Influence of processing on starch digestibility and gut morphology in the weaned piglet

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ABSTRACT

To overcome the 'post-weaning growth check' commonly seen at weaning, the incorporation of antibiotic growth promoters (AGPs) to the diet has been a useful management tool. Recent legislation within the European Union banning the use of AGPs at sub-therapeutic levels in animal feed means that the quality of dietary ingredients used in weaner diets has assumed a much more fundamental role. In order to improve the availability of starch in the piglet diet, processing of cereals is widely practised. However, descriptions of processing techniques used in many studies are simply referred to by name, with no regard of the precise variables used. In addition, many of the feed materials are simply referred to as 'cooked' which gives little indication of nutritional value.

Five trials were conducted in order to assess the use of raw and processed cereals on diet component digestion, digesta properties and gut morphology in newly-weaned piglets. The main objective was to examine the use of precisely controlled processing variables, such that starch digestibility was maximised with benefits for the gastrointestinal environment. A second aspect of the programme of work reported was the application of a number of analytical tests commonly used in the field of human food science, to examine the physicochemical properties of starch granules, and the changes they undergo upon processing. Using this approach, a comparison could be made between *in-vitro* (rheological) results and *in-vivo* (biological) responses.

Trials 1 and 2 examined variability between raw cereals. Wheat, barley, rye and triticale were assessed in Trial 1. Wheat (identical batch), naked oats, whole oats and maize were evaluated in Trial 2. Coefficients of apparent digestibility (CAD) for starch and nitrogen revealed considerable variation between the cereals. In Trial 1, there was a strong trend ($P = 0.051$) for starch digestion to be highest for the rye diet and lowest for triticale. CAD for starch was not significantly affected by cereal type in Trial 2. Despite having more viscous intestinal digesta than other animals ($P = <0.001$), pigs fed the rye-based diet did not experience any detrimental effects to animal performance.

Trial 3 examined the use of raw wheat, of either hard or soft endosperm texture. From 5 days post-weaning, piglets fed the soft wheat diet had a tendency (P = 0.063) to have higher feed intakes. In addition, pigs fed soft wheat diets had significantly less viscous tract digesta (P = 0.029) than those animals fed the diet based on hard wheat. There was no significant difference in CAD for starch between the two dietary treatments but CAD for nitrogen was found to be significantly higher (P = 0.006) in the distal region of the small intestine for pigs fed the soft wheat diet. The results from Trial 3 suggest that endosperm texture of wheat can have an effect on nutritional value, and that wheat of soft endosperm texture is more beneficial than hard wheat for the young piglet.

Trial 4 was a 2 x 2 factorial study examining wheat endosperm texture (hard vs. soft) and degree of micronisation (high cook vs. low cook). CAD for starch was not affected by endosperm texture, although degree of cook was an important factor with significantly higher starch digestion for the high cook diets, compared to low cook (P = 0.047). The use of micronised wheat lessened the reduction in starch digestibility seen on day 4 post-weaning in the small intestine, compared against the decline seen using raw wheat diets in Trial 3. In summary, Trial 4 demonstrated that micronisation can enhance the nutritional value of wheat for the weaned piglet, with degree of cook, a more significant factor than wheat endosperm texture.

Trial 5 assessed wheat endosperm texture (hard vs. soft) and degree of extrusion (high cook vs. low cook). Raw soft wheat was used as a control. Results showed that CAD for starch in the small intestine was noticeably higher than in the other animal trials. Starch digestion was significantly affected by endosperm texture (greater coefficients for soft than hard; P <0.001) and by degree of cook (high SME greater than low SME; P <0.001). The use of extruded wheat diets almost eliminated the drop in starch digestion at the 0.5 intestinal site seen on day 4 post-weaning. Wheat of soft endosperm texture responded better to extrusion processing than hard wheat under the conditions of Trial 5.

The use of computer modelling was able to demonstrate a correlation between *in-vitro* starch parameters and *in-vivo* starch digestion in the small intestine of the piglet.

PRESENTATIONS & PUBLICATIONS

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LIST OF ABBREVIATIONS

AGP	Antibiotic growth promoter
AIA	Acid-insoluble ash
BW	Body weight
CAD	Coefficient of apparent digestibility
cm	Centimetre
CMC	Carboxymethylcellulose
cP	Centipoise
CP	Crude protein
CTTAD	Coefficient of total tract apparent digestibility
cv	Coefficient of variation
CV	Cross validation
CVR	Control animals versus the rest
DE	Digestible energy
DLWG	Daily live weight gain
DM	Dry matter
DSC	Differential scanning calorimetry
EU	European union
FCR	Food conversion ratio
FI	Feed intake
g	G-force
g	Gram
g/d	Grams per day
GE	Gross energy
$g\ kg^{-1}$	Grams per kilogram
h	Hours
HCl	Hydrochloric acid
IMS	Industrial methylated spirit
$J\ g^{-1}$	Joules per gram

J kg^{-1}	Joules per kilogram
kg	Kilogram
L	Linear
M	Molar
ME	Metabolisable energy
mg	Milligram
min	Minute
MJ	Megajoule
ml	Millilitre
mm	Millimetre
mmol l^{-1}	Millimoles per litre
m^2	square metre
Nm	Newton metre
NS	Not significant
NSP	Non-starch polysaccharide
PCA	Principal component analysis
PLS	Partial least squares
pH	log of reciprocal hydrogen ion concentration
Q	Quadratic
rpm	Revolutions per minute
RVA	Rapid visco analyser
sec	Seconds
sed	Standard error of the difference
SME	Specific mechanical energy
µg	Microgram
µl	Microlitre
µm	Micrometre
µmol l^{-1}	Micromoles per litre
VFA	Volatile fatty acid
v/v	Volume to volume
vs.	Versus

WAI	Water absorption index
Wh kg^{-1}	Watt hours per kilogram
WSI	Water solubility index
w/v	weight to volume
XRD	X-ray diffraction

CHAPTER 1: LITERATURE REVIEW

1.1. WEANING

In current production systems, weaning is a crucial step and presents the young piglet with many challenges. In the wild, weaning is a gradual process which typically takes 3 to 4 months (Jensen, 1986; Jensen and Recén, 1989). During this time, the piglet will become accustomed to foods other than milk and its digestive enzymes will gradually adapt accordingly (English *et al.*, 1988). In modern U.K. production systems, weaning is abrupt and commonly occurs at a much younger age, typically at around 21-25 days, as the technology to feed and house the piglet at this age is apparently well established. The constant drive to wean at this early age often means the piglet is regularly subjected to stress from various sources. Because of the many challenges at weaning, no matter how carefully managed, the process itself can in fact be very stressful for the young animal. Although sources of stress around weaning are numerous, the major ones can be divided into three areas:

Psychological stress - The act of removal from the sow and placing into new surroundings is a considerable source of stress. The piglet has lost the reassurance of its mother's presence and is often mixed with animals from other litters. This means it has to determine its place within a new hierarchical structure, and this often leads to an increased incidence of fighting (Friend *et al.*, 1983; Worobec *et al.*, 1999). There is evidence to suggest that this aggressive behaviour may be alleviated by allowing unfamiliar piglets to mix with each other before weaning (Hessel *et al.*, 2006).

Environmental stress - The transition from a farrowing house to a nursery can also be a major stress for the young animal. Piglets reared in a poor environment have been found to behave more aggressively and exhibit higher basal cortisol levels than those reared in an enriched environment (De Jonge *et al.*, 1996). Simple measures such as the addition of straw to the floor of the pen can provide the piglet with sufficient interest in its surroundings to help prevent stress in the form of boredom (Dybkjaer, 1992).

Nutritional stress - There is a sudden change from a warm liquid diet (milk), which the sow provided to the piglet, several times a day on a regular basis, to a dry feed which is usually only available via a feeder. This dietary change from milk (which is designed to be digested and utilised effectively) to a dry solid feed requires rapid adaptation of the piglet's digestive system. In addition, the piglet must now also learn to drink water, usually from a hard steel nipple. This itself will be an alien experience, but one that needs to be learnt in order for survival. There is some evidence to suggest that eating and drinking behaviour post-weaning is correlated with weaning weight (Dybkjaer *et al.* 2006) where larger piglets spent less time eating at the feed trough and more time drinking. The opposite was true for the smaller animals.

1.1.1. Factors limiting weaned piglet performance

1.1.1.1. Low feed intake

The most limiting factor of piglet performance at weaning is voluntary food intake. At weaning, the piglet is faced with an unfamiliar diet, which it has to recognise, and decide when and how much to consume. Bark *et al.*(1986) reported that early-weaned piglets failed to consume an adequate amount of food during the initial three days post-weaning in an unfamiliar environment. This post-weaning anorexia is common and piglets often fail to consume sufficient food to meet their energy requirements. Le Dividich and Herpin (1994) reported that the metabolisable energy (ME) requirement for maintenance was not met until the fifth day after weaning (Figure 1.1). Furthermore, it was not until the end of the second week post-weaning that the ME intake levels reached those seen pre-weaning. Regardless of weaning age, ME intake at the end of the first week post-weaning is typically between 700 and 800 kJ ME/kg $BW^{0.75}$, which equates to only 0.6 - 0.7 of ME levels derived from milk before weaning (Le Dividich and Sève, 2000). By the time the piglet has associated the dry diet with nourishment, it usually has a high level of hunger. This can lead to abnormal feed intake patterns, which often result in digestive upsets for the relatively immature digestive system (Mavromichalis and Varley, 2003).

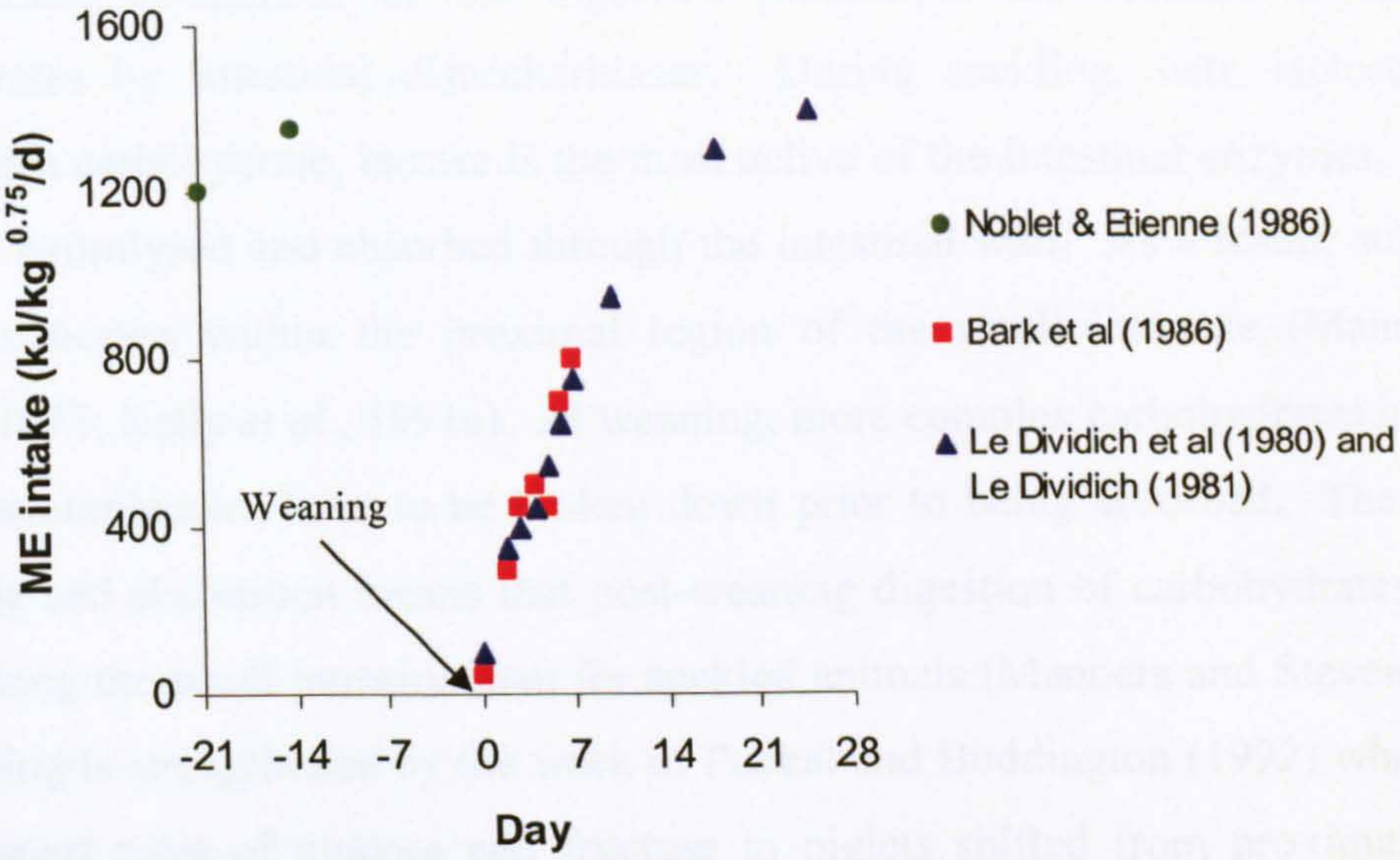


Figure 1.1: *The voluntary food intake of piglets before and after weaning (adapted from LeDividich and Herpin, 1994)*

The first few days after weaning are typically characterised by low or even zero feed intake. This inadequate intake of feed can lead to detrimental changes in gut structure and function. The important relationship between feed intake and gut morphology is described in more detail later in the chapter. As it seems possible that digestive disorders are an effect and not a cause of the problem of poor feed intake (Kelly *et al.*, 1992), every effort should be made to stimulate the piglet's appetite during this critical time in its life. In order to do this, the weaner diet should be of a composition that is both highly digestible and palatable.

1.1.1.2. Digestive enzyme development

Digestive enzymes from birth have been designed to cope well with a diet of sows' milk. High levels of the enzyme lactase are secreted, along with sufficient protease and lipase to digest the proteins and fats contained within the milk. Over the following weeks of the piglet's life, the amount and activity of these enzymes will change, in order to cope with the transition from a liquid to a solid dry diet. As the piglet grows and the nutritional composition of the diet changes, the digestive system will mature, and the ability to digest more complex proteins and carbohydrates (primarily starch) increases.

An important component of the digestive process is the breakdown of dietary carbohydrates by intestinal disaccharidases. During suckling, with lactose as the predominant carbohydrate, lactase is the most active of the intestinal enzymes. Lactose is readily hydrolysed and absorbed through the intestinal wall. As a result, subsequent absorption occurs within the proximal region of the small intestine (Manners and Stevens, 1972; Kelly *et al.*, 1991a). At weaning, more complex carbohydrates in the diet mean macromolecules have to be broken down prior to being absorbed. The delay in hydrolysis and absorption means that post-weaning digestion of carbohydrates extends further along the small intestine than for suckled animals (Manners and Stevens, 1972). This finding is strengthened by the work of Puchal and Buddington (1992) who showed that transport rates of glucose and fructose in piglets shifted from proximal to mid-intestine following weaning.

A number of studies have been carried out comparing the effect of weaning on the secretory patterns and activities of the main digestive enzymes in the young piglet:

(i) Lactase

Lactase is the enzyme responsible for the hydrolysis of the milk sugar lactose, into glucose and galactose. The general pattern of lactase secretion in the young piglet is shown in Figure 1.2a. This enzyme has been recorded in the piglet foetus as early as day 105 of gestation (Aumaitre and Corring, 1978). Consequently, high levels are present in the newborn, with levels peaking during the first week of life. Activity then gradually declines with age, and eventually reaches a relatively constant level by adulthood (Manners and Stevens, 1972; Kidder and Manners, 1980). Weaning has been reported to influence lactase activity; Miller *et al.* (1986) found the activity of lactase was significantly (2 to 5 fold) lower five days after weaning 21d old piglets, than those of similar age still left to suckle on the sow. A comparable effect was also reported in piglets weaned at two weeks of age (Kelly *et al.*, 1991a), when lactase activity was only 40% of that found in unweaned counterparts a week later.

(ii) Sucrase

Activity of this enzyme is rarely detectable at birth. Manners and Stevens (1972) were only able to detect activity in one of six newborn piglets. After the first week, however, sucrase activity was found to be present in all of the piglets. Activity typically rises progressively through to maturity (Figure 1.2b) although the process of weaning appears to have an effect on the pattern of secretion: Studies have shown that the act of weaning piglets at three weeks of age typically induces a temporary reduction in sucrase activity (Hampson and Kidder, 1986; Miller *et al*, 1986). This depression appears to be short-lived however, and enzyme levels in the post weaned piglet are typically greater in the following weeks than in animals left on the sow (Kelly *et al*., 1991a), even up to eight weeks of age (Kidder and Manners, 1980).

(iii) Maltase

This enzyme is responsible for the hydrolysis of maltose into two glucose molecules. Enzyme activity is very similar to that of sucrase and although present in low levels at birth, rises progressively with age (Figure 1.2c). Maltase activity has been shown to increase significantly (x 143) by eight weeks (Aumaitre and Corring, 1978), before reaching a plateau by around 200 days of age (Kidder and Manners, 1980). Numerous studies have shown that weaning can significantly increase activity levels in piglets, irrespective of weaning age (McCracken, 1984; Miller *et al.*, 1986; Kelly *et al.*, 1991a).

(iv) Trypsin and Chymotrypsin

Trypsin and chymotrypsin are initially secreted from the pancreas in their inactive forms (trypsinogen and chymotrypsinogen). Trypsinogen is converted into its active form (trypsin) by enterokinase in the intestinal mucosa. Trypsin is then able to activate chymotrypsinogen into chymotrypsin. These two enzymes continue the process of protein digestion, initiated in the stomach. The typical pattern of activity is shown in Figure 1.2d. It appears from the literature that enzyme levels are closely linked with pancreatic tissue development in the weeks following birth: Corring *et al* (1978) identified rapid pancreatic growth in piglets during the first week of life, and again from the fourth to the eighth week. Enzyme activities were closely linked with these two

stages of growth and development. From the third to fourth week of age, total enzymatic activities were found to markedly increase, which was attributed to the intake of solid feed by the piglets. Other authors have also reported a significant increase of enzyme levels, in relation to intake of solid feed at weaning (Efird *et al.*, 1982; Lindemann *et al.*, 1986).

(v) Pancreatic lipase

Secreted in its active form, this enzyme is responsible for the hydrolysis of dietary fats (triglycerides) into fatty acids and glycerol. In a study with unweaned piglets it was reported by Corring *et al.* (1978) that specific activity increased with age up to six weeks, when it peaked (Figure 1.2e). In weaned piglets there appears to be a temporary decrease in activity post-weaning. Lindemann *et al.* (1986) reported that the concentration of lipase in the pancreas and digesta fell by between 30 and 60% of pre-weaning levels. It is suggested that the piglet does not have the ability to increase enzyme secretion immediately after weaning (Jensen *et al.*, 1997) and utilisation of fat will therefore be limited by this reduction in pancreatic lipase activity (Thacker, 1999). This decreased secretion would correlate with a change in the piglet's diet at this time; the transition from a diet high in fat (sows' milk), to a weaner diet with lower levels would be accompanied with the usual drop in feed intake associated with weaning. These two factors would commonly result in a reduced level of dietary fat intake by the piglet around this time, which would explain reduced pancreatic lipase levels.

(vi) Pancreatic amylase

This enzyme is also secreted in its active form and hydrolyses starch into the disaccharide sugar maltose. The general pattern of amylase secretion in the young piglet is shown in Figure 1.2f. Several studies have reported that pancreatic amylase activity increases with age (Corring *et al.*, 1978; Lindemann *et al.*, 1986; Owsley *et al.*, 1986; Kelly *et al.*, 1991a). In unweaned piglets, amylase appears to undergo a two stage development of activity, consistent with other pancreatic enzymes. Corring *et al.* (1978) found specific activity at three weeks was higher (x 2.8) than at birth. This increased more rapidly up to six weeks (x 27.5), where it remained unchanged through to eight

weeks of age. Although one study has reported a marked increase in activity in unweaned piglets up to four weeks, it was suggested that the unexpectedly high amylase levels were probably due to the piglets gaining access to the sows' feed, as she was not removed from the crate during the experimental period (Lindemann *et al.*, 1986). This increase in amylase activity with age appears significantly greater in weaned piglets than in unweaned animals and has been shown to occur rapidly. Kelly *et al.* (1991a) observed a dramatic increase in total amylase activity even at three days post-weaning, which had doubled by seven days.

(vii) General patterns in digestive enzyme activity

From the studies reported above it can be concluded that, with the exception of lactase, the majority of digestive enzymes are present in the piglet at low levels at birth. Enzyme levels rise progressively with age, although the capacity of the piglet to secrete these enzymes can be significantly reduced by the process of weaning. The decrease in enzyme levels around this time is often linked to a reduction in feed intake by the piglet. It has been shown that digestive enzyme development is related to appetite, with greater feed intakes resulting in enhanced enzyme activity (Makkink *et al.*, 1994). Following the dip at weaning, enzyme levels increase again, often at a greater rate than before (Lindemann et al., 1986). There is strong evidence that this marked increase in enzyme activity is in response to the change in diet at weaning. McCracken (1984) stated that the nature of the dietary carbohydrate can alter the extent of enzyme induction in the immediate post-weaning period. Amylase activity, for example, significantly increases in order to digest starch that would now be present in significant amounts in the weaner diet. The notion of enzyme induction is further supported by the work of Kelly *et al.* (1991a) who observed dramatic increases in both maltase and amylase activities, even by three days post-weaning. There is also evidence that factors such as age, sex and weight at weaning can influence organ weight and gastrointestinal development; Cranwell *et al*, (1997) found that total pancreatic activity of most enzymes was greater in heavy and older piglets and tended to be greater in gilts, than in boars. It was suggested that this last factor may explain the difference in post-weaning performance between the two sexes.

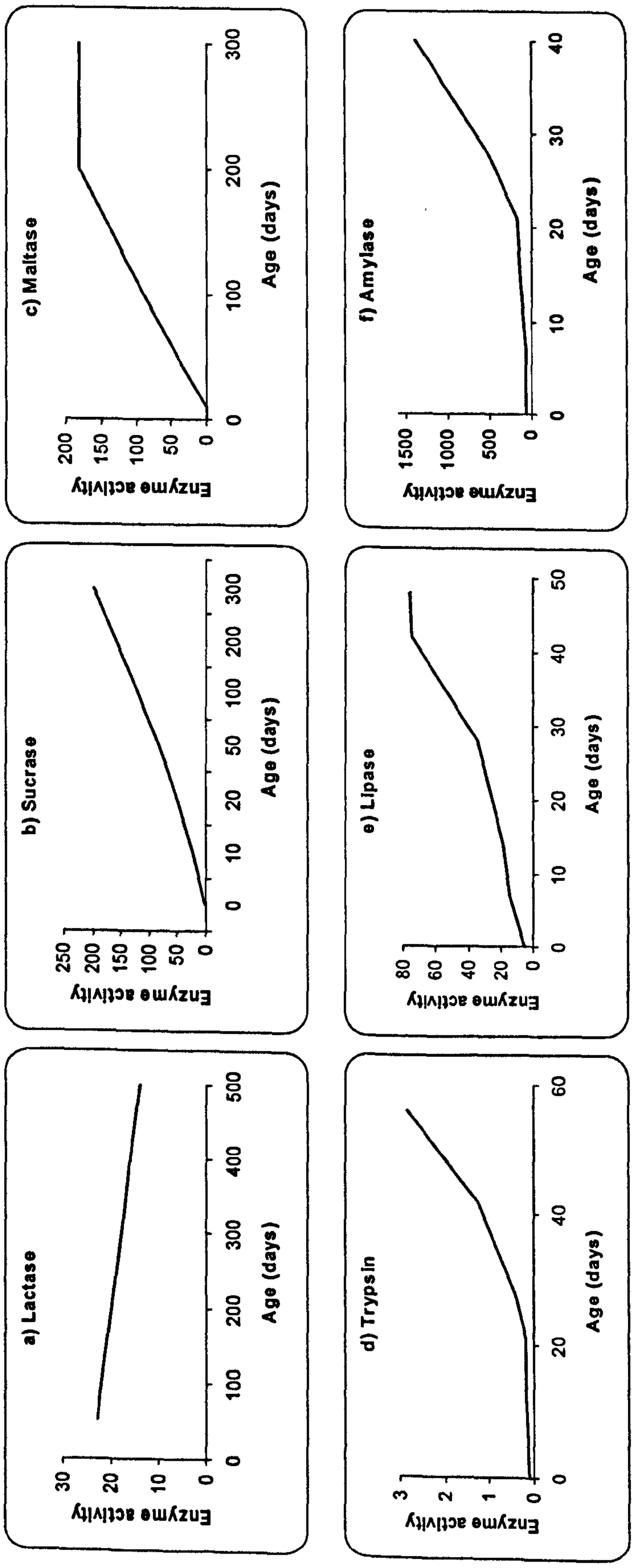


Figure 1.2: Digestive enzyme development in the young piglet (adapted from Corring et al., 1978 & Kidder and Manners, 1980) (Enzyme units for lactase, sucrase and maltase are µmol/min per g protein. Specific activities for trypsin, lipase and amylase are /mg protein)

1.1.1.3. Gut structure and function

Marked changes to the structure of the small intestine are typically observed in the immediate post-weaning period. Numerous authors have reported a decrease in villus height and an increase in crypt depth after weaning (Hampson, 1986; Miller *et al.*, 1986; Cera *et al.*, 1988; Pluske *et al.*, 1996b; Spreeuwenberg *et al.*, 2001). In a study involving piglets weaned at 21 days of age, Hampson (1986) reported that villus height was reduced by 25% within 24 hours. By the fifth day post-weaning, villus height was reduced to 50% of that seen prior to weaning. Similar decreases were observed by Miller *et al.* (1986) in pigs weaned at either four or six weeks of age (Table 1.1). Crypt depth is also known to increase although this may not be affected significantly until around five days after weaning (Hampson, 1986). Measurements of villus height to crypt depth ratio in the period immediately after weaning are therefore suggested to be primarily the result of villus shortening (Miller and Slade, 2003).

Table 1.1: Effect of age at weaning upon piglet intestinal structure

Gut morphology	*4 Weeks*		*6 Weeks*	
	Unweaned	**Weaned (+5 days)**	**Unweaned**	**Weaned (+5 days)**
Villus height (μm)	628	330	561	310
Crypt depth (μm)	169	320	268	326

(adapted from Miller et al., 1986)

The villi cell (enterocyte) originates from the division of crypt cells and is initially secretory in function. As the cell matures and migrates up the side of the villus, its function changes to become absorptive. Hampson (1986) proposed that villus atrophy at weaning was caused by a reduction in the number of enterocytes lining the villus, and not due to villus contraction. If the villi tips are damaged (as is commonly observed at weaning), the capacity to absorb nutrients is considerably reduced (Buddle and Bolton, 1992). Weaning has also been shown to affect the morphological structure of the villi; Cera *et al.* (1988) reported that suckled piglets exhibited villi that were long and slender. The result of weaning was a dramatic reduction in villus height within three days, which

lasted seven days in duration. Although villus height increased after this period, the villi exhibited a flatter 'tongue shaped' appearance. This structural change has also been reported by Hampson (1986) with three-week-old weaned piglets. It was concluded that this change in villus shape would appear to increase the luminal surface area.

It has been reported by numerous studies (Kidder and Manners, 1980; Hampson, 1986; Cera *et al.*, 1988; Pluske *et al.*, 1996a) that villus height is greatest at the proximal section of the small intestine, with a decreasing height gradient along the proximal to distal sections. It is suggested that this mirrors the nutrient concentration gradient along the gut, and therefore the requirement for absorption would be greatest at the proximal section (Cera *et al.*, 1988; Pluske *et al.*, 1996b). Hampson (1986) examined the effects of weaning on gut structure, in piglets aged between 21 and 32 days of age and found the loss of villus height was greatest at the proximal section of the small intestine. This was also noted by Spreeuwenberg *et al.* (2001) and by Boudry *et al.* (2004) where piglets weaned at three weeks exhibited greatest villus atrophy in the proximal part of the small intestine shortly after weaning (1-2d), with morphological changes in the ileum only apparent a few days later.

There is now a large body of evidence to suggest that the physical presence of food within the gastrointestinal tract plays a strong role in the integrity of the structure and function of the small intestine after weaning (Kelly *et al.*, 1991b; Pluske *et al.*, 1996b; McCracken *et al.*, 1999; Spreeuwenberg *et al.*, 2001; Vente-Spreeuwenberg *et al.*, 2003). McCracken *et al.* (1999) demonstrated that villus atrophy and inflammatory responses correlated with weaning anorexia where piglets weaned at three weeks exhibited a decrease in villus height and an increase in $CD8^+$ T-cell numbers. As appetite returned after four days, these measurements returned to day zero values. The link between luminal nutrition and gut structure is further supported with work involving gastric intubation of piglets weaned at 14 days (Kelly *et al.*, 1991b) where piglets with a restricted intake of feed, exhibited significantly greater villus atrophy, compared to those fed a continuous nutrient supply (Table 1.2).

Table 1.2: Effect of feed level on intestinal villus height (μm)

Intestinal section	Feed level		Significance
	Continuous	*Restricted*	
Proximal	546	404	**P = <0.001**
Mid	481	437	**P = <0.001**
Distal	390	385	**P = <0.001**

(from Kelly et al., 1991b)

There is evidence that the structure and function of the small intestine can be preserved by the provision of a milk diet immediately post-weaning; Pluske *et al.* (1996a) showed that feeding piglets whole cows' whole milk at regular intervals for five days after weaning maintained villus height and crypt depth integrity (Table 1.3). Further work (Pluske *et al.*, 1996b) reported that piglets weaned at 28 days of age onto a diet of ewes' milk also maintained villus height and crypt depths equal to those observed in pre-weaned animals. Significant correlations were found between feed intake, proximal villus height and body weight gain.

Table 1.3: Gut morphology of piglets fed whole cows' milk for 5 days post-weaning.

	Proportion Of intestine	Weaned	Weaned (+5 days)	Significance
Villus height (μm)	0.25	550	570	NS
	0.50	523	513	NS
	0.75	427	476	NS
Crypt depth (μm)	0.25	130	126	NS
	0.50	140	128	NS
	0.75	110	137	**P = <0.05**

NS = P>0.05 *(adapted from Pluske et al., 1996a)*

It has been suggested that the effect of dietary composition on intestinal integrity is not as important as that of low feed intake in the first four days after weaning (Spreeuwenberg *et al.*, 2001). A more recent trial (Vente-Spreeuwenberg *et al.*, 2003)

found that villus architecture in weaned piglets was not affected by carbohydrate source (glucose, lactose or starch), when fed in a liquid form.

It is apparent from these studies that weaning often presents major challenges to the digestive system of the piglet, leading to commonly observed villus atrophy and increased crypt depth within the small intestine. Although gut structure typically recovers, there is a period where the shortened villi surface area will mean reduced enzyme development, and absorptive capacity within the small intestine. It is in this period that the piglet could be predisposed to malabsorption, diarrhoea and enteric infection (Cera *et al.*, 1988; Buddle and Bolton, 1992). As nearly all non-infectious pig diseases and performance depressions are caused by intestinal microbes (Bolduan *et al.*, 1988), weaning is a critical period for the piglet. Overgrowth by the gut flora at this time can open niches for pathogenic bacteria, leading to enteric infection and even mortality.

1.1.1.4. Immunity

The piglet is born without any protective immunity and therefore is dependent upon immunoglobulins in the colostrum for protection against prevailing infection (*passive immunity)*. The three main immunoglobulins (IgA, IgG and IgM) are readily adsorbed through the gut as intact proteins during the first few hours of life. It is vital that the newborn piglet receives an adequate supply of colostrum within the first few hours of birth, as the absorption of these immunoglobulins is limited after 24 hours (English *et al.*, 1988). The predominant antibody in colostrum is IgG and makes up 0.76 of total immunoglobulins (Xu, 2003a). Following ingestion, it is adsorbed into the general circulation where it provides systemic protection against bacteria and viruses. As lactation progresses, the concentration of IgG declines rapidly, and IgA becomes the main immunoglobulin in the sows' milk (Klobasa *et al.*, 1987). IgA has a very important role for the young piglet as it protects the intestinal lining against pathogenic bacterial toxins.

Although these immunoglobulins give temporary protection, they are slowly degraded and diluted in the bloodstream so that by two weeks of age they are present only in low levels (English *et al.*, 1988). The piglet does not start to build up its own *active immunity* until around three weeks of age, and then only slowly. Therefore, the act of weaning, which typically occurs around this time, will challenge the piglet when its immune system is still at a low developmental level. The removal of the sows' milk at weaning will also deprive the piglet of the maternal supply of IgA. Without the protection afforded by this important immunoglobulin, it is probable that the piglet will be subject to damage to the gut lining, with subsequent digestive upsets (English *et al.*, 1988).

1.1.2. Post-weaning growth check

It is clear that numerous factors can present major challenges to the weaned piglet. Reduced feed intake, limited digestive enzyme development and challenges to the digestive tract can all have serious consequences for the development and survival of the young pig. These factors often lead to the post-weaning 'growth check' commonly observed during this time, which can have a major impact on subsequent performance. A reduction in voluntary feed intake is the main characteristic, as weaning anorexia has a negative impact on growth and leads to mobilisation of fat stores (Bark *et al.*, 1986). This growth check can greatly compromise the overall growing and finishing performance of the piglet, and represents a major production loss in many commercial piggeries (Pluske *et al.*, 1997). In order to minimise the effects of this growth check, the regular incorporation of antibiotic growth promoters (AGPs) as a feed additive, had, until recently, been a valuable tool in the nutritional management of the weaned piglet.

1.1.3. Antibiotic growth promoters

The role of antibiotics has traditionally been for the treatment against microbial infection. For many years it has also been recognised that when included in the diets of domestic animals, they exhibit a growth promoting effect. This was first discovered by Moore *et al.* (1946) in work with broilers. Pigs were found to exhibit a similar effect and, as a result, antibiotics had been routinely used as feed additives in their diets ever

since. The exact mode of action of AGPs remains unclear to date. This uncertainty is complicated by the large and varied range of reported effects (Table 1.4), although alteration of the gastrointestinal microflora balance is suggested to be the most likely mechanism (Li *et al.*, 2003).

Table 1.4: Summary of reported effects of AGPs

Physiological	Nutritional	Metabolic
↑ Nutrient absorption	↑ Energy retention	↑ Liver protein synthesis
↑ Feed intake	↑ Nitrogen retention	↑ Gut alkaline phosphatase
↓ Food transit time	↑ Vitamin absorption	↓ Toxic amine production
↓ Gut wall diameter	↑ Fatty acid absorption	↓ Aromatic phenols
↓ Gut wall weight	↑ Glucose absorption	↓ Bile degradation products
↓ Faecal moisture	↑ Calcium absorption	↓ Fatty acid oxidation
↓ Mucosal cell turnover	↓ Gut energy loss	↓ Faecal fat excretion
	↓ Vitamin synthesis	↓ Gut microbial urease

↑ = *increases*, ↓ = *decreases* *(from Gaskins et al., 2002)*

For many years, the use of AGPs as feed additives was a widely accepted method to increase productivity and decrease sub-clinical infections. Zimmerman (1986) showed that this antibiotic-mediated growth enhancement decreased with age (weight) of pigs. As the greatest response is exhibited early in life (Figure 1.3), almost all weaner diets were, until recently, supplemented with AGPs.

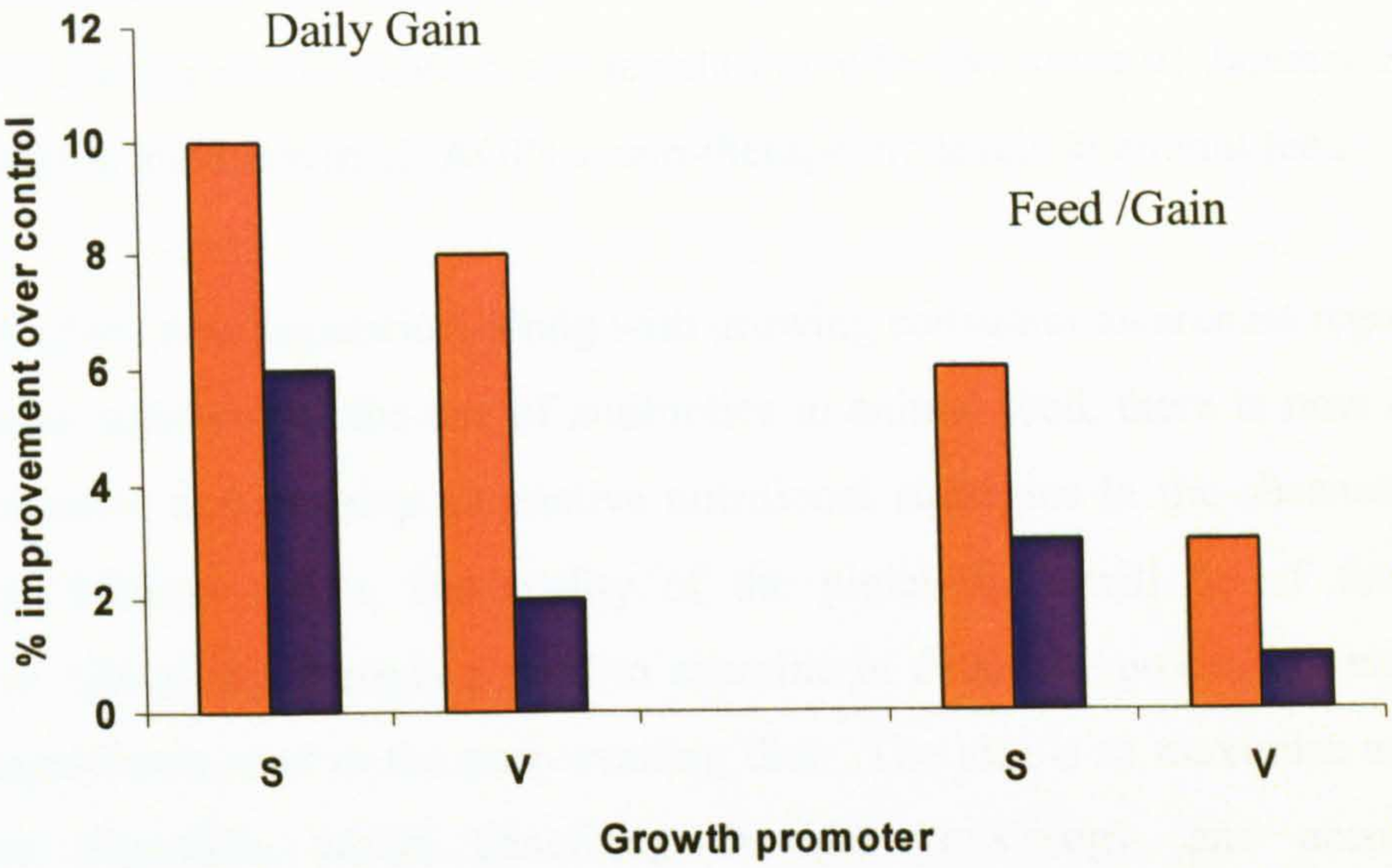


Figure 1.3: *Responses to AGPs (**S**alinomycin and **V**irginiamycin) fed during the starter (■) and grower/finisher (■) periods. (adapted from Zimmerman, 1986)*

The inclusion of AGPs in weaner diets has been shown to exert several beneficial effects: Hays (1987) reviewed a total of 937 experiments involving 12,000 weaner piglets, and reported that antibiotic supplementation increased average daily live weight gain (DLWG), feed conversion ratio (FCR) and reduced mortality significantly (15.6% vs. 3.1%) on farms under highly stressful conditions (Table 1.5).

Table 1.5: Post-weaned piglet responses to AGP supplementation

Factor	Control	+ Antibiotics	% Improvement
DLWG (kg)	0.39	0.46	17
FCR (kg/kg)	2.32	2.16	7
Mortality (%)	4.3	2.0	115

(from Hays, 1987)

1.1.3.1. Concerns over AGP use in feed

The routine inclusion of antibiotics in the feed of livestock has raised safety concerns since 1968 (Swann, 1969). There has been growing concern that widespread use could lead to increased resistance of enteric bacteria, which could theoretically pass on resistance to pathogenic microbes, thereby endangering public health. This concern has

already seen countries such as Sweden (1986) and Denmark (2000) abandon the use of all antibiotics as growth promoters. EU legislation, effective since 01 January 2006, has now banned the inclusion of all AGPs at sub-therapeutic levels in animal feed.

As a result of the new legislation, along with growing consumer awareness regarding the ethical issues surrounding the use of antibiotics in animal feed, there is now an urgent need to examine and develop alternative nutritional strategies in the absence of these antibiotics. Without AGPs, the quality of the piglet diets will be of fundamental importance. Given this there is a need to examine in detail the processing variables of the raw ingredients used in the post-weaning diet. The aim is to maximise energy and component digestion, whilst benefiting the gut physiology, and accompanying gastrointestinal microflora. When considering the post-weaned piglet, starch is the most important dietary component in terms of the provision of energy (Wiseman *et al.*, 2001).

1.2. STARCH

During photosynthesis, plants utilise carbon dioxide, water and sunlight to produce oxygen and simple sugars such as glucose. Any glucose not needed in the short-term is converted into the polysaccharide starch which forms the major storage carbohydrate of plants. Starch is used by plants as an energy reserve and is found mainly in the seeds, roots and tubers. Considerable energy is stored within the chemical bonds between the glucose units which becomes available to the plant (or, if eaten, to the animal) when the bonds are broken. The large number of bonds available for potential energy release makes starch a good store of dietary energy (Thomas and Atwell, 1999). In the diet of weaned piglets, starch is invariably provided by the inclusion of cereals.

1.2.1. Starch structure

Starch has been extensively studied over many years. Its structure (from molecular through to granular level) is now well understood, and has been described in detail in several reviews (Zobel, 1988; Buléon *et al.*, 1998; Tester *et al.*, 2004; Svihus *et al.*, 2005).

1.2.2. Starch digestion

The process of hydrolysing starch to glucose in the digestive tract of the non-ruminant begins in the mouth of the animal. Saliva secreted into the mouth is composed of roughly 0.99 water and 0.01 mucin, inorganic salts and enzymes. The principal enzyme responsible for the initial hydrolysis of starch is α-amylase. In the pig, α-amylase is present in the saliva but the activity is low and it is considered doubtful whether much starch digestion occurs here as the food is swallowed quickly and passes into the stomach (McDonald *et al.*, 1995). Optimal α-amylase activity is between pH 5.5 and 8, so the acidic environment of the stomach is considered to be unfavourable for further activity by the enzyme. It is suggested, however, that some hydrolysis may occur in the stomach since the enzyme mixes with the digesta and may therefore be partly protected from the gastric conditions (McDonald *et al.*, 1995).

The partially digested food leaving the stomach then passes into the duodenum where the majority of starch is digested by α-amylase secreted via the pancreas (Lewis and Hill, 1983). The pancreatic α-amylase has specificity for the α-1,4 links but is unable to cleave the α-1,6 branching links found in the amylopectin molecule. As a result of this specificity, when amylose (containing exclusively α-1,4 bonds) is attacked by α-amylase, the resulting products of digestion consist mainly of maltose and glucose (Flourié, 1989). By contrast, the branched α-1,6 units of amylopectin are not cleaved by the enzyme and the resulting products are composed of a number of branched and unbranched oligosaccharides (called α-limit dextrins) containing the α-1,6 bonds.

The hydrolysis of these oligosaccharides to glucose is by the actions of surface enzymes termed oligosaccharidases, which are associated with the intestinal villi. Enzymes secreted here include maltase, which breaks down maltose into two molecules of glucose, and, importantly, oligo-1,6-glucosidase which attacks the α-1,6 bonds in α-limit dextrins. The enzyme sequentially cleaves individual glucose residues in turn until finishing with a chain consisting of one or two glucose molecules which can be finally cleaved by maltase into glucose (Gray, 1992). The final product of starch digestion is glucose (Figure 1.4), which is subsequently transported by a specific glycoprotein across the intestinal membrane (Gray, 1992).

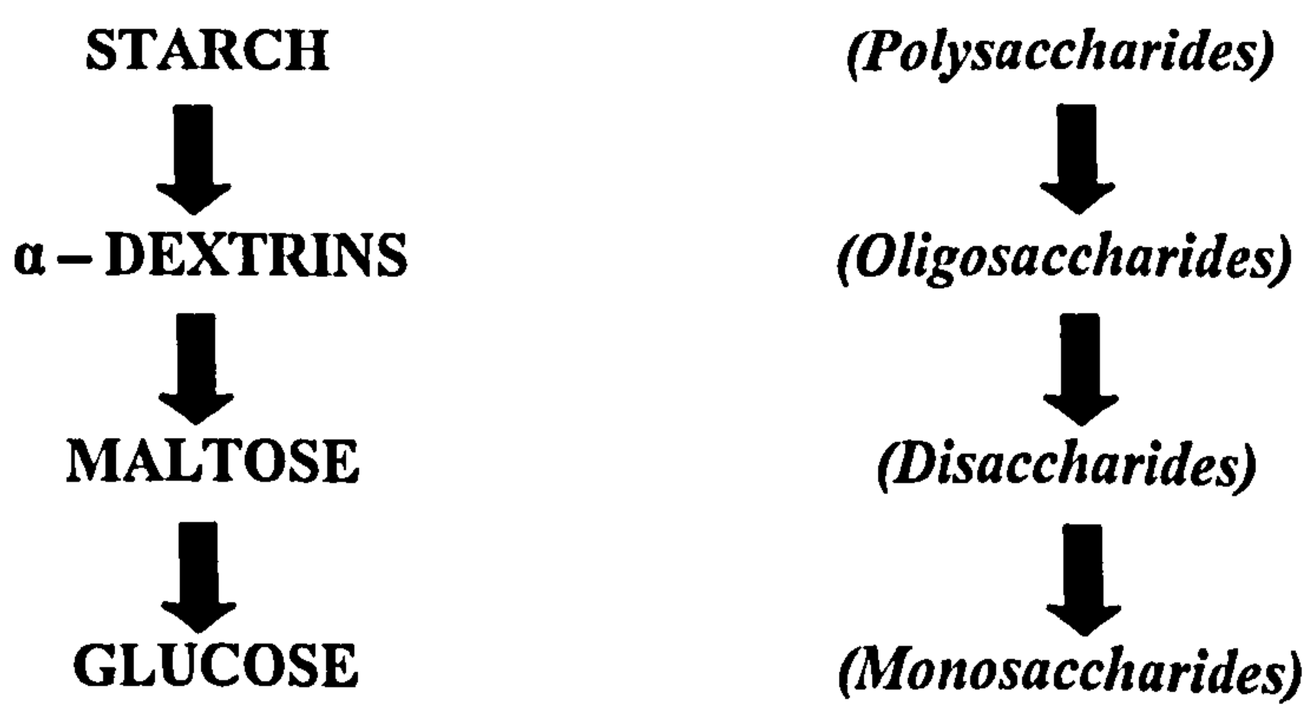


Figure 1.4: *Sequence of starch digestion products*

1.2.3. Raw starch hydrolysis

When the semi-crystalline part of the starch granule is viewed using X-ray diffraction (XRD), the helices are observed to be either densely packed (termed A-type) or less densely packed (termed B-type). Cereal starches exhibit an A-type pattern, while potato starch gives the B-type pattern (Ring *et al.*, 1988). Pea starch exhibits a mixture of both A and B-type patterns, and is termed C-type. Types B and C starches are generally less susceptible to hydrolysis by digestive enzymes than Type A (Table 1.6). Substitution of cereal starches with pea or potato starch in piglet diets has been shown to result in a reduction in ileal digestibility (Table 1.7). It has been suggested that the reason for this variability lies in the physical structure of the starch granules: Digestive enzymes attack 'weak-points' within the structure of A-type starches, 'bore' holes into the granule and effectively hydrolyse the starch from the inside out. B-type starches on the other hand, are subjected to surface hydrolysis of the granule (van Doren and van Swaaij, 1996). The molecular branching points in A-type starches are located within the crystalline regions of the granule, and are more susceptible to enzyme hydrolysis. By contrast, the branching points of B-type starches lie within the amorphous regions of the granule and are less susceptible to enzyme attack.

Table 1.6: Hydrolysis of raw granular starch by α-amylase after 24hr at 37°C

Sample	Starch type	% Hydrolysis (2 units/mg polysaccharide)	% Hydrolysis (20 units/mg)
Wheat starch	A	25	100
Pea starch	C	10	67
Potato starch	B	<5	15

(*Ring et al., 1988*)

Table 1.7: Effect of substitution of starch type upon coefficient of ileal digestibility

Feed	Starch type	Ileal digestibility	Source
Barley/Wheat	A	0.98	Gdala *et al.*, 1997
Pea	C	0.95	
Barley	A	0.96	Just *et al.*, 1987
Potato	B	0.81	

The starch granules also contain proteins and lipids, the proportions of which vary between plant species. Generally, tuber and root starches have less lipid and protein than cereal starches with the result that tubers are less flavoursome than cereals (Thomas and Atwell, 1999). The proteins exist in two types, namely *surface starch granule proteins* that are extracted relatively easily, and the *integral starch granule proteins* that are more difficult to extract. Lipids are mainly concentrated in the germ of the cereals.

1.2.4. Heating of starch

In order to increase starch availability for enzymic hydrolysis, the application of heat and moisture treatments is commonly used to create physical changes to the starch structure. Water-binding capacities and enzyme susceptibility have been shown to increase as a result of heat-moisture treatment of various starches (Kulp and Lorenz, 1981; Anguita *et al.*, 2006). These treatment methods are routinely used when formulating diets for young animals, in order to enhance both the digestibility and subsequent nutritional quality of starches. This enhanced nutritional benefit is especially important for the young pig, which typically has a less well-developed digestive system.

1.2.4.1. Gelatinisation

At room temperature, there is a slight uptake of water into the granules that results in minimal swelling. This process is reversible and there is no permanent damage to the structure as a result of this swelling. If the temperature of a starch suspension in excess water is continuously raised, the starch molecules begin to vibrate. This action leads to the breaking of the intermolecular bonds and allows water to penetrate and bind, resulting in increased swelling (Würsch, 1989). Upon further heating, this swelling becomes irreversible as the helical regions of the granules uncoil and dissociate, leading to the destruction of crystallinity and loss of birefringence. Amylose begins to leach out from the granules leading to an increased viscosity and paste formation. The temperature at which a particular starch gelatinises to form a paste is termed the gelatinization temperature and is defined as the temperature at which 0.98 loss of birefringence occurs. There is a degree of variation in the gelatinisation temperature within the same starch source, due to factors such as cultivar and ratio of amylose to

amylopectin (Morrison *et al.*, 2000; Sasaki *et al.*, 2000). Table 1.8 shows the general gelatinisation temperatures of a range of common starch sources, as identified by a number of authors.

Table 1.8: Typical starch gelatinisation temperatures

Starch Source	Temperature (°C)	Source
Wheat	60-65	Sasaki *et al.*, 2000
Potato	77-86	Morrison *et al.*, 2000
Oat	68-73	Tester and Karkalas, 1996
Rice	75-77	Patindol and Wang, 2002
Pea	75-80	Ratnayake *et al.*, 2002

1.2.4.2. Retrogradation

Upon cooling, less energy is available to keep the soluble molecules apart and, as a result, some of this gelatinised starch begins to re-associate and form crystalline structures once more. This re-association is termed retrogradation and is largely due to the re-crystallizing of amylose, which is much more rapid than amylopectin (Würsch, 1989). Amylose molecules are attracted to each other and neighbouring molecules form bundles of polysaccharide chains via hydrogen bonding. This re-association of amylose means a return to an insoluble crystalline condition. As retrogradation progresses, the starch paste becomes increasingly opaque and forms a gel (Thomas and Atwell, 1999). Despite being crystalline, this gel is still susceptible to amylolysis but a portion remains resistant to enzymic digestion and is termed *resistant starch.* Because of its resistance to enzyme attack, it is therefore considered to contribute to the dietary "fibre" component of the diet.

1.2.5. Classification of starch

For nutritional purposes, starch in foods can be classified into three broad categories: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). There is considerable interest in this latter category as starch that escapes digestion in the small intestine passes through to be fermented within the large intestine, where it is

available for fermentation (Haralampu, 2000). In a trial with three-week weaned piglets comparing raw with micronised hull-less barley, Huang *et al.* (1998) reported differences in starch digestibility at the ileum but not when calculated over the total tract, indicating considerable digestion of raw cereals may occur within the large intestine. Opinion is divided as to whether this starch fermentation in the hind gut is beneficial (creating a more acidic environment via volatile fatty acid production and their subsequent absorption by colonocytes) or detrimental (proliferation of pathogenic microbes).

Resistant starch can be further subdivided into the following subcategories:

- Starch that is physically inaccessible to digestive enzymes (e.g. locked within cell walls) is termed **RS1**
- Starch granules resistant to enzyme attack (e.g. raw potatoes, some peas) **RS2**
- Retrograded starch (see above) **RS3**

The 3 fractions are not equally susceptible to fermentation; RS1 and RS2 types are rapidly fermented, whereas RS3 undergoes a much slower rate of fermentation.

1.2.6. Resistant starch and the colon

There is growing evidence that some beneficial effects may be obtained from the consumption of resistant starch. Studies have reported an increase in the production of butyrate, from fermentation of resistant starch within the large intestine; Englyst *et al.* (1987) found that fermentation of resistant starch yielded a relatively high concentration of butyric acid (0.21 mg / mg substrate). More recent findings by Martin *et al.* (2000) have demonstrated that consumption of various sources of resistant starch induced different patterns of VFA production in growing pigs where raw potato starch resulted in a significant increase ($P = <0.001$) in the appearance of butyrate within the portal blood (Table 1.9).

Table 1.9: Mean portal blood VFA concentration (μmol/l) in pigs fed 3 diets composed of resistant starches.

VFA	Potato starch (raw)	High amylose starch	Retrograded high Amylose starch
Acetate	236.5	206.4	321.5
Propionate	47.4	34.1	38.8
Butyrate	21.5	1.5	1.7

(from Martin et al., 2000)

In addition to being a prime substrate for energy metabolism within the colonocytes, it has been suggested that butyrate is responsible for stimulating regeneration of inflamed mucosal gut lining of the large intestine (Brouns *et al.*, 2002). In respect of this, an increased production of butyrate would suggest obvious benefits in maintaining a healthy colonic environment.

1.2.7. *In-vitro* characterisation of starches

1.2.7.1. Introduction

Analysis of starchy materials such as cereals in animal feeds is often limited to a determination of their gross composition and, or when processed, the amount of gelatinised starch as a proportion of total starch (Medel *et al.*, 1999, 2004). These measurements are somewhat limited as they provide no information on the properties of the starch component, in relation to its digestibility when fed to the animal. These limitations can now be overcome with the application of reliable quantitative laboratory tests that have been commonly used in the field of human food development for a number of years. These analyses are able to assess the physicochemical properties of raw materials and the changes they undergo upon processing. By using these laboratory methods, it is possible to observe that starch is not simply 'cooked' but undergoes a series of complex changes during processing, to reach an amount of cook depending on the type of processing, and variables used. This continuum of changes during processing has been termed 'starch conversion' and involves destruction of the crystalline order of the starch granules and depolymerisation of the individual amylose

and amylopectin molecules (Cheyne *et al.*, 2005). A description of some of the more commonly used food science tests performed in order to assess the physicochemical properties of starchy materials in feed are summarised below.

1.2.7.2. Hydration properties

In order for soluble products to be released for amylolytic degradation, it is of fundamental importance for the starch component within the feed to be hydrated. Information about hydration properties can be gathered by using the following methods:

(i) Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI and WSI are commonly used to assess the degree of swelling and solubility of starches in excess water. They therefore give an indirect indication of the amount of starch conversion (ie. destruction of the structured order of the starch granules - Ollet *et al.*, 1990). WAI is affected by gelatinisation as well as by granular and molecular breakdown following processing, and represents the volume occupied by hydrated starch following swelling in excess water. WAI is therefore generally associated with starch that has been extensively gelatinised. WSI is a measure of the soluble polysaccharides released from the granules into solution and is associated with starch that has undergone a high degree of molecular breakdown.

(ii) Rapid Visco Analyser (RVA)

The RVA has been used extensively to examine pasting profiles of starch in raw materials (Zeng *et al.*, 1997) and in processed foods (Guha *et al.*, 1998). This analysis measures the resistance of starches to shearing forces under defined temperature and moisture conditions and provides a pasting profile of parameters (e.g. cold swelling peak, gelatinisation peak, final viscosity – Figure 1.5) which represent a measure of starch gelatinisation, disintegration, and gelling ability (Ravi *et al.*, 1999). The RVA is a powerful and reliable technique which can detect differences in hydration of starches, caused by different processing techniques and processing conditions. It is also able to assess the presence of endogenous α-amylase within the cereals, which may ultimately affect nutritive value (Noda *et al.*, 2003).

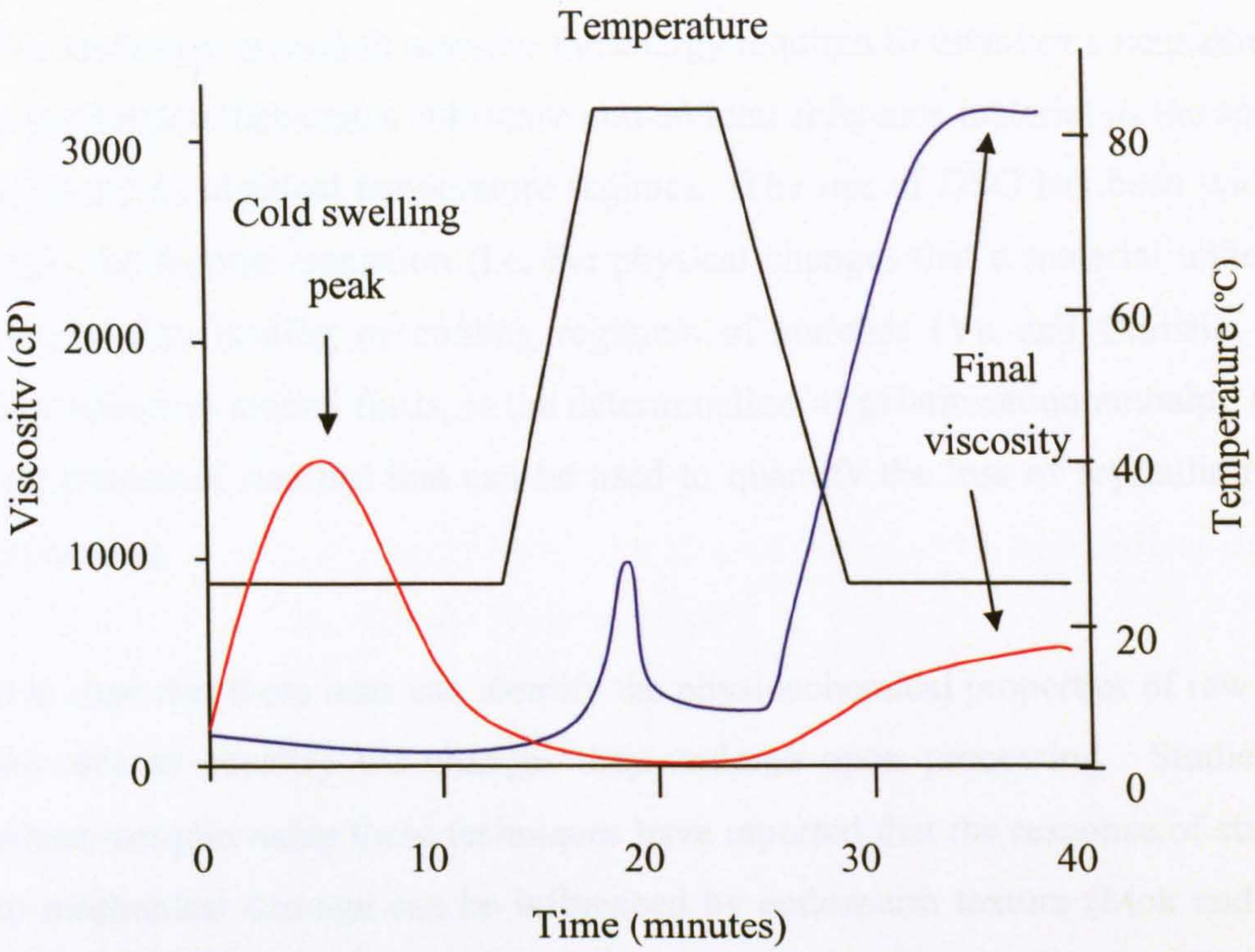


Figure 1.5: *Typical RVA starch pasting profile for raw (—) and extruded (—) wheat.*

1.2.7.3. Structural properties

In order to help explain the changes in starch hydration properties due to processing conditions, it is useful to have information relating to the structure of the granules, in order to help quantify starch conversion. Two commonly used tests are described below:

(i) X-Ray Diffraction (XRD)

As described in Section 1.2.3, XRD can be used to categorise native starches into three types of semi-crystalline materials, depending on their botanical source. The crystalline structures can be identified by their diffraction patterns. This method can also detect the loss of crystallinity due to starch conversion from processing (Zobel *et al.*, 1988). The use of XRD has already been applied to characterise micronised wheat and barley when added to weaned piglet diets (Zarkadas and Wiseman, 2001, 2002).

(ii) Differential Scanning Calorimetry (DSC)

This technique is used to measure the energy required to establish a near zero difference in temperature between a substance and an inert reference material as the specimens are subjected to identical temperature regimes. The use of DSC has been widely used to study the thermal transition (i.e. the physical changes that a material undergoes when subjected to heating or cooling regimes) of starches (Yu and Christie, 2001). Of importance in animal feeds, is the determination of gelatinisation enthalpy (ΔH) of raw and processed starches that can be used to quantify the loss of crystallinity caused by processing.

It is clear that these tests can identify the physicochemical properties of raw starches and are able to quantify the changes they undergo upon processing. Studies examining wheat samples using these techniques have reported that the response of starch granules to mechanical damage can be influenced by endosperm texture (Mok and Dick, 1991; Zeng *et al.*, 1997).

1.2.8. Endosperm texture

1.2.8.1. Introduction

Endosperm texture is one of the fundamental means of classifying wheat, and relates to the amount of compressive force needed to fracture the endosperm. Although often classified as 'hard' and 'soft', endosperm texture is a continuum and therefore not absolute. It is largely genetically determined but environmental factors, in addition to interactions within the endosperm matrix (such as between protein and starch), can exert significant effects (MacRitchie, 1980). The difference in endosperm hardness lies in the binding strength between the protein matrix and starch granules. A 15kDa protein called *friabilin* is more abundant in soft wheat than hard, and has been shown to affect wheat grain hardness (Turnbull and Rahman, 2002). It is this hardness that determines the end use of the resultant flour; hard wheat is used for breadmaking, soft wheat flour for pastries and biscuits.

In hard wheats, a continuous protein matrix entraps the starch granules within the endosperm. This makes separation difficult during the milling process, and results in larger flour particle size, increased water absorption and a greater incidence of starch granule damage (Mok and Dick, 1991).

In soft wheats, the starch granules are embedded in a friable matrix. It is thought that friabilin hinders the bonding between starch granules and the protein matrix, leading to an endosperm that is easily crushed upon milling (Svihus *et al.*, 2005). Consequently, flour from soft wheat usually exhibits a smaller particle size and has less damage to the starch granules (Turnbull and Rahman, 2002).

Wheat has been considered to be of a comparatively constant nutritional value. This is now known to be not so, and a whole range of factors including climate, location of growth, soil type, agronomic practices and post-harvest treatments can all significantly affect its nutritive value (Wiseman and Inborr, 1990; Kim *et al*, 2005b).

1.2.8.2. Influence of wheat endosperm texture on animal performance

Although genetic differences in wheats have been shown to have little impact on nutritive value in ruminants (Garnsworthy and Wiseman, 2000), studies with poultry and pigs show endosperm texture can have major implications on digestibility and performance. Trials with young broilers have reported negative correlations between wheat endosperm hardness and digestibility of amino acids (Short *et al.*, 2000) and starch (Carré *et al.*, 2005). Of the few studies examining the effect of wheat cultivar on young piglet performance, it appears difficult to establish firm conclusions from the observed results because trial design has often confounded hardness with other factors (such as the presence of the 1B/1R gene). By feeding weaned piglets on *ad-libitum* diets, differing only in the cultivar of wheat used, Pearce *et al.* (1997) reported a variation of 15% in feed intake levels which equated to an 18% difference in growth rate. Further studies examining the influence of wheat endosperm texture on piglet performance appear to report contrasting conclusions (Lewis *et al.*, 1998; Lewis *et al.*,

1999) although conclusions from both trials suggested a lack of relationship between wheat *in-vitro* viscosity and overall digestibility.

Having discussed the properties of starch and explained why it plays such an important role in the diet of the weaned piglet, the following section will broaden the discussion to consider how various methods of feed processing are used, with the aim of maximising starch availability for the young animal.

1.3. FEED PROCESSING

Feed represents a major cost in livestock production. It is important to provide feed in a form that will encourage consumption, provide adequate nutrition, and ideally, minimise wastage. The processing of feed is both time-consuming and expensive, and would not be undertaken unless considerable advantages were offered over unprocessed feed (Zarkadas, 1999). Processing methods may be hot (heat applied or created during the treatment process) or cold (temperature of the grain is not increased significantly), dry or wet, chemical or mechanical. Whether single or multiple processing methods are used, the objectives are typically to provide one or more of the following benefits: to improve palatability, reduce wastage, alter the physical form of the feed, improve digestibility and/or nutritive value, inactivate toxins/anti-nutritional factors, or improve handling characteristics (Kent and Evers, 1994).

Grinding and flaking are the most common physical treatments applied to cereals, and improve the digestibility, without modifying the chemical characteristics of the grain (Zarkadas, 1999). Although heat treatment of cereals often alters the external physical characteristics of the grain, the primary objective is to modify the cereal starch within the grain, through gelatinisation. The aim is to provide the animal with increased starch availability *in vivo*, in a form that is more readily available to the digestive enzymes, thereby providing a greater supply of energy (Lawrence, 1972). For example, treatments such as steam-flaking are often carried out on maize, where the grain is cooked and

rolled, resulting in a product of increased digestibility compared with the unprocessed grain (McDonald et al, 1995). Another popular processing method is pelleting. In this process, the feed is usually ground or rolled before being subjected to pressure, and is forced through the die of a pelleting machine. This method reduces the common problem of feed 'bridging' in the hopper, improves the handling characteristics of the diet, and reduces wastage (Kent and Evers, 1994). The following is a review of the literature from piglet trials and examines the effectiveness of three different methods used to process cereal grains (grinding, micronisation and extrusion). The review focuses on these three methods in particular, as these processing techniques were evaluated in the current research programme.

1.3.1. Grinding

This process usually involves passing the feed through a hammer mill, in order to decrease the particle size. This increases the surface area available to the digestive enzymes, thereby theoretically leading to an increase in feed efficiency (Ohh *et al.*, 1983; Medel *et al.*, 2000). Studies examining the effect of grinding appear to be somewhat contradictory. The large degree of variation in particle sizes and feeds studied makes comparison difficult. In a study with piglets weaned at 21 days, Kim *et al.* (2005a) reported that CTTAD for starch was significantly improved ($P = <0.001$) by feeding a finely (4.5 mm) compared to a coarsely (8.5 mm) ground wheat diet for three weeks. However, despite this improvement in apparent digestibility, this did not translate into improved animal performance as measured by feed intake (FI) and feed conversion efficiency (FCR) values. Goodband and Hines (1988) reported that FCR was improved in young pigs fed finely ground (635 μm) compared to medium ground (768 μm) barley. This is in contrast to Medel *et al.* (2000), where an increase in piglet performance for the first 14 days post-weaning was attributed to heat processing of barley (micronization and expansion), and not due to any change in feed particle size (Table 1.10).

Table 1.10: Effect of dietary treatment of barley on piglet performance

	Screen Size (mm)	0-14 Days		14-28 Days	
		DLWG	FCR	DLWG	FCR
Raw	4.0	208	1.00	484	1.42
Raw	2.5	204	1.04	470	1.43
Micronised	2.5	225	1.00	482	1.46
Expanded	2.5	238	0.98	502	1.42

DLWG = daily live weight gain (g) *(adapted from Medel et al., 2000)*

Any beneficial effects in performance from reduced particle size appear to be most apparent during the first two weeks after weaning: Healy *et al.* (1994) reported improvements in both DLWG and FCR values when particle size of maize was reduced from 900 μm to 300 μm. This effect, however, was only seen for 14 days post-weaning, and not thereafter. This is supported by Lawrence (1978) who found that grinding cereals at a screen size of 5 mm or less had little effect upon performance of growing and finishing pigs. This would suggest that reduced particle size is of most importance to the young piglet, and the optimum particle size increases as the animal gets older.

There are disadvantages of feeding finely ground cereals to young pigs; in the case of wheat, the gluten content can result in the feed becoming sticky and pasty in the mouth. This leads to a reduction in voluntary feed intake because of reduced palatability (Lawrence, 1978). Other problems encountered with finely ground feed can include poor flowing properties in hoppers (bridging), and also gastric ulcers (Hedde *et al.*, 1985; Fjetland and Teige, 2002). There is also an economic factor to consider when grinding feed, as the more finely a feed is ground, the higher the milling costs. Healy *et al.* (1994) found that reducing maize particle size from 900 μm to 300 μm resulted in a reduced production rate and an increase in energy required to mill the feed.

1.3.2. Micronisation

1.3.2.1. Introduction

In this processing technique, the feed is subjected to dry heat by microwaves, emitted from infrared burners. The microwaves cause the molecules within the feed to vibrate. This leads to rapid internal friction heating, and an increase in water vapour pressure. Under these conditions, the starch granules swell, fracture and gelatinise (Lawrence, 1973a), before being passed through a roller, to give the end product a flake-like appearance. This process should result in increased enzyme susceptibility and carbohydrate utilisation (Hoover and Vasanthan, 1994). Several factors can be controlled, including temperature of the grain, and density of the finished product. In attempting to review the effectiveness of micronisation from the literature, the many variables involved, including cereal varieties, and processing conditions used, means that comparison is somewhat difficult. Several studies often refer to the effectiveness of the process alone, without stating the variables used (temperature, moisture and time).

1.3.2.2. Use of micronisation processing in animal trials

An analysis of published literature suggests that a consensus of opinion has yet to be established as to whether micronisation of cereal is beneficial to animal performance. Micronised barley has been shown to improve the performance of young pigs, in terms of increased ileal and faecal digestibility of energy, and ileal digestibility of most amino acids (Huang *et al.*, 1998). Lawrence (1973b) reported that micronised maize and barley diets significantly improved growth rate and digestibility of dry matter (DM) in growing pigs. This effect was not exhibited in those animals fed a wheat-based diet. Further work (Lawrence, 1975) compared wheat at three different micronisation temperatures with cold processed (hammer-milled or rolled) samples and found there was no significant difference between micronised and raw wheat (Table 1.11).

Table 1.11: Effect of processing treatment of wheat on growth in pigs

	Hammer -milled	Rolled	Micronised (155°C)	Micronised (190°C)	Micronised (220°C)	Significance
Growth rate (g/d)	714	700	710	712	691	NS

NS = not significant (P>0.05) (*adapted from Lawrence, 1975*)

More recent work (Zarkadas and Wiseman, 2001) has also questioned the process of micronisation as an effective means of improving the nutritive value of wheat. In a trial with weaned piglets, the effect of wheat, micronised under varying dwell times (200°C for 10, 15 and 35 seconds), was examined. Micronisation reduced crystallinity and increased gelatinised starch. DM increased with increasing time spent under the microniser. There was no observed effect on DLWG and although significant, FCR was better for piglets on the raw wheat diet (Table 1.12). In a repeat of the trial, replacing wheat with barley (Zarkadas and Wiseman, 2002), micronisation yielded increases in DLWG and starch content together with significant increases in DM and feed intake levels. There was no observed benefit from a prolonged dwell time, as values for DLWG and starch content were just as beneficial for a dwell time of 10 seconds, compared to 35 seconds spent under the microniser.

Table 1.12: Chemical analysis and performance effects of feeding raw and micronised wheat under different dwell times to weaned piglets

Variable	Raw	Micronised *10 seconds*	Micronised *13 seconds*	Micronised *35 seconds*	P
DM (g/kg)	871	878	888	952	NS
DLWG (g/d)	606	548	589	570	NS
FCR (g DM/g gain)	1.34	1.43	1.47	1.47	**0.013**
Intake (g DM/d)	810	781	862	837	**0.036**

NS = not significant (P>0.05) (*adapted from Zarkadas and Wiseman, 2001*)

The effect of steeping cereal grain in water for different lengths of time, prior to micronisation at different temperatures, has also been investigated with wheat (Zarkadas and Wiseman, 2001) and barley (Zarkadas and Wiseman, 2002). Investigating wheat, the authors found significant improvements for the shorter versus longer (2 h vs. 12 h) steeping period, in terms of feed intake, DLWG and gross energy (GE) in piglets. It was concluded there was no benefit from steeping wheat for the prolonged period of time. When repeated with barley, piglet performance and digestibility values were not significantly affected by the inclusion of steeped micronised barley, regardless of length of steeping period (Zarkadas and Wiseman, 2002).

It would appear from current literature that micronisation of raw wheat is questionable as a means of improving the nutritive value for weaned piglets. Although steeping the wheat in water prior to micronisation has been reported to exhibit a beneficial effect upon FI and DLWG, there appears to be no advantage of a prolonged versus shorter steeping time (Zarkadas and Wiseman, 2001). Micronisation of barley has been shown to increase growth rate and DM digestibility (Lawrence, 1973b), amino acid digestibility (Huang *et al.*, 1998), starch content and DLWG (Zarkadas and Wiseman, 2002) in young pigs. An interesting point to note from several micronisation studies is that despite an observed increase in the proportion of gelatinised starch *in vitro,* this has not necessarily been equated with improved piglet performance *in vivo* (Vestegraad *et al.*, 1990 as cited by Medel *et al.*, 1999; Zarkadas and Wiseman, 2001; Zarkadas and Wiseman, 2002). It would appear, therefore, there is some disagreement regarding the benefits of using micronised cereal grains in piglet diets.

1.3.3. Extrusion

1.3.3.1. Introduction

This processing technique involves applying heat and pressure, by means of friction to the feed material. It is a high temperature, short time process where the material is passed through a tapered screw by an auger-like rotor. It is then forced through a narrow opening under pressure. Extrusion may be dry (no moisture added) or wet (moisture added in variable amounts). Moisture added to the material is unable to evaporate

during the process, but upon the return to sudden atmospheric pressure, flashes off as steam. During extrusion, starch granules are physically torn apart by shear forces and allow rapid entrance of water into the starch molecules (Kokini *et al.*, 1992). The use of extrusion processing produces a very homogenous, end product and has been shown to thoroughly gelatinise starch, thereby improving its digestibility, even at low moisture levels (Gomez and Aguilera, 1983). As with many other processing techniques, variables from the feed ingredients (particle size and moisture content) as well as those from the process itself (screw speed, temperature, size and shape of die etc), can have a significant effect upon the nutritive value of the end product (Dahlin and Lorenz, 1993).

1.3.3.2. Use of extrusion processing in animal trials

In evaluating the studies that have examined the use of extrusion processing in the diets of weaned piglets, conclusions have been somewhat mixed; Jansen (1992) fed diets containing either 'strongly' or 'weakly' extruded wheat and reported increased feed intake levels and live weight gains for three weeks post-weaning. Medel *et al.* (1999) found that processing of barley and maize (by micronisation and extrusion) significantly improved DLWG and FCR when compared to diets based on the raw cereals. Benefits have also been reported from feeding an extruded, rather than pelleted diet, resulting in increases in DLWG and FCR by 8% and 6% respectively (Sauer *et al.*, 1990 – Table 1.13). Other studies have reported variable effects when providing the animals with feed in an extruded form (Tomchuk, 1989; Hongtrakul *et al.*, 1998; Lizardo *et al.*, 1999). Once again, due to the lack of description of processing variables cited in some of the published literature, it can be difficult to draw conclusions when assessing the effectiveness of extrusion processing when used in young piglet diets.

Table 1.13: Summary of reported effects of weaned piglets in response to extrusion processing

Extruded fraction	Weaning age	Trial Length	Response	Author
Cereal	23 days	25 days	Improved DLWG **(P = 0.01)** Improved FCR **(P = 0.02)** FI (NS)	Medel *et al.*, 1999
Whole diet	21 days	35 days	Improved DLWG **(P = <0.05)** Improved FCR **(P = <0.05)** FI (NS)	Sauer *et al.*, 1990
Cereal	10 days	21 days	DLWG (NS) FI (NS)	Hongtrakul *et al.*, 1998

NS = P >0.05

1.3.4. Starch gelatinisation vs. piglet performance

The application of heat to a feedstuff is often reported as an effective tool for increasing starch digestibility *in vitro*. Heating has been shown to promote higher and faster hydrolysis of starch in many feed ingredients including barley (Osman *et al.*, 1970), beans (Alonso *et al.*, 2000), wheat and oats (Anguita *et al.*, 2006). By using heat to process cereals prior to their inclusion in animal diets, it is hoped that *in vivo* starch availability will be increased, and in a form that is more readily susceptible to amylolytic digestion. Several studies using heat-treated cereals in piglet diets have reported an increased proportion of gelatinised starch resulting from flaking (Lawlor *et al.*, 2003; Medel *et al.*, 2004), micronisation (Medel *et al.*, 1999, 2000; Zarkadas and Wiseman, 2001, 2002) and extrusion (Van der Poel *et al.*, 1990; Medel *et al.*, 1999). However, it is clear from this literature that increased *in*-vitro starch availability is not always equated with improved animal performance (Table 1.14). Of the literature that reports performance benefits, these advantages are often only apparent up to 14 days after weaning (Van der Poel *et al.*, 1990; Medel *et al.*, 1999, 2000, 2004).

Table 1.14: Summary of reported piglet performance from trials citing increased *in-vitro* starch availability from heat processing of cereals

Cereal	Heat treatment	Response (DLWG/FCR/FI)	Author
Wheat / Maize	Steamed & Flaked	DLWG (NS) FCR / FI (NS)	Lawlor *et al.*, 2003
Barley / maize	Steamed & Flaked	Improved DLWG **(P = 0.041)** FCR / FI (NS)	Medel *et al.*, 2004
Barley	Micronised	Improved DLWG **(P = 0.02)** FCR / FI (NS)	Medel *et al.*, 2000
Barley	Micronised	Improved FI **(P = 0.019)** DLWG / FCR (NS)	Zarkadas and Wiseman, 2002
Barley / Maize	Extruded	Improved DLWG **(P = 0.01)** Improved FCR **(P = 0.02)**	Medel *et al.*, 1999

NS = P >0.05

1.4. CONCLUSIONS

To summarise, the newly-weaned piglet is subjected to a number of stressors. In order to minimise the impact these have on the young animal, AGPs have proved to be a useful and convenient tool for the past 50 years or so. However, against a background of growing public concern regarding possible antibiotic resistance, European legislation has banned the routine inclusion of all AGPs in livestock feed at sub-therapeutic levels. Without AGPs, the quality of dietary ingredients will assume a more fundamental role in maintaining piglet health. In order to improve the availability of the starch fraction (and therefore make it more susceptible to *in-vivo* digestion), processing of cereals prior to inclusion in piglet diets is widely practised. In attempting to draw firm conclusions regarding the effectiveness of various thermal or thermo-mechanical processing techniques, the lack of cited processing variables used in the trials makes overall comparisons difficult. Moreover, description is often limited to the name of the process

alone, with no regard of the precise variables used (*e.g.* temperature, time, moisture). In many instances the feed materials are simply termed 'cooked' which gives little or no indication of nutritional value.

1.5. OBJECTIVES

The current programme aims to examine nutritional strategies for the post-weaned piglet in the absence of AGPs. The objectives are to optimise processing conditions of various dietary cereals such that nutrient density, energy and starch availability are maximised. The aim is to identify a diet that not only benefits the piglet nutritionally, but also provides the animal with a healthy gut environment.

As argued, existing literature often reports the processing methods alone, with no description of exact variables used. The current research project identifies the precise conditions and evaluates how these impact upon nutritional value. The end result will be a better understanding of the optimum conditions necessary for weaned piglet diets.
A second original aspect of this work is that it will also assess the usefulness of particular laboratory tests as a possible predictor of starch digestion *in vivo* within the animal. These laboratory analyses use food science techniques to examine how processing methods affect the physicochemical properties of starch granules. Variation in starch damage and hydration properties between raw cereals and processed wheat of differing endosperm texture are predicted to result in differences in digestibility for the weaned piglet.

A comparison of rheological (*in vitro*) and biological (*in vivo*) results will be provided in Chapter 8.

CHAPTER 2: MATERIALS AND METHODS

2.1. INTRODUCTION

Studies were undertaken to examine the digestive physiology and nutrition of the post-weaned piglet, as influenced by dietary raw materials and the processing conditions to which they were subjected. Many protocols were common to all trials and these are described.

2.2. ANIMALS AND HOUSING

For each trial, newly-weaned entire male piglets of approximately 8 to 12 kg live weight and uniform genotype (Large White x Landrace) were delivered to the university on the day of weaning (28d ± 1d). Upon arrival, the piglets were individually weighed, randomly allocated to a diet, and individually housed in an environmentally controlled (28 - 32°C, 45% humidity, 16L : 8D lighting regime) room in the pig metabolism building. Each piglet was housed in a pen 1 m^2 with slatted rubber flooring. The pens had wire mesh partitions, allowing visual and limited physical contact between piglets. Fresh water was available *ad-libitum* from two nipple drinkers per pen. Pigs used in Trial 2 shared the same genotype but were sourced from a different supplier to animals used in the other trials.

2.3. DIETS

Experimental diets for all trials were formulated on site at Nottingham (see Appendix 1 for dietary composition). None of the experimental diets contained AGPs. The wheat cultivars used throughout the trials were recognised breeds but are not named in this research programme due to commercial sensitivity. Two batches of soft wheat were used in the research programme; the first batch for Trials 1 & 2, the second for Trials 3, 4 & 5. Soft wheat batches were of the same variety, grown by the same breeder at the same location but were from two differing harvest years. Diets were fed in a dry meal form and on an *ad-libitum* basis. Feed was changed twice daily (at 08.30 h and 16.30 h), with a ready-weighed bag of feed given in the morning, and again in the evening. All refusals were removed and weighed (likewise spillages) and replaced with fresh feed so

that individual intakes could be calculated. Feed intake was closely monitored, and the amount of feed given was increased in relation to appetite, as the trial progressed. Feed contaminated with water (or urine) was dried to remove all moisture, prior to wastage being calculated on a fresh-weight basis.

2.4. EXPERIMENTAL AND SLAUGHTER PROCEDURE

All piglets were weighed on day zero (day of weaning and delivery to unit) and at seven days into the trial. In addition, piglets chosen on slaughter days were weighed to ensure that they had gained weight, prior to slaughter. The first group of piglets was slaughtered on day zero as it is assumed that these possess the same gut morphology as a suckling piglet, and therefore acted as a control (referred to from now on as control animals), when compared against the data from the other animals. Piglets were slaughtered on days 0, 2, 4, 6, 10 & 14 of the trials. Slaughter days were designed to allow careful observation of the changes in gut morphology that typically occur in the period (especially during the first week) immediately after weaning (Hampson, 1986; Miller *et al.*, 1986). Slaughter programmes for each trial are described in the relevant chapters.

Animals were slaughtered by electrical stunning, requiring a minimum of 1.3 Amps, with maximum stun-stick interval being 30 seconds. The main jugular vein was then severed, resulting in exsanguination (with 3-4 minutes being allowed to bleed out completely). At slaughter, the small intestine was removed and pieces (at approximately 0.25, 0.5 and 0.75 proportionally along the tissue) were cut and fixed in Bouin's solution, as described in the histology section, for assessment of gut morphology. Digesta were collected (details given in subsequent chapters) and immediately analysed for pH and viscosity. The pH was measured using a digital pH meter (Mettle Toledo 320; Fisher Scientific UK), calibrated prior to measurement using buffering solutions (Fisher Scientific UK). Approximately 2 g of fresh digesta from each gut region was centrifuged (9,000 x *g*, 6 min) and the viscosity was determined with 0.5 ml of supernatant using a Brookfield viscometer with a CP40 cone (see 2.7.1.). Digesta not

used for pH and viscosity analysis was frozen at –195˚C in liquid nitrogen, and stored at –20˚C for subsequent laboratory analysis.

2.5. GASTRIC ULCERATION

Because of the possibility of gastric ulcers from feed ground to the fine particle size employed (Hedde *et al.*1985; Fjetland and Teige, 2002), the stomachs from slaughtered pigs were removed, and the contents washed out. Each stomach was carefully inspected for any signs of ulceration by an experienced slaughterman (stomach observations were carried out by the same individual throughout the 5 piglet trials). Photographs of the stomachs were taken using a digital camera.

2.6. FAECAL COLLECTION

To enable analysis of digestibility, faecal samples were collected during the trial. Eight piglets were assessed over two time periods where a fresh faecal sample was removed from each animal at regular intervals throughout the day. The first collection period was from days 1-5 inclusive, the second collection period was days 9-13 inclusive. Faecal samples were frozen immediately after collection for laboratory analysis.

2.7. ANALYTICAL PROCEDURES

2.7.1. Determination of viscosity

Viscosity is a measure of a fluid's resistance to flow and was determined using a Brookfield DV-III and cone plate rotational viscometer (CP40; Brookfield Engineering Laboratories, MA, USA). A calibrated spring drives a spindle through 0.5 ml of sample fluid. The resistance of the fluid against the spindle is measured by the spring deflection, using a rotary transducer. The viscometer was connected to a water bath, with a capacity to circulate water and maintain a temperature of 39.6˚C (equivalent to a piglet's core body temperature - Ruckebusch *et al.*, 1991). All readings of viscosity are displayed in units of centipoise (cP). The fluid analysed is the resulting supernatant from centrifuging a sample of digesta (9,000 x *g* / 6 min). Prior to any analysis, the viscometer was set up as follows:

1. Viscometer and water bath set on a level surface. The air bubble indicator was centred by adjusting the levelling feet.
2. Power switched on, when prompted by display, press any key on control pad (ensuring spindle had been removed). Display read "autozero". When prompted, attached spindle (using spindle wrench to hold shaft steady).
3. Attached cup (**taking care to avoid hitting the cone**) securely using clip under cup base.
4. Viscometer switched on and set running at 12 rpm. Display should read 0%. If not, adjustment ring turned **clockwise** (*as you look down from above*) by one division. Waited six seconds, and if necessary, adjusted again.
5. Once display showed 0%, adjustment ring turned **anticlockwise**, one division at a time, waiting six seconds between adjustments. Adjustment continued until display reading jumped from 0 to 1% or higher. This is the hit point. Adjustment ring turned **clockwise** by one division.
6. The viscometer was now set up and ready for sample measurement. Ensured machine was switched to the "CP" reading, prior to commencing any analysis.
7. Calibration tested by placing 0.5 ml distilled water into the cup, and attached to cone. Set to 60 rpm. Set display to CP and switched on. Reading should be 0.75 cP.
8. Cup and spindle were cleaned and dried thoroughly between samples, using distilled water and absorbent paper.
9. During analysis of samples, display occasionally read EEEE, indicating the sample was very viscous. In such circumstances, the speed was decreased. Alternatively, if the "CP" or "?" flashed, the sample was not very viscous, and the rpm was increased.
10. Once reading had stabilised, the viscosity was recorded and the procedure was repeated for each sample in duplicate.

2.7.2. Determination of dry matter

Dry matter (DM) of the diets was determined in a laboratory drying oven (MSE) as follows: Approximately 5 g of sample was weighed accurately into pre-weighed crucibles and dried for 72 hours at 80°C. The samples were then removed from the

oven, cooled in a desiccator and re-weighed. Digesta and faecal samples were then frozen (-20°C) and dried in a freeze-dryer (Edwards Super Modulyo). After drying, the dry matter (g / kg) in the samples is calculated as follows:

Dry Matter (g / kg) = (Dry weight / Wet weight) x 1000

2.7.3. Determination of Acid-Insoluble Ash

Acid-insoluble ash (AIA) was measured using a modified version of the method described by Van Keulen and Young (1977). A sample weight of 1 g (diet, digesta or faecal) was used in each analysis, as this was found to yield results consistent with an initial assessment using larger sample quantities, confirmed using analysis of variance (ANOVA) - Table 2.1.

Table 2.1: Assessment of sample quantity needed for AIA analysis

Sample weight (g)	**AIA (g/kg)**	**ANOVA**		
		s.e.d.	**P**	**cv%**
10	18.2	0.464	0.315	3.2
5	18.3			
3	17.7			
1	17.5			

(Each sample weight performed in triplicate, AIA figures shown are mean values)

Although this method was originally used for ruminant studies, it has since been recommended for nutritional evaluation in work with young pigs (Ly *et al.*, 2002). This method was used as ashing prior to acid treatment yielded the following benefits:
1) A lower molarity acid could be used, and 2) the initial ashing burns off the organic matter, resulting in elimination of odour when treating with acid.

Each duplicate 1g sample (freeze-dried and ground) was weighed into a crucible (W_1) and then ashed overnight at 530°C in a muffle furnace. The ash was transferred into a 600 ml beaker (without spout) and 100 ml of 2M HCl was added. The mixture was

boiled for five minutes in a fumehood (condenser attached to each flask to prevent loss of HCl). The hot hydrolysate was filtered (Whatman hardened ashless) and washed free of acid with hot distilled water (85 - 100°C). The ash and filter paper were then transferred back into a crucible and ashed again overnight at 450°C.

The resulting ash was weighed (W_2) and calculated using the formula below.

$$\textbf{Acid-Insoluble ash (g/kg)} = \left(\frac{\textbf{Final ash weight } (W_2) - 0.0012}{\textbf{Initial sample weight } (W_1)} \right) \textbf{x } 1000$$

In order to test if the filter paper was completely burnt away during ashing, three papers (containing no sample) were treated in exactly the same way (subjected to hot acid/water/ashing) and any remaining residue weighed. It was found that in each case, 0.0012 g of residue was detectable. This figure was subtracted from each ashed sample, in order to give an accurate weight of ash.

2.7.4. Determination of starch

Starch was determined using a total starch assay kit (Megazyme, Ireland. AOAC method 996.11, AACC method 76.13). Using this procedure, starch is hydrolysed in two phases: Solubilisation of starch to α-dextrins is carried out using dimethyl sulphoxide (DMSO) and thermostable α-amylase. The second phase uses amyloglucosidase to hydrolyse these dextrins to glucose.

Reagents:

MOPS buffer:

(50mM, pH 7.0) plus calcium chloride (5mM) and sodium azide (0.02%) MOPS (sodium salt, 11.55 g – Sigma Chemical Co.) was added to 900 ml of distilled water. This solution was adjusted to pH 7.0 by addition of 1M HCl (approximately 17 ml was needed). Calcium chloride dihydrate (0.74 g) and sodium azide (0.2 g) were added and dissolved. Volume was adjusted to 1 litre. Buffer stored at room temperature.

Sodium acetate buffer:

(200 mM, pH 4.5) plus sodium azide (0.02%). Glacial acetic acid (11.8 ml, 1.05 g/ml) was added to 900 ml of distilled water. This solution was adjusted to pH 4.5 by addition of 1M (4 g/100 ml) sodium hydroxide solution. Approximately 60 ml was needed. Sodium azide (0.2 g) was added and the volume adjusted to 1 litre. Stored at room temperature.

Glucose determination reagent (GOPOD):

Diluted entire contents of glucose reagent buffer (concentrate 50 ml) to 1 litre with distilled water. Used this to dissolve the glucose determination reagent (GOPOD).

Enclosed standard:

Glucose standard solution (100 µg / 0.1 ml in 0.2% benzoic acid)

Enclosed control:

Regular maize starch (starch content ~960 g/kg dry weight, ~840 g/kg as is basis) 140 g moisture/kg.

Enzymes:

Thermostable α-amylase:

(10 ml, 3000 U / ml at pH 6.0 and 40°C). Diluted an aliquot (1.0 ml) to 30 ml with MOPS buffer.

Amyloglucosidase:

(10 ml, 200 U / ml at pH 4.5 and 40°C). This enzyme was used directly without dilution.

2.7.4.1. Starch Assay Procedure:

1. Ground sample to pass a 0.5 mm screen
2. Added sample (~100 mg; weighed accurately) to a glass tube. Repeated with maize starch sample as a control.
3. Wetted with 0.2 ml of aqueous ethanol (80% v/v). Stirred on vortex mixer.
4. Immediately added 2 ml DMSO (Fisher Scientific UK). Stirred on vortex mixer.
5. Incubated tubes in a boiling water bath (100°C / 5 minutes).
6. Added 3 ml of α-amylase (in MOPS buffer) and vigorously stirred tube on vortex mixer. Incubated in boiling water bath (100°C / 6 minutes) – stirred tube after 2 and 4 minutes.
7. Incubated in water bath (50°C / 5 minutes)
8. Added 4 ml sodium acetate buffer, followed by 0.1 ml amyloglucosidase (using positive displacement pipette). Stirred tube on vortex mixer and incubated in water bath (50°C / 30 min).
9. Transferred entire contents to a 100 ml volumetric flask. Rinsed out tube contents thoroughly with distilled water. Made up to 100 ml with distilled water. Mixed thoroughly. Centrifuged a 10 ml aliquot at 1881 x g / 10 min.
10. Transferred duplicate aliquots (0.1 ml) of the sample solution to glass tubes.
11. Prepared glucose control – 0.1 ml glucose standard solution.
12. Prepared reagent blank – 0.1 ml distilled water.
13. Added 3 ml GOPOD reagent to **all** tubes (sample tubes, glucose control and reagent blank).
14. Incubated in water bath (50°C / 20 min).
15. Using a spectrophotometer (Unicam Helios γ), zeroed the absorbance at 510 nm using distilled water. Measured absorbances firstly of the blanks (A_{BLK}), then standards (A_{STD}), and finally the samples (A_{SAM}).
16. Absorbance of the distilled water was read after every six samples to ensure the spectrophotometer zero had not changed. If necessary, re-zeroed the spectrophotometer.

2.7.4.2. Starch calculation

1) The amount of starch in the sample was determined as follows:

$$\textbf{STARCH g/kg}_{\textbf{(as is)}} = \Delta E \times (F / W) \times 900$$

Where:

ΔE = absorbance of sample (A_{SAM}) – absorbance of blank (A_{BLK})

F = 100 / absorbance of glucose standard (A_{STD})

W = Weight in milligrams ("as is" basis) of the sample analysed

2) Starch g/kg (dry weight basis) was calculated as:

$$\textbf{STARCH g/kg}_{\textbf{(dry wt)}} = \textbf{Starch g/kg}_{\textbf{(STD as is)}} \times 100 / (100 - \textbf{moisture content (\%)})$$

3) Finally, starch g/kg (corrected for dry matter) was calculated as follows:

$$\textbf{STARCH g/kg} = \textbf{Starch g/kg}_{\textbf{(as is)}} \times (1000 / \textbf{Starch g/kg}_{\textbf{(dry wt)}})$$

2.7.4.3. Coefficient of apparent starch digestibility (CAD_S)

For digesta samples, this was calculated as follows:

$$\textbf{CAD}_S = 1 - (\textbf{Starch}_{\textbf{dig}} \times \textbf{AIA}_{\textbf{diet}}) / (\textbf{AIA}_{\textbf{dig}} \times \textbf{Starch}_{\textbf{diet}})$$

Where:

$Starch_{dig}$ = starch in digesta (g/kg)

AIA_{diet} = acid insoluble ash in diet (g/kg)

AIA_{dig} = acid insoluble ash in digesta (g/kg)

$Starch_{diet}$ = starch in diet (g/kg)

2.7.4.4. Apparent digestible starch content

Expressed as g digestible starch / kg diet DM: this was calculated as:

$$\textbf{CAD}_S \times \textbf{starch content in the diet (g/kg)}$$

2.7.4.5. Coefficient of total tract apparent digestibility (CTTAD)

CTTAD was calculated using the same equation as for CAD_S but with substitution of faecal data

$$\mathbf{CTTAD = 1 - (Starch_{faec} \times AIA_{diet}) / (AIA_{faec} \times Starch_{diet})}$$

Where:

$Starch_{faec}$ = starch in faeces (g/kg)

AIA_{diet} = acid insoluble ash in diet (g/kg)

AIA_{faec} = acid insoluble ash in faeces (g/kg)

$Starch_{diet}$ = starch in diet (g/kg)

2.7.5. Determination of Nitrogen

Nitrogen content of the diets, digesta and faecal samples was analysed using a Nitrogen Element Analyser (Fisons, UK). This uses an automatic flash combustion method, which is based on the quantitative combustion of the sample, followed by reduction. Approximately 50 mg of each sample was loaded into a small tin capsule, sealed and loaded into the analyser.

2.7.5.1. Coefficient of apparent nitrogen digestibility (CAD_N)

For digesta samples, this was calculated as follows:

$$\mathbf{CAD_N = 1 - (N_{dig} \times AIA_{diet}) / (AIA_{dig} \times N_{diet})}$$

Where:

N_{dig} = nitrogen in digesta (g/kg)

AIA_{diet} = acid insoluble ash in diet (g/kg)

AIA_{dig} = acid insoluble ash in digesta (g/kg)

N_{diet} = nitrogen in diet (g/kg)

2.7.5.2. Coefficient of total tract apparent digestibility (CTTAD)

Once again, to calculate CTTAD of nitrogen, the digesta figures are replaced with faecal data to give the following equation:

$$\mathbf{CAD = 1 - (N_{faec} \times AIA_{diet}) / (AIA_{faec} \times N_{diet})}$$

Where:

N_{faec} = nitrogen in faeces (g/kg)

AIA_{diet} = acid insoluble ash in diet (g/kg)

AIA_{faec} = acid insoluble ash in faeces (g/kg)

N_{diet} = nitrogen in diet (g/kg)

2.7.6. Determination of caecal volatile fatty acids (VFAs)

Concentration of caecal VFAs were determined using a gas chromatograph (GC) following the method described by Franklin *et al.* (2002). Caecal digesta was centrifuged at 3345 x *g* for 15 min. The resulting supernatant (1 ml) was mixed with 200 µl of 25% metaphosphoric acid and incubated at room temperature for 30 minutes. After further centrifugation (13,000 x *g* / 10 min), 1 µl of sample was injected into the GC (Model 6890, Agilent) with a WCOT fused silica 25 m x 0.32 mm x 1 mm capillary column. A flame ionisation detector was used with an oven temperature of 140°C, and a detector temperature of 250°C. A working standard and a control (distilled water) were included in each assay run, where the working standard contained acetic, propionic, n-butyric, iso-butyric, valeric and iso-valeric acids (see Table 2.2), made up to 100 ml with 70% (v/v) ethanol. Aliquots of this were then used in each assay run.

Table 2.2: VFA concentrations of standard used in GC analysis

Acid	Volume (ml)	Density	MW	Concentration (mmol l^{-1})
Acetic	0.101	1.049	60.05	17.7
Propionic	0.101	0.993	74.08	13.6
Iso-butyric	0.101	0.950	88.11	10.9
n-butyric	0.101	0.959	88.11	11.0
Iso-valeric	0.101	0.931	102.14	9.2
Valeric	0.101	0.939	102.14	9.3

MW = Molecular weight

Individual VFA concentrations for each sample could then be determined by comparing graph peak areas as follows:

$$\text{Sample (mmol l}^{-1}) = \frac{\text{Sample area}}{\text{Standard area}} \times \text{Concentration of standard}$$

Molar proportions for each of the individual acids were then calculated by dividing the sample concentration obtained for each acid by the total concentration of all six VFAs.

2.8. HISTOLOGY

2.8.1. Introduction

Preliminary studies by Zarkadas (1999) have shown that Bouin's fixative adequately prepares piglet intestinal tissue for morphological measurements and was therefore used for all samples.

2.8.2. Fixing tissue samples

Pieces of intestinal tissue (6-8 cm in length) were ligated at distances of proportionately 0.25 (proximal), 0.50 (medial) and 0.75 (distal) along the whole length of the small intestine, from the gastric pylorus to the ileocaecal valve. For each section, both ends were doubly tied, and the pieces of tissue cut from the tract. Each was then filled by injecting Bouin's fixative, consisting of Picric acid (saturated aqueous 714.3 ml l^{-1}; Sigma-Aldrich Co), formaldehyde (formalin) (238.1 ml l^{-1}; Sigma-Aldrich Co) and glacial acetic acid (47.6 ml l^{-1}; Fisher Scientific UK) into the lumen. The sections were then submersed in Bouin's fixative for a minimum period of 15 to 20 minutes. The ends of the fixed intestinal sections were then cut, and the tissue rinsed with normal saline (0.9% w/v) to flush out the intestinal contents, before being returned to fresh Bouin's for storage.

Previous work has shown that 24 hours is sufficient to allow full penetration of the tissue by the fixative (Zarkadas, 1999) and therefore, after approximately 24 hours, the tissue

was removed from the fixative and transferred to 70% (v/v) Industrial Methylated Spirit (I.M.S.). The solution was changed a number of times in order to remove excess fixative from the tissue, and stored in 70% I.M.S. until embedding.

2.8.3. Embedding of tissue sections

A ring-shaped section of tissue (approximately 1 cm in length) was dehydrated by immersion in an ascending series of aqueous alcohol solutions (70%, 90% and 100% I.M.S.) using a histokinette (Model TP1020 Leica, Germany). Following dehydration, the tissue was cleared using toluene (Fisher Scientific UK), as this fluid is miscible with both alcohol and paraffin, therefore allowing the molten paraffin wax to penetrate the tissue. The schedule used for the samples is outlined below (Table 2.3).

Table 2.3: Embedding schedule for intestinal sections.

Step	Chemical	Duration
1	70% I.M.S.	1 hour
2	90% I.M.S.	1 hour
3	100% I.M.S. 1	1 hour
4	100% I.M.S. 2	1 hour
5	Toluene 1	1 hour
6	Toluene 2	1 hour
7	Wax 1	1 hour
8	Wax 2	1 hour

Once infiltrated with wax, the tissue was rapidly transferred into a pre-warmed embedding mould filled with molten wax, and orientated as desired. The mould was then immediately cooled in water at 15°C to prevent fracturing. Once a solidified scum had formed, the moulds were completely submerged in water, weighed down, and left overnight. The wax blocks were then broken out of the mould and stored for subsequent sectioning.

2.8.4. Sectioning of tissue samples

After embedding, the tissue was sectioned using a rotary microtome (HM 355 Microm Laborgeräte GmbH, Walldorf, Germany) with the specimen feed being set at 7 microns (μm). Zarkadas (1999) showed that intestinal villi in piglets of this age are less than 150 microns in diameter. With this in mind, for each sample, four serial transverse sections were cut, then 200 microns of tissue was fed through before the next serial sections were taken. This was repeated four times per sample, and was used to ensure that each group of transverse serial sections contained different villi. After cutting, the sections were placed on slides, stretched flat using 1% glycerin albumin (Raymond A. Lamb Ltd) on a hot plate, and baked overnight at 35°C, ready for staining.

2.8.5. Staining of tissue samples

The trichrome staining procedure was used, where the slides were run down a series of alcohols of decreasing strength, to rehydrate them prior to staining. The procedure used is outlined below (all reagents obtained from Fisher Scientific UK unless otherwise stated).

1. Xylene 1: 2 mins
2. Xylene 2: 2 mins
3. Absolute I.M.S. 1: 2 mins
4. Absolute I.M.S. 2: 2 mins
5. 95% (v/v) I.M.S. 1: 3 mins
6. 70% (v/v) I.M.S. 2: 3 mins
7. Running water: 3 mins
8. Harris haematoxylin (BDH) 15 mins
9. Wash in running water 3 mins
10. Destain in 0.5% HCl in 70% (v/v) I.M.S. for a few seconds, (sections will turn pink in colour). Transfer to ammoniated 70% (v/v) I.M.S. until sections turn blue in colour. Examine under microscope; if nuclei are too dark, repeat above procedure. When nuclei stand out as a sharp blue colour against a colourless background, rinse slides thoroughly in water for 3 mins.

11. Fix stain in 1% (w/v) phosphomolybdic acid soln:	2 $^{1}/_{2}$ mins
12. Stain in orange G (Raymond A. Lamb Ltd):	5 mins
13. Rinse in distilled water:	
14. Treat with 1% (w/v) acetic acid:	2 mins
15. Stain in fast green (Fisons Scientific):	5 mins
16. Treat with 1% (w/v) acetic acid:	3 mins
17. Rinse in 95% (v/v) I.M.S., transfer to second 95% I.M.S.	5 mins
18. Absolute I.M.S.	3 mins
19. Absolute I.M.S.	3 mins
20. Xylene 3:	3 mins
21. Xylene 4:	3 mins

22. Slides were removed one at a time from the xylene, the back of the slide quickly dried using paper towel, before a glass pasteur pipette was used to apply DPX Mounting Medium (Raymond A. Lamb Ltd). After a coverslip was added, a mounted needle was used to apply gentle pressure to remove any bubbles.

2.8.6. Histological measurements

Slide-mounted sections were measured using a computerised image analysis system consisting of a Leitz system microscope (Model Diaplan; Leitz Wetzlar Germany) fitted with a Panasonic camera (Model WV-F15E). The camera projected live images onto a computer that utilised a video frame grabber (Model Flashpoint 3D Lite; Integral Technologies Inc). Gut morphology measurements were measured using Scion Image for Windows (version Beta 4.02 Scion Corporation). This software was calibrated using a stage micrometer (Raymond A. Lamb), consisting of a 1mm scale, divided into 1000 divisions. Only straight villi that were complete from tip to base (single layer of epithelial cells) were selected for measurement. Ten of the tallest well-orientated villi were measured, as defined by Schultz (1991). Measurements taken (Figure 2.1) were villus height (distance from villus base to villus tip), villus width (one third of the distance down from the villus tip), and crypt depth (distance between villus base and muscularis layer). The calculation of villus height to crypt depth ratio was also performed.

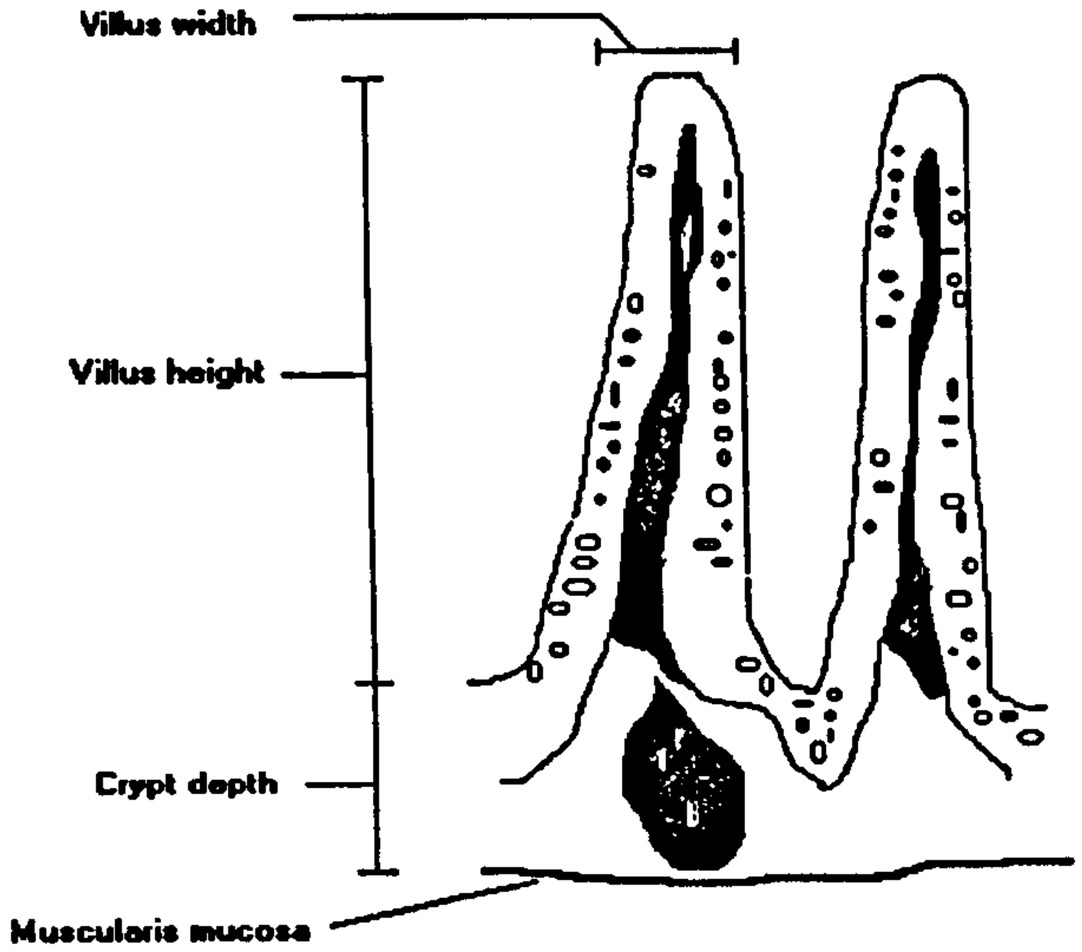


Figure 2.1: Diagram showing villus measurements taken for gut morphology analysis

2.9. PERFORMANCE PARAMETER CALCULATIONS

Although not a major objective of the trials, DLWG and FI (expressed as DE intake) values were calculated in order to provide an indication of animal performance. FCR was not determined, as the highly variable feed intake of piglets at weaning would make interpretation of FCR data limited.

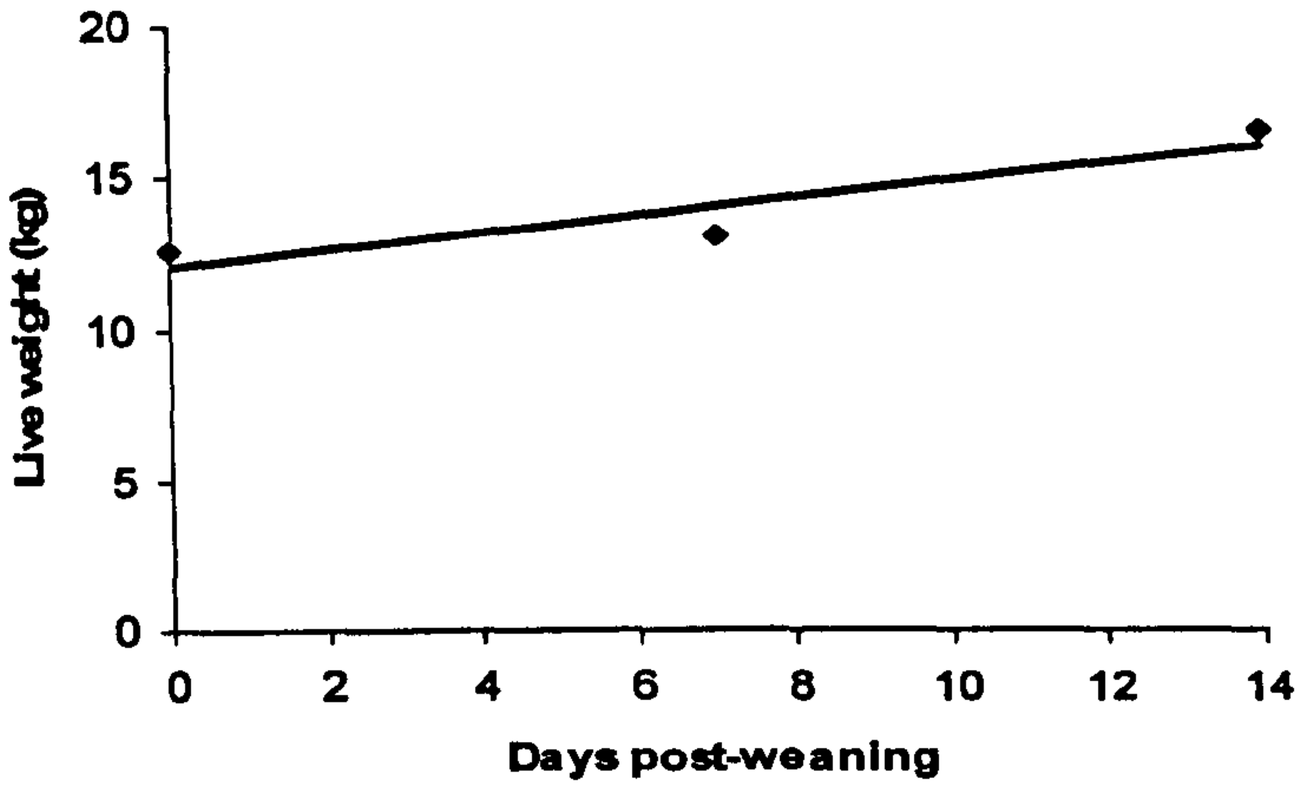
2.9.1. Daily live weight gain (DLWG)

Average DLWG was determined by performing a linear regression of live weight against time. Example: If the live weights of a piglet are as follows:

Day	Live weight (kg)
0	12.6
7	13.1
14	16.5

Live weights are then plotted against day to give a regression graph:

Using the equation **y (live weight) = a (DLWG) x (day) + b (intercept)**, this graph generated the following values for this piglet:



$$y = 0.278\,x + 12.117$$

So this piglet had an average daily live weight gain of 0.278 kg / day.

2.9.2. Daily feed intake

Throughout the trials, individual feed intake was measured for all piglets on a daily basis. This was calculated as total feed offered less any refusals (and spillages).

2.10. STATISTICAL ANALYSES

2.10.1. Individual Trial statistics

All statistical analyses were undertaken using Genstat 8.1 (Lawes, 2005) and all graphs were compiled using Excel (Microsoft, 2003). Analysis was undertaken using General Analysis of Variance and was performed in 2 parts:

- Control animals versus the rest (CVR) compares those slaughtered on day zero with the remaining experimental animals in the study.
- Dietary effects by removing control animals and comparing treatments.

In each analysis, all interactions were determined. Where day was employed as a parameter, linear (L) and non-linear (Q) contrasts were established using a polyanova routine.

2.10.2. Meta-analysis statistics

In order to determine whether any relationships were evident between the *in vitro* and *in vivo* data across the animal trials, principal component analysis (PCA) was employed. PCA is a powerful statistical tool for analysing data and uses a mathematical procedure to transform a number of (possibly) correlated variables into a smaller number of variables called *principal components*. The first principal component accounts for as much of the variability in the dataset as possible, with each succeeding component accounting for as much of the remaining variability as possible. PCA is used to reduce the dimensionality of the dataset (allowing graphical representation of the reduced number of dimensions, without much loss of information) and expresses the data in such a way as to highlight similarities and differences between groups of samples within the dataset. Following PCA, any significant correlations in the research data were then analysed by a linear regression model using partial least squares (PLS). This technique is used to find fundamental relations between two variables – describing a *predicted* variable in terms of other *observed* (measured) variables, and expresses any correlation as a linear model.

2.11. FOOD CHEMISTRY ANALYSES

In parallel with the animal work, colleagues from the department of Food Sciences at University of Nottingham performed a range of physicochemical tests on each of the diets and cereals used in the trials. These were carried out in order to examine differences in structure, hydration and digestive properties between raw starches from different sources, and between the raw and processed starches used.

2.11.1. Rapid Visco Analyser (RVA)

The pasting of starch was analysed using a RVA (Series 4, Newport Scientific, 1998), which measures the resistance of starches to shearing forces under defined hydration and temperature regimes. The process measures the viscosity of a starch-water suspension, which is stirred, heated and then cooled at a uniform rate. Paste viscosity (reflecting the physical changes in the starch granules) is recorded from the force needed to drive the stirrer through the paste.

2.11.2. Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI is a measure of the volume of a starch granule or polymer, after swelling in excess water. WSI measures the amount of free polysaccharide released from the granule, when in excess water. These analyses were carried out using a modified method of that used by Anderson (see Sriburi, 1999), and were used to provide an indication of the degree of gelatinisation and extent of molecular breakdown, during processing.

2.11.3. X-Ray Diffraction (XRD)

This method is used to detect the loss of crystallinity when native starches are heated. It can also be used to quantify the amount of converted starch due to processing of the samples. The method was carried out as described by Sriburi (1999).

2.11.4. Differential Scanning Calorimetry (DSC)

This thermo-analytical technique measures the difference in the amount of heat required to increase the temperature of a sample and inert reference material in two sealed containers. This analysis is used to monitor the degree of thermal transition in a sample, based on the principle that native starch has no loss of crystallinity (0%) and that a theoretically fully cooked sample contains 100% loss of crystallinity. Essentially, a 12 mg sample was added to water at a ratio of 1:3. Heat was applied (using a Perkin-Elmer DSC 7) raising the temperature from 10°C to 90°C (at a rate of 10°C per minute) and the gelatinisation enthalpy (ΔH, expressed in J g^{-1}) was determined by comparing the amount of heat required to keep the two containers at an equal temperature.

2.11.5. Polarised light microscopy

When observed under a microscope using polarised light, starch granules exhibit a phenomenon termed birefringence. The semi-crystalline structure of native starch is indicated by the appearance of bright 'maltese crosses'. These crosses are absent when the starch structure has been damaged by processing.

2.11.6. *In vitro* amylolytic digestion

Developed at the University of Nottingham, this method monitors the hydrolysis of each sample initiated by pancreatic α-amylase solution at 37°C using the calorimetric phenol-sulphuric acid method to determine the total amount of glucose released over time (Englyst and Cummings, 1987). For the analysis, 60 mg (dry weight) of cereal flour is incubated in a 30 ml citrate phosphate buffer solution (0.06 M dm $^{-3}$; pH 6.9) to which was added 30 µl of commercial pancreatic α-amylase (1370 units; Sigma, UK) at 37°C. One unit of enzyme is defined as liberating 1 mg of maltose from starch in 3 min at 20 °C. A variation of this protocol (*i.e.* no enzyme added) was also used to estimate the level of endogenous α-amylase in all cereals.

CHAPTER 3:

Trial 1: Examining inter-cereal variability in terms of digestibility, digesta properties and gut morphology in the post-weaned piglet: (Part 1 - Soft wheat, Barley, Rye & Triticale)

3.1. INTRODUCTION

A review of the literature shows an increasing interest in the relationship between piglet nutrition and the viscosity of intestinal digesta. Although studies with chickens have shown that viscous soluble non-starch polysaccharides (NSP) can interfere with nutrient digestion and result in decreased weight gain (Bedford and Classen, 1992; Choct and Annison, 1992a), there appears to be less information about piglets. The addition of a viscous NSP (such as guar gum) to the diet has been associated with a detrimental effect on glucose absorption in growing pigs (Rainbird *et al.*, 1984) and reduced growth rates in weaned piglets (McDonald *et al.*, 1999). Recent studies have also demonstrated that a viscous diet can lead to a reduction of villus height in chickens (Langhout *et al.*, 2000) and in weaned piglets (McDonald *et al.*, 2001). It has been suggested that newly-weaned piglets have less physical capacity to dilute viscous material within the intestinal tract (McDonald *et al.*, 2001) and therefore it would appear that a diet promoting decreased intestinal viscosity would be more beneficial for the piglet, in terms of improved nutrient absorption and gut morphology. With this in mind, two related trials were designed in order to understand the impact of different raw cereals in terms of digesta viscosity and subsequent physiological / nutritional responses. Both used soft wheat as a control. Trial 1 evaluated barley, rye and triticale, and Trial 2 looked at naked oats, whole oats and maize. The current chapter reports the results from Trial 1.

3.2. OBJECTIVE

- The objective of Trial 1 was to examine inter-cereal variability of piglet diets in terms of digestibility, digesta properties and gut morphology in newly-weaned animals. The dietary cereals evaluated in this trial were soft wheat, barley, rye and triticale.

3.3. HYPOTHESES

- Increased viscosity of tract digesta (influenced by dietary cereal) will be detrimental to weaned piglet performance.
- Starch digestibility will be reduced within the small intestine as a result of increased tract digesta viscosity.

3.4. METHOD

3.4.1. Animals and housing

Piglets (n = 44) were housed and fed as described in Chapter 2.

3.4.2. Diets

Four experimental diets were manufactured on site at Nottingham. Raw cereals (soft wheat, barley, rye or triticale) were ground through a hammer mill (screen size 1.5 – 2 mm) and incorporated into diets (see Appendix 1 for dietary specification).

3.4.3. Experimental and slaughter procedure

Slaughter procedure and sample collection was carried out as described in Chapter 2. Unfortunately upon collection, there was insufficient digesta from the 0.25 sampling site in several pigs. This meant that some of the laboratory analyses (principally for the determination of starch and nitrogen coefficients) from this region of the small intestine were unable to be performed. The slaughter programme is shown in Table 3.1.

Table 3.1: Slaughter programme for Trial 1

Group	Day	Piglets slaughtered
1	0	n = 4
2	2	n = 8 (2 per diet)
3	4	n = 8 (2 per diet)
4	6	n = 8 (2 per diet)
5	10	n = 8 (2 per diet)
6	14	n = 8 (2 per diet)

3.4.4. Faecal collection

Faecal samples from eight pigs were collected over two sampling periods as described in Chapter 2.

3.5. RESULTS

3.5.1. Gastric ulceration

No evidence of gastric ulceration was found in any of the animals studied.

3.5.2. pH

Mean digesta pH figures and analysis of variance are shown in Tables 3.2 and 3.3. Significant interactions between CVR and region and between CVR, day and region were observed. Apart from these, no other parameters involving the control pigs were significant. There was no effect of diet or day and no interaction between the two was found ($P > 0.05$). As expected, tract region was highly significant ($P = <0.001$) and an interaction between day and region was also observed ($P = 0.036, 0.027(L)$).

3.5.3. Viscosity

Mean digesta viscosity figures and analysis of variance are shown in Tables 3.4 and 3.5. There was a highly significant dietary effect ($P = <0.001$) with the greatest digesta viscosity exhibited by pigs on the rye diet. A significant interaction between diet and region also showed that the rye diet produced the most viscous digesta in all tract regions studied, with pigs on the wheat diet having the least viscous digesta ($P = <0.001$). Over time there was a general increase in viscosity ($P = <0.001, <0.003(L)$) and a significant interaction between diet and day showed the rye diet was consistently more viscous than the other cereals throughout the trial period ($P = <0.001, <0.001(L), 0.047(Q)$). Significant effects were also observed for region and the interactions between day and region, and diet, day and region. All analyses involving control animals were also found to be highly significant (Table 3.5).

Table 3.2: Mean digesta pH from Trial 1

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	Stomach	2.8						**2.8**
	0.25	6.6						**6.6**
	0.50	7.3						**7.3**
	0.75	7.5						**7.5**
	Caecal	6.8						**6.8**
Soft Wheat	Stomach		2.7	4.6	3.1	1.7	3.5	**3.1**
	0.25		6.2	6.2	6.6	6.4	5.6	**6.2**
	0.50		7.2	6.9	7.0	7.0	7.0	**7.0**
	0.75		7.5	7.4	7.4	7.6	7.4	**7.4**
	Caecal		6.9	6.8	6.7	6.2	5.3	**6.4**
Barley	Stomach		3.2	4.2	2.9	3.1	4.3	**3.5**
	0.25		6.4	6.1	6.4	6.6	6.0	**6.3**
	0.50		7.2	6.6	6.9	6.7	7.0	**6.9**
	0.75		7.8	7.9	7.2	7.2	7.7	**7.5**
	Caecal		7.0	6.4	6.5	5.9	5.5	**6.2**
Rye	Stomach		2.0	3.4	3.6	3.1	3.4	**3.1**
	0.25		6.4	6.7	6.6	6.4	6.0	**6.4**
	0.50		7.2	7.4	7.3	6.7	7.1	**7.1**
	0.75		7.5	7.8	7.0	7.2	7.4	**7.4**
	Caecal		6.9	6.6	6.1	6.2	6.1	**6.4**
Triticale	Stomach		2.9	2.5	2.9	2.6	4.1	**3.0**
	0.25		6.2	6.4	6.0	6.2	6.1	**6.2**
	0.50		7.0	7.3	7.2	7.0	7.3	**7.2**
	0.75		7.0	7.5	7.0	7.0	7.1	**7.1**
	Caecal		5.7	6.0	6.0	6.1	5.9	**5.9**

0.25, 0.5 & 0.75 = proportion along small intestine (n = 10 per diet, control pigs; n = 4)

Table 3.3: Analysis of variance of mean digesta pH from Trial 1

Factor	s.e.d.	P	cv
CVR	0.15	0.252	10.9%
CVR*Day	0.18	0.147	
CVR*Diet	0.17	0.387	
CVR*Region	0.35	**<0.001**	
CVR*Day*Diet	0.26	0.886	
CVR*Day*Region	0.40	**0.029**	
CVR*Diet*Region	0.39	0.772	
CVR*Day*Diet*Region	0.57	0.969	
Diet	0.13	0.398	11.1%
Day	0.15	0.156, 0.157(L), 0.514(Q)	
Diet*Day	0.30	0.893, 0.282(L), 0.655(Q)	
Region	0.15	**<0.001**	
Diet*Region	0.30	0.785	
Day*Region	0.33	**0.036, 0.027(L)**, 0.450(Q)	
Diet*Day*Region	0.67	0.972, 0.917(L), 0.781(Q)	

CVR = control animals versus the rest L = Linear effect Q = Quadratic effect

Table 3.4: Mean digesta viscosity (cP) from Trial 1

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	Stomach	0.9						**0.9**
	0.25	1.2						**1.2**
	0.50	1.2						**1.2**
	0.75	1.3						**1.3**
	Caecal	1.0						**1.0**
Soft Wheat	Stomach		1.2	1.6	1.4	0.9	1.4	**1.3**
	0.25		1.4	1.2	2.0	1.3	0.7	**1.3**
	0.50		1.4	1.6	1.9	1.4	1.8	**1.6**
	0.75		1.5	1.8	2.3	1.6	3.4	**2.1**
	Caecal		1.2	1.4	2.7	3.7	1.2	**2.0**
Barley	Stomach		1.2	1.4	1.1	0.7	1.4	**1.2**
	0.25		1.4	1.3	1.3	1.4	1.3	**1.3**
	0.50		0.9	1.5	1.4	1.4	3.1	**1.7**
	0.75		2.7	3.9	1.6	3.1	5.1	**3.3**
	Caecal		1.2	2.9	2.0	2.0	1.6	**1.9**
Rye	Stomach		1.2	1.8	2.1	3.2	1.8	**2.0**
	0.25		1.1	2.1	3.3	1.0	2.3	**2.0**
	0.50		1.0	3.5	4.9	2.1	6.0	**3.5**
	0.75		1.3	7.7	3.5	6.4	15.4	**6.9**
	Caecal		1.6	3.0	2.6	3.4	2.1	**2.5**
Triticale	Stomach		1.7	1.3	2.0	1.1	1.4	**1.5**
	0.25		1.4	1.5	2.4	1.4	2.1	**1.8**
	0.50		2.8	4.2	6.8	1.4	3.2	**3.7**
	0.75		3.7	5.3	6.9	2.4	4.5	**4.6**
	Caecal		2.1	4.6	2.7	2.1	1.4	**2.6**

0.25, 0.5 & 0.75 = proportion along small intestine (n = 10 per diet, control pigs; n = 4)

Table 3.5: Analysis of variance of mean digesta viscosity from Trial 1

Factor	s.e.d.	P	cv
CVR	0.31	**<0.001**	56.3%
CVR*Day	0.36	**<0.001**	
CVR*Diet	0.34	**<0.001**	
CVR*Region	0.68	**<0.001**	
CVR*Day*Diet	0.50	**<0.001**	
CVR*Day*Region	0.80	**<0.001**	
CVR*Diet*Region	0.77	**<0.001**	
CVR*Day*Diet*Region	1.13	**0.009**	
Diet	0.28	**<0.001**	57.7%
Day	0.31	**<0.001, 0.003(L)**, 0.499(Q)	
Diet*Day	0.62	**<0.001, <0.001(L)**, 0.533(Q)	
Region	0.31	**<0.001**	
Diet*Region	0.62	**<0.001**	
Day*Region	0.70	**<0.001, <0.001(L), 0.047(Q)**	
Diet*Day*Region	1.39	**0.040, <0.001(L)**, 0.381(Q)	

CVR = control animals versus the rest L = Linear effect Q = Quadratic effect

3.5.4. Starch digestibility

3.5.4.1. Starch digestibility in the small intestine

A significant effect of region showed CAD significantly increased from mid to distal sections of the small intestine (P = <0.001 – Tables 3.6 and 3.7). Starch digestion in the proximal section (0.25) was found to be negligible in those animals where sufficient digesta had been obtained. There was a significant effect of day (P = 0.004; 0.038(L); <0.001(Q)), and the interaction between day and region (P = 0.023; 0.003(Q)) showed digestibility decreased around day 6 post-weaning in both the mid and distal tract regions, before recovering throughout the rest of the trial period. Dietary differences approached significance (P = 0.051) and showed CAD was highest for the rye diet and lowest for triticale.

Table 3.6: Mean coefficients of apparent starch digestibility from Trial 1

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Soft	0.50	0.63	0.52	0.49	0.34	0.67	**0.53**
Wheat	0.75	0.86	0.93	0.83	0.80	0.94	**0.87**
Barley	0.50	0.54	0.15	0.25	0.36	0.67	**0.39**
	0.75	0.85	0.93	0.68	0.93	0.99	**0.88**
Rye	0.50	0.72	0.40	0.32	0.71	0.71	**0.57**
	0.75	0.85	0.79	0.82	0.98	0.97	**0.88**
Triticale	0.50	0.58	0.38	0.28	0.16	0.70	**0.42**
	0.75	0.72	0.81	0.66	0.92	0.69	**0.76**

0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet)

Table 3.7: Analysis of variance of mean coefficients of apparent starch digestibility

Factor	s.e.d.	P	cv
Diet	0.047	0.051	
Day	0.054	**0.004, 0.038(L), <0.001(Q)**	
Region	0.034	**<0.001**	
Diet*Day	0.107	0.333, 0.431(L), 0.850(Q)	22.8%
Diet*Region	0.068	0.310	
Day*Region	0.076	**0.023,** 0.649(L), **0.003(Q)**	
Diet*Day*Region	0.151	0.301, 0.952(L), 0.356(Q)	

L = Linear effect Q = Quadratic effect

3.5.4.2. Starch digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 3.8. Coefficients for all diets were found to be greater than 0.96. There was no effect of diet although mean coefficients increased significantly between collection periods (0.976 vs. 0.995). No interaction was observed between diet and collection period.

Table 3.8: Mean coefficients of total tract apparent digestibility for starch and analysis of variance from Trial 1

Diet	**Days 1-5**	**Days 9-13**
Wheat	0.966	0.997
Barley	0.979	0.995
Rye	0.983	0.995
Triticale	0.976	0.992

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.011	0.893	1.6%
Collection Period	0.008	**0.044**	
Diet*Collection Period	0.016	0.829	

(n = 2 pigs per diet for each collection period)

3.5.5. Nitrogen digestibility

3.5.5.1. Nitrogen digestibility in the small intestine

Mean CAD values are shown in Table 3.9, and analysis of variance in Table 3.10. A significant regional effect was observed with digestibility increasing from mid to distal region of the small intestine (P = <0.001). There was also a significant effect of day (P = 0.004; 0.002(L)), with a reduction in digestibility on day 6 post-weaning which recovered as the trial progressed. There was a highly significant dietary effect with wheat yielding the highest digestibility coefficients and barley exhibiting the lowest (P = <0.001). This dietary significance disappeared when samples were analysed over the total tract (P = >0.05). Apart from the interaction between diet and day (P = 0.032; 0.042(Q)), no other interactions between parameters were found.

Table 3.9: Mean coefficients of apparent nitrogen digestibility from Trial 1

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Soft	0.50	0.49	0.65	0.59	0.49	0.69	**0.58**
Wheat	0.75	0.52	0.75	0.75	0.78	0.85	**0.73**
Barley	0.50	*	0.05	*	0.38	0.60	**0.34**
	0.75	0.43	0.52	0.07	0.40	0.76	**0.44**
Rye	0.50	*	0.19	0.11	0.51	0.20	**0.25**
	0.75	0.58	0.26	0.63	0.77	0.71	**0.59**
Triticale	0.50	0.13	0.45	*	0.46	0.55	**0.41**
	0.75	0.73	0.80	0.27	0.27	0.66	**0.55**

** = Insufficient digesta 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet)*

Table 3.10: Analysis of variance of mean coefficients of apparent nitrogen digestibility

Factor	s.e.d.	P	cv
Diet	0.062	**<0.001**	
Day	0.070	**0.004, 0.002(L),** 0.064(Q)	
Region	0.044	**<0.001**	
Diet*Day	0.139	**0.032,** 0.218(L), **0.042(Q)**	43.8%
Diet*Region	0.088	0.376	
Day*Region	0.099	0.394, 0.210(L), 0.279(Q)	
Diet*Day*Region	0.197	0.225, 0.161(L), 0.493(Q)	

L = Linear effect Q = Quadratic effect

3.5.5.2. Nitrogen digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 3.11. There was no effect of dietary treatment but overall mean coefficients increased significantly between collection periods (0.541 vs. 0.841). No interaction between diet and collection period was observed.

Table 3.11: Mean coefficients of total tract apparent digestibility for nitrogen and analysis of variance from Trial 1

Diet	**Days 1-5**	**Days 9-13**
Wheat	0.427	0.853
Barley	0.656	0.852
Rye	0.601	0.843
Triticale	0.479	0.815

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.139	0.808	28.5%
Collection Period	0.099	**0.016**	
Diet*Collection Period	0.197	0.845	

(n = 2 pigs per diet for each collection period)

3.5.6. Gut morphology

General patterns of morphology over time are shown in Figure 3.1.

3.5.6.1. Villus height

Mean villus height measurements and analyses of variance are shown in Tables 3.12 and 3.13. There was no significant effect between mean values for control versus experimental animals (P = 0.063) although all other interactions involving control animals were highly significant (P = <0.001). For experimental pigs, there was a significant dietary effect with piglets on soft wheat diets exhibiting the highest mean villus heights, and pigs on the barley diet having the lowest (P = <0.001). A significant effect of day was observed (P = <0.001, <0.001(L), 0.013(Q)) with villus height generally increasing over time, evident for all diets (Figure 3.1a). A significant regional effect was apparent with the longest villi seen in the proximal section of the small intestine, decreasing in length toward the distal region (P = <0.001 – Table 3.12). Significant interactions were also determined between diet and day, between diet and region, between day and region and between diet, day and region (all P = < 0.001).

3.5.6.2. Villus width

Mean villus width measurements are shown in Table 3.14 and analyses of variance in Table 3.15. Experimental pigs exhibited greater overall mean values for villus width than the control animals, irrespective of diet (P = 0.018). All other interactions comparing control pigs proved significant (P <0.05). For experimental piglets, a significant dietary effect was evident with greatest mean villus width values from animals on barley based diets, with pigs on soft wheat having the shortest (P = 0.014). The interaction between diet and day was also highly significant (P = <0.001) and is shown in Figure 3.1b. Significant interactions were also determined between day and region and between diet, day and region (P - <0.001 for both). A significant interaction between diet and region (P = 0.007) showed that the shortest width measurements were found within the 0.5 region for wheat and barley diets, but within the 0.25 region for diets based on rye and triticale.

3.5.6.3. Crypt depth

Mean measurements for crypt depth are shown in Table 3.16, and analyses of variance in Table 3.17. The overall mean values for control versus experimental pigs were found not to be statistically significant, likewise for CVR and diet. However, all other interactions involving control animals proved to be significant ($P < 0.05$). No dietary effect was found for experimental animals although a significant effect of day was observed ($P = <0.001$, 0.008(L), <0.001(Q)). A significant interaction between diet and region was determined; barley, rye and triticale diets showed greatest hypertrophy within the proximal section of the small intestine, decreasing through to the distal region. However, for the wheat diet the greatest hypertrophy was seen within the mid region of the tract ($P = 0.004$). Significant interactions were also found between diet and day (Figure 3.1c), between day and region and between diet, day and region ($P = <0.001$).

3.5.6.4. Villus height to crypt depth ratio

Mean ratios are shown in Table 3.18 and analysis of variance in Table 3.19. Analyses of CVR, CVR and diet and between CVR and region, showed no significance. All other interactions involving control pigs were statistically significant ($P = < 0.001$; Table 3.19). For experimental animals on the trial there was no observed effect of dietary treatment or of tract region alone. There was a significant effect of day with a general increase over time ($P = <0.001$, <0.001(L), <0.001(Q)). Highly significant interactive effects were found between diet and day (Figure 3.1d), between diet and region, between day and region and between diet, day and region ($P = <0.001$).

Table 3.12: Mean villus height measurements (μm) from Trial 1; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	289						**289**
	0.50	310						**310**
	0.75	302						**302**
Soft Wheat	0.25		325	370	270	354	357	**335**
	0.50		312	367	321	389	448	**367**
	0.75		310	169	297	318	413	**301**
Barley	0.25		257	373	306	327	431	**339**
	0.50		261	323	266	386	430	**333**
	0.75		282	231	218	314	321	**273**
Rye	0.25		294	249	375	344	446	**341**
	0.50		298	291	337	384	382	**338**
	0.75		278	259	267	357	361	**305**
Triticale	0.25		277	371	375	418	387	**366**
	0.50		291	311	311	301	345	**312**
	0.75		257	289	331	260	296	**287**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 3.13: Analysis of variance of mean villus height

Factor	s.e.d.	P	cv
CVR	5.7	0.063	16.4%
CVR*Diet	6.0	**<0.001**	
CVR*Day	10.9	**<0.001**	
CVR*Region	9.0	**<0.001**	
CVR*Diet*Day	10.7	**<0.001**	
CVR*Diet*Region	10.0	**<0.001**	
CVR*Day*Region	13.4	**<0.001**	
CVR*Diet*Day*Region	15.9	**<0.001**	
Diet	4.4	**<0.001**	16.4%
Day	9.7	**<0.001, <0.001(L), 0.013(Q)**	
Region	3.8	**<0.001**	
Diet*Day	12.5	**<0.001, <0.001(L), <0.001(Q)**	
Diet*Region	7.6	**<0.001**	
Day*Region	11.5	**<0.001**, 0.169(L), **0.009(Q)**	
Diet*Day*Region	18.5	**<0.001, <0.001(L), <0.001(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 3.14: Mean villus width measurements (μm) from Trial 1; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	82						**82**
	0.50	85						**85**
	0.75	91						**91**
Soft Wheat	0.25		87	83	100	95	86	**91**
	0.50		89	84	92	80	99	**89**
	0.75		93	75	93	106	85	**90**
Barley	0.25		84	87	94	118	91	**95**
	0.50		89	87	96	99	80	**90**
	0.75		86	81	112	104	93	**95**
Rye	0.25		82	93	91	93	90	**90**
	0.50		93	100	91	98	86	**94**
	0.75		90	94	92	99	106	**96**
Triticale	0.25		88	90	77	88	82	**85**
	0.50		92	84	98	86	86	**89**
	0.75		86	79	94	116	109	**97**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 3.15: Analysis of variance of mean villus width

Factor	s.e.d.	P	cv
CVR	1.9	**0.001**	
CVR*Diet	1.9	**0.018**	
CVR*Day	3.6	**<0.001**	
CVR*Region	2.9	**<0.001**	18.9%
CVR*Diet*Day	3.5	**<0.001**	
CVR*Diet*Region	3.3	**0.005**	
CVR*Day*Region	4.4	**<0.001**	
CVR*Diet*Day*Region	5.2	**<0.001**	
Diet	1.5	**0.014**	
Day	3.2	**<0.001, <0.001(L), <0.001(Q)**	
Region	1.2	**<0.001**	
Diet*Day	4.1	**<0.001, 0.778(L), <0.001(Q)**	19.1%
Diet*Region	2.5	**0.007**	
Day*Region	3.8	**<0.001, <0.001(L), 0.412(Q)**	
Diet*Day*Region	6.1	**<0.001, <0.001(L), 0.018(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 3.16: Mean crypt depth measurements (μm) from Trial 1; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	225						**225**
	0.50	221						**221**
	0.75	226						**226**
Soft Wheat	0.25		256	210	233	251	192	**228**
	0.50		220	242	260	242	233	**239**
	0.75		221	191	255	212	166	**209**
Barley	0.25		262	189	259	298	215	**244**
	0.50		248	178	237	247	224	**227**
	0.75		234	169	206	263	149	**204**
Rye	0.25		242	257	220	236	249	**241**
	0.50		212	284	204	253	228	**236**
	0.75		195	223	194	184	192	**198**
Triticale	0.25		242	237	206	271	232	**238**
	0.50		238	202	235	229	212	**223**
	0.75		187	214	228	244	183	**211**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 3.17: Analysis of variance of mean crypt depth

Factor	s.e.d.	P	cv
CVR	4.9	0,439	20.4%
CVR*Diet	5.2	0.928	
CVR*Day	9.5	**<0.001**	
CVR*Region	7.8	**<0.001**	
CVR*Diet*Day	9.3	**<0.001**	
CVR*Diet*Region	8.7	**0.003**	
CVR*Day*Region	11.6	**<0.001**	
CVR*Diet*Day*Region	13.8	**<0.001**	
Diet	3.9	0.989	20.7%
Day	8.5	**<0.001, 0.008(L), <0.001(Q)**	
Region	3.3	**<0.001**	
Diet*Day	11.0	**<0.001**, 0.132(L), **0.002(Q)**	
Diet*Region	6.7	**0.004**	
Day*Region	10.1	**<0.001, 0.022(L), 0.004(Q)**	
Diet*Day*Region	16.3	**<0.001**, 0.173(L), **0.010(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 3.18: Mean villus height to crypt depth ratios from Trial 1; covariate = live weight

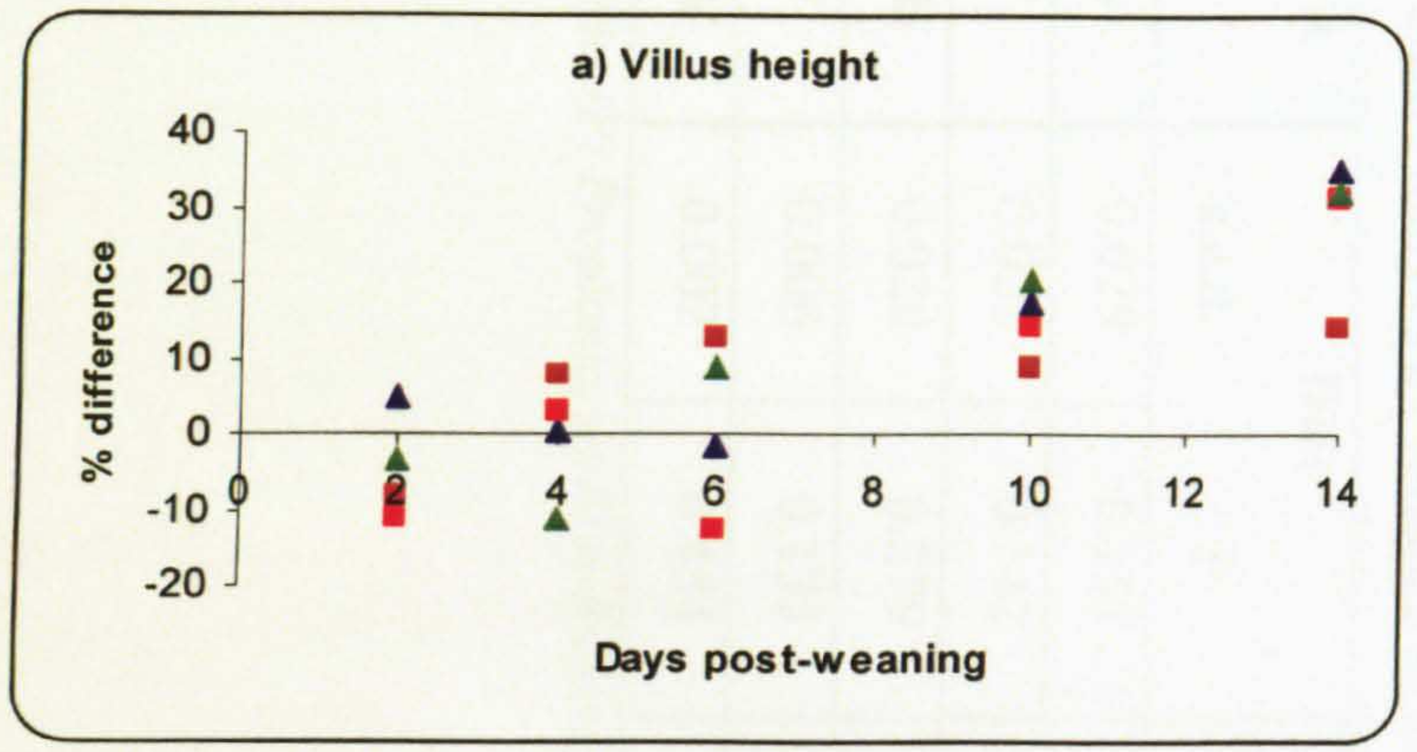
Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	1.3						**1.3**
	0.50	1.4						**1.4**
	0.75	1.4						**1.4**
Soft Wheat	0.25		1.3	1.8	1.2	1.5	1.9	**1.5**
	0.50		1.5	1.6	1.3	1.7	1.9	**1.6**
	0.75		1.4	0.8	1.2	1.5	2.3	**1.5**
Barley	0.25		1.1	2.1	1.2	1.2	1.9	**1.5**
	0.50		1.1	1.9	1.1	1.6	2.0	**1.6**
	0.75		1.3	1.4	1.1	1.3	2.0	**1.4**
Rye	0.25		1.2	1.0	1.8	1.6	1.8	**1.5**
	0.50		1.5	1.1	1.7	1.5	1.8	**1.5**
	0.75		1.5	1.2	1.5	2.0	1.8	**1.6**
Triticale	0.25		1.2	1.6	1.9	1.6	1.7	**1.6**
	0.50		1.3	1.6	1.4	1.4	1.6	**1.4**
	0.75		1.5	1.4	1.6	1.1	1.6	**1.4**

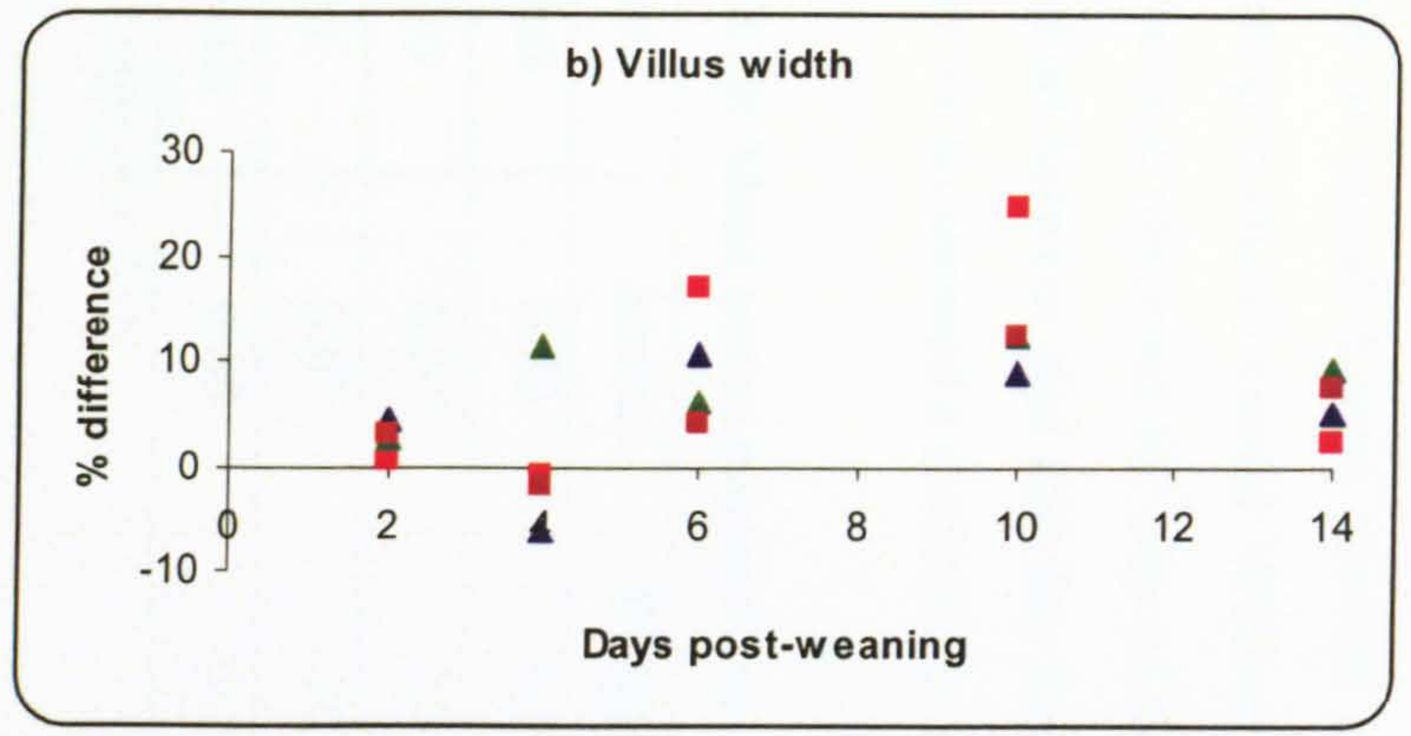
0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

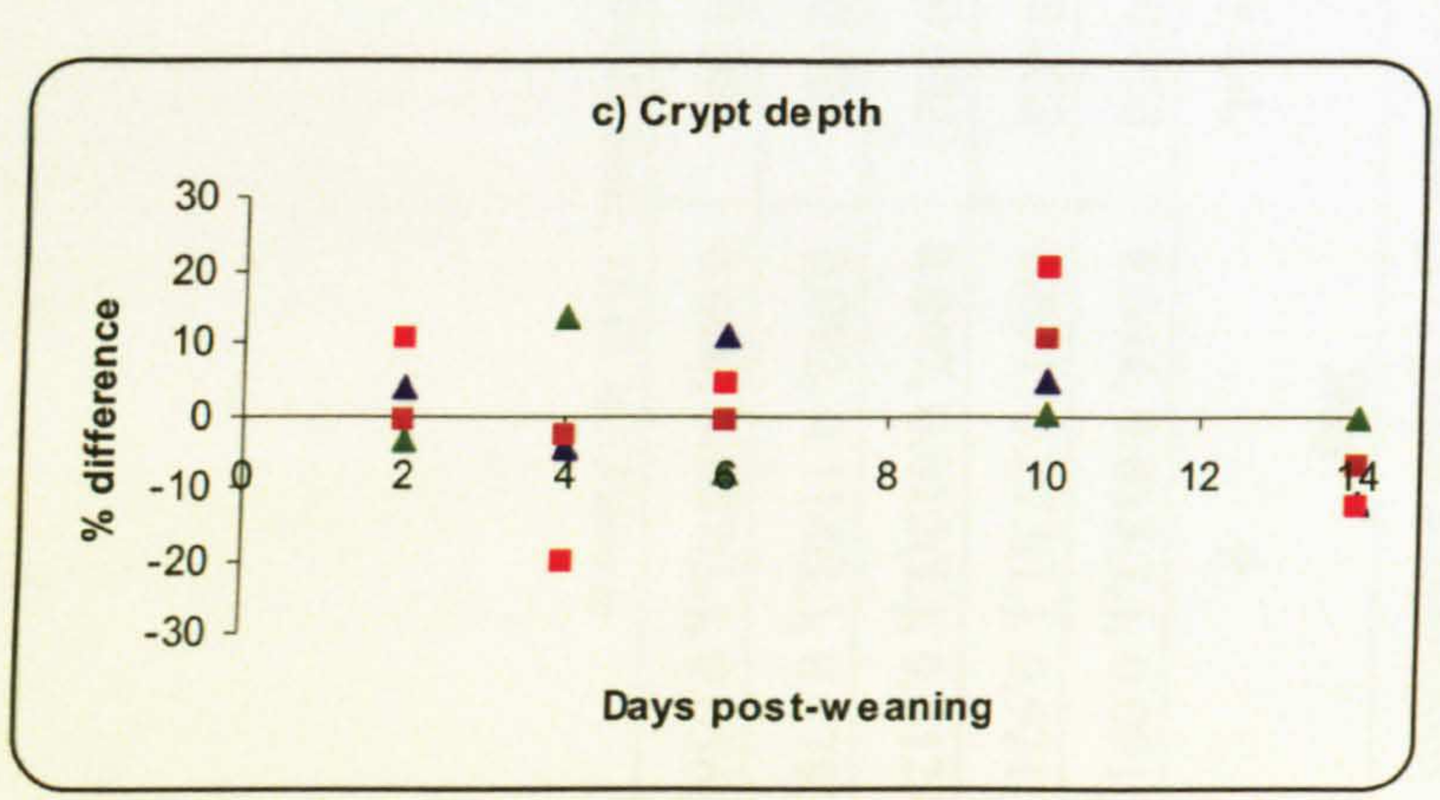
Table 3.19: Analysis of variance of mean villus height to crypt depth ratios

Factor	s.e.d.	P	cv
CVR	0.04	0.124	25.9%
CVR*Diet	0.04	0.770	
CVR*Day	0.08	**<0.001**	
CVR*Region	0.07	0.188	
CVR*Diet*Day	0.08	**<0.001**	
CVR*Diet*Region	0.07	**<0.001**	
CVR*Day*Region	0.10	**<0.001**	
CVR*Diet*Day*Region	0.12	**<0.001**	
Diet	0.03	0.205	26.4%
Day	0.07	**<0.001, <0.001(L), <0.001(Q)**	
Region	0.03	0.144	
Diet*Day	0.09	**<0.001, <0.001(L), <0.001(Q)**	
Diet*Region	0.06	**<0.001**	
Day*Region	0.09	**<0.001**, 0.067(L), **<0.001(Q)**	
Diet*Day*Region	0.14	**<0.001, <0.001(L), 0.027(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect







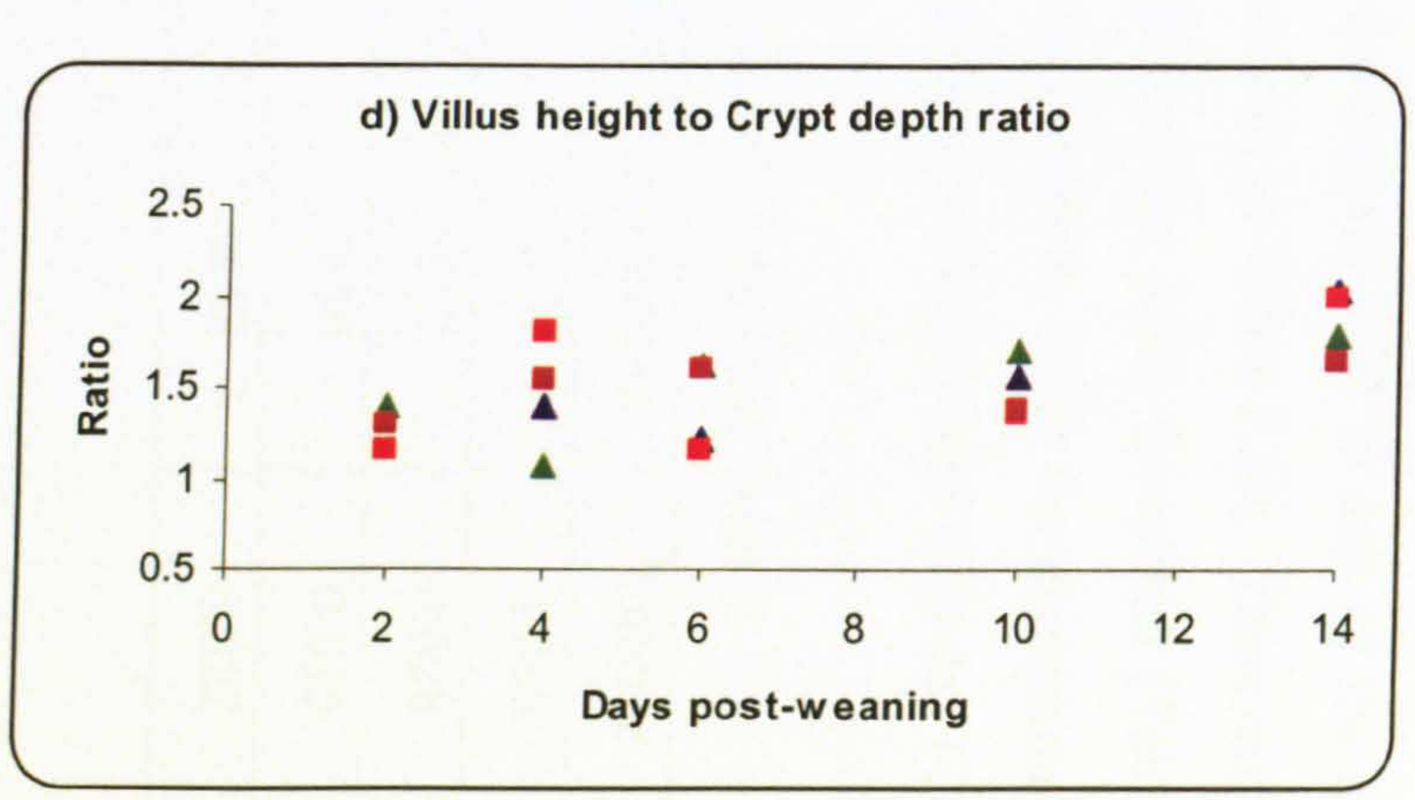


Figure 3.1: *Effect of raw cereals on mean gut morphology measurements (covariate = live weight; n = 10 piglets per diet). (▲) Soft Wheat (■) Barley (▲) Rye (■) Triticale*

3.5.7. Caecal VFA analysis

Analysis of caecal digesta showed no Iso-butyric acid in any of the samples. Mean molar proportions of caecal VFAs are shown in Table 3.20. There was no significant dietary effect observed for any of the individual acids measured (Table 3.21) and no interactions between diet and day were determined. With the exception of valeric acid, all other VFAs showed a statistically significant effect linked with day (Table 3.21).

Table 3.20: Mean molar proportions of caecal VFAs from Trial 1

Diet	Acetic	Propionic	n-Butyric	Valeric	Iso-Valeric
Wheat	0.60	0.29	0.09	0.016	0.005
Barley	0.54	0.31	0.12	0.028	0.006
Rye	0.60	0.28	0.10	0.015	0.005
Triticale	0.60	0.26	0.12	0.022	0.003

(n = 10 pigs per diet)

Table 3.21: Analysis of variance of mean caecal VFA molar proportions

VFA	Diet		Day		cv
	s.e.d.	P	s.e.d.	P	
A	0.029	0.157	0.033	**0.044, 0.013(L)**, 0.091(Q)	11.2%
P	0.021	0.142	0.023	**0.031**, 0.273(L), 0.921(Q)	16.6%
n-B	0.020	0.279	0.022	**0.007, 0.012(L), 0.012(Q)**	41.0%
V	0.006	0.135	0.006	0.092, 0.114(L), 0.478(Q)	61.1%
I-V	0.002	0.454	0.002	**0.016, 0.007(L)**, 0.056(Q)	95.3%

(A) Acetic, (P) Propionic, (n-B) n-Butyric, (V) Valeric, (I-V) Iso-Valeric

3.5.8. Performance Parameters

3.5.8.1. Feed intake

Mean DE intakes for the initial 5 days post-weaning and analysis of variance are shown in Table 3.22. Intakes increased significantly over time (P = <0.001, <0.001(L), 0.001(Q)) and pigs on the triticale diet consumed significantly more feed (P = 0.005). The pigs on the wheat diet exhibited the lowest intakes. No significant interaction was found between diet and day. Post 5 day intakes showed a significant dietary effect with pigs still preferring the triticale diet (Table 3.23). Again intakes for all pigs increased significantly over time (P = <0.001, <0.001(L)) but there was less variation in the data compared with the 0-5 day period (cv 14.5% vs. 62.6%) and no significant interaction between diet and day was determined (Table 3.23).

Table 3.22: Mean daily DE intake 0-5 days (MJ) and analysis of variance from Trial 1

	Days post-weaning						
Diet	**0**	**1**	**2**	**3**	**4**	**5**	**Mean**
Wheat	0.84	2.63	3.20	3.78	4.31	4.80	**3.26**
Barley	0.55	3.23	3.55	5.48	4.52	4.95	**3.71**
Rye	0.81	3.92	4.47	5.64	4.86	5.53	**4.21**
Triticale	0.75	5.08	5.90	6.95	6.80	6.91	**5.40**

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.612	**0.005**	
Day	0.749	**<0.001, <0.001(L), 0.001(Q)**	62.6%
Diet*Day	1.499	0.998, 0.799(L), 0.616(Q)	

L = Linear effect Q = Quadratic effect (n = 6 pigs per diet)

Table 3.23: Mean DE intake post 5 days (MJ) and analysis of variance from Trial 1

	Days post-weaning								
Diet	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**Mean**
Wheat	9.68	9.72	8.97	11.07	15.29	15.36	16.41	15.87	**12.80**
Barley	10.11	11.80	11.27	11.22	11.98	13.20	14.46	15.38	**12.43**
Rye	9.51	11.76	10.61	11.52	12.79	11.22	15.57	15.07	**12.26**
Triticale	11.10	13.94	14.31	16.46	15.84	15.80	16.98	17.52	**15.24**
Analysis of variance									
Factor		**s.e.d.**			**P**			**cv**	
Diet		0.676			**<0.001**				
Day		0.957			**<0.001, <0.001(L)**, 0.836(Q)			14.5%	
Diet*Day		1.913			0.711, 0.290(L), 0.469(Q)				

L = Linear effect Q = Quadratic effect (n = 2 pigs per diet)

3.5.8.2. DLWG

Mean DLWG throughout the trial period increased significantly with day (P = <0.001, <0.001(L)) and all pigs gained weight by day 5 post-weaning following initial weight loss. Triticale was the most beneficial cereal in terms of weight gain (Table 3.24), but not to the point of statistical significance. There was no observed interaction between diet and day.

Table 3.24: Mean DLWG (Kg) and analysis of variance from Trial 1

Diet	**Day 2**	**Day 4**	**Day 6**	**Day 10**	**Day 14**	**Mean**
Wheat	-0.18	0.08	0.21	0.24	0.56	**0.18**
Barley	-0.38	-0.05	0.13	0.34	0.68	**0.14**
Rye	-0.10	0.18	0.29	0.24	0.58	**0.24**
Triticale	-0.05	0.20	0.37	0.22	0.70	**0.29**
Analysis of variance						
Factor		**s.e.d.**		**P**		**cv**
Diet		0.057		0.094		
Day		0.064		**<0.001, <0.001(L)**, 0.431(Q)		59.7%
Diet*Day		0.127		0.497, 0.056(L), 0.862(Q)		

L = Linear effect Q = Quadratic effect (n = 10 pigs per diet)

3.6. SUMMARY OF RESULTS FROM TRIAL 1

Although it is difficult to draw firm conclusions from some of the data in the current trial, the numerous analyses suggest that digestibility, digesta viscosity and gut morphology in weaned piglets can be significantly influenced by cereal type.

CAD for starch in the small intestine was greatest for the diet based on rye (P = 0.051), whereas the soft wheat diet appeared most beneficial for the digestibility of nitrogen (P = <0.001). CTTAD values showed that any dietary differences had disappeared by the end of the large intestine, although digestibility of both starch and nitrogen increased significantly between the two collection periods. Strong regional effects were found for both starch and nitrogen, with coefficients increasing significantly along the small intestine, from the mid to distal regions (P = <0.001). Irrespective of diet, both starch and nitrogen showed a common decrease in digestibility around day 6 post-weaning. The significant interaction between day and region (P = 0.023) in the starch data revealed that this drop in apparent digestibility on day 6 was evident at both the 0.5 and 0.75 intestinal sites.

A typical pattern of villus atrophy and recovery was seen for all pigs in the days after weaning (Figure 3.1a), although the effects of gut morphology in response to diet were somewhat unclear. Villus atrophy occurred between days 2 and 6 post-weaning but a gradual but sustained recovery in the following days of the trial meant that all villi were greater than levels seen pre-weaning by day 10 of the study. Villus width showed a similar pattern with a degree of narrowing evident on day 4, followed by an increase in width up until day 10 of the trial, before reducing again for all pigs by day 14. Although significant dietary effects were determined for both villus height and villus width, there was no clear correlation with cereal type. By feeding a high viscosity carboxymethylcellulose (CMC)/cooked rice diet to weaned piglets, McDonald *et al.* (2001) reported that villus height and villus height to crypt depth ratio was significantly reduced, suggesting that excessive viscosity may be detrimental to the assimilation of nutrients through the gut. Results from the current trial revealed that pigs fed the rye-

based diet showed significantly greater digesta viscosity but did not exhibit detrimental effects to gut morphology. A possible explanation for this could be that an excessive increase in intestinal viscosity is needed before negative effects on gut morphology are seen and that in the current trial using raw cereals, this viscosity threshold was not reached.

Crypt depth data for all diets followed a similar pattern over time; despite some fluctuation on day 4, measurements were reasonably constant until day 6 of the study, when a general increase was observed on day 10. By day 14, average crypt depth had reduced considerably for all diets, indicating that intestinal morphology had largely recovered from the effects of weaning, especially as corresponding villus height data showed strong positive growth for this time period. Observational data recording incidence of scours throughout the 14 day study period revealed no there was no dietary effect.

There was no support in the current trial for the hypothesis that increased viscosity of tract digesta is detrimental to weaned piglet performance: Viscosity followed a consistent pattern along the digestive tract, showing a sustained increase from the stomach through to the distal region of the small intestine, before decreasing again in the caecum. The most viscous diet at all tract regions was rye, followed by triticale, barley and then wheat. Although the pigs fed the rye-based diet exhibited significantly more viscous digesta than the other cereals ($P = <0.001$), this was not detrimental to animal performance, as shown by the feed intake and DLWG data. Analysis of digesta pH and caecal VFA data showed no effect of diet under the conditions of the trial described.

As mentioned in the introduction, Trials 1 and 2 are closely interlinked. The following chapter (Trial 2) evaluates the use of different raw cereals, namely naked oats, whole oats and maize. In line with Trial 1, viscosity is a key focus of interest, and soft wheat was used as a control.

CHAPTER 4:

Trial 2: Examining inter-cereal variability in terms of digestibility, digesta properties and gut morphology in the post-weaned piglet: (Part 2 - Soft wheat, Naked oats, Whole oats and Maize)

4.1. INTRODUCTION

The trial discussed in the current chapter is the second of two related studies and follows on from the previous chapter. These studies examine the variability between raw cereals, in terms of their influence on digesta viscosity, and subsequent physiological and nutritional responses, when added to weaned piglet diets.

4.2. OBJECTIVE

- As the trials were closely related, the objective of Trial 2 was identical to that of Trial 1; namely, the examination of variability between raw cereals when added to diets in terms of digestibility, digesta properties and gut morphology. The current trial evaluated soft wheat (same as that used in Trial 1), naked oats, whole oats and maize.

4.3. HYPOTHESES

- Increased viscosity of tract digesta (influenced by dietary cereal) will be detrimental to weaned piglet performance.
- Starch digestibility will be reduced within the small intestine as a result of increased tract digesta viscosity.

4.4. METHOD

4.4.1. Animals and housing

Piglets (n = 44) were housed and fed as described in Chapter 2.

4.4.2. Diets

Four experimental diets were manufactured on site at Nottingham as before. Raw cereals (soft wheat, naked oats, whole oats and maize) were ground through a hammer mill (1.5 – 2 mm screen size) and incorporated into diets as before (see Appendix 1).

4.4.3. Experimental and slaughter procedure

Piglets were slaughtered with tissue and digesta collected as in chapter three. Again, a lack of digesta from the 0.25 sampling site of the small intestine from several pigs limited some analyses performed. The slaughter programme for Trial 2 is shown in Table 4.1.

Table 4.1: Slaughter programme for Trial 2

Group	Day	Piglets slaughtered
1	0	n = 4
2	2	n = 8 (2 per diet)
3	4	n = 8 (2 per diet)
4	6	n = 8 (2 per diet)
5	10	n = 8 (2 per diet)
6	14	n = 8 (2 per diet)

4.4.4. Faecal collection

Faecal samples were collected from eight piglets and analysed as described in Chapter 2.

4.5. RESULTS

4.5.1. Gastric ulceration

No evidence of gastric ulceration was found in any of the piglets.

4.5.2. pH

Mean digesta pH values and analysis of variance are shown in Tables 4.2 and 4.3. Significant interactions between CVR and day, between CVR and region and between CVR, day and region were observed (all P = <0.001). Digesta pH was found to be significant between diets (P = 0.024), with wheat showing the highest (most alkaline) overall pH value, and maize the lowest. This dietary difference disappeared when tract region was incorporated into the statistical analysis (P = 0.391). The effects of day and of tract region were both found to be highly significant (P = <0.001). The interaction between the day and region showed that pH reduced slightly in all tract regions (except the stomach) on day 6 post-weaning, before increasing in alkalinity again as the trial progressed (P = <0.001, <0.001(L), <0.001(Q)). This interaction between day and region was not affected by diet (P = 0.361).

4.5.3. Viscosity

Mean digesta viscosity figures and analysis of variance are shown in Tables 4.4 and 4.5. Piglets fed the cereal diets exhibited significantly more viscous digesta than the control pigs on sows' milk (P = 0.050). A significant interaction between CVR and region showed experimental animals had more viscous digesta than control pigs throughout all regions of the tract studied (P = <0.001). The interaction between CVR, diet and region was also significant (P = 0.025) and showed that pigs on the naked oats diet exhibited the most viscous digesta throughout all tract regions, whereas pigs on the maize diet had tract viscosities not greatly different from those of the control animals. For experimental pigs, animals on the naked oats diet had the most viscous digesta, with the pigs on the maize diet having the least (P = 0.017). This dietary difference was seen in all tract regions (P = 0.043) and the interaction with day showed these dietary differences in viscosity were evident from day 6 post-weaning (P = <0.001, <0.001(L)). There was no observed effect of day alone but the effect of tract region was highly significant, with viscosity increasing progressively along the digestive tract (P = <0.001).

Table 4.2: Mean digesta pH from Trial 2

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	Stomach	3.5						**3.5**
	0.25	6.1						**6.1**
	0.50	6.6						**6.6**
	0.75	7.3						**7.3**
	Caecal	6.7						**6.7**
Soft Wheat	Stomach		1.7	4.3	4.2	4.2	4.1	**3.7**
	0.25		7.0	6.4	6.3	6.4	6.2	**6.5**
	0.50		7.7	7.0	6.9	7.4	7.4	**7.3**
	0.75		8.1	7.9	7.2	7.5	7.8	**7.7**
	Caecal		6.6	6.9	6.4	6.3	6.2	**6.5**
Naked Oats	Stomach		1.7	4.0	3.1	2.9	4.3	**3.2**
	0.25		6.4	6.4	6.0	6.3	6.4	**6.3**
	0.50		7.3	7.2	6.2	6.9	7.2	**7.0**
	0.75		8.5	7.4	8.0	7.3	7.6	**7.8**
	Caecal		7.2	6.4	6.1	6.4	6.0	**6.4**
Whole Oats	Stomach		2.2	4.1	4.3	3.7	3.8	**3.6**
	0.25		6.6	6.4	5.9	6.3	6.4	**6.3**
	0.50		6.9	7.3	6.3	7.0	7.3	**7.0**
	0.75		7.9	7.9	6.7	8.0	8.0	**7.7**
	Caecal		7.5	6.8	6.4	6.7	6.2	**6.7**
Maize	Stomach		2.3	4.6	3.8	2.6	4.0	**3.5**
	0.25		6.4	6.4	6.0	6.4	6.2	**6.3**
	0.50		7.2	7.3	6.4	7.0	7.0	**7.0**
	0.75		7.5	7.9	6.7	7.3	7.5	**7.4**
	Caecal		7.0	6.2	5.6	6.3	6.1	**6.2**

0.25, 0.5 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 4.3: Analysis of variance of mean digesta pH from Trial 2

Factor	s.e.d.	P	cv
CVR	0.12	0.244	8.1%
CVR*Day	0.14	**<0.001**	
CVR*Diet	0.13	0.051	
CVR*Region	0.26	**<0.001**	
CVR*Day*Diet	0.19	0.911	
CVR*Day*Region	0.30	**<0.001**	
CVR*Diet*Region	0.29	0.578	
CVR*Day*Diet*Region	0.43	0.700	
Diet	0.09	**0.024**	7.3%
Day	0.10	**<0.001**, 0.848(L), **0.014(Q)**	
Diet*Day	0.20	0.825, 0.887(L), 0.357(Q)	
Region	0.10	**<0.001**	
Diet*Region	0.20	0.391	
Day*Region	0.22	**<0.001, <0.001(L), <0.001(Q)**	
Diet*Day*Region	0.45	0.386, 0.429(L), 0.524(Q)	

CVR = control animals versus the rest L = Linear effect Q = Quadratic effect

Table 4.4: Mean digesta viscosity (cP) from Trial 2

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	Stomach	0.9						**0.9**
	0.25	1.2						**1.2**
	0.50	1.0						**1.0**
	0.75	1.3						**1.3**
	Caecal	1.8						**1.8**
Soft Wheat	Stomach		0.7	1.0	1.1	1.2	1.1	**1.0**
	0.25		0.8	0.7	1.3	1.3	1.2	**1.1**
	0.50		1.8	1.2	2.0	1.7	1.7	**1.7**
	0.75		1.3	1.5	1.8	1.6	2.5	**1.7**
	Caecal		1.4	1.6	1.4	1.7	1.8	**1.6**
Naked Oats	Stomach		0.9	0.8	0.9	1.2	1.6	**1.1**
	0.25		2.1	1.3	1.3	1.2	1.5	**1.5**
	0.50		2.3	1.1	1.8	1.7	2.0	**1.8**
	0.75		1.9	1.6	1.5	2.9	2.6	**2.1**
	Caecal		2.6	1.2	1.6	2.8	2.5	**2.1**
Whole Oats	Stomach		1.5	1.1	1.0	0.8	1.2	**1.1**
	0.25		1.4	1.3	0.9	1.5	1.2	**1.3**
	0.50		1.7	2.8	1.0	1.5	1.3	**1.7**
	0.75		1.5	3.4	1.4	1.9	2.0	**2.0**
	Caecal		2.5	2.4	1.3	0.9	1.1	**1.6**
Maize	Stomach		1.2	1.2	0.7	1.1	0.9	**1.0**
	0.25		0.9	1.2	1.0	1.1	0.8	**1.0**
	0.50		1.4	1.4	1.0	1.3	1.4	**1.3**
	0.75		1.2	1.2	1.1	1.1	1.2	**1.2**
	Caecal		4.3	1.8	2.1	1.8	1.4	**2.3**

0.25, 0.5 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 4.5: Analysis of variance of mean digesta viscosity from Trial 2

Factor	s.e.d.	P	cv
CVR	0.13	**0.050**	38.3%
CVR*Day	0.16	0.091	
CVR*Diet	0.15	**0.011**	
CVR*Region	0.30	**<0.001**	
CVR*Day*Diet	0.22	**<0.001**	
CVR*Day*Region	0.35	0.191	
CVR*Diet*Region	0.34	**0.025**	
CVR*Day*Diet*Region	0.49	0.441	
Diet	0.12	**0.017**	39.3%
Day	0.13	0.117, 0.763(L), **0.036(Q)**	
Diet*Day	0.27	**<0.001, <0.001(L),** 0.380(Q)	
Region	0.13	**<0.001**	
Diet*Region	0.27	**0.043**	
Day*Region	0.30	0.261, 0.063(L), 0.505(Q)	
Diet*Day*Region	0.59	0.565, 0.325(L), 0.956(Q)	

CVR = control animals versus the rest L = Linear effect Q = Quadratic effect

4.5.4. Starch digestibility

4.5.4.1. Starch digestibility in the small intestine

Mean CAD values and analysis of variance are shown in Tables 4.6 and 4.7. There was no observed effect of diet (P = 0.098); although not significant, there was a strong trend for coefficients to increase from mid to distal regions of the small intestine (P = 0.063). The only statistically significant effect was day, with starch digestion increasing between days 2 and 10 post-weaning before beginning to decline slightly towards the end of the trial (P = 0.025, 0.018(L), 0.012(Q)).

Table 4.6: Mean coefficients of apparent starch digestibility from Trial 2

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Soft	0.50	*	*	0.42	0.77	0.66	**0.62**
Wheat	0.75	*	0.41	0.92	0.84	0.79	**0.74**
Naked	0.50	*	0.62	0.73	0.80	0.37	**0.63**
Oats	0.75	*	0.77	0.96	0.82	0.40	**0.74**
Whole	0.50	*	0.31	0.87	0.70	0.90	**0.70**
Oats	0.75	*	0.72	0.95	0.74	0.95	**0.84**
Maize	0.50	*	0.33	0.68	*	0.82	**0.61**
	0.75	0.29	0.44	0.68	0.71	*	**0.53**

** = Insufficient digesta 0.50 & 0.75 = proportion along small intestine (n = 10 per diet)*

Table 4.7: Analysis of variance of mean coefficients of apparent starch digestibility

Factor	s.e.d.	P	cv
Diet	0.069	0.098	
Day	0.077	**0.025, 0.018(L), 0.012(Q)**	
Region	0.049	0.063	
Diet*Day	0.153	0.147, 0.096(L)< 0.264(Q)	34.1%
Diet*Region	0.097	0.202	
Day*Region	0.109	0.125, 0.090(L), 0.689(Q)	
Diet*Day*Region	0.217	0.954(L), 0.989(Q)	

L = Linear effect Q = Quadratic effect

4.5.4.2. Starch digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 4.8. There was a significant dietary effect with pigs on the maize diet exhibiting the lowest coefficients. Although there was no effect of collection period alone, a significant interaction with diet showed pigs on maize had the lowest coefficients for both collection periods.

Table 4.8: Mean coefficients of total tract apparent digestibility for starch and analysis of variance from Trial 2

Diet	**Days 1-5**	**Days 9-13**
Wheat	0.994	0.989
Naked Oats	0.990	0.996
Whole Oats	0.993	0.992
Maize	0.978	0.960

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.003	**<0.001**	0.5%
Collection Period	0.002	0.092	
Diet*Collection period	0.005	**0.035**	

(n = 2 pigs per diet for each collection period)

4.5.5. Nitrogen digestibility

4.5.5.1. Nitrogen digestibility in the small intestine

Mean CAD values and analysis of variance are displayed in Tables 4.9 and 4.10. A highly significant effect of diet showed that the pigs on wheat had the highest digestibility coefficients with the animals on a diet based on whole oats yielding the lowest (P = 0.043). There was a linear effect of day (P = 0.032) but no effect of tract region was found (P = 0.565). No significant interactions were observed between any of the parameters measured (Table 4.10).

Table 4.9: Mean coefficients of apparent nitrogen digestibility from Trial 2 .

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Soft	0.50	*	*	0.78	0.72	*	**0.75**
Wheat	0.75	*	0.51	0.79	0.99	0.75	**0.76**
Naked	0.50	*	*	0.56	0.69	0.44	**0.56**
Oats	0.75	*	0.34	0.54	0.78	*	**0.55**
Whole	0.50	*	*	0.39	0.13	0.74	**0.42**
Oats	0.75	*	*	0.50	0.66	0.34	**0.50**
Maize	0.50	*	*	0.51	0.45	0.69	**0.55**
	0.75	0.19	0.16	0.42	0.61	*	**0.35**

** = Insufficient digesta 0.50 & 0.75 = proportion along small intestine (n = 10 per treatment)*

Table 4.10: Analysis of variance of mean coefficients of apparent nitrogen digestibility

Factor	s.e.d.	P	cv
Diet	0.049	**0.043**	
Day	0.055	0.073, **0.032(L)**, 0.070(Q)	
Region	0.035	0.565	
Diet*Day	0.110	0.249, 0.359(L), 0.259Q)	32.2%
Diet*Region	0.069	0.323	
Day*Region	0.077	0.061, 0.198(L), 0.057(Q)	
Diet*Day*Region	0.155	0.899(L), 0.569(Q)	

L = Linear effect Q = Quadratic effect

4.5.5.2. Nitrogen digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 4.11. There was no observed effect of dietary treatment although overall mean coefficients increased significantly between collection periods (0.630 vs. 0.755). No interaction between diet and collection period was found.

Table 4.11: Mean coefficients of total tract apparent digestibility for nitrogen and analysis of variance from Trial 2

Diet	**Days 1-5**	**Days 9-13**
Wheat	0.68	0.80
Naked Oats	0.47	0.78
Whole Oats	0.69	0.73
Maize	0.68	0.71

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.051	0.204	10.4%
Collection Period	0.036	**0.008**	
Diet*Collection Period	0.072	0.087	

(n = 2 pigs per diet for each collection period

4.5.6. Gut morphology

General patterns of gut morphology over time are shown in Figure 4.1.

4.5.6.1. Villus height

Mean villus height measurements are shown in Table 4.12 and analysis of variance is displayed in Table 4.13. Overall mean villus height was significantly longer for control animals than for experimental pigs, irrespective of diet (P = <0.001). Only pigs on the whole oat diet exhibited villi that recovered to pre-weaning heights during the trial period (Figure 4.1a), although it is difficult to determine any clear patterns in gut morphology from this interaction. All parameters involving control pigs were statistically significant (Table 4.13). For experimental animals, a significant dietary effect was detected; pigs on the wheat diet had the highest villi with those animals on the whole oats having the lowest (P = <0.001). Villus height increased with time and a significant regional effect showed that villus height was longest in the proximal region of the small intestine, decreasing in length through to the distal section (P = <0.001; Table 4.12). Significant interactions were also seen between diet and region and between day and region. The latter showed villus height was generally greatest in the 0.25 section of the tract throughout the study period, although some atrophy in the proximal region was apparent between days 5 and 7 post-weaning. All other measured parameters involving experimental animals achieved significance (Table 4.13).

4.5.6.2. Villus width

Mean measurements of villus width and analysis of variance are shown in Tables 4.14 and 4.15 respectively. For all diets, experimental animals had significantly wider villi than the control pigs (P = 0.012). With the exception of the interactions between CVR and diet and between CVR, diet and region, all other analyses involving control pigs were significant (Table 4.15). Villus width was not affected by dietary treatment (P = 0.364) but did increase over time (P = <0.001, 0.004(L), 0.008(Q)). There was also a highly significant effect of tract region with the widest villi of the experimental animals seen in the 0.75 region of the small intestine (Table 4.14). This was evident throughout the 14 day trial period (P = 0.003, <0.001(Q)). A significant interaction was also found

between diet and day (Figure 4.1b) although it is difficult to determine any clear patterns from the data.

4.5.6.3. Crypt depth

Mean measurements for crypt depth are shown in Table 4.16 with analysis of variance in Table 4.17. All crypt depth measurements involving control pigs were significant (Table 4.17). Experimental animals exhibited greater overall mean crypt depths than control pigs (P = <0.001) in all tract regions studied (P = <0.001). A significant effect of diet was determined with animals on the maize diet showing the greatest crypt depth measurements with pigs on whole oats having the lowest (P = <0.001). A highly significant effect of day showed crypt depth increased up to day 6 post-weaning before reaching a plateau (P = <0.001, <0.001(L), <0.001(Q)). However, when individual diets were plotted over time, pigs on the two oat-based diets exhibited a different crypt depth pattern compared with animals on the wheat and maize diets (Figure 4.1c). The effect of tract region was highly significant with greatest crypt depths found in the proximal section of the small intestine, decreasing towards the distal region (P = <0.001). The interaction between diet and region showed that pigs on the maize diet had the greatest crypt depth measurements in all three tract regions studied (P = 0.027). Significant effects were also found for the interactions between day and region and between diet, day and region (Table 4.17).

4.5.6.4. Vilus height to crypt depth ratio

Mean ratio measurements and analysis of variance are shown in Tables 4.18 and 4.19. Ratios were higher for control pigs, compared with experimental animals (P = <0.001) and most parameters involving control pigs were highly significant (Table 4.19). A significant effect of diet (P =<0.001) showed ratios were highest for the pigs on wheat and lowest for those on maize. Ratio measurements for all pigs decreased up to four days post-weaning before fluctuating (depending on diet) for the rest of the trial (Figure 4.1d). A significant regional effect showed ratios decreased along the intestinal tract from proximal to distal section. There was no interaction between diet and region,

although day and region, and the interaction between diet, day and region were significant.

Table 4.12: Mean villus height measurements (μm) from Trial 2; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	434						**434**
	0.50	320						**320**
	0.75	277						**277**
Soft Wheat	0.25		321	329	313	335	366	**333**
	0.50		302	303	332	273	315	**305**
	0.75		263	274	317	263	267	**277**
Naked Oats	0.25		281	285	318	374	365	**325**
	0.50		270	265	331	340	333	**308**
	0.75		228	244	243	270	240	**245**
Whole Oats	0.25		287	283	237	395	411	**323**
	0.50		272	257	242	355	347	**295**
	0.75		222	235	211	318	274	**252**
Maize	0.25		319	249	345	285	331	**306**
	0.50		301	212	368	316	309	**301**
	0.75		260	201	332	304	224	**264**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 4.13: Analysis of variance of mean villus height

Factor	s.e.d.	P	cv
CVR	5.0	**<0.001**	17.2%
CVR*Diet	5.6	**<0.001**	
CVR*Day	6.6	**<0.001**	
CVR*Region	8.6	**<0.001**	
CVR*Diet*Day	8.6	**<0.001**	
CVR*Diet*Region	9.7	**<0.001**	
CVR*Day*Region	10.4	**<0.001**	
CVR*Diet*Day*Region	14.3	**0.013**	
Diet	4.1	**<0.001**	17.0%
Day	5.4	**<0.001, <0.001(L),** 0.607(Q)	
Region	3.5	**<0.001**	
Diet*Day	9.7	**<0.001, <0.001(L), <0.001(Q)**	
Diet*Region	7.1	**<0.001**	
Day*Region	8.3	**<0.001, <0.001(L), <0.001(Q)**	
Diet*Day*Region	16.1	**0.006,** 0.089(L), **0.024(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 4.14: Mean villus width measurements (μm) from Trial 2; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None	0.25	73						**73**
(Control)	0.50	65						**65**
	0.75	68						**68**
Soft	0.25		68	69	71	74	69	**70**
	0.50		71	67	70	62	72	**69**
Wheat	0.75		81	82	67	75	86	**78**
Naked	0.25		72	76	61	72	75	**71**
	0.50		67	82	66	67	67	**70**
Oats	0.75		69	86	67	79	82	**77**
Whole	0.25		57	67	74	62	73	**67**
	0.50		67	67	64	71	80	**70**
Oats	0.75		83	71	71	75	86	**77**
	0.25		65	63	77	71	68	**69**
Maize	0.50		68	62	75	70	76	**70**
	0.75		73	68	77	71	82	**74**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 4.15: Analysis of variance of mean villus width

Factor	s.e.d.	P	cv
CVR	1.4	**0.012**	20.5%
CVR*Diet	1.6	0.434	
CVR*Day	1.9	**<0.001**	
CVR*Region	2.4	**<0.001**	
CVR*Diet*Day	2.4	**<0.001**	
CVR*Diet*Region	2.8	0.261	
CVR*Day*Region	3.0	**0.006**	
CVR*Diet*Day*Region	4.1	**0.002**	
Diet	1.2	0.364	19.7%
Day	1.5	**<0.001, 0.004(L), 0.008(Q)**	
Region	1.0	**<0.001**	
Diet*Day	2.7	**<0.001, 0.022(L),** 0.062(Q)	
Diet*Region	2.0	0.214	
Day*Region	2.4	**0.003,** 0.432(L), **<0.001(Q)**	
Diet*Day*Region	4.6	**<0.001,** 0.199(L), **0.011(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 4.16: Mean crypt depth measurements (μm) from Trial 2; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None	0.25	155						**155**
(Control)	0.50	135						**135**
	0.75	138						**138**
Soft	0.25		122	191	217	192	165	**177**
	0.50		133	167	184	208	167	**172**
Wheat	0.75		139	175	165	206	182	**173**
Naked	0.25		154	185	183	186	207	**183**
	0.50		128	198	183	183	195	**178**
Oats	0.75		109	170	171	165	191	**161**
Whole	0.25		121	190	226	158	210	**180**
	0.50		121	166	158	185	194	**165**
Oats	0.75		117	136	183	207	188	**166**
	0.25		148	170	222	249	183	**194**
Maize	0.50		129	152	224	232	185	**184**
	0.75		118	137	201	226	188	**174**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 4.17: Analysis of variance of mean crypt depth

Factor	s.e.d.	P	cv
CVR	3.4	**<0.001**	
CVR*Diet	3.8	**<0.001**	
CVR*Day	4.5	**<0.001**	
CVR*Region	5.8	**<0.001**	20.2%
CVR*Diet*Day	5.8	**<0.001**	
CVR*Diet*Region	6.6	**0.032**	
CVR*Day*Region	7.1	**<0.001**	
CVR*Diet*Day*Region	9.7	**<0.001**	
Diet	2.8	**<0.001**	
Day	3.7	**<0.001, <0.001(L), <0.001(Q)**	
Region	2.4	**<0.001**	
Diet*Day	6.7	**<0.001, <0.001(L), <0.001(Q)**	19.5%
Diet*Region	4.9	**0.027**	
Day*Region	5.7	**<0.001, <0.001(L),** 0.657(Q)	
Diet*Day*Region	11.1	**<0.001,** 0.960(L), **0.002(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 4.18: Mean villus height to crypt depth ratios from Trial 2; covariate = live weight

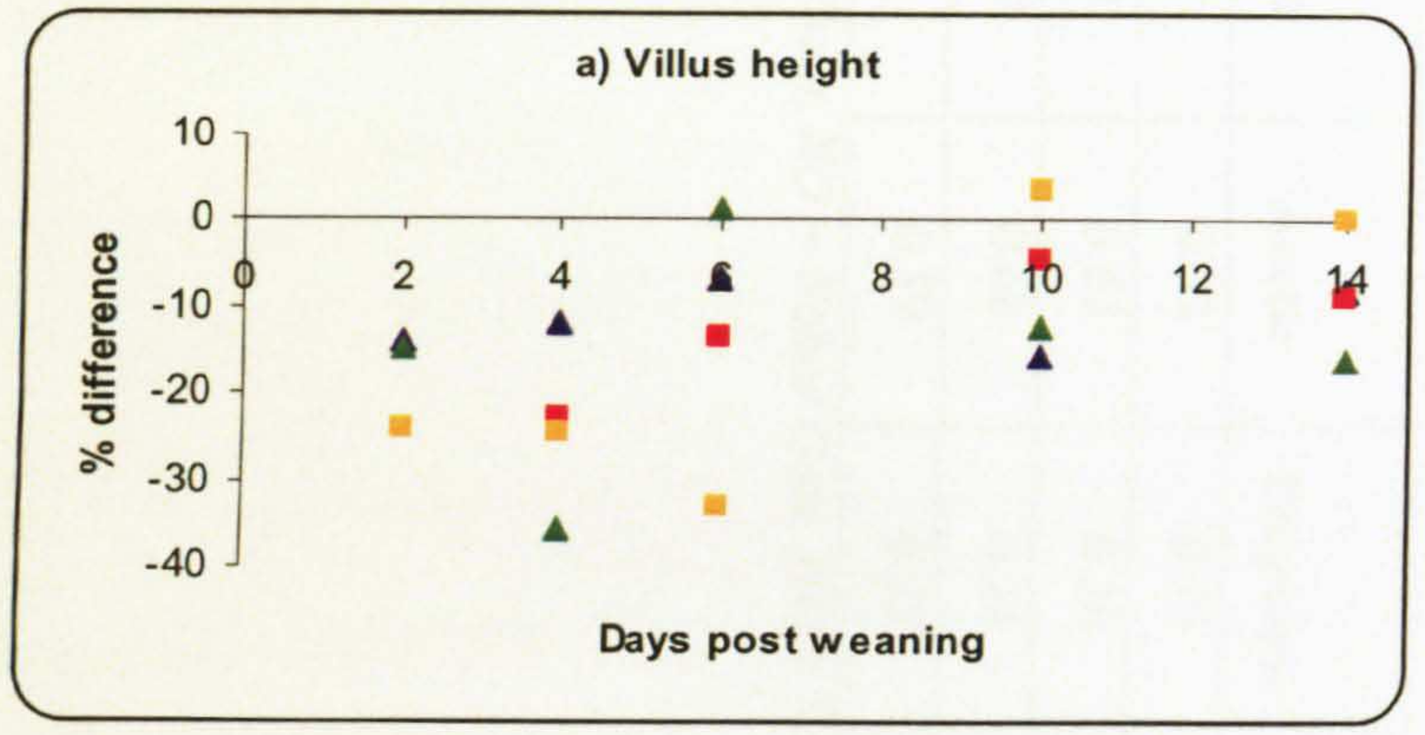
Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	3.0						**3.0**
	0.50	2.6						**2.6**
	0.75	2.3						**2.3**
Soft Wheat	0.25		2.7	1.9	1.5	1.8	2.3	**2.0**
	0.50		2.3	1.9	1.9	1.5	2.0	**1.9**
	0.75		2.1	1.7	2.0	1.4	1.5	**1.7**
Naked Oats	0.25		1.8	1.6	1.7	2.1	1.8	**1.8**
	0.50		2.1	1.4	1.9	2.0	1.7	**1.8**
	0.75		2.1	1.5	1.5	1.7	1.2	**1.6**
Whole Oats	0.25		2.5	1.5	1.0	2.6	1.9	**1.9**
	0.50		2.2	1.6	1.6	2.0	1.8	**1.8**
	0.75		1.9	1.7	1.2	1.7	1.5	**1.6**
Maize	0.25		2.2	1.5	1.6	1.1	1.9	**1.7**
	0.50		2.3	1.4	1.7	1.4	1.7	**1.7**
	0.75		2.2	1.5	1.7	1.3	1.2	**1.6**

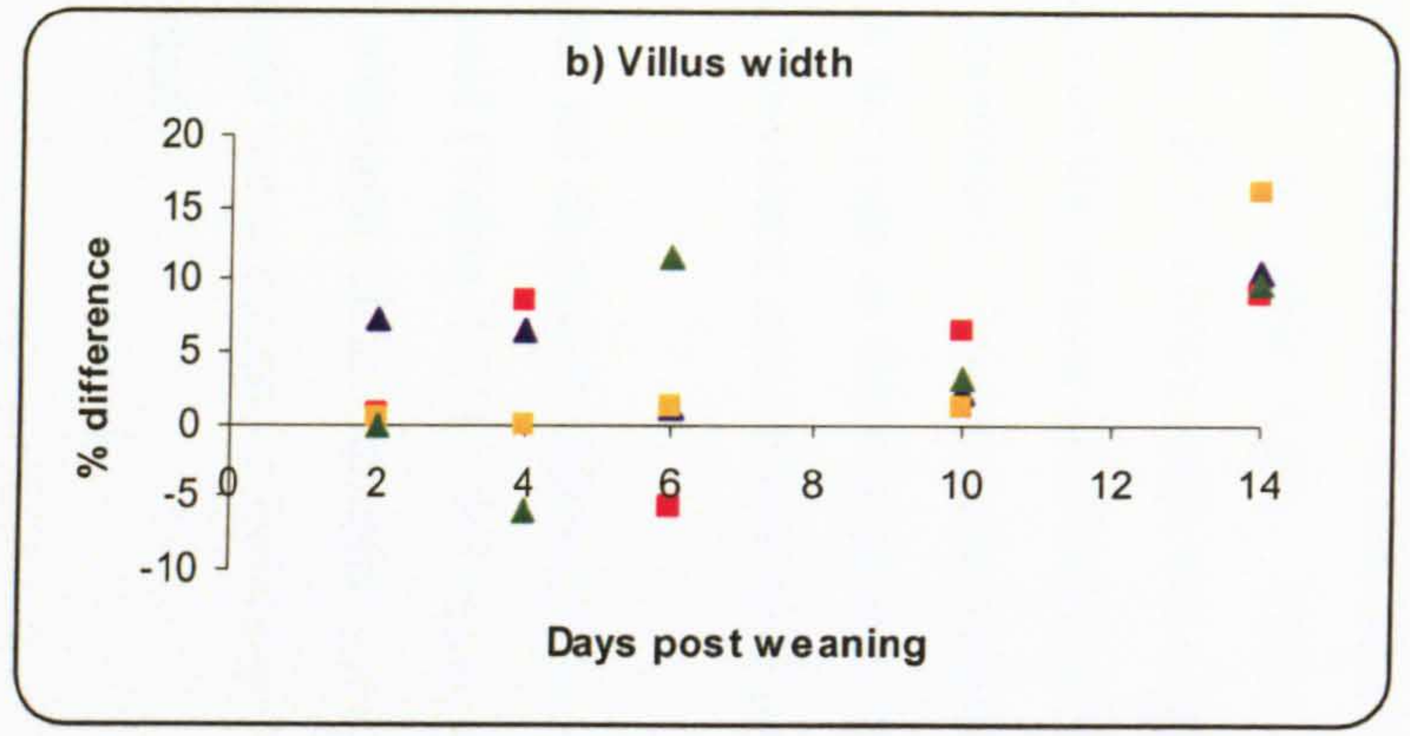
0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

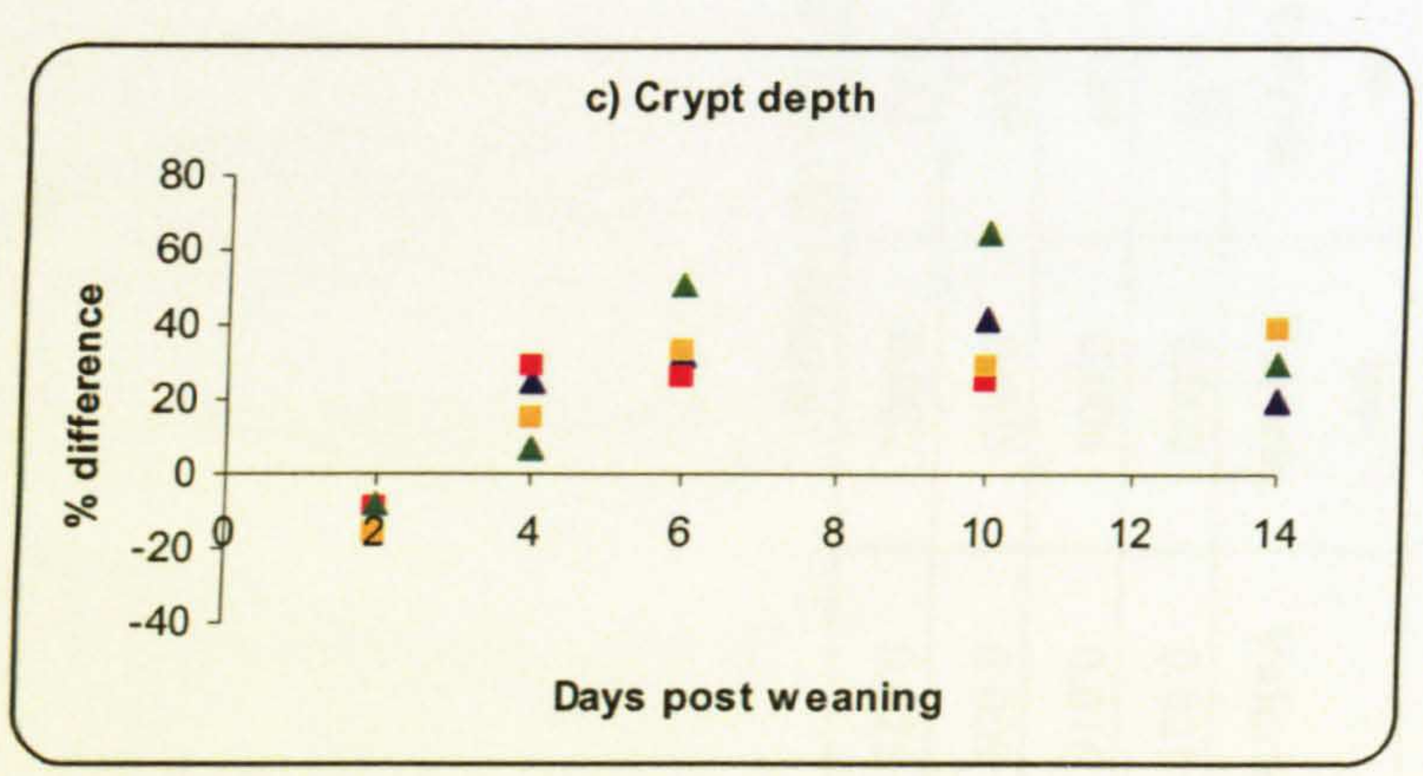
Table 4.19: Analysis of variance of mean villus height to crypt depth ratio

Factor	s.e.d.	P	cv
CVR	0.05	**<0.001**	29.2%
CVR*Diet	0.06	**<0.001**	
CVR*Day	0.07	**<0.001**	
CVR*Region	0.09	**<0.001**	
CVR*Diet*Day	0.09	**<0.001**	
CVR*Diet*Region	0.10	0.253	
CVR*Day*Region	0.11	**<0.001**	
CVR*Diet*Day*Region	0.15	**<0.001**	
Diet	0.04	**<0.001**	25.7%
Day	0.05	**<0.001, 0.152(L), <0.001(Q)**	
Region	0.03	**<0.001**	
Diet*Day	0.09	**<0.001, 0.034(L), <0.001(Q)**	
Diet*Region	0.06	0.085	
Day*Region	0.08	**<0.001, <0.001(L), <0.001(Q)**	
Diet*Day*Region	0.15	**<0.001, 0.836(L), <0.001(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect







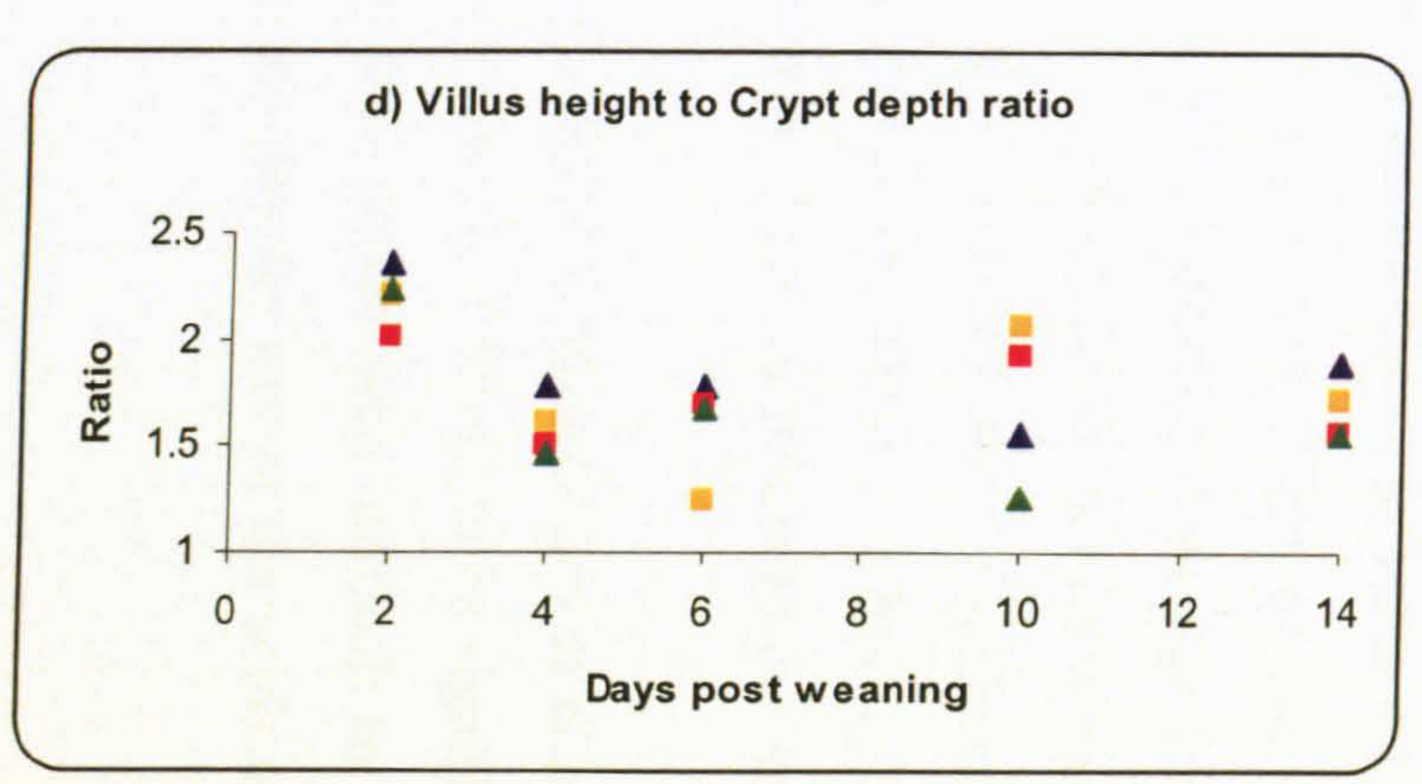


Figure 4.1: *Effect of raw cereals on mean gut morphology measurements (covariate = live weight; n = 10 piglets per diet). (▲) Soft Wheat (■) Naked Oats (■) Whole Oats (▲) Maize*

4.5.7. Caecal VFA analysis

Mean molar proportions of the measured VFAs are shown in Table 4.20, with analysis of variance in Table 4.21. There was a significant effect of diet on acetic acid production (P = 0.006), with the highest molar proportion from pigs fed the whole oat diet (pigs on the maize diet had the lowest). Propionic acid levels were not affected by dietary treatment. Molar proportions of n-butyric acid were significantly higher (P = 0.005) in the pigs on the maize diet, with animals fed whole oats having the lowest. Iso-butyric, Iso-valeric and valeric acid proportions were not affected by dietary cereal.

There was no observed affect of day on measured concentrations of either propionic or valeric acid (Table 4.21). All remaining VFAs were found to be significantly affected by day although clear patterns over the trial period were difficult to ascertain. No interactions were observed between diet and day for any of the acids measured in the current study

Table 4.20: Mean molar proportions of caecal VFAs from Trial 2

Diet	Acetic	Propionic	n-Butyric	Iso-Butyric	Valeric	Iso-Valeric
W	0.62	0.25	0.10	0.002	0.021	0.008
NO	0.63	0.26	0.09	0.004	0.016	0.008
WO	0.68	0.24	0.06	0.005	0.009	0.008
M	0.59	0.25	0.12	0.002	0.025	0.007

(W = Wheat, NO = Naked Oats, WO = Whole Oats, M =Maize)

Table 4.21: Analysis of variance of mean caecal VFA molar proportions

VFA	Diet		Day		cv
	s.e.d.	P	s.e.d.	P	
A	0.022	**0.006**	0.025	**0.034,** 0.095(L), 0.089(Q)	8.0%
P	0.016	0.651	0.017	0.401, 0.245(L), 0.608(Q)	14.0%
n-B	0.015	**0.005**	0.017	**0.005, 0.009(L), 0.019(Q)**	37.2%
I-B	0.002	0.486	0.002	0.116, 0.174(L), **0.020(Q)**	136.4%
V	0.006	0.070	0.007	0.189, 0.096(L), 0.719(Q)	76.6%
I-V	0.002	0.992	0.002	**<0.001, <0.001(L), <0.001(Q)**	49.9%

(A) Acetic, (P) Propionic, (n-B) n-Butyric, (I-B) Iso-Butyric, (V) Valeric, (I-V) Iso-Valeric

4.5.8. Performance Parameters

4.5.8.1. Feed intake

Mean DE intakes for 0-5 days post-weaning and analysis of variance are displayed in Table 4.22. A significant effect of diet was found (P = 0.005) with pigs on the maize diet consuming the most food, compared to the other animals. Intakes over this period increased significantly with time (P = <0.001, <0.001(L), <0.001(Q)) and a significant linear effect was also observed for the interaction between diet and day. After 5 days, a significant dietary effect was still evident, although during this period of the trial; it was the pigs on the naked oats that consumed the most feed (P = 0.002). Intakes for all animals continued to increase significantly over time (P = <0.001, <0.001(L)) and were found to be more uniform (cv 23.2% vs. 72.7%) throughout this period of the trial. There was no observed interaction between diet and day (Table 4.23).

Table 4.22: Mean daily DE intake 0-5 days (MJ) and analysis of variance from Trial 2

	Days post-weaning						
Diet	**0**	**1**	**2**	**3**	**4**	**5**	**Mean**
Wheat	0.11	0.14	0.49	1.02	2.85	3.25	**1.31**
N Oats	0.10	0.12	0.33	1.16	3.56	3.93	**1.53**
W Oats	0.06	0.14	0.72	1.13	1.89	2.44	**1.06**
Maize	0.08	0.09	0.43	2.20	4.50	4.41	**1.95**

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.25	**0.005**	72.7%
Day	0.31	**<0.001, <0.001(L), <0.001(Q)**	
Diet*Day	0.61	**0.110, 0.003(L), 0.619(Q)**	

L = Linear effect Q = Quadratic effect (n = 6 pigs per diet)

Table 4.23: Mean DE intake post 5 days (MJ) and analysis of variance from Trial 2

	Days post-weaning								
Diet	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**Mean**
Wheat	3.43	3.86	4.81	5.33	5.16	6.02	6.43	7.73	**5.35**
N Oats	4.11	4.28	5.46	6.87	6.91	6.38	8.22	9.88	**6.51**
W Oats	3.37	3.60	3.76	4.23	4.49	4.93	5.65	6.68	**4.59**
Maize	2.12	4.09	5.37	6.64	7.24	6.26	6.92	7.29	**5.74**

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.46	**0.002**	
Day	0.64	**<0.001, <0.001(L)**, 0.740(Q)	23.2%
Diet*Day	1.29	0.936, 0.477(L), 0.118(Q)	

L = Linear effect Q = Quadratic effect (n = 2 pigs per diet)

4.5.8.2. DLWG

There was no observed effect of dietary treatment on mean DLWG. There was a significant effect of day with pigs gaining weight as the trial progressed (P = <0.001 – Table 4.24). No interaction was found between diet and day, although all pigs gained weight after a period of initial weight loss. For animals on the wheat, naked oats and maize diets, this was achieved by day 5 post-weaning, whereas for those on the whole oats diet, weight gain was not seen until day 7.

Table 4.24: Mean DLWG (kg) and analysis of variance from Trial 2

Diet	**Day 2**	**Day 4**	**Day 6**	**Day 10**	**Day 14**	**Mean**
Wheat	-0.08	0.04	0.12	0.15	0.20	**0.09**
Naked Oats	-0.20	-0.01	0.08	0.11	0.21	**0.04**
Whole Oats	-0.15	-0.04	-0.01	0.04	0.23	**0.01**
Maize	-0.15	-0.03	0.16	0.16	0.20	**0.07**

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.036	0.230	
Day	0.041	**<0.001, <0.001(L), 0.027(Q)**	156.4%
Diet*Day	0.081	0.859, 0.716(L), 0.313(Q)	

(n = 10 pigs per diet)

4.6. SUMMARY OF RESULTS FROM TRIAL 2

As with Trial 1, a number of parameters examined in the current trial appear to be significantly influenced by raw cereal type. CAD values for nitrogen in the small intestine were significantly higher for the wheat diet (P = 0.043), although this difference was lost when apparent digestion was analysed over the total tract. However, it should be remembered that several missing values in the animal data (due to lack of sufficient intestinal digesta samples) will affect the digestion coefficients obtained. In addition to exhibiting enhanced nitrogen digestion, pigs on the wheat-based diet also displayed significantly longer villi than the other treatment groups (P = <0.001).

Starch digestion in the small intestine was not affected by cereal source although there was a trend for coefficients to increase along the small intestine from mid to distal region (P = 0.063). By the end of the large intestine, digestibility of starch was found to be significantly lower for pigs on the maize diet (P = <0.001). This was evident in both collection periods. Again, it was difficult to obtain digesta from some of the pigs, particularly on day 2 of the slaughter programme. Experimental design meant that only two piglets per diet were analysed on each slaughter day. In addition, weaned piglets typically exhibit erratic feeding patterns in the immediate days after weaning. These two factors offer an explanation as to why digesta collection was found to be insufficient for laboratory analysis during this period of the trial.

Intestinal morphology appeared to be considerably affected following weaning; throughout the trial period, the majority of villi were shorter than those seen in the control pigs on day zero. Adding the factor of diet into the analysis failed to show any effect of cereal on this pattern of atrophy (Figure 4.1a). Post-weaning atrophy of the villi was most severe at the 0.25 site and the combination of this severe shortening of the intestinal villi, together with an increase in crypt depth measurements from day 4, shows that the pigs experienced quite severe alterations to their intestinal structure in the two weeks following weaning. Villus width did not alter markedly during the trial and although variations in the data were seen between diets on each of the slaughter days, no

consistent effect of cereal type was observed. From day 4, a considerable and sustained increase in crypt depth was seen for all pigs with maximum values evident at day 10. The generally high values seen in the pigs fed the maize diet from day 6 helps to explain why a highly significant effect of cereal type was found for the crypt depth data.

Digesta pH along the digestive tract followed a typical pattern in all experimental piglets with acidic conditions in the stomach, followed by a gradual increase in alkalinity along the small intestine, before reaching more acidic levels in the caecum. Overall mean pH figures showed pigs on the maize diet had the most acidic digesta with those animals on the wheat diet having the least acidic. This dietary difference was no longer significant when each tract region was compared between pigs on the different diets.

Pigs on the maize diet consumed the most feed during the initial five days of the trial. After this period there was less variation in intake levels between treatment groups, and although a significant effect of diet was still evident, it was the pigs on the naked oats diet that consumed the greatest amount of feed after 5 days. It is likely that the erratic feeding behaviour mentioned above accounted for the high variation in intakes from 0-5 days, as appetites would have become more settled as the trial progressed. The uniformity of intakes from day 5 would most likely account for the fact that no dietary differences were evident for DLWG during the trial.

The first hypothesis stated that increased viscosity of tract digesta (influenced by dietary cereal) would be unfavourable to weaned piglet performance. The current trial does not support this hypothesis; pigs on the naked oats diet had significantly more viscous digesta than the other treatment groups in all tract regions studied ($P = 0.043$), but no detrimental effects were observed for any of the digestibility, gut morphology or performance parameters measured.

In the current trial, a number of interesting responses were observed for the animals on the whole oat diet. Piglets in this group exhibited the lowest nitrogen digestibility coefficients compared to other dietary treatments and were found to exhibit the shortest

mean villi. In addition, the DLWG data showed that these animals took on average two days longer (7 vs. 5 days) to achieve weight gain following initial weight loss after weaning. It would appear therefore that whole oats is the least favourable of the dietary cereals examined in the current trial. Observational data recording incidence of scouring over the experimental period revealed no effect of dietary treatment.

A comparison of the results obtained from the use of raw cereals used in Trials 1 and 2 will be discussed in Chapter 8. The next trial (Trial 3) is designed to examine how endosperm texture of raw wheat can influence physiological and nutritional responses in the weaned piglet.

CHAPTER 5

Trial 3: Effects of feeding diets containing raw wheat of differing endosperm texture, on digestibility, digesta properties and gut morphology in the post-weaned piglet.

5.1. INTRODUCTION

Grain hardness is one of the most important characteristics of wheat and determines the end use of the flour. It is defined using the Single Kernel Characterisation System (SKCS) which measures the force required to crush individual grains between two surfaces. As the endosperm constitutes the greatest proportion (typically >0.80) of the grain, hardness is largely determined by the properties of the endosperm (Turnbull and Rahman, 2002). As mentioned in Chapter one, results of trials examining the effect of wheat endosperm texture on animal performance have often been confounded with other factors such as the presence of the 1B/1R gene. Therefore, in order to fully evaluate the effects of wheat endosperm texture, wheats used in this trial were of similar genetic background with hardness being the only difference between them.

Variation in particle sizes and starch damage between hard and soft wheat were predicted to result in differences in digestibility for the weaned piglet. Due to differences in fracturing patterns when ground, starch granules from hard wheat typically show greater damage (Kulp *et al.*, 1980; Zeng *et al.*, 1997), leading to increased water absorbance (Mok and Dick, 1991). It was hypothesized that piglets fed the hard wheat diet would exhibit increased digesta viscosity, leading to a reduction in the rate of digestion (by limiting the mixing of digestive enzymes with the substrate in the gastrointestinal tract, thereby reducing nutrient availability). It was predicted therefore, that the diet containing hard wheat would be less favourable (as a result of reduced availability and absorption of nutrients from the viscous digesta) for the weaned piglet.

5.2. OBJECTIVE

The objective of the Trial 3 was to determine changes in digestibility, digesta properties and gut morphology in weaned piglets fed one of two diets containing raw wheat of either hard or soft endosperm texture. The diets were iso-nitrogenous and iso-energetic and were grown under the same environmental conditions. Neither of the wheat cultivars contained the 1B/1R gene.

5.3. HYPOTHESES

- Soft endosperm wheat will be more beneficial than hard endosperm wheat in terms of enhanced starch digestibility within the small intestine and reduced digesta viscosity.
- Physicochemical properties of starch from hard endosperm wheat will negatively impact upon villus architecture in the small intestine of the newly weaned piglet.

5.4. METHODS

5.4.1. Animals and housing

Due to a limited availability of wheat, 22 piglets were used for this trial. Animals were weighed and housed as previously described. Twenty of the piglets each received one of the two diets (fed in dry meal form and on an *ad-libitum* basis), with the remaining two used as control animals (not fed a trial diet).

5.4.2. Diets

Two experimental diets were manufactured on site at Nottingham, each identical apart from the cereal component; one contained hard wheat (hardness 11.2), the other, soft wheat (hardness 6.3). Both were ground through a hammer mill fitted with a 1.5 – 2 mm screen. Dietary ingredients were then incorporated to form the completed diets (see Appendix 1). Diets were iso-energetic and iso-nitrogenous (14.4 MJ digestible energy (DE) / kg and 235g crude protein (CP) / kg).

5.4.3. Experimental and slaughter procedure

Slaughter procedure and sample collection was carried out as before (Table 5.1).

Table 5.1: Slaughter programme for Trial 3

Group	Day	Piglets slaughtered
1	0	n = 2
2	2	n = 4 (2 per diet)
3	4	n = 4 (2 per diet)
4	6	n = 4 (2 per diet)
5	10	n = 4 (2 per diet)
6	14	n = 4 (2 per diet)

5.4.4. Faecal collection

Twelve piglets (six per diet) were assessed over two time periods. Samples were collected and stored as in previous trials.

5.5. RESULTS

Data were subjected to analysis of variance; when day was included in the model, variance was partitioned into linear and non-linear contrasts.

5.5.1. Gastric ulceration

At slaughter, none of the animals showed any evidence of gastric ulceration.

5.5.2. pH

As expected, gastric pH was more acidic than digesta from the rest of the tract (Table 5.2). The pH of caecal digesta was also slightly more acidic than digesta from the small intestine. Significant differences were exhibited between region (P = <0.001), CVR (P = 0.021) and the interaction between CVR and region (P = <0.001). However, no differences between diet or day were determined (Table 5.3).

5.5.3. Viscosity

A significant dietary effect was observed (P = 0.029) with piglets on the hard wheat diet exhibiting more viscous digesta throughout all regions of the digestive tract, than those on the soft wheat diet (see Tables 5.4 & 5.5). This pattern was clearly evident for the

first 10 days after weaning. Significant differences were also observed for day (P = 0.029 (L)), region (P = 0.031) and CVR (P = <0.001). There also were significant correlations between day and region (P = 0.039 (L)) and CVR and region (P = <0.001).

Table 5.2: Mean digesta pH from Trial 3

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	Stomach	4.0						**4.0**
	0.25	6.4						**6.4**
	0.50	6.8						**6.8**
	0.75	7.3						**7.3**
	Caecal	7.1						**7.1**
Hard Wheat	Stomach		3.3	3.3	3.5	3.8	3.4	**3.4**
	0.25		6.9	6.3	6.4	6.0	5.9	**6.3**
	0.50		6.6	6.8	6.8	6.7	6.7	**6.7**
	0.75		7.0	7.1	6.6	7.2	7.1	**7.0**
	Caecal		6.3	6.3	5.9	6.6	5.5	**6.1**
Soft Wheat	Stomach		3.3	3.8	4.3	3.8	4.3	**3.9**
	0.25		6.4	6.3	5.5	6.5	6.5	**6.2**
	0.50		6.4	6.9	6.6	6.9	6.9	**6.7**
	0.75		6.9	7.2	7.0	7.1	7.2	**7.1**
	Caecal		6.4	7.0	5.5	6.1	5.6	**6.1**

0.25, 0.50 &0.75 = proportion along small intestine (n = 10 per diet, control pigs; n = 2)

Table 5.3: Analysis of variance of mean digesta pH from Trial 3

Factor	s.e.d.	P	cv
CVR	0.15	**0.021**	7.6%
CVR*Region	0.34	**<0.001**	
CVR*Day*Region	0.39	0.083	
Diet	0.09	0.320	7.8%
Day	0.15	0.328, 0.788(L), 0.896(Q)	
Region	0.15	**<0.001**	
Diet*Day	0.21	0.368, 0.234(L), 0.654(Q)	
Diet*Region	0.21	0.485	
Day*Region	0.33	0.105, **0.027(L)**, 0.436(Q)	

CVR = control animals versus the rest L = linear effect Q = Quadratic effect

5.5.4. Starch digestibility

5.5.4.1. Starch digestibility in the small intestine

Virtually no starch digestion was found in the proximal (0.25) region of the tracts studied. There was no significant effect of diet (P = 0.148), however, a highly significant effect of region was seen (P = <0.001) with higher CAD exhibited in the distal, compared to mid regions of the gut (Table 5.6, Figure 5.1). A significant effect of day was also found (P = 0.015; 0.022 (L); 0.012 (Q)) and there was a non-linear effect between day and region (P = 0.028 (Q)). Statistical analysis of starch digestion (Table 5.7) showed no significant interactions between diet and day (P = 0.366), or diet and region (P = 0.611).

Table 5.6: Mean coefficients of apparent starch digestibility from Trial 3

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Hard Wheat	0.50	0.53	0.19	0.20	0.62	0.59	**0.43**
	0.75	0.84	0.87	0.92	0.84	0.95	**0.89**
Soft Wheat	0.50	0.53	0.06	0.31	0.06	0.69	**0.33**
	0.75	0.86	0.66	0.76	0.96	0.93	**0.84**

0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet)

Table 5.7: Analysis of variance of mean coefficients of apparent starch digestibility

Factor	s.e.d.	P	cv
Diet	0.046	0.148	
Day	0.073	**0.015, 0.022(L), 0.012(Q)**	
Region	0.046	**<0.001**	
Diet*Day	0.102	0.366, 0.838(L), 0.172(Q)	23.4%
Diet*Region	0.065	0.611	
Day*Region	0.102	0.139, 0.435(L), **0.028(Q)**	
Diet*Day*Region	0.145	**0.025**, 0.440(L), 0.264(Q)	

L = Linear effect Q = Quadratic effect

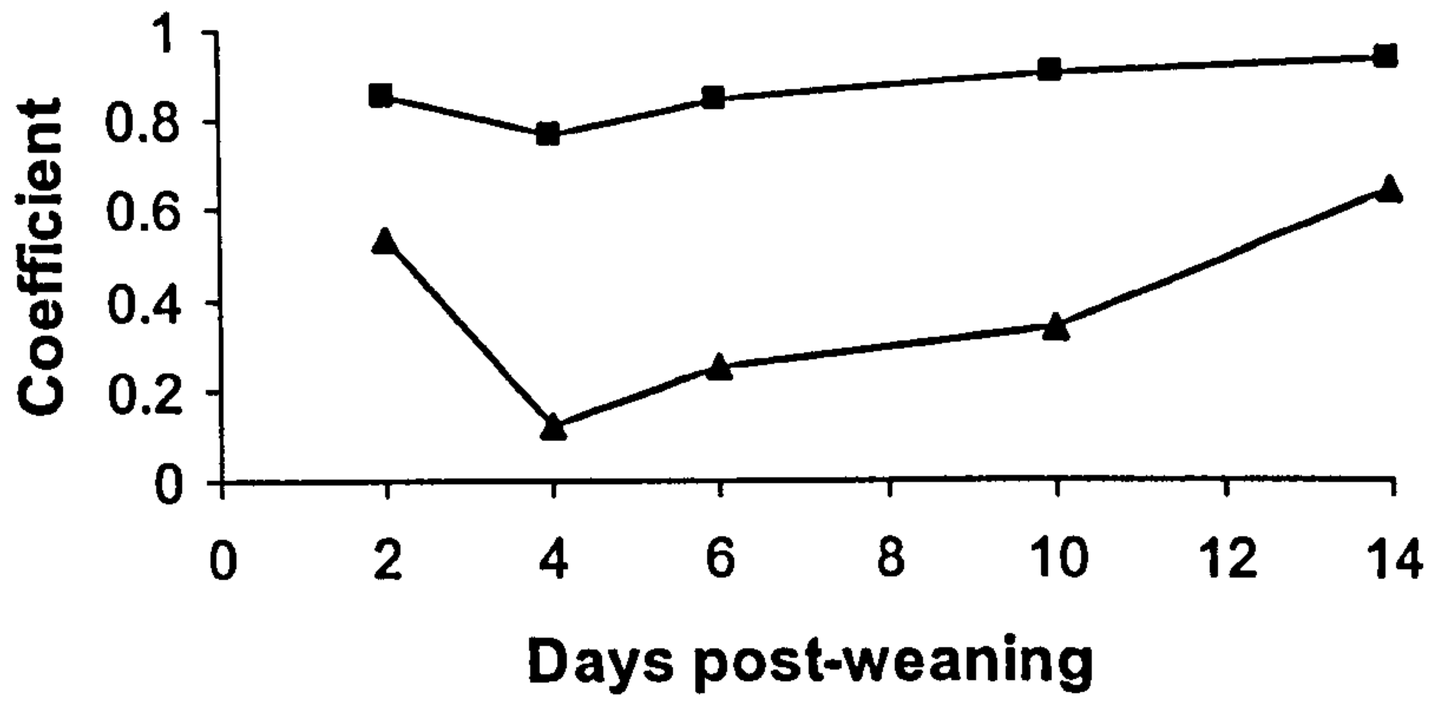


Figure 5.1: *Coefficient of apparent starch digestibility in mid (-▲-) and distal (-■-) regions of small intestine over 14 days post-weaning (n = 10 animals per diet)*

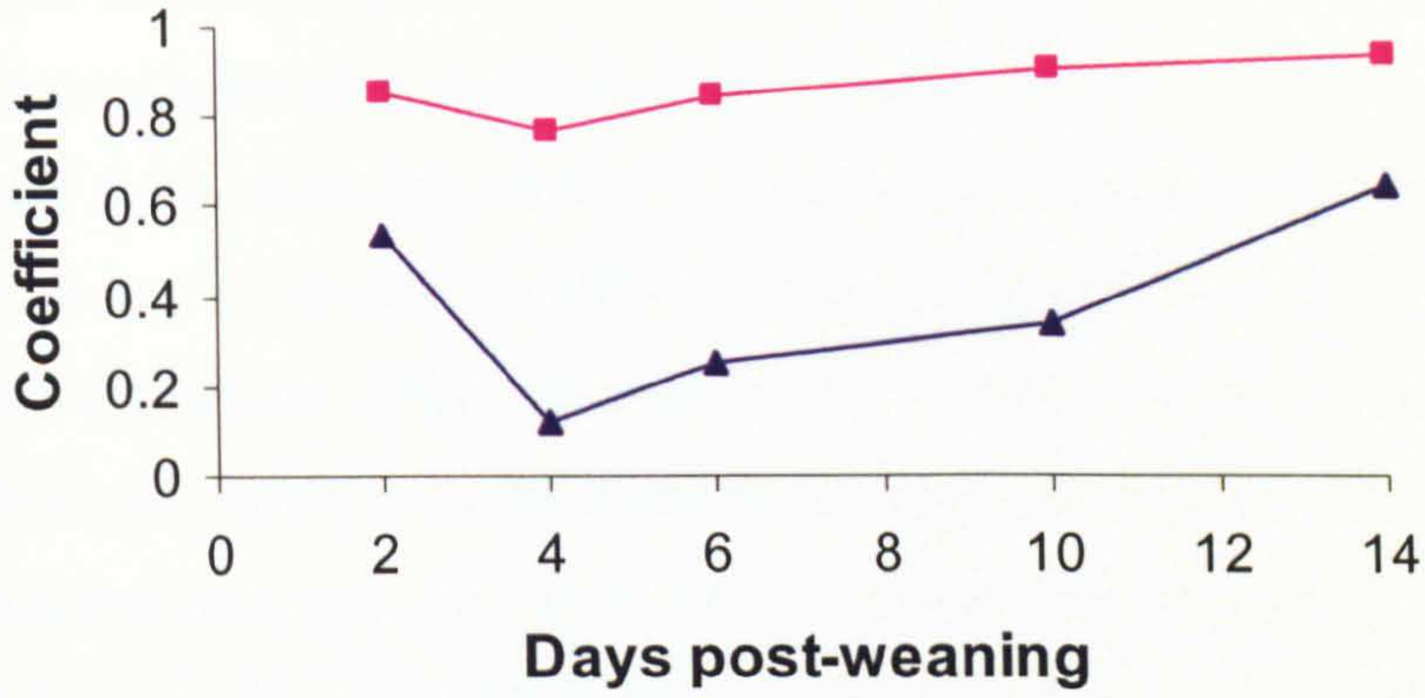
5.5.4.2. Starch digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 5.8. Piglets fed the soft wheat diet were found to have significantly higher CTTAD values than animals fed hard endosperm wheat. There was no effect of collection period and no interaction was found with diet.

Table 5.8: Mean coefficients of total tract apparent digestibility for starch and analysis of variance from Trial 3

Diet	**Days 1-5**	**Days 9-13**	
Hard Wheat	0.988	0.992	
Soft Wheat	0.998	0.998	
Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.002	**<0.001**	0.4%
Collection Period	0.002	0.293	
Diet*Collection Period	0.002	0.368	

(n = 6 pigs per diet for each collection period)



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Hard Wheat	0.988	0.992	
Soft Wheat	0.998	0.998	
Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.002	**<0.001**	0.4%
Collection Period	0.002	0.293	
Diet*Collection Period	0.002	0.368	

(n = 6 pigs per diet for each collection period)

5.5.5. Nitrogen digestibility

5.5.5.1. Nitrogen digestibility in the small intestine

Tables 5.9 and 5.10a show the effect of dietary treatment on CAD for nitrogen during the trial. There was no significant effect of diet (P = 0.416) or day (P = 0.416) but there was a highly significant effect of region (P = <0.001) with apparent digestibility increasing from the proximal to the distal section of the intestine. No interactions were observed between diet and day (P = 0.668), or day and region (P = 0.267) but the interaction between diet and region approached significance (P = 0.073). When tract regions were analysed individually, pigs on the soft wheat diet exhibited significantly higher CAD in the 0.75 region; Table 5.10b shows a significant effect of diet (P = 0.006), day (P = 0.013) and a diet day interaction (P = 0.031, 0.007 Q).

Table 5.9: Mean coefficients of apparent nitrogen digestibility from Trial 3

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Hard Wheat	0.25	0.38	0.43	0.32	0.16	0.37	**0.33**
	0.50	0.65	0.53	0.51	0.54	0.58	**0.56**
	0.75	0.57	0.71	0.61	0.65	0.72	**0.65**
Soft Wheat	0.25	0.28	0.43	0.38	0.12	0.36	**0.31**
	0.50	0.42	0.40	0.44	0.26	0.49	**0.40**
	0.75	0.58	0.79	0.85	0.78	0.71	**0.74**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 per diet)

Table 5.10a: Analysis of variance of mean coefficients of apparent nitrogen digestibility

Factor	s.e.d.	P	cv
Diet	0.035	0.416	32.3%
Day	0.055	0.416, 0.971(L), 0.137(Q)	
Region	0.049	**<0.001**	
Diet*Day	0.078	0.668, 0.706(L), 0.381(Q)	
Diet*Region	0.070	0.073	
Day*Region	0.110	0.267, 0.716(L), 0.210(Q)	
Diet*Day*Region	0.156	0.856	

L = Linear effect Q = Quadratic effect

Table 5.10b: Analysis of variance of mean coefficients of apparent nitrogen digestibility from distal (0.75) region of small intestine

Factor	s.e.d.	P	cv
Diet	0.013	**0.006**	
Day	0.020	**0.013, 0.021(L), 0.008(Q)**	4.1%
Diet*Day	0.028	**0.031**, 0.338(L), **0.007(Q)**	

L = Linear effect Q = Quadratic effect

5.5.5.2. Nitrogen digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 5.11. No effect of diet was observed but coefficients increased significantly between collection periods (overall means; 0.694 vs. 0.783). CTTAD was greater during both faecal collection periods for the pigs fed the soft wheat diet, although this interaction between diet and collection period was not statistically significant.

Table 5.11: Mean coefficient of total tract apparent digestibility for nitrogen and analysis of variance from Trial 3

Diet	Days 1-5	Days 9-13	
Hard Wheat	0.557	0.831	
Soft Wheat	0.710	0.855	
Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.067	0.198	22.1%
Collection Period	0.067	**0.005**	
Diet*Collection Period	0.094	0.342	

(n = 6 pigs per diet for each collection period)

5.5.6. Gut morphology

Graphs of the gut morphology data can be seen in figures 5.2a – 5.2d.

5.5.6.1. Villus height

Mean villus height measurements and analysis of variance are shown in Tables 5.12 and 5.13. As expected, a degree of villus atrophy was observed immediately after weaning, before a period of regeneration up to day 14 (P = <0.001; <0.001 (L); <0.001 (Q)). There was a strong interaction between diet and day (P = <0.001; 0.001 (L); 0.030 (Q)), where the piglets fed the soft wheat diet exhibited less severe villus atrophy in the period immediately post-weaning, than those animals on the hard wheat diet (Figure 5.2a). A strong regional effect (P <0.001) revealed that the longest villi were found at the 0.25 site, with the distal portion (0.75) region of the intestine exhibiting the shortest. A significant interaction between CVR and day (P = <0.001) showed that after the initial period of atrophy, villi had recovered to pre-weaning heights for both diets by day 5 of the trial. The interaction between CVR and Region (P = <0.001) revealed that villus atrophy was most severe for the experimental pigs at the 0.25 site, compared against values seen in control animals. A significant interaction was also observed between CVR and diet (P = 0.001) although no significant difference was determined between dietary treatment alone (P = 0.131).

5.5.6.2. Villus width

Mean villus width measurements and accompanying analysis of variance are shown in Tables 5.14 and 5.15. Analysis of day alone revealed a significant effect (P = 0.007) with villus width regenerating, following a short period of atrophy after weaning. When the effect of diet was included in the analysis, a strong interaction was found (P = 0.001; 0.026(Q) and it was evident that the reduction in villus width seen on day 4 post-weaning was only observed for the soft wheat diet (Figure 5.2b). There was a significant interaction between CVR and day (P = 0.005) but no effects were found for CVR, region or the interaction between CVR and region (P >0.05). In addition to this, no significant dietary effect was observed (P = 0.147).

5.5.6.3. Crypt depth

Mean crypt depth values and analysis of variance are shown in Tables 5.16 and 5.17. Once again, there is no observed effect of diet alone (P = 0.246) although highly significant effects were seen for day (P = <0.001; <0.001 (Q)), and region (P = 0.001). A significant interaction between diet and day (P = 0.001; 0.003 (L); <0.001 (Q)) revealed a similar pattern between the diets over the trial period (Figure 5.2c) although crypt depth measurements between days 6 and 14 were greater for pigs fed the hard wheat diet. A significant interaction between CVR and region (P = <0.001) revealed that mean crypt depths were greater for experimental than control pigs, with the greatest difference evident at the 0.5 and 0.75 tract sites. There were also significant interactions between CVR and day (P = <0.001), and the interaction between CVR diet and day (P = <0.001). No differences were observed between CVR alone or the interaction between CVR and diet (P = >0.05).

5.5.6.4. Villus height to crypt depth ratio

Mean ratio data and analysis of variance in Tables 5.18 and 5.19 show no significant differences between diet, region or the interaction between CVR and region (P = >0.05). There was a highly significant effect of day (P = <0.001; <0.001 (L)) with ratio measurements increasing throughout the duration of the trial. When the effect of diet was added to the model, a strong interaction between diet and day was found (P = 0.012 – Figure 5.2d). Significant effects were also determined for CVR (P = <0.001), and the interactions between CVR and diet (P = 0.045) and CVR and day (P = <0.001).

Table 5.12: Mean villus height measurements (μm) from Trial 3; covariate = live weight)

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	0.25	526						**526**
	0.50	456						**456**
	0.75	431						**431**
Hard Wheat	0.25		333	477	491	482	529	**462**
	0.50		341	436	480	499	517	**455**
	0.75		321	368	462	493	483	**425**
Soft Wheat	0.25		451	400	543	527	464	**477**
	0.50		435	424	500	484	495	**467**
	0.75		334	426	453	484	557	**451**

Region = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 2)

Table 5.13: Analysis of variance of mean villus height

Factor	s.e.d.	P	cv
CVR	10.7	**<0.001**	
CVR*Day	11.7	**<0.001**	
CVR*Diet	10.6	**0.001**	
CVR*Region	16.5	**<0.001**	15.0%
CVR*Day*Diet	13.1	**<0.001**	
CVR*Day*Region	19.2	**0.001**	
CVR*Diet*Region	17.1	0.621	
CVR*Day*Diet*Region	22.0	**<0.001**	
Diet	5.8	0.131	
Day	9.5	**<0.001, <0.001(L), <0.001(Q)**	
Region	6.9	**<0.001**	
Diet*Day	13.1	**<0.001, 0.001(L), 0.030(Q)**	15.2%
Diet*Region	9.8	0.628	
Day*Region	15.8	**0.001, <0.001(L), 0.351(Q)**	
Diet*Day*Region	22.2	**<0.001, 0.001(L), 0.160(Q)**	

CVR = control animals versus the rest L = linear effect Q = Quadratic effect

Table 5.14: Mean villus width measurements (μm) from Trial 3; covariate = live weight)

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	0.25	111						**111**
	0.50	108						**108**
	0.75	108						**108**
Hard Wheat	0.25		114	111	115	106	111	**111**
	0.50		104	115	115	122	95	**110**
	0.75		117	117	106	123	122	**117**
Soft Wheat	0.25		113	93	108	113	107	**107**
	0.50		132	89	113	108	104	**110**
	0.75		114	104	118	108	121	**113**

Region = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 2)

Table 5.15: Analysis of variance of mean villus width

Factor	s.e.d.	P	cv
CVR	3.8	0.531	
CVR*Day	4.2	**0.005**	
CVR*Diet	3.8	0.194	
CVR*Region	5.9	0.137	22.2%
CVR*Day*Diet	4.7	**<0.001**	
CVR*Day*Region	6.9	0.057	
CVR*Diet*Region	6.1	0.694	
CVR*Day*Diet*Region	7.9	**0.008**	
Diet	2.1	0.147	
Day	3.4	**0.007**, 0.716(L), 1.000(Q)	
Region	2.5	**0.039**	
Diet*Day	4.7	**<0.001**, 0.926(L), **0.026(Q)**	22.4%
Diet*Region	3.5	0.702	
Day*Region	5.7	0.067, **0.033(L)**, 0.209(Q)	
Diet*Day*Region	8.0	**0.010**, 0.634(L), **0.008(Q)**	

CVR = control animals versus the rest L = linear effect Q = Quadratic effect

Table 5.16: Mean crypt depth measurements (μm) from Trial 3; covariate = live weight)

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	0.25	274						**274**
	0.50	257						**257**
	0.75	234						**234**
Hard Wheat	0.25		269	243	269	322	262	**273**
	0.50		265	236	297	348	252	**280**
	0.75		232	219	279	299	257	**257**
Soft Wheat	0.25		275	229	289	291	301	**277**
	0.50		293	263	286	279	240	**272**
	0.75		262	248	266	268	231	**255**

Region = proportion along small intestine (n = 10 pigs per diet, control pigs n = 2)

Table 5.17: Analysis of variance of mean crypt depth

Factor	s.e.d.	P	cv
CVR	8.1	0.856	
CVR*Day	8.9	**<0.001**	
CVR*Diet	8.0	0.544	
CVR*Region	12.6	**<0.001**	19.5%
CVR*Day*Diet	10.0	**<0.001**	
CVR*Day*Region	14.6	0.054	
CVR*Diet*Region	13.0	0.541	
CVR*Day*Diet*Region	16.7	**0.014**	
Diet	4.3	0.246	
Day	7.1	**<0.001**, 0.740(L), **<0.001(Q)**	
Region	5.1	**<0.001**	
Diet*Day	9.7	**<0.001, 0.003(L), <0.001(Q)**	19.1%
Diet*Region	7.3	0.534	
Day*Region	11.7	**0.048, 0.016(L)**, 0.103(Q)	
Diet*Day*Region	16.4	**0.012, 0.003(L)**, 0.577(Q)	

CVR = control animals versus the rest L = linear effect Q = Quadratic effect

Table 5.18: Mean villus height to crypt depth ratios from Trial 3; covariate = live weight)

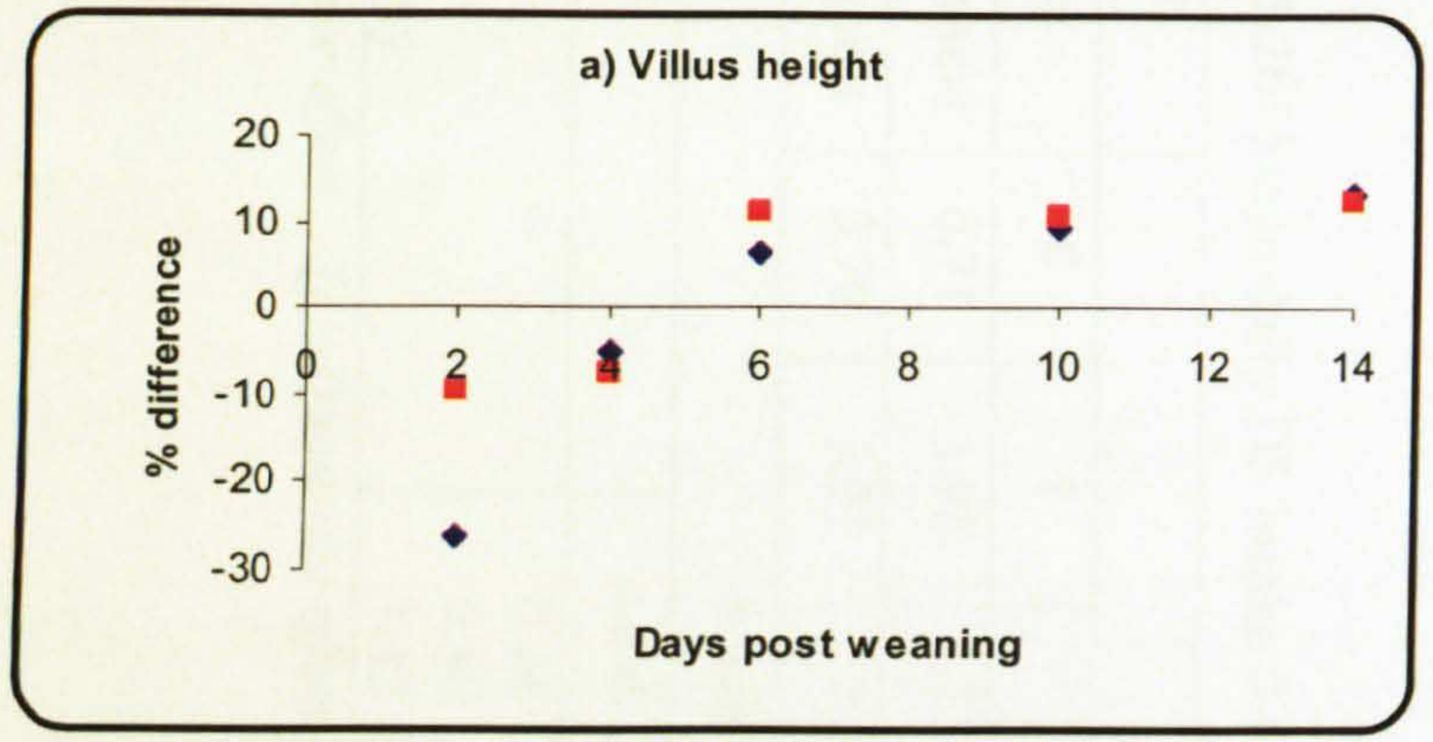
Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	0.25	2.0						**2.0**
	0.50	2.0						**2.0**
	0.75	1.9						**1.9**
Hard Wheat	0.25		1.3	2.1	1.9	1.5	2.2	**1.8**
	0.50		1.3	1.9	1.7	1.5	2.1	**1.7**
	0.75		1.4	1.7	1.8	1.7	1.9	**1.7**
Soft Wheat	0.25		1.7	1.8	2.0	1.9	1.6	**1.8**
	0.50		1.5	1.7	1.8	1.8	2.1	**1.8**
	0.75		1.3	1.8	1.8	1.8	2.5	**1.9**

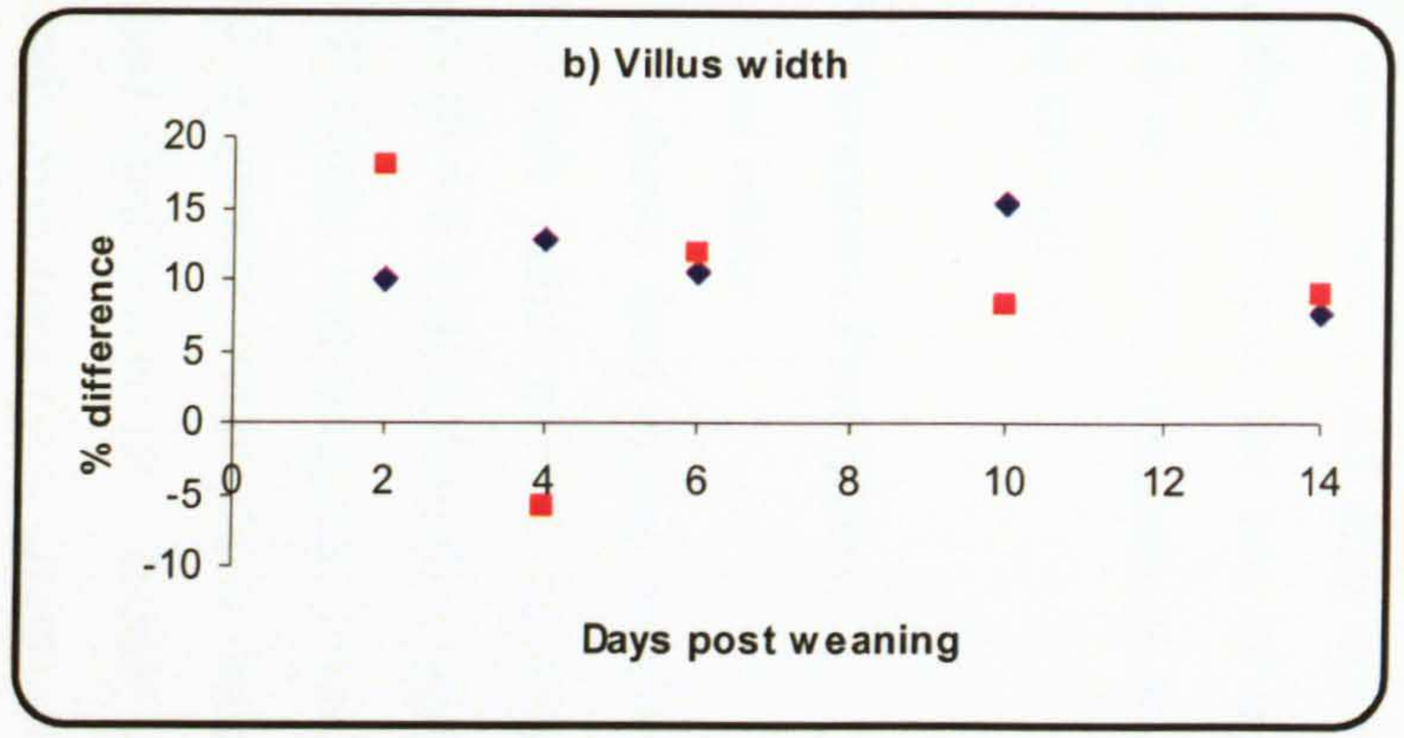
Region = proportion along small intestine (n = 10 per pigs per diet, control pigs; n = 2)

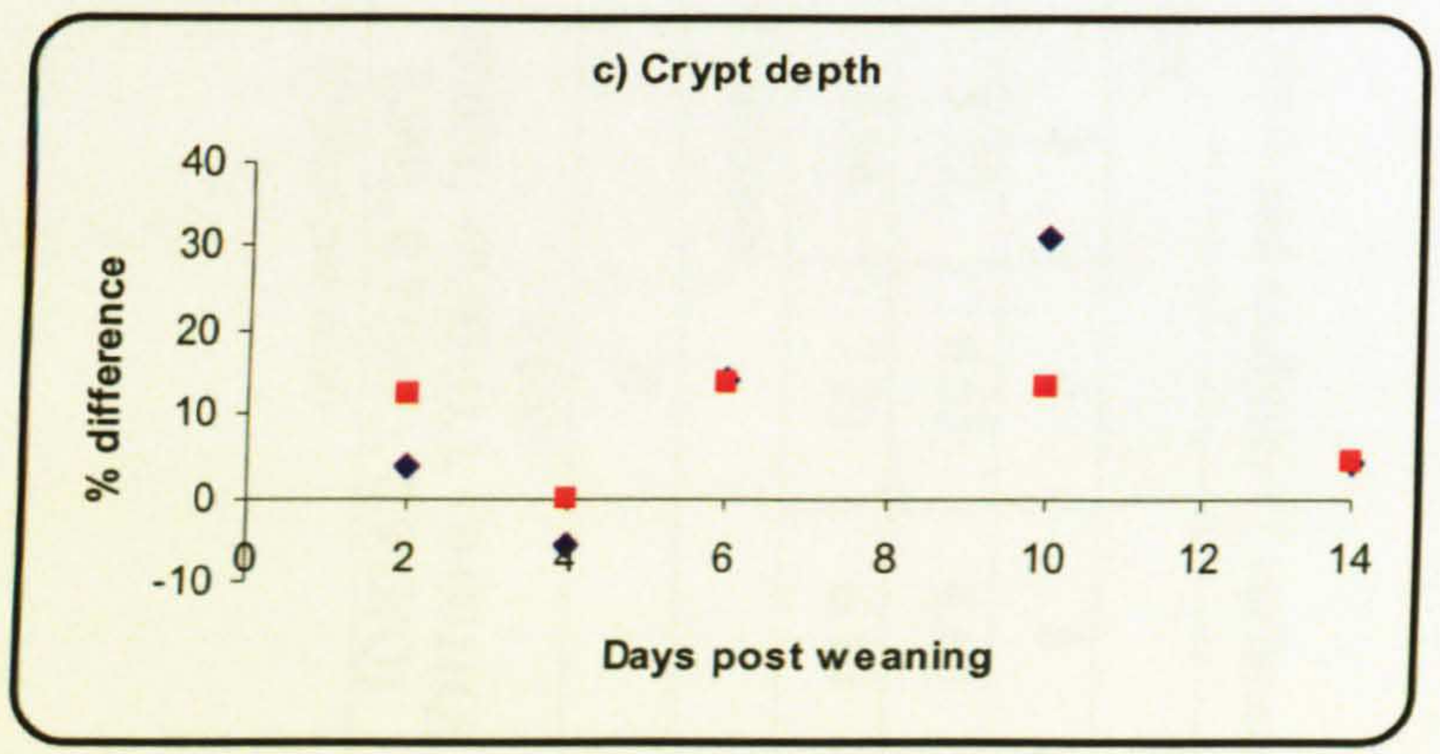
Table 5.19: Analysis of variance of mean villus height to crypt depth ratios

Factor	s.e.d.	P	cv
CVR	0.08	**<0.001**	
CVR*Day	0.08	**<0.001**	
CVR*Diet	0.07	**0.045**	
CVR*Region	0.12	0.722	26.7%
CVR*Day*Diet	0.09	**0.014**	
CVR*Day*Region	0.13	**0.013**	
CVR*Diet*Region	0.12	0.515	
CVR*Day*Diet*Region	0.15	**<0.001**	
Diet	0.04	0.143	
Day	0.07	**<0.001, <0.001(L)**, 0.188(Q)	
Region	0.05	0.341	
Diet*Day	0.09	**0.012**, 0.930(L), 0.435(Q)	26.5%
Diet*Region	0.07	0.501	
Day*Region	0.11	**0.010, <0.001(L)**, 0.073(Q)	
Diet*Day*Region	0.15	**<0.001, <0.001(L)**, 0.104(Q)	

CVR = control animals versus the rest L = linear effect Q = Quadratic effect







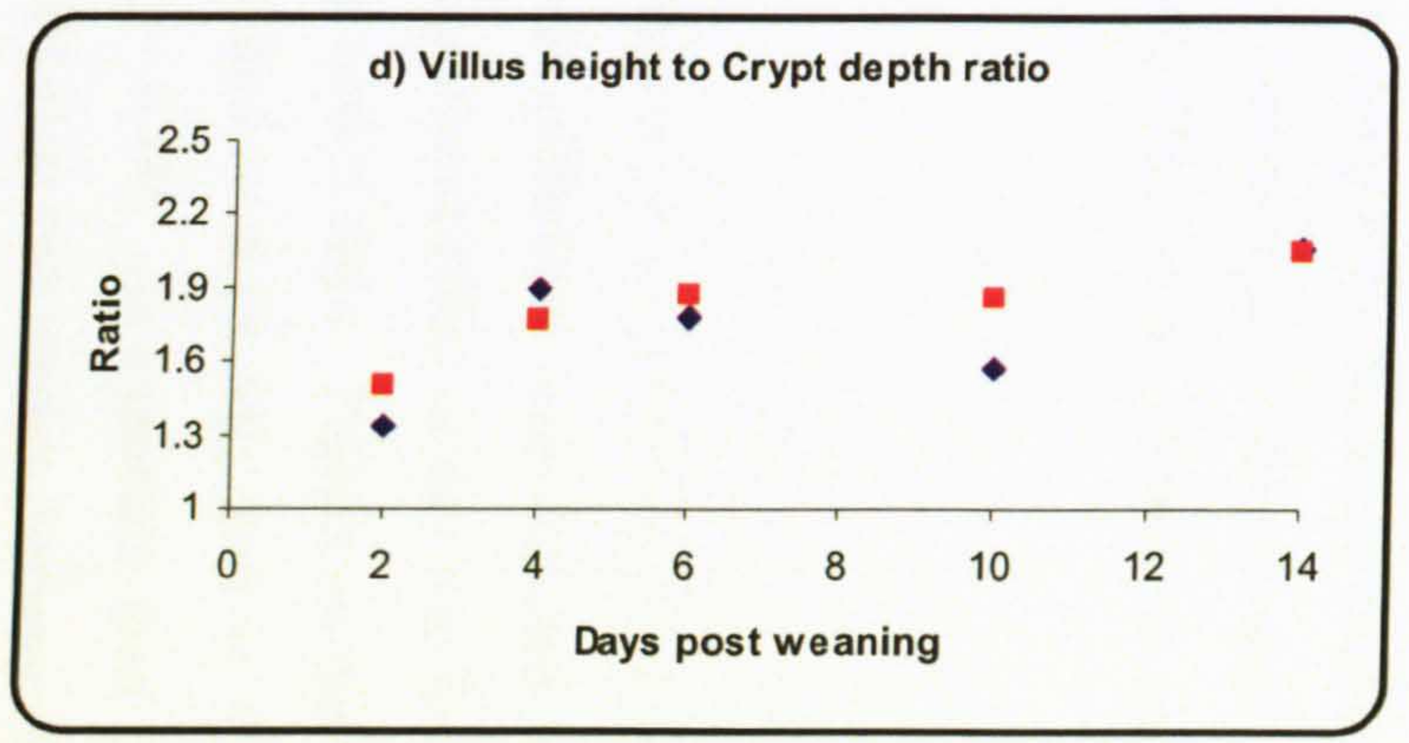


Figure 5.2: *Effect of endosperm texture of raw wheat on mean gut morphology measurements (covariate = live weight; n = 10 piglets per diet) (♦) Hard endosperm (■) Soft endosperm*

5.5.7. Caecal VFA analysis

Unfortunately, due to an error in sample storage and processing in this trial, the caecal samples were freeze-dried after collection along with the other digesta samples. As VFA analysis requires the use of freshly frozen digesta (Illman and Topping, 1985; Fleming *et al.*, 1987; Marsono *et al.*, 1993; Choct *et al.*, 1996), no analysis of caecal VFAs was carried out.

5.5.8. Performance Parameters

5.5.8.1. Feed intake

Table 5.20 shows feed intake data and analysis of variance for the first 5 days of the trial. For this initial period, no significant difference was detected between dietary treatments (P = 0.863) although a highly significant effect of day was found. Table 5.21 shows DE intake and analysis of variance post 5 days. Piglets on the soft wheat diet exhibited a greater mean daily intake (Hard = 8.48 MJ; Soft = 9.31 MJ) which approached significance (P = 0.063; s.e.d. = 0.44). As expected, feed intake increased significantly over time (P = <0.001; < 0.001 (L)) and intakes became more uniform as the trial progressed (cv 24.4% vs. 47.8%).

Table 5.20: Mean daily DE intake 0-5 days (MJ) and analysis of variance from Trial 3

	Day						
Diet	**0**	**1**	**2**	**3**	**4**	**5**	**Mean**
Hard wheat	0.71	3.98	7.79	5.72	6.53	6.60	**5.22**
Soft wheat	0.70	3.91	7.35	5.94	7.21	6.70	**5.30**
Analysis of variance							
Factor	**s.e.d.**	**P**	**cv**				
Diet	0.46	0.863					
Day	0.80	**<0.001, <0.001(L), <0.001(Q)**	47.8%				
Diet*Day	1.13	0.991, 0.719(L), 0.943(Q)					

L = Linear effect Q = Quadratic effect (n = 10 pigs per diet)

Table 5.21: Mean DE feed intake post 5 days (MJ) and analysis of variance from Trial 3

	Day								
Diet	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**Mean**
Hard wheat	5.75	7.49	7.40	6.84	7.79	9.45	11.40	11.71	**8.48**
Soft wheat	5.24	7.98	·8.21	8.90	9.22	10.00	12.40	12.56	**9.31**
Analysis of variance									

Factor	**s.e.d.**	**P**	**cv**
Diet	0.44	0.063	
Day	0.89	**<0.001, <0.001(L),** 0.294(Q)	24.4%
Diet*Day	1.25	0.925, 0.512(L), 0.280(Q)	

L = Linear effect Q = Quadratic effect (n = 6 per diet)

5.5.8.2. Daily live weight gain (DLWG)

No significant effects were established for this trial. There was no relationship with diet (P = 0.645).

5.6. SUMMARY OF RESULTS FROM TRIAL 3

The hypothesis for this trial was that soft endosperm wheat would be more beneficial than hard wheat in terms of enhanced starch digestion within the small intestine, and reduced digesta viscosity. In addition, it was proposed that hard endosperm wheat would negatively affect the villus architecture of the small intestine

It was difficult to establish any dietary influence on feed intake for the initial few days of the experiment. This was expected, as it is well known that the amount of feed consumed by the piglet in the period immediately post-weaning, is often highly variable (Bark *et al.*, 1986; Le Dividich and Herpin, 1994). However, from 5 days post-weaning, the piglets on the soft wheat diet exhibited higher feed intakes for the remainder of the trial, when compared with those on the hard wheat diet. As the diets were identical except for the cereal component, this difference in intake would suggest that the soft wheat was more palatable for the piglet.

A significant dietary effect on digesta viscosity was found; piglets on the soft wheat diet exhibited less viscous digesta throughout all sampled regions of the gastrointestinal tract, possibly as a result of reduced starch granular damage of the soft endosperm wheat influencing the hydration characteristics of the gastrointestinal digesta. This dietary difference was prevalent until at least day 10 post-weaning. The CTTAD values for nitrogen were found to be higher for the piglets fed soft wheat. This dietary difference was greatest for the first 5 days of the trial, but as the experiment progressed, became less pronounced, although still evident. Analysis of apparent nitrogen digestibility in the various regions of the tract indicated a highly significant regional effect. On further analysis, it was the distal section (0.75) of the small intestine that exhibited the greatest difference, with the piglets on the soft wheat diet showing significantly higher apparent digestibilities than those fed the hard wheat.

There were some inconclusive responses from this trial; no dietary differences were detected in apparent starch digestibility, although there were interesting regional

responses. The vast majority of starch digestion appeared to occur in the mid and distal sections of the intestine, as very little digestion was detected in the proximal section. Whilst there is literature that suggests that the jejunum is the major site for starch digestion in chickens (Osman, 1982), work with piglets is less clear (Stevens and Kidder, 1972; Kidder and Manners, 1980). Figure 5.1 shows a short-term reduction in digestion coefficients in both the mid and distal regions around 4 days post-weaning. This reduction occurs around the same time that villus atrophy is typically seen in the gut. It is reasonable to suggest that the reduced absorptive capacity of the gut when experiencing atrophy could be responsible for the temporary reduction in starch digestion.

Although diet alone had no significant effect on gut morphology, there were significant interactions between diet and day for each of the parameters measured. As expected, villus atrophy occurred in all animals following weaning but an interesting observation was that although the maximum reduction in villus height was observed by day 2, this atrophy was smaller for the piglets on the soft wheat diet than for those fed the hard wheat (Figure 5.6a). A degree of reservation should be made however when assessing the significance of this observation, due to the limited number of piglets analysed per diet on each slaughter day, due to experimental design.

Studies have shown that inadequate intake of feed around this time can lead to detrimental changes in gut structure and function (McCracken, 1984; Kelly *et al.*, 1991b; McCracken *et al.*, 1999) but this difference in degree of atrophy cannot be explained by appetite as 0-5 day intakes for the two treatment groups were not found to be significantly different. Despite this dietary variation on day 4, atrophy was short-lived, and villus height in all animals had recovered to pre-weaning levels by day 5 of the trial. There is some variation in reports regarding the duration of villus atrophy in newly-weaned piglets: Hampson (1986) reported that villus height was still only 0.5 of pre-wean levels by day 5. On the other hand, the work of Pickard (2003) is in agreement with the current trial, in observing full recovery of villus height by day 5 post-weaning. In line with Hampson's findings, degree of atrophy was most pronounced proximately.

Other measurements of villus architecture showed strong regional effects (villus width; P = 0.039, crypt depth; P = <0.001) and these were most pronounced in the proximal and medial regions of the small intestine. With the exception of the day 4 data for the soft-wheat pigs, villus width measurements remained fairly constant throughout the 14 day study and there was no sustained effect of wheat endosperm texture over time. A familiar pattern in crypt depth data was seen for both hard and soft wheat data with values increasing from day 4 up to day 10, before reducing again to day 14. A significant interaction between diet and day (P = <0.001) revealed some variation in the crypt depth data between the two diets. This variation was most noticeable on day 10 where piglets fed the hard endosperm wheat diet experienced greater crypt hypertrophy than those animals fed a soft endosperm wheat diet. Over the trial period, the incidence of scouring was not linked to dietary treatment.

In summary, Trial 3 demonstrates that endosperm texture of raw wheat may have an effect on nutritional value for the post-weaned piglet. The first hypothesis was partly supported in terms of reduced digesta viscosity from the animals fed a soft wheat diet, although no clear effects of endosperm texture influencing starch digestibility were found. The gut morphology data provided some support for the second hypothesis, in that there appeared to be a negative effect of hard endosperm wheat on villus architecture, although this observation would benefit from a repetition of this trial, on a larger scale, to verify the effects seen. Whilst some of the analyses gave inconclusive responses, other data suggest that soft wheat is more beneficial than hard. A logical progression from studying these raw wheats is to examine them under the influence of controlled processing techniques. This was the objective of the subsequent trial that examined the effect of precisely controlled micronising conditions using the same two wheats (variety and batch) as analysed in this study.

CHAPTER 6

Trial 4: Effects of feeding diets containing ground wheat, differing in endosperm texture and processed under different micronising conditions, on digestibility, digesta properties and gut morphology in the post-weaned piglet.

6.1. INTRODUCTION

Micronisation is an intensive, short time, high-temperature heat treatment, traditionally used in the human food and animal feed industries (van Zuilichem *et al.*, 1985). The dietary ingredients are exposed to high intensity infrared radiation which causes rapid internal heating (Lawrence, 1973a). Benefits from using this process include reducing anti-nutritional factors in the feed, and improved starch gelatinisation (Fasina *et al.*, 2001; Wray and Cenkowski, 2002). A consensus of opinion has yet to be established as to whether micronisation of cereal grains is beneficial to pig performance, although the wide variation in processing variables (if reported at all) may account for the disparity in the published reports. The current trial was designed to assess whether an increase in *in vitro* starch gelatinisation (as a result of processing the cereal grain by micronisation) would result in enhanced *in vivo* starch digestion within the small intestine of the young piglet. Trial 4 was designed to evaluate the use of 2 micronised wheats, differing in endosperm texture, when added to the weaned piglet diet.

6.2. OBJECTIVE

The wheats were from the same batches as used in Trial 3. The objective was to examine differences in digestibility, digesta characteristics and gut morphology in weaned piglets fed diets containing micronised wheat (processed using precisely controlled conditions). This was a 2 x 2 factorial experiment, examining endosperm texture (Hard vs. Soft) and degree of micronisation (Low Cook vs. High Cook).

6.3. HYPOTHESES

- Cooking (through micronisation) will enhance the digestibility of starch within the small intestine of the weaned piglet.

- A higher degree of cook will lead to increased gelatinisation of starch, compared against a low degree of cook, thereby improving *in vivo* starch digestibility within the piglet small intestine.
- Response to micronisation will be more beneficial for the soft endosperm wheat.

6.4. METHOD

6.4.1. Animals and housing

For this trial, piglets (n = 44) were weighed and allocated to diets as before.

6.4.2. Diets

Four experimental diets were manufactured on site at Nottingham. Diets contained hard or soft wheat (same type and batch as used in Trial 3) and were subjected to either mild (low cook) or more extreme (high cook) micronising conditions (see Table 6.1 for variables). The two cooking levels were designed to represent the extremes normally encountered when using this process in commercial feed mills and were achieved by varying the belt speed, thereby changing the length of time the grain was exposed to the heat burners. The micronised wheat was then ground through a hammer mill (1.5 – 2 mm screen size) and incorporated into diets, formulated in the same proportions and with identical ingredients as those used in other trials (see Appendix 1).

Table 6.1: Micronising variables used for Trial 4

Wheat/cook level	Raw moisture (g/kg)	Belt speed (m/sec)	Cook time (sec)	Final moisture (g/kg)
Hard/Low Cook	135	10	10	95
Hard/High Cook	135	3	30	50
Soft/Low Cook	146	10	10	107
Soft/High Cook	146	3	30	64

(Burner temp: 250°C, wheat exiting at 90 - 100°C)

6.4.3. Experimental and slaughter procedure

Slaughter procedure and sample collection was carried out as before; four piglets were slaughtered on day zero, with eight piglets (two per diet) slaughtered on each of the subsequent slaughter days (see Table 6.2). Again, insufficient digesta from the 0.25 region of the small intestine in several pigs restricted the amount of laboratory analyses performed.

Table 6.2: Slaughter programme for Trial 4

Group	Day	Number of piglets
1	0	n = 4
2	2	n = 8 (2 per diet)
3	4	n = 8 (2 per diet)
4	6	n = 8 (2 per diet)
5	10	n = 8 (2 per diet)
6	14	n = 8 (2 per diet)

6.4.4. Faecal collection

Eight piglets (two per diet) were assessed over two faecal collection periods; days 1-5 for the first collection, and days 9-13 for the second. Samples were collected and stored as before.

6.5. RESULTS

6.5.1. Gastric ulceration

One piglet (a group 4 animal) was found to exhibit a small area of reddening in one portion of the stomach. Aside from this animal, none of the other piglets showed any evidence of gastric ulceration.

6.5.2. pH

Mean digesta pH figures and analysis of variance are shown in Tables 6.3 and 6.4 respectively. There was a highly significant effect of region (P = < 0.001). Digesta pH followed a typical pattern; low pH in the stomach, rising through the regions of the intestine followed by slightly more acidic conditions in the caecum. The only other significant effect was the interaction between CVR and region (P = < 0.001). There was no effect of diet.

Table 6.3: Mean digesta pH from Trial 4

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	Stomach	3.5						**3.5**
	0.25	6.5						**6.5**
	0.50	6.8						**6.8**
	0.75	74.						**7.4**
	Caecal	5.8						**5.8**
Hard wheat + High Cook	Stomach		4.0	1.8	4.3	4.2	3.1	**3.5**
	0.25		6.5	6.0	6.3	6.3	6.6	**6.3**
	0.50		7.1	6.6	7.1	7.0	6.9	**6.9**
	0.75		7.2	7.5	7.2	7.1	6.9	**7.2**
	Caecal		7.2	6.9	6.8	5.9	6.0	**6.6**
Soft wheat + High Cook	Stomach		3.1	3.6	4.1	2.7	4.1	**3.5**
	0.25		6.7	6.3	6.8	5.9	6.5	**6.4**
	0.50		7.5	6.8	7.0	7.1	6.9	**7.1**
	0.75		7.1	6.9	7.0	7.4	7.1	**7.1**
	Caecal		6.9	6.0	5.9	7.2	6.2	**6.4**
Hard wheat + Low Cook	Stomach		3.3	3.6	3.1	3.4	3.2	**3.3**
	0.25		6.2	6.5	5.6	6.2	6.3	**6.2**
	0.50		6.6	6.8	7.3	6.8	6.6	**6.8**
	0.75		7.3	7.1	7.5	7.5	7.2	**7.3**
	Caecal		6.5	6.3	5.8	6.6	6.0	**6.2**
Soft wheat + Low Cook	Stomach		3.9	4.5	4.1	3.7	3.9	**4.0**
	0.25		6.3	6.2	6.8	6.2	6.3	**6.4**
	0.50		6.5	6.9	6.9	6.9	6.6	**6.8**
	0.75		6.9	6.9	7.2	7.3	7.4	**7.1**
	Caecal		6.3	6.2	6.3	7.0	6.1	**6.4**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n =4)

Table 6.4: Analysis of variance of mean digesta pH

Factor	s.e.d.	P	cv
CVR	0.13	0.681	
CVR*Day	0.15	0.421	
CVR*Diet	0.15	0.403	9.1%
CVR*Region	0.29	**<0.001**	
CVR*Day*Diet	0.21	0.587	
Endosperm	0.08	0.246	
Cook	0.08	0.416	
Endosperm*Cook	0.11	0.280	
Region	0.12	**<0.001**	8.7%
Day	0.12	0.377, 0.491(L), 0.598(Q)	
All other interactions		>0.05	

L = Linear effect Q = Quadratic effect

6.5.3. Viscosity

Mean data and analysis of variance are shown in Tables 6.5 and 6.6. There was a strong correlation between CVR and diet (P = 0.055), with experimental animals exhibiting more viscous digesta than control pigs. All other analyses involving CVR proved significant (P <0.05). No effect of endosperm texture was observed (P = 0.187), but there was an effect of cook level (P = 0.024) with pigs on low cook diets exhibiting more viscous digesta (1.80 vs. 1.59 cP). Regional analysis showed viscosity increased along the tract (P = <0.001). Interactions were found between day and region (P = 0.005), cook and day (P = 0.044(Q), endosperm, cook and day (P = 0.029) and between region, day and endosperm (P = 0.011(L)).

Table 6.5: Mean digesta viscosity (cP) from Trial 4

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	Stomach	0.9						**0.9**
	0.25	1.6						**1.6**
	0.50	1.3						**1.3**
	0.75	1.3						**1.3**
	Caecal	1.0						**1.0**
Hard wheat + High Cook	Stomach		1.2	1.0	1.6	1.4	1.1	**6.3**
	0.25		1.3	0.7	1.4	1.7	1.4	**1.3**
	0.50		1.1	1.9	1.7	1.4	1.3	**1.5**
	0.75		1.9	1.7	1.5	1.9	2.1	**1.8**
	Caecal		1.8	2.9	1.6	4.2	2.0	**2.5**
Soft wheat + High Cook	Stomach		1.1	1.2	1.3	1.5	1.2	**1.3**
	0.25		1.0	1.4	1.3	1.7	1.3	**1.3**
	0.50		1.6	2.1	1.4	1.5	1.1	**1.5**
	0.75		1.4	2.9	1.7	1.0	1.2	**1.7**
	Caecal		2.3	2.2	1.7	1.2	1.8	**1.8**
Hard wheat + Low Cook	Stomach		1.7	1.3	1.1	1.5	1.5	**1.4**
	0.25		1.6	1.2	1.3	1.2	1.1	**1.3**
	0.50		1.9	1.5	2.2	1.5	1.3	**1.7**
	0.75		4.6	2.3	2.1	1.2	2.5	**2.5**
	Caecal		1.7	4.4	2.4	1.3	2.1	**2.4**
Soft wheat + Low Cook	Stomach		1.5	1.6	1.8	1.2	1.3	**1.5**
	0.25		1.4	1.2	1.0	1.5	1.3	**1.3**
	0.50		1.1	1.4	1.1	2.4	1.4	**1.5**
	0.75		2.2	2.6	1.2	1.8	3.3	**2.2**
	Caecal		2.8	3.6	1.3	1.6	2.2	**2.3**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 6.6: Analysis of variance of mean digesta viscosity

Factor	s.e.d.	P	cv
CVR	0.14	**<0.001**	
CVR*Day	0.17	**0.031**	
CVR*Diet	0.16	0.055	
CVR*Region	0.32	**<0.001**	37.5%
CVR*Day*Diet	0.24	**0.017**	
CVR*Day*Region	0.38	**0.001**	
CVR*Day*Diet*Region	0.53	**0.017**	
Endosperm	0.09	0.187	
Cook	0.09	**0.005**	
Endosperm*Cook	0.13	0.869	
Region	0.15	**<0.001**	
Day	0.15	**0.049**, 0.103(L), 0.390(Q)	
Region*Day	0.33	**0.005**, 0.528(L), **0.008(Q)**	38.5%
Region*Cook	0.21	0.167	
Endosperm*Day	0.21	0.525, 0.864(L), 0.671(Q)	
Cook*Day	0.21	0.206, 0.217(L), **0.044(Q)**	
Day*Endosperm*Cook	0.29	**0.029, 0.011(L)**, 0.347(Q)	
Region*Day*Endosperm	0.46	0.084, 0.577(L), **0.044(Q)**	

CVR = control animals versus the rest L = Linear effect Q = Quadratic effect

6.5.4. Starch digestibility

6.5.4.1. Starch digestibility in the small intestine

There was a significant effect of region, with CAD increasing along the tract, from mid to distal section (P <0.001 – Tables 6.7 and 6.8). Starch digestion in the 0.25 region of the intestine was found to be negligible. No effect of day was observed although a significant interaction was found between day and region (P = 0.003). Degree of cook proved significant (P = 0.047), with CAD greater for high cook, compared with low cook processing conditions. There was no observed effect of endosperm texture alone, but there was a significant interaction between endosperm and cook level (P = 0.004) where a low degree of cook improved digestibility for soft wheat but not for hard.

Table 6.7: Mean coefficients of apparent starch digestibility from Trial 4

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Hard Wheat	0.50	0.91	0.48	0.52	0.71	0.67	**0.66**
High Cook	0.75	0.92	0.98	0.92	0.92	0.95	**0.94**
Soft Wheat	0.50	0.87	0.56	0.57	0.75	0.33	**0.62**
High Cook	0.75	0.44	0.87	0.91	0.55	0.67	**0.69**
Hard Wheat	0.50	0.65	0.17	0.55	0.35	0.55	**0.45**
Low Cook	0.75	0.85	0.91	0.67	0.43	0.75	**0.72**
Soft Wheat	0.50	0.77	0.50	0.67	0.34	0.77	**0.61**
Low Cook	0.75	0.61	0.93	0.56	0.83	0.96	**0.78**

0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet)

Table 6.8: Analysis of variance of mean coefficients of apparent starch digestibility

Factor	s.e.d.	P	cv
Day	0.063	0.282, 0.407(L), 0.055(Q)	
Region	0.040	**<0.001**	
Endosperm	0.040	0.645	
Cook	0.040	**0.047**	25.9%
Region*Day	0.089	**0.003**, 0.351(L), 0.166(Q)	
Endosperm*Cook	0.056	**0.004**	
All other interactions		>0.05	

L = Linear effect Q = Quadratic effect

6.5.4.2. Starch digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 6.9. All digestibility coefficients were found to be greater than 0.980. There were no effects of dietary treatment or collection period and no interaction was observed.

Table 6.9: Mean coefficient of total tract apparent digestibility for starch and analysis of variance from Trial 4

Diet	**Days 1-5**	**Days 9-13**
Hard Wheat/High Cook	0.995	0.987
Soft Wheat/High Cook	0.997	0.998
Hard Wheat/Low Cook	0.994	0.999
Soft Wheat/Low Cook	0.993	0.988
Analysis of variance		
Factor	**s.e.d.**	**P**
Diet	0.006	0.582
Collection Period	0.004	0.653
Diet*Collection Period	0.009	0.726

cv
0.9%

(n = 2 pigs per diet for each collection period)

6.5.5. Nitrogen digestibility

6.5.5.1. Nitrogen digestibility in small intestine

Mean CAD for nitrogen and analysis of variance are shown in Tables 6.10 and 6.11. There was a significant effect of endosperm texture, with higher CAD for the hard wheat diet (P = 0.005). Degree of cook was not significant, and no interactions were found between endosperm and cook, endosperm and day or between cook and day (all >0.05). The effect of day proved significant, as did the interaction between endosperm, cook and day (P = 0.040). A highly significant regional effect was also seen (P = <0.001) with coefficients increasing along the tract.

Table 6.10: Mean coefficients of apparent nitrogen digestibility from Trial 4

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Hard Wheat	0.50	0.71	0.09	0.52	0.48	0.45	**0.45**
High Cook	0.75	0.73	0.64	0.67	0.64	0.75	**0.69**
Soft Wheat	0.50	0.59	0.34	0.62	0.10	0.41	**0.41**
High Cook	0.75	0.46	0.57	0.67	0.36	0.59	**0.53**
Hard Wheat	0.50	0.60	0.41	0.75	0.15	0.45	**0.47**
Low Cook	0.75	0.53	0.80	0.69	0.50	0.57	**0.62**
Soft Wheat	0.50	0.30	0.22	0.51	0.05	0.60	**0.34**
Low Cook	0.75	0.18	0.57	0.44	0.42	0.71	**0.46**

0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet)

Table 6.11: Analysis of variance of mean coefficients of apparent nitrogen digestibility

Factor	s.e.d.	P	cv
Day	0.060	**0.003**, 0.916(L), 0.104(Q)	
Region	0.038	**<0.001**	
Endosperm	0.038	**0.005**	
Cook	0.038	0.236	34.2%
Region*Day	0.085	**0.008**, 0.172(L), 0.140(Q)	
Endosperm*Cook	0.054	0.525	
Endosperm*Cook*Day	0.120	**0.040, 0.016(L)**, 0.588(Q)	
All other interactions		>0.05	

L = Linear effect Q = Quadratic effect

6.5.5.2. Nitrogen digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 6.12. There was no effect of dietary treatment or collection period, and no interaction was observed.

Table 6.12: Mean coefficients of total tract apparent digestibility for nitrogen and analysis of variance from Trial 4

Diet	**Days 1-5**	**Days 9-13**
Hard Wheat/High Cook	0.827	0.651
Soft Wheat/High Cook	0.787	0.853
Hard Wheat/Low Cook	0.784	0.885
Soft Wheat/Low Cook	0.750	0.672

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.095	0.524	17.2%
Collection Period	0.067	0.752	
Diet*Collection Period	0.134	0.476	

(n = 2 pigs per diet for each collection period)

6.5.6. Gut Morphology

General patterns of morphology over time are shown in Figure 6.1.

6.5.6.1. Villus height

Mean villus height data and analysis of variance are shown in Tables 6.13 and 6.14. As with Trial 3, there was a significant effect of day (P = <0.001, <0.001(L), <0.001(Q)), with a reduction in villus architecture immediately post-weaning, which recovered as the trial progressed (Figure 6.1a). There was also a strong regional effect with the proximal section of the intestine exhibiting the longest villi (P = <0.001). Significant differences were seen both for endosperm texture (Hard; 295 vs. Soft; 274 microns, P = <0.001) and degree of cook (High cook; 276 vs. Low cook; 293 microns, P = 0.004), with the most beneficial diet appearing to be the hard wheat/low cook combination (P = <0.001). When experimental and control animals (CVR) were compared, all parameters exhibited significant differences (P = <0.001).

6.5.6.2. Villus width

Mean villus width data is shown in Tables 6.15 and 6.16. As with villus height, there appeared to be a short period of atrophy after weaning, followed by regeneration (Figure 6.1b). Although this effect of day was highly significant (P = <0.001, <0.001(L), <0.001(Q)) no regional effects were seen (P = 0.785). Significant differences between endosperm texture (Hard; 87 vs. Soft; 84 microns, P = 0.001) and cook level (High cook; 87 vs. Low cook; 84 microns, P = <0.001) were found, but for villus width the hard wheat/high cook diet appeared to be the most beneficial combination. Again, all parameters comparing control animals were found to yield significant results (P = <0.01).

6.5.6.3. Crypt depth

As shown in Tables 6.17 and 6.18, highly significant dietary effects were found between endosperm texture (Hard; 220 vs. Soft; 203 microns, P = <0.001) and cook level (High cook; 214 vs. Low cook; 209 microns, P = <0.001) with changes in crypt depth morphology appearing to be less severe in the piglets fed the soft wheat diets after day 8

(Figure 6.1c). Significant differences were found for day (P = <0.001, <0.001(L), <0.001(Q)) and also for region (P =<0.001) with the greatest increase in depth running proximal to distal along the intestine. With the exception of CVR alone (control: 203 vs. experimental: 210 microns; P = 0.903), all interactions involving control animals were highly significant (P = <0.001).

6.5.6.4. Villus height to crypt depth ratio

Mean measurements and analyses of variance are shown in Tables 6.19 and 6.20. No effect of endosperm texture was found although degree of cook was highly significant (P = <0.001) with low cook yielding a greater ratio than high cook. Animals fed the hard wheat/low cook dietary combination showed the smallest post-weaning reduction in villus height to crypt depth ratio (Figure 6.1d).

Table 6.13: Mean villus height measurements (μm) from Trial 4; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	0.25	436						**436**
	0.50	302						**302**
	0.75	296						**296**
Hard/ High Cook	0.25		279	225	355	304	318	**296**
	0.50		280	233	332	297	291	**287**
	0.75		255	177	219	236	271	**232**
Soft/ High Cook	0.25		341	277	324	327	308	**315**
	0.50		275	288	292	279	269	**281**
	0.75		241	205	251	268	244	**242**
Hard/ Low Cook	0.25		343	297	387	294	419	**348**
	0.50		343	271	297	316	381	**322**
	0.75		317	254	257	277	298	**280**
Soft/ Low Cook	0.25		296	279	223	265	366	**286**
	0.50		274	268	208	271	344	**271**
	0.75		245	217	207	278	263	**242**

Region = proportion along small intestine (n = 10 piglets per diet, control pigs; n = 4)

Table 6.14: Analysis of variance of mean villus height

Factor	s.e.d.	P	cv
CVR	4.8	**<0.001**	
CVR*Day	5.9	**<0.001**	
CVR*Diet	5.4	**<0.001**	
CVR*Region	7.7	**<0.001**	15.9%
CVR*Day*Diet	7.7	**<0.001**	
CVR*Day*Region	9.3	**<0.001**	
CVR*Diet*Region	8.8	**0.004**	
CVR*Day*Diet*Region	12.8	**<0.001**	
Endosperm	2.6	**<0.001**	
Cook	3.0	**0.004**	
Day	4.7	**<0.001, <0.001(L), <0.001(Q)**	
Region	3.1	**<0.001**	
Endosperm*Cook	4.0	**<0.001**	
Endosperm*Day	6.2	**<0.001**, 0.177(L), 0.952(Q)	
Cook*Day	6.3	**<0.001**	
Endosperm*Region	4.5	0.104	15.6%
Day*Region	7.3	**<0.001**, 0.232 (L),0.641(Q)	
Endosperm*Cook*Day	8.6	**<0.001, <0.001(L)**, 0.330(Q)	
Endosperm*Cook*Region	6.4	**0.004**	
Endosperm*Day*Region	10.2	**<0.001, <0.001(L), <0.001(Q)**	
Cook*Day*Region	10.3	**<0.001, <0.001(L), <0.001(Q)**	
Endosperm*Cook*Day*Region	10.3	0.770, 0.939(L)	

L = Linear effect Q = Quadratic effect

Table 6.15: Mean villus width measurements (μm) from Trial 4; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	0.25	100						100
	0.50	80						80
	0.75	83						83
Hard/ High Cook	0.25		85	80	90	96	99	90
	0.50		77	82	99	104	91	91
	0.75		78	69	87	95	93	84
Soft/ High Cook	0.25		77	78	91	85	88	84
	0.50		78	76	83	77	93	81
	0.75		76	75	101	91	102	89
Hard/ Low Cook	0.25		76	85	84	98	96	88
	0.50		74	70	79	96	104	85
	0.75		71	73	70	88	107	82
Soft/ Low Cook	0.25		81	71	88	86	77	81
	0.50		63	74	103	82	106	86
	0.75		70	73	78	108	90	84

Region = proportion along small intestine (n = 10 piglets per diet, control pigs; n = 4)

Table 6.16: Analysis of variance of mean villus width

Factor	s.e.d.	P	cv
CVR	1.9	**0.002**	21.7%
CVR*Day	2.4	**<0.001**	
CVR*Diet	2.2	**<0.001**	
CVR*Region	3.1	**<0.001**	
CVR*Day*Diet	3.1	**<0.001**	
CVR*Day*Region	3.8	**<0.001**	
CVR*Diet*Region	3.6	**<0.001**	
CVR*Day*Diet*Region	5.2	**<0.001**	
Endosperm	1.1	**0.001**	21.9%
Cook	1.3	**<0.001**	
Day	2.0	**<0.001, <0.001(L), <0.001(Q)**	
Region	1.3	0.785	
Endosperm*Cook	1.7	0.104	
Endosperm*Day	2.6	**0.003**, 0.054(L), 0.252(Q)	
Cook*Day	2.7	**0.017, 0.010(L)**, 0.957(Q)	
Endosperm*Region	1.9	**<0.001**	
Day*Region	3.1	**<0.001, <0.001(L)**, 0.962(Q)	
Endosperm*Cook*Day	3.7	**0.003**, 0.418(L), **<0.001(Q)**	
Endosperm*Cook*Region	2.7	**0.034**	
Endosperm*Day*Region	4.3	**0.003**, 0.223(L), **0.007(Q)**	
Cook*Day*Region	4.3	**<0.001, 0.009(L)**, 0.433(Q)	
Endosperm*Cook*Day*Region	6.1	**<0.001**, 0.573(L)	

L = Linear effect Q = Quadratic effect

Table 6.17: Mean crypt depth measurements (μm) from Trial 4; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	0.25	244						**244**
	0.50	195						**195**
	0.75	172						**172**
Hard/ High Cook	0.25		178	196	258	277	258	**234**
	0.50		195	197	264	240	255	**230**
	0.75		158	168	232	219	223	**200**
Soft/ High Cook	0.25		197	183	259	236	204	**216**
	0.50		165	177	255	210	169	**195**
	0.75		197	161	208	234	215	**203**
Hard/ Low Cook	0.25		184	184	243	271	236	**224**
	0.50		168	174	216	266	235	**212**
	0.75		192	169	191	277	253	**216**
Soft/ Low Cook	0.25		149	183	241	238	235	**209**
	0.50		146	190	210	266	229	**208**
	0.75		140	150	186	242	191	**182**

Region = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 6.18: Analysis of variance of mean crypt depth

Factor	s.e.d.	P	cv
CVR	3.8	0.903	17.3%
CVR*Day	4.6	**<0.001**	
CVR*Diet	4.3	**<0.001**	
CVR*Region	6.1	**<0.001**	
CVR*Day*Diet	6.1	**<0.001**	
CVR*Day*Region	7.3	**<0.001**	
CVR*Diet*Region	7.0	**<0.001**	
CVR*Day*Diet*Region	10.1	**<0.001**	
Endosperm	2.1	**<0.001**	17.3%
Cook	2.4	**<0.001**	
Day	3.8	**<0.001, <0.001(L), <0.001(Q)**	
Region	2.6	**<0.001**	
Endosperm*Cook	3.3	0.808	
Endosperm*Day	5.1	**<0.001, <0.001(L), 0.018(Q)**	
Cook*Day	5.2	**<0.001, <0.001(L),** 0.818 (Q)	
Endosperm*Region	3.7	0.796	
Day*Region	6.0	**<0.001,** 0.411(L), **<0.001(Q)**	
Endosperm*Cook*Day	7.1	**<0.001, 0.001(L),** 0.112(Q)	
Endosperm*Cook*Region	5.3	**<0.001**	
Endosperm*Day*Region	8.4	0.080	
Cook*Day*Region	8.4	**0.002, 0.045(L),** 0.878(Q)	
Endosperm*Cook*Day*Region	11.8	**<0.001, 0.011(L)**	

L = Linear effect Q = Quadratic effect

Table 6.19: Mean villus height to crypt depth ratios from Trial 4; covariate = live weight

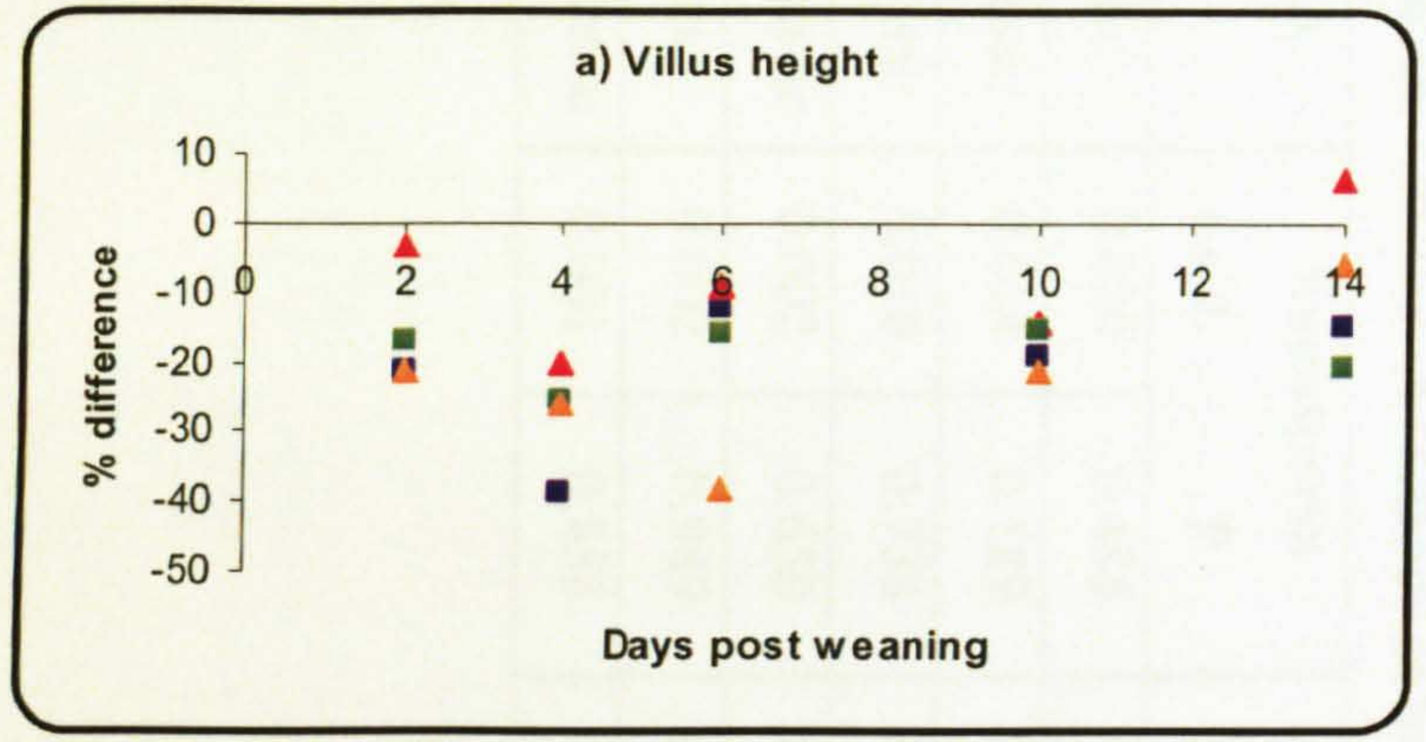
Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (control)	0.25	1.8						**1.8**
	0.50	1.6						**1.6**
	0.75	1.8						**1.8**
Hard/ High cook	0.25		1.6	1.2	1.4	1.1	1.3	**1.3**
	0.50		1.5	1.3	1.3	1.3	1.2	**1.3**
	0.75		1.7	1.1	0.9	1.1	1.2	**1.2**
Soft/ High cook	0.25		1.9	1.5	1.3	1.4	1.6	**1.5**
	0.50		1.7	1.7	1.2	1.3	1.7	**1.5**
	0.75		1.3	1.3	1.3	1.2	1.2	**1.2**
Hard/ Low cook	0.25		1.9	1.7	1.6	1.1	1.8	**1.5**
	0.50		2.2	1.7	1.4	1.3	1.7	**1.6**
	0.75		1.7	1.6	1.4	1.1	1.3	**1.4**
Soft/ Low cook	0.25		2.1	1.6	1.0	1.1	1.6	**1.5**
	0.50		2.0	1.4	1.0	1.0	1.6	**1.4**
	0.75		1.8	1.5	1.2	1.2	1.4	**1.4**

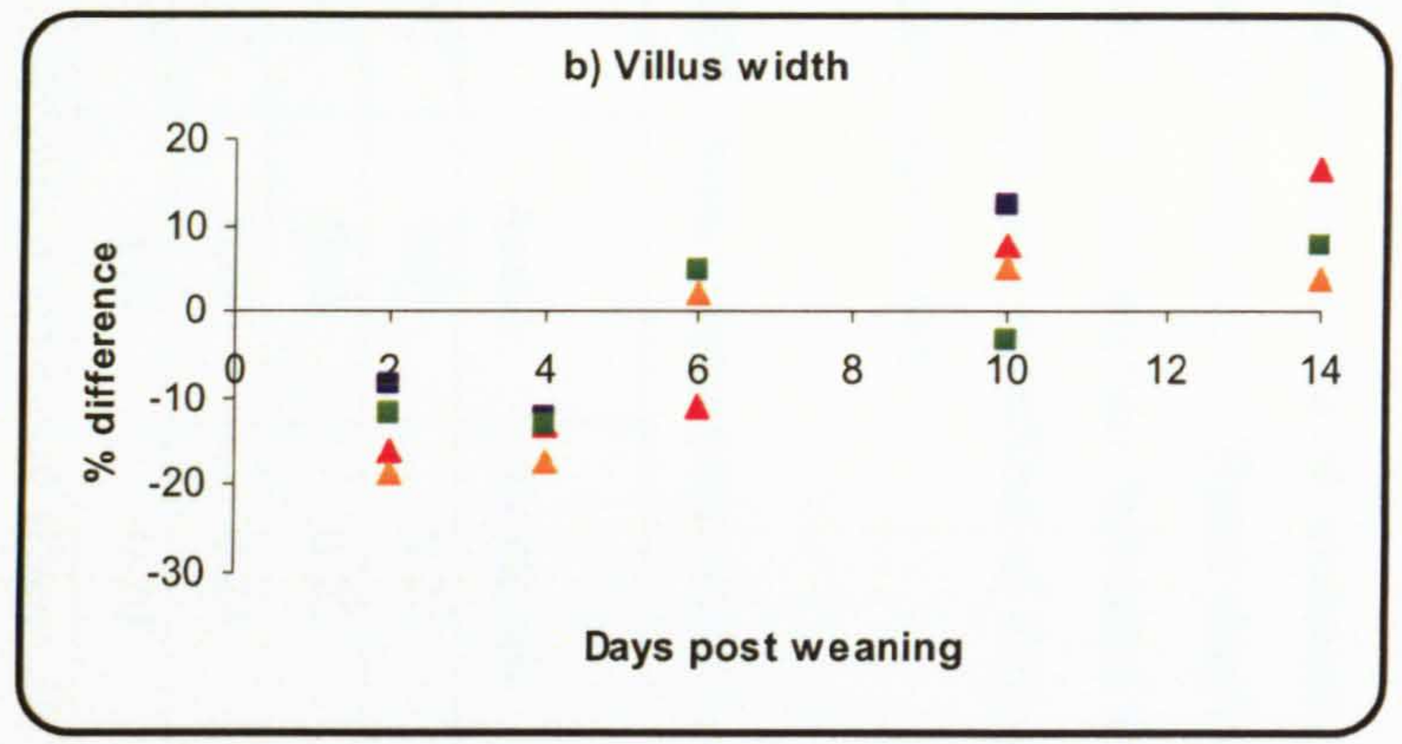
Region = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

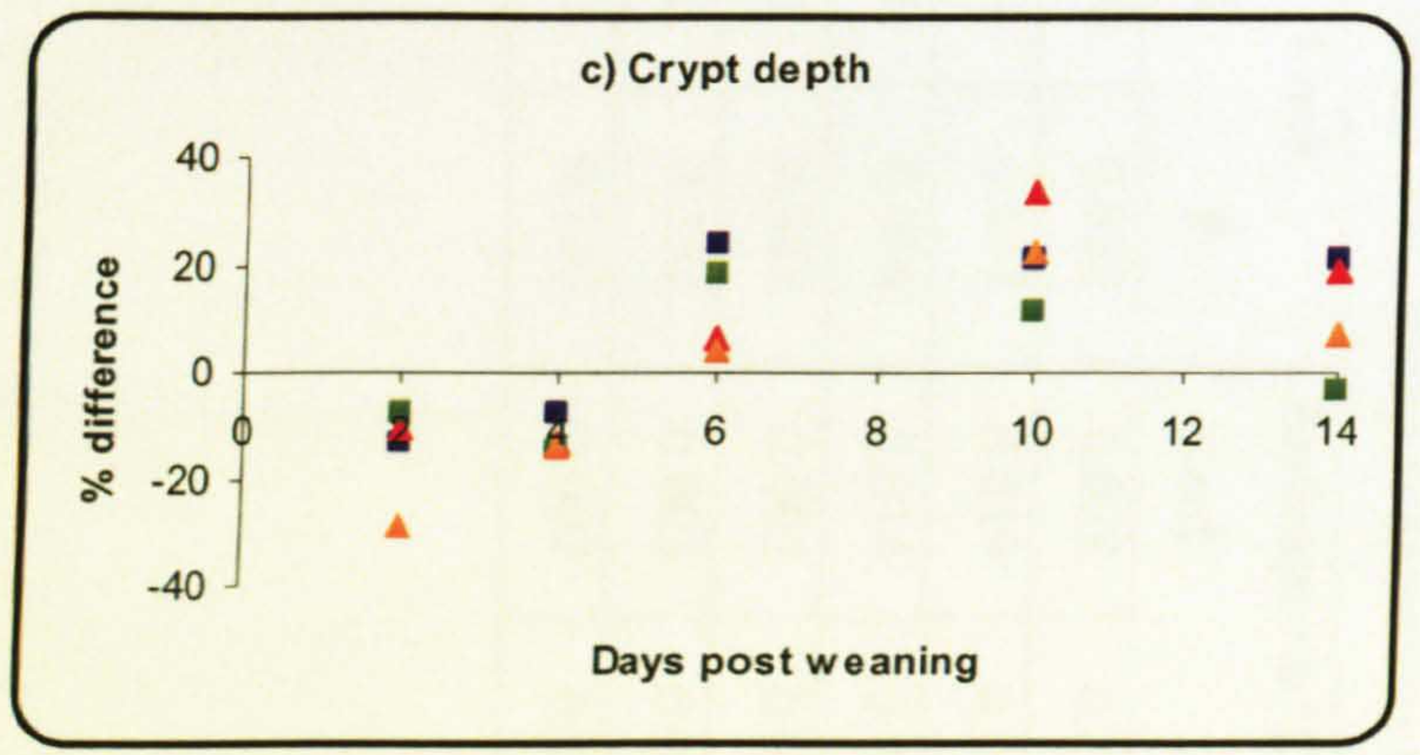
Table 6.20: Analysis of variance of mean villus height to crypt depth ratio

Factor	s.e.d.	P	cv
CVR	0.04	**<0.001**	24.5%
CVR*Day	0.05	**<0.001**	
CVR*Diet	0.04	**<0.001**	
CVR*Region	0.06	**<0.001**	
CVR*Day*Diet	0.06	**<0.001**	
CVR*Day*Region	0.07	0.069	
CVR*Diet*Region	0.07	**<0.001**	
CVR*Day*Diet*Region	0.08	**<0.001**	
Endosperm	0.02	0.362	25.0%
Cook	0.02	**<0.001**	
Day	0.04	**<0.001, <0.001(L), <0.001(Q)**	
Region	0.03	**0.020**	
Endosperm*Cook	0.03	**<0.001**	
Endosperm*Day	0.05	**<0.001**, 0.139(L), **0.005(Q)**	
Cook*Day	0.05	**<0.001, <0.001(L), <0.001(Q)**	
Endosperm*Region	0.04	0.518	
Day*Region	0.06	0.068, 0.984(L), **0.003(Q)**	
Endosperm*Cook*Day	0.07	**<0.001**, 0.992(L), **0.023(Q)**	
Endosperm*Cook*Region	0.05	**<0.001**	
Endosperm*Day*Region	0.08	**<0.001**, 0.369(L), **<0.001(Q)**	
Cook*Day*Region	0.08	**0.024**, 0.628(L), **0.006(Q)**	
Endosperm*Cook*Day*Region	0.01	**0.007**, 0.603(L)	

L = Linear effect Q = Quadratic effect







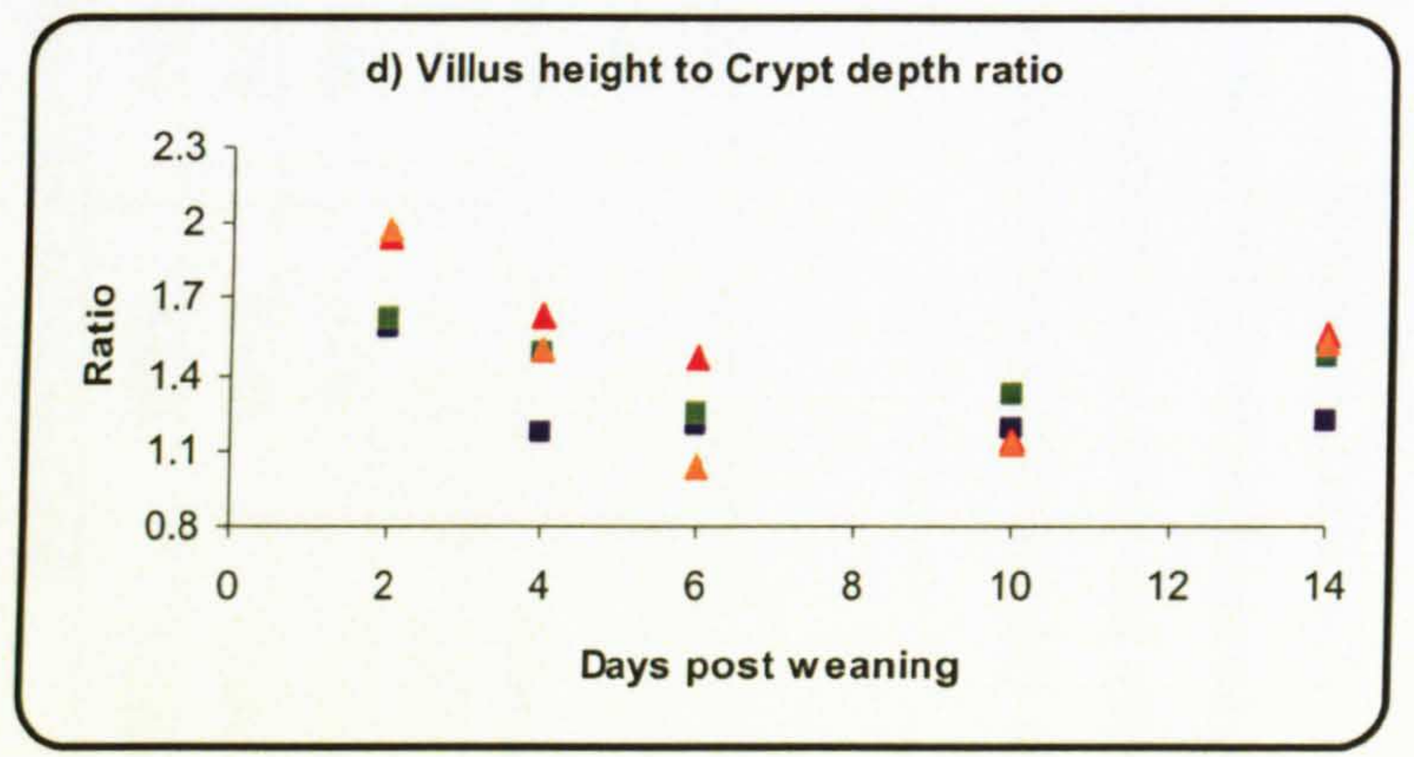


Figure 6.1: *Effect of micronised wheats on mean gut morphology measurements (covariate = live weight; n = 10 pigs per diet). (■) Hard Wheat/High Cook, (▲) Hard Wheat/Low Cook, (■) Soft Wheat/High Cook, (▲) Soft Wheat/Low Cook*

6.5.7. Caecal VFA analysis

Mean molar proportions of measured VFAs are shown in Table 6.21 with analysis of variance in Table 6.22. There was no significant effect of endosperm texture or cook level, and no interaction between the two factors was observed (Table 6.22). In addition, there was no effect of day, and no interaction involving day was found to be statistically significant.

Table 6.21: Mean molar proportions of caecal VFAs from Trial 4

Diet	Acetic	Propionic	n-Butyric	Iso-Butyric	Valeric	Iso-Valeric
HW/HC	0.69	0.22	0.07	0.005	0.010	0.005
SW/HC	0.68	0.22	0.08	0.005	0.010	0.005
HW/LC	0.68	0.24	0.08	0.003	0.008	0.002
SW/LC	0.71	0.20	0.08	0.004	0.008	0.003

(HW = Hard wheat, SW= Soft Wheat, HC = High Cook, LC=Low Cook)

Table 6.22: Analysis of variance of mean caecal VFA molar proportions for Trial 4

VFA	Endosperm		Cook		Endosperm*Cook		cv%
	s.e.d.	P	s.e.d.	P	s.e.d.	P	
Acetic	0.020	0.485	0.020	0.632	0.028	0.315	9.1
Propionic	0.014	0.189	0.014	0.677	0.019	0.175	19.6
n-Butyric	0.010	0.733	0.010	0.866	0.014	0.775	41.1
Iso-Butyric	0.002	0.650	0.002	0.382	0.002	0.685	118.6
Valeric	0.002	0.989	0.002	0.352	0.003	0.969	71.3
Iso-Valeric	0.001	0.858	0.001	0.143	0.002	0.536	115.2

6.5.8. Performance Parameters

6.5.8.1. Feed Intake

0-5 days: As expected, intakes increased with time (P = <0.001; <0.001(L), <0.001(Q)). There was no significant effect of wheat endosperm texture or cook level, although there was a significant interaction (P = 0.018 – Table 6.23). Piglets on the soft wheat/high cook diet exhibited the highest DE intakes over this initial period of the trial. Intakes after 5 days were found to increase linearly over time (P = 0.012 – Table 6.24) although there was no observed effect of endosperm texture during this time period. Degree of cook was highly significant, with piglets on low cook diets exhibiting greater mean daily DE intakes than those on the high cook diets (8.87 vs. 5.86 MJ/d; P = <0.001). A significant interaction between endosperm texture and cook level was also seen (P = 0.010) with piglets appearing to prefer the hard wheat/low cook combination during this stage of the trial (Table 6.24).

Table 6.23: Mean daily DE intake 0-5 days (MJ) and analysis of variance from Trial 4

	Days post-weaning						
Diet	**0**	**1**	**2**	**3**	**4**	**5**	**Mean**
HW/HC	0.25	2.18	4.11	4.57	4.59	4.38	**3.35**
SW/HC	0.42	2.99	4.89	4.46	5.59	5.84	**4.03**
HW/LC	0.25	2.39	3.29	4.05	5.81	6.16	**3.66**
SW/LC	0.19	1.14	3.21	4.27	4.54	5.41	**3.13**

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Day	0.44	**<0.001, <0.001(L), <0.001(Q)**	42.9%
Endosperm	0.25	0.767	
Cook	0.25	0.243	
Endosperm*Cook	0.36	**0.018**	
All other interactions		>0.05	

(HW = Hard wheat, SW= Soft Wheat, HC = High Cook, LC=Low Cook)
L = Linear effect Q = Quadratic effect (n = 6 pigs per diet)

Table 6.24: Mean DE intake post 5 days (MJ) and analysis of variance from Trial 4

	Days post-weaning								
Diet	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**Mean**
HW/HC	5.05	5.19	5.64	4.27	4.14	4.85	4.63	5.61	**4.92**
SW/HC	4.80	5.16	5.17	6.67	7.85	9.09	7.68	8.01	**6.80**
HW/LC	7.88	8.42	8.42	10.83	10.37	11.10	11.07	12.60	**10.09**
SW/LC	6.36	4.80	6.65	7.83	7.93	8.36	9.75	9.55	**7.65**

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Day	1.54	0.394, **0.012(L)**, 0.978(Q)	
Endosperm	0.77	0.721	
Cook	0.77	**<0.001**	41.7%
Endosperm*Cook	1.09	**0.010**	
All other interactions		>0.05	

(HW = Hard wheat, SW= Soft Wheat, HC = High Cook, LC=Low Cook)
L = Linear effect Q = Quadratic effect (n = 2 pigs per diet)

6.5.8.2. DLWG

Data are summarised in Table 6.25. Over the trial period, DLWG was found to significantly increase with time (P = <0.001, <0.001(L), <0.001(Q)) with all pigs gaining weight by day 4 post-weaning after initial weight loss. There was no observed effect of endosperm hardness but there was a significant difference between cook levels (low cook 0.193 vs. high cook 0.107; P = 0.022). No interaction was found between endosperm texture and cook level (P = 0.073) although a significant interaction was evident between cook level and day (P = 0.022).

Table 6.25: Mean DLWG (Kg) and analysis of variance from Trial 4

Diet	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
HW / HC	-0.10	0.03	0.23	0.11	0.12	**0.08**
SW / HC	-0.28	0.16	0.40	0.17	0.24	**0.14**
HW / LC	-0.05	0.24	0.15	0.30	0.49	**0.23**
SW / LC	-0.18	0.19	0.16	0.30	0.33	**0.16**

Analysis of variance

Factor	s.e.d.	P	cv
Day	0.054	**<0.001, <0.001(L), <0.001(Q)**	72.5%
Endosperm	0.034	0.990	
Cook	0.034	**0.022**	
Day*Cook	0.077	**0.022,** 0.069(L), 0.094(Q)	
All other interactions		>0.05	

(HW = Hard wheat, SW= Soft Wheat, HC = High Cook, LC=Low Cook)
L = Linear effect Q = Quadratic effect (n = 10 pigs per diet)

6.6. SUMMARY OF RESULTS FROM TRIAL 4.

It appears from the data that the soft wheat/high cook diet was the most palatable for the first 5 days following weaning. After this time, there appeared to be a switch in preference to the hard wheat/low cook dietary combination, which was evident for the remainder of the trial period. It is possible that this dietary observation could have been a chance effect, due to the high variability in feed intake typically seen in piglets in the first few days after weaning (Bark *et al.*, 1986; Le Dividich and Herpin, 1994) as appetite would take a few days to stabilise. The highest feed intakes after 5 days by pigs on the hard wheat/low cook diet would explain why these animals also exhibited the greatest DLWGs during the trial. Feed intake was not affected by wheat endosperm texture but there was a significant preference for a low cook diet after 5 days.

The current trial shows some support for the hypothesis that cooking (through micronisation) of wheat enhances starch digestibility for the young piglet; micronisation improved digestibility of starch in the 0.5 region of the small intestine shortly after weaning. A reduction in starch digestion was observed around day 4 in this section of the tract, although it did not appear as severe as that seen with raw wheat in Trial 3. There was also evidence that degree of cook influenced nutritional value of wheat; a high level of cook resulted in higher CAD for starch and less viscous digesta. The DLWG data appears to complicate this evidence however, as the pigs fed the low cook diets grew the fastest around this time (Table 6.25).

The effects on gut morphology in response to diet were somewhat unclear; villus atrophy occurred in the period shortly after weaning with the greatest reduction in villus height seen on day 4 for all diets except the soft wheat/low cook (greatest atrophy on day 6). An interesting observation was that only those animals on the hard wheat/low cook diet had villi that recovered to pre-weaning levels by the end of the trial period. This could be attributed to the high appetites of this group as there is strong evidence linking feed consumption with mucosal integrity and villus atrophy in weaned piglets (McCracken *et al.*, 1999; Spreeuwenberg *et al.*, 2001). A reduction in villus width was

seen in all pigs, regardless of diet during the initial four days of the trial, before a gradual and sustained increase in width was observed for the rest of the trial period. Crypt depth measurements followed a typical pattern whereby a significant increase was not appreciably seen until around day 5 post weaning (Hampson, 1986 as cited by Miller and Slade, 2003). Again the graph showing the interaction between diet and day (Figure 6.1c) revealed an S-shaped curve over time with the highest values seen on day 10 post-weaning, followed by a reduction in crypt hyperplasia by day 14 of the trial. A dietary influence on crypt depth morphology was evident in this trial, with hypertrophy appearing less severe for the soft wheat diets, irrespective of cook level. There was a general trend for villus height to crypt depth ratios to decrease, although the period of stability usually observed with this gut parameter after day 6 (Miller and Slade, 2003) was less evident, due mainly to the severe atrophy seen in the villus height data.

Strong regional effects for both nitrogen and starch digestion were seen, with CAD increasing along the small intestine from mid (0.5) to distal (0.75) regions. The current trial showed little evidence to support the hypothesis that response to micronising was more beneficial for wheat of soft endosperm texture; feed intakes, DLWG, starch digestibility (in small intestine and over total tract), digesta pH and viscosity all showed no significant effect ($P > 0.05$). It is difficult to establish any firm conclusions regarding the effect of endosperm texture on gut morphology; although significant interactions were determined between endosperm texture and day for all gut morphology parameters, the results are somewhat contradictory; hard endosperm wheat appears better than soft at a low level of cook for the villus height data, whereas soft wheat appears more beneficial for the crypt depth data. Overall CAD for nitrogen in the small intestine was significantly higher for pigs fed the hard wheat diet but any significance was lost when the effect of day was added to the analysis. As with other trials, any incidence of scouring was not found to be linked to dietary treatment.

In summary, Trial 4 appears to demonstrate that the use of micronisation can enhance starch digestibility in wheat for the young piglet. Micronising increased intestinal starch digestibility and degree of cook was an important variable, although endosperm texture

appeared less significant. Despite these observed improvements in starch digestibility in the small intestine however, any benefit was not found to be manifested in the animal performance data. This finding is in agreement with similar piglet studies examining the effectiveness of micronisation (Zarkadas and Wiseman, 2001, 2002). Taking the gross energy (GE) content of starch to be around 17.7 MJ/kg (McDonald *et a*l., 1995), an apparent digestibility of 0.4 would result in a digestible energy (DE) content of 7.1 MJ/kg. For an apparent digestibility of 0.5, this would result in a DE of 8.9 MJ/kg. These calculations suggest that despite an improvement in the digestibility coefficients, the relatively small increase in DE value associated with this improved digestibility would mean that performance improvements would be unlikely. Of more importance is the improved apparent digestibility of starch in the small intestine as a result of processing, resulting in less starch entering the hind-gut for fermentation. The topic of rapidly vs. slowly digestible starch in the piglet digestive tract is discussed in further detail in Chapter 8.

In order to assess further the effect of technological processing on wheat nutritive value, the next trial (Trial 5) was designed to examine the use of precisely controlled extrusion conditions using the same two wheat cultivars.

CHAPTER 7

Trial 5: Effects of feeding diets containing ground wheat, differing in endosperm texture and processed under different extrusion conditions, on digestibility, digesta properties and gut morphology in the post-weaned piglet.

7.1. INTRODUCTION

Extrusion processing uses the application of heat and pressure to dissipate energy through a feed material using frictional forces from a flighted screw, rotating in a tightly fitting barrel. The mechanical input used is termed Specific Mechanical Energy (SME) and shear forces arising from this process tear apart starch granules and break the 1,4 molecular bonds leading to a loss of crystallinity (Chiang and Johnson, 1977; Kokini *et al.*, 1992). Studies examining the extrusion of beans (Alonso *et al.*, 2000) and cereals (Anguita *et al.*, 2006), have reported benefits including improved *in-vitro* digestibility of starch, when compared with raw ingredients. As mentioned in Chapter 1, there is some disparity concerning the effectiveness of extrusion processing in piglet trials; whilst some studies have reported improved performance effects from feeding an extruded feed (Medel *et al.*, 1999; Sauer *et al*, 1990), others have found no benefits (Hongtrakul *et al.*, 1998). The lack of description of processing variables cited in some of the published literature makes evaluation of the process difficult. Because of this, Trial 5 was designed to evaluate the use of precisely processed extruded wheats of differing endosperm texture, when employed in diets for weaned piglets.

7.2. OBJECTIVE

Wheats were from the same batches as those used in Trials 3 and 4. The objective of Trial 5 was to examine differences in digestibility, digesta characteristics and gut morphology in weaned piglets fed diets containing extruded wheats (processed under precisely controlled cooking conditions). This trial examined the effect of endosperm texture (Hard vs. Soft) and degree of extrusion (Raw vs. Low SME vs. High SME).

7.3. HYPOTHESES

- Extrusion of wheat will enhance digestibility of starch within the small intestine of the newly-weaned piglet.
- A higher degree of extrusion will lead to enhanced gelatinisation of starch, compared against a lower extrusion cook level, thereby increasing *in vivo* starch digestibility.
- Response to extrusion will be more beneficial for the soft endosperm wheat

7.4. METHOD

7.4.1. Animals and housing

Piglets (n = 44) were individually weighed and housed as before.

7.4.2. Diets

Diets were manufactured on site at Nottingham. Three contained hard or soft wheat, subjected to mild or more extreme extrusion conditions (See Table 7.1). Raw soft wheat (same batch and variety as Trials 3 & 4), was used as a control to make a fourth diet (due to limited animal housing facilities, a hard wheat/low cook diet was not studied). Wheats were ground (screen size 1.5 – 2 mm) and incorporated into the diets with identical ingredients as before.

Table 7.1: Extrusion variables used for Trial 5

	Soft Wheat Low Cook	Soft Wheat High Cook	Hard Wheat High Cook
Screw speed (rpm)	202	401	401
Moisture* (g/kg)	262	205	197
Die Temp (°C)	134	155	154.5
SME ($W.h.kg^{-1}$)	103.3	158.9	161.9

** During processing (water added)*

SME is the measure of mechanical energy input per unit weight of material in the extrusion process and is calculated by using the following equation:

$$\text{SME (W.h.kg}^{-1}\text{)} = \frac{\text{screw torque(N.m) x screw speed(r.p.m.) x 2 x } \pi \text{ x number of screws}}{\text{Mass flow rate (kg.h}^{-1}\text{) x 60}}$$

The units of SME are $J.kg^{-1}$ or $Wh.kg^{-1}$ ($1\ Wh.kg^{-1} = 3.6\ kJ.kg^{-1}$)

7.4.3. Experimental and slaughter procedure

Slaughter procedure along with collection of tissue and digesta samples, were carried out as in previous trials. Insufficient digesta from the 0.25 region of the small intestine again restricted some analyses.

7.4.4. Faecal collection

Faecal samples from eight piglets (two per diet) were collected for each collection period as before.

7.5. RESULTS

Analysis of variance was used to compare control against experimental pigs (CVR; n = 44), the effect of endosperm texture (hard wheat/high SME vs. soft wheat/high SME; n = 20) and degree of cook (soft raw wheat vs. soft wheat/low SME vs. soft wheat/high SME; n = 30) in separate analyses.

7.5.1. Gastric ulceration

At slaughter, none of the stomachs showed any evidence of gastric ulceration.

7.5.2. pH

Mean digesta pH figures and analysis of variance are shown in Tables 7.2 and 7.3. No effect of endosperm texture or cook level was evident. Region was found to exhibit a highly significant effect (P = <0.001). For the pigs on the soft wheat diets, there was also a significant interaction between day and region (P = 0.024, 0.008(L), 0.027(Q)). None of the other parameters showed significance.

Table 7.2: Mean digesta pH from Trial 5

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	Stomach	3.2						**3.2**
	0.25	6.4						**6.4**
	0.50	6.9						**6.9**
	0.75	7.4						**7.4**
	Caecal	6.1						**6.1**
Soft wheat + Raw	Stomach		3.4	2.9	4.3	3.6	3.2	**3.5**
	0.25		6.2	6.4	7.2	6.6	6.2	**6.5**
	0.50		6.6	6.7	7.0	7.0	7.0	**6.9**
	0.75		7.8	7.2	7.2	7.4	7.3	**7.4**
	Caecal		6.4	5.3	6.1	6.1	5.8	**5.9**
Soft wheat + Low SME	Stomach		1.7	3.3	3.1	4.1	3.7	**3.2**
	0.25		6.5	6.0	6.9	6.5	6.5	**6.5**
	0.50		7.0	7.0	7.0	7.0	7.0	**7.2**
	0.75		7.7	8.0	7.7	7.6	7.6	**7.7**
	Caecal		7.2	5.6	6.3	5.8	6.0	**6.2**
Hard wheat + High SME	Stomach		3.1	3.1	2.7	3.7	2.9	**3.1**
	0.25		6.4	6.7	6.7	6.9	6.4	**6.6**
	0.50		6.9	7.0	7.3	7.7	6.7	**7.2**
	0.75		7.8	7.9	7.9	7.3	7.0	**7.6**
	Caecal		6.2	5.8	6.1	6.3	6.5	**6.2**
Soft wheat + High SME	Stomach		2.6	2.2	3.0	3.6	3.3	**2.9**
	0.25		6.7	6.2	6.7	7.7	6.4	**6.7**
	0.50		6.9	7.0	7.0	7.3	6.8	**7.0**
	0.75		8.0	8.0	7.7	6.7	7.8	**7.6**
	Caecal		6.4	6.5	6.4	5.8	5.8	**6.2**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 7.3: Analysis of variance of mean digesta pH

Factor	s.e.d.	P	cv
CVR	0.15	0.570	10.2%
CVR*Day	0.17	0.157	
CVR*Diet	0.16	0.879	
CVR*Region	0.33	**<0.001**	
CVR*Day*Diet	0.24	0.975	
CVR*Day*Region	0.38	0.063	
CVR*Diet*Region	0.37	0.723	
Endosperm	0.13	0.893	10.9%
Day	0.21	0.584, 0.893(L), 0.229(Q)	
Endosperm*Day	0.30	0.971, 0.948(L), 0.632(Q)	
Region	0.21	**<0.001**	
Endosperm*Region	0.30	0.949	
All other interactions		>0.05	
Cook	0.12	0.736	9.5%
Day	0.15	0.174, 0.700(L), 0.193(Q)	
Cook*Day	0.26	0.938, 0.780(L), 0.900(Q)	
Region	0.15	**<0.001**	
Cook*Region	0.26	0.355	
Day*Region	0.33	**0.024, 0.008(L), 0.027(Q)**	
All other interactions		>0.05	

CVR = Control animals versus the rest L = Linear effect Q = Quadratic effect

7.5.3. Viscosity

Mean viscosity figures are shown in Table 7.4 and analysis of variance in Table 7.5. Experimental pigs had more viscous digesta than control pigs (P = 0.029), in all regions of the tract (P = <0.001). Endosperm texture was highly significant; pigs on soft wheat diets had less viscous digesta than those on hard wheat (P = <0.001) for at least the first 10 days post-weaning (P =<0.001, <0.001(L), <0.001(Q)). There was also an interaction between endosperm and region for the pigs on the high cook diets, with animals fed soft wheat exhibiting less viscous digesta for all regions of the digestive tract, except the 0.25 portion of the small intestine (P = 0.003). For the pigs on soft wheat diets, there appeared to be an inverse relationship between cook level and digesta viscosity with a high cook yielding less viscous digesta than low cook, which in turn was less viscous than the raw wheat diet (P = 0.041). Viscosity showed a similar regional pattern for all pigs with digesta becoming less viscous after leaving the stomach, and then increasing along the tract with the highest values recorded in the caecum (P = <0.001).

Table 7.4: Mean digesta viscosity (cP) from Trial 5

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	Stomach	0.9						**0.9**
	0.25	1.3						**1.3**
	0.50	1.1						**1.1**
	0.75	1.9						**1.9**
	Caecal	1.8						**1.8**
Soft wheat Raw	Stomach		1.5	1.1	1.1	1.2	1.0	**1.2**
	0.25		1.3	1.0	1.2	1.4	0.9	**1.2**
	0.50		2.0	4.0	1.7	1.6	1.3	**2.1**
	0.75		5.1	2.9	2.0	2.2	2.0	**2.8**
	Caecal		3.2	2.9	2.5	2.6	4.1	**3.1**
Soft wheat + Low SME	Stomach		1.6	1.4	1.1	1.8	1.1	**1.4**
	0.25		1.3	1.2	1.2	1.0	1.3	**1.2**
	0.50		1.3	0.9	1.9	1.5	1.8	**1.5**
	0.75		2.1	4.4	2.4	3.9	3.1	**3.2**
	Caecal		1.6	1.9	3.1	5.1	2.8	**2.9**
Hard wheat + High SME	Stomach		1.7	1.7	1.8	1.7	1.3	**1.6**
	0.25		1.1	1.3	1.5	1.6	0.9	**1.3**
	0.50		1.6	1.5	1.4	2.0	0.8	**1.5**
	0.75		1.6	3.1	6.0	1.7	1.0	**2.7**
	Caecal		3.1	2.7	3.9	0.7	3.6	**2.8**
Soft wheat + High SME	Stomach		1.1	1.1	1.2	1.1	1.2	**1.1**
	0.25		1.8	1.0	1.4	1.4	1.0	**1.3**
	0.50		1.7	1.3	1.4	0.9	1.4	**1.3**
	0.75		1.5	1.1	1.6	1.6	2.4	**1.6**
	Caecal		2.0	0.9	2.1	1.6	3.3	**2.0**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 7.5: Analysis of variance of mean digesta viscosity

Factor	s.e.d.	P	cv
CVR	0.26	0.066	
CVR*Day	0.30	0.910	
CVR*Diet	0.29	**0.029**	
CVR*Region	0.58	**<0.001**	59.6%
CVR*Day*Diet	0.43	**0.041**	
CVR*Day*Region	0.67	0.735	
CVR*Diet*Region	0.65	0.451	
Endosperm	0.09	**<0.001**	
Day	0.14	**<0.001**, 0.295(L), 0.349(Q)	
Endosperm*Day	0.20	**<0.001, <0.001(L),**	
Region	0.14	**<0.001(Q)**	25.8%
Endosperm*Region	0.20	**<0.001**	
Day*Region	0.32	**0.003**	
Endosperm*Day*Region	0.45	**<0.001**, 0.120(L), **<0.001(Q)**	
		<0.001, 0.002(L), <0.001(Q)	
Cook	0.25	**0.041**	
Day	0.33	0.961, 0.823(L), 0.676(Q)	
Cook*Day	0.57	0.316, 0.158(L), 0.167(Q)	68.3%
Region	0.33	**<0.001**	
All other interactions		>0.05	

CVR = control animals versus the rest L = Linear effect Q = Quadratic effect

7.5.4. Starch digestibility

7.5.4.1. Starch digestibility in the small intestine

Mean CAD for starch is shown in Table 7.6 with analysis of variance in Table 7.7. For all pigs, starch digestion was significantly affected by tract region, with coefficients increasing along the small intestine. There was a highly significant effect of wheat endosperm texture, with greater coefficients for soft wheat than for hard (P = <0.001). This difference was evident in both the mid (0.50) and distal (0.75) tract regions (P = 0.001). There was a positive relationship between starch digestion and cook level with a higher degree of cook yielding greater coefficients (P = <0.001). A significant interaction between cook level and region (P = 0.021) showed that this difference in digestibility, due to cooking, was evident in both mid and distal regions of the small intestine.

Table 7.6: Mean coefficients of apparent starch digestibility

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Soft Wheat	0.50	0.05	0.04	0.94	0.49	0.43	**0.39**
Raw	0.75	0.77	0.84	0.73	0.89	0.81	**0.81**
Soft Wheat	0.50	0.96	0.75	*	0.60	0.89	**0.80**
Low SME	0.75	0.98	0.98	0.96	0.97	0.97	**0.97**
Hard Wheat	0.50	0.97	0.69	0.99	0.78	0.59	**0.80**
High SME	0.75	0.98	0.99	0.98	0.98	0.90	**0.97**
Soft Wheat	0.50	0.92	0.97	0.97	0.97	0.97	**0.96**
High SME	0.75	0.99	0.99	0.97	0.93	0.98	**0.97**

0.50 & 0.75 = proportion along small intestine * *= Insufficient digesta (n = 10 pigs per diet)*

Table 7.7: Analysis of variance of mean coefficients of apparent starch digestibility

Factor	s.e.d.	P	cv
Endosperm	0.006	**<0.001**	
Day	0.010	**<0.001, <0.001(L), <0.001(Q)**	
Region	0.006	**<0.001**	
Endosperm*Day	0.014	**<0.001, <0.001(L), <0.001(Q)**	2.1%
Endosperm*Region	0.009	**<0.001**	
Day*Region	0.014	**<0.001, <0.001(L), <0.001(Q)**	
Endosperm*Day*Region	0.020	**<0.001, <0.001(L), 0.014(Q)**	
Cook	0.067	**<0.001**	
Day	0.087	0.253, 0.547(L), 0.343(Q)	
Region	0.055	**0.004**	
Cook*Day	0.151	0.393, 0.323(L), 0.153(Q)	25.7%
Cook*Region	0.095	**0.021**	
All other interactions		>0.05	

L = Linear effect Q = Quadratic effect

7.5.4.2. Starch digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 7.8. Coefficients for all diets were found to be greater than 0.990. There was no effect of diet or collection period and no interaction was observed.

Table 7.8: Mean coefficient of total tract apparent digestibility for starch and analysis of variance from Trial 5

Diet	Days 1-5	Days 9-13
Soft Wheat/Raw	0.994	0.993
Soft Wheat/Low SME	0.996	0.995
Hard Wheat/High SME	0.997	0.998
Soft Wheat/High SME	0.997	0.995

Analysis of variance

Factor	s.e.d.	P	cv
Diet	0.001	0.071	0.2%
Collection Period	0.001	0.433	
Diet*Collection Period	0.002	0.378	

(n = 2 pigs per diet for each collection period)

7.5.5. Nitrogen digestibility

7.5.5.1. Nitrogen digestibility in the small intestine

Mean CAD for nitrogen is shown in Table 7.9, with analysis of variance in Table 7.10. Although not significant, there was a strong tendency for raw wheat to be more beneficial than cooked (P = 0.056). There were no significant effects of endosperm texture or tract region. The only apparent statistical significance was for the pigs fed on soft wheat diets, where there was a quadratic effect of day (P = 0.045). No significant interactions were determined between any of the factors tested (all P >0.05).

Table 7.9: Mean coefficients of apparent nitrogen digestibility

Diet	Region	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
Soft Wheat	0.50	0.33	0.57	0.54	0.57	0.55	**0.51**
Raw	0.75	0.72	0.57	0.81	0.86	0.57	**0.70**
Soft Wheat	0.50	0.43	0.24	0.36	0.65	0.45	**0.42**
Low SME	0.75	0.53	0.59	0.45	0.52	*	**0.52**
Hard Wheat	0.50	0.52	0.55	0.25	0.59	0.63	**0.51**
High SME	0.75	0.49	0.66	0.53	0.63	0.52	**0.57**
Soft Wheat	0.50	0.10	0.42	0.42	0.64	0.67	**0.45**
High SME	0.75	0.43	0.51	0.53	0.72	0.72	**0.58**

** = Insufficient digesta 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet)*

Table 7.10: Analysis of variance of mean coefficients of apparent nitrogen digestibility

Factor	s.e.d.	P	cv
Endosperm	0.074	0.748	45.0%
Day	0.117	0.238, 0.078(L), 0.529(Q)	
Region	0.074	0.249	
All interactions		>0.05	
Cook	0.069	0.056	41.7%
Day	0.089	0.134, 0.115(L), **0.045(Q)**	
Region	0.056	0.073	
All interactions		>0.05	

L = Linear effect Q = Quadratic effect

7.5.5.2. Nitrogen digestibility over total tract

Mean CTTAD values and analysis of variance are shown in Table 7.11. Digestibility coefficients for all diets were greater than 0.80. No significant dietary effect was observed and no differences between collection periods were seen. There was no interaction between diet and collection period.

Table 7.11: Mean coefficient of total tract apparent digestibility for nitrogen and analysis of variance from Trial 5

Diet	**Days 1-5**	**Days 9-13**
Soft Wheat/Raw	0.849	0.856
Soft Wheat/Low SME	0.826	0.802
Hard Wheat/High SME	0.839	0.902
Soft Wheat/High SME	0.853	0.872

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Diet	0.021	0.116	3.5%
Collection Period	0.015	0.311	
Diet*Collection Period	0.030	0.299	

(n = 2 pigs per diet for each collection period)

7.5.6. Gut morphology

General patterns of gut morphology over time are shown in Figure 7.1.

7.5.6.1. Villus height

Mean villus height data and analysis of variance are shown in Tables 7.12 and 7.13 respectively. For all pigs, the longest villi were found within the proximal section of the small intestine, decreasing in length toward the distal region (P = 0.001). Compared with control animals, experimental pigs had significantly longer villi (P = <0.001), irrespective of dietary treatment or tract region. With the exception of the interaction between CVR, diet and region, all other analyses involving control animals were highly significant (P = <0.001). For experimental animals, there was a highly significant effect of day (P = <0.001, <0.001(L), <0.001(Q)) with villus height exhibiting a typical pattern over time (Figure 7.1a). Despite a period of atrophy shortly after weaning, villi recovered to pre-weaning levels by day 5 of the trial. This recovery pattern was evident in all three regions of the small intestine. There was no effect of wheat endosperm texture alone (P = 0.092), although a significant interaction with day was observed (P = <0.001, <0.001(L)). For pigs on soft wheat diets, an inverse relationship was found between villus height and cook level. The longest villi were therefore evident in pigs on the raw wheat and the shortest in those on the high cook diet (P = <0.001). Significant interactions between day and region and between day, cook and region were also apparent (P = <0.001).

7.5.6.2. Villus width

Mean measurements of villus width are shown in Table 7.14 with analysis of variance shown in Table 7.15. Overall mean villus width data showed no significant difference between control and experimental pigs. There was no statistically significant interaction between CVR and diet or CVR and day, but all other analyses involving control pigs were shown to be significant (P = <0.05). Villus width data from experimental pigs followed a similar pattern to that seen for villus height; a period of atrophy after weaning accompanied with recovery to pre-wean levels by day 5 (Figure 7.1b). There was a highly significant regional effect with measurements increasing along the tract from

proximal to distal region (P = <0.001). This was evident for each sampling day throughout the trial. No effect of endosperm texture was observed (P = 0.739) but there appeared to be a strong interaction between endosperm and region with soft endosperm wheat more favourable than hard (P = 0.053). For animals on high cook diets, significant interactions between endosperm and day, region and day and between endosperm, day and region were apparent (P = <0.01). There was no observed effect of cook level for those pigs on the soft wheat diets (P = 0.865). With the exception of the analysis between cook level and region, all other parameters were highly significant (P =<0.001) for these pigs, although it is difficult to determine any firm patterns from the data.

7.5.6.3. Crypt depth

Mean measurements and analysis of variance are shown in Tables 7.16 and 7.17. Experimental animals experienced significantly greater hypertrophy than control pigs (P = <0.001), irrespective of dietary treatment (P = <0.001). As observed with other villus architecture measurements, crypt depth was reduced around day 4 post-weaning but then exhibited an increase in all tract regions until around day 10 (Figure 7.1c). With the exception of the interaction between CVR, diet and region, all other parameters comparing control animals were found to be highly significant (P = <0.001). Experimental pigs experienced greatest hypertrophy within the mid region of the small intestine, with least hypertrophy seen in the distal section. There was no effect of endosperm texture (P = 0.895) although significant interactions between endosperm and day, and between endosperm, day and region were evident from the analyses. Crypt depth was affected by cook level (P = 0.020) with the greatest hypertrophy seen in the pigs on the high cook diet (least hypertrophy for the low cook diet). The effect of day was significant (P = <0.001) as were the interactions between cook and day, region and day and between cook, day and region (P =<0.01).

7.5.6.4. Villus height to crypt depth ratio

Mean ratios are shown in Table 7.18 with accompanying analysis of variance in Table 7.19. Overall mean rations showed no significance between control and experimental

animals (P = 0.071). With the exception of the interaction between CVR, diet and region, all other analyses comparing control pigs showed significance. There was a highly significant effect of region, with ratios decreasing along the tract from proximal to distal end for all pigs (P = <0.001). There was no observed effect of wheat endosperm texture (P = 0.108) although a significant interaction with day showed soft endosperm wheat appearing more beneficial from day 8 of the trial (Figure 7.1d). For pigs on the high cook diets, significant interactions between day and region and between endosperm, day and region were also determined. There was a significant effect of cook level (P = <0.001) with the most beneficial ratios from the raw wheat diets (high cook was least beneficial). However, there was no observed interaction between cook level and tract region (P = 0.391). The effect of day and the interactions between cook and day, region and day and between cook, day and region were all highly significant (P = <0.001).

Table 7.12: Mean villus height measurements (μm) from Trial 5; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	334						**334**
	0.50	290						**290**
	0.75	241						**241**
Raw/ Soft Wheat	0.25		308	435	336	428	377	**377**
	0.50		263	338	325	423	354	**341**
	0.75		293	206	319	305	277	**280**
Soft/ Low SME	0.25		296	310	447	347	382	**356**
	0.50		261	289	365	303	406	**325**
	0.75		244	242	256	355	286	**277**
Hard/ High SME	0.25		297	303	365	458	248	**334**
	0.50		271	292	378	324	279	**309**
	0.75		247	232	311	201	296	**258**
Soft/ High SME	0.25		280	268	399	428	360	**347**
	0.50		255	220	341	358	339	**302**
	0.75		253	208	271	322	283	**267**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs n = 4)

Table 7.13: Analysis of variance of mean villus height

Factor	s.e.d.	P	cv
CVR	5.6	**<0.001**	
CVR*Diet	5.5	**<0.001**	
CVR*Day	6.8	**<0.001**	
CVR*Region	8.2	**<0.001**	14.9%
CVR*Diet*Day	8.2	**<0.001**	
CVR*Diet*Region	8.9	0.059	
CVR*Day*Region	9.9	**<0.001**	
CVR*Diet*Day*Region	13.2	**<0.001**	
Endosperm	4.4	0.092	
Day	9.1	**<0.001, <0.001(L), <0.001(Q)**	
Region	4.6	**<0.001**	
Endosperm*Day	10.9	**<0.001, <0.001(L)**, 0.063(Q)	15.3%
Endosperm*Region	6.8	0.087	
Day*Region	12.1	**<0.001**, 0.598(L), **<0.001(Q)**	
Endosperm*Day*Region	16.1	**<0.001, 0.014(L), <0.001(Q)**	
Cook	3.9	**<0.001**	
Day	6.8	**<0.001, <0.001(L), <0.001(Q)**	
Region	3.8	**<0.001**	
Cook*Day	9.9	**<0.001, 0.004(L), 0.035(Q)**	14.6%
Cook*Region	6.6	0.052	
Day*Region	9.6	**<0.001, <0.001(L), <0.001(Q)**	
Cook*Day*Region	15.6	**<0.001**, 0.098(L), **<0.001(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 7.14: Mean villus width measurements (μm) from Trial 5; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	79						79
	0.50	78						79
	0.75	95						95
Raw/ Soft Wheat	0.25		76	75	75.	77	84	77
	0.50		71	72	80	98	83	81
	0.75		68	85	98	103	105	92
Soft/ Low SME	0.25		85	84	77	78	75	80
	0.50		81	75	78	83	77	79
	0.75		99	70	95	89	93	89
Hard/ High SME	0.25		70	82	73	70	78	75
	0.50		80	74	69	78	90	78
	0.75		97	81	94	109	97	95
Soft/ High SME	0.25		80	93	69	67	82	78
	0.50		74	93	79	70	87	81
	0.75		81	102	103	84	87	91

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 7.15: Analysis of variance of mean villus width

Factor	s.e.d.	P	cv
CVR	2.0	0.134	20.2%
CVR*Diet	2.0	0.918	
CVR*Day	2.4	0.611	
CVR*Region	2.9	**<0.001**	
CVR*Diet*Day	2.9	**<0.001**	
CVR*Diet*Region	3.2	**0.035**	
CVR*Day*Region	3.6	**<0.001**	
CVR*Diet*Day*Region	4.8	**<0.001**	
Endosperm	1.6	0.739	20.0%
Day	3.3	**0.012**, 0.096(L), 0.811(Q)	
Region	1.7	**<0.001**	
Endosperm*Day	3.9	**<0.001, 0.001(L)**, 0.525	
Endosperm*Region	2.4	0.053	
Day*Region	4.4	**<0.001**, 0.110(L), **<0.001(Q)**	
Endosperm*Day*Region	5.8	**0.002**, 0.660(L), **0.030(Q)**	
Cook	1.4	0.865	20.1%
Day	2.4	**<0.001**, **<0.001(L)**, 0.120(Q)	
Region	1.4	**<0.001**	
Cook*Day	3.5	**<0.001**, **<0.001(L)**, 0.582(Q)	
Cook*Region	2.4	0.514	
Day*Region	3.4	**<0.001, 0.001(L), <0.001(Q)**	
Cook*Day*Region	5.6	**<0.001**, 0.072(L), **0.002(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 7.16: Mean crypt depth measurements (μm) from Trial 5; covariate = live weight

Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	182						**182**
	0.50	163						**163**
	0.75	179						**179**
Raw/ Soft Wheat	0.25		175	158	191	22	233	**196**
	0.50		149	167	196	244	240	**199**
	0.75		141	151	208	223	190	**183**
Soft/ Low SME	0.25		188	179	191	190	211	**191**
	0.50		191	153	207	176	226	**190**
	0.75		181	126	212	194	205	**184**
Hard/ High SME	0.25		226	128	199	246	176	**195**
	0.50		191	133	195	259	276	**211**
	0.75		182	141	168	262	217	**194**
Soft/ High SME	0.25		214	185	193	209	184	**197**
	0.50		202	192	217	214	192	**203**
	0.75		216	182	203	212	170	**197**

0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

Table 7.17: Analysis of variance of mean crypt depth

Factor	s.e.d.	P	cv
CVR	4.3	**<0.001**	18.5%
CVR*Diet	4.2	**<0.001**	
CVR*Day	5.2	**<0.001**	
CVR*Region	6.2	**<0.001**	
CVR*Diet*Day	6.3	**<0.001**	
CVR*Diet*Region	6.9	0.166	
CVR*Day*Region	7.6	**<0.001**	
CVR*Diet*Day*Region	10.1	**<0.001**	
Endosperm	3.5	0.895	18.4%
Day	7.2	**<0.001, <0.001(L)**, 0.310Q)	
Region	3.7	**0.002**	
Endosperm*Day	8.7	**<0.001, <0.001(L)**, 0.559(Q)	
Endosperm*Region	5.4	0.324	
Day*Region	9.6	**<0.001, <0.001(L)**, 0.506(Q)	
Endosperm*Day*Region	12.8	**<0.001, <0.001(L), 0.030(Q)**	
Cook	2.9	**0.020**	17.9%
Day	5.0	**<0.001, <0.001(L), 0.002(Q)**	
Region	2.8	**0.001**	
Cook*Day	7.3	**<0.001, <0.001(L), <0.001(Q)**	
Cook*Region	4.9	0.308	
Day*Region	7.1	**<0.001, 0.025(L), <0.001(Q)**	
Cook*Day*Region	11.5	**0.004**, 0.137(L), **0.025(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect

Table 7.18: Mean villus height to crypt depth ratios from Trial 5; covariate = live weight

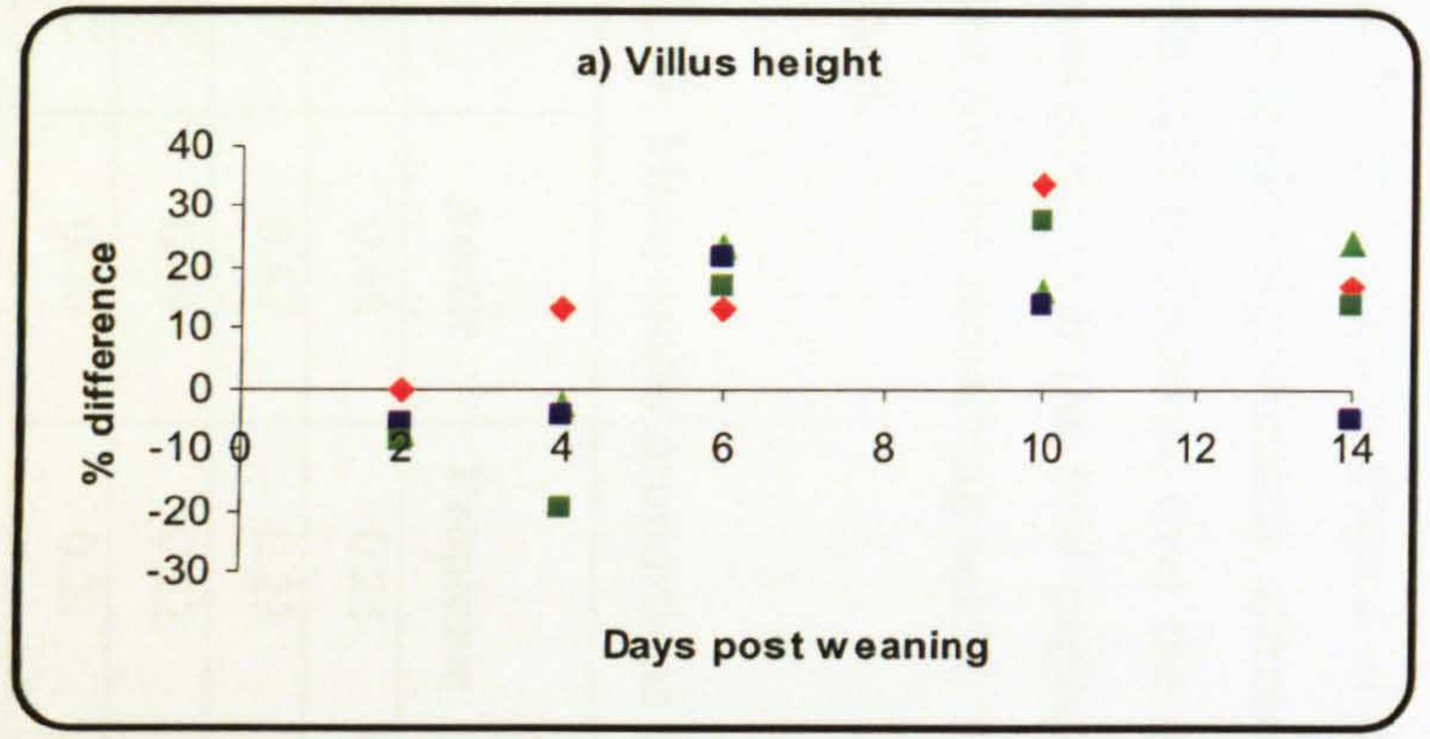
Diet	Region	Day 0	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
None (Control)	0.25	1.9						**1.9**
	0.50	1.8						**1.8**
	0.75	1.4						**1.4**
Raw/ Soft Wheat	0.25		1.8	3.0	1.8	1.9	1.6	**2.0**
	0.50		1.8	2.1	1.7	1.7	1.5	**1.8**
	0.75		2.2	1.4	1.6	1.3	1.5	**1.6**
Soft/ Low SME	0.25		1.6	1.8	2.4	1.9	1.9	**1.9**
	0.50		1.5	2.0	1.8	1.8	1.9	**1.8**
	0.75		1.4	2.0	1.3	1.9	1.4	**1.6**
Hard/ High SME	0.25		1.4	2.6	1.9	2.0	1.5	**1.9**
	0.50		1.5	2.3	2.0	1.3	0.9	**1.6**
	0.75		1.4	1.7	1.9	0.8	1.4	**1.4**
Soft/ High SME	0.25		1.4	1.5	2.1	2.1	2.0	**1.8**
	0.50		1.4	1.2	1.6	1.7	1.8	**1.5**
	0.75		1.2	1.1	1.4	1.6	1.8	**1.4**

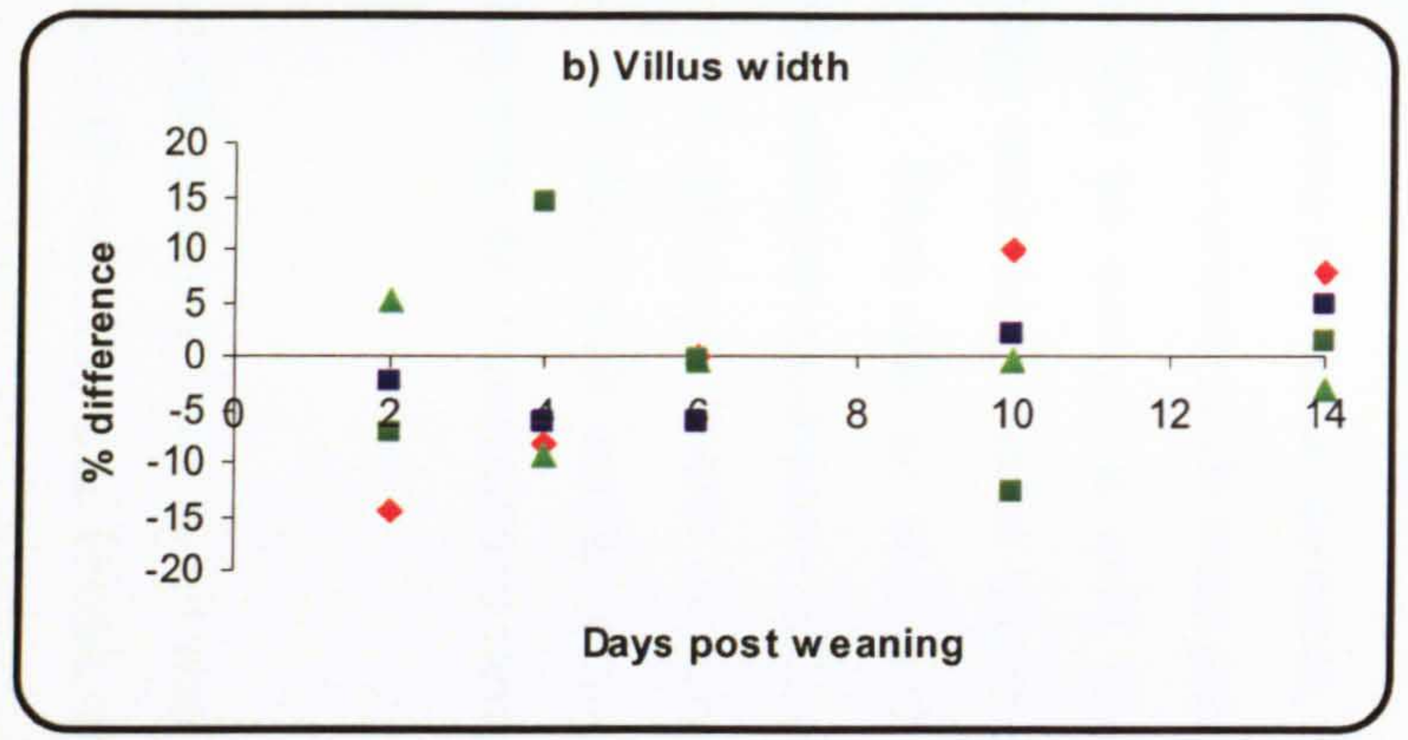
0.25, 0.50 & 0.75 = proportion along small intestine (n = 10 pigs per diet, control pigs; n = 4)

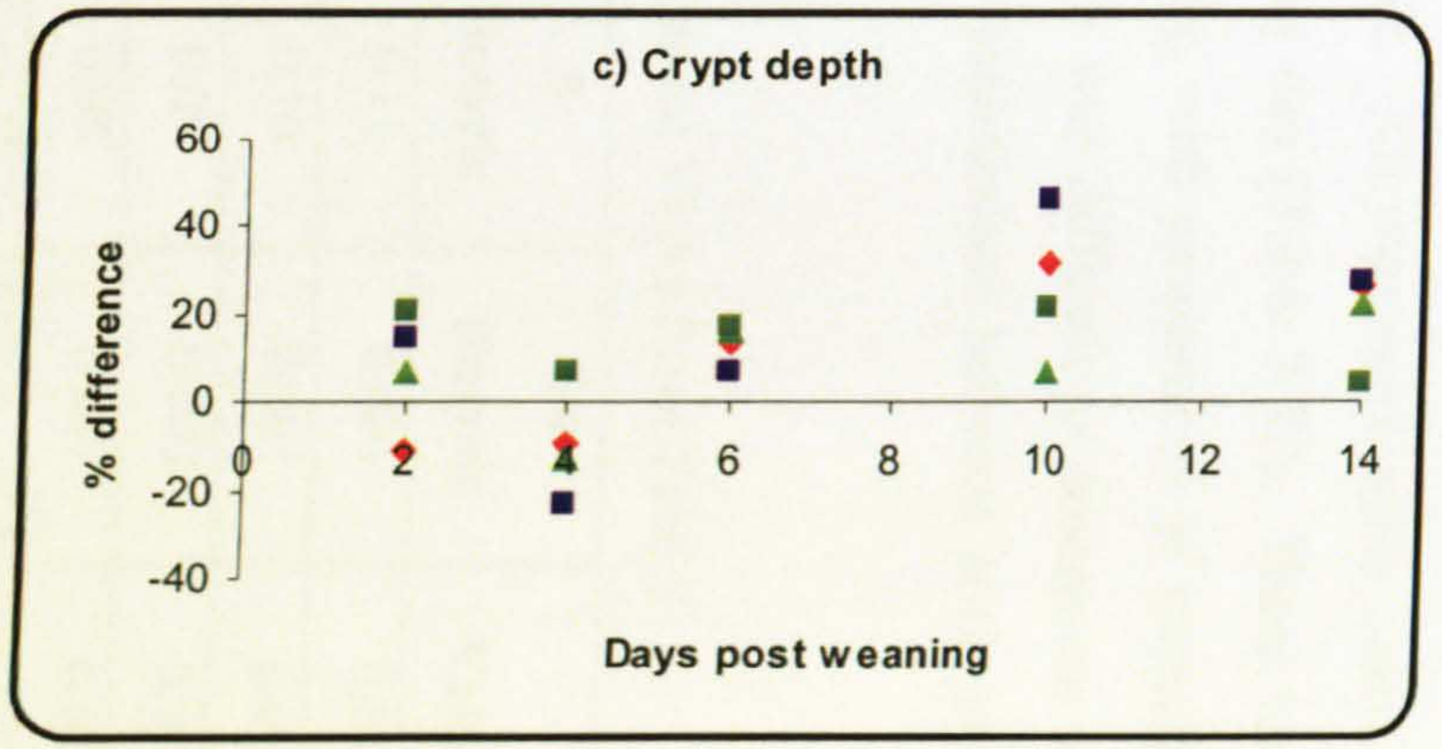
Table 7.19: Analysis of variance of mean villus height to crypt depth ratios

Factor	s.e.d.	P	cv
CVR	0.05	0.071	24.8%
CVR*Diet	0.05	**<0.001**	
CVR*Day	0.06	**<0.001**	
CVR*Region	0.07	**<0.001**	
CVR*Diet*Day	0.07	**<0.001**	
CVR*Diet*Region	0.08	0.604	
CVR*Day*Region	0.09	**<0.001**	
CVR*Diet*Day*Region	0.12	**<0.001**	
Endosperm	0.04	0.108	24.9%
Day	0.08	**<0.001, 0.285(L), <0.001(Q)**	
Region	0.04	**<0.001**	
Endosperm*Day	0.09	**<0.001, <0.001(L), 0.001(Q)**	
Endosperm*Region	0.06	0.816	
Day*Region	0.10	**<0.001, <0.001(L), <0.001(Q)**	
Endosperm*Day*Region	0.14	**<0.001, 0.023(L), 0.054(Q)**	
Cook	0.03	**<0.001**	23.7%
Day	0.06	**<0.001, <0.001(L), <0.001(Q)**	
Region	0.03	**<0.001**	
Cook*Day	0.09	**<0.001, <0.001(L), 0.041(Q)**	
Cook*Region	0.06	0.391	
Day*Region	0.08	**<0.001, 0.637(L), <0.001(Q)**	
Cook*Day*Region	0.14	**<0.001, 0.629(L), 0.011(Q)**	

CVR = control pigs versus the rest L = Linear effect Q = Quadratic effect







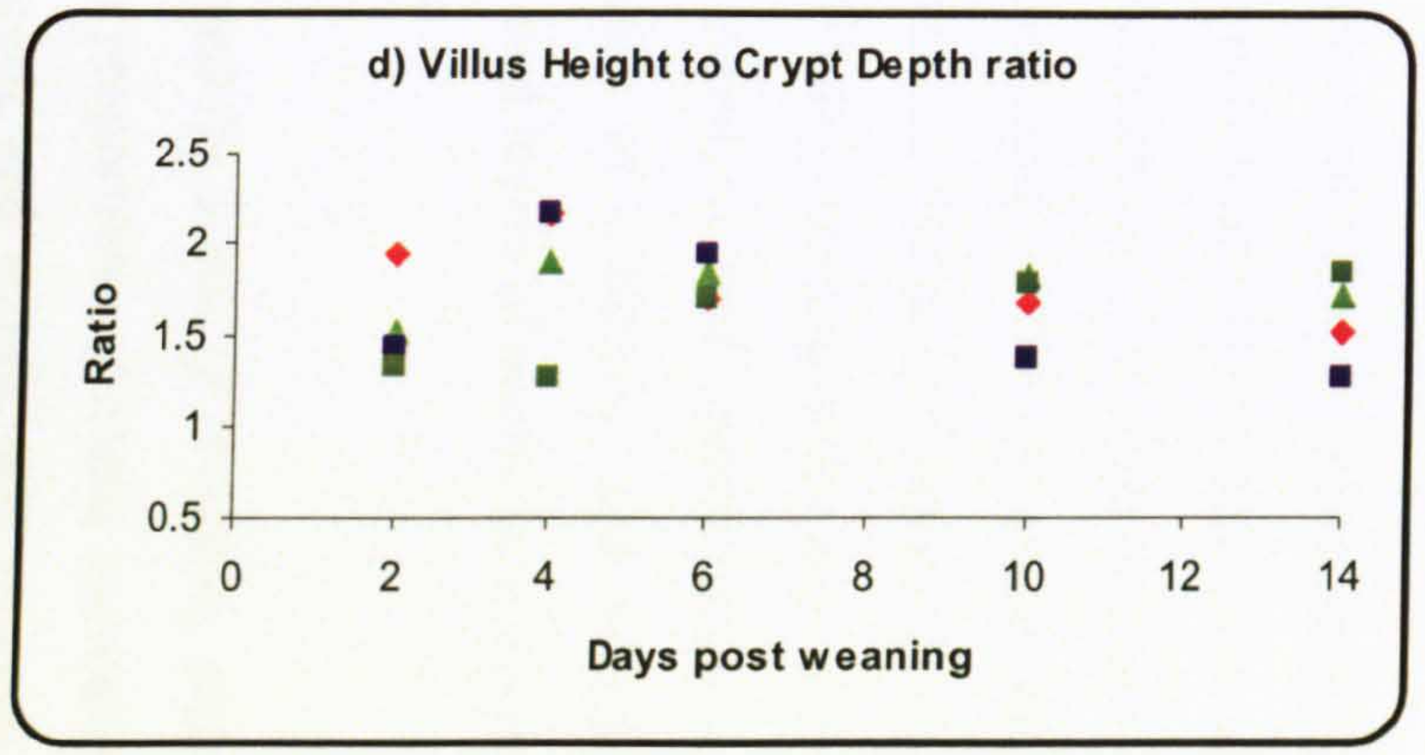


Figure 7.1: *Effect of extruded wheats on mean gut morphology measurements (covariate = live weight; n = 10 pigs per diet). (♦) Soft Raw Wheat, (▲) Soft Wheat/Low SME, (■) Hard Wheat/High SME, (■) Soft Wheat/High SME*

7.5.7. Caecal VFA analysis

When analysing the caecal contents, it was found that the concentrations of iso-butyric, valeric and iso-valeric acids were present only in small amounts (if at all) in many of the samples and no iso-valeric acid was detected in any digesta from pigs on the soft wheat/high cook diet. Mean molar proportions of the measured VFAs are shown in Table 7.20. There was no observed effect of wheat endosperm texture alone, and no interaction between endosperm texture and day was found for any of the acids measured (Table 7.21) although levels of acetic, n-butyric and valeric acids were found to be affected by day, with molar proportions of n-butyric and valeric acid generally increasing over the experimental period, and levels of acetic acid decreasing throughout the study.

A significant effect of cook level was found for acetic ($P = 0.029$), n-butyric ($P = 0.006$) and valeric ($P = 0.013$) acid levels, with proportions of acetic increasing in response to increased cook level. Conversely, n-butyric and valeric acid proportions decreased in response to an increased degree of cook. All measured acids, with the exception of valeric were also significantly affected by day (Table 7.22). There was a general trend for acetic acid to decrease over the study, with proportions of propionic and n-butyric acids increasing over the trial period. It was difficult to determine any clear patterns over time for the remaining acids. No interactions between cook level and day were determined.

Table 7.20: Mean molar proportions of caecal VFAs from Trial 5

Diet	Acetic	Propionic	n-Butyric	Iso-Butyric	Valeric	Iso-Valeric
SW/R	0.64	0.23	0.11	0.001	0.013	0.001
SW/LC	0.67	0.23	0.10	0.001	0.007	0.001
HW/HC	0.69	0.22	0.09	0.002	0.007	0.001
SW/HC	0.70	0.21	0.08	0.001	0.005	0

(R = Raw, HW = Hard Wheat, SW= Soft Wheat, HC = High Cook, LC=Low Cook)

Table 7.21: Analysis of variance of mean caecal VFA molar proportions, as affected by wheat endosperm texture

VFA	Endosperm		Day		Endosperm*Day		cv %
	s.e.d.	P	s.e.d.	P	s.e.d.	P	
A	0.018	0.377	0.028	**0.019, 0.002(L),**	0.040	0.824, 0.759(L)	5.7
P	0.016	0.725	0.026	0.302, 0.203(L)	0.036	0.547, 0.354(L)	16.8
n-B	0.007	0.397	0.011	**0.001, <0.001(L),**	0.016	0.660, 0.274(L)	19.4
I-B	0.008	0.116	0.001	0.186, 0.146(L), 0.073(Q)	0.002	0.359, 0.062(L),	147.1
V	0.002	0.356	0.003	0.160, **0.027(L)**	0.005	0.969, 0.874(L)	77.0
I-V	0.001	0.367	0.002	0.503, 0.553(L)	0.002	0.503, 0.553(L)	471.3

(A) Acetic, (P) Propionic, (n-B) n-Butyric, (I-B) Iso-Butyric, (I-V) Iso-valeric, (V) Valeric

Table 7.22: Analysis of variance of mean caecal VFA molar proportions, as affected by extrusion cook level

VFA	Cook		Day		Cook*Day		cv %
	s.e.d.	P	s.e.d.	P	s.e.d.	P	
A	0.020	**0.029**	0.026	**<0.001, <0.001(L)**	0.044	0.424, 0.866(L)	6.6
P	0.017	0.458	0.022	**0.010, 0.002(L)**	0.040	0.750, 0.554(L)	16.9
n-B	0.009	**0.006**	0.011	**<0.001, <0.001(L)**	0.020	0.120, 0.224(L)	21.8
I-B	0.001	0.639	0.001	**0.028,** 0.078(L), 0.061(Q)	0.002	0.927, 0.643(L)	204.3
V	0.003	**0.013**	0.003	0.332, 0.115(L)	0.006	0.572, 0.792(L)	69.7
I-V	0.001	0.364	0.001	**0.016, 0.024(L), 0.023(Q)**	0.002	0.424, 0.239(L)	264.2

(A) Acetic, (P) Propionic, (n-B) n-Butyric, (I-B) Iso-Butyric, (I-V) Iso-valeric, (V) Valeric

7.5.8. Performance Parameters

7.5.8.1. Feed intake:

Mean DE intakes for 0-5 days and analysis of variance are shown Table 7.23. For all pigs, intakes increased significantly over time (P = <0.001, P = <0.001(L)). Greater intakes were observed for hard endosperm wheat compared to soft (P = 0.017) although there was no observed interaction with day. Degree of cook proved highly significant (P = <0.001) with pigs on the raw wheat diet exhibiting the greatest DE intakes over this period of the trial. Pigs on the low cook diet had the lowest intakes. No interaction between cook and day was observed.

Table 7.23: Mean daily DE intake 0-5 days (MJ) and analysis of variance from Trial 5

	Days post-weaning						
Diet	**0**	**1**	**2**	**3**	**4**	**5**	**Mean**
SW/R	0.21	1.83	4.44	6.51	7.34	7.49	**4.63**
SW/LC	0.31	1.45	1.73	3.69	4.53	5.76	**2.91**
HW/HC	0.43	1.95	3.65	5.26	6.01	6.19	**3.91**
SW/HC	0.25	1.46	3.29	4.67	5.18	6.15	**3.50**

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Endosperm	0.17	**0.017**	19.3%
Day	0.29	**<0.001, <0.001(L),**	
Endosperm*Day	0.41	**<0.001(Q)**	
		0.799, 0.884(L), 0.295(Q)	
Cook	0.37	**<0.001**	42.3%
Day	0.52	**<0.001,<0.001(L), 0.073(Q)**	
Cook*Day	0.90	0.333, 0.073(L), 0.119(Q)	

(R = Raw, HW = Hard Wheat, SW= Soft Wheat, HC = High Cook, LC=Low Cook)
L = Linear effect Q = Quadratic effect (n = 6 pigs per diet)

After 5 days, pigs on the hard wheat/high cook diet had the greatest mean DE intakes, with the least preferable diet being the soft wheat/low cook combination (Table 7.24). Feed intakes increased over time for the pigs on the high cook diets, with piglets fed the hard endosperm wheat, having significantly greater DE intakes than those fed the soft endosperm wheat diet (P = 0.027). Degree of cook proved a more significant factor with pigs on the raw wheat showing the greatest intakes, and those on the low cook diet having the lowest appetites (P = <0.001). Pigs on soft wheat diets had appetites that increased significantly with time (P = 0.002), although no interaction between cook level and day was evident.

Table 7.24: Mean DE intake post 5 days (MJ) and analysis of variance from Trial 5

	Days post-weaning								
Diet	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**Mean**
SW/R	7.03	6.18	5.88	7.97	8.27	7.96	9.03	10.38	**7.84**
SW/LC	5.26	4.61	4.43	3.99	5.00	5.27	7.00	8.90	**5.56**
HW/HC	8.21	8.12	8.14	8.21	8.21	8.28	9.69	10.54	**8.67**
SW/HC	6.65	6.42	7.17	7.55	7.76	7.17	7.81	9.72	**7.53**

Analysis of variance			
Factor	**s.e.d.**	**P**	**cv**
Endosperm	0.42	**0.027**	
Day	0.85	0.120, **0.009(L)**, 0.121(Q)	14.8%
Endosperm*Day	1.20	0.980, 0.802(L), 0.600(Q)	
Cook	0.53	**<0.001**	
Day	0.86	**0.002, <0.001(L), 0.018(Q)**	21.3%
Cook*Day	1.49	0.973, 0.699(L), 0.337(Q)	

(R = Raw, HW = Hard Wheat, SW= Soft Wheat, HC = High Cook, LC=Low Cook)
L = Linear effect Q = Quadratic effect (n = 2 pigs per diet)

7.5.8.2. DLWG

There was a significant effect of day with values increasing throughout the trial period (P = <0.001, <0.001(L), <0.001(Q)) and all pigs gaining weight by day 5 post-weaning after initial weight loss. Raw soft wheat appeared most beneficial, with low cook soft wheat the least favourable (Table 7.25). There was no effect of wheat endosperm texture and no interaction was observed between endosperm and day. A significant effect of cook was observed with raw wheat appearing more beneficial than either of the two cooking levels (P = 0.048). No interaction was observed between cook and day.

Table 7.25: Mean DLWG (Kg) and analysis of variance from Trial 5

Diet	Day 2	Day 4	Day 6	Day 10	Day 14	Mean
SW / R	-0.33	0.28	0.40	0.40	0.34	**0.22**
SW / LC	-0.05	-0.06	0.20	0.22	0.15	**0.09**
HW / HC	-0.30	0.04	0.23	0.20	0.39	**0.11**
SW / HC	-0.28	0.15	0.13	0.27	0.29	**0.11**

Analysis of variance

Factor	s.e.d.	P	cv
Endosperm	0.052	0.588	
Day	0.081	**<0.001, <0.001(L), <0.001(Q)**	113.4%
Endosperm*Day	0.115	0.676, 0.285(L), 0.532(Q)	
Cook	0.047	**0.048**	
Day	0.060	**<0.001, <0.001(L), <0.001(Q)**	91.4%
Cook*Day	0.104	0.055, 0.151(L), 0.082(Q)	

(R = Raw, HW = Hard Wheat, SW= Soft wheat, HC = High Cook, LC=Low Cook)
L = Linear effect Q = Quadratic effect (n = 10 pigs per diet)

7.6. SUMMARY OF RESULTS FROM TRIAL 5

The current trial shows strong support for the hypothesis that cooking (through extrusion) enhances starch digestibility in wheat for the weaned piglet; starch coefficients within the small intestine (especially noticeable within the 0.50 region) were much higher than those observed in Trials 3 and 4. The high coefficient of the soft wheat/high SME diet within the 0.50 region suggests enhanced digestion and more rapid release of starch within this region of the tract. Extrusion appeared to arrest the decline in starch digestibility typically observed immediately after weaning. This enhanced digestibility was also evident in the CTTAD values where digestion of starch was almost totally complete (>0.990).

There was evidence that degree of cook affected *in vivo* starch digestion; in agreement with the previous trial, a high level of cook was shown to result in significantly greater starch digestibilities (P = <0.001). Digesta viscosity was also affected by level of cook with a higher degree of cook resulting in significantly less viscous tract digesta (P = 0.041). However, not all data showed an advantage of using cooked wheat; villus architecture responded less well to highly cooked wheat, compared to raw wheat diets. Feed intake data also showed that piglets preferred the uncooked wheat diet throughout the duration of the trial, which was mirrored in the DLWG data.

Some of the trial data support the hypothesis that soft endosperm wheat responded better to extrusion than hard wheat under the conditions of the trial described; starch digestibility was greater for soft wheat in both regions of the small intestine. Digesta viscosity was significantly less viscous for soft wheat than for hard (P = <0.001). Although the measured gut morphology parameters were not affected by endosperm texture alone, villus height showed a significant interaction between endosperm and day (P = <0.001) where from 7 days after weaning, soft wheat appeared more beneficial than hard (Figure 7.1a). Gut morphology data showed that piglets fed the soft raw wheat diet did not exhibit any shortening of the villi after weaning. Animals on the remaining three diets did experience a period of atrophy although mean villus height measurements for

all pigs recovered strongly by day 6 and showed a considerable degree of lengthening over control values by day 10 of the trial. It is difficult to establish any clear patterns from the villus width data as dietary effects varied between slaughter days. In common with previous trials, crypt depth data revealed an S-shaped curve over time, with values decreasing around day 4, followed by the greatest degree of crypt hyperplasia noticeable on day 10, before reducing again to day 14. The ratios between villus height and crypt depth were found to be relatively constant over time, due to the mirrored effects seen for both of the respective parameters during the trial period.

For the pigs on the high SME diets, there was no observed effect of wheat endosperm texture on apparent nitrogen digestibility within either the small intestine, or over the total tract. Analysis of CAD data for nitrogen from the animals fed soft wheat diets revealed a strong tendency ($P = 0.056$) for those given raw wheat to have higher coefficients within the small intestine than animals fed either of the cooked soft wheat diets. Although analysis of digesta pH data revealed a clear effect of tract region, no influence of either wheat endosperm texture or extrusion cook level was found. Caecal VFA concentrations were unaffected by wheat endosperm texture although in response to an increasing level of cook, molar proportions of acetic acid were found to significantly increase ($P = 0.029$). In addition, both n-butyric and valeric acids were significantly affected by cook level, with proportions decreasing in response to an enhanced level of cooking (n-butyric, $P = 0.006$; valeric, $P = 0.013$). Incidence of scouring during the experimental period was not linked to dietary treatment.

Chapter 8 discusses the findings from piglet Trials 1-5, and compares the *in*-vivo biological responses with rheological data gained from the *in-vitro* laboratory analyses.

CHAPTER 8: GENERAL DISCUSSION

The aim of the current research programme was to examine some selected dietary strategies for the weaned piglet, in the absence of AGPs. The objectives were to maximise nutritional value of dietary cereals, with a particular emphasis on starch availability, through the optimisation of processing conditions. As the provision of a healthy gastrointestinal environment was a key concern, the effects of processing on villus architecture within the piglet small intestine were also examined. The general discussion will consider the main issues emerging from the series of experiments which comprised the programme. It will also compare the results of *in-vitro* rheological analyses of starch granules using food science techniques with the *in-vivo* biological responses observed during the piglet trials through Principal Component Analysis.

8.1. Digesta pH

In all trials, a typical pattern was evident with pH rising from the stomach, through the three intestinal regions before becoming more acidic again in the caecum. Figure 8.1 shows mean gastric and caecal pH values from the five animal trials. Gastric pH values in all trials ranged from 2.8 – 4.0 indicating that sufficiently acidic conditions were maintained within the stomach for promotion of strong proteolytic activity (Xu, 2003b) and for deterring proliferation of coliforms (Brooks *et al.*, 2001); it is generally accepted that a gastric pH of around 3.5 is sufficient for both. Only the maize-based diet in Trial 2 affected overall digesta pH significantly, although this response was lost when tract regions were compared across all dietary groups. There were no other effects of raw cereal type on digesta pH. This finding is in agreement with the observations of Medel *et al.* (1999, 2004) where ground barley and maize diets fed to weaned piglets had no effect upon gastric pH levels.

Piglets fed raw cereals had a more acidic caecal environment than the comparative day zero (control) animals in each trial. This indicates that the change in dietary composition from milk to a cereal-based feed resulted in a proportion of the starch

passing through the small intestine undigested, before undergoing fermentation in the caecum. This fermentation of the undigested starch with subsequent generation of VFAs would explain the increased acidic environment observed within the caecum of the pigs. By comparison, there was no difference in caecal values between control animals and experimental piglets on the cooked cereal diets. This suggests that as the proportion of digestible starch increased with processing, there was a reduction in the amount of undigested starch reaching the hindgut, resulting in less fermentation and hence reduced generation of VFAs.

It can be seen in Figure 8.1 that throughout the five animal trials, mean caecal acidity remained around pH 6, revealing no discernable effect of heat processing on the caecal environment. This finding is not in agreement with a similar study by Medel *et al.* (2004) where the use of cooked barley and maize (steam-cooked and flaked) in piglet diets resulted in significantly higher caecal pH values. However, other studies examining barley and maize have not noted any significant variation in tract pH in response to heat-processing (Medel *et al.*, 1999, 2002).

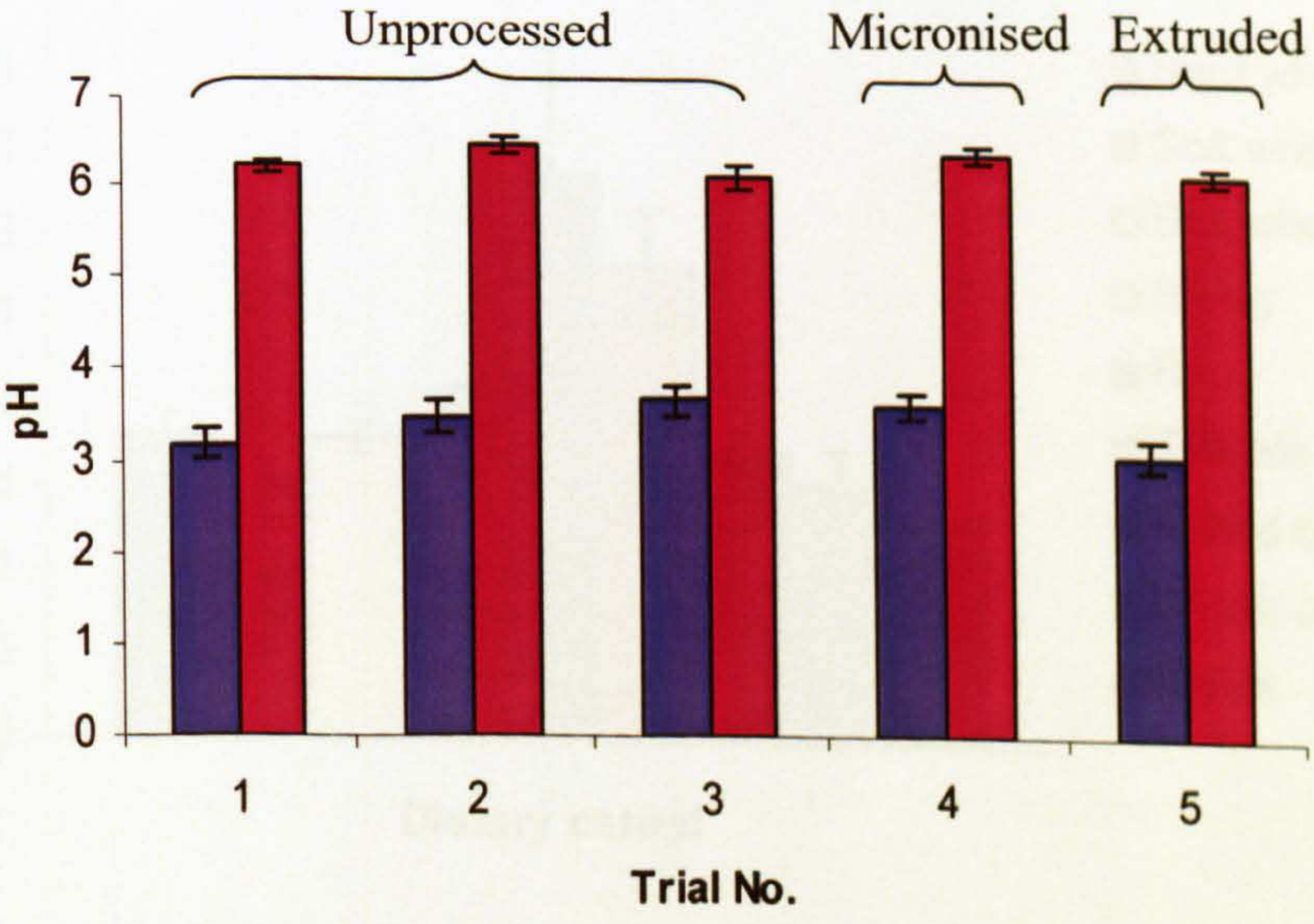


Figure 8.1: *Mean piglet stomach (■) and caecal (■) pH values from Trials 1 – 5 (Error bars represent standard error of the mean values)*

8.2. Digesta viscosity

From the series of experiments reported, viscosity of tract digesta was found to be hugely variable and largely dependent upon cereal type. Of the raw cereals analysed, average digesta viscosity was considerably greater in the piglets on the rye and triticale-based diets (Figure 8.2). Despite these animals having much higher viscosities for all regions of the digestive tract, there were no perceived detrimental effects on pig performance (feed intake and DLWG) or digestibility of dietary components (starch and nitrogen digestion coefficients). These results agree with Hopwood *et al.* (2004) where the addition of varying amounts of pearl barley to a rice-based diet significantly increased small intestinal viscosity but had no effect upon DLWG in weaned piglets. In contrast, McDonald *et al.* (2001) found the inclusion of CMC in the diet significantly increased both intestinal viscosity and DLWG, but diets used were much more viscous (equivalent to a range of 50 – 800 cP) than in the current research programme. It seems therefore that the range of raw cereals studied in the current research programme did not increase the viscosity of piglet digesta sufficiently to affect animal performance.

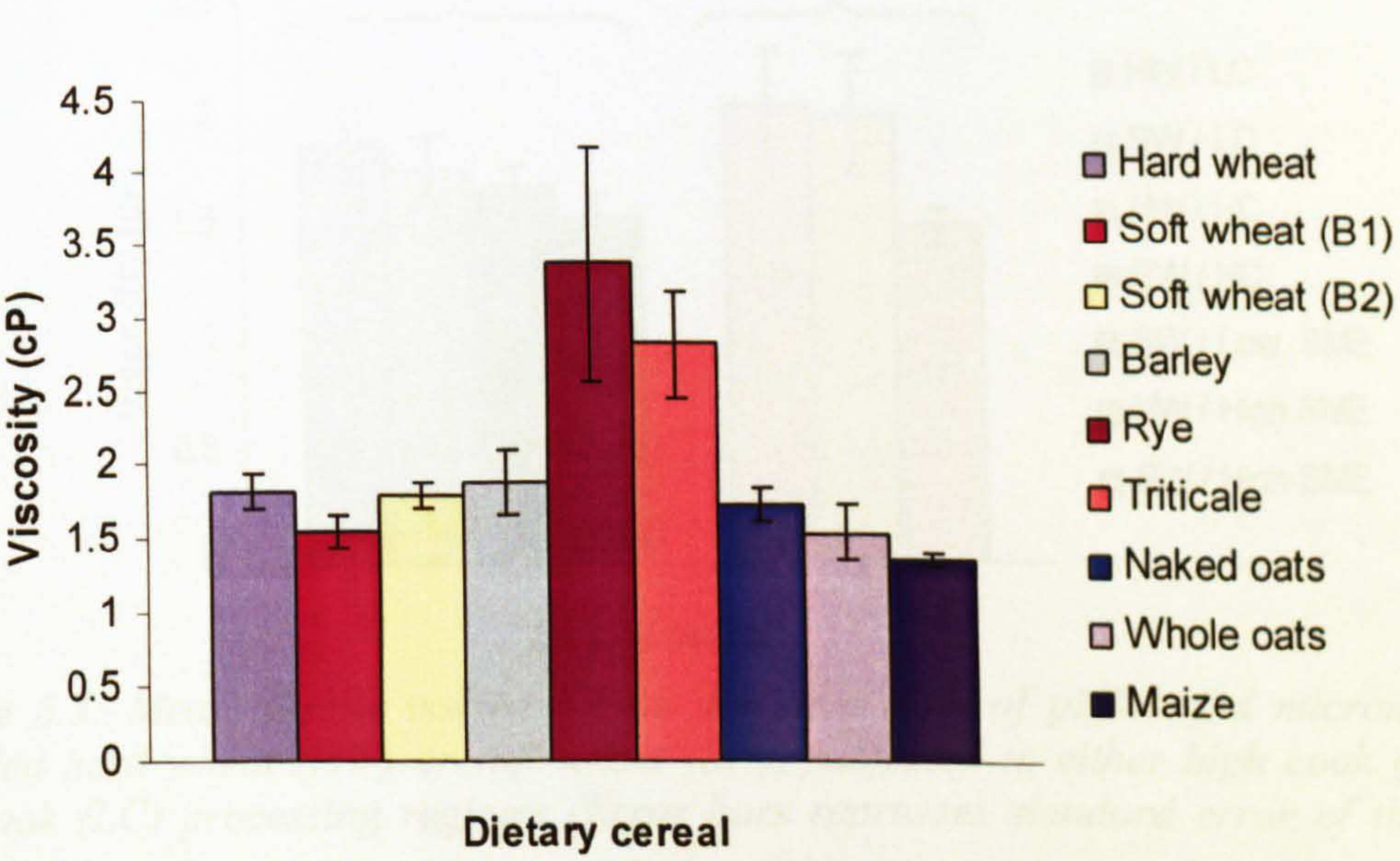


Figure 8.2: *Mean digesta viscosity from the digestive tract of piglets fed raw cereals, including two batches (B1 and B2) of soft endosperm wheat (Error bars represent standard error of the mean values)*

With the exception of Trial 3, there was a general tendency for pigs fed raw cereals to have more viscous digesta in all tract regions than comparative day-zero animals. A similar pattern of viscosity was seen for pigs fed the cooked cereal diets in Trials 4 and 5, except values at the 0.25 region were not noticeably different to control data. A general trend regarding cook level was apparent with digesta becoming less viscous in relation to an increasing level of cook for both micronisation and extrusion processing treatments (Figure 8.3). The response of micronised wheat in Trial 4 was not affected significantly by endosperm texture, although by varying the degree of cook from a moderate to a more extreme level digesta was found to be significantly less viscous ($P = 0.024$). This inverse relationship between cook level and tract viscosity was also apparent in Trial 5 where a high SME diet resulted in significantly less viscous tract digesta ($P = 0.041$) than a low SME diet, which in turn was less viscous than for the unprocessed wheat.

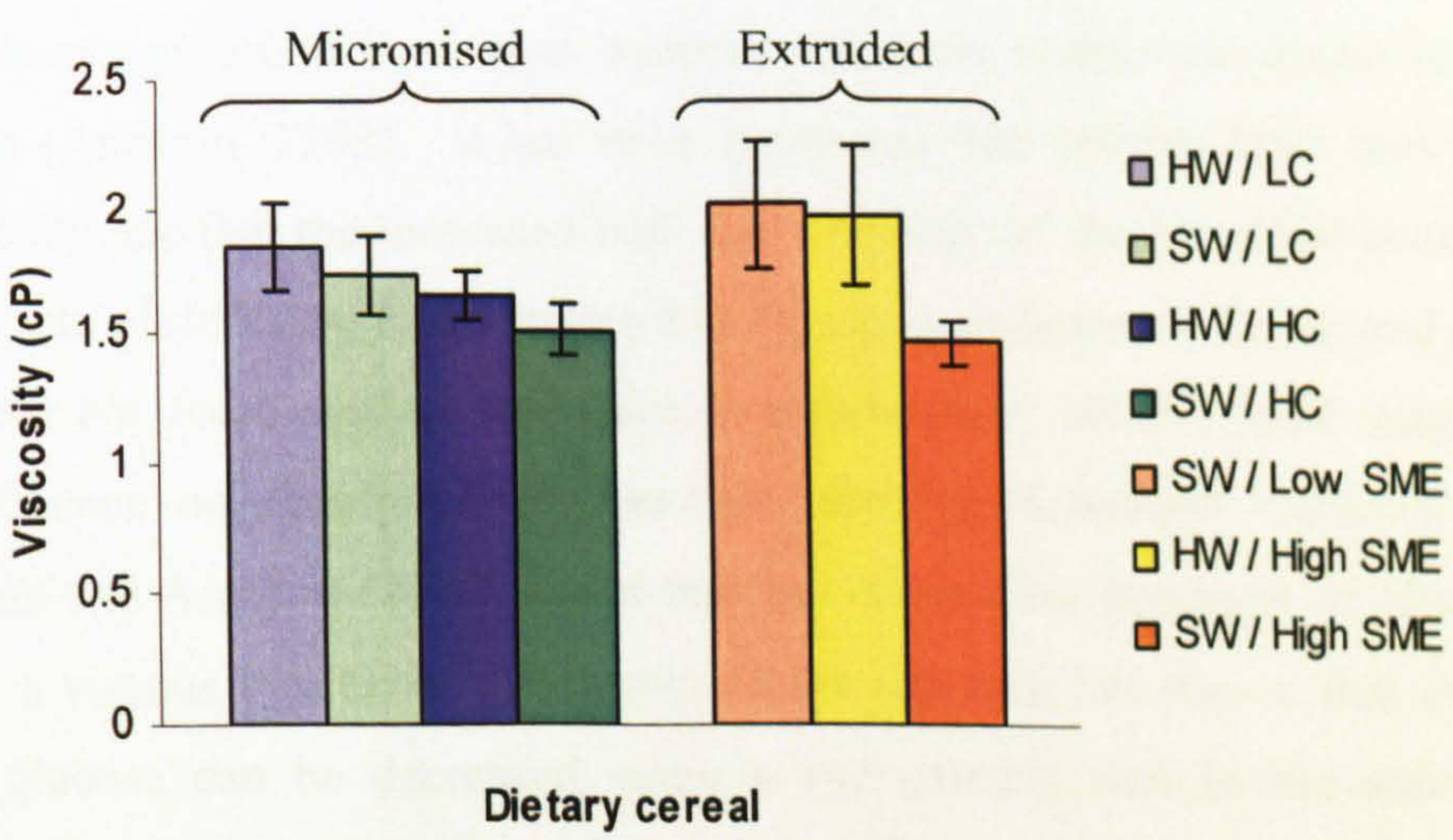


Figure 8.3: *Mean digesta viscosity from digestive tract of piglets fed micronised or extruded hard wheat (HW) or soft wheat (SW), subjected to either high cook (HC) or low cook (LC) processing regimes (Error bars represent standard error of the mean values)*

This association between cook level and viscosity within the digestive tract may be dependent upon cereal type as similar work comparing raw, micronised and extruded treatment of barley and maize failed to find a relationship between ileal viscosity and

heat treatment method (Medel *et al.*, 1999). Response of wheat to extrusion was also significantly affected by endosperm texture, with pigs on the soft endosperm wheat diet having less viscous digesta than those pigs on the hard wheat diet. This indicates that the response of wheat to thermal processing can be affected by fundamental properties of the cereal grain such as endosperm texture.

Although the main focus of this research programme concentrated on the starch fraction of the diets, consideration should also be given to the role of non-starch polysaccharides (NSP) and the possible influence that this natural component of the dietary cereals may have had on the observed viscosity results. Figure 8.2 showing the mean viscosity values obtained from within the digestive tract revealed the most viscous digesta was from pigs fed the rye-based diet, with maize-fed animals having the least viscous digesta. It is likely that the natural variations in NSP content between the raw cereals, accounted for the differences observed in the viscosity data. For instance, rye is typically high in NSP content whereas maize is a cereal that has a low NSP level. Water soluble NSPs can give rise to viscous aqueous solutions within the digestive tract of monogastrics (Annison, 1993). It has been suggested that soluble NSP may increase digesta viscosity and that the increased bulk and viscosity of the intestinal contents will decrease the rate of diffusion of substrates and digestive enzymes (Classen and Bedford, 1991). There are some studies that have examined how soluble NSP may exert a negative influence on absorption of nutrients, through increased viscosity of tract digesta; Smits and Annison (1996) found that the convective transport of glucose was impaired by a viscous *in vitro* environment. Other research has shown that the rate of dialysis of glucose can be decreased using a rye extract, rich in the soluble NSP arabinoxylan (Fengler *et al.*, 1988). In conclusion, when assessing the results of the viscosity data from the piglet trials, the influence that factors such as NSP level of the cereals may have had on the observed results, should also be taken into account.

8.3. Nitrogen digestibility

Analyses from Trials 1 and 2 showed nitrogen digestion within the small intestine was somewhat variable between the raw cereals. Generally, nitrogen coefficients increased from mid to distal regions of the small intestine, except for the diets based on naked oats (similar coefficients within the two regions) and maize (digestion decreased from 0.5 to 0.75 sections), although the resulting coefficients are complicated somewhat by the lack of sufficient digesta samples obtained on some of the slaughter days. Tywonczuk *et al.* (1994) found that rye, substituted at a rate of 300 g/kg in cereal-based diets (based on barley and wheat) for 25 kg pigs, reduced utilisation of nitrogen over the total tract by 30%. Although rye was the least well-digested cereal within the 0.5 tract region in Trials 1 and 2 of the current study, CTTAD values were not found to be negatively affected, even though in Trial 1, rye was the only cereal and was included at a higher level of 586 g/kg. For the initial 5 days of the trials, considerable variation in nitrogen digestion was apparent, indicated by CTTAD values during the first collection period. By the second collection period, CTTAD had increased for all animals and less variation was evident between dietary groups, indicating that utilisation of nitrogen had improved.

Although initial analysis of coefficients over the whole of the small intestine revealed no differences between raw hard and soft wheat diets, further statistical analysis revealed digestion within the 0.75 region was significantly greater for the soft endosperm wheat (Soft 0.74 vs. Hard 0.65; $P = 0.006$). This indicates a significant regional effect of nitrogen digestibility which is influenced by wheat endosperm texture. However, any difference between the raw wheats was no longer evident by the end of the large intestine, as indicated by the CTTAD data which suggests that digestibility of raw wheat in the large intestine of the weaned piglet may be significant. These findings are in agreement with those of Pearce *et al* (1997) where diets containing wheats of differing endosperm texture (three hard, three soft) were not found to influence CTTAD values for nitrogen.

The use of micronised wheat in Trial 4 revealed a significant effect of endosperm texture with higher nitrogen coefficients within the small intestine for the hard wheat diets,

although increasing the level of cook had no effect. CTTAD values were not affected by level of cook which is in agreement with similar work examining micronised wheat by Zarkadas and Wiseman (2001). The use of extrusion processing in Trial 5 had no significant effect on nitrogen digestibility within the small intestine although there was a strong trend for the raw wheat to have higher digestibility coefficients than the cooked diets (P = 0.056). It is likely that this reduced digestibility of nitrogen in the extruded diets was as a result of the occurrence of the Maillard reaction during processing of the cereal. Factors such as temperature, moisture content and shear forces all influence this reaction between a free amino group of lysine and a reducing sugar, forming less digestible complexes. This reaction process can also lead to a reduction in lysine availability (van Barneveld *et al.*, 1994) which would help to explain the reduced coefficients seen for the extruded diets in the current animal study. In conclusion, the process of cooking (through micronisation or extrusion) did not improve digestion of nitrogen within the small intestine, compared to raw wheat. In addition, CTTAD values for nitrogen were not significantly affected by either the type of processing method or by the severity of the cooking regimes.

8.4. Starch digestibility

Considerable variation in digestibility coefficients was found between the raw cereals in Trials 1 and 2. Apparent digestion of starch increased from 0.5 to 0.75 regions of the small intestine for all cereals with the exception of the maize diet, again somewhat affected by lack of sufficient digesta samples collected. Analysis of the other cereals revealed that the barley and triticale diets were the least well digested within the 0.5 intestinal region although these low coefficients had been alleviated by the time digestion had reached the 0.75 site. Of the raw cereals analysed, maize was the least well digested, as indicated by low digestibility coefficients at the 0.75 region of the small intestine and also significantly lower CTTAD values over both collection periods. Starch digestibility results from Trial 3 comparing raw wheats were inconclusive as there were no clear effects observed between the dietary treatments.

A series of *in vitro* analyses studying the physicochemical properties of the wheats used in Trial 4 revealed that the micronisation regimes used were very mild and much of the starch had not undergone a loss of crystallinity. The amylose and amylopectin polymers of starch are typically organised in granules as alternating semi-crystalline and amorphous layers. When the granules of raw starch are viewed under polarised light, the arrangement of these layers gives rise to the appearance of characteristic patterns, termed 'maltese crosses'. Upon processing, this defined pattern is no longer visible as a result of changes to the starch granule through swelling and gelatinisation. The technique of using polarised light microscopy is therefore a relatively quick and simple method of providing an initial assessment of the degree of starch granular damage as a result of processing. Polarised light microscopy images comparing the raw and micronised wheat samples (Figure 8.4 – A and B) revealed the appearance of maltese crosses in both the raw and processed samples, indicating that there had been little, if any disruption to the starch granules as a result of the micronisation process.

Using XRD to further analyse of the degree of disruption to the starch crystalline structure as a result of processing demonstrated that the diffraction profiles of the raw and micronised wheats were very similar. Despite this similarity, a subtle difference was apparent, but only for the soft wheat/high cook profile (Figure 8.5), indicating that the starch granules in this treatment had undergone a small degree of decreased crystallinity, as a result of processing. The fact that the remaining micronisation profiles were so similar to the raw wheat samples adds further support to the speculation that the starch in the micronised wheats had not undergone extensive gelatinisation as a result of processing.

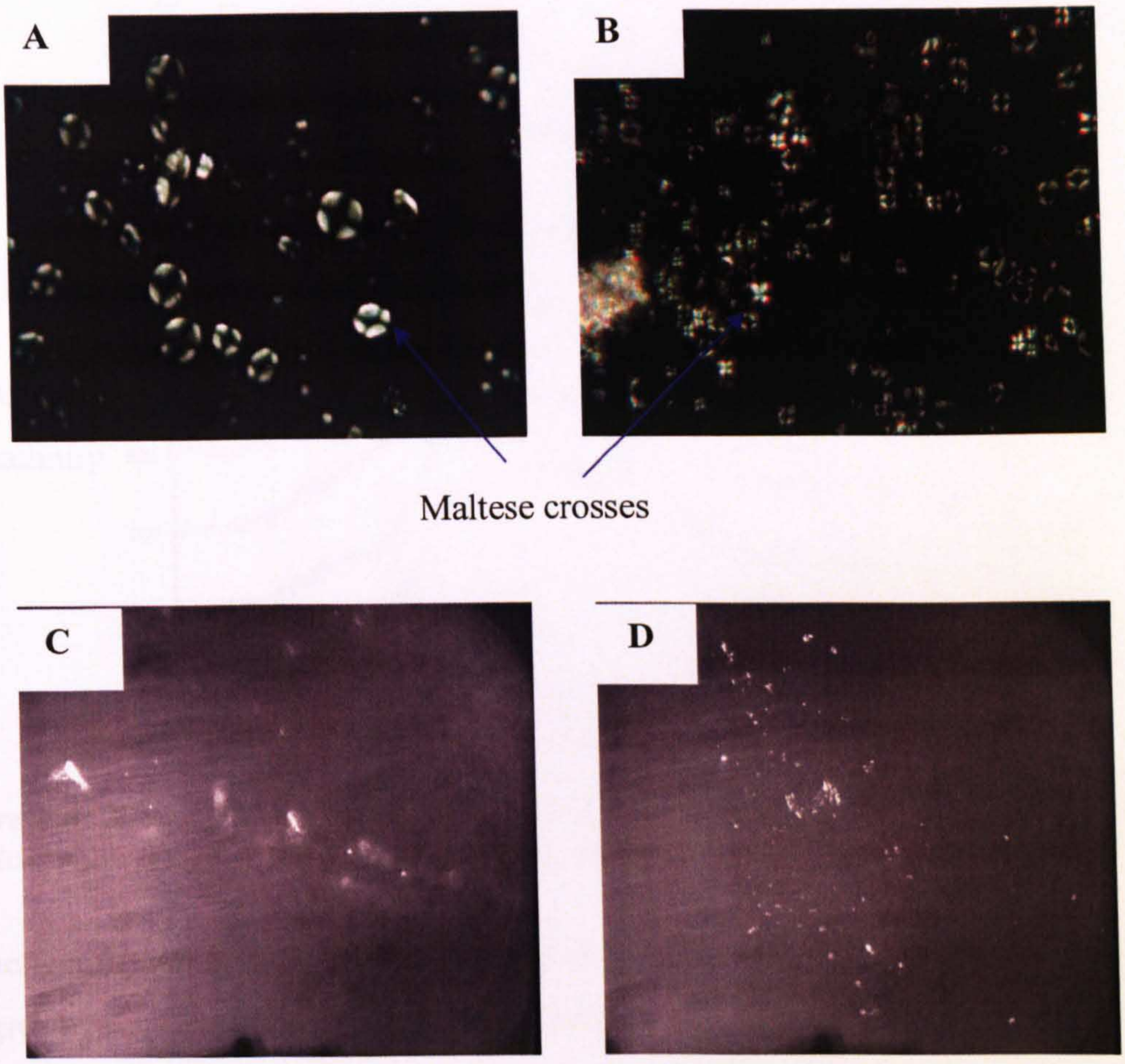


Figure 8.4: *Polarised Light Microscopy images of Raw (**A**), 'Micronised' (**B**), Extruded – Low SME (**C**) and Extruded – High SME (**D**) wheats. (Different scales)*

Table 8.1: WAI and WSI values for raw and micronised wheats

Sample	WSI (%)	WAI (g/g)
Raw Soft wheat	9.28 ± 0.12	1.07 ± 0.19
Raw Hard wheat	10.50 ± 0.55	1.01 ± 0.14
Micronised Soft wheat – High Cook	**24.73 ± 4.40**	**1.82 ± 0.09**
Micronised Soft wheat – Low Cook	9.73 ± 0.50	1.14 ± 0.16
Micronised Hard wheat – High Cook	10.89 ± 1.80	1.72 ± 0.01
Micronised Hard wheat – Low Cook	9.63 ± 1.28	1.20 ± 0.25

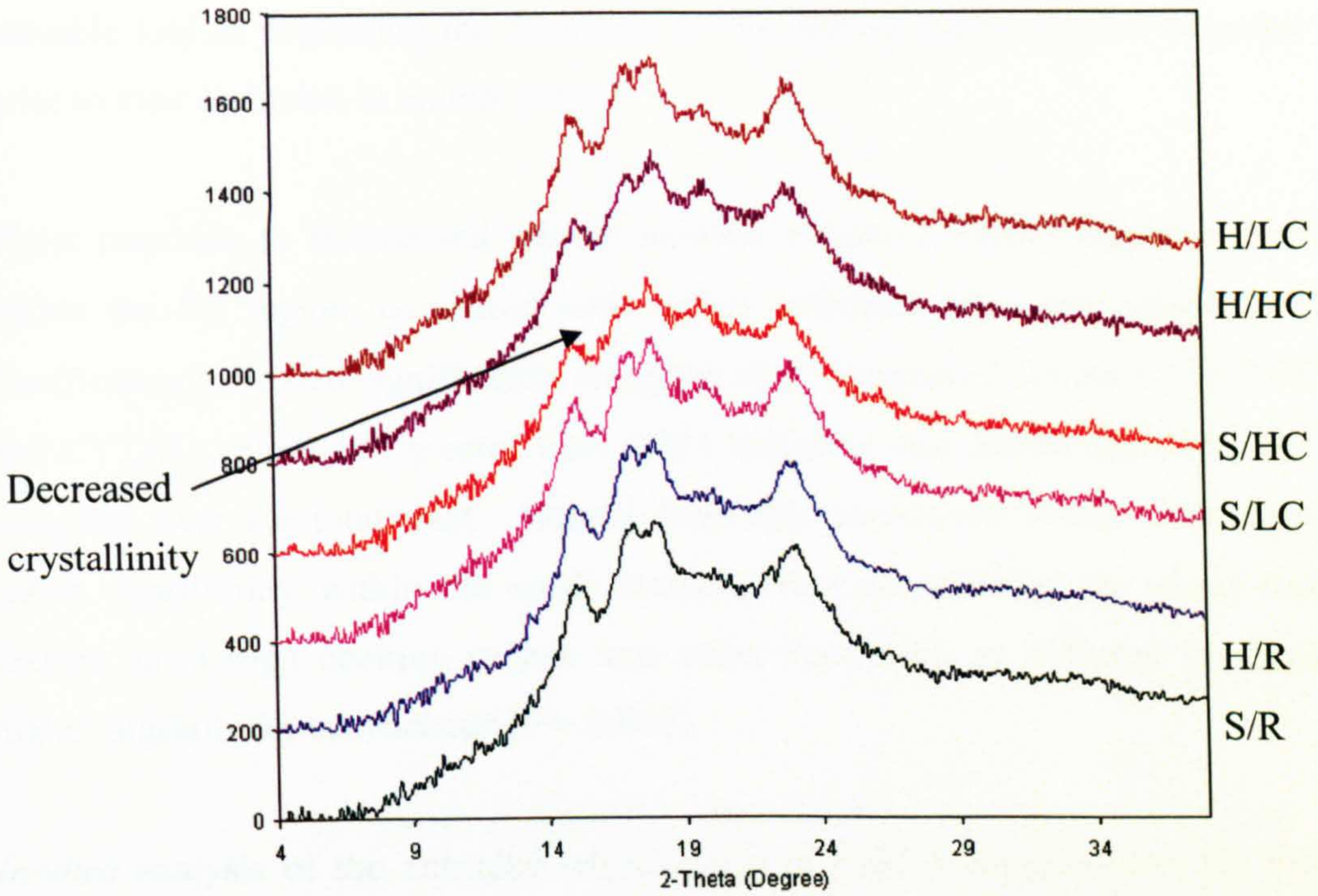


Figure 8.5: *XRD pattern of soft (S) and hard (H) endosperm wheat comparing raw (R) with low cook (LC) and high cook (HC) micronising regimes*

Further confirmation that only the starch in the soft wheat/high cook diet had undergone a degree of cooking from the micronising process was evident from the analysis of WSI and WAI values (Table 8.1). It can be seen that the soft wheat/high cook had elevated WSI and WAI values compared with the other treatments, indicating that the starch had undergone a degree of conversion, but with minimum molecular breakdown. Data for the other micronised treatment regimes were not greatly different to raw wheat values, again indicating that only minimum damage had been done to the starch granules. In conclusion, the culmination of results from the *in vitro* analyses, strongly suggest that the micronised wheat samples used in Trial 4 were not adequately processed. Only the soft wheat/high cook treatment showed evidence that the starch had undergone a degree of gelatinisation. The starch structure of the remaining micronised samples appeared very similar to that found in the raw wheat, despite being processed under typical conditions commonly employed in commercial feed mills. Because of this apparent disparity in processing, it is recommended that these *in vitro* methods could be a

valuable tool in evaluating the adequacy of processing, when applied to cereal grains, prior to their inclusion in animal diets.

Piglet response to micronised wheats showed enhanced starch digestion coefficients within the 0.5 region, compared with values obtained using raw wheats in Trial 3. Coefficients increased significantly along the small intestine from the 0.5 to 0.75 region, and CTTAD values (all greater than 0.97) indicated that starch digestion was nearly complete over the total tract. Results from the micronised wheat diets showed that starch digestibility within the small intestine was not affected by wheat endosperm texture but a high cooking regime was more beneficial, as reflected in significantly higher digestibility coefficients ($P = 0.047$).

In-vitro analysis of the extruded wheats used in Trial 5 revealed that the processing conditions had caused extensive damage to the starch granules. The RVA pasting profiles comparing the raw and extruded wheat (Figure 8.6) showed a shift from a gelatinisation peak (typical of raw starch) to a cold swelling peak (typical of extruded starch) following a progressive loss of starch integrity and a decrease in final end viscosity. Polarised light microscopy of the extruded wheats showed no evidence of maltese crosses, again indicating that the starch granules had been extensively damaged (Figure 8.4 – C and D). This loss of crystallinity was further confirmed by the XRD profiles of the extruded wheats (Figure 8.7) revealing much smoother curves than those obtained from the micronised wheats in Figure 8.5.

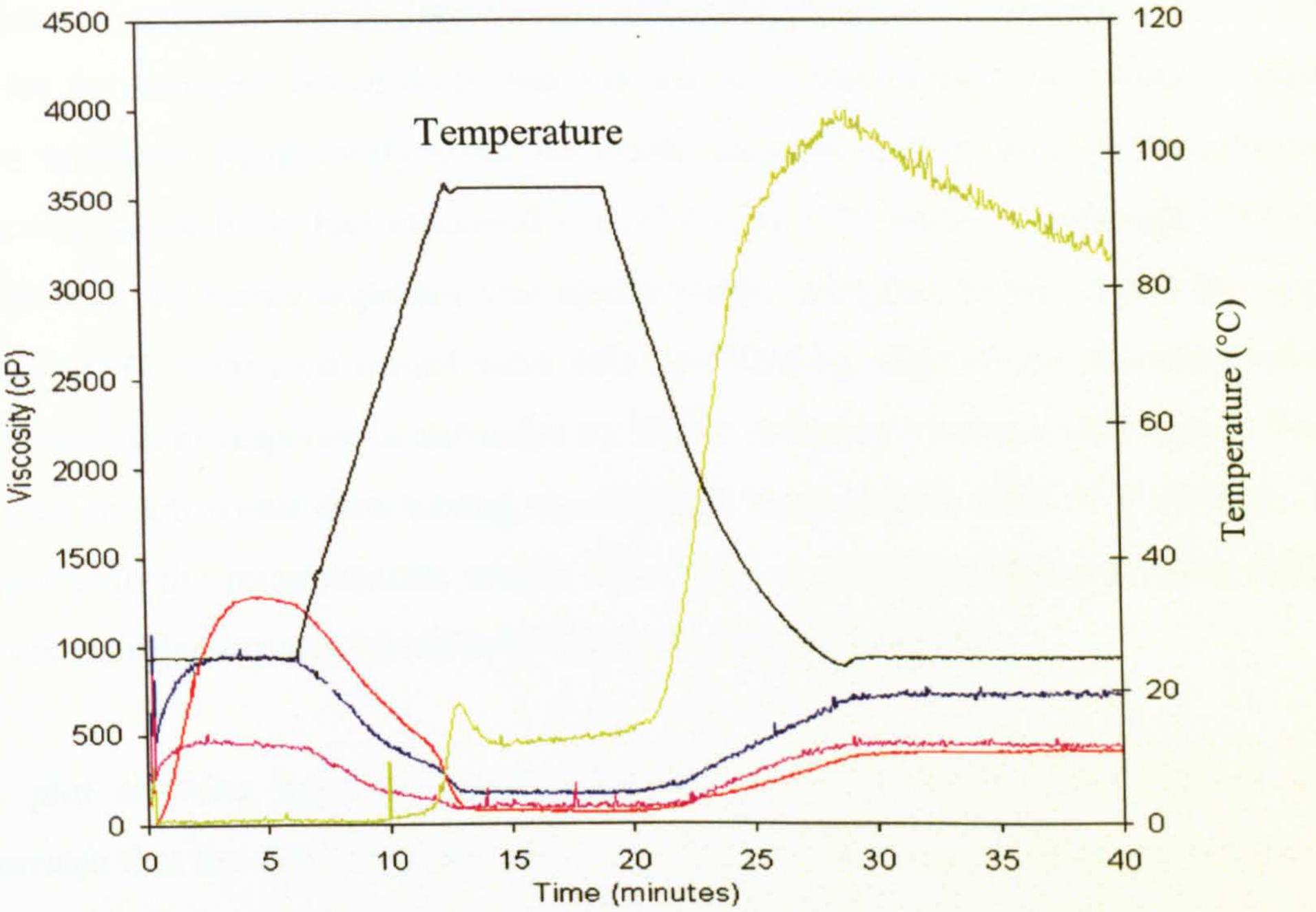


Figure 8.6: *RVA profile of soft raw (■) and extruded wheat samples. Hard/high SME (■), soft/high SME (■), soft/low SME (■)*

Figure 8.7: *XRD pattern of soft raw (S/R) and extruded wheats: soft/low cook (S/LC), soft/high cook (S/HC), hard/low cook (H/LC) and hard/high cook (H/HC)*

Coefficients of apparent starch digestibility within the piglet small intestine were much greater for the extruded wheat diets than the raw and micronised cereal diets. Starch digestion increased significantly along the small intestine and animals on the extruded diets showed digestibility had increased to 0.97 by the 0.75 region. Although CTTAD values showed that starch digestion was almost totally complete by the end of the large intestine (>0.99), enhanced values were still exhibited by pigs on the extruded wheat diets. A significant response to extrusion by wheat endosperm texture was seen in Trial 5 with pigs on soft wheat diets having significantly higher coefficients (P = <0.001). In agreement with the micronisation results from Trial 4, a higher degree of cook (High SME) was significantly more beneficial than a low cooking regime.

A box plot showing apparent starch digestion from the piglet trials (Figure 8.8) demonstrates that the choice of processing method can have a marked effect on *in-vivo* starch digestibility. A comparison of starch digestion within the mid and distal small intestine for all of the cereals reveals an overall trend for starch digestion to increase in relation to the severity of the processing technique. An increase in extrusion processing promoted high starch digestibility whereas the miconisation data were much lower and similar to those obtained using the raw cereals. Despite the factor of biological variation, it can be seen that the inter-quartile range of digestibility coefficients obtained at the 0.75 region was considerably narrower for piglets on the processed cereal diets, compared with those fed unprocessed cereals. This observation was still evident at the 0.5 site for the extruded cereals but the inter-quartile range for both the unprocessed and micronised cereals in this region of the small intestine was very similar (Figure 8.8), suggesting that micronisation did not greatly improve apparent starch digestibility within this region of the tract when compared against the raw cereals.

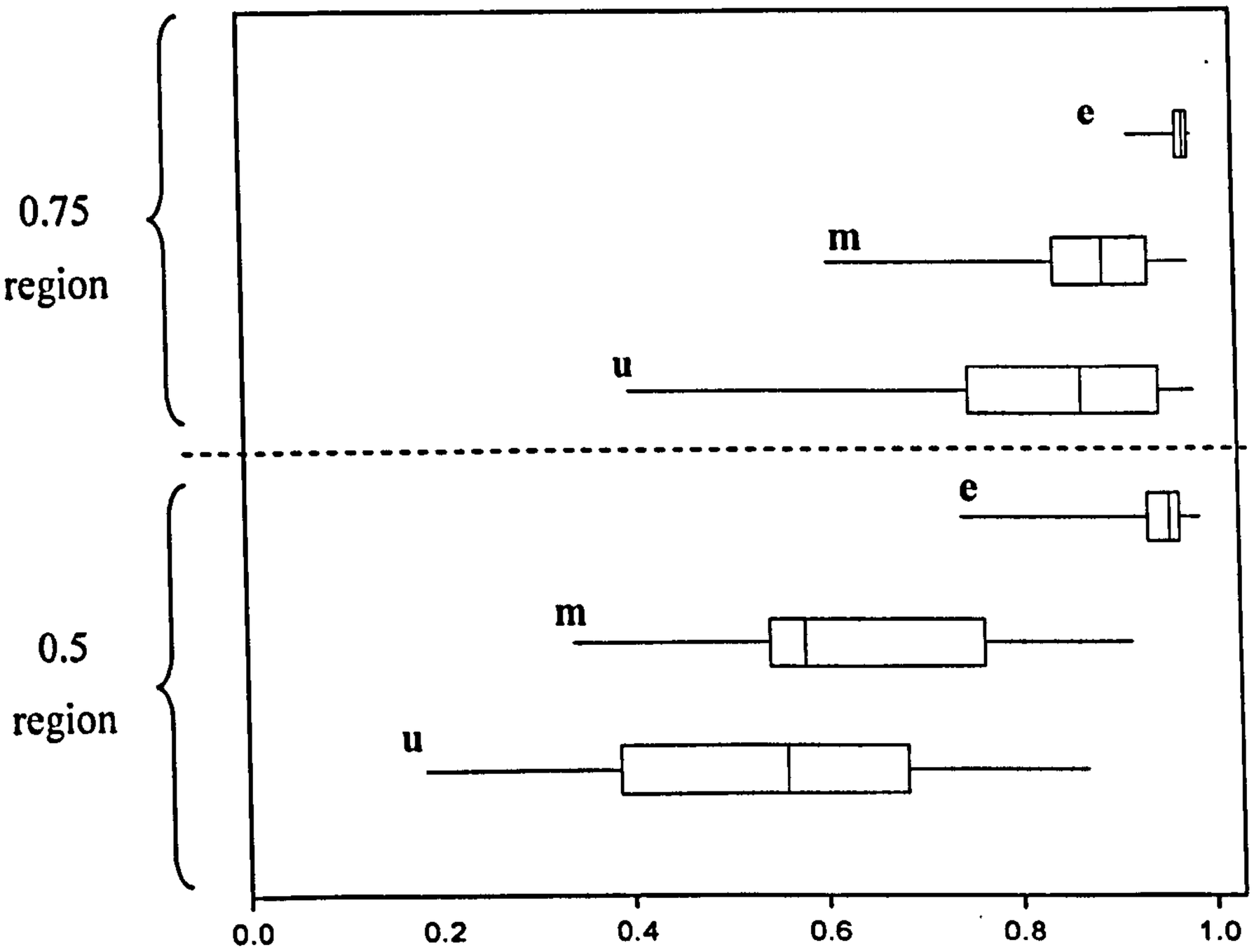


Figure 8.8: *Box plot of digestibility coefficients of starch within the 0.5 and 0.75 sites of the small intestine in weaned piglets from a range of cereals. (u) unprocessed, (m) micronised, (e) extruded*

Further evidence that the use of micronisation and extrusion improved *in-vivo* digestibility of starch in wheat can be seen in Figure 8.9. Examination of starch digestibility coefficients at the 0.5 site of the small intestine comparing raw with micronised and extruded wheat from Trials 3, 4 and 5 shows a significant improvement in starch digestion in the period following weaning. Feeding of raw wheat resulted in a drop in starch digestion around day 4 post-weaning (Figure 8.9). Although this reduction was still evident in pigs fed the micronised wheat diets, the drop in digestibility was less severe. The use of a micronised wheat diet generally elevated starch coefficients throughout the trial period, compared to piglets fed a raw wheat diet. Digestibility of starch was increased still further by the use of extrusion processing to the point where coefficients within this region of the small intestine were above 0.75 throughout the 14 day trial period (Figure 8.9). From these results, it is clear that feeding piglets a diet containing unprocessed wheat can lead to a significant reduction in

starch digestibility in the immediate period following weaning. Furthermore, this impairment in starch digestion around day 4 can be considerably lessened by the application of heat-processing to the wheat grain. In addition, there appears to be a clear relationship between the degree of starch granular damage from the processing method (extrusion vs. micronisation), and the benefits, in terms of enhanced starch digestion, in the 14 day period after weaning. Given that this reduction in digestibility on day 4 occurs around the same time that changes to piglet digestive physiology are typically seen in the weaned piglet, particularly villus atrophy (Hampson, 1986; Cera *et al.*, 1988), it would be reasonable to conclude that any improvement in starch digestion at this time when component absorption is often compromised would be beneficial in helping the piglet to overcome, in part, the post-weaning growth check.

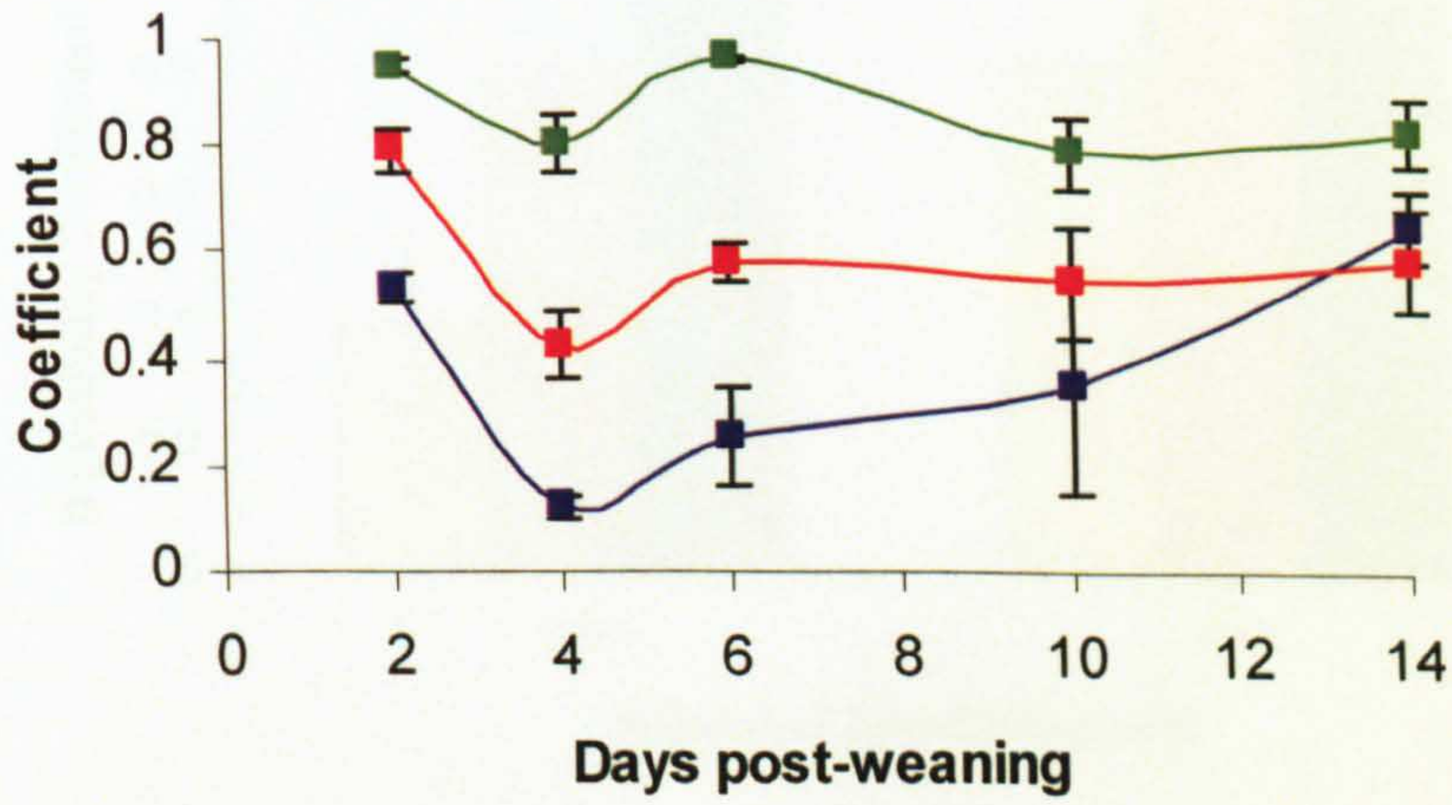


Figure 8.9: *Effect of processing treatment of raw (■), micronised (■) and extruded (■) wheat on in-vivo starch digestibility within the 0.5 region of the small intestine over 14 days post-weaning (Error bars represent standard error of the mean values)*

One of the aims of the current research programme was to highlight the inadequacy of describing thermal processing methods by name alone, with little or no stated description of the precise variables used. The importance of carefully stating the variables when subjecting cereal to a heat-treatment process is illustrated in Figure 8.10 where the effects of different extrusion parameters (Low SME vs. High SME) used in Trial 5 on the digestibility of raw soft wheat were compared within the mid and distal

regions of the piglet small intestine. A clear difference observable at the 0.5 tract site between the two extrusion regimes highlights the necessity of stating specific processing conditions used in animal trials. A positive relationship was found between starch digestibility and SME level within the 0.5 tract region and the high coefficient for the soft wheat/high SME diet suggests enhanced digestion and a more rapid release of starch within this area of the small intestine. It is unclear whether a diet containing rapidly, as opposed to slowly, digestible starch is more beneficial for the piglet, although there is evidence that the latter may lead to better overall performance in broiler chickens (Weurding *et al.*, 2003). This is an important topic in relation to the rate of starch breakdown in the weaned piglet which warrants further research.

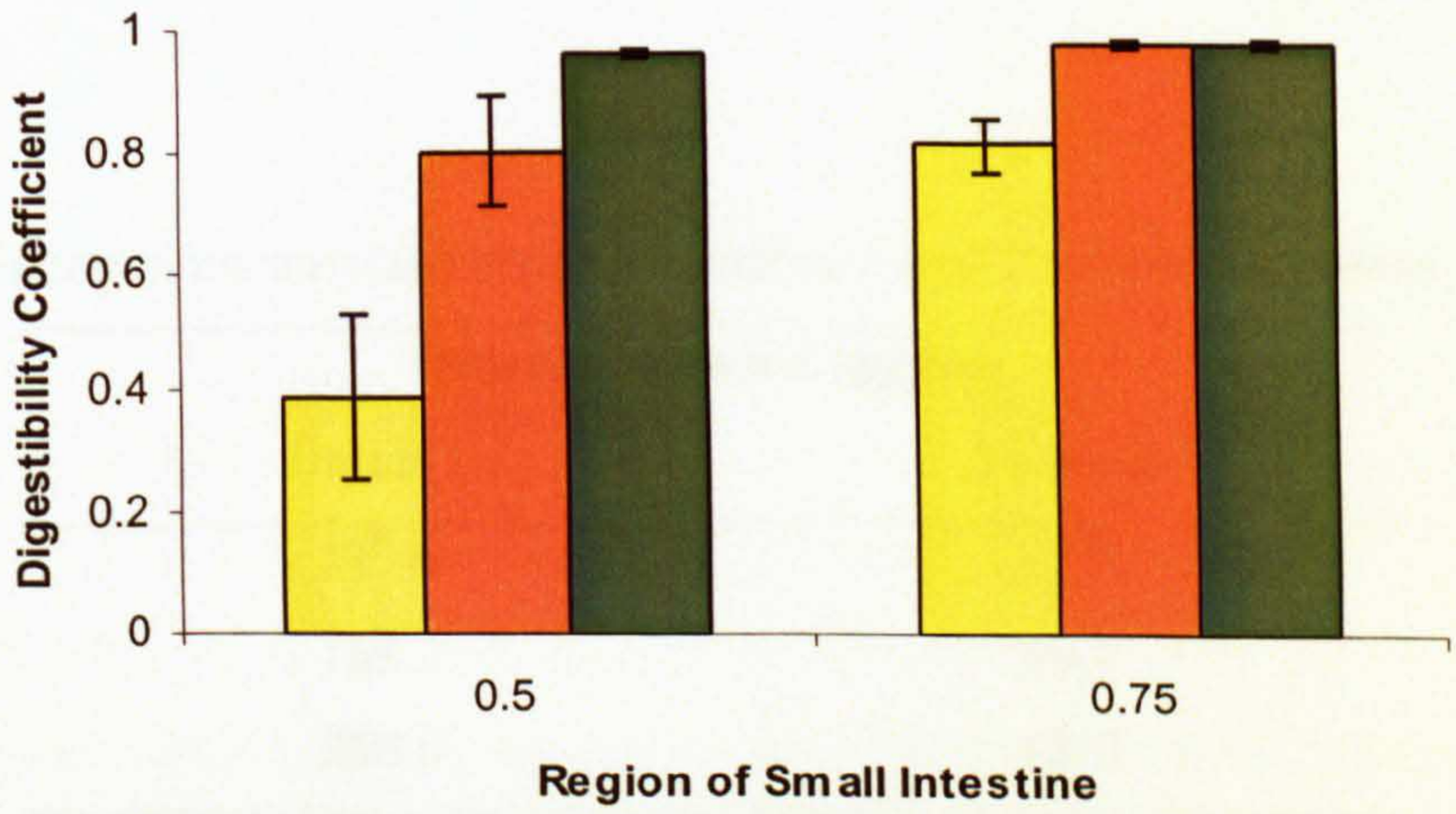


Figure 8.10: *Coefficients of apparent starch digestibility within the 0.5 and 0.75 regions of the small intestine comparing soft raw wheat (■), with soft wheat extruded under Low SME (■) and High SME (■) conditions (Error bars represent standard error of the mean values)*

8.5. Analysis of soft wheat batches

In-vitro analysis of the two soft wheat batches used in the piglet trials revealed considerable differences in their rheological properties. Although the RVA pasting curves obtained for the wheats were identical in shape, the profile of the soft wheat used in Trials 1 and 2 (batch 1) was considerably lower than that seen for the wheat used in Trials 3, 4 and 5 (batch 2). The addition of an amylase inhibitor ($AgN0_3$) to batch 1

restored the pasting profile towards that seen for batch 2, although a noticeable reduction in the gelatinisation peak was still evident (Figure 8.11). Further analysis using *in-vitro* amylolytic digestion (described in Section 2.11.6) revealed a considerable difference in endogenous amylase levels between the two wheat batches (Table 8.2), even though the two batches were the same wheat variety grown on the same farm. This variation in endogenous enzyme concentration helps to explain the differing RVA pasting profiles seen between the two wheats. The higher amylase content of batch 1 wheat promotes a greater degree of hydrolysis of the amylopectin bonds, resulting in smaller polysaccharide chains. These smaller molecular fragments lead to reduced swelling and a lower gelatinisation peak when the sample is heated. In addition, the smaller polysaccharide chains also restrict the size of molecular re-association that occurs upon cooling, with the effect of reducing the final viscosity, clearly apparent in the RVA profile.

Table 8.2: Endogenous amylase levels in batches 1 and 2 of raw soft wheat

Glucose released (µg/ml)			
	Batch 1		**Batch 2**
	187.6		17.9
	168.2		19.1
	200.0		18.9
Mean	**185.3**	**Mean**	**18.6**

(Values represent the average of 3 samples of each wheat batch, performed in triplicate)

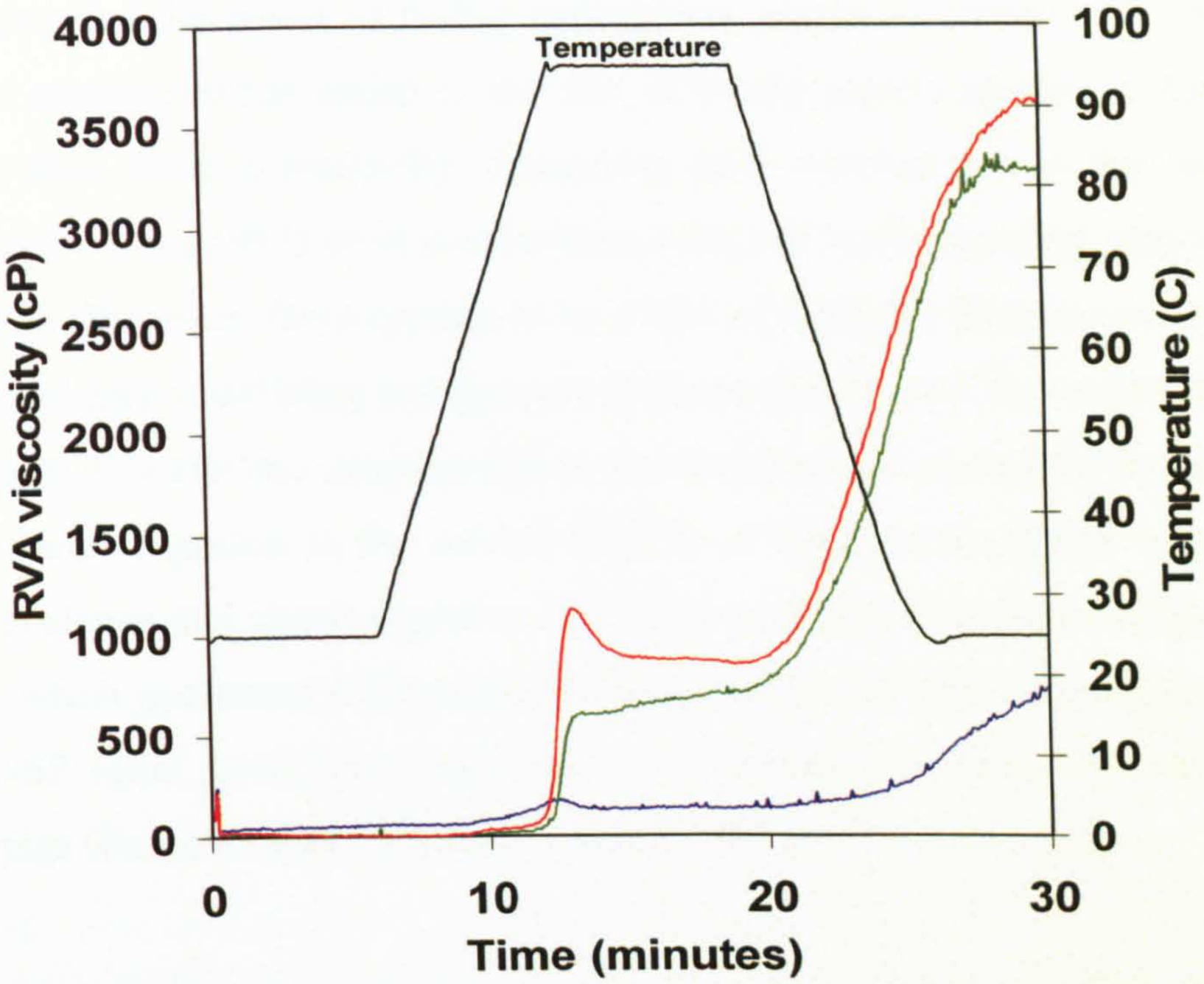


Figure 8.11: *RVA pasting profiles of soft wheat: (—) batch 1 minus $AgN0_3$, (—) batch 1 plus $AgN0_3$), (—) batch 2*

Piglet response in relation to the differing soft wheat batches was somewhat mixed. CAD of starch within the small intestine was noticeably higher for the piglets fed soft wheat from batch 1 (high amylase) compared with animals fed the second batch. This variation in starch digestibility was most noticeable at the 0.5 site. However, further examination of the data revealed considerable fluctuations in the coefficients from this region over the trial period. This fluctuation was particularly evident in both trials where pigs were fed the wheat of lower endogenous amylase content (Trials 3 and 5). This is an example of how experimental design limited the sample population (two pigs per diet) from which digesta was collected on each of the slaughter days, which may have influenced some of the mean coefficients obtained in these trials.

Examination of the data from the 0.75 site is more robust as the coefficients are more consistent over time. Pigs fed the higher amylase wheat had greater average CAD values at the 0.75 site (Trial 1 - 0.870) compared with animals fed the second wheat batch (Trial 3 - 0.840; Trial 5 – 0.810) indicating a degree of enhanced starch digestion

in the piglets fed the wheat of higher endogenous amylase content. The inclusion of exogenous amylase, when added to the diet of young piglets appears to have varying effects on ileal starch digestibility, depending upon whether fed as the only enzyme source (Gdala *et al.*, 1997) or in combination with cell wall degrading enzymes (Inborr *et al.*, 1993). However, there appears to be a lack of available literature on animal trials examining wheats of differing endogenous amylase content, and the associated effect on *in-vivo* starch digestibility. Another factor that may have accounted for the difference in apparent starch digestion is the soluble NSP level between the 2 soft wheat batches. There is evidence that starch digestion in poultry is reduced by supplementation of the diet with wheat pentosans (Choct and Annison, 1992a, 1992b). Similarly, the use of soluble NSP (guar gum) has been reported to reduce the absorption of glucose in growing pigs (Rainbird *et al.*, 1984; Ellis *et al.*, 1995).

Overall, these findings highlight the fact that a known variety of wheat, grown at the same location by the same breeder on two consecutive years can have markedly different rheological properties, owing to differences in the amount of endogenous amylase content. In light of this variation, and in the differences observed in the starch digestibility coefficients within the small intestine in the young piglet, the current research highlights the importance of collecting information about the physicochemical properties of raw cereals, prior to their inclusion in diets for use in animal trials.

8.6. Gut morphology

During the experimental studies, one animal from Trial 4, fed a soft wheat/low cook micronised diet exhibited a small area of reddening in a portion of the stomach. None of the remaining pigs in any of the trials exhibited any evidence of gastric ulceration, indicating that the finely ground particle size of the diet was not detrimental to piglet health. In addition, any incidence of scouring observed during the trials was not linked to dietary treatment and there was a considerable reduction in the incidence of scouring in the studies feeding cooked wheats (especially noticeable in Trial 5).

One of the stated objectives for this research programme was to provide the weaned piglet with a diet that maintains a healthy gut environment. Previous research by Pickard (2003), examining bacterial cultures within the hind-gut of newly-weaned piglets, noted the time-consuming nature of employing this method to assess gut health. Considering time constraints in the current research programme, it was decided that examining villus morphological structure within the small intestine, would be a more useful approach in assessing gastrointestinal health of the young piglet in the period following weaning.

Comparison of gut morphology data from the studies involving raw cereals (Trials 1-3) revealed significant changes in the measured parameters over time. Pigs in Trial 2 experienced more severe changes to their intestinal morphology in the period immediately after weaning than animals in Trial 1, in terms of a greater reduction in villus height along with increased crypt hyperplasia. An initial conclusion from these results would be that the raw cereals fed in Trial 2 were more detrimental to piglet gut morphology over the trial period than those fed in Trial 1. Complicating this assumption, however, are the differing morphology profiles from the raw wheat diet, common to both Trials 1 and 2. Although of identical genotype, the piglets in Trial 2 were sourced from a different supplier to those used in the other four studies. As corresponding feed intakes over the experimental period were noticeably less for these animals, this could well have accounted for the variation seen in the intestinal data.

The morphology results, however, do not appear to show a straightforward link between maintenance of villus architecture and feed intake after weaning; Trial 1 revealed that piglets fed the triticale diet had significantly higher feed intakes during both the 0-5 day and the post 5-day-periods. Despite this enhanced appetite, there was no observed relationship with enhanced villus architecture throughout the 14 day trial. Likewise, the strong preference ($P = 0.059$) for the maize diet in Trial 2 for the initial 5 days of the study revealed no associated benefit in morphology parameters for these animals. Analysis of the data in Trial 3 also appears to show some discrepancy between the parameters of feed intake and gut morphology; the shortening of the villi on day 2 post-

weaning was considerably worse for piglets fed the hard endosperm wheat (-26%) than those given the soft endosperm wheat diet (-10%). As feed intakes between the two groups were not significantly different during this period, this variation in appetite suggests that soft endosperm wheat offers an advantage over hard endosperm wheat in maintaining villus architecture over the first 6 days after weaning. This finding is based on a small sample size (two pigs per diet) for each slaughter day and therefore would warrant further investigation using a larger number of animals to confirm the results obtained.

The effect of heat processing on gut morphology parameters appears to be dependent upon the type of processing treatment used. The greatest degree of variation between treatment methods was seen in the villus height and width data. Micronisation appeared less favourable than the use of extrusion processing for maintaining piglet intestinal morphology, as shown by a more severe and prolonged period of villi shortening. An interesting observation from the micronised data is a clear effect between endosperm texture and crypt depth. Pigs fed the hard wheat diets had greater crypt depth measurements than those fed soft wheat. This endosperm effect was seen for both cooking levels and suggests there may be a relationship between crypt hyperplasia and endosperm texture.

Of the thermal processing treatments studied, extrusion was the most beneficial in maintaining villus architecture post-weaning. Measurements of villus height were all greater than pre-weaning levels by day 6 and were between 10% and 30% longer than control values by day 10 of the trial. There appears to be very little literature at present that has investigated the effect of heat-treated feeds on small intestinal structure in piglets. One such study that has examined the inclusion of differently micronised and extruded full fat soya beans in weaner diets concluded that small intestinal integrity was not affected to any significant extent by the use of thermal processing (Zarkadas and Wiseman, 2005). The results of Trials 4 and 5 clearly reveal different villus height profiles over time, depending upon thermal processing treatment. However, the

extrusion data is clouded somewhat by the similarity between the processed and raw wheat morphology profiles.

8.7. Caecal VFAs

As no VFAs were determined using caecal digesta from animals in Trial 3, conclusions are restricted to data collected from the remaining trials. Total VFA production was not significantly affected by individual dietary treatment in any of the trials studied. However, the total production of VFAs was noticeably reduced in the studies involving processed wheats, indicating that the application of heat treatments to the cereals resulted in less substrate being made available for fermentation within the hind gut, leading to a reduced production of VFAs by the gastrointestinal microflora.

None of the experimental trial diets showed a statistically significant effect on molar proportions of propionic acid within the caecum. Despite this, a relationship was evident whereby piglets fed processed cereals had lower molar proportions of propionic acid than those fed the raw diets (Figure 8.12), indicating that less undigested starch passed through to the large intestine, as a result of processing the cereal. This reduced production of propionic acid was not clearly affected by the severity of the processing variables used in Trial 4 although there was a trend in Trial 5 for lower propionic acid concentrations in response to the highly extruded cooking regime. The association between undigested starch in the small intestine and subsequent fermentation of the same substrate within the caecum is complicated by the results of Trial 5; despite differences in starch coefficients between the raw and low SME wheat diets at the 0.75 region of the small intestine between these two diets, this did not lead to variation in propionic acid fermentation levels within the caecum.

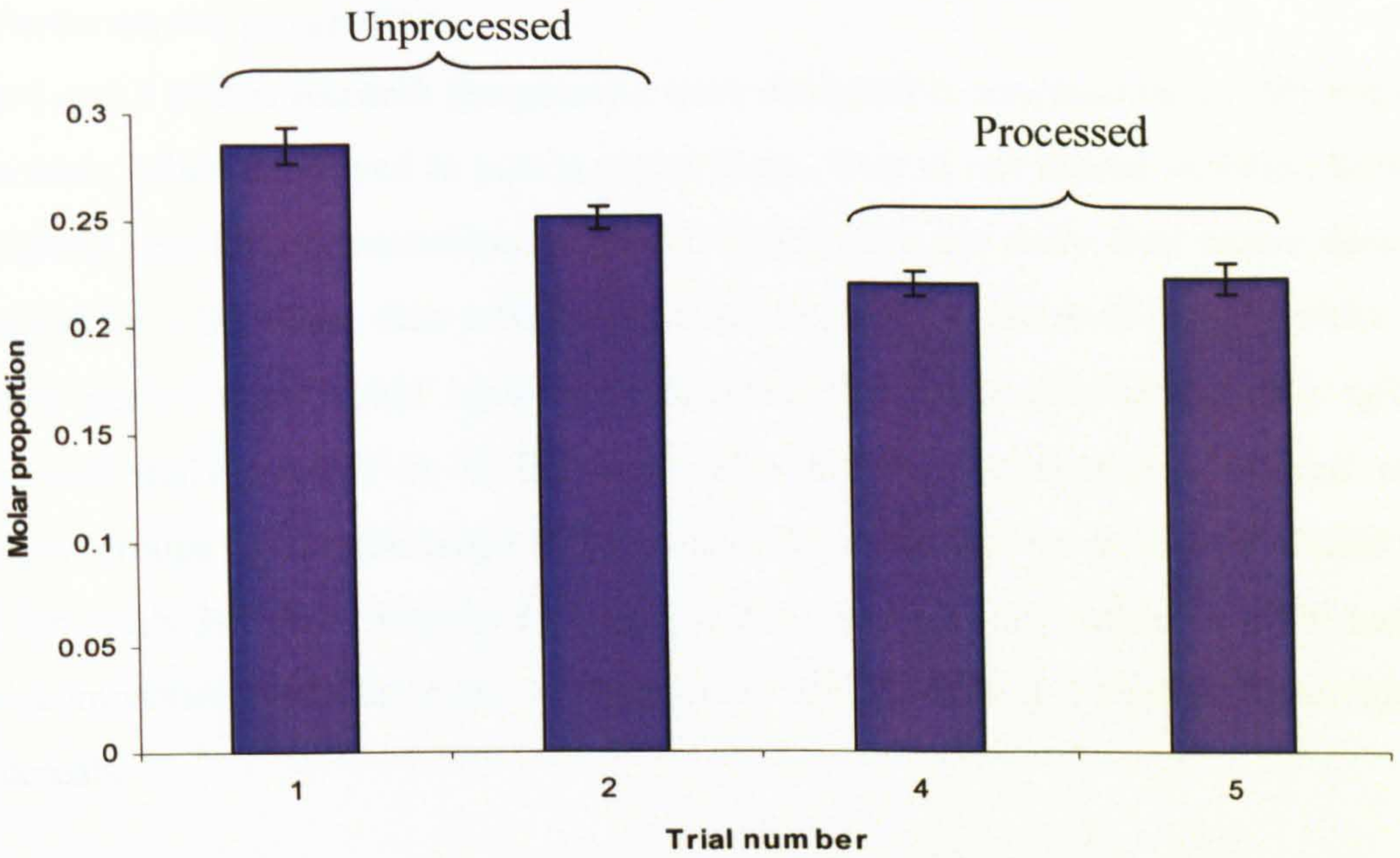


Figure 8.12: *Mean molar proportions of propionic acid (■) in the caecum of weaned piglets fed raw and processed cereals (Error bars represent standard error of the mean values)*

The presence of mainly branched-chain VFAs (isobutyric, isovaleric and valeric) in the large intestine is usually explained as a result of fermentation of protein which has escaped digestion in the small intestine (Williams *et al.,* 2001). The high apparent digestibility of nitrogen in the small intestine of pigs fed the diet based on whole oats explains the trend for the same animals exhibiting lower levels of valeric acid within the caecum. The results of Trial 5 showed that mean molar proportions of valeric acid decreased in response to an increasing severity of extrusion cook level ($P = 0.013$). This was surprising as CAD of nitrogen was also lower for the extruded wheat diets in the small intestine which would indicate a greater amount of protein being made available for subsequent fermentation. This confounding result suggests that the relationship between the amounts of protein digested and fermented within the small and large intestines respectively may be more complex than originally assumed.

8.8. Performance parameters

Trials 1 and 2 of this research programme were designed to examine variability between raw cereals, when employed in young piglet diets. Due to nutritional variation between raw cereals (eg. DE concentration, available lysine etc), the daily feed intake data was calculated on a DE basis, thus reflecting a more accurate measure of energy intake over the trial period than would have been indicated by using raw intake data (g/day). Expressing daily intakes on a DE basis also allowed comparison between cereal treatment groups to be undertaken within each trial. Although iso-energetic wheats were used for Trials 3-5, daily intakes for these studies were also expressed on a DE basis to allow comparison between these 3 wheat trials and the initial 2 studies, involving the raw cereals.

The intake of feed in the initial few days following weaning is typically hugely variable. Trials 1-5 show that appetites over the first 5 days after weaning fluctuated greatly before a regular eating pattern was established. In all trials, the variation in appetite between treatment groups was generally reduced after 5 days, as indicated by a reduction in the calculated coefficients of variation. Trial 1 revealed that pigs had a significant preference for the triticale diet which was evident throughout the 14 day trial period. The increased DE intake of pigs on the triticale diet meant that these animals also exhibited the greatest improvement in terms of DLWG compared with piglets fed the other cereals in the trial, but not to the point of statistical significance. Furthermore, the current research programme found that DLWG was not affected by any of the raw cereals examined. The results from Trial 2 agree with the work of Lynch and Zoccarato (1992), who reported no difference in DLWG between weaned piglets fed either raw milled wheat or maize diets. In Trial 3, the lack of significance between wheat endosperm texture on feed intake and DLWG values agree with the findings of Pearce *et al* (1997) where a comparison of hard and soft wheats failed to show any significant difference in terms of performance parameters.

Trials 4 and 5 demonstrated that post-5-day appetites can be significantly affected by wheat endosperm texture, dependent upon how the wheat is processed (including choice

of processing method), prior to feeding. The micronisation and extrusion trials show that choice of cooking parameters has a greater influence on performance patterns than wheat endosperm texture. The micronised wheat data from Trial 4 suggests that whilst feed intake does not appear to be significantly influenced by wheat endosperm texture, the intensity of micronisation cook level can exert a significant response, but only after the initial 5 days following weaning. The significant interactions between endosperm texture and cook level, observed for both time periods (0-5 day and post 5 day), showed a switch in both wheat endosperm texture and micronisation cook level as the trial progressed. As described in the summary of Trial 4, this switch is most likely due in part to chance; a reflection of the highly variable nature of piglet appetites at weaning, particularly as the preference remained until the end of the trial period.

The influence of cook level on DLWG in Trials 4 and 5 appeared dependent upon processing method used. For micronised wheat, the significantly greater DLWG values for the low cook diets are not in agreement with a similar study by Zarkadas and Wiseman (2001), where the effect of different micronisation temperatures of wheat found no difference in piglet performance. Trial 5 revealed that extrusion, irrespective of cooking regime, resulted in significantly worse feed intakes throughout the trial, than the comparative raw wheat diet, with DLWG following a similar pattern. This lack of benefit from feeding an extruded wheat diet to weaned piglets has also been reported by Lynch and Zoccarato (1992), where heat processing of wheat (by extrusion) failed to show any significant improvement in terms of DLWG compared to animals fed a raw wheat diet.

8.9. Meta-analysis

As a final analysis, computer modelling was employed to determine whether any relationships or interactions were evident across the five animal trials. Principal component analysis (PCA) is a powerful statistical model that is used to identify patterns in data and to highlight any similarities or differences between samples. PCA was used to give a good overview of linear relationships between variables, in addition to showing any segregation of different groups of samples within the dataset.

Despite the differences observed in the small intestinal data within the individual trials, the use of PCA on the total gut morphology data collected from Trials 1-5 did not indicate any overall relationship with diet. Similarly, analysis of viscosity and nitrogen data from the five trials did not reveal any significant relationships using PCA.

In order to allow comparison between *in-vitro* starch parameters measured using food science techniques and *in-vivo* starch coefficients from the piglet trials, a linear regression model using partial least squares (PLS) was used.

By allocating an individual number to each dietary treatment studied in the pig trials, an average CAD value for starch at the 0.5 site was calculated for each diet (Table 8.3). Each of the two raw wheat batchès were allocated a different dietary number in order to identify whether variation in endogenous amylase content could be distinguished using the model. In addition, the soft raw wheat from Trial 2 was also allocated an individual dietary number in order to determine whether animal variation (different pig supplier used for this trial) had any notable effect. Cross validation (CV) was used to determine how well the *in-vivo* data fitted the model; each dietary treatment was removed in turn from the dataset and its value predicted using the remaining 16 data points. This was repeated for each diet to produce a linear regression graph comparing predicted *in-vivo* values against those actually obtained from the animal trials. Using this approach, a strong correlation (R^2 = 0.71) was found between the predicted and actual starch coefficients, indicating a good fit of the data to the computer model (Figure 8.13).

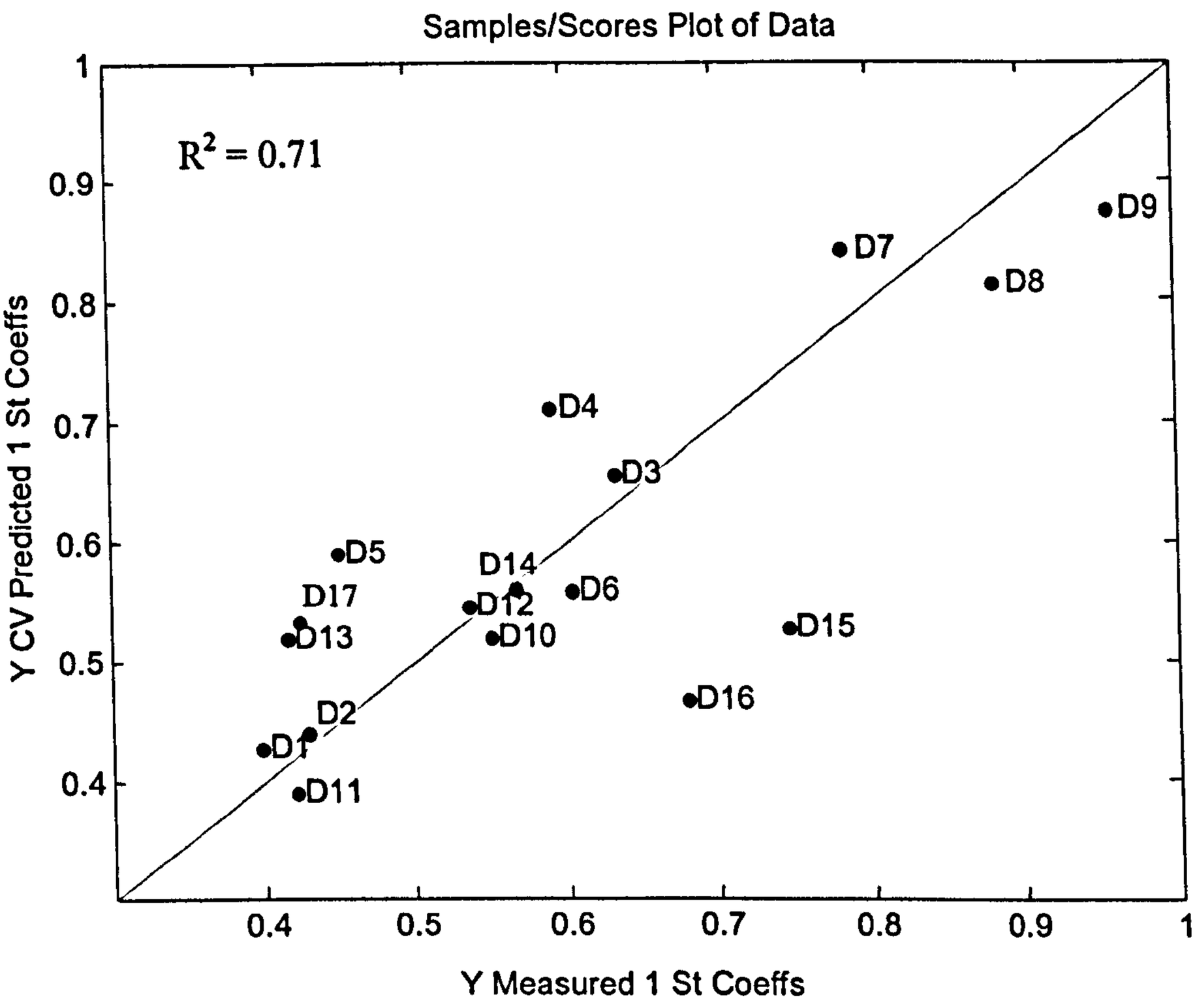


Figure 8.13: *Linear regression model using PLS showing correlation between predicted and observed in-vivo starch coefficients at the 0.5 site of the small intestine, using in-vitro starch parameters (data grouped by Diet)*

Table 8.3: Dietary numbers allocated for meta-analysis

Diet No.	Cereal	Processing Treatment	Diet No.	Cereal	Processing treatment
1	HW	Raw	**10**	SW *	Raw
2	SW	Raw	**11**	Barley	Raw
3	HW	High Micronised	**12**	Rye	Raw
4	SW	High Micronised	**13**	Triticale	Raw
5	HW	Low Micronised	**14**	SW * [y]	Raw
6	SW	Low Micronised	**15**	Naked Oats [y]	Raw
7	SW	Low Extruded	**16**	Whole Oats [y]	Raw
8	HW	High Extruded	**17**	Maize [y]	Raw
9	SW	High Extruded			

*(HW) Hard wheat, (SW) Soft wheat, * higher amylase content, [y] pigs from different supplier*

The resulting location of the data points in the regression graph revealed that the extruded wheat diets (D7, D8 and D9) were clearly separate from the other dietary values. In addition, data from the micronised wheat diets (D3, D4, D5 and D6) were not noticeably different to the values from the raw cereals, a finding that was also observed with the associated piglet studies. The model was also able to distinguish between the batch of soft wheat with a high amylase content (D10) and the second batch of wheat with a lower enzyme level (D2), revealing that a higher endogenous amylase content was generally associated with higher *in-vivo* starch coefficients at the 0.5 site. This finding supports the general conclusion obtained from the piglet trials, although fluctuating coefficients in the individual studies complicated this original assumption.

An initial analysis of the *in-vitro* starch data revealed seven variables that were either strongly positively or negatively correlated with *in-vivo* starch coefficients from the 0.5 tract region in the piglets (Figure 8.14). Table 8.4 identifies the *in-vitro* variables along with their correlated regression coefficient values.

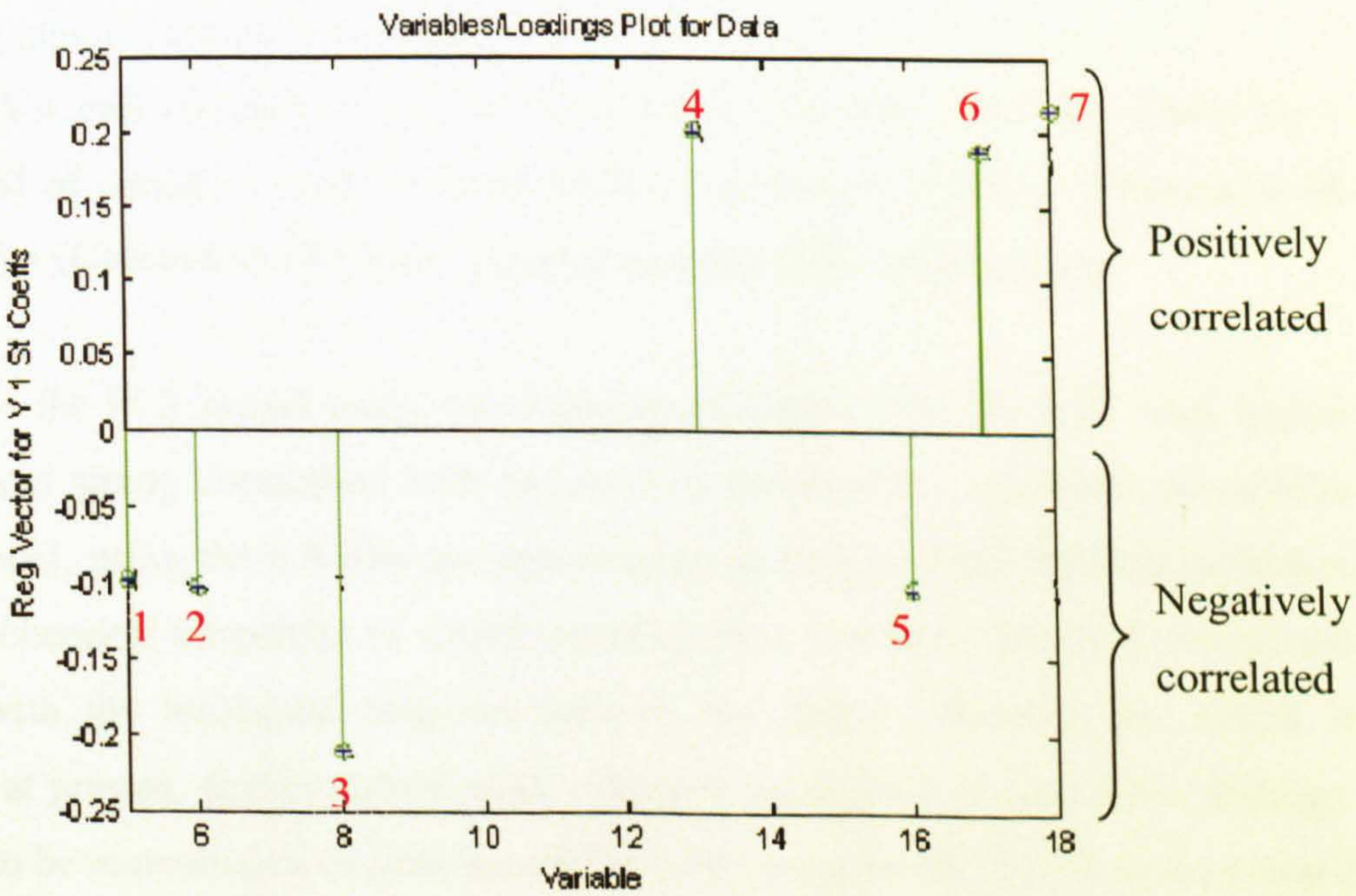


Figure 8.14: *In-vitro variables strongly correlated with in-vivo CAD of starch at the 0.5 site in the weaned piglet*

Table 8.4: Strongly correlated *in-vitro* variables and associated regression coefficients

Number	Variable I.D.	Regression coefficient
1	RVA end viscosity of diet	-0.098
2	RVA peak viscosity of diet	-0.102
3	Delta H (from DSC analysis)	-0.209
4	WSI of cereal	0.201
5	Endogenous α-amylase	-0.105
6	Glucose released at 45 min	0.187
7	Glucose released at 300 min	0.213

Using the regression coefficients obtained from each of the *in-vitro* variables in Table 8.4, a linear equation can be formulated to predict *in-vivo* starch digestibility at the 0.5 site in the piglet for an individual dietary sample.

The linear equation obtained is as follows:

- 0.098 x (RVA end viscosity) - 0.103 x (RVA peak viscosity) – 0.209 x (Delta H) + 0.201 x (WSI of cereal) – 0.105 x (Endogenous amylase) + 0.188 x (Glucose at 45 min) + 0.213 x (Glucose at 300 min) = *in-vivo* starch coefficient at 0.5 site.

Repetition of the PLS model using the starch coefficients from the 0.75 tract region revealed a less strong correlation with the *in-vitro* parameters. However, the results from the model, using the 0.5 data are encouraging as they provide tentative evidence that physicochemical properties of starch granules from raw and processed cereals are correlated with the biological response seen in the piglet. Because the model is preliminary at present, further animal trials would be needed to validate these findings. It should also be remembered that the use of the model is currently limited to diets based on cereals, included in the diet at a rate of 586 g/kg. It is clear that such an approach could be used to identify not only differences between cereal types, but also variation in endogenous amylase content between different batches of a named cereal and the effect

these parameters have on starch digestibility within the small intestine of the young piglet.

This current study has provided the first strong direct evidence highlighting the importance of collecting primary information about the properties of raw and processed cereals, and the changes they undergo upon processing, prior to their inclusion in piglet diets. The programme reported has emphasised the importance of the need to state the precise processing variables used in animal trials, and has demonstrated that reliance on a named processing method alone is an insufficient descriptor of potential nutritional value. The adoption of such an approach should provide a more accurate interpretation of animal responses to changes in diets. In addition, the use of this method will likely contribute to the determination of the optimum processing conditions necessary for weaned piglets.

8.10. General conclusions

In summary, this thesis has demonstrated that:

i) Considerable variation in digesta viscosity is evident throughout the digestive tract in piglets fed diets containing various raw ground cereals. Despite this variation in viscosity, there was no perceived detrimental effect on animal performance in the two weeks after weaning.

ii) The apparent digestibility of nitrogen at the distal (0.75) region of the small intestine can be significantly affected by wheat endosperm texture.

iii) Changes to intestinal morphology in the immediate days following weaning may not be solely attributed to variations in feed intake.

iv) The use of thermal processing of wheat through micronisation and extrusion does not offer any benefit over unprocessed wheat with regard to digestibility of nitrogen in the small intestine in the young piglet.

v) The use of micronisation improves the apparent digestibility of starch at the 0.5 tract region of young piglets, compared with animals fed an unprocessed wheat diet. Digestion coefficients at this region are improved further by the use of extrusion processing.

vi) Micronisation of wheat lessens the reduction in starch digestibility on day 4 post-weaning, typically seen at the 0.5 intestinal region in piglets fed an unprocessed wheat diet. Starch digestibility on day 4 is improved still further by the use of extrusion processing of wheat.

vii) The use of thermal processing reduces the range of starch digestibility coefficients observed at the 0.5 and 0.75 intestinal sites. The more severe the processing method, the more uniform the coefficients obtained at each tract region.

viii) The labelling of dietary ingredients as 'cooked' is wholly inadequate as a descriptor of their nutritional value.

ix) Precise processing variables must be stated in all animal trials involving the use of processed ingredients. Starch digestibility within the small intestine of

weaned piglets has been shown to be clearly affected by severity of extrusion regime.

x) Batches of wheat of known variety and grown at the same location on consecutive years can have markedly different rheological properties. Therefore a thorough *in-vitro* assessment involving techniques described in this research programme is necessary, prior to their inclusion in animal diets.

xi) Measurements of physicochemical properties of starch granules using a range of accurate and quantitative tests in the field of human food science can be used to predict starch coefficients at the 0.5 site of the small intestine in the weaned piglet.

8.11. Problems encountered

A common difficulty found throughout the animal trials was a lack of sufficient digesta for analysis at the 0.25 site of the small intestine on slaughter days. This problem has been reported previously in similar work with weaned piglets (Inborr *et al.*, 1993) where insufficient collection of digesta prevented any analysis at the proximal region from being performed. In the study by Inborr *et al* (1993), the time interval between the lights being switched on in the experimental unit and time of slaughter was at least three hours. In the current series of trials, this time difference was at least five hours, and it would appear that even though feed was provided on an *ad-libitum* basis, many of the pigs still had insufficient digesta in the proximal region at the time of slaughter. It is likely that experimental design (i.e. targeting sample collection from a specific location of the tract) coupled with the variable feed intakes of weaned piglets were contributing factors with regard to sample collection from many of the animals.

8.12. Future work

Further investigations are required in order to establish whether the rate of starch breakdown in the small intestine has an effect on weaned piglet health and performance.

Further work should be performed in order to support the findings of Trial 3 where a significant interaction between wheat endosperm texture and day was found for villus

architecture in the initial week after weaning. It is suggested that future experimental design incorporates a larger sample population on each slaughter day.

Some of the research findings indicate that wheat of soft endosperm texture is more beneficial for the weaned piglet than hard. In order to support these findings, further trials examining a greater number of wheat cultivars are necessary.

The meta-analysis findings suggest that further animal trials would be warranted in order to test the statistical model, correlating *in-vitro* starch measurements with *in-vivo* starch coefficients.

Lastly, the research programme has shown the fundamental importance of collaboration between the fields of human food science and animal nutrition. Any future work should involve the expertise of both fields, in order to determine the optimum processing conditions necessary to maximise starch digestion in trial diets.

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APPENDIX 1: Dietary specification used in all trials.

Ingredient	Inclusion (g/kg)
Cereal	586
Hipro Soya	150
Skimmed Milk	175
Vegetable Oil	50
L-Lysine	4.6
Methionine	1.6
Threonine	1.7
Tryptophan	0.3
Salt	5.0
Limestone	4.1
Dicalcium Phosphate	9.2
Vitamin/Mineral Premix	12.5