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**FEED PARTICLE SIZE, WHOLE WHEAT INCLUSION AND
XYLANASE SUPPLEMENTATION IN BROILER DIETS:
INFLUENCE ON THE PERFORMANCE, DIGESTA
CHARACTERISTICS AND DIGESTIVE TRACT
DEVELOPMENT**

A thesis presented in partial fulfillment of the requirements for the
degree of

Doctor of Philosophy in

Poultry Nutrition

at Massey University, Palmerston North,
New Zealand

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2008

ABSTRACT

The first three experiments of this thesis examined the effects of particle size reduction of grains in relation to feed form (mash vs. pellet), grain type (wheat vs. maize) and xylanase supplementation on broiler performance, energy utilisation, digestive tract development and digesta parameters. The fourth experiment examined the interaction between wheat hardness and xylanase supplementation. The effects of insoluble fibre source and whole wheat inclusion were studied in the fifth experiment.

In the first experiment (Chapter 4), pelleting reduced nitrogen-corrected apparent metabolisable energy (AMEn), but broiler performance was superior in birds fed pelleted wheat-based diets compared to those fed mash diets. Feed form had a greater effect on various measured parameters than did particle size. Pelleting evened out differences in particle size distribution between treatments and, as a result, wheat particle size had no effect on the performance of broilers fed pelleted diets. In contrast, the second experiment (Chapter 5) showed that differences in particle size distribution persisted between diets after pelleting and, as a result, coarse grinding of wheat or maize improved broiler performance compared to those fed diets based on fine particles. These results may be related, in part, to changes in size distribution following pelleting.

In mash diets, inconsistency in performance responses were found. In the first experiment (Chapter 4), coarse grinding of wheat improved weight gain and feed per gain compared to medium grinding. In the third experiment (Chapter 6), however, grinding particle size had no influence on broiler performance. The observed discrepancy suggests involvement of other factors such as wheat cultivar and grain hardness.

Data reported in Chapter 6 showed that xylanase supplementation improved feed per gain of birds fed the coarse particle size diet, but had no effect on those fed the medium particle size diet. In Chapter 7, there was a significant interaction between wheat hardness and xylanase supplementation due to the improved feed per gain and AMEn of birds maintained on hard wheat-based diet, while there was no effect of xylanase on soft wheat-based diet. These findings suggest that the efficiency of exogenous enzymes is influenced by both particle size and wheat hardness.

Data reported in Chapter 7 showed that inclusion of soft or hard whole wheat pre-pelleting produced different particle size distributions in the pelleted diets. This

suggested that hardness of the grain must be considered when choosing whole wheat for inclusion in broiler diets.

Data on the effect of feed particle size on its subsequent distribution in poultry digesta are scanty. Results reported in Chapters 4 and 5 showed that there was no effect of feed particle size within feed form on duodenal digesta particle size. On the other hand, particle size of duodenal digesta was influenced by feed form (mash vs. pellet). Wheat hardness was also found to influence the particle size of proximal (duodenum and jejunum) intestinal digesta (Chapter 7). These results indicated that the gizzard does not uniformly reduce the size of all particles. However, the gizzard appears highly efficient in grinding large particles, although some large particles escape the grinding.

The final experiment demonstrated that the effects of insoluble fibre on digestive tract development and broiler performance differed depending on the fibre source. Wood shavings, a source of coarse insoluble fibre, increased relative gizzard size and improved corrected feed per gain and ileal starch digestibility. In contrast, cellulose, a source of fine insoluble fibre, had no influence on these parameters.

In conclusion, dietary manipulations, which stimulated gizzard development, positively influenced broiler performance and starch digestibility. The findings of this thesis suggest that energy savings during feed processing could be achieved by coarse grinding of grains with no adverse effect on broiler performance and that cereals used in broiler diets can be ground more coarsely than the current practice. Wheat hardness appears to be an important criterion to consider when choosing a cultivar for whole wheat inclusion in broiler diets. Another major finding was that the effectiveness of exogenous xylanase in wheat-based diets could be improved by considering factors such as particle size and wheat hardness.

Didicated to Nrmeen (my wife)

Acknowledgments

First of all, I would like to express my profound gratitude and sincerest thanks to my chief supervisor, Professor Ravi Ravindran, for his guidance, encouragement, advices and providing expert knowledge in poultry nutrition. Without his guidance, patience, understanding and encouragement, I would not have had this achievement. Warmest thanks and appreciation is also extended to my co-supervisor Associate Professor Roger Lentle, for his valuable comments, encouragement and advice. I would like also to thank my co-supervisor, Dr. David Thomas, for his support, guidance and advice with histological measurements. Very special appreciation to Mr Donald Thomas for his technical support, assistance, suggestions and advice; Dr Patrick Morel for his advice on statistical matters.

I would also like to thank Colin Naftel and the entire staff of the Massey University Poultry Research Unit. I am thankful for the friendships we have made and that we worked together during my research.

Last and most of all, my deepest gratitude is expressed to my family, for all the support, patience, understanding and encouragement, to my wife Nrmeen Alkhiry and my children Heyam and Omar for their never-ending encouragement and love.

And to those I forgot to mention.

Publications

Studies completed during candidature, some of which are reported in this thesis have been presented in the following communications:

Refereed scientific papers

Amerah, A, M., Lentle, R.G. and Ravindran, V. (2007) Influence of feed form on gizzard morphology and particle size spectra of duodenal digesta in broiler chickens. *Journal of Poultry Science* 44: 175-181.

Amerah, A, M. Ravindran, V., Lentle, R.G. and Thomas, D.G. (2007) Feed particle size: Implications on the digestion and performance of poultry. *World's Poultry Science Journal* 63: 439-456.

Amerah, A, M. Ravindran, V., Lentle, R.G. and Thomas, D.G. (2007) Influence of feed particle size and feed form on the performance, energy utilization, digestive tract development and digesta parameters of broiler starters. *Poultry Science* 86: 2615-2623.

Conference presentations

Amerah, A, M. Ravindran, V., Lentle, R.G. and Thomas, D.G. (2005) Influence of whole wheat feeding on the performance, gizzard size and carcass characteristics of broiler chickens. *Proceedings of the Massey Technical Update Conference, Vol. 7. Massey University, Palmerston North. pp. 63-68.*

Amerah, A, M. Ravindran, V., Lentle, R.G. and Thomas, D.G. (2005) Feeding of whole wheat for broilers: Influence on performance, gizzard size and carcass characteristics. *Proceedings Nutrition Society of New Zealand. 30:164-170, Massey University, Palmerston North.*

Amerah, A, M. Ravindran, V., Lentle, R.G. and Thomas, D.G. (2006) Influence of particle size and feed form on the performance and energy utilisation of broilers. *Proceedings of the Massey Technical Update Conference, 8: 58-65 Massey University, Palmerston North.*

- Amerah, A, M. Ravindran, V., Lentle, R.G. and Thomas, D.G. (2006) Particle size and broiler performance. New Zealand Poultry Industry Conference 8:58-66, Massey University, Palmerston North.
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- Amerah, A, M. Ravindran, V., Lentle, R.G. and Thomas, D.G. (2007) Effects of fibre source and whole wheat inclusion on the performance, starch digestibility and gut parameters of broiler chickens. Asia Pacific Journal of Clinical Nutrition 16 (Suppl.): 59 (Abstract).

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List of abbreviations

AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of variance
DGGE	Denaturing gradient gel electrophoresis
DM	Dry matter
DNA	Deoxyribonucleic acid
GE	Gross energy
GLM	General linear model
GMD	Geometric mean diameter
GSD	Geometric standard deviation
h	Hours
HI	Hardness index
MJ	Mega joule
mm	millimetre
µm	Microns
N	Nitrogen
NIR	Near-infrared reflectance
NSP	Non-starch polysaccharide
PCR	Polymerase chain reaction
PDI	Pellet durability index
PSI	Particle size index
SEM	Standard error of mean
SKCS	Single-kernel characterisation system
Ti	Titanium
UV	Ultra violet
XU	Xylanase unit

CHAPTER 1

General introduction

The term 'particle size' refers to the average diameter of individual particles of feed, or simply the 'fineness of grind' of feed. The extent of milling whole grains is known to influence a number of aspects of poultry production, including bird performance and digestive tract development. In addition, the reduction of feed particle size is the second largest energy cost after that of pelleting in the broiler industry (Reece *et al.*, 1985) and likely to be the largest user of energy in the layer industry where feeds are not pelleted (Deaton *et al.*, 1989). Accordingly, any reduction in energy consumption from grinding could significantly lower feed cost.

Despite its importance, studies examining the effects of particle size in the different cereal grains used in poultry feeds are limited. In recent years, this issue has become more topical, as the industry continues to search for ways of optimising feed utilisation and improving production efficiency. Renewed attention in the area of particle size is also driven partly by increasing commercial interest in the feeding of whole grains. Recommendations regarding optimum particle size, however, have been contradictory, as the results from feeding trials are confounded by a number of factors including physical form of the feed, complexity of the diet, grain type, endosperm hardness, grinding method, pellet quality and particle size distribution.

Majority of the feed used in the production of broilers is fed as pelleted or crumbled feed, where the effect of feed particle size on feed intake and the performance becomes less important. However, the interaction between feed physical form and particle size must be given due consideration in any decision regarding optimum particle size. Evidence suggest that the grain particle size influences broiler performance to a greater extent when birds are fed mash diets than when they are fed pelleted or crumbled diets (Section 2.6.1).

The particle size of milled feed is also influenced by grain type. Several studies have shown that grinding different grains with the same mill under similar conditions gives products with different particle sizes. These observations suggest that, during grinding, different screen sizes may have to be used according to the grain type to obtain the desired particle size distribution. But it must be noted that even within a grain type, grinding in the same mill type under similar conditions may result in different particle sizes due to variations in endosperm hardness. Lentle *et al.* (2006) reported that

the grinding of grains from three cultivars of wheat in a hammer mill through the same screen size produced different particle size distributions. However, limited studies have been carried out comparing between the effects of different grain types on particle size distribution after pelleting, digestive tract parameters and broiler performance.

The variability in the apparent metabolisable energy (AME) of wheat for broiler chickens is generally attributed to the soluble non-starch polysaccharides (NSP) in wheat (Annison and Choct, 1991). The negative effects of soluble NSPs may be ameliorated by the supplementation with exogenous xylanases that degrade NSPs and improve nutrient utilisation. Currently, NSP degrading enzymes are routinely included in wheat-based poultry diets. It has been suggested that the physical form of the diet (mash, pellet or whole grain) may affect the efficacy of exogenous enzymes in poultry diets (Acamovic, 2001). Hardness is one of the major physical characteristics of wheat grain that determines the particle size distribution in the milled products. However, there have been no studies examining the interaction between enzyme supplementation and feed particle size or wheat hardness in broiler chickens.

Cereals and legumes used in poultry feeds contain considerable amounts of insoluble fibre. In addition to insoluble fibres from the feed, broilers also ingest wood shavings from the litter (Hetland *et al.*, 2004). Insoluble fibre is generally considered as a nutrient diluent in poultry diets but the effects are not well understood. In general, inclusion of moderate levels of insoluble NSPs has no effect on digesta viscosity but improves starch digestibility and feed per gain (Section 2.2.4). Studies examining the effect of particle size of insoluble fibres on performance, gut measurements and nutrient digestibility are limited. Consequently, studies are required to investigate the effects of insoluble fibre sources and the size of fibre particles in broiler diets.

This thesis consists of nine chapters. The first two chapters discuss the framework of the experimental research with Chapter 1 giving a general introduction to the thesis. Chapter 2 reviews the research conducted into the optimum particle size of different feeds for efficient poultry production and highlights the various issues relating to feed particle size. Chapter 2 also presents a brief review of current literature with an emphasis on the effects of exogenous enzymes in poultry diets and the relationship between wheat hardness and the nutritive value of wheat for broiler chickens. The general materials and methods used in the experimental work of this thesis are described in Chapter 3. Chapters 4 through 8 present the experimental work of this thesis. Each

chapter includes an abstract, introduction, materials and methods, results and discussion. The objectives of the experiments conducted in this thesis include,

1. To investigate the influence of feed particle size and feed physical form on the performance, energy utilisation, digestive tract development and digesta parameters of broiler fed wheat-based diets (Chapter 4).
2. To investigate the influence of feed particle size and grain type on the performance, energy utilisation, digestive tract development and digesta parameters of broilers fed either wheat- or maize-based diets (Chapter 5).
3. To investigate the influence of particle size and xylanase supplementation on the performance, energy utilisation and, gross morphology and histology of the digestive tract of broilers fed wheat-based diets (Chapter 6).
4. To investigate the influence of wheat hardness and xylanase supplementation on the performance, energy utilisation, digestive tract development and digesta parameters of broiler fed wheat-based diets (Chapter 7).
5. To investigate the influence of insoluble fibre source and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens (Chapter 8).

Chapter 9 is a general discussion of the experimental results. This chapter addresses the major findings and draws some conclusions from the data generated.

CHAPTER 2

Review of Literature

2.1. Feed particle size: Implications on the digestion and performance of poultry¹

2.1.1. Introduction

The first stage in the manufacture of commercial poultry feed involves the blending of ground seeds with protein meals. The feed industry has little control over the particle size of protein meals, such as soybean meal and meat meal, as they are supplied pre-ground by the processor. However, the particle size of seeds, including cereals and legumes, may vary at the feed mill.

Particle size reduction is a two-step process involving the disruption of outer seed coat and the exposure of endosperm. Continued reduction increases both the number of particles and the surface area per unit volume allowing greater access to digestive enzymes (Goodband *et al.*, 2002). Other benefits include increased ease of handling and easier mixing of the ingredients (Koch, 1996). However, there are practical limits to the degree of particle size reduction, as poultry of different ages may encounter difficulties in consuming very coarse or very fine particles.

In recent years, the interest in feed particle size has increased, as the industry continues to search for ways of optimising feed utilisation and improving production efficiency. Recommendations regarding optimum particle size, however, have been contradictory, as the results from feeding trials are confounded by a number of factors including as feed physical form, complexity of the diet, grain type, endosperm hardness, grinding method, pellet quality and particle size distribution.

The extent of milling these seeds is known to influence a number of aspects of poultry production, including bird performance and digestive tract development. The aim of this review is to investigate the published data on the influence of feed particle size on these aspects and, to highlight its implications for gut health and functionality. Data regarding the quantitative effects of grinding on energy cost and pellet quality are also discussed.

¹ Section 2.1 was published in World's Poultry Science Journal (2007) 63: 439-456.

2.1.2. Measurement of particle size

Size can be defined as the average diameter of individual particles of feed or simply the “fineness of grind” of the feed. Early researchers (Eley and Bell, 1948; Davis *et al.*, 1951) used the general terms ‘fine, medium and coarse’ to describe particle size, but these terms prevent meaningful comparisons of data. To overcome these limitations, the American Society of Agricultural Engineers (1983) developed methods to describe particle sizes more specifically. The average particle size is given as the geometric mean diameter (GMD), expressed in millimetres (mm) or microns (μm) and the range of variation is described by the geometric standard deviation (GSD), with a larger GSD representing lower uniformity. GMD and GSD are accurate descriptors only when the natural logs of the proportions of particles retained on each of a series of sieves in a geometric size sequence, are distributed normally (Lucas, 2004).

Feed particle size is typically determined by dry sieving a 100-gram representative sample (Baker and Herrman, 2002). The feed sample is passed through a sieve stack on a shaker for 10 minutes. The amount of particles retained on each screen size is then determined, and the GMD and GSD of the sample calculated using standard formula or computer software. Particle size distribution may also be determined by wet sieving, but this method is used more often on digesta and excreta samples. In the wet sieving method, feed samples are suspended in 50ml of water and left to stand for 30 minutes prior to sieving to ensure adequate hydration (Lentle *et al.*, 2006). The sample is then washed through a set of sieves and the material retained on each sieve, plus elute, is subsequently filtered and dried for 24h at 80°C. The weight of the retained particles from each sieve are then expressed as percent of total dry matter recovered.

2.1.3. Methods of particle size reduction

Two processing methods are commonly used to reduce the particle size of the grains; the hammer mill and the roller mill (Koch, 1996; Waldroup, 1997).

Hammer mill

This mill comprises a set of hammers moving at high speed in a grinding chamber, which reduce the size of the grains until the particles are able to pass through a screen of designated size. Thus, the size and spectrum of particles produced depends on the screen size and the hammer speed (Koch, 1996). The efficiency of the hammer mill is

influenced by a number of factors, including grain type, grain moisture content, screen size, screen area, peripheral speed, hammer width and design, number of hammers, hammer tip to screen clearance, feed rate, power of the motor and speed of air flow through the mill (Martin, 1985).

Roller mill

The roller mill comprises of one or more pairs of horizontal rollers in a supporting frame, the distance between which can be altered according to the particle size required. The grains size is reduced by a constant compression force as they pass between the rotating rollers.

Roller mills are more efficient and require less energy for grinding than the hammer mill. The roller mill produces a more uniform particle size distribution with a lower proportion of fines (GMD, < 0.5 mm) than the hammer mill (Nir and Ptichi, 2001), although the particle size and spectrum may vary with the type of corrugation on the rollers and the type of the grain (Martin, 1985). Undersized grains may escape the grinding process in the roller mill (Douglas *et al.*, 1990) and the shape of the particles produced is more irregular, being cubic or rectangular (Koch, 1996) compared to those from a hammer mill, which tend to be spherical with more uniform shape (Reece *et al.*, 1985). Commercially, hammer mills are more commonly used for grinding grains because they are easier to use and maintain.

The particle size of the milled product is influenced by grain type. Several studies have shown that grinding different grains with the same mill under similar conditions gives products with different particle sizes (Table 2.1). These observations suggest that, during grinding, different screen sizes may have to be used, according to the grain type, to obtain the desired particle size distribution. However, it must be noted that even within a grain type, grinding in the same mill type under similar conditions may result in different particle sizes due to variations in endosperm hardness. Lentle *et al.* (2006) reported that grinding grains from three cultivars of wheat in a hammer mill through the same screen size produced different particle size distributions.

Table 2.1. The effect of grain type on particle size analysis¹

Grain	GMD (mm)	GSD	Reference
Hammer mill			
Maize	0.947	2.07	Douglas <i>et al.</i> (1990)
Sorghum	0.841	1.77	
Sorghum	0.628	1.88	Nir <i>et al.</i> (1995)
Wheat	0.681	2.29	
Roller mill			
Maize	1.470	1.82	Douglas <i>et al.</i> (1990)
Sorghum	1.800	1.40	
Sorghum	1.413	1.76	Nir <i>et al.</i> (1995)
Wheat	2.170	1.65	

¹ In each study, the grains were ground in the same mill under similar conditions.

2.1.4. Effect of grinding method on broiler performance

Hammer and roller mill grindings had no effect on broiler performance, when diets of similar geometric mean particle diameter were compared (Nir *et al.*, 1990). However, GMD is an accurate descriptor of particle size distribution only when log particle size is normally distributed (Lucas, 2004). As the hammer mill grinding produces a greater amount of fine particles (Reece *et al.*, 1985), this may have confounded the results.

2.1.5. Effect of particle size and particle size distribution on performance

It must be recognised that not only the size of the feed particles, but also uniformity of particles size, is relevant in determining the influence of particle size on bird performance. Both particle size and shape may influence bird performance (Axe, 1995). Birds distinguish the differences in feed particle size by mechanoreceptors located in the beak (Gentle, 1979). Chickens are known to have a preference for larger feed particles (Schiffman, 1968), which is observed at all ages (Portella *et al.*, 1988) and particle size preference is thought to increase with age (Nir *et al.*, 1994b). This may be related to the size of the bird's 'gape' (the width of the beak). Whilst beak width increases with age (Gentle, 1979), there are no published data relating preferred particle size to gape. It is possible that the particle size might increase with age for optimum poultry performance.

A more uniform diet will reduce the time spent searching for and selecting larger particles, with beneficial effects on performance. Using maize-soy diets in mash form, Nir *et al.* (1994a) showed that diets with lower GSD gave better weight gain and feed efficiency. Despite the practical importance of this factor, only limited studies have

been carried out on the effects of particle size uniformity for different cereal grains in poultry feeds. This lack of interest may be due to the fact that the majority of the feed used in the production of broilers is fed as pelleted or crumbled feed, where there is no opportunity for selection of particles of different sizes (Reece *et al.*, 1986a).

2.1.6. Effect of physical form of feed - Mash versus Pellets

Interaction between particle size and feed physical form in broiler diets for weight gain and feed intake is well documented. Available data clearly suggest that grain particle size is more critical in mash feeds, than in pelleted or crumbled feeds (Reece *et al.*, 1985; 1986a; Cabrera, 1994; Hamilton and Proudfoot, 1995a; Nir *et al.*, 1995; Svihus *et al.*, 2004a; Peron *et al.*, 2005).

Pelleting is known to improve weight gain, feed intake and feed efficiency in broilers regardless of the grain source (Calet, 1965; Douglas *et al.*, 1990; Nir *et al.*, 1995; Jensen, 2000; Nir and Ptichi, 2001). These improvements have been attributed *inter alia* to higher density, improved starch digestibility resulting from chemical changes during pelleting, increased nutrient intake, changes in physical form, reduced feed wastage and decreased energy spent eating (Calet, 1965; Jensen, 2000). Similar improvements have been reported in laying hens (Morgan and Heywang, 1941). In contrast, Hamilton and Proudfoot (1995b) reported that layers fed a mash diet performed better than those fed a crumbled diet.

2.1.7. Effect of grain particle size on bird performance in mash diets

Most studies on the effects of particle size reduction in mash diets have been conducted with maize or sorghum (Table 2.2). One of the earliest reports suggested that maize-based mash diets of larger particle size increased feed consumption and decreased feed wastage, without influencing weight gain (Eley and Bell, 1948). In contrast, another early work showed that cracked maize was selected in preference to the rest of the ration, which resulted in significantly poorer growth and feed efficiency (Davis *et al.*, 1951). Subsequent studies, however, have confirmed the beneficial effects of medium or coarse grinding in mash diets (Reece *et al.*, 1985; Reece *et al.*, 1986a,b; Nir, 1987; Proudfoot and Hulan, 1989; Nir *et al.*, 1990; Nir *et al.*, 1994a,b; Hamilton and Proudfoot, 1995a; Nir *et al.*, 1995). Reece *et al.* (1985) fed maize-based mash diets ground by hammer mill (GMD, 0.814mm) and roller mill (GMD, 1.34 mm) to broiler starters, and observed better performance in birds fed coarse particle size in terms of

weight gain and feed efficiency. Similar results have been reported by Proudfoot and Hulan (1989) and Hamilton and Proudfoot (1995a) who found that weight gain was improved when birds were fed mash diets containing coarse or very coarse maize particles compared to those containing fine particles.

Table 2.2. Effect of particle size on the performance of broiler fed mash diets

Grain	Age (days)	Particle size (mm)	Gain (g/bird)	Feed intake (g/bird)	Feed/gain (g/g)	Reference
Maize	1-21	0.814	582 ^a	-	1.43 ^a	Reece <i>et al.</i> (1985)
		1.343	635 ^b	-	1.40 ^b	
Maize	1-21	0.947	521 ^a	-	1.49 ^a	Douglas <i>et al.</i> (1990)
		1.470	488 ^b	-	1.55 ^b	
Sorghum	7-21	Fine	364 ^b	532 ^b	1.46 ^a	Nir <i>et al.</i> (1990)
		Medium	376 ^a	548 ^{ab}	1.46 ^a	
		Coarse	382 ^a	561 ^a	1.47 ^a	
Maize	7-21	0.897	522 ^a	725	1.37 ^a	Nir <i>et al.</i> (1994a)
		1.102	463 ^b	716	1.54 ^b	
		2.010	473 ^b	740	1.60 ^b	
Maize, wheat and sorghum	1-21	Fine	357 ^b	591 ^b	1.65 ^b	Nir <i>et al.</i> (1994b)
		Medium	427 ^a	662 ^a	1.55 ^a	
		Coarse	401 ^a	645 ^a	1.60 ^{ab}	
Maize and wheat	7-42	Fine	1942 ^a	-	1.91 ^a	Hamilton and Proudfoot (1995)
		Medium	1982 ^b	-	1.92 ^a	
		Coarse	2004 ^c	-	1.92 ^a	

^{a,b,c} Within each reference, values in a column with different superscripts are significantly different ($P < 0.05$).

Similar effects are reported with sorghum grain, ground in hammer and roller mills to produce particle size fractions with GMD of 0.536-0.574 mm (fine), 0.671-0.773 mm (medium) and 0.871-0.905 mm (coarse) and fed as mash diets (Nir *et al.*, 1990). Feed intake increased on the coarser particle diets as did weight gain, with no deleterious effect on feed efficiency. Nir *et al.* (1995) reported that broilers fed wheat and sorghum mash diets with coarser particles had heavier body weights and better feed efficiency compared to those fed the finely ground diets.

In contrast, Douglas *et al.* (1990) reported that mash feeding of coarser particle diets of either sorghum or maize that were ground in a roller mill (GMD, 1.47-1.80 mm) depressed weight gain and feed efficiency in broilers compared with those fed diets with finely hammer milled grains (GMD, 0.833-0.947 mm). The poor performance of birds fed large particles was attributed to the preferential selection of large particles but, as noted previously, may equally have resulted from differences in the particulate spectra from the two methods of milling.

It appears that the effects of feed particle size may be influenced by age of birds. When Nir *et al.* (1994b) compared maize, wheat or sorghum based diets that were ground in a hammer mill to differing particle sizes but nearly similar GSD, no significant differences in performance were observed in 7-day old chicks, whilst performance was improved in 21-day old birds that consumed medium and coarse mash diets compared with those that were fed the fine mash diet.

There is some evidence that very coarse particles may adversely effect the performance in young birds. Particle sizes larger than 1 mm are thought to be too large for chicks to utilise efficiently, as passage through the gizzard is slower (Lott *et al.*, 1992), possibly because the undeveloped gizzard is physically unable to break down large grains (Covasa and Forbes, 1996). This may explain the poor performance observed when chicks are fed mash diets based on cracked maize (Davis *et al.*, 1951) and very coarse particles (Douglas *et al.*, 1990) from day 1 post hatch. A parallel situation occurs when whole grains are fed, and it has been observed by Ravindran *et al.* (2006) that young chicks have difficulties in swallowing whole wheat during the first few days of life. These researchers found that, when chicks are fed diets with whole wheat from day 1, the birds grow slower and eat less feed compared to those fed ground wheat diets. Thus, optimum particle size probably varies with the rate of development of the digestive system, the beak dimensions and the gape (Portella *et al.*, 1988). Once adequate gizzard development is attained, improvements in performance associated with the feeding of coarser, more uniform particles may be partly explained by the lower energy input required by birds when they ingest coarser particles. It is known that the number of pecks to consume a given amount of feed is reduced when particle size increases (Jensen *et al.*, 1962). In general, published data suggest that medium and coarse grindings are advantageous in improving the performance of broilers fed mash diets and that these beneficial effects are greater in diets with better particle uniformity. General recommendations for final grain GMD from hatch to market weight for broilers

have been suggested by Nir and Ptichi (2001), and these values are presented in Table 2.3. However, it should be noted that uniformity of the diet should also be considered and that fine particles less than 0.600 mm GMD should be avoided at all ages (Waldroup, 1997). Based on available published data, it may be concluded that the optimum feed particle size lies between 0.600 and 0.900 mm for broiler diets based on maize or sorghum.

Table 2.3. Suggested GMD of cereal grains for broiler chickens¹

Age (days)	GMD (mm)
1-7	0.900-1.100
7-21	1.100-1.300
21-market	1.300-1.500

¹Adapted from Nir and Ptichi (2001).

Although laying hens are usually fed mash diets, few studies have examined the effects of feed particle size on their performance. Early work suggested that neither egg production nor body weight gain were influenced by particle size (Berg and Bearse, 1948). Subsequent work showed no difference in layer performance between hens fed hammer milled maize diets with particle GMD ranging from 0.814 to 0.873 mm or with roller milled maize diets with GMD ranging from 1.34 to 1.50 mm (Deaton *et al.*, 1989). Hamilton and Proudfoot (1995b) similarly found that the effect of grain particle size in layer diets was of lesser importance than the physical form of the feed. In contrast, Cabrera (1994) found that layers performed better on diets containing coarse maize, but the reverse was true for sorghum, suggesting a particle size x grain type interaction. Goodband *et al.* (2002) also concluded that, for layers, medium and coarse grindings are preferable for maize and that there is no advantage in reducing particle size below 0.800 mm.

2.1.8. Effect of grain particle size on broiler performance in pelleted diets

Although there is a degree of inconsistency, most data suggest that any effect of feed particle size on broiler performance is reduced when offered in the crumble or pelleted diets (Table 2.4). However, there has been sustained interest in studying the effects of particle size in pelleted feeds on the basis that the pellets dissolve in the crop after

consumption, and hence that the effect of feed particle size may be maintained even after pelleting (Nir *et al.*, 1995).

Table 2.4. Effect of particle size on the performance of broiler fed pelleted diets

Grain	Age (days)	Particle size (mm)	Gain (g/bird)	Feed intake (g/bird)	Feed/gain (g/g)	Reference
Maize	0-46	0.910	1998 ^a	-	1.934 ^a	Reece <i>et al.</i> (1986a)
		1.024	2001 ^a	-	1.931 ^a	
Maize	0-42	0.679	1820 ^a	3447 ^b	1.894 ^a	Reece <i>et al.</i> (1986b)
		0.987	1754 ^b	3347 ^a	1.908 ^b	
		1.289	1800 ^a	3400 ^{ab}	1.889 ^a	
Maize	1-21	0.679	748 ^a	1048 ^a	1.40 ^a	Lott <i>et al.</i> (1992)
		1.289	729 ^b	1032 ^a	1.42 ^b	
Maize	0-16	0.869	386 ^a	-	1.35 ^a	Kilburn and Edwards (2001)
		2.897	382 ^b	-	1.39 ^b	
Wheat	1-42	Fine	2007 ^a	3512 ^a	1.78 ^a	Engberg <i>et al.</i> (2002)
		Coarse	1997 ^a	3478 ^a	1.78 ^a	
Wheat	11-30	0.600	1361 ^a	2133 ^a	1.57 ^a	Svihus <i>et al.</i> (2004a)
		1.700	1315 ^a	2114 ^a	1.58 ^a	
Wheat	7-15	0.300	440 ^a	380 ^a	1.28 ^a	Peron <i>et al.</i> (2005)
		0.955	444 ^a	375 ^a	1.25 ^a	

^{a,b} Within each reference, values in a column with different superscripts are significantly different ($P < 0.05$).

A number of studies have shown that feed particle size has no significant effect on broiler performance in pelleted feeds. Reece *et al.* (1986a) found no effect on performance using maize of differing particle sizes to formulate broiler starter diets in crumble form. Similarly, Svihus *et al.* (2004a) showed no difference in any of the performance parameter when broilers were fed pelleted feeds made from wheat ground in hammer and roller mills to a range of particle sizes, and concluded that pelleting evened out differences in particle size distribution. Peron *et al.* (2005) also found no effect on the performance of broilers fed pelleted diets made from wheat of two particle sizes (GMD, 0.380 and 0.955 mm), although differences in particle size distribution persisted after pelleting.

In contrast, a number of researchers have demonstrated effects of particle size in pelleted feeds that suggest variation between grain types. Lentle *et al.* (2006) fed broilers with pelleted feeds based on three cultivars of wheat that each produced different particle size spectra as a result of hammer milling, and found that diets with a higher relative proportion of coarser particles resulted in better feed efficiency. Other investigators, using maize-based pelleted diets, have suggested that fine grinding has beneficial effect on performance compared to coarse grinding. Lott *et al.* (1992) found that broilers fed pelleted feeds made from coarse hammer mill ground maize (GMD, 1.19 mm) had a significantly lower weight gain and poorer feed efficiency at 21 days when compared with those fed pellets made from finer ground maize (GMD, 0.679 mm). Similarly, Kilburn and Edwards (2001) reported that performance and true metabolisable energy were improved when the broilers were fed pelleted diets that included fine maize (GMD, 0.869 mm) compared to that made from coarse maize (GMD, 2.89 mm).

On the other hand, Reece *et al.* (1986b), in an extensive comparison between maize based diets, found that both fine (GMD, 0.679 mm) and coarse (GMD, 1.28 mm) grindings improved both weight gain and feed efficiency, relative to those fed medium ground maize (GMD, 0.987 mm) even though there were no differences in feed intake. No significant difference was observed between performances of the birds maintained on the former two diets. However, birds maintained on pellets formulated with one-half of finely ground maize and one-half of coarse maize, with an average GMD 0.908 mm, performed better than those maintained on medium ground maize, although the GMD was somewhat similar.

Such contradictory evidence from studies comparing pelleted diets formulated from different grain particle sizes may be related, in part, to changes in size distribution following pelleting. These changes appear to be dependent on grain type and cultivars. The particle size of milled products is known to be affected by grain hardness (Dobraszczyk *et al.*, 2002; Carre, 2004). However, the effect of grain hardness on particle size distribution after pelleting remains to be investigated. When particle size differences persist in wheat-based diets after pelleting, those with coarser particles were found to improve feed efficiency of broilers (Lentle *et al.*, 2006). On the other hand, no effect on performance was observed when pelleting evened out any differences in particle size distribution (Engberg *et al.*, 2002; Svihus *et al.*, 2004a).

2.1.9. Effect of whole wheat inclusion in broiler diets

Feeding whole wheat is a common practice in many countries where there is a high production of wheat. The main advantages of whole wheat feeding for broilers are reduced feed costs, due to reduced handling and processing costs of the feed (Svihus *et al.*, 2004b), improved gut health (Gabriel *et al.*, 2003; Bjerrum *et al.*, 2005) and improved nutrient digestibility and AME (Preston *et al.* 2000; Hetland *et al.*, 2002).

Most studies have examined the effects of whole wheat post-pelleting, however, studies examining the effects of whole wheat pre-pelleting are limited and have produced inconsistent results. Wu *et al.* (2004) found that 200g/kg whole wheat inclusion pre-pelleting improved feed per gain but had no effect on gut measurements. On the other hand, Taylor and Jones (2004) found no effect of whole wheat inclusion pre-pelleting on broiler performance but an increase in relative gizzard weights. Similar results were observed by Svihus *et al.* (2004b) when they included 500 g/kg whole wheat pre-pelleting. Further studies are required to explain the inconsistency in research results and to understand the factors that determine the effects of whole wheat inclusion pre-pelleting on broiler performance.

2.1.10. Effect of particle size on digestive tract development and physiology

The development of digestive tract of poultry, especially the gizzard, is known to be influenced by feed particle size, which is evident in chickens at 7 days of age. Nir *et al.* (1994b) reported greater gizzard development and lower gizzard pH in 7-day old chicks fed medium or coarse particle size diets compared with those fine particulate diets.

The gizzard is a muscular organ that reduces the particle size of ingested foods and mixes them with digestive enzymes (Duke, 1986). The mechanical pressure applied in grinding by the gizzard may exceed 585 kg/cm² (Cabrera, 1994). When such grinding is carried out by feed mills, this has negative effects on gizzard size and gut function. As a result, the gizzard is relatively underdeveloped and the proventriculus becomes enlarged when broilers are fed finely ground, processed diets (Taylor and Jones, 2004). Under these conditions, the gizzard functions as a transit rather than a grinding organ (Cummings, 1994). The relative weights of both the gizzard (Nir *et al.*, 1995; Engberg *et al.*, 2002) and the small intestine (Nir *et al.*, 1995) have been shown to decrease when birds are fed pelleted rather than mash diets.

Feed particle size is positively correlated to relative gizzard weight (Nir and Ptichi, 2001) when diets are fed as mash. In pelleted diets, however, this is likely to depend on the particle size distribution after dissolution in the crop. Svihus *et al.* (2004a) found no effect of particle size pre-pelleting on gizzard weight when a hard wheat (hardness index 64, measured in a SKCS 4100), with GMD varying between 0.600 and 1.70 mm, was used. The explanation for this finding probably lies in the lack of differences in particle size distribution after pelleting in this study. In contrast, Peron *et al.* (2005) found that particle size differences remained even after pelleting in wheat-based diets made from a very hard wheat (hardness value 83, measured using near infrared reflectance spectrometry), with GMD values of 0.380 and 0.955 mm, and that pelleted diets made from coarse particles significantly increased gizzard weights compared to those made from fine wheat. This was probably due to resistance of the hard particulate material to reduction by the pelleting process.

A large, well-developed gizzard improves gut motility (Ferket, 2000) through increasing the levels of cholecystokinin release (Svihus *et al.*, 2004b), which stimulates the secretion of pancreatic enzymes and the gastro-duodenal refluxes (Duke, 1992; Li and Owyang, 1993). Coarse particles may slow the passage rate of digesta through the gizzard (Nir *et al.*, 1994b), increasing the exposure time of nutrients to digestive enzymes, which in turn, may improve energy utilisation and nutrient digestibility (Carre, 2000). It has been reported that a lower pH of gizzard contents may increase pepsin activity (Gabriel *et al.*, 2003) and therefore improve protein digestion. It has also been suggested that a lower pH of gizzard contents may reduce the risk of coccidiosis (Cumming, 1994) and feed-borne pathogens (Engberg *et al.*, 2002).

Feed particle size has been shown to influence the development of other segments of digestive tract in birds fed mash diets. Nir *et al.* (1994b) reported the hypertrophy of the small intestine and lowering of intestinal pH when fine mash diets were fed. Similar results were reported by Nir *et al.* (1995), who found lower relative duodenal weights in birds fed coarse particle diets compared to those fed fine particle diets. Interestingly, a similar pattern was also been recorded in the birds fed whole-wheat diets (Gabriel *et al.*, 2003). The significance of lower duodenal weights associated with coarse feed particles is unclear.

2.1.11. Effect of feed particle size on digesta particle size

Data on the effect of feed particle size on its subsequent distribution in poultry digesta are scanty. Hetland *et al.* (2004) stated that the gizzard has a remarkable ability to grind all organic constituents of feed to a consistently fine size regardless of the original particle size of the feed. The digesta passing through the gizzard had a consistent particle size distribution, with the majority of particles being smaller than 40 μm in size regardless of the original feed structure (Hetland *et al.*, 2002). In contrast, Lentle (2005) speculated that an increase in the proportion of coarser particles in the diet caused greater amounts of coarser particles to transit the gizzard but that these particles increased digestive efficiency as a result of increasing the permeability of digesta to digestive enzymes. This speculation was based on the observations that digesta contains a mixture of large and small particles, with small particles occupying the spaces between larger particles, which resulted in a reduction of mean void space radius available for permeation (Wise, 1952; Deresiewicz, 1958). Thus an increase in the numbers of coarser particles may lead to a local increase in permeation of digestive fluids through sites where there is a greater proportion of larger particles (Lentle, 2005; Lentle *et al.*, 2006).

2.1.12. Effect of particle size on nutrient utilisation

Although particle size reduction is said to improve digestion of nutrients by increasing the surface area available to digestive enzymes, studies which relate particle size to digestibility of nutrients are limited and, in the case of grains, equivocal. Kilburn and Edwards (2001) reported that fine grinding of maize increased the true metabolisable energy values in mash diets, but the opposite effect was observed with pelleted diets. Peron *et al.* (2005) found that fine grinding wheat improved starch digestibility and the AME compared to coarse grinding. On the other hand, coarse grinding of maize has been reported to increase the efficiency of nitrogen and lysine retention in broilers fed mash diets (Parsons *et al.*, 2006). Svihus *et al.* (2004a) found no effect of wheat particle size on the AME.

The results for dicotyledonous seeds are less equivocal. Coarse grinding of grain legumes has been shown to lower energy utilisation and, digestibility coefficients of nutrients. A discussion on the effects of particle size on starch digestibility in grain legumes has been published (Carre, 2004). In general, fine grinding of peas was

reported to improve the total tract digestibility of starch and protein when they were fed as mash diets (Carre *et al.*, 1998; Daveby *et al.*, 1998; Carre, 2000). Similarly, fine grinding of peas has been shown to improve the apparent ileal protein digestibility (Crevieu *et al.*, 1997). All of these effects may be attributed to the increased accessibility of nutrients in fine legume particles. Fine grinding of faba beans had no effect on the total tract digestibility of protein (Lacassagne *et al.*, 1991), but these contradictory results are possibly related to the differences in site of measurement (ileal versus total tract). The variable and modifying effects of caecal microflora on protein digestion have been recognised in recent years (Ravindran *et al.*, 1999) and it is now generally agreed that the analysis of ileal digesta rather than excreta is the preferred method for assessing nutrient digestibility in poultry (Ravindran and Bryden, 1999).

Interestingly, in terms of mineral availability, coarse grinding appears preferable over fine grinding. Large maize particle size has been shown to significantly improve calcium, total phosphorus and phytate phosphorus utilisation in broilers (Kasim and Edwards, 2000; Kilburn and Edwards, 2001). Bone ash and plasma phosphorus levels of broilers were improved after feeding of coarse (GMD, 1.23 mm) rather than fine (GMD, 0.891 mm) soybean meal (Kilburn and Edwards, 2004). Similar findings have been reported with maize-soy diets by Carlos and Edwards (1997). It was hypothesised that larger particle size led to a longer transit time allowing more time for mineral digestion and absorption. These benefits were reduced when the diet was fed in pelleted or crumbled form (Kilburn and Edwards, 2004), presumably as a result of concomitant degradation of larger particles during the pelleting process.

2.1.13. Effect of particle size on passage rate

It is hypothesised that rapid passage rate reduces the time available for digestion and absorption, whilst slow passage rate limits the intake of feed (Svihus *et al.*, 2002). The passage rate of the digesta is usually measured using insoluble (solid phase) coloured markers such as chromic or ferric oxide, but it must be borne in mind that results may be confounded by preferential retention of particles of particular size in particular segments of the gut, by adherence to other particles or by dissolution. Furthermore, in a number of species, soluble nutrients traverse the gut at faster rates than particulate matter (Lentle, 2005).

In broiler chickens, solid phase markers appear in the excreta 1.6 to 2.6 hr after ingestion (Denbow, 2000). Several factors are known to affect the passage rate of solid

phase markers, including the strain of the chicken (Denbow, 2000), age of the bird (Shires *et al.*, 1987), dietary content of non-starch polysaccharides (Almirall and Esteve-Garcia, 1994), the fraction of water insoluble non-starch polysaccharides (Hetland and Svihus, 2001), dietary fat level (Sell *et al.*, 1983) and environmental temperature (Denbow, 2000). In general, larger particles are retained longer than finer particles in the digestive tract (Nir *et al.*, 1994b; Denbow, 2000), prolonging mean residence time. Thus the proportion of coarse fibre in the gizzard is double that in the feed (Hetland *et al.*, 2005) possibly reflecting selective retention of coarse particles (Hetland *et al.*, 2004; 2005).

It should also be mentioned, however, that overall retention time does not increase when birds are fed whole grains (Svihus *et al.*, 2002; Svihus *et al.*, 2004a; Wu and Ravindran, 2004). Hetland *et al.* (2005) speculated that there is rapid dissolution of starch granules and protein from the whole grains in the low pH environment of the gizzard so that the particle size is rapidly reduced with no effect on passage rate.

2.1.14. Effect of particle size on pellet quality

Good pellet quality is defined as the ability to withstand mechanical handling (bagging, transport etc) without breaking up, and to reach feeders without generating a high proportion of fines. Pellet quality is determined by two physical parameters, the pellet durability index (PDI) and pellet hardness. The PDI measures the proportion of fines generated during standardised mechanical handling (Behnke, 2001), typically in a tumbling can (ASAE, 1987) or Holman Pellet Tester (Holman Chemical Ltd, Norfolk, UK). Pellet hardness is determined in a spring hardness tester, as the static force (in kg) required to break the pellet.

There is a positive correlation between pellet durability and feed efficiency (Carre *et al.*, 2005). Higher pellet durability lowers fine formation and reduces feed wastage and selection of larger particles by the birds. Pellet durability is thought to be inversely related to particle size (Angulo *et al.*, 1996), with smaller particles having more contact points with each other because of their larger surface area per unit volume ratio (Behnke, 2001). However, there is little scientific evidence to support this, and a number of studies having shown that fineness of grind of grain has no effect on pellet durability (Reece *et al.*, 1986b; Koch, 1996). Moreover, contradictory results have been obtained from the limited studies that relate particle size to pellet durability (Table 2.5).

Table 2.5. Effect of particle size on pellet durability

Grain	GMD (mm)	Pellet durability index	Reference
Maize	0.910	91 ^a	Reece <i>et al.</i> (1986a) ¹
	1.024	91 ^a	
Maize	0.679	91 ^a	Reece <i>et al.</i> (1986b) ¹
	0.987	91 ^a	
	1.289	92 ^b	
Wheat	0.600	88 ^a	Svihus <i>et al.</i> (2004a) ¹
	0.930	81 ^a	
	1.700	80 ^a	
Wheat	0.380	25 ^a	Peron <i>et al.</i> (2005) ²
	0.955	25 ^a	

¹ Pellet durability was measured using Holmen Pellet Tester.

² Pellet durability was measured using Euortest rotary mill.

^{a,b} Within each reference, values in a column with different superscripts are significantly different ($P < 0.05$).

Carre *et al.* (2005) reported a positive correlation between pellet durability and wheat hardness. It was stated that this hardness effect is independent of the particle size. Other factors shown to effect pellet durability include dietary protein and oil contents (Briggs *et al.*, 1999), mash conditioning, die specifications, and cooling and drying (Behnke, 1996). Thus, any effect of particle size on pellet durability may be confounded by other dietary or milling parameters.

High starch gelatinisation has also been reported to improve pellet durability (Cramer *et al.*, 2003). Therefore, it has been suggested that poor quality of the pellets associated with coarse particles is due to the low starch gelatinisation in coarse particles compared to pellets made with fine particles (Svihus *et al.*, 2004a).

2.1.15. Effect of particle size on energy cost

Feed constitutes the greatest single cost in poultry production. The reduction of feed particle size is the second largest energy cost after that of pelleting in the broiler industry (Reece *et al.*, 1985) and likely to be the largest user of energy in the layer industry where pelleting is not performed (Deaton *et al.*, 1989). Dozier (2002) estimated that utility usage comprised 25 to 30% of the manufacturing cost of broiler feed. Reducing feed particles to a finer size requires greater energy use and lowers the

production rate. Thus, any reduction in energy consumption from grinding could significantly lower feed cost.

Reece *et al.* (1986a) reported that energy savings of 27% could be achieved by increasing the screen size of a hammer mill from 4.76 to 6.35 mm. However, the relationship between screen size and energy consumption is not linear. The energy consumption during milling of maize with a hammer mill from a GMD of 0.600 mm to one of 0.400 mm is double that required to reduce particle size from 1.00 to 0.600 mm (Wondra *et al.*, 1995). Production rates are similarly non-linear (Wondra *et al.*, 1995). Moreover, several studies have shown that fineness of grind of grain has no effect on the rate/efficiency of pelleting (Martin, 1985) or power consumption during pelleting (Martin, 1985; Svihus *et al.*, 2004a). Hence, any gain in productivity of birds from the reduction of particle size must be sufficient to offset the higher cost of fine grinding.

2.1.16. Conclusions

This review highlights the limited amount of research conducted in establishing the optimum particle size of different grains for efficient poultry production. This limited body of work is likely due to the current industry practice of using highly processed, pelleted diets, which clearly masks the influence of particle size.

Although it is generally believed that finer grinding will increase and coarse grinding will decrease the substrate surface area for enzymatic digestion, data reviewed herein suggest that coarser grinding to a more uniform particle size improves the performance of birds maintained on mash diets. This counter-intuitive effect may result from the positive effect of feed particle size on gizzard development. A well developed gizzard is associated with increased grinding activity, resulting in not only increased gut motility and greater digestion of nutrients, but also in greater reduction in particle size entering the small intestine and increasing the accessibility to digestive enzymes. It must be noted that both GMD and GSD are important in maintaining bird performance on mash diets. The effects of particle size on pellet quality remains unexplored and warrant urgent attention. Further systematic investigations on the relationship between feed particle size and bird performance, gut health, and pellet quality are required if production efficiency is to be optimised in respect of the energy expenditure of grinding.

2.2. Exogenous enzymes and the nutritive value of wheat

2.2.1. Introduction

Wheat is one of the major feed ingredients in poultry diets. However, wheats vary widely in AME and this variation is believed to be caused by non-starch polysaccharides (NSP) which have negative impact on the digestion and absorption of nutrients, and consequently on bird performance (Annison and Choct, 1991). It is well documented that these negative effects can be overcome by the addition of glycanases (Bedford and Schulze, 1998). Therefore, enzyme supplementation to poultry diets based on wheat is currently a common practice.

2.2.2. Carbohydrates in wheat

Starch is the most abundant carbohydrate component in wheat which is a polymer of glucose linked by α (1-4) or α (1-6) glucosidic linkage. The other carbohydrates in wheat are called NSP. The main NSP in wheat is arabinoxylan which is composed of β (1-4) xylan backbone with side chain of arabinose (Figure 2.1). The types and level of NSPs found in wheat are summarised in Table 2.6. Unlike starch, NSPs are not digested by endogenous enzymes and also have a great ability to absorb water.

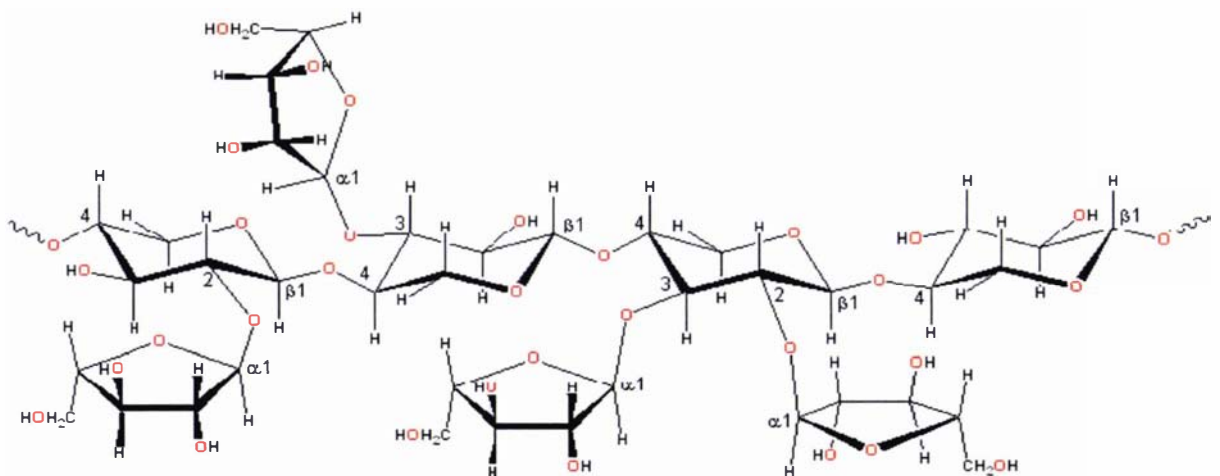


Figure 2.1. Arabinoxylans consist of β (1 \rightarrow 4)-linked D-xylopyranose backbone and α -L-arabinofuranose residues attached as branch-points (Source: Chaplin, 2007).

Table 2.6. The types and level of NSP present in wheat (g/kg dry matter)

	Arabinoxylan	β -Glucan	Cellulose	Galactose	Uronic Acid	Total
Soluble	18	4	0	2	0	24
Insoluble	63	4	20	1	2	90

Source: Modified from Choct (1997).

2.2.3. Anti-nutritional effects of NSP

Soluble and insoluble NSPs have different properties and consequently different effects on digestion in poultry. It is believed that soluble NSPs are largely responsible for the anti-nutritional effects of NSP. Two major mechanisms have been proposed to explain the effects of NSP (Bedford and Schulze, 1998). The first mechanism is associated with the fact that starch and protein are encapsulated by the cell wall in wheat endosperm cells (Figure 2.2). Cell wall composed mainly of cellulose, hemicelluloses (primarily xyloglucans and glucuronarabinoxylans), pectins and lignin (Figure 2.3). It is noteworthy that most of the arabinoxylans in cereal grains are insoluble in water because they are anchored to the cell walls by alkali-labile ester-like cross links (Choct, 1997). Therefore, in addition to diluting nutrients in the diet, insoluble NSP can act as a physical barrier to digestive enzymes. Since the endogenous enzymes in the birds are unable to hydrolyse the cell wall, so the starch and the protein surrounded by cell wall may escape digestion.

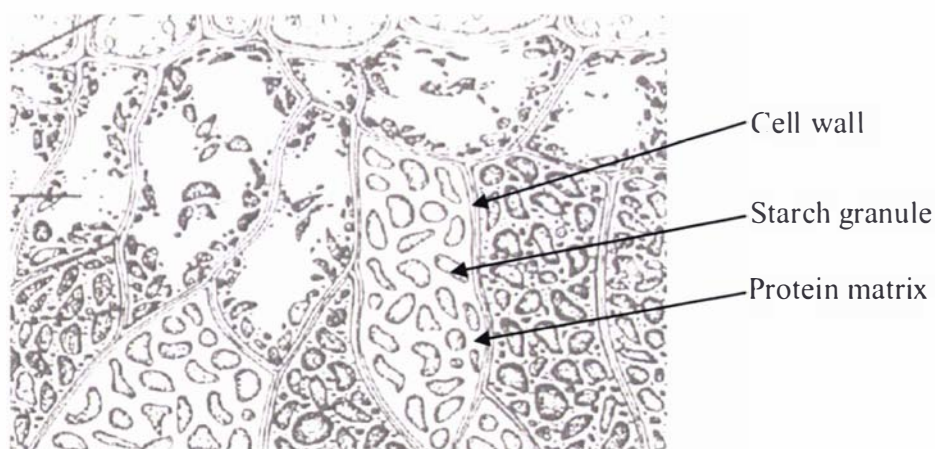


Figure 2.2. Wheat endosperm cells (Source: Knudsen, 2005).

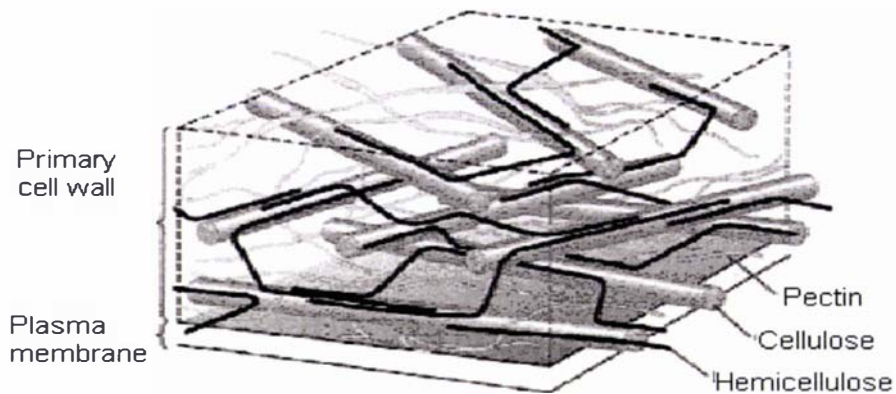


Figure 2.3. Cell wall structure of wheat endosperm cell (Source: the Internet Encyclopaedia of Science, accessed July 2007).

The second mechanism relates to the viscous nature of digesta caused by the soluble NSPs. The degree of viscosity depends on the solubility of the NSP which in turn depends on the size of the molecule, whether it is branched or linear, the presence of charged groups, the surrounding structure and the concentration of the NSP. The effects of high intestinal digesta viscosity include (Smits and Annison, 1996; Choct, 1997; Bedford and Schulze, 1998) (1) reduction in passage rate; (2) reduced mixing of digestive enzymes with substrate nutrients; (3) increased secretion of endogenous enzymes and modification of the endogenous secretion of water, proteins, electrolytes and lipids which may increase the endogenous losses; (4) increased secretory response of mucus, which may increase the resistance for transport of nutrients through the unstirred water layer adjacent to the epithelial surface by increasing mucus layer thickness; and (5) interaction with gut microflora (Smits and Annison, 1996). In addition, relative weights of digestive organs increase with higher digesta viscosity (Choct, 1997) which may increase the metabolic cost of maintaining the gut. A summary of the effects of wheat NSP on gut and metabolic parameters is shown in Figure 2.4.

It is noteworthy, that digesta viscosity less than 10 mPa.s does not limit bird performance when wheat-based diets are fed (Bedford and Schulze, 1998). The effectiveness of exogenous xylanases in improving bird performance (Bedford and Classen, 1992; Choct and Annison, 1992; Preston *et al.*, 2000; Silva and Smithard, 2002) and nutrient digestibility (Preston *et al.*, 2000; Silva and Smithard, 2002) of wheats with lower digesta viscosity is well known.

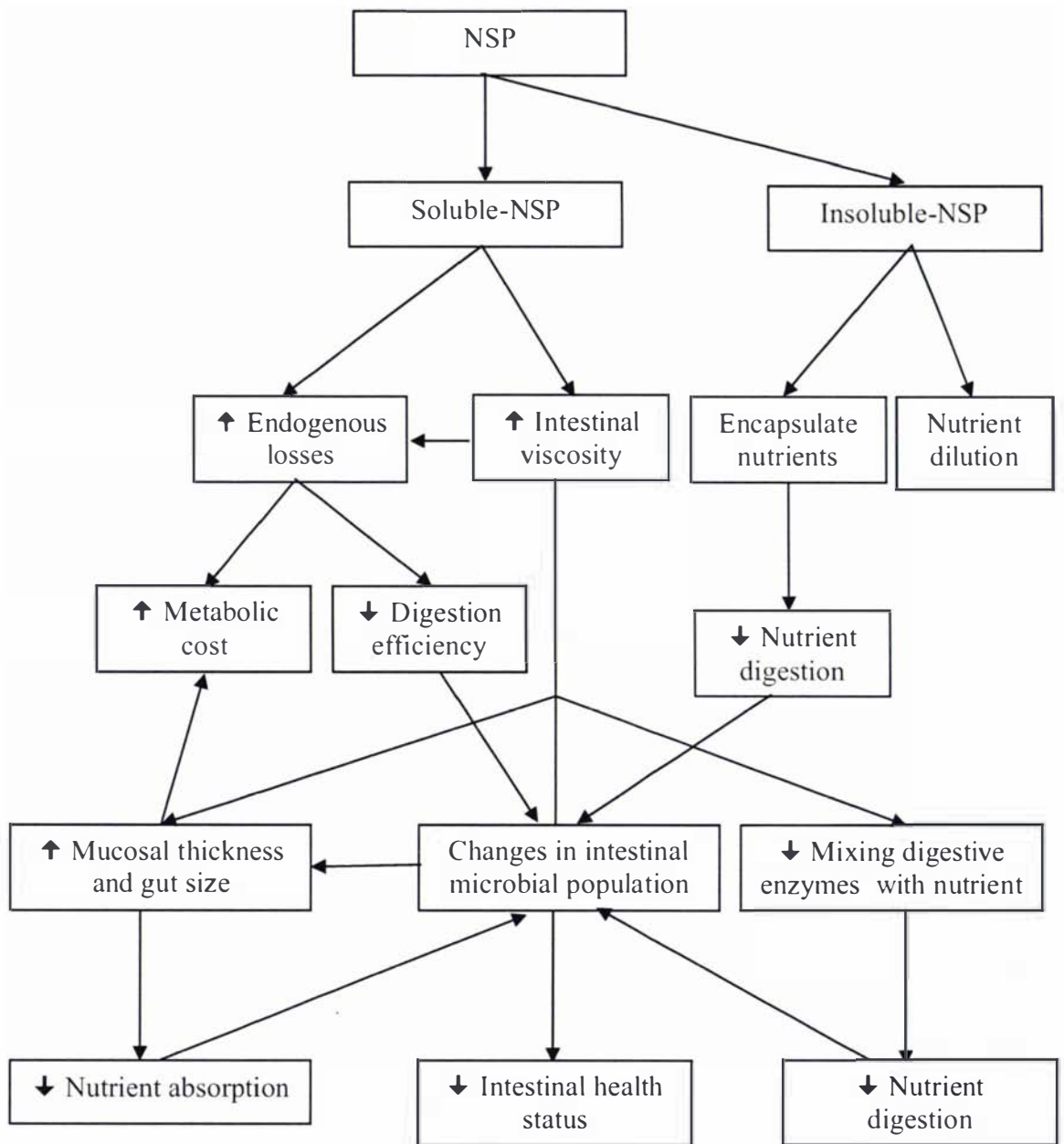


Figure 2.4. Proposed modes of action of wheat NSP.

Interestingly, Choct *et al.* (2006) found that the type of xylanase which is responsible for solubilising insoluble NSP resulted in higher jejunal and ileal digesta viscosity with no adverse effect on feed per gain or AME. Previously, Choct and Annison (1992) reported higher digesta viscosity when depolymerised pentosan was added to sorghum-based diet with no effect on broiler performance. Surprisingly, the improvement in feed per gain with whole wheat feeding was concurrent with increased digesta viscosity (Engberg *et al.*, 2004; Wu *et al.*, 2004). These results suggested that reduction in digesta viscosity is not the only mechanism associated with enzyme supplementation. Although most of the research supports the latter mechanism and it has been discussed extensively in the literature, it is reasonable to suggest that both mechanisms are likely to be involved (Bedford, 2006; Cowieson *et al.*, 2006).

2.2.4. Effects of insoluble-NSP on broiler chickens

The effects of insoluble NSP in poultry diets are not well understood. In general, inclusion of moderate levels of insoluble NSPs had no effect on digesta viscosity (Svihus and Hetland, 2001; Shakouri *et al.*, 2006) but resulted in improved starch digestibility (Rogel *et al.*, 1987; Hetland and Svihus, 2001; Svihus and Hetland, 2001) and feed per gain (Hetland *et al.*, 2003). The suggested mechanisms by which the birds maintained normal weight gain when fed wheat-based diets diluted with insoluble fibre are either by increasing capacity of the digestive system and/or faster passage through the digestive tract (Hetland and Svihus, 2001). Coarse insoluble fibres were found to improve starch digestibility (Rogel *et al.*, 1987; Hetland *et al.*, 2003). Gonzalez-Alvarado *et al.* (2007) found lower gizzard digesta pH and improvements in total tract apparent retention of nutrients when insoluble fibre was added to maize or rice-based diets and the effect was more evident in the rice-based diet. Few studies have examined the effect of insoluble fibre particle size on gut measurements and nutrient digestibility.

It has been suggested that the effect of insoluble fibres on the composition and the quantity of gut microflora to be insignificant (Hetland *et al.*, 2004). Nevertheless there is no scientific evidence to support this suggestion. In contrast, a recent study has shown that inclusion of 30g/kg cellulose in broiler diet modified the intestinal microbial population (Shakouri *et al.*, 2006). Therefore, further studies are required to examine the effects of insoluble fibre on intestinal microbial population using modern culture-independent techniques.

2.2.5. Types of xylanases

Xylanases have the ability to degrade either the soluble or insoluble arabinoxylans. In general, commercial xylanase preparations have broad spectrum activity on both soluble and insoluble arabinoxylans in the feed. It has been found that xylanases obtained from the same organism differ markedly in their catalytic activities on various xylans (Bedford and Schulze, 1998). Choct *et al.* (2004) compared three xylanase products, namely, xylanase A with affinity for both soluble and insoluble NSP, xylanase B is known to solubilise insoluble NSP and xylanase C with affinity for soluble NSP only. It was found that xylanase A increased the soluble NSP levels in the jejunum, but had no effect on digesta viscosity which suggested that while the enzyme attacked the insoluble cell walls to release soluble NSP into the digesta, it also hydrolysed the released soluble NSP. Xylanase B, increased the soluble NSP levels in both the jejunum and ileum and elevated digesta viscosity while xylanase C reduced digesta viscosity.

2.2.6. Mode of action of NSP degrading enzymes

The proposed mechanisms by which enzymes improve energy and nutrient utilisation of wheat based diets include degradation of NSP in the cell wall matrix and the release of encapsulated nutrients, lowering of digesta viscosity in the intestinal tract, increased accessibility of nutrients to endogenous digestive enzymes, stimulation of intestinal motility and improved feed passage rate or indirectly by enhancing cell wall breakdown through stimulation of the gizzard (Bedford and Schulze, 1998). As a result, enzyme addition has been shown to improve performance and nutrient digestibility, reduce the incidence of sticky droppings and modify the microflora of the distal gut (Cowieson *et al.*, 2006). Broiler responses to xylanase supplementation, however, have not been consistent. Factors that may cause variation in response include the type of xylanase, quality of wheat and breed and age of birds (Bedford, 1997). Some studies have shown that differences in the response of enzyme supplementation might be related to hindgut microflora (Choct *et al.*, 1996; 2006). Other factors suggested to explain variation between individual birds within the same treatment group include diurnal rhythms, water intake patterns and pancreatic serous secretion output (Bedford and Schulze, 1998).

2.2.7. Feed processing and enzyme supplementation

According to Acamovic (2001) the physical form of the diet (mash, pellet and whole grains), diet ingredients and processing methods may affect the efficacy of exogenous enzymes in poultry diets. The beneficial effects of including whole wheat were shown to be further enhanced with supplemental xylanase (Wu and Ravindran, 2004). This additive effect was attributed to higher grinding activity of the larger gizzard in birds fed whole grains leading to enhanced mixing of the substrate with the supplemental enzyme in the gizzard.

Feed particle size may also have an effect on digesta viscosity, but studies investigating this aspect are limited. Feeding wheat-based diets with a fine particle size was found to increase digesta viscosity compared to birds fed medium, coarse or whole-wheat diets, with no effect on overall feed per gain (Yasar, 2003). In contrast, whole-wheat feeding increased digesta viscosity and improved feed per gain (Engberg *et al.*, 2004; Wu *et al.*, 2004).

In pigs, it has been found that the efficacy of xylanase in wheat-based diets is likely to be influenced by degree of grinding (Kim *et al.*, 2003). However, the interaction between wheat particle size and enzyme supplementation in broilers has not been previously examined.

2.2.8. Effect of enzyme supplementation on digestive tract development

Inclusion of exogenous enzymes may be expected to alter the gross morphology of the different gut segments compared to birds fed an unsupplemented diet. The relative reduction in the overall length and weight of the intestine of birds fed diets supplemented with enzymes has been reported in a number of studies (Brenes *et al.*, 1993; Wang *et al.*, 2005), but the underlying cause of this adaptive mechanism is not well understood. Wang *et al.* (2005) speculated that gut length and weight are stimulated by the presence of arabinoxylans, which increase the viscosity of the digesta and inhibit digestive enzyme substrate interaction, causing the structure and function of intestine and organs to change. The increase in the size of the gut components with unsupplemented diets might be an adaptive response to an increased need for enzymes (Brenes *et al.*, 1993). Enzyme supplementation of wheat-based diets, which increase the degradation of NSP, may ease the demand on secretory function of organs and intestinal segments and may decrease organ sizes as a result (Brenes *et al.*, 1993; Wang *et al.*,

2005). Enzyme supplementation has been reported to reduce the thickness of gizzard glands and the tissues lining the gizzard in wheat-based diets (Yasar and Forbes, 2000), and indirectly enhance the ability of the gizzard to grind and breakdown of endosperm cell walls (Bedford and Schulze, 1998; Bedford, 2006).

Enzyme supplementation has also been shown to increase mean villus height in turkeys (Ritz *et al.*, 1995) and broilers (Viveros *et al.*, 1994; Mathlouthi *et al.*, 2002). These effects may result from changes in digesta flow (Caspary, 1992). Viveros *et al.* (1994) reported morphological changes including shortening, thickening and atrophy of the villi and an increase in the number and size of goblet cells in the small intestine of birds fed barley-soy diets compared to those fed corn-soy diet or barley diet supplemented with enzymes. Similar results have been observed with rye-based diets (Mathlouthi *et al.*, 2002). These changes may be related to modification of the gut microflora with higher digesta viscosity increasing the numbers of harmful bacteria which may irritate the intestinal mucosa (Wu, 2003). It has been suggested that the metabolic cost of the gut mucosal maintenance can have a significant impact on nutrient supply to the whole animal (Iji *et al.*, 2001). Therefore, the deleterious performance effects of wheat arabinoxylans may occur, in part, via the higher metabolic cost of thicker gut mucosa impacting significantly on overall efficiency. Enzyme supplementation may reduce this metabolic cost.

2.2.9. Conclusions

Xylanase supplementation to wheat-based poultry diets is a common practice currently. The exact mode of action of the enzymes remains unclear, but a number of mechanisms have been proposed, including degradation of NSP within the cell wall matrix to release encapsulated nutrients, lowering of intestinal digesta viscosity and increased accessibility of nutrients to endogenous digestive enzymes. Response to the enzyme supplementation in broilers, however, has not been consistent. Wheat particle size may be a factor contributing to variations in enzyme responses, but this has not been previously investigated.

2.3. Endosperm hardness and the nutritive value of wheat

2.3.1. Definition of endosperm hardness

Hardness is one of the important characteristics of wheat grain that determines the end use of the wheat flour. Two main theories have been proposed to explain hardness or softness of wheat grain (Turnbull and Rahman, 2002). Firstly, hardness is determined by the continuity and the strength of the protein matrix surrounding the starch granules in the endosperm cells. Secondly, hardness depends on the amount of adhesion between the protein matrix and the starch granules. However, most of the research to date supports the latter theory.

Although there is no universally accepted definition of hardness, the physical definition of hardness appears to be preferred (Dobraszczyk *et al.*, 2002) due to the variation in the other factors that effect wheat hardness. Thus, the relative hardness or softness of a grain has been defined as the relative resistance of grains to deformation or crushing when an external force is applied (Turnbull and Rahman, 2002).

2.3.2. Genetic basis of wheat hardness

Hardness is a genetic trait determined by the Hardness (Ha) locus on chromosome 5D that encodes for the puroindolines a and b (also called friabilin or grain softness protein) (Sourdille *et al.*, 1996). This dominant gene is found in soft wheat cultivars and is absent or weakly expressed in hard cultivars (Turnbull and Rahman, 2002). It has been suggested that friabilin acts as a ‘non-stick’ agent between starch granules and the surrounding protein matrix (Anjum and Walker, 1991). In support of this hypothesis, Swan *et al.* (2006) found no interaction between the protein matrix and starch granules in soft cultivars, indicating that friabilin may directly control grain softness by reducing the interaction between starch granules and the surrounding protein matrix (Figure 2.5).

2.3.3. Major factors affecting wheat hardness

Among the various factors influencing wheat hardness, four important ones, namely, environmental conditions, protein content, quantity and quality of pentosans and moisture content, are discussed below.

1. Environmental conditions are believed to have a major effect on hardness (Stenvert and Kingswood, 1977). By changing environmental and drying conditions it is possible to have a genetically soft wheat which is physically hard or to make a genetically hard wheat soft (Dobraszczyk *et al.*, 2002).
2. Protein content is thought to be positively correlated with hardness (Cornell and Hoveling, 1998). However, high protein content in softer wheats has also been reported (Turnbull *et al.*, 2002).
3. Soluble and insoluble pentosan content may be positively correlated with wheat hardness. However, some studies have shown that pentosan quality is more important than quantity in determining hardness (Turnbull and Rahman, 2002).
4. Grain moisture content affects the absolute and the relative hardness of wheat and generally higher moisture levels means softer wheat (Turnbull and Rahman, 2002).

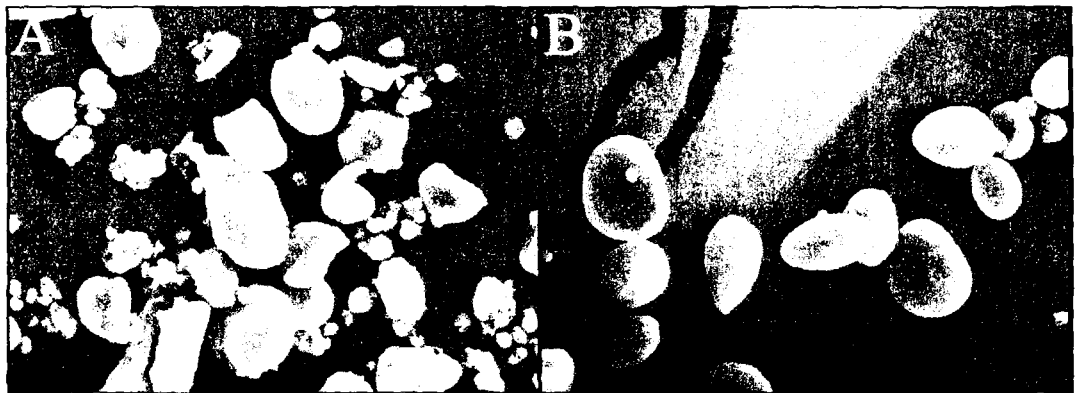


Figure 2.5. Scanning electron microscope analysis of purified starch granules taken at 1000 \times magnification. A) Hard wheat starch granules are clumped together along with protein bodies, oblong, and rough in texture with cracks on the surface of the large granules. B) Super-soft wheat starch granules are single and discrete, smooth on the surface, and have virtually no adhering protein.

Source: Swan *et al.* (2006).

2.3.4. Measurement of wheat hardness

Different methods have been used to measure wheat hardness. Some are indirect methods (Dobraszczyk *et al.*, 2002) and they are usually based on the particle size distribution of the ground meal of whole wheat (GreVeuille *et al.*, 2006). In these methods, hardness of a grain sample is determined as the percentage of fine particles

obtained after grinding, with a higher percentage of fine particles indicating a lower hardness (Dobraszczyk *et al.*, 2002; Carre *et al.*, 2005). The three indirect methods are,

1. Particle size index (PSI), wherein relative hardness is determined by grinding 22 g sample of wheat through a laboratory mill, using either a burr mill (AACC, 1995) or Cyclone mill fitted with 1 mm screen size (Canadian Grain Commission, 2004) and 10 g of the ground meal is sieved through 75µm sieve (AACC, 1995). The % PSI is determined using the following formula:

$$\text{PSI \%} = (\text{Weight of throughs}/\text{Sample weight}) \times 100.$$

2. Near-infrared reflectance (NIR) spectroscopy: In this method, relative hardness is determined by grinding 15 g sample of wheat through a laboratory mill. The spectral characteristics vary with particle size distribution of the ground whole grain meal. Harder wheat results in increased particle size and the signal absorption increases (AACC, 1995).
3. The energy or time taken to produce a given volume of flour under standardised grinding conditions (Pomeranz and Williams, 1990).

The Perten single-kernel characterisation system (SKCS) 4100 is a direct method to measure the physical hardness of wheat (USDA-ARS Grain Marketing and Production Center and Perten Instruments, Reno, NV, USA). The SKCS method measures the force-deformation characteristics during the crushing of a single kernel and the average force for crushing 300 kernels is reported, in terms of a hardness index (HI). The SKCS HI depends on the moisture, kernel size, weight and the force-deformation curve derived from crushing individual kernels. Table 2.7 summarises a proposed classification, in terms of both HI and PSI. The SKCS-4100 can complete a test in about 3 minutes, and simultaneously reports mean and standard deviation data for kernel weight, diameter, and moisture content, as well as the HI (Williams, 1998).

2.3.5. Effect of wheat hardness on milling products

Endosperm hardness is an important aspect in determining the milling outcomes of wheat cultivars. A hard endosperm gives larger irregular shaped particles (Rose *et al.*, 2001) which are easy to handle (GreVeuille *et al.*, 2006). Higher hardness is also known to increase the energy required to grind wheat (GreVeuille *et al.*, 2006) and to increase starch damage which is a favourable characteristic for bread making flour. On the other hand, flours from soft wheats are more suited to making biscuits and cookies.

Table 2.7. Classification of wheat types according to kernel texture

	PSI Burr mill	PSI Cyclone mill	Perten SKCS HI
Extra hard	Up to 7	Up to 35	90 - 100+
Very hard	8 - 11	36 - 44	80 - 89
Hard	12 - 15	45 - 55	65 - 79
Medium hard	16 - 20	56 - 62	50 - 64
Medium soft	21 - 25	63 - 66	40 - 49
Soft	26 - 30	67 - 70	30 - 39
Very soft	31 - 36	71 - 75	15 - 29
Extra soft	37 +	76 +	Up to 14

PSI = Particle Size Index; HI = Hardness index.

Source: modified from Williams (1998).

2.3.6. Endosperm hardness and broiler performance

Despite the wide use of wheat in poultry diets, studies on the effect of wheat endosperm hardness on broiler performance are limited. As previously noted, harder wheat produces larger particle size flours, which may account for the better broiler performance reported using mash diets based on hard wheats (Rose *et al.*, 2001; Pirgozliev *et al.*, 2003). In pelleted diets, Uddin *et al.* (1996) compared two wheat cultivars selected to be similar in nutrient composition but differing in hardness value. They found no effect of wheat hardness on broiler performance either in ground or whole grain form. Similarly, Hetland *et al.* (2007) found no relationship between wheat hardness and broiler performance.

2.3.7. Effect of wheat hardness on nutrient digestibility in broilers

Research data which relate wheat hardness to digestibility of nutrients are limited and equivocal. It has been suggested that the higher amount of damaged starch in hard wheat makes starch easily hydrolysed by α -amylase (Tester *et al.*, 2004). This is related to a higher capacity of the damaged starch to absorb water and consequently increased susceptibility to amylolysis (Kent and Evers, 1996). In contrast, Wiseman (2006) stated that 'because hydration patterns of any substrate are crucial to enzyme action and hence digestibility, it is possible that soft wheats, with better hydration patterns, may be more digestible than hard wheats'.

A negative relationship between wheat hardness and the digestibility of starch in pelleted diets has been reported (Carre *et al.*, 2002; 2005; Peron *et al.*, 2006). This effect of hardness was attributed to larger particulate size reducing the surface area and

accessibility for digestive enzymes (Carre *et al.*, 2005). In support of this theory, Peron *et al.* (2005) found that fine grinding of hard wheat improved starch digestibility in broilers fed pelleted diets. Similarly, Short *et al.* (2000) found that the hard wheat endosperm was associated with lower amino acid digestibility. It has been suggested that the interaction between the starch granules and the surrounding protein matrix may act to obstruct enzyme hydrolysis of starch in hard wheat (Guerrieri *et al.*, 1997; Peron *et al.*, 2006). Conversely, Uddin *et al.* (1996) found that the AME of pelleted wheat diets was not affected by endosperm hardness in broilers. This lack of a relationship between grain hardness and AME or starch digestibility in wheat-based mash diets has been reported by several workers (Rogel *et al.*, 1987; Rose *et al.*, 2001; Pirgozliev *et al.*, 2003). Therefore, further studies are required to fully understand the factors that may effect nutrient utilisation in relation to wheat hardness.

2.3.8. Conclusions

Hardness is one of the major characteristics of wheat grain that determines the milling outcomes of wheat cultivars. The beneficial effect of higher wheat hardness on the performance of broilers fed mash diets is related to diet particle size, but the effect of hardness on nutrient digestibility is less clear.

CHAPTER 3

General Materials and Methods

All experimental procedures described in this thesis were approved by the Massey University Animal Ethics Committee (Anon, 1992) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes (2001).

3.1. Birds and housing

For all experiments, day-old male broilers (Ross 308) were obtained from a commercial hatchery, individually weighed and assigned to 24 cages (8 birds per cage) in electrically heated battery brooders so that the average total bird weight per cage was similar. The birds were transferred to grower cages on day 14 and were maintained on the same diets until day 21. The birds were maintained under constant fluorescent illumination. The battery brooders and grower cages were housed in an environmentally controlled room. The temperature was maintained at 31 °C on d 1, and was gradually reduced to 22 °C by 21 d of age. Body weights and feed intake by cage were recorded at weekly intervals throughout the trial. Mortality was recorded daily. Feed per gain values were corrected for the body weights of any birds that died during the course of the experiment.

3.2. Determination of the geometric mean diameter (GMD) and the geometric standard deviation (GSD) of feed particles

A set of sieves (Endocot, London, UK) sized 2, 1, 0.5, 0.25, 0.106 and 0.075 mm and a Tyler Ro-Tap testing sieve shaker were used to separate the particles into different sized fractions as described by Baker and Herrman (2002). Briefly, the feed sample was passed through a sieve stack on a shaker for 10 minutes. The amount of particles retained on each sieve was determined and, the GMD and GSD of the sample were calculated using the below formulae. These calculations were based on the assumption that the weight distribution of ground cereal grains is logarithmically normal (Martin, 1985). Two replicates per sample were analysed for particle size determination.

$$d_i = (d_u \times d_o)^{0.5}$$

$$\text{GMD} = \log^{-1} \left[\frac{\sum (W_i \log d_i)}{\sum W_i} \right]$$

$$\text{GSD} = \log^{-1} \left[\frac{\sum W_i (\log d_i - \log \text{GMD})^2}{\sum W_i} \right]^{0.5}$$

Where,

d_i = diameter of i^{th} sieve in the stack.

d_u = diameter opening through which particles will pass (sieve proceeding i^{th}).

d_o = diameter opening through which particles will not pass (i^{th} sieve).

W_i = weight fraction on i^{th} sieve.

3.3. Apparent metabolisable energy determination

In all experiments, the feed intake and total excreta output of each cage were measured from day 17 to 20 posthatch. The pooled excreta from each cage was mixed into a slurry in a blender and sub-sampled for determination of dry matter (DM), gross energy (GE) and nitrogen content. Each sub-sample was freeze-dried, ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at -4°C pending analysis. The DM content, total nitrogen and GE were determined in samples of diet and excreta.

3.3.1. Dry matter

Dry matter content was determined using standard procedures (method 930.15, AOAC, 2005). Duplicate samples were weighed and placed in a drying oven for 24 h at 105°C and the weight was recorded after two hours of cooling in a desiccator.

3.3.2. Gross energy

Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid.

3.3.3. Nitrogen

Nitrogen was determined by combustion (method 968.06; AOAC, 2005) using a CNS-200 carbon, nitrogen, and sulphur auto analyser (LECO Corporation, St. Joseph, MI, USA). Pre-weighed samples were placed into a furnace at 850°C with excess oxygen (O_2) and totally combusted. The combustion products, mainly carbon dioxide (CO_2), water (H_2O), nitrous oxides (NO_x) and nitrogen gas (N_2) were passed through a series of columns to remove H_2O , convert NO_x to N_2 , and to remove the remaining oxides and excess O_2 . The gaseous N_2 , carried by helium, was then measured by thermal conductivity and expressed as a percentage of the sample.

3.3.4. Calculation

The AME values were calculated using the following formula with appropriate corrections made for differences in DM content.

$$\text{AME (MJ/kg diet)} = \frac{(\text{Feed intake} \times \text{GE}_{\text{diet}}) - (\text{Excreta output} \times \text{GE}_{\text{excreta}})}{\text{Feed intake}}$$

Nitrogen-corrected AME (AMEn) values were calculated by correction for zero nitrogen retention by simple multiplication with 36.54 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958).

$$\text{AMEn (MJ/kg diet)} = \text{AME} - ((36.54 \times \text{N retained g/kg DM intake})/1000)$$

3.4. Digestive tract measurements

In all experiments, two birds from each replicate cage, with body weights closest to the mean weight of the cage, were sacrificed by cervical dislocation on day 21.

The live weight and weight of digestive tract segments from crop to caeca of each bird were determined. The length of each intestinal segment was determined with a flexible tape on a wet glass surface to prevent inadvertent stretching. The length (± 0.1 mm) of the duodenum (from the pyloric junction to the distal-most point of insertion of the duodenal mesentery), the length of the jejunum (from the distal most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), the length of the ileum (from the junction with Meckel's diverticulum to ileocaecal junction) and the sum of the lengths from ostium to tip of each caeca were determined. Following division and freeing of each of these components from any adherent mesentery, their full and empty weights (± 0.1 g) were determined along with those of the crop, proventriculus and gizzard.

3.5. Intestinal morphology measurements

In experiments reported in Chapters 4, 5 and 6 where intestinal morphology was determined, two more birds from each replicate cage were sacrificed by cervical dislocation. Sections from the middle of the duodenum and jejunum (about 5 cm in length) were excised and flushed with ice-cold saline, and immediately placed in Bouin's fluid. Samples were transferred into 70% ethanol after 24 h. Each fixed sample was then embedded into wax, and sectioned at a thickness of 7 μm , stained with alcian

blue/haematoxylin-eosin and examined by light microscopy using a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany). Visual measurements of villus height, epithelial cell layer and crypt depth were made on 10 villi at 100x and 200x magnifications using imaging software (Image Pro Plus, Version 4.1.0.9, Media Cybernetics, Silver Spring, MD USA). The following variables were measured: villus height (the distance from the apex of the villus to the junction of the villus and crypt) crypt depth (the distance from the junction to the basement membrane of the epithelial cell at the base of the crypt) and the total extent of the mucosal layer (the distance from the apex of the villus to the basement membrane of the epithelial cell at the base of the crypt).

3.6. Determination of particle size distribution in the diets, duodenal and colonic digesta by wet sieving

In experiments reported in Chapters 4, 5 and 7, two more birds per cage were euthanased by intravenous injection of sodium pentobarbitone on day 21. Digesta was removed from the duodenum by simple drainage and from the colon by manual expression and pooled within a replicate cage for each segment. Samples from the two birds within a cage were pooled, giving a total of six digesta samples per dietary treatment. The particle size spectra of various samples were determined by wet sieving using the method described by Lentle *et al.* (2006). Briefly, weighed samples of the diet, duodenal digesta and colonic digesta were each divided into two sub-samples. One was oven dried at 80 °C in a forced draft oven for 24 h to determine the DM content and the other was suspended in 50 ml of water before washing through a set of sieves (Endocot, London, UK) sized 2, 1, 0.5, 0.25, 0.106 and 0.075 mm. The samples of diet were suspended in 50ml of water and left to stand for 30 min prior to sieving to ensure adequate hydration. The contents of each of the sieves were subsequently washed onto dried, pre-weighed filter papers, dried for 24 h in a forced draft oven at 80 °C for 24 h and re-weighed. The dry weight of particles retained by each sieve and of fines remaining in the bottom pan were expressed as percent of total DM recovered.

3.7. Determination of digesta transit time

In experiments reported in Chapters 4 and 5, feed was withdrawn from each cage for 2 h on day 15 and identical diets containing chromic oxide (1.0 g/kg) were then offered for 15 min before being replaced with original unmarked diets. Transit time was determined

as the lapsed time from the introduction of the diets to the time of first appearance in the droppings of green chromic oxide colouration.

3.8. Pellet durability

Pellet durability was determined in a Holmen Pellet Tester (New Holmen Pellet Tester, TekPro Ltd., Norfolk, UK) using the method described by Svihus *et al.* (2004a). Pellet samples (100 g) were circulated pneumatically through a closed pipe for 30 seconds before being passed through a 3 mm sieve. The Pellet Durability Index (PDI) was calculated as the relative proportion by weight of pellets retained on the 3 mm sieve.

3.9. Statistical analysis

In experiments reported in Chapters 4, 5, 6 and 7, the performance data were analysed by two-way analysis of variance (ANOVA) using the General Linear Model procedure of SAS (1997). Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

In experiments reported in Chapters 4, 5, 6 and 7, gross and microscopic measurements of the digestive tracts of individual birds were first subjected to log transformation to achieve near normal distribution on graphic analysis SYSTAT software (Wilkinson, 1990). Two-way ANOVA was then used to identify any interaction between factor 1 and factor 2 on the microscopic morphometric parameters of gut components. For gross digestive tract measurements, a three-way ANOVA was used to identify any interaction between factor 1, factor 2 and the various digestive tract components (crop, proventriculus, gizzard, duodenum, jejunum, ileum and caeca) so as to understand how factor 1 and factor 2 influence the overall tract development. A similar model was used for analysis of (log transformed) digesta content, with the digesta content of the various segments of the small intestine being the third factor. In each case, the significance of individual components within the three levels was explored by *post hoc* testing with Bonferroni correction. Factors 1 and 2 are identified in each chapter.

CHAPTER 4

Influence of feed particle size and feed form on the performance, energy utilisation, digestive tract development and digesta parameters of broiler fed wheat-based diets²

4.1. Abstract

The influence of feed particle size and feed form on the performance, energy utilisation, digestive tract development and digesta parameters of broiler fed wheat based diets was studied. The experimental design was a 2 x 2 factorial arrangement of treatments evaluating two wheat particle sizes (medium and coarse) and two feed forms (mash and pellet). The two particle sizes were achieved by grinding the whole wheat in a hammer mill to pass through 3mm and 7mm screens. Broiler starter diets, based on wheat and soybean meal, were formulated and each diet was fed to six cages of eight male broilers each from day 1 to 21 posthatch. Broiler performance was superior ($P < 0.05$) in birds fed pelleted diets compared to those on mash diets. Pelleting evened out differences in particle size distribution between treatments and, as a result, wheat particle size had no effects ($P > 0.05$) on the performance of broilers fed pelleted diets. In mash diets, coarse grinding of wheat increased ($P < 0.05$) the weight gain and lowered feed per gain compared to medium grinding. Pelleting had a negative effect ($P < 0.05$) on AMEn. The improvement in bird performance with pelleting was accompanied by a decrease in the relative length of all components of the digestive tract. Conversely, the extent of the mucosal layer was greater ($P < 0.05$) in both the duodenum and jejunum of birds fed pelleted feeds. Feed form influenced ($P < 0.05$) the particle size of duodenal digesta whereas particle size had no effect ($P > 0.05$). Overall, the results showed that feed form had a greater influence on the different measured parameters than did the particle size.

² Poultry Science (2007) 86: 2615-2623.

4.2. Introduction

The reduction of particle size of grains involves disruption of the outer seed coat and fracture of the endosperm. Finer reduction increases the number and size of particles, and hence the overall surface area per unit volume, which may allow greater access to digestive enzymes and increase digestive efficiency (Goodband *et al.*, 2002). Other benefits of particle size reduction include generally greater ease of handling and ease of mixing of ingredients (Koch, 1996). Interest in the effects of feed particle size has increased in recent years, as the poultry industry searches for ways to optimize utilisation of feed and improve production efficiency.

The anticipated ban in the use of antibiotic growth promoters in animal feeds has encouraged nutritionists to explore alternative feed management strategies to improve the health and the digestive efficiency of broiler chickens. Promotion of the development of the gizzard is one such nutritional strategy, which can be achieved by manipulating feed particle size (Nir *et al.*, 1995; Engberg *et al.*, 2002). A well developed gizzard is associated with improvement in gut motility (Ferket, 2000) and may prevent pathogenic bacteria from entering the small intestine (Bjerrum *et al.*, 2005), thus reducing the risk of coccidiosis and other enteric diseases (Cumming, 1994; Engberg *et al.*, 2002, 2004; Bjerrum *et al.*, 2005). Part of the beneficial effects of feed particle size may arise due to their influence on intestinal morphometry, but there is no published data on this aspect. Data on the effect of feed particle size on particle size distribution of intestinal digesta in poultry are also scanty. It has been speculated that greater proportions of coarser particles in the diet will result in greater numbers of coarser particles transiting the gizzard, which may increase the permeability of digesta to enzymes and improve digestive efficiency (Lentle, 2005).

The feeding of coarser grain size is sound from the viewpoint of energy expenditure. Grinding of whole grain for broiler feeds constitutes the second greatest energy expenditure after pelleting (Reece *et al.*, 1985). Increasing screen size in the hammer mill from 4.76 to 6.35 mm has been reported to reduce the total energy consumption by 27% (Reece *et al.*, 1986a). However, coarse grinding is believed to reduce pellet quality, although there is no objective evidence to this claim.

In any study examining the effects of feed particle size in broiler diets, the physical form of the diet (mash versus pellets) should also be considered. Evidence suggest that the

large grain particle size influences broiler performance to a greater extent when birds are fed mash diets than those fed pelleted or crumbled diets (Nir and Ptichi, 2001). The objectives of the present experiment were to compare the effects of particle size and feed form on the performance, energy utilisation, digestive tract development, intestinal villus morphology and, duodenal and colonic digesta particle size spectra of broilers fed wheat-based diets.

4.3. Materials and methods

4.3.1. Birds and Housing

192 day-old male broilers (Ross 308) were obtained from a commercial hatchery. Housing conditions were as described in Section 3.1.

4.3.2. Diets

Whole wheat was ground in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) to pass through a screen size of 3 or 7 mm for medium and coarse grades, respectively. The GMD and GSD were then determined as described in Section 3.2. Two broiler starter diets, each based on one of the milled wheat grades plus soybean meal, were formulated to meet or exceed the NRC (1994) recommendations for major nutrients for broiler starters (Table 4.1). Half of each formulated diet was fed in mash form and the other half was cold pelleted using a pellet mill (Richard Size Limited Engineers, Orbit 15, Kingston-upon-Hull, England) capable of manufacturing 180 kg of feed/h and equipped with a die ring (3 mm holes and 35-mm thickness). Conditioning time of the mash was 90 s to a temperature near 70 °C. Each of the four dietary treatments was fed *ad libitum* to six cages, each housing eight birds. Water was freely available throughout the trial.

4.3.3. AME determination

The AME determination was carried out between day 17 to 20 posthatch as described in Section 3.3.

Table 4.1. Composition and calculated analysis (g/ kg as fed) of the basal diet

Ingredient	
Wheat ¹	648
Soybean meal	295
Soybean oil	13.1
Dicalcium phosphate	16.2
Limestone	16.5
L-lysine. HCl	1.9
DL-methionine	3.8
Salt	2.5
Trace mineral-vitamin premix ²	3.0
Xylanase ³	+
Calculated analysis	
Metabolisable energy, MJ/kg	12.4
Crude protein	226
Lysine	11.5
Methionine + cystine	9.4
Calcium	10.9
Available phosphorus	4.5

¹ Two particle sizes were obtained by grinding the whole grain in a hammer mill to pass through 3 and 7 mm screens.

² Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl- α -tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

³ Kemzyme, Kemin (Asia) Pte Ltd, Singapore.

4.3.4. Digestive tract measurements

Two birds per cage were euthanased and, digestive tract measurements and gut morphological examinations were carried out as described in Sections 3.4 and 3.5, respectively.

4.3.5. Determination of particle size distribution in the diets, duodenal and colonic digesta

Two more birds per cage were euthanased by intravenous injection of sodium pentobarbitone on day 21. The particle size distributions of duodenal digesta, colonic digesta and the diets were determined as described in Section 3.6.

4.3.6. Determination of digesta transit time

The transit time was determined on day 15 as described in Section 3.7.

4.3.7. Pellet durability

The pellet durability index was measured as described in Section 3.8.

4.3.8. Data analysis

The cage means were used to derive performance data. For digestive tract measurements, individual birds were considered as the experimental unit. Data were analysed as described in Section 3.9, where factor 1 was feed form and factor 2 was particle size.

4.4. Results

The GMD of wheat ground through 3 and 7 mm screen sizes were determined to be 0.839 and 1.164 mm, respectively, with corresponding GSD values of 1.69 and 1.54.

Graphic comparisons of the particle size distributions of mash and pelleted diets, (Figure 4.1) obtained by wet sieving showed that pelleting reduced the relative proportion of particles > 1 mm, and increased the proportion of fine particles < 0.075 mm in coarse feeds so that the particle size distributions of the two feeds were almost identical.

Pelleting of the diet increased ($P < 0.05$) the weight gain and feed intake and improved feed per gain compared to those obtained from birds fed mash diets (Table 4.2). In mash diets, coarse grinding resulted in higher ($P < 0.05$) weight gain and feed intake and lower ($P < 0.05$) feed per gain than medium wheat particle size. No differences ($P > 0.05$) were observed between the particle size treatments within pelleted diets for any of the performance parameters.

Pelleting reduced ($P < 0.05$) the overall AMEn compared with that of birds maintained on mash diets (Table 4.2). However, there was no effect ($P > 0.05$) of feed particle size on AMEn or significant interaction of feed form with particle size.

Digesta transit time did not vary ($P > 0.05$) with any of the dietary treatments.

The PDI values of pellets made from diets based on medium (32.7 ± 1.8) and coarse (34.2 ± 1.8) ground wheats were not significantly ($P > 0.05$) different.

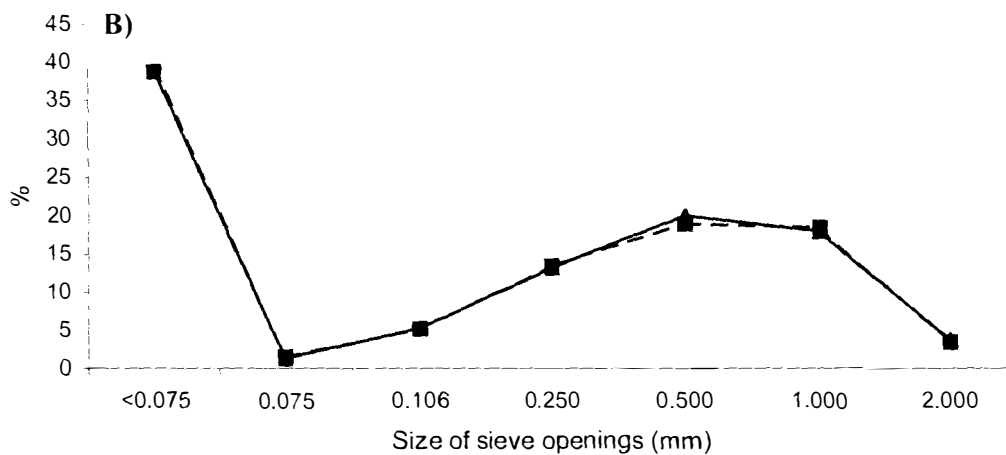
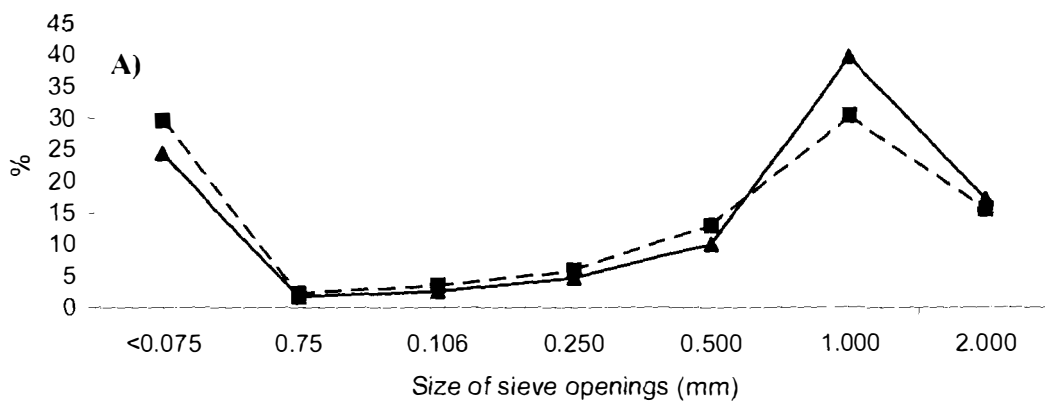


Figure 4.1. Particle size distribution of mash (A) and pelleted (B) diets
 (-■- Medium · -▲- Coarse)

Table 4.2. Influence of particle size and feed form on the weight gain (g/bird), feed intake (g/bird), feed per gain (g/g), AMEn (MJ/kg dry matter) and digesta transit time (min.) of broilers (1-21 days posthatch)¹

Form	Particle size	Weight gain	Feed intake	Feed per gain	AMEn	Transit time
Mash	Medium	453 ^a	777 ^a	1.717 ^a	12.44	151
	Coarse	539 ^b	877 ^b	1.629 ^b	12.39	149
Pellet	Medium	834 ^c	1271 ^c	1.525 ^c	11.69	148
	Coarse	824 ^c	1253 ^c	1.521 ^c	11.90	148
SEM ²		15	20	0.017	0.12	4
Main effects						
Feed form						
	Mash	496	827	1.673	12.53 ^a	150
	Pellet	829	1262	1.523	11.79 ^b	148
Particle size						
	Medium	643	1024	1.621	12.06	150
	Coarse	681	1065	1.575	12.08	149
Probability, P <						
	Feed form	***	***	***	***	NS
	Particle size	*	NS	*	NS	NS
	Feed form x Particle size	***	*	*	NS	NS

NS, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

¹ Each value represents the mean of six replicates (8 birds per replicate).

² Pooled standard error of mean.

^{a,b,c} Means in a column not sharing a common superscript are significantly different (P < 0.05).

The effects of feed form and particle size on the relative tissue weights, digesta contents and relative lengths of the various intestinal components are shown in Table 4.3. The relative empty weights of all components were greater (P < 0.05) in birds fed mash diets than those fed pelleted diets. Although there was no main effect of wheat particle size on the wet tissue weight of gut components, there was interaction (P < 0.05) between particle size with feed form due to the weight of all gut components being higher in birds fed medium particle mash than in those fed coarse particle mash. There was also a significant (P < 0.05) interaction between feed form and the weights of the various gut segments due to birds fed mash feed having a relatively heavier gizzard and caeca and birds fed pelleted diets having a heavier jejunum.

Table 4.3. Influence of particle size and feed form on gross morphology of the digestive tract of broilers maintained on wheat based pelleted and mash diets¹

	Mash		Pellet	
	Medium	Coarse	Medium	Coarse
Relative empty weights (g/kg BW)^{3, 5, 6}				
Proventriculus	5.8±0.31	5.1±0.21	5.24±0.46	5.5±0.65
Gizzard	22.0±0.74	20.1±0.71	10.7±0.66	11.3±0.72
Duodenum	6.3±0.34	5.5±0.13	6.3±0.20	6.5±0.30
Jejunum	13.1±0.52	11.8±0.47	13.2±0.62	12.6±0.64
Ileum	9.5±0.33	8.3±0.50	8.7±0.31	9.1±0.44
Small intestine ²	28.9±0.87	25.7±0.88	28.3±0.87	28.3±0.96
Caeca	2.3±0.14	2.1±0.11	1.5±0.06	1.7±0.11
Relative digesta content (g/kg BW)^{3, 5, 6}				
Proventriculus	0.8±0.24	0.3±0.069	1.5±0.86	2.3±1.35
Gizzard	15.5±1.34	12.4±1.05	3.7±0.99	5.7±1.95
Small intestine ²	40.6±2.85	39.3±1.91	44.9±3.35	42.1±1.59
Caeca	3.8±0.26	3.3±0.38	2.4±0.27	2.9±0.52
Relative length (cm/kg BW)^{3, 4, 5}				
Duodenum	36±1.89	30.5±1.12	27.6±0.76	26.8±0.53
Jejunum	95±4.35	83.1±3.66	70.5±1.94	69.5±1.81
Ileum	96.7±4.61	85.4±2.00	71.4±2.99	72.3±1.82
Small intestine ²	227±10.33	199±7.46	169.5±4.2	168.8±3.36
Caeca	24±0.99	20.9±0.97	16.7±0.83	17.5±0.69

¹ Each value represents the mean ± SE of 12 birds.

² Small intestine = duodenum + jejunum + ileum.

³ Feed form effect (P < 0.05).

⁴ Particle size effect (P < 0.05).

⁵ Feed form x particle size (P < 0.05).

⁶ Feed form x gut component (P < 0.05).

A significant interaction (P < 0.05) between feed form and particle size was observed for relative digesta content weights. This was due to the overall digesta content being higher (P < 0.05) in birds fed medium particle size than in those fed coarse size particles in mash form, but not different between those fed the medium and coarse size particles in pelleted form. There was an interaction (P < 0.05) between individual gut components with feed form due to the greater digesta content of the gizzard in birds fed mash diets than in those fed pelleted diets, with no effect of feed form on digesta content of other gut components. The main effect of feed form was significant (P < 0.05) for relative digesta content weights, with greater digesta contents in the mash treatments.

An interaction ($P < 0.05$) between feed form and particle size was noted for the relative length of gut components. All gut components were shorter in birds fed coarse compared with those fed medium in mash form but there was no difference in gut length between those fed coarse and those fed medium in pelleted form. The relative length of all gut components was shorter ($P < 0.05$) in birds fed pelleted diets. Similarly, all gut components were shorter ($P < 0.05$) in birds given feeds containing coarse particles. Although there were overall differences ($P < 0.05$) in the relative lengths of the gut components with feed form and particle size and there were differences ($P < 0.05$) in the lengths of individual components, there was no interaction ($P > 0.05$) between the lengths of the individual components either with feed form or with particle size. Thus, whilst the form and particle size of the feed influenced the overall length of all gut components, it did not change the pattern of their individual lengths.

The morphometry of the duodenum and jejunum are shown in Table 4.4. In both segments, birds fed pelleted diets had a greater ($P < 0.05$) mucosal extent, villus height and crypt depth measurements compared to those fed mash diets. The main effect of particle size and the two-way interactions were not significant ($P > 0.05$) for these morphological parameters.

Table 4.4. Influence of particle size and feed form on total extent of the mucosal layer (μm), villus height (μm) and crypt depth (μm) of the duodenum and jejunum of broilers¹

Treatment	Mash		Pellet		SEM ²	Feed form	PS ³	Feed form x PS
	Medium	Coarse	Medium	Coarse				
Duodenum								
Extent of the mucosal layer	1052±24	1102±21	1297±23	1335±25	33	***	NS	NS
Villus height	962±20	1014±28	1197±24	1234±27	67	***	NS	NS
Crypt depth	89±7	87±7	100±5	102±7	6.7	***	NS	NS
Jejunum								
Extent of the mucosal layer	515±27	610±29	773±27	747±28	49	***	NS	NS
Villus height	477±27	528±29	676±27	659±26	46	***	NS	NS
Crypt depth	72±7	82±7	99±6	95±8	6.8	***	NS	NS

NS, not significant; ***, $P < 0.001$.

¹ Each value represents the mean \pm SE of 12 birds.

² Pooled standard error of mean.

³ PS = particle size.

In both mash and pelleted diets, the discriminant analysis failed to show distinction ($P > 0.05$) between particle size distribution of both the duodenal and colonic digesta from birds fed diets with different particle sizes (Table 4.5). When particle size treatments within feed forms were combined and mash and pelleted diets were compared, discriminant analysis of the particle size distribution of duodenal and colonic digesta showed successful distinction ($P < 0.01$) between pelleted and mash diets (Table 4.6). Thus the duodenal digesta of the birds fed mash diets had a lower proportion ($P < 0.01$) of particles 0.5 mm and higher proportion of particles >1 mm. Similarly, there was a significant ($P < 0.0001$) difference between the colonic digesta of birds fed pelleted and those fed mash diets, with birds fed mash diets having significantly lower proportions of particles 0.25, 0.5 mm and fines (< 0.075 mm) than pelleted diets.

Table 4.5. Influence of particle size and feed form on the proportion of particle size classes in the duodenal and colonic digesta (on a dry weight basis) of broilers¹

	Mash		Pellet	
	Medium	Coarse	Medium	Coarse
Duodenal digesta²				
<0.075mm	0.386±0.089	0.436±0.037	0.497±0.087	0.410±0.034
0.075-0.106mm	0.050±0.014	0.100±0.042	0.066±0.014	0.055±0.012
0.106-0.25mm	0.090±0.021	0.117±0.013	0.150±0.027	0.139±0.014
0.25-0.5mm	0.296±0.110	0.150±0.017	0.238±0.021	0.199±0.031
0.5-1 mm	0.095±0.020	0.098±0.010	0.151±0.024	0.134±0.008
1-2 mm	0.069±0.015	0.070±0.008	0.047±0.010	0.059±0.011
>2 mm	0.014±0.006	0.028±0.008	0.000	0.004±0.004
Colonic digesta²				
<0.075mm	0.055±0.016	0.045±0.008	0.095±0.027	0.103±0.016
0.075-0.106mm	0.022±0.003	0.019±0.004	0.034±0.005	0.042±0.013
0.106-0.25mm	0.038±0.003	0.052±0.009	0.082±0.008	0.059±0.008
0.25-0.5mm	0.146±0.012	0.143±0.013	0.271±0.021	0.253±0.019
0.5-1 mm	0.289±0.008	0.274±0.009	0.320±0.022	0.300±0.010
1-2 mm	0.368±0.019	0.386±0.024	0.181±0.016	0.205±0.029
>2 mm	0.083±0.012	0.082±0.010	0.028±0.008	0.038±0.010

¹ Each value represents the mean ± SE of six replicates.

² The discriminant analysis failed to show distinction ($P > 0.05$) between particle size distribution of both the duodenal and colonic digesta from birds fed diets with different particle sizes.

Table 4.6. Influence of feed form on the proportion of particle size classes in the duodenal and colonic digesta (on a dry weight basis) of broilers¹

Particle size class	Duodenal digesta ²		Colonic digesta ³	
	Mash	Pellet	Mash	Pellet
<0.075mm	0.411±0.046	0.453±0.046	0.050±0.009	0.099±0.015
0.075-0.106mm	0.075±0.022	0.061±0.009	0.021±0.002	0.038±0.007
0.106-0.25mm	0.104±0.012	0.144±0.014	0.045±0.005	0.070±0.006
0.25-0.5mm	0.223±0.057	0.219±0.018	0.144±0.008	0.262±0.014
0.5-1 mm	0.097±0.010	0.143±0.012	0.281±0.006	0.310±0.012
1-2 mm	0.069±0.008	0.053±0.007	0.377±0.015	0.193±0.016
>2 mm	0.021±0.005	0.002±0.002	0.082±0.007	0.033±0.006

¹ Each value represents the mean ± SE of 12 replicate samples.

² The discriminant analysis of the particle size distribution digesta showed successful distinction ($P < 0.05$) between pelleted and mash diets.

³ The discriminant analysis of the particle size distribution digesta showed successful distinction ($P < 0.001$) between pelleted and mash diets.

4.5. Discussion

The feeding of pelleted diets increased weight gain and feed intake and improved feed per gain ratio compared to those birds maintained on mash diets as has been reported in a number of studies (Nir *et al.*, 1995; Engberg *et al.*, 2002; Svihus *et al.*, 2004). These improvements have been attributed variously to increased nutritional density, improved starch digestibility resulting from chemical changes during pelleting, increased nutrient intake, changes in the physical form of the feed, reduced feed wastage and decreased energy expenditure in eating (Calet, 1965; Jensen, 2000). Although pelleting had a negative effect on AMEn, broiler performance measured by feed per gain was improved relative to that of mash diets. This anomaly could be explained by the higher feed intake in the pelleted treatments. It would appear that the lower AME is more than compensated by increasing feed intake in excess of maintenance requirements.

The present data showed that the relative gizzard weight and content were lower in birds fed pelleted feeds than in those fed mash feeds. Similar findings have been reported by Nir *et al.* (1995). These results may suggest that pelleting decreased the grinding requirement of the gizzard so that its function was reduced to that of transit.

It is noteworthy that AMEn did not vary with particle size either in mash and pelleted diets, a finding that is similar to that of Svihus *et al.* (2004) for pelleted diets. In contrast, Peron *et al.* (2005) reported that the AME was lower with pelleted diets made from coarse ground wheat compared to that made from fine ground wheat.

Nitrogen-corrected AME of birds that were fed pelleted diets was reduced compared with that of birds fed mash diets. It has been hypothesised that the higher intake of pelleted wheat-based diets increases the starch load in the gut and lowers the digestibility of starch (Svihus and Hetland, 2001). This hypothesis is supported by the finding of this study that the extent of the mucosal layer of the duodenum and jejunum is increased in birds fed pelleted diets, which could be viewed as indicating a general increase in digestive and absorptive capacity of the proximal small intestine in response to the greater flow of nutrients.

The reduction in the relative length of the intestine of birds fed pelleted diets is less readily explained, as it does not appear to optimise digestion. The fact that this lengthwise reduction is of a general nature and not limited to specific gut compartments indicate that it may result from a general metabolic effect rather than from redundancy of the digestive action of a particular gut compartment. This raises the question whether there can only be concerted lengthwise growth of the various components of the avian digestive tract and that responses to changing nutrient availability are limited to increases in the extent of the mucosal layer. Such a mechanism may account for the reduction of AME as, given limited radial mixing in the small intestine (Lentle, 2005), a high intake of feeds of high nutritional density would allow nutrient flow to outstrip local absorptive capacity.

With regard to the feeding of mash diets, a reduction in feed per gain in birds fed coarse ground mash compared to those fed medium ground mash has also been reported in a number of other studies and generally attributed to the preference for larger sized particles (Proudfoot and Hulan, 1989; Nir *et al.*, 1995) and the lowering of viscosity of the liquid phase of digesta (Yasar, 2003). The current study showed that the gut components of birds fed coarse mash were relatively shorter than those of birds fed medium ground mash, but that this was accompanied by a significant relative increase in feed intake. This suggests that, at some stage of development, gut component length may vary inversely with intake whilst the weight and the extent of the mucosal layer of individual gut components varies with nutrient flow.

In the present study, the effect of grinding by the gizzard on the relative proportions of particles of different size in duodenal digesta was examined. Birds fed mash diets had a significantly larger mass of gizzard, but a higher proportion of large particles (1-2 mm) were found in the duodenal digesta in birds fed mash diets than in those fed pelleted diets. These results indicate that the larger gizzard size was not

sufficient to uniformly reduce the size of all particles, contrary to the suggestion of Hetland *et al.* (2004). It is noteworthy that the relative proportion of fines did not differ between the diets and was not a major basis of discrimination in the analysis. This difference may account for conclusions of Hetland *et al.* (2004) as their data were based only on finer particles (<0.88 mm). These data suggest that the gizzard is highly efficient in grinding large particles, but some large particles still escape the grinding as has been previously reported by Lentle *et al.* (2006). Previous studies with whole cereals found that moderate inclusion of whole cereals, which was accompanied by larger gizzard, did not affect the proportion of particles smaller than 0.100 mm in duodenal digesta (Svihus *et al.*, 1997).

It is generally believed that there is an inverse relationship between particle size and pellet durability (Angulo *et al.*, 1996). Smaller particles produce more durable pellets because they have more contact points with each other due to a larger surface area to unit volume ratio (Behnke, 2001). However, in the present study, wheat particle size had no effect on pellet durability. In contrast, Reece *et al.* (1986b) found that pellets made from coarse corn particles gave significantly more durable pellets than those made from fine particles. On the other hand, Svihus *et al.* (2004) showed numerical improvements in pellet durability when made from fine particles, which was attributed to higher starch gelatinisation in the diets made with fine ground wheat. The contradictory results in the literature concerning the effect of particle size on pellet durability may be explained by confounding factors such as protein and oil contents of the diet (Briggs *et al.*, 1999) and hardness of the grain (Carre *et al.*, 2005).

In the present study, it was found that the pelleting process further reduced the size of coarse particles thus minimizing the differences in the particle size distributions of coarse and medium grindings. During the pelleting process, the feed is passed through steam, which softens the feed particles before they are pressed through the die by the rolls in the pellet press, causing additional grinding effect (Engberg *et al.*, 2002; Svihus *et al.*, 2004). This may explain the lack of differences in performance between birds fed pelleted diets based on medium and coarse grinds. Previous research conducted using pelleted diets have also reported no effect of particle size on broiler performance (Reece *et al.*, 1986 a,b; Proudfoot and Hulan, 1989; Svihus *et al.*, 2004; Peron *et al.*, 2005).

4.6. Conclusions

The data showed that feed form had a greater influence on the different measured parameters than did the particle size. Pelleting reduced the relative proportion of larger particles and increased that of smaller particles in the diet. The reductions in feed per gain in pelleted diets were accompanied by a decrease in the length of all components of the digestive tract. Conversely the extent of the mucosal layer increased in both duodenum and jejunum in birds fed the pelleted diets. The feeding of coarse ground wheat in mash diets improved intake, weight gain and lowered feed per gain compared with when medium ground wheat was fed. Pellet durability was not affected by wheat particle size. It would therefore appear that energy savings could be achieved by coarsely grinding wheat grain with no adverse effect on broiler performance.

CHAPTER 5

Influence of feed particle size and grain type on the performance, energy utilisation, digestive tract development and digesta parameters of broilers

5.1. Abstract

The influence of feed particle size and grain type on the performance, energy utilisation, digestive tract development and digesta parameters of broilers fed either wheat or maize based diets were studied. The experimental design was a 2 x 2 factorial arrangement of treatments evaluating two particle sizes (fine and coarse) and two grain types (wheat and maize). The two particle sizes were achieved by grinding the whole grains, in a hammer mill to pass through 1 and 7 mm screens, respectively. Each dietary treatment was pelleted and fed *ad libitum* to six cages of eight male broilers each from day 1 to 21 posthatch.

The results showed that the differences in particle size distribution still existed between diets after pelleting especially in the proportion of coarse particles (1 mm and over). There was a tendency ($P = 0.07$) for a grain type x particle size interaction for weight gain, with increased weight gains observed only in birds fed coarse ground maize-based diets. Feed intake was influenced ($P < 0.001$) by grain type, with the intake of wheat-based diets being greater than maize-based diets. Birds fed maize-based diets had a lower ($P < 0.001$) feed per gain compared to those fed wheat-based diets. Coarse grinding also lowered ($P < 0.001$) feed per gain of broilers compared to fine grinding. Maize-based diets had higher ($P < 0.05$) AMEn compared to wheat based diets. There was however, a tendency ($P = 0.06$) for treatment interactions, with coarse grinding improving the AME only in wheat-based diets. A three-way interaction ($P < 0.05$) between particle size, grain type and the weights of the various gut segments was found due to birds fed coarse particle size having a relatively heavier gizzard in birds fed maize-based diets but this effect was not found in birds fed wheat-based diets. Gut morphological examinations showed no effect of particle size and grain type on villus height, crypt depth and epithelial thickness. Grinding in the gizzard evened out differences in particle size between the diets, so no difference ($P > 0.05$) in particle size distribution in duodenal digesta was found in birds fed fine and coarse particles.

5.2. Introduction

Available evidence suggest that grain particle size is more critical in mash feeds, and less so in pelleted or crumbled feeds (Reece *et al.*, 1985, 1986a; Nir *et al.*, 1995; Svihus *et al.*, 2004a; Peron *et al.*, 2005). This is surprising since the effect of particle size on broiler performance may be expected to be maintained even after pelleting, given that pellets dissolve in the crop after ingestion.

Grain type is known to influence the particle size spectra of milled products. Passing different grains through the same screen size opening in a hammer mill will give different particle size distributions. This suggests that, during hammer mill grinding, different screen sizes dependant on grain type must be used to obtain the desired particle size distribution. Nir *et al.* (1995) reported that grinding wheat with the same hammer or roller mill under the same conditions gave a higher GMD compared to sorghum.

Studies examining the effect of pelleting on diets particle size distribution have produced inconsistent results. Peron *et al.* (2005) and Lentle *et al.* (2006) showed that the differences in particle size distribution existed even after pelleting. Whilst Engberg *et al.* (2002) and Svihus *et al.* (2004a) reported that pelleting reduced the differences in particle size distribution, and resulted in grain particle size having no effect on broiler performance. Similarly, studies examining the effects of feed particle size in pelleted diets on broiler performance also produced inconsistent results. Peron *et al.* (2005) found no significant differences in growth performance with different wheat grinding. In contrast, Lentle *et al.* (2006) found that diets with the highest relative proportion of coarse particles resulted in the lowest feed per gain in broiler chickens.

The objectives of the present experiment were to investigate the effects of particle size and grain type on the performance, energy utilisation, gut parameters and digesta parameters of broilers. The influence of particle size and grain type on pellet durability was also investigated.

5.3. Materials and methods

5.3.1. Animals and housing

192 day-old male broilers (Ross 308) were obtained from a commercial hatchery. Housing conditions were as described in Section 3.1.

5.3.2. Diets

The experimental design was a 2 x 2 factorial arrangement of treatments testing two grain types (wheat and maize) and two particle sizes (fine and coarse). The two particle sizes were achieved by grinding the whole wheat and maize in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) to pass through 1 and 7 mm screens, respectively. The GMD and GSD were then determined as described in the Section 3.2. Two diets based on wheat or maize, were formulated to meet or exceed the NRC (1994) recommendations for major nutrients for broiler starters (Table 5.1). All diets were cold pelleted (70 °C) using a 3 mm die. Each of the four diets was fed *ad libitum* to six cages, each housing eight birds. Water was freely available throughout the trial.

Table 5.1. Composition and calculated analysis (g/kg as fed) of the basal diet

Ingredient	Wheat diet	Maize diet
Wheat ¹	657.6	-
Maize ¹	-	586.2
Soybean meal	273.4	351.8
Soybean oil	27.3	17.8
Dicalcium phosphate	20.3	21.7
Limestone	3.4	7.8
Salt	1.4	2.3
L-lysine. HCl	3.4	1.8
DL-methionine	2.4	2.5
L-threonine	1.2	0.3
Sodium bicarbonate	3.6	1.8
Trace mineral-vitamin premix ²	3.0	3.0
Xylanase ³	+	-
Calculated analysis		
AME, MJ/kg	12.5	12.5
Crude protein	220	220
Lysine	13.5	13.5
Methionine + cystine	9.5	9.5
Calcium	9.5	9.5
Available phosphorus	4.8	4.8

¹ Two particle sizes were obtained by grinding the whole grain in a hammer mill to pass through 1 and 7 mm screens.

² Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl- α -tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

³ Kemzyme, Kemin (Asia) Pte Ltd, Singapore.

5.3.3. Determination of digesta transit time

The transit time was determined on day 15 as described in Section 3.7.

5.3.4. AME determination

The AME determination was carried out between day 17 to 20 posthatch as described in Section 3.3.

5.3.5. Digestive tract measurements

Two birds per cage were euthanased and, digestive tract measurements and gut morphological examinations were carried out as described in Sections 3.4 and 3.5, respectively.

5.3.6. Determination of particle size distribution in the diets, duodenal digesta and excreta

Two more birds per cage were euthanased by intravenous injection of sodium pentobarbitone on day 21. The particle size distributions of duodenal digesta, colonic digesta and the diets were determined as described in Section 3.6.

5.3.7. Pellet durability

The pellet durability index was measured as described in Section 3.8.

5.4. Data analysis

The cage means were used to derive performance data. For digestive tract measurements, individual birds were considered as the experimental unit. Data were analysed as described in Section 3.9, where factor 1 was grain type and factor 2 was particle size.

5.5. Results

Particle size distributions of the ground grains and pelleted diets, determined by wet sieving, are shown in Figures 5.1 and 5.2, respectively. The GMD of wheat ground through 1 and 7 mm screen sizes were determined to be 0.284 and 0.890 mm, respectively, with corresponding GSD values of 1.95 and 2.19. The GMD of maize

ground through 1 and 7 mm screen sizes were determined to be 0.297 and 0.528 mm, respectively, with corresponding GSD values of 1.81 and 2.33. Graphic comparisons of the particle size distributions of the pelleted diets (Figure 5.2) obtained by wet sieving showed that the differences in particle size distribution persisted after pelleting notably in the proportion of coarse particles (1 mm and over).

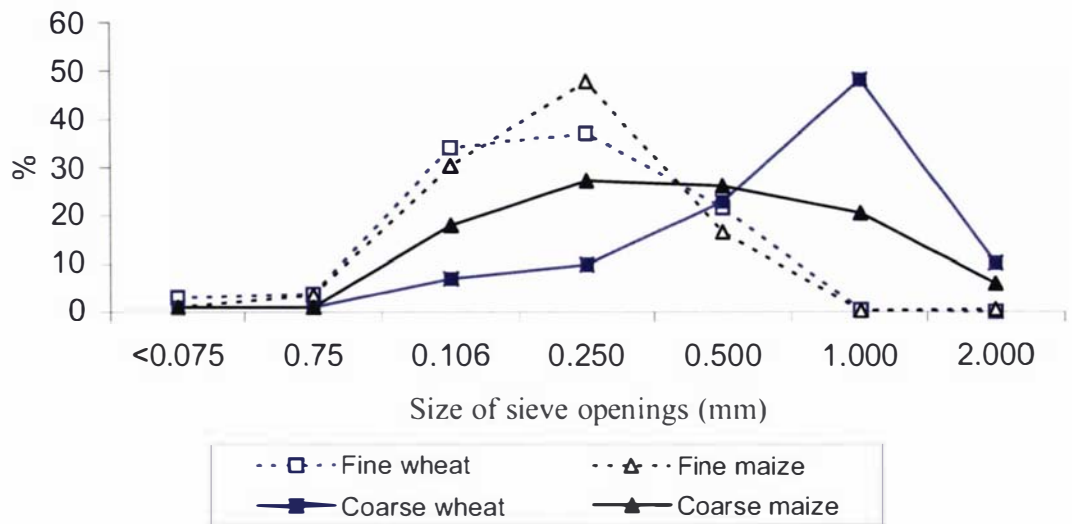


Figure 5.1. Particle size distribution of wheat and maize used in diet formulations.

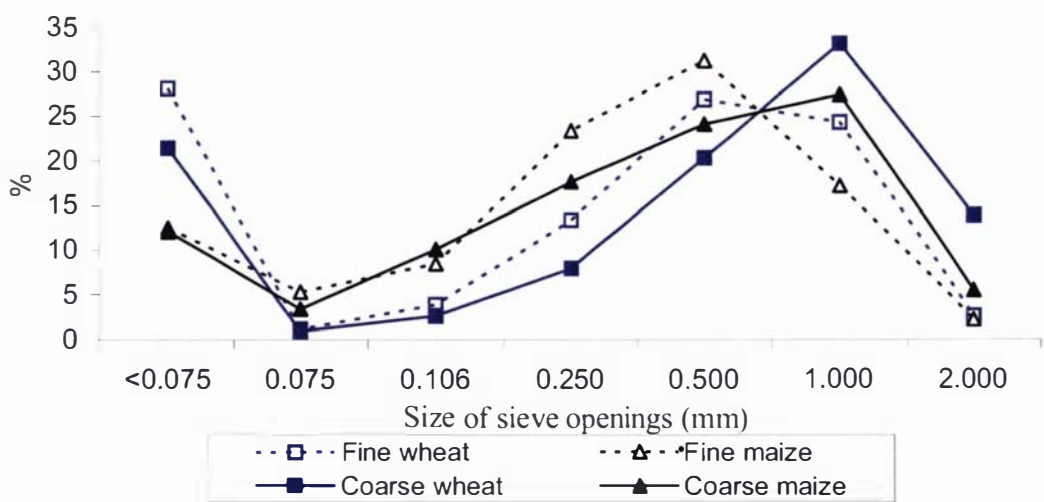


Figure 5.2. Particle size distribution of pelleted diets.

The influence of dietary treatment on the performance of broilers is summarised in Table 5.2. Grain type tended ($P = 0.06$) to influence weight gain, with birds fed wheat-based diets having a greater gain than those fed maize-based diets. There was however, a tendency ($P = 0.07$) for a grain type x particle size interaction, with coarse grinding increasing gains in birds fed maize-based diets only. Feed intake was influenced ($P < 0.001$) by grain type, with the intake of wheat-based diets being greater than those of maize-based diets. Feed intake was also influenced ($P < 0.05$) by particle size, with the intake of fine ground diets being greater than those of coarse ground diets. Birds fed maize-based diets had a lower ($P < 0.001$) feed per gain compared to those fed wheat-based diets. Coarse grinding lowered ($P < 0.0001$) feed per gain of broilers compared to fine grinding. No interaction ($P > 0.05$) was observed for feed intake and feed per gain.

Table 5.2. Influence of particle size and grain type on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broilers (1-21 days posthatch)¹

Grain type	Particle size	Weight gain	Feed intake	Feed per gain
Wheat	Fine	888	1357	1.528
	Coarse	872	1262	1.467
Maize	Fine	823	1191	1.448
	Coarse	870	1173	1.360
SEM ²		17	22	0.013
Main effects				
Grain type				
Wheat		880	1310 ^a	1.497 ^a
Maize		846	1182 ^b	1.404 ^b
Particle size				
Fine		855	1274 ^a	1.488 ^a
Coarse		871	1218 ^b	1.414 ^b
Probabilities, $P \leq$				
Grain type		.06	***	***
Particle size		NS	*	***
Grain type x Particle size		.07	NS	NS

NS, not significant; *, $P < 0.05$; ***, $P < 0.001$.

¹ Each value represents the mean of six replicates (8 birds per replicate).

² Pooled standard error of mean.

^{a,b} Means in a column not sharing a common superscript are significantly different ($P < 0.05$).

The influence of dietary treatments on the AMEn, digesta transit time and pellet durability index is summarised in Table 5.3. Grain type influenced ($P < 0.001$) AMEn, with maize-based diets increasing AMEn compared to wheat-based diets. There was however, a tendency ($P = 0.06$) for treatment interactions, with coarse grinding improving the AME only in wheat-based diets. Transit time was not influenced ($P > 0.05$) by grain type or particle size.

Pellet durability was influenced by grain type and particle size. Pellets made with maize had better ($P < 0.0001$) pellet durability than those made with wheat. Fine grinding resulted in a better ($P < 0.05$) pellet durability than coarse grinding. No treatment interactions ($P > 0.05$) were observed for pellet durability.

Table 5.3. Influence of particle size and grain type on the AMEn (MJ/kg dry matter) and transit time (min.) of broilers and pellet durability index (%)¹

Grain type	Particle size	AMEn	Transit time	PDI
Wheat	Fine	12.07	136	93.0
	Coarse	12.41	142	91.5
Maize	Fine	13.25	139	94.6
	Coarse	13.04	139	92.1
SEM ²		0.13	3.2	.033
Main effects				
Grain type				
Wheat		12.24	139	92.2
Maize		13.15	139	93.4
Particle size				
Fine		12.66	137	93.8
Coarse		12.73	140	91.8
Probabilities, P <				
Grain type		***	NS	***
Particle size		NS	NS	***
Grain type x Particle size		.06	NS	NS

NS, not significant; ***, $P < 0.001$.

¹ Each value represents the mean of six replicates.

² Pooled standard error of mean.

The effects of particle size and grain type on the relative weights, digesta contents and lengths of the various intestinal components are shown in Table 5.4. Although there were no significant ($P > 0.05$) main effects of particle size and grain type on the relative empty weight of gut components, there was an interaction ($P < 0.05$) was noted between individual gut components and grain type due to the greater gizzard

weight in birds fed maize-based diet. There was also an interaction ($P < 0.05$) between individual gut components and particle size. This was due to the greater gizzard weight in birds fed coarse particle size diets. A three-way interaction ($P < 0.05$) between particle size, grain type and the weights of the various gut segments was found due to birds fed coarse particle size having a relatively heavier gizzard in birds fed maize-based diets but this effect was not found in birds fed wheat-based diets.

Table 5.4. Influence of particle size and grain type on gross morphology of the digestive tract of broilers¹

	Wheat		Maize	
	Fine	Coarse	Fine	Coarse
Relative empty organ weights (g/kg BW)^{5,6,7}				
Crop	3.04±0.12	3.05±0.08	2.90±0.11	3.30±0.13
Proventriculus	3.84±0.13	3.95±0.23	4.62±0.16	4.28±0.22
Gizzard	9.03±0.27	10.0±0.27	9.40±0.39	12.6±0.76
Duodenum	5.12±0.23	5.20±0.24	5.25±0.29	5.24±0.18
Jejunum	11.6±0.47	11.7±0.39	10.8±0.48	10.5±0.37
Ileum	7.9±0.31	7.6±0.40	7.5±0.34	6.9±0.33
Small Intestine ²	24.7±0.91	24.6±0.89	23.5±0.95	22.7±0.77
Caeca	1.48±0.05	1.63±0.07	1.59±0.08	1.42±0.07
Relative digesta contents (g/kg BW)^{5,6}				
Proventriculus	0.98±0.16	0.92±0.11	0.74±0.21	1.06±0.20
Gizzard	3.50±0.39	5.17±0.39	2.50±0.73	8.15±1.94
Small intestine ²	47.4±3.01	41.9±2.03	42.4±1.78	38.6±1.66
Caeca	2.17±0.27	2.88±0.42	1.95±0.50	1.52±0.30
Relative length (cm/kg BW)^{3,4}				
Duodenum	22.4±0.46	23.8±0.66	25.4±0.53	23.3±0.56
Jejunum	61.7±1.27	59.8±2.00	69.2±2.04	60.2±1.96
Ileum	60.5±1.61	60.7±2.38	67.0±1.90	62.9±1.96
Small intestine ²	144±2.91	144±4.65	161±4.15	146±4.10
Caeca	13.9±0.48	14.1±0.37	15.1±0.67	13.9±0.48

¹ Each value represents the mean± SE of 12 birds.

² Small intestine = duodenum + jejunum + ileum.

³ Grain type effect ($P < 0.05$).

⁴ Grain type x particle size ($P < 0.05$).

⁵ Grain type x gut component ($P < 0.05$).

⁶ Particle size x gut component ($P < 0.05$).

⁷ Grain type x particle size x gut component ($P < 0.05$).

There were no significant main effects of particle size and grain type on the relative digesta contents of different gut components. An interaction ($P < 0.05$) was noted between individual gut components and grain type due to the greater digesta content of the gizzard in birds fed maize-based diet. There was an interaction ($P < 0.05$) between individual gut components and particle size. This was due to the greater digesta content of the gizzard in birds fed coarse particle size diets. There was also a tendency ($P = 0.09$) for three-way interaction between particle size, grain type and digesta weight of the various gut segments due to birds fed coarse particle size having a relatively greater digesta content of the gizzard in birds fed maize-based diet but this effect was not observed in birds fed wheat-based diets.

An interaction ($P < 0.05$) between particle size and grain type was noted for the relative length of gut components. All gut components were shorter in birds fed coarse particle sizes compared with those fed fine particle sizes in maize-based diets but there was no difference in gut length between different particle sized wheat-based diets. The relative length of all gut components was generally shorter ($P < 0.05$) in birds fed wheat-based diets compared to those fed maize-based diets.

Total extent of the mucosal layer, villus height, crypt depth and epithelial cell thickness in the duodenum were not influenced ($P > 0.05$) by grain type and particle size (Table 5.5). However, there was a tendency ($P = 0.06$) for birds fed maize-based diets to have deeper crypts.

Table 5.5. Influence of particle size and grain type on villus height-crypt base, villus height (μm), crypt depth (μm), and epithelial thickness (μm) of the duodenum of broilers¹

Grain type	Particle size	Extent of the mucosal layer	Villus height	Crypt depth	Epithelial thickness
Wheat	Fine	1230	1128	109	20
	Coarse	1084	984	99	20
Maize	Fine	1184	1072	112	21
	Coarse	1229	1103	126	21
SEM ²		79	73	7	0.93
Main effects					
Grain type					
Wheat		1157	1056	104	20
Maize		1207	1087	119	21
Particle size					
Fine		1207	1100	111	21
Coarse		1157	1044	113	21
Probabilities, P \leq					
Grain type		NS	NS	.06	NS
Particle size		NS	NS	NS	NS
Grain type x Particle size		NS	NS	NS	NS

NS, not significant.

¹ Each value represents the mean of 12 replicates.

² Pooled standard error of mean.

Discriminant analysis failed to show any distinction between particle size distributions of the duodenal digesta of birds fed fine and coarse particle sized wheat- and maize-based diets (Table 5.6). Similarly, the discriminant analysis failed to show distinction between particle size distributions of the colonic digesta of birds fed fine and coarse particle sized wheat- and maize-based diets (Table 5.6).

Table 5.6. Influence of particle size and grain type on the proportion of particle size classes in the duodenal and colonic digesta (on a dry weight basis) of broilers¹

	Wheat		Maize	
	Fine	Coarse	Fine	Coarse
Duodenal digesta²				
<0.075mm	0.535±0.058	0.463±0.040	0.334±0.086	0.397±0.090
0.075-0.106mm	0.056±0.010	0.077±0.013	0.100±0.025	0.119±0.023
0.106-0.25mm	0.167±0.021	0.184±0.031	0.251±0.035	0.202±0.051
0.25-0.5mm	0.134±0.025	0.151±0.026	0.210±0.040	0.214±0.042
0.5-1 mm	0.094±0.048	0.067±0.014	0.089±0.015	0.078±0.014
1-2 mm	0.013±0.002	0.046±0.010	0.016±0.006	0.034±0.009
Colonic digesta²				
<0.075mm	0.174±0.031	0.146±0.022	0.082±0.006	0.160±0.058
0.075-0.106mm	0.048±0.005	0.048±0.010	0.076±0.031	0.037±0.010
0.106-0.25mm	0.124±0.008	0.115±0.010	0.147±0.012	0.136±0.012
0.25-0.5mm	0.266±0.014	0.238±0.006	0.345±0.025	0.287±0.016
0.5-1 mm	0.294±0.018	0.267±0.009	0.279±0.021	0.292±0.046
1-2 mm	0.095±0.014	0.154±0.020	0.071±0.009	0.102±0.018

¹ Each value represents the mean ± SE of six replicates.

² The discriminant analysis failed to show distinction ($P > 0.05$) between particle size distribution of both the duodenal and colonic digesta from birds fed diets with different particle sizes within grain type.

5.6. Discussion

Particle size determination of ground grains showed differences in particle size distribution between wheat and maize passed through the same screen size in the same hammer mill. Grinding wheat resulted in coarser particles compared to maize. Nir *et al.* (1995) similarly showed that grinding wheat with the same mill under the same conditions resulted in coarser particles compared to sorghum. These results may be due to differences in endosperm hardness between the grain types, since the particle size of milled grains is effected by grain hardness (Dobraszczyk *et al.*, 2002).

Wet sieving of the pelleted diets indicated differences in particle size distribution between diets after pelleting, especially in the proportion of coarse particles over 1mm. The coarse wheat diet consisted of 46.8% of particles over 1mm compared to 26.6% in the fine wheat diet. Similarly, the coarse maize diet had 33.7% of particles over 1mm compared to 19.2% in the fine maize diet. Similar findings have been reported by Peron *et al.* (2005). In contrast, data reported in Chapter 4 showed that pelleting evened out the differences in particle size distribution of diets consisting of medium or coarse ground wheat. The inconsistency between research results may be

due to the differences in grain type and/or cultivars used which may affect the distribution of feed particles after pelleting.

Particle size influenced both feed intake and feed per gain, with coarse grinding reducing feed intake and improving feed per gain in both wheat- and maize-based diets. However, previous research using maize-based diets have shown that birds fed medium ground maize in pelleted form performed better than those fed coarse or very coarse particles (Lott *et al.*, 1992; Kilburn and Edwards, 2001). Lott *et al.* (1992) found that maize ground through a hammer mill screen opening of 9.59 mm, which resulted in coarse particles (GMD, 1.20 mm), significantly reduced 21-day body weights and increased feed per gain compared with maize ground through a screen opening of 3.18 mm (GMD, 0.68 mm). Kilburn and Edwards (2001) reported similar improvements in performance and true metabolisable energy when the diet included medium ground maize (GMD, 0.87 mm) compared to that made from very coarse maize (GMD, 2.90 mm). In contrast, Reece *et al.* (1985; 1986 a,b) found that the effect of maize particle size (GMD, 0.68 vs. 1.29 mm) was not evident in crumbled or pelleted diets. However, the results of the current study, suggest that fine maize particles (GMD, 0.29 mm) should be avoided to optimise broiler performance in pelleted diets.

With regard to pelleted wheat-based diets, previous research findings on the effect of particle size on broiler performance are conflicting. Peron *et al.* (2005) reported a tendency for lower feed per gain in birds given coarse wheat (GMD, 0.96 mm) versus those fed fine ground wheat (GMD, 0.39 mm) and this enhancement was associated with a greater relative gizzard weight. Similarly, when using three wheat cultivars which resulted in different particle size distributions, Lentle *et al.* (2006) found that the diet with the highest relative proportion of coarser particles resulted in the lowest feed per gain in broiler chickens. Both Peron *et al.* (2005) and Lentle *et al.* (2006) found that the differences in particle size distribution between treatments still existed even post-pelleting. On the other hand, Engberg *et al.* (2002) and Svihus *et al.* (2004a) reported that pelleting evened out the differences in particle size distribution in pelleted diets which resulted in a lack of a wheat particle size effect on broiler performance in their studies. It can be suggested, therefore, that the effect of wheat particle size on broiler performance in pelleted diets may depend on particle size distribution post-pelleting, with coarse particles remaining post-pelleting having beneficial effects.

Three different explanations for the improvement in feed efficiency in birds fed diets with coarse particles have been suggested (Lentle *et al.*, 2006). Firstly, that a greater proportion of coarse particulate matter resulted in a longer residence time within the gizzard and small intestine leading to more complete digestion and thus better feed efficiency. Secondly, that a greater proportion of coarse particulate matter might stimulate greater gizzard activity leading to more efficient grinding with production of greater quantities of finer particles that are more readily digested. Thirdly, the converse of the second, that with greater requirement for gizzard action to reduce particle size, efficiency is reduced, a greater proportion of coarser material enters the small intestine and that this coarser particulate material in some way stimulates digestive efficiency and is itself digested more readily. However, the results of the current study do not support the third possibility as discriminant analysis failed to show any distinction between particle size distribution in the duodenal digesta of birds fed fine and coarse particles of both wheat and maize based diets. The improvement in feed per gain found in the birds fed coarse particle diets in the current study may be explained, in part, by the larger relative gizzard and digesta weights observed in these birds. The higher digesta content observed in the gizzard of the birds fed coarse particles may have stimulated the gizzard to increase its weight. Several beneficial effects of a larger gizzard have been suggested. A well-developed gizzard has been reported to improve gut motility (Ferket, 2000) through increased levels of cholecystokinin release (Svihus *et al.*, 2004b), which in turn stimulates the secretion of pancreatic enzymes and the gastro-duodenal refluxes (Duke, 1992; Li and Owyang, 1993). Coarse particles may slow the passage rate of digesta through the gizzard (Nir *et al.*, 1994) increasing the exposure time of nutrients to digestive enzymes, which in turn, may improve energy utilisation and nutrient digestibility (Carre, 2000). Furthermore, it has been reported that a lower pH of gizzard contents may increase pepsin activity (Gabriel *et al.*, 2003) and improve protein digestion.

Fine particles increased the relative length of all gut components in birds fed maize-based diet but not in birds fed wheat-based diets. This may be an adaptive mechanism to increase the absorptive surface area in order to optimise nutrient utilisation due to the lower digestion rate associated with fine grinding of maize (Weurding *et al.*, 2001).

In the present study, birds fed maize-based diets had a lower feed per gain compared to those fed wheat-based diets. These results may be explained by the lower

AMEn observed in wheat-based diet. Wheat particle size had no effect on AMEn, which is in agreement with the findings of Lentle *et al.* (2006).

In the current study, there was an interaction between grain type and the weights of the various gut segments due to birds fed maize-based diets having a relatively heavier gizzard than those fed wheat-based diets. Nir *et al.* (1994b) also found that the relative weights of gizzard and its contents were greater in birds fed maize-based diets compared to those fed wheat-based diet. The reasons for these findings are unclear.

The lack of difference in digesta particle size distribution between treatments in the birds suggests that the grinding activity of gizzard evened out the differences in particle size distribution of the diets. Similar results also observed in Chapter 4, and suggest that the gizzard is efficient in grinding large particles.

In general, it is believed that there is an inverse relationship between particle size and pellet durability (Angulo *et al.*, 1996), which is based on the fact that smaller particles have more contact points with each other because of their larger surface area per unit volume ratio (Behnke, 2001). This is in agreement with the findings of the current study where pellets made from fine particles gave better durability compared to those made from coarsely ground grains. However, the lower PDI of the coarse diets did not adversely influence feed per gain of broilers, which may be explained by the good pellet durability in all diets, PDI of over 90%. These results are consistent with those of Svihus *et al.* (2004a) who reported a tendency for better pellet durability in diets made from fine particles. This finding was attributed to higher starch gelatinisation in fine ground wheat. Reece *et al.* (1986b) in contrast, found that pellets made from coarse particles gave significantly more durable pellets than those made from fine particles. In contrast, the data reported in Chapter 4 showed the PDI values were not influenced by wheat particle size.

5.7. Conclusions

Under the conditions of the current study, grinding wheat and maize under the same milling conditions resulted in different particle size distributions. Coarse grinding lowered feed per gain when compared to fine grinding regardless of the grain type. This improvement in feed efficiency with coarse ground diets was concomitant with higher relative gizzard weights. The gizzard evened out dietary particle size differences which resulted in a similar particle size distribution in the duodenal digesta of the birds fed fine and coarse particles. Birds fed maize-based diets had a relatively heavier gizzard

than those fed wheat-based diets. Total extent of the mucosal layer, villus height, crypt depth and epithelial thickness of the duodenum were not influenced by either grain type or particle size. Pellets made from fine particle sizes were more durable than those made from coarse ground grains, however, good pellet durability was determined for all diets. The results of the present study suggest that coarse grinding is advantageous in terms of broiler performance.

CHAPTER 6

Influence of particle size and xylanase supplementation on the performance, energy utilisation and, gross morphology and histology of the digestive tract of broilers fed wheat-based diets

6.1. Abstract

The aim of the present experiment was to examine the influence of particle size and enzyme supplementation on the performance, energy utilisation and, gross morphological and histological parameters of the digestive tract of broilers fed wheat-based diets. The experimental design was a 2 x 2 factorial arrangement of treatments evaluating two wheat particle sizes (medium and coarse) and two levels of xylanase supplementation (without or with). The two particle sizes were achieved by grinding the whole wheat in a hammer mill to pass through 3 and 7 mm screens. Broiler starter diets, based on wheat and soybean meal, were formulated and each diet was fed *ad libitum* to six cages of eight male broilers each from day 1 to 21 posthatch.

Wheat particle size had no effect ($P > 0.05$) on any of the measured performance parameters. Xylanase supplementation had no effect ($P > 0.05$) on weight gain and feed intake. However, a significant ($P < 0.05$) particle size x xylanase interaction was observed for feed per gain with xylanase lowering feed per gain only in the coarse particle size diet. Xylanase supplementation improved ($P < 0.05$) the AME at both particle sizes.

Xylanase supplementation decreased ($P < 0.05$) the weight and length of all gut components. Villus height and crypt depth and total extent of the mucosal layer were unaffected ($P > 0.05$) by both wheat particle size and xylanase supplementation. Viscosity of the digesta in the proximal small intestine was not also influenced ($P > 0.05$) by xylanase addition or particle size.

In conclusion, the findings of this study suggest that the efficacy of exogenous xylanase is influenced by wheat particle size and the effectiveness of exogenous xylanase could be improved by considering particle size.

6.2. Introduction

The variability in the AME of wheat for broiler chickens is generally attributed to the soluble NSP levels (Annison and Choct, 1991). Two key mechanisms have been proposed for the anti-nutritional effects of soluble NSP (Bedford and Schulze, 1998). The first mechanism is based on the fact that starch and protein are encapsulated by the cell wall in wheat endosperm cells. The second mechanism is related to higher digesta viscosity caused by the soluble NSPs which reduces the diffusion of digestive enzymes and consequently lowers the utilisation of nutrients and energy. Although most published research are in favour of the latter mechanism, it is reasonable to suggest that both mechanisms are likely to be involved (Bedford, 2006; Cowieson *et al.*, 2006). The negative effects of soluble NSPs may be ameliorated by supplementation with exogenous xylanases that degrade NSPs and improve nutrient utilisation. The proposed mechanisms by which enzymes improve nutrient utilisation of wheat based diets include degradation of NSP in the cell wall matrix and the release of encapsulated nutrients, lowered digesta viscosity in the intestinal tract, increased accessibility of nutrients to endogenous digestive enzymes, stimulation of intestinal motility and improved feed passage rate (Bedford and Schulze, 1998).

Acamovic (2001) suggested that the physical form of the diet (mash, pellet or whole grain) may affect the efficacy of exogenous enzymes in poultry diets. However, there has been no previous study examining the interaction between enzyme supplementation with feed particle size in poultry. In pigs, Kim *et al.* (2005) found no effect of wheat particle size, enzyme supplementation or any interaction of particle size and enzyme on growth performance. It was, however, observed that enzyme supplementation increased digestible energy to a greater extent in diets with larger particle size than those with finer particle size.

The present experiment investigated the effects of particle size and xylanase supplementation on the performance, AME, digesta viscosity and intestinal morphology of broiler starters fed wheat-based diets.

6.3. Materials and methods

6.3.1. Birds and housing

192 day-old male broilers (Ross 308) were obtained from a commercial hatchery. Housing conditions were as described in Section 3.1.

6.3.2. Diets

Whole wheat was ground in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) to pass through a screen size of 3 or 7 mm for medium and coarse grades, respectively. The GMD and GSD were then determined as described in Section 3.2.

Two broiler starter diets (Table 6.1), each based on one of the milled wheat grades and soybean meal, were formulated to meet or exceed the NRC (1994) recommendations for major nutrients for broiler starters. Half of each formulated diet was supplemented with xylanase (1000 XU/kg diet, Kemzyme, Kemin (Asia) Pte Ltd, Singapore) and the other half was not supplemented with xylanase. Each of the four dietary treatments was fed in mash form *ad libitum* to six cages. Water was freely available throughout the trial.

Table 6.1. Composition and calculated analysis (g/kg as fed) of the basal diet

Ingredient	
Wheat ¹	648
Soybean meal	295
Soybean oil	13.1
Dicalcium phosphate	16.2
Limestone	16.5
L-lysine. HCl	1.9
DL-methionine	3.8
Salt	2.5
Trace mineral-vitamin premix ²	3.0
Xylanase ³	+
Calculated analysis	
Metabolisable energy, MJ/kg	12.4
Crude protein	226
Lysine	11.5
Methionine + cystine	9.4
Calcium	10.9
Available phosphorus	4.5

¹ Two particle sizes were obtained by grinding the whole grain in a hammer mill to pass through 3 and 7 mm screens.

² Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl- α -tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

6.3.3. AME determination

The AME determination was carried out between day 17 to 20 posthatch as described in Section 3.3.

6.3.4. Digestive tract measurements

Two birds were euthanased, and digestive tract measurements and gut morphological examinations were carried out as described in Sections 3.4 and 3.5, respectively.

6.3.5. Rheological measurements

The apparent viscosity of digesta was determined using the method described by Lentle *et al.* (2005) with minor modifications. On day 21, two birds from each cage were euthanased by intravenous injection of sodium pentobarbitone and the intestinal tracts were carefully excised and frozen. The tissues were subsequently thawed and the contents of the proximal intestine (defined as the length of gut from the start of the duodenal loop to Meckel's diverticulum), were allowed to drain into containers. The contents were then frozen for subsequent analysis.

Digesta were thawed and mixed before the variation of apparent viscosity with shear rates over the range of 1.0-100 reciprocal seconds was determined using a stress-controlled Paar-Physica rheometer USD200 (Paar-physica, Stuttgart, Germany) with a plate-plate geometry consisting of a MP31 50-mm diameter probe and a TEK 180 with the plate-plate gap of 2 mm. The range of rotational frequencies was chosen to span the shear rates likely to be experienced by the digesta *in vivo*. All measurements were carried out at a constant temperature of 40 °C maintained by an inbuilt Peltier heating system. In each case, the volume of digesta placed on the lower plate were just sufficient to occupy the space between the two plates at the 2mm plate gap.

6.3.6. Data analysis

The cage means were used to derive performance data. For digestive tract measurements, individual birds were considered as the experimental unit. Data were analysed as described in Section 3.9, where factor 1 was wheat particle size and factor 2 was enzyme supplementation. The relationship between viscosity and physiological shear rate was explored by ANCOVA of log transformed data.

6.4. Results

The GMD of wheat ground through 3 and 7 mm screen sizes were determined to be 0.618 and 0.882 mm, respectively, with corresponding GSD values of 2.63 and 2.42, respectively. Particle size distribution of the two wheat grinds and diets are shown in Figure 6.1.

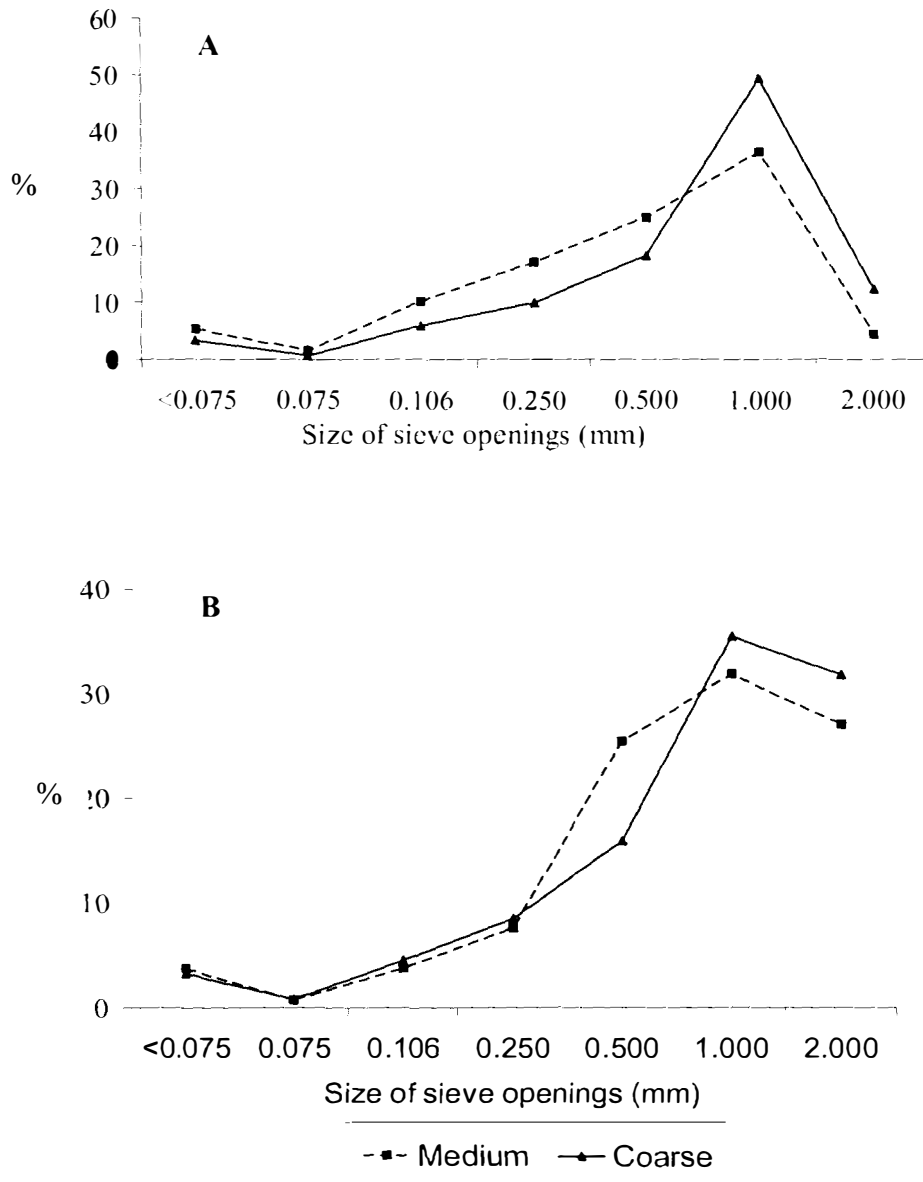


Figure 6.1. Particle size distributions of the two grinds of wheat (A) and diets (B) used in this experiment

The influence of treatments on the performance of broilers is summarised in Table 6.2. The main effects of particle size and xylanase supplementation on weight gain and feed intake were not significant ($P > 0.05$). Feed per gain of birds fed xylanase-supplemented diets was lower ($P < 0.05$) than that of those fed unsupplemented diets. However, there was an interaction ($P < 0.05$) between particle size with enzyme supplementation due to the lowered feed per gain of birds maintained on coarse diet, while there was no effect of enzyme supplementation on medium particle size diet.

Nitrogen-corrected AME was greater ($P < 0.05$) with xylanase supplementation compared with that of unsupplemented diets (Table 6.2). However, there was no effect ($P > 0.05$) of particle size on AMEn or significant interaction of particle size and xylanase supplementation.

Table 6.2. Influence of particle size and xylanase supplementation on the weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) and AMEn (MJ/kg dry matter) of broilers (1-21 days posthatch)¹

Particle size	Xylanase	Weight gain	Feed intake	Feed per gain	AMEn
Medium	-	815	1103	1.375 ^{ac}	12.52
	+	806	1063	1.365 ^{bc}	12.72
Coarse	-	772	1065	1.396 ^a	12.56
	+	813	1093	1.344 ^b	13.26
SEM ²		13.0	20.0	0.009	0.18
Main effects					
Particle size					
Medium		810	1102	1.370	12.62
Coarse		786	1079	1.370	12.91
Xylanase					
-		786	1093	1.386	12.54 ^b
+		810	1088	1.354	12.99 ^a
Probabilities, P <					
Particle size		NS	NS	NS	NS
Xylanase		NS	NS	***	*
Particle size x Xylanase		NS	NS	*	NS

NS, not significant; *, $P < 0.05$; ***, $P < 0.0001$.

^{a,b,c} Means in a column not sharing a common superscript are significantly different ($P < 0.05$).

¹ Each value represents the mean of six replicates (8 birds per replicate).

² Pooled standard error of mean.

The relationship between viscosity and physiological shear rate (Figure 6.2) showed that apparent viscosity of whole digesta at physiological shear rates in the four treatment groups showed no significant effect ($P > 0.05$) of xylanase supplementation or particle size.

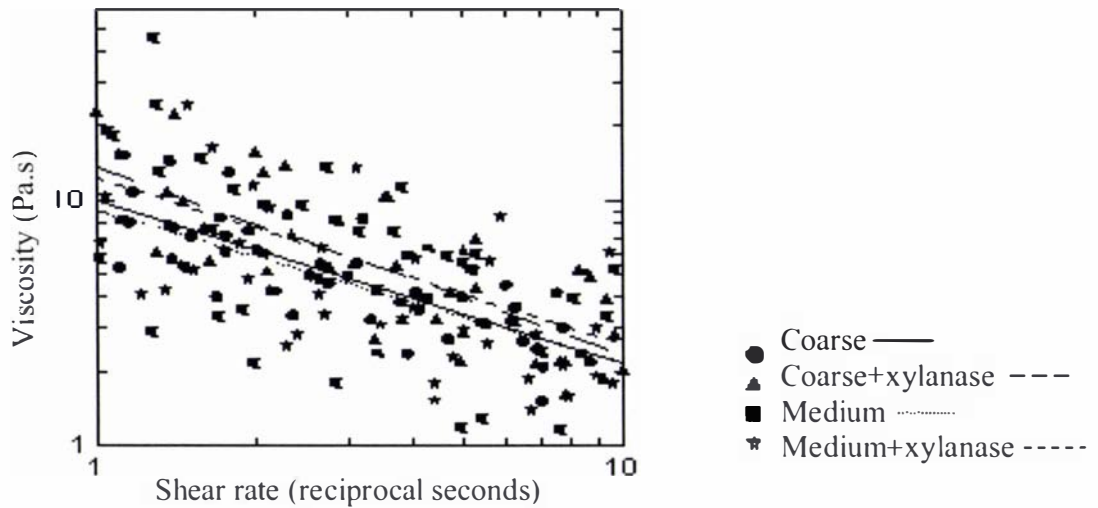


Figure 6.2. Flow curves of digesta of broilers fed coarse ground wheat, coarse ground wheat supplemented with xylanase, medium ground wheat and medium ground wheat supplemented with xylanase

The morphometry of the duodenum and jejunum are shown in Table 6.4. Wheat particle size and enzyme supplementation had no effect ($P > 0.05$) on the total extent of the mucosal layer, villus height and crypt depth in the intestinal segments.

Table 6.4. Influence particle size and xylanase supplementation on total extent of the mucosal layer (μm), villus height (μm) and crypt depth (μm) of the duodenum and jejunum of broilers¹

Treatment	Medium		Coarse		SEM ²	Xylanase	PS	Xylanase x PS
	-	+	-	+				
Duodenum								
Extent of the mucosal layer	1759	1778	1775	1708	54	NS	NS	NS
Villus height	1588	1615	1608	1558	54	NS	NS	NS
Crypt depth	172	166	166	152	9.4	NS	NS	NS
Jejunum								
Extent of the mucosal layer	879	860	874	851	38	NS	NS	NS
Villus height	753	732	756	744	36	NS	NS	NS
Crypt depth	126	128	118	107	8.2	NS	0.09	NS

NS, not significant.

¹ Each value represents the mean of 12 birds.

² Pooled standard error of mean.

PS= Particle size.

6.5. Discussion

In the present study, wheat particle size had no influence on the measured performance parameters. Based on the determined GMD of wheat grinds, differences in bird performance were anticipated. However, the differences in particle size distribution between medium and coarse diets were found to be not large enough to cause any differences in performance parameters. It is noteworthy that previous studies, comparing medium and coarse grinding, have also reported similar results in mash diets (Nir *et al.*, 1990; 1994).

Digesta viscosity in chickens is usually measured using a supernatant of the intestinal content. Solid particles are known to be responsible for digesta rheological characteristics and contribute to the viscoelastic property of gut contents (Takahashi and Sakata, 2002) and it is not feasible to speculate on the diffusion rate across the gut or on the mobility of gut contents, based on the apparent viscosity of the supernatant fraction alone (Takahashi *et al.*, 2004). Only one study has reported the measurement of intestinal viscosity in poultry without the removal of solid digesta particles (Takahashi *et al.*, 2004). Thus, in the current study, the apparent viscosity of whole digesta was determined over the physiological range of shear rates. It was observed that the digesta behaved in a non-Newtonian manner which means that digesta viscosity varied with different shear rates. However, dietary treatments were found to have no effect on digesta viscosity and this finding was due, in part, to the excessive variation in rheological profiles between individual birds. High individual bird variability in digesta viscosity has been previously reported (Choct *et al.*, 2006) and intestinal viscosity is known to be subjected to diurnal rhythms, individual bird water intake patterns and pancreatic secretion output at slaughter time (Bedford and Schulze, 1998). It is also noteworthy that digesta viscosity is not the only limiting factor determining the bird performance when fed wheat-based diets, especially if the digesta viscosity is less than 10 mPa.s (Bedford and Schulze, 1998).

In the present study, enzyme supplementation had no effect on digesta viscosity, but still improved the AME in both particle size diets and the feed per gain of birds fed the coarse diets. The finding that the apparent viscosity of whole digesta in the physiological range of shear rates was unaffected by added xylanase does not support the popular mechanism based on enzymatic thinning. The second proposed mechanism in the literature, which is based on the physical barrier of endosperm cell wall and nutrient encapsulation, also does not provide an explanation as enzyme supplementation

improved the feed efficiency of birds fed coarse diets and no effect was observed with medium ground diets. Based on the result reported in this thesis a third mechanism may be suggested. It is possible that enzyme supplementation acts, in part, on gel barriers formed by partial solubilisation of NSPs on digesta particles. The smaller effect of enzymatic supplementation in improving digestive efficiency seen in medium particulate feeds may be related to a higher surface area available for gel formation which causes increased *in situ* gel formation and lowers the effectiveness of enzyme. It has been previously suggested that enzyme does not directly physically disrupt the cell walls, but rather, peels away a layer (Bedford and Schulze, 1998). Alternatively, the presence of larger particles may increase the digestive efficiency of enzyme by increasing the permeability of digesta (Lentle, 2005) by increasing the void spaces between adjacent particles (Dullen, 1979). However, in Chapter 4, the gizzard evened out differences in particle size distribution between birds fed medium and coarse particle size in mash diet. Kim (2003) suggested that the cell walls of coarsely ground wheat are less disrupted, thus the efficacy of cell wall degrading enzymes are greater than in more finely ground wheat. Further studies are warranted to explain the improved xylanase efficiency with coarse grinding. The results reported here, however, broadly concur with that of previous studies in broiler chickens, which found that the substitution of whole grain wheat for ground wheat improved feed efficiency and that this increase could be further augmented by supplementation with xylanase (Wu and Ravindran, 2004). Therefore, in broiler starter diets, enzyme efficacy appears to be affected by wheat particle size. There are no previous studies examining the interaction of wheat particle size and enzyme supplementation.

In the present study, wheat particle size and enzyme supplementation had no effect on villus height, crypt depth and the total extent of the mucosal layer. Similar results have been previously reported (Iji *et al.*, 2001). In contrast, enzyme supplementation has been shown to increase mean villus height in turkeys (Ritz *et al.*, 1995) and broilers (Mathlouthi *et al.*, 2002). The lack of an enzyme supplementation effect on histological measurements in this study may be explained by the similar lack of an enzyme effect on digesta viscosity. Digesta viscosity may cause the morphological changes indirectly via its effect on microbial population (Smits and Annison, 1996).

Birds fed diets supplemented with xylanase had shorter lighter gut components than those fed unsupplemented diets. The results are similar to previous studies insofar as inclusion of exogenous enzymes brought about a relative reduction in the overall

length and weight of the intestine (Brenes *et al.*, 1993; Wang *et al.*, 2005). Similarly, Yasar and Forbes (2000) found that enzyme supplementation reduced the thickness of gizzard glands and of the tissues lining the gizzard. The metabolic cost of gut mucosal maintenance can have remarkable impact on the nutrient partitioning for the growth of the whole animal (Cant *et al.*, 1996). Thus the findings of the current study lend support to the hypothesis that the deleterious effect of wheat NSPs can be partially explained by the higher metabolic cost of larger gut components impacting on overall feed efficiency. Wang *et al.* (2005) speculated that the reduction in the weight and length of gut components following xylanase supplementation in wheat-fed broilers was due to reversal of the viscoelastic effects of wheat arabinoxylans resulting in a reduction in mixing requirement and in requirements for the secretion of endogenous enzymes. Hence xylanase supplementation of the wheat-based diet may have eased the secretory function of the responding organs and intestinal segments, resulting in reduction in the weight and length of gut components (Brenes *et al.*, 1993; Wang *et al.*, 2005).

6.6. Conclusions

The hypothesis that the efficiency of exogenous xylanase will be greater in coarse particle-sized diets than in medium particle-sized diets was tested in the present study. Particle size and xylanase supplementation had no effect on weight gain and feed intake of the broilers, but the interaction between particle size and xylanase the feed efficiency supports the original hypothesis. Xylanase responses in feed efficiency were observed only in coarse particle-sized wheat diets. Overall, enzyme supplementation improved broiler performance measured by feed per gain but that, this effect was found only in coarse based diet. The findings of this study suggest that the efficacy of supplemental xylanase is influenced by the particle size of wheat and that the effectiveness of the enzyme could be improved by considering particle size in diet formulations.

CHAPTER 7

Influence of wheat hardness and xylanase supplementation on the performance, energy utilisation, digestive tract development and digesta parameters of broilers fed wheat-based diets

7.1. Abstract

The aim of this study was to examine the influence of wheat hardness and xylanase supplementation on the performance, energy utilisation, digestive tract development and digesta parameters of broilers fed wheat-based diets. The experimental design was a 2 x 2 factorial arrangement of treatments evaluating two wheat hardness (soft and hard) and two levels of xylanase supplementation (without or with). Two diets based on soft or hard wheat, were formulated. Whole wheat 200 g/kg from the same wheat batch replaced ground wheat prior to pelleting in each diet. Each diet was fed *ad libitum* to six cages of eight male broilers each from day 1 to 21 posthatch.

The distribution of particle size differed between diets, with the hard wheat diet having 32.7% of particles greater than 1mm in size compared to 18.7% in the soft wheat diet. Interactions ($P < 0.05$) were observed between wheat hardness and enzyme supplementation for weight gain, feed intake, feed per gain and AMEn. Enzyme supplementation increased ($P < 0.05$) weight gain in the soft wheat-based diet but not in the hard wheat diet. Enzyme supplementation also increased ($P < 0.05$) feed intake in the soft wheat-based diet, but reduced ($P < 0.05$) intake in the hard wheat diet. Feed per gain and AMEn were improved ($P < 0.05$) by enzyme supplementation in the hard wheat-based diet, while no enzyme effect was observed in the soft wheat diet.

Feeding the hard wheat-based diet increased ($P < 0.05$) the relative gizzard weight, and this was associated with a general increase in finer particles in the proximal intestinal digesta when compared to those present in the diet. These results suggest that wheat hardness may be an important criterion when selecting whole wheat for inclusion prior to pelleting in broiler diets and that the response of broilers to xylanase supplementation is influenced by wheat hardness.

7.2. Introduction

The relative hardness or softness of a grain is defined as the relative resistance of the grain to deformation or crushing when an external force is applied (Turnbull and Rahman, 2002). Hardness is one of the important characteristics of a wheat grain that determines the end use of the wheat flour. A hard endosperm gives larger particles with more irregular shape (Rose *et al.*, 2001) which are easy to handle (GreVeuille *et al.*, 2006). Increased hardness is also known to increase the milling energy required to grind the wheat (GreVeuille *et al.*, 2006).

In recent years, there has been a renewed interest in whole grain feeding, which is driven by the need to reduce feed costs while improving bird health and welfare. Feed cost savings can be achieved by combining whole wheat with pellet-processed feed in the broiler diet, due to reduced handling and processing. Most published studies have examined the effects of whole wheat added post-pelleting (See Section 2.1.9), with studies examining the effects of whole wheat inclusion pre-pelleting limited and producing inconsistent results (Svihus *et al.*, 2004b; Taylor and Jones, 2004; Wu *et al.*, 2004). Although wheat hardness may be an important factor contributing to the variable responses achieved with whole wheat feeding, this aspect has attracted little attention. It could be expected that harder wheat would require greater grinding activity, which may increase gizzard size. A well developed gizzard is known to have beneficial effects on bird performance and gut health (See Section 2.1.10).

The beneficial effects of including whole wheat were shown to be further enhanced with supplemental xylanase (Wu and Ravindran, 2004). Furthermore, in the previous study (Chapter 6), enzyme supplementation improved feed per gain only in birds fed the coarse particle size diet. Thus it may be hypothesised that hard wheat can have beneficial effects on gizzard development and bird performance, and these benefits can be further improved with xylanase supplementation.

The aim of the current experiment was to test the interaction between wheat hardness and enzyme supplementation on the performance, energy utilisation, digestive tract parameters and digesta parameters of broiler starters when 200 g/kg whole wheat replaced ground wheat pre-pelleting.

7.3. Materials and methods

7.3.1. Wheat samples

Two wheat cultivars known to differ in endosperm hardness (Clare soft and Monad hard) were obtained from a commercial source (Goodman Fielder, Mount Maunganui, New Zealand). Upon receipt at the research unit, the hardness of the wheat samples were measured as described below. The N content of the wheat samples was determined as described in Section 3.3.3. The crude protein content of the samples was calculated by multiplying the N value by 5.83 and iso-nitrogenous diets were formulated. Analysed crude protein content of the hard wheat was found to be 139 g/kg and for the soft wheat 108 g/kg.

7.3.2. Measurement of wheat hardness

Wheat hardness was determined using the particle size index (PSI) method with minor modification (AACC, 1995, method 55-30). AACC Method No. 55-30 was modified by using a laboratory mill (Retsch ZM 100, Retsch GmbH, Haan, Germany) fitted with 1 mm screen size (Canadian Grain Commission, 2004) instead of using a burr mill. Relative hardness was determined by grinding a 22 g sample of wheat, and a 10 g sample of the ground meal was sieved through a 75µm sieve. The % PSI was determined using the following formula,

$$\text{PSI \%} = (\text{Weight of throughs} / \text{Sample weight}) \times 100.$$

Grains were found to be extra hard (PSI % = 29) for the hard wheat cultivar and medium soft (PSI % = 63) for the soft wheat cultivar (Canadian Grain Commission, 2004).

7.3.3. Animals and housing

192 day-old male broilers (Ross 308) were obtained from a commercial hatchery. Housing conditions were as described in Section 3.1.

7.3.4. Diets

The experimental design was a 2 × 2 factorial with two wheat cultivars of differing hardness without or with xylanase supplementation. The two wheats were ground in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) to pass through 3

mm sieve. The GMD and GSD were then determined as described in Section 3.2. Two broiler starter diets (Table 7.1), each based on one of the two wheats and soybean meal, were formulated to meet or exceed the NRC (1994) recommendations for major nutrients for broiler starters. Whole wheat (200 g/kg) from the same wheat batch replaced the ground wheat before pelleting in each diet. Half of each formulated diet was supplemented with xylanase (1000 XU/kg diet, Kemzyme, Kemin (Asia) Pte Ltd, Singapore) and the other half was unsupplemented. All diets were cold pelleted (70 °C) using a 3 mm die. Each of the four dietary treatments was fed *ad libitum* to six cages of eight birds each. Water was freely available throughout the trial.

Table 7.1. The composition and calculated analysis (g/kg as fed) of the basal diet¹

Ingredient	Hard wheat	Soft wheat
Hard wheat	664.3	-
Soft wheat	-	609.2
Soybean meal	266	303
Soybean oil	27.3	18.2
Dicalcium phosphate	18.2	18.2
Limestone	8.5	8.0
Salt	1.4	2.3
L-lysine. HCl	3.6	3.6
DL-methionine	3.8	1.6
L-threonine	1.9	1.2
Sodium bicarbonate	3.6	3.6
Trace mineral-vitamin premix ²	3.0	3.0
Calculated analysis		
Metabolisable energy, MJ/kg	12.7	12.6
Crude protein	220	220
Lysine	12.6	12.8
Methionine + cystine	9.6	9.7
Calcium	9.9	10
Available phosphorus	4.8	4.8

¹ Adjustment was made to account for the differences in protein and amino acid concentrations of the wheat samples. Amino acid concentrations were calculated from protein contents using equations provided by Degussa (Degussa AMINODat™ 2.0). Apparent metabolisable energy was considered to be similar.

²Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl- α -tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

7.3.5. AME determination

The AME determination was carried out between day 17 to 20 posthatch as described in Section 3.3.

7.3.6. Digestive tract measurements

Two birds per replicate cage were euthanased, and digestive tract measurements were carried out on day 21 as described in Section 3.4.

7.3.7. Determination of particle size distribution in the diets and proximal small intestine digesta

Two more birds per cage were euthanased by intravenous injection of sodium pentobarbitone on day 21. The particle size distributions of the proximal intestinal digesta (from the proximal of duodenal loop to Meckels diverticulum) and the diets were determined as described in Section 3.6.

7.3.8. Data analysis

The cage means were used to derive performance data. For digestive tract measurements, individual birds were considered as the experimental unit. Data were analysed as described in Section 3.9, where factor 1 was wheat hardness and factor 2 was enzyme supplementation.

7.4. Results

The GMD of the hard wheat was determined to be 0.637 with a corresponding GSD value of 2.77. The GMD of the soft wheat was determined to be 0.560 with a corresponding GSD value of 2.68.

Comparisons of the particle size distribution of the ground grains and pelleted diets, determined by wet sieving, are shown in Figures 7.1 and 7.2, respectively. The particle size distribution of the ground wheats and the pelleted diets indicated a higher proportion of coarse particles (1 mm and over) associated with the hard wheat.

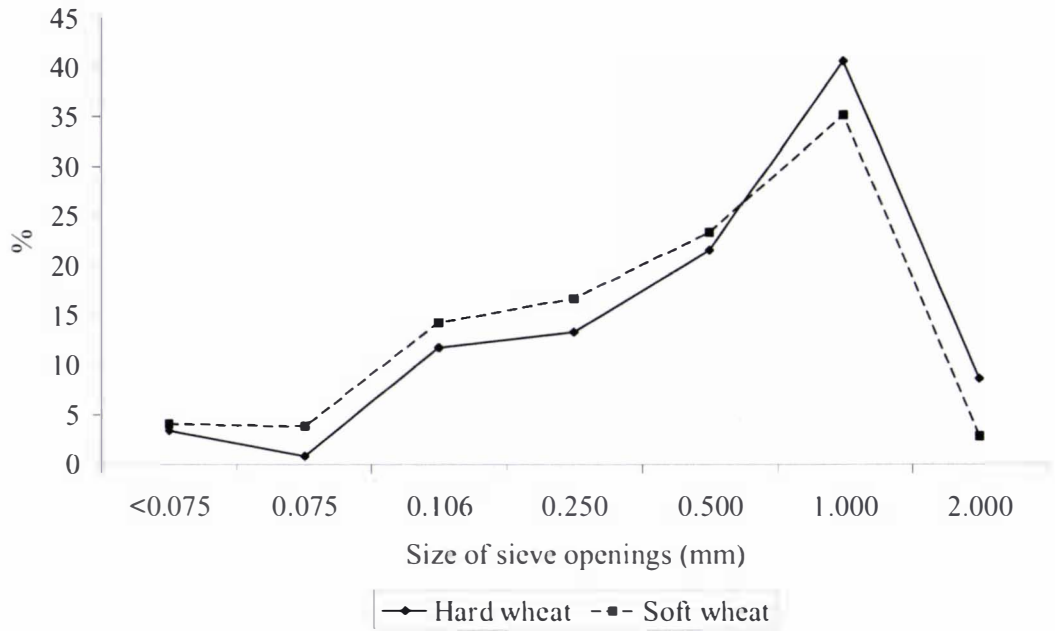


Figure 7.1. Particle size distribution of ground soft and hard wheats

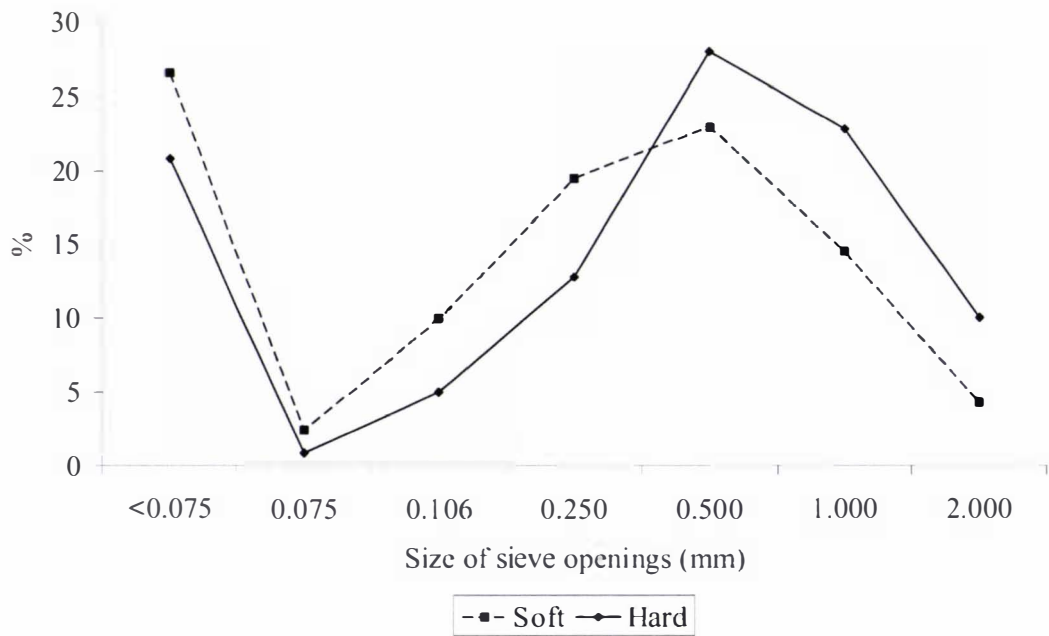


Figure 7.2. Particle size distribution of pelleted diets

The influence of the treatments on the performance of broilers is summarised in Table 7.2. Significant ($P < 0.05$) interactions between wheat hardness and xylanase supplementation were observed for weight gain, feed intake and feed per gain. Xylanase supplementation increased ($P < 0.05$) weight gain in the soft wheat diet but not in the

hard wheat diet. Xylanase supplementation also increased ($P < 0.05$) feed intake in the soft wheat diet, but reduced ($P < 0.05$) feed intake in the hard wheat diet. Feed per gain was lowered ($P < 0.05$) by enzyme supplementation in hard wheat diet, while there was no effect in the soft wheat diet.

There was an interaction ($P < 0.05$) between wheat hardness and xylanase supplementation on AMEn. Nitrogen-corrected AME was improved ($P < 0.05$) by enzyme supplementation in the hard wheat diet, while there was no effect in the soft wheat diet.

Table 7.2. Influence of wheat hardness and xylanase supplementation on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) and AMEn (MJ/kg dry matter) for broilers (1-21 days posthatch)¹

Wheat hardness	Enzyme	Weight gain	Feed intake	Feed per gain	AMEn
Soft wheat	-	912 ^b	1261 ^c	1.389 ^a	13.59 ^a
	+	958 ^c	1338 ^a	1.397 ^a	13.45 ^a
Hard wheat	-	991 ^a	1401 ^b	1.414 ^a	13.51 ^a
	+	977 ^a	1319 ^a	1.351 ^b	13.84 ^b
SEM ²		12	16	0.013	0.08
Main effects					
Wheat hardness					
		935	1300	1.395	13.52
		984	1360	1.382	13.67
Xylanase					
		951	1331	1.402	13.55
		967	1329	1.374	13.65
Probabilities, P ≤					
		NS	NS	*	NS
		***	***	NS	0.08
		*	**	*	**

NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

¹ Each value represents the mean of six replicates (8 birds per replicate).

² Pooled standard error of mean.

^{a,b} Means in a column not sharing a common superscript are significantly different ($P < 0.05$).

The effects of wheat hardness and enzyme supplementation on the weights, digesta contents and lengths of various intestinal components are shown in Table 7.3. The relative weights of overall gut components were lower ($P < 0.05$) in birds fed soft wheat than those maintained on hard wheat-based diets. The main effect of enzyme

supplementation was not significant ($P > 0.05$). There was an interaction ($P < 0.05$) between wheat hardness and the weights of the various gut segments due to birds fed the hard wheat having a relatively heavier gizzard ($P < 0.05$). There was also an interaction ($P < 0.05$) between enzyme and the weights of the various gut segments due to birds fed diet supplemented with xylanase having a relatively heavier gizzard.

Table 7.3. Influence of wheat hardness and enzyme supplementation on gross morphology of the digestive tract of broilers¹

	Soft		Hard	
	-	+	-	+
Relative empty organ weights (g/kg BW)^{3,5}				
Proventriculus	4.38±0.31	4.11±0.13	4.48±0.12	4.26±0.10
Gizzard	9.94±0.30	9.85±0.37	11.9±0.66	13.8±0.54
Duodenum	4.55±0.26	4.35±0.18	4.26±0.23	4.35±0.17
Jejunum	8.57±0.26	8.42±0.18	8.65±0.55	9.15±0.30
Ileum	6.45±0.15	6.61±0.25	6.65±0.30	6.37±0.16
Small Intestine ²	19.5±0.57	19.3±0.41	19.5±0.95	19.8±0.50
Caeca	1.43±0.07	1.27±0.06	1.24±0.05	1.32±0.05
Relative digesta contents (g/kg BW)^{5,6}				
Proventriculus	0.81±0.14	0.92±0.09	0.88±0.11	0.66±0.14
Gizzard	3.15±0.28	3.91±0.47	7.20±1.67	10.2±0.87
Small intestine ²	45.8±1.19	42.4±1.10	48.5±2.43	43.7±1.23
Caeca	2.93±0.47	2.33±0.28	2.62±0.29	2.54±0.28
Relative length (cm/kg BW)⁴				
Duodenum	23.7±0.66	22.4±0.60	22.3±0.58	22.1±0.48
Jejunum	63.6±1.62	59.6±1.50	61.4±1.27	60.7±0.96
Ileum	63.3±1.75	59.5±1.49	61.3±1.70	59.7±0.98
Small intestine ²	150.8±3.80	141.5±3.22	145±3.02	143±2.13
Caeca	15.3±0.72	14.0±0.35	13.2±0.45	13.6±0.31

¹ Each value represents the mean± SE of 12 birds.

² Small intestine = duodenum + jejunum + ileum.

³ wheat hardness effect ($P < 0.05$).

⁴ Wheat hardness x enzyme ($P < 0.05$).

⁵ Wheat hardness x gut component ($P < 0.05$).

⁶ Wheat hardness x enzyme x gut component ($P < 0.05$).

There were no significant ($P > 0.05$) main effects of wheat hardness and enzyme supplementation on the relative digesta contents of gut components. The interaction between wheat hardness and individual gut components was significant ($P < 0.05$) due to the greater gizzard digesta content of birds fed the hard wheat-based diet. There was a three-way interaction ($P < 0.05$) between wheat hardness, enzyme supplementation and

the weights of the various gut components due to birds fed hard wheat supplemented with enzyme having a relatively greater digesta content of the gizzard compared to other dietary treatments.

There was a significant interaction ($P < 0.05$) between wheat hardness and enzyme supplementation on the relative lengths of gut components due to birds fed soft wheat supplemented with enzyme having a relatively shorter gut than those fed the unsupplemented diet.

The discriminant analysis of the particle size distribution of digesta from the proximal small intestine showed successful distinction ($P < 0.05$) between different treatments (Table 7.4). Discriminant analysis showed that the birds fed hard wheat had a higher proportion ($P < 0.01$) of particles < 0.075 mm and a lower proportion ($P < 0.05$) of particles 0.5 mm compared to those fed soft wheat. Similarly, when particle size within wheat hardness were combined and soft and hard wheat-based diets were compared (Table 7.5), discriminant analysis of the particle size distribution of the proximal small intestine digesta showed successful distinction ($P < 0.01$) between soft and hard wheat-based diets. The proximal intestinal digesta of the birds fed hard wheat diets had a higher proportion ($P < 0.01$) of particles < 0.075 mm and a lower proportion ($P < 0.05$) of particles 0.5 mm. Conversely, when digesta particle size within diets, without or with supplemented xylanase, were combined and compared (Table 7.5), the discriminant analysis failed ($P > 0.05$) to show distinction between particle size distributions of digesta.

Table 7.4. Influence of wheat hardness and enzyme supplementation on the proportion of particle size classes in the proximal small intestine digesta (on a dry weight basis) of broilers^{1,2}

	Soft		Hard	
	-	+	-	+
<0.075mm	0.187±0.033	0.228±0.026	0.325±0.036	0.280±0.019
0.075-0.106mm	0.028±0.004	0.040±0.003	0.033±0.004	0.039±0.005
0.106-0.25mm	0.171±0.028	0.136±0.019	0.143±0.013	0.148±0.012
0.25-0.5mm	0.239±0.018	0.237±0.022	0.187±0.013	0.225±0.007
0.5-1 mm	0.216±0.016	0.216±0.020	0.165±0.013	0.167±0.007
1-2 mm	0.127±0.013	0.122±0.009	0.108±0.010	0.094±0.006

¹ Each value represents the mean ± SE of six replicates.

² The discriminant analysis of the particle size distribution of digesta showed successful distinction ($P < 0.05$) between treatments.

Table 7.5. Influence of wheat hardness and enzyme supplementation on the proportion of particle size classes in the proximal small intestinal digesta (on a dry weight basis) of broilers¹

	Wheat hardness ²		Enzyme ³	
	Soft	Hard	-	+
<0.075mm	0.208±0.021	0.303±0.020	0.256±0.031	0.254±0.017
0.075-0.106mm	0.034±0.003	0.036±0.003	0.030±0.002	0.040±0.003
0.106-0.25mm	0.153±0.017	0.145±0.009	0.157±0.015	0.142±0.011
0.25-0.5mm	0.238±0.013	0.206±0.010	0.213±0.013	0.231±0.012
0.5-1 mm	0.216±0.012	0.166±0.007	0.190±0.012	0.191±0.012
1-2 mm	0.124±0.007	0.101±0.006	0.117±0.008	0.108±0.006

¹ Each value represents the mean ± SE of 12 samples.

² The discriminant analysis of the particle size distribution of digesta showed successful distinction ($P < 0.05$) between soft and hard diets.

³ The discriminant analysis failed to show distinction ($P > 0.05$) between the particle size distribution of digesta between supplemented and unsupplemented diets.

Since the two wheat cultivars differed in hardness and consequently particle size distribution in the diets, it was important to check whether differences in the relative proportions of proximal small intestinal digesta particles simply reflected differences in the relative proportions of particles ingested or resulted from other changes such as the mechanical activity of a relatively enlarged gizzard. To do this, the proportions of the various particle size classes in the digesta were divided by those present in the diet. Thus, if the relative proportion of a particular size class in digesta was unchanged from that in feed, the ratio will be one. If there was relative enrichment in a particular particle size class in digesta, the ratio will be greater than one and if there was relative reduction in the proportion of a particular particle size class in the digesta the ratio will be less than one. As the parameters are interdependent, changes in the relative proportion of a particle size class could be brought about either by reduction of the amount in that class or by an increase in the relative amount of material in other size classes.

Discriminant analyses (Table 7.6) of changes in the relative proportions of the various particle size classes present in digesta with respect to those present in the diet showed that there was a general increase ($P < 0.05$) in particles of size class 0.5 mm and below with a corresponding decrease in particles greater than 0.5 mm after passage of both feeds through the gizzard. However, there were significant ($P < 0.001$) differences between the birds fed soft and hard wheat diets. The proportions of particles in the size range 0.075-0.106 mm and of fines (less than 0.075 mm) were significantly greater in the birds fed the hard wheat diets.

Table 7.6. Relative differences¹ in of particle size classes between diet and proximal small intestinal digesta of broilers fed soft and hard wheat-based diets

Particle size class	Soft wheat-based diet	Hard wheat-based diet	Discriminant function
<0.075 mm	0.783±0.080	1.458±0.101	1.980
0.075-0.106 mm	1.430±0.135	4.315±0.438	0.548
0.106-0.25 mm	1.541±0.175	2.930±0.193	0.773
0.25-0.5 mm	1.225±0.071	1.618±0.083	1.663
0.5-1 mm	0.940±0.055	0.593±0.027	0.161
1-2 mm	0.857±0.054	0.443±0.030	0.659
>2mm	0.628±0.22	0.433±0.062	-

¹ Ratio of percentage dry weight in feed and digesta: ratios of less than 1.0, indicating a relative diminution of the particle size class following its passage through the gizzard, are shown in bold type.

Jack-knifed classification: Soft wheat diet 100% correct, hard wheat diet 100% correct. Wilkes lambda 0.060 F = 44.10; df, 6, 17; P = 0.0001.

7.5. Discussion

Particle size spectra of the ground wheats showed that there were differences between soft and hard wheat cultivars when passed through the same screen size in the same hammer mill with grinding of hard wheat resulting in coarser particles compared to soft wheat. Similar results have been reported by Rose *et al.* (2001).

Wet sieving of the pelleted diets indicated differences in particle size distribution between diets after pelleting, especially in the proportion of coarse particles over 1mm. The hard wheat diet had 32.7% of particles with sizes over 1mm compared to 18.7% in the soft wheat diet. This effect of endosperm hardness on the size of particles in pelleted feed may explain, at least in part, the inconsistent results of the previous studies examining the effect of whole wheat inclusion pre-pelleting. Those findings suggested that the hardness of the grain must be considered when choosing whole wheat for pre-pelleting inclusion in broiler diets.

In the current study, it was hypothesised that hard wheat will have beneficial effects on gizzard size and bird performance. Although hard wheat increased weight gain, feed intake and relative gizzard weight, it had no effect on feed per gain in broilers. It was expected that hard wheat would improve feed per gain due to coarser particles (Chapter 5) and greater gizzard development. A well developed gizzard is known to have positive effects on nutrient digestibility and bird performance (Svihus *et al.*, 2004b). This lack of hardness effect on feed per gain and AMEn in the present study may be related to a higher energy requirement of the gizzard to grind harder wheat.

Xylanase supplementation reduced feed intake and feed per gain, and improved AMEn in the hard wheat diet, but not in the soft wheat diet. The beneficial effect of xylanase supplementation of the hard wheat-based diet may be related to greater grinding activity and mixing in the gizzard as indicated by the higher relative weight and digesta content. Wu and Ravindran (2004) suggested that the additive effect of xylanase supplementation with whole wheat feeding is a consequence of the increased grinding activity of the larger gizzard and enhanced mixing of the substrate with the supplemental enzyme. The greater digesta content of the gizzards of birds fed hard wheat suggests that feed remained longer in the gizzard, thus giving more time for enzyme-substrate interaction. Sufficient time for enzyme and substrate incubation in the gastrointestinal tract was suggested to be an important factor to greater cell wall dissolution (Bedford and Schulze, 1998). On the other hand, the lower digesta content in the gizzard of birds fed soft wheat suggest that feed passed quickly through the gizzard, therefore mixing less with exogenous enzymes.

The lower feed intake of birds fed the unsupplemented soft wheat diet may be due to the higher digesta viscosity caused by the fine particle size (Yasar, 2003), which is known to reduce feed passage rate (Bedford and Schulze, 1998) and feed intake (Almirall and Estive-Garcia, 1994). The higher digesta viscosity may also explain the longer relative length of all gut components in these birds, which may be considered as adaptive mechanism to increase the surface area required for absorption. However, when soft wheat was supplemented with exogenous xylanase this may have lowered digesta viscosity, resulting in increased feed intake and shorter gut length.

Interestingly, the birds fed diets supplemented with xylanase had a relatively heavier gizzard. It has been suggested that xylanases indirectly enhance cell wall breakdown through the stimulation of gizzard/stomach function (Bedford and Schulze, 1998). However, there was no effect of enzyme supplementation on the particle size distribution of proximal intestinal digesta.

Discriminant analyses of the changes in relative proportions of the various particle size classes present in the proximal intestinal digesta, compared to those present in the diet, showed significant differences between the groups fed hard and soft wheat diets with a general increase in finer particles in the proximal intestinal digesta in birds fed the hard wheat-based diet. A possible explanation for this is that hard wheat stimulated gizzard development leading to more efficient grinding and production of greater quantities of finer particles. However, a recent study by Peron *et al.* (2007)

showed a greater proportion of coarser particles in the ileal digesta of birds fed hard wheat compared to those fed soft wheat. This apparent inconsistency between the current study and that of Peron *et al.* (2007) may be related to the site of measurements. The current study measured particle size distribution present in the proximal intestinal digesta, meanwhile, Peron *et al.* (2007) measured particle size distribution present in the ileal digesta.

7.6. Conclusions

These results showed that grinding wheat cultivars differing in endosperm hardness under similar conditions produced samples with different particle size distributions. The hypothesis that efficacy of exogenous xylanase will be greater in hard wheat-based diets than that in the soft wheat diets was tested in this study. Xylanase supplementation reduced feed intake and feed per gain and improved AMEn in the hard wheat diet, but had no effect in the soft wheat diet. This improvement in performance with xylanase supplementation of a hard wheat diet was concomitant with higher relative weight and digesta content in the gizzard, which may have caused longer and better mixing of the xylanase with the substrate. Soft wheat feeding resulted in lower relative gizzard weights and digesta content, suggesting that feed passed quickly through the gizzard. A higher proportion of fine particles was also observed in the proximal intestinal digesta in birds fed hard wheat-based diets further supporting the idea that gizzard function was improved on hard wheat diets. The results of the current study suggest that the response of broilers to xylanase supplementation is influenced by wheat hardness.

CHAPTER 8

Influence of insoluble fibre source and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens

8.1. Abstract

The objectives of this experiment were to examine the effects of diluting wheat-based diets with insoluble fibre sources and whole wheat inclusion on the performance, starch digestibility, digestive tract development and ileal microbiota profile of broiler chickens. The experimental treatments were as follows: Treatment 1, control diet based on ground wheat; Treatment 2, where 200 g/kg whole wheat replaced the ground wheat pre-pelleting; Treatment 3, where the control diet was diluted with cellulose at a ratio of 6:100 (w/w); and Treatment 4, where the control diet was diluted with ground wood shavings at a ratio of 6:100 (w/w). The wheat and the wood shavings were both ground using a hammer mill through a 3 mm screen size.

Feed per gain was not influenced ($P > 0.05$) by inclusion of whole wheat or wood shavings, but increased ($P < 0.05$) with cellulose inclusion. However, when feed per gain of birds fed diets diluted with cellulose and wood shavings was corrected by subtracting the amount of cellulose and wood shavings from the total feed consumption, it was found that the inclusion of wood shavings lowered ($P < 0.05$) feed per gain, while cellulose inclusion had no effect ($P > 0.05$). Wood shavings increased ($P < 0.05$) the relative gizzard weight and improved ($P < 0.05$) ileal starch digestibility compared to other dietary treatments. All gut components were shorter ($P < 0.05$) in birds given diets containing cellulose and wood shavings compared to those fed the control and whole wheat diets. Pelleting process evened out the differences in the particle size distributions between the control and whole wheat diets. Overall, these results suggest that the effects of insoluble fibre on broiler performance differed depending on the fibre source.

8.2. Introduction

Insoluble fibre is generally considered as a nutrient diluent in poultry diets (Hetland *et al.*, 2004) although effects are not well understood. Inclusion of moderate levels of insoluble fibre has been reported to have no effect on digesta viscosity (Svihus and Hetland, 2001; Shakouri *et al.*, 2006) and weight gain of broilers (Hetland and Svihus, 2001) but improved starch digestibility (Rogel *et al.*, 1987; Hetland and Svihus, 2001; Svihus and Hetland, 2001) and feed per gain (Hetland *et al.*, 2003) in birds fed wheat-based diets. Jorgensen *et al.* (1996) comparing three fibre source (pea fibre, wheat bran or oat bran) reported that the effects of fibre on digestibility and digestive tract development were related to source.

The ban in the use of antibiotic growth promoters in animal feeds has encouraged nutritionists to explore alternative feed management strategies to improve the health and digestive efficiency of broiler chickens. Promotion of gizzard development by whole wheat feeding is one such strategy which has been found to prevent potentially pathogenic bacteria from entering the intestinal tract (Engberg *et al.*, 2004). Dietary inclusion of coarse insoluble fibre has also been reported to promote gizzard development (Hetland *et al.*, 2003; 2004), but the effect of insoluble fibre on the intestinal microbial population in broiler chickens is unknown. Hetland *et al.* (2004) suggested that the effect of insoluble fibres on the composition and the quantity of gut microflora may be relatively insignificant. However, previous *in vitro* studies indicated that bacterial populations were altered on the basis of different fibre source fermented with poultry caecal inocula (Lan *et al.*, 2005; Dunkley *et al.*, 2007). The effects of insoluble fibre and whole wheat on ileal microflora composition in broiler chickens have been poorly studied.

Most studies examining the effects of whole wheat feeding and insoluble fibre on the microbial ecology of the digestive tract used media culture techniques (Engberg *et al.*, 2004; Shakouri *et al.*, 2006). However, this technique has selectivity of bacteria to culture conditions, in addition to being time-consuming and labour-intensive (Tannock, 2002; Li *et al.*, 2003). It has been found that up to 99% of the bacteria in many environments fail to grow under media culture conditions (Apajalahiti *et al.*, 2004). Culture-independent molecular techniques, such as polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), have been recently used to investigate the microbial ecology of the digestive tract (Knarreborg *et al.*, 2002; Zhu *et al.*, 2002; Hume *et al.*, 2003) and advantages of this technique such as rapidity and

sensitivity are discussed (Muyzer *et al.*, 1993; Tannock, 2002; Hume *et al.*, 2003). Polymerase chain reaction-based DGGE can be successfully used to investigate the effects of digestive tract development and diet on digestive microbial communities in chickens (Hume *et al.*, 2003).

The objectives of the present experiment were to investigate the effects of diluting the diet with different sources of insoluble fibre (wood shavings and cellulose) and whole wheat inclusion on the performance, digestive tract development and ileal microbial composition of broiler chickens.

8.3. Materials and methods

8.3.1. Animals and Housing

192 day-old male broilers (Ross 308) were obtained from a commercial hatchery. Housing conditions were as described in Section 3.1.

8.3.2. Wheat sample

Whole wheat was obtained from a commercial source (Denver Stock feeds, Palmerston North, New Zealand). Upon receipt at the research unit, hardness of the wheat sample was measured as described in Section 7.3.2. The wheat hardness was measured and found to be medium soft (PSI % = 65) (Canadian Grain Commission, 2004).

8.3.3. Diets

Broiler starter diets, based on wheat and soybean meal, were formulated to meet or exceed the NRC (1994) recommendations for major nutrients for broiler starters (Table 8.1). The experimental treatments were as follows: Treatment 1, control diet based on ground wheat; Treatment 2, where 200 g/kg whole wheat replaced the ground wheat pre-pelleting; Treatment 3, where the control diet was diluted with cellulose at a ratio of 6:100 (w/w); and Treatment 4, where the control diet was diluted with ground wood shavings at a ratio of 6:100 (w/w). Titanium oxide (0.3%) was included in all diets as a dietary marker. The wheat and the wood shavings were ground using a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) through a 3mm sieve. All diets were cold pelleted (70 °C) using a 3 mm die.

Table 8.1. Composition and calculated analysis (g/kg as fed) of the control diet¹

Ingredient	
Wheat ¹	648
Soybean meal	295
Soybean oil	13.1
Dicalcium phosphate	16.2
Limestone	16.5
L-lysine. HCl	1.9
DL-methionine	3.8
Salt	2.5
Trace mineral-vitamin premix ²	3.0
Titanium oxide	3.0
Xylanase ³	+
Calculated analysis	
Metabolisable energy, MJ/kg	12.4
Crude protein	226
Lysine	11.5
Methionine + cystine	9.4
Calcium	10.9
Available phosphorus	4.5

¹ Treatment 1, control diet based on ground wheat; Treatment 2, wherein 200 g/kg whole wheat was included replacing ground wheat pre-pelleting; Treatment 3, diet containing cellulose wherein the control diet was diluted with cellulose in the ratio of 6:100 (w/w); and Treatment 4, diet containing wood shavings was made by diluting the control diet with ground wood shavings in the ratio of 6:100 (w/w).

² Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl- α -tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

³ Kemzyme, Kemin (Asia) Pte Ltd, Singapore.

8.3.4. AME determination

The AME determination was carried out between day 17 to 20 posthatch as described in Section 3.3.

8.3.5. Excreta scores

On day 19 and 20, excreta were scored for consistency on a scale of 1 to 5, with a score of 1 representing normally formed excreta and 5 representing pasty and very sticky excreta.

8.3.6. Digestive tract measurements

Two birds per replicate cage were euthanased, and digestive tract measurements were carried out on day 21 as described in Section 3.4.

8.3.7. Digesta collection

On day 21, four more birds per replicate were euthanased by intravenous injection with sodium pentobarbitone. Immediately after euthanasia, the ileum was excised and divided into two parts, the anterior and posterior ileum. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileo-caecal junction. Contents of the posterior ileum were collected by gently flushing with distilled water into plastic containers. Digesta were pooled within a cage, lyophilised, ground to pass through a 0.5 mm screen size, and stored at -4 °C in airtight containers until laboratory analysis.

Starch content was measured using an assay kit (Megazyme, Boronia, VIC, Australia) based on the thermostable α -amylase and amyloglucosidase (McCleary *et al.*, 1997). Samples of diets and ileal digesta were assayed for titanium on a UV spectrophotometer following the method of Short *et al.* (1996), and apparent ileal starch digestibility calculated using the following formula

$$\text{Apparent ileal starch digestibility} = \frac{(\text{Starch/Ti})_d - (\text{Starch/Ti})_i}{(\text{Starch/Ti})_d}$$

where $(\text{Starch/Ti})_d$ = ratio of starch to titanium in the diet, and
 $(\text{Starch/Ti})_i$ = ratio of starch to titanium in the ileal digesta.

8.3.8. Determination of particle size distribution in the diets

The particle size distributions of the diets were determined as described in Section 3.6.

8.3.9. Ileum DNA isolation and PCR-DGGE analysis

On day 21, eight birds from each dietary treatment were euthanased by intravenous injection of sodium pentobarbitone. Approximately 1 ml digesta was obtained from the middle part of the ileum and stored at -80° C until DNA extraction. Bacterial DNA was extracted from each sample using QIAamp Stool DNA Mini Kit (QIAGEN, Valencia, CA, USA). The content from the ileum samples were vortexed in sterile phosphate

buffered saline solution (pH 7.2) for 10 min with glass beads (5 mm diameter) until all of the solid material was taken up into the aqueous phase. From this solution, 200µl was taken and 1.4ml of ALT buffer was added. The solution was vortexed and kept at 70 °C for 15min. The solution was then centrifuged at 10000g for 1 min and transferred the supernatant to a clean eppendorf. An InhibitEX tablet (QIAGEN, Valencia, CA, USA) was added, before the mixture was vortexed again and incubated at room temperature for a minute. The solution was centrifuged at 10000g for 6 min. Then 20µl Proteinase K and 200µl of AL buffer was added to 200µl supernatant from the previous step and incubated at 70 °C for 20 min. 200µl of ethanol (96-100%) was added to the lysate and mixed by vortexing. The extracted DNA was then purified using a column method provided by the manufacturer, and finally eluted in 200µl of nuclease free water. For PCR-DGGE analysis, each DNA sample was amplified using primers specific for conserved sequences flanking the variable V2–V3 region of the 16S ribosomal DNA, as described by Muyzer *et al.* (1998). Each reaction mixture contained 36 µL of nuclease free water, 50 µL of Go Green Master mix (PROMEGA GO, Madison, WI, USA), Primers (50 pmol of each per reaction mixture; primer 2, 5'-ATTACCGCGGCTGCTGG-3', and primer 3 with a 40-base G-C clamp 5'CGCCCGCCGCGCGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGA GGCAGCAG- 3') and 8 µL of DNA template 3 µL primer (Invitrogen, Carlsbad, CA, USA). Thirty cycles of 94 °C melting for 30 sec, 57 °C annealing for 1 min and 72 °C for 30 Sec performed in thermal cycler (Bio-Rad, Hercules, CA, USA). Nine cycles were then performed using an extension of 72 °C for 7 min.

Denaturing gradient gel electrophoresis was performed using the Bio-Rad D-Code System (Bio-Rad, Hercules, CA, USA) as described previously (Simpson *et al.*, 1999). To separate the PCR fragments, DNA-denaturing gradients (100% denaturant is equivalent to 7 mol/L urea and 40% deionised formamide) were formed in 6% polyacrylamide gels using a Bio-Rad Gradient Former (Bio-Rad, Hercules, CA, USA). Polymerase chain reaction products were loaded in each lane and electrophoresis was performed at 130 V for 24 h at 60 °C. Bacterial reference ladders representing known bacterial strains were loaded to allow standardisation of the bands. After electrophoresis, gels were stained using SYBR Safe™ Staining (Sigma Chemical Company, St. Louis, MO, USA). Then the two prepared gels were then photographed under BioRad Gel 2000 Documentation System (Bio-Rad, Hercules, CA, USA) and the

DGGE patterns were analysed using a software package (Phoretics 1D Advanced, Non-linear Dynamics, Newcastle, UK).

Comparison of samples were based on the Dice similarity coefficient and the unweighted pair group method using arithmetic means for clustering (Hume *et al.*, 2003). The relative similarities of the groups were determined by comparing the patterns of a relatedness tree (dendrogram). The amount of similarity was indicated by the percentage coefficient bar located below each dendrogram. Values between 0 and 1 represented an arithmetic determination of the degree to which banding patterns were similar.

8.3.10. Data Analysis

Cage means were used to derive performance data. The performance data, starch digestibility and AME were analysed by a one-way ANOVA using the General Linear Model procedure of SAS (1997) in a completely randomised design. Differences were considered to be significant at $P < 0.05$, and significant differences between means were separated by the Least Significant Difference test. Excreta score data were analysed as categorical data using the general model (GENMOD) procedure of SAS (1997).

Gross measurements of the digestive tracts of individual birds were first subjected to a log transformation to achieve near normal distribution on graphic analysis in SYSTAT (Wilkinson, 1990), and a two-way ANOVA was used to identify any interaction between dietary treatment and the various digestive tract components (proventriculus, gizzard, duodenum, jejunum, ileum and caeca) so as to understand how dietary treatment influence the overall tract development. A similar model was used for analysis of (log transformed) digesta content. In each case, the significance of individual components within the two levels was explored by *post hoc* testing with Bonferroni correction.

8.4. Results

Comparison of the particle size distributions of the experimental diets obtained by wet sieving (Figure 8.1), show that particle size distributions of the control and cellulose treatments were almost identical. Whole wheat treatment had relatively a higher proportion of 0.5 mm particles compared to the other dietary treatments. The wood shavings treatment had relatively a higher proportion of 1 mm particles compared to the other dietary treatments.

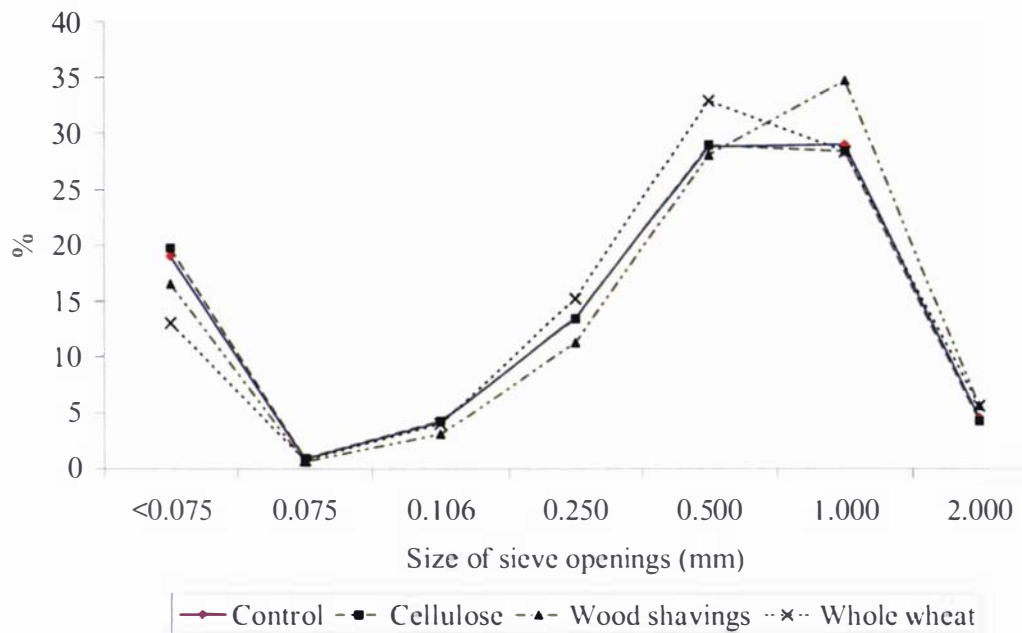


Figure 8.1. Particle size distribution of experimental diets

The influence of the four treatments on bird performance, AMEn and excreta score are summarised in Table 8.2. Weight gain was unaffected ($P > 0.05$) by the dietary treatments. Cellulose inclusion increased ($P < 0.05$) feed intake and feed per gain compared to the other dietary treatments. Whole wheat and wood shavings had no effect ($P > 0.05$) on feed intake and feed per gain compared to the control diet. However, when the feed per gain of birds fed diets diluted with cellulose or wood shavings were corrected for by subtracting the amount of cellulose and wood shavings from the total feed consumption, it was found that the inclusion of wood shavings improved ($P < 0.05$) feed per gain, while cellulose had no effect ($P > 0.05$). There was no effect ($P > 0.05$) of the dietary treatments on AMEn. Excreta scores were improved ($P < 0.05$) with wood shavings treatment compared to other treatments.

The inclusion of wood shavings improved ($P < 0.05$) starch digestibility (Table 8.3), while cellulose or whole wheat had no effect ($P > 0.05$).

Table 8.2. Weight gain (g/bird), feed intake (g/bird), feed per gain (g/g), AMEn (MJ/kg dry matter) and excreta score of broilers as influenced by the inclusion of whole wheat and insoluble fibres (1-21 days posthatch)¹

	Control	Whole wheat	Cellulose	Wood shavings	SEM ²
Weight gain	960	961	1002	979	13.6
Feed intake	1268 ^a	1271 ^a	1398 ^b	1290 ^a	18.3
Feed per gain	1.388 ^a	1.382 ^a	1.455 ^b	1.374 ^a	0.008
Corrected Feed per gain ³	1.388 ^a	1.382 ^a	1.368 ^a	1.292 ^b	0.008
AMEn	13.63	13.59	13.66	13.84	0.16
Excreta score	2.87 ^a	3.08 ^a	3.20 ^a	1.79 ^b	0.20

^{ab} Means in a row not sharing a common superscript differ ($P < 0.05$).

¹ Each value represents the mean of six replicates (8 birds per replicate).

² Pooled standard error of mean.

³ Data were corrected for by subtracting the amount of added cellulose and wood shavings from the total feed consumption (Hetland *et al.*, 2003).

Table 8.3. Ileal starch digestibility of broilers as influenced by the inclusion of whole wheat and insoluble fibres¹

	Control	Whole wheat	Cellulose	Wood shavings
Mean value ²	0.85 ^a	0.88 ^a	0.87 ^a	0.94 ^b
Minimum value	0.81	0.84	0.83	0.87
Maximum value	0.88	0.91	0.90	0.97
Standard deviation	0.013	0.008	0.011	0.015

¹ Each value represents the mean of six replicates.

² Pooled standard error of mean = 0.012

The effects of the dietary treatments on the relative weights, digesta contents and lengths of the intestinal components are shown in Table 8.4. The relative weights of gut components were different ($P < 0.05$) between treatments. There was an interaction ($P < 0.05$) between treatments and gut components due to higher gizzard weight in birds fed diets with wood shavings compared to those fed other treatments. The relative weight of digesta in all gut components were different ($P < 0.05$) between treatments. There was an interaction ($P < 0.05$) between treatment and gut components due to higher gizzard digesta content in birds fed wood shavings compared to those fed the other treatments.

The relative lengths of gut components were different ($P < 0.05$) between treatments. Relative lengths of gut components were shorter ($P < 0.05$) in birds fed diets containing cellulose or wood shavings compared to those fed control and whole wheat diets.

Table 8.4. Influence of dietary treatments on gross morphology of the digestive tract of broilers (21 day posthatch)¹

	Control	Whole wheat	Cellulose	Wood shavings
Relative empty weights (g/kg BW)^{3,4}				
Proventriculus	3.98±0.18	4.11±0.08	4.24±0.30	4.99±0.32
Gizzard	9.92±0.51	10.2±0.59	9.57±0.27	13.8±1.50
Duodenum	4.28±0.12	4.42±0.17	3.98±0.13	4.05±0.21
Jejunum	10.2±0.55	10.6±0.44	9.75±0.40	8.91±0.37
Ileum	7.41±0.35	8.02±0.36	7.32±0.22	6.65±0.24
Small intestine ³	21.9±0.87	23.0±0.82	21.0±0.66	19.6±0.72
Caeca	1.55±0.06	1.48±0.07	1.43±0.10	1.41±0.06
Relative digesta content (g/kg BW)^{3,4}				
Proventriculus	0.64±0.10	0.54±0.06	0.70±0.15	1.39±0.30
Gizzard	3.81±0.97	3.12±0.46	2.75±0.32	10.8±1.44
Small intestine ²	46.7±1.54	43.6±2.12	42.7±2.07	38.3±2.63
Caeca	3.41±0.38	2.30±0.28	2.52±0.37	2.98±0.34
Relative length (cm/kg BW)³				
Duodenum	23.2±0.5	22.9±0.4	21.7±1.0	21.3±0.4
Jejunum	62.0±2.3	62.7±1.2	60.8±2.2	58.3±1.3
Ileum	63.5±1.8	63.1±1.5	58.7±2.1	59.0±1.4
Small intestine ²	149±4.0	149±2.2	141±5.0	139±2.1
Caeca	15.1±0.5	14.7±0.2	13.9±0.6	13.1±1.2

¹ Each value represents the mean ± SE of 12 birds.

² Small intestine = duodenum + jejunum + ileum.

³ Treatment effect ($P < 0.05$).

⁴ Treatment x gut component ($P < 0.05$).

The effect of the dietary treatments on the composition of the ileal microbiota is shown in Figures 8.2 and 8.3. Treatments were grouped into two main clusters based on visual comparison of the DGGE band patterns. The two groupings showed a similarity between birds fed the control and cellulose diets and a similarity between birds fed the whole wheat and wood shavings diets.

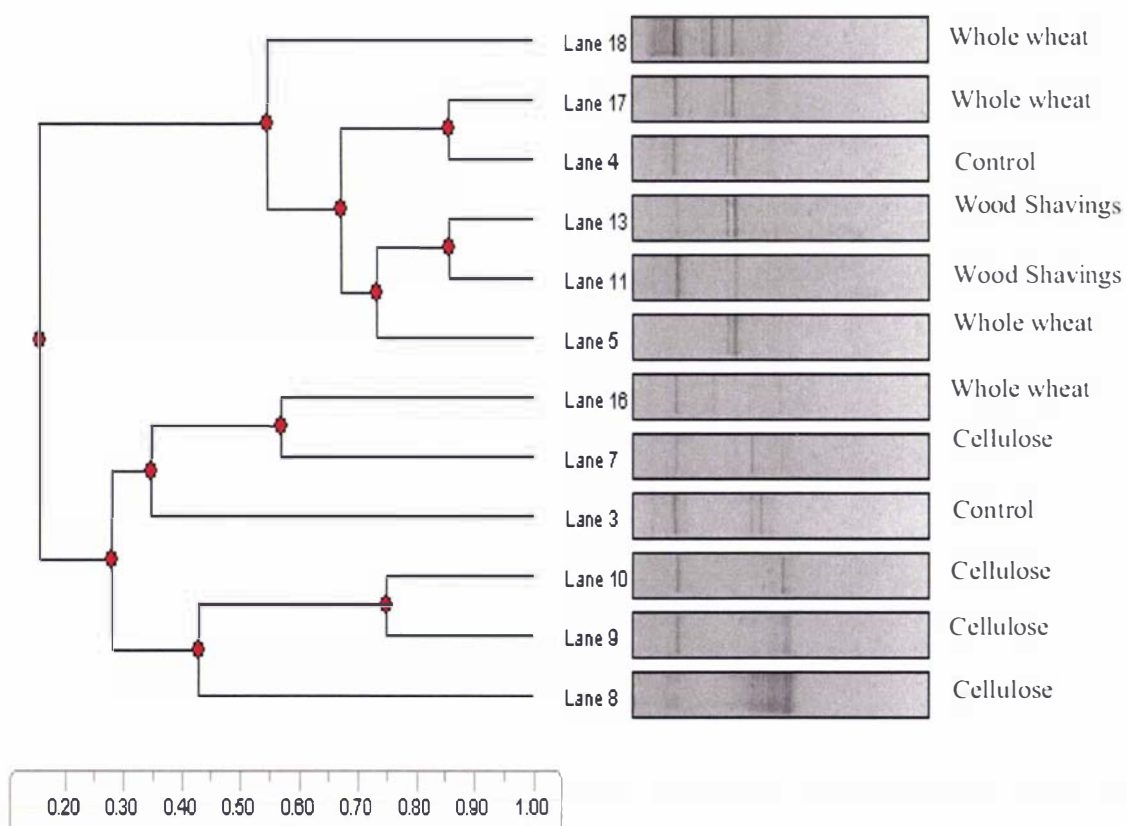
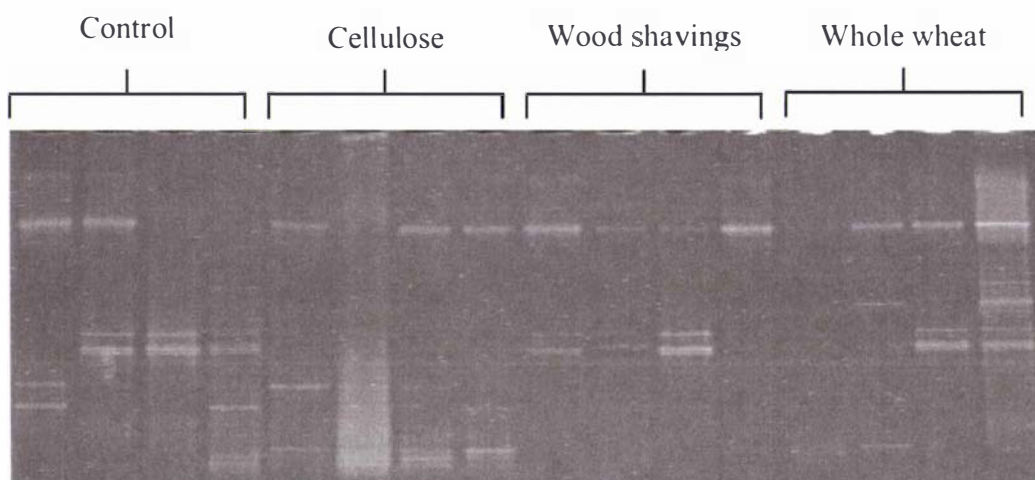


Figure 8.2. Denaturant gradient gel electrophoresis profile (gel 1) from ileal digesta of broilers as influenced by the inclusion of whole wheat and insoluble fibres. Relative similarity of band patterns of two denaturing gradient gel electrophoresis gels is indicated by their grouping on the dendrogram and the percentage coefficient (bar).

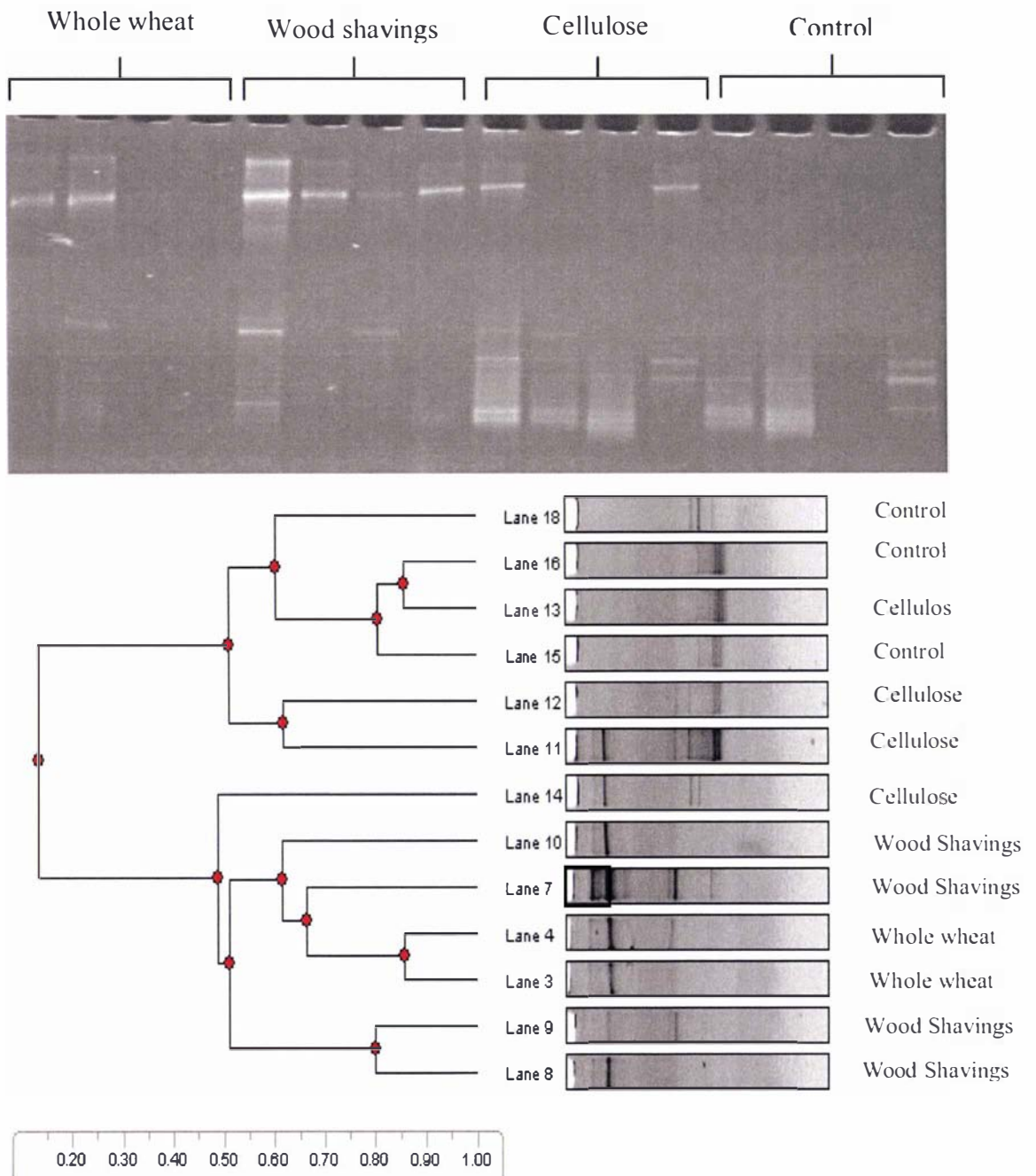


Figure 8.3. Denaturant gradient gel electrophoresis profile (gel 2) from ileal digesta of broilers as influenced by the inclusion of whole wheat and insoluble fibres. Relative similarity of band patterns of two denaturing gradient gel electrophoresis gels is indicated by their grouping on the dendrogram and the percentage coefficient (bar).

8.5. Discussion

In the present study, feed per gain was not influenced by the inclusion of whole wheat or wood shavings, but was increased with cellulose inclusion. However, when feed per gain was corrected for the consumption of cellulose and wood shavings, it was found that the inclusion of wood shavings improved feed per gain, while cellulose inclusion had no effect. Hetland *et al.* (2003) also found that diluting a wheat-based diet with oat hulls had no influence on the weight gain and feed intake but improved the corrected feed per gain. Similarly, in turkeys, diluting a maize-based diet with 4:100 (w/w) wood shavings improved the feed per gain during the critical first seven days posthatch period and by the end of the experiment (28 d) treatment birds had about 8% lower feed per gain than birds fed the control diet (Santos *et al.*, 2006). The findings of the current study suggest that the fibre effect on broiler performance is related to fibre source.

Diluting the diet with cellulose resulted in a higher feed intake. In agreement, Shakouri *et al.* (2006) found that diluting the diet with cellulose 3:100 (w/w) increased feed intake. Svihus and Hetland (2001) also found that diluting the diet with cellulose 10:100 (w/w) tended to increase feed intake. It has been suggested that the birds are able to maintain normal weight gain when fed diets diluted with insoluble fibre by the increased capacity of the digestive system and/or faster passage through the digestive tract (Hetland and Svihus, 2001). However, the fibre effect on passage rate depends on the particle size of the fibre with finer particles increasing feed passage rate and coarse particles accumulating in the gizzard and decreasing passage rate (Hetland *et al.*, 2004). Consequently, the higher feed intake in birds fed diets diluted with cellulose in the present study may have resulted from faster gut emptying (Almirall and Estive- Garcia, 1994; Shakouri *et al.*, 2006).

Differences in particle size distribution between whole wheat and the control diet in the present study were expected. However, the pelleting process reduced the size of the whole wheat grains and minimised differences in particle size distributions between the whole wheat and control diets, so that the particle size distributions were almost identical. This result may explain, in part, the lack of effect of whole wheat inclusion on performance, starch digestibility and gut parameters in the study. Previous studies examining the effects of whole wheat added pre-pelleting have produced inconsistent results. Wu *et al.* (2004) found that 200g/kg whole wheat inclusion pre-pelleting lowered the feed per gain with no effect on digestive tract measurements. In contrast, Taylor and Jones (2004) found no effect of adding 200g/kg whole wheat pre-

pelleting on broiler performance but increased relative gizzard weights. Similar results were obtained by Svihus *et al.* (2004b) adding 500g/kg whole wheat pre-pelleting. The differences between research results may be explained, partly, by the differences in the wheat hardness which has been found to affect wheat milling outcome and particle size distribution after pelleting (Chapter 7).

In the current study, wood shavings increased the relative weight of the gizzard. Large amounts of wood shavings were observed to accumulate in the gizzard and this could be attributed to the physical structure of the shavings. The relative digesta content of the gizzard of birds fed wood shavings was almost three times the amount found in birds on other treatments. This accumulation of shavings may cause a mechanical stimulation of the gizzard and an increase in size. Hetland *et al.* (2003) reported that fibre levels of gizzard contents were about twice that of the feed in layers fed wood shavings.

Ileal starch digestibility was 9% higher in birds fed diet was diluted with wood shavings. Previous studies have also found that inclusion of coarse insoluble fibres (Rogel *et al.*, 1987; Hetland *et al.*, 2003) and whole wheat inclusion (Svihus and Hetland, 2001; Hetland *et al.*, 2002, Svihus *et al.*, 2004b) improved starch digestibility. The results reported here can be explained by the larger gizzard sizes observed in birds fed wood shavings. A large, well-developed gizzard improves gut motility (Ferket, 2000), through increasing the levels of cholecystokinin release (Svihus *et al.*, 2004b), which in turn stimulates the secretion of pancreatic enzymes and gastro-duodenal refluxes (Duke, 1992; Li and Owyang, 1993). Hetland *et al.* (2003) reported that the total amount of bile acids in the gizzard increased in layers with access to wood shavings indicating increased gastro-duodenal reflux. A recent study by Gonzalez-Alvarado *et al.* (2007) reported improvements in the total tract apparent retention of nutrients when soy hulls or oat hulls were added to maize- or rice-based diets. Svihus (2006) hypothesised that the modern broiler strains overconsume feed, which results in the overloading of the digestive system and a reduction in nutrient digestibility. On the other hand, a well-developed gizzard functions as a regulator of feed intake and will improve nutrient digestibility (Svihus, 2006). Svihus and Hetland (2001) also stated that the gizzard may be the key site for the prevention of starch overload in the digestive tract. The findings of the current study support this hypothesis and it appears that adding moderate levels of coarse insoluble fibre to poultry diets will have beneficial effects on

digestive tract development and starch digestibility, and consequently feed efficiency of diets.

The lack of an effect of cellulose inclusion on starch digestibility contradicts the findings by Svihus and Hetland (2001) who reported that diluting the diet with 10:100 (w/w) cellulose increased starch digestibility from 0.79 to 0.93. These researchers hypothesised that the concentration of wheat starch in the intestinal chyme is inversely correlated to starch digestibility. The inconsistency between the current study and that of Svihus and Hetland (2001) may be related to the level of cellulose inclusion, in the present study the control diet was diluted 6:100 (w/w) cellulose, meanwhile, Svihus and Hetland (2001) diluted the diet with 10:100 (w/w) cellulose.

One of the objectives of this experiment was to investigate whether the inclusion of whole wheat and insoluble fibre influences the ileal microbial composition of broilers. The DGGE band patterns (Figures 2 and 3) showed that ileal microbiota composition was influenced by fibre, which is in agreement with *in vitro* studies in poultry caeca (Lan *et al.*, 2005; Dunkley *et al.*, 2007). Factors which may have contributed to these changes in microbiota profile in the current study include the physical structure of the fibre which has been shown to influence gizzard function and retention time at low pH (Engberg 2002; 2004, Bjerrum *et al.*, 2005), the concentration of fermentable nutrients in the small intestine (Engberg *et al.*, 2004) and digesta viscosity (Langhout, 1999). However, in the current study, the lack of similarity between whole wheat and the control treatments was surprising, given that particle size distribution was similar between the diets post-pelleting. One possible explanation is the difference in the method of particle size reduction between the two treatments. In the control diet, all the wheat was hammer milled using a 3 mm screen size, however, in the whole wheat treatment, 200 g/kg was crushed during the pelleting process. This may have influenced the structure of the wheat fibre and consequently microbial composition. It may be suggested, therefore, that method of particle reduction may influence intestinal microbiota profile. Further studies are warranted in this area to identify the bacterial species as influenced by fibre source.

Inclusion of insoluble fibres in the diet reduced the relative length of the small intestine, which is in agreement with previous studies (Rogel *et al.*, 1987; Gonzalez-Alvarado *et al.*, 2007). A shorter small intestine in birds fed diets with insoluble fibres may be explained by the lower nutrient density, which reduces the surface area required for absorption. Alternatively, insoluble fibres may increase villus length (Moran, 2006)

reducing the need for a longer small intestine. Santos *et al.* (2006), however, reported that intestinal length was not affected by dietary inclusion of wood shavings in turkeys.

Excreta scores were improved by the inclusion of wood shavings. Coarse fibres can hold large amounts of water, thus function to reduce the solubilisation of NSPs (Choct, 1997), which in turn may reduce digesta viscosity and improve excreta quality. Lower digesta viscosity with enzyme supplementation in wheat-based diet has been reported to improve excreta score (Wu *et al.*, 2004).

8.6. Conclusions

The effects of insoluble fibre on digestive tract development and broiler performance differed depending on the source of fibre. Inclusion of wood shavings in the diet increased relative gizzard size and improved corrected feed per gain and ileal starch digestibility. On the other hand, cellulose inclusion in the diet increased feed intake and had no effect on starch digestibility. Whole wheat inclusion (200g/kg) pre-pelleting had no effect on broiler performance and starch digestibility. Inclusion of wood shavings in the diet improved excreta quality. This study shows that the ileal microbiota profile is influenced by fibre inclusion and fibre source.

CHAPTER 9

General discussion

9.1. Introduction

Feed constitutes the greatest single cost in poultry production comprising 60% of the total cost of production. Dozier (2002) estimated that energy usage comprised between 25 to 30% of the manufacturing cost of broiler feed. The reduction of feed particle size is the second largest energy cost after that of pelleting in the broiler industry (Reece *et al.*, 1985). Reducing feed particles to a finer size requires greater energy use and lowers the production rate. Thus, any reduction in energy consumption used for grinding could significantly lower the cost of feed manufacture. Reece *et al.* (1986a) reported that energy savings of 27% could be achieved by increasing the screen size of a hammer mill from 4.76 to 6.35 mm. In New Zealand, medium grind using a 3 mm screen size in a hammer mill is typically used for grinding grains, with the primary aim of maintaining good pellet quality. The general findings of this thesis suggest that coarsely grinding of cereal grains using 7 mm hammer mill screen size does not adversely influence pellet quality or broiler performance. The work reported herein also showed that particle size distribution of the milled product is influenced by grain type (Chapter 5) and endosperm hardness (Chapter 7). These data suggest that, during grinding in a hammer mill, different screen sizes may have to be used according to the grain type to obtain the desired particle size distribution.

9.2. Effects of the physical form of the feed - mash versus pellets

Data reported in Chapter 4 showed that the physical form of the feed had a greater influence on the different measured parameters than did the particle size. The feeding of pelleted diets increased both weight gain and feed intake, and improved feed efficiency compared to birds maintained on mash diets as has been previously reported in a number of studies (Nir *et al.*, 1995; Engberg *et al.*, 2002; Svihus *et al.*, 2004a). However, whilst pelleted diets improved broiler performance relative to mash diets, they also reduced AMEn. This may be explained by the higher feed intake of birds fed pelleted diets compared to those fed mash. It would appear that the lower AME is more than compensated by increasing feed intake in excess of maintenance requirements.

9.3. Effect of wheat particle size on broiler performance in mash diets

Data reported in Chapter 4 showed that wheat particle size influences broiler performance to a greater extent when birds were fed mash diets than when they were fed pelleted diets. Improvements in weight gain, feed intake and feed per gain in birds fed coarsely ground mash compared to those fed medium ground mash have been reported in a number of other studies (Proudfoot and Hulan, 1989; Nir *et al.*, 1995). These results are generally attributed to the preference for larger sized particles (Proudfoot and Hulan, 1989; Nir *et al.*, 1995) and the lowering of viscosity of the liquid phase of digesta in wheat-based diets (Yasar, 2003). However, in the third experiment (Chapter 6) using the same hammer mill screen sizes under similar conditions (same hammer mill, screens, operator, etc) as in the first experiment (Chapter 4), but using a different batch of wheat, no effect of coarse grinding on broiler performance was observed. In this experiment (Chapter 6) the differences in particle size distribution between medium and coarse diets were found to be not sufficiently large enough to cause any differences in performance parameters. This inconsistency may be related to differences in the physical characteristics of wheat. It can be suggested, therefore, that the physical characteristics of wheat may influence the differences in the particle size distribution between the milling outcomes using different screen sizes. These results also suggest that, it is important to specify the wheat physical characteristics in any study examining the effects of wheat particle size in poultry diets.

9.4. Effect of wheat particle size on broiler performance in pelleted diets

A number of studies have shown that feed particle size has no effect on performance when pelleted feeds were fed to broilers (Reece *et al.*, 1986a; Svihus *et al.*, 2004a; Peron *et al.*, 2005), while others have demonstrated an effect (Lott *et al.*, 1992; Kilburn and Edwards, 2001; Lentle *et al.*, 2006). The work reported in this thesis similarly showed inconsistent effects of feed particle size in pelleted diets on broiler performance. When particle size differences persisted after pelleting, birds fed coarsely ground grains had better feed efficiency compared to those fed finely ground grains (Chapter 5). On the other hand, no effect on performance was observed between birds fed medium and coarse ground wheat when pelleting process evened out the differences in particle size distribution (Chapter 4). As discussed above, such contradictory results appear to be related to grain physical characteristics. The particle size of milled products is known to be affected by grain hardness (Dobraszczyk *et al.*, 2002; Carre, 2004), and wheat

hardness was found to influence diet particle size distribution post-pelleting in this thesis (Chapter 7). Other factors influencing diet particle size distribution post-pelleting may include conditioning temperature, mash residence time within the conditioner, die specifications and gap distance between the die and the roller and these potential effects need to be elucidated in future studies.

9.5. Effect of whole wheat inclusion in broiler diets

Feeding whole wheat to poultry is a common practice in many countries. Whole wheat feeding reduces feed cost and may improve gut health (Gabriel *et al.*, 2003; Bjerrum *et al.*, 2005), nutrient digestibility and AME (Preston *et al.* 2000; Hetland *et al.*, 2002). However, studies examining the effects of whole wheat inclusion pre-pelleting on broiler performance are limited and those that have been carried out have produced inconsistent results (See Section 2.1.9). Results reported in this thesis also showed similar inconsistent results. Data presented in Chapter 8 showed that the inclusion of 200g/kg soft whole wheat pre-pelleting had no effect on broiler performance and gizzard development. This may be due to the effect of the pelleting process which reduced the size of the whole wheat and minimised the differences in the particle size distributions between the whole wheat and the control diet, so that the particle size distributions were similar. On the other hand, data reported in Chapter 7 showed that the inclusion of 200g/kg hard wheat increased weight gain, feed intake and gizzard weight compared to soft wheat-based diet. In this experiment (Chapter 7), 200g/kg whole wheat inclusion of soft or hard wheat pre-pelleting produced different particle size distributions in the pelleted diets. Therefore, differences between studies may be explained, at least in part, by the differences in the wheat hardness. Results from this thesis clearly demonstrate that grain hardness must be considered when choosing whole wheat for inclusion in broiler diets.

9.6. Wheat particle size, wheat hardness and xylanase supplementation in broiler diets

Xylanase supplementation of wheat-based poultry diets is a common commercial practice. Although improvement in bird performance with xylanase supplementation is well documented (Bedford and Schulze, 1998), the response varies according to factors such as type of xylanase, wheat quality and the breed and the age of the birds (Bedford, 2002). Feed particle size may also affect the efficacy of exogenous enzymes, but no

previous study has examined the interaction between xylanase supplementation and feed particle size. Data reported in Chapter 6 showed that xylanase supplementation improved feed efficiency in birds maintained on a coarse particle-sized diet, but had no effect on a medium particle-sized diet. Three suggestions have been proposed to explain this finding (See Section 6.4). First, it is possible that enzyme supplementation acts, in part, on gel barriers formed by partial solubilisation of NSPs on digesta particles. Thus, the finer the particles the higher the surface area available for gel formation which causes increased *in situ* gel formation and lowers the effectiveness of enzyme. Second, the presence of larger particles may have increased the efficiency of xylanase by increasing the permeability of digesta (Lentle, 2005). Third, the cell walls of coarsely ground wheat are less disrupted, thus the efficacy of cell wall degrading enzymes are greater than in more finely ground wheat (Kim, 2003). However, further studies to determine the underlying reasons for the better enzyme efficiency with coarse particles will be of practical interest.

Data reported in Chapter 7 demonstrated an interaction between wheat hardness and xylanase supplementation, with lower feed intake and feed per gain, and higher AMEn in hard wheat-based diets. In contrast, such positive effects were not observed in soft wheat-based diets. It is likely that the beneficial effect of enzyme supplementation in hard wheat-based diets may be related to greater grinding activity and mixing in the gizzard as indicated by heavier gizzard weights and greater digesta content. Wu and Ravindran (2004) suggested that the additive effect of xylanase supplementation and whole wheat feeding is a consequence of increased grinding activity of the larger gizzard and enhanced mixing of the enzyme and substrate. The higher relative digesta weights in the gizzards of birds fed hard wheat-based diets indicated that feed remained longer in the gizzard, providing more time for enzyme and substrate interaction. On the other hand, the lower digesta content in the gizzard of birds fed soft wheat suggest that feed passed quickly through the gizzard, thus mixing less with exogenous enzymes. Sufficient time for enzyme and substrate interaction in the gastrointestinal tract was suggested to be an important factor to greater cell wall dissolution (Bedford and Schulze, 1998).

9.7. Factors influencing the development and function of the digestive tract of broilers

Throughout this thesis, the influence of several factors on digestive tract development was studied. Mechanical processing of feed in feed mills has negative effects on gizzard size and function (See Section 2.1.10). In addition, modern broiler strains overconsume feed, resulting in digestive system overload and reduction in nutrient digestibility (Svihus, 2006) which may be mitigated by a well-developed gizzard. A larger gizzard may function as a regulator of feed intake and will improve nutrient digestibility (Svihus, 2006). The data reported in Chapter 8 lend support to this suggestion, with the addition of 60 g/kg wood shaving, as a source of coarse insoluble fibre, resulting in a larger gizzard which had beneficial effects on starch digestibility, and consequently feed per gain.

Feed particle size has been shown to be positively correlated to relative gizzard weight (Nir and Ptichi, 2001) when diets are fed as mash. However, published data on the effects of feed particle size in pelleted diet on gizzard development are inconsistent (Engberg *et al.*, 2002; Svihus *et al.*, 2004a; Peron *et al.*, 2005). Conflicting results were also found in the two studies reported in the thesis (Chapters 4 and 5). This inconsistency appeared to be due to differences in particle size distribution after pelleting. Coarse particles that persisted after pelleting stimulated gizzard development (Chapter 5), but when the differences in particle size were evened out by pelleting, no effect on gizzard development was observed (Chapter 4). The beneficial effects of a well developed gizzard are discussed in Section 2.1.10.

The effect of xylanase supplementation on digestive tract measurements of broilers fed wheat-based diets showed inconsistent results in studies reported in this thesis. In the experiment reported in Chapter 6, birds fed a diet supplemented with xylanase had shorter, lighter gut components compared to those fed unsupplemented diets, but this effect was not found in the experiment reported in Chapter 7. This variation in response of digestive tract to xylanase supplementation may be related, partly, to the physical form of feed (mash in Chapter 6 vs. pellet in Chapter 7) or the characteristics of the wheat used.

It has been hypothesised that the higher intake of pelleted wheat-based diets increases the starch load in the gut and lowers starch digestibility when compared to mash diets (Svihus and Hetland, 2001). This hypothesis is supported by results in Chapter 4 showing that the extent of the mucosal layer of the duodenum and jejunum is

increased in birds fed pelleted diets compared to those fed mash diets. This may be suggestive of a general increase in digestive and absorptive capacity of the proximal small intestine in response to the greater flow of nutrients. However, this was concurrent with a reduction in the relative length of the intestine in birds fed the pelleted compared to those fed the mash diets. In contrast, data reported in Chapters 4 and 5 showed that the duodenal and jejunal morphology were not influenced by grain particle size and grain type, but intestinal length was longer in birds fed medium compared with those fed coarse particle size diets in mash form (Chapter 4), and those fed fine compared with those fed coarse particle size in maize-based diet but not in birds fed wheat-based diets (Chapter 5). The increase in the relative length of the intestine in these birds appears to be an adaptive mechanism to optimise nutrient absorption. The intestinal surface area available for absorption and intestinal length have been reported to be positively correlated (Mitjans *et al.*, 1997). To the author's knowledge, the effect of feed particle size on intestinal morphology of broilers and its relationship with intestinal length has not been previously investigated. However, although this thesis does not give a definite conclusion with regard to the relation between feed particle size, intestinal morphology and the intestinal length due to the confounding effects of different wheat samples used in different experiments, the present data showed that the development of the small intestine lengthwise was influenced by pelleting (Chapter 4), grain particle size (Chapters 4 and 5), nutrient dilution (Chapter 8) and xylanase supplementation (Chapter 6).

Xylanase supplementation had no influence on villus height, crypt depth and the total extent of the mucosal layer. The lack of xylanase effect on histological measurements in Chapter 6 is probably related to the lack of an enzyme effect on digesta viscosity. Changes in digesta viscosity have been thought to be responsible for the morphological changes associated with experimental xylanase in wheat-based diets (Smits and Annison, 1996).

9.8. Factors effecting digesta particle size

The gizzard is a muscular organ that reduces the particle size of ingested feed (Duke, 1986). The mechanical pressure applied during grinding by the gizzard may exceed 585 kg/cm² (Cabrera, 1994). Data on the effect of original feed particle size on subsequent particle size distribution in intestinal digesta are limited. It has been suggested that the gizzard has an ability to grind all organic constituents of feed to a consistently fine size

regardless of the original particle size of the feed (Hetland *et al.*, 2004). Hetland *et al.* (2002) reported that the digesta passing through the gizzard had a consistent particle size distribution, with the majority of particles being smaller than 40 μm regardless of the original feed structure. In contrast, Lentle (2005) speculated that an increase in the proportion of coarser particles in the diet caused greater amounts of coarser particles to transit the gizzard. Results reported in Chapters 4 and 5 showed that there was no effect of feed particle size within same feed form (mash or pellet) on the particle size of duodenal digesta. Similarly, results reported in Chapter 7 showed that there was no effect of xylanase supplementation on the digesta particle size in proximal intestine. However, with regard to feed form, gizzard mass was greater in birds fed mash diets and that the duodenal digesta contained a higher proportion of large particles (1-2 mm) compared with birds fed pelleted diets. In this study, whilst the gizzard enlarged in response to an increase in the coarse fraction of the diet, it did not uniformly reduce particle size. In Chapter 7, changes in proportions of the various particle size classes present in the proximal intestinal digesta, relative to those present in the diet, showed differences between the groups fed hard and soft wheat based diets and there was a general increase in finer particles in the hard wheat based diet. It would appear that the physical structure of hard wheat stimulated the gizzard leading to more efficient grinding and the production of greater quantities of finer particles. Thus, the results of this thesis do not support the theory that the gizzard has the ability to grind feed to a fine consistent particle size irrespective of the original particle size of the feed. Factors such as feed form and endosperm hardness were found to influence the particle size distribution of the intestinal digesta.

9.9. Effect of particle size on pellet durability index

Higher pellet durability lowers the formation of fines and, reduces feed wastage and selection of larger particles by the birds. Pellet durability is thought to be inversely related to particle size (Angulo *et al.*, 1996), due to smaller particles having more contact points with each other because of their larger surface area per unit volume (Behnke, 2001). However, there is little scientific evidence to support this view. A limited number of studies have shown that fineness of grind of grain has no effect on pellet durability (Reece *et al.*, 1986b; Koch, 1996), which are similar to the findings of the first experiment of the thesis (Chapter 4). Results of the second experiment (Chapter 5) showed that pellets made from fine particles gave better durability compared to those

made from coarse ground grains. However, all diets showed good pellet durability and the lower PDI of coarse particle size diets did not adversely influenced feed per gain of broilers. It is difficult to draw a definite conclusion from the work reported in this thesis due to the confounding effects of factors such as dietary protein and oil contents (Briggs *et al.*, 1999), mash conditioning, die specifications, cooling and drying (Behnke, 1996) and endosperm hardness (Carre *et al.*, 2005). More research is required to verify the interaction between these factors, degree of grinding and pellet quality.

9.10. Summary and main conclusions

The work reported in this thesis identified some factors that contribute to the understanding of the complexity of the subject of feed particle size including feed physical form, grain type, endosperm hardness, pellet quality and particle size distribution post-pelleting. Nutritional strategies to promote gizzard development were reported in this thesis and the effects of several factors effecting digestive tract development were also studied. The interaction between feed particle size and aspects of the pelleting process needs further study.

In summary, gizzard stimulation through the feeding of coarse ground cereals positively influenced broiler performance. The current practice of feeding broilers highly processed feed has a negative effect on gizzard size and function. Feed form had a greater influence on the different measured parameters than did particle size. The effect of grain particle size in pelleted diets on broiler performance and gizzard development was related, in part, to changes in size distribution following pelleting. Broilers have high ability to adapt to various diet structures and composition by modulating digestive tract development. The general findings of this thesis suggest that cereals used for broilers can be ground more coarsely than is currently practiced with no adverse effect on performance. Furthermore, it appears that the additional energy consumption during grain milling required to obtain a fine particle size to improve pellet quality is not justified by the resultant performance data observed in this thesis. The efficacy of xylanase was found to be influenced by wheat hardness and degree of grinding. Endosperm hardness should also be considered when selecting a wheat cultivar for whole wheat inclusion pre-pelleting in broiler diets. The intestinal digesta particle size distribution was found to be influenced by feed form and wheat hardness and the gizzard did not uniformly reduce particle size.

Overall, the data reported in this thesis suggest that the current practice in the feed industry of particle size reduction of whole grains may require re-evaluation from two perspectives, namely, the energy costs associated with feed processing and the degree of gizzard development in the birds.

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