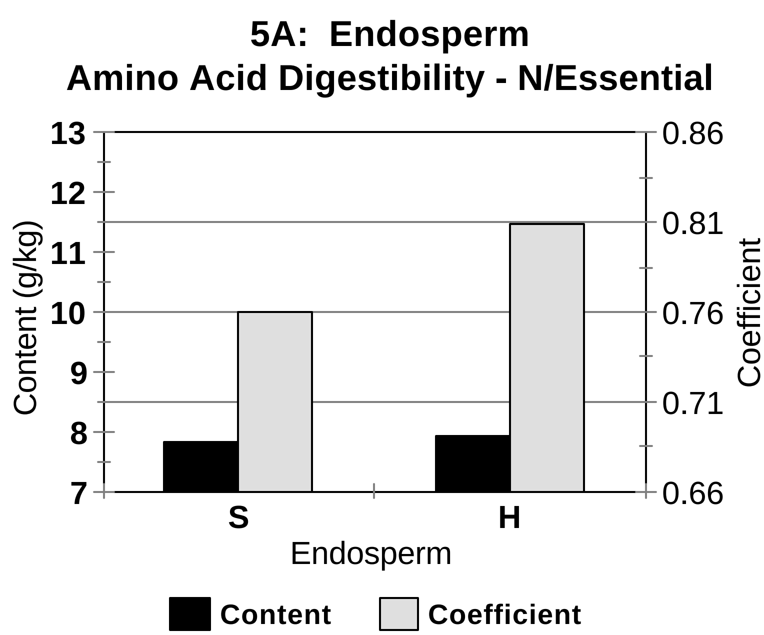
Table 25: Mean data / analysis of variance
Table 26: Linear regressions and solutions

Specific protein backgrounds appear to influence the expression of the effects of endosperm texture which have been recorded previously. However, neither of these two trials recorded major differences; trial 3B had drawn a similar conclusion.

Trial 5 Figures

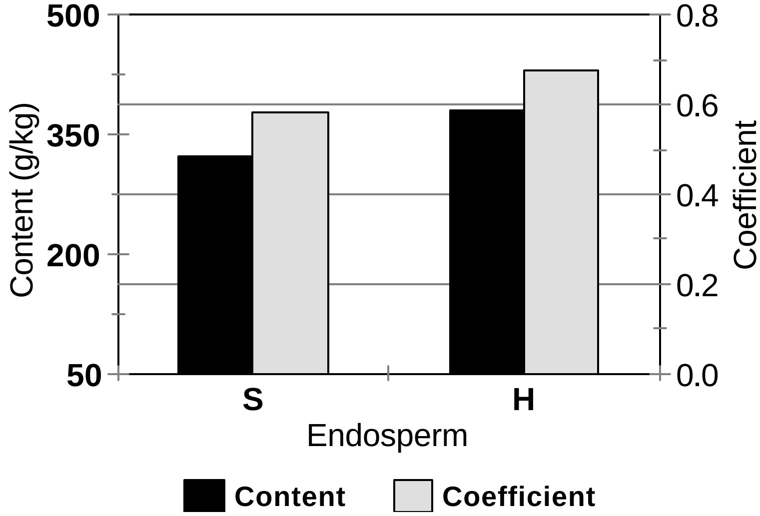




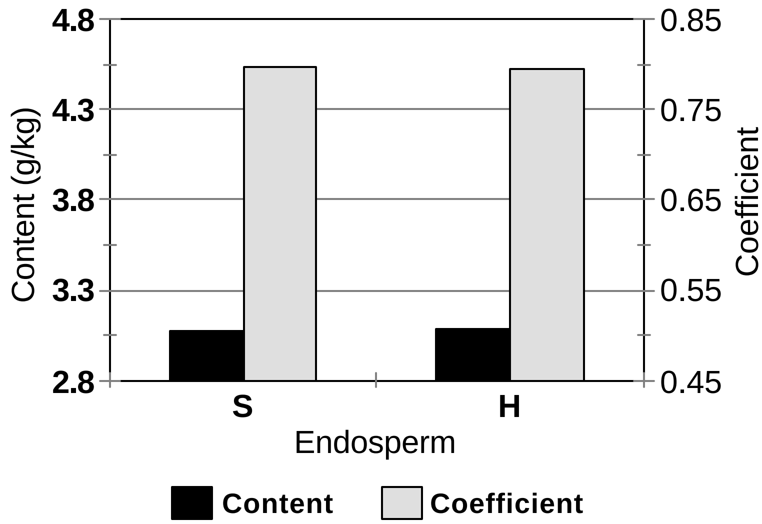


Trial 5 Figures

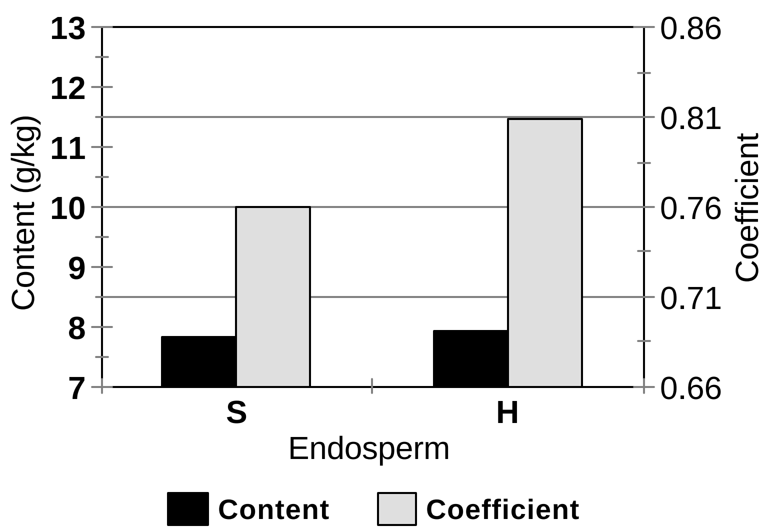
5B: Endosperm
Starch Digestibility



Trial 5B - Influence of Endosperm
Amino Acid Digestibility - Essential



5B: Endosperm
Amino Acid Digestibility - N/Essential



Trial 6A/6B. Assessment of Sicco isogenics containing high molecular weight sub-units

6A Results (Tables in appendices, figure p54):

Table 27: Mean data / analysis of variance
Table 28: Linear regressions and solutions

6B Results (Tables in appendices, figure p54):

Table 29: Mean data / analysis of variance
Table 30: Linear regressions and solutions

The overall conclusions to be drawn are that the trial is inconclusive since 5+10 controls are different, suggesting other effects in the production of seed are being involved

Trial 6C - Assessment of lines differing in amylose content

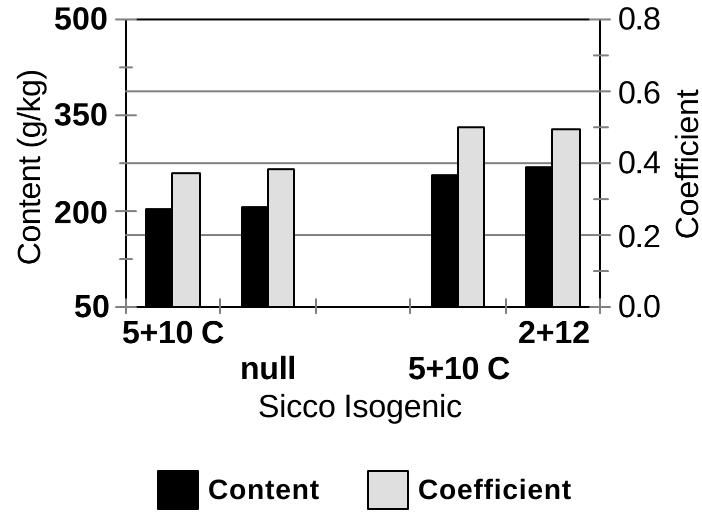
Results (Tables in appendices, figure p55):

Table 31: Mean data / analysis of variance
Table 32: Linear regressions and solutions

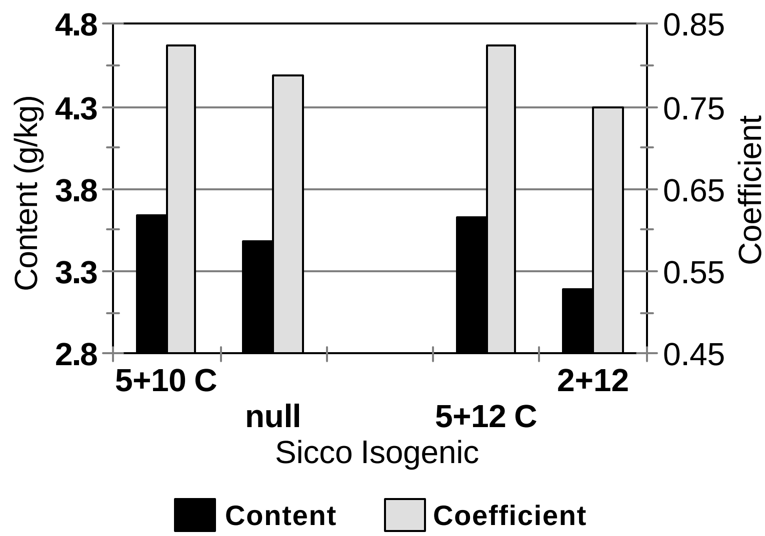
Development of wheats with differing amylose / amylopectin ratios is becoming of interest. The data generally indicated that there were no important differences. Duplicate lines (B and D) are slightly different indicating that there is no genetic effect of differences in amylose content

Trial 6 Figures

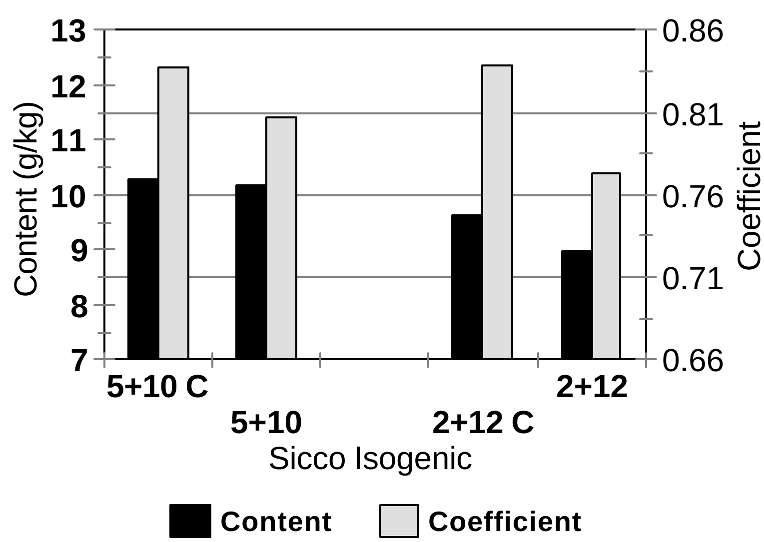
6A / B: Sicco Isogenics
Starch Digestibility



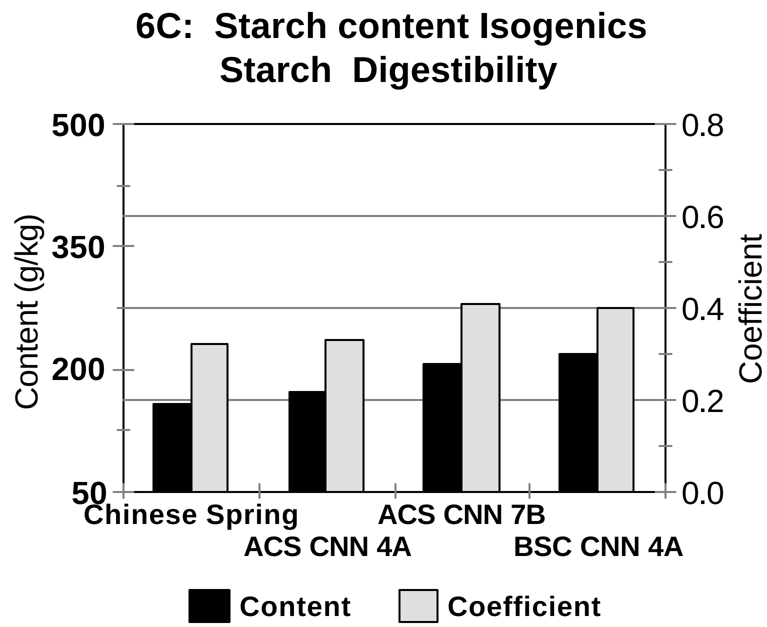
6A / B: Sicco Isogenics
Amino Acid Digestibility -Essential

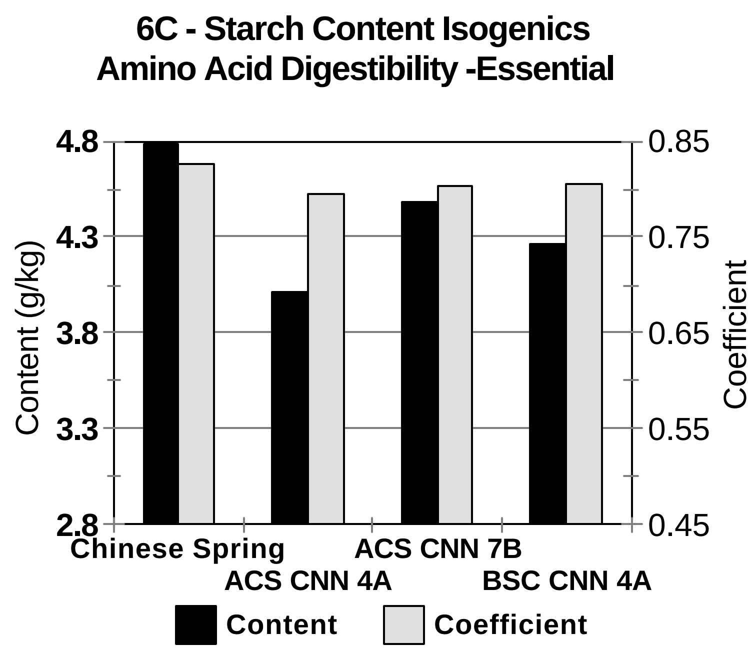


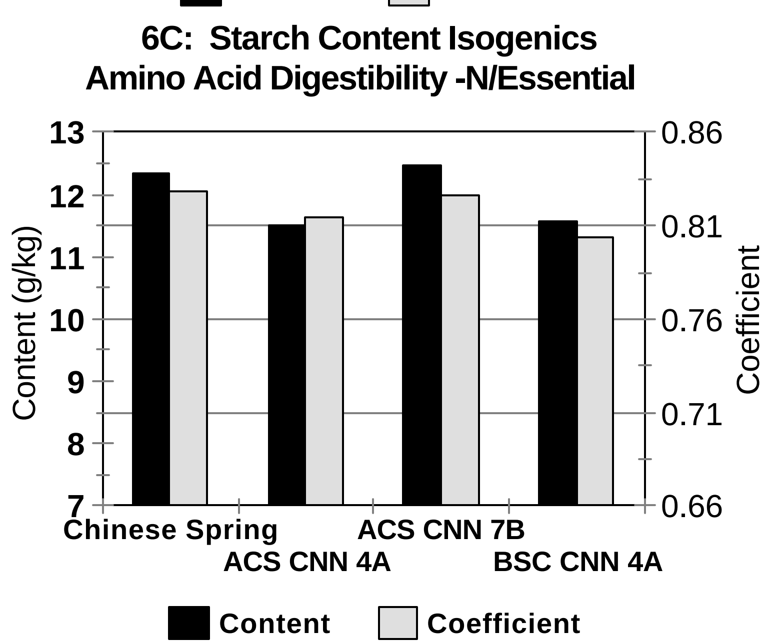
6A / B: Sicco Isogenics
Amino Acid Digestibility -N/Essential



Trial 6 Figures







Trial 7A - Assessment of grain colour (Red grain isogenics)

Results (Tables in appendices, figure p57):

Table 33: Mean data / analysis of variance
Table 34: Linear regressions and solutions

The data indicate considerable differences between individual wheat lines with respect to starch digestibility, but with red generally worse than the white control. These data are interesting, but trial needs to be repeated before definitive conclusions may be drawn

Trial 7B - Assessment of high molecular weight (Sicco isogenics)

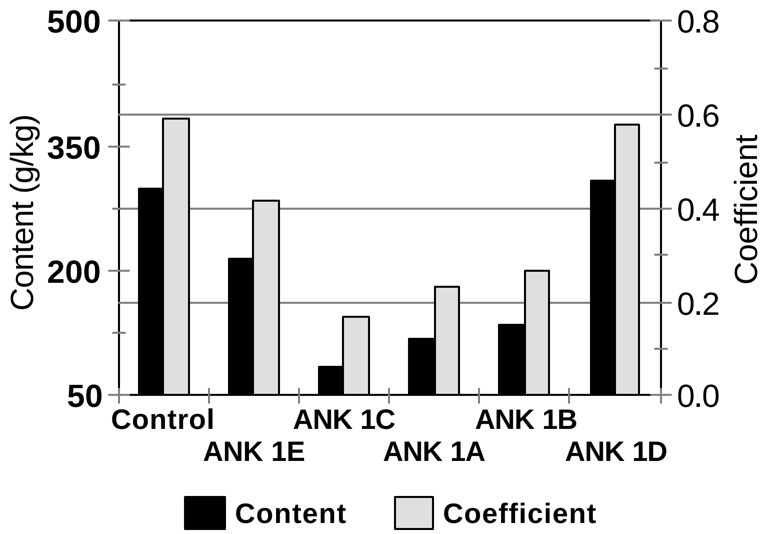
Results (Tables in appendices, figure p57):

Table 35: Mean data / analysis of variance
Table 36: Linear regressions and solutions

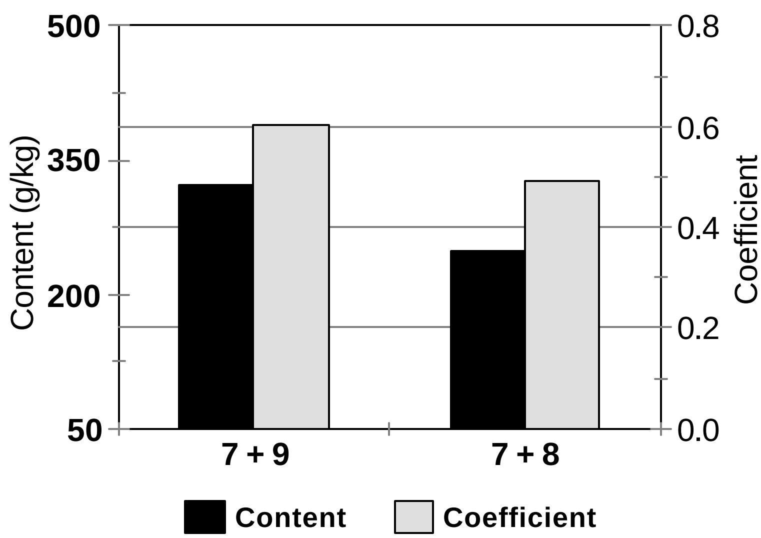
The data for starch digestibility appear to suggest that the better subunit 7+9 for bread-making quality may be associated with a superior nutritional value.

Trial 7 Figures

7A: Red Grain Isogenics
Starch Digestibility



7B: Sicco Isogenics
Starch Digestibility



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Wiseman, J. and McNab, J.M. 1993.Nutritive value of wheat fed to non-ruminants. Project No. 111, Home Grown Cereals Authority

Wiseman, J. Short, F.J. Boorman, K.N. and Stringer, S. 1996. True digestibility of wheat protein and amino acids in broilers. Project No. 170, Home Grown Cereals Authority

Appendices

These contain tables of results

Table 1. Trial 1A. Assessment of IB/IR and Agronomy

a. Mean data - g digestible starch / kg experimental diet

		Time		
	1	2	Mean	
	488	466	477	
			Agronomy	
		First	Second	Mean
IBIR	N	491	495	493
	Y	508	414	461
Mean		500	454	477

b. Analysis of variance

Factor	Degrees of Freedom	Mean Square	SEd	P=
Time	1	7901	16.1	0.175
Agronomy	1	325705	16.2	0.007
IB/IR	1	16051	16.2	0.056
Agronomy * IB/IR	1	38953	22.9	0.004
Residual	51(4)	4180		

Table 2. Trial 1A Linear regression equations and solution for starch

Wheat sample	Characteristics		Linear Regression (Y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
	IBIR	Agronomy	b	a	R^2	Solution when x =1000		
1	Y	One	770 ±106.0	-0.443 ±0.172	0.485	328 ±77.4	623	0.53 ±0.124
2	Y	Two	867 ±81.2	-0.767 ±0.143	0.822	100 ±71.6	588	0.17 ±0.121
3	N	One	698 ±33.3	-0.304 ±0.056	0.802	393 ±26.7	536	0.73 ±0.050
4	N	Two	801 ±75.6	-0.564 ±0.122	0.772	236 ±55.1	535	0.44 ±0.103
5	N	One	764 ±74.8	-0.550 ±0.126	0.720	215 ±60.0	535	0.40 ±0.112
6	N	Two	787 ±63.6	-0.491 ±0.103	0.784	296 ±46.4	574	0.52 ±0.081
7	Y	One	762 ±51.9	-0.469 ±0.088	0.798	293 ±41.6	529	0.55 ±0.079
8	Y	Two	840 ±94.7	-0.793 ±0.160	0.771	47 ±76.0	533	0.09 ±0.142

Table 3. Trial 1B. Assessment of VPM eyespot resistance and Agronomy

a. Mean data - g digestible starch / kg experimental diet

		Time		
	1	2	Mean	
	502	484	493	
		Agronomy		
		First	Second	Mean
VPM Eyespot Resistance	N	526	482	504
	Y	455	509	482
Mean		491	495	493

b. Analysis of variance

Factor	**Degrees of Freedom**	**Mean Square**	**SEd**	**P=**
Time	1	2393	19.0	0.374
Agronomy	1	172	19.0	0.801
VPM	1	3877	19.0	0.260
Agronomy * VPM	1	19502	26.9	0.017
Residual	21(2)	2895		

Table 4. Trial 1B Linear regression equations and solution for starch

Wheat sample	Characteristics	Linear Regression (Y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
	VPM Eyespot resistance	b	a	R^2	Solution when x =1000		
3	Y	698 ±33.3	-0.304 ±0.056	0.802	393 ±26.7	536	0.73 ±0.050
4	Y	801 ±75.6	-0.564 ±0.122	0.772	236 ±55.1	535	0.44 ±0.103
5	N	764 ±74.8	-0.550 ±0.126	0.720	215 ±60.0	535	0.40 ±0.112
6	N	787 ±63.6	-0.491 ±0.103	0.784	296 ±46.4	574	0.52 ±0.081

Table 5. Trial 2A. Assessment of endosperm hardness

a. Mean data - g digestible component / kg diet

Component	**Endosperm**			**Time**	
	Soft	Hard	Mean	1	2
Starch	522	512	517	521	512
CYS	1.86	1.69	1.78	1.78	1.77
MET	0.96	0.65	0.95	0.95	0.95
ASP	2.50	2.27	2.38	2.35	2.42
THR	1.51	1.30	1.40	1.37	1.44
SER	2.13	1.96	2.04	1.94	2.14
GLU	13.54	13.25	13.39	13.36	13.42
GLY	1.93	1.77	1.85	1.80	1.90
ALA	1.82	1.71	1.76	1.75	1.78
VAL	1.72	0.74	1.73	1.70	1.76
ILEU	1.52	1.52	1.52	1.52	1.52
LEU	3.46	3.40	3.43	3.43	3.472
PHE	2.22	2.08	2.15	2.15	2.14
HIS	1.53	2.14	1.84	1.79	1.88
LYS	1.38	1.23	1.30	1.27	1.34
ARG	2.66	2.57	2.62	2.58	2.66
PRO	5.97	5.87	5.91	5.95	5.87

Table 5 (continued). Trial 2A

b. Analysis of variance of wheat characteristics
(In no case was effect of time significant)

Component	Endosperm				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	443	14.6	0.487	11	854
CYS	1	0.11092	0.036	<0.001	10(1)	0.005
MET	1	0.0008	0.030	0.645	10(1)	0.00355
ASP	1	0.20242	0.109	0.065	10(1)	0.04716
THR	1	0.1729	0.081	0.028	10(1)	0.02638
SER	1	0.10755	0.139	0.266	10(1)	0.07746
GLU	1	0.3310	0.175	0.131	10(1)	0.1224
GLY	1	0.10374	0.096	0.338	10(1)	0.03718
ALA	1	0.04703	0.083	0.222	10(1)	0.02769
VAL	1	0.00144	0.116	0.873	10(1)	0.05399
ILEU	1	0.00023	0.060	0.900	10(1)	0.01426
LEU	1	0.01623	0.078	0.434	10(1)	0.02438
PHE	1	0.08232	0.055	0.026	10(1)	0.01215
HIS	1	1.47755	0.063	<0.001	10(1)	0.01568
LYS	1	0.097628	0.046	0.007	10(1)	0.008560
ARG	1	0.03245	0.099	0.383	10(1)	0.03905
PRO	1	0.05909	0.114	0.310	10(1)	0.05174

Table 6. Trial 2A Linear regression equations and solution for starch and amino acids

Wheat sample	Characteristics Endosperm	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
1	Soft	Starch	689 ±24.2	-0.296 ±0.041	0.881	392 ±19.4	569	0.69 ±0.034
2	Hard		777 ±38.0	-0.473 ±0.064	0.884	305 ±30.5	546	0.56 ±0.056
1	Soft	CYS	-0.302 ±0.079	0.0038 ±0.00013	0.992	3.54 ±0.064	4.2	0.843 ±0.015
2	Hard		-0.121 ±0.083	0.0032 ±0.00013	0.990	3.10 ±0.061	4.2	0.739 ±0.014
1	Soft	MET	-0.034 ±0.049	0.0018 ±0.00008	0.985	1.73 ±0.039	2.0	0.866 ±0.020
2	Hard		-0.006 ±0.087	0.0017 ±0.00014	0.960	1.69 ±0.064	2.0	0.843 ±0.032
1	Soft	ASP	-0.449 ±0.267	0.0052 ±0.00045	0.950	4.79 ±0.214	6.0	0.798 ±0.036
2	Hard		-0.219 ±0.217	0.0044 ±0.00035	0.963	4.22 ±0.158	5.7	0.740 ±0.028
1	Soft	THR	-0.351 ±0.197	0.0033 ±0.00033	0.933	2.96 ±0.158	3.8	0.778 ±0.042
2	Hard		-0.272 ±0.173	0.0028 ±0.00028	0.943	2.53 ±0.126	3.6	0.704 ±0.035
1	Soft	SER	-0.771 ±0.377	0.0054 ±0.00064	0.902	4.38 ±0.303	5.2	0.842 ±0.058
2	Hard		-0.085 ±0.277	0.0037 ±0.00045	0.916	3.58 ±0.202	4.9	0.731 ±0.041
1	Soft	GLU	-0.306 ±0.344	0.0246 ±0.00058	0.966	24.30 ±0.276	26.8	0.907 ±0.010
2	Hard		-0.900 ±0.460	0.0252 ±0.00075	0.995	24.26 ±0.336	26.4	0.919 ±0.013
1	Soft	GLY	-0.202 ±0.242	0.0038 ±0.00041	0.924	3.58 ±0.194	4.8	0.746 ±0.040
2	Hard		-0.292 ±0.200	0.0037 ±0.00032	0.855	3.38 ±0.146	4.5	0.751 ±0.032

Table 6. (continued)

Wheat sample	Characteristics Endosperm	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
1	Soft	ALA	-0.094 ±0.207	0.0034 ±0.00035	0.930	3.30 ±0.166	4.4	0.751 ±0.038
2	Hard		-0.234 ±0.153	0.0035 ±0.00025	0.970	3.22 ±0.112	4.2	0.767 ±0.027
1	Soft	VAL	-0.653 ±0.322	0.0042 ±0.00054	0.895	3.56 ±0.258	4.7	0.758 ±0.055
2	Hard		-0.370 ±0.135	0.0038 ±0.00022	0.980	3.39 ±0.099	4.6	0.736 ±0.021
1	Soft	ILEU	-0.406 ±0.145	0.0034 ±0.00024	0.966	3.02 ±0.116	3.6	0.840 ±0.032
2	Hard		-0.220 ±0.116	0.0031 ±0.00019	0.978	2.87 ±0.085	3.5	0.819 ±0.024
1	Soft	LEU	-0.305 ±0.155	0.0067 ±0.00026	0.989	6.39 ±0.124	7.4	0.863 ±0.017
2	Hard		-0.259 ±0.202	0.0065 ±0.00033	0.985	6.23 ±0.148	7.3	0.854 ±0.020
1	Soft	PHE	-0.448 ±0.122	0.0047 ±0.00021	0.987	4.29 ±0.098	5.2	0.825 ±0.018
2	Hard		-0.219 ±0.127	0.0041 ±0.00021	0.985	3.86 ±0.092	4.9	0.787 ±0.019
1	Soft	HIS	-0.280 ±0.156	0.0032 ±0.00026	0.955	2.94 ±0.125	3.6	0.817 ±0.035
2	Hard		-0.143 ±0.147	0.0041 ±0.00024	0.980	3.93 ±0.107	4.7	0.836 ±0.023
1	Soft	LYS	-0.234 ±0.132	0.0029 ±0.00022	0.959	2.64 ±0.106	3.2	0.825 ±0.033
2	Hard		-0.088 ±0.077	0.0023 ±0.00013	0.983	2.26 ±0.056	2.9	0.778 ±0.019
1	Soft	ARG	-0.045 ±0.269	0.0048 ±0.00045	0.941	4.77 ±0.216	5.8	0.822 ±0.037
2	Hard		-0.048 ±0.144	0.0047 ±0.00023	0.985	4.62 ±0.105	5.5	0.840 ±0.019
1	Soft	PRO	-0.302 ±0.210	0.0112 ±0.00036	0.993	10.85 ±0.169	12.0	0.904 ±0.014
2	Hard		-0.242 ±0.322	0.0108 ±0.00052	0.986	10.58 ±0.235	11.7	0.904 ±0.020

Table 7. Trial 2B. Assessment of IB/IR rye translocation

a. Mean data - g digestible component / kg diet

Component	IB/IR			Time		
	N	Y	Mean	1	2	Mean
Starch	467	428	447	457	438	447
CYS	1.64	1.59	1.61	1.62	1.60	1.61
MET	0.93	1.00	0.96	0.94	0.99	0.96
ASP	2.14	2.12	2.13	2.02	2.24	2.13
THR	1.28	1.29	1.28	1.22	1.35	1.28
SER	2.12	2.20	2.16	2.13	2.18	2.16
GLU	13.14	14.14	13.64	13.54	13.74	13.64
GLY	1.57	1.59	1.58	1.53	1.62	1.58
ALA	1.48	1.47	1.47	1.43	1.52	1.47
VAL	1.40	1.44	1.42	1.38	1.46	1.42
ILEU	1.38	1.48	1.43	1.42	1.44	1.43
LEU	3.19	3.48	3.33	3.26	3.4	3.33
PHE	1.79	2.27	2.03	1.94	2.11	2.03
HIS	2.09	2.02	2.05	2.03	2.08	2.05
LYS	1.11	1.18	1.15	1.14	1.16	1.15
ARG	2.18	2.21	2.19	2.14	2.24	2.19
PRO	4.77	4.94	4.85	4.65	5.05	4.85

Table 7 (continued). Trial 2B

b. Analysis of variance of wheat characteristics
(In only case was effect of time significant)

Component	Endosperm				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	6231	32.6	0.261	8(3)	4258
CYS	1	0.00931	0.055	0.400	9(2)	0.01196
MET	1	0.018171	0.025	0.024	9(2)	0.002492
ASP	1	0.00151	0.104	0.855	9(2)	0.04300
THR	1	0.00042	0.079	0.900	9(2)	0.02486
SER	1	0.02452	0.080	0.199	9(2)	0.02570
GLU	1	4.0457	0.193	<0.001	9(2)	0.1490
GLY	1	0.00190	0.098	0.828	9(2)	0.03819
ALA	1	0.00025	0.082	0.925	9(2)	0.02660
VAL	1	0.00712	0.138	0.767	9(2)	0.07613
ILEU	1	0.04364	0.112	0.376	9(2)	0.05035
LEU	1	0.34504	0.094	0.013	9(2)	0.03566
PHE	1	0.93179	0.064	<0.001	9(2)	0.01651
HIS	1	0.02042	0.057	0.243	9(2)	0.01307
LYS	1	0.01974	0.072	0.352	9(2)	0.02050
ARG	1	0.00293	0.107	0.806	9(2)	0.04553
PRO	1	0.1151	0.377	0.663	9(2)	0.5688

Table 8. Trial 2B Linear regression equations and solution for starch and amino acids

Wheat sample	Characteristics IBIR	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
1	N	Starch	737 ±49.7	-0.480 ±0.094	0.835	257 ±49.7	432	0.59 ±0.115
2	Y		696 ±95.2	-0.479 ±0.154	0.591	217 ±69.4	468	0.46 ±0.148
1	N	CYS	-0.186 ±0.124	0.0032 ±0.00021	0.971	3.05 ±0.100	3.9	0.783 ±0.026
2	Y		-0.075 ±0.120	0.0030 ±0.00020	0.977	2.88 ±0.096	4.0	0.719 ±0.024
1	N	MET	-0.110 ±0.075	0.0018 ±0.00013	0.968	1.74 ±0.060	2.0	0.869 ±0.030
2	Y		-0.065 ±0.039	0.0019 ±0.00007	0.994	1.84 ±0.031	2.2	0.835 ±0.014
1	N	ASP	-0.548 ±0.278	0.0048 ±0.00047	0.936	4.23 ±0.223	5.8	0.729 ±0.038
2	Y		-0.173 ±0.269	0.0041 ±0.00045	0.943	3.97 ±0.216	5.9	0.672 ±0.037
1	N	THR	-0.615 ±0.213	0.0034 ±0.00036	0.925	2.75 ±0.171	3.7	0.743 ±0.046
2	Y		-0.192 ±0.166	0.0027 ±0.00028	0.947	2.45 ±0.133	3.8	0.652 ±0.035
1	N	SER	-0.473 ±0.207	0.0046 ±0.00035	0.961	4.13 ±0.166	5.3	0.779 ±0.031
2	Y		-0.052 ±0.123	0.0040 ±0.00021	0.987	3.96 ±0.099	5.6	0.707 ±0.018
1	N	GLU	-0.532 ±0.527	0.0242 ±0.00089	0.991	23.77 ±0.423	26.7	0.890 ±0.016
2	Y		0.257 ±0.236	0.0247 ±0.00040	0.999	25.00 ±0.190	29.0	0.862 ±0.007
1	N	GLY	-0.197 ±0.226	0.0031 ±0.00038	0.905	2.94 ±0.181	4.4	0.668 ±0.041
2	Y		0.153 ±0.227	0.0026 ±0.00038	0.899	2.73 ±0.182	4.7	0.581 ±0.039

Table 8. (continued)

Wheat sample	Characteristics IBIR	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
1	N	ALA	-0.125 ±0.176	0.0029 ±0.00030	0.928	2.73 ±0.142	4.0	0.682 ±0.035
2	Y		0.047 ±0.225	0.0026 ±0.00038	0.899	2.61 ±0.181	4.2	0.621 ±0.043
1	N	VAL	-0.337 ±0.340	0.0031 ±0.00057	0.799	2.74 ±0.273	4.3	0.638 ±0.064
2	Y		0.001 ±0.249	0.0026 ±0.00042	0.880	2.58 ±0.200	4.6	0.560 ±0.043
1	N	ILEU	-0.004 ±0.289	0.0025 ±0.00049	0.778	2.45 ±0.232	3.4	0.721 ±0.068
2	Y		0.024 ±0.152	0.0026 ±0.00026	0.953	2.62 ±0.122	3.7	0.709 ±0.033
1	N	LEU	-0.323 ±0.241	0.0062 ±0.00041	0.971	5.92 ±0.193	7.2	0.822 ±0.027
2	Y		-0.080 ±0.212	0.0064 ±0.00036	0.984	6.29 ±0.170	7.8	0.807 ±0.022
1	N	PHE	-0.338 ±0.162	0.0038 ±0.00027	0.965	3.44 ±0.130	4.6	0.747 ±0.028
2	Y		-0.194 ±0.220	0.0044 ±0.00037	0.966	4.23 ±0.177	5.5	0.770 ±0.032
1	N	HIS	-0.153 ±0.141	0.0040 ±0.00024	0.976	3.84 ±0.113	4.7	0.816 ±0.024
2	Y		-0.026 ±0.119	0.0037 ±0.00020	0.985	3.63 ±0.095	4.7	0.772 ±0.020
1	N	LYS	-0.173 ±0.180	0.0023 ±0.00030	0.889	2.12 ±0.144	3.0	0.705 ±0.048
2	Y		0.060 ±0.112	0.0020 ±0.00019	0.957	2.06 ±0.090	3.1	0.665 ±0.029
1	N	ARG	-0.245 ±0.213	0.0043 ±0.00036	0.953	4.06 ±0.171	5.2	0.782 ±0.033
2	Y		0.270 ±0.307	0.0035 ±0.00052	0.898	3.74 ±0.247	5.4	0.693 ±0.046
1	N	PRO	-1.640 ±1.060	0.0114 ±0.00178	0.851	9.75 ±0.848	10.7	0.911 ±0.079
2	Y		0.148 ±0.347	0.0086 ±0.00059	0.977	8.78 ±0.278	11.0	0.798 ±0.025

Table 9. Trial 2C. Assessment of VPM eyespot resistance

a. Mean data - g digestible component / kg diet

Component	VPM Eyespot Resistance			Time		
	Y	N	Mean	1	2	Mean
Starch	482	462	472	485	459	472
CYS	1.70	1.80	1.75	1.73	1.78	1.75
MET	0.98	0.97	0.97	0.94	1.00	0.97
ASP	2.44	2.46	2.45	2.40	2.50	2.45
THR	1.33	1.34	1.34	1.29	1.39	1.34
SER	2.28	2.24	2.26	2.23	2.30	2.26
GLU	13.87	14.23	14.05	14.00	14.11	14.05
GLY	1.84	1.98	1.91	1.89	1.93	1.91
ALA	1.58	1.74	1.66	1.63	1.69	1.66
VAL	1.65	1.62	1.63	1.57	1.70	1.63
ILEU	1.52	1.54	1.53	1.50	1.55	1.53
LEU	3.47	3.70	3.59	3.56	3.62	3.59
PHE	2.32	2.28	2.30	2.26	2.34	2.30
HIS	1.92	2.20	2.06	2.12	2.00	2.06
LYS	1.21	1.24	1.22	1.21	1.23	1.22
ARG	2.37	2.48	2.42	2.40	2.44	2.42
PRO	5.93	5.83	5.88	5.79	5.97	5.88

Table 9. Trial 2C

b. Analysis of variance of wheat characteristics
(In no case was effect of time significant)

Component	VPM Eyespot Resistance				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	1680	22.0	0.377	9(2)	1943
CYS	1	0.03715	0.051	0.085	11	0.01039
MET	1	0.000342	0.041	0.827	11	0.006867
ASP	1	0.00053	0.112	0.092	11	0.05051
THR	1	0.00045	0.067	0.876	11	0.01772
SER	1	0.00701	0.109	0.709	11	0.04775
GLU	1	0.5293	0.212	0.115	11	0.1804
GLY	1	0.07009	0.118	0.284	11	0.05527
ALA	1	0.10644	0.099	0.127	11	0.03896
VAL	1	0.0041	0.184	0.865	11	0.1356
ILEU	1	0.00093	0.094	0.875	11	0.03565
LEU	1	0.21599	0.078	0.012	11	0.02428
PHE	1	0.00865	0.071	0.525	11	0.02007
HIS	1	0.3321	0.187	0.153	11	0.1405
LYS	1	0.00336	0.055	0.610	11	0.01220
ARG	1	0.04698	0.119	0.382	11	0.05674
PRO	1	0.04368	0.098	0.310	11	0.03850

Table 10. Trial 2C Linear regression equations and solution for starch and amino acids

Wheat sample	Characteristics VPM Eyespot Resistance	Component	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
			b	a	R^2	Solution when x =1000		
1	Y	Starch	727 ±25.6	-0.435 ±0.043	0.935	292 ±20.6	499	0.59 ±0.041
2	N		699 ±96.2	-0.432 ±0.148	0.6	266 ±61.4	524	0.51 ±0.117
1	Y	CYS	-0.083 ±0.123	0.0032 ±0.00021	0.971	3.09 ±0.10	4.0	0.773 ±0.025
2	N		-0.187 ±0.104	0.0035 ±0.00018	0.983	3.35 ±0.084	4.2	0.796 ±0.020
1	Y	MET	-0.002 ±0.060	0.0017 ±0.00010	0.977	1.74 ±0.048	2.1	0.827 ±0.023
2	N		0.063 ±0.124	0.0016 ±0.00021	0.892	1.67 ±0.100	2.1	0.795 ±0.047
1	Y	ASP	-0.045 ±0.188	0.0044 ±0.00032	0.965	4.37 ±0.151	6.1	0.718 ±0.024
2	N		-0.036 ±0.300	0.0044 ±0.00051	0.915	4.39 ±0.241	6.3	0.697 ±0.038
1	Y	THR	-0.106 ±0.120	0.0026 ±0.00020	0.958	2.45 ±0.097	3.7	0.663 ±0.026
2	N		-0.221 ±0.187	0.0278 ±0.00032	0.916	2.56 ±0.150	3.8	0.672 ±0.040
1	Y	SER	0.000 ±0.176	0.0041 ±0.00030	0.964	4.06 ±0.141	5.4	0.751 ±0.026
2	N		-0.145 ±0.287	0.0042 ±0.00048	0.916	4.10 ±0.230	5.6	0.731 ±0.041
1	Y	GLU	0.254 ±0.588	0.0242 ±0.00100	0.988	24.46 ±0.472	27.8	0.880 ±0.017
2	N		-0.305 ±0.277	0.0258 ±0.00047	0.998	25.54 ±0.223	28.4	0.899 ±0.008
1	Y	GLY	0.100 ±0.326	0.0031 ±0.00055	0.815	3.20 ±0.262	4.7	0.681 ±0.056
2	N		-0.128 ±0.147	0.0037 ±0.00025	0.97	3.61 ±0.118	5.0	0.727 ±0.024

Table 10 (continued)

Wheat sample	Characteristics IBIR	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
1	Y	ALA	0.148 ±0.281	0.0025 ±0.00047	0.799	2.69 ±0.226	4.1	0.657 ±0.055
2	N		-0.018 ±0.155	0.0031 ±0.00019	0.974	3.11 ±0.092	4.4	0.707 ±0.021
1	Y	VAL	-0.010 ±0.306	0.0029 ±0.00052	0.819	2.94 ±0.246	4.5	0.653 ±0.055
2	N		0.082 ±0.481	0.0027 ±0.00081	0.596	2.81 ±0.386	4.7	0.598 ±0.082
1	Y	ILEU	0.045 ±0.184	0.0026 ±0.00031	0.910	2.67 ±0.148	3.6	0.741 ±0.041
2	N		-0.065 ±0.224	0.0028 ±0.00038	0.888	2.78 ±0.180	3.7	0.751 ±0.049
1	Y	LEU	0.115 ±0.203	0.0060 ±0.00034	0.978	6.08 ±0.163	7.6	0.800 ±0.021
2	N		-0.086 ±0.134	0.0067 ±0.00023	0.992	6.65 ±0.107	8.0	0.831 ±0.013
1	Y	PHE	-0.105 ±0.127	0.0043 ±0.00022	0.983	4.21 ±0.102	5.4	0.780 ±0.019
2	N		-0.157 ±0.189	0.0043 ±0.00032	0.963	4.17 ±0.152	5.5	0.758 ±0.028
1	Y	HIS	0.265 ±0.553	0.0029 ±0.00093	0.559	3.20 ±0.444	4.6	0.695 ±0.097
2	N		-0.020 ±0.172	0.0040 ±0.00029	0.964	3.93 ±0.138	4.9	0.803 ±0.028
1	Y	LYS	0.017 ±0.163	0.0021 ±0.00028	0.893	2.13 ±0.131	3.0	0.711 ±0.044
2	N		-0.090 ±0.040	0.0024 ±0.00007	0.994	2.27 ±0.032	3.1	0.731 ±0.010
1	Y	ARG	0.127 ±0.262	0.0040 ±0.00044	0.920	4.11 ±0.210	5.4	0.762 ±0.039
2	N		0.007 ±0.250	0.0044 ±0.00042	0.939	4.40 ±0.201	5.7	0.772 ±0.035
1	Y	PRO	-0.028 ±0.260	0.0106 ±0.00044	0.988	10.57 ±0.209	11.9	0.888 ±0.018
2	N		-0.118 ±0.222	0.0106 ±0.00038	0.991	10.45 ±0.178	11.9	0.878 ±0.015

Table 11. Trial 3A Assessment of endosperm hardness

a. Mean data - g digestible starch / kg experimental diet				b. Analysis of variance				
Component	**Endosperm**			**Factor**	**⁰ F**	**Mean Square**	**SEd**	**P=**
	Hard	Soft	Mean	**Endosperm**	1	373	48.8	0.846
Starch	494	484	489	**Residual**	14(1)	4180		

Table 12. Trial 3A Linear regression equations and solution for starch

Wheat sample	Characteristics Endosperm	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
1	Hard	Starch	787 ±46.5	-0.521 ±0.784	0.86	266 ±37.3	555	0.479 ±0.067
2	Soft		704 ±32.7	-0.373 ±0.053	0.89	331 ±23.9	518	0.639 ±0.046

Table 13. Trial 3B Assessment of protein content

a. Mean data - g digestible starch / kg experimental diet				b. Analysis of variance				
Component	**Protein content**			**Factor**	**⁰ F**	**Mean Square**	**SEd**	**P=**
	High	Low	Mean	**Protein**	1	22529	46.2	0.129
Starch	566	491	528	**Residual**	14(1)	8556		

Table 14. Trial 3B Linear regression equations and solution for starch

Wheat sample	Characteristics Protein content	Component	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
			b	a	R^2	Solution when x =1000		
1	High	Starch	710 ±37.4	-0.245 ±0.061	0.720	465 ±27.3	607	0.765 ±0.045
2	Low		784 ±57.7	-0.521 ±0.097	0.798	263 ±46.3	556	0.472 ±0.083

Table 15. Trial 4A Comparison of:

A - hard endosperm without IB/IR rye translocation
B - soft endosperm with IB/IR rye translocation

a. Mean data - g digestible component / kg diet

Component	Wheat			Time	
	A	B	Mean	1	2
Starch	554	489	522	505	539
CYS	1.95	1.75	1.85	1.82	1.87
MET	1.03	0.86	0.94	0.94	0.95
ASP	2.37	2.17	2.27	2.24	2.30
THR	1.20	1.06	1.13	1.09	1.16
SER	2.48	2.06	2.27	2.22	2.31
GLU	16.8	14.5	15.7	15.6	15.7
GLY	1.76	1.80	1.78	1.74	1.81
ALA	1.59	1.69	1.64	1.62	1.66
VAL	2.01	1.97	1.99	1.96	2.03
ILEU	1.61	1.64	1.62	1.60	1.65
LEU	3.63	3.40	3.51	3.51	3.52
PHE	2.29	2.29	2.29	2.30	2.28
HIS	1.56	1.99	1.77	1.76	1.79
LYS	1.33	1.87	1.60	1.57	1.63
ARG	2.39	2.20	2.29	2.27	2.31
PRO	5.39	6.40	5.89	5.84	5.95

Table 15 (continued). Trial 4A Comparison of:

A - hard endosperm without IB/IR rye translocation
B - soft endosperm with IB/IR rye translocation

b. Analysis of variance of wheat characteristics
(In only two cases was effect of time significant)

Component	Wheat				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	16805	11.2	<0.001	11	503
CYS	1	0.15665	0.02	<0.001	10(1)	0.002
MET	1	0.1203901	0.01	<0.001	10(1)	0.0007
ASP	1	0.15848	0.05	0.003	10(1)	0.01017
THR	1	0.073001	0.04	0.006	10(1)	0.00615
SER	1	0.722361	0.04	<0.001	10(1)	0.00735
GLU	1	21.54196	0.07	<0.001	10(1)	0.01869
GLY	1	0.0057	0.04	0.382	10(1)	0.00681
ALA	1	0.045166	0.04	0.015	10(1)	0.005293
VAL	1	0.005246	0.04	0.385	10(1)	0.006369
ILEU	1	0.003342	0.02	0.249	10(1)	0.002227
LEU	1	0.207986	0.04	<0.001	10(1)	0.004943
PHE	1	0.000262	0.04	0.855	10(1)	0.007491
HIS	1	0.737092	0.02	<0.001	10(1)	0.002286
LYS	1	1.157962	0.03	<0.001	10(1)	0.003976
ARG	1	0.135187	0.04	<0.001	10(1)	0.005860
PRO	1	4.06805	0.06	<0.001	10(1)	0.01499

Table 16. Trial 4A Linear regression equations and solution for starch and amino acids

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	Starch	738 ±28.4	-0.327 ±0.048	0.866	411 ±22.8	591	0.697 ±0.039
B		768 ±35.6	-0.496 ±0.060	0.906	273 ±28.6	498	0.547 ±0.057
A	CYS	-0.085 ±0.057	0.0036 ±0.00010	0.995	3.53 ±0.046	4.1	0.860 ±0.011
B		0.016 ±0.072	0.0031 ±0.00012	0.992	3.10 ±0.052	3.8	0.817 ±0.014
A	MET	-0.005 ±0.033	0.0018 ±0.00005	0.994	1.83 ±0.026	2.1	0.873 ±0.012
B		-0.018 ±0.030	0.0016 ±0.00005	0.994	1.54 ±0.022	1.8	0.854 ±0.012
A	ASP	-0.032 ±0.147	0.0043 ±0.00025	0.977	4.24 ±0.118	5.6	0.756 ±0.021
B		-0.026 ±0.067	0.0039 ±0.00011	0.995	3.89 ±0.049	5.4	0.720 ±0.009
A	THR	-0.113 ±0.098	0.0023 ±0.00017	0.966	2.21 ±0.079	3.2	0.692 ±0.025
B		-0.030 ±0.101	0.0019 ±0.00016	0.959	1.92 ±0.074	3.1	0.619 ±0.024
A	SER	-0.038 ±0.112	0.0045 ±0.00019	0.988	4.44 ±0.090	5.5	0.807 ±0.016
B		-0.011 ±0.120	0.0037 ±0.00019	0.984	3.68 ±0.088	4.9	0.750 ±0.018
A	GLU	0.014 ±0.183	0.0299 ±0.00031	0.999	29.94 ±0.147	32.0	0.936 ±0.005
B		0.007 ±0.127	0.0258 ±0.00021	1.000	25.83 ±0.093	28.1	0.919 ±0.003
A	GLY	0.024 ±0.113	0.0031 ±0.00019	0.974	3.11 ±0.090	4.2	0.740 ±0.022
B		-0.025 ±0.084	0.0032 ±0.00014	0.990	3.22 ±0.061	4.4	0.732 ±0.014
A	ALA	0.046 ±0.094	0.0027 ±0.00016	0.977	2.78 ±0.076	3.8	0.733 ±0.020
B		0.028 ±0.073	0.0030 ±0.00012	0.991	2.99 ±0.053	4.1	0.730 ±0.013

Table 16 (continued)

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	VAL	0.068 ±0.111	0.0035 ±0.00019	0.980	3.52 ±0.089	4.6	0.765 ±0.019
B		-0.025 ±0.084	0.0036 ±0.00014	0.991	3.54 ±0.062	4.6	0.769 ±0.013
A	ILEU	0.024 ±0.067	0.0028 ±0.00011	0.989	2.84 ±0.054	3.5	0.811 ±0.015
B		0.003 ±0.058	0.0029 ±0.00009	0.994	2.91 ±0.042	3.6	0.809 ±0.012
A	LEU	-0.001 ±0.094	0.0065 ±0.00016	0.996	6.45 ±0.075	7.5	0.860 ±0.010
B		0.008 ±0.051	0.0060 ±0.00008	0.999	6.04 ±0.037	7.2	0.839 ±0.005
A	PHE	-0.203 ±0.117	0.0044 ±0.00020	0.986	4.24 ±0.094	5.2	0.815 ±0.018
B		-0.004 ±0.055	0.0041 ±0.00009	0.997	4.06 ±0.040	5.2	0.781 ±0.008
A	HIS	0.045 ±0.059	0.0027 ±0.00010	0.991	2.74 ±0.047	3.4	0.805 ±0.014
B		0.013 ±0.057	0.0035 ±0.00009	0.996	3.53 ±0.042	4.2	0.840 ±0.010
A	LYS	0.090 ±0.097	0.0022 ±0.00016	0.963	2.29 ±0.078	3.0	0.765 ±0.026
B		0.025 ±0.049	0.0033 ±0.00008	0.997	3.31 ±0.035	4.0	0.827 ±0.009
A	ARG	0.011 ±0.099	0.0042 ±0.00017	0.989	4.23 ±0.080	5.2	0.814 ±0.015
B		0.002 ±0.076	0.0039 ±0.00012	0.994	3.92 ±0.055	5.1	0.768 ±0.011
A	PRO	0.079 ±0.197	0.0094 ±0.00033	0.991	9.51 ±0.158	10.7	0.889 ±0.015
B		0.026 ±0.045	0.0113 ±0.00007	1.000	11.37 ±0.033	12.5	0.909 ±0.003

Table 17. Trial 4B Assessment of IB/IR rye translocation (in hard feed wheat background)

a. Mean data - g digestible component / kg diet

Component	IBIR			Time	
	N	Y	Mean	1	2
Starch	541	535	538	527	548
CYS	1.55	1.59	1.57	1.58	1.56
MET	0.92	0.88	0.9	0.91	0.89
ASP	2.32	2.37	2.34	2.39	2.30
THR	1.17	1.21	1.19	1.24	1.15
SER	2.37	2.37	2.37	2.41	2.32
GLU	14.3	14.5	14.4	14.5	14.3
GLY	1.61	1.73	1.67	1.71	1.63
ALA	1.47	1.55	1.51	1.55	1.46
VAL	1.95	1.61	1.78	1.79	1.77
ILEU	1.28	1.26	1.27	1.30	1.24
LEU	3.11	3.07	3.09	3.13	3.06
PHE	1.97	2.10	2.04	2.08	2.00
HIS	1.65	1.49	1.57	1.58	1.56
LYS	1.35	1.47	1.41	1.41	1.41
ARG	2.39	2.20	2.29	2.27	2.31
PRO	5.39	6.40	5.89	5.84	5.95

Table 17. Trial 4B

b. Analysis of variance of wheat characteristics
(In no case was effect of time significant)

Component	IB/IR				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	166	37.3	0.866	10 (1)	5557
CYS	1	0.007604	0.040	0.299	11	0.006412
MET	1	0.007966	0.022	0.070	11	0.001980
ASP	1	0.00834	0.101	0.660	11	0.04089
THR	1	0.00520	0.078	0.654	11	0.02452
SER	1	0.00010	0.063	0.939	11	0.01591
GLU	1	0.1790	0.160	0.221	11	0.1066
GLY	1	0.05242	0.071	0.135	11	0.02018
ALA	1	0.02878	0.070	0.251	11	0.01961
VAL	1	0.47015	0.075	<0.001	11	0.02254
ILEU	1	0.002932	0.035	0.449	11	0.004761
LEU	1	0.00539	0.093	0.699	11	0.03433
PHE	1	0.06700	0.085	0.156	11	0.02896
HIS	1	0.099052	0.038	0.002	11	0.005716
LYS	1	0.06157	0.055	0.046	11	0.01222
ARG	1	0.135187	0.038	<0.001	11	0.005860
PRO	1	4.06805	0.061	<0.001	11	0.01499

Table 18. Trial 4B Linear regression equations and solution for starch and amino acids

Wheat IBIR	Component	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
		b	a	R^2	Solution when x =1000		
N	Starch	788 ±59.2	-0.435 ±0.096	0.766	352 ±43.2	624	0.565 ±0.069
Y		770 ±98.0	-0.419 ±0.165	0.437	351 ±78.7	587	0.598 ±0.134
N	CYS	-0.264 ±0.110	0.0032 ±0.00019	0.977	2.96 ±0.088	3.4	0.870 ±0.026
Y		-0.092 ±0.056	0.0030 ±0.00009	0.993	2.90 ±0.045	3.4	0.853 ±0.013
N	MET	-0.035 ±0.054	0.0017 ±0.00009	0.980	1.67 ±0.044	1.9	0.877 ±0.023
Y		-0.073 ±0.043	0.0017 ±0.00007	0.987	1.80 ±0.034	1.8	0.898 ±0.019
N	ASP	-0.341 ±0.291	0.0047 ±0.00049	0.929	4.39 ±0.234	5.5	0.798 ±0.042
Y		-0.181 ±0.127	0.0045 ±0.00021	0.985	4.35 ±0.102	5.4	0.805 ±0.019
N	THR	-0.315 ±0.232	0.0026 ±0.00039	0.865	2.33 ±0.186	3.2	0.729 ±0.058
Y		-0.717 ±0.098	0.0025 ±0.00016	0.969	2.28 ±0.078	3.1	0.737 ±0.025
N	SER	-0.304 ±0.181	0.0048 ±0.00031	0.972	4.45 ±0.145	5.3	0.840 ±0.027
Y		-0.196 ±0.100	0.0046 ±0.00017	0.999	4.36 ±0.080	5.2	0.838 ±0.015
N	GLU	-0.357 ±0.466	0.0260 ±0.00079	0.994	25.68 ±0.374	27.4	0.937 ±0.014
Y		-0.167 ±0.209	0.0261 ±0.00035	0.999	25.91 ±0.168	27.5	0.942 ±0.006
N	GLY	-0.170 ±0.197	0.0032 ±0.00033	0.928	3.00 ±0.158	3.9	0.769 ±0.040
Y		-0.101 ±0.112	0.0032 ±0.00019	0.977	3.15 ±0.090	4.0	0.787 ±0.023
N	ALA	-0.186 ±0.205	0.0029 ±0.00035	0.91	2.75 ±0.165	3.6	0.764 ±0.046
Y		-0.064 ±0.097	0.0029 ±0.00016	0.978	2.81 ±0.078	3.6	0.780 ±0.022

Table 18. (Continued)

Wheat IBIR	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
N	VAL	-0.206 ±0.183	0.0038 ±0.00031	0.956	3.63 ±0.147	4.5	0.806 ±0.033
Y		-0.104 ±0.136	0.0030 ±0.00023	0.962	2.94 ±0.109	3.8	0.773 ±0.029
N	ILEU	-0.019 ±0.102	0.0023 ±0.00017	0.963	2.30 ±0.082	3.0	0.766 ±0.027
Y		-0.046 ±0.067	0.0023 ±0.00011	0.984	2.27 ±0.054	2.8	0.811 ±0.019
N	LEU	-0.200 ±0.263	0.0059 ±0.00044	0.962	5.68 ±0.211	6.6	0.861 ±0.032
Y		-0.090 ±0.118	0.0056 ±0.00020	0.991	5.53 ±0.094	6.4	0.864 ±0.015
N	PHE	-0.268 ±0.255	0.0040 ±0.00043	0.924	3.71 ±0.205	4.6	0.807 ±0.045
Y		-0.193 ±0.078	0.0041 ±0.00013	0.993	3.86 ±0.063	4.7	0.827 ±0.013
N	HIS	-0.069 ±0.099	0.0031 ±0.00017	0.980	2.99 ±0.079	3.5	0.853 ±0.023
Y		-0.074 ±0.062	0.0028 ±0.00010	0.990	2.71 ±0.050	3.2	0.847 ±0.015
N	LYS	-0.024 ±0.139	0.0024 ±0.00024	0.939	2.42 ±0.112	3.0	0.806 ±0.037
Y		-0.056 ±0.093	0.0027 ±0.00016	0.977	2.66 ±0.075	3.1	0.859 ±0.024
N	ARG	-0.146 ±0.196	0.0043 ±0.00033	0.959	4.12 ±0.157	4.9	0.840 ±0.032
Y		-0.070 ±0.097	0.0047 ±0.00016	0.991	4.59 ±0.078	5.3	0.865 ±0.015
N	PRO	-1.033 ±0.636	0.0085 ±0.00107	0.897	7.42 ±0.511	8.0	0.928 ±0.064
Y		-0.165 ±0.147	0.0081 ±0.00025	0.993	7.92 ±0.118	8.7	0.911 ±0.014

Table 19 Trial 4C. Comparisons of endosperm texture and IB/IR rye translocation

A - soft endosperm without IB/IR rye translocation
B - hard endosperm with IB/IR rye translocation

a. Mean data - g digestible component / kg diet

Component	Wheat			Time	
	A	B	Mean	1	2
Starch	487	454	470	460	481
CYS	1.8	1.62	1.71	1.72	1.7
MET	1.01	1.06	1.03	1.02	1.05
ASP	2.51	2.57	2.54	2.5	2.57
THR	1.16	1.07	1.12	1.11	1.12
SER	2.24	2.19	2.21	2.22	2.21
GLU	15.56	14.37	14.97	14.94	15
GLY	1.93	1.78	1.86	1.84	1.87
ALA	1.74	1.55	1.64	1.64	1.64
VAL	1.96	1.74	1.85	1.83	1.87
ILEU	1.68	1.55	1.61	1.59	1.64
LEU	3.49	3.36	3.42	3.4	3.45
PHE	2.15	3.36	2.76	2.75	2.76
HIS	1.71	1.55	1.63	1.62	1.65
LYS	1.49	1.6	1.54	1.5	1.58
ARG	2.67	2.56	2.61	2.54	2.68
PRO	5.12	5.03	5.08	5.05	5.11

Table 19 (continued) Trial 4C. Comparisons of endosperm texture and IB/IR rye translocation

A - soft endosperm without IB/IR rye translocation
B - hard endosperm with IB/IR rye translocation

b. Analysis of variance of wheat characteristics
(In no case was effect of time significant)

Component	Wheat				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	4337	27.4	0.260	9(2)	3002
CYS	1	0.137763	0.028	<0.001	9(2)	0.003187
MET	1	0.007997	0.017	0.025	9(2)	0.001107
ASP	1	0.01308	0.066	0.407	9(2)	0.01732
THR	1	0.02920	0.052	0.133	9(2)	0.01073
SER	1	0.006773	0.050	0.428	9(2)	0.009841
GLU	1	5.67624	0.096	<0.001	9(2)	0.03714
GLY	1	0.084177	0.041	0.007	9(2)	0.006843
ALA	1	0.150244	0.047	0.002	9(2)	0.008650
VAL	1	0.20075	0.075	0.015	9(2)	0.02230
ILEU	1	0.058615	0.034	0.006	9(2)	0.004576
LEU	1	0.06681	0.055	0.043	9(2)	0.01208
PHE	1	5.85685	0.066	<0.001	9(2)	0.01754
HIS	1	0.101640	0.035	0.001	9(2)	0.004786
LYS	1	0.049290	0.043	0.029	9(2)	0.007301
ARG	1	0.04970	0.075	0.171	9(2)	0.02249
PRO	1	0.036077	0.048	0.082	9(2)	0.009384

Table 20. Trial 4C Linear regression equations and solution for starch and amino acids

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	Starch	745 ±50.4	-0.460 ±0.085	0.801	286 ±40.4	588	0.49 ±0.069
B		759 ±95.5	-0.532 ±0.147	0.707	227 ±61.0	539	0.42 ±0.113
A	CYS	-0.073 ±0.077	0.0033 ±0.00014	0.990	3.26 ±0.068	3.9	0.836 ±0.018
B		0.008 ±0.050	0.0029 ±0.00008	0.995	2.87 ±0.036	3.7	0.775 ±0.010
A	MET	-0.003 ±0.031	0.0018 ±0.00005	0.995	1.80 ±0.027	2.1	0.859 ±0.013
B		0.034 ±0.050	0.0018 ±0.00008	0.988	1.86 ±0.037	2.2	0.843 ±0.017
A	ASP	-0.039 ±0.146	0.0045 ±0.00026	0.981	4.50 ±0.129	6.0	0.750 ±0.021
B		0.023 ±0.171	0.0045 ±0.00028	0.978	4.55 ±0.125	6.2	0.734 ±0.020
A	THR	-0.085 ±0.119	0.0022 ±0.00021	0.948	2.13 ±0.105	3.3	0.644 ±0.032
B		-0.082 ±0.117	0.0021 ±0.00019	0.951	1.97 ±0.086	3.2	0.616 ±0.027
A	SER	-0.107 ±0.124	0.0042 ±0.00022	0.984	4.06 ±0.109	5.2	0.780 ±0.021
B		-0.069 ±0.099	0.0040 ±0.00016	0.991	3.95 ±0.072	5.2	0.760 ±0.014
A	GLU	0.026 ±0.221	0.0276 ±0.00039	0.999	27.66 ±0.195	29.8	0.928 ±0.007
B		0.056 ±0.228	0.0255 ±0.00037	0.999	25.52 ±0.166	27.9	0.915 ±0.006
A	GLY	0.022 ±0.090	0.0034 ±0.00016	0.987	3.42 ±0.079	4.6	0.743 ±0.017
B		0.041 ±0.103	0.0031 ±0.00017	0.983	3.14 ±0.075	4.4	0.715 ±0.017

Table 20. (continued)

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	ALA	0.053 ±0.124	0.0030 ±0.00022	0.969	3.05 ±0.109	4.2	0.726 ±0.026
B		0.060 ±0.080	0.0026 ±0.00013	0.986	2.70 ±0.059	3.9	0.692 ±0.015
A	VAL	-0.058 ±0.176	0.0036 ±0.00031	0.957	3.54 ±0.155	4.7	0.753 ±0.033
B		0.089 ±0.169	0.0029 ±0.00027	0.950	3.03 ±0.124	4.3	0.704 ±0.029
A	ILEU	0.004 ±0.068	0.0030 ±0.00012	0.990	2.98 ±0.060	3.7	0.806 ±0.016
B		0.008 ±0.097	0.0028 ±0.00016	0.981	2.76 ±0.071	3.5	0.789 ±0.020
A	LEU	0.045 ±0.134	0.0061 ±0.00024	0.991	6.17 ±0.118	7.4	0.834 ±0.016
B		0.031 ±0.124	0.0059 ±0.00020	0.993	5.95 ±0.091	7.2	0.827 ±0.013
A	PHE	-0.145 ±0.178	0.0041 ±0.00032	0.965	3.94 ±0.157	5.1	0.772 ±0.031
B		-0.220 ±0.112	0.0064 ±0.00018	0.995	6.15 ±0.082	7.3	0.842 ±0.011
A	HIS	-0.024 ±0.054	0.0031 ±0.00009	0.994	3.07 ±0.047	3.7	0.829 ±0.013
B		0.067 ±0.104	0.0026 ±0.00017	0.976	2.71 ±0.076	3.5	0.776 ±0.022
A	LYS	0.077 ±0.085	0.0025 ±0.00015	0.979	2.60 ±0.075	3.3	0.787 ±0.023
B		0.096 ±0.140	0.0027 ±0.00023	0.959	2.78 ±0.102	3.5	0.794 ±0.029
A	ARG	0.038 ±0.120	0.0047 ±0.00021	0.988	4.73 ±0.106	5.7	0.830 ±0.019
B		0.190 ±0.261	0.0042 ±0.00042	0.943	4.42 ±0.190	5.7	0.775 ±0.033
A	PRO	-0.054 ±0.114	0.0092 ±0.00020	0.997	9.16 ±0.100	10.3	0.889 ±0.010
B		0.115 ±0.123	0.0087 ±0.00020	0.997	8.86 ±0.090	10.2	0.869 ±0.009

Table 21. Trial 4D. Assessment of dough extensibility in background of Galahad and Galatea

A - 'normal' dough characteristics
B - very extensible dough

a. Mean data - g digestible component / kg diet

Component	Wheat			Time	
	A	B	Mean	1	2
Starch	500	496	498	481	515
CYS	2.55	2.20	2.37	2.43	2.32
MET	1.46	1.42	1.44	1.44	1.44
ASP	3.55	3.45	3.5	3.54	3.46
THR	1.71	1.67	1.69	1.73	1.65
SER	3.13	2.99	3.06	3.08	3.04
GLU	20.97	20.46	20.71	20.77	20.65
GLY	2.38	2.00	2.19	2.21	2.17
ALA	2.25	2.04	2.15	2.18	2.11
VAL	2.76	2.61	2.68	2.71	2.66
ILEU	2.42	2.39	2.40	2.41	2.39
LEU	4.85	4.83	4.83	4.87	4.81
PHE	3.12	3.27	3.19	3.22	3.17
HIS	2.28	2.6	2.44	2.45	2.43
LYS	2.01	1.96	1.99	1.99	1.99
ARG	3.87	3.49	3.68	3.65	3.71
PRO	5.51	7.64	6.57	6.64	6.51

Table 21 (continued). Trial 4D. Assessment of dough extensibility in background of Galahad and Galatea

A - ‘normal’ dough characteristics
B - very extensible dough

b. Analysis of variance of wheat characteristics
(In no case was effect of time significant)

Component	Dough extensibility				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	0.03	22.6	0.866	11	2043
CYS	1	0.49120	0.056	<0.001	11	0.01272
MET	1	0.009269	0.027	0.097	11	0.002812
ASP	1	0.03812	0.131	0.471	11	0.06840
THR	1	0.00583	0.093	0.690	11	0.03487
SER	1	0.08546	0.090	0.133	11	0.03245
GLU	1	1.0182	0.193	0.024	11	0.1492
GLY	1	0.60666	0.091	0.001	11	0.03322
ALA	1	0.16997	0.095	0.054	11	0.03636
VAL	1	0.08467	0.093	0.145	11	0.03450
ILEU	1	0.00516	0.061	0.568	11	0.01486
LEU	1	0.00252	0.105	0.813	11	0.04283
PHE	1	0.08935	0.086	0.111	11	0.02971
HIS	1	0.39898	0.056	<0.001	11	0.01253
LYS	1	0.00971	0.064	0.457	11	0.01635
ARG	1	0.57982	0.139	0.019	11	0.07756
PRO	1	0.57982	0.082	<0.001	11	0.07756

Table 22. Trial 4D Linear regression equations and solution for starch and amino acids

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	Starch	815 ±48.8	-0.561 ±0.082	0.866	255 ±39.2	502	0.51 ±0.078
B		762 ±57.6	-0.473 ±0.097	0.765	289 ±46.2	505	0.57 ±0.092
A	CYS	-0.060±0.096	0.0046 ±0.00016	0.992	4.58 ±0.077	5.3	0.864 ±0.015
B		-0.046 ±0.170	0.0040 ±0.00029	0.965	3.94 ±0.137	4.6	0.857 ±0.030
A	MET	-0.059 ±0.022	0.0027 ±0.00004	0.999	2.65 ±0.017	2.9	0.913 ±0.006
B		0.014 ±0.078	0.0025 ±0.00013	0.981	2.51 ±0.062	2.8	0.895 ±0.022
A	ASP	-0.152 ±0.144	0.0066 ±0.00024	0.991	6.43 ±0.115	7.9	0.814 ±0.015
B		0.037 ±0.376	0.0061 ±0.00063	0.929	6.11 ±0.301	7.6	0.804 ±0.040
A	THR	-0.166 ±0.117	0.0033 ±0.00020	0.976	3.17 ±0.094	4.3	0.738 ±0.022
B		-0.084 ±0.267	0.0031 ±0.00045	0.871	3.04 ±0.214	4.1	0.742 ±0.052
A	SER	-0.133 ±0.118	0.0058 ±0.00020	0.992	5.67 ±0.095	6.8	0.834 ±0.014
B		-0.073 ±0.249	0.0054 ±0.00042	0.960	5.36 ±0.200	6.4	0.838 ±0.031
A	GLU	-0.220 ±0.209	0.0377 ±0.00035	0.999	37.44 ±0.168	39.6	0.946 ±0.004
B		0.098 ±0.557	0.0362 ±0.00094	0.995	36.30 ±0.447	38.5	0.943 ±0.012
A	GLY	-0.113 ±0.110	0.0044 ±0.00019	0.988	4.33 ±0.088	5.4	0.801 ±0.016
B		0.016 ±0.254	0.0035 ±0.00043	0.904	3.53 ±0.204	4.6	0.768 ±0.044

Table 22. (continued)

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	ALA	-0.083 ±0.100	0.0041 ±0.00017	0.989	4.06 ±0.080	5.1	0.797 ±0.016
B		0.057 ±0.277	0.0035 ±0.00047	0.889	3.59 ±0.223	4.7	0.764 ±0.047
A	VAL	-0.210 ±0.113	0.0053 ±0.00019	0.991	5.07 ±0.091	6.0	0.844 ±0.015
B		-0.046 ±0.263	0.0047 ±0.00044	0.941	4.68 ±0.211	5.7	0.821 ±0.037
A	ILEU	-0.095 ±0.073	0.0045 ±0.00012	0.995	4.37 ±0.059	5.0	0.876 ±0.012
B		0.017 ±0.170	0.0042 ±0.00029	0.968	4.23 ±0.137	4.9	0.863 ±0.028
A	LEU	-0.183 ±0.102	0.0090 ±0.00017	0.997	8.77 ±0.082	9.8	0.895 ±0.008
B		0.049 ±0.302	0.0085 ±0.00051	0.975	8.54 ±0.243	9.7	0.881 ±0.025
A	PHE	-0.213 ±0.094	0.0059 ±0.00016	0.995	5.71 ±0.075	6.8	0.840 ±0.011
B		-0.119 ±0.249	0.0060 ±0.00042	0.967	5.90 ±0.200	6.9	0.855 ±0.029
A	HIS	-0.122 ±0.067	0.0043 ±0.00011	0.995	4.15 ±0.054	4.7	0.882 ±0.011
B		-0.027 ±0.157	0.0047 ±0.00026	0.978	4.63 ±0.126	5.2	0.891 ±0.024
A	LYS	-0.062 ±0.102	0.0037 ±0.00017	0.985	3.62 ±0.082	4.2	0.862 ±0.020
B		0.072 ±0.164	0.0034 ±0.00028	0.954	3.43 ±0.132	4.1	0.837 ±0.032
A	ARG	-0.013 ±0.083	0.0069 ±0.00014	0.997	6.89 ±0.066	7.9	0.872 ±0.008
B		0.104 ±0.418	0.0060 ±0.00071	0.911	6.12 ±0.336	7.3	0.839 ±0.046
A	PRO	-0.249 ±0.080	0.0102 ±0.00013	0.999	9.99 ±0.064	10.9	0.917 ±0.006
B		-0.037 ±0.263	0.0136 ±0.00044	0.993	13.60 ±0.211	14.6	0.932 ±0.014

Table 23. Trial 5A Assessment of endosperm hardness (in a high protein background)

a. Mean data - g digestible component / kg diet

Component	Endosperm			Time	
	S	H	Mean	1	2
Starch	478	449	463	493	434
CYS	1.61	1.60	1.60	1.56	1.65
MET	0.91	0.92	0.92	0.91	0.92
ASP	2.23	2.34	2.28	2.28	2.29
THR	1.13	1.19	1.16	1.16	1.16
SER	2.11	2.50	2.31	2.30	2.31
GLU	14.0	13.9	14.0	14.0	14.0
GLY	1.56	1.63	1.59	1.56	1.63
ALA	1.38	1.43	1.41	1.29	1.52
VAL	1.63	2.11	1.87	1.84	1.90
ILEU	1.34	1.52	1.43	1.42	1.45
LEU	3.02	3.11	3.07	3.01	3.13
PHE	1.79	1.63	1.71	1.69	1.73
HIS	1.69	1.76	1.73	1.71	1.74
LYS	1.24	1.49	1.36	1.36	1.37
ARG	2.35	2.17	2.26	2.32	2.19
PRO	5.45	3.77	4.61	4.58	4.64

Table 23. Trial 5A Assessment of endosperm hardness (in a high protein background)

b. Analysis of variance of wheat characteristics
(In only two cases was effect of time significant)

Component	Endosperm				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	6917	21.1	0.175	25(2)	3549
CYS	1	0.00076	0.080	0.904	26(1)	0.05145
MET	1	0.00066	0.047	0.848	26(1)	0.01745
ASP	1	0.09164	0.111	0.342	26(1)	0.09782
THR	1	0.02459	0.069	0.428	26(1)	0.03795
SER	1	1.21145	0.075	<0.001	26(1)	0.04479
GLU	1	0.183	0.363	0.680	26(1)	1.052
GLY	1	0.04248	0.109	0.509	26(1)	0.09496
ALA	1	0.01805	0.107	0.660	26(1)	0.09136
VAL	1	1.84056	0.076	<0.001	26(1)	0.04580
ILEU	1	0.25489	0.061	0.007	26(1)	0.02973
LEU	1	0.0733	0.124	0.448	26(1)	0.1232
PHE	1	0.18205	0.075	0.054	26(1)	0.04470
HIS	1	0.03082	0.060	0.308	26(1)	0.02847
LYS	1	0.4713	0.114	0.043	26(1)	0.1046
ARG	1	0.25356	0.096	0.075	26(1)	0.07391
PRO	1	22.635	0.379	<0.001	26(1)	1.148

Table 24. Trial 5A Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
S	Starch	1	580 ±67.7	-0.154 ±0.114	0.104	426 ±54.3	594	0.72 ±0.092
S		8	711 ±61.8	-0.442 ±0.104	0.708	269 ±49.6	556	0.48 ±0.089
H		3	693 ±59.2	-0.436 ±0.099	0.784	256 ±47.5	516	0.42 ±0.077
H		5	595 ±98.6	-0.258 ±0.166	0.168	336 ±79.1	536	0.63 ±0.148
S	CYS	1	-0.114 ±0.070	0.0035 ±0.00012	0.992	3.36 ±0.056	4.1	0.821 ±0.014
S		8	-0.021 ±0.177	0.0025 ±0.00029	0.924	2.43 ±0.129	3.3	0.737 ±0.039
H		3	-0.203 ±0.152	0.0031 ±0.00026	0.954	2.89 ±0.122	3.7	0.782 ±0.033
H		5	-0.198 ±0.093	0.0033 ±0.00016	0.984	3.10 ±0.075	3.7	0.839 ±0.020
S	MET	1	0.007 ±0.034	0.0018 ±0.00006	0.993	1.83 ±0.028	2.1	0.871 ±0.013
S		8	0.047 ±0.067	0.0013 ±0.00011	0.960	1.36 ±0.049	1.7	0.799 ±0.029
H		3	-0.040 ±0.053	0.0016 ±0.00009	0.977	1.51 ±0.042	1.8	0.841 ±0.024
H		5	-0.019 ±0.044	0.0018 ±0.00007	0.989	1.81 ±0.035	2.0	0.906 ±0.018
S	ASP	1	-0.070 ±0.136	0.0045 ±0.00023	0.982	4.45 ±0.109	5.8	0.767 ±0.019
S		8	0.215 ±0.318	0.0031 ±0.00052	0.856	3.33 ±0.232	5.2	0.641 ±0.045
H		3	-0.135 ±0.158	0.0041 ±0.00027	0.971	3.96 ±0.127	5.4	0.734 ±0.023
H		5	-0.311 ±0.201	0.0050 ±0.00034	0.969	4.69 ±0.161	5.6	0.837 ±0.029

Table 24 (continued). Trial 5A Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
			b	a	R^2	Solution when x =1000		
S	THR	1	-0.127 ±0.088	0.0023 ±0.00015	0.972	2.18 ±0.071	3.20	0.682 ±0.022
S		8	-0.186 ±0.372	0.0023 ±0.00060	0.685	2.08 ±0.272	3.10	0.669 ±0.088
H		3	-0.260 ±0.204	0.0024 ±0.00034	0.870	2.12 ±0.164	3.20	0.661 ±0.051
H		5	-0.269 ±0.118	0.0028 ±0.00020	0.966	2.51 ±0.094	3.30	0.762 ±0.029
S	SER	1	-0.129 ±0.150	0.0041 ±0.00025	0.974	3.99 ±0.120	5.00	0.798 ±0.024
S		8	-0.079 ±0.179	0.0038 ±0.00029	0.965	3.67 ±0.131	4.90	0.749 ±0.027
H		3	-0.273 ±0.221	0.0047 ±0.00037	0.957	4.40 ±0.177	5.40	0.814 ±0.033
H		5	-0.399 ±0.197	0.0054 ±0.00033	0.974	5.02 ±0.158	5.60	0.896 ±0.028
S	GLU	1	0.010 ±0.265	0.0268±0.00045	0.998	26.83 ±0.213	28.80	0.932 ±0.007
S		8	0.745 ±0.687	0.0216 ±0.00111	0.984	22.32 ±0.501	25.60	0.872 ±0.020
H		3	-0.056 ±0.289	0.0242 ±0.00049	0.997	24.19 ±0.232	26.60	0.909 ±0.009
H		5	-0.321 ±0.261	0.0258 ±0.00044	0.998	26.90 ±0.209	26.90	0.946 ±0.008
S	GLY	1	-0.035 ±0.143	0.0033 ±0.00024	0.963	3.22 ±0.115	4.30	0.750 ±0.027
S		8	0.277 ±0.341	0.0019 ±0.00055	0.649	2.15 ±0.249	3.80	0.564 ±0.065
H		3	-0.088 ±0.210	0.0027 ±0.00036	0.894	2.66 ±0.169	4.00	0.665 ±0.042
H		5	-0.149 ±0.123	0.0035 ±0.00021	0.976	3.32 ±0.099	4.20	0.792 ±0.024

Table 24 (continued). Trial 5A Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
			b	a	R^2	Solution when x =1000		
S	ALA	1	-0.184 ±0.210	0.0032 ±0.00035	0.921	3.03 ±0.168	3.9	0.777 ±0.043
S		8	0.283 ±0.434	0.0015 ±0.00070	0.384	1.81 ±0.317	3.5	0.518 ±0.091
H		3	-0.029 ±0.270	0.0024 ±0.00046	0.789	3.70 ±0.217	3.7	0.634 ±0.059
H		5	-0.150 ±0.093	0.0030 ±0.00016	0.981	2.87 ±0.075	3.7	0.777 ±0.020
S	VAL	1	-0.081 ±0.102	0.0033 ±0.00017	0.981	3.23 ±0.082	4.3	0.751 ±0.019
S		8	0.157 ±0.219	0.0023 ±0.00035	0.876	2.49 ±0.160	4.0	0.623 ±0.040
H		3	-0.094 ±0.214	0.0037 ±0.00036	0.938	3.64 ±0.171	5.0	0.728 ±0.034
H		5	-0.234 ±0.121	0.0043 ±0.00021	0.984	4.10 ±0.098	4.9	0.836 ±0.020
S	ILEU	1	-0.023 ±0.086	0.0027 ±0.00015	0.980	2.67 ±0.069	3.3	0.808 ±0.021
S		8	0.107 ±0.140	0.0019 ±0.00023	0.921	2.02 ±0.102	2.9	0.697 ±0.035
H		3	-0.103 ±0.118	0.0027 ±0.00020	0.964	2.62 ±0.095	3.3	0.794 ±0.029
H		5	-0.077 ±0.058	0.0030 ±0.00010	0.993	2.92 ±0.045	3.4	0.860 ±0.014
S	LEU	1	-0.049 ±0.104	0.0060 ±0.00018	0.994	5.95 ±0.084	7.1	0.838 ±0.012
S		8	0.137 ±0.359	0.0045 ±0.00058	0.909	4.68 ±0.262	6.3	0.743 ±0.042
H		3	-0.123 ±0.199	0.0054 ±0.00034	0.974	5.27 ±0.160	6.6	0.799 ±0.024
H		5	-0.168 ±0.128	0.0062 ±0.00022	0.992	6.02 ±0.102	6.9	0.873 ±0.015

Table 24 (continued). Trial 5A Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
			b	a	R^2	Solution when x =1000		
S	PHE	1	-0.097 ±0.115	0.0037 ±0.00019	0.981	3.56 ±0.093	4.6	0.774 ±0.020
S		8	0.135 ±0.263	0.0026 ±0.00043	0.859	2.74 ±0.192	4.1	0.668 ±0.047
H		3	-0.244 ±0.152	0.0033 ±0.00026	0.958	3.01 ±0.122	4.0	0.753 ±0.030
H		5	-0.250 ±0.122	0.0034 ±0.00021	0.975	3.18 ±0.098	3.9	0.816 ±0.025
S	HIS	1	-0.031 ±0.054	0.0034 ±0.00009	0.995	3.33 ±0.043	4.0	0.832 ±0.011
S		8	0.059 ±0.130	0.0026 ±0.00021	0.962	2.65 ±0.095	3.5	0.757 ±0.027
H		3	-0.052 ±0.110	0.0031 ±0.00019	0.976	3.08 ±0.088	3.9	0.790 ±0.023
H		5	-0.133 ±0.079	0.0034 ±0.00013	0.990	3.31 ±0.064	3.7	0.870 ±0.017
S	LYS	1	0.048 ±0.105	0.0024 ±0.00018	0.963	2.46 ±0.085	3.2	0.768 ±0.026
S		8	0.197 ì0.162	0.0016 ±0.00026	0.851	1.75 ±0.118	2.8	0.626 ±0.042
H		3	0.003 ±0.108	0.0021 ±0.00018	0.948	2.06 ±0.086	2.9	0.711 ±0.030
H		5	-0.115 ±0.095	0.0034 ±0.00016	0.985	3.31 ±0.077	3.8	0.871 ±0.020

Table 24 (continued). Trial 5A Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
			b	a	R^2	Solution when x =1000		
S	ARG	1	0.035 ±0.142	0.0046 ±0.00024	0.981	4.62 ±0.114	5.5	0.839 ±0.021
S		8	0.134 ±0.102	0.0034 ±0.00017	0.986	3.53 ±0.074	4.6	0.768 ±0.016
H		3	-0.036 ±0.131	0.0037 ±0.00022	0.975	3.65 ±0.105	4.6	0.794 ±0.023
H		5	-0.084 ±0.146	0.0042 ±0.00025	0.977	4.15 ±0.117	4.8	0.864 ±0.024
S	PRO	1	-0.089 ±0.130	0.0105 ±0.00022	0.997	10.39 ±0.104	11.5	0.904 ±0.009
S		8	0.171 ±0.262	0.0087 ±0.00042	0.986	8.89 ±0.191	10.4	0.855 ±0.018
H		3	-0.097 ±0.197	0.0047 ±0.00033	0.966	4.60 ±0.158	5.9	0.780 ±0.027
H		5	-0.160 ±0.131	0.0092 ±0.00022	0.991	9.00 ±0.105	9.8	0.919 ±0.011

Table 25. Trial 5B. Assessment of endosperm hardness (in a low protein background)

a. Mean data - g digestible component / kg diet

Component	Endosperm			Time	
	S	H	Mean	1	2
Starch	481	492	486	499	474
CYS	1.51	1.48	1.49	1.43	1.56
MET	0.87	0.89	0.88	0.86	0.89
ASP	2.30	2.20	2.25	2.24	2.25
THR	1.11	1.04	1.08	1.03	1.12
SER	2.46	2.27	2.37	2.34	2.39
GLU	12.4	12.9	12.7	12.6	12.7
GLY	1.65	1.65	1.65	1.58	1.72
ALA	1.51	1.46	1.48	1.37	1.60
VAL	2.03	2.11	2.07	2.01	2.13
ILEU	1.31	1.42	1.36	1.31	1.42
LEU	2.80	2.94	2.87	2.79	2.94
PHE	1.64	1.53	1.58	1.56	1.61
HIS	1.46	1.57	1.52	1.48	1.56
LYS	1.33	1.51	1.42	1.40	1.44
ARG	2.27	2.09	2.18	2.22	2.14
PRO	3.88	3.81	3.85	3.81	3.88

Table 25 (continued). Trial 5B. Assessment of endosperm hardness (in a low protein background)

b. Analysis of variance of wheat characteristics
(In only two cases was effect of time significant)

Component	Endosperm				Residual	
	⁰F	MS	SEd	P=	⁰F	MS
Starch	1	1034	20.3	0.581	25(2)	3307
CYS	1	0.01073	0.045	0.421	27	0.01604
MET	1	0.001522	0.032	0.671	27	0.008268
ASP	1	0.07941	0.073	0.182	27	0.04238
THR	1	0.03896	0.063	0.281	27	0.03213
SER	1	0.3071	0.169	0.255	27	0.2272
GLU	1	1.2914	0.150	0.013	27	0.1821
GLY	1	0.0001	0.121	0.977	27	0.1174
ALA	1	0.02236	0.096	0.586	27	0.07375
VAL	1	0.0624	0.120	0.468	27	0.1151
ILEU	1	0.08504	0.072	0.161	27	0.04093
LEU	1	0.15611	0.078	0.084	27	0.04845
PHE	1	0.09231	0.059	0.081	27	0.02817
HIS	1	0.09562	0.070	0.130	27	0.03924
LYS	1	0.28005	0.090	0.048	27	0.06520
ARG	1	0.24651	0.047	<0.001	27	0.01762
PRO	1	0.040	0.436	0.873	27	1.522

Table 26. Trial 5B Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
S	Starch	2	628 ±71.2	-0.225 ±0.120	0.333	373 ±57.2	578	0.65 ±0.099
S		7	737 ±54.6	-0.463 ±0.092	0.777	274 ±43.8	524	0.52 ±0.084
H		4	605 ±52.1	-0.276 ±0.092	0.573	328 ±45.9	508	0.65 ±0.090
H		6	670 ±41.8	-0.236 ±0.068	0.650	434 ±30.5	616	0.70 ±0.050
S	CYS	2	-0.321 ±0.204	0.0031 ±0.00034	0.922	2.83 ±0.164	3.5	0.807 ±0.047
S		7	-0.157 ±0.141	0.0031 ±0.00024	0.960	2.93 ±0.113	3.6	0.813 ±0.031
H		4	-0.150 ±0.197	0.0029 ±0.00033	0.913	2.72 ±0.158	3.5	0.776 ±0.045
H		6	-0.228 ±0.040	0.0031 ±0.00007	0.997	2.83 ±0.032	3.5	0.808 ±0.009
S	MET	2	-0.096 ±0.071	0.0019 ±0.00012	0.972	1.76 ±0.057	2.0	0.880 ±0.029
S		7	-0.020 ±0.096	0.0014 ±0.00016	0.918	1.43 ±0.077	1.7	0.839 ±0.045
H		4	-0.039 ±0.039	0.0017 ±0.00007	0.990	1.68 ±0.031	1.9	0.883 ±0.017
H		6	-0.042 ±0.028	0.0016 ±0.00005	0.994	1.53 ±0.022	1.8	0.850 ±0.012
S	ASP	2	-0.552 ±0.298	0.0051 ±0.00050	0.936	4.53 ±0.239	5.7	0.795 ±0.042
S		7	-0.233 ±0.183	0.0045 ±0.00031	0.968	4.25 ±0.147	5.5	0.772 ±0.027
H		4	-0.168 ±0.206	0.0044 ±0.00035	0.958	4.22 ±0.165	5.4	0.782 ±0.031
H		6	-0.256 ±0.158	0.0042 ±0.0027	0.972	3.92 ±0.127	5.2	0.754 ±0.024

Table 26 (continued). Trial 5B Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
S	THR	2	-0.464 ±0.242	0.0027 ±0.00041	0.859	2.23 ±0.194	3.2	0.697 ±0.061
S		7	-0.229 ±0.229	0.0025 ±0.00039	0.850	2.26 ±0.184	3.3	0.684 ±0.056
H		4	-0.208 ±0.130	0.0024 ±0.00022	0.945	2.21 ±0.104	3.2	0.690 ±0.033
H		6	-0.276 ±0.098	0.0021 ±0.00017	0.960	1.87 ±0.079	2.9	0.644 ±0.027
S	SER	2	-0.452 ±0.237	0.0043 ±0.00040	0.943	3.86 ±0.191	4.6	0.840 ±0.041
S		7	-0.223 ±0.174	0.0056 ±0.00029	0.981	5.42 ±0.140	6.4	0.847 ±0.022
H		4	-0.300 ±0.204	0.0051 ±0.00034	0.969	4.76 ±0.164	5.6	0.849 ±0.029
H		6	-0.209 ±0.107	0.0039 ±0.00018	0.985	3.70 ±0.090	4.7	0.787 ±0.018
S	GLU	2	-0.679 ±0.409	0.0227 ±0.00069	0.994	22.04 ±0.328	23.8	0.926 ±0.014
S		7	-0.494 ±0.363	0.0236 ±0.00061	0.995	23.14 ±0.291	25.1	0.921 ±0.012
H		4	-0.190 ±0.345	0.0229 ±0.00058	0.995	22.68 ±0.277	24.5	0.926 ±0.011
H		6	-0.387 ±0.223	0.0238 ±0.00038	0.998	23.46 ±0.179	25.4	0.924 ±0.007
S	GLY	2	-0.383 ±0.245	0.0029 ±0.00041	0.877	2.56 ±0.196	3.6	0.711 ±0.055
S		7	-0.157 ±0.259	0.0039 ±0.00044	0.918	3.74 ±0.208	4.9	0.762 ±0.042
H		4	-0.138 ±0.185	0.0033 ±0.00031	0.941	3.18 ±0.149	4.3	0.740 ±0.035
H		6	-0.215 ±0.066	0.0032 ±0.00011	0.991	2.96 ±0.053	4.0	0.739 ±0.013

Table 26 (continued). Trial 5B Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
S	ALA	2	-0.378 ±0.269	0.0029 ±0.00045	0.848	2.50 ±0.216	3.5	0.713 ±0.062
S		7	-0.137 ±0.353	0.0034 ±0.00060	0.819	3.27 ±0.284	4.5	0.723 ±0.063
H		4	-0.103 ±0.176	0.0029 ±0.00030	0.931	2.81 ±0.142	3.9	0.719 ±0.036
H		6	-0.148 ±0.070	0.0027 ±0.00012	0.987	2.57 ±0.056	3.7	0.694 ±0.015
S	VAL	2	-0.437 ±0.259	0.0038 ±0.00044	0.912	3.30 ±0.208	4.3	0.768 ±0.048
S		7	-0.158 ±0.311	0.0045 ±0.00052	0.913	4.36 ±0.249	5.5	0.793 ±0.045
H		4	-0.102 ±0.208	0.0040 ±0.00035	0.949	3.90 ±0.167	5.1	0.765 ±0.033
H		6	-0.228 ±0.113	0.0041 ±0.00019	0.985	3.87 ±0.091	5.0	0.774 ±0.018
S	ILEU	2	-0.248 ±0.144	0.0028 ±0.00024	0.948	2.50 ±0.115	2.5	0.807 ±0.037
S		7	0.024 ±0.383	0.0023 ±0.00065	0.628	2.34 ±0.307	3.2	0.730 ±0.096
H		4	-0.025 ±0.232	0.0026 ±0.00039	0.860	2.58 ±0.186	3.4	0.757 ±0.055
H		6	-0.233 ±0.146	0.0029 ±0.00025	0.951	2.66 ±0.118	3.3	0.805 ±0.036
S	LEU	2	-0.491 ±0.275	0.0056 ±0.00046	0.954	5.12 ±0.221	6.2	0.826 ±0.036
S		7	-0.056 ±0.323	0.0053 ±0.00054	0.931	5.25 ±0.259	6.6	0.795 ±0.039
H		4	-0.193 ±0.214	0.0055 ±0.00036	0.970	5.29 ±0.172	6.5	0.814 ±0.026
H		6	-0.316 ±0.134	0.0059 ±0.00023	0.990	5.54 ±0.108	6.7	0.827 ±0.016

Table 26 (continued). Trial 5B Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
S	PHE	2	-0.389 ±0.286	0.0036 ±0.00048	0.888	3.23 ±0.230	4.1	0.789 ±0.056
S		7	-0.557 ±0.153	0.0039 ±0.00026	0.970	3.32 ±0.123	4.0	0.831 ±0.031
H		4	-0.319 ±0.182	0.0032 ±0.00031	0.941	2.93 ±0.146	3.7	0.791 ±0.039
H		6	-0.311 ±0.142	0.0033 ±0.00094	0.965	3.00 ±0.114	3.9	0.770 ±0.029
S	HIS	2	-0.215 ±0.143	0.0027 ±0.00024	0.948	2.51 ±0.115	3.1	0.809 ±0.037
S		7	-0.098 ±0.138	0.0030 ±0.00023	0.960	2.94 ±0.111	3.6	0.815 ±0.031
H		4	-0.119 ±0.115	0.0033 ±0.00019	0.976	3.19 ±0.092	3.8	0.838 ±0.024
H		6	-0.117 ±0.032	0.0027 ±0.00005	0.997	2.59 ±0.026	3.2	0.809 ±0.008
S	LYS	2	-0.170 ±0.130	0.0027 ±0.00022	0.955	2.53 ±0.104	3.2	0.789 ±0.033
S		7	-0.131 ±0.103	0.0026 ±0.00017	0.968	2.42 ±0.083	3.1	0.780 ±0.027
H		4	-0.100 ±0.107	0.0034 ±0.00018	0.980	3.29 ±0.086	3.9	0.844 ±0.022
H		6	-0.101 ±0.067	0.0023 ±0.00011	0.984	2.24 ±0.054	2.9	0.773 ±0.018

Table 26 (continued). Trial 5B Linear regression equations and solution for starch and amino acids

Endosperm	Component	Wheat	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
			b	a	R^2	Solution when x =1000		
S	ARG	2	-0.166 ±0.161	0.0043 ±0.00027	0.972	4.11 ±0.129	5.0	0.822 ±0.026
S		7	-0.129 ±0.127	0.0043 ±0.00021	0.983	4.18 ±0.102	4.9	0.854 ±0.021
H		4	-0.086 ±0.182	0.0039 ±0.00031	0.959	3.85 ±0.146	4.6	0.838 ±0.033
H		6	-0.255 ±0.161	0.0041 ±0.00027	0.970	3.85 ±0.130	4.6	0.836 ±0.028
S	PRO	2	-0.162 ±0.104	0.0050 ±0.00018	0.991	4.84 ±0.084	5.8	0.835 ±0.014
S		7	-0.388 ±0.139	0.0098 ±0.00024	0.996	9.39 ±0.112	10.2	0.921 ±0.011
H		4	-0.257 ±0.159	0.0057 ±0.00027	0.985	5.43 ±0.128	6.3	0.862 ±0.020
H		6	-0.229 ±0.109	0.0087 ±0.00018	0.997	8.50 ±0.088	9.5	0.895 ±0.009

Table 27. Trial 6A. Assessment of Sicco isogenics containing high molecular weight sub-units

A - Sicco 5 + 10 control
B - Sicco isogenic null 5 + 10

a. Mean data - g digestible component / kg diet

Component	Wheat			Time	
	A	B	Mean	1	2
Starch	503	440	471	455	487
CYS	1.73	1.76	1.75	1.71	1.78
MET	1.05	0.96	1.01	0.98	1.03
ASP	2.53	2.49	2.51	2.37	2.65
THR	1.44	1.33	1.39	1.31	1.46
SER	2.70	2.79	2.75	2.66	2.83
GLU	17.73	18.07	17.90	17.58	18.23
GLY	2.01	1.79	1.90	1.80	2.00
ALA	1.57	1.52	1.55	1.44	1.65
VAL	2.30	2.24	2.27	2.19	2.36
ILEU	1.79	1.81	1.80	1.76	1.84
LEU	3.64	3.69	3.66	3.58	3.75
PHE	2.43	2.54	2.49	2.35	2.62
HIS	1.91	1.88	1.90	1.86	1.93
LYS	1.38	1.18	1.28	1.23	1.33
ARG	2.40	2.28	2.34	2.24	2.44
PRO	6.78	6.28	6.53	6.37	6.69

Table 27 (continued). Trial 6A. Assessment of Sicco isogenics containing high molecular weight sub-units

A - Sicco 5 + 10 control
B - Sicco isogenic null 5 + 10

b. Analysis of variance of wheat characteristics
(In no case was effect of time significant)

Component	Wheat				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	16017	25.4	0.030	11	2582
CYS	1	0.002559	0.048	0.609	11	0.009241
MET	1	0.032690	0.027	0.006	11	0.002891
ASP	1	0.00587	0.144	0.796	11	0.08332
THR	1	0.04961	0.066	0.119	11	0.01740
SER	1	0.03406	0.102	0.383	11	0.04125
GLU	1	0.4577	0.391	0.405	11	0.6102
GLY	1	0.18992	0.115	0.083	11	0.05242
ALA	1	0.01069	0.108	0.641	11	0.04645
VAL	1	0.01405	0.103	0.575	11	0.04203
ILEU	1	0.00120	0.055	0.760	11	0.01226
LEU	1	0.01220	0.131	0.681	11	0.06822
PHE	1	0.04843	0.116	0.364	11	0.05397
HIS	1	0.00244	0.067	0.720	11	0.01810
LYS	1	0.14782	0.105	0.093	11	0.04365
ARG	1	0.04908	0.124	0.390	11	0.06124
PRO	1	1.00841	0.138	0.004	11	0.07664

Table 28. Trial 6A Linear regression equations and solution for starch and amino acids

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	Starch	887 ±52.0	-0.684 ±0.087	0.895	203 ±41.7	551	0.37 ±0.076
B		741 ±64.0	-0.536 ±0.108	0.772	205 ±51.4	545	0.38 ±0.094
A	CYS	-0.321 ±0.089	0.0037 ±0.00015	0.988	3.33 ±0.071	3.8	0.877 ±0.019
B		-0.134 ±0.130	0.0034 ±0.00022	0.971	3.23 ±0.105	3.9	0.828 ±0.027
A	MET	-0.073 ±0.062	0.0020 ±0.00011	0.981	1.92 ±0.050	2.2	0.874 ±0.023
B		-0.052 ±0.068	0.0018 ±0.00012	0.972	1.75 ±0.055	2.1	0.831 ±0.026
A	ASP	-0.574 ±0.303	0.0055 ±0.00051	0.943	4.94 ±0.243	6.4	0.771 ±0.038
B		-0.444 ±0.404	0.0052 ±0.00068	0.892	4.77 ±0.324	6.5	0.734 ±0.050
A	THR	-0.442 ±0.134	0.0033 ±0.00023	0.969	2.91 ±0.108	3.8	0.765 ±0.028
B		-0.267 ±0.197	0.0028 ±0.00033	0.911	2.57 ±0.158	3.7	0.695 ±0.043
A	SER	-0.672 ±0.180	0.0060 ±0.00030	0.982	5.32 ±0.145	6.3	0.845 ±0.023
B		-0.272 ±0.292	0.0054 ±0.00049	0.946	5.18 ±0.234	6.5	0.796 ±0.036
A	GLU	-0.705 ±0.527	0.0328 ±0.00089	0.995	32.07 ±0.423	34.4	0.935 ±0.012
B		-1.440 ±1.220	0.0347 ±0.00205	0.976	33.25 ±0.976	35.6	0.934 ±0.027
A	GLY	-0.469 ±0.238	0.0044 ±0.00040	0.945	3.94 ±0.191	5.0	0.787 ±0.038
B		-0.344 ±0.308	0.0038 ±0.00052	0.882	3.45 ±0.247	4.7	0.734 ±0.053

Table 28. (continued)Trial 6A .

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	ALA	-0.355 ±0.218	0.0034 ±0.00037	0.924	3.07 ±0.175	4.1	0.749 ±0.043
B		-0.346 ±0.311	0.0033 ±0.00052	0.848	2.97 ±0.249	4.1	0.725 ±0.061
A	VAL	-0.470 ±0.261	0.0049 ±0.00044	0.947	4.46 ±0.209	5.5	0.811 ±0.038
B		-0.282 ±0.226	0.0045 ±0.00038	0.952	4.21 ±0.182	5.5	0.765 ±0.033
A	ILEU	-0.224 ±0.122	0.0036 ±0.00021	0.977	3.36 ±0.098	4.0	0.839 ±0.025
B		-0.178 ±0.134	0.0035 ±0.00023	0.972	3.35 ±0.108	4.1	0.818 ±0.026
A	LEU	-0.419 ±0.231	0.0072 ±0.00039	0.980	6.79 ±0.186	8.1	0.838 ±0.023
B		-0.482 ±0.353	0.0074 ±0.00060	0.957	6.94 ±0.284	8.3	0.835 ±0.034
A	PHE	-0.447 ±0.174	0.0051 ±0.00029	0.977	4.67 ±0.140	5.7	0.820 ±0.025
B		-0.544 ±0.395	0.0055 ±0.00067	0.905	4.94 ±0.317	6.0	0.824 ±0.053
A	HIS	-0.311 ±0.119	0.0039 ±0.00020	0.982	3.63 ±0.096	4.2	0.865 ±0.023
B		-0.169 ±0.178	0.0036 ±0.00030	0.954	3.48 ±0.143	4.2	0.828 ±0.034
A	LYS	-0.255 ±0.183	0.0029 ±0.00031	0.926	2.64 ±0.147	3.4	0.778 ±0.043
B		-0.125 ±0.273	0.0023 ±0.00046	0.778	2.20 ±0.219	3.2	0.688 ±0.068
A	ARG	-0.344 ±0.286	0.0049 ±0.00048	0.935	4.53 ±0.230	5.8	0.780 ±0.040
B		-0.322 ±0.303	0.0047 ±0.00051	0.921	4.32 ±0.243	5.7	0.758 ±0.043
A	PRO	-0.505 ±0.261	0.0129 ±0.00044	0.992	12.44 ±0.210	13.3	0.936 ±0.016
B		-0.500 ±0.436	0.0120 ±0.00074	0.974	11.55 ±0.350	12.6	0.917 ±0.028

Table 29. Trial 6B. Assessment of Sicco isogenics containing high molecular weight sub-units

A - Sicco 5 + 10 control
B - Sicco isogenic 2 + 12

a. Mean data - g digestible component / kg diet

Component	Wheat			Time	
	A	B	Mean	1	2
Starch	474	497	485	483	488
CYS	1.75	1.85	1.80	1.80	1.81
MET	1.07	1.26	1.17	1.18	1.16
ASP	2.80	2.71	2.76	2.73	2.79
THR	1.51	1.42	1.46	1.44	1.49
SER	2.96	2.71	2.84	2.81	2.87
GLU	18.53	17.58	18.05	18.03	18.08
GLY	2.35	1.95	2.15	2.13	2.16
ALA	1.96	1.56	1.76	1.74	1.79
VAL	2.67	2.25	2.46	2.47	2.44
ILEU	1.76	1.56	1.66	1.68	1.65
LEU	3.88	3.48	3.68	3.67	3.69
PHE	2.52	2.12	2.32	2.29	2.35
HIS	1.95	1.78	1.86	1.87	1.86
LYS	1.41	1.22	1.32	1.31	1.32
ARG	2.27	2.07	2.17	2.17	2.16
PRO	3.53	4.01	3.77	3.73	3.82

Table 29 (continued). Trial 6B. Assessment of Sicco isogenics containing high molecular weight sub-units

A - Sicco 5 + 10 control
B - Sicco isogenic 2 + 12
b. Analysis of variance of wheat characteristics
(In no case was effect of time significant)

Component	Wheat				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	1	2105	19.8	0.271	11	1566
CYS	1	0.036953	0.021	<0.001	11	0.001724
MET	1	0.1446284	0.015	<0.001	11	0.0008419
ASP	1	0.03183	0.051	0.108	11	0.01041
THR	1	0.031759	0.036	0.030	11	0.005094
SER	1	0.251871	0.050	<0.001	11	0.009969
GLU	1	3.58668	0.096	<0.001	11	0.03683
GLY	1	0.635070	0.046	<0.001	11	0.008611
ALA	1	0.638874	0.040	<0.001	11	0.006450
VAL	1	0.71495	0.055	<0.001	11	0.01218
ILEU	1	0.161419	0.036	<0.001	11	0.005130
LEU	1	0.66036	0.066	<0.001	11	0.01732
PHE	1	0.634148	0.046	<0.001	11	0.008565
HIS	1	0.110197	0.035	<0.001	11	0.005039
LYS	1	0.153074	0.028	<0.001	11	0.003229
ARG	1	0.154806	0.049	0.002	11	0.009688
PRO	1	0.91703	0.091	<0.001	11	0.03323

Table 30. Trial 6B Linear regression equations and solution for starch and amino acids

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	Starch	754 ±37.2	-0.498 ±0.063	0.899	256 ±29.9	510	0.50 ±0.059
B		790 ±47.2	-0.521 ±0.080	0.857	269 ±37.9	546	0.49 ±0.069
A	CYS	-0.089 ±0.047	0.0033 ±0.00008	0.996	3.18 ±0.037	3.7	0.862 ±0.010
B		0.021 ±0.043	0.0033 ±0.00007	0.997	3.27 ±0.035	4.0	0.818 ±0.009
A	MET	-0.012 ±0.021	0.0019 ±0.00004	0.998	1.91 ±0.017	2.2	0.870 ±0.008
B		0.020 ±0.042	0.0022 ±0.00007	0.993	2.23 ±0.003	2.6	0.857 ±0.013
A	ASP	-0.204 ±0.125	0.0053 ±0.00021	0.989	5.14 ±0.100	6.5	0.791 ±0.015
B		0.104 ±0.108	0.0046 ±0.00018	0.989	4.74 ±0.087	6.7	0.708 ±0.013
A	THR	-0.117 ±0.079	0.0029 ±0.00013	0.985	2.77 ±0.063	3.7	0.749 ±0.017
B		-0.026 ±0.086	0.0026 ±0.00014	0.978	2.54 ±0.069	3.8	0.669 ±0.018
A	SER	-0.130 ±0.076	0.0055 ±0.00013	0.996	5.37 ±0.061	6.4	0.838 ±0.009
B		-0.127 ±0.143	0.0050 ±0.00024	0.984	4.92 ±0.115	6.3	0.781 ±0.018
A	GLU	-0.243 ±0.223	0.0334 ±0.00038	0.999	33.12 ±0.179	35.2	0.941 ±0.005
B		0.447 ±0.191	0.0305 ±0.00032	0.999	30.90 ±0.154	34.1	0.906 ±0.005
A	GLY	-0.080 ±0.090	0.0043 ±0.00015	0.991	4.23 ±0.072	5.3	0.799 ±0.014
B		0.009 ±0.111	0.0034 ±0.00019	0.980	3.46 ±0.089	4.8	0.720 ±0.019

Table 30. (continued)

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	ALA	-0.090 ±0.091	0.0037 ±0.00015	0.988	3.56 ±0.073	4.5	0.791 ±0.016
B		0.102 ±0.093	0.0026 ±0.00016	0.975	2.70 ±0.075	4.0	0.675 ±0.019
A	VAL	-0.161 ±0.112	0.0050 ±0.00019	0.990	4.87 ±0.090	5.9	0.826 ±0.015
B		0.028 ±0.127	0.0039 ±0.00021	0.980	3.97 ±0.102	5.4	0.736 ±0.019
A	ILEU	-0.135 ±0.071	0.0034 ±0.00012	0.991	3.24 ±0.057	3.8	0.853 ±0.015
B		0.016 ±0.085	0.0027 ±0.00014	0.981	2.76 ±0.068	3.6	0.768 ±0.019
A	LEU	-0.181 ±0.130	0.0072 ±0.00022	0.994	7.05 ±0.104	8.1	0.870 ±0.013
B		0.195 ±0.153	0.0058 ±0.00026	0.987	6.03 ±0.123	7.7	0.783 ±0.016
A	PHE	-0.160 ±0.107	0.0048 ±0.00018	0.990	4.59 ±0.086	5.6	0.820 ±0.015
B		0.038 ±0.105	0.0037 ±0.00018	0.984	3.73 ±0.084	5.2	0.718 ±0.016
A	HIS	-0.085 ±0.048	0.0036 ±0.00008	0.997	3.53 ±0.038	4.1	0.860 ±0.009
B		-0.052 ±0.097	0.0033 ±0.00016	0.983	3.20 ±0.078	4.0	0.801 ±0.019
A	LYS	-0.055 ±0.074	0.0026 ±0.00012	0.984	2.55 ±0.059	3.3	0.774 ±0.018
B		0.089 ±0.047	0.0020 ±0.00008	0.989	2.09 ±0.038	3.1	0.676 ±0.012
A	ARG	-0.004 ±0.108	0.0040 ±0.00018	0.986	4.03 ±0.087	5.3	0.761 ±0.016
B		0.274 ±0.103	0.0032 ±0.00017	0.980	3.47 ±0.083	5.2	0.667 ±0.016
A	PRO	-0.220 ±0.230	0.0067 ±0.00039	0.977	6.45 ±0.185	7.40	0.872 ±0.025
B		0.014 ±0.176	0.0071 ±0.00030	0.988	7.12 ±0.141	8.40	0.848 ±0.017

Table 31 Trial 6C Assessment of starch composition

A - Waxy wheat (Chinese Spring) control
B/D - Waxy wheat ACS (CNN 4A) lower amylose
C - Waxy wheat BSC (CNN 4A) lower amylose

a. Mean data - g digestible component / kg diet

Component	Wheat					Time	
	A	B	C	D	Mean	1	2
Starch	433	425	436	469	441	466	416
CYS	2.00	1.98	2.06	1.91	1.99	1.98	1.99
MET	1.24	1.14	1.26	1.10	1.18	1.15	1.21
ASP	3.33	2.80	3.22	3.02	3.09	3.04	3.15
THR	1.82	1.63	1.72	1.70	1.72	1.68	1.76
SER	3.44	3.30	3.36	3.40	3.37	3.38	3.37
GLU	21.92	20.10	24.47	22.76	22.31	22.13	22.49
GLY	2.58	2.45	2.75	2.61	2.60	2.62	2.57
ALA	2.20	1.96	2.27	2.22	2.16	2.14	2.19
VAL	2.78	2.63	3.21	2.91	2.89	2.87	2.90
ILEU	2.28	2.03	2.28	2.19	2.19	2.19	2.20
LEU	4.83	4.38	4.88	4.65	4.68	4.67	4.69
PHE	3.60	3.28	3.36	3.33	3.39	3.34	3.44
HIS	2.83	2.08	2.40	2.24	2.39	2.39	2.39
LYS	2.63	1.53	1.65	1.64	1.86	1.83	1.89
ARG	3.04	2.75	2.69	2.84	2.83	2.78	2.88
PRO	7.91	7.88	5.68	4.77	6.56	6.44	6.68

Table 31 Trial 6C Assessment of starch composition

b. Analysis of variance of wheat characteristics
(In only one case was effect of time significant)

Component	Wheat				Residual	
	^{0}F	MS	SEd	P=	^{0}F	MS
Starch	3	3006	46.9	0.795	23	8784
CYS	3	0.03288	0.057	0.082	23	0.01297
MET	3	0.04076	0.067	0.109	23	0.01807
ASP	3	0.43495	0.157	0.013	23	0.09814
THR	3	0.05003	0.097	0.292	23	0.03792
SER	3	0.02722	0.139	0.787	23	0.07712
GLU	3	26.4253	0.373	<0.001	23	0.5553
GLY	3	0.11698	0.120	0.137	23	0.05742
ALA	3	0.15752	0.099	0.020	23	0.03945
VAL	3	0.48950	0.140	0.003	23	0.07840
ILEU	3	0.11145	0.077	0.011	23	0.02399
LEU	3	0.41142	0.142	0.008	23	0.08071
PHE	3	0.16852	0.099	0.015	23	0.03882
HIS	3	0.83215	0.076	<0.001	23	0.02316
LYS	3	2.10619	0.070	<0.001	23	0.01944
ARG	3	0.18465	0.117	0.036	23	0.05493
PRO	3	20.19052	0.129	<0.001	23	0.06643

Table 32. Trial 6C Linear regression equations and solution for starch and amino acids

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	Starch	787 ±64.6	-0.629 ±0.109	0.822	158 ±51.9	494	0.32 ±0.105
B		748 ±169.0	-0.574 ±0.286	0.303	173 ±136.0	523	0.33 ±0.260
C		731 ±70.6	-0.525 ±0.119	0.724	207 ±56.7	506	0.41 ±0.112
D		791 ±91.9	-0.573 ±0.155	0.644	218 ±73.8	551	0.40 ±0.134
A	CYS	-0.046 ±0.076	0.0036 ±0.00013	0.991	3.59 ±0.061	4.3	0.835 ±0.014
B		-0.085 ±0.160	0.0037 ±0.00027	0.963	3.59 ±0.128	4.3	0.835 ±0.030
C		-0.138 ±0.157	0.0039 ±0.00026	0.969	3.77 ±0.126	4.5	0.838 ±0.028
D		-0.141 ±0.081	0.0036 ±0.00014	0.990	3.50 ±0.065	4.2	0.833 ±0.016
A	MET	0.054 ±0.070	0.0021 ±0.00012	0.978	2.15 ±0.057	2.5	0.862 ±0.023
B		-0.052 ±0.055	0.0021 ±0.00009	0.987	2.07 ±0.044	2.4	0.864 ±0.018
C		0.066 ±0.271	0.0021 ±0.00046	0.741	2.16 ±0.218	2.7	0.801 ±0.081
D		-0.161 ±0.106	0.0022 ±0.00018	0.957	2.08 ±0.085	2.4	0.866 ±0.035
A	ASP	-0.154 ±0.288	0.0062 ±0.00049	0.959	6.05 ±0.231	7.8	0.775 ±0.030
B		-0.177 ±0.320	0.0053 ±0.00054	0.931	5.12 ±0.257	7.0	0.732 ±0.037
C		-0.277 ±0.506	0.0062 ±0.00085	0.881	5.93 ±0.406	7.9	0.751 ±0.051
D		-0.248 ±0.222	0.0058 ±0.00037	0.972	5.56 ±0.178	7.5	0.741 ±0.024

Table 32. (continued)

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	THR	-0.136 ±0.183	0.0035 ±0.00031	0.947	3.35 ±0.147	4.5	0.744 ±0.033
B		-0.214 ±0.193	0.0033 ±0.00033	0.935	3.07 ±0.155	4.2	0.731 ±0.037
C		-0.200 ±0.325	0.0034 ±0.00055	0.844	3.22 ±0.261	4.5	0.716 ±0.058
D		-0.271 ±0.126	0.0035 ±0.00021	0.975	3.23 ±0.101	4.4	0.733 ±0.023
A	SER	-0.199 ±0.227	0.0065 ±0.00038	0.976	6.26 ±0.182	7.7	0.813 ±0.024
B		-0.152 ±0.416	0.0061 ±0.00070	0.915	5.98 ±0.334	7.4	0.808 ±0.045
C		-0.205 ±0.330	0.0063 ±0.00056	0.949	6.14 ±0.265	7.7	0.797 ±0.034
D		-0.231 ±0.191	0.0064 ±0.00032	0.983	6.22 ±0.154	7.7	0.808 ±0.020
A	GLU	-0.010 ±0.524	0.0390 ±0.00088	0.996	38.97 ±0.421	41.9	0.931 ±0.010
B		0.039 ±0.651	0.0357 ±0.00110	0.993	35.70 ±0.523	38.9	0.918 ±0.013
C		0.140 ±1.270	0.0432 ±0.00214	0.983	43.39 ±1.020	47.2	0.919 ±0.022
D		0.203 ±0.753	0.0401 ±0.00127	0.993	40.31 ±0.604	44.1	0.914 ±0.014
A	GLY	-0.090 ±0.230	0.0047 ±0.00039	0.955	4.65 ±0.185	6.0	0.775 ±0.031
B		-0.099 ±0.286	0.0045 ±0.00048	0.926	4.44 ±0.229	5.8	0.765 ±0.040
C		-0.554 ±0.338	0.0059 ±0.00057	0.937	5.31 ±0.271	6.1	0.871 ±0.044
D		-0.136 ±0.178	0.0049±0.00030	0.974	4.74 ±0.143	6.2	0.765 ±0.023

Table 32. (continued)

Wheat	Component	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
		b	a	R^2	Solution when x =1000		
A	ALA	-0.068 ±0.214	0.0040 ±0.00036	0.946	3.97 ±0.172	5.2	0.764 ±0.033
B		-0.053 ±0.204	0.0036 ±0.00035	0.938	3.52 ±0.164	4.8	0.734 ±0.034
C		-0.226 ±0.275	0.0044 ±0.00046	0.929	4.22 ±0.221	5.4	0.781 ±0.041
D		-0.114 ±0.174	0.0041 ±0.00029	0.966	4.03 ±0.140	5.4	0.746 ±0.026
A	VAL	-0.103 ±0.217	0.0051 ±0.00037	0.965	5.02 ±0.174	6.4	0.785 ±0.027
B		-0.096 ±0.388	0.0049 ±0.00065	0.885	4.76 ±0.311	6.2	0.767 ±0.050
C		-0.252 ±0.377	0.0062 ±0.00064	0.930	5.91 ±0.302	7.3	0.810 ±0.041
D		-0.264 ±0.194	0.0056 ±0.00033	0.977	5.38 ±0.156	6.7	0.803 ±0.023
A	ILEU	-0.050 ±0.128	0.0041 ±0.00022	0.981	4.09 ±0.103	4.9	0.835 ±0.021
B		-0.019 ±0.186	0.0036 ±0.00031	0.950	3.62 ±0.150	4.5	0.805 ±0.033
C		-0.138 ±0.232	0.0043 ±0.00039	0.945	4.16 ±0.186	5.0	0.832 ±0.037
D		-0.140 ±0.101	0.0041 ±0.00017	0.988	4.00 ±0.081	4.8	0.833 ±0.017
A	LEU	-0.100 ±0.291	0.0088 ±0.00049	0.978	8.66 ±0.234	10.1	0.858 ±0.023
B		-0.048 ±0.274	0.0079 ±0.00046	0.976	7.82 ±0.220	9.4	0.831 ±0.023
C		-0.315 ±0.422	0.0093 ±0.00071	0.960	8.91 ±0.339	10.4	0.857 ±0.033
D		-0.199 ±0.223	0.0086 ±0.00038	0.987	8.42 ±0.179	10.1	0.834 ±0.018

Table 32. (continued)

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	PHE	-0.200 ±0.207	0.0068 ±0.00035	0.982	6.56 ±0.166	7.7	0.852 ±0.022
B		-0.150 ±0.229	0.0061 ±0.00039	0.973	5.94 ±0.183	7.3	0.813 ±0.025
C		-0.286 ±0.280	0.0065 ±0.00047	0.964	6.19 ±0.225	7.5	0.825 ±0.030
D		-0.077 ±0.178	0.0061 ±0.00030	0.983	5.97 ±0.143	7.5	0.797 ±0.019
A	HIS	-0.059 ±0.135	0.0051 ±0.00023	0.986	5.08 ±0.109	5.9	0.861 ±0.018
B		-0.005 ±0.230	0.0037 ±0.00039	0.928	3.71 ±0.185	4.6	0.806 ±0.040
C		-0.068 ±0.172	0.0044 ±0.00029	0.970	4.32 ±0.138	5.2	0.830 ±0.027
D		-0.075 ±0.100	0.0041 ±0.00017	0.988	4.04 ±0.081	4.9	0.824 ±0.016
A	LYS	-0.034 ±0.123	0.0047 ±0.00021	0.987	4.69 ±0.099	5.6	0.838 ±0.018
B		-0.059 ±0.146	0.0028 ±0.00025	0.949	2.76 ±0.118	3.7	0.746 ±0.032
C		-0.176 ±0.221	0.0032 ±0.00037	0.914	3.07 ±0.178	4.0	0.767 ±0.044
D		-0.099 ±0.118	0.0031 ±0.00020	0.972	2.99 ±0.095	4.0	0.748 ±0.024

Table 32. (continued)

Wheat	Component	Linear Regression (y = b + ax) b	a	R^2	Solution when x =1000	Level in wheat g/kg	Coefficient of Apparent Digestibility
A	ARG	-0.037 ±0.251	0.0055 ±0.00042	0.959	5.43 ±0.202	6.9	0.787 ±0.029
B		-0.049 ±0.240	0.0050 ±0.00041	0.955	4.92 ±0.193	6.5	0.757 ±0.030
C		-0.157 ±0.373	0.0051 ±0.00063	0.901	4.91 ±0.300	6.5	0.756 ±0.046
D		-0.140 ±0.134	0.0053 ±0.00023	0.987	5.16 ±0.107	6.7	0.770 ±0.016
A	PRO	-0.065 ±0.336	0.0142 ±0.00057	0.989	14.12 ±0.270	15.5	0.911 ±0.017
B		-0.290 ±0.211	0.0145 ±0.00036	0.996	14.24 ±0.169	15.3	0.931 ±0.011
C		0.216 ±0.434	0.0097 ±0.00073	0.962	9.92 ±0.348	11.8	0.841 ±0.029
D		-0.127 ±0.263	0.0087 ±0.00044	0.982	8.58 ±0.211	10.1	0.849 ±0.021

Table 33 Trial 7A. Assessment of red grain

A - Control
B - ANK 1E
C - ANK 1C
D - ANK 1A
E - ANK 1B
F - ANK 1D

a. Mean data - g digestible component / kg diet

Component	Wheat						Time	
	A	B	C	D	E	F	1	2
Starch	468	451	370	424	397	455	418	437

b. Analysis of variance of wheat characteristics
(The effect of time was not significant)

Component	**Wheat**				**Residual**	
	^{0}F	**MS**	**SEd**	**P=**	**^{0}F**	**MS**
Starch	5	11455	26.3	0.005	34(1)	2775

Table 34. Trial 7A Linear regression equations and solution for starch

Wheat	Component	Linear Regression (Y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
		b	a	R^2	Solution when x =1000		
A 5	Starch	685 ±51.3	-0.385 ±0.087	0.729	300 ±41.2	507	0.591 ±0.081
B 1		756 ±24.3	-0.542 ±0.041	0.961	214 ±19.5	512	0.418 ±0.038
C 2		734 ±119	-0.650 ±0.192	0.634	84 ±86.7	494	0.170 ±0.175
D 3		817 ±38.8	-0.700 ±0.065	0.942	118 ±31.1	504	0.234 ±0.062
E 4		733 ±69.3	-0.597 ±0.117	0.782	136 ±55.7	507	0.269 ±0.110
F 6		642 ±42.0	-0.333 ±0.071	0.752	309 ±33.7	534	0.579 ±0.063

Table 35. Trial 7B. Assessment of high molecular weight Sicco isogenics

A - High Molecular Weight 7 + 9
B - High Molecular Weight 7 + 8

a. Mean data - g digestible component / kg diet

Component	**Wheat**			**Time**	
	A	B	Mean	1	2
Starch	464	430	447	430	464

b. Analysis of variance of wheat characteristics
(The effect of time was not significant)

Component	**Wheat**				**Residual**	
Starch	**^{0}F**	**MS**	**SEd**	**P=**	**^{0}F**	**MS**
	1	4702	20.7	0.125	11	1708

Table 36. Trial 7B. Trial 4A Linear regression equations and solution for starch

Wheat	Component	Linear Regression (y = b + ax)				Level in wheat g/kg	Coefficient of Apparent Digestibility
		b	a	R^2	Solution when x =1000		
A	Starch	646 ±52.6	-0.324 ±0.089	0.638	323 ±42.2	536	0.602 ±0.079
B		664 ±45.8	-0.415 ±0.077	0.799	248 ±36.8	505	0.492 ±0.073