PROJECT REPORT No. 274

WHEAT AND BARLEY IN PIG AND POULTRY DIETS: EFFECTS OF PHYSICAL PROCESSING AND ENZYMES; ASSESSMENT OF NUTRITIVE VALUE

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WHEAT AND BARLEY IN PIG AND POULTRY DIETS: EFFECTS OF PHYSICAL PROCESSING AND ENZYMES; ASSESSMENT OF NUTRITIVE VALUE

by

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ABSTRACT

Knowledge of the effects of processing on the nutritive value of cereals is of direct relevance to the animal feed industry. Recognition of the importance of optimal combinations of mechanical and thermal inputs in grain processing and the identification of appropriate combinations of process and enzyme supplement are likely to lead to developments in systems of feed preparation which can be incorporated directly into industrial practice.

In examining the nutritional consequences of processing the primary focus was on digestibility and the secondary focus on growth performance. The rationale for this was that processing would alter the chemical and physical structures of the grain and thereby increase or decrease the amounts of nutrients released in digestion and that these changes would be the primary cause of any alterations in growth performance. For the most part this supposition proved correct. However, the effects observed differed in magnitude according to the nutrient examined. Furthermore, some changes associated with heat processing were detected, the effects of which were not confined to the gastrointestinal tract but could also affect post-absorptive metabolism. In particular, the effect of thermal energy on protein was found to have opposing effects: on the one hand, increased digestion and absorption due to the disruption of cellular structures; on the other, heat damage to protein, with a reduction in the availability of lysine.

There were other effects of processing which went beyond alterations in digestibility. The most significant general effect of processes involving heat treatment was the increased solubilisation of non-starch polysaccharides leading to an increase in the viscosity of the diet, and of digesta. These changes led not only to reduced digestibility, but (in chicks) to reductions in feed intake and growth rate as well. In the more extreme cases these changes were accompanied by sticky beaks and sticky droppings. The addition of supplementary enzymes generally had the effect of at least partially reversing the deleterious effects of heat treatment and at best enhancing the recovery of nutrients to an extent not achieved by either processing or enzyme addition alone.

In the final part of the project four methods of processing were compared and the interaction between processing and enzyme supplementation examined in whole diets based on a mixture of wheat and barley. This confirmed in practical diets what had previously been shown with single cereals alone.

The project has highlighted the need for further and more detailed investigation of the alterations in grain structure and chemical composition resulting from mechanical and thermal processing, in particular the effects on the cell walls of the aleurone layer.

EXECUTIVE SUMMARY

In examining the nutritional consequences of processing the primary focus was on digestibility and the secondary focus on growth performance. The rationale for this was that processing would alter the chemical and physical structures of the grain and thereby increase or decrease the amounts of nutrients released in digestion and that these changes would be the major cause of any alterations in growth performance. For the most part this supposition proved correct. However, the effects observed differed in magnitude according to the nutrient examined:

- Minimal processing (hammer-milling) of grain enabled pigs and poultry to achieve starch digestibilities of 97-100% regardless of cereal fed. Protein digestibility was substantially lower with values around 65-70% at the terminal ileum giving scope for improvement.

- The Rapid Visco Analyzer (RVA) provided a quantitative description of effects of processing on starch granule structure and the subsequent gelation properties of the released starch polymers significantly related to the immediate accessibility of starch polymers to amylolytic attack.

- Combined thermal and mechanical processing destroyed granule structure and reduced gelation capacity to an extent directly related to temperature but independent of moisture content within the range tested (18-30%). Puffing was the most effective process, completely destroying granule structure and gelation capacity, followed by extrusion. Pelleting was considerably less effective than extrusion at comparable temperatures. Thermal processing alone (micronisation) had little effect on granule structure and tended to promote, rather than reduce, the gelation capacity of the released polymers.

- No evidence was found of starch retrogradation following any of the treatments.

- Physical chemical methods based on nitrogen gas adsorption provided reproducible measures of surface area (1-2 m^2 g^{-1}), pore sizes (0.5 -5 nm radius) and pore size distribution in grain. Although highly statistically significant changes were observed after processing, none could be related to process temperature or to moisture content.

- All processing methods examined increased the solubilisation of NSP. Amounts of mixed-linked glucan and arabinoxylan released by combined thermal and mechanical processes were directly related to process temperature and, in the case of mixed-linked glucan, negatively related to moisture content. Puffing caused the greatest release, followed by extrusion at temperatures >100°C. Pelleting and extrusion at lower temperatures produced similar results. There was a similar positive relationship between temperature and release of NSP on micronisation. Flaking significantly promoted the release of mixed-linked glucan from barley.

- The more extreme cases of high NSP release resulted in depressed digestibility, reduced feed intake, and sticky beaks and sticky droppings when the processed grain was fed to broilers. In untreated grain, over half of the soluble mixed-linked glucan and arabinoxylan originated from the endosperm and virtually all of the remainder from the bran fraction. The ratio arabinose:xylose differed in xylans from the two botanical sources and a change in ratio in xylans solubilised by processing gave an indication of the predominant source of any additional polymers released.

- There was limited evidence to suggest that the rheological properties of released NSP may be modified by endogenous enzyme activity and that low temperature conditioning of the grain before any high temperature processing may be beneficial.

- The addition of supplementary enzymes (principally xylanases to wheat and β-glucanase to barley) generally had the effect of at least partially reversing the deleterious effects of heat treatment and, at best, enhancing the recovery of nutrients to an extent not achieved by either process or enzyme addition alone.

- A number of processes involving pelleting, low temperature extrusion or high temperature extrusion with added enzymes were identified where an improvement of 7 ileal nitrogen digestibility units was achieved for wheat and grain fed to pigs. Responses were less in broiler chicks although significant improvements were obtained.

- The more extreme thermal processes led to the formation of Maillard compounds which reduced the availability of lysine in particular. High temperature micronisation was the most damaging process.

In the final part of the project four methods of processing were compared and the interaction between processing and enzyme supplementation examined in whole diets based on a 2:1 mixture of wheat and barley. This confirmed in practical diets what had previously been shown with single cereals alone. In terms of digestibility, beneficial effects of both increased energy input and enzyme supplementation were seen although they were not always additive. Highest digestibility values were generally achieved with the combination of single pelleting and enzyme addition rather than any of the more thermally intensive processes. Chick performance in the growth trial generally reflected these trends. There were only small, non-significant differences between processes in terms of feed intake, but significant differences in weight gain and the efficiency of feed conversion. Extrusion, in particular, led to lower weight gain and feed conversion and was associated with the highest *in vivo* digesta viscosity. Enzyme addition reduced digesta viscosity in all treatments to approximately the same value.

OBJECTIVES

The aims of this project are:

- **to develop and evaluate novel methods of physical processing, alone and in combination with enzymes, on the nutritional quality of the selected feed ingredients;**
- **to develop improved methods for assessing nutritive value and the effects of processing**

Selected UK-produced crop materials will be subjected to processes (mechanical, thermal, enzymic and combinations of these) designed to improve their nutritional value and reduce the problems of waste disposal. The crop products to be used are whole wheat, whole barley and selected by-products, rapeseed and extracted rapeseed meal. The aim of the processes used will be to disrupt the plant cell wall matrix sufficiently to allow the entry of mammalian digestive enzymes, the release of the soluble nutrients contained in those cells and such partial breakdown of the cell wall materials as will maximise their subsequent susceptibility to microbial degradation. These changes are also expected to increase the susceptibility of the NSP to selective degradation by exogenous enzymes. The processes used will include hammer-milling, wet milling, extrusion, expansion and micronisation with selective use of exogenous enzymes. Improvements will be assessed first by *in vitro* laboratory methods. Measurements of porosity will be used to indicate the extent to which the plant cell wall matrix has been disrupted by the treatment and the increased susceptibility to both mammalian and exogenous enzymes. At the same time the susceptibility of starch and protein to digestion will be assessed *in vitro*. These measurements will be used to optimise processing. Materials optimally treated will be evaluated in digestibility trials with rats and those showing significant improvement in digestibility will be evaluated by direct measurement of absorbed nutrients with pigs and poultry. Finally, those materials showing significant improvement through processing will be included in complete diets which will be evaluated in feeding trials with pigs and poultry.

Achievements

Developed the use of: the Rapid Visco Analyzer for evaluating the effects of processing on starch structure; improved *in vitro* digestibility assays for rapidly assessing the effects of processing on the nutritive value of cereals.

Demonstrated that an approximately 7% enhancement in the recovery of cereal protein is possible through optimal processing.

Aims not achieved

The development of improved processing technologies. Reason: failure to secure the involvement of feed processing machinery manufacturers and process engineers.

The use of rats as a model for digestibility in pigs and chicks. Reason: highly variable data due to feed selection and inconsistent intake.

PREFACE

This final report to the Consortium summarises and discusses the main results of the project, the aims of which were:

1. to develop and evaluate novel methods of physical processing, alone and in combination with enzymes, and to examine their effects on the nutritional quality of the selected feed ingredients;

2. to develop improved methods for assessing nutritive value and the effects of processing.

Several new processes have been developed in recent years in the food and animal feed industries. A number of these were designed primarily for purposes not related to nutrition, such as the alteration of texture, and their effects on the structure and chemical composition of different foods are largely unknown. Although some have found a limited use in the animal feed industry, for example, the use of extruders to produce shaped pet food products, or to reduce microbial numbers in finished feeds but others have been used only for human food, for example, the puffing of cereals for breakfast foods.

Most previous assessments of the effects of processing have concentrated on just one particular aspect, such as the physical changes, the digestibility of nutrients, *in vitro* or *in vivo,* or the performance of animals. The processing equipment has been designed by engineers and many of the operating parameters of processing equipment have been chosen with very little knowledge of their effects on nutritional parameters. In this project an attempt has been made to understand the effects of these processing techniques on cereals, exploring the ways in which thermal or mechanical work may alter the physical structure, or the chemical composition, or both, and how these changes in turn affect the way in which the material is digested by animals.

It was envisaged that the beneficial effects of improved processing would include:

- the rupture or perforation of plant cell walls, allowing the release of readily digested proteins, oils and polysaccharides contained within them, or the entry of host enzymes allowing digestion to take place within the cells;

- the accelerated digestion of less soluble compounds, resulting in the absorption of their end-products from the small intestine rather than fermentation in the large intestine

At the same time, processing could have antinutritional effects, including:

- accelerated solubilisation of non-starch polysaccharides, such as β-glucans, leading to increased viscosity of feed and digesta;

- the formation of chemical complexes, especially those involving proteins and reducing sugars, resulting in reduced availability of nutrients.

The project concentrated on wheat and barley, reflecting the overwhelming importance of these two cereal grains in pig and poultry feeding.

In most of the processes examined a number of operating parameters could be varied, any or all of which could alter the effects of the process, giving rise to a large number of possible permutations. In order to understand the effects of these parameters it was considered desirable to produce for investigation material processed under a wide range of conditions and numerous combinations of conditions. Recognising that *in vivo* measurements are costly and time-consuming, it was clearly not feasible to carry out animal experiments on all such materials. Therefore, from the outset, a hierarchical strategy was adopted for evaluating the effects of processing. It was decided to use the more rapid laboratory based methods to make initial assessments of the general direction and magnitude of changes due to altered processing conditions, then to use that information to select conditions suitable for producing the larger quantities of material needed for animal experiments.

The major methods used for the assessment of physical change were surface area or porosity, viscosity and electron microscopy while the main assessments of changes in chemical composition were by measurement of non-starch polysaccharides and their constituent sugars. The third arm of the laboratory methodology was the measurement of digestibility *in vitro,* using simple enzyme digestions and measuring the solubilisation of substrate.

The *in vivo* assessment of the effects of treatments centred on measurements of digestibility. In order to distinguish as far as possible between digestion by host enzymes and microbial fermentation, measurements were made of digestibility proximal to the terminal ileum as well as over the entire gastrointestinal tract. The main test species were pigs and chicks. It was initially hoped that rats might prove a useful and convenient model for both pigs and chicks and accordingly, in the first experiment, all three species were used. It was found, however, that food selection by rats resulted in biased results, and they were therefore not used in any further experiments.

INTRODUCTION

Animal feed is the biggest single market for grain produced in the UK, consuming some five million tonnes of wheat and four million tonnes of barley, over 40% of total production. Despite the importance of cereals in animal feeding the requirements of the animal feed industry have been little considered when setting quality standards for cereals; the overriding concerns have been the much more clearly voiced needs of the milling and brewing trades. Due partly to the limitations of cereals and the lack of positive steps to promote their improvement for animal feeding, imported alternatives have appeared increasingly in compound feeds. Within the animal feed sector quality requirements differ according to the species considered. Although pigs and poultry differ in some aspects of their digestive processes their needs are broadly similar and contrast with those of the ruminant species. This project has been concerned only with non-ruminants.

Cereals are not by themselves an ideal diet for pigs and poultry , being deficient in a number of important nutrients, especially protein and particularly certain essential amino acids. The protein sources which have been mixed with cereals to form the basis of practical diets have traditionally been imported soya bean meal and animal by-products. More recently home-grown crops, especially rape, have been developed as an alternative source. In addition to deficiencies in content, many of the nutrients in cereals are poorly available to animals. Typically, only 70% of the amino acids and 80% of the energy of cereal grains is absorbed. The poor availability of nutrients may arise from several sources. First, the nutrients may be inherently resistant to dissimilation by mammalian digestive enzymes. The glucose residues of cellulose and the phosphorus present as phytate are conspicuous examples. Second, the nutrients, though themselves susceptible to attack by mammalian digestive enzymes, may be enclosed by resistant cell wall material and inaccessible to enzyme attack. The high quality proteins in the aleurone layer of cereal grains are largely unavailable for this reason. Third, the nutrients, though accessible and susceptible to hydrolytic enzymes, may not be released and absorbed because of interference by other substances such as trypsin inhibitors or viscous polysaccharides. These problems are all of concern in cereals, cereal byproducts and rapeseed, as well as in other home-grown crops such as peas and field beans.

The unavailability of nutrients in animal feeds is not only a nutritional but an environmental problem. The major nuisance of intensive animal production is in waste disposal; typically 20-25% of the organic matter fed to pigs appears in the slurry. The development of processes which would both increase the nutritional value of feeds and reduce the volume of solid waste would be of enormous value to the industry.

In addition to the whole grain which is included in animal feeds, cereal by-products, such as milling offal and bran, brewers' and distillers' grains and solubles, as well as gluten and other fractions from the starch and cereal syrup industries, further augment the volume of relatively indigestible material included in animal feeds.

Traditionally, feed crop materials have had little if any processing before they are included in diets. Whole cereal grain is normally hammer-milled, a process which can vary greatly in its effects, depending upon the design and operation of the equipment. More recently, other processes have been introduced. Some grain, especially that intended for young animals, may be micronised; rapeseed may be extruded and complete diets may be substantially heated by pelleting. Other physical treatments, whether mechanical, thermal or both, have been developed for human foods but have been little examined in the context of the animal feed industry .

The products used were:

- whole wheat
- whole barley
- wheat feed.

The processes

1. Hammer-milling was regarded as the 'standard' process. 'Standard' is in quotation marks because its effects can vary considerably. It was therefore necessary to investigate a range of milling conditions as part of the experimental plan as well as setting, for each material, fixed conditions to serve as the basis of comparison with other processes.
2. Expanders use a screw(s) to force material through a chamber, usually with the addition of water or steam. Expanders are often used for preconditioning feed prior to pelleting.
3. Extruders use a screw(s) to force the feed material through a chamber and out through a die. The increased temperature and pressure as the material passes through, and the sudden release of pressure as it leaves the die, produce similar effects to expanders.
4. Micronisation has been used in the animal feed industry for a number of years, mostly applied to cereals to improve their quality for young animals in which rapid gut transit and a relatively immature digestive system combine to limit the extent to which unprocessed cereals can be utilised. The internal heating of micronisation causes disruption of cell structure, improving the digestibility of starch.
5. Puffing has been used in the production of cereal products for many years. The process involves heating the material under pressure, usually in the presence of steam,

then rapidly reducing the pressure. This causes explosive volatilisation of the water within the material, disrupting the water-bearing structures, including cell walls.

6. Pelleting is standard in the production of many finished feeds, but the conditions used can vary widely. In addition to the physical parameters of the pellet mill, the addition of steam, pretreatment by expansion, or other adjunct processes can produce a range of effects.

Enzyme addition can form an adjunct to any of these processes. Proteases and various polysaccharidases, have been introduced in pig and poultry diets. The value of breaking down the soluble {ß-glucans of barley has consistently been shown to be of value in poultry but for pigs the benefits of enzyme treatment are less clear. One reason is the supposition that all the constituents of non-starch polysaccharides would be more useful to the pig in monomeric form. However, the poor utilisation of pentose sugars and uronic acids by pigs means that arabinoxylans and pectic substances may be of equal or greater value to the pig if digested by microbial activity in the lower digestive tract than if hydrolysed further up the gut. The use of enzymes in conjunction with processing needs therefore to be targeted precisely, with clearly specified aims.

The rationale for choice of assessment methods

From a purely nutritional perspective the cereal grain can be considered as consisting of three parts: the endosperm rich in starch granules and protein; the thick walled aleurone which contributes smaller amounts of protein but of higher nutritional quality; and the seed coat (plus pericarp/testa) formed from lignified cell walls and of limited nutritional value to non-ruminants.

The endosperm

Minimal disruption of the outer layers of the grain is sufficient to allow water and host digestive enzymes to penetrate the endosperm. Although starch and protein are protected by the cellular nature of the endosperm, the chemistry of the endosperm wall allaws its ready disruption. Unlike cell walls with a support function, the endosperm remains thin (only 3-4 polymer widths) and retains a 'juvenile' structure in which cellulose, which normally replaces the first formed mixed-Iinked glucan, is essentially absent. Processing, other than the minimum necessary to disrupt the grain, would be expected to hold little advantage and may have the negative effect of resulting in a greater solubilisation of the two polysaccharides (arabinoxylan and mixed-Iinked glucan) which make up 90% of the barley and wheat endosperm wall.

Starch granules possess a common basic form in which the core of the granule consists of disc-shaped, radially-directed amylopectin molecules intertwined into double helices with the reducing end of the main chains directed inwards. Amylose in association with monoacyl

lipids is generally restricted to the outer parts of the granule. The alternating concentric rings of amorphous and crystalline regions evident in sections of the granule prepared for electron microscopy appear to relate to absence and presence respectively of grape-like clusters of side chains, 'hanging' from the main chain of the amylopectin molecule. Penetration of the amorphous region by water and by enzymes is more rapid than penetration of the crystalline region and probably provides the means of entry and the rapid mobilising of the starch granule. Any form of thermal processing in which temperatures exceeds -60°C (the glass transition temperature of cereal starch) will result in the disruption of granule structure and gelatinisation, making the starch more immediately available to host amylolytic enzymes. As a result methods geared to examining the properties of intact starch granules, such as differential scanning calorimetry (DSC) and X-ray diffraction, provided only limited information about processed grain. Although DSC did find application in this work for the detection of retrograded starch, the Rapid Visco Analyser, designed to monitor changes to starch during flour processing, provides a better routine means of monitoring the extent of disruption of starch granules and the gelation properties of the released starch polymers.

Starch utilisation by young pigs and poultry is known to be high and while processing was expected to make the starch more immediately available it was not expected to significantly improve the extent of utilisation. The ready access to the starch granules by endogenous enzymes after minimal processing implies an equal bioavailability of endosperm protein. Thus utilisation of this fraction of the total grain protein was similarly thought unlikely to be improved by processing.

The aleurone

The aleurone cells, located as a single row underlying the testa/pericarp in the case of wheat and as a two or three row layer in the case of barley, are the source of the autolytic enzymes released on the breaking of dormancy. As a result they contain a relatively high concentration of protein whose nutritional value is superior to that found in the endosperm. They also provide a valuable supply of lipids, sucrose, phytate and B-group vitamins. The aleurone have the same developmental origin as the endosperm and their walls have a similar chemical structure. However aleurone walls are considerably thicker and are more able to resist processing and mastication. Thermal processing was expected to kill the aleurone cells and to lead to the disruption of their membrane structure. However, the ability of the cell contents to leach out of the aleurone or host digestive enzymes to access aleurone contents in cells which remain entire is also determined by the porosity of the cell wall. The entirety of the cell is likely only to be disrupted by process which included the application of sheer forces.

Spaces within the three-dimensional polymer network that forms the cell wall define the pore structure of the wall and act as sieve, allowing compounds of lesser dimensions to diffuse through and retarding those compounds, such as proteins, whose Stokes radius is greater than the spacing between the wall polymers. There are a number of published methods for measuring the porosity of cell walls, but those based on the use of polymers of known dimensions were considered to be insufficiently sensitive. Physical chemical methods are commonly used in industry for monitoring similarly porous materials such as catalysts and provide a means of detecting pores of the dimensions encountered in grain cell walls (and starch granules). The method selected, gas adsorption, has not previously been applied to biological systems. With this method, information about surface features is derived from the amount of gas which physically adsorbs as a monolayer onto the surface of the cell wall sample measured at various partial pressures. From this volume measurement, values for total surface area, pore sizes and pore size distribution can be obtained. Since most surfaces available to very small probes, such as nitrogen, are associated with pores defined by the spacing within and between the polymers which comprise the starch granules and the cell wall, any change to pore size or distribution is indicative of changes to these structures. It was expected that any processing which disrupted cell wall structure sufficiently to increase the bioavailability of protein and other nutrients held within the aleurone would be detected as an increase in overall porosity and the dimensions of wall-associated pores.

The outer layers of the grain

The outer layers of the grain consist almost entirely of lignified cell wall material and associated waxes and have very little nutritional value for non-ruminants. There is a need for this coat to be disrupted by some mechanical process to allow digestive enzymes access to the endosperm, otherwise this fraction of the grain is seen purely in negative terms. However, a not inconsiderable part of the protein fraction exists as an integral part of wall structure. This protein is not normally available to non-ruminants and only partially available to ruminants where it accounts for most of the ADF-N fraction.

The wall structure of the cells of the outer layers more closely resembles the normal pattern associated with vegetative tissues and is based on a network of cellulose and xylans. Although this structure is more resistant to disruption that the aleurone walls it is possible that the more severe forms of mechanical and thermal processing could sufficiently damage the wall to release or make accessible this nitrogen fraction. Again this would be reflected in a demonstrable change to pore volume and the distribution of pores of various diameters in the wall (the pore regime).

The sequence of assessment

The effects of the processing treatments were evaluated in four stages. First, the disruption of the cell wall structure was assessed by chemical analysis and by measurement of porosity and the breakdown of cell wall materials by the extent of the solubilisation of non-starch polysaccharides. Physical disruption of feeds and feed ingredients was evaluated using gas adsorption-desorption methods and mercury porosimetry. These methods were used to monitor changes after processing in the surface area available for attack by host enzymes or exogenous enzymes added as a process aid or as a dietary supplement.

Second, the potential release of major nutrients was assessed by *in vitro* tests based on simple simulations of mammalian systems, using pepsin/HCl or amylase/amylopectin digestions.

Third, those treatments which appear to have been effective, as judged by *in vitro* testing, were evaluated in digestibility trials. Digestibility of various carbohydrate and protein fractions were measured both proximal to the caecum and over the whole digestive tract. Initially, rats were evaluated as a possible model for pigs, as well as chicks. However, rats did not prove suitable for coarse materials because of their inclination to select particular fractions of the diet and all materials were therefore evaluated in chicks and pigs. For the assessment of ileal digestibility in chicks and rats, the animals were killed and the ileal digesta from several animals pooled. The pigs had simple T-cannulas in the terminal ileum.

Finally, the most effective treatments were evaluated in a practical growth trial in which the overall efficacy of complete diet treatment can be subjected to cost-benefit analysis.

Composition and nutritional value of the milled grain

Two 3 tonne batches of wheat (cv Riband) and barley (cv Manitou) were provided by Dalgety Agriculture. The first batch delivered early in the project was used for Experiments 1-4 and the second batch delivered in 1996 used to prepare the processed grain for Experiment 5 onwards (Table 1). The wheat was chosen to be of high bushel weight. All received grain was bagged and stored under ambient conditions in the dry until required. A single batch of wheat middlings (ex Riband) was also supplied and used throughout this work.

Table 1. Proximate analysis of the bulk wheat and barley samples supplied. Values (g kg-1) for the various analytical fractions are given on a dry matter basis. The dry matter content is as received.

Fraction	Barley		Wheat	
	Batch 1	Batch 2	Batch 1	Batch 2
Dry matter	892.4 ± 0.9	890.0 ± 0.4	887.9 ± 2.6	897.0 ± 09
Ash	21.5 ± 2.1	29.0 ± 0.4	16.3 ± 0.4	17.7 ± 13
Starch	609.3 ± 3.9	552.8 ± 0.0	681.9 ± 17.5	634.2 ± 76
NDF	143.2 ± 4.7	162.3 ± 5.6	89.5 ± 4.1	88.0 ± 11
ADF	56.3 ± 1.9	67.4 ± 4.5	30.0 ± 0.1	34.2 ± 08
ADL	9.1 ± 0.3	10.0 ± 0.4	8.9 ± 0.6	9.9 ± 01
AEE*	22.5 ± 0.7	21.1 ± 0.4	23.3 ± 0.9	26.1 ± 06
Crude protein -1	109.5 ± 1.4	130.2	106.9 ± 2.0	125.3 ± 22
Crude protein -2	99.9 ± 1.3	118.8	97.4 ± 1.8	114.3 ± 20
Crude fibre	44.1 ± 2.5	57.3 ± 2.5	21.1 ± 1.9	26.9 ± 16

AEEF* acid ether extractives. Crude protein 1 - N x 6.25, crude protein 2 - N x 5.7

Pigs and broiler chicks have a high capacity for starch utilisation and, provided the grain is sufficiently disrupted and the endosperrn accessible, overall digestibility values between 95-98% would be expected. The observed values for the milled grain, which are shown in Table 2, confirmed this expectation.

Table 2. Percentage ileal and overall organic matter (OM), starch and nitrogen digestion by broiler chicks and pigs fed the experimental wheat or barley. (Data is based on a numerical analysis of the values obtained for the milled cereals in Experiments 1-4).

Component		Wheat		Barley		SEM
		Chicks	Pigs	Chicks	Pigs	
OM	- ileal	0.69	0.76	0.60	0.67	0.020
	- overall	0.71	0.86	0.62	0.73	
Starch	- ileal	0.97	0.98	0.97	0.97	0.012
	- overall	0.95	1.00	1.00	1.00	
Nitrogen	- ileal	0.70	0.67	0.65	0.62	0.019
	- overall	0.65	0.82	0.64	0.72	

Although, as these values show, there is little room for improvement in starch digestibility, even a 1-2% increase can usefully improve the economics of production. However, there was greater scope for improving nitrogen utilisation in both species and with both cereals. Thus the aim of processing was to maintain or, ideally, improve slightly the utilisation of starch while significantly increasing the accessibility of protein and minimising any release of NSP with antinutritional properties.

Starch properties and utilisation

Milling alone was sufficient to disrupt the grain and ensure starch accessibility. Any very limited improvement to starch accessibility, perhaps by increasing the uniformity of treatment, would be unlikely to justify the additional energy inputs required. However, any thermal inputs beyond the glass transition of the starch granule (onset -56°, mid-point -60°) would also be expected to lead to gelatinisation and the faster solubilisation of starch from treated grain, with possible beneficial consequences for intake and the rate of weight gain.

The effectiveness of the various processing treatments on the *in vitra* and *in viva* properties of starch were assessed by:

- Enzyme degradability to measure short-term availability (30 and 120 min); .
- Enzyme degradability (24 hours) as predictor of the *in viva* value;
- Rapid Visco Analyzer (RVA) curves to measure the extent of gelatinisation and gel-forming capacity;
- Differential scanning calorimetry (DSC) to confirm loss of granule structure and absence of retrograded starch ;
- Scanning electron microscopy of grain and starch-granule structure;
- *In viva* starch digestibility (ileal and whole tract) in broiler chicks and pigs.

Processing gelatinisation and the immediate availability of starch Use of a Rapid Visco Analyzer (RVA) produces so-called *pasting curves* which describe the changes in viscosity of a continuously stirred suspension of treated grain while it undergoes a preselected heating and cooling regimen. The resultant curve reflects a sequence of events starting with the initial swelling of the starch granules, gelatinisation and the exudation of starch from granules as the temperature is increased giving an initial peak viscosity value. As the disintegration of the granules progresses, shear thinning occurs and the viscosity drops although the temperature is held constant. Finally, as the operating temperature is reduced, a second increase in viscosity produced by gelation occurs (Figure I). Although these events are driven largely by the starch content of the grain it is probably more accurate to consider them as an endosperm phenomenon (Figure 1). Almost certainly the protein and NSP content of the endosperm contribute to the observed viscosity.

All the processed wheat samples were examined by RVA using a constant temperature profile and data was collected from three points on the curve representing the maximum viscosity of the first peak, the minimum viscosity of the trough and the maximum viscosity of the second peak. In each case the time and temperature taken to reach this value were also recorded (Appendix IX). As might be expected, the three viscosity values were very highly correlated as shown in Table 3, with a particularly strong relationship between the trough value and peak 2. Only a limited number of pasting curves were produced for the barley samples, since the pattern appeared to match that of wheat. However, as a rule, viscosity was greater with barley than with wheat and maximum values were reached a little earlier.

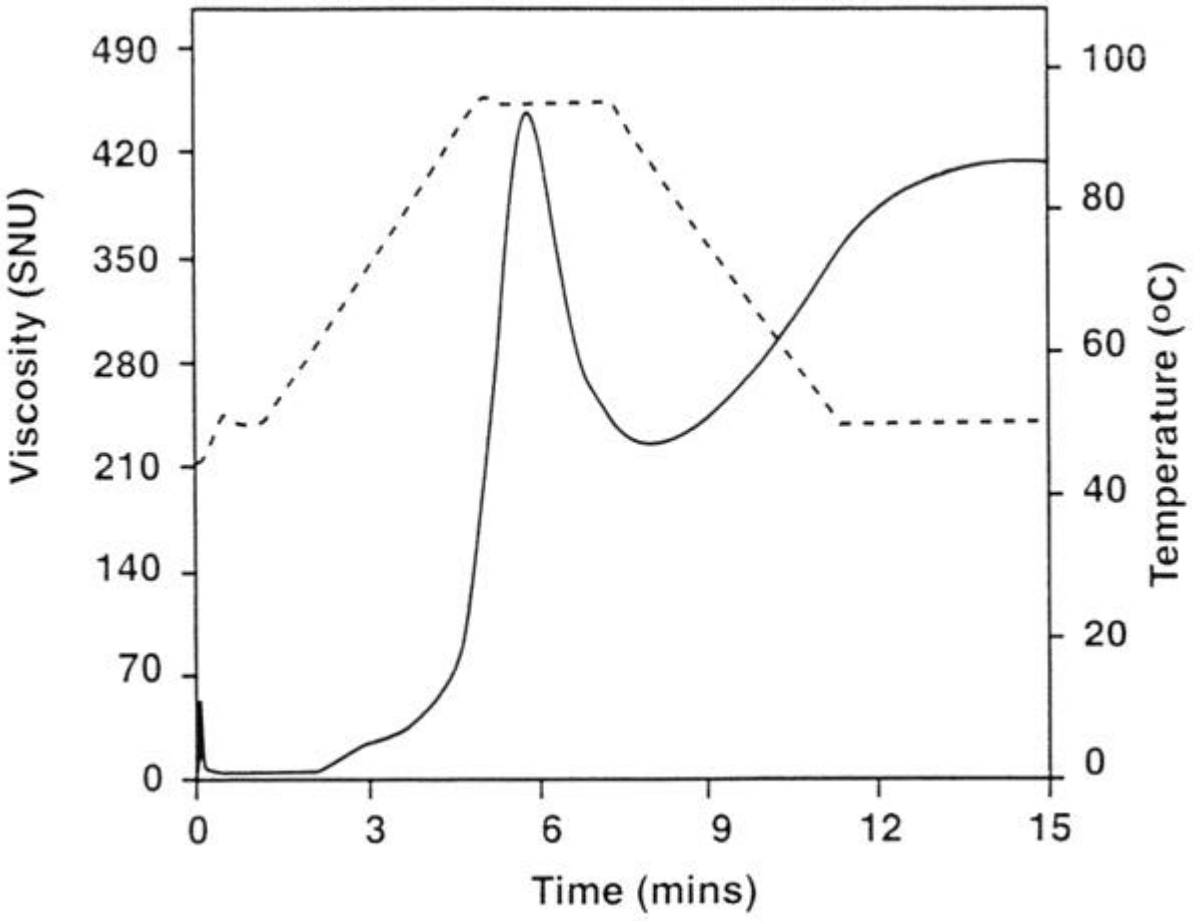

Figure I. Pasting curve for wheat endosperm. The temperature changes applied are shown as the dotted line.

Table 3. Correlation matrix for the three viscosity values produced by the RVA analysis of the processed wheat samples (excluding puffed samples).

	Peak 1	Trough	Peak 2
Peak 1	1.000		
Trough	0.953	1.000	
Peak 2	0.932	0.988	1.000

Extrusion of wheat led to a substantial fall in all three viscosity values compared to the unprocessed grain (Figure 2) with the greatest reduction associated with the highest

temperature (1200). Viscosity did not appear greatly influenced by moisture content at processing within the range tested (18-30%) except for the Peak 2 values at 120°. These were between 30-50% of the untreated grain and showed an almost linear response to moisture content, with the lowest gel-forming capacity occurring at the highest moisture content. This relationship did not hold for other processing temperatures. Pelleting, regardless of the form of conditioning, also led to reduced viscosity values but the response seen was substantially smaller than even that observed for the least effective extrusion conditions (60°, 30% moisture). Conditioning by expansion or steam led to significantly lower viscosity values ($P > 0.001$) compared to conditioning with water .

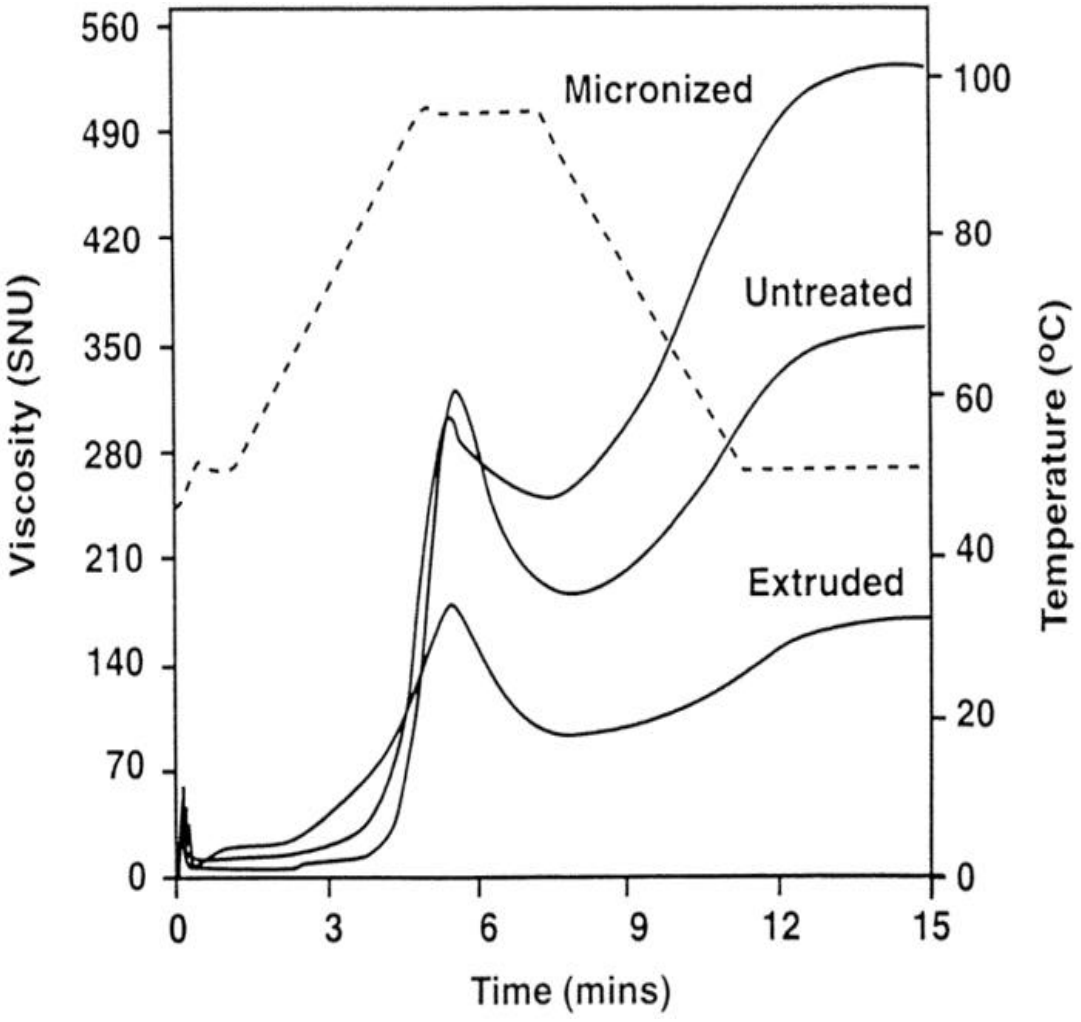

Figure 2. Pasting curve for untreated polished wheat, wheat micronised at 140°C and 15% moisture and wheat extruded at 100°C and 22% moisture. The temperature changes applied, shown as a dotted line, are the same as that shown in Figure 1.

In many ways puffing of wheat produced a similar response to extrusion, but more extreme, (Appendix IX). However, results are not wholly comparable because the wheat used in the puffing experiment was a different sample supplied by Quaker and was, of necessity, polished (pericarp removed) before treatment. However, the RVA curves for the polished wheat were similar to those of untreated Riband, differing only in the maximum viscosity of the first peak. Puffing produced a substantial response (Figure 3). The maximum viscosity of the first peak, while remaining the same magnitude as the unpuffed sample, developed within 20 sec compared to 5.8 min for the unpuffed sample and 5.6 minutes for untreated Riband. This indicated that processing had produced a total disruption of granule structure and, consequently, the immediate availability (water-solubility) of the starch. This was confirmed by simple extraction of the puffed product into water at 40° which resulted in the immediate solubilisation of 31% of the starch originally present compared to 6% from the unpuffed grain. Evidently the starch also had undergone some structural change since its subsequent capacity to gel was almost totally destroyed (Figure 3).

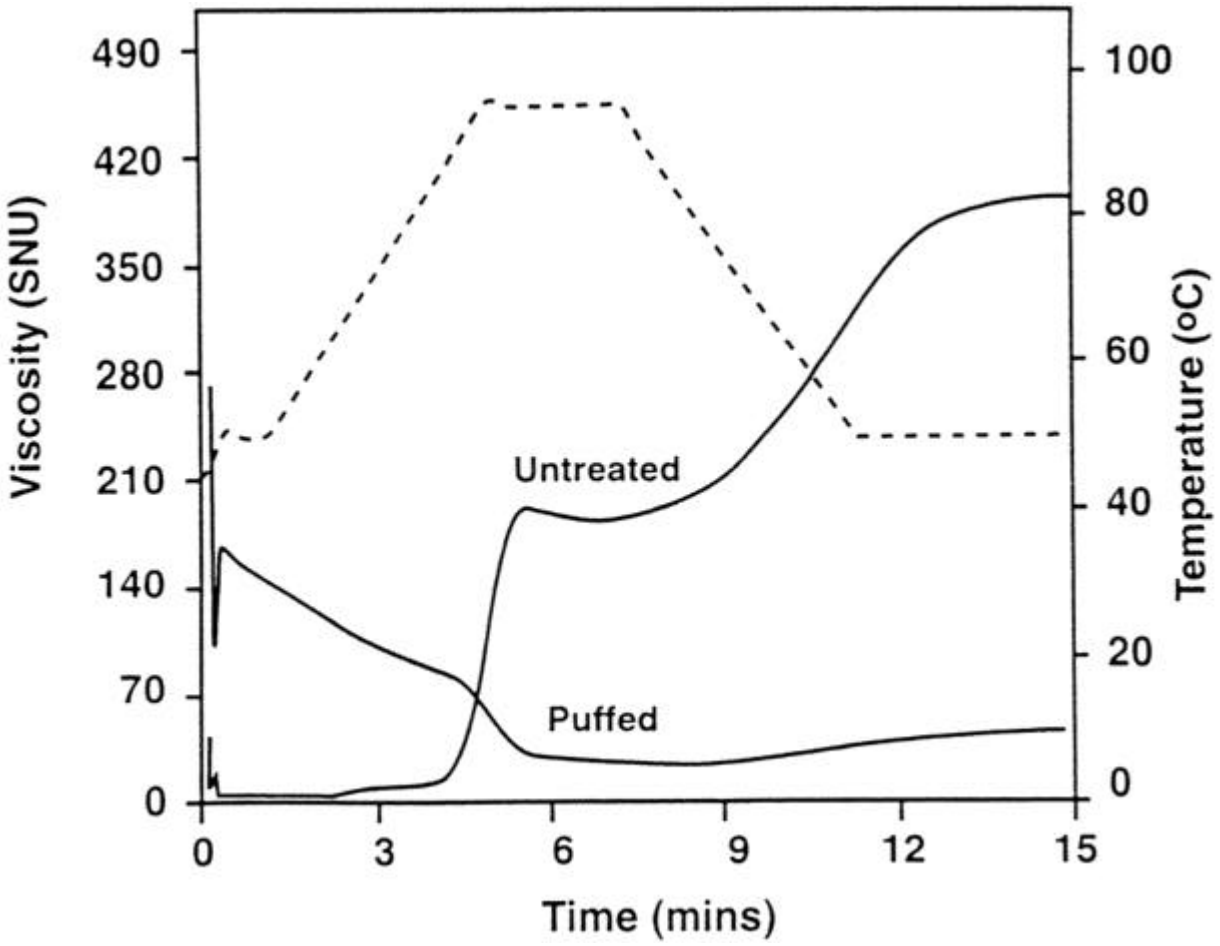

Figure 3. Pasting curves for untreated polished wheat and the same wheat after puffing. The temperature changes, shown as a dotted line, are the same as that shown in Figure 1.

At the lowest temperature examined (75°), micronisation, with or without flaking, had little effect on any of the RVA viscosity values (Figure 2). Increasing the temperature or moisture content had relatively little effect on the viscosity of the first peak but tended to promote rather than destroy the capacity for subsequent gelation (Appendix X). Micronisation involves the application of heat to the surface of the grain only and, in the absence of flaking, no mechanical treatment is involved. Since kernel integrity is maintained, the structural constraints imposed by the testa and pericarp are probably sufficient to limit the swelling and fragmentation of the starch granules. This leaves the treated material more closely resembling the untreated grain compared to the samples which are processed under similar moisture and temperature conditions but in which the grain is first disrupted by milling. Consequently, when analysed in the RV A, providing sufficient energy has been applied to change granule structure, granules may become more resistant to shear leading to the significantly higher trough and peak 2 viscosity values observed.

Extrusion, pelleting and puffing involve the application of both heat and mechanical energy. Such processes would be expected to lead to the partial or complete destruction of starch granules during the cooking process and the subsequent formation of starch structures with different types and degrees of crystallinity. Since starch granule destruction has already been initiated, the initial viscosity values from the pasting curve would be expected to be lower than with the untreated wheat. Although this was observed no clear relationship between moisture content and temperature could be established. This may have been because the control exercised on both of these parameters was limited as shown when repeat extrusions, nominally made under the same operating conditions, produced material which was demonstrably different. In addition, no attempt was made to control, or account for, mechanical energy inputs.

Any change to granule structure would be expected to affect the accessibility of the starch to amylolytic attack. However, this may be evident only in the short term. With longer exposure to moisture and body heat starch granules swell and rupture in any case, as is shown when milled grain is fed, and any advantage introduced by processing would no longer be detectable. In order to assess the effects of processing on the immediate availability of starch to enzyme attack, 30 min and 120 min incubations were made with porcine amylase and the release of reducing sugar measured (Appendix X). A regression analysis of the relationship between sugar release after 30 min and the RVA parameters demonstrated a highly significant ($P < 0.001$) negative relationship with the magnitude of the first peak of the pasting curve. As would be expected, because of the correlation between all three viscosity values, this relationship also held for the trough and second peak values, although the regression coefficients were lower. Adding into the first equation values for the trough and second peak accounted for very little of the remaining variation. Although the

relationship between sugar release after 120 min and the first peak remained highly significant ($P < 0.001$), the correlation was considerably weaker indicating that any additional benefit gained by processing was unlikely to be seen beyond this period.

Evidently processes such as extrusion, which combine heat with mechanical work, lead to the partial break-up of starch granules, to a lower peak 1 value and to the more immediate accessibility of the starch. Heat alone, as in the case of micronisation, is considerably less effective unless the grain is also flaked. Visual examination of treated grains using scanning electron microscopy generally supported these conclusions. Micronisation tended to produce little visible change in starch granule or grain structure; extrusion was intermediate in effect and caused some loss of granule structure, while puffing completely destroyed any semblance of structure within the starch granule or endosperm (see Appendix XI).

Starch retrogradation

Initial concerns, introduced by an inability to fully hydrolyse starch with a thermostable amylase (Termamyl), that thermal processing might lead retrogradation of some of the starch and thus reduce its biological availability proved unfounded. Samples of all processed wheat were examined by differential scanning calorimetry (DSC) in collaboration with Dr C. Imrie, Department of Chemistry, University of Aberdeen. DSC is a technique specifically designed to monitor the structural properties of polymer molecules and the effect of temperature on phase transitions within the material. It has been shown to be particularly effective in detecting the highly organised crystalline form of amylose referred to as retrograded starch (Figure 4) which has parallels with crystalline cellulose in its highly ordered state and its consequent resistance to hydration and thus to hydrolytic attack. None of the samples examined had evidence of an endothermic transition around 150° typical of retrograded starch. Retrogradation often develops because of cycling between heating and cooling of starch samples and the single cycle to which wheat was exposed during most processing was probably insufficient for retrogradation to occur to any detectable extent. Manufacture of retrograded starch in the laboratory , even when starting with amylose alone, often requires several cycles of heating and cooling.

The glass transition marks the process of gelatinisation in the starch and, in the milled wheat, becomes evident at -59° and reaches a midpoint at -62°. This endothermic transition remains evident in samples extruded at 60-80° at all moisture contents but is increasingly difficult to detect at higher temperatures. Similarly the transition is evident in samples previously micronised at 75 and 100° but absent in those processed at 120°. Since glass transition is essentially an effect of temperature alone on the starch polymers, moisture or mechanical flaking made no detectable difference.

Puffing and high temperature extrusion bring about the same effect and the endothermic transition around 60° is no longer detectable (Figure 5).

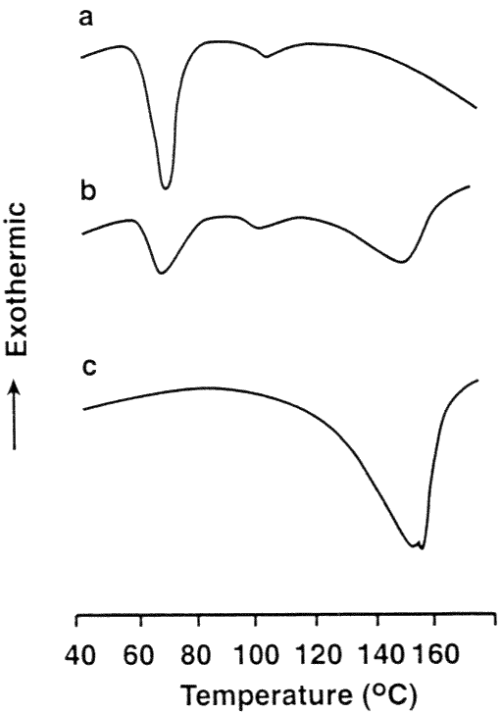

Figure 4. DSC thermograms of retrograded and normal wheat starch showing the glass transition of normal starch at -60°C and the endothermic transition -150°C typical of retrograded starch. (a) normal starch, (b) 1:1 mix of normal and resistant starch, (c) resistant starch.

In vivo and in vitro digestibility of starch

In vitro estimates of wheat and barley starch digestibility made by first pre-treating samples with pepsin/HCl and then incubating with pancreatin for 22 hours invariably produced results which closely approximated to 100%. The method lacked the sensitivity to discriminate within the very narrow range of digestibility values observed *in vivo*. The samples of wheat middlings which had been extruded under varying conditions gave a slightly greater range of degradabilities from 90.5-100% (Appendix XIII). No significant relationship between these values and the energy inputs during extrusion could be demonstrated.

The high *in vivo* digestibility of starch by chicks and pigs allowed little room for improvement and statistically significant gains were difficult to detect. The exception was hammer-milling of wheat followed by extrusion compared to milling alone where extrusion resulted in a significant improvement in whole tract and ileal utilisation of starch by chicks (Experiment 3, Appendix III).

In other experiments where starch digestion was measured, treatments did not significantly affect starch utilisation in chicks or pigs fed wheat, but extrusion consistently depressed the utilisation of barley starch (Experiments 2 and 3, Appendices II and III). Depression was greatest with chicks and not always seen in pigs.

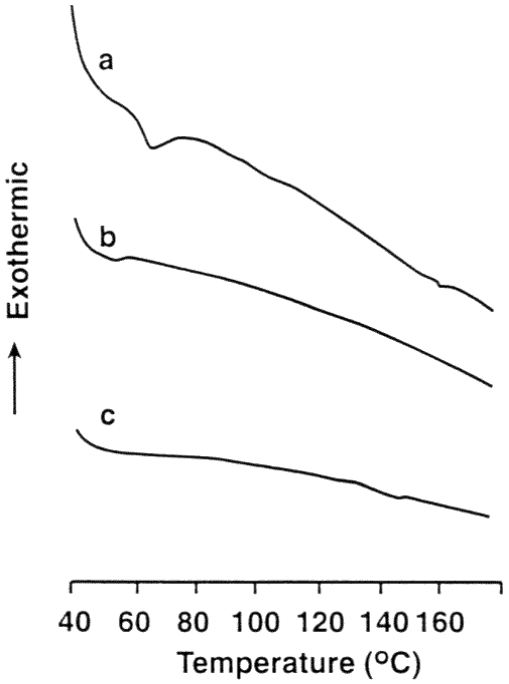

Figure 5. DSC thermograms of (a) untreated wheat, (b) wheat following puffing and (c) wheat after extrusion at 120°C and 18% moisture.

Effects of treatment on the cell wall fraction

Most processing technologies involve heating cereals in the presence of moisture coupled with some form of mechanical work designed to generate shear forces able to fragment the grain. Any plant cell wall exposed to these conditions undergoes a mild acid hydrolysis as a result of the immediate release of acidic groups, particularly acetyl groups, which cause a fall in pH. Hydrolytic cleavage of the more labile bonds within the wall may encourage the partial solubilisation of wall components and changes to the architecture of the wall. Mechanical forces might then be expected to further distort wall structure. The nutritional consequences of such changes were envisaged as potentially both beneficial and detrimental to livestock. Benefits might accrue through disruption of aleurone and endosperm walls allowing more complete access to nutrients, from the increase in potentially fermentable substrates in the pig hindgut and from better organic matter digestibility and increased intake. The negative consequences of processing were expected to be the added release from cell walls of non-starch polysaccharides (NSP) capable of increasing digesta viscosity and depressing the availability of the major nutrients.

The potential for NSP release was estimated directly in extracts made under conditions designed to reflect the digestive tract and coupled with measurement of the intrinsic viscosity of released polysaccharide. Changes to cell wall properties and architecture were investigated indirectly by a physical method based on gas adsorption.

Gas adsorption and cell wall properties

Physico-chemical techniques based on movement of gas or mercury into a structure provide quantitative methods for the determination of available surface area and pore regimes. Both probes measure pore distributions by sequentially filling the pores, but mercury porosimetry scans from large to small pore size while gas adsorption scans in the other direction. However, it was found early in the programme that, because of the limits of detection and the high pressures involved (up to 372 MPa), mercury porosimetry was an inappropriate method for cell wall studies and all subsequent work was based on gas adsorption.

The pore regime associated with cell walls was derived from the adsorption isotherm obtained at relative pressures (P/Po) up to 0.9 which represented a maximum pore radius of 10 nm. Although there was substantial uptake of gas at higher relative pressures the 'pores' measured were of sufficient size to include interstitial spaces and the cell lumen. Assumptions have to be made about pore structure when calculating results from adsorption measurements. All pores are assumed to be cylindrical or able to be treated as a series of cylinders with the greatest radius exposed at the cell wall surface. Any pores which are, for example, bottle shaped will lead to an underestimate of pore volume. In biological materials pore shapes will always deviate from the ideal. However, the shape of the adsorption/desorption curve is a reasonable guide to the magnitude of errors of this type; the larger the disparity between the adsorption and desorption curves, the more the pore structure is likely to deviate from the ideal. It can be seen from Figure 6 that relatively little hysteresis was seen with grain samples and it is reasonable to conclude that errors derived from the distortion of pores shapes were negligible.

Table 4. Pore volume and total surface area of untreated cereal grain determined by gas adsorption

	Barley	Wheat
Pore volume (x 10^{-3} cm3 g-l)	1.59	2.22
Volume of pores < 5 nm radius (x 10^{-3} cm^3 g-l)	0.89	1.41
Surface area (m^2 g-l)	1.39	0.95

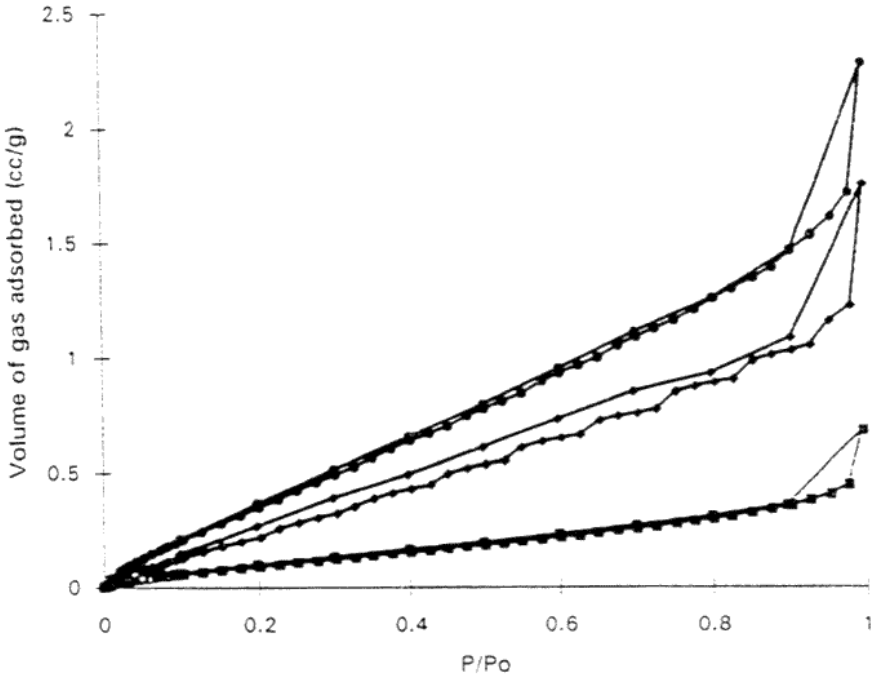

Figure 6. Sorption isotherm of wheat bran (♦) endosperm (•) and extruded whole wheat (▽). The curves with the majority of observations are the adsorption curves from which data was derived, and those with fewer observations, the desorption curves.

The pore size and pore distribution of the untreated grain were very similar (Table 4, Figure 7). The same group of pores ranging in radius from 0.5-5 nm, heavily skewed towards the smaller size and occurring in approximately the same proportions, were present in both cereals. A discrete pore regime was found superimposed on a more amorphous background produced by a continuous range of pore radii. Two milling fractions, the endosperm representing 76.5 % of the whole grain and the finished bran fraction representing 8.2% of the whole grain dictated the overall pore regime (shown for wheat in Figure 7). Examined in isolation, the bran fraction gave a well defined pore regime while, in contrast, the endosperm fraction, dominated by its starch content, gave a much more diffuse pattern with only a few discrete populations superimposed (from endosperm walls) and little evidence of structure (Figure 8). This was evident after high temperature extrusion cooking of wheat under conditions which resulted in starch gelatinisation. The pore regime of the extruded sample was indistinguishable from that of the pure bran sample, and all features attributable to the endosperm were lost (Figure 9).

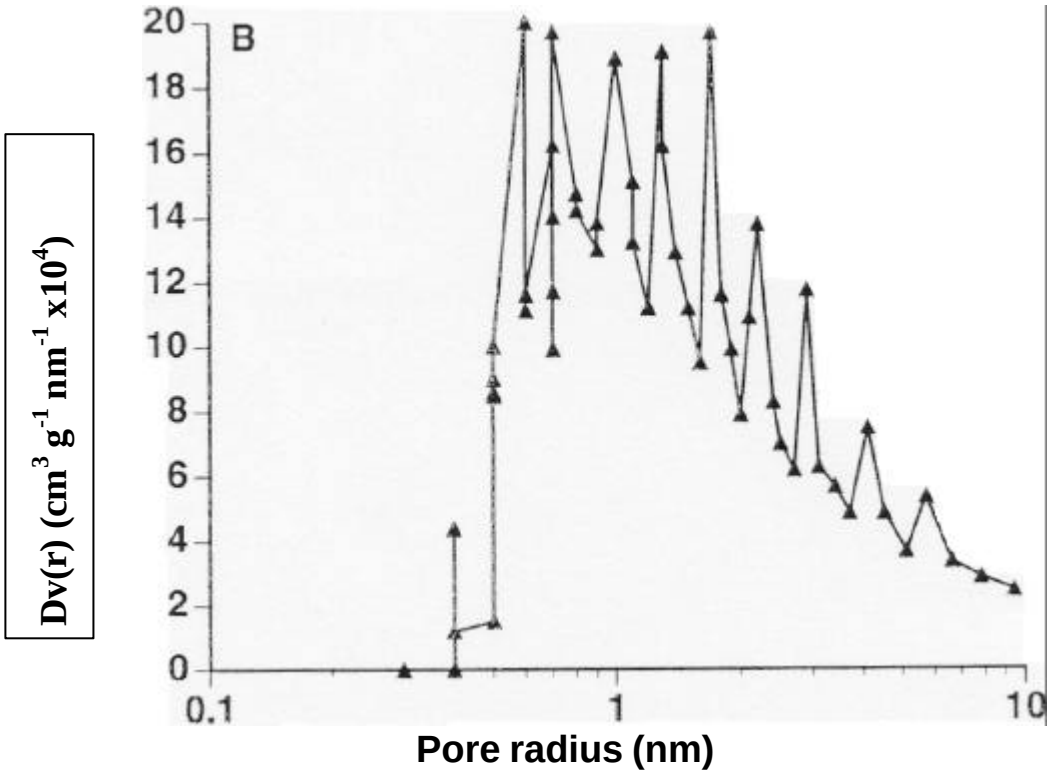

Figure 7. The relationship in milled wheat between pore radius and Dv(r), the volume-based pore size distribution, which provides a better estimate of relative pore numbers.

The effects of extrusion, micronising, puffing and pelleting under various conditions on total pore volume and surface area are given in full in Appendix XII. Although highly statistically significant variations in pore volume and surface area were observed, differences were rarely consistent with changes to process parameters. Thus the surface area of the treated grain samples was significantly related to moisture and temperature ($P<0.05$ to 0.001) and, in the case of micronisation, to flaking ($P< 0.001$). Pore volume, particularly the volume associated with pores of < 10 nm radius, was primarily related to moisture ($P<0.001$) in the case of extruded samples, but more to temperature and flaking in the case of the micronised grains. However, in no case did changes to the physical properties of the processed grain follow a pattern which could be readily attributed to changes in process conditions. This may be because process conditions were not sufficiently accurately monitored and that total thermal and/ or mechanical inputs were not available.

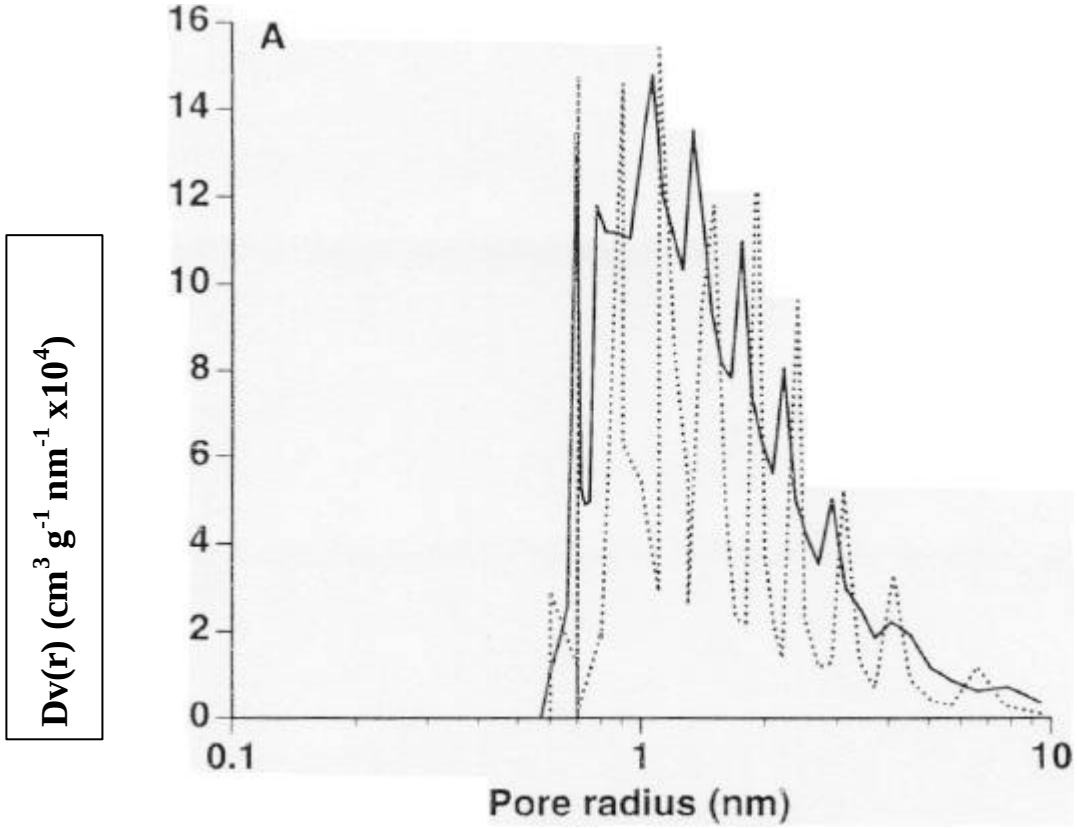

Figure 8. Pore size distribution of bran (dotted line) and starchy endosperm (solid line) fractions of wheat grain

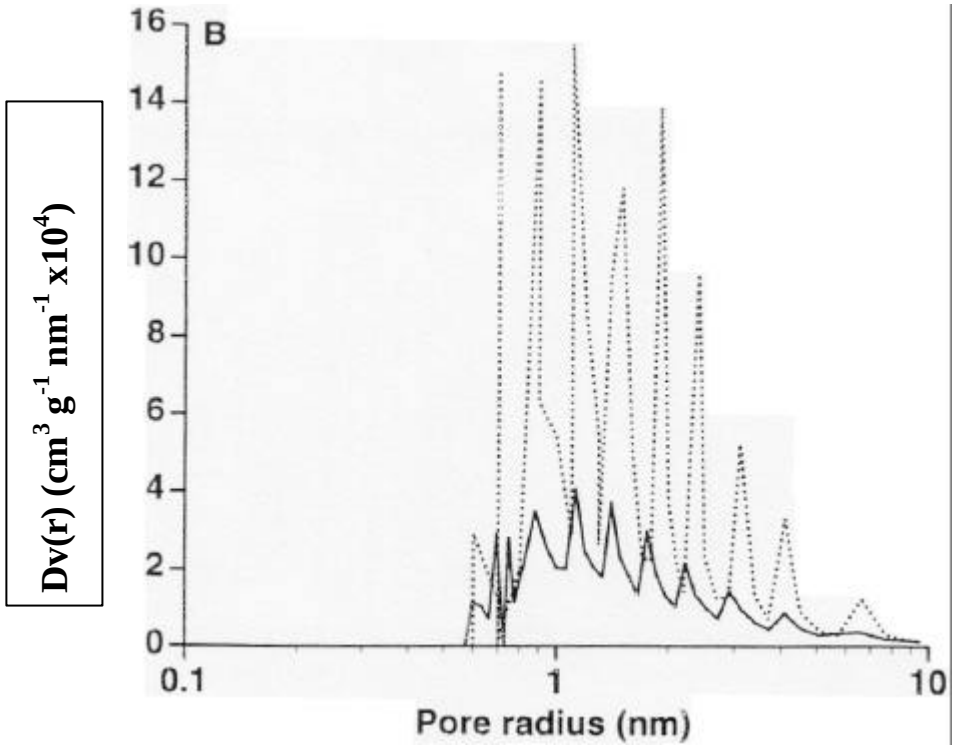

Figure 9. Pore size distribution in bran (dotted line) and in whole grain after extrusion at lOO°C and 30% moisture (solid line).

Alternatively, there may well have been processing factors which were not considered. The multivariate analysis invariably showed a highly significant degree of interaction between those process conditions that were measured regardless of the grain property monitored.

In both the extruded and micronised samples, the discrete elements of pore size distribution remained remarkably constant regardless of treatment and only the ill-defined 'peak' associated with starch was reduced. The extent of reduction reflected the RVA results and was greater for the extruded samples than for those which had been micronised.

Puffing, the most extreme of the treatments in terms of the destruction of the endosperm (see 10, Appendix XI), produced surprisingly little change in surface area or pore volume and distribution (Table 5). The small reduction in total surface area *(P<0.005)* and the volume of pores of < 10 nm radius *(P<0.005)* was, however, significant. Since most of the structure associated within the outer grain layers was absent in these polished wheat samples, starch structure would have dominated. Evidently, although granule morphology had disappeared, the spacing between the starch polymers (and within endosperm and aleurone walls) must have remained largely unchanged.

Table 5. Summary of gas absorption data on puffed and unpuffed wheat samples.

	Unpuffed wheat	Puffed wheat	*sem**
Total surface area ($m^2 g^{-1}$)	2.16	1.71	0.05
Total pore volume ($x 10^{-3} cm^3 g^{-1}$)	2.50	2.56	0.51
Pore volume < 10 nm radius ($x 10^{-3} cm^3 g^{-1}$)	1.55	1.24	0.03

*Standard error of the mean

Chemical treatments of cell walls such as the application of alkali lead to bond cleavage within the wall, to a loosening of wall structure and, as the wall becomes progressively more hydrated, to a visible increase in the dimensions of the cell wall. Such changes are readily detected by gas adsorption methods in terms of a significant increase in mean pore size and total pore volume. None of the processing methods studied produced changes of this nature and it can only be assumed that, despite microscopic evidence of delamination and the evident solubilisation of some wall polymer, the molecular architecture of the bulk of cell walls remained essentially unchanged.

Solubilisation of NSP

An estimate of the amount of NSP likely to be readily solubilised in the digestive tract was made *in vitro* by extracting grain samples into buffer at pH 4.0, 40°, treating the extract with

a thermostable amylase at 90° to hydrolyse the starch and then precipitating any intact (NSP) polymer by addition of ethanol. Mixed-linked glucan present in the precipitated material was measured by a specific enzymatic method and the arabinose/xylose content after hydrolysis determined by GC as their alditol acetates.

Origin of soluble NSP

Milling fractions of the barley and wheat samples prepared by the Campden and Chorleywood Food Research Association were used to determine the origin of soluble NSP in untreated grain. Six fractions were obtained from the Riband wheat and seven from the Manitou barley (Table 6). The milling machine used was configured for wheat and it dealt only imperfectly with barley. In order to achieve clean preparations of endosperm and aleurone, some mixed endosperm/aleurone had to be discarded. This is referred to as offal.

Table 6. Content and contribution to whole grain of the milling fractions from the wheat and barley samples.

Fraction	Content	Proportion of whole grain (%)	
		Barley	Wheat
Straight run flour	Endosperm	40.6	76.5
Finished flour 1	Aleurone-enriched	3.2	1.6
Finished flour 2	Aleurone-enriched	1.2	0.6
Finished flour 3	Aleurone-enriched	0.7	0.4
Unfinished bran	Bran/aleurone	2.9	15.3
Finished bran	Almost pure bran	20.9	8.2
Offal	Endosperm/bran/aleurone	30.5	none

Analysis of milling fractions for soluble and total NSP allowed the contribution of each part of the grain to the total amount of mixed-linked glucan and arabinoxylan that could be solubilised from the grain to be calculated (Tables 7 and 8). Mixed-linked glucan is characteristic of very juvenile cell walls and, in most tissues, is displaced by cellulose early in cell-wall development. It is only retained to any extent in those cell walls in which mechanical strength is not required such as the endosperm where very little cellulose synthesis occurs. In wheat, the endosperm contributed some 40% of the grain total and over half of the total soluble mixed-linked glucan while, in barley, the endosperm (defined as endosperm + offal) provided over 80% of the total and 85% of the soluble ß-glucan. The remaining source of ß-glucan was the endosperm in both cereals. Aleurone, although containing mixed-linked glucan, represents so little of the overall mass of the grain that its contribution is negligible.

Arabinoxylan is more extensively distributed throughout the grain where it can account for 30% or more of the cell wall (Table 8). Approximately 13% of the total amount of arabinoxylan in wheat was provided by the endosperm, but a disproportionate 65% of the soluble xylan came from this source. The barley endosperm similarly contributed a disproportionate amount of arabinoxylan to the total soluble value although the data are somewhat confused by the inclusion of offal as a milling fraction. Offal contains all fractions of the grain, although endosperm predominates.

Table 7. Percentage contribution of milling fractions to the total and soluble mixed-linked glucan content of barley and wheat grain

Fraction	Barley		Wheat	
	Total	Soluble	Total	Soluble
Endosperm	29.5	33.5	39.6	53.9
Finished flour 1	0.5	0.9	0.8	1.0
Finished flour 2	0.5	0.9	0.4	0.6
Finished flour 3	0.8	0.9	0.5	0.8
Unfinished bran	1.6	1.8	36.0	28.7
Finished bran	10.0	9.8	22.6	15.0
Offal	57.1	52.2		

Table 8. Percentage contribution of milling fractions to the total and soluble arabinoxylan content of barley and wheat grain

Fraction	Barley		Wheat	
	Total	Soluble	Total	Soluble
Endosperm	8.7	35.4	13.4	64.2
Finished flour 1	3.8	2.5	0.3	1.2
Finished flour 2	3.8	1.0	0.4	0.7
Finished flour 3	0.3	0.8	0.4	0.6
Unfinished bran	5.4	3.1	51.5	21.4
Finished bran	51.1	25.3	34.0	11.9
Offal	26.8	31.9		

Xylan associated with primary cell walls tend to be highly substituted with arabinose side chains compared to the xylan laid down during secondary thickening and lignification. Both cereals exemplified this with an arabinose:xylose ratio approaching unity in the endosperm and falling to half or less of this value in the lignified bran (Table 9)

The ratio of arabinose: xylose in soluble arabinoxylan from the endosperm reflected the structure of the total polymer, but there was evidence from barley at least that the xylan solubilised from the bran was more heavily substituted than the fraction from which it derived. Thus any shift in arabinose:xylose ratio in soluble xylan recovered after processing provided some indication of the botanical source of the additional material solubilised. A shift towards unity implied a greater solubilisation of endosperm, while a reduction in ratio suggested that the origin was predominately bran.

Table 9. Ratio of arabinose:xylose in total and soluble arabinoxylan from barley and wheat

Grain fraction		Barley	Wheat
Endosperm	total	1.05	0.77
	soluble	0.98	0.94
Finished bran	total	0.34	0.58
	soluble	1.43	0.57
Whole grain	total	0.52	
	soluble	0.80	0.72

Effect of treatment on the release of NSP

The amount of NSP released from both cereals was primarily dependent on the temperature of treatment. Where there was an effect of moisture content, the trend was to reduced NSP solubility for reasons which are not immediately apparent. Flaking of micronised samples promoted additional solubilisation at temperatures up to and including 100° but had little effect at higher temperatures. The mean values for the amount of arabinoxylan and mixed-linked glucan released at each temperature and for each treatment are summarised in Table 10 and given in full in Appendix XIII.

Table 10. Summary of the effect of processing temperature on the release of mixed-Iinked glucan and arabinoxylan (g kg-l) from barley and wheat and on the ratio of arabinose:xylose.

Temperature	Barley			Wheat		
	Glucan	A + X	A/X	Glucan	A + X	A/X
Untreated grain	0.1	0.3	0.80	0.0	0.8	0.72
Pelleted						
Water conditioned	5.8	2.9	0.88	0.4	1.9	1.00
Steam conditoned	7.8	4.0	0.93	0.5	5.2	1.07
Expander conditioned	11.8	5.6	0.89	0.8	4.5	0.91
Micronised						
75 ^{O} C	6.1	3.6	0.72	0.4	4.9	0.76
100^{O} C	5.7	3.4	0.70	2.9	5.9	0.69
130^{O} C	10.4	6.5	0.97	17.8	11.6	0.79
Extruded						
60 ^{O} C	9.7	3.4	0.81	0.6	3.1	0.78
80 ^{O} C	13.5	3.8	0.79	0.9	3.2	0.83
100^{O} C	15.5	4.0	0.80	1.5	4.8	0.95
120^{O} C	17.7	4.1	0.87	1.6	4.8	0.96
Puffed						
Unpuffed				0.5	4.0	1.17
Puffed				5.6	19.7	0.73

A + X, sum of arabinose and xylose which closely approximates to total arabinoxylan

A/X, ratio of arabinose to xylose which provides a measure of degree of branching.

Extrusion. Release of mixed-linked glucan was significantly promoted by increased temperature in both wheat *(P<0.001)* and barley *(P<0.05)* but was negatively affected by increasing moisture content *(P<* 0.05; Table 11). While there was no evidence of any interaction between moisture and temperature, moisture effects were most evident at two lowest temperatures examined. Extrusion released mixed-linked glucan from barley in amounts ranging from 9.7 g kg-1 at 60° to 17.7 g kg^{-1} at 120°. Some depression of feed value would be expected in the presence of soluble ß-glucans at these concentrations. While pelleting also promoted release of mixed-linked glucan, amounts from steam-conditioned barley were less than that released by extrusion at the lowest temperature examined. Temperature also significantly increased the amount of arabinoxylan released from wheat *(P<* 0.05), causing a 5-10-fold increase in release compared to the untreated grain. However the amounts released, which were similar in barley, were comparable to the amount solubilised by pelleting and, in wheat at least, any antinutritional effects would be no greater than those associated with pelleted feed. No significant effects of moisture were detected, although increasing moisture did appear to reduce solubilisation of wheat arabinoxylan at 60 and 80°C. In wheat, but not barley, increased temperature or moisture caused a significant change in the arabinose:xylose ratio although the direction of the response differed. Increasing the temperature significantly increased the A/X ratio *(P<* 0.001), implying more solubilisation of arabinoxylan from the endosperm while increasing the moisture content reduced the ratio *(P<0.05)* implying a greater contribution of arabinoxylan from the bran fraction.

Table 11. Effect of temperature and moisture content on the solubilisation of mixed-linked glucan (g kg^{-1}) from extruded wheat and barley grain

Cereal	Moisture (%)	Temperature (^{O}C)			
		60	80	100	120
Wheat	18	1.29	1.94	1.72	1.87
	22	0.69	0.80	1.31	1.24
	26	0.29	0.32	1.44	1.04
	30	0.14	0.35	1.37	1.41
				*sem**	*0.31*
Barley	18	19.63	15.87	18.14	18.83
	22	7.35	16.06	12.66	20.48
	26	8.27	10.93	15.85	12.85
	30	3.37	10.95	15.40	18.49
				*sem**	*2.86*

*sem** Standard error of the mean

Material prepared in bulk for feeding trials under the same notional conditions of extrusion failed to match exactly the experimental material produced in smaller batch sizes. However, the extruded barley samples prepared for Experiments 2 and 3 had soluble glucan contents of 13.8 (22% moisture, 100°), 15.5 (Experiment 2, 30% moisture, 120°) and 16.7 (Experiment

3, 30% moisture, 120°), which was of similar magnitude to that expected. Extrusion of barley severely depressed organic matter, starch and nitrogen digestibility in broiler chicks (Appendices II and III), with the greatest effect being shown by the treatment at the higher temperature. As would be expected from the lesser amounts of soluble NSP released, any negative consequences of extrusion of wheat were far less evident and were non-significant in chicks and absent in pigs.

Micronisation. There was a highly significant relationship between temperature and solubilisation of mixed-linked glucan in both cereals ($P < 0.001$), although at temperatures of 100° and below, micronising resulted in substantially less solubilisation of mixed-linked glucan from either cereal than extrusion at corresponding temperatures. At these temperatures micronising would be unlikely to introduce any antinutritional effects not seen in pelleted feed. No significant effects of moisture content were seen in either cereal but flaking significantly promoted solubilisation of mixed-linked glucan in barley ($P < 0.001$) but not wheat. There also was evidence of a strong interaction ($P < 0.005$) between flaking and temperature in barley with effects of flaking most apparent at 75 and l00°C and much reduced at 140°C.

Release of arabinoxylan was not significantly promoted by either varying the moisture content or by flaking. The cereals, however, showed a significant response to increased process temperature ($P < 0.001$) both in the amount of arabinoxylan solubilised and in a changed A/X ratio. Increasing the temperature to 130° doubled the amount of water-soluble ß-glucan and arabinoxylan released. The pronounced shift in arabinose:xylose ratio indicated that the source of the additional soluble arabinoxylan, and presumably the ß-glucan, was the endosperm.

The likely consequences for nutritional performance of micronising at temperatures around 130° were the subject of Experiment 4 (Appendix IV). Again problems were encountered scaling up treatments to provide sufficient material for trials. The bulk low temperature (75°) treatments gave products with soluble NSP contents equal to or greater than that of the experimental samples, but high temperature treatment of wheat (130°) failed to release the predicted amounts of mixed-linked glucan and arabinoxylan (Table 12). High temperature treatment (140°) of barley was more effective, although the greatest solubilisation of glucan curiously appeared possible only in the presence of enzyme (16.5 g kg^{-1} released after high temperature micronisation in the presence of supplementary enzyme).

Table 12. Soluble mixed-Iinked glucan and arabinoxylan content (g kg^{-1}) wheat and barley micronised in bulk under low and high temperature conditions. Pilot values refer to the original small-scale processing results.

Soluble NSP	Barley		Wheat	
	Low	High	Low	High
Mixed-linked glucan	10.3	8.7	1.1	1.0
(pilot value)	5.8	10.8	0.2	16.4
Arabinoxylan	4.7	7.4	5.3	5.1
(pilot value)	3.7	6.5	3.8	12.0

Micronisation failed to produce any improvement in nutrient digestibility by pigs compared to the hammer-milled control. With barley in particular there were serious problems of low intake associated with feed refusal. This is described in more detail in Appendix IV. Low temperature micronisation tended to increase intake of the barley diet by chicks, but high temperature processing had the opposite effect. With wheat, micronisation resulted in lower intakes and severely reduced weight gain over the experimental period.

Puffing. Since only polished wheat was puffed, results are not directly comparable with the other treatments. Puffing increased the soluble mixed-linked glucan content 10-fold and soluble arabinoxylan from 4 g kg^{-1} in the unpuffed sample to nearly 20 g kg^{-1} after puffing ($P < 0.001$). The nutritional consequences of this large amount of soluble arabinoxylan was not tested but would be expected to depress performance. The puffed sample had a significantly lower arabinose:xylose ratio than the unpuffed wheat ($P < 0.001$) which, in whole wheat, might be explained by a preferential solubilisation of arabinoxylan from the testa and pericarp. Since this is not possible in polished grain, it is possible that the severity of the treatment resulted in the loss of some of the more labile arabinofuranosyl units. This may also be a consequence of some of the other more extreme treatments examined.

Pelleted samples. There were no statistically significant differences between amounts of mixed-Iinked glucan, or arabinoxylan released from the variously conditioned pelleted samples.

NSP viscosity

A consensus view of the antinutritional effects of soluble NSP is that the gel-forming (or viscosity-enhancing) properties of mixed-linked glucan and arabinoxylan contribute to the increased viscosity of the gut contents. This is believed to be particularly acute at the wall of the upper gut, where any increase in viscosity promotes the size and stability of an unstirred layer. This layer slows the diffusion of nutrients from the gut lumen towards the wall and point of absorption and may also hinder the mixing of digestive enzymes with the digesta.

The net result is that a portion of all major nutrients are carried beyond their normal area of absorption and into the hindgut.

The viscosity and structural behaviour (gel-forming characteristics) of extracts from a variety of treatments were measured in conjunction with the Biotechnology and Food Group of VTT , Finland (Table 13). Samples, wherever possible, were prepared as a 14% w/v solution, since concentrations >10% are generally considered as 'high concentration' solutions in which differences in behaviour are easier to resolve. The selected samples examined are ranked in order of viscosity in Table 13 starting with the lowest value recorded.

Although initial work suggested a simple relationship between the ß-glucan content of extracts and solution viscosity, this was found not to hold when a greater variety of processed samples was examined. This is hardly surprising since it is known that the rheological properties of a molecule are a product of molecular weight and structure in addition to concentration. This is particularly evident when the two pelleted barley samples prepared at Wageningen are considered. Despite a low solution concentration and low NSP content, both samples recorded the highest viscosity value and showed clear gel-forming properties. The implication of this finding (not confirmed) is that the NSP in both samples was of higher molecular weight than in other samples. One reason for this may be the effect of grain endogenous enzymes during processing. If the natural role of these enzymes is to initiate the breakdown of endosperm cell wall polysaccharides during germination, then it would follow that any inhibition of their activities would act to preserve polymer length. The steam-based pretreatments used in the Wageningen pelleting process may have provided sufficient conditions for the rapid denaturation of grain enzymes and the preservation of viscosity-enhancing properties. Thus it may be possible to encourage endogenous activity with lower temperature conditioning of grain before pelleting and so reduce the need for subsequent enzyme treatment.

Because of the number of factors involved in determining rheological behaviour, simple viscosity measurements made *in vitro* of extracts of treated grain hold no predictive value. Better results have been obtained with measurements made directly on digesta samples. This, however, is not a routine option and was used here only as part of the final chick growth trial (Appendix VII). This showed clearly that expansion resulted in a significantly higher digesta viscosity (13.4 cP) than pelleting or micronising (7.6-7.9 cP) and was associated with the generally poorer daily gain of the birds on this diet.

Table 13. The viscosity of wheat (W) and barley (B) soluble extracts.

Sample	Solution (w/v)	Viscosity (Pascal sec)	Components			Gel properties
			Mixed-linked glucan (%)	Starch (%)	Arabinose + xylose (%)	
Barley						
*B26/60**†	0.05	3.39×10^{-3}	0.73	2.64	0.27	
B22/60†	0.03	3.40×10^{-3}	0.57	1.43	0.17	
B18/60†	0.05	4.87×10^{-3}	0.73	2.82	0.19	
B22/100†	0.04	6.00×10^{-3}	0.51	2.36	0.14	
BE20/100 (flaked)§	0.02	8.00×10^{-3}	0.37	1.58	0.16	
BF20/140 (flaked)§	0.14	3.62×10^{-2}	0.45	11.37	0.27	
BC15/140§	0.14	4.00×10^{-2}	0.44	13.11	0.35	
B30/100†	0.13	7.00×10^{-2}	1.94	10.96	0.48	
B26/100†	0.11	8.85×10^{-2}	1.49	6.56	0.37	
B18/100†	0.14	0.336	1.62	10.03	0.46	?
B18/120†	0.14	0.490	1.77	8.91	0.33	
B18/80†	0.07	1.17	1.19	3.73	0.26	y
B (expanded material - Wageningen)	0.03	2.44	0.82	2.77	0.48	y
B (expander conditioned - Wageningen)	0.04	29.7	1.03	4.03	0.49	y
Wheat						
W (water conditioned - Wageningen)	0.06	4.80×10^{-3}	0.09	4.73	0.45	
W15/140§	0.14	5.00×10^{-3}	0.81	11.64	0.57	
W18/80†	0.14	7.63×10^{-3}	0.15	7.74	0.38	
W20/140 (flaked)§	0.13	1.16×10^{-2}	0.82	11.99	0.52	

* % moisture/temperature °C; † extruded; § micronised

Protein utilisation

The starting point for any improvement in the digestibility of cereal protein was the value found in untreated (milled) grain. The ileal digestibility of the nitrogen of barley in pigs was 0.62 and in wheat 0.67. The corresponding values for chicks were 0.03 higher. It must be borne in mind that these are apparent digestibility values and, as the grain was fed alone, at such low dietary protein concentrations, the influence of the endogenous secretions would be considerable. The true digestibility values would obviously have been much higher. These values should therefore be taken as relative and do not indicate a potential to recover a further 30-38% of the protein. The truly undigested portion of the grain protein is more likely to be of the order of 15-20%.

As discussed above, the digestibility of starch was never more than a few percentage units from 100%. It is therefore likely that the protein contained in the endosperm was also well digested. Endosperm protein tends to have rather high concentrations of glutamate and glutamine, and low concentrations of lysine and other essential amino acids. The aleurone protein is of better quality but, because of the thicker cell walls, is less accessible. A major aim of processing was to disrupt the aleurone cell walls sufficiently to increase the ileal digestibility of the aleurone protein. It was not possible to examine the effects of processing on the utilisation of aleurone protein alone but an approach to this question was made in Experiment 6. The aim was to study the effects of extrusion under various conditions on the utilisation of wheat middlings on the basis that the predominant protein fraction was that of the aleurone layer. Unfortunately, little usable data were obtained *in vivo* and the analysis of protein digestibility *in vitro* showed that processing parameters accounted for only 25% of the variance in protein solubilisation. Clearly, these treatments had only limited effects on the disruption of aleurone cell walls.

The extent to which protein digestibility was improved by processing can be assessed by comparing the ileal nitrogen digestibility of milled grain with the most effective treatment or combination of treatments in each experiment. This is done in Tables 14 and 15. In most cases, within a species, the same treatment was the most effective for both cereals but there were exceptions. On average, across the experiments in which cereals, fed alone, were processed by methods that improved digestibility and for which complete data were obtained, an improvement of 7 percentage units was achieved for both wheat and barley, in both species. These results give an indication that the original proposal of a 5% enhancement in nutrient recovery could be achievable in practice.

Table 14 Improvements in the ileal nitrogen digestibility of cereals by chicks

Experiment	Milled	'Best' process
Wheat		
1	0.64	0.70 (pelleting)
2	0.72	0.78 (low temperature extrusion)
3	0.67	0.74 (Milled plus enzyme)
4	0.74	0.79 (high temperature extrusion)
5	0.78	0.85 (steam pelleted)
Mean	0.71	0.77
Barley		
1	0.59	0.65 (extrusion)
2	0.73	No improvement
3	0.62	0.74 (extruded plus enzyme)
4	0.74	No improvement
5	0.76	No improvement
Mean*	0.61	0.70

* For those processes which gave an improvement

Table 15. Improvements in the ileal nitrogen digestibility of cereals by pigs.

Experiment	Milled	'Best' process
Wheat		
1	0.63	0.69 (pelleting)
2	0.65	0.79 (low temperature extrusion)
3	0.69	0.71 (Extruded plus enzyme)
4	0.71	No improvement
5	0.70	0.76 (expanded/pelleted)
Mean	0.67	0.74
Barley		
1	0.64	0.67 (pelleting)
2	0.56	0.64 (low temperature extrusion)
3	0.58	0.72 (Extruded plus enzyme)
4	No data	
5	0.65	0.69 (steam pelleted)
Mean*	0.61	0.68

- Excluding Experiment 4

One of the limits to improvement in protein digestibility must be the very poor accessibility of the pericarp protein which comprises, in barley, some 20% of the total grain protein and

8% in wheat. As discussed above, none of the treatments sufficiently disrupted cell wall structures to improve the accessibility of the protein of this fraction.

Heat damage to proteins

One of the dangers of thermal processing, especially with the combination of sugars and moisture present, is heat damage to proteins. Although a number of amino acids can be damaged, lysine is the most susceptible and of the most nutritional significance. Damage is caused by the formation of Maillard compounds by the reaction of the 6-amino group of lysine with free sugars. Although early Maillard damage may be reversed in digestion late Maillard products, though they may be absorbed, are irreversibly bound and are nutritionally unavailable. To assess the extent of such damage the available lysine in a number of samples was assessed. The methods are given in Appendix XIV and the results in Table 16.

Table 16. Availability of lysine (%) in heat-treated cereals, compared with milled samples. In each case, the thermal process is specified

Experiment	Process	Barley	Wheat
1	High temperature extrusion	83	93
4	High temperature micronisation	54	61
5	Steam conditioning + pelleting	90	86

These results echo both the *in vitro* and *in vivo* conclusions as to the relative severity of the treatments and also explain why chick growth was affected more than could be explained by reduction in digestibility alone. In these cereal-only diets the amount of available lysine would be the critically limiting nutrient for growth.

Use of supplementary enzymes

Enzymes were incorporated into only two of the feeding experiments (3 and 4) and as a treatment in the final large-scale trial made with broiler chicks. In the first experiment, extrusion of hammer-milled wheat and barley was compared to hammer-milling alone (Appendix III). Extrusion of barley, but not wheat, significantly depressed nitrogen digestibility at the ileum in pigs compared to the hammer-milled control. This depression was still evident when digestibility overall was calculated. Addition of enzyme reversed this position and significantly improved the ileal digestibility of nitrogen in both milled ($P<0.01$) and extruded ($P<0.001$) diets. Organic matter digestibility of barley was also significantly improved by enzyme ($P<0.001$), but little effect was seen with wheat. When fed to birds, addition of enzyme significantly increased ileal nitrogen digestibility ($P<0.05$ in both wheat and barley, but, overall, the effect was confined to milled wheat and to extruded barley. The general conclusion was that extrusion alone did not improve the digestibility of nitrogen in wheat and tended to reduce that of barley. The latter effect, predominantly a product of soluble ß-glucan release, was correctable by enzyme addition. However, it should be noted

that the combination of extrusion and enzyme improved nitrogen digestibility in both cereals by about 7% over the milled cereal with no loss of starch digestibility. Intake was greatest with extruded barley plus enzyme and this was reflected in the weight gain of the chicks over the first two weeks.

The second feeding experiment, also made with pigs and poultry, compared milled cereal with cereal subjected to high- and low-temperature micronisation followed by flaking (Appendix IV). Although pigs ate all of the wheat diets without problems, they were reluctant to consume micronised barley. Intake was poor and cannula blockage was frequent on the two barley diets. The digestibility of the micronised wheat diets was less than that of the milled control and was not significantly improved by enzyme addition.

Chicks were also reluctant to eat the micronised diets and sticky droppings were evident with micronised barley in the absence of enzyme. Micronisation depressed feed intake and weight gain of broiler chicks fed the wheat-based micronised diets, the effect being greatest with the higher temperature process. Enzyme addition had no effect on wheat micronised at low temperature and greatly exacerbated problems of poor intake and weight gain when applied to the high-temperature processed wheat. It is possible that some toxic agent was formed on micronisation at high temperature and that its release from the cereal was aided by the enzyme treatment. Only with barley subjected to low-temperature micronisation was any benefit seen in terms of a production response. Low-temperature micronisation increased intake compared to the milled barley and this was further improved by enzyme addition. The growth response that ensued appeared wholly driven by this increase in intake.

In the final experiment with broiler chicks made partly at Barhill Farm and partly at the Rowett, diets were prepared from a singe cereal source using a 2:1 mixture of wheat to barley. The four treatments used were pelleting under 'industry standard conditions', pelleting after expander conditioning, low temperature micronisation and extrusion at 100°C. Enzyme addition was also included as a variable. The Barhill trial confirmed the observation made in the Experiment 3 that performance on extruded diets is significantly poorer than on pelleted diets and that enzyme addition corrects this depression. The added measurement of gut viscosity lends support to the view that any depression in performance as a result of extrusion is a product of additional soluble NSP release. Digesta viscosity was greatest in birds on the extruded diet and enzyme addition significantly reduced this viscosity ($P < 0.001$).

Digestibility studies made with the same materials showed that ileal starch digestibility remained high with all treatments and values ranged only between 98 and 100%. Ileal nitrogen digestibility varied significantly according to process ($P < 0.001$) and enzyme

addition ($P < 0.05$). In the absence of enzyme, expansion and micronisation gave the highest ileal nitrogen digestibility values but, in the presence of enzyme, the highest value was recorded for simple pelleting. Overall, enzyme supplementation had a dramatic effect on nitrogen digestibility ($P < 0.001$), substantially improving values for all treatments, and significantly improved overall organic matter digestibility. The best results in each case tended to be simple pelleting + enzyme. Performance data (14-28 days), however, suggested that expander-conditioning before pelleting was slightly more effective than simple pelleting, although both pelleting processes gave significantly better performance figures in the presence of enzyme than either micronisation or extrusion.

DISCUSSION

The main aim of this project was to explore the links between the processing of cereals and their utilization by animals. From the start it was considered important to adopt a two-stage approach. The first stage was to examine the effects of processing in terms of the alterations it produced in the physical structure and chemical composition and, the second stage to measure the ways in which animals responded to those changes, both in terms of the digestibility of macronutrients and to the extent to which they were reflected in animal performance. This approach to the elucidation of the mechanisms by which processing alters animal performance necessarily involved very different strategies and measurements. In particular, the ability to examine a relatively large number of samples *in vitro* allowed a much larger number of treatments to be examined in this way than could be submitted to animal experimentation. In this two-stage strategy, therefore, small samples of processed materials were examined *in vitro*. Based on these findings further larger quantities were prepared for nutritional testing. In the event, it proved more difficult than expected to reproduce the processing conditions. In this regard it was unfortunate that, despite strenuous efforts at the beginning of the project, no processing machinery manufacturer was involved. Had this been so, it might have been possible to deal better with this difficulty. Despite working with pilot scale plant under carefully controlled conditions material ostensibly processed in the same way was sometimes clearly very different. This must be borne in mind when relating one set of measurements to the other.

The changes in the chemical and physical characteristics of cereal grains brought about by processing

Starch. Because starch is by far the most important source of metabolisable energy in cereal grains, changes in the structure of starch and the starch granule, its accessibility and its digestion were routinely monitored. The Rapid Visco Analyser was found to be a valuable method for assessing the extent to which processing led to gelatinisation and to changes to starch polymer structure which influenced its subsequent gel-forming capacity. Thus extrusion, which led to the rapid disruption of granules and to gelatinisation without affecting the capacity of the released polymers to gel, produced quite different effects to micronisation which had far less obvious effect on granule structure but marked effects on the gel-forming capacity of the starch polymers. However, while processes which led to gelatinisation also led to starch being more immediately accessible to amylolytic attack, as shown by short-term enzyme incubation, it is questionable whether this had any great implications for the host. The advantage of pre-gelatinisation was soon lost (~ 2 h) and its was almost impossible to demonstrate any increase in the extent of degradation *in vivo* given the almost total availability of starch in minimally (hammer-milled) processed grain.

Protein. Determination of protein digestibility *in vitro* has the potential to reveal the extent to which the disruption of cell walls by processing allows access of digestive enzymes into

cells and egress of peptides out of them. The disruption of cell walls was considered particularly important in increasing access to the proteins of the aleurone layer and these measurements were therefore made with the extruded middlings (Experiment 6). Although there was some association between the processing parameters and the *in vitro* protein digestibility it was not strong, accounting for only a quarter of the observed variance. This was disappointing, and somewhat surprising, but suggests that there are factors at work in the extrusion process which are not fully described by the measured machine parameters. In fact, it was observed in other instances, with extrusion in Experiment 2 and micronisation in Experiment 4, that simply repeating a process ostensibly under the same operating conditions, did not necessarily result in a product with the same physical and chemical characteristics.

Cell wall structure. The gas adsorption method selected to provide a quantitative measure of the surface area/pore structure of grain proved capable of distinguishing pore sizes at the 0.5-5 nm radius required. This is the first reported use of this method applied to plant material. However, because of the relatively low surface area found associated with grains and the consequent need for large sample sizes, the method was working close to its limits. The loss of structure associated with starch granule dissolution could be readily demonstrated, and significant shifts in pore volume and surface occurring during processing were detected. Unfortunately, no obvious trends were evident. None of the changes in pore regime, pore volume or surface area were incrementally affected by either increasing temperature or moisture content. This was despite the very clear effect of increasing temperature on the solubilisation of cell wall constituents which might have been expected to alter polymer spacing in the wall and thus its pore regime and volume. It is possible that any change to the porosity of endosperm walls from which the greatest amount of NSP was solubilised, was too small to be detected against the far greater contribution made by the outer grain layers or that solubilisation tended to lead to the total dissolution of the endosperm. As a result solubilisation of NSP provided a better measure of the effect of thermal processing than any changes to the physical attributes of the wall. In addition, the ratio arabinose: xylose tended to reflect the botanical origin of the NSP. Xylan solubilised from the bran tended to have a lower degree of branching than that from the endosperm and so a lower water-holding capacity but greater potential for gel formation. This may have some physiological significance as well as reflecting the severity of processing.

The amount of NSP solubilised provided a poor indication of likely effects on viscosity and factors other than concentration are evidently important. Molecular weight (polymer length) is known to contribute to gel-forming capacity and there was some indication that the nature of processing could influence the capacity of released NSP to contribute to intestinal viscosity through this route. Viscosity was greatest in extracts of grains exposed immediately

to high processing temperatures. It seems likely that rapid heating inactivated endogenous enzymes able to partially reduce the molecular weight of NSP in grain subject to lower temperatures or a more gradual increase in temperature. It is also possible that differences in exogenous NSP - degrading activity is the distinction between a 'problem' wheat and those in which depression of ME is uncommon and may also underlie the age effect in which the need for exogenous enzymes in 'problem' wheat decreases with storage. However, much of this is supposition and further experimental evidence is required. If proved correct then adjustment of processing conditions could reduce a need for exogenous enzymes.

The nutritional consequences of changes in cereal grains due to processing

Digestibility measurements. The measurements of digestibility with pigs are for the most part considered reliable and accurate, as witnessed by the small standard errors. The exception was in Experiment 6, with wheat middlings, which is discussed later. With chicks, the measurements of ileal digestibility had standard errors in the same range as those with pigs and are not considered to be subject to bias. The overall digestibility measurements with chicks, however, involved additional sources of error. The collection of digesta from the trays under the cages required their separation from spilled feed and from epidermal debris. Although this was done meticulously it is difficult to be sure all contamination is removed. Also, for the estimation of nitrogen digestibility uric acid had to be separated from the digesta and although this was done by an established method it inevitably increased the possibility of error. This is reflected in the fact that, in some instances, digestibility was higher at the ileal level than overall, which, although possible for nitrogen, is most unlikely to be true for organic matter. For this reason, whereas direct comparisons between ileal and overall digestibility can be made for pigs, and deductions made about the extent of fermentation, these calculations cannot be made for chicks and the overall digestibility values should be regarded as comparable only amongst themselves, for the comparison of treatment effects.

Starch digestibility. In both pigs and chicks ileal starch digestibility was affected by many of the treatments. However, although such changes were often significant their magnitude tended to be small and the statistical significance was as much a result of the small standard errors as the extent of the effect. Because of the predominant contribution of starch to the digestible and net energy in cereal grains even small differences in starch digestion could be important in affecting animal performance.

In pigs overall digestibility of starch was always virtually complete and little affected by processing treatments. The same was not true for chicks, in which larger and more significant changes in starch digestibility were seen as a result of processing. It is interesting that some treatments which appeared, by their severity, to depress the digestibility of

nitrogen and organic matter, compared with the milled grain, did not have this effect on starch digestibility.

Incomplete digestion of starch in the upper digestive tract of pigs coupled with its virtually complete digestion overall indicates the extent of starch fermentation in the caecum and colon. It is generally considered that the energy made available by the fermentation of starch (and other carbohydrates) fermented in the large intestine amounts to approximately half the energy of the sugars fermented, the remainder being accounted for by the heat of fermentation, the energy in microbial biomass and the increased energy consumption of the gastrointestinal tract. Any process which reduces ileal digestion of starch, even if it is eventually fully digested, therefore represents a loss of net energy.

Protein. Although the protein content of cereals is relatively low, the large amounts of cereals included in pig and poultry rations mean that they commonly supply half or more of the total dietary protein and any improvement in the digestibility of cereal protein has an important effect on the utilization of the whole diet. Most importance was attached to ileal digestibility, which provides an estimate of the proportion of the dietary protein absorbed as amino acids in the upper gastrointestinal tract.

Ileal nitrogen digestibility was improved by one or more treatments in all experiments, compared with simple milling. The treatment which resulted in the highest ileal nitrogen digestibility was usually different for wheat and barley. In particular, treatments with a large input of thermal energy were more likely to produce deleterious effects with barley than with wheat. These effects were usually partly reversed by the addition of beta glucanases.

Notwithstanding the importance of ileal digestibility the overall nitrogen economy of the animal is determined by the total amount of nitrogen absorbed, so all the digestibility experiments included also measurements of nitrogen digestibility overall.

The very different reaction of wheat and barley to processing, especially the deleterious effects of high temperature treatments on barley, raises the question of the extent to which separate processing of wheat and barley might be desirable in commercial feed milling.

The examination of the effects of extrusion on middlings was not originally intended to include *in vivo* digestibility studies, at least not in its first phase, but the availability of more feed than expected, and of cannulated pigs, made it seem useful to carry out a preliminary experiment with a subset of the samples. The results were uninterpretable but the reasons for this are not at all clear. One difference from the normal procedure is that the experimental periods were of only four days' duration instead of the usual seven. Although a shorter

period of adaptation could have made the overall digestibility values less reliable than usual it seems unlikely that this would be true of the ileal values because the transit time from mouth to ileum is measured in hours rather than days. Besides, the similarity of one diet to the next also seems to make it unlikely that incomplete adaptation could explain the extreme variability of the data. It seems more likely that the explanation lies in the unusual consistency and volume of the ileal digesta. Although usually regarded as beneficial to good sample, a large volume of digesta could result in greater separation of marker from feed residues. To obtain reliable data with a feed such a middlings fed alone, it might be necessary to mordant the metal oxide onto the middlings rather than to another marker matrix, as here.