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VARIABILITY IN THE CHEMICAL COMPOSITION OF WHEAT AND ITS UTILISATION BY YOUNG POULTRY

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VARIABILITY IN THE CHEMICAL COMPOSITION OF WHEAT AND ITS UTILISATION BY YOUNG POULTRY

by

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CHAPTER 1 INTRODUCTION

The use of wheat as a livestock feed throughout history has been less important than its role in human food and considerable information, which is employed in quality control and is also important in breeding programmes for the screening of new cultivars of potential value, has accumulated on the biochemical characteristics of wheat varieties destined for this purpose. In contrast, however, scant attention has been paid to the variability in the nutritive value of wheat for inclusion into the diets of farm livestock, particularly poultry and pigs.

The nutritional value of wheat for livestock has, in the past, been considered fairly consistent and, as a result, feed compounders have used a single value for wheat metabolisable energy (ME) in diet formulations. However recent studies have demonstrated that considerable variability in ME does exist both within and between different wheat varieties, which is becoming of greater significance as dietary inclusion levels are increasing. This is particularly important in the current UK economic climate where a fairly small change in the ME value (as little as 0.5 $MJ.kg^{-1}$ DM) can make a difference between profit and loss for poultry producers (Holmes, 1992). Not surprisingly, there has been increasing concern expressed by both the feed and broiler industries alike over the variability in quality of this important raw material. In the UK wheat, particularly soft varieties, is used extensively as an energy source in diets for poultry with levels in excess of $500g.kg^{-1}$. The broiler industry therefore represents an important market for wheat.

Variability in the nutritive value of wheat has resulted in fluctuations in the productive performances of broilers often at considerable economic cost. Of particular concern has been the somewhat inconsistent occurrence of wheats with low ME values (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Wiseman and Inborr, 1990; Choct and Annison, 1990; Annison, 1990, 1991). The variability

in ME values that occurs has not been confined to any specific variety, and considerable variation can exist within the same variety. The view is held, particularly in Australia (Annison, 1990, 1991) and within the compound animal feed trade in the UK (McNab, 1993), that variability in the nutritive value of wheat as determined chemically, may be fairly inconsistent. Accordingly, with rising demands for a greater efficiency of production, low-ME wheats have three major economic implications:

a) Increased feed cost due to poor utilisation and failure to reach target performance levels.

b) Elevated litter problems due to the incidence of larger volumes of wetter excreta and the practical problems associated with pollution and proper disposal. This also has serious implications for carcass quality.

c) Inaccuracy in diet formulation.

The causes of such intermittent variations in the quality of wheat have not been identified, and much of the evidence available is empirical or even anecdotal.

A number of factors have been proposed as being responsible for the inferior quality of some wheats including chemical composition and anti-nutritional factors within the grain such as proteinaceous α-amylase inhibitors and soluble non-starch polysaccharides affecting viscosity of the digesta (Choct and Annison, 1990; Annison 1990, 1991). Bird factors, such as age and intestinal microflora, have also been implicated to various extents in this problem (Untawale and McGinnis, 1979; Campbell *et al.*,1983; Fuller, 1984; Wiseman and Inborr, 1990).

The erratic occurrence of these low-ME wheats has precluded structured in-depth studies to identify the underlying causes. Indeed, correlations between different measured parameters and ME values have been fraught with uncertainty due to the inconsistency of the variability. Much of the variation in ME may be due to ranges in starch digestibility (Mollah *et al.*, 1983). Starch is the principal energy-yielding component of wheat and over half the energy in broiler diets will be derived from this source. It has been assumed that cereal starches are highly and uniformly digestible. Considerable variation between varieties and within the same variety can, however, occur and cereal starch utilisation by non-ruminants is receiving increasing attention.

Considerable emphasis has been placed on the effects of anti-nutritional factors (ANF), such as non-starch polysaccharides (NSP) on ME values and starch digestibility in poultry (Mollah *et al.*, 1983; Rogel *et al.*, 1987). The pentosans, (arabinoxylans), are the major components of NSP which are located primarily in the endosperm of cereals. Cell walls of wheat endosperm, on a weight basis, contain 140-150g protein and 750g polysaccharide.kg^{-1}, of which 0.85 is arabinoxylan, while the remainder comprises equal amounts of β-glucan and β-glucomannan (Mares and Stone, 1973). These components are believed to reduce digestibility and hence nutrient availability in the chick, through increased viscosity of the intestinal contents (Annison and Choct, 1991; Choct and Annison, 1992a). The water soluble pentosans form highly viscous aqueous solutions and can absorb up to 10 times their own weight of water (Voragen *et al.*, 1992; Izdorczyk and Biliaderis, 1992). Although a tenuous inverse relationship has been found between the digestibility of nutrients to chicks and the content of pentosans in the diet (Annison and Choct, 1991; Choct and Annison, 1992a) no direct evidence has been obtained in support of this.

Pentosan digestion in the chicken is limited by the lack of suitable endogenous enzymes, although microbial degradation may contribute (Wiseman and Inborr,

1990). The arabinoxylans, through their viscous properties, limit the accessibility of digestive enzymes and decrease transit time (Mulder *et al.*,1991; Bedford and Classen, 1992).

The availability of a rapid assay would greatly facilitate the screening of wheat cultivars currently being used in poultry diets to the benefit of feed compounders and plant breeders.

OBJECTIVES OF THE CURRENT RESEARCH PROGRAMME

The programme was designed to examine a number of key issues associated with the nutritional value of wheat, to include an extensive review of the literature so that data generated could be placed alongside previous reports:

1. Establish the frequency of occurrence of low nutritional value wheats in the UK (as influenced by variety, location of growth and storage post harvest) through extensive poultry metabolism trials.

2. Identify those factors that might be responsible for poor quality wheats. In particular, the following were addressed:

    a). Variations in digestibility of starch (and other major components of wheat, including pentosans) to include patterns of starch digestibility within the gastro-intestinal tract as influenced by, for example, granule morphology.

    b). Extensive biochemical characterisation of the NSP complex

    c). Assessment of the role of viscosity in reducing digestibility.

3. Develop a rapid *in vitro* biochemical assay which could be used to screen wheats prior to their incorporation into poultry diets and which would also be of value to plant breeders.

CHAPTER 2 - LITERATURE REVIEW

2.1 CEREAL CROPS

Cereals are grown over 0.735 of the global harvested area and contribute over 0.60 of the total world food production. Wheat, maize, rice and barley constitute about 0.90 while sorghum, oats, millet and rye make up the remaining 0.10 of the world cereal grain production. In developed countries, more than 0.70 of the total cereal production is fed to animals, whilst in less developed countries, 0.68-0.98 of the cereals produced are directly used for human consumption (FAO, 1980).

This chapter will limit discussion only to cereals for animal production, and will concentrate on wheat fed specifically to poultry although other cereals and pigs (the other major non-ruminant species) will be mentioned.

2.2. WHEAT

Feed wheat is used extensively in the diets of broiler chickens with incorporation levels of up to 700 - 800g.kg^{-1} to meet the energy requirements of finishing broilers (Longstaff and McNab, 1986; Wiseman and Inborr, 1990). Comparative diets in many European countries contain about 630g.kg^{-1} wheat (Holmes, 1992). The energy value of wheat in relation to its price makes it very competitive in diet formulations. The need for accurate information on the nutritional value of wheat is however critically important.

Wheat initially was considered to have high and consistent nutritional value, but variable metabolisable energy (ME) values are now recorded. For example, approximately 0.25 of wheat samples in Australia had low ME (Mollah *et al.*, 1983). ME values of wheat are dependent upon cultivar, grain sample,

environment, chemical composition, presence of anti-nutritional factors, intrinsic properties of starch and processing conditions. Bird factors such as digestive capacity which is species dependent, but is also affected by age, sex, previous diet, endogenous enzyme production, microbial status and environmental conditions will also affect utilisation. The degree of starch digestibility, which is crucial as this may be associated with wheats of poorer nutritional value, may be influenced by the presence of non-starch polysaccharides (NSP), anti-nutritional factors (ANF) and processing methods (e.g. pelleting, extruding, expanding) (Scott, 1996).

Wheats are classified according to their hardness, which is considered to be a function of the degree of adhesion between the starch and protein of the endosperm cell wall. Soft wheat varieties have a lower overall adhesion between the two components (Barlow *et al.*, 1973), but a specific protein complex in soft wheats may prevent this adhesion (Greenwell and Schofield, 1986). Weather (growing conditions) and drying conditions are of great importance. Poor weather prior to or during harvest can result in reduced wheat quality due to α-amylase activity on starch. This enzyme is present in the mature grain in small quantities but, on germination (i.e. warm, moist conditions), levels rise dramatically which increases the yield of water-soluble NSP and decreases their molecular weight and viscosity. However, Hong *et al.* (1989) reported a genotypic variance of 1.6 times that of the environment. Pentosans, by the very nature of their highly branched structure and high molecular weight, have also been implicated in affecting grain hardness.

Wheat has undergone and will continue to undergo changes in physiology, morphology and genetic composition. Greater crop diversity may in turn influence nutritional value which is now assuming greater importance.

2.2.1 Structure of the wheat grain

Wheat belongs to the genus *Triticum* (Stoskopf, 1985). Typically, wheat grains are ovoid in shape, between 5- 8 mm in length, 2.5-4.5 mm in width with an average weight of 37mg, although this depends on cultivar and location on the ear (Kent and Evers, 1994). Grains vary in hardness and colour which is due to the pigmentation of the seed coat.

Wheat grains consist of five anatomical regions which comprise the following proportions (by weight) of the grain (from Wiseman and Inborr, 1990): starchy endosperm (0.82 - 0.86), aleurone layer (0.05 - 0.08), embryo (0.02 -0.03), seed coat (testa - 0.02 - 0.03) and pericarp (0.03 - 0.06).

2.2.2 Endosperm

The endosperm consists of thin walled cells packed with starch and protein that are mobilised to support growth of the embryonic axis during germination. The starch exists as granules which are surrounded by a protein matrix, comprised mostly of gluten. In wheat, granules exist as a bimodal population of large (or A type) granules and small spherical (or B type) granules.

In cereals, the protein content of the endosperm increases towards the periphery. Cell size diminishes towards the outside of the seed but cell wall are thicker (MacMasters *et al.*1971). The cell walls in wheat starchy endosperm comprise mainly of arabinoxylans, whereas in barley and oats (1→3) and (1→4)-ß-D-glucans predominate (Fincher and Stone, 1986).

2.2.3 Aleurone

The aleurone consists of one to three layers of thick-walled cells with dense

contents and prominent nuclei. In wheat, rye, oats, maize and sorghum there is a single layer of cells in the aleurone, whereas in barley and rice there are three (Kent and Evers, 1994). Aleurone cells are rich in protein (0.20) and lipid (0.20). Other major constituents include minerals (0.20), sugar (0.10) and nicotinic acid. Aleurone cells contain a large nucleus and many aleurone grains. These grains have a complex composition of minerals and phytic acid (Austin, 1997). Aleurone cells are important during germination for the synthesis of the hydrolytic enzymes involved in the solubilisation of reserves.

2.2.4 Embryo

The embryonic axis (rudimentary root and shoot) and scutellum together constitute the embryo. The former develops into the new wheat plant, while the latter functions as a storage, secretory and absorptive organ, serving the requirements of the embryonic axis during germination. The scutellum is rich in protein, sugar, oil, enzymes, and the B and E vitamins. It consists mainly of thin-walled parenchymatous cells, each containing a nucleus, dense cytoplasm and oil bodies.

Exchange of water and solutes, for the breakdown of starch and protein, between scutellum and starchy endosperm is extremely rapid. Secretions of hormones and enzymes and absorption of solubilised nutrients occurs across the boundary during germination.

2.2.5 Pericarp

The pericarp (or fruitcoat) is a multi-layered structure surrounding the grain, which at maturity consists of largely empty cells, some being lignified. Its main function is to protect and support the growing endosperm and embryo during development.

The innermost layer of the pericarp is the inner epidermis or epicarp which has elongated cells (tube cells) with lignified walls and is often an incomplete layer in many cereals. The overlying lignified cross cells with pitted walls are closely joined side by side in rows.

The outer pericarp in wheat consists of the thin walls of empty and crushed parenchymatous cells and lignified cells and epidermis. The outer epidermis has a cuticle which controls water movement in growing grains and is relatively impervious. Hairs or trichomes present at the apex (non-embryo end) of the grain in wheat, rye, barley, triticale and oats are thick walled with a high silica content and are collectively known as the 'brush' (Kent and Evers, 1994).

2.3 GROSS COMPOSITION OF WHEAT

2.3.1 Carbohydrate: Starch

Wheat consists mainly of carbohydrate, with the major component starch accounting for between 0.60 and 0.75 grain weight. The starch content is affected by a number of factors including cultivar, environment and growing season. Starch is deposited in discrete, roughly sherical, insoluble granules (Greenwood, 1970, 1979; FAO, 1980), within cellular organelles called amyloplasts (Tester, 1997) which can account for 0.80 of certain varieties (Greenwood, 1970). The morphology of the starch granules is characteristic of the species and each granule within a population possesses individuality (FAO, 1980).

The starch has a so-called helical structure which permits individual molecules to be tightly packaged together and accumulate as concentrated deposits, whilst at the same time being sufficiently accessible to degradative (and synthetic) enzymes (Smith *et al.*, 1983).

Starch contains two polymers of α-D-glucopyranose, amylose (α-D-1-4 linkages, essentially linear) and amylopectin (α-D-1-4, and α-D-1-6 branched linkages). Wheat starch contains approximately 0.25 amylose and 0.75 amylopectin (e.g. Berry *et al.*,1988) and is independent of granule size (Bathgate and Palmer, 1972). A third, minor component and intermediate polysaccharide fraction similar to a short chain amylose has been identified which, like amylose and amylopectin, varies in concentration depending on variety (Greenwood, 1979).

The molecular weight and fine structure of both amylose and amylopectin may vary with cultivar and stage of maturity of the plant. The polymers in the granule are hydrogen bonded and aligned in an ordered radial manner (Young, 1984), as shown by the birefringent properties when viewed under polarized light (French, 1984; Blanshard, 1987). The strong, characteristic concentric Maltese crosses, together with X-ray diffraction properties, provides evidence for the highly ordered, microcrystalline structure of the granules (Galliard and Bowler, 1987). For instance, cereal starch is arranged in concentric layers (Scott, 1985). However, not all granules exhibit any anisotropy, ie. many of the granules in high amylose starch are non-birefringent (Blanshard, 1979) and materials such as cellulose are crystalline but non-birefringent.

Although granules are composed primarily of starch, other minor components include proteins and lipids (French, 1984; Blanshard, 1987; Würsch, 1989; Southgate, 1989; Tester, 1997), phosphate and ash (French, 1984) and water (Tester, 1997) which affect the uses to which the starch is put in various applications. Greenwood (1979) reported that the granules have a lipoprotein outer membrane, whereas Würsch (1989) proposed a protein-starch complex on the granule surface.

(i) *Amylose*

Essentially, amylose is a linear molecule with D-glucopyranose units linked via α-(1→4) bonds (Bohinski, 1976). Studies have indicated a limited degree of branching (α-(1→6) bonds) in most amylose wheats (Whistler *et al.*, 1984; Tester, 1997). The molecules of amylose are relatively small, tending to take up a helical-coiled conformation (Bohinski, 1976) with molecular weight ranging from a few thousand to about 500,000 Da depending on botanical source and severity of treatment used in its isolation (Tester, 1997).

The number of chains in amylose molecules from different sources ranges from 2 to 11 with the individual chain lengths containing from 250 to 670 glucose units (Morrison and Karkalas, 1990). Amylose is amorphous in normal starch granules. Recent research has indicated that in cereal starches there are in fact two amorphous forms of amylose: lipid free amylose and lipid complexed amylose (Morrison *et al.*,1993a,b). The presence of such lipid complexes within the starch granules makes the amylose less susceptible to hydrolysis by α-amylase (Karkalas *et al.*,1992).

Amylose readily forms complexes with fatty acids, low molecular weight alcohols and iodine to form a characteristic blue-black complex (Lyne, 1976; Young, 1984). Amylose is insoluble in cold water but gels on heating with individual amylose chains self-associating on cooling to form crystallites (Miles *et al.*,1985). This process is known as retrogradation (Manners, 1979) the rate of which is dependent on time, temperature, pH, concentration, method used to solubilise the amylose (Young, 1984) and degree of polymerisation. The amylose content of the starch influences digestibility (Berry, 1986), solubility, lipid binding and other functional properties (Reddy *et al.*,1984).

The biosynthesis of starch is affected by environmental factors including

temperature during growth (Tester *et al.*,1995). High temperatures depress starch deposition in wheat, resulting in reduced yields as a consequence of fewer and small granules being synthesised (Tester, 1997) although the proportion of amylose tends to increase with growth temperature (Tester *et al.*, 1995). The location of amylose with granules is uncertain (Tester, 1997).

(ii) *Amylopectin*

Amylopectin spans from the hilum (centre) to the periphery of starch granules (Tester, 1997). Most of the linkages between the D-glucopyranose are of the α-(1→4) type as in amylose, but, in addition, 0.04 to 0.05 of the glucans are combined in α-(1→6) linkages (non-reducing end groups) at intervals of about 25-to-30 glucose residues, giving rise to a branched structure (Greenwood, 1970). There is also evidence that a small amount of branching occurs through α-D-(1→3) linkages (Wolfrom and Thompson, 1956). Amylopectins have much larger molecular weights than the amyloses, ranging from several hundred thousand to many million Da (Manners, 1979; Aykroyd and Doughty, 1982; Tester, 1997). No preferred conformation has been suggested, although recent evidence indicates that A- and B-glucan chains exist. The most peripheral (exterior) chains which contain no other chains bonded to themselves are described as A-chains. These chains are bonded to B-chains which are classified by Hizukuri (1986) as B_1-B_4 depending on the number of clusters which they span radiating out from the hilum of the granule. According to this model, the average chain length of the A-, B_1-, B_2-, B_3- and B_4-chains is 12-16, 20-24, 42-48 and 69-75 glucans respectively. There is just one C-chain per amylopectin molecule and it contains the only free reducing group. This chain is in essence the 'backbone' of the molecule with B- and some A-chains α-(1-6) bonded to it (Tester, 1997).

The A- and small B-chains of amylopectin are found in discrete clusters within the molecule and are held together at the base of these clusters by the α-(1-6)

bonds. The unit chains within the clusters readily form double helices during starch deposition which are stabilised by inter-chain hydrogen bonding. These double helices pack together in an ordered fashion to form concentric crystalline laminates, which are interspersed with amorphous material created by α-(1-6) branching regions (Tester, 1997). It has been suggested that the forces holding starch granules together are mainly at the level of the double helices and that crystallinity functions as a means of achieving dense packing rather than as a primary provider of structural stability (Cooke and Gidley, 1992).

The order within starch granules can be measured by a number of methods which essentially measure short, medium or long-range regularity. Wide angle X-ray scattering measures order at the level of crystallite unit cells throughout starch granules and has been used to classify the helical forms (polymorphs) as A-, B- or C-type (Blanshard, 1987). These helical forms (although C-type is really an intermediate form of A- and B-type diffraction patterns) are characteristic of cereal, tuber and legume starches respectively. Longer range order as a consequence of the semi-crystalline nature of the granules has traditionally been investigated using a polarising microscope. With this technique, native starch granules appear as distorted spherocrystals with a characteristic dark (Maltese) cross. The apparent intensity of the birefringence is dependent on the thickness of the starch granules as well as the degree of crystallinity and orientation of the crystallite (French, 1984).

The molecule does not form stable complexes with iodine solution but produces a pale red-brown colour (Scott, 1985) which may be used in qualitative analysis. Amylopectin is insoluble in cold water but forms stable gels on heating. It is thought to be responsible for the solubility of starch granules (Reddy *et al.*, 1984).

(iii) *Other Starch Components*

In addition to amylose and amylopectin, starch may contain up to 0.10 of an intermediate fraction (reviewed by Banks and Greenwood, 1975). This fraction contains the same type of glucosidic bonds as amylopectin but differs from both in molecular size, degree of branching, β-amylolysis limit and iodine binding capacity. This intermediate material is larger than amylose but smaller than amylopectin. ß-amylolysis limits and iodine binding capacity are the inverse (Banks and Greenwood, 1975)..

(iv) Growth Rings

Growth rings have been identified in many starches using microscopy and comprise relatively large concentric amorphous shells interspersed with relatively dense shells containing laminates of alternating crystalline and amorphous material (French, 1984). Large starch granules show pronounced growth rings when they are fully hydrated, but with smaller granules, the growth rings are much less obvious. When starch granules develop in wheat plants subject to normal diurnal variation, there is one growth ring per day, although these may be absent if the plants are grown under constant light (Buttrose, 1960; French, 1984). According to French (1984), growth rings represent periodic growth and daily fluctuations in carbohydrate available for starch deposition. Growth rings have been studied by light microscopy, scanning electron microscopy (SEM) and small angle X-ray scattering, and are readily visible by SEM following enzymatic treatment.

(v) *Minor Components*

Small amounts of phospholipid and protein are frequently associated with the starch granule normally on the granule surface. These components are readily

extractable and the amounts may provide an indication of starch purity (Galliard and Bowler, 1987).

Non-waxy cereals contain significant amounts of monoacyl lipids (free fatty acids and lysophospholipids) within the granules ("true" starch lipids). Extraction of such lipid present as inclusion complexes is effected by the use of strong solvents such as n-propanol or water-saturated n-butanol at high temperatures (90-100°C). Total lipid contents of wheat granules has been estimated as 0.77-1.2g.100g^{-1} dry starch (Morrison *et al.*, 1993).

Cereal starches also contain significant levels of granule protein. Lowy *et al.*, (1981) reported 7g.kg^{-1} in large lenticular wheat starch granules, the majority of which was thought to be associated with the granule interior. Granule proteins may be responsible for impeding amylose-amylose complexes *in situ* since double helical associations occur readily *in vitro*.

Low levels of phosphorous have been detected in isolated starches, eg. 0.9g.kg^{-1} for wheat, mainly present as lysophospholipids. Certain metal ions of Ca, K, Na and Mg are also found in granules (Galliard and Bowler, 1987).

2.3.2 Cell wall polysaccharides: Non-starch polysaccharides

The cell walls of cereals are composed primarily of complex carbohydrates which are loosely termed NSP. Dietary fibers are chemically defined as NSP plus lignin. These nutritionally important components are, in cereals, found in the cell walls. The predominant constituents are the arabinoxylans (pentosans), mixed-linked (1→3),(1→4)-ß-D-glucans, cellulose and lignin. Small amounts of heteromannans, glycoproteins containing arabinogalactans and non-carbohydrate substituents, such as esterified phenolic acids, are also present.

Whole wheat is comparatively low in NSP with, for example, ranges between 105-138 and 100-106 $g.kg^{-1}$ grain (DM) respectively for the total NSP content of 12 spring and 12 winter wheats (Åman, 1988).

(i) Arabinoxylans

Arabinoxylans are polymeric chains of the pentose sugar xylose together with arabinose as branched units. They may exist in the free form or linked to other polysaccharides or proteins (Kulp, 1968). They account for some 0.85 of the polysaccharide components of the wheat cell wall.

The arabinoxylans from various sources differ in their chemical composition and properties. Even within wheat varieties, differences in the chemical composition of the NSP have been demonstrated. Structural differences in terms of ferulic acid content, ratio of constituent monosaccharides and molecular size have all been shown among arabinoxylans of different wheat varieties (D'Appolonia and MacArthur, 1976; Lineback *et al.*,1977; Ciacco and D'Appolonia, 1982; Izydorczyk *et al.*, 1991).

The structure of arabinoxylan in wheat has been studied extensively (Fincher and Stone, 1986). The arabinoxylans of wheat are similar to those of rye, consisting of a main linear backbone chain made up of (1→4)-ß-D-xylopyranosyl units to which substituents are attached to varying degrees through the O-3 or O-2 and O-3 positions of the xylose residues in a non-random manner (Voragen *et al.*, 1992). The attached constituent is mainly a single arabinose unit (terminal α-L-arabinofuranosyl residues), however some may also contain hexoses, uronic acids, short oligosaccharides, phenolic or acetyl substitutes (Fincher and Stone, 1986). Also feruloyl groups esterified to arabinose residues are present in this polysaccharide (Izydorczyk and Biliaderis, 1992). They exhibit a considerable variation in molecular mass, in xylose to arabinose ratio, in unbranched to

branched xylose ratio as well as in 2,3,4 tri-substituted to 3,4 di-substituted xylose (Vliegenthart *et al.*, 1992).

Wheat flour pentosans (23 - 30g.kg^{-1} flour; Lineback *et al.*, 1977) can be broadly classified in terms of their solubility. As such, two forms of pentosan exist within the grain, soluble and insoluble. Both fractions represent a mixture of arabinoxylans and polysaccharide-protein complexes. Generally the insoluble pentosans are present in greater amounts than the soluble pentosans (e.g. Saini and Henry, 1989) although Kulp (1968) considered that up to 0.50 of pentosans were soluble in water. Solubility may be influenced by molecular size and/or degree of branching (Cerning and Guilbot, 1974). It has been recognised that differences in properties between the soluble and insoluble arabinoxylan flour fractions stem from differences in molecular structure (Ciacco and D'Appolonia, 1982). Some studies have failed to detect a difference in the degree of branching between soluble and insoluble wheat arabinoxylans (e.g. Longstaff and McNab, 1986). The non-endospermic tissues, particularly the hull, have high contents of pentosans (up to 0.48) because they consist mostly of cell wall material.

Pentosans have been associated with anti-nutritional effects of some wheat varieties in poultry owing to their viscous properties and the inability of the chick to degrade them.

Soluble Arabinoxylans

The water-soluble NSP account for approximately 0.33 of the pentosan fraction and consist of a mixture of arabinoxylans and arabinogalactan-peptides (Shogren *et al.*,1987; Vliegenthart *et al.*, 1992). The water-soluble wheat flour arabinoxylan (WSP) (5 - 8g.kg^{-1} wheat flour; Izydorczyk *et al.*, 1991) is a high molecular weight fraction and has high water-absorbing capacity. The relatively

high ratio of arabinose to xylose confers rigid rod-like conformation to the molecule and it is partly responsible for the high viscosity of arabinoxylan solutions via covalent bonding (Andrewartha *et al.*, 1970; Hoffmann *et al.*, 1991).

Insoluble Arabinoxylans

The water-insoluble pentosans (WIP) of wheat also contribute to the water-binding properties exhibited (Hoseney, 1984; Amado and Neukom, 1985; Meuser and Suckow, 1986). They comprise primarily of L-arabinose, D-xylose and D-glucose and amount to about 20g.kg^{-1} wheat flour (Shogren *et al.*, 1987). The WIP are similar to the WSP except they have a higher degree of branching and a considerably higher molecular mass (Vliegenthart *et al.*, 1992). The ratio of arabinose to xylose differs in different wheat varieties but reports of 0.58-0.59 for Canadian winter and spring varieties have been made.

(ii) (1→3) (1→4)-ß-D-Glucans

Although the mixed-linked ß-glucans are ubiquitous amongst the cereals, these polysaccharides occur in highest amounts in barley (30 - 110 g.kg^{-1} DM; Prentice *et al.*,, 1980; Fincher and Stone, 1986) and oats (25 - 70 g.kg^{-1} DM; Andersson *et al.*, 1978; Prentice *et al.*, 1980). The ß-glucan distribution is usually more uniform in barley than in oats, but there is considerable variation between cultivars (Wood, 1984). β-glucan in wheat has received less attention despite reports indicating as much as 10g ß-glucan.kg^{-1} in certain wheat varieties (Beresford and Stone, 1983). Wheat ß-glucan is found in the inner aleurone cell wall and sub-aleurone endosperm cell walls, but considerable amounts are found in the crease area (Wood, 1984).

Cereal ß-glucan is composed entirely of glucose units, as are amylose,

amylopectin, cellulose, dextran and other glucans, but the distinction lies in the nature of the linkages between the units. Most research has concentrated on structural studies of ß-glucan on barley and, despite the overall general structural similarities with other cereal ß-glucans, there are distinctive differences in terms of physical properties such as viscosity and solubility (Wood, 1984).

One of the most important characteristics of cereal ß-glucans is viscosity. These polysaccharides are highly soluble, capable of forming highly viscous solutions or gels. This property has long been known to present problems for the malting and brewing industries as well as poultry feed manufacturers. In poultry, for example, ß-glucans give rise to low ME of barley when fed which has been attributed to the inhibition of nutrient digestion by the ß-glucan component (White *et al.*, 1981).

(iii) Cellulose

Cellulose is the major structural polysaccharide in plants, present in all cell walls. It is a linear molecule composed of ß-1-4-linked D-glucopyranoside units. The molecular weights are usually very high, about 0.5 - 1 million Da. The linear nature allows the molecule to associate closely with itself through strong hydrogen bonding rendering it insoluble and, in its native state, it is partially crystalline. The high degree of order and its insolubility, together with its ß-linkages makes the polymer resistant to attack by many organisms (e.g. poultry). Cellulose is a major component of straw and hulls constituting about 0.40 - 0.50 The pericarp of cereal grain is also quite rich and may contain up to 300g cellulose.kg^{-1}. It is usually found associated with lignin and other NSP.

(iv) Lignin

Lignin is a non-carbohydrate component of the cell walls and comprises a group

of polyphenolic compounds formed by the condensation of aromatic alcohols (cinnamyl, guaicyl, syringyl) of widely ranging molecular weight. The complex random polymers generally contain about 40 oxygenated phenylpropane units including coniferyl, sinapyl, and p-coumaryl alcohols that have undergone a complex dehydrogenative polymerisation process (Theander and Åman, 1979). Naturally occurring lignin is highly varied in its structure and composition and is condensed with a wide variety of non-phenolic compounds, particularly carbohydrates and protein. The lignin infiltrates into the polysaccharide matrix expanding the wall volume as the wall matures. An increase in the proportions of cellulose and lignin are thus associated with maturation of the plant cell. Most of the lignin occurs in the outer layers of the wheat grain. Lignin is very inert owing to its strong intramolecular bonding which includes carbon-to-carbon linkages, and it contributes to the structural rigidity of the plant cell wall. It demonstrates greater resistance to digestion than any other naturally occurring polymer (McPherson Kay, 1982). It also has the capacity to inhibit breakdown by bacterial enzymes of other types of cell wall carbohydrate such as NSP and non-cellulosic polysaccharides. This inhibition is most likely brought on by a physical encapsulation of the carbohydrate preventing access by the bacteria. Lignin may also have specific inhibitory or "toxic" properties that inhibit bacterial action.

(v) Pectic substances

Pectic substances are a complex group of polysaccharides in which D-galacturonic acid is a principal constituent. They are structural components of the cell walls and also act as intercellular cementing substances. Included are a water-soluble parent compound, proto-pectin, as well as pectinic acids, pectic acids and pectin. The backbone structure of pectin is an unbranched chain of axial-axial-(1→4)-linked-D-galacturonic acid units. Long chains of galacturonan are interrupted by blocks of L-rhamnose-rich units that result in bends in the

molecule. Many pectins have neutral sugars covalently linked to them as side chains, mainly arabinose and galactose, and to a lesser extent, xylose, rhamnose and glucose. The carboxyl groups of the galacturonic acids are partially methylated and the secondary hydroxyls may be acetylated. Pectin is highly water-soluble and is almost completely metabolised by colonic bacteria (McPherson Kay, 1982).

2.3.3 Protein

The protein content of wheat is variable. Protein can be classified in terms of its solubility (Osborne, 1907): water soluble proteins are described as 'albumins', saline soluble as 'globulins', aqueous alcohol soluble as 'prolamins' (or gliadins) and those remaining insoluble in neutral aqueous solutions, saline solutions or alcohol, as 'glutenins'. The albumins and globulins account for 50-100 $g.kg^{-1}$ protein and are functional proteins. The largest protein complex in wheat is gluten. The gliadins and glutenins (often referred to as gluten) make up 800 $g.kg^{-1}$ wheat protein and are mainly within the starchy endosperm of the grain. These proteins serve as a storage function. The gliadins are a large group of proteins with similar properties, they are single chained, have a molecular weight of around 40,000 Da and they are extremely sticky when hydrated. The glutenins are also a heterogeneous group of proteins but are multi-chained and possess molecular weights in the order of 100,000 to several million Da (Austin, 1997). It is the gluten that gives wheat its characteristic viscoelastic properties. Gluten also contains about 50 - 10g $lipids.kg^{-1}$ and 100 -150g $carbohydrates.kg^{-1}$ (Kasarda *et al.*, 1971). Fractionation of wheat proteins on the basis of solubility remains important today.

The two major amino acids in gluten are glutamic acid (approximately 0.33 of total amino acid residues) and proline (of the order of 0.16) which together make up almost half of the protein. Other amino acids are present in much smaller

quantities. The nutritional value of wheat flour as a single ingredient is limited because of low levels the essential amino acid lysine, together with some other essential amino acids.

2.3.4 Lipids

The lipids in wheat which contribute to the diet have received little attention in comparison to the carbohydrate and protein fractions, perhaps because they represent only a small proportion of the total grain. Lipids in wheat consist of a complex mix of non-polar lipid material (0.70), glycolipids (0.20) and phospholipids (0.10), which are distributed throughout the grain. The germ contains, in proportion, about 0.28, the endosperm about 0.015, the bran about 0.054 and the aleurone layer about 0.08 lipid.

2.4 OTHER COMPONENTS OF THE WHEAT GRAIN

2.4.1 Mono and oligosaccharides

Wheat contains a small amount of free sugars. Literature values vary depending on method of analysis and variety examined, but rarely account for more than 40 $g.kg^{-1}$ grain (DM) (Cerning and Guilbot, 1974). These simple sugars include the monosaccharides glucose and fructose, together with di-, tri- and tetrasaccharides based upon them and galactose (found in sucrose, raffinose and glucofructosans). The free sugars are not evenly distributed throughout the grain; the embryo has the highest concentration (Kent and Evers, 1994). About 0.25 of the sugar is found in the germ which contains mainly sucrose and raffinose. These two sugars also predominate in the bran. The glucofructosans appear to be concentrated in the endosperm and are absent in both the germ and the bran.

2.4.2 Mineral matter

About 0.95 of the minerals within the grain consists of phosphates and sulphates of potassium, magnesium and calcium. Potassium phosphate is probably present in wheat mainly in the form of KH_2PO_4 and K_2HPO_4. Some of the phosphorous is present as phytic acid. Important minor elements include iron, manganese and zinc, present at a level of 1-5 $mg.100g^{-1}$, and copper at about 0.5 $mg.100g^{-1}$. Other elements are also present in trace quantities (Kent and Evers, 1994). Most of the minerals are present in the aleurone layer.

2.4.3 Vitamins

Wheat is a good source of vitamins such as thiamine, niacin, riboflavin, pyridoxine, pantothenic acid and tocopherol. These vitamins can be found in both the aleurone and the scutellum of the wheat grains.

2.4.4 Enzymes

Wheat grains contain a variety of enzymes. During maturation of the grains most enzyme activity is concerned with synthesis, particularly in the synthesis of storage products. Hydrolytic enzymes involved in the breakdown of starch and protein stored in the pericarp may persist during maturity (Fretzdorf and Weipert, 1990). In the mature grain, enzyme levels are relatively low if the grain is sound and dry. If damaged, as in milling, lipids become exposed to lipase. As germination initiates on wetting of the grains, enzymes concerned with solubilisation are produced. Cell walls are hydrolysed, permitting greater access to storage products by enzymes which hydrolyse starch and protein.

The most abundant of the enzymes are the amylases (α and ß), proteases, lipases (relatively low activity in wheat), lipoxygenase, phytase and xylanase

(Kent and Evers, 1994).

(i) Amylases

The two types of amylase present within the wheat grain, α-amylase and ß-amylase, act synergistically to catalyse the hydrolysis of starch molecules. ß-amylase gains greater access to the substrate through the activity of α-amylase. Their modes of action are quite different; α-amylase is endo-acting while ß-amylase is exo-acting (Kent and Evers, 1994). Amylase is inactive on granular starch but is capable of rapid action when the substrate is in solution. Grain quality is influenced more by the α-enzyme as its amount is more variable according to the condition of the grain. ß-amylase is present in resting grain and increases only a few fold on germination through release of a bound form. α-amylase is actually synthesised during germination and its activity increases progressively, as germination proceeds, by several hundred fold. In different cereals, the site of synthesis of α-amylase varies; in wheat, rye and barley it occurs first in the scutellum and later in the aleurone, whereas in maize only the scutellum is involved.

Several isoenzymes of the α-amylase type exist in most cereals and they fall into two distinct groups depending upon their isoelectric points. The *Triticeae* cereals contain two groups while other cereals have only one (Kruger and Reed, 1988).

Even the combined action of the amylases cannot completely digest solubilised starch since neither the α- nor the ß-amylase can catalyse hydrolysis of the α-(1→6)-linkages, and hence the branch points remain intact. Also, those α-(1→4)-bonds close to the branch points resist hydrolysis. As such, only about 0.85 of the starch is converted to smiple sugars. For commercial purposes, a de-branching enzyme, such as amyloglucosidase, may be used to increase the yield of sugars from the starch.

(ii) Proteases

Proteolytic enzymes consist of both proteinases and peptidases, found at relatively low levels of activity. Their function is to break down proteins and peptides into their constituent amino acids.

(iii) Lipid modifying enzymes

Enzymes of two types are important in catalysing the breakdown of lipids: lipase and lipoxidase. Both are capable of causing rancidity in cereals, thus both hydrolytic and oxidative rancidity are recognised.

Lipoxidase can only catalyse the degradation of free fatty acids and monoglycerides and therefore allows lipolysis. Lipolysis occurs slowly in the dry state; enzymic oxidation occurs rapidly on wetting (Kent and Evers, 1994). Potential storage problems can be avoided in starchy endosperm if it is separated from other grain components where enzyme and substrate are concentrated. This is common practice in the case of wheat and rice in which the aleurone layer has a high lipid content. In wheat, lipases activities in the embryo and aleurone layer are 10-20 fold that of the endosperm (Kruger and Reed, 1988).

Lipases catalyse the hydrolysis of triglycerides to produce diglycerides and free fatty acids, diglycerides to give monoglycerides and free fatty acids, and monoglycerides to give glycerol and free fatty acids.

(iv) Lipoxygenase

Lipoxygenase catalyses the peroxidation of polyunsaturated fat. The unsaturated fatty acids are converted to hydroperoxides which, in turn, are changed to hydroxy acids by lipoxygenase, lipoperoxidase and other enzymes, as well as by

non-enzymic processes (Youngs, 1986).

(v) Phytase

Phytase catalyses the hydrolysis of phytic acid (inositol hexaphosphoric acid) to inositol and free orthophosphate. In wheat its activity increases six-fold on germination and more activity is found in hard wheats than soft wheats (Kruger and Reed, 1988).

(vi) Catalase and peroxidase

Catalase and peroxidase are haemoproteins. Peroxidase is involved in the degradation of aromatic amines and phenols by hydrogen peroxidase. Its activity is greater in wheat than in other cereals. Catalase catalyses the degradation of hydrogen peroxide to water and oxygen. Its physiological function is not clearly understood but it increase during germination (Kruger and Reed, 1988).

(vii) Phenol oxidases

In the mature wheat grain several polyphenol oxidases are present in the starchy endosperm, more concentrated in the bran. On germination they increase (Kent and Evers, 1994).

(viii) Xylanases and ß-glucanase

These enzymes degrade arabinoxylans and (1→3) (1→4)-ß-D-glucan in the cell walls. The latter group of enzymes assume greatest importance in barley in which the ß-glucans contribute some 0.70 of the cell walls (Kent and Evers, 1994). Xylanases are more important in wheat and rye.

2.5 METABOLISABLE ENERGY

The energy concentration of poultry feeds and the energy requirements of poultry are generally described in terms of metabolisable energy (ME) which constitutes the proportion of gross energy available to the bird for metabolic processes.

2.5.1 Measurement of ME

A number of rapid techniques have been developed for the measurement of ME in poultry such as single dose feeding, rapid broiler assays (Farrell, 1978), intubation directly into the crop using adult cockerels (Sibbald, 1976; McNab and Blair, 1988) and indirect methods such as rapid response respiration calorimetry (Lundy *et al.*, 1978; MacLeod *et al.*,1985). The intubation procedures include a correction for that portion of excreta which is metabolic and endogenous in origin and measures what is termed true metabolisable energy (TME). The ME values determined by these procedures are often adjusted to nitrogen equilibrium. Although these methods generally have the advantages of speed, precision in the measurement and timing of intake, reduced amounts of feed required and of allowing the feeding of substances which, although nutritious, may be unpalatable at high concentrations, they are subject to errors due to residues from the previous diet remaining in the digestive tract of the birds and being excreted during the balance period, resulting in the underestimation of digestibility coefficients. Also, incomplete evacuation of undigested feedstuffs within the time allocated for collection (usually 48hr) results in an overestimation of the digestibility (McNab, 1993). The method of Farrell (1978), for example, as recommended, uses adult cockerels and does not equate with normal broiler feeding management. The differences in some wheat ME values observed between the classical and Farrell bioassays (Mollah *et al.*,1983) suggest that caution should be exercised if values obtained for wheat,

and some other ingredients, by the latter procedure are to be applied to growing broilers.

A problem with trying to relate published ME values is that there are 4 types of ME value and unfortunately it is not always apparent which type is being reported (Sibbald, 1979). In addition, values may vary according to the specific assay procedure used for the determination. In the current study, standardisation of the assay procedure between trials was implemented as far as possible.

ME, sometimes referred to as classical (uncorrected) ME, is a function of the amount of gross energy consumed in the feed by birds fed *ad libitum* over several days minus that voided in the corresponding excreta plus urine which, in poultry, are combined as a single excreta and combustible gases. The energy lost as gaseous products of digestion is insignificant and can therefore be almost always ignored. This convention is followed in the current study. The actual term derived is referred to as 'apparent' (AME) because only part of the energy excreted is derived directly from the food consumed (ie. excretion of undigested and unmetabolised dietary residues). The other part of the excreta energy is derived from endogenous losses (EEL, ie. that arising from the bird). EEL are reported to consist primarily of sloughed-off gut lining, bile excretions and unabsorbed enzymes, which are faecal in origin; and the excretory products of nitrogen metabolism, which are of urinary origin (McNab, 1990).

It is assumed that the experimental birds begin and end the collection period with the same quantity of undigested residues in the gut. This assumption may not always be valid but suggestions that a period of starvation be imposed at the start and finish of the collection period, or that an inert marker be included in the feed to colour the excreta and identify the start and finish of the bioassay, may pose some difficulties.

It is usually assumed that the AME values of dietary components are additive and unaffected by the nature of the diet (Wallis *et al.*,1985). Since the AME of dietary ingredients can be determined and/or are available within the literature, values for AME of the test ingredient (in this case, wheat) can therefore be estimated. Unfortunately, these assumptions have not always proved to be valid. For example, it has been shown (Payne, 1976) that the AME of wheat depends on its inclusion level within the diet. Mollah *et al.* (1983) revealed low AME values for many Australian wheats when included in broiler diets at high rates of inclusion. Furthermore, evidence is available which indicates that adding maize or oat hulls to the diet can improve both the AME value and the digestibility of starch (Mollah and Annison, 1981; Mollah, 1982).This clearly indicates that dietary AME values can be influenced by the presence of, and the interaction with, other cereals. For the purposes of the current study, however, the AME values of all dietary components were assumed to be additive.

It is common practice to 'correct' ME values for changes in the nitrogen status of the bird during the balance period of the trial. Nitrogen corrected AME (AME_n) is the most commonly used estimate of ME. Correcting to zero nitrogen retention (nitrogen equilibrium) provides one measure of standardisation for comparative purposes which acts as a common base for the expression of all ME values in order to eliminate the variable influence of nitrogen retention, and thus allows for more meaningful results between laboratories. The use of nitrogen-corrected ME may, however, be questioned because the amount of nitrogen retention will depend on a number of factors including the quantity and quality of protein in the diet, as well as the age and physiological state of the test bird. The 'nitrogen-correction' is easily obtained from nitrogen balance data. Hill and Anderson (1958), working with chicks, introduced a correction factor of 34.39 $kJ.g^{-1}$ nitrogen retained. The numerical effect of the correction is, however, relatively small.

Provided time is not a major consideration the classical AME assay over a period of days is the most appropriate technique which is widely accepted as being an effective means of establishing the ME of diets. Recent studies (Bourdillon *et al.*, 1990a,b) have indicated that AME_n, although a biological value and subject to many sources of error, is highly reproducible. The method is, however, slow, has a high labour requirement and requires a large feed sample. Where feed intake is low, as when diets high in fibre are fed (Sibbald, 1976), metabolic and endogenous energy may contribute substantially to the total energy excreted, thus producing an underestimate of ME. Other difficulties may arise with large volumes of excreta collected and the need for this to be re-sampled.

2.5.2 Energy value of diets

(i) Choice of Bird

ME has been determined for birds of both sexes and at all ages. Peterson *et al.*, (1976) tested 13 ingredients with male and female chickens from 14 to 28 days of age but were unable to show any influence of sex on ME values. Age may exert an effect on the AME_n value of diets and of raw materials. The principle source of variation is associated with fat. Young birds digest fat with a reduced efficiency compared with adults owing to a deficiency in bile salt secretion (Larbier and Leclercq, 1994). This relationship is the reverse for proteins where digestibility falls as the birds age. Excreta output has been measured most frequently in groups of growing chickens aged from 1 to 3 weeks.

The importance of using birds at the correct age for ME bioassays has been demonstrated. Studies have identified wheat cultivars with unusually low AME values when included at high levels in diets for growing broilers (Mollah *et al.*, 1983).The AME_n values of these wheats was significantly improved (9-14%) when fed to adult cockerels (Mollah *et al.*, 1983). This suggests that, with

wheat-based diets, ME determinations should be carried out with stock of a specified age to which the feed is to be given.

(ii) Influence of processing treatment

It is accepted that certain varieties of wheat contain starch which is poorly digested if not heat treated, and steam pelleting at 80°C is often sufficient to improve digestibility and dietary energy value. This change is probably bought about by gelatinisation of the starch molecules thus increasing accessibility to the digestive enzymes, and/or a denaturing of anti-nutritional factors present. It is, however, impossible to give a fixed and systematic value for the improvement arising from pelleting.

2.6 THE ANATOMY AND DIGESTIVE PHYSIOLOGY OF THE CHICK

The alimentary tract of the chick (Larbier and Leclercq, 1994) may be five to six times the external length of the bird. The length of various parts of the tract vary with size of bird, type of food eaten, and other factors. Birds eating coarse, fibrous food tend to have especially large digestive tracts, and grain-eating birds have larger tracts than carnivores (Sturkie, 1976).

Diets for poultry are generally a mixture of raw materials of diverse origin and complex chemical composition which are usually subjected to a series of physical and chemical processes to enhance digestibility. Digestive physiology constitutes the collective processes of digestion and absorption. The passage of digesta is extremely rapid, generally around 10 hours, though it may even be as little as 4 hours, which implies highly efficient mechanisms of digestion and absorption. The mechanical, chemical and enzymatic processes take place throughout the gastro-intestinal (alimentary) tract. Absorption takes place essentially in the small intestine (Larbier and Leclercq, 1994).

2.6.1 Anatomy of the alimentary tract

The gastro-intestinal tract can be distinguished from mammals in terms of the following features:

- a beak;
- existence of two successive and distinctive stomachs, the proventriculus (glandular or 'chemical' stomach) and the gizzard ('mechanical' stomach);
- the cloaca, which acts both as the rectum and exit for the urino-genital system.

At hatching the tract represent up to 0.25 of the live-weight, but falls to less than 0.05 by 8 weeks of age (Larbier and Leclercq, 1994).

(i) The buccal cavity

Some mechanical breakdown of feeds may be accomplished by the beak but most foods are taken into the mouth whole without undergoing any significant transformation. The foods appear to be propelled rapidly to the pharynx by a combined movement in which the head is momentarily raised and jerked forward (Hill, 1976). Water is imbibed, the beak is then closed, and after raising the head, the fluid appears to flow passively into the oesophagus. The tongue and other parts of the buccal cavity coordinate such movements. The tongue is a narrow, pointed organ, triangular in cross section, which lies within the lower beak. It is a rigid organ and has few intrinsic muscles. The hyoid bone onto which it is attached confers considerable mobility which assists in the movement of food together with the water to the oesophagus.

Taste appears to play only a minor role in feed consumption by chickens, perhaps because the taste buds are relatively few in number (Hill, 1976; Scott *et*

al., 1976). Salivary glands are numerous and scattered through the mouth and pharynx. In adults, salivary fluid is rich in mucous which moistens and lubricates the food bolus and assists its passage into the oesophagus. During feeding there is an increased secretion of saliva which can vary from 7 to 30 ml per day depending on the feeding conditions. Its composition is poorly understood, but has an alkaline pH and contains amylase and a high concentration of bicarbonate ions. In this respect, it is probably similar to that of other species (Hill, 1976; Larbier and Leclercq, 1994).

Saliva may play a small role in the conversion of starch into sugars, but the majority is degraded within the intestines.

(ii) Oesophagus and crop

The oesophagus of the mature bird is about 20cm long containing mucous glands (Sturkie, 1976; Hill, 1976). It lies between the pharynx and the proventriculus and consists of two parts, the upper (cervical or cranial) and the lower (intra-thoracic or caudial) oesophagus. Between these two regions lies the crop. The crop has essentially the same structure as the oesophagus except that mucous glands are only present at the juncture of the oesophagus (Sturkie, 1976; Hill, 1976). The crop of certain grain-eating birds, such as the chicken, are well developed. The crop is used to store food temporarily and thus acts as a reservoir which regulates the transit time of the digesta, and allows the bird to consume fairly large quantities of food at any one time. Accumulated food in the crop is softened and moistened by mixing with saliva and water which aids the subsequent digestion process. Three types of muscle in the crop enable the organ to contract, mix the food, and pass it on towards the proventriculus when required. When the gizzard is empty, food by-passes the crop and moves directly into the proventriculus. The empty crop contracts at approximately 1 to 1.5 minute intervals but less frequently when containing food. Emptying the crop

plays an important role in regulating the rate of passage of digesta and therefore the efficiency of the digestive process. It depends on a number of factors such as the capacity of the crop, how full the gizzard is, the particle size of the food, and the degree of moistening. It is apparent that those factors which assist rapid passage of digesta from the gizzard, e.g. moist or wet food and finely ground food, also accelerates crop emptying (Hill, 1976; Larbier and Leclercq, 1994). The crop appears to play a minor role in enzymic digestion and absorption.

(iii) Proventriculus and gizzard

The stomach is divided into two parts: the proventriculus and the gizzard. These two regions have complementary roles; the former has a secretory role, the latter a mechanical role. The proventriculus (or glandular stomach) is an ovoid structure surrounded by a thickened wall, lying between the lower end of the oesophagus and the gizzard the size of which varies with species. The gastric glands of chickens contain only one type of cell (chief cells), which produces both hydrochloric acid and the proteolytic enzyme pepsinogen. Passage of food into the proventriculus is dependent on the crop and lower oesophagus, which in turn are regulated by the gizzard. Movement of ingesta through the proventriculus is bought about by regular rhythmical contractions (1 min intervals). There is no appreciable residence time for ingesta in the proventriculus, although there may be oscillation of contents between the gizzard and proventriculus before the gizzard is in the receptive stage (Hill, 1976).

Under *ad libitum* feeding conditions, the contents of the proventriculus and gizzard are predominately acid and reflects the continuous secretion of gastric juice by the proventriculus in response to nervous and chemical stimulation (Hill, 1976; Larbier and Leclercq, 1994). Due to the rapid movement of food through this part of the stomach, no digestion takes place until it reaches the gizzard.

The gizzard is a flattened sphere about 5 cm in diameter and 2.5 cm thick. It is a thickened biconvex organ and is characterised by its extensive muscle development and thick tough lining which relates to its function as a grinding chamber and site of peptic hydrolysis. The presence of insoluble mineral grit (small siliceous stones) in the gizzard, which is ingested by the bird, is important for optimum digestion since it helps to grind the food through its abrasive contact and increases motility by producing contractions of greater amplitude, which is beneficial with coarse feed and whole-grain diets (Hill, 1976). The hydrochloric acid produced in the proventriculus continues its action within the gizzard (pH 2-3.5) in order to solubilise mineral salts (calcium carbonate and phosphates), to ionise electrolytes and to destroy tertiary structures of dietary proteins (Larbier and Leclercq, 1994). The grit is not attacked by the hydrochloric acid present. The ground food is mixed with water to form a paste (chyme) and peptic digestion takes place thereafter. The chyme is then passed onto the intestines.

(iv) Small intestine

The small intestine is of similar structure throughout its length and in adult birds, the total length is about 120cm which is conventionally divided into three regions, being the duodenum, the jejunum and the ileum.

The duodenum (20-24 cm) is a U-shaped loop which encloses the pancreas (Hill, 1976; Larbier and Leclercq, 1994) which secretes further digestive enzymes which are concerned with the breakdown of the three main components protein, carbohydrate and lipid. The pancreatic juice has an alkaline pH and contains appreciable amounts of bicarbonate ions to assist in neutralisation of gastric acid. Absorption of the end products of digestion mostly takes place in the duodenum. The opening from the gizzard to the duodenum (gizzard-duodenal junction) acts as a filter and prevents large particles, including grit, from entering

the duodenum. Birds do not contain the typical mucous-secreting cells, Brunner's glands, within the duodenum, but instead the function is carried out by other glands, goblet cells or crypts of Lieberkuhn. Bile, which is synthesised in the liver, is carried to the duodenum through two ducts. It is slightly acidic (pH 6) and contains bile salts and lipids (cholesterol and phospholipids). Its secretion is dependent upon cholecystokinin-pancreozyme (CCK-PZ). Lipids are emulsified by bile to facilitate the action of pancreatic lipase. Synthesis and secretion of bile develops with age of the bird, which explains why young birds are unable to digest lipids adequately, particularly if the contain saturated fatty acids (Larbier and Leclercq, 1994). Thus, the addition of bile salts to the diets of young broilers may improve fatty acid digestibility.

Secretion of pancreatic juice is influenced by secretin, a peptide hormone that is formed in the walls of the intestine, in addition to CCK-PZ. Pancreatic juice has a high concentration of bicarbonate which facilitates the increase in pH of gastric chyme in order to ensure the activity of the pancreatic enzymes. The enzymes are secreted in the form of pro-enzymes into the intestinal lumen, and are stimulated to change to the active enzymic state in the presence of ingesta.

In addition to the pancreatic and bile secretions, intestinal juice contains enzymes secreted by the brush border of the small intestine. These are, in particular, enzymes which specialise in the hydrolysis of oligosaccharides.

The bile and pancreatic ducts enter the latter (caudal) end of the ascending limb of the duodenum, which marks the point of commencement of the jejunum. This is approximately 50 cm long.

Meckel's Diverticulum is regarded as the beginning of the ileum. Structurally the jejunum and the ileum differ little and functionally they constitute the major absorptive surface of the digestive tract. The ileum is as long as the jejunum and

leads to a ringed valve before branching out into two caeca. The small intestine is lined with small finger-like projections called villi. These are made up of blood vessels and vessels of the lymphatic system and are covered by a delicate absorptive tissue through which the chemical breakdown products of the food are absorbed. Typical peristaltic and segmentation movements occur in the small intestine and are responsible for the onward propulsion of ingesta.

(v) Large intestine (caeca and colon)

The caeca (20 cm each in the adult) are paired, blind-ended sacs present at the junction of the small and large intestine. The role of the caeca is not clear. They seem to be concerned with the absorption of water and produce a limited amount of the B vitamins. They also contain micro-organisms which decompose undigested nutrients although little, if any, of the products are absorbed. Fermentation products may be absorbed from the caeca (Hill, 1976). The caeca lead directly to the rectum which is approximately 7 cm in length, the colon being virtually absent. The ileo-caeco-colonic junction (sphincter) controls the flux of chyme between the colon and the caecae. It relaxes on distension of the ileum to allow movement towards the colon, and contracts during colonic distension. During colonic contraction, ingesta are therefore directed towards the caecae or cloaca depending upon the direction of peristalsis. Caeca filling takes place at regular intervals during *ad libitum* feeding conditions. Evacuation of the caeca appears to result from a strong contraction which starts at the base of them. Frequency of emptying, as assessed by the production of caecal droppings, varies with the diet, the degree of caecal distension, the concentration of hydrogen ions present and their electrolyte activity. Digestion of food within the large intestine is minimal. Micro-organisms decompose many of the undigested food particles and form faeces which pass to the cloaca (Sturkie, 1976; Hill, 1976; Larbier and Leclercq, 1994).

(vi) Cloaca

The cloaca is divided into three sections (the coprodeum, the urodeum and the proctodeum) by two transverse membranes. Discharge of cloacal contents occurs at frequent intervals and is caused by rapid contractions of the coprodeum (where faecal material is accumulated). As a consequence of the convergence of the digestive and urinary tracts in the region of the cloaca, urine from the kidneys arriving *via* the ureters (which enter into the urodeum) may ascend up to the caeca where water and electrolytes are absorbed from the cloacal-colonic area. Urine becomes concentrated as insoluble urates and is voided in the form of a paste covering the excrements in a white layer (Sturkie, 1976; Hill, 1976; Larbier and Leclercq, 1994).

2.7 CONCEPT OF DIGESTIBILITY

Measurements of digestibility consist of the sum of activities within the gastro-intestinal tract, including some enzyme action, absorption, transit and activity of microflora. These measurements are essential in order to define bioavailability of nutrients. For a given raw material, digestibility of constituent nutrients depends upon a number of factors, some based on the composition of the material itself and any processing treatments, and others on the bird.

Digestibility may be defined in terms of apparent or true. Apparent digestibility is the relationship between that which disappears from the intestine and that which is contained within the diet. True digestibility, which gives higher values, removes that part of the excreta which is of endogenous origin, i.e. not arising directly from the diet.

Evaluation of endogenous carbohydrates is not strictly important since, apart from those which are present within the structure of microbial glycoproteins or

gastric and intestinal mucous (glucosamine, galactosamine), no starch, saccharose, lactose or fibre is found within the digestive secretions. Thus, no distinction between apparent and true digestibility of carbohydrates can be made (Larbier and Leclercq, 1994).

Actual digestibility values may also differ depending upon whether the birds were fed *ab libitum*, fed on a restricted basis or force-fed, and depending on the rate of inclusion of the test ingredient.

2.8 WHEAT AS A DIETARY ENERGY SOURCE FOR CHICKENS

Investigations into the energy value of wheat for poultry feed using AME methodologies have found that when wheat is included into the diet in high concentrations, some of the samples have poorer nutritional values than others (Rogel *et al.*, 1987; Choct and Annison, 1990; Annison, 1991). There appears to be a wide variation between individual birds in their ability to digest high-wheat diets and research and this, at least in part, may be related to this variation, with higher intakes being associated with poorer nutritive values. However, investigations employing the TME method have not observed the same variability (Longstaff and McNab, 1986). These differences may be explained in terms of wheat variety variation (certain varieties showing poor nutritional value) and in terms of bird age - older birds tend to be less variable in terms of ME values than younger birds.

2.9.1 The role of starch

Starch represents the major source of energy in wheat and poultry diets. The effectiveness of starch utilisation is therefore an important component of poultry feeding. There have been numerous studies evaluating the digestibility and availability of starch from wheat in poultry diets. Although starch is generally

well digested, there are occasions when this is not the case. There are a number of reasons for lower starch digestibility that vary with feed ingredient and animal being fed. One interpretation is that it is not the starch *per se* that is poorly utilised but other factors, such as NSP, present within the wheat grain are capable of forming a viscous environment, thereby impeding the accessibility by digestive enzymes and thus reducing starch digestibility overall (Wiseman and Inborr, 1990). There is, however, no direct evidence that increased viscosity *per se* is responsible for the inhibition of digestion. Although wheat pentosans have been implicated in poor feeding value of some wheats (e.g. Choct and Annison, 1992a) there is again no direct evidence that reduced starch digestibility is completely attributable to the pentosans.

(i) Starch granule digestion

The susceptibility of crystallised starch to enzymic degradation is influenced by the chemical integrity and permeability of the amyloplast membrane; disruption of this membrane facilitates hydrolytic breakdown of starch to glucose (Sowokinos *et al.*,1987). The extent of starch granule breakdown is dependent on a number of factors both intrinsic and extrinsic. Those intrinsic to the plant include the chemical nature of the starch, its physical form, the presence of phospholipid and protein coating the starch granule and the distribution of starch in relation to the cell wall content of the feed. Extrinsic factors include the method of feed processing (Dreher *et al.*, 1984; Williams and Chesson, 1989; Chesson, 1990). In general, provided the grains are partially disrupted, uncooked cereal starch is digested with high efficiency by poultry. The starch granule is readily attacked and ruptured by a number of factors including enzymes, physical damage, heating and drying.

(ii) ***Starch Degrading Enzymes***

Complete enzymic digestion of starch to its breakdown product D-glucose is accomplished by a number of intestinal enzymes acting in combination (Bohinski, 1976; Robyt, 1984). Starch-degrading enzymes are widely distributed and are produced by bacteria, animals, plants and fungi (Robyt, 1984; White and Kennedy, 1988). Enzymes that catalyse the hydrolysis of starch are numerous and include α-amylase (EC 3.2.1.1, 1,4-α-D-glucan 4-glucanhydrolase), β-amylase (EC 3.2.1.2, 1,4-α-D-glucan maltohydrolase), glucoamylase (EC 3.2.1.3, 1,4-α-D-glucan glucohydrolase), isoamylase (EC 3.2.1.68, 1,6-α-gluconase) and pullulanase (EC 3.2.1.41, α-dextrin 6-glucanohydrolase). The properties and mode of action of these enzymes have been comprehensively studied and reviewed by numerous authors including Robyt (1984) and Manners (1985).

(iii) ***Enzymes of the GI Tract***

Alpha-amylase (1,4-α-D-glucan 4-glucanhydrolase)

α-Amylases are secreted by salivary glands and by the pancreas (Stryer, 1981; Robyt, 1984). Porcine pancreatic α-amylase has been used for *in vitro* studies of starch digestion. In birds, α-Amylases are endoglycosidases showing an equal preference for all α-D-(1→4) non-terminal linkages in both linear and branched glucans. Although enzymic attack follows a multiple random-splitting mechanism, the (1→6) and (1→3) linkages are not hydrolysed by α-amylase (Fogarty, 1983). Breakdown products of amyloses and amylopectins are similar - maltose, maltotriose, maltotetraose and smaller amounts of glucose - although amylopectin degradation also produces α-limit dextrins, oligosaccharides made up of 4 or more glucose molecules containing at least one branched α-D-(1→6) linkage (Fogel and Gray, 1973; Manners, 1979; Fogarty, 1983; Robyt, 1984;

Gray, 1992). The result is that the α-amylase is unable to hydrolyse α-(1→4) bonds adjacent to such a α-(1→6) linkage due to steric hindrance, producing α-limit dextrins. No significant difference has been noted in enzyme action between salivary α-amylase and pancreatic α-amylase (Manners, 1979; Modi and Kulkarni, 1976).

All α-amylases are calcium-dependent enzymes, requiring one calcium atom per molecule for activity (Fogarty, 1983). Molecular weights vary considerably, depending on the source; most range between 47,000 and 52,000 Da (Fogarty and Kelly, 1980). The pH optima is normally between pH 4.8 and 6.5 but is dependent on enzyme origin. Some enzymes are heat-stable, eg. Termamyl, and allow starch hydrolysis at up to 100°C.

Alpha-dextrinase (Isomaltase)

This enzyme, secreted by the brush border cells of the lining of the small intestine (Dreher *et al.*, 1984) and by the pancreas (Gray *et al.*,1979), attacks the α-(1→6)-interchain linkages and provides a means of debranching α-limit dextrins produced from amylolysis (Manners, 1979; Stryer, 1981; Dreher *et al.*, 1984). There are at least four isomaltases (glucosidases), of which α-dextrinase (Maltase Ia) (Dahlqvist, 1962) accounts for the major part of the activity and attacks isomaltose and limit dextrins; Maltase Ib (sucrase) degrades sucrise. Maltase II appears to be a glucoamylase (Manners, 1979) and hydrolyses maltodextrose, starch, isomaltose, limit dextrins, maltosucrose, as does its heat-stable iso-enzyme maltase III (Dahlquist, 1962. Although this enzyme is much less active towards starch than pancreatic α-amylase (Kelly and Alpers, 1973), it may act on high linear maltosaccharides such as maltohexaose and on α-dextrins (Manners, 1979). The optimum pH for activity occurs at pH 6.0 (Dreher *et al.*, 1984).

Alpha-glucosidase (α-D-glucoside glucohydrolase)

The neutral α-glucosidase appears to be the final enzyme involved in the hydrolysis of starch to glucose (Fogarty, 1983). Like α-dextrinase, it is also secreted by the mucosal cells of the small intestine and by the pancreas. α-Glucosidase catalyses the hydrolysis of terminal, non-reducing (1→2)-, (1→3)-, (1→4)- and (1→6)-linked α--D-glucosyl groups of carbohydrates (Fogarty, 1983). It could play a part in degrading α-limit dextrins into glucose (Manners, 1979). The rate of hydrolysis decreases rapidly with increasing substrate molecular weight (Fogarty, 1983). The optimum pH depends on the source, and ranges between pH 3.5-7.5.

(iv) ***Starch Digestion in Non Ruminants***

Digestion in the upper gastro-intestinal tract (GIT)

Starch must be completely hydrolysed to glucose before it can be absorbed in the small intestine. Starch digestion is initiated in the mouth by the action of salivary α-amylase although the food does not remain in the mouth long enough for the α-amylase to have any major effect prior to its inactivation in the acidic environment of the stomach. Poultry saliva is not, however, thought to contain α-amylase in any quantity, but some starch hydrolysis may occur in the crop due to microbial action (Bolton, 1965). The main site of digestion is the small intestinal lumen (Fogel and Gray, 1973; Manners, 1979; Dreher *et a*l., 1984; Longland, 1991; Annison and Topping, 1994) and the glycocalyx fibers of the microvilli (Dreher *et al.*, 1984) chiefly due to the action of pancreatic α-amylase. The pH of the gastric digesta flowing into the duodenum is gradually raised to a level suitable for carbohydrase activity by the secretion of alkaline pancreatic juice, bile and the products of Brunner's glands (not present in birds).

The major carbohydratases produced in the brush border of the small intestinal mucosa are the four maltases, lactase and trehalase. Lactase and trehalase are not, however, produced in birds. Pancreatic amylase is produced in very large amounts and is capable of hydrolysing 5-10 times the amount of substrate ingested (Longland, 1991). Fowl pancreatic α-amylase activity acts truly randomly on the internal linkages of starch molecules (Longland, 1991). The end products of digestion are composed largely of maltose, α-limit dextrins, and small amounts of glucose and maltosaccharides (Dreher *et al.*, 1984; Annison and Topping, 1994).

Glucose is absorbed directly through the intestinal mucosa, whereas the oligosaccharides are acted upon by membrane-bound glucosidases. These are further degraded to glucose by a series of additional, inducible carbohydrases (Levin, 1989) in the brush border of the small intestine. These enzymes hydrolyse branched linkages and convert linear maltosaccharides to glucose. By removing successive glucose units from the extended α-glucan chains, maltose is converted to glucose. The sucrase enzymes have a higher affinity for maltose and its higher oligosaccharides which are cleaved by the sucrase α-dextrinase complex (sucrase isomaltase). This is thought to complete the end stages of hydrolysis (Gray, 1992). Sucrose is split into glucose and fructose. The free glucose, thus released, is actively transported by a carrier-mediated process across the mucosal membrane into the body (Dreher *et al.*, 1984) which is driven by sodium/potassium ATPases. Carbohydrate molecules larger than monosaccharides cannot pass the intestinal barrier (Levin, 1989). For poorly digested starches amylolysis may be the rate-limiting step (Sugimoto *et al.*, 1976; Manners, 1979); the luminal digestion phase is thought to be the rate-limiting step overall (Fogel and Gray, 1973).

Fermentation in the lower GIT: Role of Intestinal Microflora

Assumptions that starch is completely digested and absorbed in the small intestine, while NSP passes straight through unchanged into the colon to be fermented, have been based upon two theories: (i) the presence of starch hydrolysing enzymes in pancreatic secretions in amounts thought to be far in excess of those needed for the complete degradation of starch *in vitro* (ii) the failure to demonstrate the presence of endogenous enzymes capable of breaking down NSP material. However, significant amounts of starch escape digestion in the small intestine of nonruminant animals including pigs (e.g. Chesson, 1990) and to a lesser extent, poultry. Starch that remains undigested in the upper GIT, together with the non-starch polysaccharide component, constitutes a readily available substrate for the colonic microflora (Englyst and MacFarlane, 1986; Shetty and Kurpad, 1986). The products formed during fermentation are mainly short-chain organic (fatty) acids (SCFA), mainly acetic, propionic and butyric acids (representing 0.85-0.95 of the total SCFA), lactate and gases (carbon dioxide, methane and hydrogen) (Cummings, 1985; Cummings and Englyst, 1987; Björck and Siljeström, 1992).

Conventionally, the hindgut is considered to be the site of microbial fermentation, but there is increasing evidence that a significant amount of microbial activity is found in the lower part of the ileum (Millard and Chesson, 1984; Graham *et al.*,1986). Although it is of little nutritional consequence whether volatile fatty acid (VFA) production occurs in the upper (fore-) or lower (hind-) part of the intestines, since the nutritive value of the VFA remains constant, the site of fermentation may be of nutritional significance. A well established foregut microflora can compete directly with the host for potentially absorbable substrates if the absorption rates are reduced by the presence of other nutrients, such as gel-forming polysaccharides. Measurement of the disappearance of carbohydrate beyond the terminal ileum makes no allowance

for foregut fermentation and estimations of fermentative activity derived by this method are therefore likely to be underestimated.

While the mechanism of polysaccharide degradation and the structural factors determining the extent of hydrolysis are independent of the host species and of the site within the GIT, the microbial population directly or indirectly involved in polysaccharide fermentation can show considerable variation (Chesson, 1990). The pattern of SCFAs production depends on the substrate being fermented. All substrates produce acetate as the major end product but the relative amounts of propionate and butyrate vary. Xylan and pectin lead to very little propionate and butyrate whereas arabinogalactan produces more propionate (Cummings and Englyst, 1987).

(v) Factors affecting digestion of starch

For raw starches, research has shown that variation in the rate and extent of starch digestion may occur as a result of a number of factors including the physical nature of the starch (Anderson *et al.*,1981; O'Dea *et al.*, 1981), its chemical properties, physical distribution of starch in relation to other components (Hale, 1973; Snow and O'Dea, 1981) which bring about mechanical barriers in the food matrix and complex formations such as protein-starch interactions (Liener, 1969; McNeill *et al.*, 1975; Anderson *et al.*, 1981), amylose-lipid interactions (Holm *et al.*,1983) or the presence of dietary fibre components such as cellulose, hemicellulose and lignin (Anderson and Chen, 1979; Jenkins *et al.*,1980; Snow and O'Dea, 1981; Dreher *et al.*, 1984). Any chemical or physical interferences with enzyme action (Dreher *et al.*, 1984), type of processing, presence or absence of anti-metabolic factors, lectins (Liener, 1982), phytates (Sharma *et al.*,1978) and enzyme inhibitors (Griffiths, 1979), in particular, α-amylase inhibitors (Shainkin and Birk, 1970; Wolever *et al.*,1983; Yoon *et al.*, 1983), particle size (Snow and O'Dea, 1981), drying method

(Kayisu and Hood, 1979), as well as frozen and refrigerated storage (Kayisu and Hood, 1979), and an excess intake of starch in a less available form, as in unmilled grains (Snow and O'Dea, 1981) may all affect starch digestibility.

The structure of starch resisting hydrolysis in the small intestine depends on the nature of the food ingested but also on several 'external' factors depending on the environment of starch in the gut.

Amylase concentration and transit time are thought to affect the amount of starch reaching the colon (Booher *et al.*, 1951). Although pancreatic α-amylase activity is relatively low at birth, newly hatched chicks have sufficient α-amylase (Rogel *et al.*, 1987) (and protease) which is produced during embryonic growth and which increases substantially with age (Longland, 1991; Nitsan *et al.*,1991). This finding correlates with the fact that digestibility of nutrients by the young chick increases with age (e.g. Rao and Clandinin, 1970; Bartov, 1988) and that the specific activity of the enzyme reaches a maximum 1 day post-hatching. More likely, evidence indicates that the specific activity of amylase (trypsin and lipase) in the pancreas decreases during the first 3 to 6 days after hatching, and increases afterwards to between 10 and 20% higher than that at hatching on day 11 for amylase (14 and 21 for trypsin and lipase respectively), and the specific activity of small intestinal amylase within the chick rises from day 2 to day 17 (Nitsan *et al.*, 1991) when it is about 5-fold that at hatching. Considerable evidence is available confirming that even the young chick secretes pancreatic α-amylase into the small intestine in excess of its requirements (Moran, 1982; Rogel *et al.*, 1987; Longland, 1991). The greatest activity is in the jejunum (Osman, 1982) and starch digestion occurs even when most of the pancreas is removed (Ariyoshi *et al.*, 1964). Levels of α-amylase are therefore believed not to be linked with poor digestibility values of starch in young broilers, although Nitsan *et al.*(1991) suggested that this might represent a limiting factor to early digestion. The role of the gut microflora in starch

digestion might be significant.

Pancreatic enzyme synthesis and secretion appear to be regulated to meet the particular needs of the diet. Alterations in dietary starch levels lead to quantitative changes in α-amylase released from the pancreas which would accommodate complete digestion if no other factors (intrinsic or extrinsic) are involved.

Intrinsic factors and digestibility

The digestibility of starch granules *per se* may be affected by a number of factors.

(a) Physical barriers

Food particle size will affect availability of nutrients for digestibility (Robertson, 1988; Annison and Topping, 1994). Particle size can affect starch digestion by amylase as a result of surface area because smaller particles (which have a larger surface area to volume) are digested more rapidly than larger ones. During transit, particles can change in size, shape, porosity and number. The outcome is to increase the surface area available and accessibility of the particles to digestion (Robertson, 1988). Feeds containing high amounts of cell wall material, which are considered indigestible in the small intestine, can influence the rate of digestion by acting as physical barriers and encapsulating, for example, the starch granules. Particle size is unlikely to be the rate-limiting step in non-ruminant digestion although it could affect the rate of digestion by affecting nutrient availability (Kahlon *et al.* 1986). Disruption of cell walls is a prerequisite for efficient digestion. All cereals are more susceptible to hydrolysis in the finely ground form (Snow and O'Dea, 1981). Processes such as milling and grinding act in several ways: firstly they eliminate physical barriers, secondly

they increase starch granule accessibility, and thirdly they mechanically damage the starch granules through the impact and shear. The smooth surface of the native starch granules becomes roughened and cracked after milling (Stark and Yin, 1986) and may even be split or fragmented. Such a process may increase the susceptibility of starch-containing materials by up to several fold (Wong and Traianedes, 1985; Schweizer *et al.*,1988), although there is conflicting evidence and research has shown that feeding whole wheat grains to poultry results in higher nutritive values (Mollah *et al.*, 1983).

Under some processing conditions, the long α-glucan chains can form inclusion complexes with fatty acids (Holm *et al.*, 1983). These complexes can effect digestibility.

(b) Starch structure

Digestibility of starch is highly dependent upon starch source (i.e. cereal, variety, plant species) and can depend on starch type (i.e. normal versus waxy varieties) (Classen 1996). Intact granular starches are more resistant to attack by α-amylase than are starch solutions (Sugimoto *et al.*,1976; Manners, 1979) and studies *in vitro* have shown that smaller starch granules are more susceptible to hydrolysis by α-amylase than larger starch granules (Sugimoto *et al.*, 1976; Franco and Ciacco, 1992). Although the source of starch largely determines the susceptibility to α-amylolysis, each granule within the population differs in susceptibility to degradation owing to their individuality (Greenwood, 1979).

(c) α-Amylase inhibitors

Amylase inhibitors are known to be present in wheat at high concentrations, to be active against chicken pancreatic amylases (Buonocore, 1977 cited by McNab, 1993) and are thought to affect the rate of starch digestion. It seems

unlikely, however, that they will have much nutritional significance in practice since the α-amylase inhibitors from wheat are believed to be destroyed and inactivated by pepsin in the gizzard (Kneen and Sandstedt, 1946; Macri *et al.*, 1977). Macri *et al.* (1977) showed that these inhibitors fed to chickens did not result in growth rate reduction, and only if exceptionally large amounts of the inhibitor are used in feeding experiments is the availability of energy from dietary starch affected. However, evidence has indicated that large amounts of the inhibitor may overcome gastric digestion. Starch has been known to escape digestion in rats when wheat containing high amylase inhibitor activity was fed (Lajolo *et al.*,1984). This may be indicative of the potency of this factor *in vivo* and explain, in part, why some wheat varieties are poorly digested.

Two types of α-amylase inhibitor have been identified. The type of amylase inhibitor found in wheat (rye and oats) is fairly common and is of protein or glycoprotein nature. It is non-competitive, non-dialysable, α-amylase specific and relatively heat labile (except possibly in oats and rye) (Dreher *et al.*,1984). Kneen and Sandstedt (1946) reported that the inhibitor concentration increases with wheat maturation and it is present in similar amounts in all wheat types. The inhibitor is mainly located in the endosperm associated with the starch granules (Sandstedt and Beckford, 1946) and little is present within the bran portion (Kneen and Sandstedt, 1946). The activity of the inhibitor in wheat is destroyed after 30 min at 121 °C (Kneen and Sandstedt, 1946) and by a number of reducing agents such as ascorbic acid and oxidising agents such as hydrogen peroxide (Marshall, 1975).

External factors and digestibility

In addition to the intrinsic properties of starch granules themselves, external factors may account for some of the resistance of some starch granules to digestion.

(a) Processing

Processing alters the starch by converting it from a crystalline to a gel structure, which promotes efficient interaction with the α-amylases in the small intestine. When the starch granules are heated above a characteristic temperature (50-70°C) the granules lose their degree of order (measured by birefringence and X-ray crystallinity), the hydrogen bonds break and the granules swell in size (Colonna *et al.*,1992). For example, gelatinisation of barley starch granules, which occurs at 60°C, brings about a concomitant large increase in α-amylolysis. However, bird age may exert a large effect on the degree of improvement gained from processing; for younger birds with less well developed digestive tracts, processing may prove more beneficial (Bayley *et al.*, 1968). However, the presence of water during processing is crucial since dry heating of starch has very little effect on α-amylase digestibility *in vitro*. Indeed, in some cases, heating cereals grains may be ineffective or even have a detrimental effect on digestibility and nutritive value (Herstad and McNab, 1975; Antoniou and Marquardt, 1981). The combination of grinding and steam treatment appears to be less effective in modifying the granule integrity than steam heating together with physical pressure exerted by extrusion, which was particularly effective in facilitating digestion (Mercier and Guilbot, 1974).

(b) Addition of supplementary enzymes

Dietary supplementation with microbial enzyme preparations capable of hydrolysing endosperm cell walls has increased performance of broiler chickens receiving cereal-based diets (Burnett, 1966; White *et al.*, 1981; Hesselman and Åman, 1986; Pettersson and Åman, 1988; Friesen *et al.*, 1992). The increased nutritive value is brought about by a reduction of intestinal viscosity, the effects which are influenced by the content of wheat, or other cereal, in the diet (Veldman and Vahl, 1994). The high molecular weight pentosans and ß-glucans

present in the cell walls of cereals are water-soluble and create a viscous environment within the intestine. Enzymes which break down these complexes allow greater accessibility by the digestive enzymes to the nutrients.

(vi) Enzymic Attack of Starch Granules

Enzyme attack follows a specific degradation pattern, characteristic for each starch species and, is dependent upon enzyme source (Sugimoto *et al.*, 1980). The pattern of digestion that develops when native wheat starch granules ('A' type), for example, are exposed to α-amylase is one of a general surface attack and the equatorial groove is particularly susceptible. Enzymic attack by glucosidase on the other hand has a significantly greater effect on granule morphology than attack by α-amylase (Paramahans and Tharanathan, 1982). For example, glucosidase can hydrolyse about 0.90 of native legume starch compared with 0.24 hydrolysis with α-amylase.

Scanning electron microscopy (SEM) of unmodified or undamaged starch granules shows that the granule surface is relatively smooth and free from pores, cracks or fissures; enzyme-digested starch granules erode and crack open, exposing a defined layer structure (Dronzek *et al.*, 1972; Sugimoto *et al.*, 1980) on the inner portions. SEM on rat faeces has revealed that different starches are attacked in different patterns and, variations in resistance to enzyme action within the same granule; the most readily hydrolysed are the amorphous regions (Greenwood, 1970; Blanshard, 1987), and amylopectin is more resistant to enzymic attack (Yin and Stark, 1988). Studies on wheat starch granules have shown that the centre region of the granule is less resistant to α-amylolysis than other areas (Evers and McDermott, 1970; Evers *et al.*, 1971). The hydrolysis of granules from different starch sources has been examined by SEM and TEM (transmission EM) (Gallant *et al.*,1973) and, in the case of wheat starch granule degradation by pancreatin proceeded both tangentially and radially

(with the apparently random formation of channels) and a 'saw tooth' pattern emerged where the amorphous regions were preferentially attacked in the tangential direction. A similar but more rapid process leading to granule fragmentation was reported for waxy and normal maize. The morphology of starch granules resistant to amylolysis is unaffected, and is similar to the intact granules. However, those attacked by enzymes show a pitted surface with pores penetrating deep into the granule interior (Fuwa,1977; Fuwa *et al.*,1978,1979).

Granules of normal maize starch show surface circular pits, followed by internal hydrolysis when exposed to amylases (Greenwood, 1979; Sugimoto *et al.*, 1980; Dreh*er et al.*, 1984). Waxy cereal varieties show point hydrolysis and, oat starch generally disappears rather than exhibiting pit formation (Dreher *et al.*, 1984). Similarly, variability in the susceptibility of starch granules isolated from different sources has been demonstrated by *in vitro* studies on starch hydrolysis by SEM using pancreatic α-amylase, pancreatin (Fuwa *et al.*, 1979; Sugimoto *et al.*,1979) and bacterial α-amylase (Cone and Wolters, 1990; Ring *et al.*,1988).

SEM studies (rat feeding trials) have shown that enzymes from the enterobacteria in the large intestine are also involved (Sugimoto *et al.*, 1976).

(vi) Susceptibility of Starch Granules to Hydrolysis by α-Amylases

Although α-amylase is able to degrade the intact starch granule, the processing of cereals significantly improves this process because of alterations to the structure of the starch granules structure. Susceptibility of starch granules to hydrolysis by amylases is known to be dependent on several other factors, although the factors affecting the digestibility of wheat, and other cereals grains, are not clearly known.

Despite the general belief that cereal starches are readily and completely

digestible, studies using porcine amylase have shown that raw cereal starches, although almost completely digested *in vivo* (Booher *et al.*,1951; Holm *et al.*, 1983), seem to be poorly digested *in vitro* (Mercier and Feillet, 1975; Delort-Laval and Mercier, 1976; Sugimoto *et al.*, 1980; Björck *et al.*,1984). At present, measurement of starch hydrolysis *in vivo* remains the method of choice (Dreher *et al.*, 1984). Simple *in vitro* systems can, however, be useful when comparing starch from different sources or following different methods of feed preparation.

2.9.2 The nutritional role of wheat cell wall polysaccharides

The dietary fibre fraction, present in the cell wall, consists mainly of NSP which includes all the non α-glucan carbohydrate fractions such as hemicelluloses, cellulose, pectins, gums, ß-glucans and the non-cellulosic polysaccharides (e.g. Englyst and Cummings, 1987). Burnett (1966) demonstrated that the highly viscous, water-soluble ß-glucans in barley and oats are the primary growth-depressing factors limiting the bioavailable energy of these cereal grains. Similarly, Annison (1991) has shown that it is the water-soluble pentosans in wheat which exert similar effects when fed to poultry, and in both cases resulting in the production of wet, sticky droppings (Burnett, 1966; Annison, 1992).

The NSP are resistant to digestion in the gastro-intestinal tract of birds because of the lack of suitable endogenous enzymes (Wiseman and Inborr, 1990). In addition, microbial degradation of NSP in the hindgut of poultry is minimal (Carré and Leclercq, 1985; Longstaff and McNab, 1986, 1989). Digestibility coefficients of pentosans have been reported as ranging between 0.33 and 0.43 for adult birds when fed wheat as the sole dietary constituent *ad libitum* (Bolton, 1955a,b; Thorburn and Wilcox, 1965).

Wheat pentosans are believed to contribute to the low-AME phenomenon

(Annison, 1990) by exerting their effects on the digestibility of starch (Annison and Johnson, 1989). The soluble fraction of the arabinoxylans are considered to be of major importance in determining the utilisation of starch by poultry. The effects of this fraction may be described in terms of two mechanisms of action: (i) after ingestion, the arabinoxylans (and ß-glucans) solubilise, resulting in increased digesta viscosity (Salih *et al.*, 1991; Teitge *et al.*,1991); (ii) the cell walls act as a barrier preventing or slowing access of endogenous enzymes to nutrients.

The exact effect of viscosity has not been established but it is known to be a major factor influencing the nutritional value of rye, barley and oats, and more recently similar evidence has become available for wheat (Bedford and Classen, 1992b). Possible consequences for the increase in digesta viscosity include reduced rates of diffusion of endogenous enzymes and nutritional substrates and increased feed passage time. Changes in digesta flow rate might result in increased microbial numbers in the small intestine (Salih *et al.*, 1991). Digesta viscosity also plays a role in the secretion of pancreatic enzymes. As viscosity increases, the level of a number of pancreatic enzymes in the small intestine lumen, including amylase, decreases. Although this does not necessarily indicate a deficiency of the enzyme, it does indicate a potential for reduced grain digestibility when digesta viscosity increases (Classen, 1996).

The cell walls containing arabinoxylans and ß-glucans also may act as a physical barrier to endogenous enzymes and therefore reduce the utilisation of starch and protein encapsulated within endospermal cells (Hesselman and Åman, 1986). This effect could reduce total starch digestion or delay digestion of starch until the distal portion of the small intestine. This may be explained (Classen, 1996) since the breakdown of ß-glucan *via* enzyme addition results in a more anterior disappearance of starch in the small intestine, which may result in more efficient starch utilisation through reduced microbial digestion.

In general, feeding cereal grains containing relatively high levels of soluble arabinoxylan or ß-glucan, including wheat, reduces nutrient assimilation, growth rate and the efficiency of feed utilisation (Edney *et al.*,1989; Rotter *et al.*,1990; Friesen *et al.*, 1992). The effect on digestibility is general rather than being specific to starch.

2.9.3 Use of supplementary enzymes in chick diets

Since poultry do not produce suitable enzymes capable of digesting cellulose, arabinoxylans, ß-glucans or other cell wall components, the use of processing techniques or exogenous enzymes added to the diets has been employed to try and improve the nutritional value of diets containing high levels of such components. In order to reduce the intestinal viscosity it is necessary to break down the polymer complex sufficiently such that the polymers cannot associate in large networks (Bedford, 1995). Processing techniques such as pelleting and extrusion are not always adequate to bring about such conversions in some diets containing high levels of rye and barley (Bedford, 1992). Polymer breakdown has, however, been achieved with the use of enzymes supplemented in poultry feeds. The level of hydrolysis required to improve the nutritional value of cereal grains is important is terms of type and amount of enzyme to be used. Based on viscosity and/or encapsulation as the major effects of such cell wall polysaccharides, only minor disruption of the larger molecular weight substrates to shorter chains is required to achieve the majority of the improvement (White *et al.*,1983; Campbell *et al.*,1986). Cleavage of relatively few linkages leads rapidly to chain shortening and subsequent loss of gel-forming properties. Thus, enzymes which cleave the central structure of the polysaccharide are of potential value. Microbial enzymes are now used extensively to improve the nutritional value of cereals (Classen, 1996).

Arabinoxylan hydrolysis is primarily accomplished by end-1,4-ß-xylanase activity,

which cleaves (1→4)-linkages of the xylan backbone. Studies on broilers fed wheat and rye based diets have shown that the addition of pentosanases and glucanases reduces intestinal viscosity and improves the AME value of the feed (Annison, 1992; Bedford and Classen, 1992; Brenes *et al.*, 1993; Marquardt *et al.*, 1994; Choct *et al.*, 1995).

The increase in AME depends largely on the AME value prior to enzyme addition. Choct *et al.* (1995) found that wheat samples with low AME could be improved by up to 24% upon enzyme supplementation but only 2% improvement in 'normal' wheats could be achieved. It might be that a combination of enzymes is required to bring about the desired effects.

2.9.4 Prediction of nutritive value and ME

The availability of a rapid, indirect assay for viscous polysaccharides or AME / digestibility coefficients would greatly facilitate the screening of wheat cultivars for potential nutritional value.

(i) Prediction of ME from chemical composition

The AME of feed ingredients can be predicted with variable precision depending upon which method is employed. Equations have shown increasing improvements in their accuracy over the past few years, with correlations as high as $r^2 = 0.985$ (Fisher, 1982). Three different methods have been cited (Janssen, 1976), each having their drawbacks. The first depends on chemical analysis using the Weende system of proximate analysis and the determined digestibility of the component in a feedstuff. Secondly, there is the application of average digestibility coefficients to the chemical components of the feedstuff or diet (Nehring and Haenlein, 1973). Since poultry excrete faeces and urine together, the second method is probably more appropriate than the first and

generally depends on the determination of sugars and starch. The method of Carpenter and Clegg (1956), based on starch, sugar, crude protein, ether extract and dry matter has been viewed as the most precise equation, although lower AME values may arise for ingredients such as maize, wheat feed and wheat middlings (Davidson and Graham, 1981). In the third method, simple relationships are derived from one or more chemical or physical characteristics of a feed ingredient. Since fibre is poorly digested by poultry, there is generally an inverse relationship between crude fibre and ME of feedstuffs and diets (e.g. Moir and Connor, 1977). This method may not, however, prove to be satisfactory. For example, these equations are unable to predict the low AME of wheats containing starch that may be incompletely digested by chickens (Coates *et al.*, 1977; Davidson *et al.*, 1978; Mollah *et al.*,1983). AME was not well correlated with starch content, suggesting incomplete digestion of starch in the bird (Mollah and Annison, 1981). Due to the difficulties thus associated with single-prediction equations, replacement by multiple linear regression has allowed greater degrees of significance in the values of coefficients assigned to variables (Härtel, 1979).

Most of the equations predicting AME_n are based on chemical measurements of crude nutrients. Theoretically, therefore, these equations cannot take account of variations in digestibility of nutrients (Carré, 1990). In spite of this, equations predicting 0.97-0.98 of the variation in AME_n values (adult) of compound feeds have been reported (Fisher, 1982; Carré *et al.*, 1984). These figures may not be surprising in view of the fact that nutrients with low digestibility values are normally included in diets at low levels. Equations predicting AME_n are divided into those assigned to compound feeds and those for raw materials because, in contrast to mixed diets, digestibility of nutrients in raw materials can be wide ranging.

It has been suggested (Härtel, 1979) that the time and cost of chemical analyses

may not be justified in view of the rapid, biological methods of determining ME that are now available (Sibbald, 1976; Farrell, 1978).

(ii) Extract viscosity

According to Campbell *et al.*, (1989), extract viscosity is an important determinant of the feeding value of barley. Techniques that measure the viscosity of barley extracts as an indirect indication of the soluble ß-glucan content (Greenberg and Whitmore, 1974; Morgan and Gothard, 1977; Aastrup, 1979) and as an index of anti-nutritional activity (Hesselman and Åman, 1986) are routinely used in feed-barley breeding programmes. Aastrup (1979; 1983) found that the viscosity of an acid extract of barley was closely related to the content of soluble ß-glucan ($r^2 = 0.99$), whereas, Bhatty (1987) reported a value of $r^2 = 0.91$ for the soluble and $r^2 = 0.83$ for the total ß-glucan content, which may serve as a screening method to detect low total ß-glucan levels in barley genotypes (Aastrup, 1983). Genotypic differences with respect to extract viscosity were an important determinant of feeding value. Broiler chicks fed on diets containing low extract viscosity genotypes of barley performed better than broilers given diets with high viscosity types. Enzyme supplementation improved the feeding value of barley and eliminated differences in the feeding values of barleys with high extract viscosity. Burnett (1966) also demonstrated that feeding barleys with highly viscous aqueous extracts resulted in effective chick performances only with the addition of suitable enzyme preparations to the diets.

Soluble NSPs are believed to increase digesta viscosity which, in turn, inhibits nutrient digestion and absorption (Choct and Annison, 1992b). Extract viscosity is said to account for more than half of the variability of AME, and according to Choct *et al.* (1992b), may help to assess wheat AME values indirectly, but it cannot be used to predict AME precisely. This may be due to the inadequacies

of the current *in vitro* viscosity assay to accurately predict *in vivo* conditions, but it also may highlight the fact that viscosity is only one factor involved and that other mechanisms may play a significant role.

Bengtsson *et al.* (1992) found that extract viscosity of rye was correlated with content and structural characteristics of arabinoxylans. Boros *et al.* (1993) also revealed that extract viscosity may be used as an indirect assay for quantifying the water-soluble pentosan content in rye. The simple, sensitive and reliable procedure also indicated a positive relationship between viscosity of the extract and the total amount of arabinose, xylose and non-carbohydrate components, although most of the viscosity of the water-soluble extracts could be attributed to the soluble arabinoxylans.

Bedford and Classen (1993) investigated the possibility of using an *in vitro* system to predict accurately the intestinal viscosity of chicks fed rye-based diets to serve as a rapid screening method for potentially useful enzyme preparations. Results from their studies demonstrated a reliable assay for predicting the intestinal *in vivo* viscosity and growth-promoting ability of an enzyme in chicks fed rye-based diets.

CHAPTER 3 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Wheat Samples

Details of individual wheat varieties analysed and used in the chick bioassays, together with information regarding their origin and year of production, are given in Table 3.1.

Wheats used in experimental poultry diets were milled (Christy and Norris hammer mill) to pass through a 3.0mm screen. Milled samples were stored in paper sacks in rodent-proof containers in a cool room until required (usually 1-2 weeks).

Wheats for biochemical analysis were milled using a Fritsch high speed rotor mill Pulverisette 14 (Christison Scientific Equipment, Limited, Gateshead) to pass through a 0.5mm screen. Ground samples were stored at 1°C in air-tight containers.

3.1.2 Constituents of the Experimental Diets

Details of the diets used in each chick bioassay are presented in Table 3.2. Soya-protein isolate (PP 500E) was from Protein Technologies International, St. Louis, Mo). Blended vegetable oil (Nurdin and Peacock, Raynes Park, London) was obtained from Simpsons Farm Suppliers, Shepshed, Leicestershire. The mineral-vitamin premix was purchased from Ian Hollows, Whitchurch, Shropshire and was desigend to meet the macro and micro minreal and vitamin requirements of the young broiler when included at a level of $50g.kg^{-1}$

3.1.3 Starch Granules

Starch granules were extracted from certain wheat varieties (Table 3.1), intestinal contents and excreta samples according to the method outlined in section 3.3.9. Commercially isolated starch granules from wheat were purchased from Sigma Chemical Company, Poole, Dorset.

3.1.4 Chemicals, Biochemicals and Enzymes

Unless otherwise stated, all reagents used were of analytical grade quality and were obtained from Fisons Scientific Equipment Plc, Loughborough. Fine chemicals, biochemicals and enzymes were obtained from Sigma Chemical Company Ltd, Poole, Dorset, unless stated otherwise. Pancrex V capsules (Paines and Byrne Ltd, Greenford, Middlesex) were purchased though a local pharmaceutical chemist. Termamyl (120L) and Amyloglucosidase (300 AGU.ml^{-1}) were supplied by Novo Nordisk Bioindustries Ltd, Farnham, Surrey. Glucose test kits (Glucose Oxidase) were obtained from Boehringer Mannheim UK, Lewes, East Sussex.

3.2 METHODS

3.2.1 Determination of Wheat AME Values

(i) Diet Formulation

The composition of diets used in the chick bioassays are presented in Table 3.2. Preliminary assays using experimental diets with wheat incorporated at levels of 900g.kg^{-1} (diets A and B), to try and accentuate nutritional differences between the wheats, resulted in physiological problems in the chicks characterised by copious production of creamy excreta. All subsequent bioassays utilised dietary

incorporation levels of wheat of 750g.kg^{-1}. This level was considered optimal for differentiating between wheats on the basis of nutritional value.

Diets were mixed thoroughly in a Kenwood Major mixer for 20 minutes and stored at 4-5°C until required. For control diets, a wheat of known high AME value was used.

(ii) Bird Husbandry

Day old, Ross I male commercial broiler chicks (Dove Valley Poultry Limited, Ashbourne, Derbyshire) were used in bioassays I to VI inclusive. Day old PM3 male broiler chicks (Premier Poultry Limited, Dalton Chicken Hatchery, Thirsk, North Yorkshire) were used in bioassays VII and VIII. Birds were housed in wire-bottomed metabolism cages (4 per cage) located in metabolism rooms with artificial lighting, heating and ventilation. The photoperiod throughout was 23hr per day and the temperature was maintained at 33°C ± 1°C for the first 4 days, after which it was reduced at 1°C per day until 27°C was achieved when the chicks were 10d of age. For the first 7 days after arrival, the birds were fed on a commercial chick starter diet. On day 8, birds were transferred and housed two per cage with mean bird weight per cage being approximately equal. The birds were divided into groups and allocated to the experimental diets in a fully randomised design (six replicates per dietary treatment). Feed (mash form) and fresh water were supplied *ad libitum*.

(iii) Determination of Apparent Metabolisable Energy (AME) values

AME experiments were conducted according to the classical balance (total collection) method. Metabolism cages enabled the collection of excreta on pre-weighed foil-lined trays. Experimental diets were offered for 3 days in an adaptation period followed by a 3 day total collection (balance) period. Feed

intakes and excreta were recorded daily and corrected for feed spillage. Fresh excreta were frozen immediately, freeze-dried and weighed. Dried excreta were milled to a fine powder in a coffee grinder and stored in air-tight containers at 1°C. Analyses were carried out on the pooled excreta collected per cage in the balance period.

Gross energies of excreta and dietary ingredients were determined by conventional bomb calorimetry. From this data, dietary AME values ($MJ.kg^{-1}$ DM) were calculated by means of the equation:

$$AMEdiet = \frac{GEIntake - GEOut}{DMIntake(kg)} [MJ.kg-1DietaryDM]$$

where GEin and GEout = Gross Energy expressed in $MJ.kg^{-1}$ DM

Nitrogen corrected AME_n values were calculated as follows where N correction = N retention x 34.39:

$$AMEndiet = \frac{GEIntake - (GEOut + Ncorrection)}{DMIntake(kg)} [MJ.kg-1DietaryDM]$$

Excreta energy was calculated using the equation:

$$ExcretaEnergy = \frac{GEexcreta \times DMOut}{DMIntake} [MJ.kg-1DM]$$

The N correction value is obtained by multiplying the value obtained for N retention by a factor of 34.39 $MJ.kg^{-1}$ (Hill and Anderson, 1958). This is necessary because not all the gross energy of protein retained can be utilised. It is therefore convenient to adjust to zero nitrogen retention by adding to the excreta energy the amount of uric acid equivalent to the nitrogen retained per kg of feed (34.39 MJ. kg^{-1} of uric acid nitrogen). The numerical impact of the

correction is relatively small (Scott *et al.*, 1976).

Nitrogen retention (per kg diet) was calculated using the following equation:

$$Nretention = \frac{NIntake - NOutput}{DMIntake}[kg]$$

The AME value of the wheats was calculated assuming that the AME of all dietary ingredients is additive. Thus,

$$AMEwheat = AMEdiet - \sum AMEindividualdietarycomponents[MJ.kg-1DM]$$

The AME value (MJ.kg^{-1} DM) of dietary oil was taken as 36.560 from Wiseman *et al.* (1991). AME values for soya protein isolate, dl-methionine and l-lysine were taken as 18.006, 21.004 and 17.028 respectively from Bourdon *et al.* (1987).

(iv) Digesta Viscosity Measurements

In later trials, following the balance period, birds were slaughtered on the following day by carbon dioxide asphyxiation and the digesta recovered for viscosity measurements and compositional analysis.

Complete intestines from jejunum to the ileo-caecal junction were removed and weighed. The contents of the foregut (gizzard to Meckel's Diverticulum - jejunal or proximal samples) and hindgut (from Mickel's Diverticulum to the ileo-caeco-colic junction - distal samples) were recovered separately. The foregut and hindgut samples for two birds constituting a replicate (cage) were combined, centrifuged at 10,000xg for 3min and the supernatants were removed for viscosity measurements. The pellets were frozen (-18°C) until required for compositional analysis. Viscosity measurements were carried out immediately in

duplicate or triplicate (0.5ml aliquots of the supernatant) using a Brookfield digital viscometer (LV TDV-II-CP, Brookfield Engineering Laboratories Inc., Stroughton, MA) at 41°C (core body temperature of the young broiler chick) at a shear rate of between 22.5 and $450s^{-1}$ with a 0.8° cone spindle. The average time interval from slaughter to viscosity measurement (2 birds) was approximately 60 minutes (for both foregut and hindgut samples). All remaining supernatants were frozen for subsequent analysis.

(v) Determination of Apparent Digestibilities

The apparent digestibilities of DM, starch, lipid, nitrogen and non-starch polysaccharides (NSP) were calculated as follows:

$$Coefficient of Apparent Digestibility = \frac{Intake - Output}{Intake}$$

Starch was completely removed from samples to eliminate errors in the estimations of the apparent digestion of NSP in the small intestine. Errors in analysis resulting from the contamination of droppings with spilled food were minimised by maintaining the smallest amount of feed available in the feed hoppers, commensurable with *ad libitum* feeding. Most of the spilled feed could be easily brushed off the dry excreta before collection.

3.3 ANALYTICAL METHODS

3.3.1 Sampling

All measurements were carried out in duplicate unless otherwise stated.

3.3.2 Dry Weights

All samples were dried at 100-105°C to constant weight in an oven for the determination of moisture content. All data are expressed on a dry weight basis.

3.3.3 Measurement of Starch

Methods for the determination of starch have proved inconsistent and unreliable. An enzymic method for starch analysis was developed (see Chapter 4).

3.3.4 Determination of Gross Energy

The gross energy refers to the energy released on the complete combustion under oxygen of the material and is indicative of the total potential energy of a feed.

Gross energy values for wheat (1g), diet (1g), soya-protein isolate (0.6g), vegetable oil (0.25g) and excreta (1g) were determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, MO) following Standard Operating Procedures and corrected for dry matter. Benzoic acid standards were used daily to ascertain the value of the bomb constant (correction factor).

3.3.5 Determination of Nitrogen

The nitrogen content of wheats, soya-protein isolate, diets and excreta was determined by a standard Kjeldahl procedure employing a Tecator digestion system 20 (Tecator 1015 digester) together with a steam distillation unit (Tecator Kjeltec system 1002). All values were corrected for dry matter. Samples were corrected for blanks and wheat protein contents were obtained by multiplying the nitrogen content by 5.83 (Jones, 1931).

3.3.6 Determination of Lipid

The total crude fat content of wheat, soya-protein isolate, diets and excreta together with appropriate blanks was determined by the Weibul method based on that of Osborne and Voogt (1978). The lipid-containing sample (2g) was boiled with acid (3M HCl) and keiselguhr (filtration aid) for 1h. After cooling, the digest was filtered through double filter paper (Whatman No 541) and the residue was washed several times with warm distilled water until the filtrate was neutral (tested with litmus pH indicator paper). After drying, the residue was extracted with light petroleum ether by a conventional Soxhlet method for 6h. The fat content was calculated from the weight of petroleum ether soluble extract and expressed as oil (w/w) corrected for dry matter.

3.3.7 Determination of Non-Starch Polysaccharides (NSP)

The individual sugar components of the NSP were determined using a modified Englyst procedure (Englyst *et al.*, 1982; Englyst and Cummings, 1984). To ensure the complete estimation of the constituent monosaccharides present in the NSP, following the gelation of the sample in the presence of dimethyl sulphoxide (DMSO), acid washed sand (150mg; 40-100 mesh, inert) was included with the sample at the outset to facilitate complete mixing and hydrolysis. The loss of sugars during the solubilisation of the NSP with 12M H_2SO_4 was variable between runs. To enable such losses to be corrected, the internal standard, allose, was added to the sample prior to solubilisation and subsequent hydrolysis rather than between reduction and acetylation.

NSP represent the sum of neutral sugars plus uronic acids. For rhamnose, however, a correction has to be made for the incomplete release of these sugars during hydrolysis and acetylation. A maximum yield of rhamnose was obtained after hydrolysis with IM H_2SO_4 at 100°C for 5-6h, but this prolonged hydrolysis

resulted in the partial destruction of other sugars and, therefore, as a compromise, experimentally determined values for rhamnose obtained after 2h hydrolysis were multiplied by 1.7 (Englyst *et al.*, 1982).

The polymeric arabinoxylans were assumed to be comprised of a xylan backbone with arabinose side-chains. The levels of arabinoxylans (pentosans) were calculated from the sum of anhydro-arabinose and -xylose in the NSP (experimentally determined values for L-ara and D-xyl x 0.887; Choct and Annison, 1992). Other polysaccharides (α-galactan, ß-glucan and ß-mannan) were calculated using the relationship: polysaccharide = 0.9 x monosaccharide (Choct and Annison, 1992). The uronic acids were estimated colorimetrically and expressed as glucuronic acid according to the method of Scott (1979). Soluble and insoluble NSP and cellulosic and non-cellulosic polysaccharides (NCP) were determined by the Englyst procedure with the modifications listed above.

(i) Alditol Acetate Preparation

Neutral sugars were determined as alditol acetates following acid hydrolysis. Alditol acetates were prepared according to the methods of Englyst *et al.* (1982) and Englyst and Cummings (1984) with slight modification. The allose was added at an earlier stage of the process, prior to acid hydrolysis, to compensate for destructive losses. The remainder of the protocol was followed without further modification.

(ii) Gas Chromatography

The alditol acetates were quantified by gas chromatography (Pye Unicam, Pye series 104 chromatograph) equipped with a flame ionisation detector. The sugars were separated on either:

(a) A fused silica capillary column (BPX70, 0.16μ thickness), 25m x 0.32mm i.d. (SGE, Milton Keynes). Helium was used as the carrier gas at a flow rate of 7.3ml.min^{-1}. The column temperature was operated isothermally at 195°C with detector and injector temperatures of 280°C;

or

(b)A Supelco glass capillary column (coated with SP2330, 0.25μ thickness), 30m x 0.75mm i.d. The flow rate of the carrier gas (helium) was 5.3ml.min^{-1}. The column temperature operated under a controlled programme and ran between 200°C and 240°C (2min hold at 200°C thereafter increasing at a rate of 4°C per minute to 240°C). Both the injector and the detector were maintained at 250°C.

Peak areas were obtained by means of using a Pye Unicam PU4810 computing integrator.

3.3.8 Measurement of the Monosaccharide Content and Composition of Chick Digesta Samples

NCP of digesta were determined by the Englyst procedure with the slight modifications listed above (Englyst *et al.*, 1982; Englyst and Cummings, 1984). Aliquots of digesta (refer to section 2.2.1 iv) supernatant (0.1ml) and pellet (100-150mg) were placed into small screw-capped tubes and hydrolysed with 0.1ml 2M H_2SO_4 and 1ml 1M H_2SO_4 respectively, for 2h in a boiling water bath. Aliquots of the hydrolysate (0.1-0.2ml) were made up to 1ml with distilled water and the individual neutral sugar components determined as alditol acetates. Compensation was made in the method for the reduced volumes used. Results are expressed as mg anhydro sugar.g^{-1} DM.

3.3.9 Isolation of Wheat Starch Granules

Starch granules were isolated from samples of wheat, excreta and chick foregut digesta according to the method of Faulks *et al.* (1989) with slight modification (C. Linnecar, personal communication). Starch-containing samples were suspended in 2% NaCl (1:3 w/v) and allowed to stand overnight at 1°C. The slurry was filtered through a sieve (125μm mesh) and the residue was washed with distilled water until the filtrate was devoid of starch granules (as tested by 0.02% I_2/KI). The filtrate containing the starch granules was centrifuged at 1000xg for 10min at 4°C and the pellet retained. The pellet was washed by resuspending in water (approx. 1:10 w/v) and centrifuging as before. Using this procedure 3 layers were obtained, namely:

(1) Top layer containing only small granules (B granules);

(2) Middle layer containing both large and small granules (A and B granules);

(3) Lower layer containing only large granules (A granules).

Contaminating material was removed from the small granule fraction by resuspending in water and aspirating off the upper layer of the suspension settling in a tall beaker. Each fraction (1, 2 & 3) was washed several times by resuspension and centrifugation, and finally passed through the 125μm sieve and combined. The granule suspensions were transferred to form thin layers in plastic trays and placed in a sterile lamina flow cabinet to dry at room temperature (RT). The dry starch granules were passed through a 125μm mesh sieve and stored in air-tight containers at 1°C. Such granules were found to contain no less than 0.98 starch (Table 3.3).

3.3.10. Hydrolysis of Isolated Starch Granules by Porcine Pancreatic α-Amylase (PPA) *in Vitro*

(i) Routine Assays on Wheat Starch Granule Hydrolysis by PPA

Measurement of starch hydrolysis by PPA *in vitro* was carried out using a routine assay procedure employed (C. Linnecar, personal communication) which was based on methods of Hoover and Sozulski (1985) and Englyst and Cummings (1987). Assays were performed in duplicate or triplicate with appropriate starch controls and enzyme-buffer blanks. Samples of ground wheat (particle size <0.5mm), isolated wheat starch, or commercial wheat starch (Fisons Scientific Equipment) (containing 0.25g starch on a dry weight basis) were mixed with 45ml 0.1M citrate-phosphate buffer, pH 6.9. Sodium chloride (0.35g.l^{-1} buffer) was added to achieve optimum enzyme activity. Samples were incubated at 37°C with 80μl PPA (Sigma 2 x crystallised, 34.02 U.μl^{-1}) (one unit of enzyme liberates 1mg maltose from starch in 3min at pH 6.9 and 20°C). The incubation was carried out in a shaking (reciprocating) water bath (90 strokes.min^{-1}). Duplicate aliquots (1.0ml) were removed from each flask after 1, 2, 4, 6 and 8hr incubation, cooled to 0°C and centrifuged at 1500xg for 2-3 min at room temperature (RT). Supernatants were removed and assayed immediately for total soluble carbohydrate content (glucose equivalents) using the procedure of Dubois *et al.* (1956).

Total soluble carbohydrate was measured as glucose equivalents in the supernatants using the phenol-sulphuric acid method (Dubois *et al.*, 1956). A standard curve was prepared over a glucose range (0-1mg.ml^{-1}) with each determination. A_{490} of standards and samples were read on a Pye Unicam PU 8610 UV/VIS Kinetics Spectrophotometer. Starch granule digestion was expressed as the percentage hydrolysis over time. Comparisons of the susceptibility of starch granules from different sources to hydrolysis by PPA,

were made using the time (hr) required by PPA to hydrolyse 0.50 of the starch. Assays were repeated in quadruplicate.

(ii) Starch Granule Hydrolysis by PPA in the Presence or Absence of Aqueous Extracts from Wheat

Two experiments were conducted to:

(i) determine the direct effect of wheat water-solubles on starch granule hydrolysis by PPA,

(ii) determine the viscosity effects of wheat water-solubles on starch granule hydrolysis by PPA.

Direct Effect of Aqueous Extract

Two wheats with contrasting AME values [Riband (high) and Spark (low)] were selected from Trial VII. Wheat flour (0.4g) was suspended in 45ml 0.1M citrate-phosphate buffer, pH 6.9, in a shaking water bath at 37°C for 2h. These suspensions were treated in one of the following 3 ways:-

(a) Centrifuged at 1500xg for 5min and the supernatant discarded. The pellet was resuspended in the same volume of 0.1M citrate-phosphate buffer, pH 6.9, prior to the addition of PPA (80μl).

(b) PPA (80μl) was added directly to the suspension (control treatment).

(c) Production of the pellet as in (a) above. The pellet was resuspended in supernatant obtained from centrifuging a suspension of 0.8g wheat meal in 45ml citrate-phosphate buffer, pH 6.9, incubated at 37°C for 2h. PPA

(80μl) was added after resuspension.

Duplicate flasks of each treatment were incubated at 37°C, agitated and samples withdrawn at 1, 2, 4 and 8h, cooled immediately to 0°C and centrifuged at 1500xg for 2-3min at RT. Total soluble carbohydrate was determined on the supernatant by the phenol-sulphuric acid method (Dubois *et al.*, 1956).

Viscosity Effects of Aqueous Extracts

Three treatments were employed as above except wheat flour (0.5g) was extracted in 7.5ml citrate-phosphate buffer, pH 6.9 and the pellet obtained from treatment (c) was resuspended in a supernatant obtained from suspending 1g wheat flour in 7.5ml buffer. Duplicate flasks of each treatment, after the addition of PPA (80μl), were incubated in a shaking water bath at 37°C and samples withdrawn at 1, 2 and 4h. Total soluble carbohydrate was determined as described above and viscosity measurements were performed using a Brookfield viscometer (LV TDV-II-CP, Brookfield Engineering Laboratories Inc., Stroughton, MA).

(iii) Hydrolysis of Commercial Wheat Starch (CWS) using a Crude Enzyme preparation from Chicken Foregut Digesta in Vitro

Chicks fed wheats of contrasting AME values in Trials VII and VIII were slaughtered (section 3.2.1.iv) and the supernatant collected and retained from the foregut digesta.

Wheat starch (80mg, Sigma Chemical Co.) was suspended in 12ml 0.1M citrate-phosphate buffer, pH 6.9 and 80μl digesta supernatant was added to duplicate flasks and incubated at 37°C in a shaking water bath. Samples were withdrawn

at 1, 2, 4 and 8h for determination of starch hydrolysis as indicated by the soluble carbohydrate (glucose equivalents, Dubois *et al.*, 1956).

3.3.11. Measurements Relating to Aqueous Extracts of Wheat and Excreta

(i) Native Wheat

To determine the effect of extraction time on aqueous extract viscosity, ground samples (4) of wheat from Trials III and VII were extracted in 0.1M citrate-phosphate buffer, pH 6.9 (1:3 w/v) for 1, 2 or 4hr in a shaking water bath (90 strokes.min^{-1}) at 37°C. Extracts were centrifuged at 1500xg for 10min at RT and the viscosity of the extracts from wheats used in Trial III was determined by conventional viscometry using a capillary 'U' tube viscometer (Schott-Geraté, Camlab). Values presented are the mean time (sec) for a known volume (2ml) of extract supernatant to pass between 2 points on the viscometer at 37°C and are presented relative to water under the same conditions of temperature. The results were obtained after 3min equilibration to 37°C ± 0.1. The viscosity of extracts from Trial VII wheats were obtained by means of a Brookfield digital viscometer (LV TDV-II-CP, Brookfield Engineering Laboratories Inc., Stroughton, MA) at 37°C using a 0.8° cone spindle and a shear rate of 225-450s^{-1}. Results were recorded for each sample after 1min operation. The monosaccharide composition of extracts was determined following hydrolysis with 1M H_2SO_4 for 2hr at 100°C and GC analysis (sections 3.3.7 i and ii).

(ii) Chick Excreta Samples

A selection of excreta samples from Trial VII and all samples from Trial VIII were extracted (1:5 w/v) in 0.1M citrate-phosphate buffer, pH 6.9, for 1hr at 37°C in a reciprocating water bath (90 strokes.min^{-1}). The suspension was centrifuged at 1500xg for 2-3min at RT. The viscosity of the supernatants was determined using a Brookfield digital viscometer as above (i).

3.3.12. Particle Size Determination of Isolated Starch Granules

The Coulter Counter Model TA II (Coulter Electronics Ltd, Harpenden, Herts) was used to characterise the granule size distributions of starch granules isolated from different wheat samples, chick digesta and excreta. The counter was fitted with a 70μm aperture tube which made it possible to determine particle sizes between 0.79μm and 32.0μm (16 channels). Measurements were carried out in the interval between 4.0μm and 32.0μm to eliminate the likelihood of inaccurate counts, mainly in channels 1-7 (particle size $<$ 4.0μm in diameter), caused by signal noise and fine non-starch particulates. The counting solution (electrolyte) used was Isoton II (azide-free, balanced; Coulter Electronics Ltd, Luton). This solution was passed through the apparatus 5-6 times prior to sample analysis to give a satisfactorily low background count. The Instrument was calibrated using a standard suspension of P.D.V.B. Latex with an average particle size diameter of 10μm to give a concentration reading of between 2 and 5 per cent.

As each particle passes through the orifice, across which a potential difference is maintained, the conductivity changes according to the amount of electrolyte which is displaced. The number of particles within each preset channel are automatically counted and displayed.

A known quantity (2-3mg) of starch (weighed using a Mettler MT/UMT balance [Mettler Toledo Ltd., Leicester]) was suspended in electrolyte (5ml) and subjected to ultrasonication for 60sec using an Ultrawave U300 Sonicator (Radleys, Saffron Waldon, Essex). The volume adjusted with further electrolyte (150-180ml) as necessary to obtain a concentration reading between 2 and 5 per cent. Measurements (n = 4-5) were recorded for each subsample of starch in each of the 9 size channels after baseline correction. Counts were made on 4-7 replicate subsamples. Particle size measurements were recorded as the number of granules in each of the different channels depending on their volume. Results

were expressed either on a number basis (number.20μg dry weight starch^{-1}) according to the exact weights and volumes used, or as a percentage of the total number of particles present. Assuming that all granules are spherical, mean granule diameters, surface areas and volumes were calculated. Data are plotted in the form of a multiple line graph for comparative purposes.

3.3.13. Microscopic Examination of Starch Granules

(i) Light Microscopy

Isolated granules were mounted in water, or stained with either 0.2% I_2 in 2% KI or congo red (0.3%) on slides and viewed (400 x magnification) under normal light or polarised light microscopy (Nikon Labophot-2, Badhoevedop, The Netherlands) fitted with a camera (Nikon F-80-LS).

(ii) Scanning Electron Microscopy (SEM)

Preparation of isolated starch samples for SEM were made according to the method described above (section 3.3.9). Small aliquots starch granules were scattered onto double-sided tape attached to stubs and coated with gold/palladium using a cool splutter coater operated at 20mA for 90sec. This resulted in a coat thickness of approximately 150Å. Samples were viewed in a Cambridge Stereoscan 100 scanning electron microscope using 10kV accelerating voltage. Facilities for this work were kindly provided by the Electron Microscopy Unit, Department of Biology, University of Leicester with the technical assistance of Stefan Hyman.

3.3.14. Statistical Analysis

The statistical significance of difference was determined by Analysis of Variance

(ANOVA) using the software package Genstat5 (Manual Reference 1987, Oxford University Press, NY). ANOVA was carried out on all results to assess the variability between samples. The p-values shown are a measure of statistical significance; p values less than 0.05 indicate that variation is significant; p-values less than 0.01 indicate that the variation is significant at the 99% confidence interval. Significance between two treatments was evaluated using student's t-test with the appropriate standard errors of difference between treatment means. For studies with more than two treatments means, the Duncan's multiple comparison test was used following ANOVA. Results are expressed as mean values ± SEM.

Table 3.1. Production Sites and Harvest Dates of Wheat Varieties used

Wheat Variety	Growth Site	Harvest Year	Chick Trial	Biochemical Analysis
Source: University of Nottingham Farms:				
Hornet	SB	1990	+	+
Riband	SB	1990	+	+
Mercia	SB	1990	+	+
Pastiche	SB	1990	+	+
Angler	BPF	1994	+	-
Avalon	BPF	1994	+	-
Avalon[a]	SB	1994	+	+
Avital	BPF	1994	+	-
Beaver[a]	BPF	1994	+	+
Brigadier	BPF	1994	+	-
Cadenza	BPF	1994	+	-
Haven	BPF	1994	+	-
Huntsman	BPF	1994	+	-
Lynx[a]	BPF	1994	+	+
Mercia[a]	BPF	1994	+	+
Recital	BPF	1994	+	-
Rialto[a]	BPF	1994	+	+
Riband[a]	BPF	1994	+	+
Riband[a]	SB	1994	+	+
Soissons	BPF	1994	+	-
Spark[a]	BPF	1994	+	+
Wigeon	BPF	1994	+	-

[a] Starch granules extracted
SB University of Nottingham, Sutton Bonington
BPF University of Nottingham, Bunny Park Farm

Table 3.1 (continued).

Wheat Variety (Sample No.)	Growth Site	Harvest Year	Chick Trial	Biochemical Analysis
Source: Institute of Animal Physiology and Genetics Research (IAPGR), Edinburgh				
not known (18)	MA	1990	+	+
not known (20)	A	1990	+	+
Brock (22)	M	1990	-	+
Brock (35)	D	1990	-	+
Brock (85)	G	1990	-	+
Fortress (23)	M	1990	+	+
Galahad (37)	D	1990	+	+
Hornet (38)	D	1990	-	+
Jonic (103)	B	1990	-	+
Mercia (26)	M	1990	-	+
Mercia (88)	Do	1990	+	+
not known (98)	C	1990	+	+
Source: Plant Breeding International, Cambridge (Growth site not known):				
Admiral	1	1991	+	-
Admiral	2	1991	+	-
Avalon[a]	1	1991/2	+	+
Avalon	2	1991	+	+
Beaver	1	1991	+	-
Beaver	2	1991	+	-
Haven	1	1991	+	-
Haven	2	1991	+	-
Hereward[a]	1	1991	+	+
Hereward	2	1991	+	+
Mercia[a]	1	1991/2	+	+
Mercia	2	1991	+	+
Riband[a]	1	1991	+	+
Riband	2	1991	+	+
Tara	1	1991	+	-
Tara	2	1991	+	-

MA - Mears Ashby (Northants), A - Ardleigh (Essex), M - Morley (Derbyshire), D - Debenham (Suffolk), G - Glympton (Oxon), B - Bridgets (Hants), Do - Dorchester (Dorset), C - Combs (Suffolk).

Samples codes from IAPGR given in brackets.

1 and 2 signify different production sites - locations unknown.

[a] Starch granules extracted

Table 3.2. Composition of experimental diets used in the determination of wheat AME values ($g.100g^{-1}$)

Ingredient	Diet A	Diet B	Diet C
Wheat	89.80	89.63	75.00
Soya Protein Isolate	-	-	15.00
Vegetable Oil	5.00	5.00	5.00
Mineral/Vitamin Premix	5.00	5.00	5.00
DL-Methionine	0.20	-	-
L-Lysine	-	0.37	-

See section 3.1.2 for details of suppliers

Table 3.3. Starch Content of Highly Purified Isolated Starch Granules*

Origin of Starch Granules	Used in Trial	Purity (% starch, dwb)
Commercially extracted wheat starch (Sigma)		100.20 ± 0.99
Wheat Variety		
Avalon	III-V	98.95 ± 0.60
Hereward	III	98.19 ± 0.63
Mercia	III-V	98.49 ± 1.04
Riband	III, V	98.46 ± 0.48
Avalon	VII & VIII	99.78 ± 0.35
Beaver	VII & VIII	98.56 ± 1.08
Lynx	VII & VIII	99.69 ± 0.20
Mercia	VII & VIII	100.16 ± 0.18
Rialto	VII & VIII	99.51 ± 0.71
Riband (SB)	VII & VIII	99.43 ± 0.60
Riband (BPF)	VII & VIII	99.61 ± 0.23
Spark	VII & VIII	98.89 ± 1.59

* Mean values displayed (n = 4-5).
Starch granules isolated from intestinal contents and excreta were not assayed for purity because insufficient material was available.

CHAPTER 4 DETERMINATION OF STARCH

4.1 DEVELOPMENT OF AN ENZYMATIC METHOD FOR THE MEASUREMENT OF STARCH

Starch is the major component of cereal grains and is extensively used in the food and feed industries. It represents the primary energy-yielding component in diets for poultry. A major difficulty in determining the extent of digestion of dietary polysaccharides, especially starch, in the small intestine is the accurate measurement of such components, both in raw materials, feed, intestinal contents and excreta. The accurate analysis of starch is essential for most laboratories concerned with food/feed analysis.

4.2 ESTIMATION OF STARCH USING ACID HYDROLYSIS

Methods based on the direct hydrolysis of starch by mineral acids and the estimation of glucose values have been widely used in the past since such methods routinely give theoretical yields of glucose (Southgate, 1991). To demonstrate the complete hydrolysis of commercial soluble starch to free glucose units, a direct acid hydrolysis was performed. In common with other laboratories, heating with 1M H_2SO_4 at 100°C for 60 minutes was found to be adequate for the complete hydrolysis of soluble starch to glucose (Table 4.1).

The disadvantage of using acid hydrolysis to estimate the starch content is the lack of specificity of the method, with the non-cellulosic polysaccharides, such as pectins and hemicelluloses, also being susceptible (Rose *et al.*,1991; Southgate, 1991). With the use of a specific enzyme method for the measurement of glucose in the hydrolysates, e.g. the glucose oxidase method, some of these inaccuracies are reduced, but glucose from the hydrolysis of β-linked glucans, which are present in many cereals, will also be assayed resulting

in over-estimated starch values (Southgate, 1991).

4.3 ENZYMATIC ESTIMATION OF STARCH

The major advantage of methods using purified enzymes is their absolute specificity for starch. The availability of starch hydrolytic enzymes has enabled the development of specific procedures for starch analysis in a wide range of biological materials. In general, such methods (e.g. Batey, 1982; Holm *et al.*, 1986; Henry *et al.*,1990; McCleary *et al.*, 1994) involve solubilisation and gelatinisation of the starch followed by its hydrolysis by means of α-amylases (salivary, pancreatic or bacterial), often in conjunction with pullulanase, and production of glucose from the degradation products by amyloglucosidase (AMG).

Other factors may affect the accuracy and reproducibility of enzymatic methods for starch, for example Batey (1982) expressed concern that wheat products with high levels of wheat protein, for example whole wheat and wheat flour, upon dispersion resulted in the formation of glutenous aggregates associated with the cohesive properties of proteins. These reduced the accessibility of the starch to the enzymes and resulted in the incomplete hydrolysis of starch into glucose.

At the outset of the current study the existing enzymic methods in use proved unreliable. Recoveries as low as 0.60 were occasionally obtained with standard starches. Priority was given to the development of an accurate and highly reproducible enzymatic assay for the measurement of starch in wheat. The method was to be rapid, convenient and simple to use for routine analysis. The accuracy of this method was evaluated using a number of different materials.

The parameters investigated in the development of the method are listed below

using a standard commercial soluble starch preparation. In addition, resistant starch was estimated using a commercial source (Novelose).

(1) *Effect of Assay Conditions on Starch Recovery - Englyst Procedures*

(a) Enzymes - source, quantity, stability
(b) Sample weight
(c) Incubation time, temperature and pH
(d) Use of a bacteriostat (toluene)
(e) Constant or occasional stirring

(2) *Utilisation of Thermostable Enzymes in Starch Assays*

(a) Preliminary experiments with Termamyl and amyloglucosidase (AMG)
(b) Efficiency of starch hydrolysis using thermostable α-amylase and AMG) under different experimental conditions
(c) Enzyme blanks

(3) *Estimation of Resistant Starch (RS) using Thermostable Enzymes*

(a) Starch solubilisation (use of dimethyl sulphoxide - DMSO - , 2M KOH or 0.1M MES buffer, pH 6.5)

(4) *Optimisation of Glucose-Oxidase-Peroxidase (GOD-Peroxidase) for the estimation of glucose*

(a) Absorption Maxima
(b) Use of microplate reader (automated absorbance reading)
(c) Interference of glucose estimation by other sugars

4.3.1 Effect of Assay Conditions - Englyst Procedure

(i) Source of AMG, Incubation Time and the Effect of Stirring

The method employed in the first instance was an adaptation of the procedure for starch removal developed for the determination of non-starch polysaccharides (NSP) in foods of plant origin (Englyst *et al.*, 1982; Englyst and Cummings, 1984). The method used was as follows:- 100mg of test sample was boiled in screw-top test tubes for 1hr in 2.0ml DMSO and, without cooling, 8.0ml 0.1M sodium acetate (NaAC) buffer pH 5.2 at 50°C added immediately. Suspensions were incubated at 42°C for 3min, 0.5ml α-amylase (Pancrex V capsules dissolved in 4.5ml water) and 0.1ml pullulanase (diluted 1:100) added and incubated for 16-18hr at 42°C. 0.1ml amyloglucosidase (5000units) was added with stirring, and the suspension was incubated at 55°C for 3hr. Hydrolysates were centrifuged at 1500xg for 5min, the volume adjusted to 250ml and glucose estimated by the glucose oxidase method, A_{420} (refer to section 4.5.2).

Recoveries of soluble starch were low and variable both between and within runs (Table 4.2). Amyloglucosidase from Sigma resulted in better recoveries of glucose (ca. 5-10%) than that from BDH, and neither source was affected by incubation time (>3h) or stirring.

The optimum time for the development of the coloured complex with GOD-peroxidase was found to be 35-40min and not 25min as frequently stated (Table 4.3) and this was used in all subsequent work.

(ii) Sample Weight

Preliminary work revealed that smaller samples (50mg) were more effectively

hydrolysed than larger (100mg) samples under the conditions employed, with no effect of using a bacteriostat, toluene (Table 4.3).

In a more detailed study, different sample weights (15-75mg) were checked for recoveries with AMG using incubation conditions of 55°C for 6hr and 60°C for 30 and 90min respectively (Table 4.4) as detailed by Karkalas, 1985; Holm *et al.*, 1986; Henry *et al.*,1990. Recoveries of starch with sample weights (40-75mg) was identical, but recoveries for samples below 40mg were considerably lower. Sample weights for analysis in subsequent starches were 40-55mg.

(iii) AMG Stability as Affected by Temperature and Time

With increasing temperature and occassional stirring the AMG (Sigma or BDH) was precipitated out of solution above 45°C producing a turbid suspension with a prominent layer of froth (Table 4.5). Small opaque particles appeared, which were assumed to be gelatinised starch aggregates, retrograded starch or denatured enzymic protein. Generally, the extent of precipitation, turbidity and froth increased with temperature. These effects were dependent upon the presence of the enzyme but occurred in the enzyme/buffer mixtures with or without starch. It was concluded that the enzyme became insoluble with time and this process was unaffected by the presence or absence of $CaCl_2$.

The stability of the enzyme as affected by time and temperature was investigated using only BDH AMG. A 95% recovery of glucose was obtained with the control (no pre-incubation of AMG/buffer). Results are presented (Table 4.6) and represent the percentage activity remaining compared with the control.

There was an inverse relationship between incubation temperature and residual activity of AMG, independent of time. This was also reflected in the relative turbidities of the enzyme digests. Generally, the longer the incubation time, the

lower the recovery of glucose. The protein content of the pellet was estimated following centrifugation of the digest (Table 4.6).

AMG was shown to be relatively unstable and affected by both time and temperature of incubation. Evidence was obtained indicating that the enzyme is denatured and precipitated out of solution in an inactive state particularly at higher temperatures (above 45°C) (Table 4.6). This finding was correlated with the increase in protein content of the pellet (Table 4.7). At 40°C, the amount of denatured protein was relatively low up to 46hr. Denaturation and inactivation were highest in the incubations left overnight (22-46hr) at 55°C.

In conclusion, it appeared unlikely that a reliable procedure for the estimation of starch would be developed using α-amylase (Pancreatin), pullulanse and AMG.

4.3.2 Use of Thermostable Enzymes in the Enzymatic Analysis of Starch

A number of workers have used Novo enzymes (Novo A/S, Copenhagen, Denmark) including a thermostable α-amylase, Termamyl, and Amyloglucosidase (AMG) either for the analysis or solubilisation of starch (Batey, 1982; Holm *et al.*, 1986; Henry *et al.*, 1990; McCleary *et al.*, 1994). Holm *et al.* (1986) observed that Termamyl effectively solubilised all the starch in assay systems such that the solutions were clear and could be transferred quantitatively. This enabled starch analysis to be performed more accurately. Notwithstanding the efficacy of these thermostable enzymes, some workers maintain that, in quantitative assays, pretreatment of the starch with pullulanase is still necessary (Englyst *et al.*,1992; Wolters *et al.*, 1992). Preliminary experiments in this laboratory revealed that an overnight incubation with Pancrex V (α-amylase) and pullulanase, followed by Termamyl and AMG the following day, proved promising with starch recoveries of up to 94%.

In preliminary assays using Termamyl (120L) and AMG (300L) alone, the following procedure was used:- 40-50mg soluble starch in 2ml 0.1M MES buffer (pH 6.5), or DMSO, was incubated at 100°C for 60-75min with or without Termamyl (10μl). 10μl Termamyl was added to all digests and the incubation was continued at 90°C for 60-75min. After adjusting the pH to 4.6 (0.1M NaAC) 0.1ml AMG was added and the digests incubated at 58°C for 60, 120 or 180min before dilution and analysis of glucose (Table 4.8).

A single addition of Termamyl during the solubilisation stage together with incubation at 90°C (60min), followed by hydrolysis using AMG for varying periods resulted in starch recoveries of around 96% (Table 4.8, treatments A1[ii], [iii]). The length of the AMG treatment was not critical above 2h. Highest recoveries were obtained with MES buffer when Termamyl was included during the solubilisation / gelatinisation stage at 100°C followed by additional enzyme at 90°C for 75 minutes (treatment B1). Starch recoveries of approximately 98% were obtained. The use of DMSO considerably reduced the recoveries of starch compared to that of MES buffer.

Thermophilic α-amylases were found to degrade wheat starch immediately after gelatinisation (59-65°C) (Osman, 1967). Batey (1982) suggested that the initial hydrolysis of starch with Termamyl in the gelatinisation step prevented the formation of retrograded starch, which could be resistant to subsequent hydrolysis with AMG.

Although DMSO is a widely used solvent for starch and many water-insoluble materials (Libby, 1970; Englyst and Cummings, 1984; Berry, 1986; Henry *et al.*, 1990), recoveries using this solvent for starch dispersion and subsequent hydrolysis using Termamyl and AMG (Table 4.8, treatments A3 and A4) were inferior to other solubilisation processes (treatments A1, A2 and B1) with mean values ranging 84 to 91%. This may have been due to gelation of the sample at

room temperature rather than at 55°C (Libby, 1970) or in combination with acid (8M HCL) at 60°C (Boehringer Mannheim, 1987) although this latter method of extraction is not advised due to the partial degradation of non-starch polysaccharides (Karkalas, 1985; Southgate, 1991).

Longer incubation times with Termamyl at 100°C (90min) improved starch recoveries to 99% and above. The recoveries for a range of starches was often in the range 96-101%.

Samples of commercial starch granules were included at this stage to confirm the recoveries of the procedure (Table 4.9). The overall analysis time was reduced from 21-24hr (using pullulanase and α-amylase) to 5-6hr, thereby enabling more samples to be analysed in a given time. The recoveries obtained were highly consistent for all starches studied.

It is essential that enzyme blanks are prepared with each starch assay since both Termamyl and AMG contain glucose as a preservative which has to be corrected for in all estimations. Harris *et al.* (1984) detected non-dialysable mannose and glucose in AMG from Boehringer (cat. no. 208469) at extremely low levels. Karkalas (1985) reported that the glucose content of α-amylase (Sigma A-3403, from *Bacillus licheniformis*) and AMG (Boehringer Mannheim 208469, from *Aspergillus niger*) did not exceed 0.3μg.ml^{-1}. In the current work, the combination of Novo enzymes, Termamyl (120L) and AMG (300L), contained considerable amounts of glucose (mean 0.1 mg.ml^{-1}) together with smaller amounts of mannose (mean 0.04 mg.ml^{-1}) and galactose (mean 8.7μg.ml^{-1}) as determined by GC analysis.

The current work led to the conclusion that almost complete recovery of native starches could be obtained using this method through the use of thermostable enymes, namely Termamyl and AMG.

4.3.3 Enzymic Methods for the Estimation of Starch including Resistant Starch

The method developed in 4.3.2 above was extended to include the estimation of resistant starch. Dimethyl sulphoxide (DMSO) (Libby, 1970; Englyst *et al.*, 1982; Englyst and Cummings, 1984, 1988; Berry *et al.*, 1988; McCleary *et al.*, 1994) and alkalis such as KOH (Englyst and Cummings, 1984; Berry, 1986; Berry *et al.*, 1988; Englyst *et al.*, 1992) and NaOH (Karkalas, 1985; Kennedy and Cabalda, 1993) have been used to disperse resistant starch (RS) and, as a consequence increasing its susceptibility to amylolytic enzymes. Although DMSO has been used to solubilise the starch in the Englyst method for the determination of NSP content (Englyst *et al.*, 1982), it was excluded from the current study because of the low starch recoveries obtained earlier. DMSO is not a good solvent for resistant starch.

Preincubation with 2M KOH (60min constant stirring at RT) effectively dispersed the resistant starch present in Novelose prior to hydrolysis with amylolytic enzymes. This initial solubilisation step enabled the quantitative conversion of starch to glucose (Table 4.10). Further improvements may be possible if the KOH treatment is heated (Kennedy and Cabalda, 1993). KOH treatment had no effect on the determination of starch in wheat flour (RS absent). This finding is in agreement with that of Holm *et al.* (1986).

The addition of 50% (v/v) ethanol (1ml) to the soluble starch for 10min at RT is thought to 'prepare the starch' for an effective enzyme attack by wetting it, thereby aiding solubilisation of the starch in preparation for AMG hydrolysis (McCleary *et al.*, 1994). Ethanol proved to be ineffective in the recovery of starch from Novelose. Indeed, its use was slightly detrimental to the recoveries.

Comparisons were made between several different wheat varieties and other starch preparations to confirm the effectiveness of the KOH solubilisation in the

estimation of total starch. Samples were wetted (0.5ml water) for 10min prior to the addition of KOH (0.5ml, 4M KOH) to minimise gelation. For comparison, starch was estimated in the same samples using the procedure listed in section 4.3.2 above [available starch]. Total starch was measured as the glucose released by complete enzymic hydrolysis of starch after pre-treatment with KOH. Available starch is that measured without initial treatment with KOH. Resistant starch is the difference between total starch and available starch (Table 4.11). Due to the absence/limited amounts of resistant starch present, the procedure for available starch was used for all future analyses of native wheat starch.

4.4 OPTIMISATION OF GOD-PEROXIDASE METHOD FOR THE ESTIMATION OF GLUCOSE

Various aspects of this procedure were examined because of problems and uncertainties associated with the the published method (Boehringer Mannheim; Glucose / GOD-Perid Method, test kit number 124028 / 124036).

4.4.1 Absorption Maximum of the GO Chromogen

Routinely, 420nm was used in this laboratory to read the absorbance of glucose in both standard and test solutions, although results were generally variable and often low. Although this was partly attributable to the current method used for starch hydrolysis, the method employed for the estimation of glucose was not achieving optimal results. An absorption spectrum of glucose revealed that the coloured complex had a broad absorption range over 400 to 680 nm with a maximum absorption at 420nm, with a second high at ca. 650nm. In addition, the standard calibration graph (glucose concentrations 0-0.75mg.ml^{-1}) of glucose was not linear above 0.2mg.ml^{-1} when measured at an absorbance wavelength of 420nm and was too sensitive. At 600nm the standard calibration graph was linear across the range up to 0.75mg.ml^{-1}. An absorption wavelength of 600

(630 for microplate technique, see section 4.4.ii) was used throughout the current work and gave consistently higher and reproducible results (Table 4.12). It should be noted however that, despite which wavelength is used for measurement, the test results will always be relative to the standard controls used in the test as long as the linear part of the calibration curve is used.

4.4.2 Routine Analysis using a Microplate Reader

Glucose content was determined using the microplate method rather than conventional spectroscopy because of the convenience and economies in cost and assay time. The absorbance readings were undertaken at A630 since this was the nearest filter available to the A600 recommended. The absorbance of glucose at 630nm was slightly higher than that given at 600nm (Figure 4.1) but both gave a linear calibration graph up to a glucose concentration of 0.75mg.ml^{-1} ($r^2 = 1.00$).

4.4.3 Interference of Glucose Estimation by Standard Sugars

The possible interference of glucose estimation by the presence of other sugars either singly or in combination was examined. An aliquot (10μl) of each sugar solution (1 mg.ml^{-1}) was added to the GOD-peroxidase reagent (250μl) and the A_{630} determined. The colour production compared with glucose was as follows (as per cent glucose): lactose 0.05%, fructose 0.07%, arabinose 0.11%, sucrose 0.22%, maltose 0.75%, galactose 1.52%, xylose 1.57% and mannose 4.31%. Since the concentration of these sugars used was 10 times that of glucose normally determined (0.1mg.ml^{-1}) it was concluded that mannose would affect glucose estimations but only when present at extremely high concentrations. For wheat, this does not present a problem because mannose is only present in very small amounts. These results correlate well, with the exception of sucrose, with those published by Karkalas (1985) who obtained the

following values (per cent glucose): Lactose 0.03%, maltose 0.56%, xylose 0.82%, galactose 1.20% and sucrose 7.47%. In these studies, mannose was confirmed as being the primary sugar of importance for interference with the glucose assay if present in sufficiently large amounts, by reacting the GOD-Peroxidase reagent with a mixture of glucose (0.2, 0.5 or 1mg.ml^{-1}) and either mannose, galactose, maltose or xylose (1mg.ml^{-1}), in equal proportions. Mannose increased the glucose equivalent value by approximately 20% whereas the other sugars affected the glucose recovery by no more than 2%.

4.5 ROUTINE PROCEDURE FOR THE ENZYMATIC ANALYSIS OF STARCH

The starch analysis procedure finally adopted for the current work is presented in full. This new procedure enabled reproducible and highly accurate results to be obtained quickly, easily, and relatively cheaply when used in conjunction with the micro-plate reader. The method is simple, rapid and utilises highly purified enzymes to ensure specific hydrolysis and measurement of starch. It offered a convenient, alternative procedure to the previous assay. It has now been used routinely to determine the content of 'available' starch in a wide range of materials including wheat, barley (and other cereals), legumes, isolated starches, animal feeds, faeces and excreta. The method may also be used to estimate resistant starch when an additional step involving treatment with 2M KOH is included.

4.5.1 Hydrolysis Procedure

Starch (ca. 50mg) was mixed with 2ml 0.1M 2[N-Morpholino] ethane-sulphonic acid (MES) buffer (pH 6.5). and stirred, taking care not to splash the sample up the tube, and incubated at 100°C for 90min following the addition of 10μl Termamyl (1.2 KNU). A further 10μl Termamyl were added and the contents of the tube stirred prior to incubation at 90°C for 90min. The temperature was

reduced to 58°C and the pH of the solution was adjusted to 4.6 by the addition of a predetermined amount (ca. 2.8ml) of 0.1M acetate buffer (pH 4.1) at 58°C. It is essential that the tubes remain in the water bath throughout, since heating-cooling-reheating cycles result in retrogradation of the starch, which is impairs accessibility of α-amylases and AMG (Southgate, 1991).

Amyloglucosidase (0.1ml, 30AGU) was added and the contents mixed before incubation at 58°C for 2h. The hydrolysate was adjusted to 100ml with distilled water and thoroughly mixed before centrifuging an aliquot (ca. 10-15ml) for 3-4min at 1500xg. Glucose assays were performed on the supernatant with appropriate dilutions as necessary.

Three enzyme blanks (minus starch source) were included, one of which was used to determine the exact volume of buffer required to adjust the pH of the system to 4.6 (usually 2.8-2.9ml). Controls (minus enzymes) were also included.

4.5.2 Estimation of Glucose

Glucose content was determined by the glucose oxidase method (Boehringer Mannheim test kit no. 124036).

Conventional spectroscopy (600nm) and microplate (630nm) methods were observed to be equally accurate although the advantage of using the latter was the speed at which the samples could be read and analysed and the reduction in cost with the use of smaller volumes of the GOD-Peroxidase reagent.

Aliquots (10μl) of sample (including a water blank and standard glucose solution provided in the kit) were each placed into 4 wells in a microplate. Glucose oxidase-peroxidase solution (1.4g.100ml^{-1}) was added to each (250μl) and allowed to incubate for 35-40min at room temperature (20-25°C). The plates

were shaken for 10sec before being read on a Dynatech Ultrascan MR5000 microplate reader at 630nm (Dynatech Laboratories Ltd, Bullinghurst, West Sussex) using the software package Biolinx 1.1b. Starch contents were obtained after suitable correction for the blanks, controls and initial dry weight of starch by multiplying the glucose concentration by 0.9, which is the accepted factor (AACC, 1962) to account for the molecular weight difference between a glucose monomer in starch and the glucose produced.

4.5.3 Calculation of Starch Content

The starch determined (anhydro-glucose obtained from the sample by enzymatic hydrolysis) is expressed as a proportion (%) of total sample weight (dry-weight basis) according to the following equation:

$$StarchContent = \frac{\Delta E(s)/\Delta E(g) \times (9.1/100) \times V}{(100/W) \times (162/180)} [g.100g^{-1}DM]$$

$$\therefore StarchContent = \Delta E(s)/Abs(g) \times 9.1 \times (V/W) \times 0.9 [g.100g^{-1}DM]$$

where ΔE (s) is the absorbance (630nm) of the starch sample, control or enzyme blank corrected for the reagent blank absorbance; ΔE (g) is the absorbance of the standard glucose solution (Boehringer test kit) also corrected for the reagent blank; 9.1/100 is a factor for conversion of the standard glucose solution ($9.1mg.100ml^{-1}$); V is a dilution factor (volume, ml) to account for the final volume to which the hydrolysate was made up; 100/W is a factor to express starch as a percentage of the sample weight; 162/180 is a factor to convert free glucose to anhydroglucose, as occurs in starch.

4.6 CONCLUSIONS

The variability of results from the different methods of starch analysis generally arises from the incomplete dispersion or solubilisation of starch during the

analysis. The enzymes used prior to the availability of the thermostable α-amylase and AMG were denatured and precipitated out of solution at higher temperatures and longer incubation times were not adequate to achieve complete recovery.

The revised procedure described has been applied to a number of other materials containing varying amounts of starch and has produced consistent results between workers. The method has been found to be highly reproducible, accurate and simple to use. This procedure enables one person to analyse up to 70 samples in 2 days, although in some circumstances it may be more convenient to reduce this number so that the analyses may be completed in an 8hr day. Further, the starch hydrolysates may be immediately frozen for analysis of glucose on the following day or later without loss. Batey (1982) reported approximately a 5% reduction in the glucose content of starch hydrolysates when refrigerated storage overnight at 4°C but suggested freezing would reduce such losses. This method thus compares favourably with other published methods (Table 4.13).

This procedure detailed above was thus subsequently used for all starch determinations in the current programme.

Table 4.1. Effect of Heating Time (100°C) on the Hydrolysis of Soluble Starch with 1M H_2SO_4

Incubation Time (min)	Starch Hydrolysis* (%)
30	90.67 ± 0.43
60	99.93 ± 0.07
120	100.40 ± 0.05

* Mean values based on n = 2 replicates

Table 4.2. The Effect of Amyloglucosidase Source, Incubation Time and Stirring on Starch Hydrolysis*

Condition	Incubation Time (h)	Starch Hydrolysis (%) (Soluble Starch)
Amyloglucosidase (BDH)[1]		
Stirred	3	79.70 ± 1.28[a]
	6	81.26 ± 0.55
	16-18	82.07 ± 0.55
Unstirred	6	80.93 ± 0.43
	16-18	81.38 ± 0.33
Amyloglucosidase (Sigma)[2]		
Stirred	3	86.78 ± 1.08[a]
	6	89.65 ± 0.35
	16-18	89.70 ± 0.42
Unstirred	6	89.62 ± 0.48
	16-18	90.31 ± 0.36

[1] n = 8 replicates; [2] n = 5 replicates except for the samples denoted with superscript 'a' where n = 4 replicates.

* Pretreatment of sample as given in Table 4.1

Table 4.3. Effect of Sample Weight, Addition of Toluene and Reaction Time of GOD Peroxidase on the Estimation of Starch*

Treatment	Sample Weight (mg)	Reaction Time (min)	
		25	40
Sample unstirred; AMG (BDH) incubated at 55°C, 16-18hr:			
Toluene	50	84.00 ± 0.92	87.02 ± 0.72
	100	76.85 ± 1.36	79.45 ± 1.10
None	50	85.55 ± 1.92	88.70 ± 2.04
	100	75.68 ± 0.86	79.31 ± 0.67

* Mean values of n = 4 replicates.

Table 4.4. Effect of Sample Weight and Incubation Conditions of AMG on Soluble Starch Recoveries

Sample Weight (mg)	Incubation Conditions of AMG (BDH) 55°C, 6h	60°C, 30min	60°C, 90min
15	73.42 ±0.65 (5)	82.38 ±0.42 (6)	nd
25	83.73 ±0.85(5)	83.62 ±0.38 (6)	85.01 ±1.37 (6)
40	92.81 ±0.12 (5)	91.88 ±2.38 (6)	92.75 ±1.45 (6)
55	nd	91.80 ±0.45 (3)	92.62 ±0.06 (6)
75	nd	nd	89.67 ±0.47 (3)

Figures in brackets refer to number of replicates
nd, not determined

Table 4.5. The Appearance of Enzyme Digests following Prolonged Incubation (16-18h) at Different Temperatures.

Incubation Temperature (°C)	AMG Source BDH[1]	Sigma[2]
Enzyme/Buffer		
40	Clear	Clear
45	Some Precipitation; Some froth on top layer.	Little Precipitation; No froth on top layer.
50	Cloudy Solution; More Precipitation; More froth on top layer.	Less turbidity and precipitation than BDH. Little froth.
55	Very Turbid; Substantial precipitation; Thick layer of froth on top.	More turbidity and precipitation than at 50°C but less than BDH. Some froth.
Enzyme/Buffer plus $CaCl_2$ to act as a stabiliser		
45	Slight precipitation and solution turbidity, no froth.	Very little precipitation, no froth.
55	Substantial precipitation, more turbidity, large froth.	Some precipitation, little froth.
Soluble Starch + Enzyme/Buffer		
45	Slight precipitation, turbidity and froth.	Slight precipitation, slightly cloudy, but no froth.
55	Substantial precipitation, more turbid and more froth on top layer.	More precipitation, turbidity and froth on top layer.
Soluble Starch Controls (buffer, no enzyme)		
45	Slight amount of precipitation and turbidity, but no froth.	
55	Insignificant precipitation, less turbidity, no froth.	

[1] AMG (1000 EU/ml). IEU is the amount of AMG that is required to produce 1μmol glucose per min from starch at 37°C in acetate buffer, pH 4.6

[2] AMG (6100units/ml). I unit will liberate 1.0mg glucose from starch in 3min at 55°C, pH 4.5

Table 4.6. Effect of Time and Temperature on AMG (BDH) Hydrolytic Activity (maltose hydrolysis). Data refer to % values of controls.

Incubating Temperature (°C)	Length of Incubation (h) 2	5	22	26	46
40	83.74	80.32	78.19	74.09	68.77
45	80.23	74.91	62.24	60.99	60.42
50	69.64	67.40	38.64	32.27	26.31
55	55.81	47.15	10.63	7.94	5.07

Moisture content of maltose, 6.14g/100g maltose.
Equal volumes of 0.1M NaAC, pH 4.6, and AMG were incubated at the temperatures indicated in time concise experiments. At the appropriate times, 5.0ml of each digest was transferred to tubes containing 513mg maltose and incubated at 25°C for 30min. The glucose release was measured and expressed as a percentage of that compared to controls.

Table 4.7. Distribution of Protein in AMG (BDH)-Buffer Digests following Incubation at Different Temperatures and Times (Results expressed in mg BSA equivalents).

Incubating Temperature (°C)	Length of Incubation (h) 2	5	22	26	46
40	1.88	1.76	1.25	1.15	1.11
45	0.85	1.19	4.67	5.95	6.47
50	4.52	7.03	16.58	16.89	18.76
55	8.54	11.70	24.45	26.04	27.23

Enzyme digests were centrifuged at 1500xg for 15 min. The absorbance (A_{280}) of the supernatant was measured immediately. The pellet was resuspended in 3.0ml 0.1M NaOH and A_{280} measured. Samples were read against buffer (0.1M NaOH) and standard protein, BSA (12.5mg in 50ml 0.1M NaOH).

Table 4.8. : Efficiency of Starch Hydrolysis using Thermostable Termamyl and AMG under Different Experimental Conditions*

Experimental Treatment			Starch Hydrolysis (%)
A Samples incubated at 100°C 60min; followed by incubation at 90°C 60min.			
1 SS + MES (1 x 10μl [1]Termamyl):			
	(i)[2] AMG 1h		94.64 ± 0.22
	(ii) AMG 2h		95.99 ± 0.39
	(iii) AMG 3h		95.93 ± 0.32
2 SS + MES (2 x 10μl Termamyl):			
	AMG 2h	Run1	97.96 ± 0.23
		Run 2	97.75 ± 0.24
3 SS + DMSO (1 x 10μl Termamyl):			
	AMG 2h	Run 1	84.49 ± 0.63
		Run 2	87.28 ± 0.85
4 SS + DMSO (2 x 10μl Termamyl):			
	AMG 2h	Run 1	88.41 ± 1.03
		Run 2	90.77 ± 0.41
B Incubation times increased from 60min to 75min; AMG 2h:			
1 SS + MES (2 x 10μl Termamyl):		Run 1	98.18 ± 0.46
		Run 2	97.53 ± 0.71
		Run 3	97.86 ± 0.65

[1] Termamyl (120KNU/g). 1 unit is the amount of enzyme which breaks down 5.26g soluble starch per hour at 37°C, pH 5.6 (Ca^{2+} content of solvent, 0.0043M)

[2] AMG (300AU/ml). 1 unit is the amount of enzyme which hydrolyses 1μmol maltose per min under the following conditions: 25°C, acetate buffer pH 4.3, in 30min.

* Mean data for n = 3-7 replicates.

Table 4.9. Effect of Longer Incubation Times (2h) with Termamyl on the Recoveries from Commercial Starch (%)

Starch Source	% Starch Hydrolysis
Soluble Starch (n = 14)	99.32 ± 0.21
Commercial Wheat Starch (n = 7)	99.52 ± 0.45
Commercial Rice Starch (n = 9)	98.63 ± 0.41
Commercial Potato Starch (n = 8)	99.34 ± 0.28

Initial incubation period with Termamyl at 100°C was extended to 90min.
Termamyl (10μl) added at 90°C for 90min.
AMG (0.1ml) added at 58°C for 2h.

Table 4.10. : Recovery of Starch from Novelose following pretreatment with KOH and/or Ethanol in the Assay Procedure.

Treatment	Starch Recovered from Novelose (% dwb)
No pretreatment	75.54 ± 0.63
plus Ethanol	72.03 ± 0.32
plus 2M KOH	94.55 ± 1.74
plus Ethanol + 2M KOH	81.12 ± 0.77

Each value represents the mean of 4 replicates

Table 4.11. Effect of Alkali Solubilisation (KOH) on the Estimation of Starch in a range of Wheat Flours [g starch.100g^{-1} DM]

Sample	Starch Content*		
	'Available'	'Total'	RS[a]
Soluble Starch	99.05 ±0.55	99.00 ±0.34	0
Novelose	75.81 ±0.38	95.51 ±0.62	19.7
Wheat 1	66.43 ±0.43	66.51 ±1.15	0.08
Wheat 2	68.31 ±0.27	65.86 ±1.24	0
Wheat 3	67.58 ±0.38	67.41 ±1.46	0
Wheat 4	71.41 ±0.10	69.42 ±2.02	0
Wheat 5	65.33 ±0.90	66.34 ±0.73	1.01
Wheat 6	66.27 ±0.56	67.23 ±0.60	0.96
Wheat 7	64.84 ±1.07	65.61 ±0.20	0.77

*Mean values of n=3 replicates
[a] Resistant starch

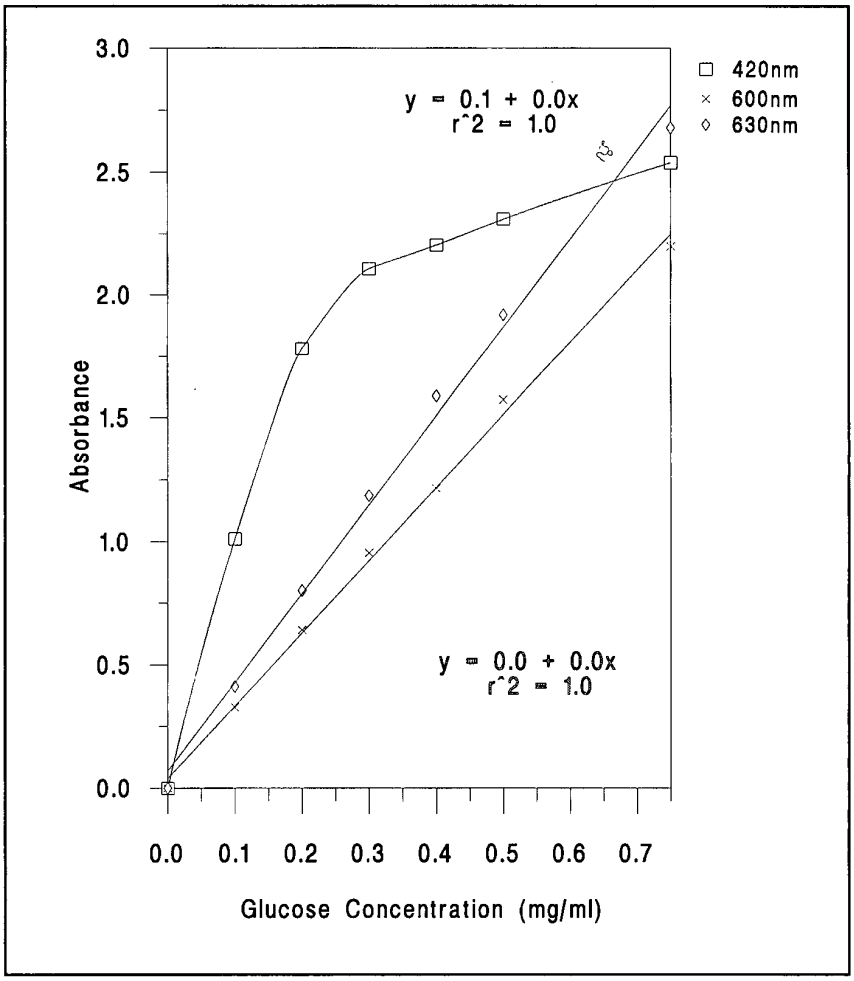
Table 4.12. : Effect of Absorption Wavelength on Starch Recovery as Glucose

Absorption Wavelength for Glucose (nm)	Starch (standard) Recovery (%)
420	78.81 ± 1.80 (n=12)
600	89.35 ± 0.93 (n=12)

Table 4.13. : Comparison of Various Starch Analysis Methods

Method	Starch Analysis
Libby, 1970	**8 samples per day**
Batey, 1982	**16 samples per day (8h)**
Karkalas, 1985	**4h**
Holm *et al.*, 1986	**30 samples per day**
McCleary *et al.*, 1994	**20 samples in 3h; a single assay can be performed in 2h**

Figure 4.1: Absorption of GOD-Peroxidase Coloured Complex



CHAPTER 5 APPARENT METABOLISABLE ENERGY (AME) VALUES OF WHEAT

5.1 DATA GENERATED FROM CURRENT PROGRAMME

The nutritional value of wheat in poultry diets varies particularly with respect to apparent metabolisable energy (AME) values. The sporadic occurrence of wheats with low-AME values is of particular concern to the animal feed industry and previously published reports illustrating this variability are presented in Table 5.1. In the current study, a number of different wheat samples were examined to assess the variability in AME values. The inconsistent availability of low-AME wheats, for which there was unequivocal evidence, presented difficulties throughout the research programme. One particular problem was that the AME value of specific varieties varied considerably with respect to season and site. Low AME wheats were identified in the current programme but considerable variability in AME was obtained ranging from 2.90 to 15.59 $MJ.kg^{-1}$ DM ($p<0.001$; Table 5.2). Coefficients of the metabolisability of gross energy (AME/GE) are also presented to eliminate variations caused by minor fluctuations in GE contents of wheats. Values were affected by wheat dietary inclusion level.

The low-AME wheat samples were, unfortunately, only available in limited quantities and other supplies covering a range of AME values were difficult to obtain. Because the determination of AME is sensitive to the method of evaluation, the data obtained are appropriate for comparative studies only, and cannot be regarded as absolute.

5.2. FACTORS AFFECTING AME VALUE OF WHEAT

Differences in AME values may be due to one, or a combination, of the following: diet, variety, growth site, harvest year and/or bird effects. In the

current study the majority of trials were conducted with birds over the same age range and similar live weights except for trial III, where birds of 2 ages were employed and where a marginal superiority for older birds was recorded.

5.2.1. Diet and rate of inclusion of wheat

A disadvantage with single ingredient assays (feeding the test material, wheat, alone) is that the bird may be consuming an unbalanced diet with respect to nutrient content (Sibbald, 1979). McIntosh *et al.* (1962) reported that individuals showed a high degree of variation which cast doubts on the use of the single ingredient assay type, while Lockhart *et al.* (1963) found that feeding wheat alone is acceptable. Initial trials in the current study used rates of inclusion of wheat of 900g.kg $^{-1}$ together with oil (to improve palatability) and a mineral / vitamin premix to meet requirements, both at 50g.kg^{-1}. Subsequent trials employed wheat at a lower rate of inclusion of 750g.kg^{-1} with soya protein isolate at 150g.kg^{-1} providing a reasonably balanced protein source. Sibbald *et al.* (1960) pointed out that the higher the amount of test ingredient included in the diet, the more accurate the AME value. It is possible to use substantial proportions of cereals in the test diets, provided the dietary protein level can be maintained (Leeson *et al.*, 1974).

The presentation of the wheat in the diet is known to be important in determining AME values. and evidence exists which suggests that feeding whole wheat to chickens results in significantly higher AME values ($p<0.01$) than the coarse, medium or fine ground forms which have similar AME values (McIntosh *et al.*, 1962). Throughout the current programme, all feed wheats were ground to pass through a 3mm sieve. This comparatively coarse grinding of wheat was used to minimise problems associated with 'beak impaction' which has been associated with feeding excessively fine ground cereals. Although pelleted diets may have reduced feed wastage, it was important that the physical structure of

the wheat starch granules was unaffected by, for example, steam processing (which occurs during the pelleting process) to avoid any possible changes to digestibility.

Trials I and II were based on high inclusion levels of wheat (900g.kg^{-1}). Although in practice such inclusion levels are never used, the rationale employed in the experimental design was that any deleterious effects with individual samples would be accentuated and would enable a wide range in nutritional values to be obtained. The difference between diet A and B was that the former contained supplementary methionine and the latter lysine. Both are nutritionally essential amino acids and it was felt that they could be deficient in diets with such high levels of inclusion of wheat. However these trials in general produced low results for AME and it was evident that such high inclusions levels could not be employed routinely thereafter although trial III compared these high levels with those of 750g.kg^{-1} in an attempt to reduce the uniformly poor responses obtained at the high levels whilst at the same time highlighting any differences that might have been attributable to variety.

Results from *Trial III* confirmed that the young broiler chick cannot utilise wheat at high dietary inclusion levels irrespective of variety. Low and erratic AME values were obtained for birds fed wheat at 900g.kg^{-1} (mean values ranged 2.90-10.10 MJ.kg^{-1} DM; $p<0.001$; Table 5.2). At lower wheat incorporation levels (750g wheat.kg^{-1}) within the diets, more consistent results were obtained, with wheats being utilised more efficiently with significantly ($p<0.001$) higher AME values (range of AME values 7.59-13.67 MJ.kg^{-1} DM) being obtained. Since the gross energies of such wheats did not vary to any great extent between the varieties tested, AME/GE values followed a similar trend to the AME values.

It is unlikely that the adverse influence of the high rate of inclusion was

attributable to nutrient deficiencies as the trials were designed to examine wheat digestibility over a comparatively short time scale rather than performance which is responsive to nutrient inadequacy but which is evaluated over considerably longer time periods.

In conclusion, the effect of diet is apparent in that significantly higher AME values were obtained for specific wheat cultivars when incorporated into diets at an inclusion of 750g.kg^{-1} compared with 900g.kg^{-1}. The higher dietary incorporation levels also resulted in considerably increased variation between individual birds on the same treatment which was associated with copious amounts of wet, cream-coloured excreta. These findings compare favourably with those of Payne (1976) who showed that high dietary inclusion levels of wheat for chicks resulted in low-AME values.

In the current study, lower rates of inclusion (750g.kg^{-1}) allowed for greater uniformity between replicates whilst still allowing individual wheat varieties to be ranked according to nutritional value and these rates were employed for all subsequent trials.

5.2.2 Wheat Variety

The effect of wheat variety on AME has been demonstrated as being important, but variety in isolation was not always sufficient to account for the large differences encountered in the current study. Although some wheat samples do vary in their ME content, there are few differences in ME between autumn sown wheat varieties produced in the UK (McNab, 1991).

In *Trial I*, four winter wheats were randomly selected to include 2 feed varieties (Hornet and Riband) and 2 milling varieties (Mercia and Pastiche). Varietal differences were apparent, although not significant, and mean AME values

ranged from 11.53-15.00 $MJ.kg^{-1}$ DM. However, variability between replicates was high and the lower mean AME values could be attributed to individual broilers reacting adversely to the high rates of inclusion of wheat ($900g.kg^{-1}$). Hornet and Mercia were considered to be low-AME varieties using this bioassay. AME values were related to feed and milling properties of the wheats; the milling varieties had significantly ($p = 0.006$) higher AME values compared to the feed varieties.

Little evidence for extreme variation in the nutritive value of varieties was obtained in *Trial II* and it was thought that differences obtained were likely to be a factor of the level of inclusion employed. Notwithstanding this, wheat samples 20 (unknown variety), 23 (Fortress) and 88 (Mercia) were considered to be of lower-AME value than average values. AME values for the low-AME wheats ($n = 3$) were significantly lower than values for the intermediate types ($n = 3$) ($p = 0.016$)

Although in *Trial III* a lower rate on inclusion of wheat was employed ($750g.kg^{-1}$), a considerable range in AME values was obtained (range of mean values 7.59-13.67 $MJ.kg^{-1}$ DM, $p < 0.001$), with 2 varieties (Avalon and Hereward) being identified as low-AME types. Admiral, Beaver, Haven, Mercia and Tara also showed low-AME values from one growth site but this finding was not replicated at the second growth site. However in all cases, except for Admiral, the second growth site gave lower AME values.

Two possible low-AME wheats were identified, in *Trials VII and VIII*, namely Rialto and Spark, although individual variation was high with birds fed these wheats. Rialto showed consistency between trials as being a low-AME type and Spark, whilst being marginally lower in AME value than the trial average, tended to be more of an intermediate type, but certainly was not regarded as being a wheat variety of high AME. Values for those wheats identified as being of high

AME were associated with lower standard errors.

Large differences in AME were obtained with varieties in particular trials, but this was not consistent between trials. Thus the same variety from different growth sites or harvest years behaved differently in different bioassays. It is evident that factors other than variety alone are important in the production of low-AME values. None of the wheats evaluated demonstrate low AME values consistently over the growth years. Environmental factors, such as climate and site, would therefore appear to be of major importance in the production of low AME wheats, although certain varieties may be predisposed to these factors. The data certainly demonstrate the variability in the AME values of wheats over successive harvest years.

Although the wheats are designated as being either low, intermediate or high-AME types, they are such ranked as such only to provide a comparative classification. There is in fact much overlap between wheats in the low-intermediary AME category and the intermediary-high AME category. Wheats with AME values less than 12 $MJ.kg^{-1}$ DM were regarded as being low AME types Table 5.3.) wheats with AME values greater than 14 $MJ.kg^{-1}$DM were considered as high AME types and those wheats with AME values in between 13 and 14 $MJ.kg^{-1}$ DM were thought to be intermediate. These categories are similar to those of Annison *et al.* (1987) who suggested that wheats with AME values $<13 MJ.kg^{-1}$ DM are considered as being low.

5.2.3 Effect of Growth Site

The growth site markedly affects the chemical composition of wheat but information on AME values is scarce. In *Trial III*, a difference in AME value of 2.65 $MJ.kg^{-1}$ DM was obtained for Avalon (low AME variety) grown on 2 sites. The same variety grown at different sites in subsequent years had normal AME

values. The effect of growing site was marginal but, although Wiseman and Inborr (1990) maintained that the effect of growing site was insignificant, a variety x site interaction was thought to be more important.

The effects of variety and growing site are difficult to define since harvest year (climatic effects) is possibly equally important. Notwithstanding these variables, differences are apparent for AME values for both variety and site. Considerable variability was experienced with respect to growth site (e.g. $p<0.001$ *Trial III*, Table 5.4.). For example, Mercia is generally a high / intermediate AME wheat, but the results of *Trials I and II* do not support this observation. The AME values for Riband were fairly constant across all trials with mean values in the range of 13.33 to 14.85 $MJ.kg^{-1}$ DM, and differences were possibly a result of variations in site and year. Avalon, which was reported as being a low AME wheat variety in *Trial III* (2 growth sites), produced high AME values in subsequent bioassays after a storage period, and *Trial VII* confirmed this. Mollah *et al.* (1983) reported a possible effect of location but the study was not designed specifically to examine the effect of growth site and data presented did not show any firm trends.

5.2.4 Effect of Harvest Year

Trials were not designed specifically to examine the effects of the environment (e.g. light intensity, temperature and precipitation) on AME values. Although Trial III examined the effect of site, the only valid comparisons that can be made when looking at the effect of harvest year are between those varieties grown at Nottingham University and used in *Trials I, V, VI and VII*, where Mercia was incorporated into *Trials I, V and VI*, and Riband was included in *Trials I and VII*. *Trials IV, V and VI* investigated the effect of wheat storage on AME, although developmental factors from season to season could not be ruled out (Table 5.2.).

The AME value of Mercia appeared to differ between harvest years with mean values ranging from 11.53 (1990) to 13.97 (1993) MJ.kg^{-1} DM, although the results obtained in 1990 were largely influenced by a number of individual birds behaving amomalously. The AME value for Riband, on the other hand, was fairly consistent with mean values (MJ.kg^{-1} DM) of 14.53 (1990) and 14.77 (1994). It seems likely from the data set available that the effect of growing year is probably not a major consideration when viewed alone, but in combination with site and varietal differences, the effects can be considerable.

5.2.5 Effect of Storage

Trials IV, V and VI were designed to investigate the effect of storage on AME values. Wheats from 2 consecutive harvest years were used in an attempt to identify this effect.

Three wheats (Avalon, Mercia and Riband) obtained from PBI in 1991 were stored ground under dry conditions and ambient temperatures (<18°C) for 20 months before a repeat bioassay. Avalon and Mercia supplied by PBI in 1992 were stored under the same conditions of temperature, light and ventilation for an additional period of 10 months after harvest in an unground state.

The results obtained from the first study (*Trials IV and V*) indicated that whole or ground wheats with inferior AME values at the outset inproved during prolonged storage at ambient temperature. Although the AME values (MJ.kg^{-1} DM) of Mercia (13.64 and 14.75) and Riband (13.67 and 13.33) remained relatively constant, those of Avalon improved markedly (7.59 to 14.32 [*Trial IV*] and to 13.35 [*Trial V*] MJ.kg^{-1} DM).

Further AME values (MJ.kg^{-1} DM) of Avalon (8.43) and Mercia (12.63) showed

an improvement to 14.24 and 15.06 $MJ.kg^{-1}$ DM respectively after 10 months storage (*Trial VI*). The time required for these changes to take place appeared to be longer than 6 months (both the 1991 and 1992 wheats were assayed within 6-7 months after harvesting), but within 10 months. Since conditions immediately prior to, and following, harvest are associated with changes in both chemical and structural composition of the wheat, bioassays were not performed immediately after harvest but rather approximately 1-2 months post harvest. From these studies it would appear that there could be an advantage in storing feed wheats post harvest for 10 months prior to feeding to guarantee uniformity and consistently highAME values.

Improvements in the nutritive value occurred both in the whole grains and meals when stored for 10 months at moisture contents of 110-113$g.kg^{-1}$ and temperatures less than 18°C. The nature of these changes have neither been investigated nor identified.

Recently, Choct *et al.* (1994) described similar effects with wheats reassayed 2-3 years after storage in rodent-proof containers in cool dry conditions; it was suggested that the gradual improvement in the AME values of the wheats may be due to an increase in the activity of the endogenous enzymes which degrade or destroy anti-nutritional components. Certain wheats contain a relatively high proportion of arabinoxylans which are heterogeneous with respect to the degree of branching and molecular weight. If the viscous high molecular weight species of these arabinoxylans were degraded to a lower molecular weight with correspondingly less viscous-promoting potential, the accessibility of α-amylases to the starch could be improved and, as a consequence, the nutritive value and AME would increase.

5.2.6 Bird Effects

AME values may be influenced by a number of bird factors including the species (Slinger *et al.*, 1964; Bayley *et al.*, 1968; Fisher and Shannon, 1973; Leeson *et al.*, 1974; Sugden, 1974), strain (Sibbald and Slinger, 1963; Slinger *et al.*, 1964; Foster, 1968a,b; Proudman *et al.*,1970; March and Biely, 1971) and age (Renner and Hill, 1960; Lockhart *et al.*,1963; Bayley *et al.*, 1968; Zelenka, 1968; Lodhi *et al.*, 1969, 1970; Rao and Clandinin, 1970). Many of the bird differences observed in the literature were either small or insignificant. Small differences in assay procedures can contribute to data variability (Sibbald, 1979).

11-14 day old Ross I broilers were used for the AME determinations in *Trials I to VI* but the effect of age was investigated in *Trial IV* where AME was assayed using PM3 broilers at 22-25 days of age. No overall differences in the AME values were obtained using the older and different bird types.

A large variation in the response of individual birds in to a specific diet was encountered *Trials I, II and III*. No individual wheat variety was identified which produced uniformly adverse effects in all the chicks, although birds receiving the higher AME wheats behaved more consistently. This indicates variability between individual broilers, and may involve factors such as feed intake, microflora load (numbers and types), and/or bird enzyme levels and activities. The different bird responses may represent an interaction between a variable component within the wheat and the individual bird especially since the responses of birds receiving the lower AME wheats were also more variable. Autopsy of a random selection of birds from *Trial III* revealed no evidence of pathological abnormalities and it was concluded that the bird responses could be attributed to high wheat contents of the diets.

Bird responses were also variable, but to a lesser extent, for individual wheat varieties at the 750g.kg^{-1} dietary inclusion level. Again, variation was greatest in those birds fed on the lower AME samples (SED 0.136- 1.136 MJ.kg DM). Feed intakes and excreta outputs were significantly increased ($p<0.001$) in those birds fed the poorer quality wheats (Avalon and Hereward, sites 1 and 2). Often, such birds responded with the production of copious quantities of wet and cream-coloured excreta usually within 3 days of feeding the experimental diets.

The effect of bird variation in *Trials IV, V and VI* was reduced in these experiments where the AME of poorer quality wheats improved significantly on prolonged storage. A maximum difference of 3.056 MJ.kg^{-1} DM was observed across all 3 trials and varieties. The effect of bird age was marginal although apparent in *Trial IV*, where older birds were used (22-25d) and AME values were between 0.59 and 0.85 MJ.kg^{-1} DM higher for Avalon and Mercia respectively. The higher AME values associated with older birds correlates with the findings of other workers (e.g. Fisher and Shannon, 1973). Zelenka (1968) showed that the ME declined from 3 to 7 days of age and then rose to a peak at 15 or 16 days.

Variation in AME values in *Trials VII and VIII* was again high with certain wheat varieties, Rialto (range of 3.04 -14.20 MJ.kg^{-1} DM), Spark (11.50-14.50 MJ.kg^{-1} DM) and Lynx (13.32-15.65 MJ.kg^{-1} DM). This finding was confirmed in *Trial VIII* when Rialto and Spark were re-assayed and found to have equally variable AME values (MJ.kg^{-1} DM) between 12.35-14.78 and 10.61-14.77 respectively. The variability of values from feeding higher AME wheats was considerably less.

Choct *et al.* (1996) showed that different wheat samples may give rise to variations in the proliferation of fermentative organisms in the digestive tract when they are fed to broilers. Although gut fermentation may increase the measured AME value of the diet, the volatile fatty acids produced by

fermentation are poorly utilised by poultry. This would have the effect of reducing the net availability of metabolisable energy.

Considerable variation was observed in DM intakes within diets (same variety) and between diets (based on different wheat varieties). Generally the variability was greater with higher intakes associated with specific wheat varieties, usually of low AME value (Table 5.5). Dietary wheat inclusion level had an important effect on feed intakes. Generally, the higher the dietary inclusion level, the higher the feed intake and the lower the AME value.

Hill and Anderson (1958) found AME to be independent of the food intake in the range from 0.3 to 1.0 of *ad libitum* intake. Brown *et al.* (1967) however demonstrated a statistically significant effect upon classical ME determinations due to restricting the feed intake of pullets to approximately 0.6 of their *ad-libitum* consumption.

Guillaume and Summers (1970) and McNab (1990) showed, however, that AME and AME_n values would vary with feed intake and the endogenous energy losses (EEL) per unit of food intake, according to the equation:

$$AME = TME - \frac{EEL}{FoodIntake} [MJ.kg-1DM]$$

where TME is the true metabolisable energy, and EEL is the endogenous energy losses.

TME would be independent of this variable (Guillaume and Summers, 1970; Sibbald, 1975). Guillaume and Summers (1970) showed that above a certain intake value, the influence of the plane of nutrition on TME is very small, i.e. it reaches a plateau. Below this value, however, the influence on ME is quite

marked, according to the formula:

$$y = \frac{ax - E}{x}$$

where y = ME (classical or corrected for nitrogen)
x = food intake
E = endogenous and metabolic energy
a = TME value

This theoretical approach agrees very well with the study of Hill and Anderson (1958) who could not find any influence of plane of nutrition on ME value. Indeed, the chicks ate approximately 1.2 to over 3.0 of their maintenance requirement for energy. In such a range, the difference in AME value is very small.

5.3 CONCLUSIONS

The AME values of wheat evaluated in the current study are extremely variable with mean values ($MJ.kg^{-1}$ DM) as low as 7.59 and as high as 15.59 being obtained. No definite pattern has emerged which enables a specific variety, growing site or harvest year to be identified with the production of these low AME wheats. Production site and harvest season appeared to be less important than variety in determining AME value, but no firm conclusions could be drawn from the data available. Composition of the diet is of paramount importance as evidenced by the inability of the young chick to utilise high dietary inclusion levels of wheat. Although wheat was included at $900g.kg^{-1}$ in an attempt to identify a range of wheats with differing AME values, such levels were associated with lower AME values. Variability does exist between individual birds and this probably accounted for some of the variation in AME values observed.

Although Wiseman and Inborr (1990) excluded extremely low AME values obtained from feeding young broiler chicks aged between 22 and 25 days of age from calculation of means, all replicates were included in the calculations in the current study, and this resulted in lower mean values and larger SED values in some instances due to the presence of some anomalous individual birds. The reasons for the individual variations are unknown. It is possible that a variable component within the wheat samples is interacting with a variable factor within the birds, such as enzyme activities or microflora inhabitation, to bring about this effect.

Prolonged storage of wheats at ambient temperatures resulted in an increase in the AME values of previously determined low AME samples. A period of between 6 and 10 months is required to bring about such changes. The reasons for these improvements and the processes involved are unclear. Since the process is time related, it is likely that some wheat component, possibly an anti-nutritive factor, is converted to another form until a critical level is reached below which it no longer exerts its effects.

Feed intakes are evidently variable between different individual birds, even when fed the same dietary treatment. Higher mean values are generally correlated with a greater degree of variability between individual birds fed certain low-AME varieties. In general, low mean AME values are associated with the consumption of higher amounts of feed although this was not always the case. Disproportionately high intakes correlated well with those individual birds that had extremely low AME values. Although a comparison between trials is not strictly valid, mean feed intakes overall range from 113 to 349 $g.3d^{-1}$ and 178 to 285 $g.3d^{-1}$ for the 900$g.kg^{-1}$ and 750$g.kg^{-1}$ dietary wheat inclusion levels respectively. This indicates the greater variability for high wheat inclusion levels within diets.

Table 5.1. Published AME Values for Wheat with Young Chicks

Term	Value ($MJ.kg^{-1}$ DM)	Sample Size	Reference
AMEn	12.35 -16.59	25	Sibbald and Slinger (1962)
AMEn	12.05 -13.47	7	Schumaier and McGinnis (1967)
AMEn	13.31 -15.86	33	March and Biely (1973)
AME	13.60 -15.06	16	Coates *et al.* (1977)
AMEn	13.10 -14.00	16	Davidson *et al.* (1978)
AME	11.00 -15.90	22	Mollah *et al.* (1983)
AME	10.35 -14.81	38	Rogel *et al.* (1987)
AME	14.43 -14.72	10[a]	Wiseman and Inborr (1990)
AME	13.04 -15.32	20[b]	Wiseman and Inborr (1990)
AME	9.52 -14.08	16	Choct *et al.* (1992)
AMEn	14.42 -16.46*	72	McNab (1995)

[a] 5 samples, 2 harvest dates in 1989; diet in mash form only.

[b] 5 samples, 4 different growth sites

* Results from 1991 trials

Table 5.2. Determined AME values for wheats (Table 3.1 for details of wheats)

Wheat Variety		AME	AME/GE
Trial I (diet A)			
Hornet	L	12.07 (1.63)	0.67 (0.09)
Mercia*	L	11.53 (1.25)	0.64 (0.07)
Pastiche	H	15.00 (0.12)	0.83 (0.01)
Riband	H	14.53 (0.25)	0.82 (0.01)
Analysis of Variance:			
F pr		0.063	0.052
s.e.d. min.rep		1.539	0.085
max.min		1.474	0.082
max.rep		1.405	0.078
Analysis of Variance: Low vs High AME			
F pr		0.006	0.005
s.e.d.		0.972	0.054
Trial II (diet B)			
Unknown (18)*	I	13.46 (0.17)	0.77 (0.01)
Unknown (20)*	L	12.05 (0.50)	0.70 (0.03)
Fortress (23)**	L	12.81 (0.53)	0.74 (0.03)
Galahad (37)**	I	13.05 (0.17)	0.74 (0.01)
Mercia (88)**	L	10.98 (1.46)	0.63 (0.08)
Unknown (98)**	I	13.15 (0.35)	0.75 (0.02)
Analysis of Variance:			
F pr	0.100	0.148	
s.e.d. min.rep	0.936	0.054	
max.min	0.837	0.048	
max.rep	0.725	0.042	
Analysis of Variance: Low vs Intermediate AME			
F pr	0.016	0.045	
s.e.d	0.491	0.029	

Table 5.2. Continued.

Trial III (diets B & C)				
Admiral 1	B	L	7.01 (1.67)	0.40 (0.10)
	C		12.63 (0.48)	0.72 (0.03)
Admiral 2	B*	I	10.10 (0.85)	0.58 (0.05)
	C		13.47 (0.17)	0.77 (0.01)
Avalon 1	B	L	5.02 (2.28)	0.29 (0.13)
	C		7.59 (0.42)	0.43 (0.02)
Avalon 2	B	L	4.48 (1.43)	0.26 (0.08)
	C		10.24 (1.02)	0.59 (0.06)
Beaver 1	B	I	9.02 (0.79)	0.52 (0.05)
	C		13.15 (0.40)	0.75 (0.02)
Beaver 2	B	L	5.75 (1.03)	0.33 (0.06)
	C		12.02 (0.84)	0.69 (0.05)
Haven 1	B	I	7.09 (0.76)	0.40 (0.04)
	C		13.36 (0.59)	0.74 (0.03)
Haven 2	B	L	6.97 (1.48)	0.39 (0.08)
	C		10.95 (0.98)	0.62 (0.06)
Hereward 1	B	L	2.90 (0.58)	0.17 (0.03)
	C		10.94 (1.06)	0.62 (0.06)
Hereward 2	B	L	5.15 (0.97)	0.30 (0.06)
	C		9.74 (1.14)	0.56 (0.07)
Mercia 1	B	I	4.75 (1.88)	0.27 (0.11)
	C		13.64 (0.32)	0.76 (0.02)
Mercia 2	B	L	6.21 (1.31)	0.35 (0.07)
	C		12.94 (0.66)	0.73 (0.04)
Riband 1	B	I	7.74 (1.37)	0.43 (0.08)
	C		13.67 (0.14)	0.76 (0.01)
Riband 2	B	I	6.06 (1.01)	0.33 (0.06)
	C		13.49 (0.56)	0.74 (0.03)
Tara 1	B	I	4.81 (1.75)	0.27 (0.10)
	C		13.46 (0.20)	0.75 (0.01)
Tara 2	B	L	9.38 (0.86)	0.52 (0.05)
	C		11.76 (0.93)	0.66 (0.05)
Analysis of Variance:				
F pr	Variety		<0.001	<0.001
	Site		0.789	0.703
	V x S		0.119	0.092
	Diet		<0.001	<0.001
s.e.d.	Variety		0.778	0.044
	Site		0.389	0.022
	V x S		1.100	0.062
	Diet		0.389	0.022

Table 5.2. Continued

Trial IV (diet C)				
Avalon 1	P1*	H	14.32 (0.12)	0.78 (0.01)
	P2		14.90 (0.16)	0.81 (0.01)
Mercia 1	P1*	H	14.75 (0.19)	0.81 (0.01)
	P2		15.59 (0.24)	0.85 (0.01)
Analysis of Variance:				
	P1			
F pr			0.094	0.031
s.e.d.			0.227	0.012
	P2			
F pr			0.039	0.018
s.e.d.			0.290	0.016
Trial V (diet C)				
Avalon 1		I	13.35 (0.43)	0.71 (0.02)
Riband 1		I	13.33 (0.45)	0.720 (0.03)
Mercia [SB, 1993]		I	13.18 (0.17)	0.72 (0.01)
Analysis of variance:				
F Pr			0.942	0.912
s.e.d.			0.528	0.028
Trial VI (diet C)				
Avalon 1		H	14.24 (0.21)	0.77 (0.01)
Mercia 1*		H	15.07 (0.29)	0.82 (0.02)
Mercia [SB, 1993]		H	13.97 (0.11)	0.79 (0.01)
Analysis of Variance:				
F PR			0.004	0.040
s.e.d.			0.283	0.015

Table 5.2. Continued.

Trial VII (diet C)			
Avalon* [SB, 1994]	H	14.68 (0.15)	0.79 (0.01)
Beaver	I	13.65 (0.39)	0.73 (0.02)
Lynx	I	13.69 (0.58)	0.74 (0.03)
Mercia	H	14.59 (0.14)	0.78 (0.01)
Rialto	L	11.75 (1.76)	0.63 (0.09)
Riband	H	14.19 (0.16)	0.76 (0.01)
Riband [SB, 1994]	H	14.77 (0.17)	0.79 (0.01)
Spark*	I	13.76 (0.59)	0.74 (0.03)
Analysis of Variance:			
F Pr		0.107	0.108
s.e.d.		1.016	0.054
Analysis of Variance (High vs Low AME Wheats):			
F Pr		0.010	0.012
s.e.d.		0.507	0.027
Trial VIII (diet C)			
Rialto*	I	13.62 (0.48)	0.73 (0.03)
Riband	H	14.85 (0.06)	0.80 (0.01)
Spark	I	13.23 (0.66)	0.69 (0.03)
Analysis of variance:			
F Pr		0.061	0.014
s.e.d.		0.647	0.034
Analysis of Variance (High vs Intermediate AME Wheats):			
F Pr		0.019	0.006
s.e.d.		0.547	0.030

AME values represent mean values (n = 6 replicates); * (n = 5 replicates); ** (n = 3 replicates). Standard error values are given in parentheses

'L, I and H refer to low, intermediate and high - AME samples.

A, B and C refer to rates of inclusion of wheat of 898, 896.3 and 750g.kg^{-1}

P1, chicks 11-14 days of age; P2, chicks 23-26 days of age.

Table 5.3. Wheats identified as being of low-AME types (< 13.0 MJ.kg^{-1} DM)

Trial	Year	Growth Site	Wheat
I	1990	SB	Hornet, Mercia
II	1990	IAPGR	20 (unknown), 23 (Fortress), 88 (Mercia)
III	1991	PBI	Admiral 1, Avalon 1 & 2, Haven 2 Hereward 1 & 2, Beaver 2, Tara 2
VII / VIII	1994	BPF	Rialto

Table 5.4. Effect of year and growth site on AME Values (MJ.kg^{-1} DM).

Variety	Year	Growth / Harvest Site				
		1	2	3	4	5
Avalon	1991	7.59 L	10.24 L	.	.	.
	1991	.	.	14.32 H	.	.
	1992	.	.	14.24 H	.	.
	1994	.	.	.	.	14.68 H
Mercia	1990	.	.	.	.	11.53 L
	1990	.	.	.	10.98 L	.
		.	.	.		.
	1991	13.64 I	12.94 I	.	.	.
	1991	.	.	14.75 H	.	.
	1992	.	.	15.07 H	.	.
	1993	.	.	.	.	13.18 I
		.	.	.	.	13.97 H
	1994	.	.	.	.	14.59 H
Riband	1990	.	.	.	.	14.53 H
	1991	13.67 I	13.49 I	.	.	.
	1994	.	.	.	.	14.19 H
		.	.	.	.	14.77 H

Sites 1, 2 and 3 - Plant Breeding International (PBI); Site 4 - Institute of Animal Physiology and Genetics Research (IAPGR);
Site 5 - Nottingham University Frams.
L = Low AME (<13 MJ.kg^{-1} DM); I = Intermediate (13-14 MJ.kg^{-1} DM); H = High AME (>14 MJ.kg^{-1} DM).

Table 5.5. Relationship between AMEn of wheat and feed intake.

Variety	Trial	AME Category	DM Intake (over 3d) at wheat inclusion: 900g.kg^{-1}	750g.kg^{-1}
Hornet	I	L	165.84 ± 26.01 (6)	
Mercia	I	L	171.79 ± 23.20 (5)	
Riband	I	H	113.06 ± 6.26 (6)	
Pastiche	I	H	121.85 ± 9.01 (6)	
Unknown (18)	II	I	148.04 ± 4.54 (5)	
Unknown (20)	II	L	149.82 ± 9.12 (5)	
Fortress (23)	II	L	138.19 ± 12.77 (3)	
Galahad (37)	II	I	123.52 ± 3.77 (3)	
Mercia (88)	II	L	149.61 ± 11.54 (3)	
Unknown (98)	II	I	143.75 ± 7.07 (3)	
Admiral 1	III	L	262.96 ± 45.11 (6)	231.93 ± 14.04 (6)
Admiral 2	III	I	186.75 ± 14.48 (5)	215.99 ± 6.06 (6)
Avalon 1	III	L	349.17 ± 35.27 (6)	264.86 ± 18.66 (6)
Avalon 2	III	L	284.68 ± 35.24 (6)	235.09 ± 21.22 (6)
Beaver 1	III	I	215.07 ± 23.47 (6)	235.06 ± 15.67 (6)
Beaver 2	III	L	277.08 ± 24.84 (6)	219.02 ± 26.69 (6)
Haven 1	III	I	298.00 ± 34.51 (6)	232.13 ± 13.34 (6)
Haven 2	III	L	288.14 ± 27.26 (6)	259.54 ± 17.72 (6)
Hereward 1	III	L	349.40 ± 30.07 (6)	247.71 ± 28.15 (6)
Hereward 2	III	L	295.40 ± 15.07 (6)	230.43 ± 18.89 (6)
Mercia 1	III	I	250.45 ± 30.72 (6)	215.02 ± 9.96 (6)
Mercia 2	III	L	308.08 ± 29.47 (6)	219.64 ± 6.85 (6)
Riband 1	III	I	249.74 ± 31.37 (6)	220.62 ± 8.62 (6)
Riband 2	III	I	300.53 ± 33.53 (6)	213.13 ± 15.22 (6)
Tara 1	III	I	278.30 ± 36.66 (6)	188.34 ± 11.44 (6)
Tara 2	III	L	199.11 ± 8. 94 (6)	218.80 ± 11.97 (6)
Avalon 1	IV (1)	H		192.00 ± 10.78 (5)
	IV (2)			242.37 ± 8.08 (6)
Mercia 1	IV (1)	H		190.29 ± 5.64 (5)
	IV (2)			244.91 ± 7.18 (6)
Avalon 1	V	I		284.66 ± 17.88 (6)
Riband 1	V	I		252.05 ± 18.80 (6)
Mercia	V	I		236.00 ± 15.98 (6)
Avalon 1	VI	H		264.14 ± 18.18 (6)
Mercia 1	VI	H		274.85 ± 22.75 (5)
Mercia (SB)	VI	H		279.41 ± 11.87 (6)
Avalon	VII	H		202.90 ± 4.07 (5)
Beaver	VII	I		180.60 ± 11.38 (6)
Lynx	VII	I		178.71 ± 13.58 (6)
Mercia	VII	H		216.33 ± 7.61 (6)
Rialto	VII	L		199.15 ± 26.41 (6)
Riband	VII	H		200.09 ± 13.91 (6)
Riband (SB)	VII	H		179.85 ± 5.92 (6)
Spark	VII	I		197.30 ± 17.10 (5)
Rialto	VIII	I		207.99 ± 14.11 (5)
Riband	VIII	H		215.02 ± 8.80 (6)
Spark	VIII	I		248.28 ± 15.02 (6)

Values in parentheses refer to the number of replicates per treatment.

CHAPTER 6 CHEMICAL COMPOSITION AND DIGESTIBILITY OF COMPONENTS OF WHEAT

6.1 INTRODUCTION

Generally, the nutritive value of cereals is defined in terms of chemical composition and digestibility. Wheats vary widely in their chemical composition as a result of varietal, genetic, environmental and cultural factors. The cereals are usually incorporated into livestock feeds on the basis of average composition (Coates *et al.*, 1977). In some circumstances, this may result in lower than average performances within flocks due to the presence of low-AME varieties.

Not all of the potentially digestible fractions of wheat are in fact completely digested by animals. The factors controlling digestibility include particle size of the feed, level of feeding, chemical composition, animal species and age, and intestinal microflora (Minson, 1976).

Environmental factors such as climate and weather damage, location of growth and harvest year, may play a significant role in determining nutritive value, particularly as conditions immediately prior to, and following, harvest are associated with changes in both chemical and structural composition of wheat (Wiseman and Inborr, 1990).

It is the objective of this chapter to examine the correlations between previously determined AME values (see Chapter 5) and gross chemical composition together with the digestibility of other major components.

6.2 GROSS CHEMICAL COMPOSITION OF WHEATS

The chemical composition of wheat is subject to variation and, according to

Bolton and Blair (1974), wheat has the most variable composition of any of the cereals. This is believed to have significant implications on the nutritive value for young poultry. The chemical structure of wheat is an important consideration when attempting to identify those chemical components that may influence nutritive value. The value of wheat in animal feeding is thought to be affected by the interactions between the individual chemical components within the grain. Evidence linking the variability in nutritive value to differences in chemical composition is somewhat limited. Some evidence, largely anecdotal (Coates *et al.*, 1977), has suggested that differences in chemical composition between different samples of wheat has a predictable effect upon their AME value. Indeed, Mollah *et al.* (1983) implied that the chemical composition of wheat is related to the AME value. However, Hill *et al.* (1960) found that in spite of a wide variation in wheat composition, particularly in protein content, 10.5 to 17.2 $g.100g^{-1}$, all the varieties of wheat tested had similar ME values averaging 15.29 $MJ.kg^{-1}$ DM. It might be expected that small differences in AME values may be reflected, at least in part, by the differences in gross energy measurements. Evaluation of the metabolisability of gross energy (AME/GE) were included in the current study to eliminate such discrepancies, although differences between samples were still observed.

Within certain varieties, some unidentifiable factors are believed to exhibit anti-nutritive properties. To examine the possible factors involved and to elucidate their mechanism of action, detailed chemical characterisation studies on various wheats were performed to investigate the low-AME phenomenon. Biochemical assessments of the carbohydrate fraction were undertaken to include starch and the composition of the non-starch polysaccharides (NSP). Protein and fat were also determined. Trials are examined on an individual basis although general conclusions will also be drawn.

6.2.1 Trial I

Mean results for gross compositional analysis are shown in Table 6.1. The wheats were found to be composed predominantly of starch with lower amounts of protein, fat and non-starch polysaccharides. Differences between samples were confined primarily to the contents of starch (range 60.8 - 71.3 $g.100g^{-1}$ DM) and digestible starch (range 49.5-69.7 $g.100g^{-1}$ DM). Although starch represents the main source of energy in wheat, total starch contents were not correlated with variations in AME, although only a small number of samples were analysed. This finding does, however, corroborate the observations of Mollah *et al.* (1983) and Rogel *et al.* (1987), and would indicate that factors other than total starch content are important. A good correlation ($r^2 = 0.83$) was, however, observed between the contents of digestible starch and the AME. Evaluation of protein content ($g.100g^{-1}$ DM) showed a significant range of values between 9.88 and 14.49. The lipid content for all wheats was small (range of mean values between 2.25 and 2.48 $g.100g^{-1}$ DM) and differences between varieties were minimal. Determination of the dietary fibre content as NSP gave a range of 133 to 140 $mg.g^{-1}$ DM for the wheats evaluated. No relationship was observed between the protein, lipid or total NSP contents and the AME values. Values for uronic acids are shown but again are too small (mean range of 2.63 - 4.57 $mg.g^{-1}$ DM) to bear any significant relationship to AME.

6.2.2 Trial II

Wheats had compositional variation although such differences were not correlated with AME values. Significant differences were found between the contents starch (range 64.9 - 74.4$g.100g^{-1}$, $p<0.001$), protein (9.5 - 12.9 $g.100g^{-1}$, $p<0.001$) and NSP (103 - 116 $mg.g^{-1}$, $p<0.001$) There was a slight

indication that the NSP content in the milling varieties (Riband and Pastiche) was higher than that of the feed varieties (Hornet and Mercia). No relationship was observed between the AME or starch and the total NSP content. No significant differences were observed in the lipid content of the wheats. No differences were observed in the contents of GE, starch, protein or fat between the milling or feed types.

6.2.3 Trial III

Variations in chemical composition lay primarily in the contents of starch (64.7 - 73.2 g.100g^{-1} DM) and protein (8.3 - 13.6 g.100g^{-1} DM). Selected wheats (four varieties) which had provided an extreme range of AME values from Trial III were evaluated biochemically. Starch content differed significantly ($p<0.001$), but overall (eight varieties, two growth sites) the degree of variation was not responsible for the wide range of AME values obtained (overall there was a poor correlation between starch content and AME, $r^2=0.36$). A factor contributing to this failure to predict AME was the wide variation in starch digestibility (see Table 6.2). Accordingly, a more appropriate procedure, as pointed out by Longstaff and McNab (1986) is to correlate AME with the content of digestible starch (i.e. total starch content multiplied by the coefficient of digestibility of starch). Results (range of 45.3 - 70.9 g.100g^{-1} DM) were highly variable (SEd = 4.39, $p<0.001$). AME values were highly correlated with the contents of digestible starch ($r^2=0.88$) and negatively correlated with the content of indigestible starch ($r^2=0.99$).

6.2.4 Trials IV and V

Since wheats used in these trials were those used in Trial III, detailed futher analyses were not undertaken. Correlations between chemical composition and AME were hampered by the lack of variation in AME values (which were all high)

between the wheat samples, despite ranging widely in Trial III (samples were chosen on the basis of having the highest and the lowest values). Variations in diet and starch digestibility were also limited.

The effect of bird age (Trial IV) was evident but marginal and not surprising in view of the AME values recorded. Feed intakes and thus NSP intakes were higher in older birds and AME values were higher. This finding might suggest that the older birds can either utilise dietary NSP more efficiently or have a superior colonisation of microflora which can cope with these elevated intakes.

6.2.5 Trial VI

This trial was designed to investigate the possibility of improvement in AME values of certain wheats on storage and was not conducted to assess chemical composition and its relationship with AME.

6.2.6 Trial VII

Differences in the gross chemical composition (Table 6.1) of eight wheats tested in this trial were marginal despite relatively large differences in wheat AME. None of the measured parameters were correlated with AME values. The range of starch contents extended from 65.6g.100g^{-1} to 67.5g.100g^{-1} DM and was independent of AME value. Mean protein contents ranged from 12.8 to 14.1 g.100g^{-1} DM although were too small to have any effect on nutritive value or AME. Mean lipid contents were significantly different for the varieties tested but these were not correlated with AME. Mean values ranged between 2.1 and 3.4g.100g^{-1} DM. Total NSP contents (mg.g^{-1} DM excluding uronic acids) were variable between 100.2 and 118.2 but would not appear to be related with AME values obtained.

These findings indicate that gross chemical composition is not closely related to the nutritional value of wheat although the content of digestible starch is strongly correlated with wheat AME ($r^2 = 0.94$).

The improvement in AME over prolonged periods of time when certain low-AME wheats are fed (Table 5.1) may be influenced by the content of starch (or content of digestible starch) which would appear to change with time during storage. Table 6.3 shows, albeit to a small extent, the increase in starch content of Avalon and Hereward (low-AME varieties). This change in chemical composition (and/or digestibility) may be significant enough to enhance the nutritive value of such wheats when fed to young chicks. The reason for such a change is not known and may merely represents a discrepancy between evaluations, although this may provide one possible explanation for the improvement in nutritive value and AME over time and, since starch represents the main energy source, this difference could be enough to account for the variation observed. The other probably more plausible explanation for such an improvement is one of NSP destruction by endogenous enzymes within the grain.

6.2.7 Conclusions

AME does not appear to be associated with gross biochemical measurements. Although the greatest proportion of energy contributed to AME is from starch, there is only a poor correlation between the two. It is difficult to recognise whether poor nutritive value is due to either the intrinsic properties of the starch granule itself, as opposed to total contents, or as a consequence of extrinsic factors. Certainly, protein, lipid and total NSP would not appear to have any marked effect on the potential nutritive value of wheats for broilers.

6.3 COMPOSITION OF WHEAT NSP

Variation in NSP content of wheat is thought to relate to the variability in nutritive value and AME (Choct and Annison, 1990), although reports have been contradictory. Specifically, it is believed that high levels of wheat pentosans, accounting for between 0.5 - 0.8 of the NSP, and consisting mainly of arabinose and xylose (Annison, 1990), exhibit anti-nutritive activity (Choct and Annison, 1990), and are the cause of the poor nutritive value. Annison and Choct (1991) and Choct and Annison (1992) have obtained a tenuous, inverse relationship between digestibility and the pentosan (arabinoxylan) level in the grain. These pentosans were found to reduce the digestibility of all components, and as a consequence, lower the AME; however the studies were conducted with NSP isolates.

In the current study, analyses on selected wheat samples revealed some variation in the NSP content and composition although the differences were unlikely to be the major influential factor on nutritive value since no significant correlations could be made with AME or energy metabolisability.

The composition of NSP are presented in table 6.2. Pentosans represent between 0.59 and 0.69 of the total NSP. The insoluble NSP are present in higher amounts in wheat than the soluble fraction and generally represent between 0.51 and 0.88 of the total NSP. The major components of each fraction are arabinose, xylose and glucose, with mannose and galactose present in much smaller amounts. As before, individual trials will be considered prior to a general discussion.

6.3.1 Trial I

The results reveal differences between the wheat varieties, although major

effects were mainly in the content of arabinoxylans (range of 82.79 - 88.71 $mg.g^{-1}$ DM) and the insoluble fraction of the NSP (range of 71.69 - 90.94 $mg.g^{-1}$ DM). Ratios of soluble to insoluble NSP were not correlated with AME. The pentosan fraction of the NSP contained greater quantities of xylose than arabinose (ratio of ara:xyl is 0.62-0.69) but no differences in content were apparent between the different wheat types. Compositional analysis of the total NSP (Table 6.4) revealed differences between the wheats primarily in the contents of glucose with an indication that higher glucose contents may be linked with higher AME values ($r^2 = 0.76$). No relationship was observed between the arabinose or xylose contents and AME.

6.3.2 Trial II

No relationship was observed between the AME or starch and NSP content in this study. Fractionation of the NSP into soluble and insoluble components revealed a variable ratio of between 0.30 and 0.47. No significant correlation existed between either of these two variables and the AME. The insoluble NSP appeared to be biologically inactive in the young chick. No correlation was evident between the non-cellulosic polysaccharides (NCP) of wheat and AME. Contrary to expectation, the arabinoxylan content was not correlated with AME. This was probably attributable to the relatively narrow range of arabinoxylan contents amongst the wheat samples. A strong negative correlation ($r^2 = 0.92$) existed between the AME value and the ratio of arabinose-to-xylose in the NSP.

6.3.3 Trial III

The four wheat samples were chosen to include a low- (Avalon), an intermediate- (Hereward), and two high- (Mercia and Riband) AME varieties (Table 6.2).

Variations in the contents and compositions on NSP were evident but minimal. No relationship was observed between the AME values and the total contents of NSP. Previous studies indicating a strong negative correlation between the ratio of arabinose-to-xylose in NSP and AME ($r^2 = 0.92$) are not supported in this instance. Fractionation of the NSP into soluble and insoluble components revealed a variable ratio of between 0.33 and 0.46, although varietal differences were insignificant and data could not be correlated with AME. Ratios of arabinose-to-xylose in the soluble fraction are inversely associated with AME ($r^2 = 0.80$). It may be that a specific molecular weight fraction within the NSP complex could be important. A weak negative relationship was apparent between the insoluble NSP fraction and the AME. It is possible that conditions within the intestines could bring about marked solubilisation of the initially insoluble hemicelluloses by virtue of freeing them from isolated starch and protein or through the action of cytolytic enzymes in the gastrointestinal tract (Burnett, 1966). The water-soluble component had no effect on AME.

6.3.4 Trial VII

Compositional analysis of NSP for the eight wheats tested, ranging in AME value between 11.75 to 14.77 $MJ.kg^{-1}$ DM, revealed variability between the samples (Table 6.2). Total pentosan contents ranged from 59.1 to 74.4 $mg.g^{-1}$ DM. A weak, negative relationship existed between total pentosan content and AME value of the wheat ($r^2 = 0.46$), indicating that as the pentosan content in the wheat increased the AME values decreased. The relatively high contribution of the water-insoluble NSP fraction to the total NSP compares with other wheat samples tested. Values for insoluble NSP ranged 77.9 to 92.0 $mg.g^{-1}$ DM and differences were highly significant. The content of soluble NSP ranged 13.0 to 40.4 $mg.g^{-1}$ DM and differences were highly significant. Neither of these variables could be related strongly with variations in AME, although a weak link between the soluble NSP and AME values might suggest that as the content of

soluble NSP increased, the AME value decreased. The ratio of soluble to insoluble NSP in the wheat varied between 0.14 and 0.52 although these differences were only loosely correlated (negatively) with AME. Non-cellulosic polysaccharide (NCP) contents varied between 75.9 and 100.0 $mg.g^{-1}$ DM. Differences were significant but only tenuously related to AME in a negative relationship. Cellulose does not appear to exert any effect on the AME value and contents ranged 10.8 to 25.3 $mg.g^{-1}$ DM.

6.3.5 Discussion

Evidence is available which indicates that wheat NSP (and components of NSP) may be indirectly associated with nutritive value. It is possible that a combination of factors are responsible for the low-AME of some varieties. Perhaps a threshold value (critical value) exists for the anti-nutritional factor within wheat, below which the deleterious effects are not manifested. Certainly bird factors play an important role since some birds are affected more so than others. This may be a consequence of differences in feed intake (i.e. increased concentration of the anti-nutritive factor) since intakes, in general, were loosely and negatively correlated with AME ($r^2 = 0.52$). Correlation between feed intake and dry matter digestibility was, however, weak ($r^2 = 0.37$, inverse relationship). It would seem that the pentosans have the greatest effect on the AME values and it is possible that with higher contents of pentosan within the wheat grain (e.g. 67-70 $mg.g^{-1}$ DM in this case), concentrations are such to bring about the reduction in AME values seen with certain varieties when fed to young chicks. Certainly a reduction of between 0.80 and 2.81 $MJ.kg^{-1}$ DM is observed in AME values at these high pentosan levels when compared with those varieties with AME values greater than 14.0 $MJ.kg^{-1}$DM. Pentosan levels below this concentration may limit the exent of anti-nutritive activity. It is possible however that this critical value for pentosan content may, in actual fact, vary from sample to sample since wheats in previous trials with equal or higher pentosan

contents did not have the same effect on the AME value.

Observations of Choct and Annison (1990) and Annison (1991) demonstrated that the AME of different cereals was negatively correlated with total NSP contents. It seems apparent from the present study however that this relationship does not hold for different wheat varieties, although only a small number of samples were analysed. Choct and Annison (1990) suggested that other types of soluble NSP contribute to the anti-nutritive activity. Annison (1991) later showed that a strong negative correlation existed between the AME of wheat and the levels of neutrally-extracted NSP (which comprised mainly of arabinoxylan) when added as isolates. It was concluded that the water-soluble NSP of wheat possesses anti-nutritive activity in the gastrointestinal tract of young birds, causing a general, non-specific inhibition of nutrient digestion. Choct and Annison (1992) subsequently demonstrated that both water-extractable and alkali-extractable pentosan-rich fractions, when added to experimental diets as purified isolates, reduced AME. These findings, however, relate only to indirect measurements and may not represent the situation *in situ*.

6.3.6 Conclusions

Although the gross chemical composition of wheat varied between varieties, evidence linking this to differences in nutritive value is limited. The bioassay responses were, in general, highly variable and lower mean values were the result of some individual broilers reacting adversely to individual wheats.

The data obtained would indicate that varietal differences are of greater importance, since the influence of growing site on AME (Trial III) was of little importance. The possibility of a site x variety interaction requires more definitive testing.

Results might suggest that the intrinsic properties of the starch granules together with a component of the NSP, possibly a specific molecular weight fraction, may react interactively within the digestive tract to bring about variations in AME. It is possible that the variation in NSP determined is too small for the anti-nutrititive properties to be established, although it cannot be ruled out that a specific component could be inducing subtle changes within the gastro-intestinal tract of the birds to bring about these effects.

Generally, published studies have relied on isolating, purifying and then adding pentosans back to experimental diets (e.g. Choct and Annison, 1990). It is possible that such isolates do not behave in the same manner as the fraction *in situ*. It is also possible that the isolation process itself may induce changes which lead to a detrimental nutritional response.

6.4 DIGESTIBILITY OF COMPONENTS OF WHEAT

The term AME (see Table 5.1 for values obtained for wheats in the current study) assesses the metabolisability of gross energy (GE) but it is also a reflection of the degree to which components are digested. Thus for example poor starch digestibility has been used to explain the existence of low-AME wheats (Mollah *et al.*, 1983). Typically, cereal starches are regarded as being readily digestible. Starch digestibility, however, may vary considerably between different wheat varieties, and evidence is presented which also indicates variability within varieties when fed to broilers (high SEM values). Table 6.5 presents data on dry matter digestibility and, in those trials where it was evaluated, digestibility of starch, fat, NSP and pentosans. As overall dry matter digestibility is closely correlated with digestibility of other components, the latter (which are more complex to estimate chemically) were not routinely evaluated.

Dry matter (DM) digestibility measurements (Trial 1) revealed significant

differences between varieties in accordance with variations in AME, although no differences were observed overall between the feed (Hornet, Mercia) and milling (Riband, Pastiche) types. DM and starch digestibilities were highly correlated with AME values obtained ($r^2 = 0.99$ and 0.96 respectively). DM digestibilities were also strongly correlated in Trial II with AME and AME/GE ($r^2 = 0.93$ and 0.97 respectively. In trial III, Significant correlations ($r^2 = 0.98$ and 0.97 for diets B and C respectively) were also obtained between overall dry matter digestibility and AME . Significant correlations ($r^2 = 0.98$ and 0.97 for diets B and C respectively) were obtained between overall dry matter digestibility and AME even with lower dietary incorporation levels of wheat (750g.kg^{-1}). Although the majority of birds responded normally, apparent starch digestibilities were highly variable (range of 69.9-97.4 g.100g^{-1} DM; $p < 0.001$), depending on variety fed, and correlated with AME ($r^2 = 0.86$). AME values are closely related to the contents of digestible starch ($r^2 = 0.86$). In both cases, varietal differences were highly significant ($p < 0.001$) but site alone was not an influential factor.

Although starch is generally well digested, there are occasions when this is not the case which indicates that the availability of starch *per se* is an important controlling factor. It might be suggested that the very young bird is unable to utilise such high incorporation levels of wheat efficiently due to an inadequacy of digestive enzymes. Despite evidence confirming that even the young chick secretes α-amylase in excess of its requirements (Moran 1982; Rogel *et al.*, 1987), data are available which demonstrate that the specific activity of the small intestinal amylase increases from day 2 to 17 when it is 5-fold that at hatching. This may possibly present a limiting factor to early digestion in the very young chick (Nitsan *et al.*, 1991).

Apparent starch digestibility appeared to be independent of the NSP level or composition (Trials I, II and III); however the digestibility of NSP and pentosans (including uronic acids), which were generally low indicating that the bird is

unable to utilise these components to any appreciable degree, indicates that there was variation which tended to follow that of AME in terms of ranking of wheat samples. Digestibility of component monosaccharides of the NSP complex (Table 6.6) showed that glucose was the most efficiently digested residue overall (mean coefficient of digestibility of 0.46). Arabinose and xylose appeared to be digested equally well with an average coefficient of 0.34. However, the pentosans of the milling wheat types (Mercia and Pastiche) were more digestible than those of the feed wheat types (Hornet and Riband).

Digestibility of the NSP fraction is somewhat limited and generally restricted to less than 0.50. Digestibility of the pentosan complex itself is hampered by the lack of suitable endogenous enzymes secreted by the chicken. However, microbial activity could be responsible for a degree of degradation of this complex. Thorburn and Wilcox (1965) commented that, as the crop and caeca are periodically and regularly emptied, the build-up of a significant microbial population may well be precluded. Nevertheless, digestibility coefficients for pentosans of between 0.370 and 0.432 were recorded for adult birds which were reduced to between 0.327 and 0.339 in caecectomised birds. These figures compare favourably with a figure of 0.326 obtained by Bolton (1955a). Longstaff and McNab (1986) subsequently obtained coefficients of digestibility of pentosans of 0.24 with adult birds, although it was not possible to clarify whether hydrolytic products were the component monosaccharide groups themselves or volatile fatty acids. Although the contribution of such activity to overall energy balance will be very small, it should not be forgotten that even a relatively modest disruption of the cell wall would allow access of enzymes and a more complex hydrolysis of the important nutrients contained within the cell (Wiseman and Inborr, 1990).

Wheat starch (Trial I) was almost completely digested (>0.90) in the milling types with higher AME values with the indication that the availability of starch

per se is the most influential factor. These findings corroborate those of Wiseman and Inborr (1990). It was concluded that the digestibility of starch was most closely linked with variations in AME

Starch digestion was found to be independent of NSP content or composition. This finding might suggest that factors other than the total NSP content present in the grain are responsible for variations in nutrient utilisation. This could be an artefact due to the comparatively narrow range of arabinoxylan contents and the presence of a few seemingly anomalous birds. Furthermore, no correlation was obtained between the arabinose-to-xylose ratio in the NSP and the AME which would indicate that the degree of branching, or more specifically the structure within the pentosans, rather than the contents is not important in determining starch digestibility.

6.5 GENERAL CONCLUSIONS

It is clear that there is a direct link between the digestibility of all components within the grain and the AME, but the causes of this are unclear. Thus, overall dry matter digestibility and that of starch varies with AME. Information suggests that isolated wheat starch is almost completely digested in poultry, at least by adult birds (Longstaff and McNab, 1986), and even the young bird is supposed to have sufficient endogenous α-amylase present to digest dietary starch (Moran, 1985). However, it would seem that wheat starch *in situ* is not completely digested by young poultry and, furthermore, digestion is variable. The primary factor controlling the digestibility of wheat is the cell wall. Removal of this barrier by physical or chemical methods should increase the digestibility by enhancing the accessibility of the nutrients by enzymes. Addition of exogenous enzymes to the diets of low-AME wheats can largely eliminate the adverse effects in broiler chickens; Choct *et al.* (1994) demonstrated improvements in weight gain, feed conversion ratio and AME by 15-34% by use

of enzyme supplementation. These improvements were due largely to increased starch digestion in the small intestine ($r^2 = 0.68$ for the correlation between AME and starch digestibility).

Variations in the nutritive value of feed wheats for broilers do exist and continue to be a subject of major importance to the industry. The chemical composition of wheats is subject to variability but no consistent pattern has emerged that enables a specific variety or growing site to be linked with the low-AME values. The utilisation of energy from wheat is not related in a simple correlation to the content of granular starch. Indeed, no single parameter was consistently superior for predicting AME values which might imply that more complex systems or a combination of systems are involved in the determination of AME

Despite extensive biochemical analyses of the NSP fraction, it has not been possible to evaluate the influence the NSP has on the digestion of certain wheats. A simple and credible hypothesis is that the NSP by vitue of their location, obstruct the release of starch and other nutrients contained in the endosperm to the digestive processes in the alimentary tract of the bird (Dreher *et al.*, 1984; McNab and Smithard, 1992). Supporting evidence for this proposal is limited since few correlations have been established between NSP contents *in situ* and AME or digestibility.

Table 6.1. Gross Chemical Composition of Wheat Samples (DM)

Wheat Variety	Gross Energy ($MJ.kg^{-1}$)	Starch ($g.100g^{-1}$) [digestible]	Protein[1/] ($g.100g^{-1}$)	Fat ($g.100g^{-1}$)	NSP[2/] [Uronics] ($mg.g^{-1}$)
Trial I					
Hornet	17.988 (0.063)	64.57 (1.55)	12.17 (0.27)	2.25 (0.06)	133.37 (2.19) [4.57 (0.31)]
Mercia	18.080 (0.034)	65.55 (0.75)	13.25 (0.30)	2.48 (0.04)	138.64 (2.72) [2.63 (0.21)]
Riband	17.676 (0.078)	71.30 (0.57)	9.88 (0.30)	2.25 (0.14)	139.52 (3.39) [4.36 (0.86)]
Pastiche	18.075 (0.061)	60.80 (0.62)	14.69 (0.33)	2.34 (0.07)	139.11 (2.29) [3.63 (0.12)]
Trial II					
Unknown (18)	17.323 (0.149)	64.96 (0.74)	12.92 (0.19)	2.35 (0.06)	103.38 (2.22) [2.30 (0.03)]
Unknown (20)	17.178 (0.136)	64.89 (1.79)	11.64 (0.07)	2.39 (0.11)	104.49 (2.12)) [2.14 (0.01)]
Fortress (23)	17.080 (0.244)	68.78 (2.29)	12.48 (0.20)	2.15 (0.01)	107.24 (1.55) [1.91 (0.09)]
Galahad	17.590 (0.149)	67.29 (0.58)	11.02 (0.10)	2.32 (0.00)	116.46 (3.60) [3.29 (0.29)]
Mercia	17.379 (0.057)	68.72 (0.81)	10.27 (0.10)	2.44 (0.11)	107.08 (2.75) [2.02 (0.17)]
Unknown (98)	17.650 (0.095)	74.42 (4.55)	9.54 (0.03)	2.53 (0.17)	109.46 (5.55) [3.24 (0.19)]
Trial III					
Avalon 1	17.532 (0.132)	64.70^{a} (1.23) [45.25^{a} (1.23)]	12.86 ( )	2.19^{ab} (0.09)	112.26 (2.86) [3.74 (0.29)]
Avalon 2	17.268 (0.136)	68.07^{ab} (1.33) [57.55^{bc} (4.13)]	10.72 ( )	2.42^{b} (0.40)	nd
Hereward 1	17.596 (0.154)	67.36^{ac} (0.96) [55.83^{b} (3.38)]	11.44 ( )	1.77^{a} (0.04)	108.31 (3.35) [3.99 (0.35)]
Hereward 2	17.349 (0.136)	65.29^{a} (2.41) [50.92^{ab} (3.94)]	13.59 ( )	1.99^{ab} (0.14)	nd
Mercia 1	17.915 (0.152)	73.21^{bd} (1.29) [70.94^{d} (0.87)]	8.34 ( )	2.30^{b} (0.17)	107.19 (3.34) [3.57 (0.55)]
Mercia 2	17.762 (0.129)	69.27^{bc} (0.39) [65.15^{cd} (1.57)]	12.29 ( )	2.40^{b} (0.11)	nd
Riband 1	18.037 (0.158)	71.29^{d} (0.92) [69.45^{d} (0.57)]	11.63 ( )	2.06^{ab} (0.10)	112.97 (0.75) [3.99 (0.43)]
Riband 2	18.235 (0.132)	69.29^{bc} (1.27) [65.23^{cd} (2.57)]	10.55 ( )	2.09^{ab} (0.01)	nd

Table 6.1. (Continued)

Wheat Variety	Gross Energy ($MJ.kg^{-1}$)	Starch ($g.100g^{-1}$) [digestible]	Protein[1/] ($g.100g^{-1}$)	Fat ($g.100g^{-1}$)	NSP[2/] [Uronics] ($mg.g^{-1}$)
(Trial IV and V used same wheats as Trial III, Trial VI evaluated wheats following storage)					
Trial VII					
Avalon	18.661 (0.060)	65.92 (0.49)	13.66 (0.04)	2.06 (0.04)	104.20 (2.20)
					nd
Beaver	18.602 (0.019)	66.44 (0.09)	12.82 (0.03)	2.07 (0.06)	104.98 (1.86)
					nd
Lynx	18.614 (0.063)	66.94 (0.15)	13.09 (0.00)	2.08 (0.09)	114.58 (1.75)
					nd
Mercia	18.700 (0.006)	67.47 (0.05)	12.86 (0.06)	2.33 (0.04)	100.18 (3.08)
					nd
Rialto	18.676 (0.055)	65.61 (0.68)	13.44 (0.04)	2.26 (0.10)	114.15 (2.53)
					nd
Riband	18.571 (0.018)	66.55 (0.27)	12.81 (0.04)	2.16 (0.06)	105.68 (0.34)
					nd
Riband	18.706 (0.005)	66.79 (0.39)	12.76 (0.01)	3.43 (0.09)	104.92 (1.46)
					nd
Spark	18.546 (0.117)	65.80 (0.71)	14.07 (0.01)	2.07 (0.05)	118.22 (1.01)
					nd

Data represent mean values with standard error of mean (SEM) given in parentheses
[1/] Protein content calculated as Nitrogen x 5.83 (Jones, 1931)
[2/] excluding uronic acids
Within columns, significant differences are represented by different superscripts ($p<0.05$).
nd not determined

Table 6.2. Composition of Wheat Non Storage Polysaccharides (NSP)

Wheat Variety	Pentosan ($mg.g^{-1}DM$) [ara:xyl]	Insoluble NSP ($mg.g^{-1}DM$)	Soluble[1/] NSP ($mg.g^{-1}DM$)	Sol:Insol Ratio	NCP2/ [Cellulose][3]/ ($mg.g^{-1}DM$)
Trial I					
Hornet	82.98 (1.22) [0.63 (0.00)]	77.88 (0.54)	55.50 (0.54)	0.71 (0.01)	116.58 (2.00) [12.91 (0.19)]
Mercia*	88.71 (0.83) [0.69 (0.01)]	76.25 (5.48)	62.40 (5.48)	0.83 (0.13)	106.43 (2.86) [10.74 (1.12)]
Riband	82.79 (1.69) [0.67 (0.03)]	71.69 (4.62)	67.83 (4.62)	0.95 (0.13)	126.02 (2.38) [10.07 (1.48)]
Pastiche	85.92 (2.88) [0.62 (0.00)]	90.94 (3.34)	48.18 (3.34)	0.53 (0.06)	121.32 (9.86) [14.58 (3.45)]
Trial II					
Unknown* (18)	66.21(0.39) [0.60 (0.01)]	79.62 (2.69)	23.76 (2.69)	0.30 (0.04)	84.10 (0.07) [16.33 (3.56)]
Unknown* (20)	66.88 (0.56) [0.64 (0.01)]	81.94 (9.06)	22.55 (9.06)	0.31 (0.16)	85.99 (1.57) [14.89 (0.95)]
Fortress**	67.47 (0.60) [0.64 (0.05)]	78.23 (1.35)	29.01 (1.35)	0.37 (0.02)	87.36 (0.65) [17.60 (1.78)]
Galahad**	75.56 (3.55) [0.61 (0.03)]	81.18 (8.36)	35.27 (8.36)	0.47 (0.15)	102.75 (0.70) [14.37 (2.23)]
Mercia**	69.63 (4.20) [0.65 (0.01)]	78.88 (2.05)	28.21 (2.05)	0.36 (0.04)	83.13 (1.22) [13.11 (2.60)]
Unknown* (98)	69.53 (5.15) [0.62 (0.01)]	78.65 (2.25)	30.81 (2.25)	0.39 (0.04)	81.64(4.05 [17.91 (5.27)]
Trial III (n = 5)					
Avalon 1	77.97 (1.78) [0.70 (0.01)]	84.61[a] (1.14)	27.64 (2.17)	0.33 (0.03)	89.93 (1.90) [17.96 (1.79)]
Hereward 1	74.60 (1.80) [0.71 (0.01)]	79.55[b] (1.14)	28.76 (3.28)	0.36 (0.04)	81.14 (2.36) [19.40 (1.43)]
Mercia 1	72.16 (1.61) [0.70 (0.02)]	73.27[c] (1.47)	33.92 (2.14)	0.46 (0.02)	78.46 (2.11) [20.05 (1.74)]
Riband	77.77 (0.94) [0.65 (0.02)]	79.66[b] (2.07)	33.31 (1.78)	0.42 (0.03)	82.64 (1.57) [20.45 (0.51)]

Table 6.2. Continued

Wheat Variety	Pentosan ($mg.g^{-1}DM$) [ara:xyl]	Insoluble NSP ($mg.g^{-1}DM$)	Soluble[1/] NSP ($mg.g^{-1}DM$)	Sol:Insol Ratio	NCP2/ [Cellulose][3]/ ($mg.g^{-1}DM$)
Trial VII					
Avalon*	62.95 (1.09) [0.68 (0.01)]	85.96 (1.92)	18.24 (0.28)	0.21 (0.00)	75.88 (0.94) [23.59 (0.61)]
Beaver	67.31 (1.23) [0.66 (0.02)]	91.71 (1.28)	13.27 (0.58)	0.15 (0.00)	84.82 (1.75) [22.09 (0.07)]
Lynx	72.99 (0.03) [0.63 (0.02)]	83.51 (4.63)	31.08 (2.88)	0.38 (0.06)	84.16 (0.62) [25.32 (1.72)]
Mercia	59.10 (3.01) [0.74 (0.04)]	87.15 (0.08)	13.03 (3.16)	0.15 (0.04)	76.23 (1.81) [24.12 (0.16)]
Rialto	73.29 (0.68) [0.70 (0.01)]	80.47 (1.38)	33.68 (1.15)	0.42 (0.01)	93.82 (2.06) [14.77 (1.05)]
Riband	66.23 (0.07) [0.71 (0.01)]	83.88 (0.76)	21.80 (0.42)	0.26 (0.01)	92.73 (0.14) [11.38 (1.27)]
Riband	66.54 (0.96) [0.71 (0.00)]	91.96 (2.06)	12.96 (0.60)	0.14 (0.01)	80.87 (0.95) [24.78 (0.07)]
Spark*	74.43 (1.07) [0.67 (0.02)]	77.85 (2.11)	40.37 (1.10)	0.52 (0.03)	99.99 (0.44) [10.75 (0.31)]

[1/] Soluble NSP calculated by difference: Total NSP minus insoluble NSP

[2/] NCP, non-cellulosic polysaccharides.

[3/] Cellulose calculated as the difference between total NSP glucose and NCP glucose.

Values represent mean data (n = 6 replicates; * = 5 replicates; ** = 3 replicates) with SEM given in brackets.

Table 6.3. Effect of storage on the starch content (g.100g^{-1} DM) of wheat

Wheat Variety[1/]	Storage Time (months)			
	0	22	39	**Mean**
Avalon 1	64.7 (1.2)	65.9 (0.6)	68.7 (0.9)	**66.4 (1.2)**
Hereward 1	67.4 (1.0)	67.7 (0.7)	71.9 (0.6)	**69.0 (1.5)**
Riband 1	71.3 (0.9)	67.6 (0.6)	70.7 (0.9)	**70.6 (1.3)**
Mercia 1	73.2 (1.3)	69.7 (0.5)	68.9 (0.8)	**69.9 (1.2)**

1/ Wheat samples used in Trial III initially.
Data represent mean values of 3 determinations with SEM given in brackets.

Table 6.4. Composition of NSP in Wheat (mg.g^{-1} DM) Trial 1

Variety	Neutral Sugar				
	Ara	Xyl	Man	Gal	Glu
Hornet	32.09^{a} (0.39)	50.89 (0.49)	4.44 (0.24)	3.20 (0.26)	42.75^{ac} (0.19)
Mercia	36.34^{b} (0.13)	52.37 (0.46)	3.93 (0.16)	4.23 (0.07)	41.77^{b} (1.25)
Riband	33.13 (0.01)	49.66 (1.38)	4.13 (0.39)	4.20 (0.51)	48.40^{c} (0.14)
Pastiche	32.84 (0.82)	53.08 (1.21)	2.83 (0.41)	4.49 (0.10)	45.87^{ab} (0.08)

Within columns, values denoted with different superscripts are significantly different: For glucose in NSP, 'a' is significantly different from 'b' at the 5% level; for arabinose, 'a' is significatly different from 'b' ($p<0.05$) and 'b' from 'c' ($p<0.01$).

Table 6.5. Apparent Digestibilities of Components

Wheat Variety	Dry Matter	Starch	Fat	NSP1/	Pentosan
Trial I (diet A)					
Hornet	0.662 (0.082)	0.825 (0.097)	nd	0.278 (0.033)	0.249 (0.031)
Mercia*	0.624 (0.063)	0.787 (0.083)	nd	0.342 (0.008)	0.324 (0.013)
Riband	0.808 (0.012)	0.997 (0.008)	nd	0.383 (0.027)	0.316 (0.039)
Pastiche	0.801 (0.005)	0.987 (0.006)	nd	0.401 (0.008)	0.359 (0.013)
Trial II (diet B)					
Unknown* (18)	0.772 (0.007)	0.976 (0.010)	nd	nd	nd
Unknown* (20)	0.729 (0.021)	0.925 (0.025)	nd	nd	nd
Fortress **	0.750 (0.029)	0.959 (0.030)	nd	nd	nd
Galahad**	0.744 (0.011)	0.953 (0.019)	nd	nd	nd
Mercia**	0.670 (0.066)	0.857 (0.067)	nd	nd	nd
Unknown** (98)	0.754 (0.015)	0.965 (0.012)	nd	nd	nd

Table 6.5. Continued

Wheat Variety	Dry Matter	Starch	Fat	NSP1/	Pentosan
Trial III (diets B & C)					
Admiral	0.568 (0.069)	nd	nd	nd	nd
	0.733 (0.019)	nd	nd	nd	nd
Admiral*	0.694 (0.030)	nd	nd	nd	nd
	0.768 (0.008)	nd	nd	nd	nd
Avalon	0.503 (0.088)	nd	nd	nd	nd
	0.564 (0.025)	0.699 (0.058)	0.502 (0.051)	0.047 (0.024)	0.155 (0.035)
Avalon	0.501 (0.057)	nd	nd	nd	nd
	0.672 (0.041)	0.846 (0.061)	0.631 (0.058)	nd	nd
Beaver	0.648 (0.035)	nd	nd	nd	nd
	0.767 (0.016)	nd	nd	nd	nd
Beaver	0.518 (0.048)	nd	nd	nd	nd
	0.716 (0.038)	nd	nd	nd	nd
Haven	0.551 (0.034)	nd	nd	nd	nd
	0.749 (0.021)	nd	nd	nd	nd
Haven	0.551 (0.059)	nd	nd	nd	nd
	0.671 (0.037)	nd	nd	nd	nd
Hereward	0.419 (0.030)	nd	nd	nd	nd
	0.674 (0.041)	0.829 (0.050)	0.656 (0.073)	0.081 (0.064)	0.113 (0.070)
Hereward	0.504 (0.033)	nd	nd	nd	nd
	0.634 (0.049)	0.780 (0.060)	0.568 (0.080)	nd	nd
Mercia	0.466 (0.080)	nd	nd	nd	nd
	0.766 (0.012)	0.969 (0.012)	0.771 (0.024)	0.061 (0.018)	0.111 (0.03)
Mercia	0.543 (0.048)	nd	nd	nd	nd
	0.747 (0.021)	0.941 (0.023)	0.780 (0.023)	nd	nd
Riband	0.585 (0.056)	nd	nd	nd	nd
	0.761 (0.007)	0.974 (0.008)	0.791 (0.012)	0.021 (0.010)	0.054 (0.023)
Riband	0.505 (0.044)	nd	nd	nd	nd
	0.753 (0.021)	0.941 (0.037)	0.749 (0.033)	nd	nd
Tara	0.471 (0.073)	nd	nd	nd	nd
	0.761 (0.009)	nd	nd	nd	nd
Tara	0.647 (0.039)	nd	nd	nd	nd
	0.703 (0.033)	nd	nd	nd	nd

Table 6.5. Continued

Wheat Variety	Dry Matter	Starch	Fat	NSP1/	Pentosan
Trial IV					
Avalon*	0.754 (0.004)	0.970 (0.008)	nd	nd	nd
	0.772 (0.007)	0.988 (0.008)	nd	nd	nd
Mercia *	0.783 (0.007)	0.983 (0.008)	nd	nd	nd
	0.804 (0.009)	0.993 (0.004)	nd	nd	nd
Trial V					
Avalon	0.701 (0.017)	0.955 (0.037)	nd	nd	nd
Riband	0.699 (0.006)	0.988 (0.002)	nd	nd	nd
Mercia	0.708 (0.009)	nd	nd	nd	nd
Trial VI					
Avalon	0.760 (0.007)	nd	nd	nd	nd
Mercia*	0.776 (0.006)	nd	nd	nd	nd
Mercia	0.774 (0.004)	nd	nd	nd	nd
Trial **VII**					
Avalon*	0.763 (0.006)	0.988 (0.004)	0.833 (0.008)	nd	nd
Beaver	0.729 (0.017)	0.956 (0.029)	0.776 (0.008)	nd	nd
Lynx	0.721 (0.018)	0.939 (0.042)	0.788 (0.013)	nd	nd
Mercia	0.758 (0.005)	0.974 (0.012)	0.844 (0.005)	nd	nd
Rialto	0.668 (0.055)	0.871 (0.086)	0.727 (0.062)	nd	nd
Riband	0.753 (0.007)	0.989 (0.004)	0.812 (0.008)	nd	nd
Riband	0.763 (0.004)	0.993 (0.005)	0.834 (0.009)	nd	nd
Spark*	0.727 (0.027)	0.946 (0.030)	0.807 (0.018)	nd	nd
Trial VIII					
Rialto*	0.721 (0.021)	0.930 (0.032)	nd	nd	nd
Riband	0.761 (0.012)	0.993 (0.004)	nd	nd	nd
Spark	0.710 (0.027)	0.898 (0.049)	nd	nd	nd

Values represent mean data (n = 6; *n = 5; **n = 3) with SEM given in brackets; nd, not determined.
1/ excluding uronic acids
Source of wheat samples: PBI, Plant brreding International, Cambridge.
A, diet A (898g.kg^{-1} wheat); B, diet B (896.3g.kg^{-1} wheat)

Table 6.6. Digestibility of NSP in Wheat ($mg.g^{-1}$ DM) Trial 1

Variety	Neutral Sugar				
	Ara	Xyl	Man	Gal	Glu
Hornet	0.254 (0.028)	0.246 (0.042)	0.429 (0.094)	0.126^{a} (0.064)	0.390^{a} (0.042)
Mercia	0.380 (0.046)	0.366 (0.044)	0.537 (0.02.)	0.177^{b} (0.088)	0.451^{bc} (0.032)
Riband	0.343 (0.42)	0.299 (0.44)	0.502 (0.019)	0.363^{a} (0.027)	0.513^{b} (0.009)
Pastiche	0.349 (0.020)	0.365 (0.015)	0.375 (0.078)	0.413^{bc} (0.022)	0.500^{bc} (0.009)

Within columns, values with different superscripts are significantly different: For galactose digestibility, 'a' is significantly different from 'b' ($p<0.05$) and 'c' ($p<0.01$); for glucose digestibility, 'a' is significantly different from 'c' ($p<0.01$).

CHAPTER 7 RELATIONSHIP BETWEEN CHARACTERISTICS OF STARCH AND AME VALUES IN WHEAT CULTIVARS

7.1 SUSCEPTIBILITY OF STARCH GRANULES TO HYDROLYSIS BY PANCREATIC α-AMYLASE *IN VITRO*

Variations in starch digestibility of wheat may be linked to differences in AME values (see Chapters 5/6). A rapid assessment of the nutritional value of wheat would be of considerable value both to nutritionists and to plant breeders in selecting / screening new cultivars. The current chapter considers measurements of starch hydrolysis *in vitro* employing porcine pancreatic amylase (PPA) assessed as a potential means of predicting the digestibility of wheat starch *in vivo* for poultry.

7.1.1 Wheat Meals and Isolated Wheat Starch Granules.

The *in vitro* digestibility of starch from wheats of known and variable AME value was investigated using meals and isolated granules. The hydrolytic enzyme employed was porcine pancreatic α-amylase (PPA). Wheat varieties were selected from Trial III (n = 6) and Trial VII (n = 8). The initial rate of hydrolysis (mg starch.min^{-1}.g^{-1}) was used as an index of starch susceptibility to PPA (Table 7.1).

The susceptibility of the starch in wheat meals to PPA, in general, reflected the AME values obtained *in vivo*. The starch of wheats with lower AME values was hydrolysed at a slower rate *in vitro* than the starch from wheats of higher AME values. Hydrolysis of the high-AME starches was complete in 8h, whereas only 0.90 was hydrolysed after the same time for the low-AME starches. Hydrolysis of starch in wheat meals was significantly different ($p = 0.02$) between varieties (Trial VII) and starch from high-AME wheats was hydrolysed significantly faster

than starch from the lower-AME wheats ($p<0.001$). It cannot of course be assumed that the rates of starch hydrolysis observed under the conditions employed in this study accurately reflect the rates of digestion *in vivo*. These observations act solely as an indication of the possible nutritional value and, as such, indicate that the *in vitro* hydrolysis of wheat starch might, therefore, provide a useful assay for the assessment and prediction of the nutritive value of wheat.

Isolated starch granules from the low- and high-AME wheats were hydrolysed at identical rates by PPA (Table 7.2). In addition, the total starch hydrolysis achieved over 6 hours was identical for each wheat type. No significant differences were obtained . This observation indicates that the poor digestibility and low AME values cannot be attributed to the intrinsic properties of the starch granules of these wheats, but rather the result of some non-starch factor present in the wheat grain adversely affecting the digestibility of starch.

Isolated starch granules were more susceptible to hydrolysis by PPA than the starch in meals. This finding corroborates those of Björck *et al.* (1984). Rogel *et al.* (1987) reported that isolated wheat starch was readily digested *in vitro* by chick pancreatic α-amylase, even that starch isolated from wheat with relatively low-AME values.

Batey (1982) observed that the complete enzymic degradation of starch in wheat flour was prevented by the formation of aggregates involving the gluten. Similarly, Anderson *et al.* (1981) suggested that starch-gluten interactions were responsible for some starch escaping degradation in the small intestine.

Starch digestion *in vivo* and hydrolysis *in vitro* can be affected both by chemical and physical factors. In addition, the accumulation of end products of digestion (EPD), namely maltose, maltotriose and α-limit dextrins, may inhibit amylase. In

the current study, the rate of hydrolysis of isolated wheat starch granules *in vitro* was unaffected as hydrolysis proceeded for any variety. It was concluded that the EPD did not exert any influence on the α-amylolysis of starch under the conditions employed. Any decrease in hydrolysis could be attributed to starch remnants being more resistant to amylolysis. The factors responsible for the low AME values of wheat were thought to be remote from the granule and possibly associated with subtle compositional differences affecting the viscosity of the digesta.

7.1.2 Starch Granule Hydrolysis as Affected by Aqueous Extracts from Wheat

Starch granules from both high and low AME wheats were equally susceptible to α-amylolysis by PPA. It would therefore seem likely that some other factor(s) was responsible for the differences in starch digestibility *in vivo*.

To be effective, these factors would have to be water-soluble. To test the effect of water-soluble factors on the hydrolysis of starch granules, aqueous extracts from both a high- and a low-AME wheat (Riband and Spark, Trial VII) were included in the α-amylolysis digestion systems. Three tests were performed as described in Chapter 3, sections 3.3.10, and the initial rates of hydrolysis are presented in Table 7.3.

Control samples were completely hydrolysed in 8h, but the presence of water soluble material had a significant effect on the overall rate of starch hydrolysis. A 2 fold concentration of water soluble material significantly depressed the overall hydrolysis, resulting in a maximum of 0.34 after 8h, irrespective of wheat (treatment c). Hydrolysis was faster in those extracted meals resuspended in buffer only (samples devoid of water soluble material), with over 0.90 of the starch being hydrolysed in both high and low AME types within 4h. Differences between the low- and high-AME samples were negligible.

It is clear from these experiments that aqueous soluble non-starch material can affect the hydrolysis of starch in meals by PPA *in vitro* which, together with the evidence that starch granules from high and low AME wheats were equally sensitive to PPA , indicates that the intrinsic properties of the granules are not responsible for the poor digestibility of the starch from low-AME wheats. Factors extrinsic to the starch granules must be responsible, possibly a component or components present in the aqueous soluble material. At this stage these factors cannot be identified and their mode of action cannot be defined. It has been suggested that water soluble arabinoxylans have adverse effects on AME values through increased viscosity when added to broiler diets. The effects of aqueous soluble extracts on the *in vitro* action of PPA may be mediated in a similar manner. The water soluble material may be acting indirectly by increasing the viscosity of the environment and thereby slowing the hydrolytic process by restricting the access of the PPA to the starch. This may explain the differences observed *in vivo* between the different wheats tested, since not only were a range of NSP and soluble NSP determined but also a range in extract and digesta viscosities also evident.

In conclusion, the aqueous soluble material from wheat has a marked effect on starch hydrolysis. This observation may indicate that such material may be partially responsible for the low AME values obtained although differences between the high- and low-AME samples were not sufficient to explain the range in AME entirely. Moreover, the improvement in AME value upon prolonged storage at ambient temperatures would have masked any significant differences.

7.1.3 Hydrolytic Capacity of Chick Foregut Digesta Preparations

An investigation into the amylolytic activity within the upper small intestine (foregut) was undertaken to establish the effects of feeding different wheat varieties on enzyme production, secretion and/or activity. It was speculated that

feeding wheats of higher AME value (and, accordingly, higher digestible starch content) might result in an increase in enzyme release and/or enzyme activity compared with feeding wheats of a lower AME content.

The hydrolytic activity of the digesta supernatant, collected from the foregut by centrifugation of the small intestine contents to the Meckels Diverticulum, was determined by incubating commercial wheat starch granules with digesta supernatant *in vitro* for 8h. Digesta samples were obtained from chicks fed either high- or low- AME wheats in Trials VII and VIII. Differences in the hydrolytic capacity of chick digesta were obtained. The initial rates of starch hydrolysis are presented in Table 7.4. Digesta preparations from chicks fed high-AME wheats hydrolysed starch more efficiently than that from chicks fed low-AME wheats. Under the conditions employed, digesta samples obtained from Trials VII and VIII hydrolysed between 0.21 - 0.48 and 0.21 - 40 of the starch granules respectively. Hydrolytic capacity was greatest in digesta samples obtained from birds fed high-AME wheats. This observation may also be explained in terms of viscosity effects. It is possible that feeding birds with higher-AME wheats may result in a less viscous digesta, containing less antinutritive water soluble material and thereby allowing for a better accessibility by the hydrolytic enzymes.

7.1.4 Discussion

It was the objective of this section to try and define the underlying reasons for the production of low-AME values of wheat cultivars, with particular reference to the susceptibility of starch granules, both in wheat meals and isolated, to hydrolysis by PPA *in vitro*. Although the rate of digestion of unmodified starch is dependent upon a number of factors, native starch granules from different origins vary in their susceptibility to the action of amylolytic enzymes. Moreover, the extent of hydrolysis overall *in vitro* is dependent on the specific system used

and the enzyme source employed.

A simple *in vitro* method has been developed in which the rate of starch (isolated granules and wheat meals) hydrolysis can be measured. This assay procedure enabled differences in the susceptibility of wheat starch to be detected. Positive correlations between starch digestibility *in vitro*, by the above procedure, and *in vivo* have been obtained. The mean digestibility figures obtained *in vitro* were not significantly different from the corresponding *in vivo* data (Table 7.5). The best agreement was obtained with the high-AME wheat samples. Therefore, and in view of the data presented herein, it is possible that measurement of starch hydrolysis *in vitro* employing PPA may provide a rapid, accurate and economical method for assessing and predicting the bioavailability of wheat starch *in vivo*, and thus the nutritional value of wheat for poultry.

Generally, varietal differences were more pronounced the longer the incubation period (4-6hr) showing a good correlation between digestibility and AME values, and the hydrolysis by PPA *in vitro* under the conditions employed. Low-AME wheats generally were less susceptible to hydrolyis by PPA *in vitro* than wheats of high-AME. This difference in susceptibility could not be attributed to the intrinsic properties of the starch granules since isolated wheat starch granules were, in general, hydrolysed more readily *in vitro* than those present in wheat meals, irrespective of variety and AME value. This observation leads to the conclusion that it is not the starch *per se* that is poorly utilised in some samples but that other factors within the wheat may be reducing starch digestibility. Certainly, Palmer (1972) reported that, in contrast to barley, the small starch granules in wheat were resistant to attack, at least during malting. These results therefore indicate that the structure of the endosperm and the presence of other extrinsic factors, such as cell wall material (NSP), could reduce the accessibility of digestive enzymes. Indeed, variability in NSP contents of the wheats was evident (Table 6.2). Some materials, such as guar and pectin act directly to

reduce the hydrolysis of polysaccharides and hence intestinal absorption of monosaccharides by increasing the viscosity of the intestinal contents and thus reducing enzyme-substrate contact. Although cereal NSP may exert a similar effect, the mechanism is unclear. In the current study, the water soluble material acted indirectly to retard starch hydrolysis, presumably by restricting the access of PPA to the starch. Such limitations might contribute to the susceptibility of the starch to enzymic degradation. Digestibility of starch granules may also be influenced by the nature of the physical processing techniques used during their isolation and purification, such that results obtained may not be completely representative of that *in situ*.

The protein matrix that surrounds the starch granules in wheat flour may inhibit the rate of starch hydrolysis in the lumen of the small intesine. Removal of the gluten from wheat flour resulted in an increased rate of amylolysis *in vitro* and an enhanced glycemic response *in vivo* (Jenkins *et al.*, 1987). Further, the susceptibility of raw and boiled wheat to α-amylase was increased substantially (22 and 27% respectively) after preincubation with pepsin (Holm *et al.*,1985). Microscopic examination revealed that the protein matrix in which the starch granules are embedded was degraded after pepsin incubation and the proportion of free granules increased. Also, in boiled wheat, numerous aggregates enclosing swollen starch granules remained after α-amylase incubation but were removed by prior digestion with pepsin. The disintegration of these encapsulating structures increased the total granule surface area exposed to enzyme action and, as a consequence, the amount of starch accessible to hydrolysis by α-amylase. It has been suggested that the protein matrix in wheat adheres to the starch granules with water-soluble material acting as a cementing substance (Barlow *et al.*,1973) and a protein which inhibited wheat starch breakdown *in vitro* has been identified. However, Englyst and Cummings (1985) reported that neither protein nor amylase inhibitors appeared to affect starch digestion significantly *in vitro*. Earlier, Shainkin and Birk (1970) concluded that

the wheat amylase inhibitors were of no significance in the digestion of wheat starch *in vivo*.

Starch granule hydrolysis by PPA *in vitro* was similar to that obtained *in vivo,* particularly for the high-AME wheats (Table 7.5). The relative order of susceptibilities was similar for the high-AME wheats between the two systems, i.e., the digestibility of starch granules decreased in the same order. However, no correlation in the order of digestibility was evident for the low AME wheats between the *in vitro* and *in vivo* systems. The reason for this variation is probably due to the high variability observed between replicates fed the low-AME wheats as opposed to those birds fed the high-AME types.

Although this system bears little resemblance to the intestinal environment during digestion, except for the presence of α-amylase, these observations nevertheless show a good correlation between the *in vitro* hydrolytic rates and the digestibilities obtained *in vivo* for the low- and high-AME wheat varieties. These results would therefore indicate that the proposed method (employing the assay system described) may be suitable for use as an indicative tool in predicting the nutritive value of wheat for young broiler feed. This finding corroborates the work of several other authors who have shown a significant correlation between starch availability *in vitro* and *in vivo* (O'Dea *et al.*,1981; Jenkins *et al.*,1982; Brand *et al.*,1985; Thorburn *et al.*, 1987; Holm *et al.*, 1988; Wolever *et al.*,1988; Bornet *et al.*,1989; François, 1989).

7.2 INFLUENCE OF AQUEOUS WHEAT EXTRACTS ON VISCOSITY

Linking aqueous extract viscosity with starch hydrolysis *in vitro* was one possible method used to explain the data observed in section 7.1. Any differences in extract viscosity might give useful information regarding the

relationship between the water-soluble material in wheat and its role in the nutritional value of wheat. The erratic occurrence of low-AME wheats has emphasised the need for a rapid predictive test for wheat quality. It was also the objective of the current section to establish whether the viscosity of aqueous extracts of wheat could be used as a reliable predictive assay for the assessment of the potential nutritive value.

Wheat and excreta samples were extracted in buffer to investigate whether any relationship exists between the soluble carbohydrate fraction of wheat, the relative viscosity and the determined nutritional value when fed to young chicks. Preliminary studies revealed greater variability between the wheats with an increase in solute concentration in the order of 1 in 100 < 1 in 10 < 1 in 4 < 1 in 3.

7.2.1 The relationship between Wheat AME and the Viscosity of Aqueous Wheat Extracts

Preliminary experiments on the viscosity of aqueous extracts were undertaken using 4 wheats with contrasting AME values (Trial III). The effect of extraction time (1-4 hours) on viscosity was investigated and results are expressed relative to water at the same temperature (37°C) (Table 7.6). Viscosity measurements were recorded using a capillary 'U' tube viscometer (Schott-Geraté, Camlab). Values represent the time (sec) for a known volume of extract (2ml) to pass between two points on the capillary tube and are presented relative to water (1.00) under identical conditions.

The results revealed that the low AME wheats had significantly higher relative viscosities ($p<0.001$) than the high AME wheats. Extraction times over 1 to 4 hours had minimal and variable effects on the viscosity of aqueous extracts. Moore and Hoseney (1990) reported that relative viscosity of aqueous extracts

of wheat flour increased up to 90min extraction, although the increase between 60 and 90min was small. The increase in viscosity observed with time was attributed to the slow solution of the pentosan fraction. Boros *et al.* (1993) also employed an extraction period of 60min for raw rye grain since longer times resulted in reduced viscosity through the action of endogenous arabinoxylanases which reduced the molecular size of the polymers.

The viscosity of the aqueous extracts was negatively but highly correlated with apparent AME values ($r^2 = 0.96$) and apparent starch digestibility values ($r^2 = 0.98$) (Trial III). Such relationships may provide indications concerning the factor(s) responsible for such differences in AME values.

Prolonged storage at ambient temperatures (10-20°C) of wheat grains resulted in an increase in AME value of low-AME wheats. The viscosity of aqueous extracts of the 4 wheats used above but stored as a meal for 16 months at 1°C were assayed using a Brookfield digital viscometer (Table 7.7). Storage of wheat meals resulted in a significant decrease of aqueous extracts and the differences between high and low AME types were reduced.

It may be concluded that (i) AME values of wheats appear to be negatively correlated with the viscosity of aqueous extracts, (ii) storage of wheat meals results in an improvement in the nutritional value of wheats of previously low AME and this may be related to a decrease in the viscosity of the aqueous extracts of such wheats.

The above findings were verified using wheats with varying AME values (Trial VII). Three wheats were selected. The effect of extraction time on aqueous extract viscosities was confirmed. Measurements were made after 30min, 1, 2 and 4hr incubation at 37°C. Results (Table 7.8) are expressed relative to water at the same temperature and corroborate those findings reported earlier. As a

general rule, an extraction time of 60 minutes produced optimal results and hence was used in these experimental studies. Using this method, the relative viscosities of 5 additional wheats from Trial VII were determined in a more detailed study.

The negative correlations between the AME values ($r^2 = 0.45$) and starch digestibility ($r^2 = 0.40$) with the viscosity of the aqueous extracts of wheat are weak, although the data support and verify the relationship described earlier. These results may be explained in view of the lack of extreme AME values and as such might highlight the possibility of using extract viscosity as a contributing determinant of nutritional value of wheat varieties for young poultry.

A weak correlation ($r^2 = 0.39$) was obtained between the extract viscosity and the wheat NSP concentration (1994 - Trial VII). The relationship between the soluble NSP and viscosity of the aqueous extracts, however, was weaker ($r^2 = 0.24$). Clearly the viscosity of the aqueous extracts of wheat may be due to specific polymers not readily identifiable in a simple analytical approach. Furthermore, although the lower-AME wheats tended to possess higher contents of soluble NSP, the lack of correlation was partly due to the wide range of soluble NSP values obtained for the samples tested. In addition, certain cultivars tested had high extract viscosities but also high AME values. The changes effected within the grains upon prolonged ambient storage made correlations between such variables very difficult. Such differences in viscosity measurements may indicate that water soluble material might play a role *in vivo* but wheats with known extreme AME values require testing.

7.2.2 Hydrolysis of Starch in Wheat Meal Suspensions by PPA in Relation to Suspension Viscosity

The effect of aqueous wheat extract viscosity on starch hydrolysis was investigated *in vitro*. The concentration of wheat suspensions was increased (1:3 w/v) to exaggerate the differences in viscosity and starch hydrolyses. Samples of wheat to include a range of AME values were incubated at 37°C with 45ml 0.1M citrate-phosphate buffer, pH 6.9. Viscosity and total soluble carbohydrates were measured on aliquots withdrawn after 1, 2, 4 and 8 h. The results revealed a trend which indicated that higher viscosities were associated with lower AME wheats which, in turn, were hydrolysed more slowly. Under the dilute conditions employed, however, the effects were not significant. Hydrolysis time was correlated with the total NSP content of the wheats in Trial VII. This observation supported the findings of Gee and Johnson (1985) who showed a significant relationship between the half-time hydrolysis and the total dietary fibre content of a range of common starch-rich foods. The mechanisms underlying these differences in the rate of starch hydrolysis remain to be fully elucidated and, in particular, the role of dietary NSP needs to be defined. Certainly, in the present context, the extract viscosity, which is likely to slow the release of starch from the granules and impede the access of amylase, is probably of greater significance than the total content of NSP, although some association between the soluble NSP content and the hydrolysis of starch may be indicated. Indeed, Sollars (1969) suggested that the water-soluble NSP may be responsible, in part, for the varietal differences in viscosity.

The randomness of the occurrence of low-AME wheats has emphasised the need for a rapid predictive assay for wheat starch digestibility. The current studies have shown that AME value is correlated to starch digestibility. It is therefore reasonable to assume that *in vitro* assays could be useful to predict starch digestibility and availability *in vivo*. Indeed, studies utilising extract

viscosity as a predictor of the nutritive quality of wheat in poultry have found that over half the variability in AME can be attributed to extract viscosity (Choct *et al.*, 1992). Further studies need to be conducted to determine the precise effects of extract viscosity on starch digestibility and AME.

7.2.3 Viscosity of Aqueous Extracts of Chick Excreta and its Relationship to Starch Digestibility and AME Values

The relative viscosity of excreta samples was measured to examine the relationship with AME. Further, it was hoped to ascertain whether these components would pass through the gut in an unmodified form.

Samples of excreta were selected to include wheats with a range of AME values namely, Rialto (low-intermediate AME), Riband (high-) and Spark (intermediate-) (Trials VII & VIII). Significant differences were apparent in the relative viscosities of excreta samples. Significantly higher viscosities were obtained with faecal extracts of birds fed low- compared with high-AME wheats. Excretal viscosity was found to be strongly correlated with AME Results are presented relative to water (Table 7.9).

7.2.4 Conclusions

The results provide some evidence linking excreta viscosity with feed intake ($r^2 = 0.5$) and, in turn, AME values can be correlated in a negative relationship with excreta viscosity ($r^2 = 0.7$) as presented in Figures 7.1 and 7.2.

The viscosity of aqueous extracts of wheat could in part be responsible for the variability observed in the AME values of wheats tested although correlations were relatively weak. Clearly, more observations are necessary using a selection of wheats with contrasting AME values before viscosity measurements could be

recommended as a predictive test for the nutritional value of wheat. Other factors may be involved in determining the AME value of wheats. A similar conclusion has also been drawn by other workers (Moore and Hoseney, 1990; Choct *et al.*, 1992; Bedford and Classen, 1993; Boros *et al.*, 1993). The relationship between the viscosity of aqueous extracts and those of gut contents of chicks is unknown. Digesta viscosity may, in part, be an effect of concentration related to feed intake. Certainly, evidence is available showing a degree of correlation between feed intake and viscosity of the excreta. Indeed, viscosity of aqueous extracts of chick excreta are also correlated well with diet (and wheat) AME values providing support for the hypothesis that the viscous components are not digested within the chick alimentary tract, but are excreted possibly in an unchanged form. It seems likely that these viscous components are involved in determining the nutritive value of wheats for young broilers.

7.3 STARCH GRANULE SIZE DISTRIBUTION IN ISOLATES FROM WHEAT, CHICK DIGESTA AND EXCRETA

Measurement of the dimensions of hydrated starch granules isolated and prepared from 12 wheat samples, 10 foregut digesta samples and 2 excreta samples were evaluated to establish whether starch granule size was important in determining rate of granule degradation.

Particle size distributions of starch granules isolated from wheat, excreta and chick foregut digesta were characterised using a Coulter Counter (see section 3.3. *et seq.*). Wheat samples were selected to include a range of AME values and digestibilities *in vivo*. Four wheats from Trial III, Avalon, Hereward, Mercia and Riband, and eight wheats from Trial VII, Avalon, Beaver, Lynx, Mercia, Rialto, Riband, Riband-SB and Spark were analysed. A selection of birds were chosen for analysis of foregut (Trials V and VII) and excretal (Trial III) starch granule populations. Results are presented in Tables 7.10 and 7.11.

Results demonstrate that each starch granule population contains a range of different granule sizes. Small granules fall within the range of 4-10μm and large granules range between 10μm and 32μm in diameter (channels 12-16). For all wheat starches tested, the majority of granules were small ($<10\mu$m). Varietal differences were small and insignificant. Wheats both within and between trials displayed similar patterns of granule size and number. In Trial III, the number of granules per unit weight (20μg) was significantly different ($p<0.001$) between the wheats although differences between low-AME and high-AME wheats were insignificant. The low-AME sample, Avalon, contained a higher proportion of the smallest sized granules (4μm) which may have been responsible for the higher viscosity values of the aqueous extracts recorded for the wheats. Starches with a high proportion of small granules produce a more viscous paste. Results from Trial VII corroborate this finding since lower-AME wheats contained a higher proportion of the smallest starch granules in general. However, no significant differences in granule size were evident between the populations of high- and low-AME wheats. For native wheats, no significant differences were observed for mean granule diameter, mean granule surface area, proportional volume / surface area between low- and high-AME types.

Differences in the starch granule size profiles for foregut digesta were observed between the two wheat types fed. Since digesta samples were not taken from Trial III, the same wheats fed to birds in a later trial were analysed from the foregut digesta. However, an added complication occurred since there had been an improvement in the AME value of the low-AME types. It would, nevertheless, appear that the digesta of birds fed the lower-AME wheat (Avalon) contains more starch granules per unit weight (20μg) compared with the higher-AME wheat (Riband) ($p=0.033$). The former also contains fewer small granules and more large granules as a proportion of the whole compared with the latter. The mean surface area of granules was significantly different between wheat types fed; the surface area of the starch granules in the foregut digesta of Avalon-fed

birds was significantly higher than that of Riband-fed birds ($p = 0.007$) which might indicate that a higher number of 'B' granules were digested in the former, and the surface area of the 'B' granules from the digesta of birds fed Avalon was lower than that of feeding Riband; although this difference was not significant ($p = 0.053$), there is evidence of a relationship. Hence, the surface area of the 'A' granules was greater in the former indicating less degradation of the large starch granules when Avalon was fed. This in turn might suggest either (a) more digestion occurred in the low-AME wheat with respect to 'B' granules since the native wheat contained a greater amount of small starch granules compared with the high-AME wheat, thus shifting the population pattern increasing the proportion of larger granules present, or (b) the results could represent more aggregation of the granules in solution.

The latter explanation is probably the more likely in view of the previous findings relating to the digestibility of high- and low-AME wheats. Using digesta isolates as a means of assessing starch granule digestion is perhaps not ideal due to aggregation of the granules in the gut, which are possibly not separated by sonication for 1min. Overall, the mean diameter of granules in the foregut digesta were not significantly different between feeding Avalon and Riband. The proportional volume of the 'B' granules was significantly lower ($p = 0.028$) in the digesta when Avalon was supplied compared with when Riband was supplied, and hence the volume of the 'A' granules was significantly greater in the former indicating less digestion of the large granules.

Excreta samples isolated from Trial III revealed some differences between the low- and the high-AME fed wheats. The number of granules excreted from birds fed the high-AME wheat (Mercia) was significantly lower ($p = 0.002$) than that amount excreted from birds fed the low-AME wheat (Avalon). The mean diameter of starch granules excreted from birds fed the lower AME variety was significantly ($p < 0.001$) lower (7.85μm) than the mean granule size excreted

from birds fed the higher AME wheat (9.78μm) signifying less digestion of the 'B' type granules. These differences were also reflected in the volume and SA results. The proportional surface area of the small starch granules excreted from the birds supplied with the low-AME wheat was significantly higher ($p<0.001$) than that excreted from birds supplied with the high-AME sample indicating that relatively fewer small starch granules had been digested. The volume of small starch granules excreted on the low-AME wheat was significantly higher ($p=0.003$) than that from the high-AME wheat.

The lack of variation in the *in vivo* data for the starch granule characteristics obtained in Trial V was reflected in data as shown by the smaller difference between the two wheat types fed. A shift in the starch granule populations (size and number) between the high- and the low-AME wheats was observed in those isolated from the excreta. Relatively, there was a far greater excretion of the smaller sized granules when birds were fed the low-AME wheat (Avalon) compared with being fed the higher AME wheat (Mercia). This observation supports the hypothesis proposed earlier that the smaller sized granules in the low-AME wheats are more resistant to digestion, or rather they are digested at a slower rate than the equivalent in the high-AME wheats. More large granules were found in the excreta of the high-AME wheat fed birds in Trial III indicating that the smaller granules were digested preferentially, but alternatively this may be an artifact of the isolation and purification processes, and/or aggregation of the smaller granules during the drying process. Certainly, starches isolated from the excreta cannot be purified to the same extent as wheat starches and will always contain some non-starch contaminants. The results indicate that greater digestion occurred when Mercia was fed compared with the Avalon although possible contamination from spilled feed could be a problem.

Trial VII also showed little difference overall in the starch granule sizes between the various wheats tested. Both low- and high-AME wheats revealed similar

patterns with respect to granule number and size, although there was some evidence which indicated the lower-AME wheats had more of the smallest sized granules.

A range in mean granule diameters was observed for the 8 wheats tested ranging from 6.92 to 8.18μm with the lower AME wheats generally possessing the smaller mean granule diameters (average data are 7.49μm and 7.71μm respectively for the low- and high- AME wheats).

These differences were reflected in the differences observed in the foregut isolates. Similar population patterns were again measured for both high- and low-AME wheats fed, with very little difference between the two types with respect to granule number and size. This is perhaps surprising in view of the fact that reasonable differences in AME value and apparent starch digestibility were recorded *in vivo*. There is, however, a shift in the pattern observed with a general decrease in the number of small granules and an increase in the number of large granules present compared with the original wheats, ie. the proportion of small-to-large granules increased.

A comparison of excretal starch granules for both wheat types fed was not possible since very few granules were excreted from birds fed the high-AME sample (Riband), and those granules which were observed were surrounded by other material which could not easily be removed.

Data pertaining to isolated granule numbers per unit weight of starch (20μg) indicate minor differences between the isolated wheat starches in Trial III. Riband, and Mercia to a lesser extent, generally contained more of the larger sized granules than Avalon, but fewer of the smallest sized granules. Overall the total number of granules per unit weight was similar. Starch granules isolated from the foregut were highly aggregated, possibly due to the presence of

mucous, since a higher number of the larger sized granules were evident compared with the original wheat starch. In addition, the data were difficult to interpret because there was a difference in the total number of granules per unit weight between the samples indicating a difference in the density of the granules.

Starch granules isolated from the excreta are perhaps more accurate although problems remain with feed contamination. The total number of granules per 20μg was similar to wheats in Trial III and they were not too dissimilar to the original wheat starches. The presence of a higher number of the smaller granules in Avalon was expected since the wheat starch also contained a higher number compared with the Mercia sample. The actual number of small granules excreted for both wheats was therefore also similar, although there was some evidence suggesting that the proportion of small granules excreted relative to the original wheat starch was much higher for the lower-AME wheat. The higher number of large granules recorded from excreted material compared with the native wheat starch was probably due to the aggregation of the granules.

Excreta from Trial VIII could not be compared for the low- and high-AME wheats fed since the starch from the high-AME wheats was almost completely digested and very few granules could be isolated or separated free from contaminating material.

7.3.1. Discussion

Measurement of starch granule particle size within the small intestine represents perhaps the most obvious method for assessing differences between wheat varieties, although samples are subject to a high amount of aggregation. Further studies investigating the effect of sonicating for longer periods may provide further information regarding the accurate measurement of particle sizes and

number.

Ratios of small to large granules, although an indication of their susceptibility to digestion, can be misleading. The proportion of large to small granules changes on movement through the digestive tract as a higher number of smaller sized granules are digested, thus the relative proportion of larger sized granules will increase. A high proportion of small granules could represent a high resistance to digestion to this fraction, or it could result from the production of fragments from partially digested granules.

Granule numbers per unit weight of starch were variable and could be markedly affected by variations and problems during isolation.

7.3.2. Conclusions

The susceptibility of starches within a population of granules to digestion (and fermentation) in the bird are shown to vary between wheats. Low-AME wheats contain a higher proportion of resistant starch (or starch that is more slowly digested) than do high-AME varieties. Whilst the smaller granules appear to be more susceptible to digestion, the larger granules are degraded to varying degrees. Overall, the rate at which the starch from the low-AME wheats was digested within the gastro-intestinal tract of the bird was slower than that of the high-AME types. This observation also holds true for excretal measurements. The proportion of large and small granules in samples clearly demonstrates differences between samples.

Although these studies compared only single contrasting wheat samples, the results show quite clearly the differences between the extremes in AME observed. Moreover, because the samples tested in each trial were grown in the same area under the same environmental conditions and treatments, any

differences observed would be genetic rather than environmental.

Differences in the aqueous extract viscosities of wheats are probably not related to starch granule size even though the low-AME wheats contain a higher proportion of the lowest sized starch granules, since there is very little difference between the wheat types in general. The data recorded correlate well with SEM observations.

7.4. STARCH GRANULE MORPHOLOGY

Evidence from digestibility studies *in vivo* have revealed that the digestion of starch is reduced in low-AME wheats. Microscopic examination of starch granules isolated from wheat flour, foregut digesta and excreta were undertaken to determine the pattern of digestion of such granules. The enzyme employed in *in vitro* assays is important in determining the type and extent of enzymic degradation of starch granules, with bacterial α-amylase differing from pancreatic α-amylase (Leach and Schoch, 1961), thus to reveal the true pattern of digestion, starch granules were isolated from *in vivo* experiments rather than *in vitro* assays.

7.4.1. Light Microscopy

Excretal samples from birds supplied with low-AME wheats (e.g. Avalon and Hereward, Trial III; Rialto and Spark, Trials VII & VIII) contained large numbers of starch granules. Most granules appeared entire from the birefringence pattern under polarised light, indicating that the starch passed through the intestinal tract largely unchanged. Weak birefringence seen in small numbers of starch granules reflected the loss of crystallinity due to partial granule degradation. Generally, starch granules were almost absent from the excreta of chicks fed high-AME wheats, and those that were present lacked birefringence. Thus in

low-AME wheats, the digestion of starch is reduced.

7.4.2. Scanning Electron Microscopy (SEM)

The changes that occur to wheat starch granules when degraded by intestinal amylases were investigated in more detail by means of SEM. Granule surface characteristics, size distribution and the extent of degradation were examined following exposure to digestive amylases. Investigations included wheats, digesta isolates and excreta isolates from wheats of varying AME value (Table 7.12).

SEM of the isolated and purified starch granules from wheat grains revealed the two characteristic but distinct types of granule, namely lenticular and prolate large 'A' granules and small, more spherical or polyhedral 'B' granules. Both granule types were smooth, although numerous small depressions or dimples were observed on the large granules which may represent either the contact site with the smaller starch granules or protein bodies during endosperm development, or may present weakened areas for initial enzyme attack. The large granules possessed the characteristic equatorial groove. The groove appears as a depression rather than a cleft. No distinct varietal differences with respect to the morphology and sizes of the starch granules were identified.

Small granules appeared to be degraded preferentially (compared with original wheat starch), although numerous intact small starch granules were excreted in the case of the low-AME wheats. These granules (Avalon, Trial III; Spark, Trial VIII) appeared largely entire although degradation within the granule cannot be completely excluded since only a restricted part of the granule surface could be viewed. The pattern of granule ('A' type) erosion was variable. Partial digestion of the low-AME wheat starch granules ('A') occurred as shallow depressions ranging through surface pits to deeper pinholes, with the region of the equatorial

groove more susceptible. Only a few large granules were extensively digested, the rest appeared entire.

Granules of type 'A' isolated from the excreta of birds fed the high-AME wheat (Mercia, Trial III) were digested to varying degrees and relatively few 'B' type granules were isolated compared with that of Avalon or Spark. The digestion of Mercia starch granules was generally more extensive and ranged from deep circular holes passing through several growth rings to the interior of the granule to almost complete degradation. Overall granule erosion varied between and within starch granule types. Areas of preferential attack may correspond to the shallow depressions observed on the original intact granules ('A' type), but the region of the equatorial groove appeared to be very susceptible. Almost no starch granules were isolated from the excreta of birds fed the high-AME wheat (Riband, in Trial VIII) and those that were recovered were heavily digested. Evers and McDermott (1970) reported mild, but general, erosion over the whole surface of the wheat granule by α-amylase *in vitro*, but penetration was confined to certain areas, particularly the equatorial groove. Following digestion within the gastro-intestinal tract, the surface was rougher and pitted and the groove was accentuated indicating a preferential attack in the vicinity of this region, at least on the surface layers. Only a few completely intact granules were found. Enzyme attack was generally similar for both 'A' ($<10\mu$) and 'B' ($\geq 10\mu$) type granules. The variation in attack between the granule sizes may be explained by the degree of resistance to hydrolysis, the two granule types being more ('A') or less ('B) resistant. Variation in attack within each granule population size may represent different stages of the degradation within the small and large intestines of the birds. The reason why some large granules isolated from the excreta were substantially more degraded and others virtually intact is not clear. It is probable that granule attack is a property of each individual granule. It could be due to the transit time through the gastro-intestinal tract or the concentration of granules which may limit enzyme-

substrate interactions. Indeed, low-AME varieties do contain a higher proportion of 'B' granules.

It would appear, therefore, that granules found in low-AME wheat varieties are generally more resistant to attack by intestinal α-amylases and this alone may be responsible for the low-AME values.

Since excreta samples may unavoidably be contaminated by spilled feed, starch granules isolated from the digesta of the proximal small intestine (foregut) possibly offers a more accurate assessment of the susceptibility to digestive enzymes and the relative amount of degradation. Samples were examined from birds in Trials V and VIII fed high- and low-AME wheats.

Numerous intact and partially degraded large granules were present in the digesta of chicks fed low-AME wheats, eg. Avalon (Trial V) and Rialto (Trial VIII). Comparatively, the small granules were mostly digested. Degradation of the large granules ranged from extreme pitting to superficial erosion. Digestion was general over the granule surface although the region of the equatorial groove appeared particularly susceptible to attack. Surface erosion and internal digestion seemed to be more extensive on the starch isolates from the foregut of birds fed the high-AME wheats, Riband in Trials V and VIII, although the overall AME values obtained in Trial V were not too dissimilar. Again, the equatorial groove was particularly susceptible to digestion. Fewer small starch granules seemed to be present compared with the Avalon- and Rialto-extracted samples. Although the digesta starch isolates from Trial V displayed similar digestion patterns to those observed in Trial VIII, whilst the range in AME values was smaller, this might have reflected differences in the rate of digestion. In Trial V, the low-AME wheats may have been slower to digest initially, but overall produced a similar digestibility to that of the higher-AME wheats. Moreover, greater fermentative activity in the lower intestine may have

compensated for the lower digestibilities observed with the lower-AME wheats, although this was not investigated as part of the current studies. These observations indicate differences in the digestion occurring in the small intestine of chicks between different wheat types.

7.4.3. Discussion

Scanning electron microscopy (SEM) was used to examine some of the experimental findings *in vivo*. SEM enabled a more detailed study on the specific surface features of starch granules. The grains were heterogeneous with respect to digestion, an inherent feature of individual granules within a population. The digestion pattern that develops when native wheat starch granules ('A' type) are exposed to α-amylase reveals a general surface attack but the region of the equatorial groove is especially susceptible. The small granules were generally more susceptible to enzymic attack since the presence of such granules isolated from the excreta and foregut digesta was far lower compared with the original wheat sample. This may be due to the higher surface area of the small granules relative to their volume. Although apparently intact, the more resistant granules from the low-AME samples may have been hydrolysed within the interior of the grain following enzyme access via a single pore. If this was the case, it was probably rare since the 'all-round' scanning of numerous starch granules failed to reveal this type of erosion pattern. Nevertheless, penetration of the enzyme to the interior of the granule and extensive digestion therein cannot be ruled out completely. In a few cases, however, pitting was restricted to a small area of the large granules signifying partial hydrolysis.

Since the differences observed *in vivo* were not significant, it was likely that large differences in the digestion patterns of the starch granules would not be found, and thus it is very difficult to establish with certainty whether wheat samples were of high- or low-AME value based solely on the digestion patterns

observed by SEM.

7.4.4. Conclusions

Notwithstanding problems associated with the isolation and purification process of starch granules, it was clear from light microscopy and scanning electron microscopic evidence that digestion of the starch granules from high- and low-AME wheat varieties differed in the chick alimentary tract. Certain factors which may be intrinsic or extrinsic to the starch granule may affect their susceptibility to enzymic degradation and thereby starch digestion and AME values of wheat.

SEM revealed a considerable variation in the size distribution of the starch granules in wheat. Results indicated that granules isolated from the low-AME varieties contained more of the smaller sized 'B' granules when compared with those isolated from high-AME varieties. This may be surprising in view of the fact that small granules are known to be more susceptible to amylolytic degradation. Small granules were predominantly smooth while large granules had more surface contours.

Starches were degraded to varying degrees in the gastro-intestinal tract of the birds. Some granules appeared completely resistant to endogenous enzyme activity (small intestine) and microbial fermentation (excreta), whilst others were almost completely susceptible. Generally, starch granules isolated from the lower-AME wheats were incompletely digested by young broilers. Small granules were digested preferentially due to their higher surface area-to-volume ratio. The incomplete digestion of the starch granules could represent a major factor contributing to the low digestibilities and AME values of certain wheats. The underlying reasons for these low values in such wheats have not been fully identified and are complicated by the lack of uniformity within the granule population of a given wheat with respect to enzyme degradation.

Table 7.1. Susceptibility Index of Wheat Starches - within meal

Wheat Variety	Trial	AME Type[1]	Initial Hydrolysis (mg starch.min^{-1}.g^{-1})
Avalon 1	III, VI, V	L	3.48 (0.19)
Hereward 1	III	L-I	3.69 (0.14)
Admiral 2	III	H	4.40 (0.23)
Mercia 1	III, IV	H	3.71 (0.06)
Riband 1	III, V	H	4.00 (0.26)
Tara 1	III	H	3.95 (0.13)
Beaver	VII	L	4.09 (0.19)
Lynx	VII	L	5.60 (0.14)
Rialto	VII, VIII	L	4.81 (0.02)
Spark	VII, VIII	L	4.64 (0.10)
Avalon	VII	H	5.90 (0.20)
Mercia	VII	H	6.34 (0.17)
Riband	VII, VIII	H	5.11 (0.17)
Riband-SB	VII	H	5.28 (0.14)

[1] L refers to low-AME wheat (<13.0MJ.kg^{-1} DM); I- intermediate AME (13.0-14.0 MJ.kg^{-1} DM); H- high AME (>14.0 MJ.kg^{-1} DM)

Table 7.2. Susceptibility Index of Wheat Starches - as isolates

Wheat Variety	Trial	AME Type[1]	Initial Hydrolysis (mg starch.min^{-1}.g^{-1})
Beaver	VII	L	7.93 (0.16)
Lynx	VII	L	8.05 (0.06)
Rialto	VII	L	8.14 (0.05)
Spark	VII	L	8.04 (0.17)
Avalon	VII	H	8.20 (0.15)
Mercia	VII	H	8.96 (0.59)
Riband	VII	H	9.05 (0.55)
Riband-SB	VII	H	8.52 (0.50)

[1] L refers to low-AME wheat (<13.0MJ.kg^{-1} DM); H- high AME (>14.0 MJ.kg^{-1} DM)

Table 7.3. The effect of aqueous soluble material from wheat meals on the hydrolysis of starch in wheat meals by porcine pancreatic amylase (PPA).

Variety	ME Type[1]	Initial Rate of Hydrolysis (mg starch/min/g)
Riband (C)	H	4.68 (0.16)
Spark (C)	L	5.18 (0.30)
Riband (+)	H	2.34 (0.33)
Spark (+)	L	2.81 (0.27)
Riband (-)	H	8.53 (0.02)
Spark (-)	L	8.27 (0.01)

C Control Treatments, + Plus 2x water solubles, - Water soluble material absent.

[1] L refers to low-AME wheat (<13.0MJ.kg^{-1} DM); H- high AME (>14.0 MJ.kg^{-1} DM).

Table 7.4. Susceptibility Index of Commercial Wheat Starch to Chick Foregut Digesta *Preparations In Vitro*

Wheat Variety	Trial	AME Type[1]	Initial Hydrolysis (mg starch.min^{-1}.g^{-1})
Huntsman (21)	VII	L	1.67 (0.01)
Zentos (10)	VII	L	2.76 (0.11)
Riband (28)	VII	H	4.18 (0.03)
Riband-SB (36)	VII	H	4.65 (0.01)
Rialto (4)	VIII	L	2.26 (0.20)
Rialto (16)	VIII	L	2.67 (0.04)
Spark (1)	VIII	L	1.63 (0.12)
Riband (5)	VIII	H	3.65 (0.03)
Riband (8)	VIII	H	3.13 (0.21)
Riband (14)	VIII	H	3.27 (0.09)

[1] L refers to low-AME wheat (<13.0MJ.kg^{-1} DM); H- high AME (>14.0 MJ.kg^{-1} DM)

Table 7.5. Relationship between *In Vitro* and *In Vivo* Starch Granule Digestion from Wheat Meals.

Wheat Variety[1]	Total Starch Hydrolysis	
	In Vitro[2]	*In Vivo*
Beaver	0.910 (0.033)	0.956 (0.029)
Lynx	0.937 (0.038)	0.939 (0.042)
Rialto	0.883 (0.025)	0.871 (0.086)
Spark	0.877 (0.023)	0.946 (0.030)
Avalon	0.992 (0.031)	0.988 (0.004)
Mercia	0.992 (0.042)	0.974 (0.013)
Riband	0.993 (0.022)	0.989 (0.004)
Riband-SB	0.997 (0.027)	0.993 (0.005)

[1] Wheat samples from Trial VII

[2] Samples significantly different ($p = 0.02$); Low-AME wheats significantly lower than high-AME wheats ($p < 0.001$).

Table 7.6. Relative Viscosity of Aqueous Extracts of Wheat and Effect of Extraction Time

Wheat Variety		Extraction Time (hr)		
		1[a]	2[b]	4[b]
		Relative Viscosity		
Avalon	(L)	2.38 (0.03)	2.42 (0.06)	2.12 (0.06)
Hereward	(I)	2.08 (0.04)	1.93 (0.02)	2.06 (0.01)
Mercia	(H)	1.76 (0.02)	1.78 (0.03)	1.60 (0.01)
Riband	(H)	1.72 (0.01)	1.89 (0.01)	1.91 (0.00)

Wheats from Trial III (site 1).

L, I, H refer to low, intermediate and high AME values respectively.

Values measured using a 'U' capillary viscometer (Schott-Geraté, Camlab) and presented relative to water (mean experimental value of 1.00 at 37°C) under the same conditions of temperature.

Viscosity and extraction were carried out at 37°C.

Figures in brackets refer to the standard errors about the means.

[a] Mean of $n = 6$ replicates; [b] mean of $n = 3$ replicates

Table 7.7. Effect of Storage of Wheat Meals on the Viscosity of Aqueous Extracts

Wheat Variety		Viscosity (cp)	
		Pre-Storage	Post-Storage
Avalon	(L)	5.12 (0.14)	3.02 (0.10)
Hereward	(I)	4.63 (0.02)	2.53 (0.10)
Mercia	(H)	3.55 (0.13)	2.18 (0.09)
Riband	(H)	3.83 (0.12)	2.28 (0.01)

Values determined using a Brookfield digital viscometer, LV TDVII-CP at 37°C.
Viscosity of water at 37°C had a mean value of 0.76
Wheats stored for 16 months at 1°C.

Table 7.8. Relative Viscosity of Aqueous Wheat Extracts from Trial VII

Variety		Extraction Time (hr)			
		0.5	1	2	4
		Relative Viscosity (cp)			
Rialto	L	6.05 (0.38)	5.59 (0.30)	5.90 (0.42)	5.13 (0.31)
Riband	H	2.88 (0.08)	2.92 (0.05)	2.81 (0.02)	2.54 (0.06)
Spark	I	4.31 (0.32)	4.22 (0.12)	3.82 (0.09)	3.27 (0.13)
Avalon	H	nd	3.40 (0.14)	nd	nd
Beaver	I	nd	5.52 (0.23)	nd	nd
Lynx	I	nd	7.34 (0.13)	nd	nd
Mercia	H	nd	2.69 (0.03)	nd	nd
Riband (SB)	H	nd	2.74 (0.04)	nd	nd

Values represent mean data for n = 2 determination ± SE; nd not determined.
Viscosity measured using a Brookfield digital viscometer, LV TDVII-CP at 37°C and are presented relative to water (0.78) under equal conditions.
1/ L refers to low-AME (< 13.0MJ.kg^{-1} DM) wheat type; I refer to a wheat of intermediate AME (13.0-14.0 MJ.kg^{-1} DM); H refers to a hig-AME wheat (> 14.0 MJ.kg^{-1} DM).

Table 7.9. Viscosity of Aqueous Extracts of Chick Excreta

Variety[1]		Relative Viscosity[2]
Rialto	I	4.25 (0.99)
Riband	H	3.01 (0.13)
Spark	I	4.53 (1.25)

[1] Samples collected from Trial VIII. Mean values of n=5-6 replicates.

[2] Mean values ± standard error. Viscosity measurements were performed on extract supernatants (1:5 w/v) after 1hr at 37°C with a Brookfield digital viscometer.

Table 7.10. Starch Granule Characteristics of Trial III Wheats: Comparison with Digested Granules

Wheat Variety	Mean Diameter (μm)	Mean Surface Area ($m^2.g^{-1}$)		Volume Proportion	Surface Area Proportion
Native Wheat					
Avalon	6.82 (0.06)	0.39 (0.01)	$<10\mu$m	0.185 (0.007)	0.426 (0.009)
L			$\geq 10\mu$m	0.815 (0.007)	0.574 (0.009)
Hereward	7.18 (0.08)	0.38 (0.00)	$<10\mu$m	0.175 (0.001)	0.389 (0.002)
I			$\geq 10\mu$m	0.825 (0.001)	0.611 (0.002)
Mercia	7.03 (0.10)	0.35 (0.02)	$<10\mu$m	0.175 (0.012)	0.407 (0.020)
H			$\geq 10\mu$m	0.826 (0.012)	0.594 (0.020)
Riband	6.94 (0.04)	0.43 (0.01)	$<10\mu$m	0.209 (0.007)	0.452 (0.010)
H			$\geq 10\mu$m	0.791 (0.007)	0.548 (0.010)
Analysis of Variance:					
F pr	0.055	0.005		0.038	0.051
s.e.d.					
min.rep	0.157	0.027		0.018	0.028
max.min	0.133	0.023		0.015	0.024
max.rep	0.103	0.018		0.012	0.018
Analysis of Variance: Low vs High-AME Types					
F pr	0.525	0.917		0.358	0.416
s.e.d.	0.091	0.018		0.010	0.016
Excreta					
Avalon	7.85 (0.08)	0.38 (0.01)		0.113 (0.006)	0.279 (0.010)
				0.887 (0.006)	0.721 (0.010)
Mercia	9.78 (0.18)	0.34 (0.03)		0.067 (0.005)	0.164 (0.010)
				0.933 (0.005)	0.836 (0.010)
Analysis of Variance:					
F pr	<0.001	0.246		0.003	0.001
s.e.d.	0.199	0.027		0.007	0.014

Table 7.10 Continued. Starch Granule Characteristics of Trial VII/VIII Wheats

Wheat Variety	Mean Diameter (μm)	Mean Surface Area ($m^2.g^{-1}$)		Volume Proportion	Surface Area Proportion
Native Wheat					
Avalon-SB H	7.84 (0.02)	0.43 (0.01)	$<10\mu$m	0.112 (0.001)	0.285 (0.002)
			$\geq 10\mu$m	0.888 (0.001)	0.715 (0.002)
Beaver L	7.82 (0.06)	0.37 (0.01)	$<10\mu$m	0.116 (0.004)	0.287 (0.007)
			$\geq 10\mu$m	0.884 (0.004)	0.713 (0.007)
Lynx L	7.38 (0.04)	0.41 (0.01)	$<10\mu$m	0.140 (0.004)	0.336 (0.006)
			$\geq 10\mu$m	0.860 (0.004)	0.664 (0.006)
Mercia H	8.18 (0.04)	0.40 (0.01)	$<10\mu$m	0.009 (0.001)	0.246 (0.003)
			$\geq 10\mu$m	0.901 (0.001)	0.755 (0.003)
Rialto L	7.64 (0.18)	0.44 (0.01)	$<10\mu$m	0.121 (0.009)	0.305 (0.018)
			$\geq 10\mu$m	0.879 (0.009	0.695 (0.018)
Riband H	7.12 (0.11)	0.42 (0.01)	$<10\mu$m	0.161 (0.009)	0.378 (0.015)
			$\geq 10\mu$m	0.839 (0.009)	0.622 (0.015)
Riband-SB H	7.90 (0.05)	0.36 (0.03)	$<10\mu$m	0.123 (0.015)	0.307 (0.024)
			$\geq 10\mu$m	0.877 (0.015)	0.693 (0.024)
Spark L	6.92 (0.03)	0.49 (0.01)	$<10\mu$m	0.177 (0.002)	0.405 (0.003)
			$\geq 10\mu$m	0.823 (0.002)	0.595 (0.003)

Table 7.11. Starch Granule Characteristics of Trial III wheats as fed in Trial V: Foregut Digesta Samples

Wheat Variety	Mean Diameter (μm)	Mean Surface Area ($m^2.g^{-1}$)	Volume Proportion	Surface Area Proportion
Avalon	10.45 (0.38)	0.47 (0.01)	$<10\mu$m 5.73 (0.31)	13.88 (1.15)
			$\geq 10\mu$m 94.28 (0.31)	86.12 (1.15)
Riband	9.50 (0.25)	0.29 (0.03)	$<10\mu$m 8.55 (0.89)	19.17 (1.58)
			$\geq 10\mu$m 91.46 (0.89)	80.83 (1.58)
Analysis of Variance:				
F pr	0.104	0.007	0.028	0.053
s.e.d.	0.451	0.035	0.008	0.020

Table 7.12. Starch Granules examined by SEM

Wheat Variety	AME Type	Trial	Wheat	Foregut	Excreta
Avalon	L*	III, IV, V	+	+	+
Hereward	L	III	+	-	-
Mercia	I-H	III, IV	+	-	+
Riband	I	III, V	+	+	-
Rialto	L-I	VII, VIII	+	+	+
Riband	H	VII, VIII	+	+	+
Riband-SB	H	VII	+	+	-
Spark	I	VII, VIII	+	+	+

* AME low in Trial III, pre-storage.

Figure 7.1 (3 anomalous data points removed from equation)

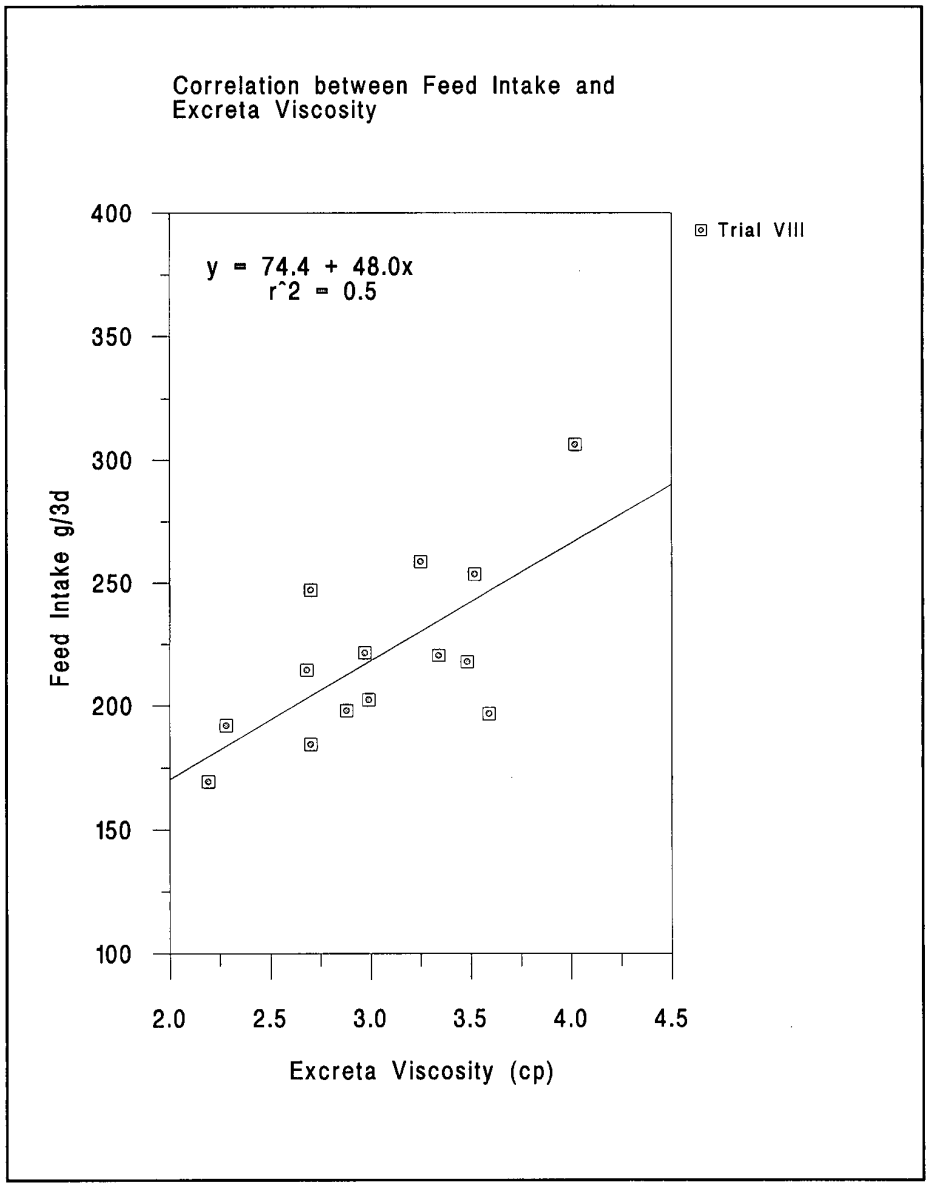
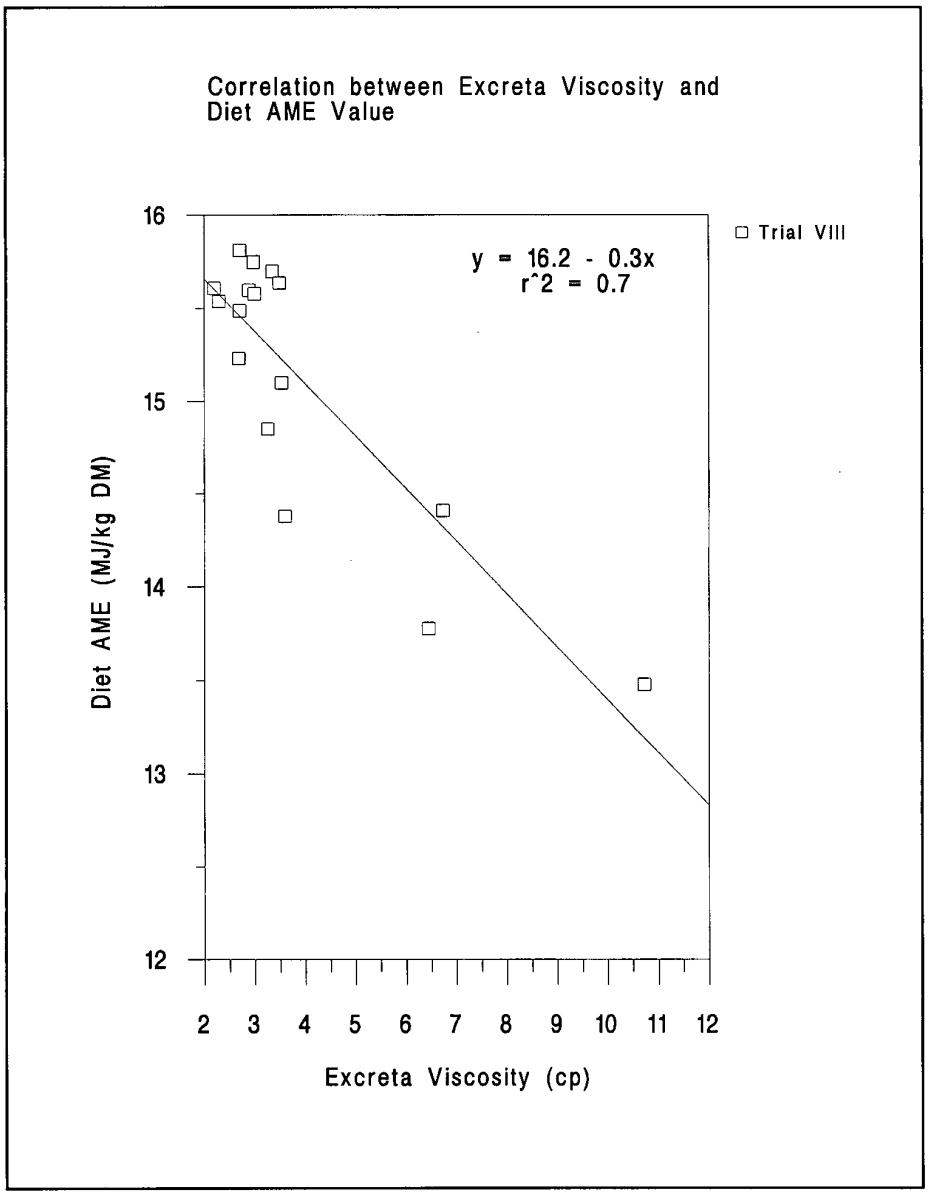


Figure 7.2 (one anomalous value removed).



CHAPTER 8 GUT VISCOSITY AND AME

8.1 VISCOSITY OF INTESTINAL CONTENTS

It is generally considered that increased digesta viscosity is one mode of action by which antinutritional factors, such as arabinoxylans in wheat, may reduce the digestion and absorption of nutrients in broilers. The hypothesis that production of sticky droppings and poor performance of chicks fed on certain wheat varieties of low AME value may be related to such factors was tested. The relative viscosity of intestinal samples was examined in later trials to investigate whether observed differences in AME values could be related to changes in digesta viscosity and whether arabinoxylan content and/or composition was important.

Subsequent to excreta collection for the determination of AME values, birds were slaughtered at 15 days of age (26 days of age in Trial IV part b) for the determination of the viscosity of intestinal digesta in both the foregut and hindgut. Viscosity measurements were carried out at 41°C to mimic core body temperature of the birds at that age. Although viscosity is inversely proportional to the temperature at which it is measured, it was considered important to relate the results to the actual conditions within the bird. Measuring the digesta viscosity at temperatures lower than body temperature would have accentuated any differences between different wheat varieties fed, but this could not be related accurately to the extent of the effects observed. According to Peterson (1995), however, the difference in measured viscosity between 20° and 40°C is only of the order of 10-20%.

Comparisons were also made between aqueous extract viscosities measured *in vitro* and gut viscosity measured *in vivo* to determine whether any relationship existed between them and with AME.

8.2 EFFECT OF CHICK AGE ON DIGESTA VISCOSITY (TRIAL IV)

8.2.1 Part I - Chicks 15 days of age

Two wheats selected from Trial III which had contrasting AME values ($MJ.kg^{-1}$ DM), Avalon 1 (7.59) and Mercia 1 (13.64) were included at rates of $750g.kg^{-1}$ into experimental diets which were each fed to six replicates of young broiler chicks (2 per cage) from 8-15 days of age.

Results from the bioassay showed that AME values were high and ranged from 14.19 to 14.94 $MJ.kg^{-1}$ DM. Varietal differences for AME/GE were not significant ($p = 0.053$). Mean data pertaining to intestinal viscosity measurements are presented (Table 8.1). Differences in viscosity between varieties were observed but did not reach significance. Variability between individual birds was evident. Hindgut (distal section of the small intestine) viscosities were higher than foregut (proximal section) viscosities. The results revealed no significant differences ($p > 0.05$) in digesta viscosity values for the foregut or the hindgut for either wheat variety fed.

Although the results indicate no significant difference between the two wheats fed, there is a tendency for Avalon to produce slightly higher digesta viscosities in both segments of the intestine compared with Mercia. These results do not however, suggest that AME is independent of intestinal viscosity as the range in values is too narrow.

Caecal measurements were undertaken (Table 8.2) as it is possible that lower AME values might be associated with increased caecal fermentation. Differences in caeca weight are difficult to interpret because of the narrow range of AME values obtained and differences in the amount of feed consumed prior to slaughter. Differences in feed intakes and apparent dry matter digestibility were

small which although there was an indication of a greater amount of fermentable material in Avalon compared with Mercia. Thus Avalon-fed birds has significantly longer caeca and there was a trend for greater weight and volume than Mercia-fed birds.

It has been demonstrated that the AME of wheat is negatively correlated to levels of water-soluble NSP (Annison, 1991). Notwithstanding the narrow range of AME and digesta viscosity values obtained, compositional analysis of sugars within the foregut and hingut sections of the small intestine were determined. Compositional analysis of the contents are given (Table 8.3). Total sugar contents increased from foregut to hindgut mainly due to a reduction in glucose, presumably through starch digestion. Pentose sugar content of the supernatants increased. The foregut aqueous phase (supernatant) of birds fed Avalon-based diets was richer in arabinose, xylose and glucose than that from the Mercia-fed birds. On passing through the digestive tract, the ratio of arabinose-to-xylose decreased indicating preferential utilisation of arabinose residues and possibly an alteration in the degree of branching. The ratio of arabinose-to-xylose was higher for Mercia samples, but the decrease on passage through the tract was greater for Avalon. Glucose was the major component in the samples, this being thought to be derived mainly from starch. The results indicate a tenuous link with the intestinal viscosity measurements. The relationship between such data and AME values is rather obscure since the variation in AME values is small.

A very small proportion of the pentosans were detected in the aqueous phase of the ileal contents. It was noted that, after centrifugation, the sedimented material from the contents of the intestines appeared layered with a thin, gel-like layer on top of a solid pellet. A more watery supernatant formed the upper-most layer in the centrifuge tube, and this was taken for the viscosity measurements and analysis of sugar contents. The gel-like material was recovered with the rest of the pelleted material. It is possible that this material contained 'soluble' NSP

material, and as such should have been collected with the supernatant fraction. This finding may explain some of the experimental losses and variability in viscosity determinations between individuals. This finding agrees with that of Annison (1992) who explained that polysaccharides often exist partially in solution in either a gel network or as microaggregates which may be sedimented by centrifugation. This thicker layer of aqueous material may have contained the bulk of the pentosan material which subsequently appeared as part of the pellet analysis.

GC analysis of the digesta solids are given in Table 8.4. There is a greater concentration of all sugars within the digesta solids compared with the aqueous phase of the small intestinal contents. Total sugar contents are greater for the Avalon-fed birds in both the fore- and hindgut samples but differences are not significant due to large residual errors. Pentosan contents were significantly higher in the proximal section of the Avalon-fed birds compared with the Mercia equivalents. The concentration of pentosans increased in the hidgut section but differences between diets were not significant. Arabinose-to-xylose ratios did not alter on passage through the alimentary tract and the ratio was higer for Mercia samples. Glucose in the foregut digesta was believed to be mainly due to the presence of undigested and insoluble starch. In the hindgut, glucose levels were considerably reduced due to digestion, and any glucose remaining had probably arisen from cellulose.

8.2.2 Part II - Chicks 26 days of age

The above experiment was extended by feeding the same wheats (diets) to a second group of birds (collected at the same time as those birds used above) to examine the effect of bird age on gut viscosity. Diets were fed to six replicates (1 bird per cage) from 8-26 days of age.

Comparative studies using older birds showed similar trends to those obtained with younger birds. Mean intestinal viscosity results are presented in Table 8.5. Variability between individual birds was great, particularly in the hindgut section of the small intestine. Hindgut viscosity values were higher than foregut viscosity values. Within both the foregut and the hindgut section of the small intestine, digesta viscosity measurements were not significantly different ($p > 0.05$).

Although no statistically significant differences between the two wheat varieties in terms of digesta viscosity values were obtained, Avalon appears to create a more viscous environment within the intestines compared with Mercia. However, the variation in AME was too small to assess whether such variations might have an impact on wheat AME.

Individual bird variation was high regardless of diet. Hindgut viscosities of the older birds appears to be greater than that in the younger birds, but the variability is also greater between replicates. The foregut viscosities appear to be lower in the older birds with similar variability. This observation corroborates that of Philip *et al.* (1995) who showed that the viscosity of the intestinal fluid declines between day 14 and day 35. Change in digesta viscosity with age has been noted previously (Salih *et al.*, 1991) but speculation has tended to ascribe this change to microbial glucanse in the small intestine. Other studies investigating the activity of β-glucanse in the crop (Philip *et al.*, 1995) do not, however, support this idea and suggest that mechanisms of adaptation other than changes in gut microflora, such as increased intestinal secretion, may need to be considered.

Caecal measurements were made as before. The results indicate that caecal dimensions are not a good indicator of the indigestible nature of the diet, although the data do reflect the AME values obtained.

The contents of pentose sugars and glucose (Table 8.6.) were considerably higher in both sections of the small intestine for birds fed Avalon. The difference in xylose contents in the foregut was significant ($p = 0.028$). Arabinose-to-xylose ratios varied less on moving through the digestive tract in the older birds compared with the young broilers, although the trend was similar. The ratio was higher in both sections of the small intestine for Mercia fed birds. The reasons for this are unclear.

Digesta pellets (Table 8.7) contained a higher proportion of arabinoxylans in the Mercia samples compared with the Avalon counterparts in both sections of the small intestine. Glucose values were, however, consistently lower. Although total sugar contents in the distal section were equal, Mercia samples contained more pentose sugars and less glucose. Again, the glucose fraction may represent differences in starch digestion. These results might be due to a simple concentration of the indigestible pentose sugars as the starch material is digested. Although the average feed intake for birds on each diet was similar, Avalon-fed birds consumed less starch (lower starch content in wheat) and show a greater glucose residue in the small intestine compared with the Mercia-fed birds. This may be due to differences in the intrinsic properties of the starch granules and their susceptibility to attack by the amylolytic enzymes of the tract. Intakes of NSP and arabinoxylans were also marginally higher for the Avalon-fed birds. The ratio of arabinose-to-xylose of the pellet on passage through the gastro-intestinal tract was lower for Mercia fed birds compared with Avalon fed birds. Differences overall were not significant due to large between-bird variability. The significance of these compositional differences is not obvious in light of the AME values obtained.

8.2.3 Conclusions

The results indicate an improvement in the nutritive value of wheat on storage. Improvements were more marked for the low-AME wheat than the high-AME wheat. This gradual improvement seen in AME values precluded conclusions with respect to factors responsible for the production of low-AME wheats.

AME values were, in general, lower in younger birds compared with birds of 3-4 weeks old. Individual bird variation was high regardless of diet and bird age. Small differences in viscosity reflected AME values. Feed intakes and thus intakes of NSP were higher in older birds despite AME values being higher. These findings indicate that older birds can either utilise dietary NSP more efficiently or have a superior colonisation of microflora which can cope with the elevated intakes. In addition, lower glucose residues remained in the intestines of the older birds suggesting better utilisation of dietary starch, possibly explaining the marginally higher AME values.

The arabinoxylans are the major NSP of wheat flour, although they constitute only a minor proportion of the wheat flour. Due to their hydrocolloid nature, the arabinoxylans have stimulated considerable interest due to their water absorption, viscosity enhancing and gelling properties (Izydorczyk and Biliaderis, 1992). Compositional analysis of the intestinal digesta would suggest that the glucose residue is more important although there is some evidence for an association with pentosans in solution. Differences were evident in the initial rate of starch hydrolysis, providing further evidence supporting the claim that differences in the mechanism of starch digestion between high- and low-AME wheats are operating, possibly due to differences in the inherent properties of the starch granules. From the evidence gathered to date, it is impossible to state whether the poor nutritive value of some wheats is due to the intrinsic properties of the starch granules or whether it is a consequence of another

component (anti-nutritional factor) within the wheat, associated perhaps with the surface of the starch granule. Certainly, these compositional differences suggest that the glucose fraction may be linked with starch digestibility and AME.

8.3 EFFECTS OF DIGESTA VISCOSITY ON AME (TRIAL V)

Trial IV was repeated using young broilers to confirm the results obtained. Riband 1 (from Trial III) was used in place of Mercia 1 as a high AME wheat (AME of 13.67 $MJ.kg^{-1}$ DM) due to a lack of sufficient material available for bioassay. Avalon was included as before. The dietary regime employed was the same as that used in Trial IV with an incorporation level of wheat of $750g.kg^{-1}$. Six replicates per dietary treatment were used in a fully randomised design. Mercia from the University Farm (1993) was included to serve as a control for future trials.

The results obtained were similar to those in Trial IV. The AME values for Avalon and Riband were not significantly different (Table 5.2). The improvement in the AME value of Avalon from Trial III (7.59 to 13.35 $MJ.kg^{-1}$ DM) meant that the digesta viscosities measured (Table 8.8) were not appropriate and any differences found could not be related to AME. Such changes may be due to subtle changes within the grain with time either with the starch or some non-starch component affecting the digestibility of the starch and diet as a whole. Despite a tendency for the gut viscosities attained with feeding Avalon to be higher than those found with feeding Riband, the differences between the wheat varieties were not significant.

Caecal measurements (Table 8.9) were generally higher for Avalon-fed birds; caecal length represented the best measure of difference between the two dietary treatments.

Characterisation of the carbohydrates in the digesta supernatant (Table 8.10) revealed differences between the wheats. The total carbohydrate content in the hindgut was significantly higher in the Avalon-fed birds compared with those fed Mercia ($p<0.05$). Differences lay mainly in the content of glucose, particularly in the distal section of the small intestine, although such differences were not significant because bird variation was high. The content of pentose sugars was also higher in the Avalon-fed birds, especially the hindgut, which may have been associated with differences in viscosity obtained. The ratio of arabinose-to-xylose did not differ considerably upon transit through the gastro-intestinal tract or between wheats fed. As with previous determinations, the higher AME wheats tended to have a higher ratio of arabinose-to-xylose.

Results may indicate a difference in the rate of starch hydrolysis between the Avalon-fed birds and the Riband-fed birds owing to the pattern of changes observed in the glucose contents between the foregut and the hindgut. The data suggest that birds fed Avalon digest wheat starch *per se* at a slower rate and/or lower extent in the small intestine than birds fed Riband which may be due to differences in the intrinsic properties of the starch granules.

8.3.1 Conclusions

AME values obtained in this trial reflected data recorded in the previous trial. The AME values of both varieties improved, although the extent of the low-AME wheat improvement was far greater. Such changes might be due to subtle changes in the grain with time affecting the starch or non-starch component, which in turn affects starch digestion.

Although the whole tract digestibility of starch and dry matter was not different for the two wheat varieties fed, this might reflect differences in the gut microflora of the two sets of birds, since small intestinal digestibility appeared to

be less efficient for the Avalon-fed birds. It is possible that these birds exhibited greater fermentable activity in the lower gut.

8.4 EFFECT OF WHEAT STORAGE ON DIGESTA VISCOSITY (TRIAL VI)

Due to an inability to obtain reproducible AME values for identified low-AME wheats following prolonged storage, a chick bioassay was undertaken utilising wheat samples with low-AME values identified in the previous 10 months. The objective of this trial was to re-examine the possibility of improvement in nutritive value of wheats over time and secondly to try and identify samples with low AME values. Wheats used in this study had the lowest and the highest AME values as determined in a parallel study at the University also funded by H-GCA (Project No. 0057/1/91A). The wheats (same varieties) were harvested in 1992 from the same sites as those wheats tested in Trial III (1991) in the current research programme. Accordingly, Avalon (site 1) and Mercia (site 1) revealed the most contrasting AME values (8.43 and 12.63 $MJ.kg^{-1}$ DM respectively) in the previous study carried out on young broilers (11-14 days of age) 6-7 months after harvest. Wheats for use in this trial had been stored for an additional 10 months in an unground state. Mercia harvested from the University Farm (1993) was included as a control.

Digesta viscosity measurements were undertaken following the AME bioassay (Table8.11). The results showed no significant differences between AME values obtained. An improvement in AME values occurred for both wheat varieties fed, although the improvement for Mercia was less marked compared with that for Avalon (increase of 2.44 and 5.81 $MJ.kg^{-1}$ DM respectively). The mean AME value of Mercia (1993; control variety) also increased marginally by 0.78 $MJ.kg^{-1}$ DM. Viscosity measurements of the intestinal contents revealed differences between the two varieties, although such differences were not significant and could not be correlated with AME in view of the extreme variability of the data.

Caecal analysis (Table 8.11) showed that birds fed Avalon had larger caeca than birds fed Mercia but the differences were not significant. This finding corresponds with the small differences in AME.

8.4.1 The relationship between digesta viscosity and AME - Trial VI

The AME values obtained in Trial VI agree with those of the previous studies. Neither wheat tested proved to have a low-AME value despite the fact that Avalon had an AME value of only 8.43 MJ/kg DM a few months previously evaluated using identical conditions. This observation together with those from Trials IV and V above strongly imply that wheats with inferior AME values improve on prolonged storage at ambient temperature both in the ground and unground state. The time period for this improvement appears to be not less than 6-7 months. The nature of these changes have not been identified. Choct *et al.* (1994) described similar effects with wheats reanalysed from their 1991/92 survey. The AME of a low-ME wheat increased considerably from 9.5 to 12.02 MJ/kg DM whilst there was no change in the nutritive value of a 'normal' wheat during storage. It was suggested that the gradual improvement in nutritive value of the wheats may have been due to storage and/or assay conditions although, more probably, due to an increase in the activity of the endogenous enzymes within the grain which degrade the antinutritional components. It is possible that by reducing the degree of polymerisation of these components the colloidal characteristics would diminish. Specifically, it is thought that the high molecular weight arabinoxylans are broken down over time to lower molecular weight components to bring about this increase in nutritive value which is manifested in reduced digesta viscosity.

8.4.2 Conclusions

The improvement in AME values observed in this trial together with results from

the previous trials indicate that wheats with inferior AME values improve on storage at ambient temperatures. A time period of not less than 7 months is required for this improvement. The nature of these changes is uncertain but it is possible that a change in the content of digestible starch occurs to even out the variation between varieties over time. Four varieties used initially in Trial III namely Avalon 1, Hereward 1, Mercia 1 and Riband 1 had widely differing AME values. A period of 2 years was required to minimise the difference between the varieties, after which time the differences were less pronounced ($p = 0.041$) and certainly the differences between the low- and high-AME types had disappeared.

It might be speculated that an increase in the activity of the endogenous enzymes within the grains of the lower AME wheats may, in addition, bring about a disruption of the anti-nutritional components. However, such an assay was not conducted in these studies.

8.4.3 Relationship between chick digesta viscosity and wheat AME - Trial VII)

Due to the inconsistencies with AME data obtained to date, a new set of wheat samples were screened and analysed for AME to try and establish whether digesta viscosity was an important parameter. Wheats (18 in total) from the 1994 harvest from the University Farms were selected and screened for their nutritive value just one month after harvest using dry matter digestibility studies. It was considered that any differences between the wheats should be more pronounced in these early stages after harvest before any storage period was enforced.

Certain birds were selected from a bioassay, based on an assessment of their excreta production, to include a range of samples for further assessment and evaluation of digesta viscosity and AME (Table 8.13). Viscosity measurements were conducted on the intestinal contents of these birds selected the following

day after completion of the total collection studies at a time when, therefore, actual AME data would not have been available.

The results show a large degree of variation in digesta viscosities in both foregut and the hindgut sections of the small intestine. Viscosities were not correlated with DM digestibility. AME values were determined for 8 wheat varieties in total which were not necessarily correlated with the viscosity measurements undertaken. AME values are given for those samples above if measured. It was assumed that DM digestibility would reflect the AME values likely to be encountered. AME was highly correlated with DMD ($r^2 = 0.95$). A weak inverse relationship between the foregut viscosity and AME values was shown ($r^2 = 0.49$). Generally, as the foregut viscosity increased, the AME value decreased, although only a small number of observations were made. There was no correlation between AME and hindgut viscosity values.

8.4.4 Relationship between wheat AME and digesta viscosity - Trial VIII

Large numbers of birds in the previous trial precluded in depth studies being made with respect to digesta viscosity and its relationship with AME. As such, three wheats were selected from Trial VII to include a range in AME values ($MJ.kg^{-1}$ DM), Rialto (11.745), Riband (14.19) and Spark (13.76).The effects of digesta viscosity on AME were investigated. Results pertaining to intestinal viscosities are presented (Table 8.14).

The results show a wide range between wheat varieties fed. Riband appears to be the most consistent variety producing relatively low intestinal viscosity values. Rialto would appear to create the most viscous environment within the small intestine of the young chicks, although Spark exerts similar effects, particularly within the distal section (hindgut). The hindgut region of the intestine appears to be affected to the greatest extent by the type of wheat

consumed, but variability between chicks is high. Viscosities attained within the foregut correlate inversely with the DM digestibilities of each wheat variety ($r^2 = 0.64$) but the relationship with the hindgut viscosity and the DM digestibility is less pronounced.

Wheat AME values obtained in this trial are less widely ranging than those encountered in the previous trial (VII), although a similar trend may be observed with Riband exhibiting the highest AME value (14.85 MJ/kg DM) and Rialto and Spark significantly lower (13.62 and 13.23 respectively). An improvement in the AME value of Rialto by 1.9 $MJ.kg^{-1}$ DM from Trial VII to Trial VIII can be observed.

AME values are highly correlated with DM digestibility values ($r^2 = 0.95$). A weak negative relationship between foregut viscosities and AME is shown ($r^2 = 0.49$). Despite a weak positive correlation between foregut viscosity and hindgut viscosity ($r^2 = 0.53$) no correlation is evident between the hindgut viscosity and AME values obtained.

It is generally regarded that the soluble arabinoxylans in wheat act as antinutritional factors when high levels are contained within broiler diets to increase to viscosity of the intestinal contents, thereby affecting nutrient absorption (e.g. Campbell *et al.*, 1989; Annison, 1991; Bedford *et al.*, 1991; Choct and Annison, 1992). Levels of NSP, pentosans and soluble NSP in the current set of wheat samples were higher in the lower AME wheats, Rialto and Spark compared with Riband. Levels of soluble NSP were significantly higher in those lower AME wheats, although the NSP and arabinoxylans in general were not. The higher intestinal viscosities generated are related to those wheats fed containing higher levels of such antinutritional components, but since feed intakes do not appear to be correlated with digesta viscosity in the current series of experiments, it indicates that although soluble NSP may be important in

terms of creating a viscous environment within the small intestines, there is possibly a critical level of consumption above which no further effects can manifest. Thus, no correlation exists between the levels of NSP consumed and the intestinal viscosity, but for birds given Riband containing less NSP, the viscosity of the digesta was always lower. Non-cellulosic polysaccharides, cellulose and the insoluble NSP do not appear to be related to intestinal viscosity.

It has been suggested that any increase in the bulk and viscosity of the intestinal contents decreases the rate of diffusion of substances and digestive enzymes and hinders their interaction at the mucosal surface (Edwards *et al.*, 1988). Viscous polysaccharides such as pentosans, and β-glucans, might also directly complex with digestive enzymes and reduce their activity (Ikeda and Kusano, 1983).

8.5 EXTRACT VISCOSITY AS A PREDICTOR OF DIGESTA VISCOSITY

Wheat flour contains a small percentage of NSP which are soluble in cold water. These carbohydrates are said to be responsible for the high viscosity of aqueous flour-extracts (Neukom and Markwalder, 1975). Extract viscosity of wheats employed in this experiment are directly and highly correlated with the digesta viscosities measured. As such, aqueous extract viscosities might be important as an indirect measure of intestinal viscosity. This measure might be used to screen out at an early stage wheat varieties of potentially low nutritive value as has happened with barley, although similar procedures have not yet been developed for quantifying the viscous carbohydrates in wheat. Boros *et al.* (1993) found that extract viscosity of rye could be used as an indirect assay for water-soluble pentosan content. With the current small sample size, a positive correlation was made between the pentosan content of the wheats and the extract viscosity ($r^2 = 0.63$).

8.6 CONCLUSIONS

Inconsistencies with wheat quality have made correlations between gut viscosity and AME difficult. Prolonged storage periods of greater than 7 months at ambient temperature were sufficient to improve the AME values of wheat, and as such removed the variability between wheat samples being tested. Generally, these experiments demonstrated adverse effects in terms of DM digestibility, AME and digesta viscosity when young chicks are fed diets containing particular wheat varieties. The absence of significance in these trials between such correlations was hampered by the variability which existed between individual birds under bioassay and also to the small difference between wheat AME values.

Differences in intestinal viscosity were found between wheat varieties fed. It was generally observed that with extremes of DM digestibility and AME values, excreta score was poor being largely wet and cream-coloured, indicative of large quantities of undigested starch material, and the intestinal viscosities were high. Although the effect of viscosity appears to be a significant factor, insufficient evidence is available from these studies to state whether it is a major controlling factor of AME values.

The soluble pentosans are particularly important due to their water absorbing capacity. The side groups of single α-L-arabinofuranose residues are attached randomly to the xylan and are responsible for the water solubility of arabinoxylans (Amado and Neukom, 1985). As in the current trial, previous research (Salmonsson *et al.*, 1984) has shown that the main constituents in the water soluble fraction are glucose, arabinose and xylose, mainly representing β-glucan and the arabinoxylans. The main constituents of the water insoluble polysaccharides were also glucose, arabinose and xylose, where glucose mainly represented cellulose and to a minor extent β-glucan. Small amounts of

rhamnose, mannose- and galactose-containing polysaccharides were also present.

Molecular weight of wheat arabinoxylans is an important determinant of their physical properties in an aqueous environment. Positive linear relationships between rigidity of cross-linked arabinoxylan and intrinsic viscosity of arabinoxylan fractions have been found. The effectiveness of that cross-linking with increasing molecular size of the arabinoxylan fractions (Izydorczyk and Biliaderis, 1992). Izydorczyk and Biliaderis (1993) subsequently found that with decreasing molecular mass of arabinoxylans, the xylose-to-arabinose ratio, ferulic acid and singly substituted xylose decreased, whereas the amount of xyloses with more than one branch point increased. The relative amounts of high, medium and low molecular mass populations of arabinoxylans seem to vary greatly with the source of these polysaccharides (i.e. wheat class, cultivar, growth location). Thus the overall effect of the arabinoxylans may vary widely.

Dietary pentosans do appear to play a contributory role in the anti-nutritive effects observed, albeit to a small extent. Certainly considerable evidence is available which indicates that the anti-nutritive effect of pentosans may also be directly or indirectly mediated by the gut microflora. There is much evidence that viscous polysaccharides can increase the residence time of digesta (Gohl and Gohl, 1977), which may result in increased microbial growth and activity within the intestine. The dietary addition of antibiotics has been shown to improve the performance of chickens fed cereal-based diets, especially those containing rye (MacAuliffe and McGinnis, 1971; Misir and Marquardt, 1978a,b). Unfortunately, strong correlations between such parameters as pentosans and intestinal viscosity and AME were not possible in this series of experiments due to the improvement in wheat nutritive value and AME observed and high variability between replicates.

It is now well established that the soluble arabinoxylans of wheat (Antoniou *et al.*, 1981; Annison, 1991; Choct and Annison, 1992a,b) elicit anti-nutritive activities and are closely related to AME values (Annison, 1991) when high concentrations of certain varieties are present in broiler diets. The NSP cause a general inhibition of absorption of the macronutrients (Annison, 1993) and probably the micronutrients (van der Klis, 1993). Certainly these data support the hypothesis that NSP are a factor in the low-AME wheat phenomenon. Whether they are the causative factor remains to be seen.

Annison *et al.* (1995) demonstrated that the viscous nature of wheat arabinoxylans is an important factor in their anti-nutritive activity. Viscosity of digesta could be important in controlling accessibility of the substrate to digestion. Factors associated with the digesta viscosity are complex and may involve transit time, mixing of luminal contents and inhibition of enzyme transport (Robertson, 1988).

The arabinose to xylose ratio in the NSP does not appear to have any bearing on the relationship with viscosity. The ratio in the soluble fraction of the NSP ranges from 0.47 to 0.51 and is not related with wheat AME. Annison *et al.* (1995) stated that an important factor in the formation of viscous polysaccharide solutions is the strength of the interaction between these molecules. In solutions of arabinoxylans the strongest interactions occur between the unsubstituted sections of the xylan main chain which can come into close association through the formation of inter-chain hydrogen bonds (Morris, 1986). The presence of arabinose side chains inhibits these interactions (Annison *et al.*, 1995). Thus more highly branched structures (higher arabinose to xylose ratios) should, according to this theory, be less viscous due to the reduced between-polysaccharide chain interactions, and thus have higher nutritive values when fed to chicks. Unfortunately, differences between these wheats in terms of branching are minimal and would not therefore have

contributed to any differences observed in these studies.

Water-soluble polysaccharides may be responsible for part of the varietal differences in viscosity, although their role in viscosity is still far from being fully understood. Certainly, extract viscosity may be an important parameter in predicting the nutritive value of wheat for poultry. A strong correlation exists between the aqueous extract viscosities of these wheats and the intestinal viscosities measured. Wheats with high extract viscosity appear to be more detrimental to chickens than those with low extract viscosity. The role that starch viscosity might play in contributing to extract viscosity may not be ruled out since this was not investigated in the current study.

From the results of these trials, the arabinoxylans cannot yet been conclusively and solely linked to decreased bird performance even though there is evidence to suggest that they make a significant contribution (Choct and Annison, 1990; Wiseman and Inborr, 1990; Annison and Choct, 1991). From the current study, it may be suggested that it is possibly more important to concentrate on the processes occurring within the chicken (i.e. degree of solubilisation of NSP) and not just total levels in the feed itself.

Table 8.1. Digesta Viscosity (centipoise, cp) Trial IV young birds

Wheat Variety	Foregut (proximal)	Viscometer Speed	Hindgut (distal)	Viscometer Speed
Avalon (L)	10.30 (1.19)	12	23.08 (2.71)	6/12
Mercia (H)	8.60 (0.74)	12	20.14 (3.77)	12

Values represent mean data of 6 replicates.
Values in parentheses represent the standard error of mean values (SEM).
L refers to a low AME wheat; H refers to a high AME wheat, as identified in Trial III.
Mean viscosity of water at 41°C is 0.70

Table 8.2. Measurements of caeca Trial IV young birds

Wheat Variety	Full caeca Weight (g)	Length (cm)	Width (cm)	Volume (cm^3)
Avalon 1	7.93 (0.76)	8.66 (0.43)[a]	0.68 (0.05)	3.76 (0.61)
Mercia 1	6.88 (0.41)	7.34 (0.38)[b]	0.72 (0.03)	3.08 (0.27)

Values are mean values of 6 replicates (12 birds).
SEM are shown in brackets.
For caeca length, the value denoted with the superscript 'a' is significantly higher than that value denoted 'b' ($p<0.05$)

Table 8.3. Monosaccharide composition (mg.g^{-1} DM) in the digesta supernatant of the small intestine of broiler chicks fed different wheat diets Trial IV young birds.

Monosaccharide	Avalon		Mercia	
	Foregut	Hindgut	Foregut	Hindgut
Rhamnose	1.3 (0.4)	0.7 (0.4)	0.7 (0.5)	0.8 (0.2)
Arabinose	1.6 (0.1)	1.8 (0.8)	1.2 (0.9)	1.7 (0.1)
Xylose	1.2 (0.1)	2.0 (1.0)	0.9 (0.6)	1.5 (0.1)
Mannose	1.9 (0.3)	0.7 (0.3)	1.8 (0.5)	0.7 (0.0)
Galactose	0.7 (0.2)	1.3 (0.5)	1.0 (0.3)	1.4 (0.1)
Glucose	58.0 (11.2)	5.9 (3.0)	47.5 (13.0)	6.0 (2.4)
Total	**64.7 (10.9)**	**12.4 (5.9)**	**53.1 (13.0)**	**12.1 (2.4)**
Pentosans	2.8	3.8	2.1	3.2
Ara:Xyl	1.33	0.90	1.33	1.13

Values are mean data for 6 replicates.
SEM are shown in brackets.

Table 8.4. Monosaccharide composition (mg.g^{-1} DM) in the digesta solids of the small intestine of broiler chicks fed different wheat diets Trial IV young birds.

Monosaccharide	Avalon		Mercia	
	Foregut	Hindgut	Foregut	Hindgut
Rhamnose	1.4 (0.3)	1.9 (0.4)	1.1 (0.3)	2.0 (0.3)
Arabinose	25.1 (1.0)	32.1 (3.7)	20.1 (1.9)	34.4 (0.9)
Xylose	38.0 (2.1)	48.8 (6.0)	28.1 (2.4)	48.1 (1.2)
Mannose	2.7 (0.2)	4.6 (0.1)	2.4 (0.2)	4.7 (0.2)
Galactose	6.2 (0.6)	9.9 (0.4)	5.0 (0.4)	9.6 (0.3)
Glucose	158.5 (10.0)	53.5 (10.9)	146.5 (14.6)	46.7 (7.1)
Total	**231.9 (9.9)**	**150.8 (9.6)**	**203.2 (19.3)**	**145.5 (7.9)**
Pentosans	63.1	80.9	48.2	82.5
Ara:Xyl	0.66	0.66	0.72	0.72

Values are mean data for 6 replicates.
SEM are shown in brackets.

Table 8.5. Digesta Viscosity (cp) Trial IV old birds

Wheat Variety	Foregut (proximal)	Viscometer Speed	Hindgut (distal)	Viscometer Speed
Avalon (L)	8.21 (0.67)	12	42.88 (7.74)	3/6
Mercia (H)	6.45 (1.23)	12	34.36 (9.75)	3-12

Data are mean values of 6 replicates.
Values in parentheses represent the standard error of mean values (SEM).
L refers to low AME wheat; H refers to high AME wheat.
Mean viscosity of water at 41°C is 0.70

Table 8.6. Monosaccharide composition (mg.g^{-1} DM) of the aqueous digesta of the small intestine of chicks (26d) fed different wheat diets. Trial IV old birds

Monosaccharide	Avalon		Mercia	
	Foregut	Hindgut	Foregut	Hindgut
Rhamnose	0.9 (0.2)	2.0 (0.6)	1.2 (1.3)	1.1 (0.3)
Arabinose	3.1 (0.5)	4.6 (1.0)	2.3 (0.3)	2.6 (0.5)
Xylose	3.0 (0.5)	4.5 (1.0)	1.8 (0.2)	2.2 (0.5)
Mannose	1.6 (0.2)	1.5 (0.3)	1.1 (0.1)	1.1 (0.3)
Galactose	3.0 (0.4)	3.1 (0.6)	2.3 (0.3)	2.3 (0.5)
Glucose	43.2 (5.4)	7.2 (1.4)	32.0 (2.7)	5.4 (1.2)
Total	**54.8 (6.4)**	**22.9 (4.6)**	**40.7 (2.7)**	**14.7 (3.2)**
Pentosans	6.1	9.1	4.1	4.8
Ara:Xyl	1.03	1.02	1.28	1.18

Values are mean data for 6 replicates.
SEM are shown in brackets.

Table 8.7. Monosaccharide composition (mg.g^{-1} DM) of the digesta solids of the small intestine of chicks (26d) fed different wheat diets. Trial IV old birds

Monosaccharide	Avalon		Mercia	
	Foregut	Hindgut	Foregut	Hindgut
Rhamnose	1.9 (0.7)	3.4 (0.6)	1.8 (0.5)	2.9 (0.4)
Arabinose	13.0 (1.7)	24.2 (2.0)	16.4 (2.1)	28.9 (2.9)
Xylose	16.1 (1.7)	31.3 (3.3)	19.9 (2.3)	38.9 (4.6)
Mannose	2.1 (0.2)	3.4 (0.3)	1.9 (0.3)	3.4 (0.2)
Galactose	4.3 (0.5)	6.9 (0.5)	3.9 (0.5)	7.1 (0.2)
Glucose	133.2 (17.1)	41.0 (16.8)	103.3 (11.4)	31.9 (4.9)
Total	**170.6 (14.7)**	**110.2 (14.3)**	**147.2 (10.9)**	**113.1 (11.6)**
Pentosans	29.1	55.5	36.3	67.8
Ara:Xyl	0.81	0.77	0.82	0.74

Values are mean data for 6 replicates.
SEM are shown in brackets.

Table 8.8. Digesta Viscosity and Wheat AME Trial V

Wheat Variety	AME (MJ/kg DM)	Digesta Viscosity Foregut	Hindgut
Avalon	13.345 (0.429)	4.76 (0.48)	21.63 (6.96)
Riband	13.329 (0.454)	3.77 (0.42)	15.43 (3.69)
	0.83 (0.01)		

Data represent means of 6 replicates.
Values in brackets refer to SEM.
Mean viscosity of water at 41°C is 0.74

Table 8.9. Measurements of caeca Trial IV young birds Trial V

Wheat Variety	Full caeca Weight (g)	Length (cm)	Width (cm)	Volume (cm^3)
Avalon 1	5.80 (0.28)	10.23^a (0.32)	0.94 (0.06)	7.33 (1.10)
Riband 1	5.50 (0.45)	9.11^b (0.21)	0.90 (0.07)	5.98 (1.04)

Values are mean values of 6 replicates (12 birds).
SEM are shown in brackets.
Values with different superscripts are significantly different ($p = 0.016$).

Table 8.10. Monosaccharide composition ($mg.g^{-1}$ DM) of the digesta supernatant of the small intestine of chicks fed different wheat diets Trial V

Monosaccharide	Avalon		Mercia	
	Foregut	Hindgut	Foregut	Hindgut
Rhamnose	0.6 (0.1)	1.9 (0.4)	0.7 (0.9)	1.8 (0.2)
Arabinose	2.0 (0.3)	15.3 (1.4)	1.9 (0.1)	12.1 (1.3)
Xylose	2.1 (0.3)	15.5 (1.4)	1.9 (0.1)	11.2 (1.2)
Mannose	0.9 (0.1)	5.1 (0.7)	0.9 (0.1)	3.8 (0.4)
Galactose	1.8 (0.1)	10.1 (1.2)	1.9 (0.1)	8.8 (1.0)
Glucose	28.2 (4.9)	35.1 (5.7)	24.7 (2.5)	21.2 (4.2)
Total	**35.6 (4.9)**	**83.0^a (7.8)**	**32.0 (2.8)**	**58.9^b (7.5)**
Pentosans	4.1	30.8	3.8	23.3
Ara:Xyl	0.95	0.99	1.0	1.08

Values are mean data for 6 replicates.
SEM are shown in brackets.
Values with different superscripts are significantly different ($p < 0.05$).

Table 8.11. Measurements of caeca Trial VI

Wheat Variety	Full caeca Weight (g)	Length (cm)	Width (cm)	Volume (cm^3)
Avalon 1	5.11 (0.36)	9.51 (0.32)	0.89 (0.07)	6.27 (1.15)
Mercia 1	4.37 (0.28)	8.97 (0.19)	0.86 (0.04)	5.29 (0.55)

Values are mean values of 6 replicates (12 birds).
SEM are shown in brackets.
Values within columns are not significantly different.

Table 8.12. Digesta Viscosity (cp) Trial VI

Wheat Variety	Foregut	Hindgut
Avalon 1	4.03 (0.37)	11.29 (2.25)
Mercia 1	**3.06 (0.45)**	8.37 (1.79)

Values in brackets refer to SEM.
Mean viscosity of water at 41°C is 0.74.
Data presented within columns are not significantly different.

Table 8.13. Relationship between diet dry matter digestibility (DMD), wheat AME and digesta viscosity (Trial VII)

Cage	DMD	AME (MJ/kg DM)	Foregut Viscosity (cp)	Viscometer Speed	Hindgut Viscosity (cp)	Viscometer Speed
3	0.723		295.0 (74.2)	0.6		
17	0.733		10.1 (0.5)	12	28.2 (3.7)	3
21	0.695		22.8 (3.8)	3		
22	0.767		59.2 (19.6)	0.6	143.0	0.6
24	0.750		4.0 (0.6)	30	21.9 (2.5)	1.5
32	0.717		6.9 (0.4)	3		
36	0.771	15.41	6.0 (0.3)	3	43.4 (3.9)	1.5
41	0.724	13.40	45.3 (5.0)	1.5	184.3 (17.1)	1.5
56	0.710		4.1 (0.2)	12	23.9 (1.5)	1.5
62	0.765	14.68	25.4 (2.2)	1.5	81.8 (12.3)	0.6
64	0.770	14.63	12.8 (0.9)	1.5	61.0 (1.0)	0.6
67	0.682		27.9 (3.1)	1.5	99.7 (20.2)	1.5
70	0.722	13.89	32.5 (2.7)	0.6	115.0 (13.7)	0.3
75	0.400	3.04	52.8 (0.5)	0.6		
78	0.548	9.84	26.5 (0.0)	1.5	92.9 (14.1)	0.3
85	0.763	14.40	6.8 (0.6)	3	33.1 (3.5)	1.5
93	0.711		57.1 (2.2)	0.6	143.0 (18.0)	0.6
102	0.766	13.26	13.9 (0.9)	1.5	37.7 (6.3)	1.5
106	0.741		32.2 (1.0)	1.5	77.4 (9.0)	1.5

Values in brackets refer to SEM
Mean viscosity of water at 41°C is 0.76.

Table 8.14. Digesta Viscosity (cp) Trial VIII

Wheat Variety	Foregut	Hindgut
Rialto	**33.2 (3.5)**	**235.8 (44.1)**
Riband	**10.0 (0.6)**	39.4 (6.8)
Spark	**23.3 (0.8)**	99.0 (23.3)

Values in brackets refer to SEM.
Mean viscosity of water at 41°C is 0.73.

Table 8.15. Content and composition of wheat NSP (mg.g^{-1} DM) Trial VIII

Wheat	NSP	Pentosans[1]	Soluble NSP
Rialto	114.2 (2.5)	73.3 (0.7)	33.7 (1.2)
Riband	105.7 (0.4)	66.2 (0.1)	21.8 (0.4)
Spark	118.2 (1.0)	74.4 (1.1)	40.4 (1.1)

Values represent the means of 4 determinations.
Values in brackets refer to SEM.
[1] Ratio of arabinose: xylose is not important. Ratio for all wheats is 0.67-0.70.

CHAPTER 9 SUMMARY

The summary and overall conclusions from the programme, to include the relevance to the industry, are presented below.

Importance of wheat to the UK poultry industry

- Wheat may supply up to 0.7 of the dietary energy requirements of poultry, principally through the starch content.
- Its price makes it an extremely cost effective raw material
- Variations in nutritional value (apparent metabolisable energy - AME - which is closely linked to starch digestibility) continue to be a major cause for concern, leading to reduced performance and litter problems which adversely influence carcass quality and waste disposal.

Variability in AME values / starch digestibility of wheat fed to poultry

- A wide range of AME values / starch digestibilities was obtained.
- The influence of site of growth and harvest year is important but the complex factors involved remain to be quantified.
- Varietal effects are inconsistent, and may interact with environmental conditions.
- The young bird is unable to utilise high rates of inclusion of wheat which is associated with the production of copious amounts of creamy excreta, although this is not linked to any evident pathological condition.
- Individual bird variability is important, the more so with wheats of low nutritional value. Poor nutritional value is associated with increased feed intake.
- The nutritional value of low AME wheats improves considerably with dry storage at ambient temperature, either in the ground form or as whole grains, between 6-10 months after harvest.

Investigations into starch digestibility

- Significant amounts of undigested starch granules were found in the digesta of birds fed low AME wheats.

- Differences in granule surface characteristics were not evident, although some evidence was obtained suggesting that low AME wheats have a higher proportion of smaller 'B' granules which, although in principle might be more susceptible to enzyme attack, might create a more viscous environment.
- Starch isolates were uniformly well-digested suggesting that it is not the starch *per se* that is poorly digested in low AME wheats but factor(s) associated with it, although both chemical and physical properties of starch can influence the degree to which it is hydrolysed.

- Starch hydrolysis *in vitro* was slower in low AME wheats

Relationship between digesta viscosity and nutritional value.

- Starch digestibility *in vitro* was slower in the presence of aqueous extracts from low AME wheats; this was also observed when aqueous extracts from digesta taken from birds fed low AME wheats were employed and thus these samples had lower amylase activity.

- The relationship between low AME wheat samples and digesta viscosity in the 'foregut', the 'hindgut' and also in the excreta was examined. High digesta viscosity (principally in the 'foregut') was associated with lower nutritional value and the fact that variations in excreta viscosity was also observed indicate that the factor(s) involved are not digested.

Relationship between nutritional value and chemical composition

- No single chemical component was responsible for the range of nutritional values observed.

- Detailed investigations of the non starch polysaccharide (NSP) fraction revealed that the relationship with AME / starch digestibility is not simple. NSP content (total, soluble or insoluble NSP) and the ratio between soluble and insoluble NSP were not correlated with nutritional value.

- The soluble NSP fraction may be associated with reduced starch digestibility and a tenuous link between this and the arabinose:xylose ratio within the soluble NSP was occasionally observed. Investigations into the structure (molecular weight, degree of branching) of arabinoxylans, which are heterogenous, are warranted.

An *in vitro test* to predict the nutritional value of wheat for broilers.

Two laboratory procedures have emerged from this programme which are of potential value in the screening of wheat samples for both the broiler industry and plant breeders:

- Viscosity of aqueous extracts of wheat
- Rate of digestion of starch *in vitro*

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