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Influence of Storage and Temperature Treatment on the Nutritional Value of Wheat for Poultry

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Contents

SUMMARY

In 2007, 787 million tonnes of wheat were produced in the world (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. High moisture content wheat at harvest is dried very carefully in order to maintain quality as far as possible; similar care is taken with such wheat if it is for seed. However, the drying conditions for feed wheats tend to be more severe, although there is little work on actual quality associated with these conditions.

The current project aimed to address three major research areas:

1. The effects of heat treatment in relation to the nutritional value of weather damaged wheat.

It appears that drying grains at 100ºC may increase the digestibility of wheat starch. However, some flour samples that were heated to 100ºC failed to demonstrate expected hydration properties that would normally be associated with increased digestibility. Therefore, an increase in coefficient of starch digestibility (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 85ºC, starch digestibility may be decreased, possibly due to changes in starch structure. Apparent Metabolisable Energy (AME) data did not follow starch digestibility.

2. 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 100ºC. These high levels of amylase activity did not appear to affect starch digestibility, presumably due to deactivation in the acidic conditions of the stomach. 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 estimate nutritional value.

3. The nutritional value of wheat after storage at ambient conditions for up to four months

There was no significant difference in AME, CAD or Feed Intake (FI).

Conclusions

The current programme of work led to the following conclusions:

- Heat treatment to dry grains at 70ºC or 100ºC, even with a starting dry matter content as low as 630g/kg (63%), does not affect AME. However, drying at 85ºC may begin to affect some of the components of the flour, in a way that is detrimental to digestibility.
- Drying at 100ºC compared to ambient conditions may increase starch digestibility, but this is possibly not a direct effect of changes in starch structure.
- For the above reasons growers could be advised that drying wheat that has been harvested wet, at 70ºC or 100ºC, in a convection oven, will probably not damage nutritional quality.
- Wheat that has a dry matter content of 630g/kg (63%) 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 100ºC reduces the pasting ability of two test wheat E (Hard) and C (Soft), but this is not due to a loss in starch crystallinity
- During two months ambient storage, there is no change in nutritional value of soft wheat variety D.
- There is no difference in nutritional quality between the hard wheat varieties E and soft wheat C. A more descriptive hardness score would be beneficial in wheat classification.
- E, a hard wheat variety, is more sensitive to heat treatment than a soft wheat, C.

INTRODUCTION

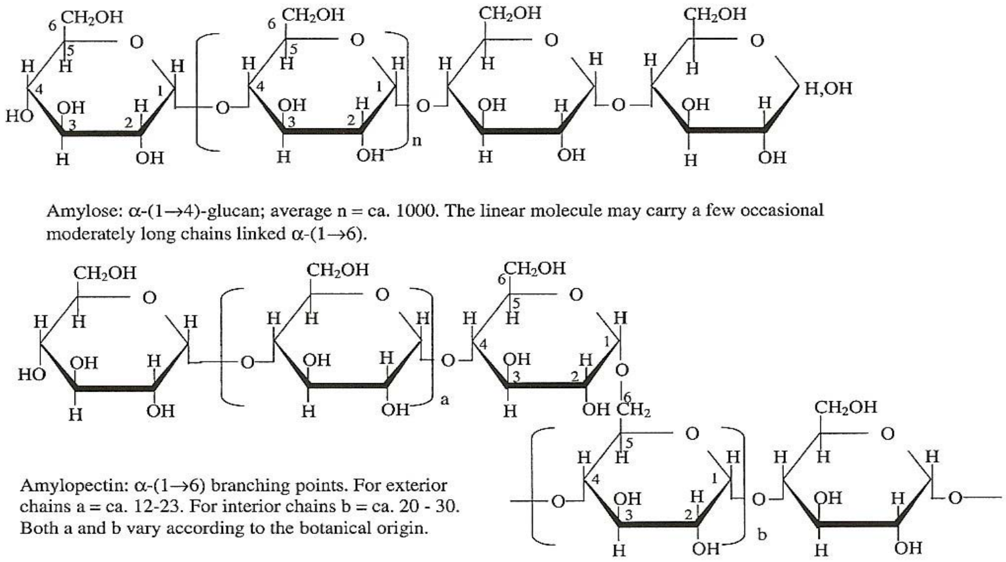
Wheat has been described as the most important food crop in the world (Giroux and Morris 1998). Animal feed is the second largest use for cereals across the globe, and their use in animal diets is growing due primarily to the high starch content that can constitute around 0.60 the diet and is of high 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 endogenous amylase levels, which can render the wheat of no value 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. Based on a moisture content of around 140 g/kg (14%), 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), 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.* 2004).



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

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).

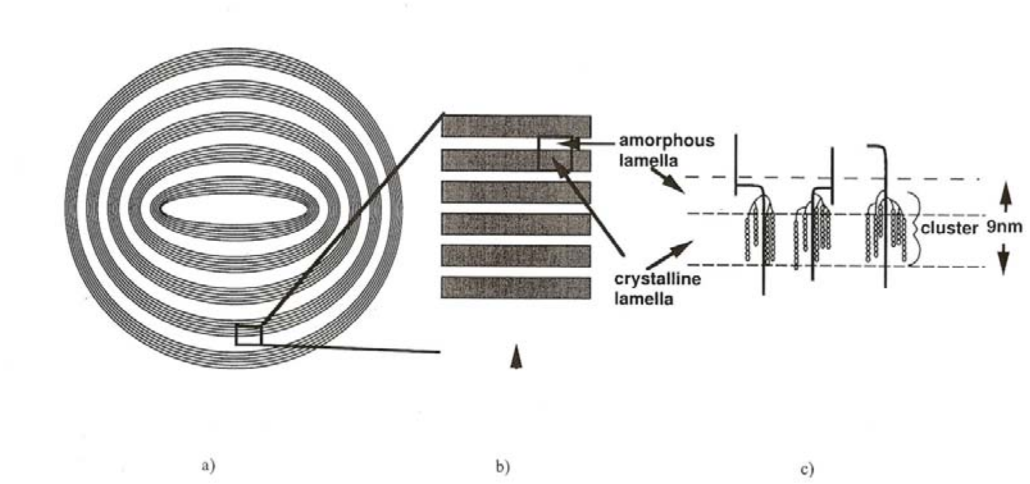


Figure 2 The semi crystalline structure of a starch granule. Modified from Donald *et al* (1997).

Starch has a semi-crystalline structure and is arranged in the granule in dark and light layers or lamellae (see figure 2). 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 2, 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.* 2004). Changes in these lamella and states of crystallisation, caused by processing, may affect gelatinisation and the extractability of amylose (Donovan *et al.* 1983).

Starch is a carbohydrate, comprised of two molecules, amylose and amylopectin, shown in figure 1. It is present in plant sources as small granules, within which molecules are laid down from the inside out (see figure 2). This structure is referred to as being crystalline. 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.

Aims and Objectives

Weather damage of wheat is a topical subject for much research. Overall, the current project aims to 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:

- 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?

MATERIALS AND METHODS

Wheat

Table 1 shows the three varieties of wheat that were used in trials, grown at various locations.

Table 1 Information regarding wheat used in trials (HGCA 2006).

Variety name	Description	Location	Trial
C	Soft, group three	Not known, provided by Nickerson (UK) Ltd*	2
D	Soft, group three	Sutton Bonington ^	4
E	Hard, group two	Not known, provided by Nickerson (UK) Ltd*	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

Chick Biosassay

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.

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. 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.

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 3 and 4 and wheat variety variations are shown in table 1. The vitamin and mineral premix contained the components shown in table 2.

Table 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 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 3 Suppliers of Dietary components.

Component	**Supplier**
Starch (purified from maize)	D F Dickens Ltd, Nottingham, UK
Glucose	D F Dickens Ltd, Nottingham, UK
Soya Oil	Central Wool Growers, Northamptonshire, UK
Vitamin and Mineral Premix (see table 2)	Target Feeds Limited, Shropshire, UK
Titanium Dioxide	Fisher Scientific, Leicestershire, UK

Table 4 Basal Dietary Composition.

Component	**Amount (g/kg diet)**
Wheat (see table 1)	750
Starch (from maize)	70
Glucose	70
Soya Oil	50
Vitamin and Mineral Premix	50
Titanium Dioxide	10

ANALYSES

Gross Energy

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

Starch Digestibility Determination

Starch digestibility was determined on a whole diet basis. Coefficient of apparent starch digestibility (CAD) refers to digestibility of whole diet starch. Although purified maize starch and glucose were 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. 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.

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).

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.

Statistical Analysis

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.

TRIAL ONE

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 (14%) 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 100ºC.

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 100ºC 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.

Diets were formulated as per table 4 in addition to wheat at a rate of 750g/kg. Wheat samples underwent the treatments as shown in table 5.

Table 5 Treatment of wheat dietary components.

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

Results

The statistical analysis of the effects of wheat variety and drying temperature on CAD is shown in table 6. The statistical model employed was a 2 (wheat cultivar) x 4 (temperature treatment) x 3 (region of the digestive tract) factorial.

There was a significant effect of temperature (P=0.007) and region (P<0.001) on CDAD, CIAD and CTTAD (CAD) (table 6). 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 E, dried at 85°C was significantly less than those fed C. However, it was the opposite in the control wheats.

These differences in starch digestibility attributed to wheat dried at 85°C were not supported by a significant difference in AME (data not displayed). However, there was a trend toward a decrease in AME with temperature (P=0.051).

There was no significant difference in FI.

TRIAL TWO

The results of the previous trial, 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. Moisture content of the wheat samples to be heat treated is perhaps equally as important as the drying temperature employed. According to the literature, advantages and disadvantages were found with heat treating wheat of very specific moisture contents (Kulp and Lorenz 1981; Mazzuco *et al.* 2002).

Wheat samples

Figure 3 shows the relationship between samples and the allocation to each diet. One variety, E, 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;

Table 6 Analysis of Variance showing the effect of temperature on the coefficient of apparent digestibility (of starch) of diets containing either C or E.

Factor			**Temperature**				**ANOVA**		
Region	**Wheat Variety**	**Control**	**70°C**	**85°C**	**100°C**	**Mean**		**P**	**sed**
Duodenum[1]	**C**	0.462	0.371	0.266	0.398	0.374	**Temperature**	0.007	0.0444
	E	0.658	0.475	0.001	0.498	0.408	**Region x Wheat Variety**	0.606	0.0449
Ileum[2]	**C**	0.738	0.728	0.724	0.743	0.733	**Region x Temperature x Wheat Variety**	0.011	0.0898
	E	0.869	0.822	0.756	0.781	0.807			
Total Tract[3]	**C**	0.937	0.959	0.810	0.921	0.907			
	E	0.852	0.947	0.954	0.952	0.926			
	Temperature Mean	0.753	0.717	0.585	0.716				
		Control	**70°C**	**85°C**	**100°C**	**Mean**		**P**	**sed**
Wheat Variety	**C**	0.712	0.686	0.600	0.687	0.671	**Wheat Variety**	0.187	0.0314
	E	0.793	0.748	0.571	0.744	0.714	**Region**	<0.001	0.0278
							Region x Temperature	0.002	0.0635
Region	**Duodenum[1]**	0.560	0.423	0.134	0.448	0.391	**Temperature x Wheat Variety**	0.525	0.0628
	Ileum[2]	0.803	0.775	0.740	0.762	0.770			
	Total Tract[3]	0.894	0.953	0.882	0.937	0.917			

[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)

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 72 hours, or at ambient temperatures. This gave eight samples for nutritional evaluation. In the previous trial 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 7.

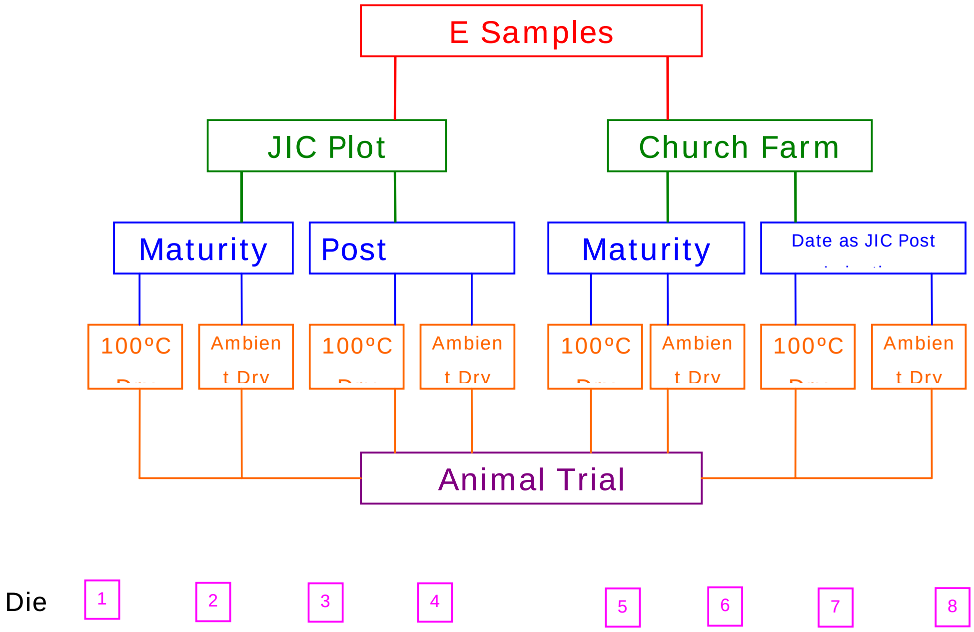


Figure 3 Description of wheat samples grown for trial two.

Table 7 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'

Results

The results of statistical analysis of the effects of harvest date and drying regime on the CAD and FI are shown in tables 8 and 9.

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 100ºC 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 100ºC 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 100ºC drying regime.

None of these effects on CAD was supported by accompanying difference in AME.

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

Table 8 Analysis of Variance showing the effect of drying regime and harvest on the coefficient of apparent digestibility of diets.

		Harvest								ANOVA		
		First				Second						
	Region	**Duodenum[1]**	**Ileum[2]**	**Total Tract[3]**	**Mean**	**Duodenum[1]**	**Ileum[2]**	**Total Tract[3]**	**Mean**	**Factor**	**P**	**sed**
Drying Regime	**Ambient**	0.331	0.719	0.862	0.637	0.529	0.854	0.935	0.773	**Harvest**	0.035	0.0177
	100°C	0.598	0.859	0.866	0.774	0.618	0.827	0.858	0.768	**Harvest x region**	0.173	0.0298
	Mean	0.464	0.789	0.864		0.574	0.841	0.897		**Drying regime x harvest**	0.028	0.0250
	Mean	0.706				0.770				**Drying regime x region x harvest**	0.438	0.0422
	Region	**Duodenum[1]**	**Ileum[2]**	**Total Tract[3]**	**Mean**							
Drying Regime	**Ambient**	0.430	0.786	0.898	0.705					**Drying regime**	0.033	0.0177
	100°C	0.608	0.843	0.862	0.771					**Region**	<0.001	0.0208
	Mean	0.519	0.815	0.880						**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)

Table 9 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

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 100ºC 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 60ºC 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 100ºC.

Alternatively, these increases in CAD with extended harvest date and 100ºC 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 one 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 100ºC 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.* 2004). 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) 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 the project thesis, Chapter 7.

Clearly, the treatments for harvest two at the two sites were not equivalent. 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 10. 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 and that the irrigation had not had an effect, moisture content prior to drying does affect CAD (P=0.004, table 10). However, this was non-linear (Plin=0.924), with 250g/kg 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.

Table 10 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**
Drying regime	**Ambient**	0.734	0.716	0.829	0.725	**Moisture content**	0.004	0.0413
	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.

TRIAL THREE

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).

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.* D) grown at Sutton Bonington. The crop was harvested at 230g/kg moisture content and dried twice, at 62ºC, 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 10-45ºC, the less the chemical changes within the wheat grain. No significant changes were found at 10ºC, after 6 months storage (Rehman and Shah 1999). Due to the lack of wheat samples taken prior to storage, the control used was variety C, known to be appropriate for poultry feeding. Diets were formulated as shown in table 4, with the addition of wheat at a rate of 750g/kg. Modifications were made as shown in table 11. The fourth diet (table 11) was a combination of both C and D in equal proportions. This was to investigate the potential amelioration of negative quality using 'standard' wheat (C).

Table 11 Diet wheat components.

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

Results

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

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 ($P<0.001$, not displayed here), 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.

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.

CONCLUSION

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 100ºC 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 100ºC. Considering both trials one and two, drying at 70ºC is appropriate and may be more economically efficient, but 100ºC 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.

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