INFLUENCE OF STORAGE AND TEMPERATURE TREATMENT ON NUTRITIONAL VALUE OF WHEAT FOR POULTRY

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Abstract

Worldwide production of wheat in 2007 was 787 million (IGC 2008). Due to its importance in the world commodity market, there has been much research into the potential problems of weather damage to wheat, particularly with reference to bread making.

The current project aimed to address three major research areas. Firstly, the effects of heat treatment in relation to the nutritional value of weather damaged wheat were investigated. It appears that drying at 100°C may increase Coefficient of Apparent Digestibility of starch (CAD). Some flour samples that were heated to 100°C failed to demonstrate expected hydration properties that would normally be associated with increased digestibility. They also appear to maintain their crystalline order. Therefore, an increase in CAD is not necessarily related to changes in starch structure and is probably more likely due to modification of non-starch components such as protein. A hypothesis is discussed, that proteins may form a film that protects the starch until the protein is digested by endogenous chick proteases. The precise drying temperature is critical, as at 85°C, digestibility may be decreased, possibly due to crystalline perfection. Apparent Metabolisable Energy (AME) did not follow starch digestibility.

Secondly, it was hypothesised that the Rapid Visco Analyser (RVA) may be able to quantify amylase activity and predict nutritional value of wheat samples. Interestingly, unexpectedly high levels of amylase were observed in some wheat samples. This activity remained despite two years in ambient storage and temperature treatment of up to 100°C. These high levels of amylase activity did not appear to affect CAD, presumably due to deactivation in the acidic conditions of the proventriculus. There were some highly significant relationships between in vivo parameters and in vitro RVA parameters, particularly between Peak Viscosity (with an amylase inhibitor) and Coefficient of Duodenal Digestibility or AME ($P<0.001$ in both cases). This suggests there is potential for the RVA to indicate nutritional value.

Lastly, the nutritional value of wheat after storage for up to four months was investigated. There was no significant difference in AME, CAD or FI.

Acknowledgements

Firstly, I would like to acknowledge the financial support, in the form of a post-graduate studentship, of the UK Home-Grown Cereals Authority.

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ABA	Abscisic Acid
ADF	Acid Detergent Fibre
AME	Apparent Metabolisable Energy
ANOVA	Analysis of Variance
BD	Breakdown
BWG	Body Weight Gain
CAD	Coefficient of Apparent Digestibility (of Starch)
CDAD	Coefficient of Duodenal Apparent Digestibility (of Starch)
CIAD	Coefficient of Ileal Apparent Digestibility (of Starch)
CP	Crude Protein
CTTAD	Coefficient of Total Tract Apparent Digestibility (of Starch)
DLWG	Daily Live Weight Gain
DM	Dry Matter
DSC	Differential Scanning Calorimetry
EV	End Viscosity
FCR	Feed Conversion Ratio
FI	Feed Intake
GA	Giberellic Acid
HFN	Hagberg Falling Number
HM	Heat Moisture Treatment
IBC	Iodine Binding Capacity
kDa	Kilo Daltons
LPL	Lysophospholipid
MC	Moisture Content
NSP	Non Starch Polysaccharide
P	Probability
pI	Isoelectric Point
PMAA	Pre Maturity α-Amylase
PoMS	Post Maturity Sprouting
PV	Peak Viscosity

RPAA	Retained α-Amylase Activity
RVA	Rapid Visco-Analyser
s.e.d	Standard error of difference
SEM	Scanning Electron Microscopy

Chapter One
Introduction

1.1 Wheat

Wheat is a cousin of the common grasses which are part of the family Gramineae (Kent and Evers 1994). The most common type in the UK (*Triticum aestivum*) is hexaploid, with three diploid pairs of seven chromosomes (Atwell 2001) which means that each characteristic is controlled by three genes. Each of these pairs of chromosomes is attributed to a different ancestor (Flintham and Gale 1988). Common in UK wheat varieties is the 1B/1R translocation. Each wheat chromosome has two 'arms', one long and one short. The 1B/1R translocation involves the replacement of the short arm of the 1B chromosome in wheat with the short arm of the 1R chromosome from rye (Short *et al.* 2000). It is thought that 1B/1R wheat varieties have broader adaptability and the translocation will often confer disease resistance. It is possible that they may also confer higher productivity (Foulkes, personal communication). Genes coding for particular glutenins, that are required for high quality bread wheat, are located on the region of the chromosome that is absent in 1B/1R wheat varieties. For this reason, these wheats tend to be feed and biscuit making varieties (Carver and Rayburn 1994). The IB/IR translocation is however associated with reduced nutritional value (Short *et al.* 2000) and is currently being bred out of UK wheats.

Three species of wheat are commonly grown around the world, with many different end uses. *Triticum aestivum* is that found in the UK; *T. compactum* and *T. durum* which is used primarily for pasta manufacture. Durum varieties have been shown to give increased growth performance when fed to chickens, compared with aestivum varieties. It is probably due to their lower productivity that they are not used in poultry diets (Pirgozliev *et al.* 2002).

Wheat is a hardy crop, and is therefore grown around the world, including in North America and Canada, Southern Australia and across Europe. It can also be grown at altitudes of over 4000m (Kent and Evers 1994; Atwell 2001).

1.1.1 Hard and Soft Categorisation

i. Measurement

Wheat can be further categorised in several ways. Of critical importance to millers, is the classification of hard or soft. The absolute classification of either hard or soft is given, in the UK, by the Home Grown Cereals Authority. It is often this classification that is quoted in studies. However, this does not allow for extremes of hard or soft to be recognised. A hardness score can be obtained using Near Infra Red Reflectance (NIR) Spectroscopy (Delwiche 1993; Carre *et al.* 2002). Scores are on a scale of one to 100; those less than 45 are soft (Greffeuille *et al.* 2006). Similarly, the system of Particle Size Index (PSI) will allow a more specific hardness rating. In this case, a score of 1-20 is hard, whereas 21 and above is soft. Both methods work on the basis of differences in milled particle size distribution. PSI measures the sieving behaviour and NIR the spectral characteristics.

With NIR, milled samples are exposed to radiation at a range of wavelengths, typically a maximum of 2500nm (Garnsworthy *et al.* 2000; Fontaine *et al.* 2002; Figueiredo *et al.* 2006). The reflectance is measured at every other wavelength and expressed as log (1/Reflectance). The resulting spectrum is compared either to a ceramic standard (Fontaine *et al.* 2002; Figueiredo *et al.* 2006) or calibrated to the reflectance of known wheat standards at 1680nm and 2230nm (Carver 1994; Garnsworthy *et al.* 2000).

In comparison to other methods, including PSI, NIR showed much less variation between measurements (Famera *et al.* 2004), and is reliable at measuring hardness (Garnsworthy *et al.* 2000).

ii. Properties

Hard wheat requires more energy to mill, and fractures along cell boundaries. This leads to coarse flour (Carre *et al.* 2002; Peron *et al.* 2006) which is easily sifted. However, although soft wheat varieties mill more easily, the resulting flour is very fine and difficult to sieve. The starch and protein is present as a matrix that is easily crumbled. Starch and protein are easily released (Short *et al.* 2000). When hard wheat varieties are milled, high proportions of starch granules are cleaved. However, the starch is trapped in a protein matrix and is difficult to extract (Short *et al.* 2000). Many more starch granules remain intact when soft wheat is milled (Rose *et al.* 2001).

Hard wheat varieties are shown to have higher protein contents than soft wheat varieties (Pirgozliev *et al.* 2002; Hetland *et al.* 2007). Endosperm hardness is

thought to be linked to the protein friabilin, which is present in or on the starch granules. In all cases of barley and wheat, small amounts are bound to the surface of the starch granule. In soft wheats higher levels are present on the surface than hard wheats, where friabilin is associated with a protein matrix inside the granule (Darlington *et al.* 2000). Friabilin is composed of two polypeptides, puroindoline A and B (PinA and PinB) and they are likely to work together (Giroux and Morris 1998). These peptides are coded for by two genes on the hardness locus (*Ha*). The genes are also named PinA and PinB (Wanjugi *et al.* 2007). It is thought that hard wheat varieties have a mutation in one of the puroindoline genes. Specifically this may be a glycine to serine mutation in the PinB gene (Giroux and Morris 1998). The presence of both genes, as the wild type, leads to a soft wheat (Wanjugi *et al.* 2007). Recently it has been suggested that the presence of PinB limits the binding of puroindoline A to starch (Swan *et al.* 2006). The presence of just one gene leads to an intermediate NIR score (Wanjugi *et al.* 2007). The genetic basis for hardness seems clear. However, Greffeuille *et al.* (2006) suggest that even if puroindolines are not directly involved in endosperm hardness, their genes provide a good marker for the characteristic.

Durum wheat varieties are classified as harder than hard common wheat varieties. In soft common wheat varieties, the endosperm is brittle whereas at the other end of the scale, durum wheat varieties are ductile. The latter can withstand twice as much stress and four times as much strain as the soft wheat varieties (Glenn *et al.* 1991). However, there is also variation in hardness indicators within hardness classes and this is correlated to Near Infra Red hardness scores (Glenn *et al.* 1991). Moisture content affects hardness, as compressive strength is negatively correlated with moisture. The more moisture in the sample, the less hard it may be. Hard wheat varieties are more sensitive to moisture than soft varieties (Glenn *et al.* 1991).

iii End Uses

Due to their milling properties, hard wheat varieties tend to be used for bread making whereas soft wheat varieties are more suitable for cakes and biscuits (Greffeuille *et al.* 2006). Hetland *et al.* (2007) and Pirgozliev *et al.* (2006) could not find any consistent difference in nutritional value of wheat varieties varying in hardness scores. However, this is a debatable issue, and many authors have found a relationship between hardness and starch digestibility (Short *et al.* 2000; Carre *et al.*

2002; Wickramasinghe *et al.* 2005; Peron *et al.* 2006; Peron *et al.* 2007). Overall starch content appears not to be related to hardness (Soulaka and Morrison 1985), although hard wheat varieties may contain more amylose (Wickramasinghe *et al.* 2005).

1.1.2 Cultivation

In terms of sowing, wheat can be divided into winter wheat and spring wheat. Winter wheat is sown in late autumn, usually between September and December depending on the previous crop (Kettlewell *et al.* 1999), in areas where the soil is unlikely to freeze. Spring wheat is sown as early as possible in spring. This method is used in areas where winter weather would be too severe (Atwell 2001). The increasing day length in spring serves to synchronise the development stages of crops sown at different times. Anthesis occurs across crops in June and gives way to ripening in July or early August. Harvest usually takes place in August although it may be later in Scotland (Kettlewell *et al.* 1999). For these reasons wheat can be found at some point in its sowing to harvest cycle somewhere in the world all year round (Atwell 2001). The quality of wheat grown in the UK is dependant on the weather throughout Europe. Kettlewell *et al.* (1999) relate quality parameters to Hagberg Falling Number (HFN) and specific weight to the North Atlantic Oscillations. If one of these parameters falls below the standard thresholds and becomes unsuitable for milling, the price of good quality wheat increases relative to the price of animal feed. This indicates that wheat for animal feed use is commonly material that has been discarded from the human food market.

1.1.3 Structure

The structure of a wheat grain is shown in figure 1.1. The wheat kernel is complex, but loosely comprises the embryonic plant or the germ, the storage section known as the endosperm, and the outer protective layers, collectively known as the bran. The kernel is between 4 and 10mm long, although this varies with variety and location of the kernel on the spikelet (MacMasters *et al.* 1971). There is a crease that runs the length of the kernel, opposite to the embryo, which may reach the middle of the kernel. The crease is unusual among cereals (Evers *et al.* 1999).

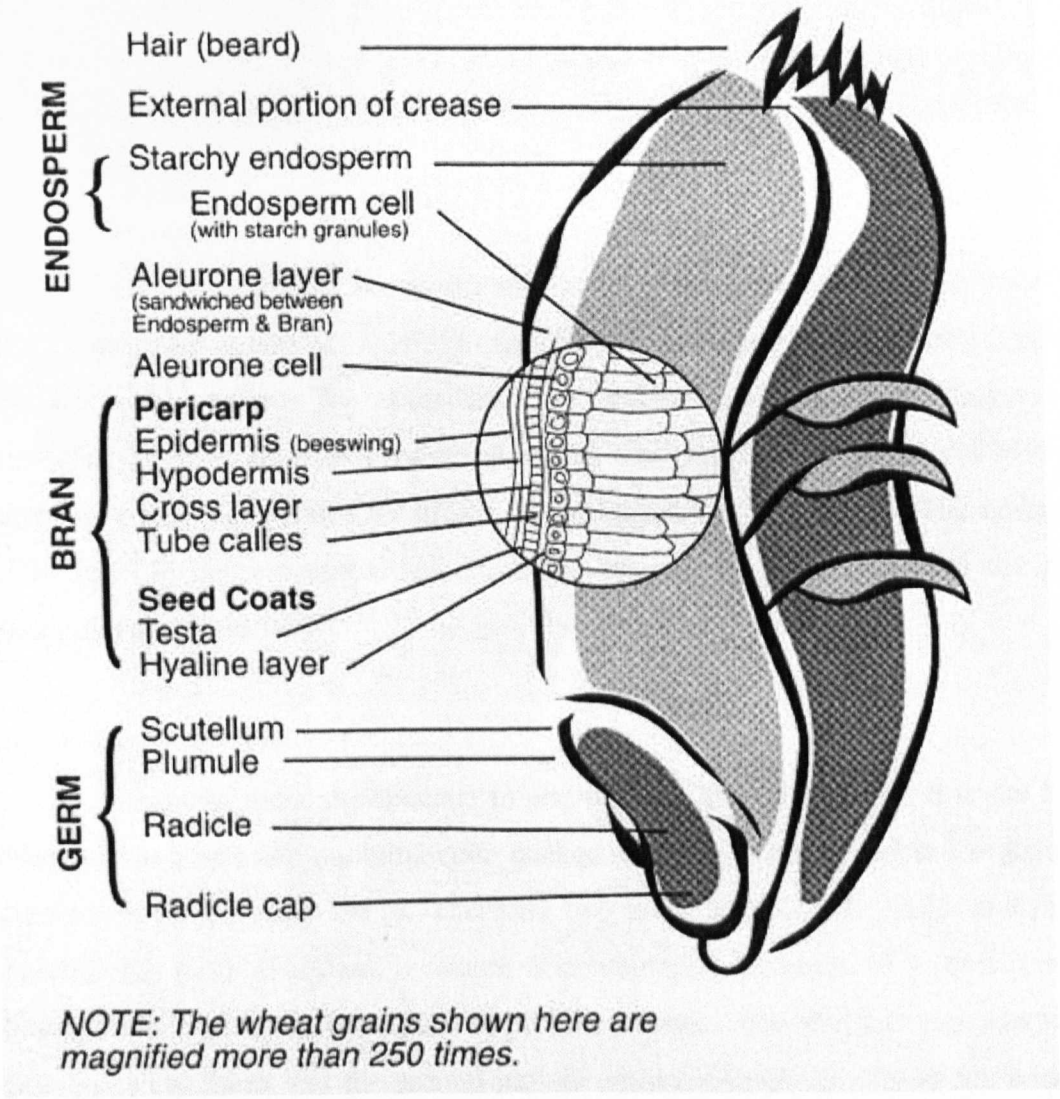


Figure 1.1 Structure of a mature wheat grain (Home Grown Cereals Authority 2007)

i. The Bran

The bran has a protective purpose and makes up approximately 0.14 of the kernel. The outer layer of the bran, the pericarp, is composed of many layers of cells and covers the whole seed. Some layers are incomplete, but during development serve to protect the embryo and endosperm (Evers *et al.* 1999). Cells of the pericarp have 42 chromosomes, all from maternal tissue (Flintham and Gale 1988). Pericarp cells are dry at maturity and are largely empty (Evers *et al.* 1999). Immediately underneath the pericarp is the seed coat, which joins with the pigment strand at the crease. The bran is rich in fibre and minerals (Atwell 2001). The bran also includes the aleurone layer, the outer layer of the endosperm. It is typically one cell thick and

covers the majority of the endosperm. The cells are roughly cubic (Evers *et al.* 1999). The aleurone cells are comprised mainly of cellulose and contain a large nucleus (MacMasters *et al.* 1971). Some would consider the aleurone to be part of the endosperm, although distinct from the *starchy* endosperm (Evers *et al.* 1999).

ii. The Germ

The germ contains the embryonic axis, or the potential root and shoot of a new plant (MacMasters *et al.* 1971). Attached to this is the scutellum which protects the embryonic axis. The scutellum also secretes hormones and enzymes and mediates the absorption of solubilised sugars and proteins (Evers *et al.* 1999). The germ may only comprise 0.03 of the whole kernel (Atwell 2001). The embryonic cells have 42 chromosomes, half from the original egg and half from the pollen (Flintham and Gale 1988).

iii. The Endosperm

Perhaps of most significance to end users is the endosperm. It is the largest tissue of the grain and contains cells packed with insoluble nutrients for growth of the embryo (Evers *et al.* 1999). There are two major components, starch and protein; starch being more abundant. Starch is contained in granules, in a protein matrix. There are thought to be two types of protein present. The first has cytoplasmic and membrane functions and the second include gluten precursors. These are important as they impact upon dough formation (MacMasters *et al.* 1971). The protein content increases moving from the centre of the endosperm outward (MacMasters *et al.* 1971). The cell size decreases toward the outside of the endosperm but the thickness of cell walls increases. The major cell wall component of wheat is arabinoxylan whereas in barley it is β-glucan (Evers *et al.* 1999). Unlike other tissues in the grain, the endosperm has 63 chromosomes, two sets from the maternal tissue, one from the paternal tissue (Flintham and Gale 1988).

1.1.4 Germination

Generally speaking, germination is a combination of physiological processes with in a seed that result in the initiation of metabolism and therefore growth (Bewley and Black 1994). Understanding the plant's life cycle and the mechanism of germination is vital for understanding some important quality issues.

Ripe grain imbibes water at a critical temperature, in an aerobic environment (Evers *et al.* 1999). Gibberellic acid (GA) is synthesised mainly in the scutellum and is associated with α-amylase production in the scutellar epithelium (Appleford and Lenton 1997). Abscisic acid (ABA) is involved in dormancy (prevention of germination) and it is detected in all grain tissues (Hilhorst 1995). Diffusion of GA from the embryo to the endosperm and the decline of ABA in the endosperm induces α-amylase gene expression (Appleford and Lenton 1997). α-Amylase and other enzymes are involved in the solubilisation of cell walls, proteins and starch which provides energy and substrates for the growth of roots and shoots of the new plant (Evers *et al.* 1999).

Resistance to premature germination (sprouting) is desirable and it is possible to select lines that genetically confer this resistance (Bassoi and Flintham 2005). Sprouting lowers starch and protein yield (Evers *et al.* 1999).

1.1.5 Chemical Composition

The chemical composition of wheat is known to be highly variable and related to variety and growing season. Some reported values for major components are shown in table 1.1.

Kim *et al.* (2003) investigated 18 Australian wheat varieties and besides the chemical composition, concluded that CP is inversely correlated to starch and soluble NSP. Acid Detergent Fibre (ADF) and lignin are negatively correlated to soluble Non Starch Polysaccharides (NSP) and free sugars are negatively correlated to soluble NSP.

There are varietal effects on the levels of protein, starch, NSP, insoluble NSP and fibre structure (Kim *et al.* 2003; Pirgozliev *et al.* 2003; Labuschagne *et al.* 2007). Starch amylose to amylopectin ratio is also influenced by cultivar (Labuschagne *et al.* 2007). Growing season is also responsible for variation in Crude Protein (CP), ADF, lignin, free sugars, starch, soluble NSP and the amylose to amylopectin ratio of starch (Kim *et al.* 2003). Starch content is reported to be related to AME (Pirgozliev *et al.* 2003; Huyghebaert and Schoner 1999). Varietal changes in CP and all fractions of NSP were also reported by Choct *et al.* (1999).

Table 1.1 Chemical composition of wheat

Component	Kim *et al.* (2003)[a]	Pirgozliev *et al.* (2003)[b]	Rose *et al.* (2001)[c]	Steenfeldt (2001)[d]
CP g/kg DM	98.1-191.0	85.0-151.0	118.0-132.0	112.0-127.0
Starch g/kg DM	585.0-737.0	594.0-732.0	629.0-662.0	658.0-722.0
Lipid g/kg DM	-	14.6-21.0	-	21.0-27.0
NSP g/kg DM	78.3-110.6	85.0-128.0	92.0-105.0	98.0-117.0
Insoluble NSP g/kg DM	68.9-101.2	66.0-85.0	68.0-87.0	73.0-94.0
Soluble NSP g/kg DM	7.0-14.1	15.0-49.0	17.0-26.0	10.0-29.0
Free sugars g/kg DM	11.2-22.7	1.7-5.5	-	-

[a]18 wheat samples, comprising 3 varieties grown in varying rainfall zones, over two seasons in Australia; measurements within one month of harvest
[b]23 wheat samples, comprising 19 varieties, grown over 3 seasons, in the UK; measurements at varying times.
[c]six wheat samples comprising six varieties, grown in one season in the UK
[d]16 wheat samples comprising 16 varieties, grown in one season in Denmark

Environmental growing conditions can affect wheat chemical composition. Location gives rise to variation in starch content. There is also an interaction with variety, with the same variety having varying starch content depending on the growth location (Labuschagne *et al.* 2007). Elevated temperatures during grain filling may decrease starch content. Amylase to amylopectin ratio may not be affected, or amylase may increase slightly and lipid may increase (Tester *et al.* 1991; Tester *et al.* 1995). The decrease in starch is probably related to a decrease in synthesis and the granules will be smaller and fewer in number (Tester *et al.* 1995).

i. Non Starch Polysaccharides (NSP)

Arabinoxylan, a polysaccharide of arabinose and xylose, is a major constituent of wheat cell walls (Annison 1993). Over the whole grain it can compose up to 0.57 of the polysaccharide content (Greffeuille *et al.* 2006). It is composed of a main β1-4 linked xylose chain, with α-arabinose groups at the O2 and O3 positions (Annison *et al.* 1991; Annison 1993). The structure is shown in figure 1.2.

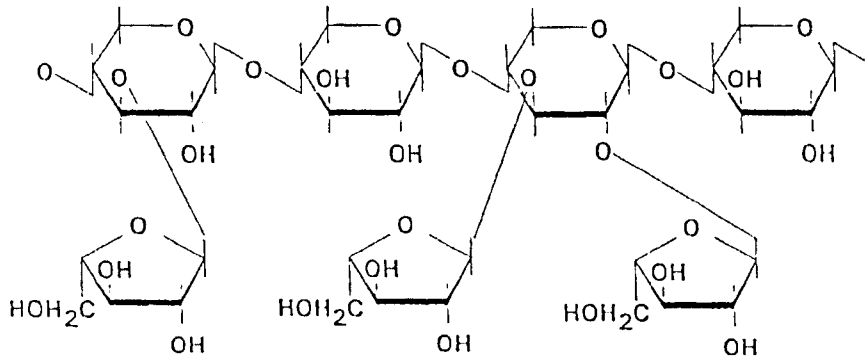


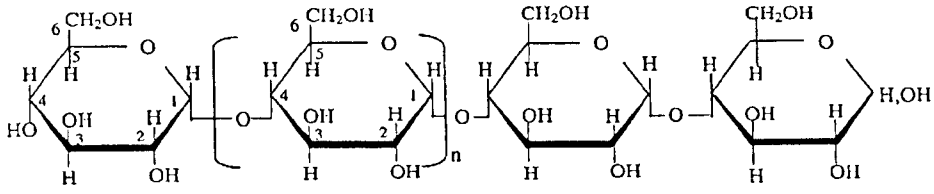
Figure 1.2 Structure of arabinoxylan. Modified from Annison (1993)

It is found that there are two fractions of NSP present in wheat, one that is water soluble and one that is not, but extractable in alkali (Annison *et al.* 1991).

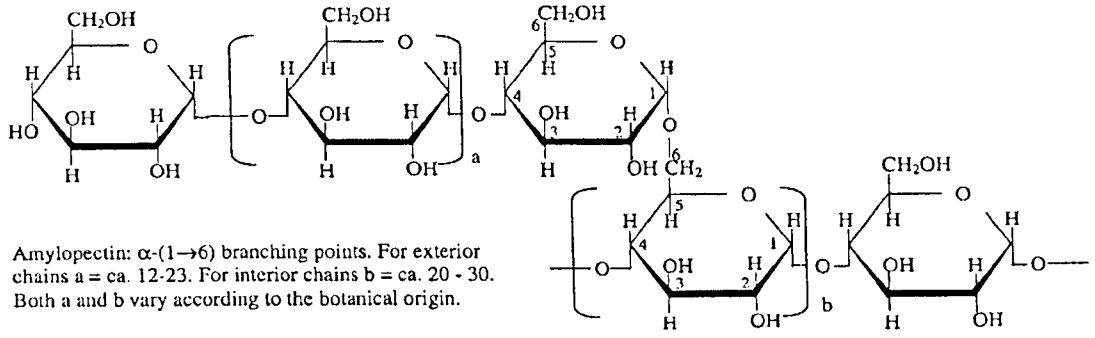
Over two consecutive seasons, the structure of arabinoxylan changed from being highly branched to less branched. The rate of synthesis of arabinoxylan was faster with higher growth temperature and greater available moisture during grain filling (Toole *et al.* 2007). Arabinoxylan content may be related to hardness, but composition of the molecule is similar irrespective of hardness (Greffeuille *et al.* 2006).

1.2 Starch

Based on a moisture content of around 140 g/kg, wheat contains around 640g carbohydrate/kg, 130g protein/kg and 20g lipid/kg (Holland *et al.* 1991). Aside from being the most abundant component of the kernel, starch is arguably one of the most important. Starch comprises, on average, 640g/kg whole wheat grain (Kent and Evers 1994) which may itself form 700g/kg of diets for broilers (Rose *et al.* 2001). It is energy-yielding and may be the sole dietary source (Moran 1982). Starch granules are present in many plant species and their physical characteristics reflect the origin. Starch is comprised of two alpha-glucans (see figure 1.3), amylose and amylopectin (Muralikrishna and Nirmala 2005) and the ratio of the two in starch granules will be characteristic of the source. Cereals can often be classified according to this ratio. For example, so called waxy starches contain less than 0.15 amylose and are therefore rich in amylopectin, whereas high amylose starches will contain >0.40 amylose (Moran 1982; Tester *et al.* 2004a).



Amylose: α-(1→4)-glucan; average n = ca. 1000. The linear molecule may carry a few occasional moderately long chains linked α-(1→6).



Amylopectin: α-(1→6) branching points. For exterior chains a = ca. 12-23. For interior chains b = ca. 20 - 30. Both a and b vary according to the botanical origin.

Figure 1.3 Structure of amylose and amylopectin. Modified from Tester *et al* (2004a)

1.2.1 Amylose and Amylopectin

Amylose is a largely linear molecule of (1-4) linked α-D-glucopyranosyl (glucose) units (Muralikrishna and Nirmala 2005). There may be some branching, resulting from α-(1-6) linkages. However, it is thought that these branches do not alter the behaviour of the molecule (Buleon *et al.* 1998). There are approximately a thousand glucose residues per molecule and the average molecular weight has been suggested to be $1x10^5$ (Buleon *et al.* 1998; Coultate 1999; Tester *et al.* 2004a). Amylose is insoluble in cold water as it mostly exists as a double helix. Each helix comprises two amylose chains, resistant to solubilisation through hydrogen bonding (Kodama *et al.* 1978).

Amylopectin is similar in that it is a polymer of α-D-glucopyranosyl residues linked with α(1-4) and α(1-6) bonds, however it is highly branched. It is a larger molecule than amylose, with a molecular weight often in excess of 1×10^8 (Buleon *et al.* 1998). Amylopectin is also involved in double-helical structures (Hedley *et al.* 2002).

The ratio of amylose to amylopectin can be determined using a lectin, Concanavalin A, which readily complexes with amylopectin. The initial starch level is calorimetrically measured after digestion to glucose using α-amylase and amyloglucosidase. Amylopectin can then be removed from an intact, equivalent

sample by addition of Concanavalin A and centrifugation. The remaining amylase is then digested and measured. The difference in the measurements account for amylopectin and therefore the ratio of the two components can be determined (Gibson *et al.* 1997).

1.2.2 Wheat Starch Granule Structure

Starch granule structure and size varies very much with botanical source (Moran 1982) and in the case of wheat, variety (Peron *et al.* 2007). Wheat starch is laid down in granules, thought to be of two types. Some are large and biconvex, type A, whereas others are smaller and spherical type B (Kent and Evers 1994; Peng *et al.* 1999; Langeveld *et al.* 2000). Type A are in the region of 12.5-35 μm in diameter whereas B type are around 2-10 μm (Soulaka and Morrison 1985; Burrell 2003; Tester *et al.* 2004a). The variation is most likely attributed to genetic differences although environment and growth location may play a part (Soulaka and Morrison 1985). It is thought that the larger, type A granules form first, with deposition initiated within 27 days. Deposition of small, type B, granules then occurs in amyloplasts containing the larger granules, at 40-53 days. The number of A type granules is set at initiation although the granules continue to grow. However, the number of B granules increases throughout development (Morrison and Gadan 1987; Morrison 1993). Type B granules are also thought to contain more lipid and probably have less amylose than type A granules (Soulaka and Morrison 1985). However, the opposite has also been reported for amylose content (Peng *et al.* 1999). The ratio of amylose and amylopectin varies with botanical origin, and the size of the granule (Moran 1982). In general the ratio is approximately 75:25 amylopectin to amylose, although certain genetic variants may be different.

In each wheat grain endosperm there is commonly an outer, vitreous portion and an inner floury portion (MacMasters *et al.* 1971). This may be related to the packing of the granules. In the vitreous area, there is close packing which may cause the granules to become misshapen, whereas in the floury area the granules appear in their natural form (Kent and Evers 1994). Temperature conditions during the grain filling period of wheat growth may affect properties of the starch. Higher temperatures may impair synthesis, particularly resulting in smaller granules and less starch in the endosperm overall (Tester *et al.* 1995).

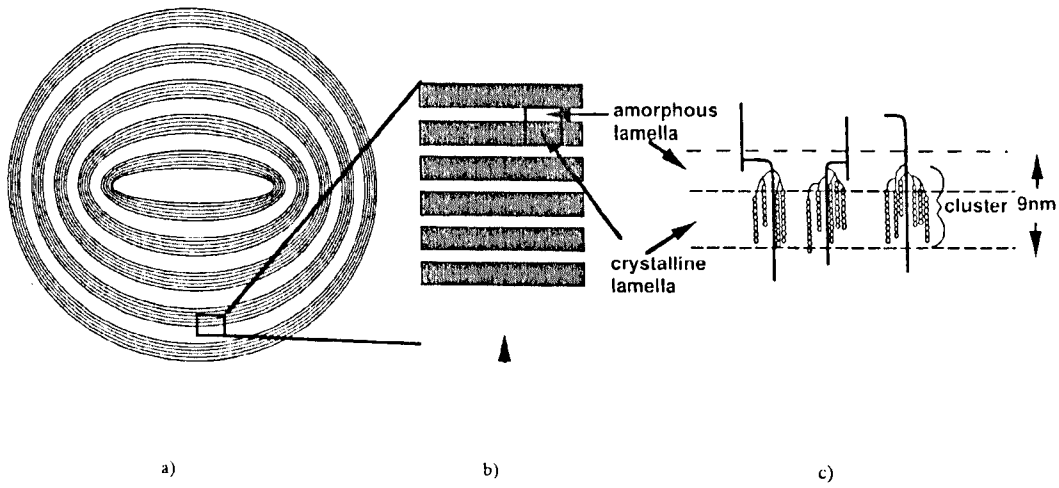


Figure 1.4 The semi crystalline structure of a starch granule. Modified from Donald *et al* (1997)

Starch has a semi-crystalline structure and is arranged in the granule in dark and light layers or lamellae (see figure 1.4, part a). These lamellae are laid down from the centre, toward the outer surface of the granules and are attributed to amylopectin and amylose chains (Robin *et al.* 1974). The dark lamellae are crystalline regions of (branched) amylopectin double helices and are interspersed by amorphous regions of amylose and the branch points of amylopectin, as illustrated in figure 1.4, b) and c) (Robin *et al.* 1974; Donald *et al.* 1997). Within these crystalline lamellae some amylopectin is in the form of a double helix and is not entirely crystalline (Gallant *et al.* 1997). The reason for this complex order is thought to be to minimise storage space and increase energy concentration (Tester *et al.* 2004a). Changes in these lamella and states of crystallisation, caused by processing, may affect gelatinisation and the extractability of amylose (Donovan *et al.* 1983).

Due to the crystalline structure native starch displays birefringence and a typical Maltese cross under polarised light (Gallant *et al.* 1997). Plane polarised light is able to pass through the granules (Moran 1982).

i. Lipid and Protein

Cereal starch granules also contain small but significant levels of lipid. It is thought however, that this is restricted to non-waxy cereals (Soulaka and Morrison 1985) and the lipid content may be between 6 and 15g/kg (on a dry matter basis) in wheat kernels (Buleon *et al.* 1998). Starch granules are thought to be covered in a lipid bilayer, about 50Å thick (Eliasson *et al.* 1981). Granule surface lipids are

commonly in the form of triglycerides whereas internal lipids tend to be in the form of lysophospholipids (LPL) with some free fatty acid (Soulaka and Morrison 1985; Tester *et al.* 2004a). The amount of these monoacyl lipids may be correlated to amylose content (Morrison *et al.* 1987; Morrison and Gadan 1987; Morrison 1993) although usually this is not evident due to environmental variation (Soulaka and Morrison 1985). LPL comprises nearly the whole lipid component of wheat (Soulaka and Morrison 1985) and some specify that lysophosphatidylcholine, palmitic and linolenic acids are the most common lipids found in wheat (Buleon *et al.* 1998). The total LPL is reported to be around 917mg/100g, and there may also be some free fatty acids, around 98mg/100g (Soulaka and Morrison 1985). Both amylose and LPL increase with maturity and larger granules show increasing amylose and LPL and you move outwards from the centre (Morrison and Gadan 1987). These monoacyl lipids can form inclusion complexes with amylose helices (Morrison 1993). Tester and Morrison (1990) suggest that although swelling is a property of amylopectin, formation of amylose-lipid complexes may hinder swelling. Therefore, there is a possibility that the presence of lipid could indirectly affect gelatinisation and therefore digestion. Amylose:lipid complexes may also decrease susceptibility to amylolysis (Holm *et al.* 1983). The review of Buleon *et al.* (1998) suggests that the presence of lipid decreases the viscosity of the resulting paste.

Similarly, small amounts of protein may be present on the surface of the granule, around 1.5-4.7mg/g. This may be in the form of gliadin or friabilin (Eliasson *et al.* 1981). This could be of importance as the granule bound proteins have been linked to grain hardness (Tester *et al.* 2004a). The mechanism for this seems unclear.

1.2.3 Changes in Wheat Starch with Storage

There has been much investigation into biochemical changes in wheat during storage, possibly due to the size of the bread making industry across the world. Some suggest that the nutritional quality is impaired as a result of storage, although this often refers to human nutrition (Rehman and Shah 1999). The requirements of individual industries are quite different. However, Posner and Deyoe (1986) suggest that storage at ambient temperature for up to 14 weeks is possible for bread wheat and Lukow *et al.* (1995) suggest up to 15 months is possible. It has even been

suggested that careful storage for up to 16 years maintains wheat for bread making (Pixton and Hill 1975). Rehman and Shah (1999) suggest that over a period of six months, pH, moisture content and amylase activity of the wheat decrease. This is particularly marked when temperatures of over 25°C are employed. Lukow *et al.* (1995) and Pixton and Hill (1975) found increases in maximum starch viscosity with storage which could be attributable to a decrease in amylase. However, it could also indicate an increased resistance of starch to amylase. Rehman and Shah (1999) found significant increases in insoluble amylose but a decrease in soluble amylose. However, there were no changes in total amylose. Rehman and Shah (1999) suggest that wheat is stored at less than 25°C, to avoid detrimental effects on nutritional quality. The potential changes in composition of cereals on storage are often considered alongside moisture content. Al-Yahya (2001) suggests that wheat is best stored at low moisture content, as moisture content is inversely related to the ability to later germinate. They do not discuss why this may be but presumably it is further evidence that with time, endogenous amylase activity falls.

Kim *et al.* (2003) found increases in soluble sugars over a period of six months but also a decrease in lignin, ADF and soluble NSP. They also found a small, but significant decrease in total starch. Jood *et al.* (2003) found similar changes in carbohydrates over a period of four months and Pixton and Hill (1975) found decreases in total sugar over eight years. All above mentioned changes in sugars may be brought about by the action of endogenous enzymes in the grain. They suggest that these changes may be beneficial to poultry.

1.2.4 Changes in Wheat Starch with Temperature

It is quite clear from the literature that the structure of cereal and other starches can be altered with thermal treatment. Often this is irreversible. These changes form the basis of several analytical methods such as Rapid Visco Analysis and Differential Scanning Calorimetry, to be discussed below. It is also clear that the extent of the temperatures used in treatments and the amount of water present, define the changes that occur.

The enzyme susceptibility of cereal starches and their solubility increases with heat/moisture treatment. This may be due to the formation of enzyme accessible regions on heating. The formation of holes on the surface of starch granules has been reported (Baldwin *et al.* 1994). The subsequent swelling power is

also reduced (Lorenz and Kulp 1982). These differences are as a result of changes in and some degradation of physical order.

When heated past a critical temperature in excess water, native starch granules begin to absorb water and swell. At temperatures of above 60°C, wheat starch begins to gelatinise (Kulp and Lorenz 1981; Walker *et al.* 1988; Allen *et al.* 1991). For maize starch the temperature may be around 66°C (Becker *et al.* 2001a). This process involves melting of the semi-crystalline structure, loss of birefringence and solubilisation and cannot happen if water is limiting (Tester *et al.* 2004b). Water permeates the granules as amylose and amylopectin chains lie perpendicular to the surface, and open regions exist. Initially, amorphous regions take up the majority of the water, whilst crystalline regions stay intact (Moran 1982; Gallant *et al.* 1997). Water aids gelatinisation penetrating between crystallites and pulling crystallites apart (Donovan 1979). It has also been described as water increasing the mobility of starch chains and aiding melting (Biliaderis *et al.* 1985). This corresponds with the beginning of the endothermic peak on the DSC curve (Gallant *et al.* 1997). It has been suggested that small crystallites melt more readily than larger crystallites (Biliaderis *et al.* 1985).

Eventually granules begin to rupture and amylose and some amylopectin leaches out (Loney and Meredith 1974). Amylose is leached more readily due to its greater solubility, but will not leach if it is complexed to lipid (Becker *et al.* 2001a). Swelling has been described as a property of amylopectin (Tester *et al.* 1991). This leaching and solubilisation can be visualised by Scanning Electron Microscopy (Gallant *et al.* 1997). Conde-Petit *et al.* (1998) found that at 80°C, durum wheat starch granules were swollen, but not disintegrated. At 95°C granules were still visible, but there was also solubilised material present. Amylose leaching can be visualised and determined by the Iodine Binding Capacity. The more leached amylose that is present, the higher the IBC (Conde-Petit *et al.* 1998). The melting of starch crystalline structure in excess water appears to occur in one phase (Liu and Shi 2006). It is concluded that high temperature drying of starch induces rearrangement of double helical structure of starch polymers (Zweifel *et al.* 2000).

If water is limiting (an intermediate level), melting occurs in two phases, evidenced using the DSC (Donovan 1979; Jang and Pyun 1996; Lui and Shi 2006). It appears that at intermediate water levels, the initial affect of heat and water is to

strip starch molecules from the surface of the granules. With further heat, water is re-distributed and complete melting occurs (Donovan 1979).

As water concentration further decreases, the gelatinisation temperature increases and the amount of energy necessary for this change in phase is increased (Donovan 1979). If water is only present in very low concentrations gelatinisation cannot occur, except when temperature and/or pressure is extremely high, as in the case of extrusion processes (Burt and Russell 1983). Burt and Russell (1983) found that samples of between 80 and 120g moisture/kg, that were heated to 120°C retained birefringence and were not swollen or disrupted. The starch of samples with a moisture content of 80g/kg was still intact at 232°C. With a moisture content of around 130g/kg, Iji *et al* (2003) found that the amylose levels decreased whilst the amylopectin content increased.

i. Protein

Protein associated with starch may also be affected by heat treatment. After treatment at 100°C, compared to a 20°C control, maize gluten content was unchanged. However, the starch yield was lower (fraction, not a definite amount) suggesting protein extractability was greater (Altay and Gunasekaran 2006). Mohammed *et al.* (2004) investigated temperature of just 30 or 50°C temperatures they expected to be too low for any starch changes. However, although no alteration was seen in the starch, major changes occurred in the protein fraction. They suggested that changes in secondary structure mean less water is absorbed by protein. This may be beneficial in terms of availability of water to starch. Amezcua and Parsons (2007) found that the amino acid content of corn was decreased on heating to 55°C or autoclaving, and that digestibility was also decreased. This was supported by research into chapati baking, during which lysine declines (Anjum *et al.* 2005). Maillard reactions are suggested to occur between reducing sugars and lysine (Amezcua and Parsons 2007).

1.2.5 Amylase

Amylases are hydrolases. That is, they specifically cleave glycosidic bonds in starch and are found in microbes, plants and animals, although the origin determines properties and mode of action. In cereals, they are very much related to germination (Greenwood and Milne 1968a; Marchylo *et al.* 1976). They can be further divided

into endoamylases, exoamylases and debranching enzymes. Endoamylases (from here on termed α-amylase) cleave α-1,4 glycosidic bonds in the middle of amylose and amylopectin molecules (Hill and MacGregor 1988; Muralikrishna and Nirmala 2005), therefore rapidly decreasing molecular weight and decreasing starch viscosity (Hill and MacGregor 1988). The initial stages of the hydrolysis involve random scission of this type of linkage within amylose molecules (Greenwood and Milne 1968b). α-amylases are single chain polypeptides, with molecular weights in the region of 41-54 kDa, depending on the isozyme (Marchylo *et al.* 1976). However, it is thought that isozymes are probably of similar sizes (Greenwood and Milne 1968a). Exoamylases (from here on termed β-amylases) however, cleave α-1,4 glycosidic bonds to release terminal maltose and glucose molecules from the non-reducing end of the starch molecule (Banks and Greenwood 1975). This results in much slower reduction in molecular weight. β-amylases may be removed from a solution also containing α-amylases by heating at 70°C with acetone (Greenwood and Milne 1968a). Glucoamylase and Pullulanase (the latter produced by the fungus *Aureobasidium pullulans*) attack α-1,6- linkages at branch points in amylose and amylopectin. These are therefore termed de-branching enzymes (Muralikrishna and Nirmala 2005).

The aleurone layer is likely to be the origin of amylase in wheat grains (Bewley and Black 1994; Evers *et al.* 1995). High levels of activity have also been found in the crease region, particularly in large grains. Evers *et al.* (1995) suggest that this is due to an excessive number of aleurone cells being produced in the crease region, during cell division. These particular cells do not then require the same stimulation as normal aleurone cells. The amount of amylase present in a wheat grain appears to be related to the size of the grain (Evers *et al.* 1995).

i. Amylase Isozymes

The number of isozymes present in a cereal depends on the cultivar, distribution in kernel and stage of development of the grain (Marchylo *et al.* 1980). There are two main groups of α-amylase that are separable on their isoelectric point (Sargeant and Walker 1978; Marchylo *et al.* 1980). Both groups are present throughout germination, although group II isoenzymes are also present at earlier stages in development (Sargeant and Walker 1978). Group I isozymes have a pI of

approximately between 6.0 and 6.5, within which there are four main components that appear on a zymogram (Marchylo *et al.* 1976; Sargeant and Walker 1978). They are the product of pre-harvest sprouting (Lunn *et al.* 2001b), but may also be produced with no visible sprouting (Evers *et al.* 1995). Group II isozymes, often termed pericarp amylases, are calcium dependant (Greenwood and Milne 1968a; Lunn *et al.* 2001b) and have a pI of closer to 4.5-5.11, and can be separated into three main components (Marchylo *et al.* 1976; Sargeant 1982). This type of amylase is degraded during development and will have almost totally disappeared at maturity (Lunn *et al.* 2001b). They make up around 0.60 of α-amylase activity in cereals (Muralikrishna and Nirmala 2005). Sargeant and Walker (1978) separated the two isozymes and investigated the adsorption of both types onto wheat starch. They concluded that for degradation to occur, the enzyme must be adsorbed onto the surface of the starch. Kruger and Marchylo (1985) disagree and suggest that adsorption is not necessarily a pre-requisite for degradation. Initially adsorption of α-amylase is greatest onto the surface of small granules, although with time, adsorption is equal on large and small granules (Marchylo *et al.* 1976). Small starch granules are preferentially degraded over large granules (Marchylo *et al.* 1976). Surface erosion seen by SEM, which Sargeant and Walker (1978) understood to indicate degradation, only occurred with group I isozymes. Following this observation, immature starch, that is starch from immature grains, was isolated. Adsorption of group I isozymes was constantly high. However, adsorption of group II began at a high level and tailed off to only 0.06 as the starch extract was from more mature grains (Sargeant and Walker 1978). The authors conclude that group I isoenzymes have a secondary action, after the initial degradation by group I isoenzymes. (Greenwood and Milne 1968a) suggest that the two enzyme isozymes have a similar mode of action.

Marchylo *et al.* (1980) studied α-amylase isoenzymes in immature wheat. They suggest that up to 22 α-amylase enzymes may be present. These isoenzymes can be characterised as group I, group II or group III isoenzymes and cultivar variation exists in all groups. During early stages of kernel development, the majority of α-amylase present is in the form of group I isoenzymes and is present mainly in the pericarp. Group III isoenzymes are suggested to be similar to those involved in germinating wheat and are predominantly present in the endosperm. It is important to point that although these terms are the same as those allocated by

authors and discussed above, it appears from the literature that the isoenzymes are different.

ii. Optimum Conditions

The optimum pH range for cereal α-amylase group I isozymes is narrower (5.5-5.7) than for group II isozymes (3.6–5.75) and at extreme pH values they may become irreversibly inactivated (Greenwood and Milne 1968a; Marchylo *et al.* 1976; Muralikrishna and Nirmala 2005). Similarly, cereal amylases have a small range of temperature optima (40-55°C) above which they are inactivated (Muralikrishna and Nirmala 2005). Group II isozymes have a much narrower optimum range. Greenwood and Milne (1968b) suggest that the temperature optimum for group II is in the range 47-49°C. Marchylo *et al.* (1976) found that after 30 minutes at 30°C, there was a rapid decrease in activity of purified group II α-amylase. However, group II isozymes, in the same time period, exposed to 60°C, only lost 10% of activity.

iii. Sprouting and Amylase Activity

Excess α-amylase activity is detrimental to baking quality (Kettlewell and Cashman 1997). A major cause of this phenomenon is pre-harvest sprouting, where ripe grain germinates whilst still on the plant in the field (Lunn *et al.* 2001b). There are thought to be four distinct routes of α-amylase accumulation. These mechanisms have, on the whole been well researched. As a result they have been repeatedly assigned different terms. For the sake of clarity, those used here are those given by Lunn *et al.* (2001b).

The first mechanism, Retained Pericarp α-Amylase Activity (RPAA), is not well recognised in the UK, but has been attributed to α-AMY-2 isozymes (Lunn *et al.* 2001b). Originally these were not thought to affect HFN scores. α-AMY-2 isozymes are less thermostable than α-AMY-1 isozymes, and therefore inactivated by the temperatures used in the HFN test. However, Lunn *et al.* (2001a) found that they may indeed prevail and therefore affect HFN scores. In the UK grains that are still green at harvest are often rejected as it is assumed that they still contain unacceptable levels of RPAA. However, Lunn *et al.* (2001b) carried out an experiment to relate green colour to RPAA and found that after green grain colour has disappeared, RPAA may still exist. However, the experiment only considered

one cultivar at two sites, on one year. As is to be discussed below, genotype may well affect amylase activity. The authors also suggest that RPAA may be the least significant of all the mechanisms discussed. Storage before use can also decrease α-AMY-2 activity (Lunn *et al.* 2001a).

The second mechanism appears much more complicated but well researched. It involves the deposition of α-AMY-1 in the endosperm cavity (see fig 1.1) (Lunn *et al.* 2001b). It has been termed Pre-Maturity α-Amylase Activity (PMAA) and is related to amylase synthesised in pre-ripe ungerminated grains (Flintham and Gale 1988). It is of significance as it is often a mechanism for increased amylase where no visible sprouting is reported (Joe *et al.* 2005). It may be affected by grain size. Evers *et al.* (1995) published observations that suggest larger grains contain more amylase, judged on their lower HFN scores. Large numbers of wheat samples grown between 1986 and 1993 were ranked in terms of HFN. Cultivars known to be larger than medium were consistently placed within the worst four scores. The same publication also presents evidence for the crease region of the grain containing a large proportion of PMAA. Genotype differences may also be responsible for PMAA (Bingham and Whitmore 1966; Gale *et al.* 1983). Grain drying rate may also play a part (Kettlewell and Cashman 1997). Gale *et al.* (1983) suggest that slow drying rate may be responsible for PMAA. Kettlewell and Cashman (1997) could not find a link between α-amylase activity or HFN and grain drying. However they do report an increase in potential evaporation with increasing HFN.

A major component of PMAA may be controlled by just one gene, which is probably expressed in the endosperm. High temperatures in the field during ripening may reduce this expression in certain varieties (Mrva and Mares 1996). However cold weather, rainfall and humidity have been suggested to encourage PMAA (Flintham and Gale 1988) and worsen HFN, which is indicative of increased amylase (Craven *et al.* 2007). However, Joe *et al.* (2005) suggest that moisture and a temperature peak in early development could also be a causative factor for PMAA. PMAA was the most frequent cause of α-amylase accumulation in a wide range of cultivars over three UK sites investigated by Lunn *et al* (2001b).

The third mechanism of α-amylase accumulation is not well recognised. What little literature that is available (Flintham and Gale 1988) suggests that it is related to germination in early development, at high moisture contents. It may be

related to extreme weather conditions and it has been termed Pre-Maturity Sprouting, PrMS. It may also be attributed to parasitic insect larvae.

The final mechanism, Post-Maturity Sprouting (PoMS), although similar to PrMS occurs after sprouting in mature grain, with moisture contents of less than 350g/kg (Lunn *et al.* 2001b). Although it falls second to PMAA in the frequency of causing α-amylase accumulation, Lunn *et al* (2001b) judge it to be the most important cause of reduced HFN in the UK. They suggest that PoMS encourages higher amylase activity than PMAA.

Grain dormancy (prevention of sprouting/germination) is controlled by a set of three genes, although many others are involved (Bassoi *et al.* 2006). These genes also control grain pigmentation. It has been shown that in red wheat varieties, such as those common in Brazil, red grain colour is a marker for sprouting resistance. For protection against pre-harvest sprouting, red coloured plants can be chosen, or better still, those that have the dominant 'R' gene (Bassoi and Flintham, 2005).

There is limited information in the literature on the effect of high endogenous amylase and the idea of sprouted wheat on poultry nutrition. Pirgozliev and Rose (2001) conducted a study using two varieties, Abbot and Equinox. Abbot was found to be 0.09 sprouted at the time of inclusion in the poultry diet. The experiment was primarily involved with storage, but found no difference in AME with between the cultivars and no storage/variety interaction (Pirgozliev and Rose 2001).

iv. Inactivation of Amylases

Amylases are inactivated by many metal ions such as those of aluminium, silver, copper, iron, mercury and lead (Greenwood and Milne 1968a; Muralikrishna and Nirmala 2005). It is also suggested that it is specifically the cations of these metals, since corresponding sodium and potassium salts had no effect (Greenwood and Milne 1968a). Since calcium ions are bound to cereal amylases, any chemical that will chelate calcium, such as EDTA (Greenwood and Milne 1968a) or EGTA, will act as an inhibitor. The lack of calcium ions will also lower the inactivation temperature, possibly the difference between 71.5 °C and 63°C (Hagenimana *et al.* 1994). Some organic acids will also inhibit amylase. Citric, ascorbic and oxalic acids are examples (Greenwood and Milne 1968a; Muralikrishna and Nirmala 2005). Barley amylases may be permanently inactivated by low concentrations of Ca^{2+} ions (Bush *et al.* 1989).

1.2.6 Rheology and Measurement of Amylase

There are many possible ways to assay for α-amylase. Iodine-based assays rely on the blue black colour formed by the association of iodine and starch. This colour is progressively lost as starch is degraded by amylase and can be measured calorimetrically. As starch is degraded by α-amylase, reducing sugars are released and can be complexed with 3,5-dinitrosalicylate to form nitroaminosalicylic acid, also measurable using calorimetry (Asp 1990).

Due to their commercial relevance and novel application in terms of poultry nutrition, this review concentrates on viscometric methods.

i. Hagberg Falling Number (HFN)

In the 1960s a simple method for measuring amylase activity in flour was devised by Hagberg (1960). This was later simplified to the HFN method (Hagberg 1961), which is still used today. Seven grams of flour and 25ml of water are stirred together in a test-tube of specific size and then immersed in boiling water to begin gelatinisation of the starch present in the sample. After a period of stirring, a weight is dropped at the top of the slurry and allowed to fall under its own weight. The time (in seconds) taken for the weight to fall 70mm is termed the HFN (Hagberg 1960; Hagberg 1961). The amount of sample used is debated. It is suggested that reducing the sample amount from 7g to 5g may increase reproducibility and decrease the coefficient of variation between replicate samples (Finney 2001).

When there is high amylase activity in a sample, gelatinisation occurs to a lesser extent, due to the degradation of starch polymers. Therefore, a low HFN is correlated with high amylase activity, as the passage of the weight through the sample is faster with less viscous slurry (Hagberg 1961; Lunn *et al.* 2001a; Johansson 2002). In the UK, wheat for bread making must have a score of over 250, and for biscuits, 180 or greater (Lunn *et al.* 2001a).

It seems there is no such standard set for wheat destined for animal feeding. The literature is unclear on the relationship between HFN and animal performance parameters. This is despite the rejection of low HFN wheat samples for food use which are instead used for animal feed purposes (Hetland *et al.* 2007). This group investigated two wheat varieties over two years, with varying HFN scores manufactured by steeping the wheat and allowing it to germinate over varying lengths of time or by delaying harvest. As expected, HFN decreased with increasing

time of germination or time in the field. The authors found no effect of HFN on FCR. They also failed to find any consistent effects on AME or starch digestibility. However, they found that as HFN began to decrease, AME was reduced. As HFN further declined, AME increased. This negative relationship was also reported by Svihus and Gullord (2002). The effect on starch digestibility was similar but non-significant. Rose *et al.* (2001) and Pirgozliev *et al.* (2003) also failed to find a link between HFN and performance parameters.

HFN (and therefore amylase) is also affected by genotype and environment (Johansson 2002). Low HFN is related to poor weather, with high rainfall. The opposite weather conditions, warm and bright often produce high quality, high HFN wheat (Johansson 2002).

The most interesting aspect of the report of Hetland *et al.* (2007) is the discussion of the reason behind this non-linear response. The authors suggest that amylase may not be entirely responsible for the change in HFN score. No change in starch was observed. They suggest that the initial reduction in AME may be as a result of increased digesta viscosity caused by an increase in soluble arabinoxylans. There is evidence that after steeping, levels of endoxylanase may be high. Initial degradation of NSP may result in increase soluble NSP, increasing digesta viscosity. With time, there may be more comprehensive degradation of NSP, leading to an increase in nutritional quality. In contrast, *insoluble* NSPs may also affect viscosity, but in other ways. They are suggested to immobilise water in a system and therefore prevent gelatinisation. The effect is concentration dependant (Tester and Sommerville 2003).

It is also suggested that HFN is affected by gluten and gliadin, that with increasing content, HFN decreases. Therefore application of nitrogen, that increases protein levels, may affect HFN (Johansson 2002).

ii. The Rapid Visco-Analyser (RVA)

As discussed above the Hagberg Falling Number test has long been used to give a qualitative assessment of amylase activity in wheat flour. It is commonly used as a screening tool when deciding flour quality. The HFN test was developed in the 1960s. In the 1980s the Rapid Visco-Analyser was developed in Australia to give a rapid, on farm measurement of flour quality. It was originally devised in response to widespread weather damage in Australian wheat crops, with particular reference to

the baking industry. The method out surpassed the Brabender visco-amylograph, due to the speed of analysis and small sample size needed for RVA analysis (Allen *et al.* 1991). The RVA is thought to give very comparable results (Deffenbaugh and Walker 1989). Originally, the RVA was designed to be a quick, 3 minute screening process but was later expanded upon to enable precise measurements (Ross *et al.* 1987). The method can also be employed to give a qualitative measure of amylase activity (Collado and Corke 1999).

As shown in figure 1.5, a typical starch pasting curve indicates how the RVA monitors the changes in starch properties with heat and moisture. Typically, when analysing a starch sample using the RVA, a heat-hold-cool program is employed. The various characteristics of that curve are dependant on the starch sample and will vary with any treatments imposed to the starch before the RVA test (Becker *et al.* 2001a). In wheat it is suggested that the large A-type granules swell more readily than the small B-type granules (Conde-Petit *et al.* 1998). The RVA can give indication of modification made to starch granule structure (Becker *et al.* 2001a). At the beginning of the test, the temperature is usually set at below the gelatinisation temperature of starch so viscosity is low. Due to hydrogen bonding (Moran 1985) and the semi-crystalline nature of starch within the granules, native starch is insoluble in cold water (Becker *et al.* 2001a).

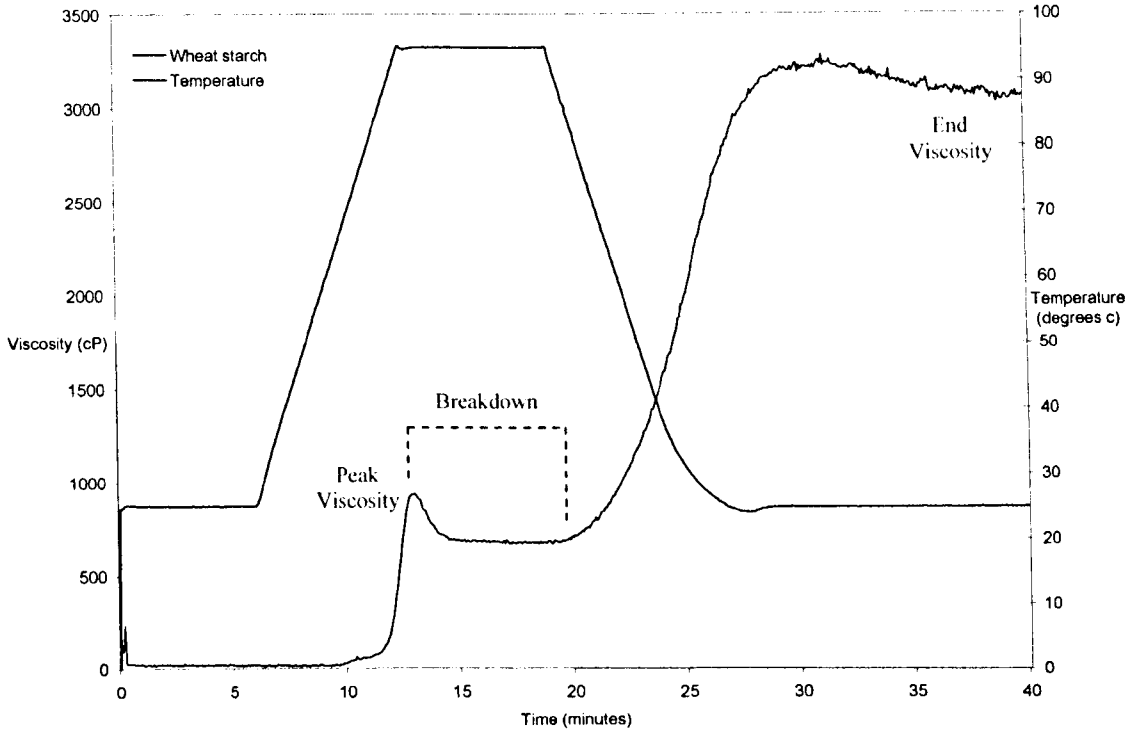


Figure 1.5 Typical RVA starch pasting profile

The RVA test exploits the behaviour of starch in excess water as already discussed. At temperatures of above 60°C, wheat starch begins to gelatinise (Kulp and Lorenz 1981; Walker *et al.* 1988; Allen *et al.* 1991). The starch melts, loses semi-crystalline structure and birefringence, and becomes soluble. Water is essential for this process (Tester *et al.* 2004b). Eventually granules begin to rupture and amylose and some amylopectin leaches out (Loney and Meredith 1974). Amylose is leached more readily due to its greater solubility, but will not leach if it is complexed with lipid (Becker *et al.* 2001b). The Peak Viscosity (PV) (figure 1.5) is reached when granule swelling and leaching are at equilibrium. This is usually past 90°C (Walker *et al.* 1988). PV is indicative of the water binding capacity of the starch (Newport Scientific 2001). The granules undergo further disruption, causing continuous leaching. Viscosity begins to drop. Often this can be attributed partly to endogenous amylase activity which has been brought into contact with its substrate. The rate and extent of the drop in viscosity (breakdown) is related to the temperature, degree of mixing and the nature of the material. As the mixture is then cooled starch molecules begin to re-associate and re-gelatinise leading to the end viscosity (Guler *et al.* 2002).

The RVA profile depends on a number of factors. The particle size of the flour is important. This is particularly true for damaged samples, such as those which have been extruded and have the characteristic of cold-swelling (Becker *et al.* 2001a). It is reported that not just the sieve fraction, but the type of mill that influence the pasting characteristics. With the same sized sieve, but different type of mill there maybe a 7% difference between pasting curves for the same sample (Becker *et al.* 2001a). Particle size is related to viscosity, the greater the particle size the greater the end viscosity (Becker *et al.* 2001a).

Free sugars that are present in the RVA slurry may also impact upon the pasting profile. Deffenbaugh and Walker (1989) investigated the effect of dextrose, sucrose and corn syrup solids (CSS) in ratios of 1:1, 2:1 or 4:1, sugar to starch, on the pasting characteristics of maize, wheat and tapioca starches. At low concentrations, peak viscosity increased in the cases of maize with dextrose, sucrose or CSS and for wheat with dextrose or sucrose. Sugar in water controls did not have an equivalent viscosity suggesting that in this case, sugars interact directly with the starch. It is suggested by that sugars can interact with starch chains to form sugar bridges between starch molecules in the amorphous area of the granule (Spies and Hoseney 1982). This may increase molecular weight and therefore viscosity. The larger the sugar, the more the starch will be stabilised. Sugars may also lower the water activity of the solution (Spies and Hoseney 1982). At higher sugar concentrations, and for all tapioca starches, sugars caused a decrease in peak viscosity. The effect of the sugar is greater, the greater its molecular weight. In all cases the pasting profile was shifted toward the right, indicating that the time to onset and peak viscosity increased (Deffenbaugh and Walker 1989).

Even in small amounts, endogenous flour α-amylase can have significant effect on pasting curves, causing variation in curves of the same flour sample. This may be alleviated by the addition of 1mM silver nitrate solution, a known inhibitor of α-amylase (Crosbie *et al.* 1999). Often unexpectedly low profiles can be attributed to high endogenous amylase within the flour (Collado and Corke 1999). Following on from these observations, it has been suggested that comparisons of RVA profiles using silver nitrate solution or water, can give an estimate of amylase activity in sweet potato flour (Collado and Corke 1999). The authors found a strong correlation ($r=0.96$, $p<0.001$) with the equation (PV2-PV1)/PV1, (where PV1 = peak viscosity

with water and PV2 = peak viscosity with 0.05mM silver nitrate solution) and the biochemical measurement of α-amylase.

Pasting properties such as peak viscosity, breakdown and pasting temperature are dependant upon hard and soft classification and variety (Wickramasinghe *et al.* 2005). Soft wheat varieties had lower pasting temperatures and increased peak viscosity. The swelling power of soft wheat varieties was also greater.

iii. Differential Scanning Calorimetry (DSC)

When starch gelatinises, the starch becomes disordered and crystallinity is lost, which is associated with an uptake of heat (Burt and Russell 1983). This peak in energy uptake can be measured using Differential Scanning Calorimetry (DSC). The operation of this equipment is discussed in chapter two. A typical DSC endotherm is shown in figure 1.6.

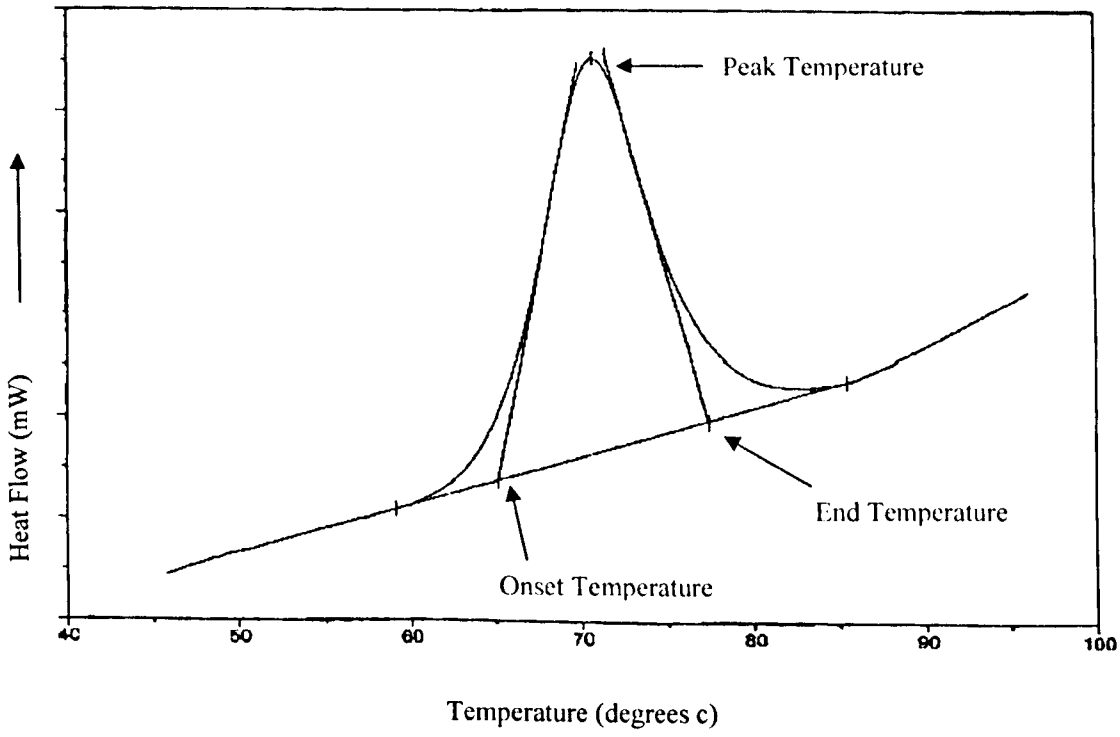


Figure 1.6 Typical DSC enthotherm, modified from Altay and Gunasekaran (2006)

Starch that has been heated in water is reported to gelatinise in two phases, the temperatures of which is dependant on the origin of the starch and extent of *previous* heat and moisture treatment. If excess water is used during the DSC,

melting of starch occurs over a very narrow temperature range and only one peak is observed (Biliaderis *et al.* 1980). The DSC heats a small sample, thoroughly mixed in excess water, at a certain heating rate. Typically this is 10°C per minute, increasing to temperatures necessary to gelatinise the starch. Gelatinisation requires heat and this is shown as a peak on a DSC endotherm (figure 1.6), the area under which is proportional to the enthalpy changes (Biliaderis *et al.* 1980). Gelatinisation enthalpy reflects crystallinity but also the loss of double helical order (Cooke and Gidley 1992). The gelatinisation temperatures (figure 1.6) are indicative of the extent of crystallinity. High temperatures represent a high degree of crystallinity (Ahmed and Lelievre 1978; Altay and Gunasekaran 2006). Interestingly, a high onset temperature is indicative of more perfect crystallites (Ji *et al.* 2004). Since gelatinisation cannot occur if crystallinity is already lost, samples that have already been heat treated and have melted, will exhibit no DSC endotherm peak.

Jang and Pyun (1996) investigated starches at various moisture contents using the DSC, that had not previously be heat treated. Their results indicate the behaviour of heat treated wheat starches at various moisture contents. They found that below 30°C no gelatinisation occurred. Between 30 and 90% moisture content, between one and four endotherms were displayed by the DSC, dependant on moisture. The first peak (G), the onset of which was routinely around 65°C, was attributed to water mediated gelatinisation of crystallites. The second (M1) was attributed to secondary crystallite melting, as described by Donovan (1979) and Lui and Shi (2006). The third, M2, appeared to be the melting of amylose:lipid complexes and the fourth (M3) the melting of amylose. Jang and Pyun (1996) found that at 300, 400 and 500 g moisture / kg, all four peaks were observed. However, at 600 and 700g moisture / kg, only G was observed indicating a singular crystalline melting phase, followed by M2. At 800 and 900 g moisture / kg the M3 endotherm was also observed. However, Altay and Gunasekaran (2006) suggested that two endotherm peaks may be explained by initial amylopectin double helical dissociation, followed by crystal melting, as opposed to two phases of melting.

In summary, the results of the DSC endotherm are indicative of the prior heat treatment of the starch. The program conditions for the DSC are important, and define the endotherm. Moisture content particularly must be kept constant between all analyses to avoid misinterpretation. For example, Altay and Gunasekaran (2006)

found that prior heat treatment of corn significantly affected the DSC endotherm at 500 or 900 but not 300 or 700 g moisture / kg .

1.3 The Chick

1.3.1 Anatomy

The avian digestive system is arranged in such a way that it is conducive to the ability to fly. The bulk of the weight is centralised in the body cavity (Klasing 1999). The regions that are of relevance to this project are outlined below.

i. The Beak, Oral Cavity and Pharynx

In avian species, the beak replaces mammalian lips and teeth. It comprises the bones of the upper and lower jaws covered by the rhamphotheca. The upper beak is then covered with a hard keratin layer (McLelland 1979; Klasing 1999). This is continuously lost and replaced with wear and tear (Klasing 1999). The oral cavity and pharynx are lined with a stratified squamous, or toughened, epithelium. Certain areas are keratinised particularly where there may be more abrasion. For example, the surface of the tongue (McLelland 1979). The tongue is attached to the floor of the oral cavity and the top surface meets with the palate when the beak is closed (McLelland 1979). Salivary glands are present in the walls of the oral cavity and pharynx and their development is related to the diet. In graniverous species the salivary glands are large compared to birds of prey for example (McLelland 1979). Often associated with the salivary glands are taste buds, although they are few. They are present on the floor of the mouth and pharynx and on the base of the tongue where the epithelia is soft and glandular (McLelland 1979; Klasing 1999). To compensate for the relatively few taste buds there are also many touch receptors on the beak, tongue and in the oral cavity (Klasing 1999).

ii. The Oesophagus and Crop

The oesophagus transports food from the pharynx to the stomach. It has longitudinal folds so is distensible and allows food to be swallowed whole (Klasing 1999). There are no sphincter muscles at either end of the oesophagus as in mammals (Denbow 2000). The crop is an expansion of the oesophagus and allows storage of food. It is placed such that it separates the upper, cervical region and the

lower, thoracic region of the oesophagus. Similarly to the oesophagus, there are longitudinal folds on the inner surface (Denbow 2000). Both the oesophagus and the crop are lined with semi-keratinised epithelia including many mucosal glands to lubricate the passage of food (Klasing 1999). These are more prolific in the thoracic region of the oesophagus than the cervical region (Denbow 2000).

The crop routinely carries *Lactobacillus* (Jozefiak *et al.* 2007). Work in the United States found the crop to contain *Salmonella, Campylobacter* and *Escherichia coli* (Smith and Berrang 2006). It has been shown that feed withdrawal, as is often the practice before slaughter, may increase the incidence of *Salmonella* in the crop (Ramirez *et al.* 1997). This could be due to proliferation of micro flora or possible ingestion of contaminated faeces through hunger (Ramirez *et al.* 1997). The crop contents weigh approximately 2.2g, measured when extracted from the carcass (Smith and Berrang 2006).

iii. The Stomach

The stomach is divided into two regions in the chicken, the proventriculus and the gizzard and is adapted for hard diets. The proventriculus is the region most analogous to the mammalian stomach (Denbow 2000) and is where digestion is initiated (Klasing 1999). Generally, it is the site of chemical digestion and secretion and is relatively small in chickens compared with carnivorous species. It is lined with a mucous membrane and papillae. There are two types of gland present; tubular, mucous secreting glands and gastric glands that secrete hydrochloric acid and pepsin (Klasing 1999; Denbow 2000). The gizzard is the site of mechanical digestion where the surface area of food is increased. It is also the site of action of secretions from the proventriculus (Klasing 1999). It is has a thick layer of smooth muscle (Kofuji and Inoue 2002) and is lined with koilin, a protective layer produced by the mucosal glands to prevent damage by acids, enzymes and hard materials (Denbow 2000). The cuticle layer is thickest under the biggest of the muscles that carry out the majority of the grinding action. Like the covering of the beak, it wears down and is continually replaced (Klasing 1999). In relation to the proventriculus, the gizzard is large and consists of two pairs of opposing circular muscles (Klasing 1999; Denbow 2000). Similarly to the crop, the gizzard contains bacteria, coliforms and *E.coli.* The population is much smaller in the gizzard than the crop because of the more hostile environment in the gizzard. For example, the pH is lower since

digesta has passed through the acid-secreting proventriculus (Smith and Berrang 2006). Feed withdrawal may have a similar effect on the gizzard as the crop, increasing the pH and therefore increasing the prevalence of *Salmonella* (Smith and Berrang 2006). The weight if the gizzard contents is around 8.4g (Smith and Berrang 2006).

iv. The Intestines

Although they are not easily differentiated, the small intestine may be divided into the duodenum, jejunum and ileum. Commonly, the Meckel's diverticulum or the vestigial yolk stalk is used to mark the jejunal-ileal junction (Denbow 2000). The intestine is surrounded by two layers of longitudinal muscle. This allows peristalsis and mixing of the digesta (Klasing 1999). In relation to carnivorous avian species the intestine is long in chickens. However, it is short in relative comparison to mammals. In chickens, villi are present that decrease in length from 1.5mm in the duodenum to 0.4-0.6mm in the ileum. In the first 10 days after hatching the number of villi decreases. Subsequently it remains constant. They are arranged in a zigzag pattern, which is believed to slow the flow of digesta (Denbow 2000).

v. The Pancreas

The pancreas is an important organ in the avian digestive tract and it lies alongside the duodenum. Digestive enzymes, similar to those produced in mammals, are produced in the tubulo-ascinar glands (Klasing 1999). These include amylase (approximately 289g/kg of enzymes produced), three chymotrypsins (200g/kg) and trypsinogen (100g/kg). Lipases, carboxypeptidases deoxyribonucleases, ribonucleases and elastases may also be present (Pubols 1991).

vii. The Caeca

In chickens the caeca are paired sacs lying alongside the small intestine and opening into at the ileo-rectal junction. In six week old birds, each caecum is around 90mm long (Ferrer *et al.* 1991). In chickens, the caeca can be divided into three regions (Ferrer *et al.* 1991). At the ileocaecal junction is the proximal portion, which composes about a third of the length. It has a smooth internal surface similar to that of the small intestine. Villi in this region are around 364μm in height with a density of 24 villi/mm^2. Mucous producing goblet cells are found in this area along with

filamentous bacteria on villi tips. In the medial region there are long folds or *plica circularis,* therefore the diameter is larger and more variable than the proximal region. In this area the villi are smaller but goblet cells are present. Again, the distal region has longitudinal but also transverse folds (Ferrer *et al.* 1991). Absorptive cells are present on the upper portions of the villi in all areas of the caeca and their structure is similar to that in the small intestine (Ferrer *et al.* 1991). There is thick musculature at the base region to prevent even small particles from entering.

The caeca is the site of microbial fermentation. Its removal slightly decreases metabolisability of diets and specifically, lower digestibility of crude fibre. It may also be involved in water and protein re-absorption (Chaplin 1989).

1.3.2 Digestion of Starch by the Chick

Nutritional quality of a feedstuff or diet for poultry is often described as a Coefficient of Apparent Digestibility (CAD) of starch and Apparent Metabolisable Energy (AME). CAD is a balance of the starch present in the diet and that which is present at various stages of the gut, or in the excreta. It refers to the amount of starch that is removed from the diet and absorbed. AME is the amount of energy from the foodstuff, available to the chick for metabolic processes, after faecal losses. The two parameters are found to be correlated (Mollah and Annison 1981; Rogel *et al.* 1987). It is debated whether or not AME is related to starch content (Rogel *et al.* 1987; Huyghebaert and Schoner 1999; Pirgozliev *et al.* 2003).

Although the rate of *in vitro* starch digestion of different wheat varieties may vary, the final coefficient of digestibility of cereal starches is high. The starch itself is highly digestible (Weurding *et al.* 2001). The changing nature of starch from source to source may affect digestibility (Moran 1982). Tuber and legume starches, for example, are more resistant to enzymic attack (Sugimoto 1980) and therefore are less well digested than cereals (Rogel *et al.* 1987; Weurding *et al.* 2001). Presumably this is due to the genetic differences in the structure of these starch granules. Cereal starches have A type granules, tubers B type and legumes C type (Weurding *et al.* 2001). Further to this, the starch of low-AME wheat varieties is well digested when extracted and fed to broilers (Rogel *et al.* 1987) suggesting problems with digestibility are not a result of the starch molecules *per se*. The protein matrix and the cell wall components are probably crucial in deciding digestibility. Weurding *et al.* (2001) found that even with well digested cereals, a small amount of starch

remains undigested by the time the digesta reaches the caeca. For cereals this may be just 0.02-0.06, and this is probably because this fraction is resistant and will not be digested in the caeca. For potato, as much as 0.67 may remain and may be substrate for microbes in the caeca (Weurding *et al.* 2001). Presumably the products of any digestion in the caeca are utilised by the microbes and are not wholly available to the chick.

The characteristics of the starch and probably the granule, are thought to be more important than total carbohydrate in deciding ME and starch digestibility. For example, the starch of waxy (high amylopectin) barley, is better digested that that of non waxy barley (Ravindran *et al.* 2007).

Chickens produce amylase in the pancreas and large amounts are present at hatching. They have the ability to digest starch immediately (Moran 1985). It has been suggested that there is no salivary amylase (Moran 1982). More recently it is reported that salivary amylase is produced but food does not remain in the mouth long enough for it to be active (Tester *et al.* 2004b). Amylase is the only enzyme endogenous to the chick that has a solely amylolytic function. It is released from the pancreas in response to starch concentration in the diet (Moran 1985). However, even after the pancreas is removed, starch digestion can still occur (Rogel *et al.* 1987). This suggests that other processes such as mechanical digestion in the gizzard and microbial fermentation in the caeca is important in starch digestion. The gizzard has a requirement for hard material, which aids the mechanical breakdown of foodstuffs. The inclusion of oat hulls in broiler diets has been found to improve *in vivo* starch digestibility (Rogel *et al.* 1987). The majority of cereal starch digestion occurs in the jejunum. For tuber and legume starches it is further through the digestive tract, or not at all (Moran 1985). Chick α-amylase is similar to that of porcine origin, and is a single chain amino acid, with three distinct domains and a crystalline structure (Moran 1982; Buisson *et al.* 1987). The most prominent form has a pH optimum of 7.5, a temperature optimum of 37°C and has a molecular weight of approximately 53,000 (Buonocore *et al.* 1977). As discussed above, α-amylase is dependant on calcium (Greenwood and Milne 1968b; Buisson *et al.* 1987; Bush *et al.* 1989; Lunn *et al.* 2001b), but also chloride ions (Moran 1982). Calcium is likely to bind to region near the active site of the enzyme (Buisson *et al.* 1987). Unlike calcium, chloride ions are not bound to the amylase molecule (Buonocore *et al.* 1977). In the first 48 hours post hatching, body weight and small intestine weight

increase, and this is correlated with increases in amylase but also trypsin and lipase (Sklan and Noy 2000).

In terms of initial attack on the starch granule itself, it is the less crystalline region of the granules that are attacked more readily (Gallant *et al.* 1997). Pancreatic α-amylase is readily adsorbed onto the surface of the starch granule (Ueda 1978). The rate of digestion is dependant on the surface area to mass ratio and the nature and crystallinity of the starch (Sugimoto 1980). Potato starches that are largely of B-type crystallinity require heating in water before they can be properly digested by the endogenous enzymes of the chick (Moran 1982). The botanical origin of the starch determines the mode of attack on the granule. With wheat, barley and rye, specific areas rapidly become pitted and then enlarge into canals (Gallant *et al.* 1997). During germination viewed by SEM, starch granules appear pitted, and may appear to have holes (Sugimoto 1980). Native, granular starch cannot be readily digested, and pre-treatment, and in the case of poultry, gizzard disruption is important (Tester *et al.* 2004b). Chick endogenous proteases may also aid starch digestion. In *in vitro* studies, commercial proteases improve the recovery of starch from cereal products (Perez-Carrillo and Serna-Saldivar 2006).

When amylose and amylopectin are digested by α-amylase, maltose/ maltotriose and other α-limit dextrins, respectively, are the first products. It is likely that the binding site of α-amylase involves two aspartic acid residues (Buisson *et al.* 1987). α-Amylase attaches randomly along the α1-4 chain of the starch molecule, and the following cleavage releases maltose. There are five sites on the amylase molecule for attachment to starch. The degree of amylopectin branching decides the speed at which it is hydrolysed (Moran 1982).

Hydrochloric acid is secreted in the proventriculus (Klasing 1999; Denbow 2000) and therefore acid digestion of starch cannot be excluded. Likewise to the mode of action of amylase, acid hydrolysis of the starch granule is dependant on crystallinity and size (Gallant *et al.* 1997). Amylose is involved in the resistance of starch granules to hydrolysis. High amylose mutants such as amylomaize are more resistant to attack then the corresponding starch with normal amylose levels.

The intestinal villi comprise enterocytes, the outer surface of which have microvilli. These secrete glycocalyx, a carbohydrate based surface coating. The microvilli trap water and mucin from goblet cells. Once diffused across this barrier, maltose, maltotriose and limit dextrins are hydrolysed by surface bound maltase and

sucrase isomaltase (Moran 1985). Glucose accumulates which builds up a concentration gradient from the lumen of the small intestine and the circulation. Sodium ions are required for absorption (Moran 1985; Sklan and Noy 2000). Although glucose can be absorbed in the duodenum and jejunum, the initial products of starch digestion cannot (Tester *et al.* 2004b).

1.3.3 Feeding Method and Feed Form

Usually, in commercial and experimental conditions, birds are fed on an *ad libitum* basis. The feed can be in the form of a mash or pellet, or a compound feed often with added components such as cereal or soya. An alternative to feeding all necessary components combined together is choice feeding (Forbes and Covasa 1995; Henuk and Dingle 2002). The distinct components can be provided in separate compartments or in one. Henuk and Dingle (2002) investigated providing components as separate entities, and that an energy source is necessary, such as a cereal; a protein source, such as soyabean meal, and vitamins and minerals, including specific calcium provision in the case of laying hens. This system allows birds to compose their own diets, allowing for environmental variation. It is accepted that birds can select for specific nutrients and minerals, especially if allowed a learning period (Forbes and Covasa 1995). The birds will spend time observing and playing with the food (Yo *et al.* 1998). After having been fed with a low protein diet, subsequent selection of either high or low protein diets is mainly of high protein, and visa versa, and balanced intake with requirement (Forbes and Shariatmadari 1994). The birds needed to actually eat the food, crop feeding did not have the same effect. The appearance and colour of the diet made no difference to their selection, nor did the position of the diet in the cage (Forbes and Shariatmadari 1994). The ability of the birds to select their diet appeared not to affect feed intake, up to six weeks of age (Yo *et al.* 1998). There is also no effect on carcass quality, although growth performance may be affected (Yao *et al.* 2006).

It is suggested that birds may also be able to select for minerals, particularly phosphorus. Barkley *et al.* (2004) did not find such an effect, although after being deprived of phosphorus (P), chicks ate less of a low P diet than a P supplemented diet, although the amounts consumed were not significantly different from equal quantities of each diet. However, when having been deprived of dietary P, the birds chose to eat significantly less calcium (Ca) supplement. It was suggested that this is

because the birds had mobilised minerals from body stores, to compensate for lack of dietary P, and therefore had a surfeit of plasma Ca (Barkley *et al.* 2004).

Choice feeding such as this can reduce costs. Mixing and grinding can be avoided and where many breeds are present, with different requirements, several different compound feeds are not required. Perhaps this system is not so relevant in experimental, or extensive commercial production systems, where temperature, light and humidity are carefully controlled. Henuk and Dingle (2002) suggest that producers in the developing world could benefit. Perhaps more relevant is giving birds the choice between a compound feed and a cereal.

There is evidence that feeding finely ground grains may decrease bird performance (Preston *et al.* 2000; Wu and Ravindran 2004). Feed Intake, FCR and weight gain may all be improved by feeding whole wheat compared to ground (Plavnik *et al.* 2002). This may be one of the reasons for research into choice feeding, where grain may be offered whole. However there is evidence that feeding whole wheat, as part of a pelleted diet, is unsuitable during the starter phase (Jones and Taylor 2001). The feed intake and FCR were worst in the case of whole triticale or wheat, in young birds, compared to ground cereal. The use of commercial feed enzymes alleviated the negative effect of whole wheat. However, at age 42 days, there was no difference in the performance of bird fed whole or ground cereal (Jones and Taylor 2001). Gizzard weight also increased with whole cereal, presumably because it had to work harder (Jones and Taylor 2001; Rutkowski and Wiaz 2001; Taylor and Jones 2004; Wu and Ravindran 2004). However, although a lack of effect in terms of FCR and BWG, starch digestibility was not measured in either study. Increased activity of the gizzard has been associated with increased starch availability and therefore greater digestibility (Hetland *et al.* 2002). Whole wheat may increase digesta viscosity compared to ground wheat (Taylor and Jones 2004), so the effect of ground versus whole cereal is clearly complex. The studies presented by Jones and Taylor (2001) and Taylor and Jones (2004), use the test cereal (milled or not) at only 200g/kg, despite the total cereal component being as much as 675g/kg of the whole diet. It is possible that the lack of any consistent significant effects is related to the low inclusion rate. Plavnik *et al.* (2002) found that rate of inclusion of whole wheat affected gizzard size. However, Wu and Ravindran (2004) found that 100g/kg or 200g/kg of whole wheat decreases FI and FCR. However, the total wheat component of the diet was slightly different in each case. Obviously this has

economic benefit, as less cereal and less preparation is necessary. It seems that feeding whole wheat will increase gizzard size, but results are inconsistent as to whether this increases performance or not.

Feeding ground cereals mixed with water has been investigated in terms of potential improvement of performance. Yasar and Forbes (1999) found that BWG was increased by feeding wheat, oats or barley mixed with more than the same weight of water. However, FI was also increased but FCR was not improved. However, digesta viscosity was reduced and the authors suggest that this may increase the accessibility of nutrients by enzymes and acids.

1.4 Variation in Nutritional Value

1.4.1 Wheat Varieties

Although AME and starch digestibility are reported to be correlated, large variation is found between different wheat varieties (Rogel *et al.* 1987; Choct *et al.* 1999; Carre *et al.* 2002; Kim *et al.* 2004). Rose *et al.* (2001) did not comment on statistical difference between the compositions of different wheat samples. However, they did find variation in chick performance when those wheat samples were used to in chick bioassays. It is reported that cultivar effects BWG, FI, FCR and AME (Rose *et al.* 2001; Steenfeldt 2001) and that the year of harvest also effects BWG, FI and AME, the later in a cultivar dependant manner (Rose *et al.* 2001). Interestingly, hardness may also be negatively correlated to FCR and positively correlated to BWG and FI (Pirgozliev 2003; Rose *et al.* 2001). McCracken *et al.* (2001) however, did not find any dramatic differences between 12 varieties, in terms of BWG, FCR or AME including 6 with the 1B/1R translocation.

It has recently been suggested, that an alternative to attempting to increase the digestibility of a diet, is to select for birds that have improved digestibility (Smith and Pesti 1998; Mignon-Grasteau *et al.* 2004). Mignon-Grasteau *et al.* (2004) found that there was a high heritability for AME_n and that it was possible to select for AME_n without affecting body weight. In further studies, those birds that were selected for improved digestibility showed lower FCR and FI and increased digestibility (Peron *et al.* 2006). Age of birds must be considered too. Younger birds are more sensitive to NSPs in the diet, probably due to an immature gut microflora (Petersen *et al.* 1999).

The relationship of physical grain measurements and nutritional value has been investigated. It appears that bushel weight and thousand grain weight are not correlated with AME and coefficients of starch digestibility in the case of pigs and poultry (Wiseman 2000).

Growing region and season affects the Digestible Energy value of wheat (Scott *et al.* 1998). In some cases, varieties may perform differently in varying environments. High rainfall appeared to decrease digestibility (Kim *et al.* 2004).

1.4.2 Non Starch Polysaccharides

It is suggested that such polysaccharides, present in cereals, may exert an anti-nutritional effect when used in broiler diets (Annison 1993; Angkanaporn *et al.* 1994). The AME of wheat has been found to be negatively correlated with total wheat NSP as well as water soluble arabinoxylan (Annison 1991). Concurrently, AME is also positively correlated with FCR (Steenfeldt 2001). There may be a dose-dependant response, that the higher the level of soluble arabinoxylan, the lower the BWG and the higher the FCR (Jozefiak *et al.* 2007). There is further evidence to support this in reports of studies that added such polysaccharides to basal broiler diets. In experiments where large NSPs considered to be similar in structure to arabinoxylan were added to inherently low NSP diets, specific polysaccharides had the ability to decrease AME, starch digestibility and LWG. The fact that not all NSPs tested had any effect suggests that there is some specificity in the mode of action of arabinoxylans (Annison 1990). When an arabinoxylan-rich wheat extract was added to a sorghum basal diet, there was a significant decrease in AME, which was greater with increasing dose (Choct and Annison 1990). The same group added an arabinoxylan-rich wheat pentosan extract to a maize basal diet and found amino acid digestibility was decreased and endogenous losses from the chick were increased (Angkanaporn *et al.* 1994). In these experiments arabinoxylan extract is added back to a basal diet. It is not arabinoxylan *in situ* in the grain that is shown to exert an effect. It is possible that endogenous arabinoxylan would not behave in the same way. Studies using Differential Scanning Calorimetry found that purified wheat arabinoxylan added back to starch, did not effect gelatinisation properties (Gudmundsson *et al.* 1991). However, the amount used was only 10-20g/kg of starch. Studies using wheat with known arabinoxylan content have failed to link AME with total, soluble or insoluble arabinoxylan (Austin *et al.* 1999). However,

Annison (1991) did find similar reductions in AME with a variety of wheat samples, and that AME was negatively correlated to the NSP (mainly arabinoxylan) content of the wheat. Other non-cereal NSPs, such as guar gum, may also reduce nutritional parameters. The effects may be less detrimental by partially hydrolysing the guar gum (Furuse and Mabayo 1996). Viscosity was reduced and therefore passage rate was increased.

The use of commercial enzymes, specifically xylanase and β-glucanase can alleviate the problem and restore AME by increasing apparent starch and arabinoxylan digestibility (Annison 1992; Friesen *et al.* 1992). Cell wall polysaccharides are literally degraded and the more complex the combination of enzymes, the better the effect on protein digestibility and FCR (Meng *et al.* 2005).

As discussed above, AME can be negatively correlated with soluble arabinoxylan. It is thought that these soluble arabinoxylans may increase viscosity in aqueous solutions (Annison 1993; Jozefiak *et al.* 2007), although negative relationships of extract viscosity and arabinoxylan content have been reported (Austin *et al.* 1999). Kim *et al.* (2004) also found a positive correlation between total and insoluble xylose, a component of arabinoxylan, and Digestible Energy. If partially hydrolysed arabinoxylan is added to a basal diet, it does not decrease AME and starch digestibility in the same way that whole arabinoxylan may and did not increase viscosity (Choct and Annison 1992). Similarly, if an equivalent weight of the constituent sugars is added to a basal diet, they do not exert the negative effects (Choct and Annison 1992). Angkanaporn *et al.* (1994) suggest that soluble NSP may interact with the gut wall, interfering with peptide hormones, and that an increased viscosity physically interrupts digestion and absorption. Other non-cereal NSPs such as guar gum, also increase viscosity, and this may affect the synthesis of gut enzymes (Iji *et al.* 2001). Non Starch Polysaccharides may also promote detrimental fermentative organisms in the small intestine (Choct *et al.* 1996; Jozefiak *et al.* 2007). In particular this may impair lipid digestion. High fibre, arabinoxylan rich diets may also lead to fermentation the caeca and caecal hypertrophy (Jozefiak *et al.* 2007). Presumably this is due to increased levels of undigested, but fermentable, starch reaching the caeca.

The work of Austin *et al.* (1999) found negative relationships with arabinoxylan and extract viscosity, contrary to the bulk of the literature. This is interesting in the context of the current project because they speculate that this is due

to storage of six months. Endogenous glycanases may be active over this period. Although this may increase the solubility of arabinoxylan, it also decreases the molecular weight, which could make arabinoxylan less able to aggregate and increase intestinal viscosity. This suggestion is mirrored by George and McCracken (2003) who found with storage, *in vitro* viscosity (IVV) of wheat that is stored whole decreases. However, if it is milled prior to storage, it may increase. Their method of determining IVV involved digestion with pepsin, hydrochloric acid and pancreatin, and continuous vortex mixing. Presumably this is designed to mimic chemical and mechanical digestion within the gut. Viscosity was measured using a standard cone and plate viscometer.

It is also suggested that birds within one experiment will respond differently to NSPs and that AME is therefore a function of the bird as well as the wheat (Hughes and Choct 1997).

1.5 Literature Summary

Wheat has been described as the most important food crop in the world (Giroux and Morris 1998). Animal feed is the second largest use for cereals across the globe, and their use in animal diets is growing. The use of cereals is due primarily to the high starch content that can constitute around 0.60 the diet and is of high dietary energy concentration. In the UK, wheat is used primarily for producing bread and cakes, and a high quality of wheat is required. There are various intrinsic and extrinsic factors that may affect nutritional value, such as chemical composition and environmental factors during growing. Cereals can easily be damaged, leading them to be inappropriate for baked products. There are several mechanisms causing damage, many of them unavoidable.

Poor weather during growing, and particularly during grain ripening, can lead to elevated amylase levels, which can render the wheat useless for bread making. There appears to be a plethora of information on adverse moisture content and the resulting amylase level. However, how this relates to poultry nutrition seems unclear. Post harvest storage is also a factor which may affect nutritional value. The majority of the literature on this subject suggests that after a minimum of six months, there may be increases in nutritional value. This may be related to changes in polysaccharide composition with time.

It is clear from the literature that whether wheat grain hardness is related to nutritional quality is a contentious issue. Variety is also an important determinant of nutritional quality, perhaps for the same reasons as hardness. The classification of wheat samples used within the current project will be carefully considered, and where possible taken into account within statistical calculations.

Starch structure, and mechanisms of damage are well documented. Excess heat and moisture may irreversibly melt the crystalline structure of the starch. Moisture content is important. When water is limiting, (approximately 200g/kg), starch structure may remain intact at temperatures of up to 232°C (Burt and Russell 1983).

Nutritional quality can be measured in terms of whole wheat starch digestibility and Apparent Metabolisable Energy (AME) of the diet. These are two parameters that are reported to be correlated and reflect the resulting performance of the bird (Mignon-Grasteau *et al.* 2004). Both parameters involve the use of a chick bioassay. There are also methods of determining starch digestibility *in vitro,* which correlate well with *in vivo* measurements.

1.6 Aims and Objectives

Since nutritional quality is commonly quantified using chick bioassays, the initial experiments aim to validate the method of Short *et al.* (2000) and Amezcua and Parsons (2007). This method excludes a protein specific ingredient from the diet, to be replaced with highly digestible starch and glucose. It is hypothesised that this may influence chick endogenous starch digestibility.

Weather damage of wheat is an area of much research. Overall, the current project aims investigate moisture provision during ripening and how subsequent high temperature drying may affect nutritional value. Extremes of heat can be particularly detrimental to starch macro and molecular structure. The heat induced damage to wheat with intermediate or high moisture content is clearly defined. However, information on the effect of heat treatment on wheat that has a relatively low moisture content especially with reference to animal feed, is lacking. The current project aims to provide information in this area and to utilise Differential Scanning Calorimetry and light microscopy to suggest changes in starch structural order. This information will be of use to wheat growers and poultry producers alike. The conclusions should aid in making decisions on whether weather damaged wheat is

positively or negatively affected by subsequent high temperature heat treatment, and therefore how it can be best used in dietary formulation. Information may also be uncovered regarding the variation in quality of differing wheat varieties.

The Rapid Visco Analyser (RVA) can be considered as a method that gives a 'fingerprint' of the wheat in question, in terms of its potential swelling and gelatinisation behaviour. The ability of a wheat to swell, may therefore indicate its nutritional value. The current project aims to develop a method using the RVA to quantify amylase levels, which has previously not been applied in a poultry nutrition context. Potentially this method could further be employed to predict nutritional quality.

These aims and objectives can be summarised as the following research questions:

- Can experimental diets that exclude protein be used to quantify wheat nutritional value?
- Does extreme heat treatment during grain drying affect swelling ability and structural order of starch and is the effect linear with temperature?
- Are these effects related to moisture content prior to drying?
- After such heat treatment, is nutritional value affected?
- What are the initial changes, if any, in nutritional value of wheat on ambient storage?
- Do different wheat varieties with varying hardness scores have different nutritional values?
- Can the RVA be successfully used to *quantify* amylase in weather damaged wheat?
- Are RVA parameters useful in predicting nutritional value of wheat for poultry?

2.1 Materials

2.1.1 Wheat

Table 2.1 shows the three varieties of wheat that were used in trials, grown at various locations.

Table 2.1. Information regarding wheat used in trials (HGCA 2006).

Variety name	Description	Location	Trial
Claire	Soft, group three	Not known, provided by Nickerson (UK) Ltd*	1,2
Deben	Soft, group three	Sutton Bonington ^	4
Einstein	Hard, group two	Not known, provided by Nickerson (UK) Ltd*	1,2
		The John Innes Centre #	3

^ Sutton Bonington Campus, The University of Nottingham, Leicestershire
* Nickerson (UK) Ltd, Market Rasen, Lincolnshire.
# The John Innes Centre (Biotechnology and Biological Sciences Research Council) Colney, Norwich, Norfolk, UK

2.1.2 Chemicals

All chemicals and reagents were purchased from Fisher Scientific, Leicestershire, UK and were of laboratory reagent grade, unless otherwise stated.

2.1 Methods

2.2.1 Chick Biosassay

i. Bird Husbandry

One-day-old male Ross strain broilers were provided by PD Hook Hatcheries Ltd, Thirsk, UK. The bioassay ran for 27 days. For this period the birds were housed in specialist metabolism rooms where temperatures, light and ventilation could be carefully monitored. For the first five days chicks were housed four per cage. On day six they were re-caged into three per cage. On day 13, chicks were weighed and re-caged into pairs, each bird within a pair weighing within 10g of each other. The cages were wire bottomed, with provision for collection of excreta. All cages used were 37cm wide by 42cm tall by 30 cm deep and contained a roost. Chicks were fed Chick Starter Crumb (Dodson and Horrell Ltd, Northamptonshire,

UK) until day 19. At this point the birds began an adaptation period, where they were fed the assigned trial diet, on an *ad libitum* basis. The trial period then took place between days 23 and 27, a total of 96 hours. During this time, feed intake was measured and excreta collected. Chicks were provided with fresh water on an *ad libitum* basis at all times. When the chicks first arrived the temperature in the metabolism room was set at 35°C. The temperature was the reduced by one degree per day until 21°C was reached. This was maintained until the end of the trial period. The air in the metabolism room was continuously circulated and humidity monitored. The birds were kept under artificial light for 23 hours per day, with one hour of dark. The birds were culled on day 27 of the bioassay; by asphyxiation with carbon dioxide and cervical dislocation to confirm death. The weight of each carcass was recorded.

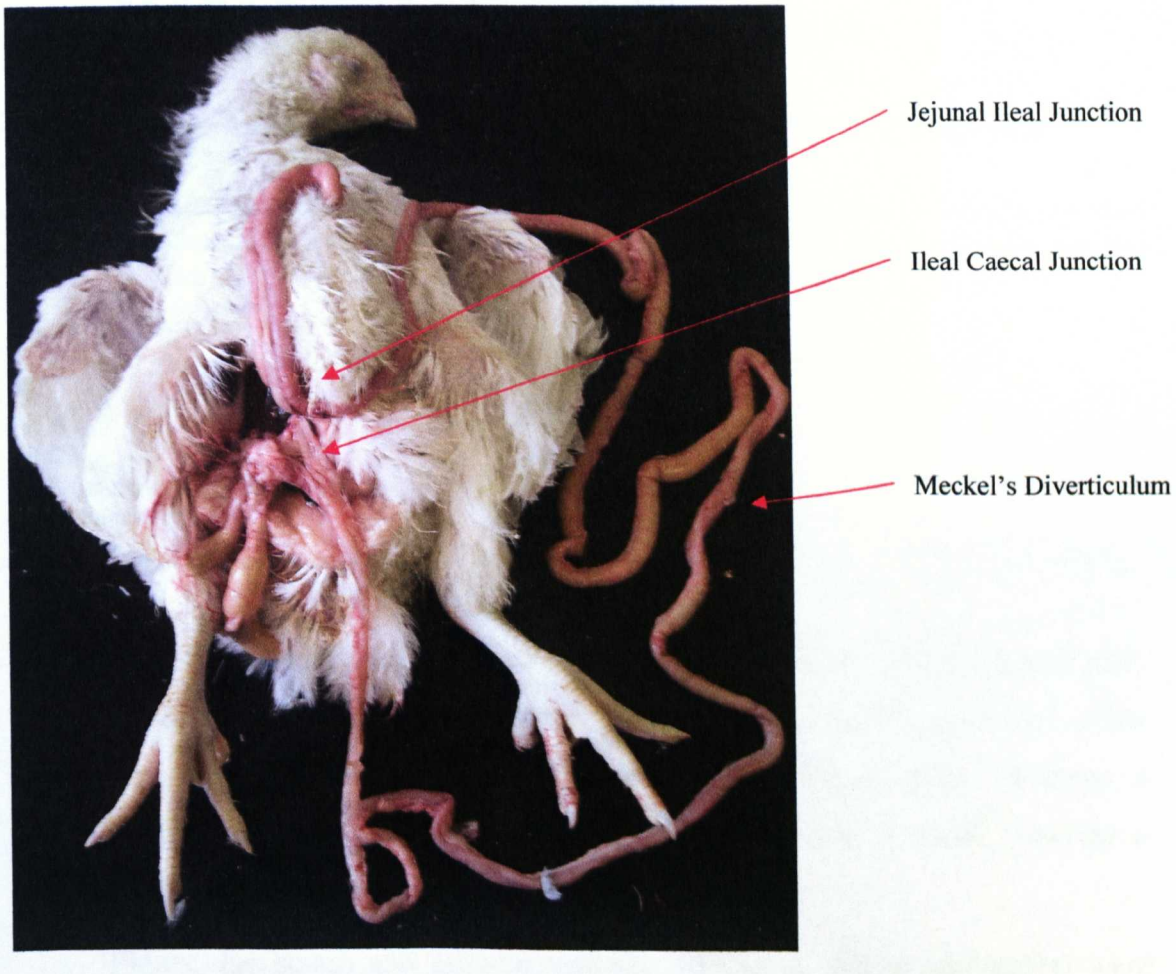


Figure 2.1 Dissection of a 27 day old Ross chick, showing the ileal regions that were removed.

The ileal region of the gut was dissected out and divided into two portions. The first of these comprised the duodenal-ileal junction to the Meckel's diverticulum and the second from the Meckel's Diverticulum to the ileal-caecal junction (see figure 2.1). Subsequently, these regions shall be referred to as the foregut and hindgut or in terms of digestibility specifically as duodenal (CDAD) and ileal (CIAD), respectively, although these terms may be considered to be anatomically incorrect.

All bird work was carried out in accordance with standard University ethical protocols.

ii. Diet Formulation

All diets were manufactured in the same way, on site at the University of Nottingham Sutton Bonington Campus. The specific components are shown in tables 2.3 and 2.4 and wheat variety variations are shown in table 2.1. The vitamin and mineral premix contained the components shown in table 2.2.

Table 2.2. The chemical analysis of Target Feeds Vitamin and Mineral Premix (information on analysis provided by Target Feeds).

Component	Concentration
Ash	640g/kg
P	100g/kg
Mg	16.6g.kg
Ca	152.0g/kg
Na	30.3g/kg
Vit A	150000 iu/kg
Vit D3	30000 iu/kg
Vit E (as alpha tocopherol acetate)	200 iu/kg
Cu (copper sulphate)	120 mg/kg
Se (Selenium BMP)	3.20 mg/kg

Wheat was ground using a Pulverisette 15 cutting mill (Fritsch GmbH, Idar-Oberstein, Germany) fitted with a 4mm screen. This type of mill has a similar action to a hammer mill, which is likely to be used in a commercial situation. However, a hammer mill is based on pivoting hammers that are rotated at speed, whereas a cutting mill has fixed blades in place of hammers.

Firstly, the starch and glucose mixture (or casein, where applicable) were mixed together with the ground wheat, titanium and vitamin and mineral premix. Once combined, the oil was added and the diet mixed on a slow speed, for 10 minutes. A commercial planetary mixer was used, similar to a Berkel PPM60

(Berkel Company, Indiana USA). All wheat was refrigerated prior to use (unless otherwise stated) and after manufacture, diets were stored at ambient temperature.

Table 2.3 Suppliers of Dietary components

Component	Supplier
Starch (purified from maize)	D F Dickens Ltd, Nottingham, UK
Glucose	D F Dickens Ltd, Nottingham, UK
Casein	Target Feeds Limited, Shropshire, UK
Soya Oil	Central Wool Growers, Northamptonshire, UK
Vitamin and Mineral Premix (see table 3.2)	Target Feeds Limited, Shropshire, UK
Titanium Dioxide	Fisher Scientific, Leicestershire, UK

Table 2.4 Basal Dietary Composition

Component	Amount (g/kg diet)
Wheat (see table 2.1)	750
Starch (from maize)	70
Glucose	70
Soya Oil	50
Vitamin and Mineral Premix	50
Titanium Dioxide	10

All diets contained the above ingredients, EXCEPT in trial one, where 140g/kg of casein directly replaced starch and glucose in diets under investigation (see chapter three, table 3.1)

2.2.2 Analyses

i. Gross Energy

Gross energy of diet and excreta samples was determined using an Adiabatic Bomb Calorimeter (Parr Instruments, Illinois, USA), using a standard protocol.

2.2.3 Starch Digestibility Determination

In the case of trials two, three and four (chapters five, six and seven respectively) starch digestibility was determined on a whole diet basis. Coefficient of apparent starch digestibility refers to digestibility of whole diet starch. Although purified maize starch (and glucose) was added to the diets it is assumed that their effect was equal across all diets, since the inclusion rate is the same and digestibility is high. However, in trial one (chapter four), starch and glucose were only added to control diets, they were replaced with casein in treatment groups. For this reason coefficient of apparent starch digestibility refers to the digestibility of wheat starch only. The amount of maize starch added to the control diets was accounted for in calculations of starch digestibility in the following way; since it is purified, maize

starch is assumed to be completely digestible, so the amount added to the control diets was subtracted from the measurement of total dietary starch.

i. Dry Matter Content

The dry matter content of all samples of wheat, diets, excreta and digesta were determined, to enable the results of all analysis to be given per weight of dry matter. For samples of wheat and diets, this was calculated from triplicate samples weighing 500mg that were dried at 100°c in a forced air convection oven. Samples are re-weighed until the dry weights were constant and the final figure used to calculate dry matter. Usually, 24 hours was sufficient. The dry matter content of excreta was determined in a similar way, although the whole sample was dried. The temperature used was 75°c to avoid cooking the sample and potentially damaging the starch content. For this reason and because of the high moisture content, it often took several days. Due to their small sample size and collection directly into plastic containers, digesta samples were frozen and then freeze dried when determining dry matter.

Other methods were investigated, for example an Infra-red balance was available. The sample was weighed, irradiated with infra-red radiation to remove water, and then re-weighed. It was found that, in comparison to the above method, the use of an infra-red balance gives unacceptably low results when measuring wheat moisture content. Presumably this is because the infra red radiation is insufficient to remove water from the middle of the grain, as it was sealed by the seed coat. A commercial hydrostatic moisture meter was also available. Although this gave results comparable to the method first mentioned above, it was thought inappropriate for excreta and digesta samples. Continuity of method used was considered important.

ii. Titanium Dioxide Determination

Titanium dioxide was added to diets as an inert marker to enable the calculation of starch digestibility. The concentration of titanium dioxide was determined in diets, digesta and excreta samples using the method described by Short *et al.* (1996). The amount of sample used for titanium dioxide analysis varied between different types of sample. The amount of diet used was approximately 1000mg, whereas digesta and excreta were 200mg and 100mg respectively. For

initial analysis of excreta, between 100 and 150mg were used but absorbencies were beyond the calibration so sample size was restricted to approximately 100mg. As the diet moves through the gut, the titanium dioxide concentration increases as dietary components are digested and absorbed, so a smaller sample size is necessary.

Samples were weighed into silica crucibles and ashed at 500°c overnight in a Carbolite CWF 1100 ashing furnace (Carbolite, Hope Valley, UK). The samples were then dissolved in 7.4M sulphuric acid, by heating to near boiling on a hotplate. The sample and crucible were then washed with distilled water through a Hardened Ashless 1100mm Circle filter paper (Whatman, Fisher Scientific Leicestershire, UK). Hydrogen peroxide (30% solution) was added to the sample which produced a yellow orange colour on reaction with the titanium dioxide. Several ions have been suggested for the production of this colour (Leone 1973; Short *et al.* 1996) and it is unclear the precise reaction involved. The absorbance of this colour at 410nm was measured using a Helios UV-Vis Spectrophotometer and SuperSipper (Thermo Fisher Scientific, Massachusetts, USA). A calibration curve was prepared using standards containing known concentrations of titanium dioxide. The slope (x coefficient) of the line is then used to calculate the concentration of titanium dioxide in the sample in the following equation.

$$\frac{\text{Absorbance}_{410nm} \times 100}{\text{X coefficient x Mass of sample (mg)}} = \text{mg } TiO_2\text{/mg sample}$$

iii. Total Starch

The Total Starch Assay Kit (Megazyme International, County Wicklow, Ireland) was used to determine starch content in wheat, diet, digesta and excreta samples. All were weighed to approximately 100mg and the weights carefully recorded. The starch was first solubilised by heating with thermostable α-amylase. In case of the presence of resistance starch the samples were pre-treated with Dimethyl Sulphoxide (DMSO) to ensure complete solubilisation. During this stage the starch was partially hydrolysed to starch dextrins by α-amylase. In the second stage these were broken down to glucose by amyloglucosidase. The absorbance at 510nm of the colour formed on reaction of glucose with Glucose Oxidase/Peroxidase

(GOPOD) reagent was measured using a Helios UV-Vis Spectrophotometer and SuperSipper (as above). The concentration of starch in the sample was then calculated taking into account the dry weight of sample, the absorbance and an adjustment for the conversion of free glucose to anhydro glucose.

2.2.4 Rheology

i. Rapid Visco Analyser

It was possible to estimate a relative measure of amylase activity using the Rapid Visco Analyser (RVA) (Newport Scientific Pty Ltd, New South Wales, Australia) and computer program used to integrate the data, Thermocline for Windows version 2 (Newport Scientific Pty Ltd). The necessary calculations and modification of the method were given by Collado and Corke (1999).

Initially, wheat flour samples were sieved before analysis on the RVA using an automatic sieve with a pan and 250μm screen (Endecotts Ltd, London, UK). Later, samples were not sieved and the development of this method is discussed in chapter eight. The following equation was used to calculate the actual amount of sample necessary for use in the RVA; 3g corrected for DM.

$$\text{Actual weight of sample (g)} = 3/(1-(mc/100)),$$

where mc= moisture content (%)

The sample was mixed with 25g of water immediately before the start of the test (Deffenbaugh and Walker 1989). The RVA was programmed as given in tables 2.5a and 2.5b. The programs were 35 or 40 minutes in duration and were therefore named Profile 35 and Profile 40 respectively. In early analysis of trial one samples, profile 35 was used. In later analysis of other trial samples, profile 40 was used. This development is discussed in chapter seven. After ten seconds of stirring at high speed the speed was reduced to a constant speed of 160 rpm. The temperature was initially 25°C but was raised to 95°C before returning to 25°C as indicated in tables 2.5a and 2.5b. The equipment used a paddle to stir the sample and water slurry at the set speed. The force necessary to measure this speed was converted to a viscosity in centipoise (cP) (Ross *et al.* 1987; Deffenbaugh and Walker 1989). The resulting

starch pasting profile allowed determination of a peak viscosity, breakdown and end viscosity, as indicated in figure 1.5 (chapter one). Breakdown is the difference between peak viscosity and the lowest estimated point in the viscosity trough. The test was then repeated using silver nitrate solution (5mM), a known alpha amylase inhibitor (Greenwood and Milne 1968a; Collado and Corke 1999). The concentration of solution used varied and is indicated and discussed in chapter seven. The difference between the peak viscosity for the experiment with (PV2) and without (PV1) the alpha amylase inhibitor can be used to calculate the relative amylase activity, using the following calculation (Collado and Corke, 1999).

$$\text{Relative Amylase Level} = (PV2-PV1)/PV1$$

All samples were analysed in duplicate, as in the protocol of Becker *et al.* (2001a). All samples were consistently milled using a bench top laboratory mill. The duration of milling per amount of sample was equal, and this method was employed since an appropriate mill with the ability to sieve fractions was not available. Inadequate amount of samples was available in all cases to sieve samples separately.

Table 2.5a RVA program details; Profile 35

Time	Speed (rpm)	Temperature (°C)
0.00.00	960	25
0.00.10	160	25
0.10.00	160	25
0.16.30	160	95
0.23.00	160	95
0.32.00	160	25
0.35.00	160	25

Table 2.5b RVA program details; Profile 40

Time	Speed (rpm)	Temperature (°C)
0.00.00	960	25
0.00.10	160	25
0.06.00	160	25
0.12.30	160	95
0.19.00	160	95
0.25.00	160	25
0.40.00	160	25

ii. Differential Scanning Calorimetry

Specific samples from trial three were analysed using Differential Scanning Calorimetry (DSC). The DSC used was a Perkin Elmer DSC 7 with associated

computer program, Pyris (all DSC equipment and consumables from Perkin Elmer, Massachusetts, USA). Approximately 3mg of sample (prepared as for the RVA) was mixed with three times the weight of distilled water. Samples were prepared the night before analysis and mixed overnight. Samples were held in Standard Aluminium Pans and sealed using a Standard Sample Pan Crimper Press. The DSC was programmed to heat the samples from 0°C to 100°C at a rate of 10°C/minute. The onset temperature, end temperature, peak height and the area under the curve, as shown in chapter one, were recorded. Samples were analysed in triplicate.

iii. Polarised Light Microscopy

The starch granules of certain samples from trial three that were analysed by DSC, were viewed using a Leitz Diaplan Triocular Compound Microscope (Leica Microsystems GmbH, Wetzlar, Germany). There was provision for normal light microscopy but also phase contrast microscopy, using a light polariser. Photographs were taken of the granules and are displayed in chapter seven. These were taken using a PixeLink Firewire Digital Camera v 3.2 and associated software (PixeLINK, Ottawa, Canada). Magnification of x400 was used for all samples and a graticule is shown alongside other photographs for scale. Slides were prepared by placing a drop of aqueous ethanol onto the slide using a Pasteur pipette. A spatula was stirred in the flour sample (prepared as above for RVA analysis) and then tapped onto the ethanol on the slide. A cover slip was placed on top.

2.2.5 Statistical Analysis

i. All Poultry Trial Data

All statistical analysis was carried out using Genstat v.9 (VSN International, UK) unless otherwise stated. Individual statistical models employed are described in the relevant chapters.

Data were analysed on a per cage basis, using pooled data for each pair of birds. In the case of Feed Intake (FI) values, data are given as a mean of diet replicates, over the 72 hour trial period. Data for cages which were highlighted on the final day of the trial period as having extremely wet excreta or where one chick had died within the balance period were excluded from statistical analysis. Figure 2.2a shows an example of excreta that would be considered normal. Figure 2.2b

shows and example of excreta that would be considered extremely poor, and where the cage would have been excluded from analysis. Only data that were highlighted independently of statistical analysis (ie not those data points which were highlighted by the statistical package as being outliers) were removed. In the case of trial three, described in chapter five, cages were given a score of one to five, one being extremely dry and acceptable and five being extremely poor and unacceptable. All cages with a score of five were excluded but also four cages that were highlighted as scoring four and also recognised by Genstat as being outliers. Table 2.6 shows where cages were removed from each trial.

Table 2.6 Information on diet and number of replicates removed from analysis. Where a diet or trial is not given, no replicates were removed.

Trial (Chapter)	Diet	Number removed
Two (four)	1	1
	3	2
	4	1
	6	1
	8	2
Three (five)	2	1
	6	1
Four (six)	1	2
	3	1

ii. Analysis of Trial Two (Chapter four)

In addition to the above analysis, the data from trial two (chapter four) was entered into a separate ANOVA with provision for polynomial regression analysis. Because of the sequential nature of the treatments, it was necessary to determine if there was a linear relationship between the potential effects of those treatments. In a polynomial ANOVA, the data for control treatments is excluded, since the comparison is between sequential treatments. This is further discussed within the relevant chapter.

The results of RVA analysis were entered into a general Analysis of Variance (ANOVA).

Figure 2.2a Excreta from a cage in trial three (chapter five) considered normal

Figure 2.2b Excreta from a cage in trial three (chapter five) considered to be extremely poor and therefore excluded from statistical analysis

iii. Analysis of Trial Three (Chapters five and seven)

In addition to the above analysis, the results of DSC analysis of two pairs of wheat samples were entered into two separate t-tests using Microsoft Excel Professional Edition, 2003 (Microsoft Corporation UK, Reading UK).

The results of RVA analysis were entered into a general Analysis of Variance (ANOVA).

3.1 Introduction

The accurate formulation of experimental diets is of vital importance during scientific animal trials. Since wheat was the ingredient under investigation during the current programme, it was important that it was included in the diets at as high a concentration as possible. This would ensure that any variability in nutritional value between samples of wheat would be identified. It had been previously shown at Nottingham that 750g/kg was an appropriate inclusion rate for the wheat component of the diet (Nichol 1999). Rates higher than this would risk copious wet excreta and poor starch digestion. This may be related to the observations of Carre *et al.* (2002) who found that increasing viscous polysaccharides, which would result from increasing wheat inclusion levels, increases water excretion in chickens. Wet litter is also an important welfare consideration. In the current diet formulation, oil was included to bind the diet, reduce dust and provide essential fatty acids. A commercial vitamin and mineral pre-mix was also included at the manufacturer's recommended rate. The remainder of the diet was made up of maize starch and glucose (Short *et al.* 2000; Amezcua and Parsons 2007). Both of these are assumed to be completely digestible. Following previous protocols, a specific protein ingredient was not included. It is thought that one of the main growth periods for chickens is 0-4 weeks (Figares *et al.* 1996). This is the age of birds used in the current trials. It has been suggested that poultry of up to 4 weeks of age have a dietary crude protein requirement of 188g/kg (Agricultural Research Council 1975). A recent report found that the crude protein contents of wheat range between 96.3 and 131.4g/kg on a dry matter basis (Anjum *et al.* 2005). Using these figures and a dry matter content of 850g/kg, previous diets could contain as little as 72.22 g CP/kg, assuming the wheat is the only protein-containing component.

As protein was not included in these previous diets, there was no need to make allowances for it in calculations. It is thought that, since it was planned to compare different wheat treatments within one trial, any problems caused by insufficient protein would be the same across each whole trial, and therefore could be

discounted in any comparison. One of the aims of the chick bioassay was to determine *in vivo* starch digestibility. This however does raise the possibility that insufficient dietary protein provision affects starch digestibility and that there could be an interaction between protein provision and wheat variety. The starch of two wheat varieties could be differently digested depending on whether or not sufficient protein was available. This could mean that results of the current programme would not necessarily be relevant in commercial situations, where diets are carefully balanced with adequate protein and amino acids. Although there may not be a direct effect on starch digestion itself, there could be a decrease in the efficiency of digestion. During previous preliminary trials, bloody excreta was occasionally observed, and this may have been some evidence of a potential problem with digestion. It is known that, in early posthatch development, feed intake initiates secretion of trypsin and amylase (Sklan and Noy 2000). It is unclear whether this is nutrient-specific or just a response to presence of diet in the tract. In later life, these enzymes are secreted in relation to feed intake (Sklan and Noy 2000). It could therefore be possible that an unbalanced diet, as with the situation of changing from the commercial starter diet to the trial diet, upsets the release of dietary enzymes. If this is the case, it raises the question as to whether the adaptation period to recover the secretion of appropriate enzymes is adequate.

Table 3.1 Modification of diets

Diet	**1**	**2**	**3**	**4**
Wheat Variety (750g/kg)	Einstein	Einstein	Clare	Clare
Casein (140g/kg)	-	√	-	√
Starch (70g/kg)	√	-	√	-
Glucose (70g/kg)	√	-	√	-
Vitamin and mineral pre-mix (50g/kg)	√	√	√	√
Soya Oil (50g/kg)	√	√	√	√
Titanium Dioxide (10g/kg)	√	√	√	√

To validate the proposed dietary formulation it was decided to run a preliminary trial. Two different wheat varieties were included in diets with or without a specific protein ingredient. The diets were formulated as per the standard,

shown in table 2.4 (chapter two), with variations shown in table 3.1. It was assumed that the starch and glucose in the control diets (diets one and three, table 3.1) are completely digestible and have a negligible, if any, effect on digestion. In the case of the diets with protein inclusion, the starch and glucose was directly replaced with casein. The level of 140 g/kg reflects the inclusion level suggested by McCracken and Stewart (2001). This rate also allowed the wheat component to remain unchanged. This was considered important to allow comparison to previous work. McCracken and Stewart (2001) also suggested that, if casein is used, arginine must also be supplemented in the diet. However, it was thought that in the current trial, this was not necessary since any potential changes in digestibility that were variety-dependant would still be seen, and starch digestibility was the main focus of the experiment.

Details of the materials and methods used are provided in chapter two. Six replicates were used of each treatment. Each replicate comprised of one cage, containing two birds.

3.2 Results

The statistical model employed was a 2 (wheat cultivar) x 2 (+/- protein) factorial (including x 3 (region of the digestive tract) when considering coefficient of digestibility).

The statistical analysis of the affects of protein inclusion on feed intake, AME and coefficient of starch digestibility is shown in tables 3.2, 3.3 and 3.4.

There was no effect of protein inclusion ($P=0.113$) or wheat variety ($P=0.510$) on the AME of the diets. There was also no significant interaction between wheat variety and protein inclusion ($P=0.128$).

There was no effect of wheat variety on the Coefficient of Apparent Digestibility of starch (CAD; $P=0.182$). There was, however, a significant interaction between variety and region of the gut on CAD ($P=0.026$). Variety Clare had a greater Coefficient of Duodenal Apparent Digestibility (CDAD). However, these differences can partially be attributed to the expected significant increase in CAD as digesta moves through the gut ($P<0.001$). There was no significant decrease in CAD with protein inclusion ($P=0.219$).

Table 3.2. Analysis of Variance showing the effect of protein (casein) inclusion on the feed intake of diets containing either wheat variety Clare or Einstein.

		Protein			ANOVA		
		+	-	Mean	Factor	sed	P
Wheat Variety	Clare	0.593	0.477	0.535	Wheat Variety	0.0398	0.288
	Einstein	0.606	0.377	0.491	Protein	0.0398	<0.001
	Mean	0.600	0.427		Wheat Variety x Protein	0.0563	0.207

Table 3.3. Analysis of Variance showing the effect of protein (casein) inclusion on the AME of diets containing either wheat variety Clare or Einstein .

		Protein			ANOVA		
		+	-	Mean	Factor	sed	P
Wheat Variety	Clare	14.85	13.17	14.01	Wheat Variety	0.488	0.510
	Einstein	14.34	14.35	14.34	Protein	0.488	0.113
	Mean	14.59	13.76		Wheat Variety x Protein	0.690	0.128

Table 3.4. Analysis of Variance showing the effect of protein (casein) inclusion on the coefficient of apparent digestibility of starch of diets containing either wheat variety Clare or Einstein.

		Clare				Einstein			
	Region	Duodenum[1]	Ileum[2]	Total Tract[3]	Mean	Duodenum[1]	Ileum[2]	Total Tract[3]	Mean
Protein	+	0.288	0.610	0.726	0.541	0.107	0.479	0.792	0.459
	-	0.171	0.783	0.788	0.581	0.046	0.634	0.928	0.536
	Mean	0.230	0.697	0.757		0.076	0.557	0.860	
	Mean	(variety)	0.561				0.498		

		Duodenum[1]	Ileum[2]	Total Tract[3]	Mean
Protein	+	0.197	0.545	0.759	0.500
	-	0.109	0.709	0.858	0.558
	Mean	0.153	0.627	0.808	

	ANOVA	
	P	sed
Protein	0.219	0.0449
Wheat Variety	0.182	0.0449
Region	<0.001	0.0508
Protein x Wheat Variety	0.706	0.0634
Protein x Region	0.047	0.0739
Wheat Variety x Region	0.026	0.0739
Protein x Wheat Variety x Region	0.892	0.1045

[1]'Duodenum' refers to measurements of Coefficient of Duodenal Apparent Starch Digestibility (CDAD) [2]'Ileum' refers to measurements of Coefficient of Ileal Apparent Starch Digestibility (CIAD) [3]'Total Tract' refers to measurements of Coefficient of Total Tract Apparent Starch Digestibility (CTTAD)

Importantly, there was no interaction between wheat variety and protein provision (P=0.706) suggesting that the response was not variety specific. There was an interaction of protein inclusion and region of the gut (P=0.047). Diets without protein were significantly better digested in the ileum. Feed intake was significantly increased with the inclusion of protein in the diet (P<0.001).

3.3 Discussion

There is very little information in the literature regarding the effect of the level of crude protein provision on starch digestibility, even less so specifically referring to poultry. What little information is available regarding non-ruminant species, specifically in pigs, is contradictory. One group suggest that the only significant difference with increasing protein is a decrease in protein digestibility (Min *et al.* 2001). Another suggests that dry matter digestibility improves with an increase in protein provision (Min *et al.* 2004). The current experiment is in agreement with the cited literature, that there is no substantial change in wheat starch digestibility.

In considering the results of the current experiment it has been assumed that the effects on protein digestibility are separate from any effects on starch digestibility. The literature also makes clear the importance of protein provision in the early days post hatch. It seems that whether or not the diet is nutritionally adequate, in the first week posthatch the yolk sac is sufficient for maturation of the gastrointestinal tract (Noy and Sklan 1999). This is of primary importance, as in the first four days post hatch, absorption of exogenous nutrients is poor due to the presence of the yolk and immaturity of the tract (Noy and Sklan 1999). After this period, adequate dietary protein is important for optimising BWG and carcass composition. The effects of varying amino acid provision are important to mention, and a key topic of discussion recently has been that of formulation of diets on the basis of ideal protein. This is the theory that the growth rate of the bird is dependant on the first limiting amino acid, in the case of poultry, Lysine. Amino acid requirements are therefore given in relation to Lysine (Wijtten *et al.* 2004).

Interestingly, feed intake is significantly increased when protein is included in the diet. This may be of interest to those formulating diets for investigations into starch digestibility. When samples of wheat to be used in such trials are in limited supply, feed intake is an important consideration. The number of bird replicates depends on the amount of diet that is available. In this case, as is common practice, birds are fed on an *ad libitum* basis. However, this is contrary to the findings of McCracken and Stewart (2001) who concluded that, with increasing protein, dry matter feed intake decreases, although, in this study with increasing protein, wheat inclusion decreases. In fact, the literature is generally contradictory to the current findings (Kidd *et al.* 2001; Smith *et al.* 1998; Smith and Pesti 1998; Sterling *et al.* 2005; Sterling *et al.* 2006). All reported that increasing protein inclusion decreases feed intake. It is well accepted than poultry have the ability to select appropriate diets, when given a choice, to ensure they are provided with sufficient protein (Forbes and Shariatmadari 1994). Presumably this is partly due to an inbuilt satiety mechanism (Nielsen 2004). The concept of ideal protein, as discussed above, was investigated by Wijtten et al. (2004) who found no significant change in feed intake with increasing ideal protein composition.

As discussed above, with protein inclusion, feed intake was significantly increased. At the same time, overall CAD was significantly decreased. This is in agreement with Svihus and Hetland (2001) who suggest that with increasing feed intake, starch digestibility decreases. However, Peron *et al.* (2005) suggest that with feed deprivation, starch digestibility actually decreases. However, both these studies are complicated by other factors such as feed form. However, subsequently, Svihus and Gullord (2002) suggest that AME is negatively correlated with feed intake.

It was concluded that a specific protein raw material could continue to be excluded from the diet. In fact, this may be beneficial as the current study suggests that this potentially increases the number of replicates possible with limited amounts of wheat.

Trial 2 - Varying Drying Temperatures of Two Wheat Varieties

4.1 Introduction

The current trial was conducted to determine if there were any effects of different drying conditions of the nutritional value of wheat with a view to building a picture of the effects of post harvest treatment on nutritional quality of wheat for poultry. If wheat is harvested after a damp period, it needs to be dried to approximately 140g moisture/kg for storage, to ensure quiescence (Bewley and Black 1994) and to prevent fungal development and potential mycotoxin production (Viera 2003). On the farm at the University of Nottingham, seed wheat is dried at 49°C for however long is necessary and wheat for bread flour is dried at 62°C. A continuous drier is used with a shallow bed setting. Temperatures given are an absolute maximum. These particular wheat categories are of high quality and care is taken when drying to ensure starch is not damaged, which may affect the quality of the end product. However, wheat for animal feed is often not treated so carefully and conditions may be harsh. At the University of Nottingham, wheat destined for the feed market may be dried quickly at temperatures as high as 100°C.

The current trial aimed to test different drying temperatures and durations, on the nutritional quality of wheat for poultry. To enable the transference of heat to the grain, it had to be soaked to reach an artificial moisture content, which may simulate the harvest of wheat after a period of rain for example.

Zarkadas and Wiseman (2001) found that, in the case of piglets, heat treatment during processing led to a significant deterioration in FCR, a negative nutritional effect in a programme that specifically investigated the process of micronization, a method of heat treatment. In the study, micronization temperature was a maximum of 200°C, although final exit temperatures were 85°C (low cook) or 110°C (high cook). Two trials were carried out that were identical, except that the second involved wheat that had been stored for 2 months. Piglets were fed diets that were identical except for heat treatment applied to wheat: low, standard or high cook micronization. In the first of the two trials, there was a significant deterioration in FCR with diets containing micronized wheat, relative to the control. The increase

was incremental: low cook<standard cook<high cook. However, the second trial, carried out in the same way, showed no such change. This potentially suggests that heat treatment decreases nutritional quality, with the higher the temperature the more severe the effect, but the effect may be ameliorated by storage. In both experiments, starch crystallinity, measured by x-ray crystallography, was decreased by heat treatment, compared to the control. The results in terms of animal performance were contrary to their findings in terms of starch structure. Gelatinisation was found to increase which would be expected to improve starch availability. However, reduced DM, starch and protein digestibility as a result of micronization have been reported, in ruminant species (Hristov *et al.* 1996) where the internal wheat temperature was reported as 90-100°C. Potential decreases in BWG have also been reported with micronization at 120°C (Niu *et al.* 1996). High temperature drying causes rearrangement of the molecular order of starch and re-orders the double helical structures (Zweifel *et al* 2000).

The third trial reported by Zarkadas and Wiseman (2001), investigated low and high cooked (micronized) wheat that had either been long (12 hours) or short-steeped (2 hours). Short steeping significantly improved BWG compared with long steeping (Zarkadas and Wiseman 2001). The maximum temperature investigated by Zarkadas and Wiseman (2001) was 200°C, the temperature of the burner in the micronizer. It has been found, with maximum micronization temperatures of 90°C, that DM, energy, starch and crude protein digestibility of barley for pigs aged 5-9 weeks, are all increased with micronization, both at an ileal and faecal level (Huang *et al.* 1997b). A similar response was obtained with wheat, although the maximum temperature used was not clear (Huang *et al.* 1997a). This is in agreement with the conclusion of Nui *et al* (1996). At a temperature of 80°C, there maybe a variety-dependant effect of heat treatment on FCR of wheat for poultry (Stewart *et al.* 1998). It appears that potential benefits of heat treatment, specifically micronization, depend on the temperature employed.

According to the literature, moisture content may also be an issue. Mazzuco *at al.* (2002) investigated feeding wheat samples that had been harvested at four moisture contents, (130, 160, 200 and 300 g/kg) and then dried at one of three temperatures (40, 70 and 100°C). The only significant improvement in AME was obtained in feeding wheat samples that had been harvested at 160g/kg and dried at 40°C. Wheat constituted approximately 540g/kg diet, which may have masked any

differences. Kulp and Lorenz (1981) also found a temperature-dependant effect of Heat Moisture (HM) treatment. They measured enzyme susceptibility of starch, an increase in which could be considered beneficial in terms of nutritional quality. It was suggested that a minimum of 210g moisture/kg is necessary for any increase in enzyme susceptibility. Presumably this is also temperature dependant and indicates a loss of crystallinity.

It is also thought that, on drying, starch granules decrease in size, due to a loss of water (Baldwin *et al.* 1994; Altay and Gunasekaran 2006). Granule size is related to enzymic degradation, with the rate of degradation of small granules being faster (Sugimoto 1980). However, if granule size is reduced on drying, this may have no impact on enzyme digestibility as all granules undergo swelling when hydrated. If crystallinity is strengthened with drying, this may affect digestibility independantly from a granule size effect.

From the cited studies it could be hypothesised that the drying temperatures that were imposed would have negative or little effect on nutritional quality. The temperatures investigated are in the same range as Zarkadas and Wiseman (2001), and Hristov *et al.* (1996) who reported a decrease in quality in at least one experiment. There is evidence of potential improvement with heat treatment (Huang *et al.* 1997b; Huang *et al.* 1997a). However, moisture content appears to be important (Mazzuco *et al.* 2002) and the effect may be incremental (Mazzuco *et al.* 2002; Kulp and Lorenz 1981) . The moisture contents of the wheat samples used in the current experiment had moisture contents similar to those investigated by Kulp and Lorenz (1981) prior to drying (See Appendix A).

However, since the current experiment employed two wheat varieties, there may be varietal differences displayed (Stewart *et al.* 1998). Furthermore, the two wheat varieties used are of different hardness ratings. Clare is classified by the HGCA as a soft wheat whilst Einstein is classified as a hard wheat (HGCA 2006). Hard wheat varieties have been reported to promote better bird performance than soft wheat varieties (Pirgozliev *et al.* 2002). However, in terms of apparent amino acid digestibility, soft wheat varieties may perform better. Short *et al.* (2000) investigated four near isogenic wheat lines that were thought to be genetically similar except in terms of endosperm texture and 1B/1R translocation. The 1B/1R translocation significantly decreased the amount of digestible amino acids, as did hard wheat varieties. However, only four wheat varieties were tested, and interestingly, although

six of the seven amino acids showed significant differences, the seventh, Lysine, did not.

In terms of starch digestibility, Peron *et al.* (2006, 2007) found that a soft wheat variety, with a low NIR score, was better digested than a hard wheat variety with a high NIR score. A tendency for a similar result was also seen by Wickramasinghe *et al.* (2005) and a significant negative correlation also reported by Carre *at al.* (2002). This may be due to more complete release of starch when soft wheat is milled (Short *et al.* 2000). With the use of hard wheat, gizzard weight is increased suggesting that it has to work harder to disrupt the wheat structure to release the starch (Peron *et al.* 2006). Other authors have failed to find any correlation between starch digestibility and hardness, in terms of a PSI score (Rogel *et al.* 1987).

As a soft wheat, it is likely that the starch of diets containing variety Clare will be better digested than Einstein (Short *et al.* 2000; Carre *et al.* 2002; Wickramasinghe *et al.* 2005; Peron *et al.* 2006; Peron *et al.* 2007)

The current experiment will represent harsh drying that occurs after wheat has become moist in the field, after maturation. It aims to investigate the effect that heat treatment at 100°C has on the wheat grain destined for feed use. However, this trial will not simulate a field situation whereby increased moisture during maturation may alter the chemical composition of the grain.

4.1.1 Preliminary experiments

Preliminary experiments were carried out to establish the soaking procedure and the subsequent drying time necessary to dry the wheat at each temperature. It was decided that the wheat should be contained in a water-permeable, woven plastic sack, in 8kg batches, and soaked in excess water at ambient temperature for two hours. After this time, the wheat had reached a constant weight and was assumed to have stopped absorbing water. It was then stored overnight to ensure the water equilibrated across the grain and was not just held in the outer layers. Absorption of water into the interior of the grain is slow, due to the boundary placed by the seed coat. It has been suggested that kernels that are simply washed and not allowed to sit

have a limited amount of water absorption into their centre (Becker 1960). The soaking protocols employed in the current trial probably best mirror a situation in the field where the environmental conditions, specifically rain over prolonged periods, are encountered. Storage was at 4°C to ensure that the grain did not begin to germinate which, amongst other changes, leads to an increase in alpha-amylase. It is suggested that, after two days at 5°C, only 0.05 of seeds will have germinated (Harrington 1923; Nyachiro *et al.* 2002).

Standard procedures for determining moisture content, that take at least 12 hours, could not be employed in the current trial. Therefore measures of changing moisture content could not be taken in real time. An infra-red balance was available which measured the weight of the sample, heated it with Infra Red radiation to drive off moisture, then re-weighed it to give a measure of moisture content. However, this was felt to be inadequate for reasons discussed in chapter two. Since wheat that had been soaked had a mean dry matter content of just 770g/kg, it was thought inappropriate to grind the wheat. This would encourage moisture loss (Becker *et al.* 2001a) and potentially damage equipment. Therefore moisture contents in both methods were taken using whole grain, which was subsequently dried overnight and reweighed. Small batches of wheat were soaked and refrigerated overnight as outlined above. They were then dried separately at the temperatures 70°C, 85°C and 100°C in a large forced air oven, on mesh bottomed metal trays. Sub samples were taken throughout and the moisture content was determined. This allowed an estimate of approximately how long was necessary to dry the wheat that was to be used to make the diets. However, only a small amount (approximately 1kg) was available to test dry the wheats whereas, due to time available, the diet wheat had to be dried in batches of 8kg. This meant that the wheats did not all attain exactly the same moisture content, although overall means were consistent. However, it was ensured that no wheat was wet for longer than 48 hours before drying and was stored at 4°C at all times. Diets were then made using the same method outlined in chapter two.

A comprehensive discussion of the materials and methods used in the experiment is presented in chapter two. For each treatment, six replicates were used. Each replicate comprised one cage containing two birds.

In terms of statistical analysis, a polynomial ANOVA was carried out in addition to a general ANOVA. The results of polynomial analysis are displayed in tables 4.2, 4.3a/b and 4.4. This ANOVA is designed to test for a linear (or quadratic) trend in treatments that follow a pattern, such as the incremental increases in temperature treatments in the current experiment. For this reason, the polynomial ANOVA excludes data from the control diets.

Diets were formulated as per table 2.4, chapter two, in addition to wheat at a rate of 750g/kg. Wheat samples underwent the treatments as shown in table 4.1.

Table 4.1 Treatment of wheat dietary components

Diet	Variety	Temperature	Duration (Hours)
1	Einstein	No Drying – Einstein Control	
2	Einstein	70	2
3	Einstein	85	1.5
4	Einstein	100	1
5	Clare	No Drying – Clare Control	
6	Clare	70	2
7	Clare	85	1.5
8	Clare	100	1

4.2 Results

The statistical analysis of the effects of wheat variety and drying temperature on AME, CAD and FI is shown in tables 4.2, 4.3 (a and b) and 4.4, respectively. The statistical model employed was a 2 (wheat cultivar) x 4 (temperature treatment) factorial (including x 3 (region of the digestive tract) when considering coefficient of apparent digestibility).

Polynomial ANOVAs (table 4.2, 4.3 a/b, 4.4) confirmed that there was no evidence of a linear effect of temperature on AME (P=0.804), Coefficient of Apparent Digestibility (P=0.976) or FI (P=0.584). There was a significant quadratic relationship between temperature and AME (P=0.017), and Coefficient of Apparent Digestibility (P=0.005). However, whether or not this is evidence of a quadratic relationship is debatable. When there is no evidence of a linear trend and there are only three treatments, the relationship, to some extent, must be quadratic.

The general ANOVAs, which include data on controls, shall be quoted in discussion below since polynomial analysis gave no evidence for a linear trend in temperature effects.

There was a significant effect of temperature (P=0.007) and region (P<0.001) on CDAD, CIAD and CTTAD (CAD) (table 4.3a). The effect of region is to be expected, since starch is increasingly well digested as it passes through the gut. The diet that contained wheat that had been dried at 85°C resulted in a decreased digestibility compared with the control, 70°C and 100°C treatments. Concurrently, there is also an interaction between region and temperature (P=0.002). Wheat that had been dried at 85°C was significantly less well digested in the duodenum, compared to the control, 70°C and 100°C treatments. This can further be described by the interaction between region, temperature and wheat variety (P=0.011). The CDAD in birds fed Einstein, dried at 85°C was significantly less than those fed Clare. However, it was the opposite in the control wheats.

There was also a significant effect of treatment on overall CAD (table 4.3b). Treatment can be described as any temperature treatment, and must be remembered that these wheat samples were also soaked prior to temperature treatment. Treated wheat had significantly lower CAD than control (non-treated) wheat (P=0.035). There was a significant interaction between treatment and region. The CDAD of treated wheat was significantly less than that of non-treated wheat (P<0.001) and this was in a variety dependant manner (P=0.026), with CDAD being significantly lower for wheat variety Clare than Einstein.

These differences in starch digestibility attributed to wheat dried at 85°C were not supported by a significant difference in AME (table 4.2b). However, there was a trend toward a decrease in AME with temperature (P=0.051). Wheat dried at 85°C had a numerically lower AME than the control, 70°C and 100°C wheat samples. There was no interaction with variety (P=0.344).

There was no significant difference in FI (table 4.4).

Table 4.2 Analysis of Variance showing the effect of drying temperature and treatment on the Apparent Metabolisable Energy (MJ/kg DM) of diets containing either wheat variety Clare or Einstein. Temperature refers to individual temperature treatments, whereas treatment refers to any temperature treatment compared to the control, non-temperature treated wheat.

Factor		Temperature					
		Control	70°C	85°C	100°C	Mean	(Polynomial) Mean
Wheat Variety	Clare	14.53	14.79	13.09	14.64	14.26	14.17
	Einstein	13.85	14.64	14.18	14.57	14.31	14.46
	Mean	14.19	14.72	13.63	14.60		

ANOVA		
Factor	P	sed
Wheat	0.887	0.330
Temperature	0.051	0.466
Wheat Variety x Temperature	0.344	0.659

		Treated v Non-Treated	
		+	-
Wheat Variety	Clare	14.71	14.53
	Einstein	14.46	13.85
	Mean	14.32	14.19

	P	sed
Treated v Non-treated	0.734	0.381
Treated vs Non-treated x Wheat Variety	0.212	0.466

Polynomial ANOVA

Factor	P	sed
Wheat	0.447	0.374
Temperature	0.052	0.459
(Linear)	0.804	
(Quadratic)	0.017	
Wheat Variety x Temperature	0.337	0.649
(Linear)	0.932	
(Quadratic)	0.146	

Table 4.3a Analysis of Variance showing the effect of temperature on the coefficient of apparent digestibility (of starch) of diets containing either Clare or Einstein.

Factor		Temperature					
Region	Wheat Variety	Control	70°C	85°C	100°C	Mean	Polynomial Mean
Duodenum[1]	Clare	0.462	0.371	0.266	0.398	0.374	0.345
	Einstein	0.658	0.475	0.001	0.498	0.408	0.325
Ileum[2]	Clare	0.738	0.728	0.724	0.743	0.733	0.732
	Einstein	0.869	0.822	0.756	0.781	0.807	0.786
Total Tract[3]	Clare	0.937	0.959	0.810	0.921	0.907	0.897
	Einstein	0.852	0.947	0.954	0.952	0.926	0.951
	Temperature Mean	0.753	0.717	0.585	0.716		

ANOVA	P	sed
Temperature	0.007	0.0444
Region x Wheat Variety	0.606	0.0449
Region x Temperature x Wheat Variety	0.011	0.0898

		Control	70°C	85°C	100°C	Mean	Polynomial Mean
Wheat Variety	Clare	0.712	0.686	0.600	0.687	0.671	0.658
	Einstein	0.793	0.748	0.571	0.744	0.714	0.687
Region	Duodenum[1]	0.560	0.423	0.134	0.448	0.391	0.335
	Ileum[2]	0.803	0.775	0.740	0.762	0.770	0.759
	Total Tract[3]	0.894	0.953	0.882	0.937	0.917	0.924

	P	sed
Wheat Variety	0.187	0.0314
Region	<0.001	0.0278
Region x Temperature	0.002	0.0635
Temperature x Wheat Variety	0.525	0.0628

Polynomial ANOVA

	P	sed
Temperature	0.019	0.0493
(Linear)	0.976	
(Quadratic)	0.005	
Region x Wheat Variety	0.422	0.0550
Region x Temperature x Wheat Variety	0.014	0.0952

	P	sed
Wheat Variety	0.468	0.0403
Region	<0.001	0.0324
Region x Temperature	0.003	0.0673
Temperature x Wheat Variety	0.593	0.0698
(Linear)	0.955	
(Quadratic)	0.005	

[1]'Duodenum' refers to measurements of Coefficient of Duodenal Apparent Digestibility (of starch) (CDAD) [2]'Ileum' refers to measurements of Coefficient of Ileal Apparent Digestibility (of starch) (CIAD) [3]'Total Tract' refers to measurements of Coefficient of Total Tract Apparent Digestibility (of starch) (CTTAD)

Table 4.3b Analysis of Variance showing the effect of treatment on the coefficient of apparent digestibility (of starch) of diets containing either Clare or Einstein. Treatment refers to any temperature treatment compared to the control, non-temperature treated wheat

		Treated v non-treated				ANOVA		
Region	**Wheat Variety**	**+**	**Mean (+)**	**-**	**Mean (-)**		**P**	**sed**
Duodenum[1]	**Clare**	0.345	0.335	0.462	0.560	**Treated v Non-treated**	0.035	0.363
	Einstein	0.325		0.658		**Treated v Non-treated x Region**	<0.001	0.0519
Ileum[2]	**Clare**	0.732	0.759	0.738	0.803	**Treated v Non-treated x Wheat Variety x Region**	0.026	0.0734
	Einstein	0.786		0.869				
Total Tract[3]	**Clare**	0.897	0.924	0.937	0.894			
	Einstein	0.951		0.852				
Wheat Variety	**Clare**	0.658		0.712		**Treated v Non-treated x Wheat Variety**	0.492	0.0513
	Einstein	0.688		0.793				
	Mean	0.673		0.753				

[1]'Duodenum' refers to measurements of Coefficient of Duodenal Apparent Digestibility (of starch) (CDAD)
[2]'Ileum' refers to measurements of Coefficient of Ileal Apparent Digestibility (of starch) (CIAD)
[3]'Total Tract' refers to measurements of Coefficient of Total Tract Apparent Digestibility (of starch) (CTTAD)

Table 4.4 Analysis of Variance showing the effect of drying temperature and treatment on the Feed Intake (kg) of diets containing either wheat variety Clare or Einstein. Temperature refers to individual temperature treatments, whereas treatment refers to any temperature treatment compared to the control, non-temperature treated wheat

Factor		Temperature					Polynomial Mean
		Control	70°C	85°C	100°C	Mean	
Wheat Variety	Clare	0.314	0.333	0.321	0.300	0.317	0.318
	Einstein	0.306	0.313	0.358	0.323	0.325	0.331
	Mean	0.310	0.323	0.339	0.312		

		Treated v Non-treated	
		+	-
Wheat Variety	Clare	0.318	0.314
	Einstein	0.331	0.306
	Mean	0.325	0.310

ANOVA

Factor	P	sed
Wheat Variety	0.591	0.0144
Temperature	0.403	0.0203
Wheat Variety x Temperature	0.346	0.0288
Treated v Non-treated	0.376	0.0166
Treated v Non-treated x Wheat Variety	0.535	0.0235

Polynomial ANOVA

Factor		P	sed
Wheat Variety		0.450	0.0169
Temperature		0.420	0.0207
	(Linear)	0.584	
	(Quadratic)	0.234	
Wheat Variety x Temperature		0.364	0.0293
	(Linear)	0.309	
	(Quadratic)	0.321	

4.3 Discussion

There appears not to be one single and conclusive effect of high drying temperature treatment on nutritional quality for poultry. Although an effect of treatment compared to control was seen, an incremental difference in nutritional value, with increasing temperature, was not seen as was reported in the literature (Zarkadas and Wiseman 2001). There was a decrease in digestibility of wheat treated at 85°C only and this can be attributed to poor digestibility within the duodenum (CDAD). Samples of wheat varieties Einstein and Clare used in the current experiment arrived from the grower with moisture contents of 137.1 and 145.5 g/kg, respectively. To enable them to be heat-treated without burning, they needed to be soaked. The process is outlined above and was identical for each equivalent variety batch. At least in terms of time, it was similar to the process used in the third experiment of Zarkadas and Wiseman (2001), which they termed short steeping. They found that short steeping improved the nutritional quality, (in terms of Daily Live Weight Gain, DLWG) of heat treated (micronized) grain in comparison to long steeped. Although DLWG was not measured in the current trial (that was designed specifically to examine digestibility), it is possible that the soaking process has in some way negated any negative effects on nutritional quality.

Lunn *et al.* (2001a) suggest that, with heating at just 55°C for 5 days, the amylase content of un-ripened wheat grains falls by 35-70%. However, the same decrease was seen with ambient air drying for the same time period so potentially this decrease in amylase is a factor of time, not solely temperature. This would be advantageous to the bread making industry where a low amylase activity is required (Lunn *et al.* 2001a). Presumably, the opposite, an increase in amylase would be beneficial when considering *in vivo* starch digestion in the chick. Specifically α-AMY-2 isozyme was measured in the study of Lunn *et al.* (2001a). At harvest the majority, if any, of α-amylase is present as the isozyme α-AMY-1, and it is known that group I isozymes are a lot less sensitive to heat than group II, which may maintain up to 90% of original activity after heating to 60°C (Marchylo *et al.* 1976). Since temperatures in the current experiment were a maximum of 100°C, storing and the heating the grain may have prevented any beneficial activity of amylase.

Lorenz and Kulp (1981) carried out a study investigating Heat/Moisture (HM) treatment and baking potential. They did not investigate amylase *per se* but

concluded that HM treatment decreased bread making potential. If considering bread making, this disagrees with Lunn *et al.* (2001a). Lorenz and Kulp (1981) investigated 100°C as opposed to 55°C in the experiments of Lunn *et al.* (2001a). The same group investigated the effect of HM treatment, specifically moisture contents of 180, 210, 240 or 270 g/kg and 100°C for 16 hours, on wheat starch (Kulp and Lorenz 1981). They found that with, increasing severity of treatment, starch solubility and enzyme susceptibility increased. This would presumably be of benefit in formulating diets for chicks. However, it appears that the increasing solubility and enzyme susceptibility are a factor of moisture content, not temperature which was constant (100°C) in each case, only moisture content varied. In the current experiment, moisture content was fairly constant. In samples that were subjected to drying, moisture varied between 226.40g/kg and 233.50g/kg, a much smaller range than investigated by Kulp and Lorenz (1981). However, the temperatures in the current study are within the same order as those of Kulp and Lorenz (1981). In the study of Kulp and Lorenz (1981), in all cases, HM did increase both parameters relative to the control, except, interestingly, 180g/kg moisture. At this moisture content, enzyme susceptibility was not improved relative to the control. From their study it could be concluded that heat affects enzyme susceptibility, in a moisture dependant way, with at least 210g/kg moisture necessary for the beneficial effect. The authors do not state the moisture content of the control, but it is implied that it is less than 180 g/kg and it was not heated in any way. Potentially beneficial effects of heat treatment due to the moisture content may have been observed.

However, the significant effect seen in the current experiment was only in one treatment group and can be attributed to CDAD, for wheat variety Einstein, dried at 85°C. Mazzuco *et al* (2002) found that the only increase in AME came with a moisture content of 160g/kg and drying at 40°C. This was compared with any other treatment combination of 130, 160, 200 or 300 g/kg and 40, 70 or 100°C. This effect may be enzyme-related. Kulp and Lorenz (1981) found 210g moisture/kg is necessary for increased alpha-amylase susceptibility. However, beyond 210g/kg, there was no change in susceptibility. Why the reverse would be seen in the current experiment is difficult to explain. The reason that the only temperature effect observed in the current experiment was with variety Einstein, could agree with the variety-dependant effect reported by Stewart *et al.* (1998) who found that FCR deteriorated for wheat variety Consort but improved with variety Hunter. It is

interesting to note that Einstein, dried at 85°C also had the highest moisture content (measured after the drying treatment) across the whole experiment (179.6g/kg). The moisture content before drying was comparable to all others, so heat moisture effects should not be the cause, *per se* (see Appendix A). It is also important to note that in the current experiment, each temperature treatment involved a different drying time. The lower temperatures underwent a shorter time period and *vice versa*, as it was felt important that wheat entering the diet were of equivalent moisture contents. It is possible that no differences in CAD were seen between treatments 70°C and 100°C, as 70°C, for two hours, may have a similar effect as 100°C for one hour, for example.

Clare is a soft wheat variety and, as such, was expected to outperform Einstein in terms of starch digestibility (Short *et al.* 2000; Carré *et al.* 2002; Carré *et al.* 2005; Wickramasinghe *et al.* 2005; Peron *et al.* 2006; Peron *et al.* 2007). There was no significant difference between the overall performances of the two varieties. This could be because they have Near Infra Red (NIR) hardness scores that are similar, despite being classified by the Home Grown Cereals Authority (HGCA) as hard and soft. The actual NIR scores for these two variety samples were measured by an external organisation, using the Single Kernel Characterisation System (SKCS), which is based upon NIR spectroscopy. This is described in chapter one. Clare had a score of 21.8 and Einstein a score of 50.5. This confirms the previous classifications; Clare is clearly soft, having a score of less than 45 on a scale of 100 (Greffeuille *et al.* 2006). However, Einstein is around the lower boundary of what is considered hard. Potentially this is why there was no difference in their overall performance. It is important to take into account NIR hardness score, as opposed to simple classification when comparing starch digestibility with hardness. It seems that, when a large range of hardness scores are investigated, a positive correlation is found between starch digestibility and hardness (Carré *et al.* 2002; Carré *et al.* 2005; Peron *et al.* 2006). On the other hand, Rogel *et al.* (1987) investigated a very small range of Particle Size Index (PSI) scores, in the middle of the scale (34.5 to 63.6) and failed to find any relationship of hardness and starch digestibility. There is the suggestion that hardness is positively correlated with pentosan content (Turnbull and Rahman 2002). This is interesting as it could be more evidence of the mechanism of the relationship of starch digestibility and hardness. Arabinoxylan, a class of pentosan, is known to reduce starch digestibility.

Endosperm hardness is not consistently correlated with AME (Garnsworthy *et al.* 2000), even over a large range of scores, although, Hetland *et al.* (2007) found that a soft wheat (score 35.5) performed better in terms of AME and starch digestibility than a hard wheat (77.3) in one of two experiments.

It seems that effects of heat treatment, as imposed in the current experiments, were difficult to predict. The effects of heat and moisture are complicated to separate in the literature, as experiments impose heat and moisture treatments together, without individual controls. However, the results of the current experiment are largely in agreement with the literature and hypothesis, that heating to 100°C should not affect nutritional quality. However, it is difficult to explain why the 85°C treatment had a significant negative effect on starch digestibility. Hoover and Manuel (1995) and Hoover and Vasanthan (1994) investigated heat and moisture treatment of cereal, legume and tuber starches, specifically 100, 200 or 300g/kg moisture, and 100°C for 16 hours. They hypothesised that bread and cake baking qualities decrease with heat and moisture treatment, the opposite of what Lunn *et al.* (2001a) would suggest, but in agreement with Lorenz and Kulp (1981). It has been found that the shape and size of starch granules is not affected by HM treatment (Hoover and Vasanthan 1994; Hoover and Manuel 1995). The authors did, however, find that amylose content of maize was decreased and suggest that this is due to the formation of amylose/lipid complexes that are dependant on the level of lipid present in the native granule. Furthermore, it was also found that *in vitro* starch hydrolysis by porcine pancreatic amylase is decreased by HM treatment and that is due to the amylose/lipid complex.

Often, the requirements of the bread making and animal feed markets could be considered to be opposites in terms of starch, but it seems that with starch structure this is not the case. In the current experiment, the decrease in CDAD, with wheat dried at 85°C is particularly marked in Einstein (although significant in both varieties compared to the controls). It is possible that this specific batch of wheat had areas that were unusually wet, or became particularly hot whilst in the oven. Although the oven has circulated air to prevent hot spots within the oven itself, the trays of wheat may have benefited from more constant and consistent agitation during the drying period. If this was the case and areas reached 300g moisture/kg the effect may have been in line with Hoover and Manuel (1995) and amylose/lipid complexes may have occurred. It has been found that amylose/lipid complexes are

less well digested than free amylose, during *in vitro* studies (Holm *et al.* 1983). After ten minutes incubation, only 0.17 of amylose (complexed to lipid) was digested at a concentration of 350U amylase per gram of amylose and only 0.34 digested at a concentration of 3000U of amylase per gram of amylose. A unit (U) is defined as the amount of amylase that can liberate 1.0mg of maltose from starch, in 3 minutes, at pH 6.9 at 20°C (Holm *et al.* 1983). The authors suggest that this is due to a new V-helical structure of amylose, rendering it less soluble (Holm *et al.* 1983). The type of lipid was also important. Amylose was better digested when complexed with amylose/oleic acid but still less well than free amylose. Holm *et al* (1983) suggest this is due to the unsaturated nature of oleic acid, making the complex more soluble.

Elevated moisture content in wheat samples is known to encourage advantageous endogenous glycanase activity (Choct and Hughes 1997). Presumably, in the short time between steeping and drying, and the fact that samples were refrigerated in the current experiment, this type of enzymic activity can be ruled out.

In summary, there was a significant decrease in CAD with heat treatment in comparison to control, non-treated wheat samples. However, there was no linear relationship between temperature treatments, with 85°C having the greatest effect. Moisture content seems to be an important consideration. Even slightly elevated moisture levels may cause a decrease in starch digestibility, particularly in the duodenum and this may be related to the formation of amylose/lipid complexes which may hinder amylose hydrolysis by amylase. It is possible that, during the drying period, localised areas of particularly wet grain occurred. Care should be taken to ensure this does not happen and in the case of the current experiment, it was probably unlikely since every effort was made to spread the grain out evenly on the drying trays.

Chapter Five

Trial 3 - Two Drying Regimes with Wheat Grown at Two Sites

5.1 Introduction

The results of trial two (chapter four), based on artificial wetting and drying regimes, were somewhat inconclusive. Accordingly, it was decided to design another trial in collaboration with the John Innes Centre in Norwich, Norfolk in which samples were grown as part of a larger agronomy trial looking into post harvest sprout resistance in a large range of wheat varieties. The aim of the animal trial was to investigate the effects of moisture provision during the ripening stage of wheat growth and the resulting drying process on AME and starch digestibility. As discussed in chapter four, moisture content of the wheat samples to be heat treated is perhaps equally as important as the drying temperature employed. Advantages and disadvantages were found with heat treating wheat of very specific moisture contents both in the experiment described in chapter four and the literature (Kulp and Lorenz 1981; Mazzuco *et al.* 2002).

The relationship between sprouting in grain and the resulting increases in endogenous amylase activity has been widely reported. It can have a seriously detrimental effect in bread making quality. The different mechanisms behind amylase activity are discussed in chapter two. At least three (Flintham and Gale 1988) or four mechanisms (Lunn *et al.* 2001b) are commonly accepted. Of particular relevance to the current chapter are Pre-Maturity α-Amylase Activity (PMAA) and Post-Maturity Sprouting (PoMS). July 2006 was particularly warm with temperatures in the range of 12.3-26.3°C as opposed to the average of 11.6-21.1°C (Buxton 2006). Warm weather during ripening has been suggested to reduce amylase expression in some varieties (Mrva and Mares 1996) and cold weather has been suggested to increase activity (Flintham and Gale 1988). However, as a whole, the month of July 2006 was also particularly dry, with just 34.1mm of rain throughout the month as opposed to the average of 51.6mm (Buxton 2006). Flintham and Gale (1998) suggested that high rainfall and humidity would encourage PMAA.

Considering the analysis of trial two (chapter four) it is possible that wheat that was dried with the 100°C regime may have decreased overall CAD of starch than that which was dried under ambient conditions. This will not necessarily be supported by a decrease in AME. Also discussed in chapter four is the influence of moisture content. Samples that had high moisture content (table 5.1) may result in lower CDAD, but this would generally not be expected at these moisture levels.

The current wheat samples may have exhibited low and consistent amylase levels, since the weather during ripening was particularly warm and dry. Wheat samples that were grown at JIC and harvested on the second date were irrigated. This may result in elevated α-amylase levels. It is noted, however, that there was no visible sprouting in any of the samples. As discussed in chapter two, it is not clear how increased amylase may affect nutritional quality. There may be a negative relationship with Hagberg Falling Number (HFN) and AME suggesting that, with increasing amylase, AME also increases (Svihus and Gullord 2002). Other authors have failed to find any correlation (Rose *et al.* 2001; Pirgozliev *et al.* 2003).

5.1.1 Materials and methods

Details are presented in chapter two. For each treatment, six replicates were used. Each replicate comprised one cage containing two birds

i. Wheat Samples

Figure 5.1 shows the relationship between samples and the allocation to each diet. One variety, Einstein, was grown at two locations. Half was grown at the John Innes Centre (JIC), and half at Church Farm (CF), two and a half miles from JIC. Half of the samples were harvested at maturity (17/7/06 in the case of JIC; 19/07/06, CF). The JIC plot was then irrigated using an overhead system for two, two-hour periods each day. The remaining JIC samples were then harvested at 270g moisture/kg (1/8/06). The remaining CF samples were harvested at approximately the same time (31/07/06) but did not undergo irrigation treatment. Each of these four samples was then dried *either* at 100°C for 72hours, or at ambient temperatures. This gave eight samples for nutritional evaluation. In trial two (chapter four) wheat samples were soaked prior to heating, which resulted in varying moisture contents. Elevated moisture contents were potentially responsible for decreased CDAD. The samples in the current experiment were either irrigated during ripening or not, and

this may highlight any differences in starch digestibility in a context that is more relevant to a commercial situation. The full details of the treatment of samples used in the current experiment are shown in table 5.1.

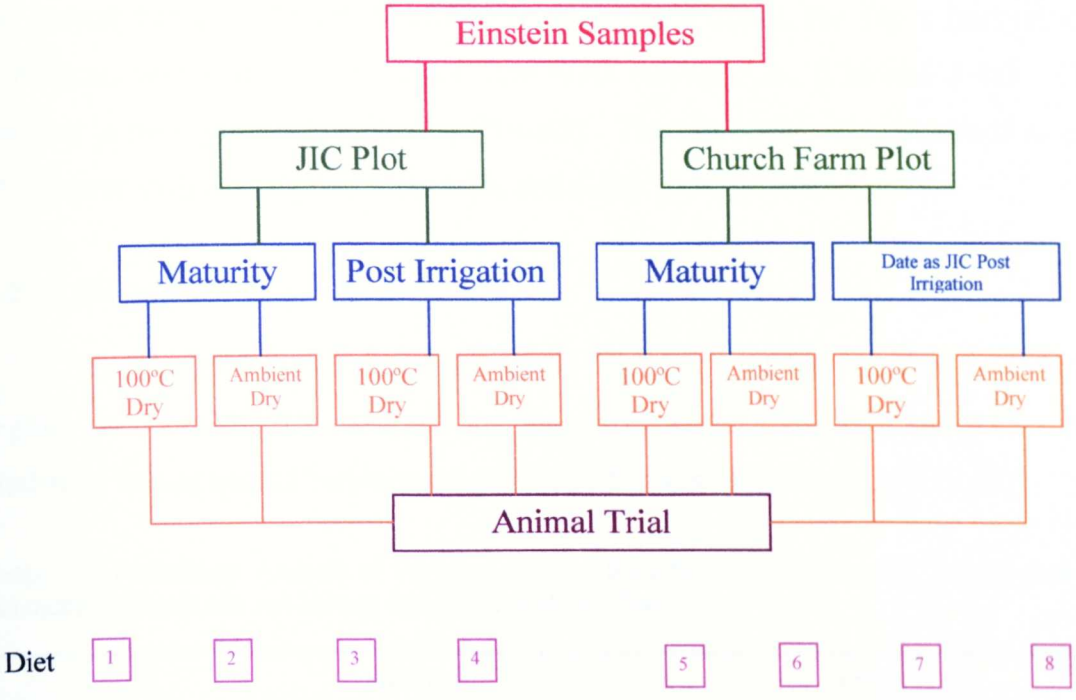


Figure 5.1 Description of wheat samples grown for trial four

Table 5.1 Specific harvest, treatment and moisture content (MC) information for wheat samples

Diet	Location*	Date of harvest	First MC**	Drying regime	Second MC***
1	JIC	17/07/2006	250 g/kg	100°C	111.9g/kg
2	JIC	17/07/2006	250 g/kg	Ambient	118.4 g/kg
3	JIC	01/08/2006	270 g/kg	100°C	105.5 g/kg
4	JIC	01/08/2006	270 g/kg	Ambient	122.4 g/kg
5	CF	19/07/2006	370 g/kg	100°C	123.1 g/kg
6	CF	19/07/2006	370 g/kg	Ambient	108.4 g/kg
7	CF	31/07/2006	122 g/kg	100°C	93.8 g/kg
8	CF	31/07/2006	122 g/kg	Ambient	122.0 g/kg

* JIC = John Innes Centre; CF= Church Farm
** = moisture content when harvested
*** = moisture content when 'dry'

ii. Statistical Analysis

The data were analysed by Analysis of Variance (as discussed in chapter two), as a full factorial split plot design. There were two factors, harvest date and

drying regime. In the case of Coefficient of Apparent Digestibility of starch, there was also a third factor: gut region. The two sites (JIC and CF) were replicate plots. The eight dried samples were the main plots.

In order to determine whether the difference in irrigation treatment prior to the second harvest date could have had a detectable effect, the site x harvest date interaction was tested within initial ANOVAs (tables 5.2a, 5.3a and 5.4a). This resulted in no significant difference (P>0.05). Therefore, site was reinstated as part of the error variance (figures 5.2b, 5.3b, and 5.4b).

5.2 Results

The results of statistical analysis of the effects of harvest date and drying regime on the AME, FI and CAD are shown in tables 5.2b, 5.3b and 5.4b. Any statistical values quoted herein were generated by these secondary ANOVAs.

Table 5.2a Preliminary Analysis of Variance showing the effect of drying regime, harvest date and interaction between site and harvest date on the AME of diets

		Harvest			ANOVA		
		1st	2nd	Mean	Factor	P	sed
Drying regime	Ambient	16.17	15.56	15.86	Drying regime	0.055	0.071
	100°C	15.60	15.55	15.58	Harvest	0.044	0.071
	Mean	15.88	15.56		Drying regime x harvest	0.057	0.101
Site*	CF	15.76	15.68		Site x harvest	0.068	0.101
	JIC	16.01	15.43				

* JIC = John Innes Centre; CF= Church Farm

Table 5.2b Analysis of Variance showing the effect of drying regime and harvest date on the AME of diets

		Harvest			ANOVA		
		1st	2nd	Mean	Factor	P	sed
Drying regime	Ambient	16.17	15.56	15.86	Drying regime	0.169	0.160
	100°C	15.60	15.55	15.58	Harvest	0.132	0.160
	Mean	15.88	15.56		Drying regime x harvest	0.174	0.226

Table 5.3a Preliminary Analysis of Variance showing the effect of drying regime, harvest date and interaction between site and harvest date on the FI of diets

		Harvest			ANOVA		
		1st	2nd	Mean	Factor	P	sed
Drying regime	Ambient	0.407	0.323	0.365	Drying regime	0.100	0.712
	100°C	0.316	0.314	0.315	Harvest	0.128	0.712
	Mean	0.362	0.319		Drying regime x harvest	0.142	0.243
Site*	CF	0.365	0.315		Site x harvest	0.715	0.243
	JIC	0.358	0.322				

* JIC = John Innes Centre; CF= Church Farm

Table 5.3b Analysis of Variance showing the effect of drying regime and harvest date on the FI of diets

		Harvest			ANOVA		
		1st	2nd	Mean	Factor	P	sed
Drying regime	Ambient	0.407	0.323	0.365	Drying regime	0.041	0.146
	100°C	0.316	0.314	0.315	Harvest	0.060	0.146
	Mean	0.362	0.319		Drying regime x harvest	0.069	0.207

Table 5.4a Preliminary Analysis of Variance showing the effect of drying regime, harvest date and interaction between site and harvest date on the coefficient of apparent digestibility of diets

		Harvest							
		First				Second			
	Region	Duodenum[1]	Ileum[2]	Total Tract[3]	Mean	Duodenum[1]	Ileum[2]	Total Tract[3]	Mean
Drying Regime	Ambient	0.331	0.719	0.862	0.637	0.529	0.854	0.935	0.773
	100°C	0.598	0.859	0.866	0.774	0.618	0.827	0.858	0.768
	Mean	0.464	0.789	0.864		0.574	0.841	0.897	
	Mean		0.706				0.770		

	Region	Duodenum[1]	Ileum[2]	Total Tract[3]	Mean
Drying Regime	Ambient	0.430	0.786	0.898	0.705
	100°C	0.608	0.843	0.862	0.771
	Mean	0.519	0.815	0.880	

	Harvest	First	Second
Site*	CF	0.700	0.776
	JIC	0.712	0.764

ANOVA		
Factor	P	sed
Harvest	0.084	0.0200
Harvest x region	0.173	0.0312
Drying regime x harvest	0.070	0.0281
Drying regime x region x harvest	0.438	0.0441
Drying regime	0.080	0.0200
Region	<0.001	0.0208
Drying regime x region	<0.001	0.0312
Site x harvest	0.609	0.0281

[1]'Duodenum' refers to measurements of Coefficient of Duodenal Apparent Starch Digestibility (CDAD)
[2]'Ileum' refers to measurements of Coefficient of Ileal Apparent Starch Digestibility (CIAD)
[3]'Total Tract' refers to measurements of Coefficient of Total Tract Apparent Starch Digestibility (CTTAD)
* JIC= John Innes Centre; CF= Church Farm

Table 5.4b Analysis of Variance showing the effect of drying regime and harvest on the coefficient of apparent digestibility of diets

		Harvest							
		First				Second			
	Region	Duodenum[1]	Ileum[2]	Total Tract[3]	Mean	Duodenum[1]	Ileum[2]	Total Tract[3]	Mean
Drying Regime	Ambient	0.331	0.719	0.862	0.637	0.529	0.854	0.935	0.773
	100°C	0.598	0.859	0.866	0.774	0.618	0.827	0.858	0.768
	Mean	0.464	0.789	0.864		0.574	0.841	0.897	
	Mean		0.706				0.770		

	Region	Duodenum[1]	Ileum[2]	Total Tract[3]	Mean
Drying Regime	Ambient	0.430	0.786	0.898	0.705
	100°C	0.608	0.843	0.862	0.771
	Mean	0.519	0.815	0.880	

ANOVA		
Factor	P	sed
Harvest	0.035	0.0177
Harvest x region	0.173	0.0298
Drying regime x harvest	0.028	0.0250
Drying regime x region x harvest	0.438	0.0422
Drying regime	0.033	0.0177
Region	<0.001	0.0208
Drying regime x region	<0.001	0.0298

[1]'Duodenum' refers to measurements of Coefficient of Duodenal Apparent Starch Digestibility (CDAD)
[2]'Ileum' refers to measurements of Coefficient of Ileal Apparent Starch Digestibility (CIAD)
[3]'Total Tract' refers to measurements of Coefficient of Total Tract Apparent Starch Digestibility (CTTAD)

There was a significant effect of drying regime (P=0.033) and harvest date (P=0.035) on overall CIAD, CDAD and CTTAD (Coefficient of Apparent Digestibility, CAD). CAD was higher with the 100°C drying regime and the second harvest date. There was a drying regime x harvest date interaction (P=0.028). At the first harvest date, CAD was significantly increased with the 100°C drying regime, whereas at the second harvest date, CAD was not significantly different. As expected, there was an effect of region on the CAD (P<0.001). Starch was progressively well digested as it passed through the gut. Critically, there was also an interaction between drying regime and region (P<0.001), with CDAD and CIAD being significantly higher with the 100°C drying regime.

None of these effects on CAD was supported by accompanying difference in AME (table 5.2b).

Drying regime had a significant effect on FI (P=0.041). Significantly less feed was consumed with the 100°C drying regime.

5.3 Discussion

The significant effect of harvest date on CAD may be explained by increasing levels of amylase as the grain was left on the plant (Hetland *et al.* 2007). Such an improvement in CAD has not been reported in the literature, but an increase in AME has (Svihus and Gullord 2002). CAD was also improved following the 100°C drying regime. This is interesting as it suggests that, if amylase is responsible for improving CAD, the improvement occurs before the wheat is subjected to drying, and does not exert an effect within the bird. Cereal amylases have an optimum temperature range of 40-55°C (Muralikrishna and Nirmala 2005) although the optimum may be slightly higher at 60°C in the case of bacterial amylases (Özbek and Yüceer 2001). Presumably amylases are still active below the optimum, at which they were stored, but are less likely to be active at temperatures of 100°C.

Alternatively, these increases in CAD with extended harvest date and 100°C drying regime could be unrelated to amylase. If irrigation had caused a marked increase in amylase levels, as would be expected, it did not have a nutritional impact since there was no interaction between site and harvest date. The effects of treatment on amylase are discussed further in chapter eight. The results of trial two (chapter

four) suggest that temperature-treated samples were less well digested than control samples. The opposite appears to be the case in the current experiment, although the current experiment is in agreement with Huang *et al.* (1997a and1997b).

Interestingly it has been found that with micronization, with a maximum temperature of 90°C, digestion and absorption is shifted toward the small intestine and away from microbial fermentation in the large intestine (Huang *et al.* 1997a; Huang *et al.* 1997b). This is supported by significantly increased CDAD and CIAD, and unchanged CTTAD with the 100°C drying regime in the current study. The majority of cereal starch digestion occurs in the jejunum and in a well digested diet nutrient residues in the hind gut are low (Choct *et al.* 1996). For tuber and legume starches, which are poorly digested, it is further through the digestive tract, or not at all (Moran 1985). This indicates that wheat starch, when efficiently digested, is digested cranially from the caeca. Glucose is efficiently absorbed in the duodenum and jejunum (Tester *et al.* 2004b). If digestion is shifted toward the caeca, one could assume that absorption was less efficient and that there may be effects on the endogenous microflora.

Heat and moisture treatment increases the enzyme susceptibility and solubility of starches, as discussed in chapter four. It is possible that this is the mechanism for increased digestibility. All the wheat samples used in the current experiment, bar one, were above the 210g moisture/kg threshold (before drying, table 5.1) suggested by Kulp and Lorenz (1981). If this is the case it may be discernable using the RVA. If enzyme susceptibility is much increased by the heat treatment and amylase is active, and solubility is increased then the RVA will display a reduced peak and end viscosity. Rheological analyses are presented in Chapter 7.

The results presented in table 5.2a were not significant. However, values for P were less than 0.1. The preliminary ANOVAs displayed in table 5.2a, 5.3a and 5.4a were designed to investigate a site x harvest difference, potentially highlighting whether irrigation had an effect. However, clearly, the treatments for harvest two at the two sites were not equivalent. As a result of the trends seen in table 5.2a and 5.4a, the effect of moisture content (prior to drying) on AME, FI and CAD was also investigated using polynomial ANOVA. There was no significant effect on AME or FI, nor were there any interactions with drying regime ($P>0.1$ in all cases, not tabulated here). Trends and significant effects on CAD are shown in table 5.5. There was no interaction between moisture content and region ($P>0.1$ not tabulated

here). Although it appeared that there was no site x harvest interaction (P>0.05, tables 4.2a, 4.3a and 4.4a) and that the irrigation had not had an effect, moisture content prior to drying does affect CAD (P=0.004). However, this was non-linear (Plin=0.924), with 250°C being significant higher than 370g/kg and 270g/kg being significantly higher than 122g/kg and g/kg. There was a trend towards an interaction between drying regime and moisture content (P=0.063). This result is partly in agreement with Kulp and Lorenz (1981) who suggest that a moisture content of at least 210g/kg is necessary for an increase enzyme susceptibility and presumably an increase in digestibility. They did not, however, see any difference beyond 210g/kg. It is difficult to explain why the highest moisture content in the current experiment did not result in the same response. Muzzuco *et al.* (2002) also found that with increasing moisture content, beyond 160g/kg, and 100°C, there was no difference in digestibility. Hoover and Manuel (1995) suggest that above 300g/kg, amylase may complex with lipid, which decreases digestibility (Holm *et al.* 1983). It is possible that this is the cause of the decrease in digestibility at 370g/kg.

Table 5.5 Analysis of Variance showing the effect of drying regime and moisture content on coefficient of apparent digestibility of diets

		Moisture Content (g/kg)				ANOVA		
		122	250	270	370	Factor	P	sed
	Ambient	0.734	0.716	0.829	0.725	Moisture content	0.004	0.0413
Drying regime	100°C	0.693	0.869	0.839	0.679	(Linear)	0.924	
	Mean	0.713	0.793	0.824	0.702	(Deviation)	0.002	
						Drying regime x Moisture content	0.063	0.0584
						(Linear)	0.978	
						(Deviation)	0.027	

In summary of the current experiment, the coefficient of apparent digestibility was increased in the cranial portions of the gut, suggesting increased efficiency of digestion and absorption, with the 100°C drying regime. Interestingly, less feed was also consumed with this treatment. It is possible that starch has been structurally altered, although unlikely as the moisture content is low. It may be that starch susceptibility to enzyme degradation has increased. Moisture content prior to drying may have an effect, albeit non-linear. Increased digestibility was seen with moisture contents of 250g/kg and 270g/kg although at 370g/kg digestibility may decrease, potentially due to amylose:lipid interactions.

6.1 Introduction

A previous project carried out at the University of Nottingham suggested that the nutritional value of low-AME wheat varieties may improve during storage. It was suggested by Nichol (1999) that storage at ambient temperatures for between seven and 10 months improved the AME of certain wheat varieties. However, wheat varieties considered to be of low AME had the greatest improvement (Choct and Hughes 1997; Nichol 1999). Wheat varieties are considered to be of low AME if they have a value of less than 13MJ/kg (Annison and Choct 1991). An investigation by Choct and Hughes (1997) reported that a wheat variety with an AME of just 9.2MJ/Kg had improved to 12MJ/Kg after one year's storage at ambient temperature. They also suggest that improvement in AME and Feed Conversion Ratio (FCR) may occur after just three or four months. It is likely that this is due to an increase in enzyme activity which degrades endogenous anti-nutritional factors that otherwise decrease nutritional value (Nichol 1999; Kim *et al.* 2003). Austin *et al.* (1999) suggest that, although solubility of arabinoxylan may be increased over six months, molecular weight is decreased, decreasing viscosity potential. Specific arabinoxylan-degrading enzymes have been isolated, and their activity is a function of time (Cleemput *et al.* 1997). Choct and Hughes (1997) support this suggestion with the further improvement of a wheat variety already bettered by storage following the use of exogenous dietary glycanases. It has been suggested that, with barley, storage for six months at high moisture levels (600 kg/DM) may reduce soluble β-glucan, a known Non Starch Polysaccharide Anti-Nutritional Factor (NSP-ANF), and therefore reduce digesta viscosity (Svihus *et al.* 1997). Where it may be assumed that this is due to increased enzyme activity, the authors suggest that, because steaming and enzyme inoculation had no further effect on the changes in β-glucan, perhaps endogenous wheat enzymes were not wholly responsible. However, such an effect on wheat with high moisture storage was not reported.

Nutritional improvements for poultry have also been reported with a time period of six months. Scott and Pierce (2001) reported a highly significant decrease

in excreta DM and an increase in FI, both of which may be considered negative. However, coupled with an increase in day 17 live weight, this resulted in an improvement in FCR. There was, however, no change in AME (Scott and Pierce 2001). AME has been found to be positively correlated with starch content and concurrently a lack of variation in starch content with storage leads to a constant AME (Huyghebaert and Schoner 1999; Pirgozliev *et al.* 2003). It has also been reported that, over six months, AME may significantly increase, only to achieve the original value after the total six months (Pirgozliev and Rose 2001; Pirgozliev *et al.* 2006). In variety Abbot, AME was greater after six weeks than the control, non-stored equivalent. However, after 18 or 24 weeks, the AME was not significantly different from the control (Pirgozliev *et al.* 2006). In an earlier study, Pirgozliev and Rose (2001) had found a similar, non-linear relationship between storage and AME. For variety Abbot, AME had increased after 12 and 18 weeks storage, relative to the control, but was not significantly different after 24 weeks, for either of two varieties. Ravindran *et al.* (2001) reported a significant increase in AME after just three months, although they did not measure the AME at any later point. In a study focusing on the effect of wheat micronization for piglet diets, Zarkadas and Wiseman (2001) found that micronization increased FCR, but this problem was ameliorated by storing the wheat for just two months. However, the FCR of all stored diets was numerically higher than the control in the non-stored set of diets. This may suggest that storage decreases treatment effects but may worsen nutritional parameters such as FCR.

A so called 'new crop syndrome' has also been suggested by Scott and Pierce (2001) who concluded that the use of newly harvested wheats may compromise performance but could be improved with storage. This has also been reported by the bread making industry (Posner and Deyoe 1986; Wang and Flores 1999).

There is evidence of significant chemical and composition changes over time and with varying temperatures. Total free sugars within wheat grains increase with storage of up to six months, at ambient conditions of 25°C (Zia-Ur-Rehman 2006). Starch has also been found to decrease, over a period of six months, suggesting that endogenous carbohydrases are active (Kim *et al.* 2001; Kim *et al.* 2003). Jood *et al.* (1993) also report the same changes in the balance of sugars and starch over a period of at least 4 months. Presumably this is responsible for a potential increase in nutritional value, as starch requires degradation before absorption, whereas simple

sugars do not. However, this suggestion is contrary to the view of Huyghebaert and Schoner (1999). Over eight years of storage α-amylase has been found to decrease (Pixton and Hill 1975).

Available lysine decreases at storage temperatures of 10, 25 and 45°C in a stepwise fashion, over a period of six months (Zia-Ur-Rehman 2006). Earlier work by Rehman and Shah (1999) suggest there is no significant change at 10°C, and that the change at 25°C is slower than that at 45°C. Protein has been reported to increase over six months, although it was non-significant (Kim *et al.* 2001).

With this information, it was felt that it was important to establish a preliminary picture of the potential improvement of wheat during the first few months of storage.

The aim of the current trial was to test for any differences in AME and starch digestibility between two samples of wheat which had been stored for two and four months. Both samples were of the same crop (*var.* Deben) grown at Sutton Bonington. The crop was harvested at 230g/kg moisture content and dried twice, at 62°C, until approximately 150g/kg was reached. Samples were taken after two months subsequent storage at ambient temperature on the farm and frozen at -20°C until analyses and trial. Samples were the taken again at four months and frozen. It was assumed that freezing the samples suspends activity within the grain and is similar to the method employed by Pirgozliev *et al.* (2006) and Pirgozliev and Rose (2001) who found no change in AME having frozen and defrosted two wheat cultivars. Rehman and Shah (1999) suggested that the lower the temperature between 10-45°C, the less the chemical changes within the wheat grain. No significant changes were found at 10°C, after 6 months storage (Rehman and Shah 1999). Due to the lack of wheat samples taken prior to storage, the control used was variety Clare, known to be appropriate for poultry feeding. Diets were formulated as shown in table 2.4 (chapter two), with the addition of wheat at a rate of 750g/kg. Modifications were made as shown in table 6.1. The fourth diet (table 6.1) was a combination of both Clare and Deben in equal proportions. This was to investigate the potential amelioration of negative quality using 'standard' wheat (Clare).

Table 6.1. Diet wheat components

Diet	Wheat Variety	Wheat Treatment	Inclusion Rate
1	Clare	None	750 g/kg
2	Deben	Stored for 2 months	750 g/kg
3	Deben	Stored for 4 months	750 g/kg
4	Clare	None	375 g/kg
	Deben	Stored for 4 months	375 g/kg

The literature is not clear on whether wheat nutritional quality changes with storage, particularly over periods of less than 6 months, although within three or four months, a significant increase has been shown (Choct and Hughes 1997; Ravindran *et al.* 2001). Certainly the length of time and storage temperature is important. With storage of between two and four months, it is also possible that a decrease in AME will be seen, although it may not be statistically significant (Pirgozliev *et al.* 2006). It is likely that any change in AME will be accompanied by a change in starch. The direction of this change is not clear. Some would suggest that with an increase in AME it would be an increase in starch (Huyghebaert and Schoner 1999; Pirgozliev *et al.* 2003) whilst others would suggest a decrease accompanied by an increase in free sugars highlighting enzymic degradation (Jood *et al.* 1993; Kim *et al.* 2001; Kim *et al.* 2003; Zia-Ur-Rehman 2006). Free sugars were not measured in the current study.

Details of materials and methods used in the current experiment are presented in chapter two. For each treatment, six replicates were used. Each replicate comprised one cage containing two birds

6.2 Results

In the analysis of coefficient of apparent digestibility the statistical model employed was a 4 (wheat treatment) x 3 (gut region) factorial.

The statistical analysis of the effects of storage on AME, CAD and FI is shown in tables 6.2, 6.3 and 6.4 respectively.

There was no significant benefit of storage of four months after harvest, compared to two months in terms of AME, CAD or FI. The bread making industry have suggested mixing new crop wheat with previous season wheat, to ameliorate

negative effects of the newly harvested wheat (Posner and Deyoe 1986). In terms of poultry nutrition, there was no advantage with this method. The only significant difference seen was in CAD in the varying regions of the gut ($P<0.001$), as is expected. However, there was no interaction between treatment and region, indicating that the progressive CAD did not vary across for all four diets.

Table 6.2 Analysis of Variance showing the effects of storage of wheat for two or four months on the AME of diets (MJ/Kg DM)

		ANOVA		
Wheat Treatment	**Mean**	**Factor**	**P**	**sed**
Variety Clare	14.40	**Wheat treatment**	0.430	0.416
2 Months Storage	13.80			
4 Months Storage	13.88			
50 Clare : 50 Deben after 4 months storage	13.78			

Table 6.3 Effects of storage of wheat for two or four months on the coefficient of apparent digestibility of starch

	Region				ANOVA		
Wheat Treatment	**Duodenum[1]**	**Ileum[2]**	**Total Tract[3]**	**Mean**	**Factor**	**P**	**sed**
Variety Clare	0.678	0.911	0.951	0.847	**Wheat treatment**	0.679	0.0473
2 Months	0.581	0.895	0.961	0.812	**Region**	<0.001	0.0249
4 Months	0.589	0.844	0.985	0.806	**Wheat treatment**		
50 Clare : 50 Deben after 4 months storage	0.691	0.889	0.981	0.854	**x region**	0.385	0.0623
Mean	0.635	0.885	0.970				

[1]'Duodenum' refers to measurements of Coefficient of Duodenal Apparent Digestibility (of starch) (CDAD)
[2]'Ileum' refers to measurements of Coefficient of Ileal Apparent Digestibility (of starch) (CIAD)
[3]'Total Tract' refers to measurements of Coefficient of Total Tract Apparent Digestibility (of starch) (CTTAD)

Table 6.4 Effects of storage of wheat for two or four months on the FI of diets

		ANOVA		
Wheat Treatment	**Mean**	**Factor**	**P**	**sed**
Variety Clare	0.339	**Storage**	0.413	0.0434
2 Months	0.323			
4 Months	0.368			
50 Clare : 50 Deben after 4 months storage	0.294			

A simple linear regression found no correlation between starch and AME (P=0.330). This suggests that both parameters did not change relative to each other as a result of the treatments.

6.3 Discussion

The current results are in agreement with the majority of the cited literature in that, before six months storage, there is little improvement in nutritional value in terms of AME (Huyghebaert and Schoner 1999; Scott and Pierce 2001; Pirgozliev *et al.* 2006). However, the current results seem to be in agreement with Rehman and Shah (1999) who, although they were not investigating any bird parameters, failed to find any variation in composition that is reported to be correlated with changes in AME. For example, they report a decrease in soluble sugars and an increase in insoluble amylose attributable to a shift towards increased crystallinity that is thought to be related to a decrease in starch digestibility (Zarkadas and Wiseman 2001). It has been found that HFN values increase with storage over 15 months (Lukow *et al.* 1995). Although this is considerably longer than the time period used in the current experiment, Lukow *et al.* (1995) found that the increase in HFN was immediate and incremental over the 15 months. They suggest that this change is either due to a decrease in amylase activity or a change in starch susceptibility. Interestingly, Rehman and Shah (1999) reported a decrease in amylase activity after 4 months at 25°C, approximately ambient temperature, at which current samples were stored. In addition to this finding, there may also be chemical changes that resulted in a decrease in susceptibility of starch and protein to enzymic degradation. Starch digestibility decreases, in an incremental manner, over six months (Zia-Ur-Rehman 2006). At 25°C, protein digestibility decreased after three months, although after that point it remained constant. At 45°C there was a incremental decrease over six months' storage (Zia-Ur-Rehman 2006).

Elevated intestinal viscosity has been linked to poor starch digestibility associated with non-starch polysaccharides, particularly arabinoxylan, in wheat. The current results are in agreement with a study of changes in *in vitro* viscosity with storage. It was found that over a period of eight weeks no change was seen in the extract viscosity of wheat that was stored at room temperature (George and McCracken 2003). After a period of six months, the viscosity had decreased although it was statistically insignificant. This suggests that there would be no

concurrent change in nutritional value over a period of four months. Presumably this is due to room temperature being conducive to the enzyme activity. George and McCracken (2003) suggested endo-acting glycanases that degrade NSPs in the mid-molecule region bringing about a rapid change in molecular weight and therefore viscosity. This is in agreement with Kim *et al.* (2003). Unexpectedly, wheat that had been milled before storage at room temperature had a significant increase in viscosity (George and McCracken 2003). This has also been reported by other authors who suggest changes in starch, as opposed to enzymes, result in increased viscosity (Loney and Meredith 1974). However, George and McCracken (2003) suggest that milling may damage enzymes and arabinoxylan may become oxidised with time. The difference in viscosity with whole or ground wheat with storage has also been reported in oats by Zhou *et al.* (1999) who suggested that increases in viscosity with storage may be related to lipid starch interactions.

It is possible that one reason no improvement in nutritional value was seen is that, as suggested by Nichol (1999), wheat varieties of intrinsically low AME show the greatest improvement, if any. Although a time zero baseline was not employed, it could be assumed from the AME value at the two month stage, that Deben does not have an AME of much less than 13MJ/kg DM, before any treatment. It is suggested that the decrease in β –glucan and viscosity with high moisture storage in the case of barley and oats is not seen in wheat because wheat has inherently low β-glucan content before any treatment (Svihus *et al.* 1997).

It appears from this trial that within four months post harvest ambient storage there is no variation in nutritional quality. There was no significant difference in coefficient of apparent digestibility, AME or FI between treatments. This is in agreement with Scott and Pierce (2001) and Nichol (1999) who suggest at least six months or seven months, respectively, is required for any improvement. The wheat samples used in this trial were harvested at high moisture. Flour samples were pasted using the RVA to investigate amylase levels, which were expected to be high. This is discussed in the following chapter.

One of the aims of the work described in this thesis was to investigate, using chick bioassays, whether extreme heat treatment is detrimental to nutritional value. Some common rheological techniques are discussed in chapter one and the current chapter discusses whether those laboratory based techniques can be used to predict nutritional value without the need for a chick bioassay.

7.1 Trial 4 – The Effects of Storage

The potential effects of storage of wheat for poultry are discussed in chapter six. In agreement with the literature, experiments found that short term storage of two or four months had no effect on the AME, CAD or FI for poultry. Subsequently, it would be expected that there would be no difference in the starch pasting characteristics. However, the wheat samples were harvested at a DM content of 680g/kg. This suggests that rainfall was high prior to harvesting, which may have resulted in elevated α-amylase levels (Flintham and Gale 1988).

The samples investigated in trial four (chapter six) were analysed using the RVA in January 2005 and again in June 2007. Between sampling on the farm and RVA analysis in 2005 samples were stored at ambient temperature, on the farm at Sutton Bonington. Between 2005 and 2007, samples were stored at 5°C. Samples were run on the RVA using profile 35 as outlined in chapter two.

7.1.1 Results and Discussion

i. Amylase

It can be seen from the RVA profiles (figure 7.1 A and B) that extremely high levels of amylase were present; the profiles of samples run using distilled water are hardly visible and do not exhibit the typical pasting curve (chapter one, figure 1.5). Their profiles are also below the lower levels of accuracy of the RVA. Initially, samples were then pasted in excess 5mM silver nitrate solution ($AgNO_3$) which inhibited amylase and allowed the starch to follow the normal gelatinisation pattern. However, samples showed high levels of variability in all parameters. As will be

seen in later examples, the RVA data are typically highly reproducible, so much so that it is generally accepted that duplicate sample runs are adequate (Wickramasinghe *et al.* 2005). In an attempt to increase the reproducibility of the profiles, samples were run in 10mM $AgNO_3$. It is possible that 5mM was not sufficient to inhibit endogenous amylase. In the case of the wheat stored for two months, reproducibility was greatly enhanced and the profile was shifted upward toward greater viscosities. This indicates that amylase activity was potentially greater than indicated by 5mM solution. However, in the second wheat sample, that had been stored for four months, no such change was seen. Interestingly, the profiles for both wheat samples using the 10mM solution exhibited a peak during the cooling stage, at approximately 50°C. Often this peak is attributed to amylose/lipid interactions (Hill, personal communication).

It can be concluded that extremely high amylase levels were present, since the profile using water was so low. According to Flintham and Gale (1988), this can probably be attributed to high rainfall during ripening, indicated by the high moisture content at harvest. A relative measure of amylase was calculated using the method given in chapter two (section 2.2.4.i). However, because there were no discernable peaks in the profiles of samples run in distilled water, this was deemed to be inaccurate and only to give an indication of amylase levels. These results are discussed in chapter eight and not presented here.

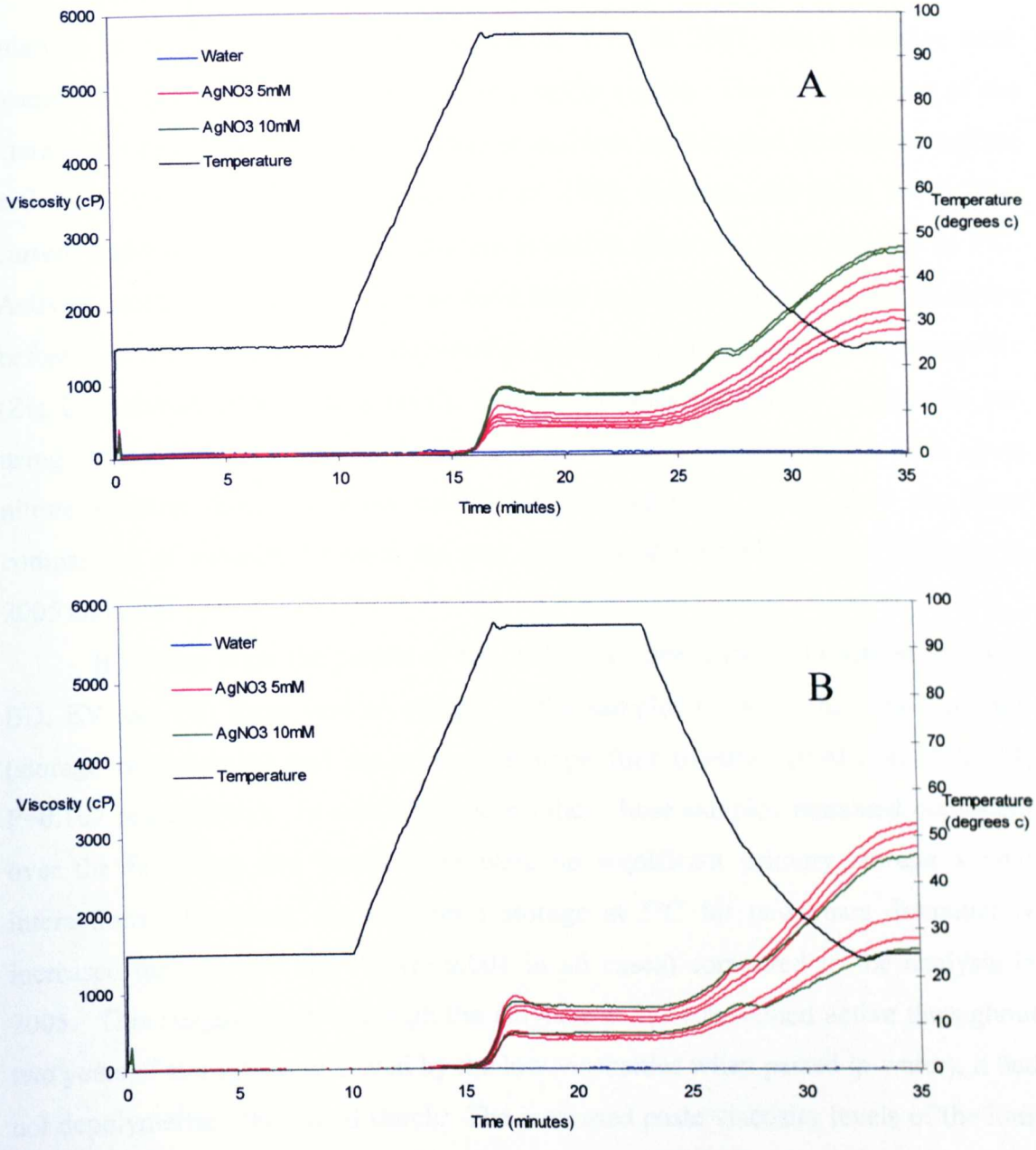


Figure 7.1 The RVA profiles of wheat stored for two (A) or four (B) months prior to sampling; analysed in 2005. Sample size of 3g (on a dry matter basis). Multiple lines of the same colour represent replicates.

ii. Pasting Characteristics

Breakdown (BD), End Viscosities (EV) and Peak Viscosities (PV) for analysis in 2005 and 2007 are shown in table 7.1. In 2007, when samples were reanalyzed, the profile using water was still hardly visible. This is interesting as the literature is clear that, over time, storage at ambient temperature decreases amylase activity (Pixton and Hill 1975; Lukow *et al.* 1995; Rehman and Shah 1999). The current experiment suggests that amylase is active after two years storage at 5°C. Activity could be maintained, or may have been so high in 2005 than any decrease before 2007 was not identified. Potentially, starch may also become less susceptible (Zia Ur Rehman 2006). As a result, the data presented are those of samples run using 5mM $AgNO_3$ solution. Although only having data for samples run with silver nitrate solution does not allow calculation of α-amylase levels, they do allow comparison of samples between the two sampling dates and between analysis in 2005 and 2007.

It is clear from the results of the ANOVA (see Table 7.1) that in terms of BD, EV and PV, there was no change in the samples between the first sampling (storage two months) and the second (storage four months) ($P=0.174$; $P=0.114$; $P=0.107$ respectively). In relation to each other, these samples remained consistent over the following two years; there were no significant primary storage x year interactions. However, the long term storage at 5°C for two years dramatically increased the BD, EV and PV ($P<0.001$ in all cases) compared to the analysis in 2005. This suggests that, although the amylase activity remained active throughout two years of storage (as indicated by the low viscosities when pasted in water), it had not depolymerised the stored starch. The increased paste viscosity levels of the long term stored samples, when amylase was inactivated, may indicate starch changes during storage or changes in the grain that are then reflected in the milling, hydration and finally swelling properties of the materials.

Table 7.1 Statistical Analysis of the Breakdown, End Viscosities and Peak Viscosities of wheat samples that have been stored for two or four months prior to sampling and analysed in 2005 or 2007. Results are a mean of two sample runs.

RVA Parameters									
	Breakdown (Silver, cP)			End Viscosity (Silver, cP)			Peak Viscosity (Silver, cP)		
Storage	**Two Months**	**Four Months**	**Mean**	**Two Months**	**Four Months**	**Mean**	**Two Months**	**Four Months**	**Mean**
Year									
2005	77	116	96	2053	2547	2300	524	685	605
2007	351	400	376	4294	4776	4534	1324	1523	1424
Mean	155	197		2693	3184		753	925	
ANOVA									
	P	**sed**		**P**	**sed**		**P**	**sed**	
Storage	0.174	28.7		0.114	283.1		0.107	97.0	
Year	<0.001	31.8		<0.001	313.3		<0.001	107.4	
Storage x Year	0.872	45.0		0.986	443.1		0.866	151.8	

iii. RVA Method Development

Initially samples were sieved before use in the RVA to a fraction less than 250μm. In 2005, in a further attempt to increase reproducibility samples were sieved to a small fraction, between 212 and 250μm. As seen in table 7.2 the reproducibility was increased. The variability of the EV is reduced when samples are sieved to particle sizes of between 212 and 250μm.

Table 7.2 Mean and Standard Deviation of the End Viscosities (cP) of samples stored for two or four months and sieved to one of two fractions. Results are the mean of two sample runs.

	Fraction			
	212-250μm		<250μm	
Storage	Mean	SD	Mean	SD
Two Months	4289	117.1	2053	340.9
Four Months	3504	311.3	2547	668.3

Sieving the flour before use in the RVA has the advantage in that particle size can be partially excluded as having an effect on the swelling behaviors of the starch (Becker *et al.* 2001a). However, sieving to this extent has two disadvantages. Huge portions of the wheat grain are lost and cannot be reused. This was pertinent as in later trials only a small amount of sample remained after the chick bioassays. It also would mean that the RVA profile was not representative of the whole grain. It is thought that amylase originates in the aleurone layer (Bewley and Black 1994) with high levels also present in the crease region (Evers *et al.* 1995). When samples are milled and sieved using the methods discussed here, it appears that the bran remains in larger particles and is largely excluded from the sample during sieving. Being that the aleurone is immediately inside the bran (see chapter one, figure 1.1) which also includes the crease, a sieved sample may underestimate amylase levels. Later samples run in 2007 were also sieved to fractions below 250μm to allow comparison with those run in 2005. Some 2007 samples were also run using whole flour (figure 7.2, note profile 40 was used) and, unexpectedly, this resulted in higher reproducibility. The whole flour analysis (figure 7.2) still suggested high amylase. With the sieved samples, it is possible that the α-amylase could still have been partly responsible for the lack of reproducibility if it was not represented in the fraction in a direct proportion to the level present (Crosbie *et al.* 1999). For this reason, during

later analyses, whole flour and profile 40 was used. Where $AgNO_3$ was used, the solution was of 5mM concentration. The use of 10mM was thought not to have alleviated the problem of reproducibility and may have altered the swelling and solubility of the materials in the samples in other ways, as evidenced by the later peak (figure 7.1).

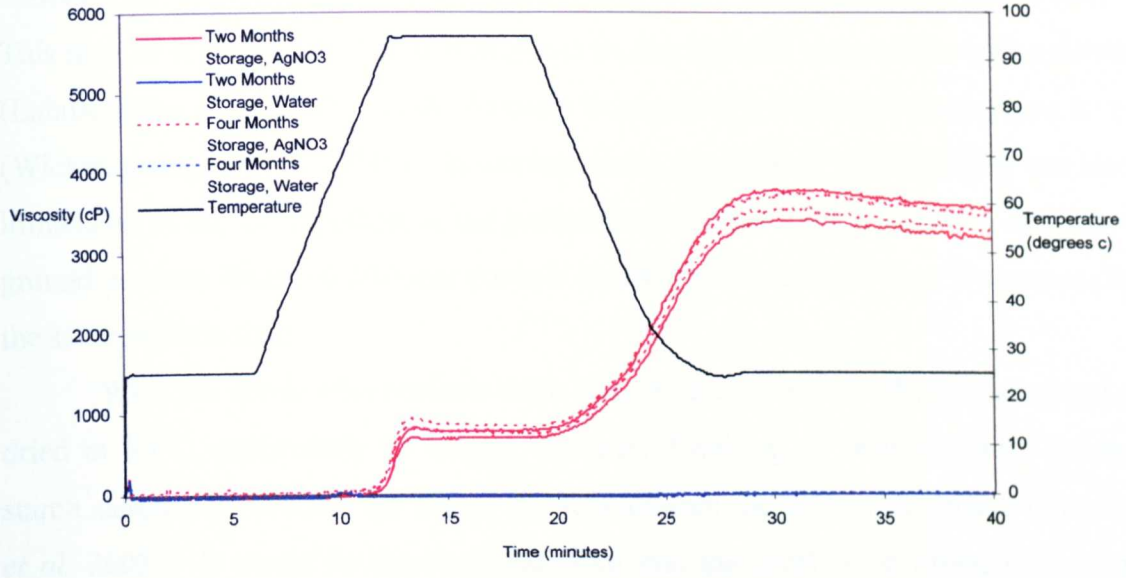


Figure 7.2 The RVA profile of the whole flour of samples stored for two or four months

7.2 Trial 2- Varying Drying Temperatures and Two Wheat Varieties

The individual treatment of these wheat samples is discussed in detail in chapter four (section 4.1 and table 4.1). In summary, two wheat varieties were used; Clare and Einstein. Control samples were not treated in any way, whereas treated samples were briefly soaked (to approximately 220g moisture/kg) and then dried at 70, 85 or 100°C before being used to formulate diets.

As discussed, the temperature-treated wheat samples were significantly less well digested in the chick than the control, non-treated samples. Wheat dried at 85°C was significantly less well digested in the duodenum, particularly wheat variety Einstein. Although there was no significant effect on AME, there was a trend that supported the observation in starch digestibility; wheat treated at 85°C had a numerically lower AME than all other treatments. Despite being soaked before

drying, all samples were of very similar moisture contents (Appendix A) so whether this was the cause of the decreased coefficient of digestibility with 85°C is unlikely.

It would be expected that the RVA profiles of wheat varieties Einstein and Clare would be different. Einstein is a hard wheat, whereas Clare is soft. Although the RVA profile reflects the viscosity of the starch wheat variety is known to affect RVA profile. Hard wheat varieties may have lower peak viscosities than soft wheat varieties; the swelling power of soft wheats is greater (Wickramasinghe *et al.* 2005). This may be related to the fact that amylose to amylopectin ratio varies with cultivar (Labuschagne *et al.* 2007). Peak viscosity is negatively correlated to amylose level (Wickramasinghe *et al.* 2005). However, water penetration and swelling are also influenced by the composition of the particulates and the hard and soft grains when ground produce flours of different particle characteristics even if they are nominally the same particle size.

With the above observations there may be differences in the wheat samples dried at 85°C, particularly of variety Einstein. Swelling of heat moisture treated starch samples is reduced, the higher the temperature the greater the effect (Zweifel *et al.* 2003). It would be expected therefore that the profiles of Einstein samples heated at 100°C would show the lowest RVA parameters.

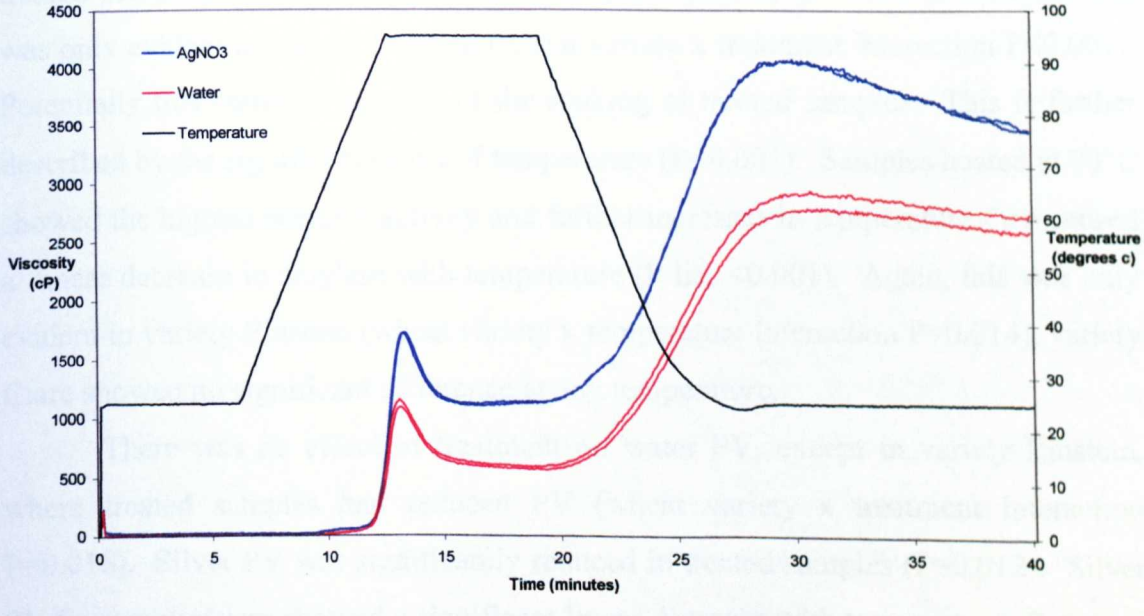


Figure 7.3 The RVA profile of untreated wheat, variety Clare, as used in trial three. Sample size of 3g (on a dry matter basis). Multiple lines of the same colour indicate replicates.

Figure 7.3 shows the RVA profile of untreated wheat variety Clare as used in trial two. This indicates the high reproducibility of the RVA.

7.2.1 Results and Discussion

The statistical analysis of variety, temperature and temperature treatment versus non treated controls, on amylase, PV, EV and breakdown is shown in tables 7.3 to 7.9 inclusive.

i. Variety

There was a significant effect of wheat variety on all RVA parameters, as expected. Einstein has significantly higher amylase activity than Clare ($P<0.001$). This was further evidenced by lower water PV and EV in Einstein ($P<0.001$ in both cases), but a higher silver nitrate PV in Einstein ($P<0.001$). The larger the difference between RVA profiles, the larger the amylase activity. Breakdown was greater for Einstein in both water and silver ($P<0.001$ in both cases).

ii. Treatment and Temperature

Any extent of temperature treatment (termed 'treatment') as compared with non-treated controls had a significant effect on RVA parameters. Surprisingly, treated samples had significantly higher amylase activity ($P=0.007$), although this was only evident in variety Einstein (wheat variety x treatment interaction $P<0.001$). Potentially this could be because of the soaking of treated samples. This is further described by the significant effect of temperature ($P<0.001$). Samples heated at 70°C showed the highest amylase activity and further increases in temperature then caused a linear decrease in amylase with temperature (P lin. <0.001). Again, this was only evident in variety Einstein (wheat variety x temperature interaction $P=0.014$), variety Clare showed no significant difference at any temperature.

There was no effect of treatment on water PV, except in variety Einstein, where treated samples had reduced PV (wheat variety x treatment interaction $P=0.016$). Silver PV was significantly reduced in treated samples ($P=0.012$). Silver PV for variety Clare showed a significant linear decrease with temperature ($P=0.004$; P lin. $=0.007$). Water breakdown was significantly lower in treated samples

(P=0.007) and this was particularly evident in variety Einstein (wheat variety x treatment interaction P=0.037). End Viscosities in both water and silver were significantly greater in treated samples than control (P=0.005 and P<0.001 respectively). In terms of temperature, there was a linear increase in water EV with increasing temperature (P=0.001; P lin. <0.001). There was however, an interaction of temperature with variety (P=0.003), the increase was seen only in variety Einstein.

Generally, the heat treatment of wheat has significantly reduced the peak viscosities of the flour, when pasted with amylase inhibitor. Breakdown and end viscosities were increased by heat treatment and the effect on EV appears to be greater with increasing temperature. The two wheat varieties performed quite differently. As predicted, Einstein has lower water PV and EV and this is agreement with the literature (Wickramasinghe *et al.* 2005). Einstein is a hard wheat and the continuous protein matrix entraps starch making the leaching process difficult (Short *et al.* 2000). Einstein seems to be particularly sensitive to heat treatment as often effects are seen with Einstein that were not seen with Clare. It is also likely that the increased moisture content prior to drying has encouraged damage to the starch crystalline structure, reducing peak viscosity. It is possible that the moisture content may have also increased amylase activity, although the temperature used to dry the wheat would be expected to inactivate the amylase.

The RVA viscosities reflect hydration and swelling of the particles of wheat. The higher protein levels in Einstein may reduce the initial swelling of the samples, thus reducing PV. However, the strength of the swollen particulate could protect the macromolecular structure from breakdown, hence EV are proportionally higher. Heat treatment would seem to be having a more noticeable effect on the protein than the starch itself. The change in the hydration pattern of the particulates may influence digestion as enzymic breakdown requires hydration.

It is known that when flour is heated, starch and protein components may be altered. When the temperature and moisture content are high, the starch may become cooked, as is discussed in chapters four and five. However, there is a suggestion that here may be a stage of crystal perfection, whereby at certain temperatures, the crystalline structure is actually strengthened rather than disrupted, by heat treatment (Jacobs *et al.* 1997). This specific temperature may be just below temperatures at

which the starch becomes cooked and may be related to mobilisation of amylopectin (Slade and Levine 1988). This would presumably result in an increased peak viscosity and a decreased starch digestibility.

The RVA is designed to investigate properties of starch and is based on ingression of water and the resulting swelling of the starch granules. However, wheat flour contains many other components aside from starch (see table 1.1) and it is likely that an RVA profile will be affected by changes in protein for example. As discussed above, it is possible that these changes in protein structure will not be linear with temperature, hence the unexpected result with Einstein flour heated at 85°C.

iii. Amylase

Table 7.3 Effect of temperature and treatment on endogenous relative amylase levels amylase. Means are of two replicates.

	Drying Regime					Treatment		
Variety	**Control**	**70**	**85**	**100**	**Mean**	**Variety**	**+**	**-**
Clare	0.53	0.53	0.52	0.41	0.50	**Clare**	0.49	0.53
Einstein	0.56	1.00	0.85	0.59	0.75	**Einstein**	0.89	0.56
Mean	0.54	0.77	0.69	0.50		**Mean**	0.65	0.54

ANOVA			Polynomial ANOVA			
	P	**sed**			**P**	**sed**
Wheat Variety	<0.001	0.026	**Wheat Variety**		<0.001	0.031
Treatment	0.007	0.030	**Drying Regime**		0.001	0.038
Drying Regime	<0.001	0.037		**Linear**	<0.001	
Wheat Variety x Treatment	<0.001	0.042		**Quadratic**	0.167	
Wheat Variety x Drying Regime	0.012	0.052	**Variety x Drying Regime**		0.024	0.054
				Linear	0.008	
				Quadratic	0.908	

Table 7.4 Effect of temperature and treatment on peak viscosity (cP) in water. Means are of two replicates.

	Drying Regime					Treatment		
Variety	**Control**	**70**	**85**	**100**	**Mean**	**Variety**	**+**	**-**
Clare	1122	1231	1143	1162	1164	**Clare**	1179	1122
Einstein	1142	939	1116	1150	1086	**Einstein**	1067	1142
Mean	1132	1084	1130	1156		**Mean**	1123	1132

ANOVA			Polynomial ANOVA			
	P	**sed**			**P**	**sed**
Wheat Variety	0.003	18.8	**Wheat Variety**		<0.001	18.7
Treatment	0.702	21.7	**Drying Regime**		0.050	22.9
Drying Regime	0.069	26.5		**Linear**	0.020	
Wheat Variety x Treatment	0.016	30.6		**Quadratic**	0.636	
Wheat Variety x Drying Regime	0.001	37.5	**Variety x Drying Regime**		0.001	32.3
				Linear	<0.001	
				Quadratic	0.019	

Table 7.5 Effect of temperature and treatment on breakdown (cP) in water. Means are of two replicates.

	Drying Regime					Treatment		
Variety	**Control**	**70**	**85**	**100**	**Mean**	**Variety**	**+**	**-**
Clare	546	585	519	494	536	**Clare**	532	546
Einstein	649	513	629	578	592	**Einstein**	573	649
Mean	597	549	574	536		**Mean**	553	597

ANOVA			Polynomial ANOVA			
	P	**sed**			**P**	**sed**
Wheat Variety	<0.001	10.8	**Wheat Variety**		0.011	11.8
Treatment	0.007	12.4	**Drying Regime**		0.086	13.9
Drying Regime	0.096	15.2		**Linear**	0.386	
Wheat Variety x Treatment	0.037	17.6		**Quadratic**	0.041	
Wheat Variety x Drying Regime	<0.001	21.5	**Variety x Drying Regime**		0.001	19.7
				Linear	0.001	
				Quadratic	0.005	

Table 7.6 Effect of temperature and treatment on peak viscosity (cP) in silver nitrate solution. Means are of two replicates.

	Drying Regime					Treatment		
Variety	**Control**	**70**	**85**	**100**	**Mean**	**Variety**	**+**	**-**
Clare	1720	1884	1744	1642	1748	**Clare**	1757	1720
Einstein	1782	1880	2064	1836	1891	**Einstein**	1927	1782
Mean	1752	1882	1904	1739		**Mean**	1752	1842

ANOVA			Polynomial ANOVA			
	P	**sed**			**P**	**sed**
Wheat Variety	<0.001	24.1	**Wheat Variety**		<0.001	20.3
Treatment	0.012	27.8	**Drying Regime**		0.001	24.9
Drying Regime	0.003	34.0		**Linear**	0.001	
Wheat Variety x Treatment	0.088	39.3		**Quadratic**	0.005	
Wheat Variety x Drying Regime	0.004	48.1	**Variety x Drying Regime**		0.002	35.2
				Linear	0.007	
				Quadratic	0.002	

Table 7.7 Effect of temperature and treatment on breakdown in silver nitrate solution. Means are of two replicates.

	Drying Regime					Treatment		
Variety	**Control**	**70**	**85**	**100**	**Mean**	**Variety**	**+**	**-**
Clare	609	694	584	521	601	**Clare**	600	609
Einstein	800	799	927	739	816	**Einstein**	822	800
Mean	704	746	756	630		**Mean**	711	704

ANOVA			Polynomial ANOVA			
	P	**sed**			**P**	**sed**
Wheat Variety	<0.001	19.3	**Wheat Variety**		<0.001	18.0
Treatment	0.783	22.3	**Drying Regime**		0.002	22.0
Drying Regime	0.003	27.3		**Linear**	0.002	
Wheat Variety x Treatment	0.511	32.0		**Quadratic**	0.012	
Wheat Variety x Treatment x Drying Regime	0.008	38.6	**Variety x Drying Regime**		0.005	31.1
				Linear	0.043	
				Quadratic	0.003	

vi. End Viscosity (Water)

Table 7.8 Effect of temperature and treatment on end viscosity (cP) in water. Means are of two replicates.

	Drying Regime					Treatment		
Variety	**Control**	**70**	**85**	**100**	**Mean**	**Variety**	**+**	**-**
Clare	2668	2846	2804	2886	2801	**Clare**	2845	2668
Einstein	2180	2095	2284	2730	2322	**Einstein**	2370	2180
Mean	2424	2470	2544	2808		**Mean**	2607	2424

ANOVA			Polynomial ANOVA			
	P	**sed**			**P**	**sed**
Wheat Variety	<0.001	41.8	**Wheat Variety**		<0.001	38.9
Treatment	0.005	48.3	**Drying Regime**		<0.001	47.2
Drying Regime	0.001	59.1		**Linear**	<0.001	
Wheat Variety x Treatment	0.902	68.3		**Quadratic**	0.058	
Wheat Variety x Treatment x Drying Regime	0.003	83.6	**Variety x Drying Regime**		0.002	66.7
				Linear	<0.001	
				Quadratic	0.442	

vii. End Viscosity (Silver Nitrate)

Table 7.9 Effect of temperature and treatment on end viscosity (cP) in silver nitrate solution. Means are of two replicates.

	Drying Regime					Treatment		
Variety	**Control**	**70**	**85**	**100**	**Mean**	**Variety**	**+**	**-**
Clare	3470	3658	3616	3574	3579	**Clare**	3616	3470
Einstein	3263	3552	3558	3690	3516	**Einstein**	3600	3263
Mean	3366	3605	3587	3632		**Mean**	3608	3366

ANOVA			Polynomial ANOVA			
	P	**sed**			**P**	**sed**
Wheat Variety	0.101	34.5	**Wheat Variety**		0.637	32.2
Treatment	<0.001	39.8	**Drying Regime**		0.551	39.4
Drying Regime	0.664	48.7		**Linear**	0.523	
Wheat Variety x Treatment	0.043	56.3		**Quadratic**	0.390	
Wheat Variety x Treatment x Drying Regime	0.113	68.9	**Variety x Drying Regime**		0.066	55.7
				Linear	0.030	
				Quadratic	0.397	

7.3 Trial 3 –Two Drying Regimes and Wheat Grown at Two Sites

The individual treatment of these wheat samples is discussed in detail in chapter five (section 5.1.1 and figure 5.1). In summary, one wheat variety was used; Einstein. Samples were harvested from two sites, the John Innes Center and neighbouring Church Farm, each on two dates. Samples were then dried under ambient conditions or at 100°C.

As discussed in chapter five, drying wheat at 100°C may have an effect for poultry nutrition, as opposed to drying at ambient temperature. Coefficient of Starch Digestibility (CAD) was improved with harsh drying, especially if the wheat was harvested immediately after maturity. During high heat moisture treatment, wheat starch may begin to melt and lose its crystalline structure. Becker *et al.* (2001b) found that with heating for increasing lengths of time at 140°C and 500g moisture/kg, crystallinity was progressively lost. It should be noted that this represents much higher temperature and moisture than used in the current experiment. However, if starch crystallinity changes had occurred in the case of the current samples, it would suggest that a decrease in crystallinity, caused by 100°C temperature treatment, increases nutritional value. The RVA was used to determine the level of 'cook'. If the samples that had been heated had lost crystallinity, compared to those dried at ambient temperatures, the peak viscosity and end viscosity as measured by the RVA pasting profile would be greatly reduced. Damaged granules do not exhibit the same swelling properties as native granules (Becker *et al.* 2001b). It is also likely that if crystalline order has been lost the starch will swell before the onset of heating and a cold swelling peak will be seen. The observation that the second harvest date had a significantly increased CAD could be related to an increase in endogenous amylase levels present in the wheat. As wheat remains in the field, amylase levels increase (Hetland *et al.* 2007). This will also be determinable using the RVA. The profile of the sample using water will be lower than the equivalent sample run using silver nitrate, the difference being indicative of the amylase level.

7.3.1 Results and Discussion

Figures 7.4 and 7.5 show examples of the RVA profiles of four of the wheat samples. Figure 7.4 shows wheat grown at JIC, harvested on the first date, and dried

at either ambient conditions (A) or 100°C (B). Figure 7.5 shows equivalent samples, grown at CF. There is no evidence of a cold swelling peak in these samples. The statistical analysis of the effects of drying regime, harvest date and site on peak viscosity and end viscosity, in water and silver nitrate solution is shown in table 7.10, 7.11, 7.12 and 7.13. Since the RVA profiles did not follow a typical curve in the temperature treated samples and there was no discernable trough, breakdown and amylase levels were not calculated.

Table 7.10 Effect of drying regime, harvest date and field site on peak viscosity (cP) in water. Means are of two replicates

	Harvest	1		2		
Drying Regime	**Site**	**CF**	**JIC**	**CF**	**JIC**	**Mean**
Ambient		926	808	550	133	604
100°		160	173	463	152	237
Mean		517		324		

	Site	CF	JIC	Harvest	1	2
Drying Regime	**Ambient**	738	470		867	341
	100°	312	162		166	307
Harvest Date	**1**	543	490			
	2	506	142			
	Mean	525	316			

ANOVA

	P	sed
Drying Regime	<0.001	25.8
Harvest Date	<0.001	25.8
Site	<0.001	25.8
Drying Regime x Harvest Date	<0.001	36.5
Drying Regime x Site	0.005	36.5
Harvest Date x Site	<0.001	36.5
Drying Regime x Harvest Date x Site	0.804	51.7

Table 7.11 Effect of drying regime, harvest date and field site on peak viscosity (cP) in silver nitrate solution. Means are of two replicates

	Harvest	1		2		
Drying Regime	**Site**	**CF**	**JIC**	**CF**	**JIC**	**Mean**
Ambient		1134	904	850	1078	991
100°		182	199	731	134	312
Mean		605		698		

	Site	CF	JIC	Harvest	1	2
Drying Regime	**Ambient**	992	991		1019	964
	100°	457	166		191	432
Harvest Date	**1**	658	552			
	2	791	606			
	Mean	724	578			

ANOVA

	P	sed
Drying Regime	<0.001	32.7
Harvest Date	0.021	32.7
Site	0.002	32.7
Drying Regime x Harvest Date	0.002	46.2
Drying Regime x Site	0.002	46.2
Harvest Date x Site	0.262	46.2
Drying Regime x Harvest Date x Site	<0.001	65.3

As expected from the RVA profiles, drying regime had a significant effect on both PV and EV. In both the case of water and silver nitrate, when treated at 100°C, the PV and EV were reduced (in all cases, $P<0.001$).

Harvest date also affects PV and EV. With water run samples, PV and EV were reduced at the second date whereas with silver nitrate run samples, PV and EV were increased at the second site. A decrease in water profile suggests increased amylase, which then leads to an increase in the silver nitrate profile as an increased level of amylase is inhibited. This is evidence that amylase increases with time as the grain is left on the plant in the field (Hetland *et al.* 2007).

There are significant site effects for PV and EV, with samples grown at Church Farm (CF) having significantly higher PV and EV. However, this is the case with water and silver nitrate run samples, indicating that the site effect is not related to changes in amylase levels.

Table 7.12 Effect of drying regime, harvest date and field site on end viscosity (cP) in water. Means are of two replicates

	Harvest	1		2		
Drying Regime	**Site**	**CF**	**JIC**	**CF**	**JIC**	**Mean**
Ambient		3016	3041	2869	187	2278
100°		974	847	2639	747	1302
Mean		1970		1610		

	Site	CF	JIC	Harvest	1	2
Drying Regime	**Ambient**	2942	1614		3028	1528
	100°	1806	797		910	1693
Harvest Date	**1**	1995	1944			
	2	2754	467			
	Mean	2374	1206			

ANOVA

	P	sed
Drying Regime	<0.001	65.6
Harvest Date	<0.001	65.6
Site	<0.001	65.6
Drying Regime x Harvest Date	<0.001	92.7
Drying Regime x Site	0.041	92.7
Harvest Date x Site	<0.001	92.7
Drying Regime x Harvest Date x Site	0.007	131.1

Table 7.13 Effect of drying regime, harvest date and field site on end viscosity (cP) in silver nitrate solution. Means are of two replicates

	Harvest	1		2		
Drying Regime	**Site**	**CF**	**JIC**	**CF**	**JIC**	**Mean**
Ambient		3503	3267	3764	3260	3451
100°		1059	821	3526	922	1582
Mean		2165		2868		

	Site	CF	JIC	Harvest	1	2
Drying Regime	**Ambient**	3633	3268		3390	3512
	100°	2293	872		940	2224
Harvest Date	**1**	2281	2049			
	2	3645	2092			
	Mean	2963	2070			

ANOVA

	P	sed
Drying Regime	<0.001	77.4
Harvest Date	<0.001	77.4
Site	<0.001	77.4
Drying Regime x Harvest Date	<0.001	109.4
Drying Regime x Site	<0.001	109.4
Harvest Date x Site	<0.001	109.4
Drying Regime x Harvest Date x Site	<0.001	154.7

The RVA profiles show hydration or swelling properties of the wheat, but do not provide definitive information on the crystallinity of the starch. Differential Scanning Calorimetry will show the temperatures and amount of energy required to melt the crystals in the starch. Low energy would indicate that the starch was exposed to heat and moisture sufficient to cause the native crystalline structure of the starch granules to be lost. DSC was therefore carried out on the samples that are shown in figures 7.5 and 7.6, according to the method described in chapter two. The means and standard deviation for the DSC endotherm parameters are shown in table 7.14. The onset temperatures, end temperatures, peak heights and delta H were not affected by heat treatment (P= 0.12, 0.34, 0.32 and 0.46 respectively). This indicates that the starch granules still maintain the native semi-crystalline structure and the time, temperature and moisture regimes which the wheat samples were exposed to during drying were not severe enough to disrupt the starch.

Table 7.14 Means, standard deviations and P value (t-test)* for DSC endotherm parameters

Site	Drying Regime	Onset (°C)			End (°C)			Peak (°C)			Delta H (J/g)		
		Mean	SD	P	Mean	SD	P	Mean	SD	P	Mean	SD	P
JIC	100°C	60.20	0.34	0.12	75.45	1.13	0.34	66.09	0.52	0.32	7.54	0.48	0.46
CF	100°C	61.84	0.10		74.79	0.87		68.17	0.44		7.36	0.16	
JIC	Ambient	60.78	0.39		73.08	0.20		65.99	0.35		6.65	0.84	
CF	Ambient	60.94	0.83		77.06	0.54		69.17	0.33		8.66	0.13	

* P value refers to a t-test comparing the replicates of those dried at 100°C to those dried at ambient temperature.

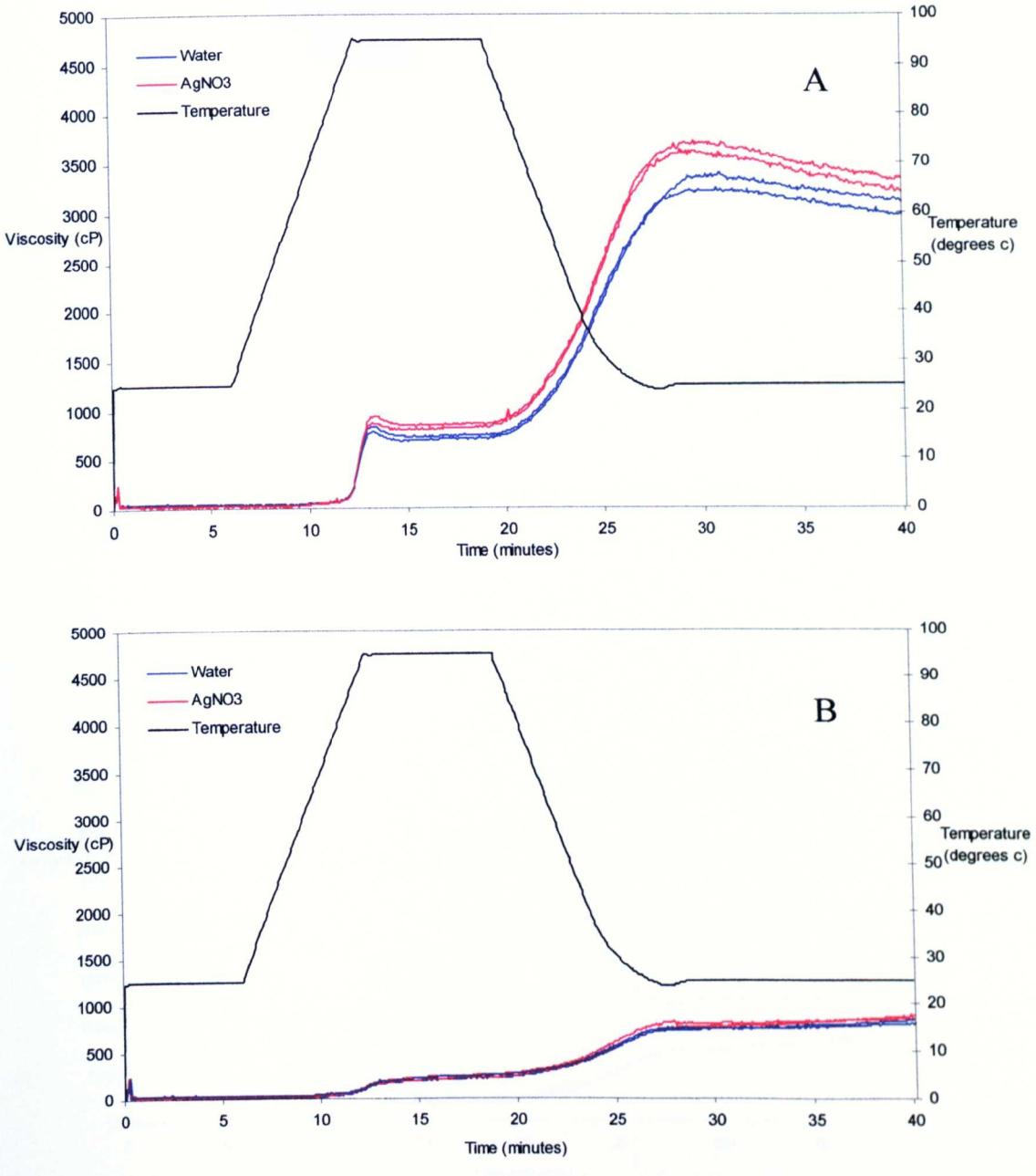


Figure 7.4 The RVA profiles of wheat grown at JIC, harvested at first date, and dried under ambient conditions (A) and 100°C (B). Sample size of 3g (on a dry matter basis). Multiple lines of the same colour indicate replicates.

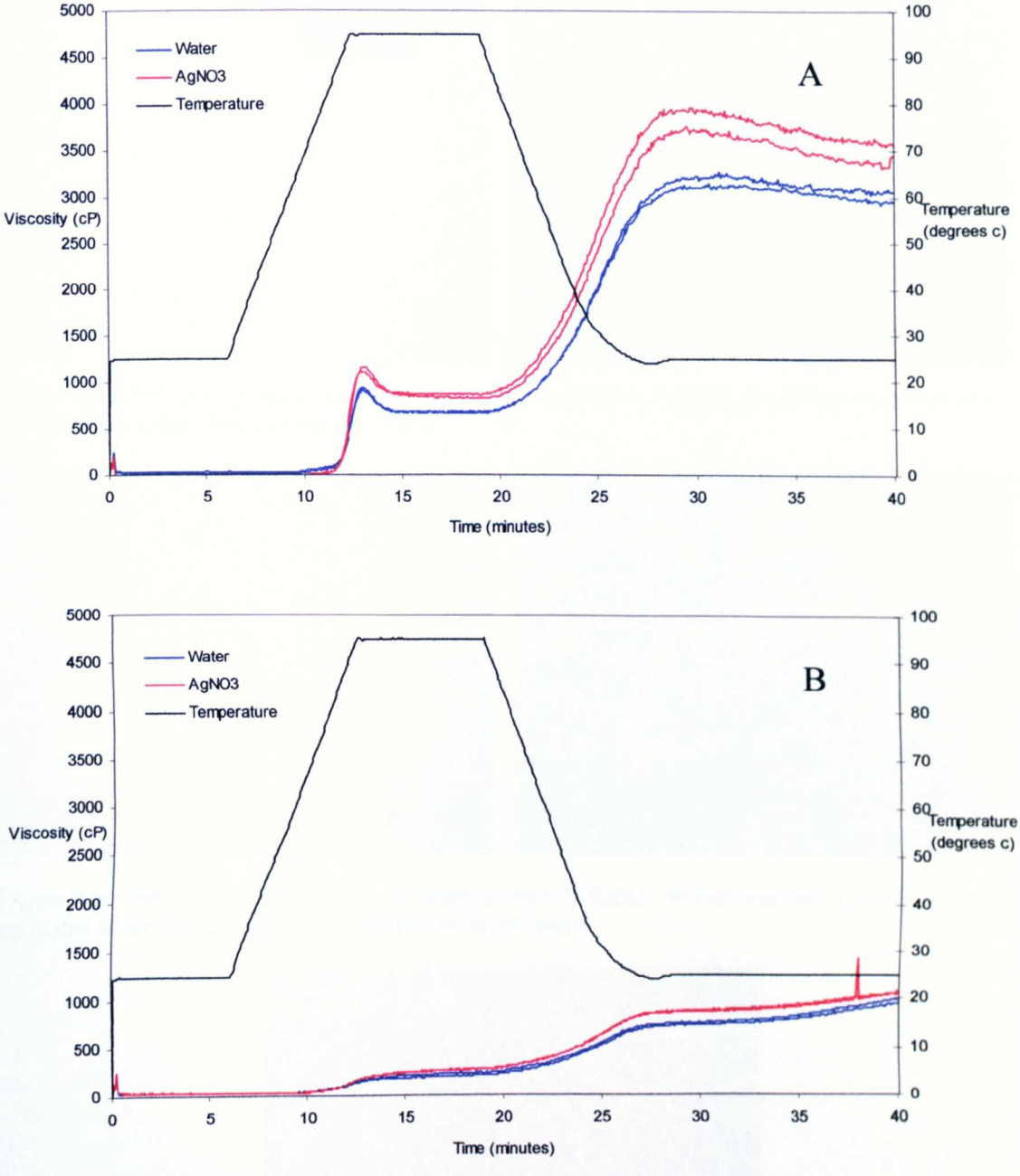


Figure 7.5 The RVA profiles of wheat grown at CF, harvested at first date, and dried under ambient conditions (A) and at 100°C (B). Multiple lines of the same colour indicate replicates

Photographs of starch granules taken under normal and polarised light are shown in figure 7.6. The same samples were examined under the microscope as were analysed using the DSC. In all cases under polarised light, the 'Maltese cross' can be seen, as indicated (*) in figure 7.6 B. This is further indication that the starch maintains its crystalline order (Baldwin *et al.* 1994; Hug-Iten *et al.* 1999). A graticule, photographed in the same way and is shown as the same size, is given for scale. The scale used is μm x 10; each mark on the graticule represents 10 μm.

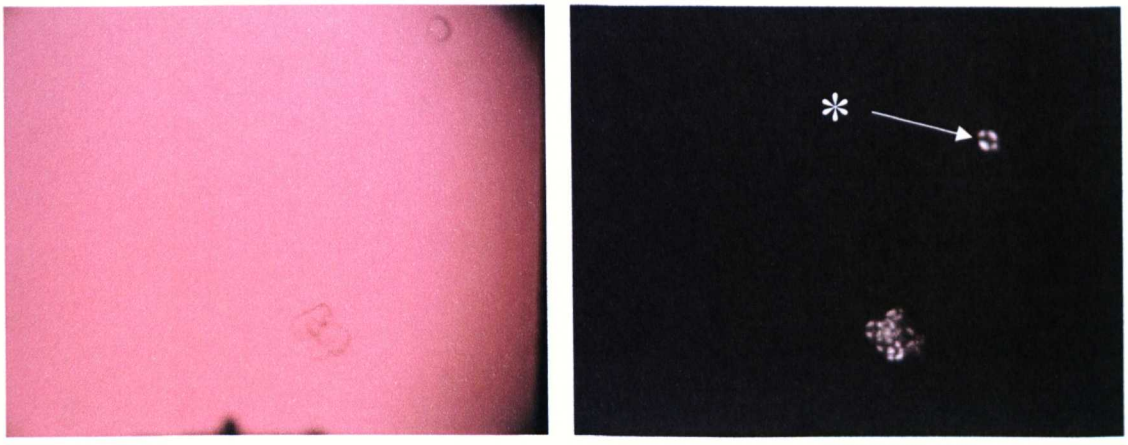


Figure 7.6 a (left, normal light) and 7.6b (right, polarised light). Wheat that had been grown at JIC, harvested at the first date and dried at 100°C

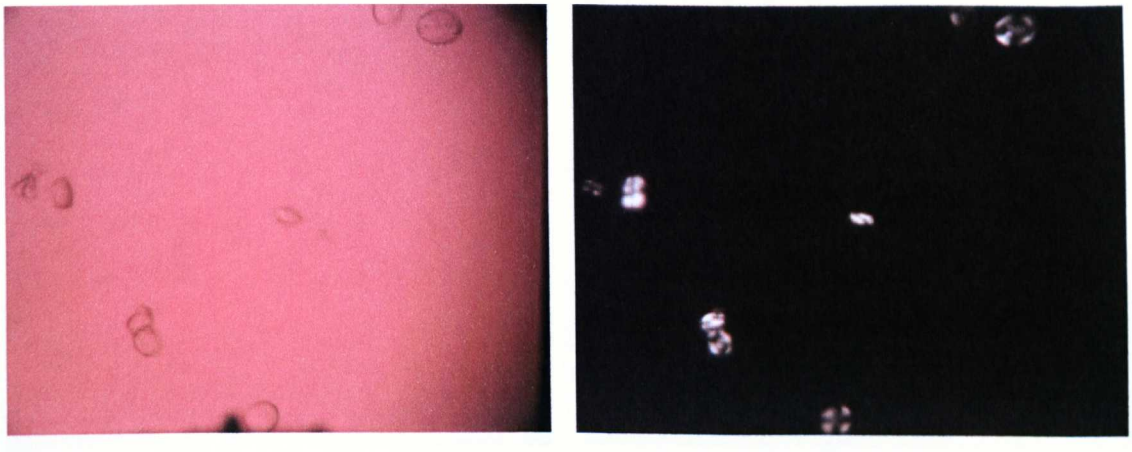
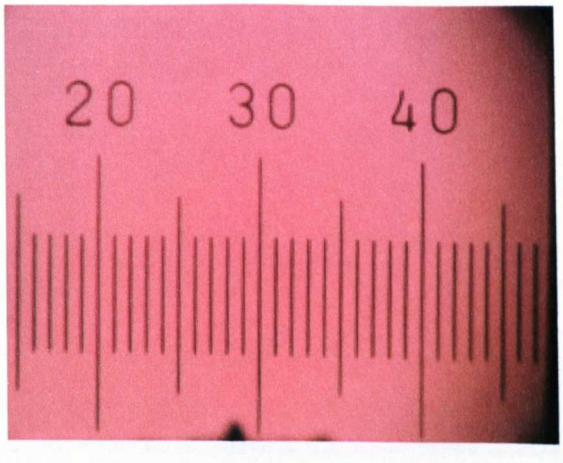


Figure 7.6c (left, normal light) and 7.6d (right, polarised light). Wheat that had been grown at JIC, harvested at the first date and dried at ambient temperature



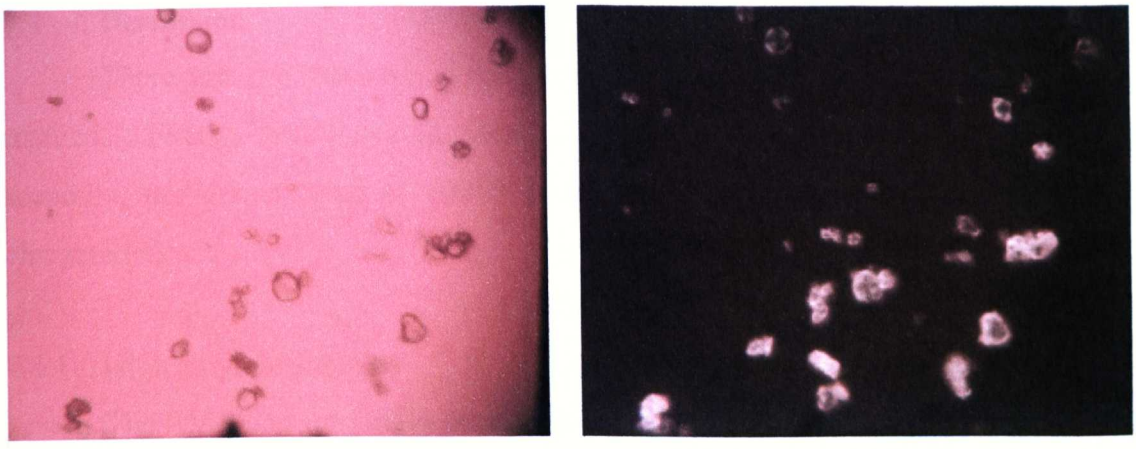


Figure 7.6e (left, normal light) and 7.6f (right, polarised light). Wheat that had been grown at CF, harvested at the first date and dried at 100°C

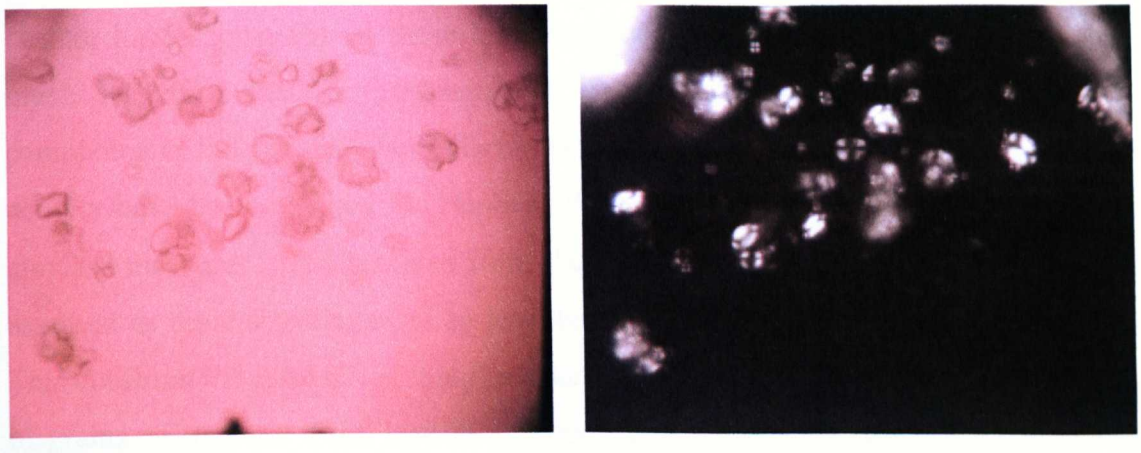
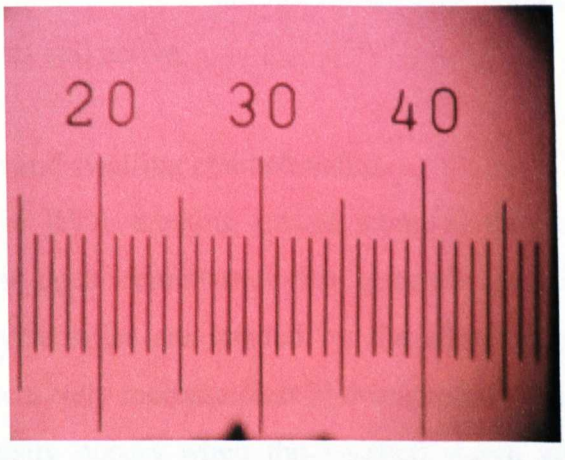


Figure 7.6g (left, normal light) and 7.6h (right, polarised light). Wheat that had been grown at CF, harvested at the first date and dried at ambient temperature



7.4 Discussion

There are two features of the grains used in the current trial that are very marked. Firstly, grains would seem to exhibit very high amylase levels and secondly, the grains dried at 100°C in trial 3 showed very low water swelling characteristics.

7.4.1 High amylase samples

The RVA was originally created to be a robust instrument for the measure of amylase activity by comparison of the pasting curves with and without the silver ions, as the metal is known to inhibit amylase activity. The results discussed in this chapter have shown that at these high amylase levels results from the RVA were much less reproducible than normally anticipated from this method. Since completion of the practical work discussed in this thesis the grains with high amylase activity have been assessed for Hagberg Falling Numbers, the standard method for amylase measures. The samples from the second harvest date again could not be assessed as the viscosities were so low that they could not be measured accurately. This confirms the assumption that these samples from the 2004 harvest were atypical of grains. However, it did lead to an opportunity to demonstrate that the amylases were still very potent after storage for two years. Also, the pre-heat treatment of the grains at ambient and at 100°C also did not much affect change in the activity and the induced enzyme was still active.

7.4.2 Heat treatment and swelling characteristics

As well as the RVA creating pasted samples that can be compared for amylases activities, it does demonstrate the general viscosity profile of pasted starches. The high viscosities are created by the starch granule gelatinising and imbibing water to extensively increase their hydrodynamic volume. For whole wheat samples a high viscosity occurs when the swollen starch granules swell to their maximum. The granules are then broken down by the shearing of the paddle in the RVA and the viscosity will decrease a little. The macromolecules, being released from the granules, entangle and hence retain the viscous nature of the paste. When the sample is cooled the viscosity will increase.

The wheat flours which were dried under ambient conditions, in the presence of the amylase inhibitor silver nitrate, show the typical RVA pasting curves expected

for wheat flours. This is not the case for the samples that had been dried at 100°C for trial 3. It is evident from figures 7.4 and 7.5, that the pasting properties of the wheat starch were altered by the harsh drying regime, compared with the samples dried at ambient conditions. The low viscosity pasting curves indicated that the harsh drying had a marked effect on the pasting curves. Examples are given in figures 7.4 and 7.5, although not all data is graphically presented here; parameters are given in table 7.10, 7.11, 7.12 and 7.13. It was the samples with the low pasting profiles that showed an increase in digestibility (chapter five).

As discussed above and in section 1.2.6, the RVA curves are indicative of viscosity, which is due to gelatinisation of native starch and the swelling of the granules. Low levels of native starch in the sample would give rise to low viscosity curves. Hence the first consideration is the amount of starch in the sample. All samples were adjusted to have the same dry matter content when pasted in the RVA and therefore the starch in each sample should be the same, what ever the drying condition used. However, the nature of the starch could be altered. If the starch had undergone gelatinisation in the initial heat treatment, it could be expected to swell in cold water and reduce the amount of native starch that increases in volume at the gelatinisation point. It does not take much precooked starch to alter an RVA pasting profile. In addition, pre cooking of the starch would be expected to increase its rate of digestion.

There are several ways that the status of starch can be investigated. One of the most effective is to view the starch granules under plain polarised light. When starch crystallinity is lost, the birefringence pattern, a feature of plain polarised light passing through the concentric crystalline regions of the native starch granules, is not observed (Moran 1987). The typical "Maltese cross" pattern is seen clearly in samples that were grown at JIC and dried at ambient temperature (figure 7.6d) and 100°C (figure 7.6b). It is also seen clearly in the samples grown at Church Farm and dried at ambient temperature. The phenomenon is seen, although maybe not as clearly, in the equivalent sample dried at 100°C. Therefore all the samples appear to show some order, as shown by the presence of birefringence.

Another method of investigating order in the samples is to measure the energy required to melt out the helical order of the amylopectin. Major loss of crystalline order is not supported by the DSC results (table 7.14), where the average

values are ~7.5 J/g for the 100°C samples and 6.65 and 8.66 J/g for the non heated samples. These values are all relatively low for wheat flours and show poor reproducibility. These facts may be associated with the samples having high amylase levels. The samples were run in water rather than silver nitrate solution and the low values might reflect amylase attack during the assay. However, the DSC and the polarised light photographs show no marked changes in the native structures of the starch. It would also be surprising if the heating regime had altered the starch to a marked extent. The melting temperature of the starch crystallites is dependent on the water content. A typical state diagram for starch would indicate that to enable the crystallites to melt, the water content would have to be in excess of 500g/kg. Although the moisture content of the grains coming from the field may have been high (320g/kg), this would not have been sufficient to plasticise the starch to allow it to melt when heated at 100°C. In a study during which durum wheat semolina was heated to 80°C or 100°C, birefringence was retained in 0.80 of granules and only completely lost in 10% of granules (Guler *et al.* 2002). They too observed DSC endotherm peak. The authors suggest that at less than 300g/kg, the moisture content was too low to allow loss of crystallinity. The study of Becker *et al.* (2001b) reported loss of crystallinity in maize starch at moisture content of 500g/kg.

However, the concept of limited water being at the surface of the grains and on heating the starch granules closest to the edge of the grains having sufficient water to loose their crystalline is feasible. Some starch may have been cooked, but the great majority should have been unchanged and this is supported by the DSC and microscopy evidence. The proposed low level of cook for the samples would not correspond to the RVA patterns observed, nor give an explanation for the change in digestibility that occurred.

The current work has clearly demonstrated that some of the current understanding on drying whole wheat and its subsequent digestion is flawed and further study would be required to identify the interactions between high amylase, heat treatment and digestion.

The results of this rheology work is discussed and concluded in the following chapter, in relation to the results of the chick bioassays.

Chapter Eight

General Discussion

The aim of the current project was to investigate the effects of adverse simulated climatic / peri and post harvest storage conditions on the nutritional quality of wheat for poultry. The objectives were to test some common features that may affect quality of wheats used for animal feeds, such as high moisture prior to harvest and the extreme temperatures that are commonly used to prepare wheat harvested under damp conditions for storage. Particular emphasis was on starch digestibility, as determined by a chick bioassay, and the wheat quality as measured by viscosity of the pasted ground wheat. The latter technique required the pasting profile analysis using a development of a Rapid Visco Analyser. The present chapter discusses these aims and objectives, and presents the results of statistical analysis of the relationship between RVA data and chick trial parameters, which relate *in vitro* data to *in vivo* data.

There were two interesting and unanticipated observations concerning the wheat samples used. Firstly, several samples of wheat displayed unusually high levels of alpha amylase. In some cases, the levels were beyond those which could be estimated using the RVA technique. Amylase activity remained despite high temperature treatment and ambient storage. Secondly, the sample of one of the heat-treated wheats failed to exhibit expected hydration behaviour. However, the starch appeared not to have been structurally altered compared to control samples. These two general observations will be discussed here in more detail.

8.1 Alpha amylase

The samples of wheat used in trial four (chapter six) were found to have extremely high levels of amylase. The level was so high that it could not be estimated accurately using the RVA; there was no discernable peak when pasted in water although there was a reading above the water only control. This pattern was also seen after two years of ambient storage, after which, amylase would be expected to fall.

The chicken produces relatively large amounts of amylase in the pancreas, around 0.30 of all enzymes produced (Klasing 1999). These are secreted into the small intestine for the digestion of starch. The pH of the small intestine is around 7 (Ao *et al.* 2008), which is presumably optimal for avian pancreatic amylases. It could be assumed that increases in wheat endogenous amylases would be beneficial for the digestive process. However, as discussed in chapter six, there was no difference in nutritional value of wheat samples (in terms of starch digestibility and AME) that showed extremely high amylase levels (Deben) and that which did not (Clare). The calculation of relative amylase levels suggested by Collado and Corke (1999) was used and gave the results presented in table 8.1. The calculation is as follows.

$$\text{Relative amylase level} = (PV2-PV1)/PV1$$

PV1 is peak viscosity in water and PV2 is peak viscosity in silver nitrate solution, an amylase inhibitor. Collado and Corke (1999) found this to be highly correlated with known amylase levels of various sweetpotato flours.

In the hypothetical situation that amylase levels in a flour are nil, then the peak viscosities with water and silver nitrate will be the same, assuming no other influence. Therefore, the relative amylase level according to this equation, would be 0. When there is some amylase activity, the peak viscosity in water will be lower than that with silver nitrate solution, as the silver nitrate inactivates amylase. So, according to the equation, the relative amylase level will increase from zero. Mathematically, the relative amylase level could be infinite and it appears that there are no values in the literature to compare with the current results.

The peak viscosities of the Deben samples when pasted in water were very low (trial four, chapter seven). The results in table 8.1 should be treated as an indication of relative amylase levels and would not be expected to be exact, as the equation calls for peak viscosity and there was no peak evident with water.

Table 8.1 Relative amylase levels of wheat sample used in trial four

Wheat Sample	Treatment	Relative Amylase Level
Deben	Storage for two months	51.55
Deben	Storage for four months	31.46
Clare	None	3.02

Results were calculated from duplicate runs using both water and silver nitrate, for each sample (see section 2.2.4.*i*

Table 8.1 suggests that, as expected, the samples that were harvested at 320g moisture/kg (*var.* Deben) probably had increased amylase levels compared to the Clare, which was assumed not to have been weather damaged.

It is likely that this increased level of amylase did not benefit the digestive process due to the low pH of the avian gastric region. Cereal amylases have an optimum pH range between 3.6 and 5.7, dependent on the isozyme and at extreme pH values they may become irreversibly inactivated (Greenwood and Milne 1968; Marchylo *et al.* 1976; Muralikrishna and Nirmala 2005). The avian gizzard is estimated to have a pH of approximately 3 (Ao *et al.* 2008), due to secretion of hydrochloric acid in the proventriculus; the cranially positioned region of the tract, which is analogous to the non-ruminant mammalian stomach. It is possible that the pH in the proventriculus is even lower than that of the gizzard and therefore it is likely that the cereal amylases are inactivated before they reach the region of the tract where starch digestion would be expected to take place.

If this project were continued, it would be interesting to paste the samples in the RVA using an acidic solution, to mimic the conditions in the cranial regions of the avian digestive tract. This may indicate whether or not the wheat amylases would affect the degradation of the starch *in vivo*.

8.2 Hydration Properties

The results of trial three (chapter five) suggest that heat treating wheat samples at 100°C may increase starch digestibility. It is difficult to explain why this might be, when it appears from rheological analysis that the starch is not able to hydrate and swell, hence showing increased viscosity, in the way that would be expected. For enzymatic digestion the starch would require movement of water and

enzyme into the matrix. The possible reasons for the lack of viscosity will be discussed.

8.2.1 Non Starch Components of Wheat

It should be noted that the original wheat grains were heated and then ground. It was the fine powder that was assessed for the current viscosity work, rather than purified starch. Therefore the low RVA profiles and altered digestibility could be due to the non-starch elements affecting the starch's behaviour. The lack of viscosity in the RVA indicates that water may not be able to penetrate the starch granules, at the expected gelatinisation temperature, and thus does not cause the expected swelling and increase in hydrodynamic volume. In addition to the starch in the endosperm there are other components and particularly the storage protein (gluten in the endosperm) and the lipids (especially free fatty acids) within the starch granules and those in the endosperm.

8.2.2 Amylose –lipid Complex Formation

In most studies on the swelling of heat treated grains and starches the factor most implicated in the differences in swelling behaviour is the formation of amylose:lipid complexes (Becker *et al.* 2001b). Levine and Slade (1990) suggested three separate mechanisms for amylose:lipid complexation. Amylose may complex with endogenous lipid within the granules and then crystallise. Amylose may, however, already be complexed with the lipid within the granules before it becomes crystalline, or there maybe an interaction of amylose with endogenous lipid. Amylose: lipid complexation can, although not always, be detected by DSC and wide angled X-ray scattering. For the heated samples used in this study, the only observable crystallinity was that typical for the amylopectin in cereal starch. To get observable amylose:lipid complexes the starches may need to be heated with sufficient water to mobilize the amylose. Therefore although there would be sufficient amylose and free fatty acids present to form the amylose:lipid complexes, it does not appear that this phenomenon is the reason for the low viscosity RVA profile observed in the current work.

8.2.3 Protein Changes

It is possible that the proteins have been modified during the initial heating of the grain. The formation of a protein matrix that entraps starch, limiting swelling has been suggested (Guler *et al.* 2002). Zweifel *et al.* (2003) found that drying at up to 100°C denatured proteins, but protein networks were preserved. In the current work general denaturation of the proteins is unlikely as the amylases retained their activity. However, the storage proteins may have been altered. The gliadins and gluteninins may melt at the temperatures and moisture contents to which the grains were subjected to during the heating at 100°C. Weegels *et al.* (1994a) demonstrated that gluten changed when heated at 80°C for 30mins at moisture contents of 200g/kg and Schofield *et al.* (1983) showed that temperatures of 55°C were sufficient to alter the disulfide interchanges. These prolamines are known to form films when heated and these films, once dried, are impervious to water (Schofield *et al.* 1983; Weegels *et al.* 1994a). The concept could be that on heating, in the presence of limited moisture the storage proteins melt and form a film surrounding the starch (see figure 8.1).

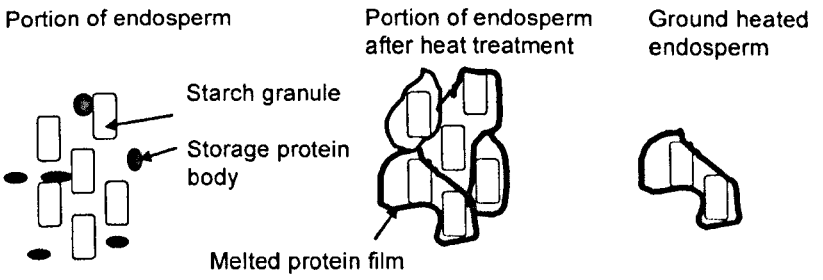


Figure 8.1 Suggested mechanism for protein changes on heating

Such a protein film could either prevent the ingress of water during the pasting within the RVA or maybe the starch granule swelling is confined by the film. This could lead to low hydrodynamic volumes being achieved and the viscosities measured by the RVA would be low, despite the starch not being cooked prior to the pasting.

Once entering the digestive tract the low pH environment and the endogenous chick proteases could rapidly break down the protein film and make the starch more readily digestible. The concept of the heat treatment of grains affecting the storage proteins to change the digestibility of the starch is novel and would require substantial work to confirm.

Part of the reasoning for the linking of protein film formation with the low pasting curves is that the variety that appears to be most sensitive is the hard wheat, Einstein. If this concept is correct there may not be a simple relationship between the protein, the heat treatment, the RVA curve and the digestibility. It was shown in trial two (chapter four) that samples heated (at 70, 85 or 100°C) had decreased overall starch digestibility compared to that of the control, non temperature treated wheat. The Einstein samples heated at 85°C had a very low Coefficient of Duodenal Apparent Digestibility, but the RVA profiles showed high viscosities, hence the hydration factors were not affected. The combination of heat and protein level may be critical for film formation and its susceptibility to breakdown. It is known that hydrated glutens decrease in their viscosity when heated up to 60°C, and then there is marked increase in viscosity when they reach 90°C, but the moisture content also affects this range of temperatures (Attenburrow *et al.* 1990; Weegels *et al.* 1994b). It must be remembered that, in the case of trial two, the temperature treated samples had prior soaking included in their treatment.

The very low RVA profiles seen on one occasion for the Einstein and the low coefficient of digestibility with normal to low RVA viscosities is interesting and requires additional work to confirm.

8.2.4 Other Factors

There are other changes that could occur on heating that may alter the digestibility of the system. It is reported that amylase inhibitors are present in native wheat (Moran 1982; Rogel *et al.* 1987). In raw wheat these remain active whereas they are removed by cooking (Macri *et al.* 1977). Presumably the presence of amylase inhibitors would have a negative effect on digestion. Macri *et al.* (1977) suggest that such inhibitors are destroyed by pepsins in the gizzard. However when the amylase inhibitors were encapsulated to protect them from the gizzard, they decreased starch digestion, particularly in the first 4 weeks after treatment (Macri *et al.* 1977). If the levels in the raw wheat used in the current experiment were so high

that they bypassed the gizzard they could potentially decrease starch digestibility. This could help to explain the decrease in digestibility and also the increase in RVA parameters in raw wheat as opposed to that dried at 100°C.

8.3 Relationship of RVA and Chick Bioassay Parameters

Further to the experiments discussed in chapter seven, linear regression analysis was performed to determine any relationship between the Rapid Visco Analyser (RVA) and chick bioassay parameter data. It is hypothesised that the RVA could be used as a predictor of the nutritional quality of wheat for poultry. The results of this analysis for trial two and trial three, where drying regime was a variable, are shown in tables 8.2 and 8.3 respectively. Significant values of P for regression analysis are highlighted in bold with the direction of that relationship shown in parentheses. The correlation coefficient, r, is also given to indicate the strength of the relationship between the two parameters.

The interpretation is that, when P is equal to or less than 0.05, the slope of the line is significantly different from zero. Therefore, there is some relationship between *x*, the RVA parameter and *y*, the chick parameter.

It is evident from this analysis that certain parameters that can be measured using the RVA have a relationship with certain indicators of nutritional value for poultry.

It appears that CDAD has a relationship with peak and end viscosities. In the case of trial two (table 8.2), there was a significant negative relationship with peak viscosity using silver nitrate (P=0.028), and this was also the case for trial three (table 8.3; P=0.024). Trial three data suggest that end viscosity in silver nitrate may also be related to CDAD (P=0.008). As discussed previously, end viscosity with silver nitrate is achieved by inhibiting endogenous α-amylase.

Table 8.2 Linear regression of RVA parameters (Amylase, Peak Viscosity and End Viscosity) against chick bioassay parameters (Coefficients of Apparent Digestibility and AME) for trial two. The wheat variables were variety (Clare or Einstein) and drying at either 70, 85 or 100°C

Linear Regression Analysis									
		Chick Parameter**							
		CDAD		CIAD		CTTAD		AME	
		r	P	r	P	r	P	r	P
RVA Parameter*	Amylase	0.084	NS	0.394	NS	0.322	NS	0.130	NS
	PV1	0.077	NS	0.447	NS	0.095	NS	0.063	NS
	PV2	0.547	**0.028 (-)**	0.122	NS	0.399	NS	0.095	NS
	EV1	0.089	NS	0.790	**<0.001 (-)**	0.045	NS	0.055	NS
	EV2	0.415	NS	0.628	**0.009 (-)**	0.365	NS	0.228	NS

*PV1, Peak Viscosity in Water; PV2, Peak viscosity in Silver Nitrate; EV1, End Viscosity in water and EV2, End Viscosity in Silver Nitrate ** CDAD, Coefficient of Duodenal Apparent Digestibility; CIAD, Coefficient of Ileal Apparent Digestibility; CTTAD Coefficient of Total Tract Apparent Digestibility and AME, Apparent Metabolisable Energy

Table 8.3 Linear regression of RVA parameters (Amylase, Peak Viscosity and End Viscosity) against chick bioassay parameters (Coefficients of Apparent Digestibility and AME) for trial three. The wheat variables were drying at ambient or 100°C and two different growing sites, JIC and CF

Linear Regression Analysis									
		Chick Parameter**							
		CDAD		CIAD		CTTAD		AME	
		r	P	r	P	r	P	r	P
RVA Parameter*	PV1	0.484	NS	0.000	NS	0.532	**0.034 (+)**	0.337	NS
	PV2	0.559	**0.024 (-)**	0.000	NS	0.152	NS	0.383	NS
	EV1	0.476	NS	0.285	NS	0.549	**0.029 (+)**	0.228	NS
	EV2	0.637	**0.008 (-)**	0.311	NS	0.155	NS	0.308	NS

*PV1, Peak Viscosity in Water; PV2, Peak viscosity in Silver Nitrate; EV1, End Viscosity in water and EV2, End Viscosity in Silver Nitrate ** CDAD, Coefficient of Duodenal Apparent Digestibility; CIAD, Coefficient of Ileal Apparent Digestibility; CTTAD Coefficient of Total Tract Apparent Digestibility and AME, Apparent Metabolisable Energy

Trial two indicated that CIAD may also be related to end viscosities in water or silver; there was a significant negative relationship in both cases ($P<0.001$ and $P=0.009$ respectively).

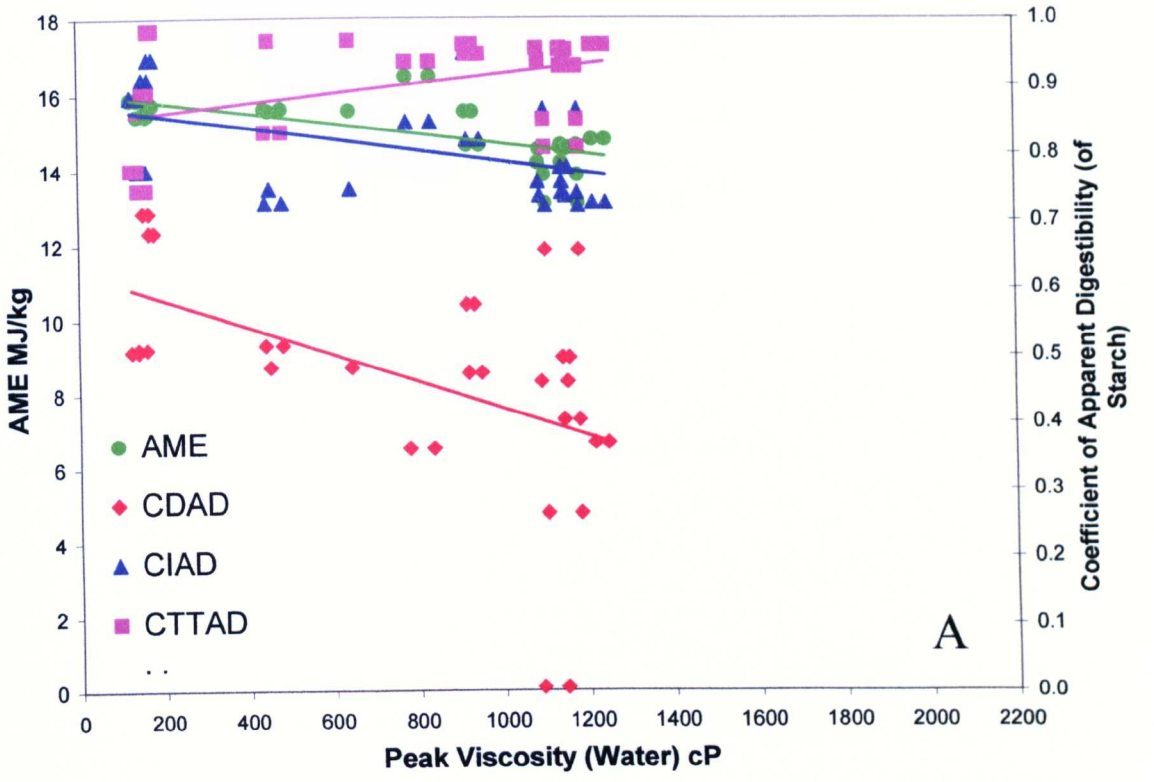
Analysis for trial three also suggested a positive relationship between peak and end viscosities in water with CTTAD ($P= 0.034$ and $P=0.029$ respectively).

With these results in mind, the data for both trials two and three were combined and analysed by linear regression. The results of linear regression are shown in table 8.4 and the data is graphically displayed in figures 8.2 and 8.3.

Table 8.4 Linear regression of RVA parameters (Amylase, Peak Viscosity and End Viscosity) against chick bioassay parameters (Coefficients of Apparent Digestibility and AME) for trials two and three combined.

Linear Regression Analysis									
		Chick Parameter**							
		CDAD		CIAD		CTTAD		AME	
		r	P	r	P	r	P	r	P
RVA Parameter*	PV1	0.508	**0.003 (-)**	0.455	**0.009 (-)**	0.396	**0.025 (+)**	0.683	**<0.001 (-)**
	PV2	0.574	**<0.001 (-)**	0.446	**0.010 (-)**	0.268	NS	0.716	**<0.001 (-)**
	EV1	0.396	**0.025 (-)**	0.482	**0.005 (-)**	0.485	**0.005 (+)**	0.303	NS
	EV2	0.506	**0.003 (-)**	0.481	**0.005 (-)**	0.232	NS	0.303	**0.048 (-)**

*PV1, Peak Viscosity in Water; PV2, Peak viscosity in Silver Nitrate; EV1, End Viscosity in water and EV2, End Viscosity in Silver Nitrate ** CDAD, Coefficient of Duodenal Apparent Digestibility; CIAD, Coefficient of Ileal Apparent Digestibility; CTTAD Coefficient of Total Tract Apparent Digestibility and AME, Apparent Metabolisable Energy



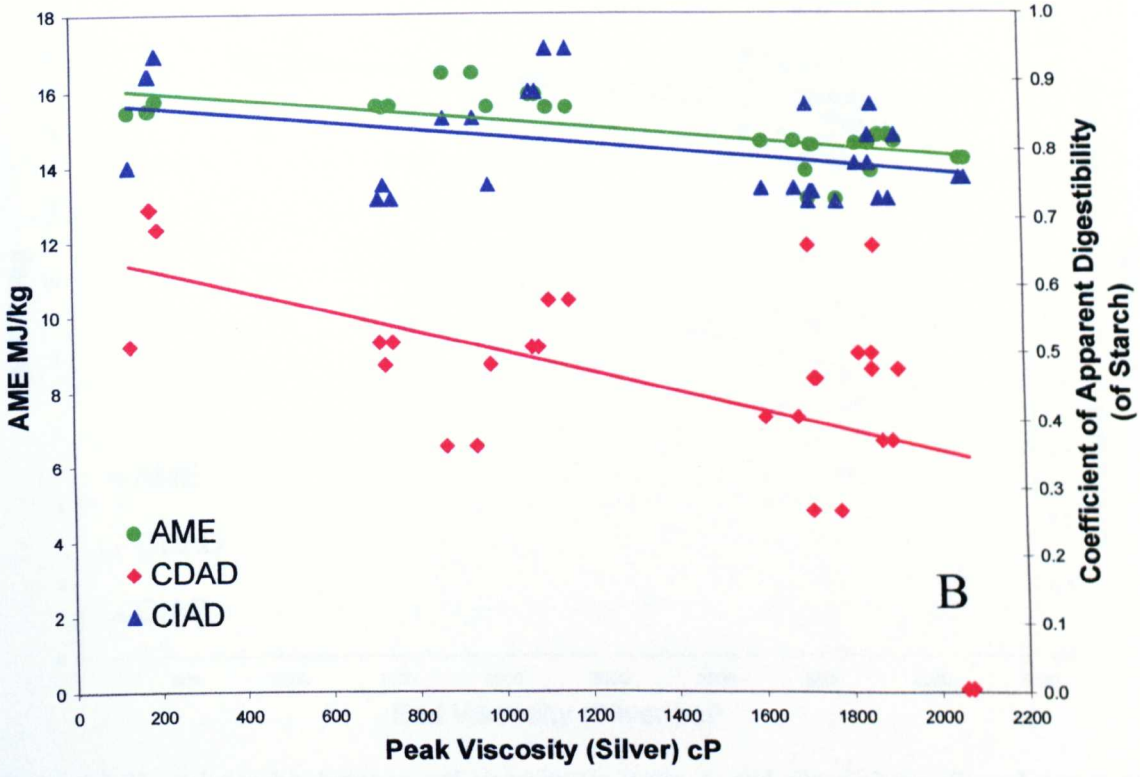


Figure 8.2 The relationship between peak viscosity (in water A, and silver nitrate, B) and Apparent Metabolisable Energy; Coefficient of Duodenal Digestibility; Coefficient of Ileal Digestibility or Coefficient of Total Tract Digestibility. Data points are the mean of six cages of two birds in case of chick parameter and t replicates in case of RVA parameter.

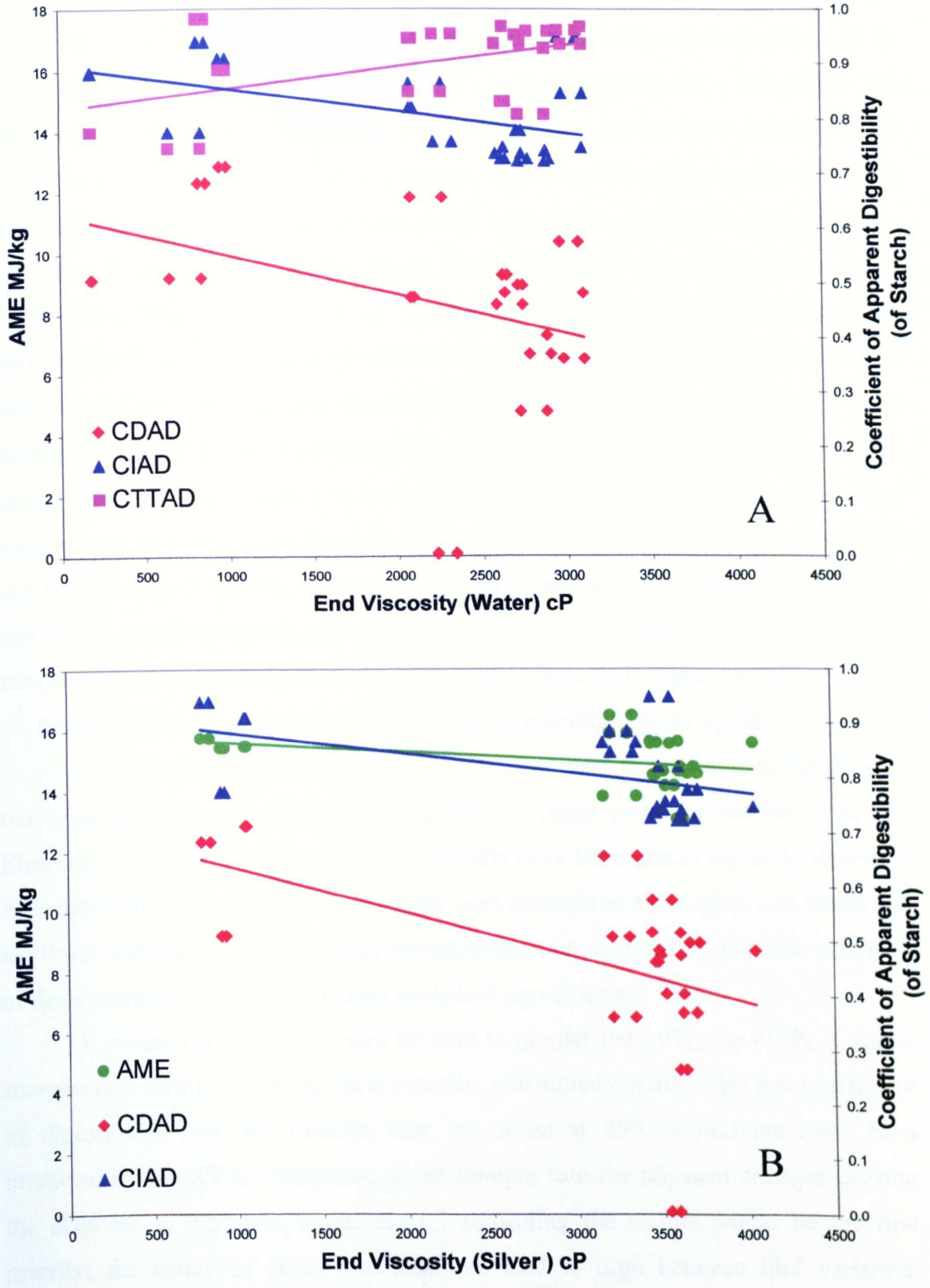


Figure 8.3 The relationship between end viscosity (in water A, and silver nitrate, B) and Apparent Metabolisable Energy; Coefficient of Duodenal Digestibility; Coefficient of Ileal Digestibility or Coefficient of Total Tract Digestibility. Data points are the mean of six cages of two birds in case of chick parameter and two replicates in case of RVA parameter.

It is clear from table 8.4 and figures 8.2 and 8.3 that when the data set, and therefore the degrees of freedom, is increased, the power of the statistical analysis is increased and all but three relationships become significant. However the relationships between the *in vitro* and *in vivo* techniques are far from definite. In all cases the relationships of *in vitro* parameters with *in vivo* measurements of CDAD and CIAD are negative. Thus indicating that lower pasting viscosities (that may be indicating less starch or that the starch structure is less robust) relates to better digestibility. Interestingly, there are also now significant negative relationships between AME and PV in water ($P<0.001$); PV in silver nitrate ($P<0.001$) and EV in silver nitrate ($P=0.048$). As the effect is the same with the silver ions present it helps support the idea that it is an intrinsic parameter of the starch rather than the presence of the amylases that is related to the coefficient of starch digestibility. There also continues to be positive relationships between CTTAD and PV in water ($P=0.025$) and EV in water ($P=0.005$). This would indicate that starch not digested in the early part of the digestive tract is still readily utilized in the hind gut. This suggests that samples with the potential to have high viscosities have their digestion shifted toward the far end of the digestive tract and microbial fermentation in the caeca.

It is noted that, in the cases of CDAD, there were two outliers in the data set that may have affected the statistical analysis. These were attributable to variety Einstein, dried at 85°C. These particular results have been discussed in detail above. Alternative methods of statistical analysis were considered although it was found that the linear correlation and associated assumptions, were appropriate and that inclusion of these points did not over-estimate statistical significance.

It seems that the RVA may be able to predict the influence of these wheat samples and their treatment on their potential nutritional quality. The low coefficient of digestibility seen for Einstein that was dried at 85°C would not have been predicted by the RVA. Therefore, some thought into the physical changes causing the decrease in digestion is necessary. Repeating the results would be the first priority; the nature of chick bioassays can lead to high between bird variation. Although the RVA measures pasted starch viscosities, these could relate to the viscosity of the materials in the gut. It is thought that increased viscosity of digesta decreases starch digestibility (Annison 1993; Jozefiak *et al.* 2007). The current data go some way to support this. Often this phenomenon is related to the content of non-starch polysaccharides in cereals. In wheat it is thought that arabinoxylan is

particularly to blame (Annison and Choct 1991), although the total amounts of these carbohydrates are much less than the starchy materials. RVA viscosity may be considered to be representative of the viscous potential of the wheat when combined in poultry diets. It has been shown by the current work that when *in vitro* RVA pasted viscosities are increased, *in vivo* starch digestibility in the small intestine (duodenum and ileum) may be reduced. It is known that accessibility of nutrients is greater if digestion occurs in the fore portion of the intestines, prior to the caeca. Further evidence is that when intestinal viscosity is increased, as potentially evidenced by increases in PV1 and EV1, CTTAD also increases. Using silver nitrate to suspend whole wheat flours appears to increase the sensitivity of the test, perhaps because amylases do not compromise the results. However, the use of water, and therefore the inclusion of amylase, would be more indicative of the *in vivo* digestive process.

The results shown above may be further indirect evidence that increased digesta viscosity is detrimental to *in vivo* starch digestion. The RVA may provide a way of predicting such viscosity without having to carry out chick experimental work. In certain situations, wheat samples that have lower peak and end viscosities are better digested in the fore portion of the gut. Clearly, this knowledge could have ethical and economic advantages. If a method of predicting quality without using live birds, which have to be culled for the purpose of the trial, can be found, this decreases the number of birds used for experimental purposes. The RVA is a rapid test with relatively low running costs and may be appropriate for cereal producers and feed manufacturers in particular. It could be used to assess individual harvests, or as part of a continual quality control system in feed manufacture. However, the results shown above must be considered as preliminary. Before this technique could be relied upon, the results of more thorough data collection and continuous statistical analysis would need to be considered.

8.4 Variety, Endosperm Hardness and Feed Form

The issue of endosperm hardness in relation to nutritional value is complicated by the method of determination and the current terminology in place. In the UK, the Home Grown Cereals Authority (HGCA) grades its recommended wheat varieties as simply hard or soft. In terms of poultry nutrition, hardness may be an important consideration. Although this is a debatable issue, the literature suggests

that soft wheat varieties are of higher nutritional quality both in terms of amino acid (Short *et al.* 2000) and starch digestibility (Carre *et al.* 2002; Wickramasinghe *et al.* 2005; Peron *et al.* 2006; Peron *et al.* 2007). However, the manner in which the wheat is fed to the birds may also be a factor. It appears that there is an interaction between feed form and endosperm hardness. Carré *et al.* (2002), who found a negative correlation between starch digestibility and endosperm hardness, highlight that their diets were in pelleted form. When wheat is fed as a mash form, hard wheat may promote increased FI leading to improved FCR and BWG (Rose *et al.* 2001; Pirgozliev *et al.* 2003; Steenfeldt *et al.* 2003). As discussed in chapter four, Rogel *et al.* (1987) investigated a small range of Particle Size Index (PSI) scores. This may have been the reason for the lack of their reported differences between the hard and soft varieties. Similarly, Sallah-Uddin *et al.* (1996) investigated wheats with a small range of NIR scores. This is possibly reflected by two of the current studies (chapters three and four), where a hard and a soft wheat did not perform differently. Their NIR scores were 21.76 (Clare) and 50.48 (Einstein), which are soft and on the borderline, respectively. Similarly, the results discussed in chapter six show that two wheat varieties of soft classification (Deben and Clare) do not exhibit different starch digestibility.

It is evident from the literature that different varieties are of different qualities for poultry and other end uses (Rogel *et al.* 1987; Choct *et al.* 1999; Carre *et al.* 2002; Kim *et al.* 2004), and various reasons are suggested. The current project found no difference between the various varieties. However, more extreme differences in hardness may have afforded different results and it is recommended that NIR or single kernel hardness scores are recorded for future studies.

The current study cannot provide any information to support the literature in terms of whether wheat endosperm hardness is relevant to poultry nutrition. It seems that a simple classification of hard or soft is inadequate for predicting starch digestibility and a distinct score would be beneficial for feed manufacturers. According to the literature the NIR and PSI systems are reliable methods that could be employed. Despite the correlation between starch digestibility and AME, the later is not predicted by hardness scores. This is presumably due to AME being more sensitive to bird parameters and enzyme susceptibility (Garnsworthy *et al.* 2000).

8.5 Drying Regimes

Jayas and White (2003) discussed in detail the protocols used around the world to dry harvested wheat, with particular reference to Canada. Most commercial growers already have the equipment to do this, as wet weather damage is an expected problem. For example, in France, maize is routinely harvested at a dry matter of just 600-700g/kg (Barrierguillot *et al.* 1993). Using ambient conditions obviously requires less input in terms of energy, a clear economic advantage, but it is slow. Hot air driers are quicker, often removing 0.06 of overall moisture in days as opposed to months. However, they require gas, oil or electricity and may be 8 times as costly. They must also allow for cooling, as wheat can retain heat whilst stored in large bins (Jayas and White 2003).

Clearly, there is a balance that is necessary in deciding an economical method of drying crops that is dependant on the harvest moisture content but also the end use. It has been recommended that seed wheat should be exposed to temperatures of no more than 43°C, commercial wheat (for bakery products) no more than 60°C but that feed wheats may be exposed to temperatures of up to 82°C (Hall *et al.* 2000). On the farm at Nottingham, temperatures of up to 100°C are regularly used for wheat harvests that have been weather damaged beyond bakery use. Whether this has any adverse effects is not clear. It is shown from the current experiments that the temperature and moisture contents are of vital importance to the end product quality.

It seems that temperature-treating feed wheat samples may change starch digestibility, but the change may be dependent on temperatures and varieties used. In fact, there may be benefit in heat treating feed wheat. Results of trial two suggest that treatment does decrease the starch digestibility, but it appears that this is as a result of the decrease in digestibility seen with samples treated at 85°C. Further work would certainly be necessary to decide whether this is an anomaly, since it is difficult to explain. The result of temperature is non-linear and drying wheat at 70°C or 100°C has no affect on digestion, but his could be due to different reasons. It may be that the effect of drying on the composition of the wheat needs to be better understood before temperatures of drying could be recommended. Usually, damage caused by moisture and heat, would be expected to increase starch digestibility, by "cooking" the starch and thus increasing its enzyme susceptibility (Kulp and Lorenz 1981).

Results of trial three suggest that very harsh heat treatment (that still did not cook the starch) as opposed to ambient drying may even increase digestibility. This is in agreement with several reports (Barrierguillot *et al.*1993; Nui *et al.* 1996; Huang *et al.* 1997a; Huang *et al.* 1997b). It is possible in the current experiment this was not related to moisture content because each samples dried at 100°C had an equivalent pair dried at ambient temperatures. The increase in digestibility is further evidenced by a shift in digestion toward the upper digestive tract, where digestion is more efficient, and away from the caeca (Moran 1985; Choct *et al* 1996; Tester *et al.* 2004b). There were effects of moisture content prior to drying, with 250g/kg and 270g/kg having increased CAD than 122g/kg or 370g/kg. When wheat is of high moisture content, it is suggested over 300g/kg (Hoover and Manuel 1995), digestibility may be decreased, due to amylose:lipid interactions.

Thus, there may be benefit in high temperature drying of wheat in terms of starch digestibility. Depending on the starting moisture content, there should not be any adverse effects, and AME should not be affected. This suggests that starch digestibility is more a factor of the wheat and the starch it contains whereas, AME, although related to starch digestibility, is more a factor of the bird. There is some evidence to suggest that particular strains of bird may be used which result in diets with increased AME (Peron *et al.* 2006; Peron *et al.* 2007). There may also be benefits in terms of mineral content. With maize, phosphorus availability is increase by decreasing phytate bound phosphorus, with heat treatment (Iji *et al.* 2003; Amezcua and Parsons 2007).

However, in terms of amino acid digestibility, there may be detrimental effects of heating and this is not well reported in the literature. With heating, Maillard reactions may occur between reducing sugars and amino acids, decreasing the bioavailability of both (Anjum *et al.* 2005, Martinez-Amezcua and Parsons 2007). For the samples used in this study no difference in colour was observed between the samples dried at different temperatures.

8.6 Experimental Design

8.6.1 Storage Experiment (Chapter Six)

The sampling process was completed prior to the start of the current project. As a result the sampling could have been carried out in a more appropriate way in terms of experimental design. Insufficient amounts of sample for an animal trial

were taken at time zero. This meant a baseline was not established. A second variety was available for sampling, but again insufficient samples were collected. However, four diets were still possible using samples of another variety, purchased from outside the University. A diet that combined stored material combined with another wheat variety allowed comparison with a practice that is common in industry.

8.6.2 Temperature Experiments (Chapters Four and Five)

During both trials that involved temperature treatments the experimental design could have been expanded to allow for more replication. In trial two (chapter four) samples were dried in individual batches that were not replicated. Samples of wheat variety Einstein that was dried at 85°C stood out as being significantly less well digested, which was unexpected. It is difficult to conclude that this is a definite effect without any replication within the trial. A repeat of the experiment would be beneficial before firm conclusions are drawn. Similarly with trial three (chapter five) sampling was not replicated at a field level, so the post harvest treatments were only carried out once (see figure 5.1). It would have been advantageous to have doubled the amount of material to allow the treatments to be duplicated.

8.6.3 Future Dietary Design

i. Proteins

The literature is very clear that low protein diets are viable and economically beneficial in terms of bird performance. However, this is only if the diet is sufficiently supplemented with essential amino acids such as lysine and methionine (Atencio *et al.* 2004; Vieites *et al.* 2004). A lack of the correct methionine and cysteine balance decreases breast meat yield (Atencio *et al.* 2004) as does a reduction in overall crude protein (Dari *et al.* 2005). It is also shown that a change in quality and/or quantity of dietary protein changes metabolism and catabolism of protein (Figares *et al.* 1996). However, Sterling *et al.* (2006) suggests that the performance of broilers in response to increasing levels of crude protein is genotype dependent. Smith and Pesti (1998) go as far as to say that feeding programs should be tailored to individual strains, for this reason. Some genotypes are more efficient at digesting starch, for example (Peron *et al.* 2006). Both fat content and deposition decrease with increasing ideal protein, and the opposite occur with protein deposition (Wijtten

et al. 2004; Sterling *et al.* 2005; Dari *et al.* 2005). Wijtten *et al.* (2004) also conclude that to achieve increased body weight gain from increased protein, amino acids must also be balanced. Clearly, when formulating diets that are either low in protein or more than sufficient, amino acids must be carefully considered and balanced to meet recommendations. Carcass content is determined by the limiting amino acid, and any supply over that which is necessary has an associated energy cost of catabolism (Sklan and Noy 2004). Quality and/or quantity of protein provision also affects the partition of nitrogenous components that are excreted (Figares *et al.* 1996) and, not surprisingly, lower dietary protein concentration leads to reduced nitrogen excretion (Kidd *et al.* 2001).

Since it is clear that low protein, amino acid balanced diets are viable, there is scope in the literature for studies into the effect this may have on starch digestibility, and therefore AME. There is little benefit in decreasing protein for economic reasons if starch digestibility and AME decrease as a result. Both BWG and FCR are adversely affected with decreasing ideal protein concentrations (Sterling *et al.* 2005; Wijtten *et al.* 2004). The current experiment suggest that overall, starch digestibility is not affected by very low levels of protein, even when amino acids were not taken into account in formulation. The overall CAD (combined CDAD, CIAD and CTTAD) did not vary with protein level nor did protein interact with variety. However, there is potential for further experimentation into the effect on digestibility in specific gut regions. There was suggestion that ileal wheat starch digestibility is increased with decreased protein provision. If total tract were found to decrease, concurrently, this would be indicative of increased efficiency of digestion and absorption. Digestion and absorption of high quality wheat varieties is significantly higher distally to the caeca (Moran 1985; Choct *et al.* 1996; Tester *et al.* 2004b).

8.7 Conclusion

Initial experiments provide evidence that the digestibility of starch is unrelated to protein provision in the diet, and that inadequate protein does not affect starch digestibility. This is valuable as little such information appears in the literature. It is an important observation as it is a relatively common practise to feed low protein diets that are supplemented with an amino acid balancer (Atencio *et al.* 2004; Vieites *et al.* 2004), and this should not affect starch utilisation. However, the current project does suggest that the protein content of the grain itself may be implicated in variation in nutritional value.

The nutritional value of a wheat variety is important information for those formulating bird rations. Therefore it is also interesting that wheat that has been dried at up to 100°C should not have decreased in nutritional value. However, moisture content prior to drying may be important, with a decrease in nutritional value seen with moisture content of 370g/kg. As far as cereal producers are concerned their commodity is valuable as a feedstuff even if dried rapidly at 100°C. Considering both trials two and three, drying at 70°C is appropriate and may be more economically efficient, but 100°C may have a benefit in terms of starch digestibility. There would need to be further work into whether or not Maillard's reaction may occur on heating. This reaction between sugars and amino acids, a process of 'browning', would have the potential to decrease the availability of lysine in the diet.

As far as the cereal producer is concerned wheat for animal feed tends not to be economically valued in terms of its nutritional quality. However, the potential improvement by heat treatment may be a factor that producers could consider.

The RVA has provided further evidence in support of the literature that implicates increased viscosity as being detrimental to digestion. Although further development of the method is required, the RVA may in future be used to predict nutritional value, using peak and end viscosity values. An increase in end viscosity may indicate a decrease in or static pre-caecal digestibility, as shown in trial two. Conversely, a reduction in end viscosity may indicate a potential improvement in pre-caecal starch digestibility. However, the actual relationships may be multi faceted and needs to be far better understood.

Previously, a reduction in swelling behaviour within the RVA would be attributable to a loss of crystallinity in the starch and an increase in digestibility. However, the current study suggests that a lack of swelling power may be

attributable to factors other than a loss of native starch structure and that this may actually be beneficial in feed formulation.

8.7.1 Summary Conclusions

In summary this thesis can be drawn to the following conclusions

- Dietary protein provision does not affect starch digestibility and therefore the described method is appropriate for determining starch digestibility.
- The current experiments suggest that heat treatment to dry grains at 70°C or 100°C, even with a starting dry matter content of 630g/kg, does not affect AME. However, drying at 85°C may begin to affect some of the components of the flour, in a way that is detrimental to digestibility.
- Drying at 100°C compared to ambient conditions may increase starch digestibility, but this is possibly not a direct effect of changes in starch structure; within chapter seven there is a discussion on how the changes may be explained by temperature effects to the storage proteins.
- For the above reasons growers could be advised that drying wheat that has been harvested wet, at 70°C or 100°C, in a convection oven, will probably not damage nutritional quality.
- Wheat that has a moisture content of 370g/kg at harvest and is subsequently dried may have decreased starch digestibility and it is hypothesised that this may be due to amylose:lipid interactions.
- Heat treatment at 100°C reduces the pasting ability of Einstein and Clare, but this is not due to a loss in starch crystallinity.
- During two months ambient storage, no change in pasting ability is seen in the flour of wheat variety Deben.
- During two months ambient storage, there is no change in nutritional value of wheat variety Deben
- There is no difference in nutritional quality of the hard wheat varieties Einstein and soft wheat Clare. A more descriptive hardness score would be beneficial in wheat classification.
- Einstein, a hard wheat variety, is more sensitive to heat treatment than a soft wheat, Clare.

- Further work into the development of the RVA for predicting nutritional value of wheat is justified.
- RVA can be used to indicate amylase levels, but in some situations the amylase content is too high to be quantified using the method of Collado and Corke (1999)
- Peak viscosity and end viscosity, as measured by the RVA, are, in some situations, negatively related to *in vivo* measurements of AME, CDAD and CIAD and positively related to CTTAD.
- End viscosity has been shown to predict *in vivo* CIAD.

Agricultural Research Council (1975) 'The Nutrient Requirements of Farm Livestock No. 1 Poultry.' (Agricultural Research Council: London)

Ahmed M, Lelievre J (1978) Effect of Various Drying Procedures on Crystallinity of Starch Isolated from Wheat Grains. *Starke* **30,** 78-79.

Allen H, Blakeney AB, Oliver JR (1991) The Measurement of Pasting Properties of Biscuit Wheats Using a Temperature Programmed RVA. (Eds Martin and Wrigley) pp. 159-160. (Royal Australian Chemistry Institute:

Altay F, Gunasekaran S (2006) Influence of drying temperature, water content, and heating rate on gelatinization of corn starches. *Journal of Agricultural and Food Chemistry* **54,** 4235-4245.

Al-Yahya, SA (2001) Effect of Storage Conditions on Germination in Wheat *Journal of Agronomy and Crop Science* **186** 273-279

Amezcua CM, Parsons CM (2007) Effect of increased heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles. *Poult.Sci.* **86,** 331-337.

Angkanaporn K, Choct M, Bryden W.L, Annison EF (1994) Effects of Wheat Pentosans on Endogenous Amino Acid Losses in Chickens. *Journal of Science of Food and Agriculture* **66,** 399-404.

Anjum FM, Ahmad I, Butt MS, Sheikh MA, Pasha I (2005) Amino acid composition of spring wheats and losses of lysine during chapati baking. *Journal of Food Composition and Analysis* **18,** 523-532.

Annison G (1990) Complex polysaccharide in poultry diets. *Proceedings of the Australian Poultry Science Symposium* 17-24.

Annison G (1991) Relationship Between the Levels of Soluble Nonstarch Polysaccharides and the Apparent Metabolizable Energy of Wheats Assayed in Broiler Chickens. *J Agric Food Chem* **39,** 1252-1256.

Annison G (1992) Commercial enzyme supplementation of wheat based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. *Animal Feed Science and Technology* **38,** 105-121.

Annison G (1993) The Role of Wheat Non-Starch Polysaccharides in Broiler Nutrition. *Aus J Agric Res* **44,** 405-422.

Annison G, Choct M (1991) Anti-nutritive Activities of Cereal Non-Starch Polysaccharides in Broiler Diets and Strategies Minimising their Effects. *World's Poul Sci J* **47,** 232-241.

Annison G, Choct M, Cheetham NW (1991) Analysis of Wheat Pentosans from a Large Scale Isolation. (Eds Martin and Wrigley) pp. 161-164. Royal Australian Chemical Institute

Ao T, Cantor AH, Pescatore AJ, Pierce JL (2008) In vitro evaluation of feed-grade enzyme activity at pH levels simulating various parts of the avian digestive tract. *Animal Feed Science and Technology* **140,** 462-468.

Appleford NEJ, Lenton JR (1997) Hormonal regulation of alpha-amylase gene expression in germinating wheat (Triticum aestivum) grains. *Physiologia Plantarum* **100,** 534-542.

Asp EH (1990) Laboratory Methods for Alpha Amylase Analysis *Cereal Foods World* **35** (9) 941-946

Atencio A, Albino LFT, Rostagno HS, Vieites FM (2004) Methionine plus cystine requirement of males broilers from 1 to 20, 24 to 38 and 44 to 56 days of age. *Revista Brasileira de Zootecnia-Brazilian Journal of Animal Science* **33,** 1152-1166.

Attenburrow G, Barnes DJ, Davies AP, Ingman SJ (1990) Rheological Properties of Wheat Gluten. *Journal of Cereal Science* **12,** 1-14.

Atwell WA (2001) An overview of wheat development, cultivation, and production. *Cereal Foods World* **46,** 59-62.

Austin SC, Wiseman J, Chesson A (1999) Influence of Non-starch Polysaccharides Structure on the Metabolisable Energy of UK Wheat Fed to Poultry. *J Cereal Sci* **29,** 77-88.

Baldwin PM, Davies MC, Melia CD (1994) Holes in Starch Granules: Confocal, SEM and Light Microscopy Studies of Starch Granule Structure. *Starch-Starke* **46,** 341-346.

Banks W, Greenwood CT (1975) *Starch and its components* Edinburgh University Press, Edinburgh, UK

Barkley GR, Miller HM, Forbes JM (2004) The ability of laying hens to regulate phosphorus intake when offered two feeds containing different levels of phosphorus. *British Journal of Nutrition* **92,** 233-240.

Barrierguillot B, Zuprizal, Jondreville C, Chagneau AM, Larbier M, Leuillet M (1993) Effect of Heat Drying Temperature on the Nutritive-Value of Corn in Chickens and Pigs. *Animal Feed Science and Technology* **41,** 149-159.

Bassoi MC, Flintham J (2005) Relationship between grain colour and preharvest sprouting-resistance in wheat. *Pesquisa Agropecuaria Brasileira* **40,** 981-988.

Bassoi MC, Flintham J, Riede CR (2006) Analysis of preharvest sprouting in three Brazilian wheat populations. *Pesquisa Agropecuaria Brasileira* **41,** 583-590.

Becker A, Hill SE, Mitchell JR (2001a) Milling - A further parameter affecting the rapid visco analyser (RVA) profile. *Cereal Chemistry* **78,** 166-172.

Becker A, Hill SE, Mitchell JR (2001b) Relevance of amylose-lipid complexes to the behaviour of thermally processed starches. *Starch-Starke* **53,** 121-130.

Becker HA (1960) On the Absorption of Liquid Water by the Wheat Kernel. *Cereal Chemistry* **37,** 309-323.

Bewley JD, Black M (1994) In 'Seeds: Physiology of development and germination'. p. 1. (Plenum Press: New York)

Biliaderis CG, Maurice TJ, Vose JR (1980) Starch Gelatinization Phenomena Studied by Differential Scanning Calorimetry. *Journal of Food Science* **45,** 1669

Biliaderis CG, Page CM, Slade L, Sirett RR (1985) Thermal-Behavior of Amylose-Lipid Complexes. *Carbohydrate Polymers* **5,** 367-389.

Bingham J, Whitmore ET (1966) Varietal Differences in Wheat in Resistance to Germination in Ear and Alpha-Amylase Content of Grain. *Journal of Agricultural Science* **66,** 197

Buisson G, Duee E, Haser R, Payan F (1987) Three dimensional structure of porcine pancreastic alpha-amylase at 2.9 A resolution. Role of calcium in structure and activity. *EMBO J* **6,** 3909-3916.

Buleon A, Colonna P, Planchot P, Ball S (1998) Starch granules:structure and biosynthesis. *International Journal of Biological Macromolecules* **23,** 85-112.

Buonocore V, Deponte R, Gramenzi F, Petrucci T, Poerio E, Silano V (1977) Purification and Properties of alpha-Amylase from Chicken (Gallus Gallus L.) Pancreas. *Molecular and Cellular Biochemistry* **17,** 11-17.

Burrell MM (2003) Starch: the need for improved quality or quantity--an overview. *J.Exp.Bot.* **54,** 451-456.

Burt DJ, Russell PL (1983) Gelatinization of Low Water Content Wheat Starch - Water Mixtures. *Starch-Starke* **35,** 354-360.

Bush BS, Sticher L, Vanhuystee R, Wagner D, Jones RL (1989) The Calcium Requirement for Stability and Enzymatic Activity of 2 Isoformsof Barley Aleurone Apha-Amylase *Journal of Biological Chemistry* **264** 19392-19398

Buxton (2006) Norfolk. Buxton-Norfolk Weather Data 2006. http://www.feedsoft.clara.net/buxton-norfolk/weather.htm . Last Accessed 9-7-2007.

Carre B, Idi A, Maisonnier S, Melcion JP, Oury FX, Gomez J, Pluchard P (2002) Relationships between digestibilities of food components and characteristics of

wheats (Triticum aestivum) introduced as the only cereal source in a broiler chicken diet. *British Poultry Science* **43,** 404-415.

Carver BF (1994) Genetic-Implications of Kernel Nir Hardness on Milling and Flour Quality in Bread Wheat. *Journal of the Science of Food and Agriculture* **65,** 125-132.

Carver BF, Rayburn AL (1994) Comparison of Related Wheat Stocks Possessing 1B Or 1Rs.1Bl Chromosomes - Agronomic Performance. *Crop Science* **34,** 1505-1510.

Chaplin SB (1989) Effect of Cecectomy on Water and Nutrient Absorption of Birds. *The Journal of Experimental Zoology Supplement* **3,** 81-86.

Choct M, Annison G (1990) Anti-nutritive Activity of Wheat Pentosans in Broiler Diets. *Brit Poul Sci* **31,** 811-821.

Choct M, Annison G (1992) Anti-nutritive activity of wheat arabinoxylans; role of viscosity. *British Poultry Science* **33,** 821-834.

Choct M, Hughes RJ (1997) The Nutritive Value of New Season Grains for Poultry. In 'Recent Advances in Animal Nutrition in Australia'. (Eds JL Corbett, M Choct, JV Nolan, and B Rowe) pp. 146-150. (The University of New England: Armidale)

Choct, M, Hughes RJ, Annison, G (1999) Apparent metabolisable energy and chemical composition of Australian wheat in relation to environmental factors *Australian Journal of Agricultural Research* **50** 447-51

Choct M, Hughes RJ, Wang J, Bedford MR, Morgan AJ, Annison G (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* **37,** 609-621.

Cleemput G, Van Laere K, Hessing M, Van Leuven F, Torrekens S, Arsene Delcour J (1997) Identification and Characterisation of a Novel Arabinoxylanase from Wheat Flour. *Plant Physiol* **115,** 1619-1627.

Collado LS, Corke H (1999) Accurate Estimation of Sweetpotato Amylase Acivity by Flour Viscosity Analysis. *J Agric Food Chem* **47,** 832-835.

Conde-Petit B, Nuessli J, Handschin S, Escher F (1998) Comparative characterisation of aqueous starch dispersions by light microscopy, rheometry and iodine binding behaviour. *Starch-Starke* **50,** 184-192.

Cooke D, Gidley MJ (1992) Loss of Crystalline and Molecular Order During Starch Gelatinization - Origin of the Enthalpic Transition. *Carbohydrate Research* **227,** 103-112.

Coultate T (1999) Polysaccharides. In: Food; The chemistry of its components. (Ed. T Coultate) pp. 29-53. (RSC: Cambridge)

Craven M, Barnard A, Labuschagne M (2007) The impact of cold termperatures during grain maturation on selcted quality paramters of wheat. *Journal of the Science of Food and Agriculture* **87,** 1783-1793.

Crosbie GB, Ross AS, Moro T, Chiu PC (1999) Starch and protein quality requirements of Japanese alkaline noodles (ramen). *Cereal Chemistry* **76,** 328-334.

Dari R, Penz A, Kessler A, Jost H (2005) Use of digestible amino acids and the concept of ideal protein in feed formulation for broilers. *Journal of Applied Poultry Research* **14,** 195-203.

Darlington HF, Tecsi L, Harris N, Griggs DL, Cantrell IC, Shewry PR (2000) Starch granule associated proteins in barley and wheat. *Journal of Cereal Science* **32,** 21-29.

Deffenbaugh LB, Walker CE (1989) Use of the Rapid-Visco-Analyzer to Measure Starch Pasting Properties. *Starch-Starke* **41,** 461-467.

Delwiche SR (1993) Measurement of Single-Kernel Wheat Hardness Using Near-Infrared Transmittance. *Transactions of the ASAE* **36,** 1431-1437.

Denbow D (2000) Gatrointestinal Anatomy and Physiology. In 'Sturkie's Avian Physiology'. (Ed. G Whittow) pp. 299-325. (Academic Press: San Diego)

Donald A, Waigh T, Jenkins P, Gidley M, Debet M, Smith A (1997) Internal Structure of Starch Granules Revealed by Scattering Studies. In 'Starch: Structure and Functionality'. (Eds P Frazier, A Donald, and P Richmond) pp. 172-179. (The Royal Society of Chemistry: Cambridge)

Donovan JW (1979) Phase-Transitions of the Starch-Water System. *Biopolymers* **18,** 263-275.

Donovan JW, Lorenz K, Kulp K (1983) Differential Scanning Calorimetry of Heat-Moisture Treated Wheat and Potato Starches. *Cereal Chemistry* **60,** 381-387.

Eliasson AC, Carlson TLG, Larsson K, Miezis Y (1981) Some Effects of Starch Lipids on the Thermal and Rheological Properties of Wheat-Starch. *Starke* **33,** 130-134.

Evers AD, Blakeney AB, O'Brien L (1999) Cereal structure and composition. *Australian Journal of Agricultural Research* **50,** 629-650.

Evers AD, Flintham J, Kotecha K (1995) Alpha-amylase and grain size in wheat. *Journal of Cereal Science* **21,** 1-3.

Famera O, Hruskova M, Novotna D (2004) Evaluation of Methods for Wheat Grain Hardness Determination. *Plant Soil and Environment* **50,** 489-493.

Ferrer R, Planas J, Durfort M, Moreto M (1991) Morphological Study of the Caecal Epithelium of the Chicken (*Gallus Gallus Domesticus L.*). *British Poultry Science* **32,** 679-691.

Figares IF, Nieto R, Aguilera JF, Prieto C (1996) The use of the excretion of nitrogen compounds as an indirect index of the adequacy of dietary protein in chickens. *Animal Science* **63,** 307-314.

Figueiredo LFDA, Davrieux F, Fliedel G, Rami JF, Chantereau J, Deu M, Courtois B, Mestres C (2006) Development of NIRS equations for food grain quality traits through exploitation of a core collection of cultivated sorghum. *Journal of Agricultural and Food Chemistry* **54,** 8501-8509.

Finney PL, (2001) Effects of Falling Number Sample Weight on Prediction of a-Amylase Activity *Cereal Chemistry* **78** (4) 485-487

Flintham J, Gale MD (1988) Genetics of pre-harvest sprouting and associated traits in wheat: review. *Plant Varieties and Seeds* **1,** 87-97.

Fontaine J, Schirmer B, Horr J (2002) Near-infrared reflectance spectroscopy (NIRS) enables the fast and accurate prediction of essential amino acid contents. 2. Results for wheat, barley, corn, triticale, wheat bran/middlings, rice bran, and sorghum. *Journal of Agricultural and Food Chemistry* **50,** 3902-3911.

Forbes JM, Covasa M (1995) Application of Diet Selection by Poultry with Particular Reference to Whole Cereals. *Worlds Poultry Science Journal* **51,** 149-165.

Forbes JM, Shariatmadari F (1994) Diet Selection for Protein by Poultry. *Worlds Poultry Science Journal* **50,** 7-24.

Friesen OD, Guenter W, Marquardt RR, Rotter BA (1992) The Effect of Enzyme Supplementation on the Apparent Metabolizable Energy and Nutrient Digestibilities of Wheat, Barley, Oats, and Rye for the Young Broiler Chick. *Poultry Science* **71,** 1710-1721.

Furuse M, Mabayo RT (1996) Effects of partially hydrolysed guar gum on feeding behaviour and crop emptying rate in chicks. *British Poultry Science* **37,** 223-227.

Gale MD, Law CN, Chojecki AJ, Kempton RA (1983) Genetic-Control of Alpha-Amylase Production in Wheat. *Theoretical and Applied Genetics* **64,** 309-316.

Gallant DJ, Bouchet B, Baldwin PM (1997) Microscopy of starch: Evidence of a new level of granule organization. *Carbohydrate Polymers* **32,** 177-191.

Garnsworthy PC, Wiseman J, Fegeros K (2000) Prediction of chemical, nutritive and agronomic characteristics of wheat by near infrared spectroscopy. *Journal of Agricultural Science* **135,** 409-417.

George J, McCracken KJ (2003) Changes in in vitro viscosity (IVV) of whole or ground wheat grain during storage and effects of storage temperature. *Journal of Cereal Science* **37,** 179-185.

Gibson TS, Solah VA, McCleary BV (1997) A procedure to measure amylose in cereal starches and flours with concanavalin A. *Journal of Cereal Science* **25,** 111-119.

Giroux MJ, Morris CF (1998) Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 6262-6266.

Glenn GM, Younce FL, Pitts MJ (1991) Fundamental Physical Properties Charcterizing the Hardness of Wheat Endosperm. *Journal of Cereal Science* **13,** 179-194.

Greenwood CT, Milne EA (1968a) Studies on Starch Degrading Enzymes Part VII. *Starch-Starke* **20,** 101-107.

Greenwood CT, Milne EA (1968b) Studies on Starch Degrading Enzymes Part VIII. *Starch-Starke* **20,** 139-150.

Greffeuille V, Abecassis J, Rousset M, Oury FX, Faye A, Bar L'Helgouac'h C, Lullien-Pellerin V (2006) Grain characterization and milling behaviour of near-isogenic lines differing by hardness. *Theoretical and Applied Genetics* **114,** 1-12.

Gudmundsson M, Eliasson AC, Bengtsson S, Aman P (1991) The Effects of Water-Soluble Arabinoxylan on Gelatinization and Retrogradation of Starch. *Starch-Starke* **43,** 5-10.

Guler S, Koksel H, Ng PKW (2002) Effects of industrial pasta drying temperatures on starch properties and pasta quality. *Food Research International* **35,** 421-427.

Hagberg S (1960) A Rapid Method for Determining Alpha-Amylase Activity. *Cereal Chemistry* **37,** 218-222.

Hagberg S (1961) Note on A Simplified Rapid Method for Determining Alpha-Amylase Activity. *Cereal Chemistry* **38,** 202-&.

Hagenimana V, Vezina LP, Simard RE (1994) Sweet-Potato Alpha-Amylase and Beta-Amylase - Characterization and Kinetic-Studies with Endogenous Inhibitors. *Journal of Food Science* **59,** 373-377.

Hall M, Jennings JP, Lewis BA, Robertson JB (2000) Evaluation of Starch Analysis Methods for Feed Samples. *Journal of the Science of Food and Agriculture* **81,** 17-21.

Harrington GT (1923) Forcing the germination of freshly harvested wheat and other cereals. *Journal of Agricultural Research* **23,** 0079-0100.

Hedley CL, Bogracheva TY, Wang TL (2002) A Genetic Approach to Studying the Morphology, Structure and Function of Starch Granules using Pea as a Model. *Starch-Starke* **54,** 235-242.

Henuk YL, Dingle JG (2002) Practical and economic advantages of choice feeding systems for laying poultry. *Worlds Poultry Science Journal* **58,** 199-208.

Hetland H, Svihus B, Olaisen V (2002) Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *British Poultry Science* **43,** 416-423.

Hetland H, Uhlen AK, Viken KH, Krekling T, Svihus B (2007) Hagberg falling number and the nutritional value of wheat in broiler chicken diets. *British Poultry Science* **48,** 12-20.

HGCA. (2006) Home Grown Cereals Authority Recommended Lists 2006/7 http://www.hgca.com/content.template/23/0/Varieties/Varieties/Varieties%20Home%20Page.mspx. Last Accessed 14-9-2006

Hilhorst HWM (1995) A Critical Update on Seed Dormancy .1. Primary Dormancy. *Seed Science Research* **5,** 61-73.

Hill RD, MacGregor AW (1988) Cereal a-Amylases in Grain Research and Technology. In 'Advances in Cereal Science and Technology'. (Ed. Y Pomeranz) pp. 217-261. (American Association of Cereal Chemists: Minnesota)

Holland B, Welch A, Unwin I, Buss D, Paul A, Southgate D (1991) In 'McCance and Widdowson's The Composition of Foods'. (Eds B Holland, A Welch, I Unwin, D Buss, A Paul, and D Southgate) (RSC: Cambridge)

Holm J, Bjorck I, Ostrowska S, Eliason A-C, Asp N-G, Larsson K, Lundquist I (1983) Digestibility of Amylose-Lipid Complexes in-vitro and in-vivo. *Starch-Starke* **35,** 294-297.

Home Grown Cereals Authority (2007). Detailed Information on Wheat. http://www.hgca.com . Last Accessed 4-7-2007

Hoover R, Manuel H (1995) The Effects of Heat-Moisture Treatment on the Structure and Physiochemical Properties of Normal Maize, Waxy Maize, Dull Waxy Maize and Amylomaize V Starches. *Journal of Cereal Science* **23,** 153-162.

Hoover R, Vasanthan T (1994) Effect of heat-moisture treatment on the structure and phsicochemical properties of cereal, legume, and tuber starches. *Carbohydrate Research* **252,** 33-53.

Hristov, A., McAllister, T. A., Xu, Z., Dong, Y., Pickard, M. D., Cheng K.J (1996) The effect of variety and micronization on the in situ digestion of wheat. *Annual Meeting of the Canadian Journal of Animal Science 1996* 548-549.

Huang SX, Sauer WC, Hargreaves L, Pickard M, Li S (1997a) Effect of micronization on energy, starch and amino acid digestibilities in wheat for young pigs. *Journal of Animal and Feed Sciences* **6,** 353-368.

Huang SX, Sauer WC, Pickard M, Li S, Hardin RT (1997b) Effect of micronization on energy, starch and amino acid digestibility in hulless barley for young pigs. *Canadian Journal of Animal Science* **78,** 81-87.

Hug-Iten S, Handschin S, Conde-Petit B, Escher F (1999) Changes in Starch Microstructure on Baking and Staling of Wheat Bread. *Lebensmittel-Wissenschaft und-Technologie,* **32,** 255-260.

Hughes RJ, Choct M (1997) Low AME Wheat or Low AME Chickens? Highly Variable Responses by Birds on the Same Low AME Wheat Diet. *Proceedings of the Australian Poultry Science Symposium* **9,** 138-141.

Huyghebaert G, Schoner FJ (1999) Influence of storage and addition of enzyme on metabolisable energy content of wheat 1. Impact of storage and enzyme addition. *Archiv fur Geflugelkunde* **63,** 13-20.

IGC (2008) Grain Market Report Number 382 *International Grains Council Online* http://www.igc.org.uk/en/downloads/gmrsummary/gmrsumme.pdf Last Accessed 7-10-2008

Iji PA, Saki AA, Tivey DR (2001) Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. *Animal Feed Science and Technology* **89,** 175-188.

Iji P, Khumalo K, Slippers S (2003) Intestinal function and body growth of broiler chickens on diets based on maize dried at different temperatures and supplemented with a microbial enzyme. *Reproduction Nutrition Development* **43,** 237-224.

Jacobs H, Eerlingen RC, Spaepen H, Grobet PC, Delcour JA (1997) Impact of annealing on the susceptibility of wheat, potato and pea starches to hydrolysis with pancreatin. *Carbohydrate Research* **305,** 193-207.

Jang JK and Pyun, YR (1996) Effect of Moisture Content on the Melting of Wheat Starch *Starch* **48** 48-51

Jayas DS, White NDG (2003) Storage and drying of grain in Canada: low cost approaches. *Food Control* **14,** 255-261.

Ji Y, Ao Z, Han JA, Jane JL, BeMiller JN (2004) Waxy maize starch subpopulations with different gelatinization temperatures. *Carbohydrate Polymers* **57,** 177-190.

Joe AFTW, Summers RW, Lunn GD, Atkinson MD, Kettlewell PS (2005) Pre-maturity alpha-amylase and incipient sprouting in UK winter wheat, with special reference to the variety Rialto. *Euphytica* **143,** 265-269.

Johansson E (2002) Effect of two wheat genotypes and Swedish environment on falling number, amylase activities, and protein concentration and composition. *Euphytica* **126,** 143-149.

Jones GPD, Taylor RD (2001) The incorporation of whole grain into pelleted broiler chicken diets: production and physiological responses. *British Poultry Science* **42,** 477-483.

Jood S, Kapoor AC, Singh R (1993) Available Carbohydrates of Cereal-Grains As Affected by Storage and Insect Infestation. *Plant Foods for Human Nutrition* **43,** 45-54.

Jozefiak D, Rutkowski A, Jensen BB, Engberg RM (2007) Effects of dietary inclusion of triticale, rye and wheat and xylanase supplementation oil growth

performance of broiler chickens and fermentation in the gastrointestinal tract. *Animal Feed Science and Technology* **132,** 79-93.

Kent NL, Evers AD (1994) 'Kent's Technology of Cereals.' (Elsevier Science: Oxford)

Kettlewell PS, Cashman MM (1997) Alpha-amylase activity of wheat grain from crops differing in grain drying rate. *Journal of Agricultural Science* **128,** 127-134.

Kettlewell PS, Sothern RB, Koukkari WL (1999) UK Wheat Quality and Economic Value are Dependant on the North Atlantic Oscillation. *Journal of Cereal Science* **29,** 205-209.

Kidd M, Gerard P, Heger J, Rowe B, Sistani K, Burnham D (2001) Threonine and crude protein responses in broiler chicks. *Animal Feed Science and Technology* **94,** 57.

Kim JC, Mullan BP, Pluske JR (2001) Storage influences the protein and carbohydrate composition of wheat. In 'Recent Advances in Animal Nutrition in Australia'. (Ed. JL Corbett) p. 34A. (The University of New England: Armidale)

Kim JC, Mullan BP, Simmins PH, Pluske JR (2003) Variation in the chemical composition of wheats grown in Western Australia as influenced by variety, growing region, season, and post-harvest storage. *Australian Journal of Agricultural Research* **54,** 541-550.

Kim JC, Mullan BP, Simmins PH, Pluske JR (2004) Effect of variety, growing region and growing season on digestible energy content of wheats grown in Western Australia for weaner pigs. *Animal Science* **78,** 53-60.

Klasing KC (1999) Avian gastrointestinal anatomy and physiology. *Seminars in Avian and Exotic Pet Medicine* **8,** 42-50.

Kodama M, Noda H, Kamata T (1978) Conformation of Amylose in Water .1. Light-Scattering and Sedimentation-Equilibrium Measurements. *Biopolymers* **17,** 985-1002.

Kofuji T, Inoue A (2002) Differentiation of smooth muscle cells in chicken gizzard - Self-catalytic mechanism for the production of smooth muscle cells. *Cell and Tissue Research* **307,** 211-223.

Kruger JE, Marchylo, BA (1985) A Comparison of the Catalysis of Starch Components by Isoenzymes from the Two Major Groups of Germinated Wheat a-Amylase *Cereal Chemistry* **62** (1) 11-18

Kulp K, Lorenz K (1981) Heat-Moisture Treatment of Starches .1. Physicochemical Properties. *Cereal Chemistry* **58,** 46-48.

Labuschagne M, Geleta N, Osthoff G (2007) The Influence of Environment on Starch Content and Amylose to Amylopectin Ratio in Wheat. *Starch-Starke* **59,** 234-238.

Langeveld S, van Wijk R, Stuurman N, Kijne J, de Pater S (2000) B-type granule containing protrusions and interconnections between amyloplasts in developing wheat endosperm revealed by transmission electron microscopy and GFP expression. *Journal of Experimental Botany* **51,** 1357-1361.

Leone JL (1973) Collaborative Study of Quantitative-Determination of Titanium-Dioxide in Cheese. *Journal of the Association of Official Analytical Chemists* **56,** 535-537

Levine H, Slade, L (1990) Influence of the glassy and rubbery states on the thermal, mechanical, and structural properties of doughs and baked products. In: H. Faridi and J.M. Faubion, Editors, *Dough Rheology and Baked Product Texture*, AVI/Van Nostrand, New York, pp. 157–330.

Liu Y, Shi Y-C (2006) Phase and State Transitions in Granular Starches Studied by Dynamic Differential Scanning Calorimetry. *Starch-Starke* **58,** 433-442.

Loney DP, Meredith P (1974) Amylograph Viscosities of Wheat Flours and Their Starches During Storage. *Cereal Chemistry* **51,** 702-705.

Lorenz K, Kulp K (1982) Cereal-Starch and Root Starch Modification by Heat-Moisture Treatment .1. Physicochemical Properties. *Starke* **34,** 50-54.

Lukow OM, White NDG, Sinha RN (1995) Influence of Ambient Storage Conditions on the Breadmaking Quality of Two Hard Red Spring Wheats. *Journal of Stored Product Research* **31,** 279-289.

Lunn GD, Kettlewell PS, Major BJ, Scott RK (2001a) Effects of pericarp alpha-amylase activity on wheat (Triticum aestivum) Hagberg falling number. *Annals of Applied Biology* **138,** 207-214.

Lunn GD, Major BJ, Kettlewell PS, Scott RK (2001b) Mechanisms Leading to Excess Alpha-Amylase Activity in Wheat (Triticum aestivum, L) Grain in the UK. *Journal of Cereal Science* **33,** 313-329.

MacMasters MM, Hinton JJC, Bradbury D (1971) Microscopic Structure and Composition of the Wheat Kernel. In 'Wheat: Chemistry and Technology'. (Ed. Y Pomeranz) pp. 51-99. (AACC: Minnesota)

Macri A, Parlamenti R, Silano V, Valfre F (1977) Adaptation of Domestic Chicken, Gallus-Domesticus, to Continuous Feeding of Albumin Amylase Inhibitors from Wheat-Flour As Gastro-Resistant Microgranules. *Poultry Science* **56,** 434-441.

Marchylo BA, Kruger JE, Irvine GN (1976) a-Amylase from Immature Hard Red Spring Wheat. I. Purification and Some Chemical And Physical Properties. *Cereal Chemistry* **53,** 157-173.

Marchylo BA, Lacroix LJ, Kruger JE (1980) alpha-Amylase Isoenzymes in Canadian Wheat Cultivars During Kernel Growth and Maturation. *Canadian Journal of Plant Science* **60,** 433-443.

Mazzuco H, Portella JA, Barioni W, Zanotto DL, de Miranda MZ, de Avila VS (2002) Influence of grain maturity stage at harvest and drying temperatures on AMEn of wheat grains for poultry. *Brazilian Journal of Animal Science* **31,** 2221-2226.

McCracken KJ, Stewart RA (2001) Importance of amino-acid and electrolyte balance in experimental diets used to determine the apparent metabolisable energy value of wheat *British Poultry Science* **42** 64-69

McCracken KJ, Bedford, MR, Stewart RA (2001) Effects of variety, the 1B/1R translocation and xylanase supplementation on nutritive value of wheat for broilers *British Poultry Science* **42** 638-642

McLelland J (1979) Digestive System. In 'Form and Function in Birds'. (Eds A King and J McLelland) pp. 69-181. (Academic Press: London)

Meng X, Slominski BA, Nyachoti CM, Campbell LD, Guenter W (2005) Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. *Poultry Science* **84,** 37-47.

Mignon-Grasteau S, Muley N, Bastianelli D, Gomez J, Peron A, Sellier N, Millet N, Besnard J, Hallouis JM, Carre B (2004) Heritability of digestibilities and divergent selection for digestion ability in growing chicks fed a wheat diet. *Poultry Science* **83,** 860-867.

Min BJ, Hong JW, Kwon OS, Lee WB, Kim YC, Kim IH, Cho WT, Kim JH (2004) The effect of feeding processed soy protein on the growth performance and apparent heal digestibility in weanling pigs. *Asian-Australasian Journal of Animal Sciences* **17,** 1271-1276.

Min T, Kim J, Tian J, Cho W, Hyun Y, Sohn K, Han I (2001) Effects of yucca extracts and protein levels on growth performance and nutrient utilisation in growing pigs. *Asian-Australasian Journal of Animal Sciences* **14,** 61-69.

Mohammed A, Rayas-Duarte P, Gordon, SH , Xu, J (2004) Estimation of HRW wheat heat damage by DSC, capillary zone electrophoresis photoacoustic spectroscopy and rheometry *Food Chemistry* **87** 195-203

Mollah Y, Annison EF (1981) Prediction of Metabolisable Energy from Wheat, Maize and Sorghum in Poultry Diets from Chemical Composition. *Proceedings of the Nutrition Society of Australia* **6,** 137.

Moran ET (1985) Digestion and Absorption of Carbohydrates in Fowl and Events Through Perinatal-Development. *Journal of Nutrition* **115,** 665-674.

Moran ET (1982) Starch Digestion in Fowl. *Poultry Science* **61,** 1257-1267.

Morrison WR (1993) Cereal starch granule development and composition. In 'Seed Storage Compounds: Biosynthesis, Interactions and Manipulation'. (Eds P Schewry and A Stobart) pp. 175-190. (Oxford University Press: Oxford)

Morrison WR, Gadan H (1987) The Amylose and Lipid Contents of Starch Granules in Developing Wheat Endosperm. *Journal of Cereal Science* **5,** 263-275.

Morrison WR, Milligan T, Azudin M (1984) A Relationship Between the Amylose and Lipid Contents of Starches from Diploid Cereals. *Journal of Cereal Science* **2,** 257-271.

Mrva K, Mares DJ (1996) Inheritance of late maturity a-amylase in wheat. *Euphytica* **88,** 61-67.

Muralikrishna G, Nirmala M. (2005) Cereal a-amylases - an overview. *Carbohydrate Polymers* **60** 163-173.

Nichol N (1999) Apparent Metabolisable Energy Values of Wheat in Chick Diets. PhD Thesis University of Nottingham.

Nielsen BL (2004) Behavioural aspects of feeding constraints: do broilers follow their gut feelings? *Applied Animal Behaviour Science* **86,** 251-260.

Niu, Z. Y, Classen, H. L., and Bennett, C. D. (1996) The Effect of Micronization, Tempering and Enzyme Addition on the Feeding Value of Wheat for Broiler Chickens. *Poultry Science* **75**[Supplement], 15

Noy Y, Sklan D (1999) Energy utilization in newly hatched chicks. *Poult.Sci.* **78,** 1750-1756.

Nyachiro JM, Clarke FR, Depauw RM, Knox RE, Armstrong KC (2002) Temperature effects on seed germination and expression of seed dormancy in wheat. *Euphytica* **126,** 123-127.

Ozbek B, Yuceer S (2001) Alpha amylase inactication udring wheat starch hydrolysis process *Process Biochemistry* **37** (1) 87-95

Peng, M, Gao, M, Abdel-Aal, E. S. M, Hucl, P, and Chibbar, R. N. (1999) Separation and Characterization of A- and B- Type Starch Granules in Wheat Endosperm. *Cereal Chemistry* **76**(3), 375.

Perez-Carrillo E, Serna-Saldivar S (2006) Cell Wall Degrading Enzymes and Proteases Improve Starch Yields of Sorghum and Maize. *Starch-Starke* **58,** 338-344.

Peron A, Bastianelli D, Oury FX, Carre B (2005) Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed pelleted diets *British Poultry Science* **46** 223-230

Peron A, Gomez J, Mignon-Grasteau S, Sellier N, Besnard J, Derouet M, Juin H, Carre B (2006) Effects of wheat quality on digestion differ between the D+ and D- chicken lines selected for divergent digestion capacity. *Poultry Science* **85,** 462-469.

Peron A, Svihus B, Gabriel I, Berot S, Tanguy D, Bouchet B, Gomez J, Carre B (2007) Effects of two wheat cultivars on physico-chemical properties of wheat flours

and digesta from two broiler chicken lines (D+ and D-) differing in digestion capacity. *British Poultry Science* **48,** 370-380.

Petersen ST, Wiseman J, Bedford MR (1999) Effects of age and diet on the viscosity of intestinal contents in broiler chicks. *British Poultry Science* **40,** 364-370.

Pirgozliev VR, Birch CL, Rose SP, Kettlewell PS, Bedford MR (2003) Chemical composition and the nutritive quality of different wheat cultivars for broiler chickens. *British Poultry Science* **44,** 464-475.

Pirgozliev, V. R. and Rose, S. P. (2001) Storage of sprouted and non-sprouted wheat samples on their metabolisable energy and broiler growth performance. *British Poultry Science* **42** S102-S103

Pirgozliev VR, Rose SP, Kettlewell PS (2006) Effect of ambient storage of wheat samples on their nutritive value for chickens. *British Poultry Science* **47,** 342-349.

Pirgozliev, V. R., Rose, S. P., Kettlewell, P. S., and Field, M. (2002) Comparison of feeding quality of durum and aestivum wheat for broiler chickens. *British Poultry Science* **43** (5), S43-S44

Pixton SW, Hill ST (1975) Long-term Storage of Wheat -III; Some Changes in the Quality of Wheat Observed During 16 Years of Storage. *Journal of Stored Product Research* **11,** 177-185.

Plavnik I, Macovsky B, Sklan D (2002) Effect of feeding whole wheat on performance of broiler chickens. *Animal Feed Science and Technology* **96,** 229-236.

Posner ES, Deyoe CW (1986) Changes in Milling Properties of Newly Harvested Hard Wheat During Storage. *Cereal Chemistry* **63,** 451-456.

Preston CM, McCracken KJ, McAllister A (2000) Effect of diet form and enzyme supplementation on growth, efficiency and energy utilisation of wheat-based diets for broilers. *British Poultry Science* **41,** 324-331.

Pubols MH (1991) Ratio of Digestive Enzymes in the Chick Pancreas. *Poultry Science* **70,** 337-342.

Ramirez GA, Sarlin LL, Caldwell DJ, Yezak CR, Hume ME, Corrier DE, Deloach JR, Hargis BM (1997) Effect of feed withdrawal on the incidence of Salmonella in the crops and ceca of market age broiler chickens. *Poultry Science* **76,** 654-656.

Ravindran V, Johnston S, Camden BJ, Thomas DV (2001) Influence of storage of New Zealand wheats on energy availability for poultry. In 'Recent Advances in Animal Nutrition in Australia'. (Ed. JL Corbett) p. 30A. (The University of New England: Armidale)

Ravindran V, Tilman ZV, Morel PCH, Ravindran G, Coles GD (2007) Influence of beta-glucanase supplementation on the metabolisable energy and ileal nutrient digestibility of normal starch and waxy barleys for broiler chickens. *Animal Feed Science and Technology* **134,** 45-55.

Rehman Z, Shah H (1999) Biochemical Changes in Wheat During Storage at Three Temperatures. *Plant Foods for Human Nutrition* **54,** 109-117.

Robin JP, Mercier C, Charbonn R, Guilbot A (1974) Lintnerized Starches Gel-Filtration and Enzymatic Studies of Insoluble Residues from Prolonged Acid Treatment of Potato Starch. *Cereal Chemistry* **51,** 389-406.

Rogel AM, Annison EF, Bryden WL, Balnave D (1987) The Digestion of Wheat-Starch in Broiler-Chickens. *Australian Journal of Agricultural Research* **38,** 639-649.

Rose SP, Tucker LA, Kettlewell PS, Collier JDA (2001) Rapid tests of wheat nutritive value for growing chickens. *Journal of Cereal Science* **34,** 181-190.

Ross AS, Walker CE, Booth RI, Orth RA, Wrigley CW (1987) The Rapid Visco-Analyzer - A New Technique for the Estimation of Sprout Damage. *Cereal Foods World* **32,** 827-829.

Rutkowski A, Wiaz M (2001) Effect of feeding whole or ground wheat grain on the weight of the gizzard and pH of digests in broiler chickens. *Journal of Animal and Feed Sciences* **10,** 285-289.

Salah-Uddin M, Rose SP, Hiscock TA, Bonnet S (1996) A comparison of the energy availability for chickens of ground and whole grain samples of two wheat varieties *British Poultry Science* **37** (2) 347-357

Sargeant JG (1982) Determination of Amylose - Amylopectin Ratios of Starches. *Starke* **34,** 89-92.

Sargeant JG, Walker TS (1978) Adsorption of Wheat alpha-Amylase Isoenzymes to Wheat Starch. *Starch-Starke* **30,** 160-163.

Schofield JD, Bottomley RC, Timms MF, Booth MR (1983) The Effect of Heat on Wheat Gluten and the Involvement of Sulfhydryl-Disulfide Interchange Reactions. *Journal of Cereal Science* **1,** 241-253.

Scott TA, Pierce AB (2001) The effect of storage of cereal grain and enzyme supplementation on measurements of AME and broiler chick performance. *Canadian Journal of Animal Science* **81,** 237-243.

Scott TA, Silversides FG, Classen HL, Swift ML, Bedford MR (1998) Effect of cultivar and environment on the feeding value of Western Canadian wheat and barley samples with and without enzyme supplementation *Canadian Journal of Animal Science* **78** (4) 649-656

Short FJ, Gorton P, Wiseman J, Boorman KN (1996) Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology* **59,** 215-221.

Short FJ, Wiseman J, Boorman KN (2000) The effect of the 1B/1R translocation and endosperm texture on amino acid digestibility in near-isogenic lines of wheat for broilers. *Journal of Agricultural Science* **134,** 69-76.

Sklan D, Noy Y (2000) Hydrolysis and absorption in the small intestines of posthatch chicks. *Poult.Sci.* **79,** 1306-1310.

Sklan D, Noy Y (2004) Catabolism and deposition of amino acids in growing chicks: effect of dietary supply. *Poult.Sci.* **83,** 952-961.

Slade L, Levine H (1988) Non-Equilibrium Melting of Native Granular Starch .1. Temperature Location of the Glass-Transition Associated with Gelatinization of A-Type Cereal Starches. *Carbohydrate Polymers* **8,** 183-208.

Smith DP, Berrang ME (2006) Prevalence and numbers of bacteria in broiler crop and gizzard contents. *Poultry Science* **85,** 144-147.

Smith ER, Pesti GM (1998) Influence of broiler strain cross and dietary protein on the performance of broilers. *Poult.Sci.* **77,** 276-281.

Smith ER, Pesti GM, Bakalli RI, Ware GO, Menten JF (1998) Further studies on the influence of genotype and dietary protein on the performance of broilers. *Poult.Sci.* **77,** 1678-1687.

Soulaka A, Morrison WR (1985) The amylose and lipid contents, dimensions and gelatinisation characteristics of some wheat starches and their A-granule and B-granule fraction. *Journal of the Science of Food and Agriculture* **36,** 709-718.

Spies RD, Hoseney RC (1982) Effects of Sugars on Starch Gelatinisation. *Cereal Chemistry* **59,** 128-131.

Steenfeldt S (2001) The dietary effect of different wheat cultivars for broiler chickens. *British Poultry Science* **42,** 595-609.

Sterling KG, Pesti GM, Bakalli RI (2006) Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. *Poult.Sci.* **85,** 1045-1054.

Sterling KG, Vedenov DV, Pesti GM, Bakalli RI (2005) Economically optimal dietary crude protein and lysine levels for starting broiler chicks. *Poultry Science* **84,** 29-36.

Stewart RA, McAllister A, McCracken KJ (1998) Effects of wheat source, heat treatment and enzyme inclusion on diet metabolisability and broiler performance. *British Poultry Science* **39,** S42-S43.

Sugimoto Y (1980) Scanning Electron Microscope Observation of Starch Granules Attacked by Enzyme. *Journal of the Japanese Society of Starch Science* **27,** 28-40.

Svihus B, Gullord M (2002) Effect of chemical content and physical characteristics on nutritional value of wheat, barley and oats for poultry. *Animal Feed Science and Technology* **102,** 71-92.

Svihus B, Herstad O, Newman CW (1997) Effect of high-moisture storage of barley, oats, and wheat on chemical content and nutritional value for broiler chickens. *Acta Agriculturae Scandinavica Section A-Animal Science* **47,** 39-47.

Svihus B, Hetland H (2001) Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion *British Poultry Science* **42** (5) 633-637

Swan CG, Meyer FD, Hogg AC, Martin JM, Giroux MJ (2006) Puroindoline b limits binding of Puroindoline a to starch and grain softness. *Crop Science* **46,** 1656-1665.

Taylor RD, Jones GPD (2004) The incorporation of whole grain into pelleted broiler chicken diets. II. Gastrointestinal and digesta characteristics. *British Poultry Science* **45,** 237-246.

Tester RF, Karkalas J, Qi X (2004a) Starch - composition, fine structure and architecture. *Journal of Cereal Science* **39,** 151-165.

Tester RF, Karkalas J, Qi X (2004b) Starch structure and digestibility enzyme-substrate relationship. *Worlds Poultry Science Journal* **60,** 186-195.

Tester RF and Morrison WR (1990) Awelling and Gelatinization of Cereal Starches. I. Effects of amylopectin, Amylose and Lipids *Cereal Chemistry* **67** (6) 551-557

Tester RF, Morrison WR, Ellis RH, Piggott JR, Batts GR, Wheeler TR, Morison JIL, Hadley P, Ledward DA (1995) Effects of Elevated Growth Temperature and Carbon-Dioxide Levels on Some Physicochemical Properties of Wheat-Starch. *Journal of Cereal Science* **22,** 63-71.

Tester RF, Sommerville MD (2003) The effects of non-starch polysaccharides on the extent of gelatinisation, swelling and alpha-amylase hydrolysis of maize and wheat starches. *Food Hydrocolloids* **17,** 41-54.

Tester RF, South JB, Morrison WR, Ellis RP (1991) The Effects of Ambient-Temperature During the Grain-Filling Period on the Composition and Properties of Starch from 4 Barley Genotypes. *Journal of Cereal Science* **13,** 113-127.

Toole GA, Wilson.R.H, Parker ML, Wellner NK, Wheeler TR, Shewry PR, Mills ENC (2007) The effect of environment on endosperm cell wall development in Triticum aestivum during grain filling: an infrared spectroscopic imaging study. *Planta* **225,** 1393-1403.

Ueda S (1978) Amylase adsorption on Raw Starch and its Relation to Raw Starch Digestion. *Journal of the Japanese Society of Starch Science* **25,** 124-131.

Vieites FM, de Moraes GHK, Albino LFT, Rostagno HS, Donzele JL, de Vargas JG, Atencio A (2004) Electrolyte balance and crude protein levels on the performance of broiler chicks from 1 to 21 days of age. *Revista Brasileira de Zootecnia-Brazilian Journal of Animal Science* **33,** 2076-2085.

Viera SL (2003) Nutritional Implications of Mould Development in Feedstuffs and Alternatives to Reduce the Mycotoxin Problem in Poultry Feeds. *Worlds Poultry Science Journal* **59,** 111-122.

Walker CE, Ross AS, Wrigley CW, McMaster GJ (1988) Accelerated Starch-Paset Characterization with the Rapid Visco-Analyzer. *Cereal Foods World* **33,** 491-494.

Wang LF, Flores RA (1999) The effects of storage on flour quality and baking performance. *Food Reviews International* **15,** 215-234.

Wanjugi HW, Hogg AC, Martin JM, Giroux MJ (2007) The role of puroindoline A and B individually and in combination on grain hardness and starch association. *Crop Science* **47,** 67-76.

Weegels PL, Degroot AMG, Verhoek JA, Hamer RJ (1994a) Effects on Gluten of Heating at Different Moisture Contents .2. Changes in Physicochemical Properties and Secondary Structure. *Journal of Cereal Science* **19,** 39-47.

Weegels PL, Verhoek JA, Degroot AMG, Hamer RJ (1994b) Effects on Gluten of Heating at Different Moisture Contents .1. Changes in Functional-Properties. *Journal of Cereal Science* **19,** 31-38.

Weurding RE, Veldman A, Veen WAG, van der Aar PJ, Verstegen MWA (2001) Starch digestion rate in the small intestine of broiler chickens differs amongst feedstuffs *Journal of Nutrition* **131** 2329-2335

Wickramasinghe HAM, Miura H, Yamauchi H, Noda T (2005) Comparison of the starch properties of Japanese wheat varieties with those of popular commercial wheat classes from the USA, Canada and Australia. *Food Chemistry* **93,** 9-15.

Wijtten PJA, Prak R, Lemme A, Langhout DJ (2004) Effect of different dietary ideal protein concentrations on broiler performance. *British Poultry Science* **45,** 504-511.

Wiseman J (2000) Correlation Between Physical Measurements and Dietary Energy Values of Wheat for Poultry. *Animal Feed Science and Technology* **84,** 1-11.

Wu YBB, Ravindran V (2004) Influence of whole wheat inclusion and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics of broiler chickens. *Animal Feed Science and Technology* **116,** 129-139.

Yao JH, Tian XY, Xi HB, Han JC, Xu M, Wu XB (2006) Effect of choice feeding on performance, gastrointestinal development and feed utilization of broilers. *Asian-Australasian Journal of Animal Sciences* **19,** 91-96.

Yasar, S, Forbes, JM (1999) Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water *British Poultry Science* **40** 65-76

Yo T, Siegel PB, Faure JM, Picard M (1998) Self-selection of dietary protein and energy by broilers grown under a tropical climate: Adaptation when exposed to choice feeding at different ages. *Poultry Science* **77,** 502-508.

Zarkadas LN, Wiseman J (2001) Influence of processing variables during micronization of wheat on starch structure and subsequent performance and digestibility in weaned piglets fed wheat-based diets. *Animal Feed Science and Technology* **93,** 93-107

Zhou M, Glennie Holmes R, Robards K, Helliwell S (1999) Effects of processing and short-term storage on the pasting characteristics of slurries made from raw and rolled oats *Food Australia* **51** (6) 251-258

Zia-Ur-Rehman (2006) Storage effects on nutritional quality of commonly consumed cereals. *Food Chemistry* **95,** 53-57

Zweifel C, Conde-Petit B, Escher F (2000) Thermal modifications of starch during high-temperature drying of pasta. *Cereal Chemistry* **77,** 645-651.

Zweifel C, Handschin S, Escher F, Conde-Petit B (2003) Influence of high-temperature drying of structural and textural properties of durum wheat pasta. *Cereal Chemistry* **80,** 159-167.

Appendix A Trial Two - Moisture content (g/kg) of wheat ingredient of diets 1-8

Wheat	Diet	Temperature Treatment °C	Moisture content Before[1]	Moisture Content After[2]
Einstein	1	0 - Control	-	137
	2	70	226	155
	3	85	230	179
	4	100	230	158
Clare	5	0 - Control	-	145
	6	70	233	174
	7	85	227	156
	8	100	233	156

[1]After soaking, before drying
[2]After drying