WHOLE GRAIN INCLUSION IN POULTRY DIETS: EFFECTS ON PERFORMANCE, NUTRIENT UTILISATION, GUT DEVELOPMENT, CAECAL MICROFLORA PROFILE AND COCCIDIOSIS CHALLENGE

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Yashpal Singh

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Abstract

Whole grain feeding has recently received renewed interest in the commercial poultry industry as a mean of lowering feed manufacturing cost. Wheat is the cereal grain of choice for whole grain feeding, despite the fact that globally maize is the most commonly cereal grain. Published data on the use of whole maize in poultry diets are scant. The size of maize grain may be the major reason for the lack of interest in feeding whole maize. The first three experiments of the thesis investigated alternative feeding strategies such as pre-pelleting inclusion or minor modifications such as cracking or coarser grinding to overcome the issue of maize kernel size. Experiment four evaluated whole wheat (WW) feding and examined the interaction between pellet diameter (3.0 vs 4.76 mm) and method of wheat inclusion (ground wheat (GW) or WW pre-and post-pelleting). The intention of using a larger pellet die was to retain the larger wheat particle size in pellets. Experiment five investigated the effect of whole wheat feeding in broilers experimentally challenged with a mixed infection of *Eimeria*.

Pre-pelleting inclusion of 0 to 600 g/kg whole maize replacing (w/w) ground maize in broiler starter diets showed that the weight gain of broilers was poorer despite improvements in gizzard development, nutrient utilisation and pellet quality (Chapter 4). Poor weight gain was due largely to reduced feed intake. Inclusion of 0 to 600 g/kg coarse maize, replacing (w/w) finely-ground maize, in broiler diets in mash form from day 11 to 35 post-hatch resulted in improvements in weight gain and gizzard weight without any negative effect on nutrient utilisation and carcass yield (Chapter 5). Increased caecal counts of beneficial bacteria *Lactobacilli* spp. and *Bifidobacteria* spp. and decreased counts of *Clostridium* spp., *Campylobacterium* spp. and *Bacteroides* spp. were also reported. Similarly, feeding diets containing 0 to 600 g/kg coarse maize to laying hens, from 39 to 62weeks of age, had no adverse effects on any production parameters and egg quality (Chapter 6). These results indicated that ground maize in broiler and layer diets could be completely replaced by coarsely ground maize with no adverse effects of bird performance. .

Data reported in Chapter 7 showed that the effect of pellet diameter on broiler performance varied depending on the form of wheat and method of WW inclusion. Larger pellet diameter increased the weight gain and lowered feed per gain of birds fed

diets with GW and post-pellet inclusion of WW. However, in birds fed diets with pre-pelleting inclusion of WW, the larger pellet diameter lowered weight gain and increased feed per gain, due largely to reduced feed intake which may be attributed partly to poorer pellet quality. Relative gizzard weight was increased by larger pellet diameter with pre-pelleting inclusion of WW, but was unaffected by diets containing GW or post pelleting inclusion of WW. Larger pellet diameter increased the apparent metabolisable energy and ileal starch digestibility, irrespective of method of WW inclusion. These results suggested that, irrespective of whether the wheat grain was milled or added whole post-pelleting, a larger diameter pellet was beneficial. On the other hand, when WW was added pre-pelleting, a smaller diameter pellet resulted in improved weight and feed per gain in broiler performance.

In the final experiment (Chapter 8), broilers fed WW either pre-or post-pelleting and experimentally challenged with a mixed *Eimeria* infection at 21 day of age showed that mortality in challenged birds was highest in those fed diets with WW post-pelleting, followed by pre-pelleted WW and GW (58, 35, and 17%, respectively). The pattern of mortality paralleled the changes in gizzard size, which suggested that WW feeding exacerbated the severity of coccidiosis infection, possibly via a mechanism involving enhanced gizzard development.

Dedicated

to

my late father

(SUJAN SINGH)

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Table of Contents

Abstract ... i
Acknowledgements ... iv
Publications ... v
Table of Contents ... vi
List of Tables ... xii
List of Figures ... xvii
List of Abbreviations ... xviii

CHAPTER 1 ... 1

General introduction ... 1

CHAPTER 2 ... 4

Review of Literature ... 4

2.1. Whole wheat feeding: Methodology and implication on the performance of poultry ... 4
2.1.1. Introduction ... 4
2.1.2. Methods of whole grain feeding ... 5
2.1.2.1. Free choice feeding ... 6
2.1.2.2. Mixed feeding ... 9
2.1.2.2.1. Pre-pelleting inclusion of whole grain ... 10
2.1.2.2.2. Post-pelleting inclusion of whole grain ... 12
2.1.2.2.3. Mixed feeding of whole grain in mash feeds ... 15
2.1.2.3. Sequential feeding ... 17
2.1.3. Effect of whole wheat feeding on digestive tract characteristics ... 19
2.1.3.1. Gizzard ... 19
2.1.3.2. Proventriculus ... 19
2.1.3.3. Pancreas ... 20
2.1.3.4. Small intestine ... 20
2.1.3.5. Morphology and enzymatic activity of gastrointestinal tract ... 21
2.1.3.6. pH of the digesta ... 21
2.1.4. Effect of whole grain feeding on feed passage rate ... 22

2.1.5. Effect of whole grain feeding on digesta particle size 23
2.1.6. Effect of whole grain feeding on carcass characteristics 23
2.1.7. Effect of whole grain feeding on digesta viscosity 23
2.1.8. Effect of whole grain feeding on nutrient utilisation 24
2.1.8.1. Apparent metabolisable energy 25
2.1.8.2. Starch digestibility 25
2.1.9. Influence of whole wheat feeding on gut microflora 26
2.1.10. Whole grain feeding to layers 27
2.1.11. Factors responsible for variable responses with whole wheat feeding . 27
2.1.11.1. Quality of wheat 27
2.1.11.2. Time of introduction of whole wheat 28
2.1.11.3. Exposure to whole grain / adaptation period before the start of trial 29
2.1.11.4. Rate of inclusion of whole grain and the length of study................ 29
2.1.11.5. Method of whole grain feeding 30
2.1.11.6. Supplementation of whole wheat diet with exogenous enzymes 31
2.1.12. Conclusions 32
2.2. Maize: Feeding of whole grains other than wheat 33
2.2.1. Introduction 33
2.2.2. Maize kernel and its types 33
2.2.3. Physical Structure of maize kernel 34
2.2.4. Chemical composition and nutritive value of maize 34
2.2.4.1. Starch 35
2.2.4.2. Protein 35
2.2.4.3. Starch-protein matrix 35
2.2.5. Preference for maize and other grains for poultry 36
2.2.6. Use of whole maize or with minimal processing for poultry 37
2.2.6.1. Practical and economic implication 37
2.2.6.2. Effect on production performance 37
2.2.7. Conclusions 38
2.3. Coccidiosis: Whole grain feeding may be an alternative strategy for control 39

2.3.1. Introduction 39
2.3.2. *Eimeria* species 39
2.3.3. Life cycle of *Eimeria* 41
2.3.4. Forms of coccidiosis in chickens 43
2.3.5. Pathophysiological effects of coccidiosis in chickens 43
2.3.6. Diagnosis of coccidiosis 44
2.3.7. Influence of whole grain feeding on coccidiosis 44
2.3.8. Conclusions 45
2.4. Overall general conclusion 45
CHAPTER 3 46
General Materials and Methods 46
3.1. Birds and Housing 46
3.2. Determination of particle size distribution of mash diets: Dry sieving method 46
3.3. Determination of particle size distribution of pelleted diets: Wet sieving method 47
3.4. Pellet durability 47
3.5. Pellet hardness 47
3.6. Apparent metabolisable energy (AME) determination 48
3.7. Ileal digesta collection 48
3.8. Chemical analysis 48
3.9. Calculations 49
3.10. Digestive tract measurements 49
3.11. Determination of digesta pH 50
3.12. Determination of digesta transit time 50
3.13. Determination of microflora count in caecal content. 50
3.14. Gas production measurement 51
3.15. Data analysis 52
CHAPTER 4 53

Influence of pre-pelleting inclusion of whole maize on performance, nutrient utilisation, digestive tract measurements and caecal microflora counts of young broilers 53
4.1. Abstract 53
4.2. Introduction 53
4.3. Material and methods 55
4.4. Results 58
4.5. Discussion 68
4.6. Conclusions 71
CHAPTER 5 72
Influence of feeding coarse maize on performance, nutrient utilisation, digestive tract measurements, carcass characteristics and caecal microflora counts of broilers 72
5.1. Abstract 72
5.2. Introduction 72
5.3. Material and methods 73
5.4. Results 77
5.5. Discussion 85
5.6. Conclusions 87
CHAPTER 6 89
Effect of feeding coarse maize on productive performance, gizzard development and energy utilisation in laying hens 89
6.1. Abstract 89
6.2. Introduction 89
6.3. Materials and methods 90
6.4. Results 94
6.5. Discussion 104
6.6. Conclusions 105

CHAPTER 7 106

Influence of method of wheat inclusion and pellet diameter on performance, nutrient utilisation, digestive tract measurements, carcass characteristics and caecal microflora counts of broilers 106

7.1. Abstract 106
7.2. Introduction 107
7.3. Material and methods 108
7.4. Results 111
7.5. Discussion 126
7.6. Conclusions 130

CHAPTER 8 131

Influence of whole wheat feeding on the development of coccidiosis in broilers challenged with *Eimeria* 131

8.1. Abstract 131
8.2. Introduction 131
8.3. Material and methods 133
8.4. Results 138
8.5. Discussion 144
8.6. Conclusions 146

CHAPTER 9 148

GENERAL DISCUSSION 148

9.1. Introduction 148
9.2. Use of whole and coarse maize in broiler diets 149
9.3. Use of coarse maize in layer diets 149
9.4. Method of whole wheat inclusion (pre- and post-pelleting) 150
9.5. Particle size distribution in pelleted feeds 151
9.6. Effect of particle size on pellet quality. 151
9.7. Whole grain feeding and gizzard development 152
9.8. Influence of whole grains on nutrient and energy utilisation 153

9.9. Whole grain feeding and gut microflora ecology. 157
9.10. In vitro excreta gas production.. 158
9.11. Whole grain feeding and coccidiosis. 158
9.12. Summary and main conclusions 160
REFERENCES 162

List of Tables

Chapter 2

Table 2.1. Influence of whole wheat feeding given in free choice feeding on performance and gizzard weight of broilers.. 8

Table 2.2. Influence of pre-pelleting inclusion of whole grains in mixed feeding systems on performance and gizzard weight of broilers 11

Table 2.3. Influence of post-pelleting inclusion of whole grains in mixed feeding systems on performance and gizzard weight of broilers 13

Table 2.4. Influence of whole grain inclusion in mash mixed feeding systems on performance and gizzard weight of broilers 16

Table 2.5. Influence of sequential feeding (SF) on performance and gizzard weight of broilers 18

Table 2.6. Differential characteristics of *E. acervulina, E. maxima and E. tenella*.......... 40

Chapter 4

Table 4.1. Composition and calculated analysis of the starter diet.................................. 56

Table 4.2. Influence of inclusion of graded levels of whole maize on the pellet durability index (PDI, %) and pellet hardness (Newton) 61

Table 4.3. Influence of pre-pelleting inclusion of graded levels of whole maize on weight gain (g/bird), feed intake (g/bird), and feed per gain (g/g) of broilers from 1-21 days posthatch 62

Table 4.4. Influence of pre-pelleting inclusion of graded levels of whole maize on AME (MJ/kg DM) and ileal nitrogen and starch digestibility coefficients for broilers 63

Table 4.5. Relative weights of proventriculus, gizzard and digestive organs (pancreas, liver and spleen) of broilers as influenced by pre-pelleting inclusion of graded levels of whole maize 64

Table 4.6. Relative weight and length of the intestinal tract of broilers as influenced by pre-pelleting inclusion of graded levels of whole maize 65

Table 4.7. Influence of pre-pelleting inclusion of graded levels of whole maize on caecal microflora counts (Log 10 cells/g caecal content) in broilers 66

Table 4.8. Influence of pre-pelleting inclusion of whole maize on pH of digesta from various segments of digestive tract and excreta of broilers 67
Table 4.9. Influence of pre-pelleting inclusion of whole maize on excreta gas production and digesta passage rate in broilers 67

Chapter 5

Table 5.1. The composition and calculated analysis of the finisher diet (g/kg as-fed basis) 74
Table 5.2. Influence of graded levels of coarse maize feeding on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) for broilers (11-35 days post-hatch) 78
Table 5.3. Influence of graded levels of coarse maize feeding on AME (MJ/kg DM) and, ileal nitrogen and starch digestibility coefficients in broilers 79
Table 5.4. Influence of graded levels of coarse maize feeding on carcass recovery (g/kg body weight), breast meat yield (g/kg body weight) and abdominal fat (g/kg body weight) of broilers 80
Table 5.5. Relative weight of digestive organs (proventriculus, gizzard, pancreas, liver and spleen) of broilers as influenced by graded levels of coarse maize inclusion 81
Table 5.6. Relative weight and length of the intestinal tract of male broilers as influenced by graded levels of coarse maize inclusion 82
Table 5.7. Influence of feeding graded levels of coarse maize on bacterial count (Log 10 cells/g of caecal content) for broilers 83
Table 5.8. Influence of graded levels of coarse maize feeding on pH of digesta from various segments of digestive tract of broilers 84
Table 5.9. Influence of different levels of coarse maize feeding on excreta gas production (ml/g) and feed passage rate (min) of broilers 84

Chapter 6

Table 6.1. Ingredient and nutrient composition (g/kg as fed basis) of diets 91
Table 6.2. Hen-day egg production of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize 96

Table 6.3. Feed intake of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize 97
Table 6.4. Egg weight of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize 98
Table 6.5. Feed efficiency of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize 99
Table 6.6. Egg mass of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize 100
Table 6.7. Shell defects in eggs of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize 101
Table 6.8. Body weight of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize 102
Table 6.9. Apparent metabolisable energy (AME; MJ/kg DM), Relative gizzard weight (g/kg body weight) and egg quality parameters[3] of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize 103

Chapter 7

Table 7.1. Composition and calculated analysis of the finisher diet (g/kg as fed basis) 109
Table 7.2. Influence of method of wheat inclusion and pellet diameter on the durability index (%) of pellets 113
Table 7.3. Influence of method of wheat inclusion and pellet diameter on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broilers, 11-35 days post-hatch 114
Table 7.4. Influence of method of wheat inclusion and pellet diameter on AME(MJ/kg DM) and apparent ileal nitrogen and starch digestibility coefficients for broilers 116
Table 7.5. Influence of method of wheat inclusion and pellet diameter on carcass recovery (g/kg body weight) and abdominal fat (g/kg body weight) for broilers........... 117
Table 7.6. Relative weight of digestive organs (proventriculus, gizzard, pancreas, liver and spleen) and gizzard contents of broilers as influenced by the method of wheat inclusion and pellet diameter 119
Table 7.7. Relative weight and length of different segments of the intestinal tract of broilers as influenced by method of wheat inclusion and pellet diameter 120

Table 7.8. Influence of method of wheat inclusion and pellet diameter on bacterial counts (Log 10 cells/g caecal content) in broilers 122
Table 7.9. Influence of method of wheat inclusion and pellet diameter on pH of digesta from segments of digestive tract and excreta of broilers 124
Table 7.10. Influence of method of wheat inclusion and pellet diameter on excreta gas production and digesta transit time of broilers 126

Chapter 8

Table 8.1. Composition and calculated analysis of basal diet (g/kg as-fed basis).......... 134
Table 8.2. Influence of method of wheat inclusion on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broilers (1 to 21 post-hatch)... 138
Table 8.3. Influence of method of whole wheat inclusion and coccidian infection on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broilers (21-28 days post-hatch).. 139
Table 8.4. Influence of method of wheat inclusion following coccidial challenge on the mortality (%) of broilers (7 days post-challenge) 140
Table 8.5. Relative weight (g/kg body weight) of organs (proventriculus and gizzard) and gizzard contents of broilers as influenced by the method of whole wheat inclusion and coccidial challenge 141
Table 8.6. Influence of method of wheat inclusion and coccidial challenge on intestinal lesion scores of broilers (at 7 days post-challenge) 142
Table 8.7. Influence of method of wheat inclusion and coccidial challenge on oocyst output (Log_{10} per g excreta) of broilers (at 7 days post-challenge) 143
Table 8.8. Influence of method of wheat inclusion and coocidial challenge on *in vitro* sporulation rate (%) of broilers (at 7 days post-challenge)... 143

Chapter 9

Table 9.1. Regression analysis to predict relationships between gizzard weight (G-WT), pancreas weight (PAN-WT), nitrogen digestibility (N), starch digestibility (S) and AME in broilers fed whole maize... 155

Table 9.2. Regression analysis to predict relationships between gizzard weight (G-WT), pancreas weight (PAN-WT), nitrogen digestibility (N), starch digestibility (S) and AME in broilers fed whole wheat 156

List of Figures

Chapter 2

Figure 2.1. Diagrammatic representation of different methods employed for feeding of whole grain in poultry 5

Figure 2.2. Maize kernel 34

Figure 2.3. Light micrograph of maize kernel endosperm matrix. PM, protein matrix; PB, protein bodies; SC, starch cavity; CW, Cell wall 36

Figure 2.4. Generalised life cycle chart of *Eimeria* 42

Chapter 4

Figure 4.1. Pellets produced with different pre-pelleting inclusion levels of whole maize and the whole maize used in the experiment 59

Figure 4.2. Particle size distribution of pelleted diets containing 0, 150, 300, 450 and 600 g/kg whole maize obtained by wet sieving method 60

Chapter 5

Figure 5.1. Particle size distribution of diets containing 0, 150, 300, 450 and 600 g/kg coarse maize (CM) obtained by the dry sieving method 77

Chapter 6

Figure 6.1. Particle size distribution of diets containing 0, 150, 300, 450 and 600 g/kg coarse maize obtained by the dry sieving method 94

Chapter 7

Figure 7.1. Particle size distribution of pelleted diets obtained by the wet sieving method 112

List of Abbreviations

AGP	Antibiotic growth promoters
AME	Apparent metabolisable energy
ANOVA	Analysis of variance
CM	Coarse maize
DM	Dry matter
FCF	Free choice feeding
FISH	Fluorescence in situ hybridisation
g	Gram
GE	Gross energy
GMD	Geometric mean diameter
GSD	Geometric standard deviation
GW	Ground wheat
h	Hours
HCL	Hydrochloric acid
kg	Kilogram
MF	Mixed feeding
Min	Minutes
MJ	Mega joule
mm	Millimetre
µm	micrometre
N	Nitrogen
NSP	Non starch polysaccharide
PDI	Pellet durability index
PP	Post-pelleting
PRP	Pre-pelleting
SF	Sequential feeding
Ti	Titanium
WW	Whole wheat

CHAPTER 1

General introduction

Feed is the single largest cost item in poultry production, accounting for 65-70% of total expenditure. Cereal grains are the major constituents of poultry diets and are generally ground and mixed into a single homogenised feed. Grinding of whole grains is the second largest energy cost after pelleting in broiler production (Reece *et al*., 1985) and is probably the highest user of energy in layer production where feeds are not pelleted (Deaton *et al*., 1989).

Whole grain feeding, through the reduction of energy consumption for grinding, could significantly lower the feed cost. Furthermore, this mode of feeding has not only shown positive effects on the performance of broilers, gut development and utilisation of feed nutrients but also meets consumer demands for a 'natural' feeding system and improved bird welfare (Gabriel *et al*., 2008). These beneficial effects have been attributed to the influence of whole grain feeding on the development and functionality of the gizzard. For these reasons, whole grain feeding has received renewed attention in the commercial poultry industry and is being increasingly used in many parts of world, especially in Europe, Australasia and Canada.

Feeding whole grains to poultry is not a new concept and was standard practice 50-60 years ago (Ewing, 1951). Despite this, research on whole grain feeding has been limited. Theoretically, there is every reason to believe that feeding whole grain is of potential value, but published data on the effects of whole grain on the performance of broilers have been contradictory. The results from feeding trials have been confounded by a number of factors, including differences in experimental methodology and variables such as type and quality of grain used, inclusion level of whole grain, the age of the birds and feeding regime.

Wheat is the cereal grain of choice for whole grain feeding. Despite maize being the most commonly used cereal grain in poultry diet formulation world-wide, little attempt has been made to feed whole maize to poultry. The size of maize grain may be the major reason for the lack of interest in feeding whole maize, although pre-pelleting inclusion or minor modifications such as cracking or coarser grinding could overcome this issue. These grinding and pelting processes may influence the digestibility of nutrients, especially of starch, and overcome issues related to digestibility of whole maize in monogastric animals. However, there have been no studies examining pre-pelleting inclusion of whole maize. Consequently, other alternative options need to be evaluated to utilise whole maize in poultry diets and to investigate its effect on digestive tract parameters, nutrient digestibility and performance of broilers. While whole grain feeding is becoming a common practice in broiler production, this application has not yet been established in layer feeding and only limited numbers of studies have been conducted with the layer as a model.

Pelleting, while moulding mash diets to macro-particles in the form of pellets, simultaneously reduces the size of micro-particles that constitute the intact pellet (Svihus *et al*., 2010b). Pellet die with a larger diameter could minimise such particle size reduction of whole grain and may further enhance the beneficial effects associated with pre-pelleting inclusion of whole grain. However, there have been no studies examining the interaction between pellet diameter and method of whole wheat inclusion in broiler chickens.

Studies have shown that the dietary inclusion of whole grain encourages the colonisation of commensal bacteria and discourage the proliferation of harmful bacteria (Bejerrum *et al*., 2005; Gabriel *et al*., 2008) and may have a beneficial effect on the prevention of coccidiosis (Cumming, 1989). The ban on the use antibiotic growth promoters (AGP) and the proposal to phase out coccidiostats as feed additives in the European Union (Regulation 1831/2003/EC; COM, 2008) have opened new dimensions of research on whole grain feeding to naturally and economically counteract coccidial-challenge and explore viable alternatives to AGPs and coccidiostats. Cumming (1989) proposed that increased gizzard size and grinding action, brought about by feeding whole wheat, might be responsible for the physical destruction of oocysts prior to reaching their sites of infection. However, beneficial effects of whole grain feeding on coccidiosis have not been confirmed in other studies (Gabriel *et al*., 2003b; 2006). Further studies are warranted to test the gizzard hypothesis and the effect of whole wheat on coccidiosis.

A total of nine chapters are reported in this thesis. The first two chapters present the frame work for the experimental research, with Chapter 1 giving a general introduction to the thesis. Chapter 2 reviews the methods of whole grain feeding and its influence on gizzard development, nutrient utilisation and performance parameters. It also highlights the various factors responsible for inconsistency in responses to whole grain feeding. The general materials and methods employed in the experimental work reported in the thesis are described in Chapter 3. Chapters 4 to 8 present the experimental work of this thesis. Each chapter includes an abstract, introduction, materials and method, results, discussion and conclusions. The objectives of the experiments conducted in this thesis include,

1. To investigate the influence of pre-pelleting inclusion of whole maize on performance, nutrient utilisation, digestive tract measurements and caecal microflora counts of young broilers (Chapter 4).
2. To investigate the influence of inclusion of coarse maize on performance, nutrient utilisation, digestive tract measurements, carcass characteristics and caecal microflora counts of broilers (Chapter 5).
3. To investigate the effect of feeding coarse maize on productive performance, gizzard development, energy utilisation and egg quality parameters in laying hens (Chapter 6).
4. To investigate the influence of whole wheat inclusion and pellet diameter on the performance, nutrient utilisation, caecal microflora, digestive tract measurements and carcass characteristics of broilers (Chapter 7).
5. To investigate the influence of whole wheat feeding on the development of coccidiosis in broilers challenged with *Eimeria* (Chapter 8).

Chapter 9 is the general discussion of the experimental results. This chapter addresses the major findings and draws conclusion and suggestions for future research.

CHAPTER 2
Review of Literature

2.1. Whole wheat feeding: Methodology and implication on the performance of poultry

2.1.1. Introduction

In recent years, whole grain feeding has received renewed attention in the scientific community and commercial poultry industry, and is being increasingly used in many parts of world, especially Europe, Australasia and Canada. The primary aim is to reduce feed costs by eliminating the grinding step. Furthermore, this also meets consumer demands for a natural feeding system and improved animal welfare (Gabriel *et al*., 2008). Whole grain feeding is thought to encourage colonisation of commensal bacteria and discourage proliferation of harmful bacteria that cause diseases or compete for nutrients in the distal intestinal tract. It has also been reported to have beneficial effects on prevention of coccidiosis (Cumming, 1989). These beneficial effects have been attributed to the influence of whole grain feeding on the development and functionality of the gizzard. Published data on the effects of whole grain feeding on performance of broilers, however, have been contradictory, with some reports showing beneficial effects, while others failing to show any advantages. The discrepancy among published reports is due to a number of confounding factors, including differences in experimental methodology, inclusion level of whole grain, type and quality of grain, age of birds, and feeding regime. Moreover, most published data are based on whole wheat and data on other grains are scarce. Despite maize being the most commonly used cereal grain in poultry diets worldwide, little attempt has been made to use whole maize in poultry diets. The aim of this chapter is to review the available data on the influence of whole wheat feeding on various aspects of production performance and to highlight alternate feeding strategies for the utilisation of whole maize in poultry diets. Factors responsible for variable responses with whole grain feeding and the potential of this strategy as a substitute for antibiotic growth promoters and coccidiostats are also discussed.

2.1.2. Methods of whole grain feeding

The three methods generally employed for feeding of whole grains (Rose *et al*., 1995) are free choice feeding (FCF; *ad libitum* choice of the whole grain with another feed in separate feeders), mixed feeding (MF; whole grain mixed with another, often pelleted or compound feed) and sequential feeding (SF; whole grain and another feed in the same feeder, but at different times).

In these feeding regimes, whole grain can be given along with another feed, either in mash or pellet form, in one of three options: (a) protein concentrate (part of a complete diet provided as a concentrate to balance nutrients provided by the whole grain); (b) balancer diet (part of a complete diet other than whole grain, rich in all nutrients except carbohydrate); or (c) complete diet. Provision of whole grain in pellet form in MF can be further sub-divided into two categories, namely pre-pelleting (PRP; whole grain is first mixed with other feed components and then pelleted) and post-pelleting (PP; first other components of feed are mixed and pelleted, and whole grain is then mixed with pelleted feed). Except for PRP, all these feeding systems allow partial control of proportions of whole grain and protein concentrate actually ingested by birds. Figure 2.1 depicts the different methods of adding the whole grain in poultry diets.

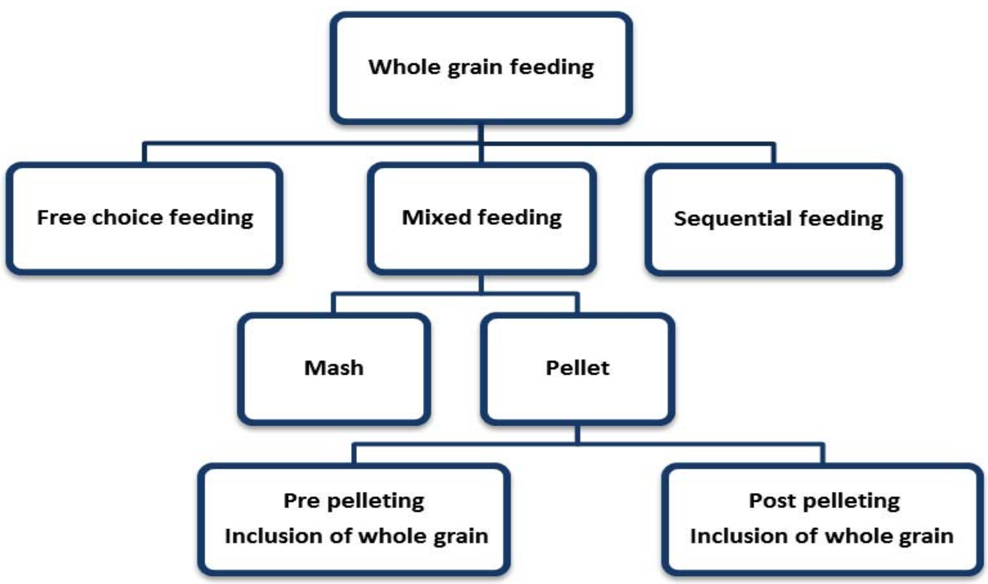


Figure 2.1. Diagrammatic representation of different methods employed for feeding of whole grain in poultry

2.1.2.1. Free choice feeding

In FCF, birds are usually offered a choice among three types of feedstuffs: an energy source, i.e., cereal grains; a protein source, e.g., soybean meal, fish meal, meat meal, etc., plus supplemental vitamins and minerals; and for laying hens, a calcium source, e.g., shell grit. The basic principle behind FCF is that individual birds are capable of consuming the required nutrients from various feed ingredients to compose their own diets according to their actual needs and production capacity. This ability, referred to as ‘nutritional wisdom’ (Forbes and Shariatmadari, 1994), is not possible when a single mixed feed is given. However, in this feeding regime, birds have a choice between the whole grain and another feed (protein concentrate, balancer or complete diet). Interest in FCF may increase in the future as a result of current concerns over welfare of caged birds and the consequent move towards use of barn and free range production systems. Birds in these latter systems are likely to have a greater range of requirements which will depend on the amount of exercise they take (Henuk and Dingle, 2002). FCF is also likely to provide an effective means whereby home-grown whole grains can be used, thus lowering transport costs in addition to lowering cost of grinding and mixing. Furthermore, with the FCF system, simple engineering techniques can be employed for feed mixing on farm (Feltwell, 1992).

The theory is that birds given a choice are able to formulate a balanced ration on their own using ‘nutritional wisdom’, but this is not always the case. A practical limitation of FCF in large poultry units is that it requires extra equipment because whole wheat and a balancer or complete diet has to be provided *ad libitum* in separate feeders. Rose *et al*. (1995) pointed out that FCF birds usually select a diet that gives rapid growth, but occasionally they will eat too much balancer and not enough whole wheat, resulting in higher feed costs.

Forbes and Covasa (1995) speculated that while birds will self-select the grain and protein concentrate, some factors can interfere with these choices. Feed form and shape are important, with birds preferring to eat larger particles as they grow older. Broiler chickens fed a mixture of mash supplement and whole grain will tend to eat the whole grain first and leave behind the mash to be eaten later. The larger, easier to manipulate grain is preferred, especially in finisher diets. Feeder design, number of birds in a pen, learning time, previous experience, and nutritional contents of the protein concentrate or balancer diet are other factors affecting eating preferences (Rose *et al*., 1986; Mastika and Cumming, 1987; Forbes and Covasa, 1995).

Published data evaluating FCF are limited and contradictory (Table 2.1). Whole wheat given in FCF resulted in increased relative weight of the gizzard, irrespective of the form of other feed (pellet or mash). Gabriel *et al*. (2003a) found no effect of whole wheat on the performance of broilers when offered free choice plus a pelleted complimentary diet. Gabriel *et al*. (2008) reported significant improvements in body weight with no effect on feed intakes and feed efficiency when whole wheat was given in FCF, with a complimentary feed in mash form. In contrast, Amerah and Ravindran (2008) reported significantly decreased body weights and feed intake, but found no effect on feed efficiency. Similarly, decreased body weight was reported when whole wheat was offered with a balancer pellet (Rose *et al*., 1995).

Table 2.1. Influence of whole wheat feeding given in free choice feeding on performance and gizzard weight of broilers

Reference	Form of other feed	Inclusion rate (g/kg)	Age (days)	% Improvement[1]			% Increase in gizzard weight
				Weight gain	Feed intake	Feed per gain	
Rose *et al*. (1995)	Balancer pellet	*Ad libitum*	24-45	-5.62*	-13.14*	-7.69	Not reported
Erener *et al*. (2003)	Standard compound feed	*Ad libitum*	7 -42	+3.61	+7.71*	+3.87	+26.0*
Gabriel *et al*.(2003a)	Pellet	400g	7-29	-7.62	0	0	+101.2**
Gabriel *et al*. (2008)	Mash	200g (1-14d) 300g (15-21d) 400g (22-44d)	8-44	+4.25 *	-0.91	-4.92	+25.8***
Amerah and Ravindran (2008)	Pellet	600-690g	7-35	-15.13*	-13.83*	+1.20	Unspecified increase

$^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$. [1] Improvement over ground grain = ((Whole grain - Control)/Control) x 100.

2.1.2.2. Mixed feeding

In MF, whole grain is either substituted for a part of the ground grain in a complete diet or added to a complete diet in the same feeder at the same time in pellet or mash form. With the former method, the only change is in the form of grain (ground or whole) and nutrient density of finished feed is unaltered. The latter method of adding whole grain to a complete diet results in a dilution of nutrients in feed, but published data on this feeding method are scant. Whole grain inclusion in MF controls the proportion of whole grain and protein concentrate actually ingested by birds by allowing limited partial selection of feed ingredients differing in form, but such selection can be eliminated by pelleting the diet after whole grain and complete diet are mixed (i.e. pre-pelleting).

Feed given in pelleted form as compared to mash is known to improve weight gain, feed intake, and feed efficiency in broilers regardless grain source (Douglas *et al*., 1990; Nir *et al*., 1995; Jensen, 2000; Nir and Pitchi, 2001). These improvements have been attributed *inter alia* to elevated nutrient density, increased nutrient intake, reduced feed wastage, and decreased energy spent eating (Calet, 1965; Jensen, 2000). However, the process of grinding is estimated to require 20 kWh/tonne of grain and further processing of feed by pelleting uses another 20kWh/tonne of feed produced. Furthermore, additional energy is required to generate steam for pelleting (Petersen, 1976). Because pelleting requires a large input of electrical energy, it adds approximately 10% to the total feed cost (Cheeke, 1999). It can be thus readily appreciated that feeding whole grain in MF produces considerable savings over a system that uses conventional feeding methods based on ground grains as mash or pellet (Karunajeewa, 1978). Another advantage of MF is that it allows for simple management without investment on additional feeders and manpower. Even with whole grain inclusion, an almost homogenous feed similar to that of a conventional, finely ground diet is assured. However, literature on the effects of MF on the performance of poultry is limited and the results show considerable inconsistency (Table 2.2 to 2.4).

2.1.2.2.1. Pre-pelleting inclusion of whole grain

Results of studies examining the effects of pre-pelleting inclusion of whole wheat have produced equivocal results (Table 2.2). Wu *et al*. (2004) found that pre-pelleting inclusion of 200 g/kg whole wheat improved feed per gain, but had no effect on gizzard weight. On the other hand, Jones and Taylor (2001) and Taylor and Jones (2004a), using the same level of whole wheat inclusion, found no effect on broiler performance, but relative gizzard weights were increased by 11 and 8%, respectively. Similarly Svihus *et al*. (2004a), with inclusion of 500 g/kg whole wheat pre-pelleting, found that relative gizzard weight increased by 26% without any effect on the performance parameters of broilers. In contrast to wheat, Jones and Taylor (2001) reported that inclusion of 200 g/kg whole triticale improved feed efficiency with significant increases in gizzard weight. These reports indicated that inclusion level and type of whole cereal may be responsible, in part, for the inconsistent results. Further studies are required to understand the factors responsible for the variable effects of pre-pelleting inclusion of whole wheat on performance and gizzard development of broilers.

Table 2.2. Influence of pre-pelleting inclusion of whole grains in mixed feeding systems on performance and gizzard weight of broilers

			% Improvement[1]			
Reference	Inclusion rate (g/kg)	Age (days)	Weight gain	Feed intake	Feed per gain	% Increase in gizzard weight
Jones and Taylor (2001)	200g (triticale)	5-42	-0.14	NR[2]	-2.29 *	+11.0*
	200g (wheat)	5-42	+0.40	NR	+1.75	+10.7*
Taylor and Jones (2004a)	200g	5-42	-1.56	NR	0	+7.79**
Wu *et al*. (2004)	200g	0 -21	+ 0.12	- 0.16	- 4.09*	0
Svihus *et al*. (2004a)	500g	11-25	+1.46	-0.16	+1.43	+26.6***

*P<0.05; **P<0.01; ***P<0.001. [1] Improvement over ground grain = ((Whole grain - Control)/Control) x 100. [2] Not reported.

2.1.2.2.2. Post-pelleting inclusion of whole grain

Most studies involving MF have evaluated post-pelleting inclusion of whole wheat and all report increased gizzard weight with no adverse effects on weight gain of broilers (Table 2.3). The only exception was that of Ravindran *et al.* (2006) who reported significantly lower weight gain in broilers. In general, this method of whole wheat inclusion either had no effect or decreased feed intake, but the response on feed efficiency was variable between studies. It is interesting to note that post-pelleting inclusion of whole wheat when substituted for a part of the ground wheat in complete diets showed either no effect (Hetland *et al.*, 2002, 2003; Svihus *et al*., 2004a) or beneficial effects on feed per gain in broilers (Wu *et al*., 2004; Wu and Ravindran, 2004; Ravindran *et al*., 2006; Amerah and Ravindran, 2008). In studies where whole wheat was substituted for part of a commercial pelleted diet, negative effects on feed per gain were observed (Bennett *et al.,* 1995; Bennett *et al*., 2002; Plavnik *et al.*, 2002). These findings indicate that addition or substitution of whole grain to a complete diet leads to dilution of nutrients, adversely affecting the performance of broilers. Rose *et al.* (1995) suggested that if high proportions of whole wheat are given then the nutrient composition of the pelleted diet needs to be rich in all nutrients except carbohydrates. This is often termed as a 'balancer' diet. In the study of Rose *et al.* (1995) where whole wheat was substituted for part of a balancer pelleted diet that contained twice the concentration of minerals and vitamins than the control diet, there were no adverse effects on any of the performance parameters of broilers.

Table 2.3. Influence of post-pelleting inclusion of whole grains in mixed feeding systems on performance and gizzard weight of broilers

Reference	Inclusion rate (g/kg)	% Improvement[1]				% Increase in gizzard weight
		Age (days)	Weight gain	Feed intake	Feed per gain	
Bennett *et al*. (1995)[a]	50g (11-21d),100g (22-31d),150g (32-41d) + standard commercial diet	11-41	-2.84	NR	+0.11	NR
	50g (11-21d),150g (22-31d),225g (32-41d) + standard commercial diet	11-41	-2.58	NR	+1.16	NR
	50g (11-21d),200g (22-31d),300g (32-41d) + standard commercial diet	11-41	-2.70	NR	+3.20*	NR
Rose *et al*. (1995)[b]	400g (24-30d), 500g (31-37d) and 600g (38-45d) + balancer diet	24-45	0.00	-0.32	-0.51	NR
Uddin *et al*. (1996)[a]	150g (24-33d) and 300g (33-44d) + Standard commercial diet (Riband variety)	24-42	-1.73	+1.28	+3.27	NR
	150g (24-33d) and 300g (33-44d) + Standard commercial diet (Haven variety)	24-42	-2.55	+1.14	+3.90	NR
Preston *et al*. (2000)	333g	14-42	-4.64	-8.15 DM intake	+3.33	+47.0***
Bennett *et al*. (2002)	0g (0-6d),50g (7-13d),200g (14-27d) and 300g (28-48d)	0-48	-1.00	NR	+1.70*	+18.2**
	0g(0-6d),50g(7-13d),350 g(14-27d) and 500g(28-48d)	0-48	-1.84	NR	+2.96*	+37.2***
	50g(0-6d),200g(7-13d),350g(14-27d) and 500g(28-48d)	0-48	-1.53	NR	+2.36*	+28.4***

Table 2.3. continued

Bennett *et al*. (2002)	0g (0-6d),50g(7-13d),500g(14-27d) and 650g(28-48d)	0-48	-1.77	NR	+4.12*	+36.5***
	50g (0-6d),200g(7-13d),500 g(14-27d) and 650g(28-48d)	0-48	-2.70	NR	+4.57*	+33.5***
Plavnik *et al*. (2002)[a]	250 g (21-45d)+ Standard commercial diet	21-45	-0.37	-4.57*	+4.46*	+30.9*
	50g (1-21d) and 150g (21-45d) + Standard commercial diet	21-45	-1.98	-5.32*	+3.65*	+23.2*
Hetland *et al*. (2002)	300g (10-29d) and 440g (29-38d)	10-38	-5.21	-10.02	+7.14	+85.7***
	125g (10-29) and 300g (29-38d)	10-38	-5.64	-8.99	+5.36	+100.0***
Hetland *et al*. (2003)	385g	11-33	+5.13	+3.05	-1.56	+35.4*
Svihus *et al*. (2004a)	375g	11-20	-2.14	-1.34	-1.45	NR
Wu *et al*. (2004)	200g	0-21	+2.12	- 3.95*	- 5.85*	+73.2*
Wu and Ravindran (2004)	100g (1-21d) and 200g (22-35d)	1-35	-2.63	-6.33***	-3.33**	+43.0***
Ravindran *et al*.(2006)	100g (1-21d) and 200g (22-35d)	1-35	-6.78*	-10.56*	-4.27*	+50.0*
Amerah and Ravindran (2008)	100g (1-21d) and 200g (22-35d)	1-35	-2.21	-3.02	-1.80	Unspecified increase

*P<0.05; **P<0.01; ***P<0.001. [1] Improvement over ground grain = ((Whole grain - Control)/Control) x 100. [2] Not reported.

[a] Whole grain added to the complete diet.

[b] Whole grain added to balancer diet

2.1.2.2.3. Mixed feeding of whole grain in mash feeds

Mixed feeding studies where whole wheat was included in mash feed have resulted in significant increases in body weight gain in broilers with no effect on feed intake, but responses in feed per gain and gizzard weight were variable (Table 2.4). Plavnik *et al.* (2002) reported that inclusion of 200 g/kg whole wheat with mash feed resulted in increased gizzard weight and improvements in weight gain and feed per gain of broilers. Low level inclusion of whole wheat (100 g/kg) resulted in similar performance responses, but failed to show any effect on gizzard weight. Similarly, Nahas and Lefrancois (2001) reported improved weight gain with inclusion of 100-350 g/kg whole wheat, but found no effect on gizzard weight of broilers.

Table 2.4. Influence of whole grain inclusion in mash mixed feeding systems on performance and gizzard weight of broilers

Reference	Inclusion rate (g/kg of feed)	Age (days)	% Improvement[1]			
			Weight gain	Feed intake	Feed per gain	% Increase in gizzard weight
Nahas and Lefrancois (2001)[a]	100g (7-21d) and 200g (22-38d)	7-38	+4.20**	+6.09	-2.00	+4.72
	100g (7-21d) and 350g (22-38d)	7-38	+5.15**	+2.24	+2.00	+0.79
	200g (7-38d)	7-38	+1.28**	+0.63	+0.17	+1.57
	200g (7-21d) and 350g (22-38d)	7-38	+2.01**	+0.58	+0.67	-0.79
Plavnik *et al*. (2002)	100g (0-46d)	6-46	+3.87**	-1.45	-4.74**	+0.66
	200g (0-46d)		+2.56**	-1.51	-3.68**	+10.0**
Erener *et al*. (2003)[a]	Standard compound feed + 5,10, 20, 30 and 40g /kg in weeks 2-6 respectively	7-42	-8.14*	+5.60	+14.36*	+26.0*

*P<0.05; **P<0.01; ***P<0.001.

[1] Improvement over ground grain = ((Whole grain - Control)/Control) x 100.

[a] Whole grain added to the complete diet.

2.1.2.3. Sequential feeding

In this feeding technique, birds are given time-limited *ad libitum* access to the whole grain followed by time-limited *ad libitum* access to a complete or balancer diet (Rose *et al*., 1995). Whole grain is offered to birds with a complete or balancer feed in the same feeder, but at different times. This feeding regime is based on the principle of choice feeding and allows birds to exercise their freedom of choice between whole grains and complete or balancer feed with restriction of time. Compared to FCF, this method is economical and efficient as it does not require offering food in two separate feeders. In addition, SF offers more control on amount of whole grain to be ingested by birds due to time restriction. However, SF remains to be validated in practice. Only two published reports are available on use of SF in poultry feeding (Table 2.5).

Rose *et al*. (1995) compared four different durations of access (4, 8, 12 and 24 h) to whole wheat and balancer diet. The balancer diet contained twice the concentration of the vitamin and mineral mixture than the complete diet as 500 g of ground wheat removed from 1 kg of complete diet and concentration of component in the remaining 500 g of mix were used to formulate the balancer diet. It was assumed that a bird that selected equal proportions of balancer and whole wheat will choose a diet with the same nutrient concentration as the complete diet. Whole wheat accounted for over 40% of the total food intake of broilers when sequential times of 8 h or more were used and 20% in the 4 h SF. Weight gain of broilers given SF was lowest in the 4 h feeding period and highest in the 8 h period. However, weight gain decreased linearly as SF period increased above 8 h. In a subsequent trial, these researchers compared 8 h SF of whole wheat and balancer diet with a complete single diet. It was found that weight gain and feed intake decreased in birds fed 8 h SF in comparison to those fed a complete single diet, but there was no effect on feed efficiency. In contrast, when Erener *et al*. (2003) offered the birds 18 h access to standard compound feed followed by 6 h access to whole wheat, there were no adverse effects on any performance parameters but an increase in gizzard weight was observed. These contradictory results may be, attributed to the amount of whole wheat ingested and, thus, the total nutrient intake.

Table 2.5. Influence of sequential feeding (SF) on performance and gizzard weight of broilers

Reference	Form of other feed	Inclusion rate (g/kg)	Age (days)	% Improvement[1] Weight gain	Feed intake	Feed per gain	% Increase in gizzard weight
Rose *et al*.(1995)	Pellet	8 h sequential of Balancer pellet [3] + Whole wheat *ad libitum*	24-45	-7.5*	-9.29*	-2.05	NR[2]
Rose *et al*. (1995)	Pellet	8 h sequential of Balancer pellet + Whole wheat *ad libitum*	24-45	-8.76*	-9.09*	0.00	NR
Erener *et al*. (2003)	Mash	Standard compound feed (18 h) + whole wheat (6 h) *ad libitum*	7-42	-4.00	+1.51	+5.52	+26*

*P<0.05.; **P<0.01; ***P<0.001.

[1] Improvement over ground grain = ((Whole grain - Control)/Control) x 100.

[2] Not reported.

[3] 500g of whole wheat removed from 1 kg of compete diet and concentration of nutrient in the remaining 500g of mixture used to formulate the balancer pellet.

2.1.3. Effect of whole wheat feeding on digestive tract characteristics

A rapid and conspicuous enlargement of the gizzard is observed when whole wheat is included in the diet, indicating that whole grain feeding influences development and, possibly, morphology and physiology of the gastrointestinal tract. The predominant hypothesis is that beneficial effects observed with whole wheat inclusion is mechanistically linked to gastrointestinal development, particularly gizzard development (Svihus *et al*., 2002; Gabriel *et al*., 2003a; Svihus, 2010a; Svihus, 2011). However, effects of whole wheat feeding on development of segments of the gastrointestinal tract other than the gizzard are inconsistent (Forbes and Covasa, 1995; Jones and Taylor, 2001; Banfield *et al*., 2002; Gabriel *et al*., 2003a; Taylor and Jones, 2004a; Engberg *et al*., 2004; Wu and Ravindran, 2004; Ravindran *et al*., 2006; Amerah and Ravindran, 2008; Gabriel *et al*., 2008).

2.1.3.1. Gizzard

The gizzard is a muscular, grinding organ made up of two muscles that reduce particle size of ingested foods and mixes them with digestive enzymes (Duke, 1986). Mechanical pressure applied in grinding by the gizzard may exceed 585 kg /cm^2 (Cabrera, 1994). However, in conventional feeding regimes where whole grain is ground before incorporation into feed, such grinding action is carried out by the feed mills. As a result, in contrast to birds fed diets containing whole wheat, ground-wheat-fed birds show dilation of the proventriculus and a relatively underdeveloped gizzard (Forbes and Covasa, 1995; Jones and Taylor, 2001; Gabriel *et al*., 2003a; Gabriel *et al.,* 2008). Thus, under conventional feeding regimes, the gizzard becomes a transit rather than a grinding organ (Cumming, 1994). When whole wheat is fed, there is development of the gizzard which has been attributed to increased frequency of contraction to reduce whole grains to fine particles (Hill, 1971; Roche, 1981). However, the amount of whole grain in the diet required to stimulate gizzard development is not known. In his latest review, Svihus, (2011) recommended that at least 200 g/kg cereal particles larger than 1.5 – 2.0 mm in size or at least 300 g/kg particles larger than 1 mm in size in diet are needed to stimulate gizzard development.

2.1.3.2. Proventriculus

Compared to whole wheat-fed birds, ground-wheat-fed birds showed a dilation of the proventriculus (Forbes and Covasa, 1995; Jones and Taylor, 2001; Gabriel *et al*., 2003a;

Gabriel *et al*., 2008). This dilation, however, did not necessarily result in any change in relative weight of this organ (Gabriel *et al*., 2003a; Gabriel *et al*., 2008). Taylor and Jones (2004a) reported reduced proventriculus proportional mass of 22 and 16%, respectively, in broilers fed diets containing either whole wheat or whole barley compared to those based on ground grains. Similarly, Jones and Taylor (2001) found a 14% reduction in relative proventriculus weight with inclusion of whole triticale in broiler diets. In contrast, some authors have failed to observe any change in relative weight of this organ with inclusion of whole wheat, (Banfield *et al*., 2002; Bennett *et al*., 2002; Gabriel *et al*., 2003a; Wu and Ravindran 2004; Ravindran *et al*., 2006; Amerah and Ravindran, 2008). Taylor and Jones (2004b) speculated that the absence of proventriculus dilation with whole wheat feeding may have positive effects on bird health by decreasing mortality due to ascites.

2.1.3.3. Pancreas

Inclusion of 100-200 g/kg whole wheat post-pelleting was reported to have no effect on relative weight of the pancreas (Ravindran *et al*., 2006; Amerah and Ravindran, 2008). In contrast, Wu *et al*. (2004) reported that pre-pelleting inclusion of whole wheat at the same level increased the relative weight of pancreas by 20%. Similarly, inclusion of 200-400 g/kg whole wheat was shown to increase relative pancreatic weight by Banfield *et al*. (2002), Engberg *et al*. (2004) and Gabriel *et al*. (2008). Reasons for these variable effects of whole grain feeding on pancreatic weight are unclear.

2.1.3.4. Small intestine

Most studies have shown no changes in the relative weight and length of intestinal segments with substitution of ground wheat for whole wheat (Preston *et al*., 2000; Jones and Taylor, 2001; Banfield *et al*., 2002; Engberg *et al*., 2004; Wu *et al*., 2004; Ravindran *et al*., 2006). However, changes in relative size of intestinal segments in response to whole wheat feeding have been observed in some studies. Gabriel *et al.* (2003a) reported 16% lower duodenal weight in birds fed whole wheat compared to those fed the ground wheat diet. Taylor and Jones (2004a) found increased duodenal length, but no differences in weight with 200 g/kg whole wheat inclusion in pelleted diets. Gabriel *et al*. (2008) found a 16% decrease in relative length of jejunum in broilers fed whole grains. Interestingly, Amerah and Ravindran (2008) reported whole wheat inclusion in MF had no effects on the relative weight and length of intestinal segments of broilers. In contrast, whole wheat in FCF resulted in increased weights of duodenum and ileum, and increased length of all intestinal segments compared to those fed the ground wheat diet. No explanation was provided for these findings.

2.1.3.5. Morphology and enzymatic activity of gastrointestinal tract

Wu *et al*. (2004) studied the effect of including 200 g/kg whole wheat on the morphology of gizzard and small intestine. No significant effects were observed on the thickness of the *tunica muscularis* layer of the gizzard or villus height, crypt depth, goblet cell number and epithelial thickness in the ileum. In addition, Gabriel *et al*. (2003a) reported that inclusion of whole wheat resulted in no changes at the cellular level; there were no differences in cell size, tissue activity, ribosomal capacity and rate of mitosis. Alkaline phosphatase activity expressed per unit of tissue weight was also not affected. In contrast, Gabriel *et al*. (2008) observed that whole wheat feeding from 8 to 44 days post-hatch resulted in increased duodenal villus to crypt length and surface ratios, due to lower crypt depth and numerically smaller crypt area. Alkaline phosphatase activity was higher in the duodenum and jejunum of whole wheat-fed birds. However, activities of leucine aminopeptidase and maltase were similar between whole and ground wheat based diets. It was speculated that with whole wheat, morphology of the upper part of the intestine improves and may contribute to better absorption of nutrients and their utilisation.

2.1.3.6. pH of the digesta

When birds were fed with coarse structured feed in comparison to finely ground feed, lower pH was recorded in the gizzard (Nir *et al*., 1994a) and proventriculus (Nir *et al*., 1995). Similarly, a significant reduction in the pH of gizzard contents of birds fed diets containing 200 g/kg whole wheat was reported by Gabriel *et al*. (2003a). However, Hetland *et al*. (2002) conducted an experiment with very high (500 g during 10-24 days and 600 g during 24-38 days), high (300 g during 10-24 days and 400 g during 24-38 days) and moderate (125 g during 10-24 days and 300 g during 24-38 days) replacement of ground wheat per kg of diet with whole wheat, barley and oats, and found that pH of gizzard contents was not conclusively affected by cereal type or form of the cereal. The effect of whole wheat feeding on pH of intestinal contents is also contradictory. Engberg *et al*. (2004) observed lower pH in duodenum and jejunum with whole wheat, whereas higher pH was reported in the duodenum with whole wheat by Gabriel *et al*. (2003a), but no effect on pH of jejunal or ileal digesta.

Taylor and Jones (2004a) reported no effect of whole wheat on the pH of contents from any intestinal segment.

2.1.4. Effect of whole grain feeding on feed passage rate

Passage rate is the time between when feed is ingested by the bird to when it is expelled as faeces. Passage rate of digesta is usually measured using an insoluble (solid phase) marker such as chromic oxide. In broiler chickens, solid phase markers appear in excreta 1.6 to 2.6 h after ingestion (Denbow, 2000), but 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 (Amerah *et al*., 2007). Passage rate of a non-structural marker is not dependent on diet structure (Svihus *et al*., 2002). Several other factors such as particle size and viscosity of digesta are known to affect the passage rate of solid phase markers. Svihus *et al*. (2002) hypothesised that rapid passage rate reduces time available for digestion and absorption, whilst slower passage rate limits feed intake. However, in general, larger particles are retained longer than finer particles in the gizzard (Nir *et al*., 1995; Denbow, 2000), prolonging the mean residence time. The proportion of coarse fibre in gizzard contents is double that present in feed, reflecting selective retention of coarse particles (Hetland *et al*., 2004; 2005) and slower digesta flow out of the gizzard. Amerah and Ravindran (2008) reported a three-fold (9.5 vs. 3.0 g/kg body weight) increase in gizzard contents of birds offered whole wheat as compared to those offered ground wheat. Moreover, coarse particles need to be ground to a certain critical size before they can leave the gizzard (Clemens *et al*., 1975; Moore, 1999). Such an effect would be expected to lengthen transit time for digesta when whole wheat is fed. Surprisingly, however, overall retention time does not increase when birds are fed whole grains (Svihus *et al*., 2002; Wu *et al*., 2004 Amerah *et al*., 2007; Amerah and Ravindran 2008). Rapid dissolution of starch granules and protein from whole grain in the low pH environment of the gizzard causes a rapid reduction of particle size and may be responsible for the lack of effect on passage rate, as speculated by Hetland *et al*. (2005). Xylanase supplementation with whole wheat is reported to reduce digesta viscosity (Yasar, 2003) and may influence feed passage rate**.** Wu *et al*. (2004), however, found no effect of xylanase supplementation on feed passage rate of birds fed diets containing whole wheat, but noted increased passage rate of those fed diets containing ground wheat.

2.1.5. Effect of whole grain feeding on digesta particle size

Hetland *et al*. (2004) stated that poultry can consume whole grains as the gizzard has a remarkable ability to grind seeds to a consistently fine size regardless of the original particle size. Hetland *et al*. (2003) reported that whole wheat inclusion had no effect on digesta particle size distribution in the duodenum. Similarly Svihus *et al*. (1997) did not find any differences in digesta particle size in the duodenum of birds fed either whole or ground barley. These studies supported the hypothesis that particles need to be ground to a certain critical size before they can leave the gizzard (Clemens *et al*., 1975; Moore, 1999). 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 (Hetland *et al*., 2002).

2.1.6. Effect of whole grain feeding on carcass characteristics

Bennett *et al*. (2002) reported that whole wheat inclusion (50, 200 and 350-650 g/kg whole wheat during 0-6, 6-13, and 27-48 days, respectively) in wheat-barley-based diets had no effect on carcass yield and abdominal fat pad weight of broilers. Similar results were reported by Wu and Ravindran (2004) and Plavnik *et al.* (2002). However, in a subsequent trial, Plavnik *et al.* (2002) found that adding 250 g/kg whole wheat in maize-wheat-based diets resulted in 12% greater relative weight of abdominal fat and 6% lower breast meat compared to diets containing ground wheat. Increases in abdominal fat of 5.3, 19, and 12% with whole grain inclusion have also been reported by Preston *et al*. (2000), Nahas and Lefrancois (2001) and Jones and Taylor (2001), respectively. In contrast, Amerah and Ravindran (2008) found that PP inclusion of whole wheat (100 and 200 g/kg whole wheat replacing ground wheat during 7-21 and 21-35 days, respectively) in wheat-soy diets significantly reduced carcass recovery by 4% and relative weight of abdominal fat by 16%, but there was no effect on breast meat yield. These conflicting results regarding the effects of whole wheat feeding may be attributed, in part, to an incorrect protein: energy ratio in the basal diet (Erener *et al*., 2003; Wu and Ravindran, 2004).

2.1.7. Effect of whole grain feeding on digesta viscosity

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 whole wheat diet (Yasar, 2003). In contrast, whole wheat feeding increased digesta viscosity and improved feed per gain (Engberg *et al*., 2004). Degree of digesta viscosity is dependent upon the amount of non-starch polysaccharide (NSP), which varies amongst cereals grains (Amerah *et al*., 2007). Digesta viscosity higher than 10 mPa·s limits bird performance by reducing passage rate and mixing of digestive enzymes with substrate nutrients (Bedford and Schulze, 1998). Negative effects of digesta viscosity can be largely overcome by the addition of exogenous glycanase enzymes (Bedford and Schulze, 1998). Wu *et al*. (2004) compared pre- and post-pelleting methods of inclusion of whole wheat and found increased viscosity of digesta in the duodenum and jejunum of birds fed with diets containing 200 g/kg whole wheat, irrespective of method of whole wheat inclusion, compared to those fed diets containing ground wheat. However, xylanase supplementation caused a significant reduction in digesta viscosity in all intestinal segments. Similar results were reported by Taylor and Jones (2004a) with pre-pelleting inclusion of 200 g /kg whole wheat supplemented with enzyme. However, neither pre-pelleting inclusion of 200 g/kg whole barley in their study nor enzyme supplementation altered viscosity of digesta beyond the duodenum. It was speculated that the presence and activity of endogenous enzymes may have contributed to this observation with whole barley. Petersen *et al.* (1999) stated that NSP associated with viscosity are not degraded by digestive enzymes and that endogenous grain enzymes may exert some effect. The additive effect of whole grain with exogenous enzyme could be attained due to greater grinding activity of a larger and more developed gizzard in birds fed whole grain leading to enhanced mixing of substrate with enzymes. However, published data on the effect of whole grain feeding with use of exogenous glycanases on digesta viscosity are limited

2.1.8. Effect of whole grain feeding on nutrient utilisation

As discussed above, whole wheat feeding is generally associated with an increase in gizzard size and it has been hypothesised that the resultant increase in grinding activity will favourably influence the bird's ability to better utilise nutrients (Svihus and Hetland, 2001). In addition to the grinding effect, an active gizzard also serves as a mixing compartment for digestive juices and substrates. Furthermore, large fibre particles seem to enhance digesta motility and backflow within the gastrointestinal tract (Williams *et al.,* 1997). These effects are consistent with results of several studies showing improvement in apparent metabolisable energy (AME) and starch digestibility with the inclusion of whole grain in broiler diets (Svihus *et al*., 2004a; Wu *et al*., 2004).

2.1.8.1. Apparent metabolisable energy

McIntosh *et al.* (1962) reported that unground wheat yielded 5 to 10% more metabolisable energy than ground wheat. Wu *et al.* (2004) compared pre and post- pelleting inclusion of 200 g/kg whole wheat in broiler diets and reported improvements in AME irrespective of method of whole wheat inclusion. Post–pelleting inclusion, however, resulted in 6% greater improvement in AME than pre-pelleting inclusion. Similarly, Svihus *et al.* (2004a) reported that AME was increased at both day 14, and 20 when 375 g/kg whole wheat instead of ground wheat was included pre-pelleting. However, in a subsequent experiment with pre-pelleting inclusion of 500 g/kg whole wheat, no effect on the AME was observed. Preston *et al*. (2000) also reported an increase in AME when ground wheat was replaced by whole wheat, whereas Uddin *et al*. (1996) found no differences when two wheat cultivars were fed ground or whole at different levels (100- 400 g/kg) to broiler chickens from 19 to 27 days.

2.1.8.2. Starch digestibility

Some studies have shown that inclusion of whole wheat resulted in improved starch digestibility (Svihus and Hetland, 2001; Hetland *et al*., 2003; Svihus *et al*., 2004a). Svihus *et al*. (2004a) reported that post-pelleting inclusion of 375 g/kg whole wheat increased the digestibility of starch at both the ileal and excreta levels. However, a subsequent experiment using pre-pelleting replacement of ground wheat with 500 g/kg whole wheat failed to show any improvement in starch digestibility. Svihus (2006) hypothesised that modern broiler strains are selected to over consume feed and, when pellet diets containing high levels of wheat are fed, this can lead to intestinal starch overload and reduced starch digestibility. Increases in gizzard size with whole wheat indicates that this organ may be the site for prevention of starch overload in the intestinal tract by reducing feed intake in WW fed birds which may be a result of satiety due to increased grinding activity (Svihus and Hetland, 2001). Moreover, the gizzard, apart from functioning as regulator of feed intake (Svihus, 2006), improves gut motility (Ferket, 2000) by increasing levels of cholecystokinin release (Svihus *et al*., 2004a) which in turn may stimulate secretion of pancreatic enzymes and gastro-duodenal reflux (Duke, 1992; Li and Owyang, 1993). Hetland *et al*. (2003) reported that total amount of bile acids in the gizzard increased in laying hens with access to wood shavings, indicating increased gastro-duodenal reflux. Scientific evidence of reverse peristalsis with whole grain feeding needs to be confirmed.

2.1.9. Influence of whole wheat feeding on gut microflora

The ban on use of antibiotic growth promoters (AGP) in poultry diets in the European Union has put tremendous pressure on the poultry industry to look for viable alternatives of AGP. It is unlikely that a single economically viable replacement for AGP will be possible (Dibner and Richards, 2005). It is evident that a multifactorial approach is needed and whole grain feeding may be a part of this strategy.

Dietary inclusion of whole grains has been shown to influence microbial ecology of the intestinal tract of poultry. Santos *et al*. (2007) conducted an experiment with broilers raised either on litter floor or cages and fed either a finely ground or whole triticale based diet from 0-42 days. They reported that the intestinal tract of birds fed the finely ground treatment had lower microbial diversity and higher salmonella prevalence than those fed the whole triticale diet. It was suggested that the combination of high dietary fibre content and increased diet coarseness in the whole triticale diets was responsible for observed beneficial effects, possibly through a competitive exclusion type mechanism. Similarly, dietary inclusion of whole wheat decreased intestinal salmonella colonisation (Bjerrum *et al.,* 2005; Santos *et al*, 2008) and *Clostridium perfringens* in broilers (Bjerrum *et al.,* 2005). Gabriel *et al.* (2003b) also reported that birds fed whole wheat had higher counts of beneficial microflora and lower counts of *Coliform* bacteria. Gabriel *et al*. (2006) showed that whole wheat given with pelleted protein concentrate led to a more beneficial microflora, higher count of *Lactobacilli* spp., and lower count of *Coliform* bacteria in broilers at 22 days compared to those fed a complete ground and pelleted diet. Bjerrum *et al*. (2005) demonstrated that whole wheat feeding influenced the development of *Salmonella* spp. infection and speculated that the gizzard has an important barrier function that prevents pathogenic bacteria from entering the distal intestinal tract. These authors found greater number of *Salmonella* spp. in the gizzard of broilers fed pelleted feed than those consuming pelleted feed supplemented with whole wheat. Furthermore, physical characteristics of feed influence pH of digesta contents in broilers (Nir *et al*., 1994a; Svihus *et al*., 2004a). Engberg *et al*. (2004) reported that the addition of whole wheat resulted in increased gizzard weight, decreased pH of gizzard contents and lowered intestinal numbers of lactose-negative *Enterobacteria* spp. and *Clostridium perfringens.* They proposed that gastric function was being stimulated through increased hydrochloric acid (HCl) secretion from the proventriculus and thorough increased grinding in the gizzard. Overall, these data suggest that whole grains may encourage colonisation of commensal bacteria and discourage pathogenic and harmful bacteria in the intestinal tract through competitive exclusion, HCl secretion or grinding action of gizzard.

2.1.10. Whole grain feeding to layers

Studies evaluating whole grain feeding in layers are limited. Faruk *et al*. (2010) fed layers equal proportions of whole wheat and a protein-mineral concentrate (balancer diet) in sequential or MF systems. Control birds were fed a complete conventional layer diet. Decreased feed intake was reported with sequential feeding of whole wheat, but egg production, egg mass and egg weight were similar among treatments resulting in improvement in efficiency of feed utilisation by 10% and 5% compared to MF and control treatments, respectively. Similarly, Maclsaac and Anderson (2007) fed layers diets containing 200 g/kg whole wheat from 20 to 64 weeks and reported no adverse effect of whole wheat feeding on production performance. Inclusion of 300 g/kg whole wheat without any enzyme supplementation (Senkoylu *et al*., 2009), and 100 g/kg whole pearl millet (Garcia and Dale, 2006) in layer diets had no adverse effect on egg production.

Feather pecking is a major welfare problem in layers (Blokhuis *et al*., 2007). Van Krimpen *et al*. (2009) supplemented layer diets with 150 g/kg oat hulls and reported that hens that were fed a standard diet had more feather damage compared with hens fed oat hull diets. In the second part of this study, improved feather condition at 49 wks of age was reported when a diluted rearing diet was fed to hens. It was suggested that with energy dilution of feed, pullets were increasingly 'imprinted' on feed as pecking substrate. This feeding strategy could be exploited with whole grain feeding for control of feather pecking. Therefore, inclusion of whole grains in layer diets has the potential not only to lower feed costs, but also of addressing welfare concerns.

2.1.11. Factors responsible for variable responses with whole wheat feeding

2.1.11.1. Quality of wheat

Variation in physical and chemical characteristics and AME that exist between the wheat used in different studies may partly explain the equivocal results in studies with whole wheat feeding. However, in none of these studies, the quality of wheat has been characterised. Studies from several parts of the world have shown that, amongst cereal grains, wheat is known to be most variable in AME content for poultry (Wiseman, 1993; Choct *et al*., 1999;

Hughes and Choct, 1999). The AME of wheat for broilers varies considerably and variations of up to 5.86 MJ/kg have been reported (Ravindran and Amerah, 2009). This variation is attributed largely to variations in the soluble NSP, which have a significant bearing on how effectively dietary components are utilised by poultry. It is well known that the quality of wheat in terms of composition and nutritive value of a given type and variety is influenced by year-to-year variation, location, agronomic conditions and climatic conditions. (McNab, 1992, 1996; Wiseman, 1993; Tester *et al*., 1995; Tester 1997). Amerah *et al*. (2009) reported physical characteristics such as endosperm hardness of wheat had a great impact on bird performance and suggested that endosperm hardness must be considered when choosing whole wheat for inclusion in broiler diets.

2.1.11.2. Time of introduction of whole wheat

Difference in the age of birds at introduction of whole wheat in different studies may have contributed to the inconsistencies reported in responses with whole grain feeding. The size of whole grain and its hardness are physical limitations to feed whole grains to newly hatched chicks. They face difficulties in breaking or swallowing of whole grain, particularly during the first few days of life. To address this problem, most researchers have delayed introduction of whole wheat until chicks are at least 5 days old (Jones and Taylor, 2001; Nahas and Lefrancois, 2001; Erener *et al*., 2003; Gabriel *et al*., 2003a; Taylor and Jones, 2004a;Amerah and Ravindran, 2008; Gabriel *et al*., 2008) to as long as 11 to 24 days old (Bennett *et al*., 1995;; Rose *et al.,* 1995; Uddin *et al*., 1996; Preston *et al.,* 2000; Plavnik *et al*., 2002; Hetland *et al*., 2002; Hetland *et al*., 2003; Svihus *et al.,* 2004a). However, some researchers (Bennett *et al*., 2002; Plavnik *et al*., 2002; Wu and Ravindran, 2004; Wu *et al*., 2004; Ravindran *et al*., 2006) have introduced the whole wheat from day 1. Ravindran *et al*. (2006) observed that chicks had difficulties in swallowing whole wheat during the first few days of life and, found significantly decreased feed intake and body weight of broilers. Similar reductions in feed intake have been reported in other studies where whole wheat was introduced from day 1, but no adverse effects on final body weight were reported (Wu and Ravindran, 2004, Wu *et al*., 2004). In studies where whole wheat was introduced at later ages, no effect was observed on feed intake. Rose *et al*. (1995) reported decreased intake when whole wheat was introduced the 24 days of age, but clearly this cannot be attributed to physical limitations. Introduction of whole grain to chicks from day 1 may affect feed intake, particularly during the first week, but any negative effects on performance are negated by the time broilers reach market weight (Jones and Taylor, 2001). Pre-pelleting inclusion of whole grain may be a practical alternative to overcome the issue of size of whole grain in the feeding of chicks.

2.1.11.3. Exposure to whole grain / adaptation period before the start of trial

Whatever the age of introduction to whole grain, chickens appear to benefit from a learning period. To adjust to whole grain feeding, Cumming (1987) emphasised the importance of experience for birds given FCF with whole grains and recommended exposing pullets during rearing to grains which may be offered later in their life. Due to differences in life span of commercial broilers and layers, broilers should be introduced to whole grains from the first week of their life, although it appears that ingestion of grains during the first 3-4 days is mainly based on innate pecking behaviour rather than nutritional selection (Forbes and Covasa, 1995). Hetland *et al*. (2002) and Svihus *et al.* (2004a) pre-exposed chicks by introducing 70 g/kg whole wheat from 6-11 days of age before final inclusion of 300 and 375 g/kg whole grain, respectively, from day 11 onwards, and reported no adverse effects on performance parameters. Similar results were reported by Hetland *et al.* (2003) who had pre-exposed whole wheat at 100 g/kg from 5-11 days of age before the start of study from day 11 with inclusion level of 385 g/kg. However, in all other studies, birds were not pre-exposed to whole grains prior to the start of the trial. It is common knowledge that sudden changes in feeding systems can lead to reductions in performance.

2.1.11.4. Rate of inclusion of whole grain and the length of study

Inclusion rates of whole wheat varied widely amongst different studies. Most studies evaluating whole wheat have used 100-200 g/kg inclusion levels (Jones and Taylor, 2001; Plavnik *et al*., 2002; Wu *et al*., 2004, Ravindran *et al*., 2006, Amerah and Ravindran, 2008). When inclusion levels are increased beyond 200 g/kg, it is tempting to speculate two scenarios. First, this may lead to a more developed gizzard with beneficial effects on bird performance, and second, there may be a need for more grinding in the gizzard resulting in increased energy requirements, which may have adverse effects on performance. Hetland *et al*. (2002) increased whole wheat inclusion to 300 and 440 g/kg and found more developed gizzards with no negative effects on production performance of broilers. Svihus *et al*. (2004a) further increased whole wheat inclusion to 500 g/kg and similarly found no difference in production parameters as compared to those fed with ground wheat. In several studies with lower inclusion levels (100-200 g/kg), a developed gizzard and improved broiler performance have been reported. Conversely, Wu *et al*. (2004) and Nahas and Lefrancois (2001), included whole wheat at 200 g/kg and 100-350 g/kg, respectively, and failed to show any effect on gizzard size, but observed beneficial effects on production performance. However, Bennett *et al*. (2002) with inclusion of 50-500 g/kg whole wheat found greater gizzard development, but with negative effects of performance. Thus variations in the inclusion level of whole grain may be partly responsible for the inconsistency in results with whole grain feeding. The minimum amount of whole grain required to enhance the functionality and size of gizzard is not known. Studies are warranted to compare the effects of different inclusion level of whole grain under similar experimental conditions.

Gizzards in young chicks are physically incapable of breaking down whole grain owing to its hardness (Covasa and Forbes, 1996). However, training or pre-exposure of birds by acclimatising them to whole grains at an early age appears to confer benefits at later stages of growth by improving their ability to select food to meet nutritional requirements and to adopt the digestive tract for better digestion (Forbes and Covasa, 1995). Most research in this area has employed very short or virtually no dietary acclimation periods. Jones and Taylor (2001) reported that the effect of whole grain feeding may only become apparent in the grower phase (22 to 42 days) of continuously fed birds. In this study, with pre-pelleting inclusion of 200 g/kg whole wheat from 5 to 42 days, no effect on relative weight of gizzard was observed at 30 d of age, but gizzard weight was increased by 11% at 42 day of age. Wu *et al*. (2004), with the same inclusion level of whole wheat pre-pelleting in broiler starter diets, failed to show any effect on the relative weight of gizzard at 21 days of age. Svihus *et al*. (2004a), on the other hand, with 500 g/kg whole wheat inclusion from 11 to 25 days observed significant increase in gizzard weight (by 27%) as compared to birds fed ground wheat based diet. Overall these data suggest that both the inclusion level of whole wheat and length of feeding are relevant for the development of the gizzard, and to the subsequent effect on bird performance.

2.1.11.5. Method of whole grain feeding

As discussed in Section 2.2, three different methods, namely FCF, MF and SF, can be used to offer whole grains to poultry. In pelleted diets, MF can be further sub-divided into two categories, namely PRP and PP. Studies comparing different methods of whole grain feeding are limited. Erener *et al*., (2003) studied the intake of whole wheat in these three different methods of whole wheat feeding and found that wheat intake was significantly higher in MF than FCF and SF. Though all three feeding methods increased gizzard weights, MF had negative effects on weight gain and feed per gain of broilers. The performance of birds on FCF and SF was similar to those fed the ground wheat diet. Amerah and Ravindran (2008) compared MF and FCF and found significant decreases in weight gain and feed intake in FCF birds compared to MF birds. Wu *et al*. (2004) compared the method of pre and post - pelleting inclusion of 200 g/kg whole wheat and found that pre-pelleting had no effect on gizzard weight, but improved AME and feed efficiency. Post-pelleting, on the other hand, improved gizzard weight (43%), feed efficiency (3%) and AME (6%) compared to pre-pelleting. Svihus *et al*. (2004a) reported post-pelleting inclusion of 375 g/kg whole wheat significantly increased AME and starch digestibility, whereas pre-pelleting inclusion of 500 g/kg of whole wheat had no effect. Differences in pre-and post-pellet method of whole wheat inclusion might be due to the fact that pelleting, whilst moulding whole grain mash diets to pellet, simultaneously reduces the size of the micro-particles that constitutes the intact pellet. Svihus *et al.* (2004b) reported the proportion of coarse particles diminished and the amount of fine increased in pelleted diets as a consequence of the pelleting process. Pre-pelleting inclusion of whole grain by use of pellet die with a larger diameter (instead of the 3.0 mm size used conventionally) may minimise the particle size reduction of whole grain. This approach might overcome the differences in these two methods of whole grain inclusion while achieving the beneficial effects with whole grain feeding. Overall these results suggest that the inconsistent results reported in different whole grain feeding studies may be attributed in part to differences in feeding method and experimental protocol.

2.1.11.6. Supplementation of whole wheat diet with exogenous enzymes

Supplementation of exogenous enzymes to poultry diets based on wheat is currently a common practice. The exact mode of action for exogenous enzymes remains unclear, but lowering of digesta viscosity is often proposed as a possible mechanism (Bedford and Schulze, 1998). Feeding wheat-based diets with whole wheat was found to decrease digesta viscosity compared to birds fed diets with finely ground wheat (Yasar, 2003). In contrast, whole wheat feeding has reported to increase digesta viscosity but improved feed per gain (Engberg *et al*., 2004; Wu *et al*., 2004). The beneficial effects of including whole wheat were shown to be further enhanced with supplemental xylanase (Wu and Ravindran, 2004). The physical form (mash and pellet) of the diet may be a factor dictating enzyme response with whole grain feeding but this has not been tested. Enzyme supplementation could be more beneficial with whole grain feeding as compared to conventional ground wheat feeding due to higher grinding activity of larger and developed gizzard leading to better mixing of the substrate with enzyme.

2.1.12. Conclusions

The available literature clearly shows that wheat is the choice of grain and studies pertaining to use of other cereal grains are scant. Overall, feeding whole grains is of potential value to lower feed costs while sustaining bird performance. This overview highlighted the limited amount of research conducted in this area and the factors contributing to the variable responses associated with whole grain feeding.

In addition, phasing out AGP in poultry diets in the European Union and recent moves towards reduction or removal of these chemicals in other parts of world have opened new dimensions of poultry research on whole grain feeding as a means to naturally and economically explore viable alternatives to control gut pathogens.

2.2. Maize: Feeding of whole grains other than wheat

2.2.1. Introduction

Globally, maize is the most commonly used cereal grain and contributes approximately 65% of the metabolisable energy and 20% of the protein in a poultry diet (Cowieson, 2005). The reason for the widespread use of maize in intensively reared poultry is its consistent and relatively high nutritional value for poultry in comparison to that of other cereal grains. The metabolisable energy value of maize is generally considered the standard with which other energy sources are compared (Ravindran, 2010). Although maize is the major grain used in poultry diets worldwide, only minimal attempts have been made to feed whole maize. The size and hardness of maize grain are probable factors for the lack of use and limited research on whole maize feeding. Pre-pelleting inclusion of whole maize or minor modifications such as cracking or coarse grinding could overcome this concern. Studies are warranted on these alternative options to utilise whole maize in diets for broilers and layers.

2.2.2. Maize kernel and its types

The maize kernel *is* botanically classified as a caryopsis (dry, indehiscent, single seeded fruit). Maize kernels are categorised into two major types based on colour (yellow or white) and hardness (flint, popcorn, flour, dent and sweet, in order of decreasing hardness) (James, 2003; Cowieson *et al*., 2011). Approximately 85% of global maize is yellow, 10-12% white, and the balance is made up of red, blue, purple and black grains. In general, yellow and white coloured maize is used for poultry and human feed, respectively (James, 2003). 'Flint' grains are hard, a shiny surface and full of horny endosperm. In contrast, 'dent' grains are made up of floury or soft endosperm that upon drying and shrinking produces a concave surface (Evers and Millar, 2002), (hence the name dent) and have an opaque appearance. About 80% of global maize production is dent or semi-dent, and is 15% flint or semi-flint. Irrespective of their pigment, shape, and hardness, kernels are genetically and nutritionally the same (James, 2003). However, in dent grain, starch granules and protein bodies are arranged in a disorganised manner within the endosperm (Wall and Bietz, 1987), whereas, in dent or vitreous maize, packing of starch granules is more organised and intergranular spaces are perfectly filled with protein bodies (Simmonds *et al.*, 1973; Gibbon and Larkins, 2005).

2.2.3. Physical Structure of maize kernel

The maize kernel is the largest cereal seed, somewhat oval and flattened in shape, weighing 250-300mg each. Maize kernels may vary from the near spherical popcorn to flattened and angular flint maize (Evers and Millar, 2002; Watson, 2003).

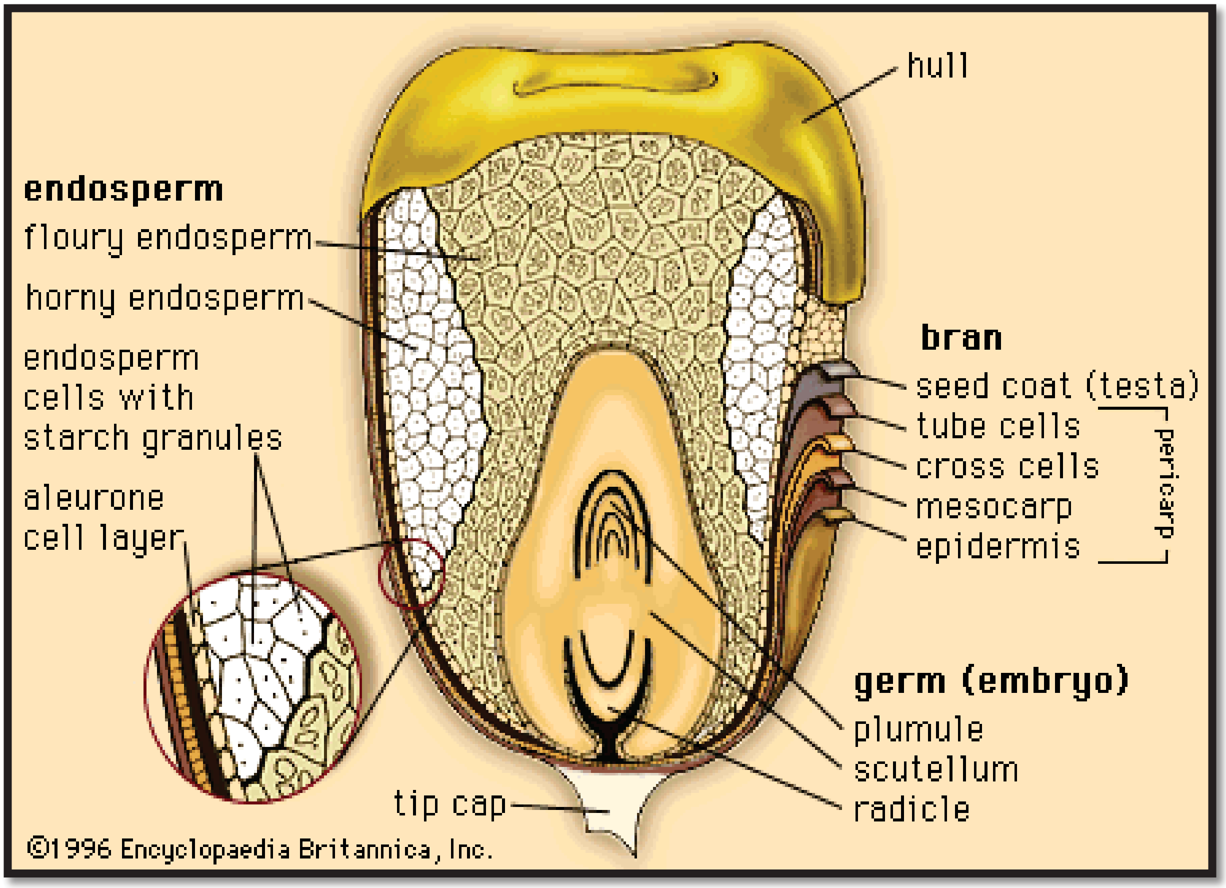


Figure 2.2. Maize kernel (source: Encyclopaedia Britannica, Inc. 1996)

A mature maize kernel is comprised of four anatomical parts: tip cap, hull or bran, embryo, and endosperm (Figure 2.2). The maize kernel largely consists of endosperm (70-80%). The endosperm consists mainly of starch-laden cells or granules having vitreous and starchy regions that are surrounded by an aleurone layer. This is a continuous layer of large cubical cells located immediately underneath the hull and contains protein granules (Watson, 2003; Sofi *et al.*, 2009).

2.2.4. Chemical composition and nutritive value of maize

Maize, on average contains 80 g/kg of crude protein and 14 MJ/kg of AME (Cowieson, 2005). In comparison to wheat, crude protein content of maize is lower but the AME for poultry is higher due to its higher starch content (Weurding *et al*., 2001a,b), lower soluble

NSP (Choct, 1997), and low concentrations of anti-nutritional factors such as phytin (Eeckhout and De Paepe,1994). However, the chemical composition and concomitant nutritional value of maize is variable and dependent on variety, growing conditions, drying temperature, starch structure and lipid/protein/starch matrices (Socorro *et al.*, 1989; Herrera-Saldana *et al.*, 1990; Leeson *et al.*, 1993; Leigh, 1994; Brown, 1996; Cromwell *et al.*, 1999; Collins and Moran, 2001).

2.2.4.1. Starch

Starch is the most abundant carbohydrate and energy yielding component of maize grain. In addition it is highly palatable and highly digestible by poultry. The starch in maize occurs as granules within the endosperm. Starch granules in maize are roughly spherical and range in size between 2–30 µm (Franco *et al.*, 1998; Tester *et al.*, 2004). Amylose and amylopectin are the semi-crystalline polymers of d-glucose found in maize starch and are differentiated by the linkages between the glucose monomers (Carre, 2004; Tester *et al.*, 2004). Average amylose content is between 160–350 g/kg and if amylose is lower or higher than the average, starches are defined as either ‘waxy’ or ‘amylo’, respectively (Moran, 1982; Tester *et al.*, 2004).

2.2.4.2. Protein

Maize is also contributes approximately 20% of the protein in broiler diets. However, the balance of amino acids in maize protein is considered nutritionally poor (Peter *et al.*, 2000). The main storage proteins in maize are zein and kafferin (McDonald *et al.*, 1990), with zein being quantitatively most important

2.2.4.3. Starch-protein matrix

The endosperm of maize kernel is made up of complex matrix of starch granules and protein bodies (Figure 2.3). Apart from cell wall thickness and degree of packing of its cellular compounds, variation in the physical structure and hardness of maize is influenced by size of cells in the endosperm storage parenchyma, thickness of proteinacious matrix in contact with starch granules, and strength of adhesion between proteinacious matrix and starch granules (Simmonds *et al.*, 1973; Abdelrahman and Hoseney, 1984; Kriz, 1987). In floury-textured maize, starch granules and protein bodies are not organised as that is in vitreous maize (Wall and Bietz, 1987Simmonds *et al.*, 1973; Gibbon and Larkins, 2005). Normally, storage proteins are responsible for the association between starch grains and endosperm matrix proteins, thus influencing grain hardness (Abdelrahman and Hoseney, 1984; Hoseney, 1987; Dombrink-Kurtzman and Bietz, 1993). The texture of maize grain is a fundamental characteristic of importance to poultry feed processors because of its relationship to kernel hardness.

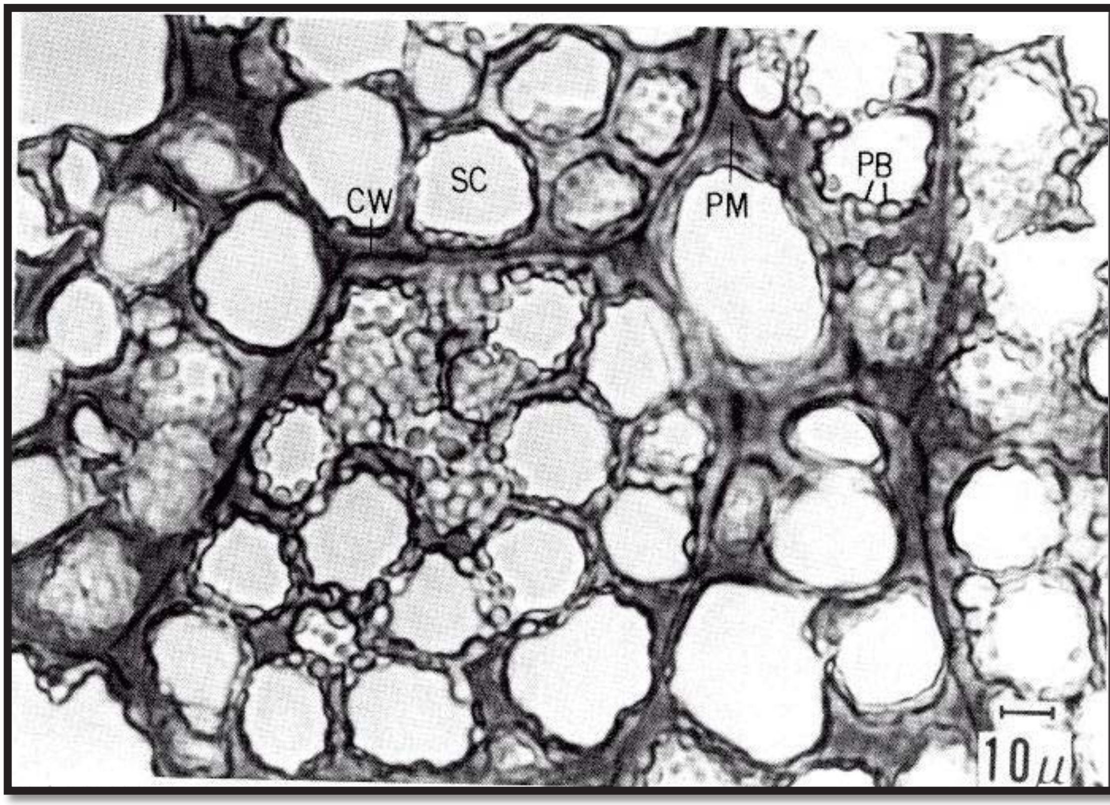


Figure 2.3. Light micrograph of maize kernel endosperm matrix. PM, protein matrix; PB, protein bodies; SC, starch cavity; CW, Cell wall (source: Watson, 2003)

2.2.5. Preference for maize and other grains for poultry

Certain grains are more acceptable for eating by poultry than others. Kempster (1916) stated that wheat was favoured, followed by maize and sorghum. Preference for maize and wheat over barley by chickens has also been reported by Fry *et al*. (1957) and Jensen *et al*. (1957). However, preference by birds towards one type of grain is difficult to access and could be related to form of presentation, palatability and metabolic consequences or, more likely, a combination of all these (Forbes and Covasa, 1995).

2.2.6. Use of whole maize or with minimal processing for poultry

Feeding birds either whole maize or after slight modifications such as cracking and coarse grinding has become more topical to determine if beneficial effects similar to those associated with whole wheat feeding can be obtained with maize. However, the literature reveals limited studies have been carried out in use of either whole maize or modified maize for poultry.

2.2.6.1. Practical and economic implication

Reece *et al*. (1986) reported a 35% reduction in energy required to grind maize using a hammer mill when the screen size was increased from 4.76 to 7.94 mm. Clark and Behnke (2004) evaluating a method using cracked maize on feed mill efficiency showed that pelleting a protein concentrate and adding cracked maize post-pellet at 130 and 280 g/kg of its formulated amount improved pelleting production rate without affecting the pellet durability index. Moreover, addition of 130 and 280 g/kg cracked maize post-pellet decreased total feed mill energy usage by 11 and 22%, respectively. However, the effect of cracked maize on the growth performance of poultry was not evaluated. Similarly Clark *et al*. (2009) reported that total finished feed output increased by 19% while overhead costs (labour, electrical consumption, etc.) decreased by addition of cracked maize to a protein concentrate pellet at the feed mill.

2.2.6.2. Effect on production performance

Jacobs *et al*. (2010) fed broilers from 0 to 21 day of age, four dietary treatments containing different maize particle sizes (GMD of 557, 858, 1210 or 1387 µm) and reported maize particle size of up to 1387 µm can be included with positive effects on the development of the gizzard without compromising growth performance and nutrient digestibility. Nir *et al*. (1994b) also showed that increasing the maize particle size from 525 to 897 µm increased broiler performance, while Nir *et al*. (1994a) found that broilers fed medium to coarse maize diets (GMD, 966 and 1332 µm) were heavier and more efficient than those fed a fine maize diet (GMD, 897µm). In contrast, Parsons *et al*. (2006) fed broilers from 3 to 6 weeks of age with maize diets varying in maize particle sizes (GMD of 781, 950, 1042, 1109, 2242 µm) and reported increased gizzard size only in broilers fed the coarsest maize (2242 µm). Performance and energy expenditure decreased when maize particle size exceeded 1042 µm (medium-coarse) but medium-coarse to coarse (GMD of 1042, 1109, 2242 µm) maize improved nutrient digestibility. Clark *et al*. (2009) fed broilers maize-based diets with different inclusion rates of 170 to 680 g/kg cracked maize replacing ground maize and blended with an appropriate concentrate pellet without altering final nutrient content. These researchers reported that up to 170 g/kg ground maize can be replaced by cracked maize without affecting broiler performance. Dozier *et al*. (2006) evaluated the effects of adding rolled maize (GMD of 1500 µm) to a pelleted feed supplement in broilers from 18 to 41 d of age. Their data indicated that broilers fed up to 230 g/kg cracked maize showed no adverse effect on any performance parameters. Dozier *et al*. (2009) reported no adverse effect on broiler performance and meat yield as up to 280g/kg cracked maize (GMD of 2691 µm) was added post pellet from 18-56 day of age. Recently, Lu *et al*. (2011) fed geese 640g (8 to 28 days) and 615g (29 to 70 days) of whole maize/kg of diet and found no negative effect in birds fed the whole maize diet compared to those fed the ground maize diet.

2.2.7. Conclusions

Evidence indicates that broilers fed diets with coarser maize particles had increased gizzard weight, which suggests that feeding cracked or coarse maize has the potential to produce beneficial effects similar to that reported for whole wheat feeding. Pre-pelleting inclusion of whole maize could be another alternative strategy. Studies are warranted on these alternative options to utilise whole maize in both broilers and layers.

2.3. Coccidiosis: Whole grain feeding may be an alternative strategy for control

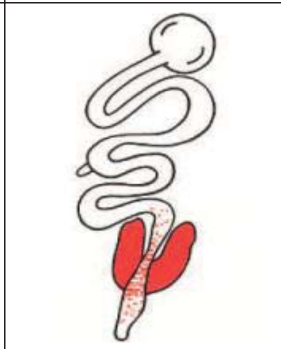
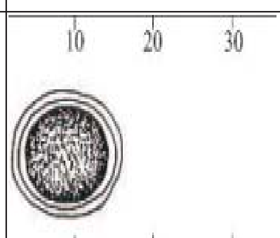
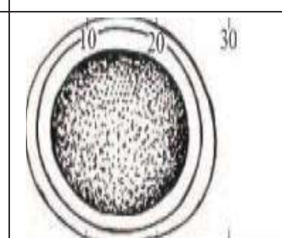
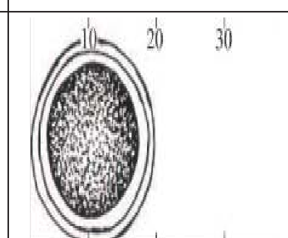
2.3.1. Introduction

Coccidiosis is one of the most common and economically important diseases of chickens worldwide. It is caused by a parasitic protozoan species belonging to the genus *Eimeria* that damages the host's intestinal system, causing loss of production, morbidity and death. Total worldwide losses are estimated to be a devastating US $ 3 billion annually (Dalloul and Lillehoj, 2006). Coccidiosis in poultry has been controlled and prevented predominately by chemotherapeutic drugs added directly to feeds (coccidiostats). However, some resistance to all coccidiostats in current use has been reported (Chapman, 1997). Furthermore, consumer concerns for fewer chemical feed additives and legislation in the European Union to phase out coccidiostats as feed additives by 31 December 2012 (COM, 2008; Regulation 1831/2003/EC) has put tremendous pressure on the poultry industry to look for alternatives. Natural and nutritional approaches, such as whole grain feeding, are well accepted by consumers and likely to play a major role in future control of coccidiosis (Brenes and Roura, 2010).

2.3.2. *Eimeria* species

Seven *Eimeria* species, namely *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis, E. necatrix, E. praecox and E. tenella*, have been identified as currently infecting poultry (Shirley, 1986).But as many as nine (Long and Reid, 1982), with two more species *E. hangi* and *E. mivati,* have also been implicated. Each species has an affinity for a specific zone within the digestive tract and exhibits a characteristic degree of pathogenicity. Of these, *E. acervulina* (duodenum), *E. maxima* (jejunum and ileum) and *E. tenella* (caeca) are the three significant species in New Zealand (Conway and McKenzie, 2007). Differential characteristics for these *Eimeria* species are depicted in Table 2.6.

Table 2.6. Differential characteristics of *E. acervulina, E. maxima and E. tenella* (Sources: Conway and McKenzie, 2007; modified after Long and Reid, 1982)

	DIFFERENTIAL CHARACTERISTICS FOR SPECIES OF CHICKEN COCCIDIA Diagnostic characteristic in red			
		E. acervulina	E. maxima	E. tenella
Macroscopic Lesions	CHARACTERISTICS ZONE PARASTISED			
	MACROSCOPIC LESIONS	Light infection: **transverse, whitish bands of occyst** Heavy infection: pacques coalescing thickened wall	Thickened walls, mucoid, blood-tinged exudate, petechiae	**Onset: haemorrahage into lumen Later: thickening, whitish mucosa, cores clotted blood**
Microscopic Lesions	MILLIMICRONS OOCYST REDRAWN FROM ORIGINLAS			
	LENGTH X WIDTH (μ) LENGTH= WIDTH=	AV.= 18.3 x 14.6 17.7-20.2 13.7-16.3	30.5 x 20.7 21.5-42.5 16.5-29.8	22.0 x 19.0 19.5-26.0 16.5-22.8
	OOCYST SHAPE AND INDEX-LENGTH /WIDTH	Ovoid 1.25	Ovoid 1.47	Ovoid 1.16
	SCHIZONT, MAX IN MICRONS	10.3	9.4	54.0
	PARASITE LOCATION IN TISSUE SECTIONS	Epithelial	Gametocytes Subepithelial	2^{nd} generation schizonts subepithelial
Life cycle	MINIMUM PREPETENT PERIOD- HR	97	121	115
	SPOROLUATION MINIMUM-HR	17	30	18

2.3.3. Life cycle of *Eimeria*

In the environments where poultry are reared, oocysts are practically ubiquitous and may be transported by the definitive host, paratenic avian hosts, rodents, flying insects, other invertebrate pests, contaminated feed, old litter, human agency and the general paraphernalia of the poultry industry (Fayer and Reid, 1982). However, coccidia are usually spread by the faecal-oral route.

A generalised life cycle of *Eimeria* is illustrated in Figure 2.4. Unsporulated oocysts are expelled from the intestinal mucosa and excreted in faeces. Excreted oocysts sporulate in about 24 h and become infective under suitable environmental conditions for which oxygen, moisture and warmth are necessary (Kheysin, 1972). The sporulated oocyst contains four sporocysts, each sporocyst contains two sporozoites. Infection occurs when a susceptible chicken ingests a sporulated oocyst. After ingestion, sporozoites are released by mechanical action of the gizzard and biochemical action in the digestive tract of the chicken (Ikeda, 1955; Farr and Doran, 1962; Reid, 1978). Liberated sporozoites penetrate epithelial cells in a specific zone of the intestine or ceca depending on the *Eimeria* species involved (Table 2.6). The sporozoite enters the host cell and transforms to a trophozoite in 12 to 48 h. Trophozoites grow by a process of asexual division of the parasite nucleus known as schizogony (merogony). The parasite at this stage is referred to as a schizont or meront (first generation), which contain merozoites. The schizont matures (3 days), ruptures, and releases merozoites (first generation). Most of these invade other epithelial cells to repeat the second generation of trophozoites and schizogonous stages. Depending on the species, some go through a third schizogonous cycle and finally male (microgametocytes) or female (macrogametocytes) gametocytes are formed. The male microgametocyte matures and ruptures, releasing a large number of minute biflagellate microgametes, while the female macrogametocyte grows and convert into a macrogamete. The macrogamete is fertilised by a microgamete and a zygote is formed (sexual part of life cycle). This stage is the young or immature oocyst. The prepatent period is the time between ingestion and excretion of oocyt, and varies with each species depending on the time required for the different stages in the digestive tract (see Table 2.6).

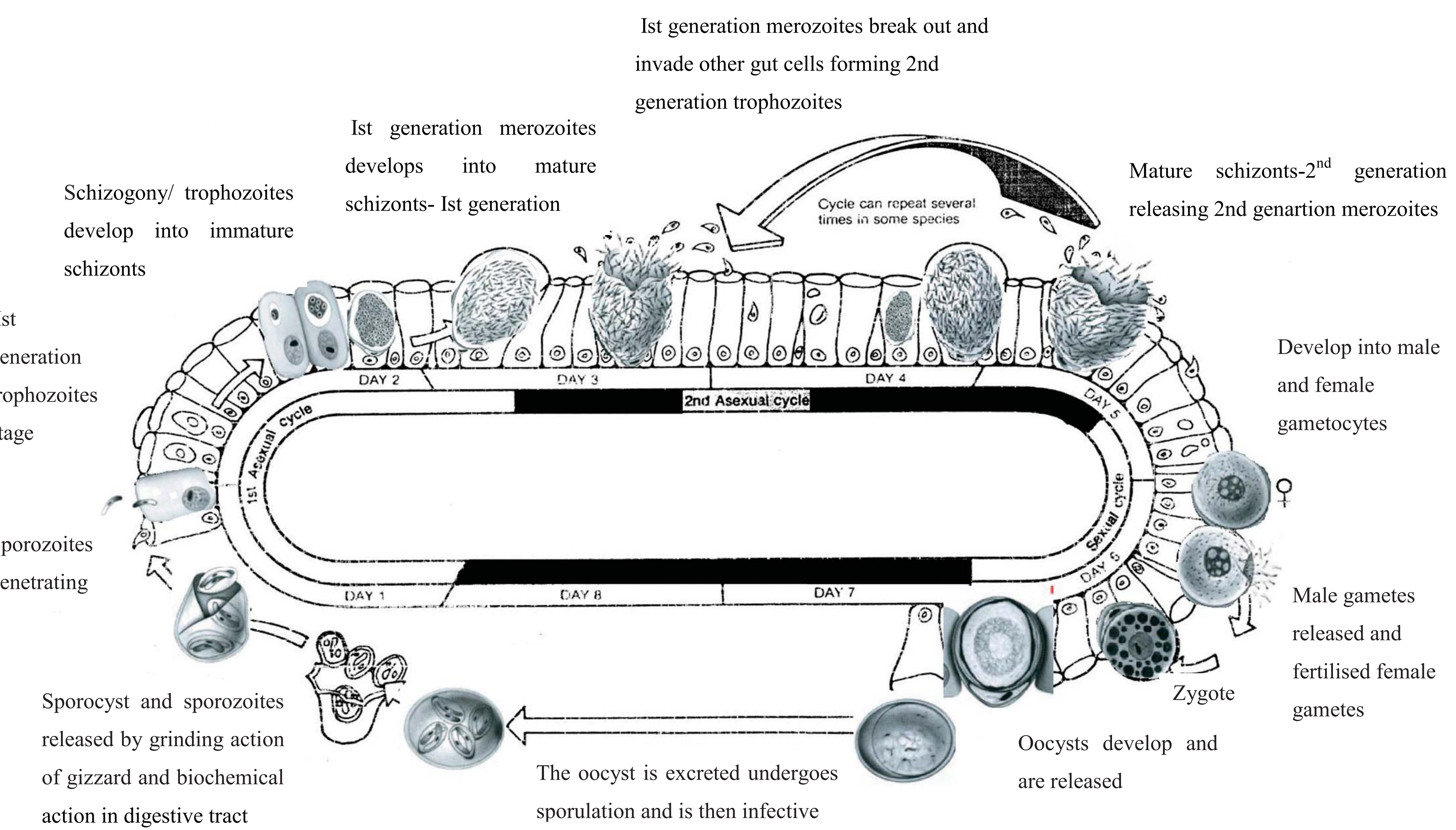


Figure 2.4. Generalised life cycle chart of *Eimeria* (Sources: modified from Philips, 1988;Conway and McKenzie, 2007)

2.3.4. Forms of coccidiosis in chickens

Coccidiosis is the clinical manifestation of the disease produced by ingestion of sporulated coccidian oocysts in sufficient numbers. Coccidia have a genetically fixed, self-limiting life cycle (Johnson, 1923; Tyzzer, 1929); therefore, severity of infection by each species is positively correlated with the number of infective oocysts ingested (Johnson, 1927; Tyzzer, 1932; Dickinson, 1941). However, infection may exhibit three increasing levels of severity (Williams, 1999): (1) coccidiosis, a mild infection, causing no adverse effects (Levine, 1961); (2) subclinical coccidiosis, resulting in slight infection with no apparent visible signs, but economically important reductions of growth and feed utilisation; and (3) clinical coccidiosis. The last category can be further subdivided into a) less severe clinical coccidiosis (caused by *E. acervulina*) and symptoms are diarrhoea, morbidity, reduction of weight gain and poor feed conversion; and, b) more severe coccidiosis (caused by *E. maxima*, and *E. tenella*) producing similar signs and with heavier infections, various degrees of intestinal haemorrhage and death (Williams, 2005).

2.3.5. Pathophysiological effects of coccidiosis in chickens

The pathophysiological effects of poultry coccidiosis are anorexia (Reid and Pitois, 1965); villous atrophy (Pout, 1967); intestinal leakage of plasma proteins (Schalm *et al*., 1975; Shane *et al*., 1985); reduced activities of digestive enzymes, such as disaccharidases associated with the epithelial brush border (Enigk and Dey-Hazra, 1976; Major and Ruff, 1978a); and amylolytic activity in the pancreas (Major and Ruff, 1978b), which occurs especially in *E. acervulina* and *E. maxima* infections caused by the sexual stages damaging mucosa (Schalm *et al*., 1975; Shane *et al*., 1985). All these effects contribute to reduced nutrient digestion (Turk, 1972) and intestinal malabsorption of nutrients (Pesti and Combs, 1976; Tyczkowski *et al*., 1991), leading to reduced weight gain and poor feed conversion efficiency (Reid and Pitois, 1965). Pathophysiological effects are influenced by host genetics, nutritional factors, concurrent diseases, and species of the coccidium. *E tenella* is the most pathogenic in chickens because schizogony occurs in caeca and causes extensive haemorrhage.

2.3.6. Diagnosis of coccidiosis

Location and appearance of lesions in the host, and size of oocysts are used to determine the *Eimeria* species, however, mixed coccidial infections are common. Coccidial infections are readily confirmed by demonstration of oocysts in feces or intestinal scrapings; however, the number of oocysts present has little relationship to the extent of clinical disease (Khan and Line, 2010). Lesion scoring is crucial to asses *Eimeria* pathogenicity (Yvore *et al*., 1980; Allen *et al*., 2000). Classical lesions of *E. tenella* are pathognomonic. While comparison of lesions and other signs with diagnostic charts allows a reasonably accurate differentiation of the coccidial species (Long *et al*., 1976; Conway and McKenzie, 2007), other diagnostic methods are adequate (Joyner and Long, 1974; Long *et al*., 1976; Joyner, 1978; Long and Reid, 1982; Conway and McKenzie, 2007).

2.3.7. Influence of whole grain feeding on coccidiosis

Cumming (1989) reported that feeding of whole grains had beneficial effects on prevention of coccidiosis. Elwinger *et al.* (1992) found lower moisture content in litter when ground wheat was replaced with whole wheat, which was suggested to reduce the risk of coccidiosis. Cumming (1989) hypothesised that increased gizzard size and grinding action brought about by an increase in fibre levels and/or the physical structure of whole grain, may physically destroy oocysts prior to reaching their sites of infection. This theory was supported by subsequent work from the author in which feeding birds with whole wheat grains in a FCF situation reduced excreta oocyst shedding and were negatively correlated with gizzard size. Faecal oocyst count was used in his study to assess the severity of infection. However, such beneficial effects of lower oocyst shedding associated with whole wheat feeding have not been confirmed in other studies (Waldenstedt *et al*., 1998; Banfield and Forbes, 2001; Banfield *et al*., 2002). Conversely, Gabriel *et al*. (2003b, 2006) studied separately the effect of whole grain inclusion on three species of Eimeria (*E. acervulina* (duodenum), *E. maxima* (jejunum and ileum) and *E. tenella* (caeca) and reported higher total occyst counts and more severe lesion scores at seven day post-infection in whole wheat fed birds in comparison to birds fed ground wheat. They also observed that with whole grain feeding, regardless of *Eimeria* species, more clinical coccidiosis during the acute phase of coccidiosis developed. In birds challenged with *E. maxima*, weight gain was decreased in whole wheat-fed birds sooner than in birds fed a ground diet (fourth day instead of fifth day post infection) (Gabriel *et al*., 2003b). Similarly, Gabriel *et al*. (2006) found the maximum oocyst excretion in birds infected with *E. tenella* occurred in whole wheat fed birds one day prior to ground wheat fed birds. It was proposed that whole wheat feeding modified the life cycle of Eimeria and thus oocyst excretion should not be used as the sole infection criterion to explore dietary impacts on coccidiosis. In addition, the presence of oocysts in excreta confirms the coccidial infection, but this has little relationship to the extent of clinical disease (Khan and Line, 2010). Lesion scoring is also crucial to assess *Eimeria* pathogenicity (Yvore *et al*., 1980; Allen *et al*., 2000). These conflicting results may be related to variation in several parameters in different studies, including parasitic dose, species and strains of coccidia, age and strain of birds, age of introduction of whole grain, quantity and type of whole grain fed, and age of inoculation.

2.3.8. Conclusions

The need to move away from chemotherapeutic control of coccidiosis has prompted research on whole grain feeding for alternatives capable of maintaining poultry health. However, the role of whole grain feeding and gizzard development in the prevention of coccidiosis is unclear. Further studies are warranted to test the hypothesis that whole grain feeding during coccidiosis will grind the oocysts in the gizzard, prior to reaching the site of infection, and prevent the incidence of coccidiosis.

2.4. Overall general conclusion

Despite the fact that maize being the most commonly used grain in poultry diet formulation world-wide, wheat is the grain of choice in whole grain feeding,. The size of maize grain may be the reason for lack of interest in feeding whole maize to poultry. To overcome this, studies are warranted using alternate feeding strategies such as pre-pelleting inclusion of whole maize or minor modifications such as cracking or coarser grinding in both broilers and layers. Furthermore, globally, ban on AGP, proposal to phase out coccidiostats in the European Union and recent moves towards reduction or removal of these chemicals in other parts of the world has renewed interest into research on whole grain feeding. Studies are warranted to explore the possibility whether whole grain feeding can be a natural and economically viable alternative to control gut pathogens and coccidiosis.

CHAPTER 3
General Materials and Methods

All experimental procedures were in accordance with the New Zealand Revised Code of Ethical Conduct for the use of Live Animals for Research, Testing and Teaching and approved by the Massey University Animal Ethics Committee (Anonymous, 2008).

3.1. Birds and Housing

Day-old male broiler (Ross 308) chicks were obtained from a commercial hatchery, individually weighed and assigned to cages (eight birds per cage) in three-tier electrically heated battery brooders in a manner that average bird weight per cage was similar. The birds were transferred to grower colony cages on day 11 and were maintained on the same experimental diets until the end of experiment. Battery brooders and grower colony cages were housed in an environmentally controlled room. Room temperature was maintained at 32±1 ^{o}C during the first week and gradually reduced to 21±1 ^{o}C by the end of the third week. Twenty hours of fluorescent lighting was provided per day throughout the experimental period. Body weights and feed intake were recorded on a cage basis at weekly intervals. Mortality was recorded daily. Any bird that died was weighed and, the weight was included in weekly weight gain data and used to adjust feed per gain.

3.2. Determination of particle size distribution of mash diets: Dry sieving method

Dry sieving method was used to determine the particle size distribution of mash diets as per the method described by Baker and Herrman (2002). A set of sieves (Endecott, London, UK), sized 2, 1, 0.5, 0.212, 0.106 and 0.075 mm, with an Endecott testing sieve shaker were used to separate feed particles into different sized fractions. Briefly, a representative weighed sample of feed was passed through the sieve stack on a shaker for 10 min. The amount of particles retained on each sieve was measured by subtracting the weight of the sieve from the final weight of sieve and sample after shaking. The geometric mean diameter (GMD) and geometric standard deviation (GSD) were then calculated using the formula shown below. These calculations were based on the assumption that the weight distribution of ground cereal grains is logarithmically normal (Martin, 1985). Two samples per diet were analysed.

$di = (du \times do)^{0.5}$

$GMD = Log^{-1}[\Sigma (Wi \log di) / \Sigma Wi]$

$GSD = Log^{-1}[\Sigma Wi (\log di - \log GMD)^2 / \Sigma wi]^{0.5}$

Where,

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

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

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

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

3.3. Determination of particle size distribution of pelleted diets: Wet sieving method

The wet sieving method was used to determine particle size distribution of pelleted diets (Lentle *et al*., 2006). Briefly, a representative sample of the pelleted diet (50 g) was divided into two portions. One half of the sample was oven-dried at 80 °C in a forced draft oven for 24 h to determine dry matter (DM) content, while the other half was suspended in 50 mL of water for 30 min before being washed though a series of sieves (Endecott, London, UK) sized 2, 1, 0.5, 0.212, 0.106 and 0.075 mm. Contents of each sieve were subsequently washed onto pre-weighed filter papers, and then dried for 24 h in a forced draft oven at 80 °C. Amount of diet retained by each sieve and fines which passed through all sieves were weighed and expressed as a percentage of total DM recovered. The measurement was made in duplicate.

3.4. Pellet durability

Pellet durability of pelleted diets was measured by the method described by Svihus *et al*. (2004b) using a Holmen Pellet Tester (New Holmen Pellet Tester, Tekpro Ltd., Norfolk, UK). Briefly, weighed samples of pellet (100 g) were circulated pneumatically through a closed chamber before being passed through a 2 mm sieve. Pellet durability index was calculated as the ratio between the amount pellets not passing through the sieve after the test to amount of whole pellets at the beginning.

3.5. Pellet hardness

Pellet hardness was measured in a Stable Micro System Texture Analyser (TA-XT Plus, Godalming, Surrey, UK) using the method as described by Svihus *et al*. (2004b). Fifteen individual pellet samples were placed between the pressure piston and bar. By increasing the pressure applied by means of the pressure piston, the force (Newton) needed to break the pellet was determined.

3.6. Apparent metabolisable energy (AME) determination

Feed intake and excreta output of each cage were measured quantitatively for four days either between days 17 and 20 or 31 and 34 post-hatch. Total excreta output was pooled within a cage, mixed well using a blender and sub-sampled. Sub-samples were then freeze-dried, ground to pass through a 0.5 mm screen, and stored in airtight plastic containers at -4 ^{o}C until analysis. Excreta and diet samples were analysed for the DM and gross energy (GE).

3.7. Ileal digesta collection

Two birds per cage, selected randomly were euthanised by intravenous injection of sodium pentobarbitone (Provet NZ Pvt. Ltd., Auckland, New Zealand) at 0.5 ml per kg body weight. Immediately after euthanasia, portion of small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileo-caecal junction (ileum) was carefully exercised. The contents of the lower half of ileum were collected by gently flushing with distilled water into plastic containers. Digesta samples were pooled within a cage, freeze-dried, ground to pass through a 0.5 mm screen size and stored in air tight container at -4 ^{o}C until laboratory analysis. Digesta and diet samples were analysed for DM, Titanium (Ti), nitrogen (N) and starch.

3.8. Chemical analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2005). Samples were weighed and placed in a drying oven for 24 h at 105 ^{o}C. Dry weight was recorded after two hours of cooling in a desiccator at room temperature.

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

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). Weighed samples were placed in a furnace at 850 ^{o}C with excess of oxygen until totally combusted. The combustion products, mainly carbon dioxide (CO_2), water (H_2O), and nitrous oxide (NO_X), were passed through a series of columns to remove H2O, convert NO_X

to N_2 and to remove remaining oxides and excess O_2. Gaseous N_2, carried by helium, was then measured by thermal conductivity and expressed as a percentage of the sample.

Titanium was determined by sulphuric acid digestion followed by colourimetric determination on a UV spectrophotometer as described by Short *et al*. (1996).

Starch was measured using an assay kit (Megazyme, International Ireland Ltd., Wicklow, Ireland) based on conversion of starch to glucose using thermostable α-amylase and amyloglucosdase (McCleary *et al*., 1997).

3.9. Calculations

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

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

Apparent ileal digestibility of starch and N was calculated based on titanium marker ratios in the diet and ileal digesta using the formula below.

$$\text{Apparent ileal nutrient digestibility} = \frac{(\text{Nutrient / Ti})\ \text{diet} - (\text{Nutrient / Ti})\ \text{ileal}}{(\text{Nutrient / Ti})\ \text{diet}}$$

Where,

(Nutrient / Ti) diet is the ratio of nutrient (N or starch) to titanium in diet, and

(Nutrient / Ti) ileal is the ratio of nutrient (N or starch) to titanium in ileal digesta.

3.10. Digestive tract measurements

For digestive tract measurements, two birds (closest to the mean cage weight) were selected from each cage. Body weights were recorded and birds were killed by cervical dislocation. The gastrointestinal tract and organs were carefully excised. Adherent mesentery and fat was removed. Empty weights of proventriculus and gizzard, and weights of pancreas, liver and spleen were recorded. Different segments of small intestine were emptied by gentle pressure and, empty weight and length of duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to 1 cm above ileo-caecal junction), and caeca (ostium to tip) were recorded. The length of various intestinal segments was determined with a flexible tape on a wet glass surface to prevent inadvertent stretching. Relative organ weights (g/kg body weight) and relative length (cm/kg body weight) were calculated.

3.11. Determination of digesta pH

To measure the digesta pH, one bird per cage was killed by cervical dislocation and the gastrointestinal tract was eviscerated immediately and placed on a stainless-steel dissection tray at room temperature and gently uncoiled without tearing or stretching. The pH was measured with a calibrated digital pH meter (model IQ120, 2075-E Corte Del Nogal, Carlsbad, CA, USA). In a sequential manner, segments of the gastrointestinal tract from crop to caecum were opened in the middle by an incision and split along the length longitudinally and then, pH was quickly recorded by inserting the pH meter directly into the digesta (German *et al*., 2009). Readings were recorded after stabilisation of value on the screen of pH meter.

3.12. Determination of digesta transit time

To determine the digesta transit time, feed was withdrawn for 2 h and experimental diets, containing chromic oxide at 1.0 g/kg were offered for 30 min and then substituted by original unmarked experimental diets. Digesta transit is the time lapsed between the introduction of marked diet to the first appearance of the green coloured droppings.

3.13. Determination of microflora count in caecal content.

Caecal microflora counts were made using fluorescence in situ hybridisation (FISH) technique as per the procedure described by Dinoto *et al*., (2006) with some minor modifications. Samples were prepared as described by Molan *et al*. (2009a). In brief, caecal contents from two birds (the same birds which were used for ileal digesta collection, section 3.7) were pooled and a representative sample caecal digesta (1 g) was mixed with 9 ml of sterile-filtered phosphate buffer solution (PBS; pH 7.2). The mixture was homogenised by vortexing with a dozen glass beads for 3 min. Caecal debris was removed by centrifugation at low speed (700 x *g*), and the bacteria containing supernatant was fixed in 4% (w/v) paraformaldehyde in PBS (pH 7.2) overnight at 4 ^{o}C. Fixed samples were washed in PBS and stored in a known volume of 50% (v/v) ethanol-PBS at -20 ^{o}C until the time of hybridisation. Aliquots (5 µl) of fixed bacterial cells were applied to Teflon-coated microscopic slides (BIOLAB, North Shore City, New Zealand; 10 wells) and air dried. Bacterial cells were then dehydrated with a series of solutions containing 50, 80, and 99.5% ethanol (3 min for each concentration). Bacterial cells fixed on glass slides were hybridised by addition of 8 µl of hybridisation buffer (0.9 M NaCl, 0.01% sodium dodecyl sulphate, 20 mM Tris–HCl, 20% deionised formamide; pH 7.2) with 0.5 µl of Cy3-labeled oligonucleotide specific probes (50 ng/µl). Slides were hybridised at 46 ^{o}C for 2 h in a plastic box containing wet sponges (soaked in hybridisation buffer). After hybridisation, the slides were rinsed with warm hybridisation buffer at 48 ^{o}C and washed in pre-warmed washing buffer (225 mM NaCl, 0.01% sodium dodecyl sulfate, 20 mM Tris–HCl; pH 7.2) for 20 min at 48 ^{o}C. After washing slides were rinsed with ice-cold distilled water and thoroughly dried. Dried slides were examined with an Olympus BX51 microscope, under 400X magnification. Images were captured using an Optronics MagnaFIRE SS99802 digital camera with MagnaFIRE frame-grabbing software on a Pentium IV computer (Manawatu Microscopy and Imaging Centre, Massey University, Palmerston North, New Zealand). Fluorescent cells were counted automatically in five randomly selected fields/slide using Image (Abramoff *et al*., 2004). Commercially synthesised and labelled fluorescent dye (Cy3) probes (Gene-Works, Hindmarsh, Australia) used in the study were: Bif164 with a sequence of 5-CATCCGGCATTACCACCC-3 (bifiobacteria); Lab158 with a sequence of 5-GGTATTAGCAYCTTCCA-3 (lactobacilli); Bac303 with a sequence of 5-CCAATGTGGGGGACCTT-3 (bacteroides); Chis150 with a sequence of 5-TTATGCGGTATTAATCTCCCTTT-3 (clostridia); and Cajej with a sequence of 5-AGCTAACCACACCTTATACCG-3 (campylobacter).

3.14. Gas production measurement

For measurement of gas production, a sub-sample of excreta prepared for AME determination (section 3.5), was used. A weighed excreta sample (8 g) per cage was put into air tight Hungate tubes (Fisher Scientific, North Shore City, New Zealand). The tubes were tightly sealed and placed in an incubator at 37 ^{o}C for 48 h. Gas measurement readings (in ml) were made precisely by using a needle and syringe. The assay was based on the principle that when excreta samples are incubated at 37 ^{o}C, the bacterial population will multiply and produce gas. A 20 gauge, 2.5 cm needle with syringe was used for measuring the gas production. At the end of the incubation period, the needle with syringe attached was inserted into the rubber top of the Hungate tube. Accumulated gas in the Hungate tube pushed the plunger of the syringe until the gas pressure equilibrated. Amount of gas produced in each tube was measured in ml.

3.15. Data analysis

In all experiments, cage means served as the experimental unit for statistical analysis of performance traits. For digestive tract measurements, individual birds served as the unit. In experiments reported in Chapters 4, 5 and 6, data were analysed using the General Linear Model procedure subjected to orthogonal polynomial contrast (SAS, 2004). In experiments 6 and 7, data were analysed by two-way analysis of variance using the General Linear Model procedure (SAS, 2004). If the F-test was significant, means were separated by least significant difference (LSD). Differences were considered significant at $P < 0.05$.

CHAPTER 4

Influence of pre-pelleting inclusion of whole maize on performance, nutrient utilisation, digestive tract measurements and caecal microflora counts of young broilers

4.1. Abstract

The objective of the present study was to examine the effects of pre-pelleting inclusion of whole maize on performance, digestive tract measurements, nutrient utilisation and caecal microflora counts in broiler starters. Five diets containing 600 g/kg ground maize or 150, 300, 450 and 600 g/kg whole maize replacing (w/w) ground maize, were formulated and pelleted at 65 ^{o}C. Each diet was offered *ad libitum* to six replicates (8 birds per replicate cage) from day 1 to 21 post-hatch. Weight gain and feed intake decreased (linear effect, $P<0.001$) with increasing pre-pelleting inclusion of whole maize. Feed per gain (quadratic effect, $P<0.05$) increased as the inclusion level of whole maize increased to 300 g/kg and then plateaued with further inclusions. Relative gizzard weight (quadratic effect, $P<0.05$) and apparent metabolisable energy (quadratic effect, $P<0.05$) increased with increasing inclusion of whole maize up to 300 g/kg and then levelled off. Ileal digestibility of starch (linear effect, $P<0.001$) and nitrogen (linear effect, $P=0.07$) increased with increasing inclusion levels of whole maize. A linear ($P<0.05$) effect was observed for caecal microflora numbers. *Lactobacilli* spp. counts increased and counts of *Clostridium* spp., *Campylobacterium* spp. and *Bacteroides* spp. decreased with increasing inclusion levels of whole maize. Pellet quality, measured as pellet durability index, (quadratic effect, $P<0.001$) increased sharply as the inclusion of whole maize increased to 150 g/kg and a further increase was observed at the inclusion level of 450 g/kg. The present data showed that, despite improvements in gizzard development, nutrient utilisation and pellet quality, weight gain of broilers were poorer with pre-pelleting inclusion of whole maize. Poor weight gain was due largely to the reduced feed intake.

4.2. Introduction

In recent years, whole wheat feeding of broilers has received renewed attention as a means of lowering feed costs and because of its reported positive effects on production performance and nutrient utilisation (Wu *et al*., 2004; Wu and Ravindran, 2004). These positive effects are often attributed to the impact of feed structure on gizzard development. In addition, there is some evidence that whole wheat feeding may influence gut microflora ecology (Santos *et al.*, 2008). This has encouraged nutritionists to explore whole grain feeding as a natural feeding strategy to improve health and production efficiency of broilers. Such an approach is becoming especially relevant in view of an anticipated global ban on use of antibiotic growth promoters as feed additives.

Wheat is the grain of choice for whole grain feeding in poultry. However, throughout the world, poultry diets are primarily based on maize. Despite this, little or no attempt has been made to use whole maize in poultry diets. The size of maize grain may physically limit feeding it whole to poultry and this may be responsible for the lack of interest to evaluate whole maize in poultry diets. Pre-pelleting inclusion of whole maize could overcome this limitation, while delivering the benefits associated with whole grain feeding.

Studies examining the influence of pre-pelleting inclusion of whole grains in poultry diets are limited and have produced equivocal results. Differences in experimental methodology and inclusion level of whole grain may be responsible for these inconsistent results. Wu *et al*. (2004) reported that pre-pelleting inclusion of 200 g/kg whole wheat had no effect on gizzard development, but improved the performance of broilers. In contrast, Jones and Taylor (2001) and Taylor and Jones (2004a) observed that inclusion of 200 g/kg whole wheat had no effect on broiler performance, but increased relative gizzard weights. Svihus *et al*. (2004a) increased pre-pellet inclusion level of whole wheat to 500 g/kg and found no differences in performance parameters, but significant increases in gizzard weight compared to birds fed ground wheat diets.

Most previous studies have evaluated a constant and low inclusion level of whole grain (Plavnik *et al*., 2002; Taylor and Jones, 2001; Wu *et al*., 2004; Ravindran *et al*., 2006; Amerah and Ravindran, 2008). Graded increases in pre-pelleting inclusion level of whole maize in broiler diets may provide a better understanding of its effects on production performance and pellet quality parameters. The aim of the present study was to examine the effects of pre-pelleting inclusion of graded levels of whole maize on performance, energy and nutrient utilisation, digestive tract development, and caecal microbial counts of broiler starters.

4.3. Material and methods

4.3.1. Hardness of maize

Maize was purchased as whole grain from a local supplier. Upon receipt, requisite samples were obtained for measurement of grain hardness. Hardness was determined using a micro hammer mill (Stenvert Hardness Tester, Glen Creston Ltd. Stanmore, UK) as per the method described by Abdollahi *et al*. (2011). Maize samples (20 g) were ground using a micro hammer mill (5,692 rpm) equipped with a 0.2 mm sieve and the energy (KJ) needed to grind the sample was determined. Measurements were made on five samples and adjusted to 140 g/kg moisture. Maize used in the experiment required 6.2 KJ energy to mill a 20 g sample, which confirmed that it was a soft maize variety, yellow dent (Li *et al*., 1996).

4.3.2. Diets

Five maize soy diets containing 600 g/kg ground maize or 150, 300, 450 and 600 g/kg whole maize replacing (w/w) ground maize were formulated to meet Ross 308 strain recommendations for major nutrients for broiler starters (Ross, 2007; Table 4.1). Whole and ground maize used were from the same lot. For the ground maize component, whole maize was ground in a single batch in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) to pass through a 4.0 mm screen. Diets were formulated to be isocaloric and isonitrogenous. All diets were mixed and cold pelleted at 65 ^{o}C using a pellet mill (Richard Size Limited Engineers, orbit, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring of 3 mm hole size and 35 mm thickness. Representative samples were collected after pelleting for the determination of particle size distribution, pellet quality (pellet durability and hardness). All diets were manufactured one week prior to the start of the experiment.

Table 4.1. Composition and calculated analysis of the starter diet

Ingredients	g/kg as fed
Maize[1]	600.0
Soybean meal	297.4
Soybean oil	20.7
Meat bone meal	59.4
Dicalcium phosphate	2.0
Limestone	8.0
L-lysine	1.0
DL-methionine	2.5
Salt	3.0
Titanium oxide	3.0
Vitamin- trace mineral premix[2]	3.0
Calculated analysis	
AME, (MJ/kg)	12.7
Crude protein	223
Lysine	12.6
Methionine + Cysteine	9.6
Threonine	9.0
Tryptophan	2.4
Calcium	10.4
Total phosphorus	6.9
Available phosphorus	4.5

[1] In treatment diets, ground maize was replaced (w/w) by 0,150, 300, 450 and 600 g /kg whole maize prior to pelleting.

[2] Nutrients supplied per kilogram of premix: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017mg; folic acid, 5.2mg; menadione, 4 mg; niacin, 35mg; 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, 60mg.

4.3.3. Birds and housing

Two hundred and forty day-old male broiler chicks (Ross 308), obtained from a commercial hatchery, were assigned to 30 cages (8 birds per cage) as described in Chapter 3, section 3.1. Each of the five dietary treatments was then randomly allocated to six replicate cages. Feed was offered *ad libitum* and water was available at all times throughout the 21-day trial Housing conditions have been described in Chapter 3, section 3.1.

4.3.4. Performance data

Performance data were recorded as described in Chapter 3, section 3.1.

4.3.5. Determination of particle size distribution

Particle size distribution of pelleted diets was determined as described in Chapter 3, section 3.3.

4.3.6. Pellet quality

Pellet durability index (PDI) and pellet hardness were measured as described in Chapter 3, sections 3.4 and 3.5, respectively.

4.3.7. Apparent metabolisable energy (AME) determination

The AME determination was carried out between days 17 and 20 post-hatch as described in Chapter 3, section 3.6.

4.3.8. Ileal digestibility determination

On day 21, two birds per cage were euthanised and, ileal digesta was collected and processed as described in Chapter 3, section 3.7.

4.3.9. Determination of caecal microflora.

Caecal microflora counts were performed only in three extreme range treatments (0, 300 and 600 g/kg) out of total five treatments. From birds used for ileal digesta collection (two birds /cage), caeca were carefully excised and stored at -20 ^{o}C until determination of microflora using a fluorescence in situ hybridization technique as described in Chapter 3, section 3.13.

4.3.10. Digestive tract measurements

On day 21, two more birds per cage were killed by cervical dislocation and digestive tract measurements were carried out as described in Chapter 3, section 3.10.

4.3.11. Determination of digesta transit time

On day 22, the birds were used to determine digesta transit time as described in Chapter 3, Section 3.12.

4.3.12. pH of digesta of different segments of digestive tract

On day 22, one bird per cage was killed and pH of digesta was measured as described in Chapter 3, section 3.11.

4.3.13. Measurement of excreta gas production

Excreta gas production was measured as described in Chapter 3, section 3.14.

4.3.14. Chemical analysis

Dry matter, nitrogen (N), gross energy, titanium, and starch contents were determined as described in Chapter 3, section 3.8.

4.3.15. Calculations

The AME and apparent ileal nutrient (N and starch) digestibility were calculated using the formulae described in in Chapter 3, section 3.9.

4.3.16. Data analysis

Cage means served as the experimental unit for performance data. For digestive tract data, individual birds were considered as the experimental unit. All data were subjected to orthogonal polynomial contrasts using the general linear model procedure of SAS (2004) to examine whether responses to increasing levels of pre-pelleting inclusion of whole maize were of a linear or quadratic nature. Differences were considered significant when $P<0.05$.

4.4. Results

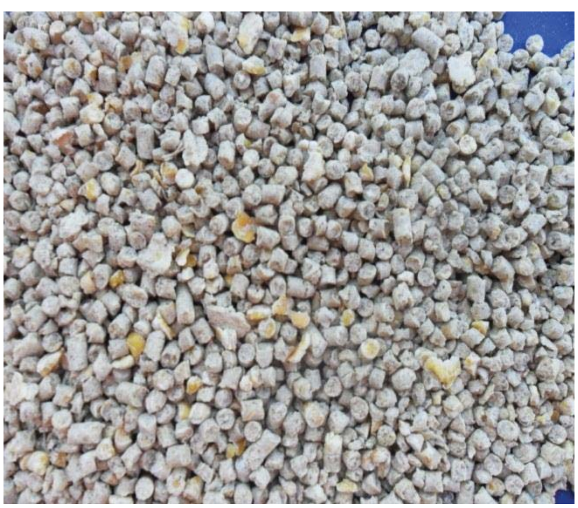
Whole maize and pellets produced at different inclusion levels of whole maize are shown in Figure 4.1.

Ground maize

150 g/kg whole maize

300 g/kg whole maize

450 g/kg whole maize



600 g/kg whole maize

Whole maize

Figure 4.1. Pellets produced with different pre-pelleting inclusion levels of whole maize and the whole maize used in the experiment

4.4.1. Particle size distribution

Particle size distribution of pelleted diets is shown in Figure 4.2. The proportion of coarse particles (> 1 mm) increased with increasing pre-pelleting inclusion of whole maize. The percentage of coarse particles were 8.9, 14.1, 16.0, 18.3 and 19.5% and those over 2 mm were 0.28, 2.1, 3.8, 4.7 and 6.0% in diets pre-pelleted with 0, 150, 300 , 450 and 600 g/kg whole maize, respectively.

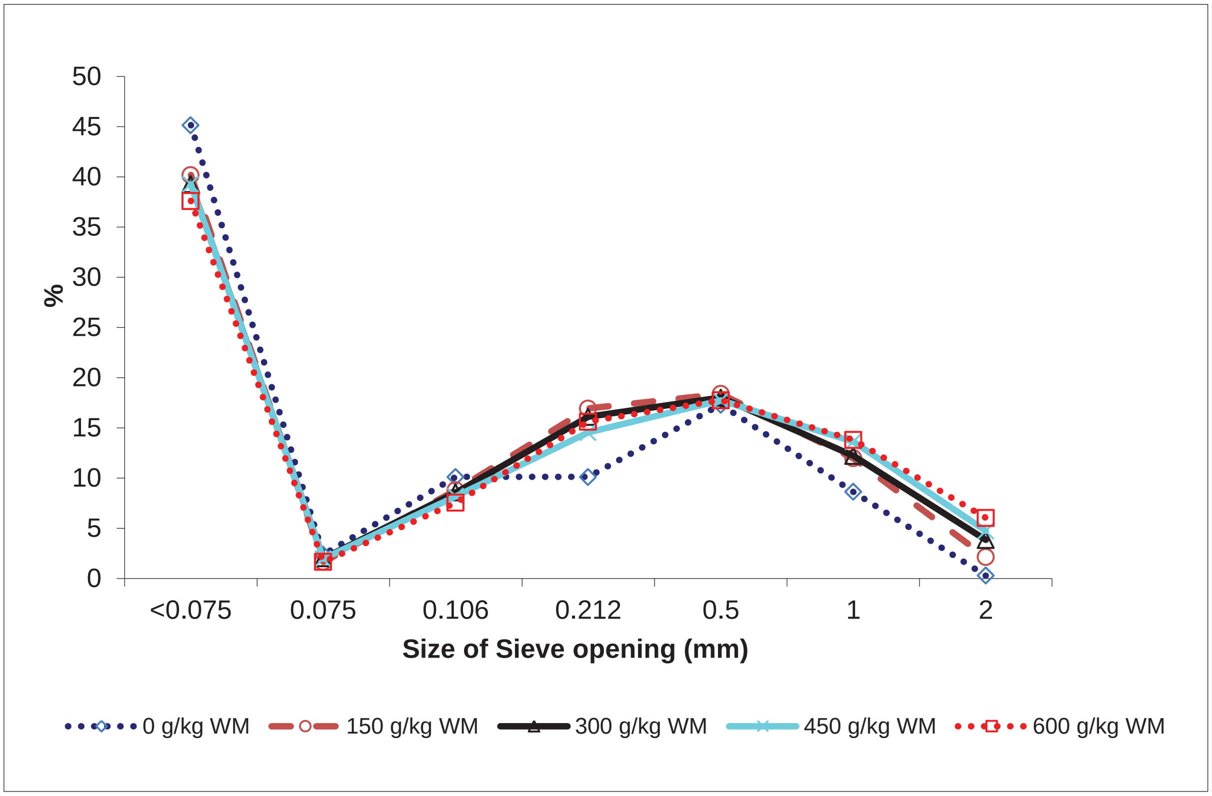


Figure 4.2. Particle size distribution of pelleted diets containing 0, 150, 300, 450 and 600 g/kg whole maize obtained by wet sieving method

4.4.2. Pellet quality

Influence of dietary treatments on the pellet durability and hardness is presented in Table 4.2. Pellet durability, measured as pellet durability index (PDI), showed a quadratic ($P<0.001$) response. Pellet durability index increased sharply as the inclusion of whole maize increased to 150 g/kg and a further increase in PDI was observed at the inclusion level of 450 g/kg. A linear ($P<0.001$) increase in pellet hardness was observed with increasing inclusion of whole maize.

Table 4.2. Influence of inclusion of graded levels of whole maize on the pellet durability index (PDI, %) and pellet hardness (Newton)

Whole maize inclusion (g/kg)	PDI[1]	Pellet hardness[2]
0	64.4	16.4
150	77.9	22.2
300	77.5	26.0
450	81.9	27.9
600	83.5	35.5
SEM[3]	0.42	1.44
Probabilities, P ≤		
Overall treatment effect	0.0001	0.001
Linear effect	0.0001	0.0001
Quadratic effect	0.0001	0.75

[1] Each value represents the mean of six samples.
[2] Each value represents the mean of 15 samples
[3] Pooled standard error of mean.

4.4.3. Performance

Mortality during the experiment was negligible. Only three birds out of 240 died and the deaths were not related to any specific treatment

Linear ($P<0.001$) decreases in weight gain and feed intake were observed with increasing inclusion of whole maize (Table 4.3). However, a quadratic ($P<0.05$) response was observed for feed per gain. Feed per gain increased with increasing inclusion levels of whole maize to 300 g/kg and then plateauing with further inclusion.

Table 4.3. Influence of pre-pelleting inclusion of graded levels of whole maize on weight gain (g/bird), feed intake (g/bird), and feed per gain (g/g) of broilers from 1-21 days posthatch[1]

Whole maize inclusion (g/kg)	Weight gain,	Feed intake,	Feed per gain,
0	1005	1303	1.304
150	990	1312	1.324
300	919	1214	1.334
450	933	1226	1.341
600	857	1136	1.341
SEM[2]	19.8	25.6	0.0056
Probabilities , P ≤			
Overall treatment effect	0.0001	0.001	0.001
Linear effect	0.0001	0.0001	0.0001
Quadratic effect	0.62	0.37	0.05

[1] Each value represents the mean of six replicates (8 birds per replicate).
[2] Pooled standard error of mean.

4.4.4. Nutrient utilisation

Effects of pre-pelleting inclusion of graded levels of whole maize on the AME and, apparent ileal N and starch digestibility coefficients are shown in Table 4.4. A quadratic effect ($P<0.05$) was observed for AME with increasing inclusion levels of whole maize. Apparent metabolisable energy increased with increasing inclusion to 300 g/kg and then decreased with further inclusions to 600 g/kg. A tendency for a linear increase ($P=0.07$) was seen for ileal nitrogen digestibility with increasing inclusion of whole maize. Starch digestibility coefficient increased linearly ($P<0.001$) as inclusion level increased.

Table 4.4. Influence of pre-pelleting inclusion of graded levels of whole maize on AME (MJ/kg DM) and ileal nitrogen and starch digestibility coefficients for broilers[1]

Whole maize inclusion (g/kg)	AME	Ileal N digestibility	Ileal starch digestibility
0	14.23	0.791	0.983
150	14.42	0.802	0.987
300	14.50	0.802	0.990
450	14.33	0.811	0.993
600	14.37	0.810	0.992
SEM[2]	0.053	0.0080	0.0014
Probabilities , P ≤			
Overall treatment effect	0.05	0.40	0.001
Linear effect	0.26	0.07	0.0001
Quadratic effect	0.05	0.65	0.09

[1] Each value represents the mean of six replicates (8 birds per replicate).
[2] Pooled standard error of mean.

4.4.5. Digestive tract and organ measurements

Influence of pre-pelleting inclusion of whole maize on the relative weights of proventriculus, gizzard and digestive organs is summarised in Table 4.5. A quadratic effect ($P<0.05$) was observed for the relative gizzard weight, with the weight increasing as inclusion of whole maize increased to 300 g/kg and then plateauing with further inclusion. Relative weights of proventriculus and pancreas showed a linear ($P<0.05$) increase with increasing inclusion of whole maize. A quadratic ($P<0.05$) response was observed for the relative weight of liver, with the weight decreasing at 150 g/kg inclusion and then plateauing with further inclusion. .Relative weight of the spleen was unaffected ($P>0.05$) by dietary treatments.

Table 4.5. Relative weights of proventriculus, gizzard and digestive organs (pancreas, liver and spleen) of broilers as influenced by pre-pelleting inclusion of graded levels of whole maize[1]

Whole maize inclusion	Relative weight (g/kg body weight)				
(g/kg)	Proventriculus	Gizzard	Pancreas	Liver	Spleen
0	3.94	11.9	2.37	31.1	0.872
150	4.33	14.4	2.93	29.6	0.938
300	4.27	15.0	2.78	28.5	0.835
450	4.57	15.7	2.93	28.5	0.800
600	4.70	15.5	2.89	29.7	0.844
SEM[2]	0.141	0.56	0.160	0.85	0.069
Probabilities , P ≤					
Overall treatment effect	0.001	0.0001	0.05	0.18	0.70
Linear effect	0.01	0.0001	0.05	0.16	0.38
Quadratic effect	0.77	0.05	0.06	0.05	0.93

[1] Each value represents the mean of 12 birds.
[2] Pooled standard error of mean.

Effects of pre-pelleting inclusion of graded levels of whole maize on the relative weight and length of different segments of the intestinal tract are shown in Table 4.6. Except for a linear ($P < 0.05$) increase in the relative weight of jejunum, no effects ($P > 0.05$) were observed for relative weights any intestinal segment or small intestine.

No effects were observed for the relative length of any intestinal segment except ileum (linear effect, $P < 0.01$) and caeca (quadratic effect, $P < 0.05$). However, a quadratic ($P < 0.05$) effect was observed for the relative length of the small intestine. As the inclusion level of whole maize was increased, a linear increase was observed for the length of the ileum. Length of caeca and overall length of small intestine was unaffected when pre-pelleting inclusion of whole maize was increased to 450 g/kg, whereas increase from 450 to 600 g/kg resulted in significant increase in relative lengths.

Table 4.6. Relative weight and length of the intestinal tract of broilers as influenced by pre-pelleting inclusion of graded levels of whole maize[1]

Whole maize inclusion	Relative weight (g/kg body weight)					Relative length (cm/kg body weight)				
(g/kg)	Duodenum	Jejunum	Ileum	Caeca	Small Intestine[2]	Duodenum	Jejunum	Ileum	Caeca	Small Intestine[2]
0	6.45	9.62	6.94	3.40	23.0	26.5	61.0	63.7	14.6	151
150	7.03	10.1	7.28	3.24	24.4	28.6	62.0	66.3	14.2	157
300	6.30	9.82	6.95	3.09	23.0	27.5	61.7	66.7	14.2	155
450	6.03	10.2	6.96	3.16	23.2	26.0	62.9	66.9	14.0	155
600	6.41	10.8	7.60	3.18	24.8	28.3	68.8	73.0	15.7	170
SEM[3]	0.28	0.35	0.295	0.120	0.69	1.06	2.06	2.10	0.47	4.6
Probabilities , P ≤										
Overall treatment effect	0.17	0.14	0.43	0.49	0.21	0.44	0.06	0.05	0.16	0.06
Linear	0.28	0.05	0.29	0.20	0.07	0.78	0.01	0.01	0.10	0.01
Quadratic	0.96	0.51	0.40	0.21	0.68	0.94	0.13	0.39	0.05	0.05

[1] Each value represents the mean of 12 birds.
[2] Small intestine = duodenum+ jejunum + ileum.
[3] Pooled standard error of mean.

4.4.6. Caecal microflora counts

Effects of inclusion of graded levels of whole maize in broiler diets on caecal microbial populations are presented in Table 4.7. With increasing inclusion of whole maize, there was a linear increase in numbers of *Lactobacilli* spp. (P<0.01) and linear decreases in numbers of *Clostridium* spp. (P<0.01), *Campylobacter* spp. (P<0.05) and Bacteroides spp. (P<0.05). Dietary treatments had no effect (P>0.05) on *Bifidobacteria* spp. counts.

Table 4.7. Influence of pre-pelleting inclusion of graded levels of whole maize on caecal microflora counts (Log 10 cells/g caecal content) in broilers [1]

Whole maize inclusion (g/kg)	*Lactobacilli* spp.	*Bifidobacteria* spp.	*Clostridium* spp.	*Campylobacter* spp.	*Bacteroides* spp.
0	6.94	6.29	7.43	6.24	6.40
300	7.21	6.40	6.93	6.10	6.17
600	7.35	6.29	6.84	5.91	6.04
SEM[2]	0.079	0.069	0.138	0.109	0.110
Probabilities, P ≤					
Overall treatment effect	0.01	0.48	0.05	0.14	0.09
Linear effect	0.01	0.99	0.01	0.05	0.05
Quadratic effect	0.53	0.24	0.23	0.84	0.75

[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.

4.4.7. pH of the digesta

Influence of pre-pelleting inclusion of whole maize on pH of digesta from various segments of the digestive tract is presented in Table 4.8. A linear (P<0.001) decrease in pH of gizzard contents was observed with increasing inclusion level of whole maize. A quadratic response (P<0.05) was seen for pH of proventriculus contents. However, no effects (P>0.05) were observed for pH of digesta from caeca or any intestinal segment. A linear (P<0.05) increase in pH of excreta was observed with increasing inclusion of whole maize.

Table 4.8. Influence of pre-pelleting inclusion of whole maize on pH of digesta from various segments of digestive tract and excreta of broilers[1]

Whole maize inclusion (g/kg)	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Caeca	Excreta
0	3.88	4.01	5.76	5.93	6.63	6.46	7.16
150	3.01	3.98	6.06	6.15	6.66	6.58	7.55
300	3.00	3.48	5.96	6.06	6.93	6.38	7.61
450	3.16	3.35	5.93	6.08	6.90	6.60	7.65
600	3.20	3.50	6.01	6.10	6.96	6.53	7.94
SEM[2]	0.231	0.134	0.086	0.080	0.125	0.080	0.208
Probabilities , P ≤							
Overall treatment effect	0.07	0.01	0.17	0.42	0.21	0.32	0.16
Linear effect	0.11	0.001	0.19	0.30	0.05	0.56	0.05
Quadratic effect	0.05	0.16	0.27	0.33	0.62	0.87	0.38

[1] Each value represents the mean of six birds.
[2] Pooled standard error of mean.

4.4.8. Excreta gas production and digesta transit time

Dietary treatments had no effects (P>0.05) on excreta gas production and digesta transit time with increasing pre-pelleting inclusion of whole maize (Table 4.9).

Table 4.9. Influence of pre-pelleting inclusion of whole maize on excreta gas production and digesta passage rate in broilers[1]

Whole maize inclusion (g/kg)	Gas production (ml/g)	Transit time (min)
0	0.450	163
150	0.500	160
300	0.502	158
450	0.460	146
600	0.555	164
SEM[3]	0.0510	5.5
Probabilities , P ≤		
Overall treatment effect	0.62	0.17
Linear effect	0.16	0.93
Quadratic effect	0.46	0.60

[1] Each value represents the mean of six replicates values.
[2] Pooled standard error of mean.

4.5. Discussion

To the author's knowledge, no published data are available on the effects of whole maize inclusion in poultry diets. In the present study, feeding broiler starter diets with increasing pre-pelleting inclusion levels of whole maize resulted in poor weight gain despite better development of the gizzard. Weight gain decreased linearly with increasing inclusion of whole maize and this paralleled the linear depression observed for feed intake.

Reasons for decreased feed intake are unclear, especially as pellet quality, measured as PDI, improved with increasing inclusion of whole maize. Benefits in broiler performance associated with pellet quality have been well documented (Calet, 1965; Doughlas *et al*., 1990; Nir *et al*., 1995; Jensen, 2000; Nir and Ptichi, 2001). Poor pellet quality reduces feed intake and bird performance (Proudfoot and Sefton, 1978; Nir *et al*., 1995). In the present study, however, feed intake was reduced despite improved PDI. This observation questions whether PDI measured using the Holmen Pellet Tester is a good measure of pellet quality when whole grains are used. This method of PDI determination is most reliable when particle size of ingredients making up the pellet is similar. In the present study, there were large differences in the particle size among ingredients because of the relatively large size of whole maize and as maize inclusion rate increased, proportion of larger particles making up the pellets increased. The Holmen Pellet Tester measures pellet durability by circulating a sample of pellets in a stream of air, forcing them into contact with each other and tester walls. An excluder screen allows smaller particles (e.g., from broken pellets) to leave the tester. The weight of material remaining within the tester is a measure of pellet durability. The current findings suggest that, when pellets contain particles too large to pass through the excluder screen and leave the pellet tester even if pellets break, the Holman Pellet Tester will not accurately measure the actual pellet durability.

From visual observations, it appeared that the selective ingestion of larger maize particles, whose proportion progressively increased in pellets with increasing pre-pelleting inclusion level of whole maize, was partly responsible for the depressions in feed intake. In agreement, Clark *et al*. (2009) also reported linear decrease in weight gain and feed intake in broilers fed diets containing 150, 300, 450 or 600 g/kg cracked maize blended with pellets during the starter phase (0 to18 days). These researchers also speculated possibly feed sorting and selection could be the reason for the observed responses.

Higher relative gizzard weights observed with pre-pelleting inclusion of whole maize is consistent with published reports on whole wheat feeding (Jones and Taylor, 2001; Taylor and Jones, 2004a; Svihus *et al*., 2004a). In the current study, however, gizzard weight plateaued at the maize inclusion level of 300 g/kg, suggesting gizzard growth may have a threshold beyond which development may be restricted. However, such a threshold may not necessarily associated with grinding efficiency and functionality of the gizzard. This thesis is supported by the finding that while gizzard weight plateaued, ileal digestibilities of starch and N linearly increased with increasing whole maize inclusions.

Increasing pre-pelleting inclusion of whole maize resulted in a greater proportion of coarse particles in the diets and, this would be expected to increase the contraction frequency of the gizzard to reduce the particle size before entry to the duodenum. Regardless of original feed structure size, particles need to be of a critical size before they can leave the gizzard (Clemens *et al*., 1975). Grinding action of the gizzard activates cholecystokinin release (Svihus *et al*., 2004a), which in turn stimulates secretion of pancreatic enzymes and gastro-duodenal reflux (Duke, 1992; Li and Owyang, 1993). This serves to re-expose digesta to pepsin, enhancing mixing of digesta with pancreatic enzymes, and improving digestion of nutrients (Ferket, 2000; Hetland *et al*., 2002; Ravindran *et al*., 2006). Increases in relative weight of the pancreas and improvements in the digestibility of nitrogen and starch observed in the present study lend support to this hypothesis. Increased relative weight of the pancreas with inclusion of whole wheat has been reported previously (Banfield *et al*., 2002; Engberg *et al*., 2004; Wu *et al*., 2004; Gabriel *et al*., 2008).

In the present study, pre-pelleting inclusion of whole maize resulted improvements in AME. However, AME increased up to 300g/kg whole maize inclusion and then leveled off with further inclusions. This observation is in agreement to previous studies with whole wheat that showed inclusion of whole wheat at levels of 200 to 375 g/kg improved the AME (Svihus *et al*., 2004a; Wu *et al*., 2004). It is noteworthy that the inclusion level of whole wheat at 500g/kg failed to show any positive impact on the AME (Svihus *et al*., 2004a).

The increase in the relative weight of the proventriculus observed in the present study with increasing inclusion of whole maize is in contrast to several studies showing that birds fed whole wheat have either reduced the weight of the proventriculus (Jones and Taylor, 2001; Taylor and Jones, 2004a) or had no effect (Banfield *et al*., 2002; Bennett *et al*., 2002; Gabriel *et al*., 2003a; Wu and Ravindran 2004; Ravindran *et al*., 2006; Amerah and Ravindran, 2008) compared to those fed ground wheat.

Examination of gizzard pH in the current trial indicates that gastric HCl secretion, and thus gastric function, was stimulated by the inclusion of whole maize. The observed increase in proventricular weight may be a further indication of greater gastric function with whole maize feeding. This suggestion is supported by the increasing ileal nutrient digestibility as inclusion of whole maize increased. Similar decreases in gizzard pH have been reported in broilers fed coarsely ground compared to finely ground maize (Nir *et al*., 1994a) and those fed whole wheat versus ground wheat diets(Gabriel *et al*., 2003a).

The more acidic environment in the gizzard may be responsible for the observed linear decreases in the populations of harmful bacteria, namely *Clostridium* spp., *Campylobacter* spp. and *Bacteroid*es spp., with progressive increase in whole maize inclusion level. Furthermore, increase in the numbers of beneficial *Lactobacilli* spp. may also have decreased the numbers of harmful bacteria through competitive exclusion. The diet is the ultimate source of substrate for the metabolism of bacteria (Savory, 1992) and, whole grain inclusion in diets encourages the colonisation of commensal bacteria and discourages the colonisation of harmful bacteria through competitive exclusion, HCl secretion or grinding action of the gizzard. The gizzard thus has an important function as a barrier organ that prevents pathogenic bacteria from entering the distal digestive tract (Engberg *et al*., 2004; Bjerrum *et al*., 2005; Santos *et al*., 2008). These results are in agreement to those of Engberg *et al*. (2002) who reported that feeding whole wheat resulted in a more acidic gizzard environment and greater numbers of *Lactobacilli* spp. and fewer harmful bacteria such as *E. coli*.

No published data on the effects of whole maize inclusion on digestive tract development of broilers are available. Published data on the effects of whole wheat feeding on digestive tract development (other than the gizzard) are not in general agreement (Jones and Taylor, 2001; Banfield *et al*., 2002; Engberg *et al*., 2004 Gabriel *et al*., 2003a; Wu and Ravindran, 2004; Ravindran *et al*., 2006; Amerah and Ravindran, 2008; Gabriel *et al*., 2008) with the findings of the present trial. However, the reduction in the relative weight of the liver with inclusion of whole maize is in agreement with pre-pelleting inclusion of whole wheat in one study (Wu and Ravindran, 2004). In the present study, the relative weight of the jejunum, and length of jejunum and ileum increased linearly with increasing inclusion of whole maize. No previous studies with whole wheat have reported changes in jejunal weight (Preston *et al*., 2000; Jones and Taylor, 2001; Banfield *et al*., 2002; Engberg *et al*., 2004; Wu *et al*., 2004; Ravindran *et al*., 2006). In broilers fed whole wheat, Amerah and Ravindran (2008) reported an increase in the relative lengths of jejunum and ileum, but, Gabriel *et al*. (2008) reported a decrease in the length of jejunum.

It is unclear why the relative length, but not weight, of the small intestine was markedly greater with a whole maize inclusion of 600 g/kg compared with lower inclusion rates in the current trial. Amerah and Ravindran (2008), however, reported a similar increase in length of all segments of small intestine in broilers fed whole wheat in a free choice feeding system as compared to those fed ground wheat. In contrast, most studies where ground wheat was substituted with whole wheat showed no changes in relative weight and length of the segment of small intestine (Preston *et al*., 2000; Jones and Taylor, 2001; Banfield *et al*., 2002; Engberg *et al*., 2004; Wu *et al*., 2004; Ravindran *et al*., 2006).

Gas production in fresh manure is not a problem, but storage of manure leads to production of harmful gases mainly by anaerobic bacteria (Canada Animal Manure Management Guide, 1979). Accordingly, antimicrobial effect of whole grain feeding strategies could reduce atmospheric emissions of volatile organic compounds from poultry manure that cause odour and also to reduce the risk of releasing disease-transmitting vectors and airborne pathogens (Laubach, 2006). However, in the present study, the observed change in the populations of the studied pathogenic bacteria did not transfer to a significant decrease *in vitro* gas production from anaerobic incubation of fresh droppings of broilers fed diets with whole maize.

The digesta transit time was similar between dietary treatments regardless of whole maize inclusion in this study. This finding is consistent with previous studies where overall digesta retention time did not change when the birds were fed whole grains (Svihus *et al*., 2002; Svihus *et al*., 2004a; Wu *et al*., 2004 Amerah *et al*., 2007; Amerah and Ravindran 2008).

4.6. Conclusions

In summary, it was thought that pre-pelleting inclusion of whole maize could overcome initial kernel size issues associated with whole maize. This strategy, however, resulted in poor weight gain in broilers due to reduced feed intake, despite enhanced gizzard development and nutrient digestion. Enhanced gizzard development with pre-pelleting inclusion of whole maize had a positive impact on beneficial bacteria and negative impact on harmful bacteria, suggesting that the gizzard acts as a barrier to prevent the entry of pathogenic bacteria into distal gastrointestinal tract.

CHAPTER 5

Influence of feeding coarse maize on performance, nutrient utilisation, digestive tract measurements, carcass characteristics and caecal microflora counts of broilers

5.1. Abstract

The objective of the present study was to examine the effects of feeding coarsely ground maize on performance, digestive tract measurements, nutrient utilisation and caecal microflora counts in broilers. Five diets containing 600 g/kg finely-ground maize (hammer milled) or 150, 300, 450 and 600 g/kg coarse maize (cracked in roller mill) replacing (w/w) finely-ground maize were formulated. Each diet in mash form was offered *ad libitum* to six replicate cages of broilers (8 birds per cage) from day 11 to 35 post-hatch. Weight gain increased (linear effect, $P<0.01$) with increasing inclusion of coarse maize. Feed intake (quadratic effect, $P<0.05$) increased at 150 g/kg coarse maize inclusion, plateaued until 450 g/kg, and then increased again to 600 g/kg. Feed per gain (quadratic effect, $P<0.05$) increased as inclusion of coarse maize increased to 300 g/kg and then decreased with further inclusion. Apparent metabolisable energy (quadratic effect, $P<0.01$) was unaffected up to 300g/kg inclusion, and then decreased with further inclusion of coarse maize. Relative gizzard weight increased (linear effect, $P<0.05$) with increasing inclusion of coarse maize. Inclusion of coarse maize had no affect ($P>0.05$) on ileal nitrogen and carcass yield, whereas, starch digestibility tended to decrease linearly ($P=0.07$) as inclusion of coarse maize increased. Breast weight decreased linearly ($P<0.05$), whereas abdominal fat increased (linear effect, $P<0.001$) with increasing inclusion of coarse maize. A linear ($P<0.05$) effect was observed for caecal microflora counts. *Lactobacilli* spp. *and bifidobacteria* spp. counts increased and counts of *Clostridium* spp., *Campylobacterium* spp. and *Bacteroides* spp. decreased with increasing inclusion of coarse maize. The present data showed that coarse maize can totally replace ground maize in broiler diets with beneficial effects on weight gain, gizzard development and gut microflora profile.

5.2. Introduction

In many regions of the world, particularly in Europe, Australasia and Canada, there is renewed interest in incorporating whole grains in broiler diets. This interest is driven by the need to reduce feed costs while improving bird health and welfare (Gabriel *et al*., 2003a).

Diets that are used in these countries are generally based on wheat, and studies focusing on use other whole grains, especially maize, are limited.

Maize is the most commonly used cereal in poultry diets throughout the world. Despite this, little attempt has been made to evaluate use of whole maize in poultry diets. The size and hardness of the grain may be physical limitations for birds and could be the reason for lack of research in whole maize for broiler diets. However, minor processing such as cracking may overcome these concerns. Feeding coarse maize has the potential to produce benefits similar to those seen in whole grain feeding (Parsons *et al*., 2006).

Beneficial effects of whole grain feeding have been attributed to enhanced gizzard function due to increased frequency of contraction to reduce whole grain to fine particles (Hill, 1971; Roche, 1981). Furthermore, increased gizzard action may help prevent entry of pathogenic bacteria into the intestinal tract (Engberg *et al*., 2004, Santos *et al*., 2008).

Identification of strategies to utilise whole or minimally processed maize in broiler diets is warranted. The objective of the present study was to examine effects of adding graded amounts of coarse maize on the performance, AME, nutrient utilisation, digestive tract development and caecal microflora counts of broilers.

5.3. Material and methods

5.3.1. Hardness of maize

Maize was purchased as whole grain from a local supplier. Upon receipt, requisite samples were obtained for measurement of grain hardness. Hardness was determined using a micro hammer mill (Stenvert Hardness Tester, Glen Creston Ltd. Stanmore, UK) as described by Abdollahi *et al*. (2011). Maize samples (20 g) were ground using a micro hammer mill (5,692 rpm) equipped with a 0.2 mm sieve and the energy (KJ) needed to grind the sample was determined. Measurements were made on five samples and adjusted to 140 g/kg moisture. Maize used in the experiment required 6.2 KJ energy to mill a 20 g sample, which confirmed that it was a soft maize variety, yellow dent (Li *et al*., 1996).

5.3.2 Diets

Five maize soy diets containing 600 g/kg ground maize or 150, 300, 450 and 600 g/kg coarse maize replacing (w/w) ground maize were formulated to meet the Ross 308 strain recommendations for major nutrients for broiler finishers (Ross, 2007). Ingredient composition and calculated analysis of diets are shown in Table 5.1. Ground maize was manufactured by grinding whole maize through a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) using a 4.0 mm screen. Coarse maize was prepared by cracking whole maize in a laboratory scale twin roller flour mill. Rollers were of 200 mm diameter fluted with a 1 mm pitch. The mill was operated with two rollers counter-rotating at differential speeds (530 and 630 rpm). Samples of ground and coarse maize were obtained from the same batch before processing. Treatment diets were formulated to be isocaloric and isonitrogenous. Representative samples of coarse maize and diets were collected for determination of geometric mean diameter (GMD) and geometric standard deviation (GSD). All diets were stored for a week prior to the start of the experiment.

Table 5.1. The composition and calculated analysis of the finisher diet (g/kg as-fed basis)

Ingredient	
Maize[1]	600.0
Soybean meal	316.8
Soybean oil	49.5
Dicalcium phosphate	14.5
Limestone	12.0
L-lysine	1.0
DL-methionine	1.6
Salt	2.4
Vitamin-trace mineral premix[2]	2.2
Calculated analysis	
AME, (MJ/kg)	13.5
Crude protein	203
Lysine	11.7
Methionine + Cysteine	8.2
Theronine	8.4
Tryptophan	2.3
Calcium	8.8
Total phosphorus	6.4
Available phosphorus	3.9

[1] In the treatment diets, 0, 150, 300, 450 and 600 g/kg ground maize replaced (w/w) by coarse maize.

[2] Nutrients supplied per kilogram of premix: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017mg; folic acid, 5.2mg; menadione, 4 mg; niacin, 35mg; 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, 60mg.

5.3.3. Birds and Housing

Two hundred and forty day-old male broiler (Ross 308) chicks, obtained from a commercial hatchery, were raised in three-tier electrically heated battery brooders housed in an environmentally controlled room until 10 day of age. During this time, birds were fed a commercial maize-based broiler starter diet. On day 11, birds were individually weighed and allocated to 30 colony cages (8 birds/cage) so that the total of all birds in each cage was equal. Each of the five dietary treatments in mash form was randomly allocated to six cages. Feed was offered *ad libitum* and water was freely available throughout the trial which lasted until day 35 post-hatch. Housing conditions have been described in Chapter 3, section 3.1.

5.3.4. Performance data

Performance data were recorded as described in chapter 3, section 3.1.

5.3.5. Determination of particle size distribution

Particle size distribution of coarse maize and diets were measured for determination of geometric mean diameter (GMD) and geometric standard deviation (GSD) as described in Chapter 3, section 3.2.

5.3.6. Apparent metabolisable energy (AME) determination

The AME determination was carried out between days 31-34 post-hatch as described in Chapter 3, section 3.6.

5.3.7. Ileal digestibility determination

On day 35, two birds per cage were euthanised and ileal digesta was collected and processed as described in Chapter 3, section 3.7.

5.3.8. Determination of caecal microflora

From the birds which were used for ileal digesta collection (two birds /cage), caeca were carefully excised and stored at -20 ^{o}C until used for determination of microflora using fluorescence in situ hybridization technique as described in Chapter 3, section 3.13.

5.3.9. Carcass measurements

On day 35 day, two more birds per cage (close to the mean cage weight) were selected, weighed and killed by cervical dislocation. Following exsanguination, feathers, viscera, shanks, and neck were removed and weights of the eviscerated hot carcass, abdominal fat and breast muscle were recorded

5.3.10. Digestive tract measurements

On day 35 day, from the birds which were used for carcass measurements (two birds /cage), digestive tract measurements were carried out as described in Chapter 3, section 3.10.

5.3.11. Determination of digesta transit time

On day 36, the birds were used to determine digesta passage rate as described in Chapter 3, section 3.12.

5.3.12. pH of digesta from different segments of digestive tract

On day 36, one bird per cage was killed and pH of digesta was measured as described in Chapter 3, section 3.11.

5.3.13. Measurement of excreta gas production

Excreta gas production was measured as described in Chapter 3, section 3.14.

5.3.14. Chemical analysis

Dry matter, nitrogen (N), gross energy, titanium, starch contents were determined as described in Chapter 3, section 3.8.

5.3.15. Calculations

The AME and apparent ileal nutrient (N and starch) digestibility were calculated using the formula described in Chapter 3, section 3.9.

5.3.16. Data analysis

For statistical analysis, cage means served as the experimental unit for performance parameters, nutrient digestibility and microflora count. For digestive tract parameters, pH and carcass characteristic individual birds served as experimental unit. All data were subjected to orthogonal polynomial contrasts using the general linear model procedure of SAS (2004) to examine whether responses to increasing levels of coarse maize were of a linear or quadratic nature. Differences were considered significant when $P<0.05$.

5.4. Results

5.4.1. Particle size distribution of coarse maize and diets

Geometric mean diameter of coarse maize was determined to be 1,695 µm with a corresponding GSD value of 1.97. Particle size distribution of diets is shown in Figure 5.1. It can be seen that the proportion of coarse particles (> 1 mm) increased with increasing inclusion of coarse maize. The percentage of coarse particles over 1 mm were 29.4, 39.9, 49.2, 56.0, and 64.3 %, while those over 2 mm were 3.1, 14.9, 26.7, 33.8, 44.6 % in diets containing 0, 150, 300 , 450 and 600 g/kg coarse maize, respectively.

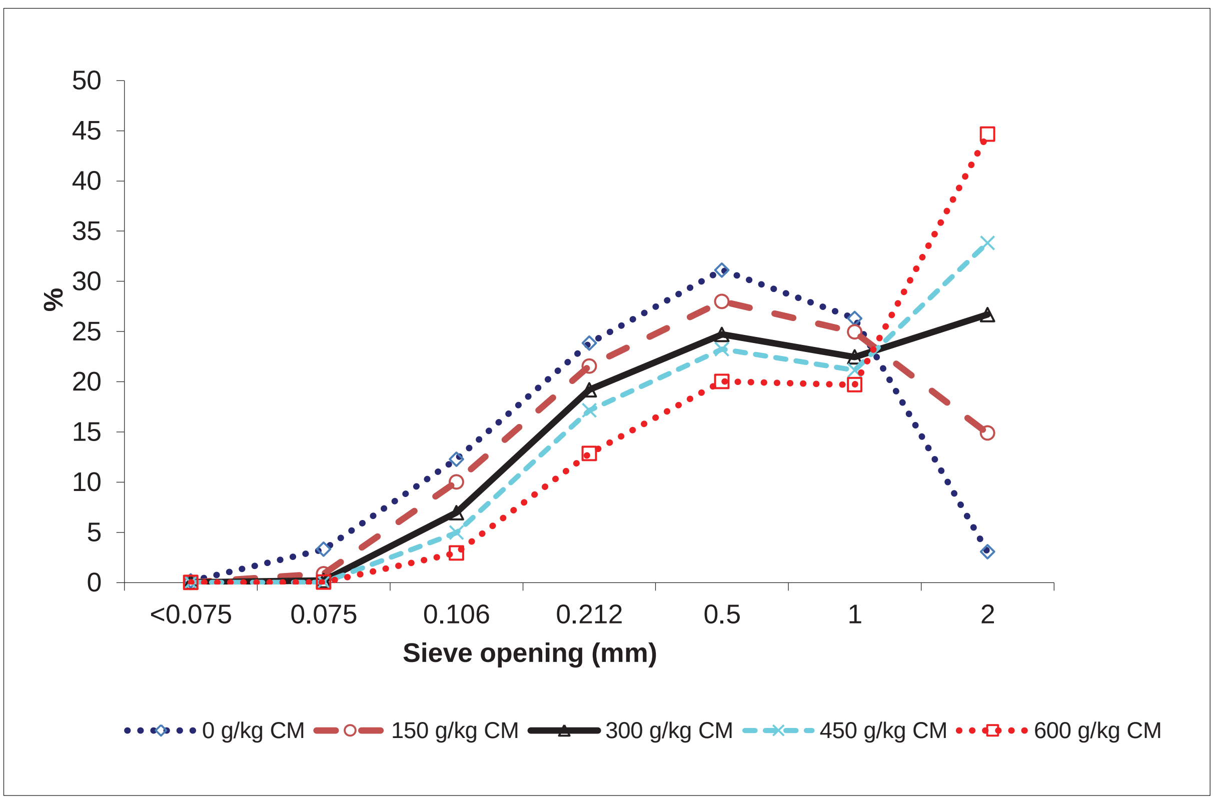


Figure 5.1. Particle size distribution of diets containing 0, 150, 300, 450 and 600 g/kg coarse maize (CM) obtained by the dry sieving method

Geometric mean diameter of diets with 0, 150, 300, 450 and 600 g/kg ground maize replaced by coarse maize were 578, 726, 877, 987, and 1172 µm, respectively, with corresponding GSD values of 2.24, 2.25, 2.24, 2.18 and 2.07, respectively

5.4.2. Performance

Mortality during the experiment was negligible. Only four birds out of 240 died and the deaths were not related to any specific treatment. A linear (P<0.01) increase in weight gain was observed as the inclusion of coarse maize increased (Table 5.2). A quadratic (P<0.05) response was observed for feed intake. Feed intake increased at 150 g/kg coarse maize inclusion, plateaued until 450 g/kg, and then increased again to 600 g/kg. A quadratic effect (P<0.05) was observed in feed per gain, with values increasing as the inclusion of coarse maize increased to 300 g/kg, and then decreasing with further inclusions.

Table 5.2. Influence of graded levels of coarse maize feeding on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) for broilers (11-35 days post-hatch)[1]

Coarse maize inclusion (g/kg)	Weight gain	Feed intake	Feed per gain
0	1595	2673	1.693
150	1648	2889	1.754
300	1707	2896	1.768
450	1787	2882	1.679
600	1733	2901	1.689
SEM[2]	37.9	42.4	0.025
Probabilities, P ≤			
Overall treatment effect	0.05	0.01	0.06
Linear effect	0.01	0.01	0.30
Quadratic effect	0.18	0.05	0.05

[1] Each value represents the mean of six replicates (8 birds per replicate).
[2] Pooled standard error of mean.

5.4.3. Nutrient utilisation

Effects of inclusion of graded levels of coarse maize on AME and, apparent ileal N and starch digestibility coefficients are shown in Table 5.3. A quadratic (P<0.01) effect was observed for the AME. The AME was not influenced up to 300 g/kg inclusion and then decreased with further increase in the inclusion level. No treatment effects were observed for ileal nitrogen digestibility, whereas starch digestibility tended (P=0.07) to response linearly as inclusion of coarse maize increased.

Table 5.3. Influence of graded levels of coarse maize feeding on AME (MJ/kg DM) and, ileal nitrogen and starch digestibility coefficients in broilers [1]

Coarse maize inclusion (g/kg)	AME	Nitrogen Digestibility	Starch digestibility
0	15.42	80.9	98.4
150	15.55	78.6	97.1
300	15.54	76.0	97.2
450	15.18	78.6	97.5
600	14.96	79.1	97.2
SEM[2]	0.085	1.93	0.35
Probabilities, P ≤			
Overall treatment effect	0.0001	0.49	0.24
Linear effect	0.0001	0.57	0.07
Quadratic effect	0.01	0.15	0.10

[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.

5.4.4. Carcass characteristics

No treatment effects were observed for carcass recovery (Table 5.4). A linear (P<0.05) decrease in breast yield and a linear (P<0.001) increase in relative abdominal fat weight was observed with increasing inclusion levels of coarse maize.

Table 5.4. Influence of graded levels of coarse maize feeding on carcass recovery (g/kg body weight), breast meat yield (g/kg body weight) and abdominal fat (g/kg body weight) of broilers [1]

Coarse maize inclusion (g/kg)	Carcass recovery	Breast meat yield	Abdominal fat
0	727	166	10.5
150	725	165	12.2
300	722	155	14.8
450	712	153	16.5
600	726	153	17.7
SEM[2]	5.4	4.9	1.50
Probabilities, P ≤			
Overall treatment effect	0.28	0.18	0.01
Linear effect	0.42	0.05	0.001
Quadratic effect	0.24	0.55	0.73

[1] Each value represents the mean of 12 birds.
[2] Pooled standard error of mean.

5.4.5. Digestive tract and organ measurements

Influence of graded increases in the inclusion of coarse maize on the relative weights of proventriculus, gizzard and digestive organs are summarised in Table 5.5. A linear ($P<0.05$) increase was observed for the relative gizzard weight. A quadratic ($P<0.05$) response was observed for relative weights of proventriculus and liver. The weight of proventriculus increased up to 450 g/kg and then decreased at 600 g/kg, whereas liver weight increased as inclusion of coarse maize increased to 300 g/kg and then decreased with further inclusion. Relative weights of the pancreas and spleen were unaffected ($P>0.05$) by the inclusion of coarse maize.

Table 5.5. Relative weight of digestive organs (proventriculus, gizzard, pancreas, liver and spleen) of broilers[1] as influenced by graded levels of coarse maize inclusion

Coarse maize inclusion (g/kg)	Relative weight (g/kg body weight)				
	Proventriculus	Gizzard	Pancreas	Liver	Spleen
0	3.18	14.2	1.89	22.9	1.33
150	3.26	15.7	1.83	23.2	1.19
300	3.59	16.5	2.03	26.6	1.23
450	3.59	16.1	1.91	24.8	1.52
600	3.32	16.4	1.82	24.2	1.32
SEM[2]	0.109	0.60	0.080	0.79	0.150
Probabilities, P ≤					
Overall treatment effect	0.02	0.05	0.36	0.05	0.57
Linear effect	0.08	0.05	0.81	0.10	0.50
Quadratic effect	0.05	0.11	0.22	0.05	0.82

[1] Each value represents the mean of 12 birds.
[2] Pooled standard error of mean.

Effects of graded levels of coarse maize on relative weight and length of different intestinal segments are shown in Table 5.6. Except for a decreasing linear ($P<0.01$) response for the relative weight of the caeca, no effects ($P>0.05$) were observed for the relative weight or relative length of any intestinal segment with increasing dietary inclusion of coarse maize.

Table 5.6. Relative weight and length of the intestinal tract of male broilers[1] as influenced by graded levels of coarse maize inclusion

Coarse maize inclusion	Relative weight (g/kg body weight)					Relative length (cm/kg body weight)				
(g/kg)	Duodenum	Jejunum	Ileum	Caeca	Small Intestine[2]	Duodenum	Jejunum	Ileum	Caeca	Small Intestine[2]
0	3.84	6.87	5.18	2.70	15.9	15.2	35.1	36.0	9.00	86.5
150	3.41	6.58	5.16	2.39	15.1	15.6	34.4	36.3	8.27	86.3
300	3.78	6.39	5.38	2.64	15.5	16.0	34.1	37.3	8.99	87.4
450	3.94	6.54	5.22	2.34	15.7	16.1	33.9	36.8	8.56	86.9
600	3.50	6.27	5.29	2.21	15.0	15.7	32.5	35.6	8.13	83.9
SEM[3]	0.171	0.215	0.176	0.101	0.40	0.50	1.15	1.74	0.277	2.67
Probabilities, P ≤										
Overall treatment effect	0.15	0.36	0.91	0.01	0.29	0.77	0.60	0.96	0.09	0.89
Linear	0.78	0.07	0.63	0.01	0.38	0.39	0.12	0.95	0.10	0.58
Quadratic	0.72	0.64	0.75	0.61	0.97	0.33	0.81	0.49	0.60	0.46

[1] Each value represents the mean of 12 birds.
[2] Small intestine = duodenum +jejunum+ ileum.
[3] Pooled standard error of mean.

5.4.6. Caecal microflora counts

Effects of inclusion of graded levels of coarse maize in broiler diets on caecal microbial counts are presented in Table 5.7. There were linear increases in the numbers of *Lactobacilli* spp. ($P<0.001$) and *Bifidobacteria* spp. ($P<0.01$), and linear decreases in the numbers of *Clostridium* spp. ($P<0.05$), *Campylobacter* spp. ($P<0.001$) and *Bacteroides* spp. ($P<0.05$) with increasing inclusion of coarse maize.

Table 5.7. Influence of feeding graded levels of coarse maize on bacterial count (Log 10 cells/g of caecal content) for broilers[1]

Coarse maize inclusion (g/kg)	*Lactobacilli* spp.	*Bifidobacteria* spp.	*Clostridium* spp.	*Campylobacter* spp.	*Bacteroides* spp.
0	7.15	7.11	7.45	7.36	6.95
300	7.72	7.56	7.16	6.81	6.35
600	7.83	7.61	7.09	6.52	6.10
SEM[2]	0.114	0.096	0.093	0.111	0.222
Probabilities, P ≤					
Overall treatment effect	0.01	0.01	0.05	0.001	0.05
Linear effect	0.001	0.01	0.05	0.0001	0.05
Quadratic effect	0.12	0.12	0.38	0.36	0.54

[1] Each value represents the mean of six replicates
[2] Pooled standard error of mean.

5.4.7. pH of the digesta

Influence of graded inclusion of coarse maize on pH of digesta of various segments of the digestive tract is presented in Table 5.8. Except for a tendency towards a linear ($P=0.08$) decrease of pH in the proventriculus, no effect was seen in pH of digesta from any other segment as the inclusion of coarse maize increased.

Table 5.8. Influence of graded levels of coarse maize feeding on pH of digesta from various segments of digestive tract of broilers[1]

Coarse maize inclusion (g/kg)	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Caeca
0	3.43	3.13	6.16	5.63	7.50	6.23
150	2.68	3.01	6.05	6.03	7.46	6.28
300	3.06	3.03	6.06	6.06	7.48	6.16
450	2.48	3.06	6.06	6.03	7.53	6.33
600	2.83	3.05	6.06	5.98	7.51	6.25
SEM[2]	0.244	0.187	0.088	0.233	0.164	0.171
Probabilities, P ≤						
Overall treatment effect	0.09	0.99	0.88	0.67	0.99	0.97
Linear	0.08	0.85	0.52	0.35	0.85	0.37
Quadratic	0.19	0.76	0.52	0.28	0.91	0.23

[1] Each value represents the mean of six birds.
[2] Pooled standard error of mean.

5.4.8. Excreta gas production and digesta transit time

The influence of coarse maize inclusion on gas production and feed digesta transit time is presented in Table 5.9. A linear ($P<0.05$) decrease in gas production was observed with increased inclusion of coarse maize. Feed transit time was unaffected ($P>0.05$) by inclusion of coarse maize.

Table 5.9. Influence of different levels of coarse maize feeding on excreta gas production (ml/g) and feed passage rate (min) of broilers[1]

Coarse maize inclusion (g/kg)	Gas production	Transit time
0	0.777	163
150	0.708	160
300	0.663	158
450	0.452	146
600	0.561	164
SEM[2]	0.091	5.5
Probabilities, P ≤		
Overall treatment effect	0.13	0.17
Linear	0.05	0.93
Quadratic	0.58	0.59

[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.

5.5. Discussion

In the present study, linear improvements in weight gain were observed with graded inclusion of coarse maize in broiler diets. This increase in weight gain was closely related to the increase feed intake. These results are in agreement with those reported by Nir *et al*. (1994a) where broilers fed medium to coarse maize diets (GMD, 996 and 1332 µm) ate more and were heavier than those fed a fine maize diet (GMD, 897 µm). Nir *et al*. (1994b) also showed that increasing the GMD of maize particle size from 525 to 897 µm in diets increased weight gain of broilers. Similar increases in weight gain in birds has been reported in previous studies in birds fed mash diets containing coarse or very coarse maize particles compared to those containing fine particles (Recce *et al*., 1985; Hamilton and Proudfoot, 1995; Proudfoot and Hulan, 1989). However, Parsons *et al*. (2006) observed increased feed intake without any effect on weight gain in broilers fed mash diets containing coarse maize (GMD, 2242 µm) compared to those fed diets containing fine, small, medium, or large maize (GMD = 781, 950, 1,042, or 1109 µm, respectively). Similarily, Jacobs *et al*. (2010) fed broilers from 0 to 21 days of age four dietary treatments containing different maize particle sizes (GMD of 557, 858, 1210 or 1387 µm) and reported maize particle size of up to 1387 µm can be included without compromising growth performance. Dozier *et al*. (2006, 2009) evaluated effects of adding increasing amounts of coarse maize (GMD of 1500 µm; 80 to 235 g/kg from 18 to 41 days, and GMD of 2691 µm; 150 to 280 g/kg from 18 to 56 day) to a pelleted feed supplement in broilers and observed that weight gain, feed intake and feed conversion were not affected. Conversely, Clark *et al*. (2009) fed broilers maize-based diets from 0 to 41 days with 170 to 680 g/kg coarse maize replacing ground maize and blended with an appropriate concentrate pellet and reported a linear decrease in weight gain and feed intake and a linear increase in feed efficiency. Increased weight gain in broilers has also been reported when whole wheat was offered in a mixed feeding system without altering the final nutrient content in mash diets at 100, 200 and 350 g/kg (Nahas and Lefrancois, 2001; Plavnik *et al*., 2002).

In the present study, the increase in gizzard weight observed with coarse maize inclusion may be attributed to increased frequency of contractions to reduce the particle size (Hill, 1971; Roche, 1981). These results are consistent with previously published data on whole wheat feeding (Nahas and Lefrancois, 2001; Plavnik *et al*., 2002; Amerah and Ravindran, 2008), inclusion of cracked maize (Clark *et al*., 2009) or feeding coarse maize (Nir *et al*., 1994a; Parsons *et al*., 2006; Jacobs *et al*., 2010). Conversely, Dozier *et al*. (2006) observed no effect on gizzard weight when coarse maize was fed in addition to pellets to broilers from 18 to 41 day. Reported benefits of a developed gizzard include increased gut motility (Ferket, 2000) and improved digestibility of nutrients through more efficient grinding and mixing (Svihus *et al*., 2004a; Amerah *et al*., 2007). In the present study, however, increased gizzard weight failed to improve digestibility of starch and nitrogen.

In the present study, except for a decrease in the relative weight of caeca, no intestinal parameter was influenced by the dietary inclusion of coarse maize; which agrees with studies on whole wheat feeding (Preston *et al*., 2000; Hetland *et al*., 2002; Gabriel *et al*., 2003a; Svihus *et al*., 2004a).

In the current study, inclusion of coarse maize had no influence on carcass yield, but breastmeat yield was decreased and abdominal fat weight increased. The latter findings cannot be readily explained. However, Plavnik *et al*. (2002) and Parsons *et al*. (2006) also reported greater abdominal fat weights and lower breastmeat yields in broilers fed diets containing whole wheat or coarse maize, respectively. Parsons *et al*. (2006) speculated that larger maize particle size may increase the proportion of feed energy utilised for gizzard development as opposed to breast muscle. However, in studies with broilers fed coarse maize at different inclusion levels, no effect was reported on carcass yield, breastmeat yield and the relative weight of abdominal fat (Clark *et al*., 2009; Dozier *et al*., 2009). Published data on the effect of whole wheat on abdominal fat weight in broilers are equivocal. Increased abdominal fat with whole grain inclusion have been reported in some studies (Preston *et al*., 2000; Jones and Taylor, 2001; Nahas and Lefrancois, 2001). In contrast, Wu and Ravindran (2004) found no effect, whereas Amerah and Ravindran (2008) found reduced abdominal fat with the inclusion of whole wheat.

In present study, AME was not influenced up to 300 g/kg inclusion of coarse maize and then decreased with further inclusions. The reason for the decrease in AME at higher inclusion levels is unclear. Jacobs *et al*. (2010) in two separate studies, using Hampshire x Columbian crossbreed or Ross 308, observed no effect of feeding coarse maize diets on nitrogen-corrected AME. Previous studies with whole wheat have produced inconsistent results. Inclusion of whole wheat at levels of 100 to 375 g/kg resulted improvement in AME (Preston *et al*., 2000; Svihus *et al*., 2004a; Wu *et al*., 2004). However, higher inclusion levels of 400 to 500 g/kg failed to show any effect on AME (Uddin *et al*., 1996; Svihus *et al*., 2004a).

In the present study, counts of beneficial bacteria, namely *Lactobacilli* spp. and *Bifidobacteria* spp., increased and those of pathogenic bacteria, namely *Clostridium* spp., *Campylobacter* spp. and *Bacteroides* spp., decreased with increasing inclusion of coarse maize. Similarly, dietary inclusion of whole wheat was shown to decrease *Salmonella* spp. colonisation (Bejerrum *et al*., 2005; Santos *et al*., 2008) and counts of *Clostridium perfringens* (Bejerrum *et al*., 2005) in broilers. Studies have also reported that diets containing whole wheat showed higher counts in beneficial *Lactobacilli* spp. and lower counts of *Coliform* bacteria (Gabriel *et al*., 2003b; Engberg *et al*., 2004). Two theories have been proposed to explain the observed effects on gut microflora profile. First, stimulation of gizzard development and increased secretion of hydrochloric acid into the gizzard reduces pH, which may exert an antimicrobial effect on pathogenic bacteria entering the distal part of gastrointestinal tract (Naughton and Jensen, 2001; Engberg *et al*., 2002). Second, whole grain feeding may encourage colonisation of commensal bacteria and thus, discourage colonisation of harmful bacteria through competitive exclusion (Bejerrum *et al*., 2005; Santos *et al*., 2008). In the present study, however, inclusion of coarse maize had no effect on gizzard pH. Jacob *et al*. (2010) similarly observed no effect of diets containing large maize particles (GMD, 557 to 1,387 um) on the pH of gizzard contents. However, increased numbers of *Lactobacilli* spp. and *Bifidobacteria* spp. may explain the decrease in the numbers of pathogenic bacteria through competitive exclusion (Bejerrum *et al*., 2005; Santos *et al*., 2008). Engberg *et al*. (2002) speculated that an increase in *Lactobacilli* spp. is usually considered to be beneficial to the host because they can prevent the colonisation of pathogenic bacteria such as *E. coli*. A decrease in pathogenic bacteria may have contributed to the lower gas production observed with inclusion of coarse maize. The positive correlation between the gas production and the population of *Clostridium* spp. may be related to the fact that these bacteria are hydrogen producers (Skillman *et al*., 2009). It has been found that in birds infected with *Clostridium perfringens*, the intestine is friable and distended with gas caused by toxins (Broussard *et al*., 1986; Porter, 1998).

5.6. Conclusions

The present data showed that increasing the inclusion of coarse maize resulted in a linear increase in weight gain of broilers. In addition, inclusion of coarse maize in broiler diets increased the weight of the gizzard similar to that reported with whole grain feeding, however, no beneficial effects on AME and digestibility of nitrogen and starch were shown. Enhanced gizzard development resulting from coarse maize had a positive impact on beneficial *Lactobacilli* spp., *Bifidobacteria* spp. and negative impact on harmful bacteria, namely *Clostridium* spp., *Campylobacter* spp. *and Bacteroides* spp. These findings suggest that gizzard can prevent entry of pathogenic bacteria into the distal gastrointestinal tract which has implications in the absence of AGP in poultry diets and in terms of food safety to prevent food borne illnesses. Overall, coarse maize can totally replace ground maize in broiler diets. Therefore, coarse maize feeding can be used as a strategy to lower the cost of processing and improve broiler performance.

CHAPTER 6

Effect of feeding coarse maize on productive performance, gizzard development and energy utilisation in laying hens

6.1. Abstract

A total of 2,200 White Leghorn layers were used to study the effect of feeding coarse maize on productive performance, gizzard weight, apparent metabolisable energy (AME) and egg quality parameters. The experiment was a completely randomised design with five treatments, each being replicated five times (88 birds per replicate). Dietary treatments included a control diet with 600 g/kg of ground maize and experimental diets with 150, 300, 450 or 600 g/kg coarse maize replacing (w/w) ground maize. Diets, in mash form, were offered from 39 to 62 weeks of age. Performance parameters were recorded and compiled at four-week intervals throughout the 24-weeks experimental period. Gizzard weight, egg quality and AME were measured during the last week of the experiment. Over the entire experimental period (39 to 62 weeks), treatments had no effect ($P > 0.05$) on any of the production parameters, except feed intake. A quadratic effect ($P < 0.05$) was observed for feed intake where intake increased at 150 g/kg coarse maize inclusion. At higher inclusion levels of coarse maize, feed intake was similar to that of the control. Dietary treatments had no effect ($P > 0.05$) on gizzard weight, AME or egg quality. The results indicate that up to 600 g/kg of coarse maize could replace ground maize in layer diets with no adverse effect on production.

6.2. Introduction

In recent years, incorporation of whole grains in broiler diets to reduce handling losses and processing costs has become a common practice. However, this application has not yet been used in commercial layer hens because of limited published data with layers. In addition to reducing feed costs, benefits of whole grain feeding may include improvements in feed efficiency, nutrient digestibility and apparent metabolisable energy (AME), as observed in broilers (Wu *et al*., 2004; Svihus *et al*., 2004a). In layers, this feeding strategy could also be exploited to address the welfare issue of feather pecking; which develops as a substitute for normal ground pecking feeding behaviour in the absence of adequate foraging incentives in conventional diets based on ground grains (Blokhuis, 1986).

Wheat is the cereal grain of choice for whole grain feeding. Despite the fact that maize is the most commonly used cereal in poultry diet formulations world-wide, little attempt has been made to feed whole maize to poultry. Poultry have been reported to show a preference for certain grains over others. Kempster (1916) stated that wheat was the favoured grain, followed by maize and sorghum with size of the wheat grain probably being a favourable factor. The preference of chickens for maize and wheat over barley has been reported by Fry *et al*. (1957) and Jensen *et al*. (1957). However, preference by birds towards one type of grain is not easy to access and could be related to form of presentation, palatability and metabolic consequences or, more likely, a combination of all these factors (Forbes and Covasa, 1995). Rather than preference, the size of grain may be the main reason for the lower acceptance of whole maize by the bird. Coarse grinding could overcome the problem of large whole grain size while achieving the beneficial effects associated with whole grain feeding. Clark *et al*. (2009) replaced finely ground maize with coarse maize in broiler diets at rates of 0 to 680 g/kg when blended with an appropriate concentrate pellet so that final nutrient content wasn't altered and reported that up to 170 g/kg coarse maize could be used without a negative response on bird performance. However, in recent work, researchers have replaced finely ground maize with coarse maize in broiler **(**Dozier *et al*., 2009) and layer diets (Safaa *et al*. 2009; Gewehr *et al*., 2011) without compromising bird performance.

The present study was conducted to investigate the effect of feeding coarsely ground maize on performance, AME, egg quality and gizzard weight in laying hens.

6.3. Materials and methods

The experiment was carried out at the M/S Sri Lakshmi Narashimha Poultry Research Farm (Rudravalli, Bibi-nagar, Ranga Reedy District, Andhra Pradesh, India) from 26 December 2010 to 16 June 2011. The experiment was divided into six periods of 4-week intervals. The ambient temperatures, which was recorded daily at 0600 and 1430 hrs, during the six experimental periods were 12.0 and 31.0, 12.0 and 32, 12.0 and 36.0, 21.0 and 37.0, 24.0 and 40.0, and 24.0 and 39.0 °C, respectively.

6.3.1 Diets

Whole maize, obtained from a local supplier, was ground in a hammer mill (Hard Case Machinery, Hyderabad, India) provided with a 6 mm sieve for the ground maize component. Whole maize from the same batch was coarsely ground using a 10 mm sieve and included at 150, 300, 450 or 600 g/kg by replacing (w/w) the ground maize in the control diet (Table

6.1). All diets contained identical nutrient contents with the only difference being ratio of ground to coarse maize. Diets were fed *ad libitum* in mash form. All diets met or exceeded the requirements for major nutrients of White Leghorn-laying hens (NRC, 1994). The ingredient composition and calculated analysis of the diets are presented in Table 6.1.

Table 6.1. Ingredient and nutrient composition (g/kg as fed basis) of diets

Ingredient	
Maize[1] (ground and/or coarse)	600.0
Soybean meal, 450 g/kg CP	258.2
Deoiled rice bran	16.79
Salt	3.50
Dicalcium phosphate	8.15
Oyster shell grit	86.72
Limestone	20.00
DL-methionine	0.98
Vitamin – trace mineral premix[2]	4.55
Phytase[3]	0.06
Sodium bicarbonate	1.00
Calculated composition	
ME (MJ/kg)	11.0
Crude protein	168
Lysine	8.9
Methionine + cysteine	6.8
Theronine	7.0
Tryptophan	1.9
Calcium	36.0
Non phytate phosphorus	3.3

[1] The control diet contained 600 g/kg ground maize. In experimental diets, ground maize was replaced (w/w) by 150, 300, 450 and 600 g/kg coarse maize.
[2]Provided (mg/kg of diet): thiamine, 1; pyridoxine, 2; cyanocobalamin, 0.01; niacin, 15; pantothenic acid, 10; α-tocopherol, 10; riboflavin, 10; biotin, 0.08; menadione, 2; retinol acetate, 2.75; cholecalciferol, 0.06; choline, 650; copper, 8; iron, 45; manganese, 80; zinc, 60; selenium, 0.18; hydrated sodium calcium aluminosilicates, 800.
[3] Ronozyme, DSM Nutritional Products India Pvt. Ltd, Thane, India.

6.3.2. Birds and Housing

A total of 2200 White Leghorn (BV 300) layers, 39 weeks of age, housed in 550 colony cages (4 birds/ cage) were used. Cages were located on elevated platforms in an open-sided house. Adjacent 22 colony cages (88 birds), with a common feeder, were considered as a replicate. Each diet was randomly assigned to five replicates and offered *ad libitum* from 39-62 weeks of age. Water was provided from a common channel water trough and was available freely throughout the trial. Fluorescent bulbs were used to provide 16 hr light period per day, including the normal daylight.

6.3.3. Determination of particle size distribution

Particle size distribution of diets, geometric mean diameter (GMD), and geometric standard deviation (GSD) were determined as described in Chapter 3, section 3.3.

6.3.4. Performance data

Feed intake and egg production were recorded and compiled at four-week intervals. Egg production was recorded twice daily and expressed on a hen-day basis. Measured quantity of feed was offered twice daily. At the end of each four-week period, feed residual was weighed and, feed intake per bird and the quantity of feed consumed to produce an egg were calculated and compiled for each four-week period. The average egg weight was recorded by weighing 60 eggs per replicate per day for three consecutive days at the end of each four-week period. Egg mass was calculated by multiplying the average egg weight with the total number of eggs produced. The number of abnormal eggs produced (defective, shell-less or broken eggs) were recorded daily and expressed in relation to the total number of eggs produced as egg shell defects (ESD). Body weight was recorded at the end of each four-week period by weighing the same 20 birds in each replicate.

6.3.5. Gizzard weight

At the end of the experiment (62 weeks of age), two birds closest to the mean body weight of the treatment were selected from each replicate, weighed and killed by cervical dislocation. Gizzards were carefully excised, surrounding fat was removed, and gizzard weight was recorded and expressed in proportion to the live weight of the bird.

6.3.6. Apparent metabolisable energy (AME) determination

During the last month of the experiment (58-62 weeks), titanium oxide (3 g/kg) was incorporated into the diets and offered to four cages per replicate. Ten days after the introduction, grab samples of excreta were collected daily for three consecutive days. Daily excreta collections from each replicate were pooled, representative samples were obtained, dried in an oven at 60 ^{o}C for 48 h and ground. Diet and excreta samples were analysed for dry matter (DM), titanium oxide (Ti) and gross energy (GE).

AME was calculated based on titanium marker ratios in the diet and excreta using the formula below.

$$AME = \frac{(GE/Ti)\ diet - (GE/Ti)\ excreta}{(GE/Ti)\ diet}$$

Where,

(GE / Ti) diet is the ratio of GE to titanium in diet, and

(GE / Ti) excreta is the ratio of GE to titanium in excreta.

6.3.7. Egg quality parameters

On three consecutive days of the last week of the experiment (62 weeks), egg quality was measured on eight randomly collected eggs/replicate/day. The eggs were individually weighed, broken and their contents were removed. Shell with the membranes, yolk and albumen were weighed, and their relative proportions were determined. Shell thickness was measured at three points (the two ends and the centre) using dial gauge micrometer (Model No. 7301, Mitutoyo, Japan).

6.3.8. Statistical analysis

For statistical analysis, cage means served as the experimental unit for performance parameters. For the gizzard weight, individual birds served as the experimental unit. Data were initially analysed using repeated measures analysis (SAS, 2004) to include age of bird (period) and identify treatment x period interactions. However, interaction between treatment and period was not significant for any parameter. Data were then subjected periodwise to orthogonal polynomial contrasts using the general linear model procedure of SAS (2004) to examine whether responses to increasing levels of coarse maize were of a linear or quadratic nature. Differences were considered significant when $P<0.05$.

6.4. Results

Particle size distribution of diets as determined by the dry sieving method is shown in Figure 6.1. The proportion of coarse particles (over 1 mm) increased with increasing inclusion of coarse maize. The percentage of coarse particles over 1 mm were 59.1, 62.1, 65.5, 69.1 and 74.0 %, and those over 2 mm were 34.9, 36.6, 40.4, 42.6 and 48.0% in diets with 0, 150, 300, 450 and 600 g/kg coarse maize, respectively. The GMD of diets with 0, 150, 300, 450 and 600 g/kg coarse maize was 905, 925, 989, 1018 and 1163 µm respectively, with corresponding GSD values of 2.80, 2.88, 2.83, 2.98 and 2.64 respectively.

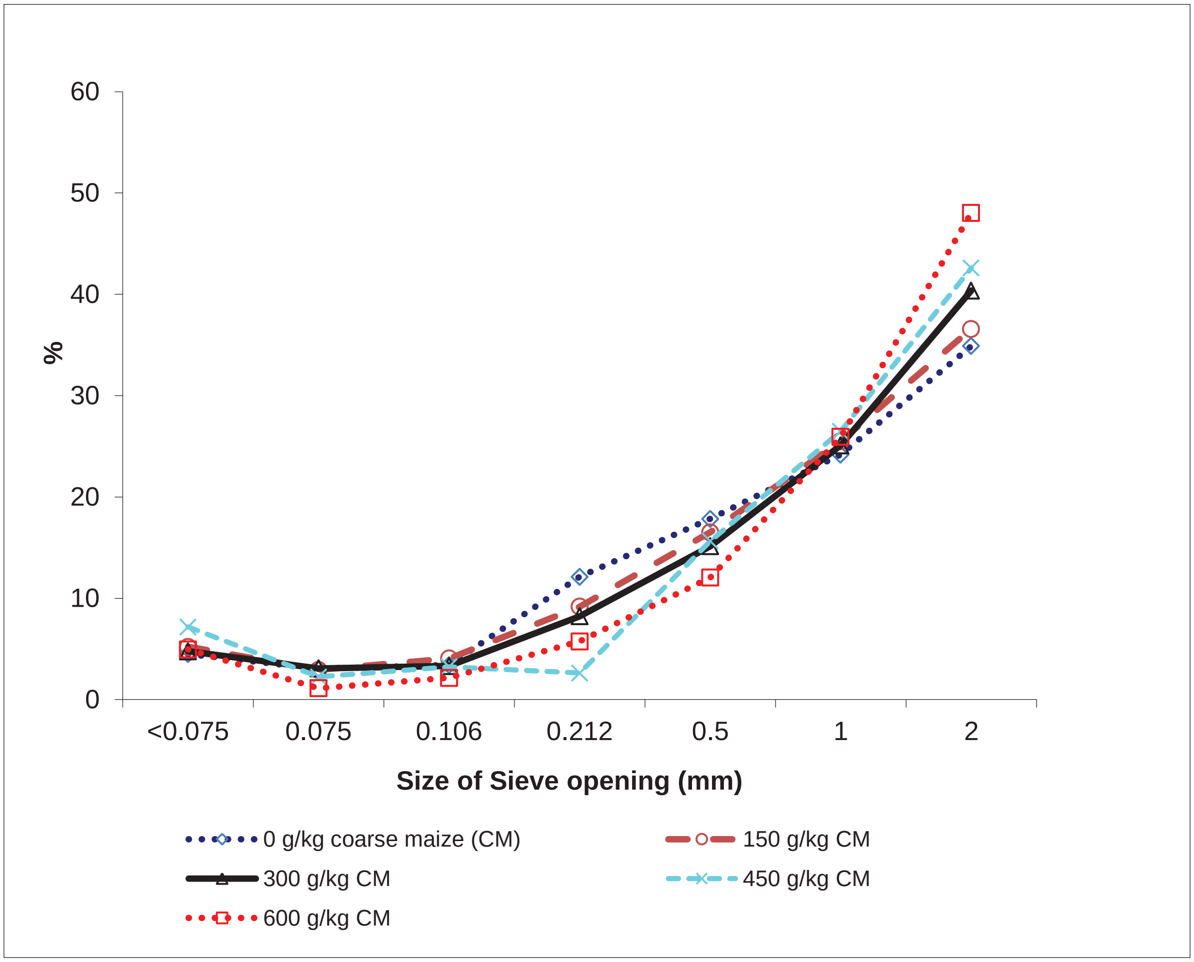


Figure 6.1. Particle size distribution of diets containing 0, 150, 300, 450 and 600 g/kg coarse maize obtained by the dry sieving method

Egg production was not affected (P>0.05) by the inclusion of coarse maize during either the different periods or over the entire experiment period (Table 6.2). Linear (P<0.01) and quadratic (P<0.01) responses were observed for feed intake during periods 3 and 4, respectively (Table 6.3). A linear (P<0.05) decrease in feed intake with increasing inclusion of coarse maize was observed in period 3. In period 4, feed intake increased with increasing level of coarse maize to 150 g/kg and then decreased with further inclusion. Feed intake was not influenced by increasing inclusion of coarse maize during periods 1, 2, 5 and 6. Over the entire trial period, however, a quadratic (P<0.05) response was observed for feed intake, where intake increased with the inclusion of coarse maize to 150 g/kg and, then decreased and plateaued with further inclusion. Differences between the feed intake of broilers fed diets containing 600 g/kg ground maize and those fed diets containing 300, 450 and 600 g/kg coarse maize were non-significant (P>0.05).

Egg weight was not affected (P>0.05) by the level of coarse maize during different periods or over the entire experiment (Table 6.4)

Feed efficiency decreased linearly (P<0.05) in response to increasing inclusion of coarse maize during period 3 (Table 6.5), but was unaffected (P>0.05) by dietary treatments during the other periods or over the entire trial.

A linear tendency (P = 0.08 to 0.11) occurred with increasing inclusion of coarse maize for egg mass during periods 1, 2, 3 and 4 (Table 6.6). Egg mass was not affected (P>0.05) by dietary treatments during periods 5 and 6 or over the entire experiment.

Inclusion level of coarse maize had no effect (P>0.05) on egg shell defects (Table 6.7) and body weight (Table 6.8) during individual periods or over the entire trial. The only exception was body weight during period 3, where a linear (P<0.05) increase with increasing inclusion of coarse maize was observed.

The AME, relative weight of gizzard, shell thickness and egg components were not influenced (P>0.05) by the level of coarse maize (Table 6.9).

Table 6.2. Hen-day egg production of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize[1]

Coarse maize (g/kg)	Hen day egg production, %						
	Period 1 (39-42 wk)	Period 2 (43-46 wk)	Period 3 (47-50 wk)	Period 4 (51-54 wk)	Period 5 (55-58wk)	Period 6 (59-62 wk)	Pooled (39-62 wk)
0	94.6	96.0	93.0	89.3	85.5	84.8	90.5
150	95.2	95.9	93.8	90.7	86.4	86.6	91.4
300	95.0	94.7	92.6	89.8	84.0	83.8	90.0
450	94.6	95.0	93.4	88.5	85.9	86.6	90.6
600	93.7	94.9	93.1	88.6	84.7	84.8	90.0
SEM	0.46	0.60	0.70	0.97	0.94	0.80	0.48
Probabilities, P ≤							
Overall treatment effect	0.23	0.45	0.79	0.51	0.45	0.09	0.25
Linear	0.13	0.11	0.91	0.27	0.5	0.99	0.24
Quadratic	0.08	0.48	0.90	0.43	0.96	0.60	0.51

[1] Each mean represents values from five replicates (88 birds/replicate).

Table 6.3. Feed intake of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize[1]

Coarse maize (g/kg)	Feed intake, g/ bird per day						
	Period 1 (39-42 wk)	Period 2 (43-46 wk)	Period 3 (47-50 wk)	Period 4 (51-54 wk)	Period 5 (55-58wk)	Period 6 (59-62 wk)	Pooled (39-62 wk)
0	115.4	117.0	105.2	101.4	93.2	93.5	104.3
150	116.7	116.5	105.1	105.0	95.8	94.9	105.7
300	114.4	116.1	102.3	104.3	96.0	94.3	104.6
450	116.6	116.6	103.7	101.7	95.7	95.0	104.9
600	115.9	117.0	102.4	100.3	93.5	92.9	103.7
SEM	0.55	0.33	0.74	1.02	1.41	1.01	0.38
Probabilities, P ≤							
Overall treatment effect	0.05	0.36	0.05	0.05	0.48	0.51	0.05
Linear effect	0.60	0.96	0.01	0.11	0.98	0.75	0.12
Quadratic effect	0.87	0.06	0.48	0.01	0.08	0.14	0.05

[1] Each mean represents values from five replicates (88 birds/replicate).

Table 6.4. Egg weight of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize[1]

Coarse maize	Egg weight, g						
(g/kg)	Period 1 (39-42 wk)	Period 2 (43-46 wk)	Period 3 (47-50 wk)	Period 4 (51-54 wk)	Period 5 (55-58wk)	Period 6 (59-62 wk)	Pooled (39-62 wk)
0	55.9	56.5	53.6	54.0	52.1	55.3	54.6
150	55.4	56.3	53.7	53.7	51.3	54.9	54.2
300	55.2	56.2	53.8	53.8	52.1	55.0	54.4
450	55.4	56.3	53.8	53.6	52.6	55.3	54.5
600	55.0	56.2	53.7	53.3	52.0	55.2	54.2
SEM	0.35	0.22	0.26	0.21	0.74	0.19	0.19
Probabilities, P ≤							
Overall treatment effect	0.50	0.85	0.99	0.43	081	0.60	0.67
Linear effect	0.15	0.34	0.86	0.10	0.67	0.56	0.59
Quadratic effect	0.66	0.68	0.60	0.96	0.99	0.36	0.80

[1] Each mean represents values from five replicates (88 birds/replicate).

Table 6.5. Feed efficiency of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize[1]

Coarse maize	Feed efficiency, g feed/egg						
(g/kg)	Period 1 (39-42 wk)	Period 2 (43-46 wk)	Period 3 (47-50 wk)	Period 4 (51-54 wk)	Period 5 (55-58wk)	Period 6 (59-62 wk)	Pooled (39-62 wk)
0	121.9	121.8	113.2	113.5	109.0	110.2	114.9
150	122.6	121.5	112.0	115.8	111.0	109.6	115.4
300	120.3	122.6	110.4	116.1	114.2	112.4	116.0
450	123.2	122.8	111.0	114.9	111.5	109.7	115.5
600	123.6	123.2	110.0	113.2	100.4	109.5	115.0
SEM	0.69	0.75	1.11	1.50	2.39	1.91	0.89
Probabilities, P ≤							
Overall treatment effect	0.05	0.21	0.30	0.56	0.65	0.80	0.91
Linear effect	0.09	0.10	0.05	0.76	0.65	0.85	0.92
Quadratic effect	0.10	0.87	0.54	0.11	0.20	0.52	0.37

[1] Each mean represents values from five replicates (88 birds/replicate).

Table 6.6. Egg mass of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize[1]

Coarse maize	Egg mass, g						
(g/kg)	Period 1 (39-42 wk)	Period 2 (43-46 wk)	Period 3 (47-50 wk)	Period 4 (51-54 wk)	Period 5 (55-58wk)	Period 6 (59-62 wk)	Pooled (39-62 wk)
0	1473	1520	1397	1352	1249	1314	1386
150	1476	1514	1413	1364	1241	1333	1390
300	1471	1491	1396	1355	1228	1293	1372
450	1469	1497	1407	1331	1265	1343	1385
600	1445	1494	1400	1328	1234	1313	1369
SEM	10.5	12.0	14.1	15.3	21.0	13.7	8.35
Probabilities, P ≤							
Overall treatment effect	0.28	0.37	0.90	0.41	0.75	0.14	0.33
Linear effect	0.08	0.09	0.10	0.11	0.94	0.85	0.17
Quadratic effect	0.44	0.45	0.73	0.47	0.97	0.87	0.73

[1] Each mean represents values from five replicates (88 birds/replicate).

Table 6.7. Shell defects in eggs of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize[1]

Coarse maize	Shell defect, %						
(g/kg)	Period 1 (39-42 wk)	Period 2 (43-46 wk)	Period 3 (47-50 wk)	Period 4 (51-54 wk)	Period 5 (55-58wk)	Period 6 (59-62 wk)	Pooled (39-62 wk)
0	0.034	0.067	0.078	0.181	0.192	0.301	0.142
150	0.025	0.025	0.061	0.151	0.188	0.151	0.101
300	0.034	0.034	0.079	0.157	0.145	0.173	0.103
450	0.042	0.034	0.078	0.092	0.185	0.194	0.104
600	0.069	0.077	0.035	0.165	0.147	0.205	0.116
SEM	0.0171	0.023	0.024	0.045	0.039	0.046	0.0154
Probabilities, P ≤							
Overall treatment effect	0.45	0.46	0.65	0.70	0.84	0.23	0.32
Linear effect	0.12	0.70	0.38	0.53	0.46	0.33	0.34
Quadratic effect	0.28	0.08	0.45	0.44	0.93	0.08	0.08

[1] Each mean represents values from five replicates (88 birds/replicate).

Table 6.8. Body weight of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize[1]

Coarse maize (g/kg)	Body weight , g						
	Period 1 (39-42 wk)	Period 2 (43-46 wk)	Period 3 (47-50 wk)	Period 4 (51-54 wk)	Period 5 (55-58wk)	Period 6 (59-62 wk)	Pooled (39-62 wk)
0	1369	1351	1322	1321	1238	1288	1315
150	1378	1408	1321	1340	1274	1363	1348
300	1375	1373	1355	1339	1250	1362	1342
450	1361	1393	1358	1295	1248	1351	1334
600	1401	1389	1377	1344	1258	1375	1358
SEM	15.5	13.5	18.9	16.8	17.4	36.2	13.4
Probabilities, P ≤							
Overall treatment effect	0.46	0.08	0.20	0.24	0.65	0.48	0.26
Linear effect	0.35	0.18	0.05	0.99	0.79	0.17	0.11
Quadratic effect	0.40	0.20	0.88	0.78	0.65	0.42	0.67

[1] Each mean represents values from five replicates (88 birds/replicate).

Table 6.9. Apparent metabolisable energy (AME; MJ/kg DM) [1], Relative gizzard weight (g/kg body weight)[2] and egg quality parameters[3] of White Leghorn layers (39-62 wk of age) fed diets containing graded levels of coarse maize

Coarse maize	AME	Relative gizzard	Proportion (%) of egg			Shell thickness
(g/kg)		weight.	Yolk	Albumin	Shell	(mm)
0	10.55	15.44	30.46	60.68	8.85	0.340
150	10.67	16.24	30.22	60.68	9.08	0.349
300	11.34	15.50	30.02	61.32	8.64	0.334
450	11.07	16.27	30.59	60.40	8.91	0.349
600	11.14	15.60	30.21	60.49	9.32	0.335
SEM	0.327	0.616	0.005	0.006	0.001	0.006
Probabilities, P ≤						
Overall treatment effect	0.41	0.77	0.95	0.86	0.16	0.35
Linear effect	0.14	0.86	0.94	0.74	0.22	0.60
Quadratic effect	0.40	0.54	0.80	0.50	0.15	0.52

[1] Each value represents the mean of five replicates.
[2] Each value represents the mean of 10 birds (two birds per replicate).
[3] Each value represents the mean of 120 eggs (24 eggs per replicate).

6.5. Discussion

In the present study, the majority of parameters (except feed intake) were unaffected by increasing inclusion of coarse maize. Similarly, Safaa *et al*. (2009) reported no effect of maize particle size on production parameters except for an increase in feed intake of layers (20-40 weeks) fed diets based on coarsely ground maize (10 mm sieve) compared to those fed diets based on either medium (8mm sieve) or finely ground (6 mm sieve) maize. Similarly, feeding diets containing whole wheat at 200 g/kg (Maclsaac and Anderson, 2007), 300 g/kg (Senkoylu *et al*., 2009) or whole pearl millet at 100 g/kg (Garcia and Dale, 2006) or equal proportions of whole wheat and protein mixture (Faruk *et al*., 2010) had no effect on the production performance of layers. In a recent study, Gewehr *et al*. (2011) reported that feeding layers (46-60 weeks) diets with different textures of maize, either whole, coarsely ground or finely ground maize (GMD of 3198, 1254 and 663 µm, respectively) had no effects on any of the performance parameters.

The quadratic effect observed for feed intake over the entire experimental period (39-62 weeks) in the current study is difficult to explain. However, it must be noted that the differences in daily intake were only around 1 g/bird and may not be of any biological significance. Safaa *et al*. (2009) also reported that daily feed intake was slightly greater for hens (20-48 weeks) fed a coarsely ground maize diet than for those fed a finely ground maize diet (110.6 vs. 107.9 g/hen per day for 10 and 6 mm screen size, respectively).

In the present study, increasing dietary inclusion of coarse maize had no influence on the relative gizzard weights. Similar to these findings, Senkoylu *et al*. (2009) did not observe any changes in gizzard weight in layers fed diets containing 300 g/kg whole wheat during a post peak laying phase (50-63 weeks of age). However, Faruk *et al*. (2010) reported increased gizzard weight in layers fed whole wheat from grower phase to peak egg production (16-46 weeks of age). An increase in gizzard weight has been consistently observed in broilers with whole wheat feeding (Cumming, 1994; Hetland *et al*., 2004; Ravindran *et al*., 2006; Amerah and Ravindran, 2008). The lack of effect on gizzard development in the present study may be attributed to the age of birds in that the gizzard is already well developed in mature birds and may not respond to changes in feed structure (Sturkie, 1965; Uni *et al*., 1999),.

Based on broiler data, it was anticipated that whole grain feeding would stimulate gizzard development and activity, and digesta motility and reflux in the gastrointestinal tract (Williams *et al.,* 1997) thus allowing better utilisation of dietary nutrients and energy. However, published data on this aspect are limited and equivocal. In some studies, improvements in nutrient digestibility in birds fed whole grains (Svihus and Hetland, 2001; Hetland *et al*., 2003; Svihus *et al*., 2004a) and coarse ingredients (Rogel *et al.,* 1987; Svihus and Hetland, 2001) were associated with increased gizzard size, and it was suggested that gizzard development may have been partly responsible for the improved AME (Preston *et al*. 2000; Svihus *et al*. 2004a). In other studies, however, an improvement in AME was not associated with gizzard development (Wu *et al*., 2004). In the present study, increasing inclusion of coarse maize had no effect on AME.

Tang *et al*. (2006) speculated that a higher proportion of fine particles in the diet may impair egg quality because nutrients supplemented in powder form, such as vitamins and trace minerals, may settle to the bottom of feeders and will not be consumed in required amounts by the hen. However, dietary treatments in the present study had no effect on various egg quality parameters. Similar to our findings, previous data indicate that the inclusion of whole wheat (Faruk *et al*., 2010) or variation in feed texture (Gewehr *et al*., 2011) or maize particle size (Safaa *et al*., 2009) in layer diets had no effect on egg quality parameters.

6.6. Conclusions

The present data suggest that total replacement of ground maize with coarse maize has no adverse effects on production parameters and egg quality of laying hens. This feeding strategy could potentially be used to lower the cost of manufacturing layer diets.

CHAPTER 7

Influence of method of wheat inclusion and pellet diameter on performance, nutrient utilisation, digestive tract measurements, carcass characteristics and caecal microflora counts of broilers

7.1. Abstract

The aim of the present study was to determine the influence of method of wheat inclusion and pellet diameter on performance, nutrient utilisation, digestive tract development and gut microflora profile and carcass characteristics of broilers. The experimental design was a 3 x 2 factorial arrangement of treatments, which included three diet forms, namely ground wheat (GW), 200g/kg whole wheat (WW) replacing GW before or after pelleting, and two pellet diameters (3.0 or 4.76 mm). Diets were offered *ad libitum* from day 11 to 35 post-hatch. A higher proportion of coarse particles (over 1 mm) was found in diets pelleted using a die of 4.76 mm diameter as compared to that of 3.0 mm diameter, irrespective of method of wheat inclusion. However, pellet durability index was better ($P<0.05$) in GW diets and deteriorated ($P<0.05$) with pre-pelleting inclusion of WW when the larger die was used. Larger pellet diameter increased ($P <0.05$) the weight gain and lowered ($P <0.05$) feed per gain of birds fed diets with GW and post-pellet inclusion of WW. However, in birds fed diets with pre-pelleting inclusion of WW, larger the pellet diameter lowered ($P<0.05$) weight gain and increased ($P<0.05$) feed per gain. Relative gizzard weight was increased ($P<0.05$) by larger pellet diameter with pre-pelleting inclusion of WW, but was unaffected ($P>0.05$) by diets containing GW or post pellet inclusion of WW. Larger pellet diameter increased ($P<0.05$) AME and starch digestibility irrespective of method of WW inclusion. Larger pellet diameter was accompanied by reductions ($P <0.05$) in the relative length and weight of all components of the digestive tract in GW fed birds, but did not affect ($P>0.05$) these parameters in birds fed WW diets. Larger pellet diameter resulted in higher ($P<0.05$) carcass recovery in the GW groups, lower ($P<0.05$) in pre-pelleting groups and no effect in the post-pelleting groups. Gizzard pH and excreta gas production were lower ($P<0.05$) in birds fed WW diets compared to those fed diets containing GW. Feeding pellets with larger diameter decreased *Clostridium* spp. *and Campylobacter* spp. counts in post-pellet and pre-pellet inclusion of WW, respectively. Overall, results showed that the effect of pellet diameter on broiler performance varied depending on the form of wheat and method of WW inclusion. Adverse effects on weight gain in the pre-pelleting WW group was due primarily to reduced feed intake which may be attributed partly to reduced pellet quality

7.2. Introduction

Rising feed cost is a major challenge faced by the poultry industry. In recent years, whole wheat (WW) feeding of broilers has received renewed attention as a means of lowering feed manufacturing costs and because of reported positive effects on production performance and nutrient utilisation (Wu *et al*., 2004; Wu and Ravindran, 2004). These positive effects are attributed to the impact of larger particle size on gizzard development and gut health. It is recognised that any change in gut health will influence the animal as a whole and consequently alter its nutrient uptake and requirements (Choct, 2009). Maintenance of gut health is essential for welfare and productivity of birds, especially when antibiotics are not allowed in the feed. Some evidence suggests that dietary inclusion of whole cereals influence the ecology of the gastrointestinal tract of poultry by increasing the resistance to colonisation by pathogens (Santos *et al*., 2008)

Chickens at all ages are known to have a preference for larger feed particles (Schiffman, 1968; Portella *et al*., 1988), and this 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) and it is common practice to feed broilers 3.0 mm diameter pellets. Genetic selection over the past two decades has resulted in increased growth rate and bird size, and consequently, the oral cavity and gape would also have been expected to have increased (Gentle, 1979). Research on the optimum pellet size required by broilers is scant.

Furthermore, studies examining the effects of including WW pre-pelleting are limited and have produced equivocal results. Wu *et al*. (2004) found that pre-pelleting inclusion of WW at 200 g/kg failed to show any effect on gizzard development, but improved feed per gain. On the other hand, Jones and Taylor (2001) reported that the same level of WW inclusion had positive effects on gizzard weight with no effect on broiler performance. Such contradictory results may be related, in part, to changes in feed particle size distribution following pelleting. When particle size differences persisted in diets after pelleting, those with coarser particles were found to improve gizzard development. On the other hand, no effect on gizzard development was observed when pelleting evened out differences in particle size (Amerah *et al*., 2007). However, the effect of whole grain inclusion on particle size distribution after pelleting remains to be investigated

Pelleting moulds mash diets to macro-particles while simultaneously reducing the size of micro-particles that constitute the intact pellet (Svihus *et al*., 2010b). Svihus *et al*., (2004b) determined the particle size of diets before and after pelleting, and reported that the proportion of coarse particles diminished and the proportion of fine particles increased as a consequence of pelleting. Larger particles in particular are affected more during the pelleting process due to the narrow pellet die diameter and this may explain why pelleting tends to reduce the particle size. Moreover, frictional forces inside the die hole can further grind coarse particles into smaller ones (Svihus *et al*., 2004b). A pellet die with a large die diameter may minimise particle size reduction of the whole grain component and may enhance the beneficial effects associated with pre-pelleting inclusion of whole grain. However there have been no studies examining the interaction between the pellet die diameter and the method of WW inclusion in broiler chickens.

The aim of present study was to determine whether using a pellet die with a larger diameter (4.76 versus 3.0 mm) during pre-pelleting of WW would be beneficial on gizzard development, gut health and performance of broilers. The intention was to maintain larger wheat particle size in pellets and maximise potential beneficial effects of WW feeding.

7.3. Material and methods

7.3.1 Diets

The experiment was designed as a 3 x 2 factorial arrangement of treatments, which included three diet forms, namely ground wheat (GW), 200g/kg WW replacing GW before or after pelleting and two pellet diameters (3.0 or 4.76 mm). Diets were formulated to meet Ross 308 strain recommendations for broiler finishers (Ross, 2007; Table 7.1) with the only differences between diets being the form of wheat and the pellet die used in pelleting. Thel wheat used in the experiment came from the same batch purchased from a local supplier. The ground wheat component used in experimental diets was ground in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) and passed through a 4.0 mm screen in single lot. Complete mash diets without or with substitution by whole wheat and mash diets (without WW added post pellet) were mixed and divided into two portions. One portion of each diet was cold pelleted at 65 ^{o}C using a pellet mill (Richard Size Limited Engineers, Orbit, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring of 3 mm hole, while the other portion was put through the same mill but with a die ring of 4.76 mm hole diameter. Representative samples were collected after pelleting for determination of particle size distribution and pellet quality (pellet durability index). All diets were manufactured a week prior to the start of the experiment.

Table 7.1. Composition and calculated analysis of the finisher diet (g/kg as fed basis)

Ingredients	
Wheat [1]	708.0
Soybean meal	231.4
Vegetable oil	25.6
Dicalcium phosphate	11.0
Limestone	15.0
L-lysine	2.5
DL-methionine	2.5
Salt	2.0
Vitamin-trace mineral premix[2]	2.0
Titanium oxide	3.0
Xylanase[3]	+
Calculated analysis	
AME, MJ/kg	13.0
Crude protein	203
Lysine	10.5
Methinine + Cysteine	7.6
Calcium	9.0
Total phosphorus	6.1
Available phosphorus	4.4

[1] In treatment diets, ground wheat was replaced by 200 g/kg whole wheat pre or post-pelleting.

[2] Supplied per kilogram of pre-mix: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017mg; folic acid, 5.2mg; menadione, 4 mg; niacin, 35mg; 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, 60mg.

[3] Avizyme, Danisco Animal Nutrition, Marlborough, UK.

7.3.2. Birds and Housing

Day-old male broilers (Ross 308) were obtained from a commercial hatchery and raised in three-tier electrically heated battery brooders housed in a environmentally controlled room until 10 days of age. Birds were fed a commercial wheat-based broiler starter diet. On day 11, birds were individually weighed and allocated by body weight to 36 cages (8 birds/ cage) housed in an environmentally controlled room. Each cage was then assigned randomly to one of the six treatments. Feed was offered *ad libitum* and water was freely available throughout the trial, which lasted until 35 days of age. Housing conditions were as described in Chapter 3, section 3.1.

7.3.3. Performance data

Performance data were recorded as described in Chapter 3, section 3.1.

7.3.4. Determination of particle size distribution in the diets

The wet sieving method used to determine particle size distribution of pelleted diets was described in Chapter 3, section 3.3.

7.3.5. Pellet quality

Pellet durability index (PDI) was measured as described in Chapter 3, section 3.4.

7.3.6. Apparent metabolisable energy (AME) determination

The AME determination was carried between days 31-34 post-hatch as described in Chapter 3, section 3.6.

7.3.7. Ileal digestibility determination

On day 35, two birds per cage were euthanised and ileal digesta was collected and processed as described in Chapter 3, section 3.7.

7.3.8. Determination of caecal microflora

From the birds used for ileal digesta collection (two birds/cage), caeca were carefully excised and stored at -20 ^{o}C until determination of microflora using the fluorescence *in situ* hybridization technique as described in Chapter 3, section 3.13.

7.3.9. Carcass measurements

On day 35, two more birds (close to the mean cage weight) were selected from each cage, weighed and killed by cervical dislocation. Following exsanguination, feathers, viscera, shanks, and neck were removed and, weights of the eviscerated hot carcass and abdominal fat were recorded.

7.3.10. Digestive tract measurements

On day 35, the digestive tract was collected from the birds used for carcass measurements (two birds /cage) and measurements were carried out as described in Chapter 3, section 3.10.

7.3.11. Determination of digesta transit time

On day 35, digesta transit time was measured as described in Chapter 3, section 3.12.

7.3.12. pH of digesta of different segments of gastrointestinal tract

On day 36, nine birds per treatment were killed and pH of digesta was measured as described in Chapter 3, section 3.11.

7.3.13. Measurement of excreta gas production

Excreta gas production was measured as described in Chapter 3, section 3.14.

7.3.14. Chemical analysis

Dry matter, nitrogen (N), gross energy, titanium, and starch contents were determined as described in Chapter 3, section 3.8.

7.3.14. Calculations

AME and apparent ileal nutrient (N and starch) digestibilty were calculated using the formulae described in Chapter 3, section 3.9.

7.3.15. Data analysis

Cage means served as the experimental unit for statistical analysis of performance data nutrient digestibility and microflora count. For digestive tract measurement, pH and carcass characteristics, individual birds were considered as the experimental unit. All data were subjected to two-way analysis of variance using the general linear model procedure of SAS (SAS, 2004) to determine the main effects (method of wheat inclusion and pellet diameter) and their interaction. If F-test was significant, then differences between means were compared by the least significant difference test. Differences were considered significant at $P < 0.05$.

7.4. Results

7.4.1 Particle size distribution

Feed particle size distribution of the four pelleted diets is shown in Fig 7.1. Notably a higher proportion of coarse particles (over 1 mm size) were observed in 4.76 mm diameter pellets as compared to that of 3.0 mm diameter pellet. The percentage of particles over 1 mm were 14.9 and 21.5 % in 3.0 and 4.76 mm diameter GW diets, respectively. The corresponding values for diets with pre-pellet inclusion of WW were 21.6 and 33.7 %, respectively.

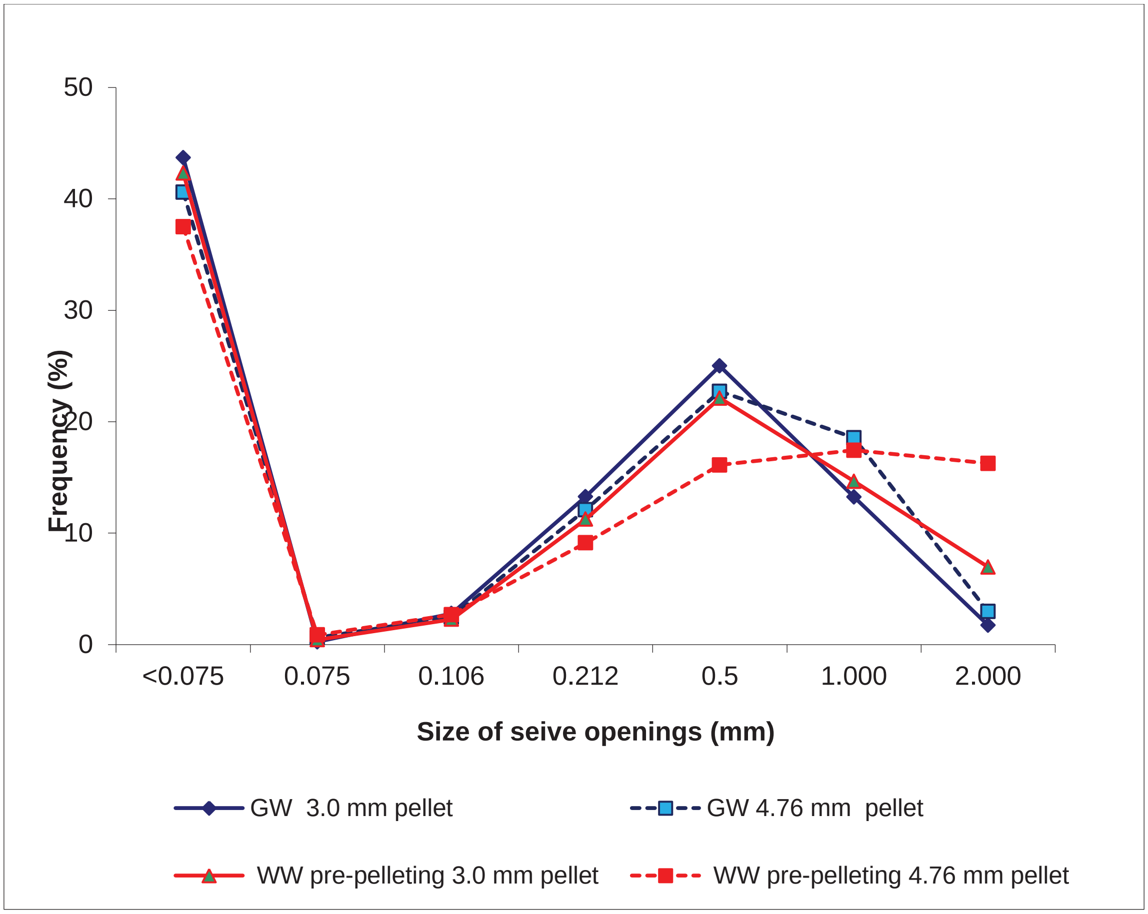


Figure 7.1. Particle size distribution of pelleted diets obtained by the wet sieving method

7.4.2. Pellet quality

A significant (P<0.05) interaction between the method of wheat inclusion and pellet diameter was observed for PDI (Table 7.2). Increasing the diameter from 3.0 to 4.76 mm increased the PDI in the GW diet, but reduced it in diets with pre-pelleting inclusion of WW.

Table 7.2. Influence of method of wheat inclusion and pellet diameter on the durability index (%) of pellets [1]

Method of wheat inclusion	Pellet diameter (mm)	PDI
GW	3.0	88.2^{c}
	4.76	91.1^{b}
Pre-pellet WW	3.0	93.9^{a}
	4.76	83.1^{d}
SEM[2]		0.522
Main effects		
Method of wheat inclusion		
GW		89.6
Pre-pellet WW		88.5
Pellet diameter		
3.0		91.1
4.76		87.1
Probabilities, P ≤		
Method of wheat inclusion		0.05
Pellet diameter		<0.0001
Method of inclusion x pellet diameter		<0.0001

[a,b,c,d] Means in a column not sharing a common superscript are significantly different (P < 0.05).
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of the mean.

7.4.3. Performance data

Four birds (out of 288) died during the experiment and the deaths were not related to any specific treatment. The influence of dietary treatments on performance of broilers is summarised in Table 7.3. Significant (P<0.05) interactions between the method employed for wheat inclusion and pellet diameter were observed for weight gain, feed intake and feed per gain. Increasing the pellet diameter from 3.0 to 4.76 mm increased (P<0.05) the weight gain of birds fed diets with GW and post-pellet inclusion of WW. This trend, however, was reversed in birds fed the diet with pre-pelleting inclusion of WW, where increasing pellet size resulted in decreased (P<0.05) weight gain.

Increasing the pellet diameter from 3.0 to 4.76 mm increased (P<0.05) the feed intake of GW diet, but reduced (P<0.05) the intake of diets with pre-pelleting inclusion of WW. Feed intake was unaffected by pellet diameter in diets with post pelleting inclusion of WW.

Feed per gain was lowered (P<0.05) by increased pellet diameter in diets with GW and post-pelleting inclusion of WW, while there was no effect of pellet diameter in diets with pre-pellet inclusion of WW.

Table 7.3. Influence of method of wheat inclusion and pellet diameter on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broilers, 11-35 days post-hatch[1]

Method of wheat inclusion	Pellet diameter (mm)	Weight gain	Feed intake	Feed per gain
GW	3.0	1665[c]	3249[b]	1.956[a]
	4.76	1999[a]	3377[a]	1.689[cd]
Pre-pellet WW	3.0	2004[a]	3371[a]	1.688[cd]
	4.76	1776[b]	3065[c]	1.725[bc]
Post-pellet WW	3.0	1625[c]	2893[d]	1.781[b]
	4.76	1794[b]	2972[d]	1.656[d]
SEM[2]		22.87	31.98	0.020
Main effects				
Method of wheat inclusion				
GW		1832	3313	1.822
Pre-pellet WW		1890	3218	1.707
Post-pellet WW		1710	2932	1.719
Pellet diameter				
3.0		1765	3171	1.809
4.76		1856	3138	1.690
Probabilities, P ≤				
Method of wheat inclusion		0.0001	0.0001	0.0001
Pellet diameter		0.0001	0.21	0.0001
Method of inclusion x pellet diameter		0.0001	0.0001	0.0001

[a,b,c,d] 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 the mean.

7.4.4. Nutrient utilisation

The effects of method of WW inclusion and pellet diameter on the AME and apparent ileal digestibility coefficients of nitrogen and starch are shown in Table 7.4. The main effects of method of wheat inclusion and pellet diameter were significant (P<0.05) for AME and starch digestibility. Both AME and starch digestibility were improved (P<0.05) with an increase in pellet diameter. Improvements in AME and starch digestibility were seen in broilers fed diets with WW, irrespective of method of inclusion, compared to those fed diets containing GW (P<0.05). Apparent metabolisable energy of diets with pre-pelleting or post-pelleting inclusion of WW was similar (P > 0.05). Starch digestibility was higher (P < 0.05) in birds fed diets with post-pellet inclusion of WW compared to those fed diets with pre-pellet inclusion of WW. Nitrogen digestibility was not influenced (P>0.05) by pellet diameter, but there was a tendency (P = 0.09) for WW diets to have higher N digestibility. No interaction (P>0.05) between method of WW inclusion and pellet diameter was observed for AME or starch and nitrogen digestibility.

Table 7.4. Influence of method of wheat inclusion and pellet diameter on AME(MJ/kg DM) and apparent ileal nitrogen and starch digestibility coefficients for broilers[1]

Method of wheat inclusion	Pellet diameter (mm)	AME	Nitrogen Digestibility	Starch digestibility
GW	3.0	12.91	0.793	0.838
	4.76	13.66	0.793	0.914
Pre-pellet WW	3.0	13.66	0.815	0.903
	4.76	13.96	0.804	0.936
Post-pellet WW	3.0	13.62	0.824	0.961
	4.76	14.2	0.831	0.983
SEM[2]		0.196	0.0151	0.0211
Main effects				
Method of wheat inclusion				
GW		13.28[b]	0.793	0.876[c]
Pre-pellet WW		13.81[a]	0.810	0.920[b]
Post-pellet WW		13.91[a]	0.827	0.973[a]
Pellet diameter				
3.0		13.40[b]	0.811	0.901[b]
4.76		13.94[a]	0.809	0.945[a]
Probabilities, P ≤				
Method of wheat inclusion		0.01	0.09	0.001
Pellet diameter		0.01	0.90	0.05
Method of inclusion x pellet diameter		0.53	0.85	0.41

[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.
[2] Pooled standard error of the mean.

7.4.5. Carcass characteristics

A significant ($P<0.05$) interaction between the method of wheat inclusion and pellet diameter was observed for carcass recovery. Increasing the pellet diameter from 3.0 to 4.76 mm resulted in increased ($P<0.05$) carcass recovery in birds fed GW diets, whereas carcass recovery decreased ($P<0.05$) in those fed diets with pre-pelleting inclusion of WW. No effect of pellet diameter was seen in birds fed diets with post-pellet inclusion of WW (Table 7.5). The main effects and the interaction were not significant ($P>0.05$) for the relative weight of the abdominal fat pad (Table 7.5).

Table 7.5. Influence of method of wheat inclusion and pellet diameter on carcass recovery (g/kg body weight) and abdominal fat (g/kg body weight) for broilers[1]

Method of wheat inclusion	Pellet diameter (mm)	Carcass recovery	Abdominal fat
GW	3.0	727[cd]	8.98
	4.76	744[ab]	8.84
Pre-pellet WW	3.0	753[a]	7.65
	4.76	738[bc]	8.99
Post-pellet WW	3.0	722[d]	9.47
	4.76	733[bd]	7.86
SEM[2]		5.0	0.791
Main effects			
Method of wheat inclusion			
GW		736	8.91
Pre-pellet WW		746	8.32
Post-pellet WW		728	8.67
Pellet diameter			
3.0		734	8.70
4.76		738	8.56
Probabilities, P ≤			
Method of wheat inclusion		0.01	0.76
Pellet diameter		0.21	0.83
Method of inclusion x pellet diameter		0.01	0.18

[a,b,c,d] Means in a column not sharing a common superscript are significantly different ($P < 0.05$).
[1] Each value represents the mean of 12 birds.
[2] Pooled standard error of the mean.

7.4.6. Digestive tract and organ measurements

Influence of pellet diameter and method of wheat inclusion on the relative weight of digestive organs and digesta content of the gizzard in broilers is presented in Table 7.6. Interaction between the method of wheat inclusion and pellet diameter was significant ($P < 0.05$) for the relative weight of gizzard. Larger pellet diameter in diets with pre-pelleting inclusion of WW increased the relative weight of gizzard, but had no effect in diets with GW or post pellet inclusion of WW. Similar trends were seen for the relative weight of gizzard digesta contents.

The main effect of method of wheat inclusion was significant ($P<0.05$) for the relative weight of proventriculus, pancreas and liver. No significant ($P>0.05$) interactions nor effect of pellet diameter were observed for these organs The relative weights of proventriculus and pancreas organs were heavier ($P<0.05$) in birds fed diets with post-pellet inclusion of WW than those fed diets with GW. However, weights of these organs in birds fed diets with pre-pelleting WW inclusion were similar to those fed diets with GW and post-pellet WW inclusion. The relative weights of liver were greater ($P<0.05$) in birds fed diets with post-pellet inclusion of WW than those fed diets with pre-pelleting WW inclusion. The liver weights were similar in birds receiving diets with GW and post-pellet inclusion of WW.

The effect of method of wheat inclusion and pellet diameter on the relative weight and length of various intestinal components are shown in Table 7.7. Interactions ($P<0.05$) between the method of wheat inclusion and pellet diameter were observed for the relative weight and length of small intestine and its various segments, with the exception of the relative weight of duodenum. Reductions in the relative weight and length of small intestine and its segments were observed with larger pellet diameter in birds fed GW diets ($P<0.05$). In birds fed diets with pre- and post-pelleting WW inclusions, larger pellet diameter had no effect on the relative weight and length of small intestine.

Table 7.6. Relative weight of digestive organs (proventriculus, gizzard, pancreas, liver and spleen) and gizzard contents of broilers[1] as influenced by the method of wheat inclusion and pellet diameter

Method of wheat inclusion	Pellet diameter (mm)	Relative weight (g/kg body weight)					
		Proventriculus	Gizzard	Pancreas	Liver	Spleen	Gizzard content
GW	3.0	2.60	7.13^{c}	1.65	23.9	2.14^{c}	2.14^{c}
	4.76	2.51	6.90^{c}	1.55	24.4	2.50^{c}	2.50^{c}
Pre-pellet WW	3.0	2.52	6.72^{c}	1.61	24.0	2.09^{c}	2.09^{c}
	4.76	2.79	8.97^{b}	1.80	21.4	5.93^{b}	5.93^{b}
Post-pellet WW	3.0	2.85	12.75^{a}	1.80	26.4	7.67^{a}	7.67^{a}
	4.76	2.89	11.85^{a}	1.92	25.4	7.41^{a}	7.41^{a}
SEM[2]		0.119	0.448	0.084	1.24	0.497	0.497
Main effects							
Method of wheat inclusion							
GW		2.56^{b}	7.01	1.60^{b}	24.1^{ab}	2.32	2.32
Pre-pellet WW		2.65^{ab}	7.85	1.71^{ab}	22.7^{b}	4.01	4.01
Post-pellet WW		2.87^{a}	12.3	1.86^{a}	25.9^{a}	7.54	7.54
Pellet diameter							
3.0		2.66	8.87	1.69	24.8	3.96	3.96
4.76		2.73	9.24	1.76	23.7	5.28	5.28
Probabilities, P ≤							
Method of wheat inclusion		0.03	0.001	0.01	0.04	0.0001	0.0001
Pellet diameter		0.46	0.31	0.29	0.29	0.01	0.01
Method of inclusion x pellet diameter		0.32	0.001	0.22	0.45	0.001	0.001

[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 12 birds.
[2] Pooled standard error of mean.

Table 7.7. Relative weight and length of different segments of the intestinal tract of broilers[1] as influenced by method of wheat inclusion and pellet diameter

Method of wheat inclusion	Pellet diameter (mm)	Relative weight (g/kg body weight) Duodenum	Jejunum	Ileum	Small Intestine[2]	Caeca	Relative length (cm/kg body weight) Duodenum	Jejunum	Ileum	Small Intestine[2]	Caeca
Ground	3.0	4.45	9.35^{a}	8.99^{a}	22.8^{a}	3.18^{a}	15.6^{a}	39.2^{a}	42.4^{a}	97.3^{a}	10.1^{a}
	4.76	3.95	7.96^{b}	6.50^{c}	18.4^{b}	2.20^{c}	12.9^{b}	30.6^{d}	32.9^{b}	76.6^{d}	8.14^{c}
Pre-pellet WW	3.0	4.16	8.47^{ab}	6.95^{c}	19.5^{b}	2.62^{c}	12.9^{b}	32.6^{cd}	33.2^{b}	78.7^{cd}	8.55^{c}
	4.76	4.08	8.42^{ab}	6.90^{c}	19.4^{b}	2.89^{bc}	15.8^{a}	34.5^{bc}	34.4^{b}	84.8^{bc}	9.02^{bc}
Post-pellet WW	3.0	4.06	8.26^{b}	8.05^{b}	20.3^{b}	2.89^{ab}	14.4^{ab}	36.5^{ab}	39.1^{a}	90.1^{ab}	9.60^{ab}
	4.76	3.94	8.78^{ab}	6.89^{c}	19.6^{b}	2.87^{c}	13.5^{b}	34.8^{bc}	34.1^{b}	82.3^{bcd}	8.47^{c}
SEM[2]		0.193	0.381	0.385	0.788	0.160	0.758	1.30	1.59	2.85	0.354
Main effects											
Method of inclusion wheat											
GW		4.20	8.65	7.74	20.6	2.69	14.3	34.9	37.7	87.0	9.15
Pre-pellet WW		4.12	8.45	6.92	20.0	2.75	14.3	33.6	33.8	81.8	8.79
Post-pellet WW		4.00	8.52	7.47	19.4	2.88	13.9	35.6	36.6	86.2	9.04
Pellet diameter											
3.0		4.22	8.69	8.00	20.9	2.90	14.3	36.1	38.2	88.7	9.44
4.76		3.99	8.39	6.76	19.1	2.65	14.1	33.3	33.8	81.2	8.54
Probabilities, P ≤											
Method of wheat inclusion		0.56	0.85	0.10	0.36	0.58	0.85	0.28	0.05	0.15	0.58
Pellet diameter		0.14	0.33	0.001	0.01	0.01	0.70	0.05	0.01	0.01	0.01
Method of inclusion x pellet diameter		0.49	0.05	0.01	0.05	0.01	0.01	0.001	0.01	0.001	0.01

[a,b,c,d] Means in a column not sharing a common superscript are significantly different ($P < 0.05$).
[1] Each value represents the mean of 12 birds.
[2] Small intestine = duodenum+ jejunum + ileum.
[3]Pooled standard error of mean.

7.4.7. Caecal microflora counts

The effects of method of wheat inclusion and pellet diameter on caecal microflora counts are shown in Table 7.8. Significant ($P < 0.05$) interactions between the method of wheat inclusion and pellet diameter were observed for counts of *Bifidobacteria* spp., *Clostridium* spp. and *Campylobacter* spp. Reductions in *Bifidobacteria* spp. were observed with larger pellet diameter in birds fed GW diets ($P < 0.05$). In birds fed diets with pre- and post-pelleting WW inclusions, larger pellet diameter had no effect on *Bifidobacteria* spp. *Clostridium* spp. count was lower ($P < 0.05$) with larger pellet diameter in birds fed WW post-pellet diets, whereas no effect was seen in birds fed either GW or WW pre-pelleting diets. Reduction in *Campylobacter* spp. was observed with larger pellet diameter in birds fed WW pre-pelleting diets ($P < 0.05$). In birds fed diets with GW and post-pelleting WW inclusions, larger pellet diameter had no effect on *Campylobacter* spp. Main effects of method of wheat inclusion and pellet diameter were significant ($P < 0.05$) for *Bacteroides* spp. and *Lactobacilli* spp., respectively. *Bacteroides* spp. counts were lower in post pelleting WW group than GW and pre-pelleting WW group ($P < 0.05$), but it was similar between GW and pre-pelleting WW groups. Reduction in the counts of lactobacilli was observed in birds fed pellets of 4.76 mm diameter in comparison to those fed 3.0 mm pellets ($P < 0.05$).

Table 7.8. Influence of method of wheat inclusion and pellet diameter on bacterial counts (Log 10 cells/g caecal content) in broilers[1]

Method of wheat inclusion	Pellet diameter (mm)	*Lactobacilli* spp.	*Bifidobacteria* spp.	*Clostridium* spp.	*Campylobacter* spp.	*Bacteroides* spp.
GW	3.0	7.39	7.40[a]	7.52[a]	5.91[a]	7.11
	4.76	7.11	6.85[b]	7.52[a]	5.85[a]	6.84
Pre-pellet WW	3.0	7.39	6.84[bc]	7.35[ab]	5.95[a]	6.93
	4.76	7.24	6.56[cd]	7.25[ab]	5.65[b]	6.85
Post-pellet WW	3.0	7.26	6.47[d]	7.35[b]	5.62[b]	6.76
	4.76	7.26	6.49[d]	6.52[c]	5.64[b]	6.57
SEM[2]		0.066	0.099	0.079	0.057	0.123
Main effects						
Method of wheat inclusion						
GW		7.26	7.14	7.54	5.92	6.95[a]
Pre-pellet WW		7.32	6.73	7.32	5.81	6.91[a]
Post-pellet WW		7.27	6.51	6.96	5.63	6.73[b]
Pellet diameter						
3.0		7.36[a]	6.92	7.43	5.85	6.95
4.76		7.21[b]	6.66	7.12	5.72	6.77
Probabilities, **P ≤**						
Method of wheat inclusion		0.56	0.001	0.001	0.001	0.05
Pellet diameter		0.01	0.01	0.001	0.05	0.08
Method of inclusion x pellet diameter		0.14	0.05	0.001	0.05	0.74

[a,b,c,d] Means in a column not sharing a common superscript are significantly different ($P < 0.05$).
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of the mean.

7.4.8. pH of the digesta

Effects of method of wheat inclusion and pellet diameter on pH of excreta and digesta from various segments of the intestine are shown in Table 7.9. Lower ($P<0.05$) gizzard pH was observed in birds fed WW post-pelleting than those fed WW pre-pelleting and GW diets. Whereas, gizzard pH was lower ($P<0.05$) in birds fed pre-pelleting WW diets compared to those fed GW diets. Significant ($P<0.05$) interaction between the method of wheat inclusion and pellet diameter was observed for duodenal pH. An increase in duodenal pH was observed with larger pellet diameter in birds fed diets with pre-pelleting WW inclusion ($P < 0.05$). In birds fed diets with GW and post-pelleting WW inclusions, larger pellet diameter had no effect on duodenal pH. No significant ($P>0.05$) main effects or interaction between the methods of wheat inclusion and pellet diameter were observed for the pH of proventriculus, jejunum, ileum, caeca and excreta.

Table 7.9. Influence of method of wheat inclusion and pellet diameter on pH of digesta from segments of digestive tract and excreta of broilers[1]

Method of wheat inclusion	Pellet Diameter (mm)	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Caecum	Exceta[2]
GW	3.0	4.30	4.67	5.91^{ab}	5.77	5.92	5.96	6.20
	4.76	4.27	4.61	5.70^{b}	5.97	6.03	5.95	7.10
Pre-pellet WW	3.0	4.03	4.42	5.55^{b}	5.97	6.17	5.86	6.41
	4.76	3.72	3.78	6.02^{a}	6.08	6.68	5.93	7.00
Post-pellet WW	3.0	3.70	3.65	5.82^{ab}	5.85	6.21	5.95	7.06
	4.76	4.20	3.80	5.80^{ab}	5.96	6.23	6.13	6.89
SEM[3]		0.182	0.170	0.108	0.117	0.228	0.149	0.378
Main effects								
Method of wheat inclusion								
GW		4.28	4.64^{a}	5.80	5.87	5.97	5.96	6.65
Pre-pellet WW		3.87	4.10^{b}	5.78	6.03	6.43	5.90	6.71
Post-pellet WW		3.96	3.72^{c}	5.81	5.91	6.22	6.04	6.97
Pellet diameter								
3.0 mm		4.01	4.25	5.76	5.87	6.10	5.92	6.56
4.76 mm		4.06	4.06	5.84	6.01	6.31	6.00	7.00
Probabilities, P ≤								
Method of wheat inclusion		0.07	0.001	0.98	0.38	0.14	0.63	0.66
Pellet diameter		0.74	0.18	0.38	0.15	0.26	0.53	0.17
Method of inclusion x pellet diameter		0.10	0.07	0.01	0.90	0.53	0.82	0.36

[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 nine birds.

[2] Each value represents the mean of six cages.

7.4.9. Excreta gas production and digesta transit time

The main effects of method of wheat inclusion and pellet diameter were significant ($P<0.05$) for gas production from the anaerobic incubation of excreta (Table 7.10). Post-pellet WW inclusion resulted in lower ($P<0.05$) gas production in comparison to pre-pelleting WW and GW diet fed birds. However, gas production was lower ($P<0.05$) in birds fed diets with pre-pellet inclusion of WW compared to those fed GW diets. Anaerobic incubation of excreta from broilers fed 4.76 mm diameter pellets led to lower ($P<0.05$) gas production than those from bird fed 3.0 mm diameter pellets.

A significant ($P<0.05$) interaction was observed for feed passage time between the method of wheat inclusion and pellet diameter. Post-pellet inclusion of WW with larger pellet diameter resulted in faster ($P<0.05$) feed passage rate, whereas diets with pre-pelleting inclusion of WW and GW had no effect ($P>0.05$) (Table 7.10).

Table 7.10. Influence of method of wheat inclusion and pellet diameter on excreta gas production and digesta transit time of broilers

Method of wheat inclusion	Pellet diameter (mm)	Gas production (ml/g)	Transit time (min)
GW	3.0	17.50	183^{ab}
	4.76	15.66	167^{bc}
Pre-pellet WW	3.0	15.33	155^{c}
	4.76	7.66	166^{c}
Post-pellet WW	3.0	7.83	193^{a}
	4.76	6.50	155^{c}
SEM[2]		1.43	6.1
Main effects			
Method of wheat inclusion			
GW		16.58^{a}	175^{a}
Pre-pellet WW		11.50^{b}	160^{b}
Post-pellet WW		07.16^{c}	174^{a}
Pellet diameter			
3.0		13.55^{a}	177^{a}
4.76		9.94^{b}	163^{b}
Probabilities, **P ≤**			
Method of wheat inclusion		0.001	0.05
Pellet diameter		0.01	0.01
Method of inclusion x pellet diameter		0.06	0.001

[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.
[2] Pooled standard error of the mean.

7.5. Discussion

Particle size distribution of pelleted feed showed that the use of a larger pellet die (4.76 vs. 3.0 mm diameter) resulted in more coarser particles over 1 mm size, irrespective of pre-pellet inclusion of wheat in ground or whole form. The GW diet pelleted with 4.76 mm die and with pre-pellet inclusion of WW using 3.0 mm die had similar percentage (21%) of particles over 1 mm size. In comparison to these diets, those with pre-pelleted inclusion of WW using a 4.76 mm die had a higher percentage (33%) of particles over 1mm size. This observation lends support to the hypothesis that reduction of feed particle size can be minimised by increasing the diameter of the pellet die. The pelleting process resulted in changes in feed microstructure due to the grinding effect in the pellet press and frictional forces inside the die hole, an effect which has been shown to be particularly prominent in diets containing coarser particles (Engberg *et al*., 2002 ; Svihus *et al*., 2004b).

In the present study, significant interactions between the method of wheat inclusion and pellet diameter were observed for performance parameters. In the GW diet, weight gain and feed efficiency were improved in birds fed 4.76 mm diameter pellets compared to those fed 3.0 mm diameter pellets. Similar effects were observed with post-pellet inclusion of WW, but the opposite trend was observed with pre-pelleting inclusion of WW, where weight gain decreased with the larger pellet diameter. Weight gain is dependent largely on feed intake and, consequently, energy and nutrient intakes. Treatments with higher weight gain were associated with higher feed intake. Feed intake in birds fed with pre-pellet inclusion of WW was markedly reduced with increasing pellet diameter resulting in lower weight gain, whereas feed intake increased with larger pellets in GW fed birds. The effect on feed intake may be attributed, in part, to the effects of larger pellet diameter on pellet quality. Pellet durability index was improved with larger pellet diameter in GW diets, but markedly reduced in diets with pre-pelleting inclusion of WW. When pellet quality is reduced, performance decreases due to reduced feed intake (Proudfoot and Sefton, 1978). Pre-pelleting inclusion of WW using the 4.76 diameter die would have resulted in uneven breakage of the WW, reducing the pellet quality. Thus it is possible that the effect of particle size may have confounded the effect of pelleting (Behnke and Beyer, 2002). Furthermore pellet durability is inversely related to particle size (Angulo *et al*., 1996) and it is thought that smaller particles increases the surface area per unit volume providing more contact points with each other (Bhenke, 2001), although there is no scientific evidence to support this claim. In addition to the size, the shape of particles may be of some significance in determining the pellet quality. The way whole grain is broken while pelleting and its effect on pellet quality needs to be examined in future studies.

In the current study, irrespective of die diameter, post-pellet inclusion of WW resulted in heavier gizzards. On the other hand, when WW was included pre-pelleting, 4.76 mm diameter pellets resulted in heavier gizzards compared to 3.0 mm diameter pellets. This finding may be related to the feed particle size distribution after dissolution in the crop. In the present study, diets with pre-pellet inclusion of WW resulted in 21 and 33% of particles over 1 mm size, respectively, when 3.0 or 4.76 mm pellet dies were used. Increased pellet die diameter in GW diets resulted in increased particle size (from 14 to 21%), but there was no effect on gizzard development. Feed particle size is positively related to relative gizzard weight when diets are fed in mash form (Nir and Ptichi, 2001). However, in pelleted diets, this is likely to be dependent on the particle size distribution of pellets after pelleting and its dissolution in the crop (Amerah *et al*., 2007). Regarding the minimal particle size of cereals required to stimulate gizzard development, Svihus (2011) recommended that at least 20-30% of particles should be larger than 1 mm in size. The weight of gizzard content in the present study indicates that when birds are fed diets with more coarser feed particles over 1 mm (33% and above), gizzard retains and actively grinds feed as opposed to simply acting as a passive transit organ, resulting in increased gizzard volume and weight. This finding may partly also explain the equivocal results reported with pre-pelleting inclusion of WW on gizzard development. Wu *et al*. (2004) found that pre-pelleting inclusion of WW had no effect on the gizzard weight. In contrast, several others (Jones and Taylor, 2001; Taylor and Jones, 2004a; Svihus *et al*., 2004a) reported increased relative gizzard weight with pre-pelleting WW inclusion. Variable effect of pre-pelleting inclusion of WW on particle size distribution may be responsible, in part, for the contradictory results observed on gizzard size.

Irrespective of the method of WW inclusion, AME and starch digestibility were improved in WW fed birds. These results are consistent with previous studies (Preston *et al*., 2000; Svihus and Hetland, 2001; Hetland *et al*., 2003; Svihus *et al*., 2004a; Wu *et al*., 2004). Since the gizzard size differed depending on the method of inclusion, these data suggest that improvements in nutrient utilisation cannot always be explained on the basis of gizzard development.

In the present study, the effect of larger pellet diameter on carcass recovery differed depending on the method of wheat inclusion. Carcass recovery increased in the GW treatment, decreased in pre-pelleting WW treatment and had no effect in post-pelleting WW treatment as pellet diameter increased. Higher carcass recovery in birds fed larger pellets in the GW group may be explained by the lighter digestive tract in comparison to birds fed smaller (3.0 mm) diameter pellets. Longer and heavier digestive tract in smaller diameter pellet-fed birds may be due to a higher percentage of particles less than 1 mm in the 3.0 mm pellet as compared to 4.76 mm diameter pellet. Birds fed a wheat-based diet containing fine particles resulted in higher digesta viscosity than those fed a diet with coarser particles (Yasar, 2003). Viscous digesta is known to reduce feed passage rate and absorption of nutrients (Bedford and Schulze, 1998), which may lead to development of relatively longer and heavier digestive tracts as an adaptive mechanism to increase the time feed is in contact with the absorptive surface in the intestine. Increase in digesta transit time (183 vs. 167 min) in birds fed 3.0 mm diameter GW pellets in comparison to 4.76 mm diameter pellet supports this argument.

Larger pellet diameter had no effect on the relative length and weight of the small intestine in birds fed diets containing WW. Inclusion of WW irrespective of the method, because of higher proportion of coarser particles, may have mitigated the effect of digesta viscosity, thus eliminating the need for any further physiological adaptation relating to nutrient digestion and absorption (Banfield *et al*., 2002). However, compared to the GW group, carcass recovery was lower in pre-pelleting WW group and similar in the post-pelleting WW group. This observation may be partly attributed to the heavier gizzard weight in the pre-pelleting group and similar gizzard weight in the post-pelleting group fed with larger diameter pellets in comparison to smaller diameter pellets. Amerah and Ravindran (2008) also reported that lower carcass recovery was associated with heavier gizzard weight. However, other researchers (Bennett *et al*., 2002; Palvinik *et al.*, 2002; Wu and Ravindran, 2004) failed to show any effect of WW feeding on carcass recovery. Inconsistency on carcass recovery associated with WW feeding of birds may in part due to differences in the method employed for WW inclusion and its effect on digestive tract.

In the present study, WW inclusion and pellet diameter had no effect on the relative weight of abdominal fat. Similar results due to WW feeding have been reported by Bennett *et al*. (2002) and Wu and Ravindran (2004). In contrast, higher relative abdominal fat weight was reported by Preston *et al*. (2000), Nahas and Lefrancois (2001), and Jones and Taylor (2001), and lower relative abdominal fat weight by Amerah and Ravindran (2008).

Gut flora profile was found to be influenced by the method of WW inclusion. Significant reduction in the numbers of pathogenic bacteria recovered from the caeca of birds fed diets containing 200 g/kg WW incorporated post-pelleting is an important finding of this study. Although the observed changes in the population of pathogenic bacteria did not translate into to performance improvements, the changes have implications in term of food safety. Intestinal pathogenic bacteria such as E. coli, *Clostridium* spp., *Campylobacter* spp., and *Bacteroides* spp. contaminate poultry carcasses during processing in slaughter houses, representing an important cause of foodborne illnesses in humans (Oosterom, 1991; Rasschaert *et al*., 2008). Accordingly, post-pelleting WW inclusion could be a useful dietary strategy to improve the safety of poultry products.

The lower gas production and population size of pathogenic bacterial species in birds fed diets with post-pelleting WW inclusion were associated with heavier gizzards. Furthermore, gizzard pH was also lower in broilers fed WW diets compared to those fed GW diets. Gabriel *et al*. (2003a) also reported that the gizzard weight was heavier in WW-fed birds and gizzard contents had a lower pH than those fed GW diet. Similarly, Bjerrum *et al*. (2005) found that the pH in the contents of the gizzard decreased as the amount of WW in the diet increased. Following infection with *Salmonella Typhimurium*, these researchers found lower numbers of Salmonella in the gizzard and ileum of birds receiving WW compared to GW. It was speculated that the feed was more exposed to the low pH and proteases in the gizzard of birds fed WW, leading to a high percentage of pathogens entering the gizzard with the feed being killed. In contrast, diets with high proportion of fine particles may be less exposed to low pH and proteases in the gizzard. According to Hill (1971), ingested feed with fine particles appears more quickly in the duodenum as a suspension of relatively unchanged particles. These observations support the conclusion that the gizzard has an important function as a barrier organ that prevents pathogenic bacteria from entering the distal digestive tract (Bjerrum *et al*., 2005).

7.6. Conclusions

The present data suggest that, irrespective of whether the grain is milled or added as whole post-pelleting, a larger diameter pellet is beneficial. On the other hand, when WW is added pre-pelleting, a smaller diameter pellet results in improved weight and feed per gain in broiler performance. WW Inclusion and larger pellet diameter improved AME and ileal digestibility of starch. However, gizzard size differed depending on the method of inclusion and the present data suggest that improvements in nutrient utilisation cannot be always explained on the basis of gizzard development alone. The hypothesis that a well-developed gizzard will improve nutrient utilisation is relevant for post-pellet inclusion of WW, but not for pre-pellet inclusion of WW, indicating the involvement of other factors.

CHAPTER 8

Influence of whole wheat feeding on the development of coccidiosis in broilers challenged with *Eimeria*

8.1. Abstract

A study was conducted to assess the effect of whole wheat (WW) feeding on performance, gizzard development, oocyst yield and intestinal lesion score of broiler chickens experimentally challenged with caecal and intestinal Eimerian species and to test the hypothesis that the inclusion of whole grain in the diet will reduce the severity of coccidial infection. Diets (ground wheat (GW) and 300g/kg WW replacing GW either before or after pelleting) were offered *ad libitum* from day 1 to day 28 post-hatching. At 21 days of age, each dietary treatment was divided into two groups, one unchallenged control and the other inoculated with mixed species of coccidia (*E. acervulina*, *E.maxima*, and *E. tenella)*. No differences (P>0.05) between dietary treatments were observed in weight gain and feed per gain during the pre-challenge period (1 to 21 day), but feed intake was lower (P<0.05) in WW fed birds. After the inoculation (21 to 28 days), challenged birds had reduced (P<0.05) weight gain and feed intake, and higher (P<0.05) feed per gain compared to unchallenged birds. Mortality in challenged birds was highest in those fed diets with WW post-pelleting, followed by pre-pelleted WW and GW (58, 35, and 17%, respectively; P<0.05). Relative gizzard weights were heavier (P<0.05) in WW fed birds, irrespective of the method of inclusion, compared to those fed GW in both challenged and unchallenged groups. Total lesion scores of challenged birds were not influenced (P>0.05) by dietary treatments. Lesion scores were, however, lower (P<0.05) in the duodenum and jejunum, and higher (P<0.05) in the caeca in WW fed birds, irrespective of the method of inclusion. Total and *E.tenella* oocyst counts in the excreta of challenged birds were higher (P<0.05) in birds fed WW, irrespective of the method of inclusion. No mortality, intestinal lesion and excreta occyst shedding were observed in unchallenged treatments. Based on increased mortality, it can be concluded that WW feeding exacerbated the severity of coccidiosis infection, possibly via a mechanism involving enhanced gizzard development.

8.2. Introduction

Coccidiosis, one of the economically important diseases in intensive poultry production, is caused by protozoan species belonging to the genus *Eimeria* and, leads to reduced growth and increased mortality in broiler chickens. Moreover, this infection predisposes the birds to secondary intestinal bacterial infections that can result in further growth reduction and economic loss (Wilson *et al*., 2005). Current prevention and control strategies rely predominantly on the use of chemotherapeutic anticoccidial drugs added directly to feeds. However, some resistance to all coccidiostats in current use has been reported (Chapman, 1997). Furthermore, consumer concerns regarding public health and food safety, and a European Union proposal to phase out coccidiostats as feed additives by 31 December 2012 (COM, 2008; Regulation 1831/2003/EC) are other reasons behind the need to find new and effective ways for controlling coccidiosis in poultry. Until the development of a less expensive and effective anticoccidial vaccine suitable for use in broiler production, there is pressure on the poultry industry for new viable alternatives to keep poultry production in line with the requirements of the market. Natural methods and nutritional approaches are likely to play an increasing role in disease control since they are well accepted by consumers (Brenes and Roura, 2010).

Feeding whole grains may be an alternative method to control coccidiosis. Cumming (1987, 1989) reported that feeding whole grain had beneficial effects on the prevention of coccidiosis. It was suggested that increased gizzard size and grinding action, brought about by feed structure of the whole wheat (WW), might be responsible for the physical destruction of oocysts prior to reaching their sites of infection. This theory was supported by subsequent work of the author in which feeding whole grains in a free-choice feeding situation reduced excreta oocyst yield and it was negatively correlated with gizzard size (Cumming 1992a, b). In this study, oocyst yield was used as a criterion for the assessment of severity of infection. However, such beneficial effects of whole grain feeding on the development of coccidiosis have not been confirmed in other studies (Waldenstedt *et al*., 1998; Banfield and Forbes, 2001; Banfield *et al*., 2002). Conversely, Gabriel *et al*. (2003b, 2006) studied the effect of WW inclusion on three of the most common species of coccidia, namely *Eimeria acervulina* (duodenum), *E. maxima* (jejunum and ileum) and *E. tenella* (caeca), in broilers and reported higher total oocyst counts and greater lesion scores at 7 days post-challenge in WW fed birds compared to ground wheat (GW) fed birds. These researchers also observed that, during the acute phase of coccidiosis, regardless of the Emeria species, WW feeding led to deleterious effects on birds, turning the subclinical coccidiosis (in GW fed birds) into clinical coccidiosis (in WW-fed birds). It was proposed that oocyst excretion should not be used as the sole infection criterion to bring about dietary effect of WW and that WW feeding modifies the life cycle of Eimeria species. In addition, the presence of oocysts in excreta confirms the coccidial infection, but this has little relationship to the extent of clinical disease (Khan and Line, 2010). Lesion scoring is also crucial to assess *Eimeria* pathogencity (Yvore *et al*., 1980; Allen *et al*., 2000). The conflicting results in the literature may be related to differences in the methodology employed in different studies, including dose, species and strains of coccidia, age and strain of birds, age of introduction of WW, quantity and type of WW fed, and age of inoculation.

The aim of present study was to evaluate the effects of WW feeding during experimental coccidial challenge with *E. acervulina*, *E. maxima* and *E. tenella* on performance, gizzard development, oocyst shedding and intestinal lesion score of broilers. The hypothesis tested was that WW feeding will lead to better development of gizzard and destroy the oocysts, prior to reaching their site of infection and lower the incidence and severity of coccidiosis.

8.3. Material and methods

Experimental procedures were approved by the Massey University Ethics Committee. Inclusion of 200 g/kg WW with 3 mm pellet die diameter (Chapter 7) failed to show any effect on gizzard development. In present study, inclusion level of WW was increased to 300 g/kg to ensure positive effects on gizzard development to test the hypothesis that better development of gizzard would destroy the oocysts, prior to reaching their site of infection and lower the incidence of coccidiosis.

8.3.1. Diets

The experimental design was a 3 x 2 factorial arrangement of treatments, which included three diet forms, namely GW, 300 g/kg WW replacing GW before pelleting, and 300 g/kg WW replacing GW after pelleting without or with coccidial challenge (challenged or unchallenged). Diets were formulated to meet Ross 308 strain recommendations for broiler finishers (Ross, 2007; Table 8.1). Treatment diets were formulated to be isocaloric and isonitrogenous, and differed only in the form and method of wheat inclusion. No coccidiostat or growth promoters were included.

WW or GW used for the manufacture of experimental diets was from the same batch. For the GW component used in experimental diets, WW was ground in single lot in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) to pass through a 4.0 mm screen. Complete mash diets without or with substitution by WW and mash diets (without WW, added post-pelleting) were mixed and cold pelleted at 65 °C using a pellet mill (Richard Size Limited Engineers, Orbit, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/hr and equipped with a die (35 mm thick and 3 mm die diameter). For the post-pelleting diet, pellets and the remaining WW were mixed for 30 seconds before being bagged. All diets were manufactured a week prior to the start of the experiment.

Table 8.1. Composition and calculated analysis of basal diet (g/kg as-fed basis)

Ingredients	
Wheat [1]	668.1
Soybean meal	270.6
Soybean oil	23.5
Dicalcium phosphate	14.2
Limestone	14.5
L-lysine	1.6
DL-methionine	2.3
Salt	2.2
Vitamin-trace mineral premix[2]	3.0
Calculated analysis	
AME, (MJ/kg)	13.0
Crude protein	210
Lysine	12.2
Methinine + Cysteine	9.4
Threonine	7.9
Tryptophan	2.7
Calcium	9.7
Available phosphorus	4.6

[1] In treatment diets, ground wheat was replaced by 300 g/kg whole wheat pre-or post-pelleting.

[2] Nutrients supplied per kilogram of premix: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017mg; folic acid, 5.2mg; menadione, 4 mg; niacin, 35mg; 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, 60mg.

8.3.2. Inoculum

The mixed culture of *Emeria* used in this study was supplied by Avivet Ltd, Palmerston North, New Zealand. The inoculum, derived from a recent field isolate contained mixed cultures of sporulated oocysts of three *Eimeria* species, namely *E. acervulina*, *E.maxima*, and *E. tenella*.

8.3.3. Birds and housing

Day old, male broiler (Ross 308) chicks were obtained from a commercial hatchery. Upon arrival, chicks were individually weighed and assigned to 36 cages (8 birds/cage), in three-tier electrically heated battery brooders housed in environmental controlled room. Cages were then randomly assigned to one of the three dietary treatments (12 replicates per treatment). In the post-pelleting group, chicks were fed GW diet up to 7 day post-hatch and then the WW diets were introduced. On day 11, the birds were transferred to grower colony cages housed in a positive-pressure environmentally controlled room to avoid any contamination and were maintained on the same treatment diets until the end of experiment. On day 21, each dietary treatment was randomly divided into two groups of six replicates each. Birds in one group were orally gavaged directly into the crop with doses of 2 and 1.5 ml at two hour interval (3.5 ml total liquid containing 87500, 3500 and 35000 sporulated oocysts of *E. acervulina*, *E. maxima* and *E. tenella*, respectively). The unchallenged control group was inoculated with the same volume of sterile distilled water in a manner identical to the challenged birds. After inoculation, birds were observed four times daily. Feed was offered *ad libitum* and water was available at all times throughout the trial, which lasted 28 days post-hatch. Housing conditions have been described in Chapter 3, section 3.1.

8.3.4. Performance data

Performance data were recorded as described in Chapter 3, section 3.1.

8.3.5. Collection of excreta samples

On day 6 post-challenge (27 days post-hatch), trays were placed under each cage and excreta samples were collected on the following day from the four corners and the middle of each tray. Excreta samples from two replicates were pooled together to give three representative samples. A portion of excreta sample was immediately used for the measurement of *in vitro* sporulation, while the remainder was stored at -4 ^{o}C for determining of oocysts counts.

8.3.6.1. Oocyst shedding

Oocyst numbers in excreta were determined using a McMaster chamber (Conway and McKenzie, 2007). Samples were processed for oocyst counts as described by Taylor *et al*. (1995). Briefly, excreta samples (3 g) were transferred into a glass beaker and mixed with 45 ml of water. The mixture was vortexed for 1 min and the resultant suspension was filtered through a double-layer of cheesecloth into a beaker. The filtrate was thoroughly mixed before transferring to a 50 ml centrifuge tube and then centrifuged for 5 min at 1000xg. After discarding the supernatant, the pellet was re-suspended in 5 ml of saturated salt solution with a vortex. Due to large number of oocysts present 50 µl of suspension was diluted with 1950 µl of saturated salt solution. After vortexing for 1 min, a sample was removed using a disposable plastic pipette and McMaster counting chamber was filled. The sample tube was again mixed before withdrawing more samples to fill the second and third McMaster chambers. The preparation was left for 5 min before counting to allow the oocysts to float. All oocysts under the grid of each chamber in the McMaster slide were counted using 10 x light microscopy and average of three counts was calculated. Results were expressed as number of oocysts per gram of excreta using the calculation, from Conway and McKenzie (2007), shown below.

Number of oocysts per gram of excreta = (N /0.15) x volume x 0.3

where,

N = number of oocysts counted,

0.15 = volume of the McMaster counting chamber,

Volume = 45 ml of water that the excreta was soaked in, and

0.3 = correction for the original 3 g of excreta sample.

8.3.6.2. *In vitro* sporulation

In vitro sporulation was determined as described by Molan *et al*. (2009b). Briefly, excreta were incubated at 29 ^{o}C for 48 h. At the end of the incubation period, samples were twice washed with tap water and stored at 4 ^{o}C until counted. The numbers of unsportulated oocysts and those with two, three or four sporocysts were counted separately for *E. acervulina, E. maxima* and *E. tenalla*. Oocysts and sporocysts were viewed using phase contrast microscopy to better illustrate the morphology as oocysts were slightly flattened under the pressure of coverslip. Percentage sporulation was estimated by counting the number of sporoluated oocysts from a total of 100 oocysts. Oocysts with four sporocysts were considered sporulated and were used to estimate the percentage of sporulation.

8.3.7. Lesion scoring

On day 7 post-challenge (28 day post-hatch), all birds (challenged and unchallenged) were sacrificed by cervical dislocation. The intestine was immediately exposed by a ventral midline incision to macroscopically examine the intestinal mucosa *in situ* for lesion score as per the method illustrated by Conway and McKenzie (2007). This evaluation was based on a lesion scoring system (Johnson and Reid, 1970), which provided a numerical ranking of gross lesions using a discrete 5-step scale (0 = no lesions; 1 = mild lesions; 2 = moderate lesions; 3 = severe lesions; 4 = extremely severe lesions or death). Lesions were scored by an experienced veterinarian, who was blind to the treatments.

8.3.8. Gizzard weight

Before sacrificing on day 28, two birds from each cage were randomly selected for the measurement of gizzard and proventriculus weights after lesion scoring. These two birds were weighed prior to killing and, full gizzard and proventriculus were removed and weighed. Relative weight of gizzard, proventriculus and gizzard contents were expressed as g/kg body weight.

8.3.9. Statistical analysis

Cage means served as the experimental unit for the statistical analysis performance parameters. For the measurement of gizzard weight, individual birds were considered as the experimental unit. Oocyst yields were logarithmically transformed ($\log_{10}$) before analysis. Performance parameters during pre-challenge phase (1 to 21 days), oocyst count, *in vitro* sporulation rate and lesion score were analysed by one-way ANOVA, whereas performance parameters during post-challenge phase (21 to 28 days) and gizzard weight were analysed by two way ANOVA, using the general linear model, (SAS, 2004). Mortality was analysed, using binomial distribution, PROC GENMODE (SAS, 2004). When treatment effects were found significant, differences between treatment means were separated using least square difference test. Differences were considered statistically different at $P < 0.05$.

8.4. Results

8.4.1. Pre-challenge performance

The performance of broilers prior to the coccidial challenge are summarised in Table 8.2. No effects (P>0.05) of method of wheat inclusion were observed for weight gain and feed per gain. The method of wheat inclusion had significant (P<0.05) effect on feed intake. Feed intake of broilers fed diets containing WW, irrespective of the method of wheat inclusion, was lower than that of birds fed with diets contain GW. However, no differences (P>0.05) in feed intake were observed between the birds fed diets containing WW either pre- or post-pelleting.

Table 8.2. Influence of method of wheat inclusion on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broilers (1 to 21 post-hatch)[1]

Method of wheat inclusion	Weight gain	Feed intake	Feed per gain
GW	834	1211[a]	1.460
Pre-pellet WW	809	1156[b]	1.441
Post-pellet WW	803	1144[b]	1.438
SEM[2]	11.2	16.7	0.0124
Probability, P ≤	0.12	0.05	0.41

[a,b] Means in column not sharing a common superscript are significantly different (P<0.05).
[1] Each value represents the mean of twelve replicates (8 birds per replicate).
[2] Pooled standard error of the mean.

8.4.2. Post-challenge performance

Post-challenge performance of broilers (21 to 28 days post-hatch) is summarised in Table 8.3. During the 7 days post-challenge, no significant interaction (P>0.05) was observed between the method of wheat inclusion and coccidial challenge for weight gain, feed intake and feed per gain. Coccidial challenge had a significant (P<0.05) effect on all performance parameters. Irrespective of the method of wheat inclusion, challenged birds had significantly (P<0.05) reduced weight gains and feed intake, and higher (P<0.05) feed per gain compared to unchallenged birds.

The method of wheat inclusion significantly (P<0.05) affected the feed intake. Feed intake was higher in birds fed the GW diet as compared to those fed WW diets. However, feed intake was similar (P>0.05) between pre and post pellet WW groups.

Table 8.3. Influence of method of whole wheat inclusion and coccidian infection on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broilers (21-28 days post-hatch)[1]

Method of wheat Inclusion	Coccidial challenge	Weight gain	Feed intake	Feed per gain
GW	Unchallenged	594	926	1.617
	Challenged	398	807	2.654
Pre-pellet WW	Unchallenged	581	904	1.587
	Challenged	294	718	2.534
Post-pellet WW	Unchallenged	558	867	1.598
	Challenged	294	746	3.429
SEM[2]		43.0	25.4	0.35
Main effects				
Method of wheat inclusion				
GW		496	866[a]	2.135
Pre-pellet WW		432	811[b]	2.060
Post-pellet WW		426	807[b]	2.514
Coccidial challenge				
Unchallenged		578[a]	899[a]	1.601[b]
Challenged		325[b]	757[b]	2.872[a]
Probabilities, P ≤				
Method of wheat inclusion		0.21	0.05	0.38
Coccidial challenge		0.0001	0.0001	0.0001
Method of wheat inclusion x coccidial challenge		0.49	0.34	0.38

[a,b] Means in 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.

8.4.3. Mortality

The mortality during coccidial challenge as influenced by the method of wheat inclusion is presented in Table 8.4.

The method of wheat inclusion had a significant effect (P<0.05) on the mortality of challenged birds. In inoculated broilers, mortality of 17, 35 and 58% was recorded in GW, pre-pellet WW and post- pellet WW, respectively. No deaths or clinical signs of infection were observed in unchallenged groups in all three dietary treatments.

The odds ratio suggests that, following coccidial challenge, mortality of birds fed WW diets, irrespective of the method of inclusion, was significantly (P<0.05) higher as compared to those fed the GW diet. Challenged birds fed diets with pre- and post-pelleting inclusion of WW were four and ten times more prone to death, respectively, than those fed the diet containing GW. Birds fed the post-pellet WW diet were 6 times more likely to die as compared to those fed pre-pellet WW.

Table 8.4. Influence of method of wheat inclusion following coccidial challenge on the mortality (%) of broilers (7 days post-challenge)[1]

Method of Wheat inclusion	Mortality[2]	Odd ratio	
GW	-1.58 ± 0.388[c] (17.0 %)[3]	Pre-pellet vs. GW	3.88
Pre-pellet WW	-0.60 ± 0.302[b] (35.4%)	Post-pellet vs. GW	10.06
Post-pellet WW	0.35 ± 0.299[a] (58.6%)	Post pellet vs. pre-pellet	6.21
Probability, P ≤	0.0001		

[a,b,c] Means in column not sharing a common superscript are significantly different (P<0.05).
[1] No mortality was observed in unchallenged birds.
[2] Non-transformed least squares mean ± standard error.
[3] Values in parentheses refer to % mortality.

8.4.4. Relative weight of gizzard

The effects of method of wheat inclusion and coccidial challenge on the relative weight of proventriculus, gizzard and gizzard contents are shown in Table 8.5. Significant (P<0.05 to 0.001) interactions between the method of wheat inclusion and coccidial challenge were observed for these parameters.

The relative weight of the gizzard in challenged birds compared to unchallenged birds was similar (P>0.05) in the GW group, increased (P<0.05) in the pre-pelleting WW group and decreased (P<0.05) in post pelleting WW group.

The relative weight of gizzard contents was similar (P>0.05) in challenged and unchallenged birds fed the GW diet. However, challenged birds fed diets with WW had heavier (P<0.05) gizzard contents, irrespective of the method of inclusion.

The relative weight of proventriculus was similar in challenged and unchallenged birds fed the GW diet, but the weight increased (P<0.05) with pre-pelleting inclusion of WW and decreased (P<0.05) with post-pelleting inclusion of WW in challenged birds compared to unchallenged birds.

Table 8.5. Relative weight (g/kg body weight) of organs (proventriculus and gizzard) and gizzard contents of broilers as influenced by the method of whole wheat inclusion and coccidial challenge [1]

Method of wheat inclusion	Coccidial challenge	Proventriculus	Gizzard	Gizzard contents
GW	Unchallenged	3.49^{b}	7.46^{e}	1.64^{d}
	Challenged	3.45^{b}	8.69^{de}	2.20^{d}
Pre-pellet WW	Unchallenged	3.56^{b}	8.88^{d}	4.33^{c}
	Challenged	3.93^{a}	10.82^{c}	8.75^{b}
Post-pellet WW	Unchallenged	3.91^{a}	15.67^{a}	8.70^{b}
	Challenged	3.47^{b}	14.01^{b}	11.14^{a}
SEM[2]		0.145	0.452	0.672
Main effects				
Method of wheat Inclusion				
GW		3.47	8.08	1.92
Pre-pellet WW		3.75	9.85	6.54
Post-pellet WW		3.69	14.8	9.92
Coccidial challenge				
Unchallenged		3.66	10.67	4.89
Challenged		3.62	11.17	7.36
Probabilities, P ≤				
Method of wheat inclusion		0.14	0.0001	0.0001
Coccidial challenge		0.73	0.17	0.0001
Method of wheat inclusion x coccidial challenge		0.05	0.001	0.05

[a-e] Means in a column not sharing a common superscript are significantly different (P<0.05).
[1] Each value represents the mean of 12 birds.
[2] Pooled standard error of the mean.

8.4.5. Intestinal lesion scores

No lesions were observed in any segment of the gastrointestinal tract of unchallenged birds. The influence of the method of wheat inclusion during coccidial challenge on intestinal lesion scores of challenged birds is summarised in Table 8.6. Total lesion scores of the challenged birds were not influenced (P>0.05) by the method of wheat inclusion. Lesion scores in the duodenum and jejunum were lower (P<0.05) and the score in caeca was higher (P<0.05) in WW fed birds, irrespective of the method of inclusion, compared to those fed the GW diet.

Lesion scores in these segments were similar (P>0.05) between pre-and post- pelleting groups. Treatments had no effect (P>0.05) on the lesion scores in the ileum.

Table 8.6. Influence of method of wheat inclusion and coccidial challenge on intestinal lesion scores of broilers (at 7 days post-challenge) [1]

Method of Wheat inclusion	Lesion scores[3]				
	Duodenum	Jejunum	Ileum	Caecum	Total score
GW	1.59[a]	1.086[a]	0.041	2.90[b]	5.61
Pre-pellet WW	1.21[b]	0.795[b]	0.021	3.31[a]	5.33
Post-pellet WW	1.19[b]	0.606[b]	0.126	3.60[a]	5.53
SEM[2]	0.095	0.1110	0.0463	0.101	0.277
Probability, P ≤	0.05	0.05	0.27	0.001	0.76

[a,b] Means in a column not sharing a common superscript are significantly different (P<0.05).
[1] Number of birds in GW, pre-pellet WW and post pellet WW groups were 47, 48, and 46, respectively.
[2] Pooled standard error of mean.
[3] No lesions were observed in unchallenged birds.

8.4.6. Oocyst output

The influence of the method of wheat inclusion on oocyst output in broilers experimentally challenged with three species of *Eimeria* is presented in Table 8.7. No oocysts were detected in the excreta of unchallenged birds. Total oocyst output of challenged birds was influenced (P<0.05) by the method of wheat inclusion. Total oocyst output was higher (P<0.05) in birds fed WW, irrespective of the method of inclusion, compared to those fed the GW diet. However, total oocyst output was similar (P>.0.05) between birds fed diets with either pre- or post-peleting inclusion of WW. In general, oocyst counts of *E. acervulina*, *E. maxima* and *E. tenella* followed a pattern similar to that of total oocyst count.

Table 8.7. Influence of method of wheat inclusion and coccidial challenge on oocyst output (Log_{10} per g excreta) of broilers (at 7 days post-challenge) [1]

Method of wheat inclusion	Oocyst output[3]			
	E. acervulina	*E. maxima*	*E. tenella*	Total oocyst
GW	5.55[b]	3.94[b]	5.28[b]	5.74[b]
Pre-pellet WW	5.87[a]	4.36[ab]	5.55[a]	6.05[a]
Post-pellet WW	5.74[ab]	4.80[a]	5.57[a]	6.00[a]
SEM[2]	0.067	0.148	0.065	0.067
Probabilities, P ≤	0.05	0.05	0.05	0.05

[a,b] Means in a column not sharing a common superscript are significantly different (P<0.05).
[1] Each value represents the mean of three replicates per treatment.
[2] Pooled standard error of mean.
[3] No oocysts were detected in the excreta of unchallenged birds.

8.4.7. *In vitro* sporulation rate

Influence of method of wheat inclusion on i*n vitro* sporulation of *Eimeria* species is summarised in Table 8.8. No treatment effect (P>0.05) was observed on oocyst sporulation rate for any of the *Eimeria* species examined.

Table 8.8. Influence of method of wheat inclusion and coocidial challenge on *in vitro* sporulation rate (%) of broilers (at 7 days post-challenge)[1]

Method of wheat inclusion	Sporulation rate[3]		
	E.*acervulina*	E.*maxima*	E. *tenella*
GW	85.3	78.3	82.3
Pre-pellet WW	85.0	77.6	84.6
Post-pellet WW	84.3	77.6	82.0
SEM[2]	0.79	0.82	1.68
Probabilities, P ≤	0.68	0.80	0.51

[a,b] Means in a column not sharing a common superscript are significantly different (P<0.05).
[1] Each value represents the mean of three replicates.
[2] Pooled standard error of mean.
[3] No oocysts were detected in excreta of unchallenged birds.

8.5. Discussion

Pre-challenge period

During the pre-challenge period (0-21 days), weight gain and feed per gain were similar among GW and WW fed birds irrespective of the method of WW inclusion. This is in agreement with previous studies where no effect on weight gain and feed per gain was observed in birds fed WW in comparison to GW fed birds (Rose *et al*., 1995; Svihus *et al*., 2004a; Amerah and Ravindran, 2008). Feed intake in the present study, however, was lower in WW fed birds, irrespective of method of inclusion, compared to GW fed birds. This observation is consistent with previous studies (Rose *et al*., 1995; Plavnik *et al*., 2002; Wu *et al*., 2004; Wu and Ravindran, 2004). Hetland *et al*. (2002) speculated that the reduced feed intake in WW fed birds may be the result of satiety due to greater gizzard activity increasing nutrient digestibility.

Post-challenge period

The primary aim of the present study was to examine the effects of inclusion of WW either pre- or post-pelleting in broilers following coccidial challenge on the development of coccidiosis. The dose of mixed sporulated oocysts was chosen to establish an experimental coccidial challenge of medium pathology, causing around 10% mortality (Personal communication, Neil Christensen, Avivet Ltd) in order to mimick natural field conditions and emphasise dietary effects of feeding whole grain on coccidial infection. The three *Eimeria* species chosen, namely *E. acervulina*, *E. maxima* and *E. tenella*, are specific to different intestinal segments, the duodenum, jejunum and ileum, and caeca, respectively (Conway and McKenzie, 2007) were used.

The reductions in weight gain and feed intake, and increases in feed per gain and mortality due to the mixed *Eimeria* infection were as expected. Coccidial infections are known to cause disruption to the intestinal mucosa and result in nutrient malabsorption and reduced performance (Dalloul and Lillehoj, 2005). Although feeding WW had no negative effect on the performance of healthy broiler chickens (Cumming 1994; Gabriel *et al*., 2003a; Enberg *et al*., 2004), detrimental effects were observed during experimental coccidiosis in both fast-growing (Banfiled and Forbes, 2001; Banfield *et al*., 2002; Gabriel *et al*., 2003b, 2006) and slow-growing broilers (Gabriel *et al*., 2007).

The working hypothesis of the current study was that better gizzard development and increased grinding activity would destroy coccidial oocysts and the infective sporozoites inside them before reaching the site of infection, and that whole grain feeding which stimulated the gizzard may be used as an alternative strategy to control coccidiosis. Previous studies (Banfield *et al*., 2002; Engberg *et al*., 2004; Gabriel *et al*., 2003a,b; Wu and Ravindran, 2004; Amerah and Ravindran, 2008; Gabriel *et al*., 2008) have consistently shown that feeding broiler chickens with WW resulted in heavier gizzards relative to those fed GW diets, and it has been proposed that WW stimulates the development of gizzard and leads to greater grinding activity. Despite heavier gizzards in WW fed birds in the present study, higher mortality was also observed in these treatment groups. Surprisingly, the pattern of mortality in different dietary treatments paralleled changes in gizzard size. These finding indicate that, contrary to the working hypothesis, a well-developed gizzard seemed to increase the severity of coccidiosis infection.

Based on the mortality rate, it was evident that WW feeding aggravated the pathogenicity of coccidial parasites. Instead of being destroyed, it appears that the coccidian sporocysts/sporozoites are liberated from the oocysts in the gizzard (Farr and Doran, 1962; Ried 1978; Fernando, 1990) by the mechanical pressure applied from grinding which may exceed 585 kg/cm^2 (Cabrera, 1994). Based on the present findings, along with previous reports (Gabriel *et al*., 2003b; Gabriel *et al*., 2006; Gabriel *et al*., 2007), it has been suggested that the well-developed gizzard in WW birds may have exposed the introduced *Eimeria* oocysts to significant grinding destroying the tough oocyst walls and liberating the enclosed sporozoites, enabling them to penetrate the villous epithelial cells of the infection site. However, further studies are warranted to confirm the role of mechanical pressure of the gizzard in destroying the oocysts and the infective sporozoites inside them.

In addition to effects on the weight and grinding activity of the gizzard, previous studies have shown WW feeding lowers the pH of gizzard contents (Nir *et al*., 1994a; Gabriel *et al*., 2003a) and increases pancreas weight (Banfield *et al*. 2002, Wu *et al*., 2004, Enberg *et al*., 2004 and Gabriel *et al*., 2008). Increased pancreas weight may in turn lead to greater pancreatic protease secretion, which is known to contribute to the excystation process, an essential step for the pathogenicity of coccidial infection (Ikeda, 1955; Farr and Doran, 1962). Grinding in the gizzard also activates cholecystokinin release (Svihus *et al*., 2004a) which in turn stimulates secretion of pancreatic enzymes and gastro-duodenal reflux (Duke, 1992; Li and Owyang, 1993). These mechanisms may further facilitate the excystation process and consequently increase the severity of the disease (Guyonnet *et al*., 1989).

The results of the present study are in contrast with those of Cumming (1987, 1992 a,b) who found lower *E. tenella* oocyst shedding in challenged birds fed WW than their counterparts fed the same, but finely GW diet. This contradiction may be related to differences in the level of WW inclusion, which was 600-700 g/kg in the studies of Cumming as against 300 g/kg in the present study. Younger birds are more susceptible to coccidiosis (Gerriets, 1961) and the difference in the age birds at the time of inoculation may be another reason for the observed differences. In the present study, birds were inoculated at day 21 of age instead of 28 days of age in Cumming's studies. Current results also in contradiction with other previous studies, which reported no difference in occyst yield between WW and GW, fed birds challenged with *E.tenella* or *E. acervulina* (Banfield *et al*., 1998; Waldenstedt *et al*., 1998; Banfield and Forbes, 2001; Banfield *et al*., 2002). The diets used in the study of Banfield and Forbes (2001) contained 200 g/kg of maize and it has been reported that maize-based diets reacted less severely to *Eimeria* infection than wheat-based diets (William, 1992). The current results, however, agree with those of Banfield *et al*. (1999) and Gabriel *et al*. (2003) who found that feeding WW to birds increased coccidia development following challenge with E. acervulina and *E. tenella* as shown by higher oocyst count compared to GW fed birds.

The number of oocysts present in exceta confirms coccidial infection, but this has little relationship to the extent of clinical disease (Khan and Line, 2010). Lesion scoring is a more meaningful indicator of the pathogensity of *Eimeria* parasites (Yvore *et al*., 1980; Allen *et al*., 2000). In the present study, no difference in overall lesion score was observed between WW and GW fed birds on day 7 post-challenge. However, caecal lesion score was higher, whereas duodenal and jejunal lesion scores were lower in WW fed compared to GW fed bird. Gabriel *et al*. (2003b) also reported higher caecal lesion scores in birds fed WW and GW diets, but no differences were reported in the duodenal or jejunal lesion scores. These differences may be due to the difference in virulence of different Eimeria species or the amount of infective doses of sporulated oocysts.

8.6. Conclusions

The present findings do not support the hypothesis that WW feeding through better development of gizzard will destroy the oocysts prior to reaching their site of infection and, lower the incidence and severity of coccidiosis. On the contrary, coccidial challenged birds fed diets with pre-pelletting and post- pelleting inclusions of WW were likely to be four and ten times more likely to die, respectively, of acute coccidiosis than those fed the GW diet. It is speculated that the heavier gizzards in WW fed birds may have exposed the inoculated *Eimeria* oocysts to greater grinding which destroyed the tough oocyst walls and enhanced excystation, thus increasing the severity of coccidiosis infection in WW fed birds.

CHAPTER 9

General discussion

9.1. Introduction

Whole grain feeding has recently received increasing attention in the commercial poultry industry as a mean of lowering feed manufacturing cost. Feed is the single largest cost in poultry production and accounts for 65-70% of total costs Cereal grains are the major constituents of poultry feed and are ground prior to mixing with other feed ingredients to make a single homogeneous feed. Grinding of whole grains is the second largest energy cost after that of pelleting in broiler production (Reece *et al*., 1985) and probably the highest user of energy in layer production where feeds are not pelleted (Deaton *et al*., 1989). Thus, eliminating or reducing the mechanical energy consumption used for grinding whole grain could significantly lower the cost of feed manufacture.

There are only few studies on whole grains other than wheat. Moreover, published data on effects of whole wheat (WW) feeding on broiler performance have been contradictory, as the results are confounded by a number of factors, including differences in experimental methodology, variables such as type and quality of grain used, inclusion level of whole grain and feeding regime (Chapter 2).

Whole grain feeding is almost always associated with the development of the gizzard, which may directly or indirectly contribute to the beneficial effects of whole grains on bird performance, nutrient utilisation and gut health. Most previous studies have used WW. Feeding strategies such as pre-pelleting inclusion of whole maize or coarsely ground/cracked maize were considered to address the limitation of the relatively large size of the maize grain in broilers (Chapters 4 and 5) and layers (Chapter 6). Results of these feeding trials using graded inclusion (0, 150, 300, 450 and 600 g/kg) of whole or coarse/cracked maize showed that the impact on gizzard development and performance varied depending on the minimal processing techniques employed, amount of whole or coarse maize in the feed, and bird age.

The work reported herein also showed that pelleting diets with WW using larger pellet die diameter (4.76 vs. 3.0 mm) modified feed microstructure (i.e., particle size distribution within pellets) and this microstructure seemed to be have a greater impact than macrostructure (i.e., pellet dimension) on gizzard development and production performance. Proportion of coarse particle in pellets can be increased by increasing the amount of whole grain added pre-pelleting and die hole diameter (Chapters 4 and 7).

In addition, inclusion of whole and coarse maize (Chapters 4 and 5) and WW (Chapter 7) positively influenced the ecology of caecal microflora. However conversely, after a challenge with *Eimeria*, WW feeding exacerbated the severity of coccidiosis in broilers (Chapter 8).

9.2. Use of whole and coarse maize in broiler diets

As noted previously in this thesis, wheat is the grain of choice for whole grain feeding in poultry and little or no attempt has been made to use whole maize. The physical limitation imposed by the size of grain appears to be responsible for the lack of interest to use whole maize in poultry diets and no published data are available on the effects of whole maize inclusion in poultry diets. In this thesis, pre-pelleting inclusion of whole maize and feeding of coarsely-ground maize to broilers were tested as potential strategies to overcome the large size of the maize kernel. These alternatives resulted in gizzard development similar to that reported in previous studies with WW in broilers (Jones and Taylor, 2001; Gabriel *et al*., 2003a; Engberg *et al*., 2004; Amerah and Ravindran, 2008; Gabriel *et al*., 2008). Increasing pre-pelleting inclusion of whole maize, however, resulted in reduced feed intake which led to poor weight gain of broilers (Chapter 4). Reasons for decreased feed intake are unclear, especially since pellet quality, measured as PDI, improved with increasing inclusion of whole maize. Increasing inclusion of coarse maize in mash diets, on the other hand, resulted in improvements in feed intake and weight gain (Chapter 5). Similar results have been reported in broilers fed a mash diet containing coarse maize (Recce *et al*., 1985; Nir *et al*., 1994a), suggesting coarser grinding of maize could be a useful strategy to lower the cost of feed manufacturing.

9.3. Use of coarse maize in layer diets

While whole grain feeding is becoming a common practice in broiler production, this application has not been established in the layer industry and only a limited number of studies have been reported in laying hens. The reasons for the lack of interest in using whole grains in layers are not clear. First, it is possible that layers may be more tolerant to changes in feed form, as compared to broilers, having the capacity to adapt to various diet structures and composition by modulating digestive tract development. Second, layers come into production much later than broilers (20 vs. 4 weeks) and, by that age, the gizzard is fully mature with little possibility for further development.

Data presented in Chapter 6 showed that dietary inclusion of coarse maize had no adverse effects on production, energy utilisation and egg quality of laying hens. Gizzard size was not affected even though coarse maize diets were fed for a period of 24 weeks. As noted above, this lack of effect on gizzard development could be attributed to the age of birds. In adult birds, the gizzard is already well developed compared to that observed during the first month after hatch (Sturkie, 1965; Uni *et al*., 1999) and may not respond to changes in feed structure. Layers fed coarse maize diets performed similarly to those fed a ground maize diet and, these findings are encouraging and may be used to lower the cost of manufacturing layer feeds. Birds prefer to eat larger particles as they grow older (Forbes and Covasa, 195) and coarser maize in diets may allow them to express their natural ground picking behaviour and achieve satiety. This strategy may also be exploited to lower the incidence of feather pecking, a major welfare issue in layers, which develops as a substitute for normal ground pecking and feeding behaviour in the absence of adequate foraging incentives in commercial diets (Blokhuis, 1986).

Studies are warranted using pullets to further explore possible advantages of feeding coarse maize in layer production. Feeding whole grains to pullets may have a positive impact on gizzard development and could improve production performance in layers.

9.4. Method of whole wheat inclusion (pre- and post-pelleting)

In the majority of studies, WW was included after pelleting, and only a few (Jones and Taylor, 2001; Svihus *et al*., 2004a; Taylor and Jones, 2004a; Wu *et al*., 2004) have examined the effect of WW inclusion prior to pelleting on the performance of broilers fed wheat-based diets. Only one study (Wu *et al*., 2004) compared the influence of method of WW inclusion (pre- vs. post-pelleting) on the performance of broilers fed wheat based diets and reported post-pelleting inclusion resulted in improved weight gain (3.9%), feed efficiency (5.8%), AME (6%) and relative gizzard weight (73%) of broilers compared to pre-pelleting inclusion of WW. Pre- and post-pelleting methods are discussed in Chapter 2, section 2.1.2.2.1 and 2.1.2.2.2, respectively. Performance differences in pre-and post-pellet method of WW inclusion might be due to the fact that pelleting, has a physical impact on feed particles. Larger particles, in particular, are more prone to reduction in particle size due to frictional forces produced inside the pellet die (Svihus, 2004b).

The study reported in Chapter 7 examined the interaction between different methods of wheat inclusion (ground, pre- or post-pelleting WW inclusion) and pellet diameter (conventional 3.0 vs. 4.76 mm). The aim of including the larger pellet diameter was to minimise reduction in particle size of WW in ground and pre-pelleted diets. Pelleting of GW diets and 200g/kg inclusion of WW pre-pelleting resulted in coarser particles over 1mm in the larger pellet compared to the smaller diameter pellet. Examination of particle size distribution of pellet confirmed that particle size reduction of whole grains was minimised by increased pellet die diameter. However, significant interactions between the method of wheat inclusion and pellet diameter were observed for performance and gizzard development. Feeding large diameter pellets resulted in greater weight gain in birds fed GW and post-pellet diets. This response was reversed, however, in birds fed diets with pre-pelleting inclusion of WW, where increased pellet diameter resulted in decreased weight gain. These findings suggest that a larger diameter pellet is more beneficial if the grain is ground first or added whole post-pelleting, while when WW is added pre-pelleting, a smaller diameter pellet results in improved weight and feed per gain.

9.5. Particle size distribution in pelleted feeds

Grain particle size is more critical in mash feeds than in pelleted or crumbled feeds (Hamilton and Proudfoot, 1995; Peron *et al*., 2005). However, there has been sustained interest in studying the effects of constituent particle size in pelleted feeds with the idea that because pellets dissolve in the crop after consumption, effects of feed particle size are maintained after pelleting (Nir *et al*., 1995). Studies on pre-pelleting inclusion of whole grain are limited and results are inconsistent (see Chapter 2, section 2.1.2.2.1**).** Reported inconsistencies may be related, in part, to changes in particle size distribution following pelleting. Pelleting has a physical impact on feed particles and these changes seem to be influenced by the amount of whole grain (Chapter 4) and the pellet die diameter (Chapter 7). An important finding of this thesis is that the pelleting process changes the resultant particle size spectrum of feed and, therefore, the particle size distribution of feed before and after pelleting will be different.

9.6. Effect of particle size on pellet quality.

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. In addition, smaller particles lead to faster penetration of heat and moisture to the core of particles which results in better binding characteristics and higher pellet quality (Behnke, 2001; Moritz and Lilly, 2010). Thomas *et al*. (1998) postulated that coarser particles result in weak points which facilitate the breakage of pellets and decrease pellet quality, but there is little scientific evidence to support this view. Published data on the effect of feed particle size on pellet quality are equivocal. In most studies, feed particle size was measured prior to pelleting. Dozier (2003) suggested that maize particle size of 650-700 μm is required to achieve good quality pellets. It must be noted, however, pelleting can reduce the size of particles that constitute the intact pellet (Svihus *et al*., 2010b). Svihus *et al*. (2004b) determined the particle size of wheat-based diets before and after pelleting, and reported that the proportion of coarser particles decreased while that of fine particles increased as a consequence of pelleting process.

Size of particles within pellets after pelleting clearly had an effect on pellet quality. Data from Chapters 4 and 7 clearly show that pellet quality increased when the proportion of coarse particles (>1 mm) in pellets increased to 210 g/kg, and deteriorated above 300 g/kg. These findings indicated that the ratio of fine (<1 mm) to coarse particles (>1 mm) of 70:30 is an important factor in determining pellet quality. However, it is difficult to draw any definite conclusions from this work especially when the conflicting effects of pellet quality and feed intake on performance reported in Chapters 4 and 7 are considered. Benefits associated with pellet quality on broiler performance have been well documented (Calet, 1965; Doughlas *et al*., 1990; Nir *et al*., 1995; Jensen, 2000; Nir and Ptichi, 2001), and when pellet quality is reduced, performance is decreased due to reduced feed intake (Proudfoot and Sefton, 1978; Nir *et al*., 1995). Performance data in Chapter 7 agree with this, but in Chapter 4, feed intake and weight gain were reduced despite improved PDI. These observations question whether PDI measured using the Holmen Pellet Tester was a good measure of pellet quality when diets are pelleted with whole grains (as discussed in Chapter 4).

9.7. Whole grain feeding and gizzard development

Inclusion of whole grain in mash and post-pelleted diets has consistently increased gizzard weights (Plavnik *et al*., 2002; Hetland *et al*., 2003; Wu and Ravindran, 2004; Amerah and Ravindran, 2008). Results observed with inclusion of coarse maize in mash and WW post-pelleted diets in Chapters 4 and 7, respectively, agree with these findings. However, when whole grains were included pre-pelleting, effects were variable as reported in Chapter 7and by various other authors (Jones and Taylor, 2001; Svihus *et al*., 2004a; Taylor and Jones, 2004a; Wu *et al*., 2004). Pre-pelleting inclusion of WW using a small diameter pellet die (3.0 mm) had no effect on gizzard weight, whereas, when a large diameter pellet die was used

(4.76 mm), birds had heavier gizzards. These variable effects can be explained by the higher proportion of coarser particles (>1 mm) in larger diameter pellets after pelleting. Svihus (2011) recommended that at least 300 g/kg cereal particles should be larger than 1 mm in size in a diet to stimulate gizzard development. This is in agreement with the results reported in Chapter 7 where broilers fed a WW-based diet pelleted through the 4.76 mm die resulted in 330g/kg particles greater than 1 mm and increased gizzard weights, whereas the same diet pelleted through the 3.0 mm die resulted only in 210 g/kg particles over >1 mm and failed to show any effect on gizzard weight. However, in Chapter 4, broilers fed whole maize-based diets pelleted through a 3.0 mm die resulted in 160 g/kg particles greater than 1 mm showed increased gizzard weights. Therefore, it can be speculated that the effect of pre-pelleting inclusion of whole grains on gizzard development is dependent on the proportion of coarse particles persisting after pelleting, and that is indirectly dependent on amount and type of cereal grain, and pellet die diameter.

It is difficult to draw firm conclusions from the work reported in this thesis concerning the amount of whole grain required to enhance gizzard development, but it seems that gizzard development depends on a number of variables, including, method of whole grain inclusion, amount of whole grain, type of grain (wheat or maize), particle size within the pellet, diet form (mash or pellet), and age of birds.

9.8. Influence of whole grains on nutrient and energy utilisation

WW feeding is generally associated with an increase in gizzard size which has been suggested to result in enhanced grinding activity and stimulation of pancreatic enzyme secretion and gastro-duodenal reflux (Duke, 1992; Li and Owyang, 1993). This serves to re-expose the digesta to pepsin, enhance mixing of digesta with pancreatic enzymes, and improve nutrient digestion and AME (Ferket, 2000; Hetland *et al*., 2002; Ravindran *et al*., 2006). Data from Chapter 7 showed that, irrespective of the method of WW inclusion (pre-or post-pelleting), AME and starch digestibilities were improved in WW fed birds. Since gizzard size differed depending on the method of inclusion, the data suggested that improvements in nutrient utilisation cannot always be explained on the basis of a larger gizzard weight. Increased pancreas weight may also be involved (Tables 9.1 and 9.2). However, data from Chapter 4 clearly showed that gizzard development and AME increased up to 300 g/kg whole maize inclusion and then levelled off, while ileal starch and nitrogen digestibility continued to increase with whole maize inclusions of up to 600g/kg. The data presented in Chapter 5 showed that the AME was not influenced up to 300 g/kg coarse maize inclusion and then decreased with further inclusion. No explanations can be provided for this decrease in AME at higher inclusion levels. Previous studies with WW have similarly produced inconsistent results. Inclusion of WW at levels of 100 to 375 g/kg improved AME (Preston *et al*., 2000; Svihus *et al*., 2004a; Wu *et al*., 2004), but, inclusion levels of 400 to 500 g/kg failed to show any effect (Uddin *et al*., 1996; Svihus *et al*., 2004a).

Regression analysis equations to predict relationship between gizzard weight, pancreas weight, nitrogen digestibility, starch digestibility and AME in broilers fed diets containing whole maize and whole wheat are presented in Tables 9.1 and 9.2., respectively. Gizzard weight has more pronounced effect on nitrogen and starch digestibility in whole wheat fed bird in comparison to whole maize fed broilers. Increases in pancreas weights may be an important contributing factor for the improvements observed in the AME and, nitrogen and starch digestibility in broilers fed whole maize.

To explain how whole grain feeding and gizzard development interact and increase nutrient utilisation, it may be hypothesised that gizzard development occurs in two stages. The first stage is typified by gizzard development without concomitant increases in gizzard muscle thickness and weight. This results in increased gizzard efficiency via increased grinding frequency, gastro-duodenal reflux and pancreatic enzyme secretion, thus improving nutrient digestibility. The second stage is characterised by increased muscle thickness and resulting increased gizzard weight. This occurs in response to whole grain in the diet, where the gizzard enlarges in order to cope with the increased mechanical load placed on it to grind larger particles. The degree of gizzard development varies depending on a number of factors discussed in section 9.7. However, there seems to be an upper limit to gizzard development and once this is reached, additional whole grain is not utilised as efficiently. Once the limit to gizzard development is exceeded, grinding efficiency starts to decrease with further whole grain addition. Higher levels of nutrient digestibility may be maintained up to a threshold, but then it starts decreasing. Well planned studies, however, are warranted to confirm this hypothesis.

Table 9.1. Regression analysis to predict relationships between gizzard weight (G-WT), pancreas weight (PAN-WT), nitrogen digestibility (N), starch digestibility (S) and AME in broilers fed whole maize

X value	Y value	No[1]	Regression equations	P value	R^2	
WMI[2]	GWT	10	G-WT =13.17+0.1291*WMI-0.000014*WMI^2	0.018	68.4	Quadratic regression
G-WT	N	10	N= -177 + 14.3* G-WT	0.161	23.0	Linear regression
PAN-WT	N	10	N= 222 – 78.1*PAN-WT	0.000	88.8	Linear regression
G-WT, PAN-WT	N	10	N= 85.2 + 8.24*G-WT – 72.8*PAN-WT	0.000	96.1	Multiple regression
G-WT	S	10	S= -223 + 18.0*G-WT	0.153	23.7	Linear regression
PAN-WT	S	10	S = 275 – 96.6*PAN-WT	0.000	87.1	Linear regression
G-WT, PAN-WT	S	10	S= 100 – 89.8*PAN-WT + 10.5*G-WT	0.000	96.2	Multiple regression
G-WT	AME	10	AME= 12.0 + 0.185*G-WT	0.166	22.5	Linear regression
PAN-WT	AME	10	AME = 17.0 – 0.917* PAN-WT	0.002	71.2	Linear regression
G-WT, PAN-WT	AME	10	AME = 15.1 + 0.115*G-WT – 0.843*PAN-WT	0.004	79.5	Multiple regression

[1] Means of pre-pellet inclusion of whole maize (Chapter 4) and inclusion of cracked maize (Chapter 5) in broilers.
[2] Whole maize inclusions.

Table 9.2. Regression analysis to predict relationships between gizzard weight (G-WT), pancreas weight (PAN-WT), nitrogen digestibility (N), starch digestibility (S) and AME in broilers fed whole wheat

X value	Y value	N[1]	Regression equations	P value	R^2	
G-WT	N	6	N = 0.767 + 0.00478*G-WT	0.000	63.2	Linear regression
PAN-WT	N	6	N= 0.664 + 0.0848*PAN-WT	0.089	55.6	Linear regression
G-WT, PAN-WT	N	6	N= 0.731+ 0.00355*G-WT + 0.0271*PAN-WT	0.210	64.6	Multiple regression
G-WT	S	6	S = 0.785 + 0.0152*G-WT	0.000	63.2	Linear regression
PAN-WT	S	6	S = 0.473 + 0.261*PAN-WT	0.105	52.1	Linear regression
G-WT, PAN-WT	S	6	S= 0.717 + 0.052*PAN-WT + 0.0128*G-WT	0.218	63.8	Multiple regression
G-WT	AME	6	AME= 13 + 0.0788*G-WT	0.334	23.2	Linear regression
PAN-WT	AME	6	AME = 10.5 + 1.84*PAN-WT	0.214	35.2	Linear regression
G-WT, PAN-WT	AME	6	AME = 10.2- 0.018*G-WT + 2.14*PAN-WT	0.518	35.5	Multiple regression

[1] Means of inclusion of WW in broilers (Chapter 7).

9.9. Whole grain feeding and gut microflora ecology.

Globally, the ban on antibiotic growth promoters (AGP) has encouraged nutritionists to explore alternative feed management strategies to improve the gut health of broilers. Manipulation of gizzard development through feeding of whole grains may be one strategy that can be used to influence microflora ecology. The effect of whole grain feeding on gut microflora composition in broiler chickens has not been widely studied (Enberg *et al*., 2004). No studies on the effect of whole maize are available. Furthermore, most studies examining the effect of WW feeding on microbial ecology of the digestive tract have used media culture-based techniques. However, these techniques are not only time consuming and labour intensive (Tannock. 2002), but also often selective, particularly for fastidious bacteria. Therefore, the majority of bacteria in many environments fails to grow under media culture conditions (Wagner *et al*., 1993; Apajalhiti *et al*., 2004). In this thesis, fluorescence in situ hybridisation (FISH), a culture-independent molecular technique, was used to determine the effect of whole grain feeding on caecal microbial communities in broiler chickens. This molecular-based approach has many advantages over culture-based methods, such as rapidity and sensitivity (Moter and Gobel, 2000; Zhu and Joerger, 2003). Inclusion of whole or coarse maize (Chapters 4 and 5) and WW (Chapter 7) resulted in decreased populations of harmful bacteria, namely *Clostridium*, *Campylobacter* and *Bacteroides*. As discussed in Chapter 5, two theories have been proposed to explain these effects on gut microflora profile. First, the stimulation of gizzard development and increased secretion of hydrochloric acid into the gizzard may have an antimicrobial effect on harmful pathogenic bacteria entering the distal part of gastrointestinal tract (Naughton and Jensen, 2001; Engberg *et al*., 2002). Second, whole grain feeding may encourage colonisation by commensal bacteria, thus discouraging colonisation by harmful bacteria through competitive exclusion (Bejerrum *et al*., 2005; Santos *et al*., 2008). The present findings lend support to the conclusion that the gizzard has an important function as a barrier organ that prevents pathogenic bacteria from entering the distal digestive tract (Bjerrum *et al*., 2005). The results of the present studies are promising, suggesting that whole grain feeding not only has the potential to be used in AGP programmes, but also has practical implications in food safety. Intestinal pathogenic bacteria such as *E. coli*, *Clostridium* species, *Campylobacter* species, and *Bacteroides* species contaminate poultry carcasses during processing in slaughter houses and represent an important cause of foodborne illnesses in humans (Oosterom, 1991; Rasschaert *et al*., 2008).

9.10. In vitro excreta gas production

Although gas production in fresh manure is not a serious problem, storage of manure leads to production of gases mainly by anaerobic bacteria (Canada Animal Manure Management Guide, 1979). Four gases, namely hydrogen sulphide (H_2S), carbon dioxide (CO_2), ammonia (NH_3) and methane (CH_4), are considered to be the most dangerous. Accordingly, strategies are needed to reduce atmospheric emissions of volatile organic compounds from poultry manure that cause odour and also to reduce the risk of releasing disease-transmitting vectors and airborne pathogens (Laubach, 2006). Anaerobic incubation of the droppings from birds fed coarse maize (Chapter 5) and WW (Chapter 7) resulted in a decrease in in vitro gas production. The lower gas production in these studies provides further confirmation of the efficacy of these dietary treatments to lower the populations of harmful pathogenic bacteria. An important finding is that treatment effects on gas production followed a pattern almost similar to the counts of *Clostridium* species. The positive association between the gas production and the populations of clostridia may be related to the fact that these bacteria are hydrogen producers (Skillman *et al*., 2009). It has been found that, in birds infected with *Clostridium perfringens*, the intestine is friable and distended with gas and gross lesion caused by toxins (Broussard *et al*., 1986; Porter, 1998). Identification of the gases produced by caecal bacteria and also the bacterial species responsible for the production of these gases is warranted.

9.11. Whole grain feeding and coccidiosis.

Coccidiosis is an economically important protozoan disease in poultry, whose prevention and control rely predominantly on use of chemotherapeutic anticoccidial drugs. However, some resistance to all currently available coccidiostats has been reported (Chapman, 1997). Furthermore, consumer concerns regarding public health and food safety and possible legislation by the European Union to phase out use of coccidiostats as feed additives in Europe by 31 December 2012 (COM, 2008; Regulation 1831/2003/EC) are other reasons behind the need to find new and effective ways of controlling coccidiosis. The aim of the study reported in Chapter 8 was to test the hypothesis that better gizzard development would destroy coccidial oocysts and the infective sporozoites inside them before reaching the site of infection, and that whole grain feeding which stimulates the gizzard may be used as an alternative strategy to control coccidiosis. Broilers were experimentally challenged with mixed sporulated oocysts (*E. acervulina*, *E. maxima* and *E. tenella)* and fed GW or WW either pre-or post-pelleting. Reduced weight gain and feed intake, and increased feed per gain in *Emeria* challenged birds in comparison to unchallenged birds, in each of the respective treatment groups, were as expected. However, mortality in challenged birds was highest in those fed the WW post-pelleting diet, followed by pre-pelleting WW and GW. Surprisingly, the pattern of mortality in different dietary treatments paralleled changes in gizzard size, suggesting that gizzard development due to WW feeding aggravated the pathogenicity of coccidial parasites. Instead of being destroyed, it seems that the coccidian sporocysts/sporozoites were liberated from the oocysts in the gizzard (Farr and Doran, 1962; Ried 1978; Fernando, 1990) by the mechanical pressure applied from grinding. Previous reports (Gabriel *et al*., 2003; Gabriel *et al*., 2006; Gabriel *et al*., 2007) also suggested that well-developed gizzards in WW birds may have exposed the introduced *Eimeria* oocysts to significant grinding which destroyed the tough oocyst walls and liberated enclosed sporozoites, which then penetrated the villous epithelial cells of the infection site. Furthermore, WW facilitated the excystation process and consequently increased the severity of disease (Guyonnet *et al*., 1989). In contrast, Cumming (1987;1992a,b) reported beneficial effects of WW based on decreased oocyst shedding, while others failed to show such effects and reported no difference in occyst yield between WW and GW fed birds challenged with *Eimeria* (Banfield *et al*., 1998; Waldenstedt *et al*., 1998; Banfield and Forbes, 2001; Banfield *et al*., 2002). The number of oocysts present in excreta confirmed coccidial infection, but this has little relationship to the extent of clinical disease (Khan and Line, 2010). Conflicting results may be related to differences in the methodology employed in different studies, including dose, species and strains of coccidia, age and strain of birds, age of introduction of WW, amount of WW fed, and age of inoculation.

Studies are warranted to confirm the role of mechanical pressure in destroying oocysts and infective sporozoites inside them, and to substantiate the role of the gizzard in coccidial pathogenicity. Other than gizzard development, coccidial pathogenicity was influenced cereal type. *E. tenella* was more pathogenic in chickens fed wheat-based diets than those fed maize-based diets probably due to the effects of soluble arabinoxylans or micronutrients (William, 1992). Studies are warranted to confirm these results using maize-based diets and to study cereal type interaction on coccidiosis development.

9.12. Summary and main conclusions

The work reported in thesis identified several factors that contribute to the inconsistent results observed with WW feeding. These include method of whole grain inclusion, amount of whole grain fed, pellet diameter, and feed particle size distribution in pellets. Alternative feeding strategies to utilise whole maize to overcome the limitation of its kernel size were also tested. Ground maize in broiler and layer diets could be completely replaced by coarsely ground maize with no adverse effects of bird performance. The practice of feeding highly processed cereal grains to broilers not only results in high feed manufacturing cost, but also has negative effects on gizzard size and functionality which may result in decreased feed utilisation. Thus, feeding coarsely ground maize is an economical and beneficial strategy to overcome the limitations imposed by the size of maize grain in broilers and layers. Pre-pelleting inclusion of whole maize, on the other hand, resulted in poor broiler performance and was not a useful approach.

Pellet diameter and size spectrum of particles in pellets influenced broiler performance depending on the method of WW inclusion. Smaller diameter pellets were found to be beneficial with pre-pelleting inclusion of WW and larger diameter pellets were better when WW was added post-pelleting. The pelleting process itself changed the particle size spectrum of feed before pelleting and resulted in a different particle size spectrum after pelleting. This microstructure (feed particle size distribution), which is dependent on a number of variables, such as grain type, amount of whole grain and pellet die diameter, seemed to be a significant factor in gizzard development and functionality, and thus, performance of birds.

Fluorescence in situ hybridisation as a culture-independent molecular technique was used to investigate the effects of whole grain feeding on caecal microbial communities in chickens. Inclusion of whole or coarse maize and WW resulted in increased gizzard size and decreased populations of harmful bacteria, namely *Clostridium*, *Campylobacter* and *Bacteroides*. These observations are suggestive of the barrier function of the gizzard that prevents pathogenic bacteria from entering the distal digestive tract. Thus, dietary inclusion of whole or modified grain could be a strategy for use in AGP-free diets, and also has practical implications for food safety. The hypothesis that enhanced gizzard development associated with WW feeding would result in the destruction of oocysts prior to reaching their site of infection and thus lower the incidence and severity of coccidiosis was not supported by findings in this thesis. On the contrary, coccidia-challenged birds fed diets with pre-pelleting and post-pelleting inclusion of WW were four and ten times more likely, respectively, to die of acute coccidiosis than those fed the GW diet. Whole wheat feeding exacerbated the severity of coccidiosis infection, possibly via a mechanism involving enhanced gizzard development.

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